Survey of infectious agents involved in acute respiratory disease in finishing pigs

W. L. A. LOEFFEN, E. M. KAMP, N. STOCKHOFE-ZURWIEDEN, A. P. K. M. I. VAN NIEUWSTADT, J. H. BONGERS, W. A. HUNNEMAN, A. R. W. ELBERS, J. BAARS, T. NELL, F. G. VAN ZIJDERVELD

Outbreaks of respiratory disease constitute a major health problem in herds of finishing pigs and their aetiology often remains unclear. In this study, 16 outbreaks of respiratory disease with acute clinical signs in finishing pigs were investigated to determine which infectious agents were involved. From each herd four diseased and two clinically healthy pigs were examined pathologically and for the presence of viruses, bacteria and mycoplasmas. In addition, paired blood samples from 10 groupmates of the diseased pigs were tested for antibodies against commonly known causal agents of respiratory disease. A clear diagnosis was possible in 12 of the 16 outbreaks. Seven were due to an infection with influenza virus and five were due to an infection with *Actinobacillus pleuropneumoniae*. A combination of influenza virus and *A pleuropneumoniae* may have caused one other outbreak, but no clear cause could be established for the other three outbreaks.

RESPIRATORY diseases are considered to be the most important health disorders in finishing pigs. They are responsible for over half of all antibiotic treatments in these pigs (Elbers and others 1990a). In addition to the costs of these treatments, respiratory diseases are also responsible for losses from mortality, decreased growth rates, reduced feed conversion efficiency and reduced carcase quality (Straw and others 1990, Paisley and others 1993).

Viruses, bacteria or mycoplasmas are usually the primary cause of respiratory disease in pigs, although the exact role of many of these agents in the often complex pathogenesis is not fully understood. Environmental factors, farm management, and the defence mechanisms of the pig may also play an important role in the onset of respiratory disease or in the severity of the clinical signs of the disease (Christensen and Mousing 1992).

The two most important primary bacterial agents involved in respiratory disease are considered to be *Mycoplasma hyopneumoniae*, which causes enzootic pneumonia (Ross 1992), and *Actinobacillus pleuropneumoniae*, which causes pleuropneumonia (Sebunya and Saunders 1983). The most important secondary agent, which becomes involved in respiratory disease only after the defence mechanisms of the lungs have been impaired, is considered to be *Pasteurella multocida* (Ciprián and others 1988, Pijoan 1992). The most important viruses in respiratory disease are swine influenza virus, Aujeszky's disease virus, porcine reproductive and respiratory syndrome (PRRS) virus and porcine respiratory corona virus (PRCV), although, especially for the last two, their exact role in respiratory disease is unclear (Laude and others 1993, Wensvoort 1993, Done and Paton 1995).

Dual infections, in which two infectious agents may cause respiratory disease after infecting the host either at the same time, or one shortly after the other, have gained more attention during the last decade. Multiple infections, involving several viruses, bacteria and mycoplasmas, have been studied under experimental conditions with inconclusive results (Fuentes and Pijoan 1987, Iglesias and others 1992, Lanza and others 1992, Amass and others 1994, Kay and others 1994, Van Reeth and Pensaert 1994, 1996, Van Reeth and others 1996a, b).

Although some field studies of the aetiological agents of respiratory disease have been published, they have usually been limited to certain pathogens. Furthermore, some of them were carried out in finishing pigs at the slaughterhouse, where there is no clear relationship with clinical problems. Studies of the association between bacterial agents and respiratory disease have been published by Awad-Masalmeh and others (1990), Castryck and others (1990), Falk and Lium (1991), Falk and others (1991) and Høie and others (1991), and serological investigations have been carried out by Callebaut and others (1986), Elbers and others (1992), Nowotny and others (1994) and Maes and others (1996). The results of a more elaborate study of five herds, combining serological investigations with the isolation of bacteria and immunofluorescence microscopy were published recently by Runge and others (1996). To the authors' knowledge, no results of extensive investigations into the infectious causes of acute respiratory disease in a substantial number of herds, during the acute phase and considering all the possible aetiological agents, have been published recently.

The main purpose of this study was to identify the infectious agents involved in acute respiratory disease in finishing pigs, preferably during the first half of the finishing period. Possible evidence of dual infections, and of their importance in the field, was also investigated.

MATERIALS AND METHODS

Selection of herds

Forty finishing or farrow-to-finish herds in the southern Netherlands were selected from approximately 200 herds with a known history of recurrent respiratory problems. The criteria for selection were: recurring respiratory problems with acute clinical signs, preferably during the first half of the finishing period; the frequent use of curative medication; and the restricted use of preventive medication. Further criteria were a herd size of more than 500 finishing pigs, with compartments containing 60 to 100 pigs, and the practice of an all-in all-out system.

Sampling

Farmers were requested to notify the Animal Health Service as soon as possible, and before they started medication, if at least one of the following clinical signs was observed in a compartment of finishing pigs with no history of respiratory disease: a decrease of feed intake by 10 per cent or more; coughing; or laboured breathing. The farms were usually visited the same day, but always within 24 hours. An outbreak was included for further investigation when there were clinical signs of respiratory disease, with several pigs having a fever of above 40°C (temperatures were taken only from pigs with clear clinical signs) and no antibiotics had been used for at least a week. Between December 1, 1995, and April 15, 1996, 16 outbreaks were reported that met these criteria. Only one outbreak was investigated in each herd.

Veterinary Record (1999) **145,** 123-129

W. L. A. Loeffen, DVM,
W. A. Hunneman, DVM, PhD,
A. R. W. Elbers, MScAg, MSc, PhD, Animal Health Service, PO Box 4, 5280 AA Boxtel, The Netherlands
E. M. Kamp, MSc,
N. Stockhofe-Zurwieden, DVM,
A. P. K. M. I. van Nieuwstadt, PhD,
J. H. Bongers, DVM,
F. G. van Zijderveld, DVM, Institute for Animal Science and Health (ID-DLO), PO Box 65, 8200 AB Lelystad, The Netherlands
J. Baars, DVM,
T. Nell, MSc, DVM, Intervet International bv, PO Box 31, 5830 AA Boxmeer, The Netherlands

From each outbreak, four diseased pigs from one compartment, and two clinically healthy pigs of approximately the same age from another compartment, were selected for pathological examination and the isolation of viruses, bacteria and mycoplasmas. The diseased and healthy pigs were kept separate while they were being transported to the laboratory.

In the compartment where the outbreak occurred, blood samples were taken from 10 groupmates of the four selected pigs, all with acute signs of respiratory disease (acute sera). The same pigs were bled again four weeks later (convalescent sera) and, if they stayed at least another three weeks on the farm, at the end of the finishing period (end sera). Blood samples were also taken from 10 randomly chosen pigs in one or two other compartments at the end of the finishing period (random end sera).

Pathology

All six pigs were anaesthetised and exsanguinated. A postmortem examination was carried out with special attention to the lungs. When present, fluid from the thoracic cavity was collected in a sterile syringe and preserved in heparin. The percentage of macroscopically affected lung tissue was estimated and the types of lung lesions were recorded. The right cranial lobe was flushed with 30 ml of sterile Eagle's minimal essential medium (EMEM) (Flow Laboratories) and the right caudal lobe was flushed with 30 ml of sterile physiological saline. The lavage fluids were examined for the presence of bacteria and mycoplasmas. Tissue specimens were taken from the right cranial lobe, the right caudal lobe and the caudal part of the left cranial lobe for histological, bacteriological and virological examination. Additional specimens were taken from pigs in which there were no pneumonic lesions at these sites. For histological examination, samples of these specimens were fixed in 4 per cent formalin, and tissue slides were stained with haematoxylin and eosin. The tonsils were collected for the isolation of viruses.

Isolation of viruses

In each outbreak approximately 20 to 25 pieces of lung tissue and tonsil were used for virus isolation. Tissue was homogenised by grinding it with sand in EMEM with 0·5 per cent lactalbumin hydrolysate and antibiotics in a mortar. The tissue homogenates were clarified at 1500 *g* for 10 minutes and passed through a 200 nm filter. The filtrate was inoculated on to monolayers of secondary pig thyroid cells and on to primary pig lung macrophages. The monolayers were incubated in a carbon dioxide-incubator at 36°C for one to two weeks and examined for cytopathic effects every day. Viruses isolated on pig thyroid cells were tested in a haemagglutination test. Haemagglutinating viruses were tested in a haemagglutination inhibition test with specific antisera against influenza virus A, subtype H1N1 (A/Swine/Netherlands/80) and H3N2 (A/Port Chalmers/1/73). Viruses isolated on pig lung macrophages were tested in an immunoperoxidase test with a specific conjugate against PRRS virus.

Isolation of bacteria

Lung tissue specimens were decontaminated for six to eight seconds in boiling water, cut aseptically into small pieces and minced in 5 ml brain heart infusion broth (Difco Laboratories) in a Stomacher 80 lab blender. Lung lavage fluids were centrifuged for 10 minutes at 1000 *g* and the supernatants were discarded.

Thorax fluids, supernatants of minced lung tissues, and the pellet from the lung lavage fluids were inoculated with a loop on to sheep blood agar and sheep blood agar supplemented with 0·1 per cent NAD. The plates were incubated at 37°C under aerobic, anaerobic, and microaerophilic conditions for at least 48 to 72 hours, and read on at least two occasions. Bacterial growth was further identified according to Cowan and Steel's (1993) manual for the identification of medical bacteria. Known pathogens were always recorded. Other bacteria were identified only when isolated in pure culture.

Isolation of mycoplasmas

Lung tissue specimens were minced as described above, except that the brain heart infusion broth contained 40 iu penicillin and 0·08 per cent thallium acetate.

Thorax fluid, supernatants of minced lung tissues, and the lung lavage fluids were inoculated immediately on to modified Edward agar and Friis agar (Friis 1975) and incubated at 37°C under microaerophilic conditions. In addition, specimens were serially diluted 10-fold to 10^{-5} in NHS25 broth, and to 10^{-2} in NHS25 containing a rabbit antiserum against *Mycoplasma hyorhinis*. The broth media were incubated at 37°C in rolling drums in which the tubes rotated once per minute. Media were inspected every other day for three weeks. Acidified cultures were transferred twice in 10-fold dilutions and then stored at –70°C until further use.

The mycoplasmas isolated were identified by using an indirect immunofluorescence test (Rosendal and Black 1972) and two polymerase chain reaction (PCR) assays (Stemke and others 1994, Mattsson and others 1995), which make it possible to discriminate between *M hyorhinis*, *M hyopneumoniae* and *Mycoplasma flocculare*.

Serological examination

All the sera collected from pigs in compartments with a reported outbreak (acute sera, convalescent sera and end sera), and all the random end sera were tested for antibodies against the following pathogens:

– Aujeszky's disease virus, by using a gE-ELISA (van Oirschot and others 1988);

– PRRS virus, by using an immunoperoxidase monolayer assay (IPMA) as described by Wensvoort and others (1991). The random end sera were tested in a 1:200 dilution, and the paired sera and end sera were tested in serial two-fold dilutions to determine the antibody titres;

– Influenza virus, by using a haemagglutination inhibition (HAI) test (Kendal and others 1982) for antibodies against H1 and H3. All the sera were tested in serial two-fold dilutions, starting at 1:9, and titres equal to or more than 18 were considered positive. Influenza virus strains A/swine/Neth/Best/96 and A/swine/Neth/St Oedenrode/96 were used to test for the H1 and H3 antibodies, respectively. Both strains were isolated in this study;

– *A pleuropneumoniae*, by using a complement fixation (CF) test (Nicolet and others 1971) with a mixture of a serotype 2 and a serotype 9 strain as antigen. The sera were tested in a 1:40 dilution. No further differentiation was made between serotypes 2 and 9;

– *M hyopneumoniae*, by using a commercially available ELISA which specifically detects an epitope of *M hyopneumoniae* without cross-reacting with *M flocculare* or *M hyorhinis* (*M hyopneumoniae* ELISA Kit; Dako).

RESULTS

Clinical signs

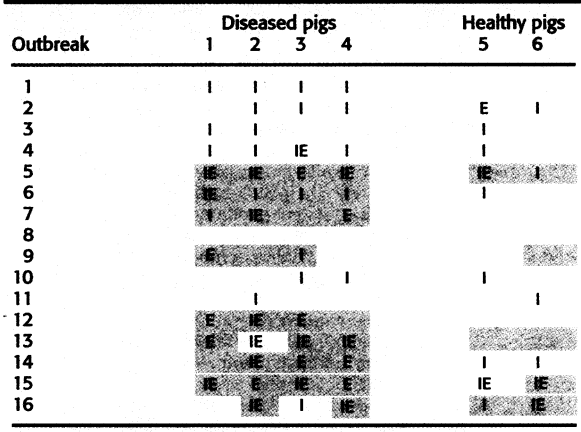
The clinical signs in the first 15 outbreaks were very similar: the pigs' feed intake decreased; they were depressed (recumbent, slow, 'sick'); and there were respiratory signs of coughing and laboured breathing. Clear signs were usually apparent in 10 to 30 per cent of the pigs at the time of the first visit. Most of the pigs with clinical signs also had a fever (40 to 42°C). Although similar, the clinical signs in the other outbreak (herd 16) were less severe and fewer pigs were affected than in the other 15 herds.

TABLE 1: Infectious agents isolated from diseased and clinically healthy pigs in 16 acute outbreaks of respiratory disease

Agent	Diseased pigs (n=64) Number (%)	Clinically healthy pigs With lung lesions (n=16) Number (%)	Clinically healthy pigs Without lung lesions (n=16) Number (%)
Viruses			
Influenza virus	32 (50)	5 (31)	3 (19)
PRRS virus	18 (28)	3 (19)	6 (38)
Bacteria			
App serotype 2	21 (33)	4 (25)	– (–)
P multocida	12 (19)	1 (6)	– (–)
H parasuis	16 (25)	3 (19)	2 (13)
B bronchiseptica	14 (22)	4 (25)	2 (13)
S suis	16 (25)	1 (6)	– (–)
A pyogenes	1 (2)	– (–)	– (–)
Mycoplasmas			
M hyopneumoniae	6 (9)	2 (13)	2 (13)
M hyorhinis	47 (73)	12 (75)	10 (63)

PRRS Porcine reproductive and respiratory syndrome, App *A pleuropneumoniae*

TABLE 2: Influenza-virus (H1 and H3) isolations and pathological findings in four diseased and two clinically healthy pigs from each of 16 acute outbreaks of respiratory disease



I Interstitial pneumonia (microscopic), E Endobronch(iol)itis (microscopic), Shaded = Influenza-virus isolated from lungs and/or tonsils (H3 in herds 7 and 12, all others H1). Virus was isolated only from the tonsils of pigs 1 and 3 from herd 9 and pigs 4 and 5 from herd 16

In herds 1 and 3 to 15, the clinical outbreaks occurred in pigs 14 to 20 weeks old, covering the second month of the finishing period. In herd 2 the pigs were 22 weeks old, and in herd 16 the pigs were only 11 weeks old.

Pathological examination

Macroscopic lung lesions were found in 61 of the 64 clinically diseased pigs. The percentages of the lungs affected ranged from 5 to 50 per cent. Most lesions were multilobular and catarrhal, sometimes with a purulent exudate. In herds 3, 9, 10 and 11 focal or multifocal haemorrhagic (pleuro)pneumonia was predominant. In herds 8 and 16 multilobular lesions were present in addition to multifocal lesions.

In most of the pigs acute lesions were predominant. However, subacute or chronic lesions were also present in the pigs from herds 2, 5, 9, 10, 11, 12, 14 and 16. In all the pigs from herd 12 there were also lesions in the liver, due to migrating *Ascaris suum* larvae.

Macroscopic lung lesions were also found in 16 of the 32 clinically healthy pigs from herds 2, 3, 5, 8, 9, 11, 12, 15 and 16. Ten pigs had pneumonia and six pigs had a chronic pleuritis. The lesions in these 'healthy' pigs were always less severe than those in the diseased pigs from the same herd. The percentage of lung affected rarely exceeded 5 per cent.

Isolation of viruses, bacteria and mycoplasmas

Influenza virus, *A pleuropneumoniae*, *S suis* and *P multocida* were isolated frequently, and more often from the diseased pigs and the clinically healthy pigs with lung lesions than from the healthy pigs without lung lesions (Table 1). The isolation of *P multocida* was usually associated with the presence of chronic or subacute lesions. *M hyorhinis*, PRRS virus, *H parasuis* and *B bronchiseptica* were also frequently isolated, but from both diseased and healthy pigs, either with or without lung lesions. *M hyopneumoniae* was not isolated very often from either the diseased or the clinically healthy pigs (Table 1).

Influenza virus was isolated from the pigs from nine herds (Table 2). Subtype H1 was predominant, but H3 was also isolated from two herds. In seven of the herds (5, 6, 7, 12, 13, 14 and 15) the virus was isolated from the lungs of three or four diseased pigs, and a histological examination revealed a high correlation with the presence of endobronch(iol)itis (Table 2). Influenza virus was isolated only from the tonsils of some of the pigs in herds 9 and 16.

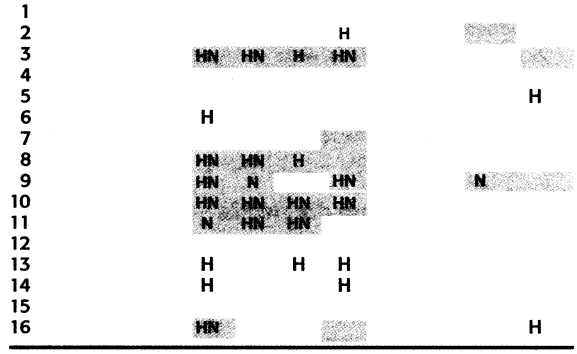
A pleuropneumoniae was isolated from the pigs from seven herds (Table 3). All the isolates were serotype 2. In five of the herds (3, 8, 9, 10 and 11) bacteria were isolated from the lungs of three or four diseased pigs. Pathological and histological examinations revealed a high correlation with haemorrhagic and, especially, necrotising pneumonia (Table 3). In herd 7, *A pleuropneumoniae* was isolated only from one pig and there was no other evidence of an infection. In herd 16, *A pleuropneumoniae* was isolated from two pigs, only one of which had pathological and histological signs of haemorrhage and necrosis.

In four herds, influenza virus or *A pleuropneumoniae* was not the single most important agent isolated. The main pathological findings and the agents isolated from each individual pig in these four herds are shown in Table 4. PRRS virus was isolated from the lungs of three or four diseased pigs from herds 1 and 4. However, it was also isolated from both the clinically healthy pigs from herd 4. Several bacteria were isolated, but none of them consistently. In herd 16, both influenza virus and *A pleuropneumoniae* were isolated from some of the pigs, from lung lesions which were typical of either of these agents. The two infections seemed to coexist, with the pigs being infected with either one or both.

Serological examination of paired sera

Most of the groupmates of the four diseased pigs in herds 5, 6, 9, 13, 14 and 15 seroconverted against influenza virus

TABLE 3: *A pleuropneumoniae* serotype 2 isolations and pathological findings in four diseased and two clinically healthy pigs from each of 16 acute outbreaks of respiratory disease



H Haemorrhage (macro- or microscopic), N Necrosis (macro- or microscopic), Shaded = *A pleuropneumoniae* isolated from lungs and/or lung lavage fluids

TABLE 4: Main pathological findings and agents isolated from all six pigs from four outbreaks (1, 2, 4 and 16) with an unclear diagnosis. Diseased pigs are numbered 1 to 4, clinically healthy pigs 5 and 6

Outbreak	Pig	Main pathological findings: Description*	% affected lung (macroscopic)	Viruses: Infl	Viruses: PRRS	Bacteria: App	Bacteria: Pm	Bacteria: Hp	Bacteria: Bb	Bacteria: Ss	Mycoplasmas: hp	Mycoplasmas: hr
1	1	Broncho-interstitial pneumonia (hist)	0	–†	±	–	–	–	+	–	–	+
	2	Catarrhal pneumonia, pleuritis	<10	–	+	–	–	+	+	–	–	+
	3	Broncho-interstitial pneumonia (hist)	0	–	+	–	–	+	+	–	–	+
	4	Catarrhal pneumonia	30	–	+	–	–	–	+	–	–	+
	5	Peribronchiolar/perivascular infiltrates (hist)	0	–	–	–	–	–	–	–	–	–
	6	Peribronchiolar infiltrates (hist)	0	–	–	–	–	–	–	–	–	+
2	1	Catarrhal purulent pneumonia	50	–	–	–	–	–	–	–	–	+
	2	Catarrhal purulent pneumonia	50	–	–	–	–	–	–	+	–	+
	3	Catarrhal purulent pneumonia	40	–	–	–	+	–	–	+	–	+
	4	Catarrhal purulent pneumonia	40	–	–	–	+	–	–	+	–	–
	5	Pleuritis	5	–	–	+	–	–	–	–	+	+
	6	Catarrhal purulent pneumonia	5	–	–	–	–	–	–	–	–	–
4	1	Catarrhal purulent pneumonia	30	–	+	–	–	+	–	+	–	+
	2	Catarrhal purulent pneumonia	15	–	+	–	–	–	+	–	–	+
	3	Catarrhal pneumonia	10	–	+	–	–	+	+	+	–	+
	4	Broncho-interstitial pneumonia (hist)	0	–	+	–	–	–	–	–	–	–
	5	Broncho-interstitial pneumonia (hist)	0	–	+	–	–	–	–	–	+	+
	6	–	0	–	+	–	–	–	–	–	–	+
16	1	Necrotising pleuropneumonia	15	–	–	+	–	–	–	–	–	+
	2	Catarrhal purulent interstitial pneumonia	25	+	–	–	–	–	–	–	–	+
	3	Catarrhal purulent pneumonia	20	–	–	–	+	–	–	–	–	+
	4	Interstitial pneumonia, pleuritis	10	±	–	+	–	–	–	–	–	+
	5	Interstitial pneumonia (hist)	0	±	–	–	–	–	–	–	–	+
	6	Catarrhal pneumonia	5	+	–	–	–	–	–	–	–	+

Infl Influenzavirus, PRRS Porcine reproductive and respiratory syndrome, App *A pleuropneumoniae*, Pm *P multocida*, Hp *H parasuis*, Bb *B bronchiseptica*, Ss *S suis*, hp *M hyopneumoniae*, hr *M hyorhinis*, * Macroscopic lesions, unless followed by (hist) = histological findings, † + Isolated from lungs, ± Isolated from tonsils only, – Not isolated

subtype H1, whereas the groupmates in herds 7 and 12 seroconverted against influenza virus subtype H3 (Table 5). This result correlated perfectly with the isolation of influenza virus subtypes H1 and H3 from the diseased pigs in the respective herds. In herd 16, however, the groupmates did not seroconvert against influenza virus, even though the virus, subtype H1, was isolated from two of the diseased pigs. Antibodies against subtype H1 were already present in the acute sera.

At least four of the 10 groupmates of the four diseased pigs in herds 3, 8, 9, 10, 11 and 16 seroconverted against *A pleuropneumoniae* (Table 5). At least nine of these groupmates were seropositive for *A pleuropneumoniae* at the end of the finishing period (data not shown). *A pleuropneumoniae* serotype 2 was isolated from the lungs of the diseased pigs from all these herds. In herds 1 and 13, at least five of the 10 groupmates tested seroconverted, although *A pleuropneumoniae* was not isolated from the four diseased pigs in these herds.

Serological examination of random end sera and end sera

The pigs in most compartments became seropositive for one or both subtypes of influenza virus, PRRS virus, and *M hyopneumoniae*. The pigs in only half of the compartments became seropositive for *A pleuropneumoniae*, and none became seropositive for Aujeszky's disease virus (Table 6).

Some of the end sera were positive for influenza virus (herds 4 and 11) or *M hyopneumoniae* (herds 6 and 16), whereas the convalescent sera were still negative for these agents. These infections apparently occurred after the investigation of the outbreaks.

Overall findings and diagnosis

Influenza virus was the most important infectious agent involved in the acute respiratory disease. In herds 5, 6, 7, 12, 13, 14 and 15, it was concluded that influenza virus was solely responsible for the outbreak of respiratory disease (Table 7). Influenza virus was isolated from three or four of the diseased pigs, endobronch(iol)itis was frequently observed, and nine or 10 of the groupmates seroconverted against the subtype isolated. Except for herd 5, few secondary bacteria were isolated.

In herds 9 and 16, influenza virus was also isolated from several pigs, but sometimes only from the tonsils. Even though endobronch(iol)itis was observed in some pigs and the groupmates in herd 9 seroconverted for the subtype isolated, it was concluded that influenza virus was not the sole or most important cause of these outbreaks.

A pleuropneumoniae was the second most important agent involved in the acute respiratory disease. In herds 3, 8, 10 and 11, *A pleuropneumoniae* serotype 2 was isolated from three or four of the diseased pigs, the pigs had haemorrhagic and necrotic lung lesions, and the groupmates seroconverted against *A pleuropneumoniae*. In herd 9, there was a concurrent infection with influenza virus, but on the basis of the isolation of the organism and the pathological findings (including histology) it was concluded that *A pleuropneumoniae* was the most important agent causing the outbreak

TABLE 5: Serological results from pairs of acute and convalescent sera taken from five pigs in 16 acute outbreaks of respiratory disease

Outbreak	Number of acute-convalescent sera that tested positive for: Infl H1	Infl H3	Auj gE	PRRS	App	Mhyo
1	9-7	10-8	1-0	10-10	0-5	0-1
2	10-10	10-10	0-0	10-10	0-0	4-9
3	9-10	1-2	0-0	10-10	0-8	3-4
4	0-1	6-6	0-0	10-10	0-0	2-5
5	0-10	6-5	0-0	10-10	0-0	6-5
6	0-10	3-4	0-0	1-10	1-0	0-0
7	4-3	1-10	0-0	9-10	0-0	0-0
8	7-6	0-0	0-0	10-10	0-7	4-6
9	0-9	10-10	0-0	9-10	4-9	2-2
10	7-9	5-4	0-0	9-9	0-4	0-1
11	9-9	0-0	0-0	10-10	2-9	4-2
12	1-4	0-10	0-0	10-10	0-0	6-7
13	1-10	0-0	0-0	9-9*	0-8	0-0
14	0-10	10-10	0-0	10-10	6-6	7-1
15	0-10	0-0	0-0	10-10	0-0	5-10
16	9-10	4-1	1-0	7-10	0-4	0-0

Infl Influenza, Auj Aujeszky's disease, PRRS Porcine reproductive and respiratory syndrome, App *A pleuropneumoniae*, Mhyo *M hyopneumoniae*, * Although nine acute sera were already positive, a four-fold rise in titre was observed in six pigs, Shaded = seroconversion (negative-positive or four-fold rise of titre) in five or more of the 10 pigs

TABLE 6: Serological results of all end and random end sera in 16 herds with a clinical outbreak of acute respiratory disease. A compartment was considered positive if at least two individual pigs were seropositive, doubtful results and results of vaccinated pigs omitted

Agent	Compartments Number Positive	Tested	(%)	Individual pigs Number Positive	Tested	(%)
Influenza H1	38	40	(95)	327	400	(82)
Influenza H3	33	40	(83)	222	400	(56)
Aujeszky-gE	0	40	(0)	0	400	(0)
PRRS	40	40	(100)	400	400	(100)
App 2/9	20	39	(51)	170	365	(47)
M hyopneumoniae	31	43	(72)	166	303	(55)

PRRS Porcine reproductive and respiratory syndrome, App *A pleuropneumoniae*

TABLE 7: History of infections in 16 compartments where an outbreak of acute respiratory disease occurred, based on serology, isolations of infectious agents and pathological findings

Outbreak	Before clinical outbreak	Cause of clinical outbreak	Concurrent infection	After clinical outbreak	Secondary bacteria
1	PRRS/Infl	Secondary infections?		App	++*
2	Mhyo/PRRS/Infl	Secondary infections?			++
3	Mhyo/PRRS/Infl	App type 2			–
4	PRRS/Infl	Secondary infections?	Mhyo	Infl H1	++
5	Mhyo/PRRS	Infl H1			+++
6		Infl H1	PRRS	Mhyo	+
7	PRRS	Infl H3			+
8	Mhyo/PRRS/Infl	App type 2			+
9	PRRS	App type 2	Infl H1		++
10	PRRS/Infl	App type 2			++
11	Mhyo/PRRS/Infl	App type 2		Infl H3	++
12	Mhyo/PRRS/Ascaris	Infl H3			+
13	PRRS	Infl H1		App	+
14	Mhyo/PRRS/App	Infl H1			+
15	Mhyo/PRRS	Infl H1			+
16	PRRS/Infl?	Infl H1/App2		Mhyo	+

* Number of isolates (*P multocida, H parasuis, B bronchiseptica, S suis*) in four diseased pigs: – none, + few (1-3), ++ moderate (4-6), +++ many (≥7)
PRRS Porcine reproductive and respiratory syndrome, Mhyo *M hyopneumonia*, Infl Influenza, App *A pleuropneumoniae*

(Table 7). In herds 1 and 13, the groupmates of the diseased pigs seroconverted, but *A pleuropneumoniae* was not isolated, and no typical lung lesions were observed. The infection with *A pleuropneumoniae* must have occurred shortly after the investigation of the outbreak.

The outbreak in herd 16 seemed to be caused by a combination of influenza virus and *A pleuropneumoniae*. The causes of the outbreaks in herds 1, 2 and 4 remained unclear (Table 7).

DISCUSSION

Influenza virus and *A pleuropneumoniae* are the most important infectious agents involved in acute respiratory disease in finishing pigs. Twelve of the 16 outbreaks in herds with recurrent acute respiratory disease could be attributed to an infection with either one of these agents. Concurrent infections with other infectious agents did occur, but the presence of these agents was not clearly correlated with the presence of influenza virus or *A pleuropneumoniae*. There was thus no clear evidence that certain specific dual infections might have been important. However, owing to the fact that only 16 outbreaks were investigated, and several combinations of dual infections may be possible, a role for dual infections can not be excluded. The aetiology of four outbreaks remained uncertain and several infectious agents were involved in them.

Influenza virus was responsible for seven of the 16 outbreaks of respiratory disease. The evidence that influenza virus was the cause of the outbreak was based on the presence of endobronch(iol)itis, which is very typical of an influenza infection (Easterday and Hinshaw 1992), the isolation of the virus, and the serology of paired sera. Furthermore, influenza virus was also involved in two other outbreaks.

Even though many clinical outbreaks can be explained by an infection with influenza virus, infections with influenza virus do not always lead to an outbreak with clinical signs. Pigs in all 16 compartments affected by an outbreak went through an infection with one or both subtypes of influenza virus. Although some of these infections caused the outbreaks investigated, most of them occurred before the clinical outbreak, at a time when no clinical signs of respiratory disease were observed by the farmer. This suggests that infections in young pigs may often remain subclinical. Furthermore, the serology of the random end sera showed a high percentage of compartments in which an infection with one or both subtypes had occurred, including many compartments in which, according to the farmer, no clinical signs of respiratory disease had been noticed. This is in agreement with the results of earlier studies in which high seroprevalences were observed in finishing pigs (Elbers and others 1990b, 1992, Van Reeth and Pensaert 1994, Maes and others 1996), even in herds without a history of respiratory disease.

A pleuropneumoniae was responsible for five outbreaks. The pathological findings of haemorrhage and necrosis were typical of an *A pleuropneumoniae* infection, and the isolation of the organism, and the serology of paired sera confirmed the infection. In some outbreaks only four or five of the 10 groupmates seroconverted within three weeks. This result is probably due either to the relatively low sensitivity of the CF test (Fenwick and Henry 1994, Sørensen and others 1996) or to a slower production of antibodies. At the end of the finishing period at least nine out of 10 pigs had seroconverted, indicating that almost all the pigs had become infected.

In one other outbreak it was concluded that *A pleuropneumoniae* was part of the cause, together with influenza virus. In two more outbreaks, an infection with *A pleuropneumoniae* probably occurred within days after the outbreak under investigation. In these two herds, clinical signs due to the infection with *A pleuropneumoniae* may have occurred, but were probably indistinguishable from the clinical signs of the initial outbreak. Infections with *A pleuropneumoniae* usually seem to lead to an outbreak with clinical signs and rarely remain subclinical.

A pleuropneumoniae infections were often preceded by infections with PRRS virus and/or influenza virus. Since most pigs go through these infections, their possible role as precursors of an *A pleuropneumoniae* infection remains unclear. However, an earlier study revealed that viral infections before an infection with *A pleuropneumoniae* may increase the severity of the clinical disease due to the bacterium (Sakano and others 1993). Secondary infections with other bacteria were slightly more common than secondary infectious after an influenza infection. This may be due to the fact that *A pleuropneumoniae* infections result in more profound lesions, thus providing secondary bacteria with a better opportunity for infecting the lungs.

Although PRRS virus was isolated frequently, sometimes from all four of the diseased pigs, there was no clear evidence that it caused respiratory disease. Because the virus can be isolated up to at least eight weeks after an infection (Meredith 1995), its isolation does not necessarily indicate a recent infection. In fact, antibodies to PRRS virus were present in the convalescent sera of all 16 outbreaks, but in at least 14 of them the infections were not recent. In these outbreaks, antibodies against PRRS virus were already present in the acute sera and there was no further increase in the antibody titres in the convalescent sera. In the IPMA, antibody titres reach a maximum at 20 to 30 days after infection (Wensvoort and others 1992) or even as late as five to six

weeks after infection (Meredith 1995); it was therefore concluded that these infections must have occurred at least three to four weeks before the onset of the clinical outbreak. A possible role of PRRS virus in dual infections has been demonstrated by Van Reeth and others (1996a, b). However, the results of one of their experiments suggested that the impairment of the lungs' defence mechanisms may be overcome within two weeks (Van Reeth and others 1996b). It was therefore concluded that it was unlikely that PRRS virus had played an important role in the infections with influenza virus or *A pleuropneumoniae* that occurred later. However, it remains unclear what the effect of persistent PRRS virus may be, once the lungs have been damaged by other infectious agents.

M hyopneumoniae was isolated only sporadically. The serological findings also indicated that *M hyopneumoniae* infections were not a major cause of acute respiratory disease. This result was not unexpected, because *M hyopneumoniae* is usually associated with chronic respiratory disease (Ross 1992, Kobisch and others 1993). Because antibodies against *M hyopneumoniae* were already present in many of the acute sera, it was concluded that infections with *M hyopneumoniae* often started well before the onset of the acute outbreak of respiratory disease. It is possible that the pigs that were subclinically infected with *M hyopneumoniae* were more susceptible to the infectious agents that caused the acute outbreaks.

The pathogenicity of *M hyorhinis* is controversial. Although some strains are able to produce pneumonia in gnotobiotic pigs (Gois and Kuksa 1974), *M hyorhinis* usually causes polyserositis and arthritis in young pigs (Ross 1992). Even though Hensel and others (1994) isolated *M hyorhinis* only sporadically from the lung lavage fluids of healthy pigs, in this study there was no difference between lungs with and without lesions. It was concluded that *M hyorhinis* is not of particular concern in acute respiratory disease.

S suis and *P multocida* were both isolated only from pigs with lung lesions, whereas *H parasuis* and *B bronchiseptica* were also isolated from pigs without lesions. The role of all these bacteria in these 16 outbreaks remains uncertain. Under conventional circumstances, they are considered to cause secondary infections after the defence mechanisms of the lungs have been impaired.

Several *Streptococcus* species, including *S suis* have been isolated from moderate to high proportions of the lungs of healthy pigs (Hensel and others 1994), as well as from pigs with lung lesions (Castryck and others 1990, Falk and others 1991). It has been established that some serotypes of *S suis* may exacerbate clinical disease after an infection with a primary agent, such as Aujeszky's disease virus, as was found by Iglesias and others (1992) for *S suis* type 2.

P multocida can cause severe pneumonia after the defence mechanisms of the lungs have been impaired (Fuentes and Pijoan 1987, Ciprián and others 1988, Amass and others 1994). In this study the bacterium was not isolated from lungs without lesions, in agreement with the results of Hensel and others (1994). *P multocida* was highly correlated with subacute or chronic lesions, as in the earlier studies of Falk and others (1991), Høie and others (1991) and Runge and others (1996). This may also explain the relatively few isolations of *P multocida*, since more than half of the pigs had only acute lesions postmortem.

It is possible that secondary bacteria may have influenced the severity of the clinical signs, and they may also have played a role in the clinical outbreaks for which no clear cause could be established. In these outbreaks there may have been a subclinical infection with an unknown primary agent (possibly PRRS virus or *M hyopneumoniae*), with clinical disease occurring only after lung lesions due to secondary infections had developed.

ACKNOWLEDGEMENTS

The authors thank all the personnel of the laboratories and the people who arranged the transport of the pigs; they all worked for many hours outside regular working hours. The authors also thank the farmers for their willing cooperation.

References

AMASS, S. F., CLARK, L. K., VAN ALSTINE, W. G., BOWERSOCK, T. L., MURPHY, D. A., KNOX, K. E. & ALBREGTS, S. R. (1994) Interaction of *Mycoplasma hyopneumoniae* and *Pasteurella multocida* infections in swine. *Journal of the American Veterinary Medical Association* **204,** 102-107

AWAD-MASALMEH, M., KÖFER, J. & SCHUM, M. (1990) On the occurrence of chronic respiratory disease at swine herds in Austria, bacteriological findings, efficacy of autogenous vaccines. *Proceedings of the International Pig Veterinary Society* **11,** 107

CALLEBAUT, P., PENSAERT, M. B., MIRY, C., HAESEBROUCK, F. & VERGOTE, J. (1986) Prevalence of influenza-, Aujeszky-, transmissible gastroenteritis and porcine epizootic diarrhea virus infections in feeder pigs. *Proceedings of the International Pig Veterinary Society* **9,** 216

CASTRYCK, F., DEVRIESE, L. A., HOMMEZ, J., CASSIMON, P. & MIRY, C. (1990) Bacterial agents associated with respiratory disease in young feeder pigs. *Proceedings of the International Pig Veterinary Society* **11,** 112

CHRISTENSEN, G. & MOUSING, J. (1992) Respiratory system. In Diseases of Swine. 7th edn. Ed A. D. Leman, B. Straw, R. Glock, W. L. Mengeling, S. D'Allaire, D. J. Taylor. Ames, Iowa State University Press. pp 138-162

COWAN, S. T. & STEEL, K. J. (1993) Manual for the identification of medical bacteria. 3rd edn. Ed E. I. Barrow, R. K. A. Feltham. Cambridge, Cambridge University Press. p 320

CIPRIÁN, A., PIJOAN, C., CRUZ, T., CAMACHO, J., TÓRTORA, J., COLMENARES, G., LÓPEZ-REVILLA, R. & DE LA GARCIA, M. (1988) *Mycoplasma hyopneumoniae* increases the susceptibility of pigs to experimental *Pasteurella multocida pneumonia. Canadian Journal of Veterinary Research* **52,** 434-438

DONE, S. H. & PATON, D. J. (1995) Porcine reproductive and respiratory syndrome – clinical disease, pathology and immunosuppression. *Veterinary Record* **136,** 32-35

EASTERDAY, B. C. & HINSHAW, V. S. (1992) Swine influenza. In Diseases of Swine. 7th edn. Ed A. D. Leman, B. Straw, R. Glock, W. L. Mengeling, S. D'Allaire, D. J. Taylor. Ames, Iowa State University Press. pp 349-357

ELBERS, A. R. W., CROMWIJK, W. A. J., HUNNEMAN, W. A. & TIELEN, M. J. M. (1990a) Log book registration of farms for slaughtering pigs in the Integrated Quality Control Project. I. Use of drugs and vaccines. *Tijdschrift voor Diergeneeskunde* **115,** 249-261

ELBERS, A. R. W., TIELEN, M. J. M., CROMWIJK, W. A. J. & HUNNEMAN, W. A. (1990b) Seroepidemiological screening of pig sera collected at the slaughterhouse to detect herds infected with Aujeszky's disease virus, porcine influenza virus and *Actinobacillus* (*Haemophilus*) *pleuropneumoniae* in the framework of an Integrated Quality Control (IQC) system. *Veterinary Quarterly* **12,** 221-230

ELBERS, A. R. W., TIELEN, M. J. M., CROMWIJK, W. A. J. & HUNNEMAN, W. A. (1992) Variation in seropositivity for some respiratory disease agents in finishing pigs: epidemiological studies on some health parameters and farm and management conditions in the herds. *Veterinary Quarterly* **14,** 8-13

FALK, K., HØIE, S. & LIUM, B. M. (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. 2. Enzootic pneumonia of pigs – microbiological findings and their relationship to pathomorphology. *Acta Veterinaria Scandinavica* **32,** 67-77

FALK, K. & LIUM, B. M. (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. 3. Serological findings and their relationship to pathomorphological and microbiological findings. *Acta Veterinaria Scandinavica* **32,** 79-88

FENWICK, B. & HENRY, S. (1994) Porcine pleuropneumonia. *Journal of the American Veterinary Medical Association* **204,** 1334-1340

FRIIS, N. F. (1975) Some recommendations concerning primary isolation of *Mycoplasma suipneumoniae* and *Mycoplasma flocculare*. A survey. *Nordisk Veterinaermedicin* **27,** 337-339

FUENTES, M. C. & PIJOAN, C. (1987) Pneumonia in pigs induced by intranasal challenge exposure with pseudorabies virus and *Pasteurella multocida. American Journal of Veterinary Research* **48,** 1446-1448

GOIS, M. & KUKSA, F. (1974) Intranasal infection of gnotobiotic piglets with *Mycoplasma hyorhinis*: differences in virulence of the strains and influence of age on the development of infection. *Zentralblad Veterinaermedicin B* **21,** 352-361

HENSEL, A., GANTER, M., KIPPER, S., KREHON, S., WITTENBRINK, M. M. & PETZOLDT, K. (1994) Prevalence of aerobic bacteria in bronchoalveolar lavage fluids from healthy pigs. *American Journal of Veterinary Research* **55,** 1697-1702

HØIE, S., FALK, K. & LIUM, B. M. (1991) An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. 4. Bacteriological findings in chronic pneumonic lesions. *Acta Veterinaria Scandinavica* **32,** 395-402

IGLESIAS, J. G., TRUJANO, M. & XU, J. (1992) Inoculation of pigs with *Streptococcus suis* type 2 alone or in combination with pseudorabies virus. *American Journal of Veterinary Research* **53,** 364-367

KAY, R. M., DONE, S. H. & PATON, D. J. (1994) Effect of sequential porcine reproductive and respiratory syndrome and swine influenza on the growth and performance of finishing pigs. *Veterinary Record* **135,** 199-204

KENDAL, A. P., SKEHEL, J. J. & PEREIRA, M. S. (1982) Concepts and procedures for laboratory-based influenza surveillance. Atlanta, US Department of Health and Human Services, Public Health Service, Centers for Disease Control

KOBISCH, M., BLANCHARD, B. & LE POTIER, M. F. (1993) *Mycoplasma hyopneumoniae* infection in pigs – duration of the disease and resistance to reinfection. *Veterinary Research* **24,** 67-77

LANZA, I., BROWN, I. H. & PATON, D. J. (1992) Pathogenicity of concurrent infections of pigs with porcine respiratory coronavirus and swine influenza virus. *Research in Veterinary Science* **53,** 309-314

LAUDE, H., VAN REETH, K. & PENSAERT, M. (1993) Porcine respiratory coronavirus – molecular features and virus host interactions. *Veterinary Research* **24,** 125-150

MAES, D., DELUYKER, H., VERDONCK, M., CASTRYCK, F., MIRY, C. & DE KRUIF, A. (1996) Epidemiological investigations on respiratory diseases in pigs in Belgium. *Proceedings of the International Pig Veterinary Society* **14,** 405

MATTSSON, J. G., BERGSTROM, K., WALLGREN, P. & JOHANSSON, K. E. (1995) Detection of *Mycoplasma hyopneumoniae* in nose swabs from pigs by in vitro amplification of the 16s ribosomal RNA gene. *Journal of Clinical Microbiology* **33,** 893-897

MEREDITH, M. J. (1995) Porcine reproductive and respiratory syndrome (PRRS). Germany, Ingelheim/Rhein. p 81

NICOLET, J., DE MEURON, P. A. & BACHMANN, P. (1971) Haemophilus infection in pigs. 4. The use of the complement fixation reaction in the diagnosis of *Haemophilus parahaemolyticus* infections. *Schweizer Archive Tierheilkunde* **113,** 191-200

NOWOTNY, N., MÖSTL, K., MADERBACHER, R., ODÖRFER, G. & SCHUH, M. (1994) Serological studies in Austrian fattening pigs with respiratory disorders. *Acta Veterinaria Hungarica* **42,** 377-379

PAISLEY, L. G., VRAA-ANDERSEN, L., DYBKJÆR, L., MØLLER, K., CHRISTENSEN, G., MOUSING, J. & AGGER, J. F. (1993) An epidemiologic and economic study of respiratory diseases in two conventional Danish swine herds. 1. Prevalence of respiratory lesions at slaughter and their effects on growth. *Acta Veterinaria Scandinavica* **34,** 319-329

PIJOAN, C. (1992) Pneumonic pasteurellosis. In Diseases of Swine. 7th edn. Ed A. D. Leman, B. Straw, R. Glock, W. L. Mengeling, S. D'Allaire, D. J. Taylor. Ames, Iowa State University Press. pp 552-559

ROSENDAL, S. & BLACK, F. T. (1972) Direct and indirect immunofluorescence of unfixed and fixed *Mycoplasma* colonies. *Acta Pathologica et Microbiologica Scandinavica* **80B,** 615-622

ROSS, R. F. (1992) Mycoplasmal diseases. In Diseases of Swine. 7th edn. Ed A. D. Leman, B. Straw, R. Glock, W. L. Mengeling, S. D'Allaire, D. J. Taylor. Ames, Iowa State University Press. pp 537-551

RUNGE, M., GANTER, M., DELBECK, F., HARTWICK, W., RÜFFER, A., FRANZ, B. & AMTSBERG, G. (1996) Etiological diagnosis of pneumonia in fattening pigs: investigation of bronchoalveolar lavage (BAL) by cultural methods and immunofluorescence microscopy, as well as serological findings. *Berliner und Münchner Tierärztliche Wochenschrift* **109,** 101-107

SAKANO, T., SHIBATA, I., SAMEGAI, Y., TANEDA, A., OKADA, M., IRISAWA, T. & SATO, S. (1993) Experimental pneumonia of pigs infected with Aujeszky's disease virus and *Actinobacillus pleuropneumoniae*. *Journal of Veterinary Medical Science* **55,** 575-579

SEBUNYA, T. N. K. & SAUNDERS, J. R. (1983) *Haemophilus pleuropneumoniae* infection in swine: a review. *Journal of the American Veterinary Medical Association* **182,** 1331-1337

SØRENSEN, V., BARFOD, K., NIELSEN, J. P., FELD, N. F. & CHRISTENSEN, J. (1996) Evaluation of a polyclonal blocking ELISA and a complement fixation assay detecting antibodies of *Actinobacillus pleuropneumoniae* serotype 2 in pigs. *Proceedings of the International Pig Veterinary Society* **14,** 191

STEMKE, G. W., PHAN, R., YOUNG, T. F. & ROSS, R. F. (1994) Differentiation of *Mycoplasma hyopneumoniae*, *M flocculare* and *M hyorhinis* on the basis of amplification of a 16s rRNA gene sequence. *American Journal of Veterinary Research* **55,** 81-84

STRAW, B. E., SHIN, S. J. & YEAGER, A. E. (1990) Effect of pneumonia on growth rate and feed efficiency of minimal disease pigs exposed to *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae*. *Preventive Veterinary Medicine* **9,** 287-297

VAN OIRSCHOT, J. T., HOUWERS, D. J., RZIHA, H. J. & MOONEN, P. J. L. M. (1988) Development of an ELISA for detection of antibodies to glycoprotein I of Aujeszky's disease virus: a method for the serological differentiation between infected and vaccinated pigs. *Journal of Virological Methods* **22,** 191-206

VAN REETH, K. & PENSAERT, M. B. (1994) Porcine respiratory coronavirus mediated interference against influenza virus replication in the respiratory tract of feeder pigs. *American Journal of Veterinary Research* **55,** 1275-1281

VAN REETH, K. & PENSAERT, M. B. (1996) A clinical and virological study in pigs infected with Aujeszky's disease virus shortly after infection with porcine respiratory coronavirus. *Proceedings of the International Pig Veterinary Society* **14,** 137

VAN REETH, K., NAUWYNCK, H. & PENSAERT, M. (1996a) Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: a clinical and virological study. *Veterinary Microbiology* **48,** 325-335

VAN REETH, K., VAN HOOF, D., WILLEMS, L. & PENSAERT, M. (1996b) A clinical study of dual infections with porcine reproductive respiratory syndrome virus and swine influenza virus administered with different time intervals. *Proceedings of the International Pig Veterinary Society* **14,** 59

WENSVOORT, G., TERPSTRA, C., POL, J. M. A., TER LAAK, E. A., BLOEMRAAD, M., DE KLUYVER, E. P., KRAGTEN, C., VAN BUITEN, L., DEN BESTEN, A., WAGENAAR, F., BROEKHUIJSEN, J. M., MOONEN, P. L. J. M., ZETSTRA, T., DE BOER, E. A., TIBBEB, H. J., DE JONG, M. F., VAN 'T VELD, P., GROENLAND, G. J. R., VAN GENNEP, J. A., VOETS, M. T., VERHEIJDEN, J. H. M. & BRAAMSKAMP, J. (1991) Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Veterinary Quarterly* **13,** 121-130

WENSVOORT, G., DE KLUYVER, E. P., LUIJTZE, E. A., DEN BESTEN, A., HARRIS, L., COLLINS, J. E., CHRISTIANSON, W. T. & CHLADEK, D. (1992) Antigenic comparison of Lelystad virus and swine infertility and respiratory syndrome (SIRS) virus. *Journal of Veterinary Diagnostic Investigations* **4,** 134-138

WENSVOORT, G. (1993) Lelystad virus and the porcine epidemic abortion and respiratory syndrome. *Veterinary Research* **24,** 117-124

ABSTRACT

Reducing bovine embryonic mortality

VETERINARY surgeons can help to reduce embryonic mortality in cattle in four ways: first, by encouraging farmers to detect cows in oestrus at the earliest possible stage; secondly, by treating repeat breeders either intravaginally with progesterone for six days starting six to eight days after oestrus, or parenterally with gonadotrophin-releasing hormone on day 11 to 13 of the cycle and/or at insemination; thirdly, by helping farmers to prevent their cows being either too fat or too thin at calving and by advising them to avoid feeding forages which are too high in nitrogen or molybdenum; and fourthly, by diagnosing pregnancy as early as possible by any method other then membrane slip.

LENAHAN, B. J. (1999) *Irish Veterinary Journal* **52,** 320

Survey of infectious agents involved in acute respiratory disease in finishing pigs

W. L. A. Loeffen, E. M. Kamp, N. Stockhofe-Zurwieden, et al.

Veterinary Record 1999 145: 123-129
doi: 10.1136/vr.145.5.123