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An Abattoir Survey of Pneumonia and Pleuritis in Slaughter Weight Swine from 9 Selected Herds.

III. Serological Findings and their Relationship to Pathomorphological and Microbiological Findings

By *K. Falk* and *B. M. Lium*

National Veterinary Institute, Oslo, Norway.

Falk, K. and B. M. Lium: An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. III. Serological findings and their relationship to pathomorphological and microbiological findings. Acta vet. scand. 1991, 32, 79–88. – Blood samples from 777 pigs, originating from 9 different herds, were collected at slaughter and examined for antibodies to *Mycoplasma hyopneumoniae* and *Actinobacillus (Haemophilus) pleuropneumoniae* by the indirect hemagglutination assay (IHA) and the complement fixation (CF) test, respectively. Results were compared to pathological and microbiological findings. Antibodies to *M. hyopneumoniae* in positive titers of 1/80 or higher were found in 62 % of the samples. The relationship between positive IHA titers to *M. hyopneumoniae* and gross findings indicative of enzootic pneumonia of pigs (EPP), histological findings indicative of EPP, the isolation of *M. hyopneumoniae* and the demonstration of *M. hyopneumoniae* by indirect immunofluorescent testing ranged from 64 % to 68 %. No correlation was noted between positive IHA titers and the isolation of *Mycoplasma flocculare*. Positive antibody titers to *A. pleuropneumoniae* of 1/10 or higher were detected in 5 % to 85 % of the samples from individual herds. Positive titers to *A. pleuropneumoniae* serotype 2 were found in 71 % to 79 % of the sampled animals from herds with high frequencies of pneumonic lesions indicative of pleuropneumonia. In herds with low frequencies of pleuropneumonia, positive titers were recorded in from 0 to 4 % of the tested pigs. However, no statistical association was found between pleuropneumonia and positive titers to *A. pleuropneumoniae* serotype 2 in individual animals. Twenty-one per cent of samples with positive CF titers to *A. pleuropneumoniae* showed antibodies to more than one serotype.

sero-survey; *Mycoplasma hyopneumoniae; Actinobacillus pleuro pneumoniae;* bronchopneumonia; pleuropneumonia.

Introduction

A slaughter house survey of pneumonia in 9 selected swine herds in the south-eastern part of Norway revealed prevalence of lesions indicative of enzootic pneumonia (EPP) ranging from 9 % to 82 % and prevalence for pleuropneumonia and/or fibrosis ranging from 0 to 69 % (*Lium & Falk* 1991). Selected samples from these lungs have been evaluated histologically (*Falk* 1988) and microbiologically (*Falk et al. 1991, Høie et al.* in prep.).

The present paper describes the prevalence of antibodies demonstrated in these pigs to *Mycoplasma hyopneumoniae* as detected by the indirect hemagglutination assay (IHA) and to *Actinobacillus (Haemophilus) pleuropneumonie* serotype as detected by the modified complement fixation (CF) test. The detection of specific antibodies is also rel-

ated to pathomorphological and microbiological findings.

Materials and methods

Herds

The investigation was initiated by the identification and selection of 9 field pig herds (herds A-D, J-K and S) of different sizes and management practices, all located in the south-eastern part of Norway. The herds were arranged into 3 groups according to the degree of respiratory problems. Group I comprised herds with severe respiratory problems (herds A-D). Group II comprised herds with subclinical to moderate respiratory problems (herds J-M) and in group III clinical information and previous abattoir recordings indicated no respiratory problems (herd S). Pathomorphological and microbiological findings from these herds have been given elsewhere (*Falk* 1988, *Falk et al.* 1991, *Lium & Falk* 1991).

Sera

Individual blood samples from 777 pigs were collected at slaughter. All the blood samples were labelled ensuring that serological, pathomorphological, and microbiological findings from each individual pigs could be compared. Sera were prepared from clotted blood and stored at – 30°C until examined.

Indirect hemagglutination assay (IHA) for the detection of antibodies to Mycoplasma hyopneumoniae

The test was essentially carried out as described by *Holmgren* (1974) with modifications made by *Meyling* (personal communication 1985) for use in the Danish SPF-scheme. Instead of formalinization, the swine erythrocytes were treated with pyruvic aldehyde (methylglyoxal). One volume of a 50 % suspension of swine erythrocytes was added to a reactant solution consisting of 1.5 volume pyruvic aldehyde (25 %), 7.0 volume sodium carbonate (1 %) and 0.7 volume 0.067 M phosphate buffer pH 8 with final adjustment to pH 7.0. The mixture was left overnight at 4°C. After coating and washing, the swine erythrocytes were resuspended to 10 % in phosphate buffered saline (PBS) pH 7.2, mixed with equal amounts of glycerin and frozen at – 80°C in 1 ml aliquots. Each batch of coated cells was tested for optimal concentration of cells to be used in the test. Before use, the glycerinated cells were washed once in phosphate buffered saline (PBS) pH 7.2 and resuspended in PBS containing 0.5 % rabbit serum. The titrations were performed in microtitration plates with "U"-shaped wells. serial 2-fold dilutions of serum ranging from 1/80 to 1/2560 were prepared in 0.05 ml volumes of PBS containing 0.5 % rabbit serum and subsequently 0.05 ml suspensions of coated cells were added. The plates were sealed with tape and incubated at room temperature overnight.

The highest dilution of serum which showed complete agglutination, was read as the end point. Serum samples with IHA titers of 1/80 or greater were considered positive for antibodies to *M. hyopneumoniae.*

The "J" strain of *M. hyopneumoniae* (NCTC 10110) was provided by Dr. Niels F. Friis, National Veterinary Laboratory, Copenhagen, Denmark. Antigen production was done in Friis' broth containing 20 % horse serum and supplemented with 0.1 % dextrose (*Friis* 1974). The *M. hyopneumoniae* strain was adapted to this broth through 30–40 passages before antigen production was carried out. Antigen was produced in 9×110 ml volumes in a roller drum and harvested when pH reached 6.4. The antigen was washed twice in PBS pH 7.2, resuspended in 18 ml aqua dest. and disintegrated in a French pressure cell whereafter

the suspension was sonicated 8 times for 10 s. Finally, the suspension was centrifugated at 12,000 × at 4°C for 20 min and the sediment was discarded. The antigen was kept at – 80°C until used.

Positive and negative reference antisera to *M. hyopneumoniae* were provided by Dr. Anders Meyling, National Veterinary Laboratory, Copenhagen, Denmark. Titrations of these sera were included in each test and the test was accepted only if the titer was within 2 dilutions of the original titer.

Complement fixation (CF) test for the detection of antibodies to A. pleuropneumoniae

The modified complement fixation (CF) test was performed as described by *Nielsen* (1982).

All serum samples were tested against type strains of the serotypes which have previously been isolated in Norway. These are serotypes 1, 2, 6, 7, 8 and 10 (*Falk,* unpublished data). *A. pleuropneumoniae* strains Shope 4071, S1536, Femø, WF83, 405, and D 13039 representing serotypes 1, 2, 6, 7, 8 and 10, respectively, were used for antigen production. Type strains as well as negative and positive pig reference sera against serotypes 1 to 10 were obtained from Dr. Ragnhild Nielsen, National Veterinary Laboratory, Copenhagen, Denmark. All antigens produced were cross titrated against all reference sera.

The highest dilution of serum which showed 30 % or less hemolysis of sheep erythrocytes was designated as the end point. Serum samples with CF titers of 1/10 or higher were considered positive for antibodies against *A. pleuropneumoniae.*

The sera were initially screened at 1/10 dilution against 3 different antigens. These antigens were (i) serotype 2 (ii) a pool of serotype 6 and 8 and (iii) a pool of serotype 1, 7 and 10. Positive sera were titrated in serial 2-fold dilutions ranging from 1/10 to 1/320 against the antigens to which they did react in the screening.

Statistical analysis

Standard microcomputer software (SAS Institute Inc., Cary, NC, USA) was used to tabulate data and to perform descriptive statistics.

Results

Antibodies to M. hyopneumoniae

Prevalence of antibodies to *M. hyopneumoniae* are shown in Table 1. Titers of 1/80 and higher were detected in 478 (62 %) of the 777 sera tested. From herds in groups I and II, prevalence of IHA antibodies ranged from 21 % to 83 %. The recorded herd prevalence in group I were all greater than 70 %. The overall prevalence in group I was nearly twice the prevalence in group II. In group III, which had no clinical indications of respiratory problems, only 1 positive

Table 1. Percentage of serum samples from swine with positive antibody titers against various *Actinobacillus (Haemophilus) pleuropneumoniea* serotypes (demonstrated by the CF test) and against *Mycoplasma hyopneumoniae* (demonstrated by the IHA test).

	A. pleuropneumoniae serotypes						*M. hyopneumoniae*
Herds	Total	2	6	7	8	10	
A (N = 135)	81	79	2	16	13	11	83
B (N = 154)	81	75	1	14	7	15	73
C (N = 199)	74	71	5	5	8	2	70
D (N = 41)	85	73	2	10	22	37	78
J (N = 49)	25	2	2	8	2	23	23
K (N = 56)	9	4	0	0	0	5	21
L (N = 58)	29	2	0	5	0	28	55
M (N = 42)	57	0	0	57	0	10	60
S (N = 43)	5	0	0	5	0	2	2
Total (N = 777)	62	51	2	11	7	12	62

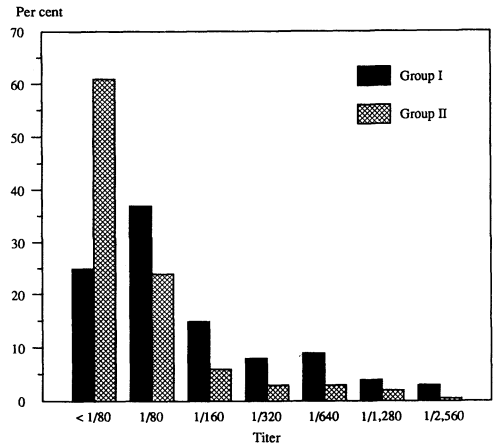

Figure 1. Percentage of antibody titers to *Mycoplasma hyopneumoniae* demonstrated by the IHA test in swine originating from 2 herd categories. Titer values below 1/80 are considered negative reactions.

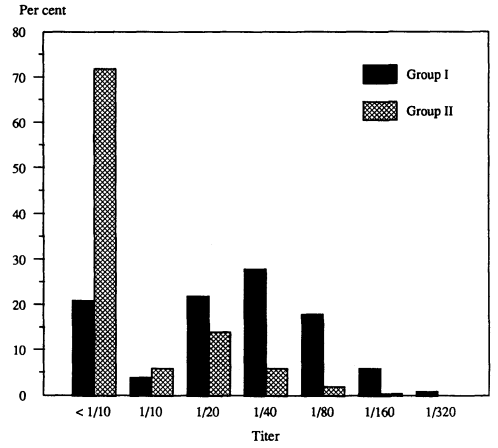

Figure 2. Percentage of antibody titers to *Actinobacillus (Haemophilus) pleuropneumoniae* demonstrated by the CF test in swine originating from 2 herd categories. Titer values below 1/10 are considered negative reactions.

sample (titer 1/80) was found. The distributions of IHA titers in group I and group II are shown in Fig. 1. In positive samples, titers ranged from 1/80 to 1/2,560 in both groups.

Antibodies to A. pleuropneumoniae

Prevalence of antibodies to *A. pleuropneumoniae* are given in Table 1. Titers of 1/10 and higher were detected in 478 (62 %) of the 777 samples. The overall prevalence in group I was nearly three times the prevalence in group II, ranging from 74 % to 85 % in group I and from 9 % to 57 % in group II. In group III, which had no history of respiratory problems, only 2 positive samples were found of which 1 reacted to serotype 7 (titer 1/20) and 1 to both serotype 7 (titer 1/20) and serotype 10 (titer 1/10). Most positive reactions were against serotype 2. In group I, 75 % of the samples reacted against serotype 2 while antibodies to this serotype were demonstrated in only 2 % of the samples in group II. Antibodies to *A. pleuropneumoniae* in group II were mainly directed against serotype 7 (herd M) and serotype 10 (herd J and L). The distribution of titers to *A. pleuropneumoniae* (all serotypes included) is shown in Fig. 2. Titers from 1/10 to 1/320 in group I and from 1/10 to 1/160 in group II.

Of 478 samples showing antibodies to *A. pleuropneumoniae,* 101 (21 %) reacted with more than 1 serotype. Frequencies of these reactions were as follows: 52 samples reacted with 2 serotypes, 3 samples reacted with 3 serotypes and 17 samples reacted with 4 or 5 serotypes. Serotype 2 reacted with 82 samples while serotypes 6, 7, 8 and 10 reacted with 15, 62, 51 and 60 samples, respectively. There was no clearcut pattern in these reactions.

Of the 777 samples investigated a total of 615 (79 %) showed antibodies to either *A. pleuropneumoniae* or *M. hyopneumoniae.* Herds in group I and II showed antibodies to one or both of these species in 501 (95 %) and 111 (54 %) samples, respectively. A total of 341 (44 %) of the sera tested carried antibodies to both agents, the prevalence in

Table 2. Percentage of serum samples from 2 groups of swine herds with positive antibody titers against *Mycoplasma hyopneumoniae* and various *Actinobacillus pleuropneumoniae* serotypes. The results are presented in relation to pathological findings in corresponding lungs indicating presence (+) or absence (–) of lesions. (EPP = enzootic pneumonia, PLF = pleuropneumonia including local fibrosis, DP = diffuse pleuritis).

	Percentage of pigs with positive antibody titers											
	Group I						Group II					
	EPP		PLF		DP		EPP		PLF		DP	
Antibodies to	–	+	–	+	–	+	–	+	–	+	–	+
M. hyopneumoniae	64	80**	71	78	69	80*	28	47*	38	57	36	59
A. pleuropneumoniae	79	79	78	80	78	80	33	25	26	57*	25	48*
A. pleurop. type 2	74	75	74	75	73	76	1	3	2	7	2	6
A. pleurop. type 7	9	11	8	13	8	12	21	11	13	43*	10	45**
A. pleurop. type 10	11	11	8	13	11	11	18	15	16	29	17	17

* $p < 0.01$
** $p < 0.001$

group I and II were 59 % and 13 %, respectively.

The prevalence of antibodies to *A. pleuropneumoniae* was not significantly higher in samples having antibodies to *M. hyopneumoniae* compared to those without antibodies to this species.

Relation between detection of antibodies and pathomorphological and microbiological findings

The relationships between serological and pathomorphological findings in the present study are summarized in Table 2. Significant correlation was observed between the detection of antibodies to *M. hyopneumoniae* compared to gross lesions indicative of EPP and compared to diffuse pleuritis. However, there was a large number of disagreements in the relationship between positive sera and gross findings. No statistical associations were noted between pathomorphological findings and the detection of antibodies against *A. pleuropneumoniae* in group I. In group II, significant correlations were noted between the overall finding of antibodies to *A. pleuropneumoniae* serotypes and the presence of pleuropneumonia as well as pleuritis. A similar statistical association was also noted for serotype 7.

There was a correlation between the bronchopneumonic score and antibody titers against *M. hyopneumoniae* ($p < 0.01$). In the various herds, high frequencies of serological reactors to *M. hyopneumoniae* were associated with both increased prevalence of gross lesions indicative of EPP ($p < 0.05$) as well as high average pneumonia scores ($p < 0.01$).

A summary of the relations between the findings of antibodies to *M. hyopneumoniae* and other diagnostic criteria for EPP is given in Table 3. The frequency of samples with positive titers to *M. hyopneumoniae* was significantly correlated to gross lesions, histological lesions i.e. lymphoreticular cuffing, to the detection of *M. hyopneumoniae* antigens by indirect immunofluorescent (IIF) testing and to the isolation of *M. hyopneumoniae*. The agreement between IHA titers and the other diagnostic criteria ranged from 64 % to 68 %. There was no correlation be-

Table 3. Frequencies of serum samples with (+) and without (–) positive IHA-titers against *Mycoplasma hyopneumoniae*. The results are presented in relation to corresponding gross lesions indicative of EPP, histological lesions i.e. lymphoreticular cuffing, demonstration of *M. hyopneumoniae* antigens by indirect immunofluorescens (IIF) testing and the isolation of *M. hyopneumoniae*, *M. flocculare* and *M. hyorhinis* in 265 swine lungs selected for diagnostic work on mycoplasmal infections.

IHA	Gross lesions		Histologic lesions		*M. hyop.* by IIF		Isolation					
							M. hyopn.		*M. floc.*		*M. hyor.*	
	–	+	–	+	–	+	–	+	–	+	–	+
–	65	61	65	61	81	45	87	39	107	19	116	10
+	12	127	24	115	53	86	69	70	121	18	79	60
Chi^2	59.16**		34.90**		18.09**		10.28*		0.25		42.20**	

* $p < 0.01$
** $p < 0.001$

tween the serological findings and the isolation of *M. flocculare*. However, the isolation of *M. hyorhinis* was significantly correlated with the findings of antibodies to *M. hyopneumoniae* ($p < 0.01$). *M. hyopneumoniae* could neither be isolated nor demonstrated by IIF in 32 lungs with lesions indicative of EPP. However, 26 (81 %) of these pigs possessed antibodies to *M. hyopneumoniae* which was a slightly higher rate compared to pigs from which the organism was demonstrated either by cultivation or by IIF.

In pigs where the *A. pleuropneumoniae* serotype 2 was isolated, 64 % showed antibodies to this serotype. Serotype 1 was isolated from 1 pig in herd A. However, no antibodies to this serotype were detected neither from this nor from any other pig in herd A.

Discussion

In the present work it was demonstrated that the prevalence of antibodies to *M. hyopneumoniae* varied from 21 % to 83 % in herds infected with this organism. Herds with the most serious pneumonia problems showed the highest frequencies of samples with positive antibody titers. In individual pigs, high bronchopneumonic scores were significantly correlated with high IHA titers.

There was a highly significant correlation between pathological lesions indicative of EPP and IHA antibodies to *M. hyopneumoniae* both in herds with severe respiratory problems (group I) and in herds with subclinical to moderate respiratory problems (group II), although the prevalence of pigs with gross lesions and no IHA antibodies, and pigs with no gross lesions and positive IHA titer was not negligible. The difference seen between the 2 groups may have been caused by differences in infection pressure and environmental conditions. Several reasons for the discrepancies between gross lesions and serological findings might be considered. *M. hyopneumoniae* does not seem to stimulate the production of high titers of serum antibodies (*Nicolet* 1987) and low serum titers might have been missed due to lack of sensitivity. *Armstrong et al.* (1983) revealed that it might take 8–9 weeks before pigs exposed by contact seroconvert and that the duration of the antibody response can be

most variable. Similar observations have also been made by *Meyling* (personal communication 1985). Another possibility is that *M. hyopneumoniae* might have been cleared from the lung leading to decreased antibody levels and that secondary microbial invaders might have caused the lesions to persist. It should also be noted that although lesions are relatively characteristic for EPP, agents other than *M. hyopneumoniae* can cause similar pneumonic changes (*Ross* 1986).

There might be 3 main reasons for the finding of antibodies, but no gross lesions. The most likely ones are that lesions are healed while the antibodies persist or that the organisms persist in the body without causing detectable lesions. The third possibility is cross reactions caused by other *Mycoplasma spp.* There is an antigenic relationship between *M. hyopneumoniae* and other mycoplasmas, particularly *M. flocculare* (*Meyling & Friis* 1972, *Ro & Ross* 1983, *Young & Ross* 1987). Cross reactions between *M. hyopneumoniae* and *M. flocculare* and also *M. hyorhinis* have been demonstrated by the enzyme-linked immunosorbent assay (ELISA) and the complement fixation (CF) test (*Freeman et al.* 1984a, *Ross* 1986). These cross reactions raise questions about the specificity of other tests as well. On the other hand, *Meyling* (personal communication 1985) did not detect any cross-reacting antibodies in the IHA test when testing 8 gnotobiotic pigs intranasally infected with *M. flocculare*. The IHA test has also been considered valuable and reliable in the Danish SPF-scheme (*Meyling,* personal communication 1985). In a recent study *Armstrong et al.* (1987) suggested that, under field conditions, *M. flocculare* does not induce sufficient levels of antibodies to interfere with *M. hyopneumoniae* in the ELISA.

In the present study, the detection of *M. flocculare* was not correlated with IHA antibodies to *M. hyopneumoniae* whereas a correlation between this serological reaction and *M. hyorhinis* was found. This finding was most probably due to concurrent infections with *M. hyopneumoniae* and *M. hyorhinis* as demonstrated elsewhere (*Falk et al.* 1991).

A highly significant correlation was found between the presence of IHA antibodies and gross findings, histological findings, demonstration of *M. hyopneumoniae* by IIF and the isolation of *M. hyopneumoniae* in samples selected for diagnostic work on mycoplasma infections. The agreement between serological findings and the other criteria of infection ranged from 64 % to 68 %. *Freeman et al.* (1984b) reported an agreement between the IHA test and other diagnostic criteria ranging from 24 % to 50 %. These discrepancies are most probably caused by the use of different test procedures.

IHA antibodies were detected in 81 % of the pigs which had pathological lesions indicative of EPP, but which were IIF as well as culturally negative for *M. hyopneumoniae.* This finding strongly suggests that *M. hyopneumoniae* either had been cleared from these lesions or the organism was not present in sufficient number to be detected by the diagnostic procedures. It also supports the opinion held by several other workers that more than one test is needed to obtain a reliable result in the diagnosis of *M. hyopneumoniae* infection (*Gois et al.* 1975, *Friis & Meyling* 1983, *Armstrong et al.* 1984).

Diffuse pleuritis was associated with antibodies to *M. hyopneumoniae* in both groups. This finding was probably due to *M. hyorhinis*, as revealed by *Falk et al.* (1991). Some strains of *Pasteurella multocida* may also induce pleuritis (*Pijoan & Fuentes* 1987). However, such a correlation was not revealed in this study.

The prevalence of antibodies to *A. pleuropneumoniae* serotype 2 were considerably higher in herds in group I than in group II. This is in agreement with the high frequencies of pleuropneumonia found in these herds, even if the organism was demonstrated only in a few samples (*Høie et al.*, in preparation). However, in group I there was a striking lack of correlation between the presence or not of pleuropneumonia or diffuse pleuritis in individual animals, and the finding or not of antibodies to *A. pleuropneumoniae* serotype 2 in the corresponding serum samples. Several explanations for this finding might be considered. One is that the pneumonic lesions were healed or were not detected when the lungs were grossly evaluated. A more likely explanation is that *A. pleuropneumoniae* resides in the upper respiratory tract, inducing antibody response independent of lung lesions in herds where the infection is prevalent, independently of lung lesions. This view is supported by the findings reported by *Kume et al.* (1984). It is also necessary to consider the possibility that chronic pleuropneumonia, which was a major finding in this study, can be caused by other agents than *A. pleuropneumoniae* even though *A. pleuropneumoniae* might have initiated the lesions. In the present survey *P. multocida* was the most commonly recovered organism from pleuropneumonic lesions (*Høie et al.*, in preparation). In our opinion, this organism may play a major role in the pathogenesis of chronical pleuropneumonia in fattening pigs. However, this organism is suggested to be unable to initiate lung lesions unless lung defence mechanisms are impaired by primary infections and/or by adverse environment or management factors. There is also evidence for virulence variations between *P. multocida* strains. According to *Pijoan & Fuentes* (1987) some *P. multocida* strains may cause clinical symptoms resembling those seen in chronic pleuropneumonia.

In group II a significant correlation between serotype 7 antibodies and pleuropneumonia was found. This was mainly due to herd M in which high prevalence both of diffuse pleuritis and of pericarditis were found.

Young et al. (1983) found that pigs which had antibodies to *M. hyopneumoniae*, more often had antibodies to *A. pleuropneumoniae* than those which were *M. hyopneumoniae* negative. No such correlation was found in this study.

In this survey there was a high prevalence of samples with antibodies to several *A. pleuropneumoniae* serotypes. Serotype 2 was the one most frequently involved. There was no uniform pattern which could explain these findings. Cross reactions between serotypes may have occurred involving common species antigens. However, this test has proved both species and type specific (*Nielsen* 1982) although cross reactions between serotype 3, 6 and 8 (*Nielsen* 1985a) and serotype 1 and 9 (*Nielsen* 1985b) have been revealed. There were some cross reactions between antigens used in the CF test and the pig reference antisera (unpublished observation), but this could hardly explain the reactions seen in field sera. A more likely explanation might be concurrent infections with other microorganisms sharing antigens with *A. pleuropneumoniae*. No serological cross reactions between *A. pleuropneumoniae* and *Haemophilus parasuis* (*Nielsen* 1982) or *Haemophilus* taxon "minor group" (*Rapp et al.* 1985) have been found. However, *Rosendal & Mittal* (1985) described an *Actinobacillus* species closely related to *Actinobacillus suis* which caused seroreaction to several *A. pleuropneumoniae* serotypes in the CF test. Other Actinobacillus species than *A. pleuropneumoniae* were not found in this study, but testing of samples from other organs

than the lungs might have given different results.

In conclusion this study shows that serological tests may be a valuable tool in the diagnosis of pneumonia on a herd basis. However, great care should be taken in the interpretation of results from single animals. The lack of type specificity of the CF test, revealed in this study, should be further investigated.

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Sammendrag

En slaktehusundersøkelse over pneumoni og pleuritt hos slaktegris fra 9 utvalgte besetninger. III. Serologiske funn og deres sammenheng med patomorfologiske og mikrobiologiske funn.

Blodprøver av 777 griser fra 9 forskjellige besetninger ble samlet inn ved slakting. Prøvene ble undersøkt for antistoffer mot *Mycoplasma hyopneumoniae* ved hjelp av indirekte hemagglutinasjonstest (IHA) og for antistoffer mot *Actinobacillus (Haemophilus) pleuropneumoniae* ved hjelp av komplementbindingstest (CF). Resultatene ble sammenholdt med patologiske og mikrobiologiske funn.

I denne undersøkelsen hadde 62 % av grisene et antistofftiter på 1/80 eller høyere mot *M. hyopneumoniae.* Sammenhengen mellom positive IHA-titre mot *M. hyopneumoniae* og patologiske funn som tydet på enzootisk pneumoni hos gris (EPP), dyrkning av *M. hyopneumoniae* og påvisning av *M. hyopneumoniae* ved hjelp av indirekte immunofluorescens test varierte fra 64 % til 68 %. Det ble ikke funnet noen sammenheng mellom positive IHA-titre og dyrkning av *Mycoplasma flocculare.*

Positive antistofftitre mot *A. pleuropneumoniae* på 1/10 eller høyere ble funnet i 62 % av prøvene. Denne frekvensen varierte fra 5 % til 85 % i de enkelte besetningene. I besetninger med høy frekvens av pleuropneumoni varierte frekvensen av griser med positive antistofftitre mot *A. pleuropneumoniae* serotype 2 fra 71 % til 79 %. I besetninger med lav frekvens av pleuropneumoni varierte frekvensen av seropositive griser fra 0 til 4 %. Det ble imidlertid ikke funnet noen statistisk sammenheng mellom pleuropneumoni og antistoffer mot *A. pleuropneumoniae* hos enkeltdyr.

Enogtyve prosent av prøvene med positive CF titre mot *A. pleuropneumoniae* hadde antistofftitre mot flere enn én serotype.

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Reprints may be requested from: Knut Falk, National Veterinary Institute, P. O. Box 8156 Dep., N-0033 Oslo 1, Norway.