

An Abattoir Survey of Pneumonia and Pleuritis in Slaughter Weight Swine From 9 Selected Herds. IV. Bacteriological Findings in Chronic Pneumonic Lesions

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Høie S, K. Falk and BM Lium: An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. IV. bacteriological findings in chronic pneumonic lesions. Acta vet. scand. 1991, 32, 395-402. - A total of 855 pig lungs were collected at slaughter and evaluated macroscopically. Bacteriological examinations were carried out on tissue samples from chronic pleuropneumonic lesions (n = 196) and from chronic bronchopneumonic lesions with suppuration (n = 14). Samples from normal lung tissue (n = 22) were also included. *Pasteurella multocida* was isolated from 54%, *Actinobacillus (Haemophilus) pleuropneumoniae* from 11%, and *Streptococcus spp.* from 14% of the pneumonic lesions, respectively. From normal lung tissue *P. multocida* was isolated from 3 (14%) of the samples, *A. pleuropneumoniae* was not recovered and streptococci were isolated from only 1 (5%) of these samples. The above mentioned bacterial species were recovered either in pure cultures or mixed with various other microbes. A total of 109 *P. multocida* strains were further characterized by capsular serotyping and testing for production of dermonecrotic toxin. Ninety-nine (91%) of the strains were capsular type A 10 (9%) were type D. Out of the type A and the type D strains 94% and 90% were toxigenic, respectively. Most of the *A. pleuropneumoniae* strains were serotype 2. Strains of serotypes 1 and 7 were also identified. The majority of the streptococci were identified as either *Streptococcus suis* or *Streptococcus dysgalactiae*. *Actinomyces pyogenes* was isolated from 14% of the lesions and anaerobic bacteria from 18%, respectively. The significance of the various bacterial species in relation to the development of chronic pneumonic lesions is discussed. Special attention is paid to *P. multocida*, and it is concluded that this bacterial species is probably of importance for the development of both types of chronic pneumonias.

lungs; bacteria; *Pasteurella multocida*.

Introduction

A number of bacterial species and several different mycoplasmas can be demonstrated in pneumonic lungs of slaughter weight swine (Gois *et al.* 1980, Osborne *et al.* 1981, Morrison *et al.* 1985, Falk *et al.* 1991). *Pasteurella multocida* is frequently isolated, while streptococci and *Actinobacillus (Haemophilus) pleuropneumoniae* are not so commonly found.

Based on capsular antigens *P. multocida* has been differentiated into various types. Capsular type A strains of *P. multocida* are much more commonly isolated from pneumonic pig lungs than type D strains (Pijoan *et al.* 1984).

Dermonecrotic toxin produced by some strains of *P. multocida* seems to be an important etiological factor in the development of atrophic rhinitis (Rutter & Mackenzie

1984). On the other hand, the significance of the dermonecrotic toxin in relation to pig pneumonia is not definitely known, but an overweight of toxigenic strains of *P. multocida* have been found in necrotic types of pneumonia (Bækbo 1986). Nevertheless, neither the toxigenic nor the nontoxigenic strains are considered primary lung pathogens (Gois et al. 1980, Morrison et al. 1985, Bækbo 1986).

Pleuropneumonia of the pig is a clearly defined pathologic entity, and it is generally agreed that *A. pleuropneumoniae* is the causative agent (Nicolet 1986). However, the isolation rate of *A. pleuropneumoniae* from chronic pleuropneumonic lesions is low even when special techniques are applied (Willson et al. 1987).

Streptococci of many serological groups are common in the respiratory tract of swine (Sanford & Ross 1986). However, certain streptococci like *Streptococcus suis* seem to deserve more attention than the other ones in relation to pneumonia. According to Erickson et al. (1984) purulent meningitis and bronchopneumonia are the two most common conditions associated with *S. suis*. In a slaughter house survey of pig lungs originating from 9 selected herds in the south-eastern part of Norway, gross lesions were found in 84% of the pig lungs, varying in frequencies from 37% in the least affected to 97% in the most affected one (Lium & Falk 1991). The microbial flora demonstrated in lesions indicative of enzootic pneumonia from pigs in these herds is described elsewhere (Falk et al. 1991). The present paper describes the bacterial flora demonstrated in samples from other lesions of these lungs with special reference to chronic pneumonic conditions. Special attention was paid to *P. multocida*, *A. pleuropneumoniae*, and *Streptococcus spp.* The capsular types of the *P. multocida* strains were identified and

their ability to produce dermonecrotic toxin was tested.

Materials and methods

Samples

A total of 855 lungs were collected at slaughter and evaluated macroscopically. Tissue samples for bacteriological examination were collected from normal lung tissue (n = 22), and from 2 categories of chronic pneumonic lesions; chronic pleuropneumonia including local fibrosis (pleuropneumonia) (n = 196) and chronic bronchopneumonia with suppuration (n = 14). The samples from healthy lung tissue were collected from the dorsal part of the caudal lobes. The 210 samples from chronic pneumonic lesions were taken from 183 pigs.

Cultivation procedures

All the primary seedings for the isolation of bacteria were done on the day of slaughter. After heat sterilization of the lung surface, a cut was made into the pneumonic tissue and a smear from the cut surface was inoculated into each of 2 blood agar (BA) plates [heart-infusion agar (Difco, Detroit, USA) with 5% bovine blood], and onto 1 bromthymol blue lactose sucrose agar (BLSA) plate (National Veterinary Institute, Oslo, Norway), and 1 Centers for Disease Control anaerobe blood agar plate (CDC) (Lennette 1980).

One BA plate and the BLSA plate were incubated aerobically. The other BA plate was streak-inoculated with a β -toxin producing *Staphylococcus aureus* as nursing strain, and incubated in a 10% CO₂ atmosphere. The CDC plate was incubated anaerobically (Gas generating box "H₂ + CO₂", bio-Mérieux, Charbonnières les Bains, France). All plates were left at 37°C and examined after 24 and 48 h.

Diagnostic procedures

Routine procedures for bacteriological diagnostic examination were followed. The occurrence of different bacteria was recorded as abundant, moderate, or sparse by the same evaluator. Sparse, concurrent growth of various bacterial species was recorded as an insignificant bacterial flora. In cases of abundant or moderate growth of 1 or more bacterial species, additional sparse occurrence of other bacteria was not considered important enough for recording.

Bacterial species were identified according to Bergey's manual of systematic bacteriology (Holt 1984, 1986) mainly with standard methods.

All except 5 *P. multocida* strains were characterized to the subspecies level as proposed by Mutters *et al.* (1985) employing fermentation of dulcitol and sorbitol as main diagnostic tests. The 5 *P. multocida* strains were identified as such on the basis of morphological and olfactorial characteristics. The capsular types A and D of *P. multocida* were identified by the hyaluronidase test (Carter & Rundell 1975) and the acriflavine flocculation test (Carter & Subronto 1973). The acriflavine test was slightly modified as colony masses from BA plates were added to the acriflavine solutions on slides (Bækbo 1986) and examined in a stereo microscope. The isolates were tested for toxin production by the embryonic bovine lung (EBL) cell culture assay (Rutter & Luther 1984).

A. pleuropneumoniae strains were identified serologically by the rapid slide agglutination test with 6 h mucoid colonies grown on modified PPLO agar (Nicolet 1971). Hyperimmune sera against serotypes 1 through 10 were produced in rabbits as described by Nielsen & O'Connor (1984). Antigens for the production of hyperimmune sera were made from strains, representing serotypes 1

through 10, kindly provided by Dr. R. Nielsen, National Veterinary Laboratory, Copenhagen, Denmark.

The *Streptococcus spp.* were characterized biochemically, serogrouped according to the Lancefield system (Lancefield 1933) and tested on the API 20 Strep system (La Balme Les Grottes, Montalieu-Vercieu, France) according to manufacturer's instructions. *S. suis* was identified according to the description by Kilpper-Bälz & Schleifer (1987) and *Streptococcus porcinus* as described by Collins *et al.* (1984).

Cultures appearing after anaerobic incubation were also tested for aerobic growth abilities and characterized by micromorphology after Gram staining.

Statistic al analysis

For statistical analysis the chi-square test for comparison of frequencies was performed using standard microcomputer software (SAS Institute Inc. 19xx).

Results

P. multocida was isolated from 54%, *A. pleuropneumoniae* from 11%, and *Streptococcus spp.* from 14% of the pneumonic lesions, respectively. From normal lung tissue *P. multocida* was isolated from 3 (14%) of the samples, *A. pleuropneumoniae* was not recovered, and *Streptococcus sp.* was isolated from only 1 (5%) of these samples.

The results of the bacteriological examination of the samples from normal lung tissue and of the 2 categories of lesions are summarized in Table 1.

P. multocida

P. multocida was isolated from 114 of the pneumonic lesions. It appeared in pure culture from 57 lesions and in mixed culture from the other 57 lesions. The following

Table 1. Bacterial isolates from normal lung tissue and from 2 categories of chronic pneumonic lesions in slaughtered swine.

Bacteria identified	Normal tissue (n = 22)	Type of lung lesions	
		Pleuro-pneumonia (n = 196)	Chronic bronchopneumonia with suppuration (n = 14)
<i>P. multocida</i>	3	102	12
<i>A. pleuropneumoniae</i>		23	1
<i>Streptococcus</i> spp.	1	27	3
Anaerobic bacteria		31	7
<i>A. pyogenes</i>		24	5
<i>S. aureus</i>		3	
Others (see text)	1	14	

bacterial species were detected together with *P. multocida*: *Streptococcus* spp. (25/57), *Actinomyces pyogenes* (25/57), *A. pleuropneumoniae* (3/57), unidentified coryneform bacteria (3/57), *Aeromonas hydrophila* (2/57), anhemolytic *Staphylococcus* sp. (1/57), *Escherichia coli* (1/57), *Proteus* sp. (1/57), *Micrococcus* sp. (1/57) and anaerobic bacteria (24/57).

Capsular serotyping of 109 strains of *P. multocida* showed that 99 (91%) were type A and 10 (9%) were type D. In 2 pigs capsular types A and D were both found in different lesions of the same lungs.

Altogether 93 out of 99 (94%) capsular type A strains and 9 out of 10 (90%) type D strains were toxigenic.

P. multocida was isolated in sparse culture from normal lung tissue from 3 pigs. Two strains were capsular type A and 1 was capsular type D. The 3 strains were all toxigenic. Two strains did not produce acid from sorbitol and were consequently identified as *P. multocida* subsp. *septica* according to *Mutters et al.* (1985). All the other biochemically examined strains produced acid from sorbitol and were identified as *P. multocida* subsp. *multocida*. One of these strains did not produce indol.

A. pleuropneumoniae

A. pleuropneumoniae was isolated from 24 of the lesions; in pure culture from 15 and in mixed culture from 9 lesions. The bacteria found together with *A. pleuropneumoniae* were: *P. multocida* (3/9), *Streptococcus* spp. (5/9), *A. pyogenes* (2/9), *Klebsiella pneumoniae* (1/9), and anaerobic bacteria (4/9). Serotyping of the *A. pleuropneumoniae* strains gave the following results: Nineteen of the 24 isolated strains proved to be serotype 2. Two strains were serotype 1 and 1 strain belonged to the serotype 7. One strain seemed not typeable and 1 was not tested.

Streptococcus spp.

The *Streptococcus* spp. were detected in mixed cultures in all but 2 cases demonstrated. The majority of the strains were identified either as *S. suis*. (10 strains) or *S. dysgalactiae* (9 strains). One streptococcal strain identified as *S. porcinus* was recovered from a pleuropneumonic lesion as part of a mixed culture. Eight strains were not identified on the species level.

Other bacteria

A. pyogenes was isolated from 29 (14%) of the pneumonic lesions and always in mixed

culture. Unidentified coryneform bacteria were demonstrated in 5 lesions and also always in mixed culture. *Staphylococcus aureus* always appeared in pure culture (3 isolates).

Anaerobic bacteria were demonstrated in 38 (18%) of the lesions, but not in samples from normal lung tissue. The anaerobic flora was mixed and consisted of Gram negative and Gram positive rods, and Gram positive cocci. The bacterial flora of only 1 lesion comprised only anaerobic bacteria, those being Gram negative rods and Gram positive cocci.

Other bacterial species demonstrated, were *Bacillus cereus* and various unidentified Gram negative aerobic cocci.

No growth of bacteria/insignificant bacterial flora

In normal lung tissue either no growth or an insignificant bacterial flora were demonstrated in 4 (18%) and 13 (59%) samples, respectively. No growth of bacteria was recorded in 13 (6%) of the pneumonic lesions and insignificant bacterial flora was found in 45 (21%) of the cases. Bacterial species that were detected in samples with insignificant bacterial flora, were among others, *P. multocida*, *Streptococcus spp.*, *A. pyogenes*, *E. coli*, *A. hydrophila*, *Proteus sp.*, and anaerobic bacteria. *A. pleuropneumoniae* was not detected in such samples.

Discussion

P. multocida was the bacterial species most frequently isolated from the chronic pneumonic lesions. Despite major differences in the studied materials, similar results were obtained by Osborne *et al.* (1981), Morrison *et al.* (1985), and by Falk *et al.* (1991). Those studies included mainly lesions »typical« of enzootic pneumonia. This might indicate that infection with *P. multocida* plays a sig-

nificant role in different categories of pneumonia.

Altogether 90% of the *P. multocida* strains isolated from chronic pneumonic lesions were identified as capsular type A and 10% as type D. In a study of catarrhal bronchopneumonic lesions reported by Falk *et al.* (1991) 96% belonged to type A and 4% to type D. Pijoan *et al.* (1983, 1984) reported 88% A and 12% D, and 97% A and 3% D from 2 different investigations, respectively. All these results strongly suggest that *P. multocida* capsular type A may be considerable more important than type D in connection with pneumonic lesions. This might be due to the large hyaluronic acid capsule in the type A strains, interfering with the phagocytosis by alveolar macrophages, as proposed by Pijoan *et al.* (1984).

In a study reported by Osborne *et al.* (1981), the isolation rate of *P. multocida* from 1 cm³ ground tissue originating from normal lung was 16%. This is in good agreement with the present study, as *P. multocida* was isolated in pure culture from 14% of the samples from normal lung tissue. These 3 strains were not different from the other *P. multocida* strains as characterized in the present study.

In the present study 94% of the *P. multocida* type A strains and 90% of the type D strains were toxigenic. Kielstein & Schimmel (1986) demonstrated toxin production in 70% of the lung strains. In the report of Bækbo (1986) the proportion of toxigenic strains was 24% even with the same method for demonstration of toxigenic ability as used in the present study. These varying proportions of toxigenic strains might be due to differences in the types of pneumonia and possibly unidentified environmental factors related to the herds, as suggested by Høie *et al.* (1990).

The low frequency of *A. pleuropneumoniae*

isolates from pleuropneumonic lesions in our investigation might be due to the fact that most of the lung samples in our material were from fibrotic lung lesions with no or very small areas of necrotic, suppurative tissue. The high frequency of *P. multocida* may also explain the low frequency of *A. pleuropneumoniae* as *P. multocida* may overgrow and mask *A. pleuropneumoniae* on BA plates.

However, the results may also indicate that *P. multocida* is essential for the development of the pleuropneumonic lesions initiated by *A. pleuropneumoniae*, thereby increasing the severity and duration of the condition. This view is supported by the serological findings of the same pigs reported elsewhere by Falk & Liim (1991). They found a striking lack of correlation between the presence or not of pleuropneumonia in individual animals, and the finding or not of antibodies to *A. pleuropneumoniae* serotype 2 in the corresponding serum samples.

The first published isolation of *S. suis* strains in Norway was done by Falk et al. (1991). A further characterization of those strains and the *S. suis* strains of the present study has been carried out by Høie et al. (1990).

The isolation of *S. porcinus* in the present study represents the first reported isolation of this species in Norway. However, Koppang & Filseth (1958) isolated *Streptococcus* sp. group E from semen of a boar.

A. pyogenes, *S. aureus*, and anaerobic bacteria were also demonstrated in pneumonic lesions. However, the significance of these organisms, and of the *Streptococcus* spp. for the development of chronic pneumonia is not clear.

The sum of frequencies of lesions with either no growth of bacteria or insignificant bacterial flora (28%), is significantly lower compared with the corresponding sum of fre-

quencies for normal lung tissue (77%) ($p < 0,01$). This indicates the occurrence of a thriving flora of bacteria in pneumonic lungs at the time of slaughter.

The findings in the present study suggest that further characterization of the various strains of *P. multocida* would be of interest to reveal virulence factors of this species as a secondary invader in swine lungs. The identification of virulence factors might also lead to a better understanding of the pathogenesis of chronic pneumonia.

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Sammendrag

En slaktehusundersøkelse over pneumoni og pleuritt har slaktegris fra 9 utvalgte besetninger. IV. Bakteriologisk funn i kroniske lungelesjoner. Lunger fra 855 slaktegriser fra 9 utvalgte besetninger ble samlet inn ved slakting og undersøkt makrosko-

pisk. Prøver fra 196 kroniske pleuropneumoniske lesjoner, fra 14 kroniske bronkopneumoniske lesjoner med suppurasjon og fra 22 normallunges ble undersøkt bakteriologisk.

Pasteurella multocida, *Actinobacillus (Haemophilus) pleuropneumoniae* og *Streptococcus* spp. ble påvist fra henholdsvis 54%, 11% og 14% av lungelesjonene. Fra normalt lungevev ble *P. multocida* isolert fra 3 (14%) av prøvene, *A. pleuropneumoniae* ble ikke påvist og streptokokker ble funnet i bare ett tilfelle. De ovenfor nevnte bakteriearter ble påvist enten i renkultur eller som del av en blandingsflora.

Kapseltyping og testing for produksjon av dermonekrotisk toksin ble utført på 109 *P. multocida* stammer. Tre av disse stammet fra normalt lungevev. Nititini (91%) av stammene var kapseltype A og 10 (9%) var kapseltype D. Av type A og type D stammene var

henholdsvis 94% og 90% toksinproduserende. To av stammene fra normalt lungevev var type A og 1 var type D. Alle 3 var positive i testen for toksinproduksjon.

De fleste av *A. pleuropneumoniae* stammene var serotype 2. Serotype 1 og 7 ble også identifisert.

De fleste streptokokkstammene ble identifisert enten som *Streptococcus suis* eller som *Streptococcus dysgalactiae*.

Actinomyces pyogenes og anaerobe bakterier ble isolert fra henholdsvis 14% og 18% av lesjonene.

Betydningen av de forskjellige bakteriearter i relasjon til utvikling av kronisk pneumoni ble diskutert. *P. multocida* ble viet spesiell oppmerksomhet, og det ble konkludert med at denne bakteriearten sannsynligvis har betydning for utviklingen av begge typer kroniske pneumonier.

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