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Incidence of antibodies to, serotypes and antibiotic sensitivity of *Haemophilus pleuropneumoniae* in Iowa swine

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INTRODUCTION

Haemophilus pleuropneumoniae, known previously as Haemophilus parahaemolyticus, is gaining increased recognition as a major respiratory pathogen of swine. The organism causes an extensive lobar pneumonia with accompanying pleuritis in swine. H. pleuropneumoniae was first identified as causing pneumonia in swine in the early 1960s in the United States. In the middle to late 1960s, European workers reported substantial economic losses with the disease. It was not until the late 1970s that significance was attached to H. pleuropneumoniae in the United States when many swine confinement units began to suffer severe economic losses from it.

The increased incidence of H. pleuropneumoniae disease is evidenced by the dramatic increase of confirmed cases in the Veterinary Diagnostic Laboratories of both Iowa and South Dakota from 1977 to 1980 (Appendix I, II). Distribution of isolations in Iowa are shown (Appendix III).

The increased diagnosis and economic losses prompted interest in determining the incidence of H. pleuropneumoniae and serotypes present in Iowa swine. With this information, it was hoped that a rational and intelligent approach to prevention and control of this disease could be developed.

This thesis deals primarily with a survey of sera from adult Iowa swine for antibodies to H. pleuropneumoniae. Iowa's annual output of 19 to 23 million slaughter hogs represents approximately one-fourth of the total United States production. Also included are results of serotyping

and antibiotic sensitivity testing of fifty isolates of H. pleuropneumoniae from swine in Iowa and surrounding states.

LITERATURE REVIEW

History

The first isolations of a member of the genus Haemophilus were made from sputa of humans affected with epidemic influenza (Pfeiffer 1892). The organism was thought to be the cause of human influenza until investigators failed to find it in many cases of the pandemic of influenza that occurred in 1918. Influenza was concluded to be of viral origin when bacteria-free filtrates of nasal exudate produced the disease. It is known that H. influenzae, as well as streptococci and pneumococci are important secondary invaders in influenza.

Murray (1920) isolated a small gram-negative coccus from swine dying with so-called "flu" and several other workers isolated a variety of organisms from the lungs of swine with influenza. In the 1930s, significant contributions were made to the understanding of the etiology of swine and human influenza. Shope (1931a,b) and Lewis and Shope (1931) isolated the swine influenza virus from pigs with this disease. H. influenzae suis (syn. H. suis) was described as a secondary invader acting synergistically with the virus to produce clinical swine influenza. These results suggested that human influenza might also be due to an interaction between the influenza virus and H. influenzae.

It is commonly thought that swine influenza is more severe when bacteria are present along with the virus; however, Easterday (1970) has pointed out that the role of Haemophilus sp. has not been considered in most recent studies on swine influenza.

Members of the genus Haemophilus are common in the respiratory tracts of animals and man and are associated primarily with respiratory disease. Species which are currently considered of importance in animal diseases are H. pleuropneumoniae, H. parasuis, H. suis, H. parainfluenzae, H. gallinarum, H. paragallinarum, H. somnus and H. equigenitalis.

Haemophilus somnus, a species of uncertain taxonomic status, and commonly associated with septicemia and secondary thromboembolic meningoencephalitis (TEME) in feedlot cattle, does not meet the criteria for for placement in the genus. Likewise H. equigenitalis, the cause of contagious equine metritis, is of questionable taxonomic status.

As mentioned, H. influenzae, the type species of the genus, is of classical importance as a secondary invader in human influenza. This species is an important cause also of meningitis, otitis, sinusitis and epiglottitis in man. Other species affecting man are H. aegyptius, a cause of conuunctivitis, and H. ducreyi the cause of soft chancre. Two other species, H. aphrophilus and H. paraphrophilus, have been isolated from endocarditis and septic conditions but are of unknown significance.

Haemophilus gallinarum and H. paragallinarum cause a disease of the of the upper respiratory tract of chickens known as infectious coryza.

Haemophilus agni (Kennedy et al. 1958) was reported to be the cause of an acute septicemic disease of sheep.

As mentioned, the genus Haemophilus contains some important species which are involved in respiratory disease of swine. The organisms are small gram-negative coccobacilli and bacilli with pleomorphic forms and a tendency to form filaments.

Haemophilus suis and Haemophilus parasuis

Haemophilus suis and H. parasuis have been considered to have an etiological role in several swine diseases. The most widely recognized is Glässer's disease, first described in Germany by Glässer (1910).

Glässer's disease is a septicemic disease of swine characterized by polyserositis, arthritis and meningitis. It occurs most often after weaning or transport (Hjärre 1958). The organism was first isolated from lesions of the disease by Schermer and Ehrlich (1922). Hjärre and Wramby (1942) were able to reproduce the disease by experimental inoculation of H. suis in swine. Likewise, Bakos et al. (1952) were able to reproduce Glässer's disease in swine and guinea pigs by intraperitoneal inoculation with certain strains of the organism. Neil et al. (1969) and Little and Harding (1971) reproduced Glässer's disease in pigs by intratracheal inoculation with encapsulated strains of H. parasuis. Hjärre and Wramby (1942), Bakos et al. (1952), and Braend and Flatla (1954) found H. suis to be the causative agent of porcine polyserositis, polyarthritis and meningitis (Glässer's disease). In addition, bronchiolitis was was observed by Hjärre et al. (1952) following intranasal infection of pigs with H. suis. When pigs were injected intraperitoneally and intravenously, the strains isolated gave rise to typical Glässer's syndrome and in one case, a focal hemorrhagic pneumonia and an acute nephritis. Bakos et al. (1952) found 3 of 28 isolates of H. suis to produce lesions of nephritis when given intraperitoneally to pigs. No other evidence that H. suis causes nephritis has appeared.

H. suis - parasuis is regarded by some investigators as part of the

normal bacterial flora in the nasopharynx of pigs. These bacteria have been isolated from a high percentage of healthy pigs (Ross et al. 1963, Harris et al. 1969, Bertschinger and Nicod 1970, Woods et al. 1972. and Nicod 1973). H. suis - H. parasuis has also been associated with atrophic rhinitis in swine (Radtke 1938, Braend and Flatla 1954, Switzer 1965, Gois et al. 1981).

H. suis was serogrouped by Bakos et al. (1952) and Bakos (1954) by means of the precipitation test into five groups, A-D and N, on the basis of type-specific antigens revealed after cross absorptions. Further evaluation by haemagglutination-inhibition and indirect haemagglutination proved H. suis to be an extremely variable antigen. No relationship was demonstrated between degree of pathogenicity and serological group.

The first isolates of H. suis were described by Shope (1931a) as requiring both X and V factors; however, Kilian (1976), in an excellent review, stated that it has unfortunately been the habit not to determine the exact growth requirements of H. suis - parasuis. Biberstein and White (1969) found that the overwhelming majority of strains designated H. suis are haemin-independent and therefore, they should be identified as H. parasuis. The prefix of the epithet of that name is in agreement with the usual convention in naming Haemophilus species (those which require only the V factor (NAD) having a prefix of para).

Haemophilus parainfluenzae

Isolates in England described as H. parainfluenzae by Pattison et al. (1957) and Matthews and Pattison (1961) were from swine with pneumonia

and pleurisy. Organisms identified as H. parainfluenzae from septicemic disease (Thomson and Ruhnke 1963) and meningitis (Radostits et al. 1963) in Canadian swine were similar to those in England. Also in England, Little (1970) reported isolating Haemophilus species from 44 of 120 pneumonic lungs collected at the slaughter house. Of the Haemophilus strains isolated, 45% were H. parasuis, 11% were H. parahaemolyticus, and 39% were nonhaemolytic isolates referred to as H. parainfluenzae. Gunnarsson (1980) suggests that H. parainfluenzae isolates may belong to a species closely related to H. pleuropneumoniae or to a separate species as recently suggested by Kilian et al. (1978).

In Norway, Odegaard (1966) produced pneumonia in swine by intranasal inoculation of H. parainfluenzae, a strain he had isolated from a pig with rhinitis. Nordstoga and Fjolstad (1967) produced a generalized Schwarzman reaction in pigs by intravenous injection of two doses of pressure-disrupted H. parainfluenzae. The isolate used was beta-haemolytic which strongly suggests that it was related to H. pleuropneumoniae (Gunnarsson 1980).

H. pleuropneumoniae (parahaemolyticus)

Pittman (1953) proposed the name H. parahaemolyticus for Haemophilus strains isolated from the human oropharnyx and subacute endocarditis. These strains required only the V factor (NAD) for growth and were hemolytic.

The first hemolytic swine isolates, described initially by Biberstein and Cameron (1961), were designated H. parahaemolyticus by Olander

(1963). The organism produced a septicaemic disease of swine which resulted in acute and peracute deaths (Olander, 1963). Shope (1964) proposed the name H. pleuropneumoniae for a hemolytic Haemophilus from swine in Argentina obtained from an outbreak of pleuropneumonia. Later it was shown that European strains of swine origin designated as H. parahaemolyticus and Shope's H. pleuropneumoniae were identical from a taxonomic point of view (Nicolet, 1968).

Members of the Haemophilus Subcommittee of the International Committee for Systematic Bacteriology (Zinnemann and Biberstein, 1974) suggested the name H. pleuropneumoniae (Shope, 1964, Kilian et al. 1978) based upon the differences between hemolytic porcine isolates and human H. parahaemolyticus strains (Kilian et al. 1978). Differences between the two groups were slight but swine H. parahaemolyticus has a predominance of coccoid forms and the absence of long filaments. It had stronger hemolyzing capacity on erythrocytes of various species and stronger fermentative activity (Nicolet, 1968). The production of urease and fermentation of mannitol, xylose, and deoxyribose differentiated H. pleuropneumoniae from other V factor dependent haemophili of porcine and human origin (Kilian, 1976). The guanine-cytosine (GC) content of H. pleuropneumoniae is significantly higher ($p > 0.01$) than human isolates of H. parahaemolyticus.

H. pleuropneumoniae has been isolated from cattle (Olander, 1963), sheep (Olander, 1963) and deer (Biberstein et al. 1977) in the United States. The significance of these infections has not been determined since so few isolations have been made from each species.

Distribution

The distribution of H. pleuropneumoniae is world-wide in swine. It has been described from the United States (Olander 1963), Argentina (Shope 1964), Switzerland (Nicolet and König 1966), Denmark (Nielsen 1970a), England (Little 1970), Sweden (Thörne and Hokanson 1970), Norway (Grøndalen 1972), Australia (Mylrea et al. 1974), Canada (Schiefer et al. 1974), German Democratic Republic (Kiupel 1975), Finland (Schulman et al. 1975), Taiwan (Hsu et al. 1976), Scotland (Morgan and Phillips 1978), Japan (Chan et al. 1978), the Netherlands (De Jong 1978) and Mexico (Stabenow et al. 1980).

Serotypes

Nicolet (1970, 1971) recognized three serotypes among isolates of H. pleuropneumoniae and numbered them 1, 2 and 3. Of 100 strains from Switzerland, 94 were assigned to serotype 2, three to serotype 3 and three were not typable. One Swedish and two Danish strains were assigned to serotype 2. One strain from England was designated serotype 3. Three Dutch strains and one Argentinean strain were assigned to serotype 1.

Nicolet (1971) isolated serotype 3 from abscesses in swine, but not pneumonia and considered it not to be significant as a respiratory pathogen. However, Gunnarsson et al. (1977) and Gunnarsson (1980) isolated serotype 3 from typical lesions of porcine contagious pleuropneumonia and considered it to be similar in pathogenicity to types 1, 2 and 5.

Gunnarsson et al. (1977) identified 2 new antigenic types (4 and 5) among isolates from Sweden and the United States. Gunnarsson (1980)

in a serological survey of 46 Swedish isolates and 22 isolates of various geographic origin of H. pleuropneumoniae found 2/3 of the 46 Swedish isolates to belong to serotype 2. Four strains belonged to serotype 3, four to serotype 4 and six in a nonmucoid phase were untypable by agglutination. Of the six isolates from the United States, five belonged to serotype 5 and one to serotype 4. Canadian isolates belong to serotype 5 and 2 (Gunnarsson 1980), the Danish isolates to serotype 2, the Swiss isolates to serotypes 2 and 3. Cultures from the German Democratic Republic were of types 2 and 5 and an Argentinian strain, type 1.

Gunnarsson et al. (1977) identified another Swedish isolate (strain 202) and initially designated it as the prototype of serotype 6; however, Kilian et al. (1978) found it to have a guanine + cytosine (G+C) content differing from H. pleuropneumoniae, suggesting that it was a new species.

In serotyping 43 strains of H. pleuropneumoniae, Kilian et al. (1978) found them to be accommodated in the serological scheme established by Nicolet (1971) and expanded by Gunnarsson et al. (1977) except for one strain (Femø). The existence of a strain untypable with available sera suggests the likelihood of additional, heretofore, unrecognized serotypes.

Understanding the geographic distribution of serotypes may be important for the preparation of serodiagnostic antigens and vaccines.

Nicolet (1971) and Gunnarsson et al. (1977) reported that only capsulated strains (M-cultures) could be serotyped by agglutination tests demonstrating that the serotype-specific determinants were located in the capsule. Gunnarsson et al. (1978) also demonstrated a reduction

and loss in some strains of serotype specificity after autoclaving the cells but also found evidence for heat-labile serotype-specific and common antigens. The cellular localization of these heat-labile antigens could not clearly be determined.

Incidence

Reports of the incidence of H. pleuropneumoniae disease in populations of swine and incidence of recovery of the organism are scanty and vary widely.

Nicod (1970), in Switzerland, found serological evidence of H. parahaemolyticus infection in 64% of the herds using the Waldmann system for swine respiratory disease control.

Nielsen and Petersen (1974) isolated H. pleuropneumoniae from 84 of 96 lungs with pleuropneumoniae from a Danish slaughter house. Diagnosis of H. pleuropneumoniae disease in Danish slaughter pigs increased from 22 percent to 37 percent of all pneumonic conditions between 1968 and 1971. This increase was attributed to an increase in intensification of the industry. Nielsen (1970a) isolated H. pleuropneumoniae from 21.8% and 26.4% of pigs with pneumonia in routine autopsies done in 1968 and 1969. Also in Denmark, Sanker (1975) reported the isolation of H. pleuropneumoniae from 133 of 171 (80%) pleuropneumonic lungs examined at slaughter. Sanker (1980), in an attempt to determine if pigs held overnight at a slaughter plant were in danger of infection with H. pleuropneumoniae, found no increased condemnations, but observed that if pigs were left for 2-3 days, for example over weekends, the condemnation rate for

pleuropneumonia rose from 1.7 per thousand to 6.68 per thousand. A distinct variation due to season was not observed. Sanker (1980) also found a 90% confirmation of the disease by isolation of H. pleuropneumoniae from pigs condemned because of pleuropneumonia.

In Sweden, Bäckström and Bremer (1976) found pleural adhesions in 6 to 8% of all slaughtered fattening pigs.

Kiupel (1975) isolated H. pleuropneumoniae from 1.4% of the lungs of 7894 swine routinely necropsied in the German Democratic Republic.

Morgan and Phillips (1978) isolated H. pleuropneumoniae from 15 of 78 pulmonary lesions (19.2%) in swine slaughtered in Scotland in July of 1977. This high rate of isolation is in agreement with Little (1973) who found 30% of sera from swine in Scotland contained complement - fixing antibodies to the organism.

In one report, Little (1970) isolated H. pleuropneumoniae from 11.4% of pneumonic lungs obtained at a slaughter house in southeast England.

Olander (1963) in a slaughter house survey of pneumonic lungs of one day's kill at a large packing plant in California in November of 1961 isolated H. pleuropneumoniae from 3 of 60 hogs. The origin of the hogs was one each from Nebraska, California, and one from the southeastern United States. In another survey of normal swine at a small packing plant in California, slaughtering only local hogs, Olander (1963) isolated H. pleuropneumoniae from the nasal passages of 2 of 69 apparently normal hogs. In the same survey, thirty lungs were cultured; no Haemophilus spp. were recovered. Harrison et al. (1978) reported H. parahaemolyticus was

isolated from 55 of 261 cases of swine pneumonia submitted to the Pennsylvania Bureau of Animal Industry Laboratory between August 1974 and May 1978. This represented a 21% infection rate in pneumonia cases presented.

Biochemical nature of Haemophilus antigens

H. influenzae, a human pathogen, and H. pleuropneumoniae, a swine pathogen, have been the most frequently isolated and among the most important species of the genus. Antigenic specificity of the soluble capsular type-specific antigens of H. influenzae types a-c has been determined to be based on different oligosaccharide units, joined through phosphoric diester linkages. For types d and e, phosphoric diester linkages have not been demonstrated (Rosenberg et al. 1961, Williamson and Zamenhof 1963, Branefors-Helander et al. 1976, Branefors-Helander 1977).

Gunnarsson and Branefors-Helander (1979) found a reaction of identity between capsular extracts of H. pleuropneumoniae-like strain 202 and H. influenzae type c.

Branefors-Helander (1973) found O-antigens with endotoxic properties (ie. LPS) in addition to serotype-specific capsular antigens in the soluble extracts of H. influenzae. These O-antigens were complex but all strains of H. influenzae shared one of the antigenic determinants (Branefors-Helander 1973).

Gunnarsson (1979a) found that phenol-extracted serotype-specific surface factors from H. pleuropneumoniae contained up to four

precipitinogens. Since the phenol-water extraction procedure is known to elute polysaccharides and lipopolysaccharides (LPS) from gram-negative bacteria, it is likely that some immunoprecipitates obtained with the extracts of H. pleuropneumoniae were produced by O-antigens of LPS nature (Gunnarsson 1979a).

Gram-negative bacteria have cell surface-associated protein antigens in addition to LPS-antigens. In H. pleuropneumoniae, Gunnarsson (1980) found heat-labile antigenic determinants which could be protein.

Häni et al. (1973) were unable to detect evidence of an endotoxin effect in pigs injected intravenously or aerosol-exposed to sonicated suspensions of pure cultures of H. pleuropneumoniae. However, when extracts of lung tissue containing H. pleuropneumoniae were given intravenously, fever and bilateral cortical necrosis developed.

Rosendal et al. (1980) demonstrated a toxic factor in sonicated H. pleuropneumoniae or a sterile filtrate of culture supernate which induced lung lesions in pigs. It was not determined whether the toxic factor was associated with the capsule or cell wall (e.g. endotoxin).

Kilian et al. (1979) found that two strains of H. pleuropneumoniae produced an extracellular enzyme that cleaved porcine secretory IgA but had no effect on human IgA. Since cleavage of IgA by such enzymes resulted in considerable loss of antibody activity, it appears probable that these IgA proteases represent a significant virulence factor.

Differentiation of swine Haemophilus

Carbohydrate fermentations appear to be of minor help in differentiating porcine Haemophilus species. In the literature, disagreement exists regarding carbohydrates fermented by porcine Haemophilus (Nicolet 1968, Nielsen 1970b, Kilian 1976, Kilian et al. 1978, Biberstein et al. 1977). Some of the discrepancies may have occurred because the organism grows poorly in most conventional media. There simply may have been insufficient growth in some carbohydrate-containing media. Furthermore, the various media used may have affected the carbohydrate fermentation reactions.

Nicolet (1978) proposed a group of tests (Table 1) for differentiation of porcine Haemophilus species.

Biberstein et al. (1977) found that hemolysin production on bovine blood agar was unreliable for identification of H. pleuropneumoniae, because there were differences in degree between strains and within strains from passage to passage. He reported that ability to produce hemolysin may be lost permanently or may be absent from freshly isolated strains that behave in other respects like typical H. pleuropneumoniae. Kilian et al. (1978) suggested that the CAMP reaction might be a better test of hemolytic activity among Haemophilus.

Gunnarsson (1980) found that all members of the H. suis - H. parasuis group required serum but that only two of fifteen strains of H. pleuropneumoniae required serum and those only in early passages. It is important then that media used for isolation of porcine Haemophilus contain serum.

Table 1. General characters of the species Haemophilus of porcine origin[a]

	Porphyrin Test*	NAD	Urease	Haemolysis	CAMP	Mannitol	Lactose
H. pleuropneumoniae	+	+	+	+	+	+	V
H. parasuis	+	+	-	-	-	-	-
H. suis	-/v[1]	+	-	-	-[2]	-[2]	v[2]
Haemophilus sp. "minor group"	+	+	+	-/w	-	-	-

1 = confirmatory test of hemin - dependence.
2 = only few strains tested.
w = weak reaction.
v = variable.
* = The porphyrin test for conversion of ß- amino-levulinic acid to porphyrin as described by Lascelles (1956) and applied to Haemophilus by Biberstein et al. (1963) and carried out according to Kilian's (1974) modification.

[a]From Nicolet 1978.

Serum dependency can also be used as an additional criterion for identification.

The mucoid-smooth-rough (M-S-R) dissociation of colony morphology has been described (Pittman 1931, Bakos 1955, Omland 1963a, b, Shope et al. 1964, Nicolet 1971) in many species within the genus Haemophilus, including H. pleuropneumoniae. Mucoid cultures have capsules, smooth cultures have only parts of capsules and rough cultures lack capsules (Omland 1963a, b, Branefors-Helander 1972).

Nicolet (1971) and Gunnarsson et al. (1977, 1978) reported that only mucoid cultures of H. pleuropneumoniae could be used for antigen in the agglutination test. Nicolet (1970) found the M-S-R dissociation to influence decisively the antigenicity of H. pleuropneumoniae.

Isolation of the organism has been enhanced by the use of 50% glycerol and 50% tap water (Shope 1964) and by a dilution method described by Little and Harding (1980). Rapid biochemical characterization of Haemophilus species by use of the Minitek system has been reported by Back and Oberhofer (1978), and the Micro-ID test system by Edberg et al. (1980).

Mode of transmission Transmission of the organism is probably effected by aerosol exposure or contact with infected carrier swine (Nicolet, 1979; Wilson, 1980). Nicolet (1971, 1979) found the most common route of infection was by direct nose-to-nose contact and thought that the boar may play an important role in spreading the disease. As few as 10^4 organisms can cause death in 1-2 days while only 10^2 organisms may provoke a lung infection but not cause death (Nicolet, 1979). In

susceptible herds the disease is capable of spreading rapidly with 50-100% of the herd being infected of which 2-50% may die (Shope 1964). Bachmann (1972) has shown that the organism resides in the tonsils of chronically infected carrier swine. Nielsen and Mandrup (1977) have shown that the organisms may persist in the tonsils for up to 4 months. They isolated it from the tonsils, lungs, pleura, and pericardium of chronically affected pigs. Bachmann (1972) found that the organism was transmitted infrequently from chronically infected carrier sows to suckling pigs. Nielsen (1975) found that pigs nursing immune dams were apparently protected by antibody acquired via colostrum during the first weeks after birth. Their resistance to H. pleuropneumoniae infection declined from 3 to 8 weeks.

Attempts to isolate the agent from the nose and throat of swine herdsmen in infected herds have failed (Nicolet 1979). The role of rodents and birds in the spread of H. pleuropneumoniae is thought to be minimal or non-existent since the organism has never been isolated from such hosts and attempts to infect such animals experimentally have not been successful (Nicolet 1979).

Serology

The first reported serological studies of H. pleuropneumoniae were performed by Olander (1963) who did cross-agglutination tests between California isolates of H. pleuropneumoniae, one of Shope's Argentinean isolates of H. pleuropneumoniae and a strain of H. suis. All isolates from California, except one, agglutinated in the same group. Neither the

Argentinean strain nor the strain of H. suis cross-agglutinated with any of the Californian strains.

Nicolet et al. (1971) found the complement fixation test the best routine method for sero-diagnosis of H. pleuropneumoniae infections. Bachmann (1972), in his comparison of the complement fixation test and the tube agglutination test, agar gel precipitation test, and immuno-electrophoresis also found it superior in specificity and sensitivity. Nielsen (1974) found the modified complement fixation test specific for H. parasuis in testing field outbreaks of polyserositis (Glässer's disease). Using either immunodiffusion or indirect hemagglutination tests, sera examined cross-reacted with H. pleuropneumoniae. Scholl et al. (1976) found cross-reactions between H. pleuropneumoniae and H. parasuis using the lymphocyte-stimulation test and leucocyte migration-inhibition test. Nicolet (1980) concluded that the enzyme-linked immunosorbent assay (ELISA) may offer an alternative to the CF test but that specific standardized antigens are needed. Until this is achieved, the CF test remains the method of choice for detection of antibodies to H. pleuro-pneumoniae in swine sera.

The methodology of the CF test, as used by Nicolet et al. (1971), Nielsen (1974), Gunnarsson (1980) and Slavik and Switzer (1972), varies slightly. Nicolet et al. (1971) and Gunnarsson (1980) eliminated the procomplementary property of swine serum by heat inactivation at 60°C. Fresh bovine serum (usually 1%) is then added to the complement. The concentration is tried at several concentrations (0.5%, 1% and 5%) in the presence of the antigen with different concentrations of the

complement (5 units, 2.5 units and 1.25 units). The serum with the weakest acceptable anticomplementarity is chosen. The bovine serum must also not modify the titer of a positive control and must give a negative reaction when used with a known negative serum. Slavik and Switzer (1972) and Nielsen (1974) reconstituted lyophilized guinea pig complement with normal unheated serum from a six to eight week-old pig. In this way the procomplementary effect of the swine serum on guinea pig complement was accounted for in the titration of the GPC. The addition of normal swine serum to GPC also restored the CF activity of heat-inactivated test serum.

Gunnarsson (1980) concluded that the multiplicity of serotypes of H. pleuropneumoniae served to stress the importance of the choice of antigens for routine sero-diagnostic work. He observed that a mixture of the five serotypes of H. pleuropneumoniae was the best screening antigen for the CF test.

Nicolet et al. (1971) first detected CF antibodies ten days after inoculation in experimentally infected pigs. Peak titers of 1:320 were detected six weeks after infection. In a spontaneous infection of an SPF herd, 100% of the animals were serologically positive after 3 months. Nielsen (1974) found CF antibodies 2 weeks following inoculation that reached peak values of 1:320 to 1:640 anywhere from 2-7 weeks post-inoculation. The titers remained high for several months. There was a tendency for IHA titers to become detectable slightly before CF titers. Yamamoto and Ogata (1980), found that agglutinating antibodies rose more rapidly than CF antibodies and declined earlier. Peak values (1:160-1:320)

were reached 1-2 weeks PI and declined gradually. In recent infections up to 100% of survivors are seropositive, but in extreme chronic situations as few as 3-4% are seropositive (Nicolet et al. 1971, Nielsen et al. 1976).

Clinical signs

Häni et al. (1973) and Mylrea et al. (1974) described three clinical syndromes in H. pleuropneumoniae transmission trials: (1) a peracute form in which death occurred within 24 hours; (2) an acute form in which there were signs of severe respiratory distress including forward protrusion of the head, abdominal respiration and a blood stained frothy exudate around the nose and mouth and death in 2-4 days; and (3) a non-fatal or chronic form in which severity of the disease was variable. Temperatures from 105-107 F occurred within 24 hours after inoculation. There was anorexia and reluctance to move. Sick pigs developed a persistent cough. The clinical signs subsided after 2-4 days. These findings were similar to those that Häni et al. (1973) described in 75 spontaneous cases. Schwartz (1979) suggested that many pigs do not "thump" or have severe abdominal respirations because they cannot breathe deeply due to fibrin deposition in the pleural cavity.

Wilson and Kierstead (1976) reported abortions associated with H. pleuropneumoniae in Canada.

Gross lesions

Mylrea et al. (1974) found that the most obvious lesions of H. pleuropneumoniae disease occurred in the thoracic cavity and consisted

of pneumonia and pleuritis.

Lesions in the lung were most frequently found in the diaphragmatic lobes but cardiac and apical lobes were also affected. The lesions were most often bilateral but were, in many cases, located in one lobe only.

The type of lesion varied considerably with the duration of infection. In peracute cases, the lungs were firm and red with the interlobular septa containing bloody fluid. Cut surfaces were red to dark red in color, wet and oozed blood-stained fluid. The pleural surfaces became roughened and had early fibrinous adhesions. As pulmonary lesions progressed they became more circumscribed, dark red to gray and with proliferation of fibrous tissue along the interlobular septa (Olander 1963, Shope 1964, White et al. 1964, Nicolet and Konig 1966). Extensive fibrous adhesions which caused difficulty in removal of the thoracic viscera were encountered at slaughter.

Bronchial lymph nodes may be swollen and blood-stained frothy exudate may be found in the trachea and larynx of pigs with peracute and acute disease. Mylrea et al. (1974) reported pericarditis with adhesions to the epicardium in some cases and, in a few field cases, they observed renal infarcts.

Microscopic lesions

Early microscopic lesions in the lungs are those of congestion, alveolar hemorrhage and proteinaceous alveolar edema. Clumps of bacteria can often be seen in the edematous fluid. There is a deposition of fibrin on the pleura. Infiltration of lymphocytes and macrophages mostly,

but occasionally neutrophils, is observed within and along the interlobular septa and peribronchiolar areas. As the lesion progresses there is a proliferation of dark, pleomorphic fibroblasts in these areas and fibrous tissue becomes thickened along the interlobular septa and pleura (Shope 1964, Nicolet and König 1966, Nielsen 1970a, Mylrea et al. 1974, Martin et al. 1977).

Lesions have been reported in tissues other than the lungs. Hsu et al. (1976) reported that the bronchial lymph nodes may be edematous and swollen. Olander (1963) reported lesions of arthritis, meningoencephalitis and vegetative endocarditis. Nielsen (1970a) found lesions of peritonitis, and as mentioned previously, Mylrea et al. (1974) found renal infarcts. These lesions have been attributed to H. pleuropneumoniae infections, but they are not as frequent nor as consistent as lung lesions.

Treatment

Schwartz (1979) reported that isolates of H. pleuropneumoniae were resistant to most antibiotics and thus medication was of little benefit in controlling outbreaks. Wilson (1980) reported that most strains of H. pleuropneumoniae were susceptible to everything that was used in their tests but that very few drugs were of value in treating the disease once clinical signs were evident.

Henry (1980) and Schultz (1981) indicated that, under field conditions, injection of all pigs in the same air space with an antibiotic to which the organism is susceptible coupled with improved husbandry was the treatment of choice. Improved husbandry, among other things, included

decreasing the number of pigs per pen, all-in-all out movement of pigs, improvement of ventilation, and prevention of extreme temperature fluctuation in the pigs' environment. A three-day regimen of therapy is often indicated. This may be accomplished with an injectable antibiotic which keeps drug plasma concentrations at a therapeutic level for 72 to 96 hours.

Henry (1980) reported that oral antibiotics often do not adequately control the disease because adequate levels of drug are not achieved in the plasma. Schultz (1981) has observed pigs receiving 400 grams of tetracycline per ton of complete feed dying from acute Haemophilus pleuropneumoniae disease. In vitro tests indicated that the organism was sensitive to tetracycline. Mylrea et al. (1974) stated that the well-encapsulated lesion with persistent infection no doubt accounts for the failure of attempts to control infection by mass medication. In spite of this, high levels of antibiotics in the feed are often used as follow-up therapy. The rationale of this treatment is to hold down other respiratory pathogens and to reduce the number of H. pleuropneumoniae organisms in tissue accessible to drug plasma concentrations.

Prevention and control

Nielsen et al. (1976) were able to avoid acute outbreaks of pleuropneumonia and decrease the incidence of chronic pleuritis from 12% to 6% and 8% to 1% in two progeny testing stations by testing incoming swine and admitting only animals seronegative to H. pleuropneumoniae.

E. Scholl (Klinik für Nutztiere und Pferde, Universität Bern,

personal communication, 1980) has instigated an effective eradication program in Switzerland among the SPF farms by testing and removing animals seropositive for H. pleuropneumoniae.

Nicolet (1979) describes a method of control in chronically infected herds by weaning pigs at 3 weeks of age and raising them far from their infected mothers and other infected stock. At 12 weeks of age such animals are seronegative.

The use of bacterins has met with a certain degree of success in Denmark, Switzerland, Canada and the United States (Nielsen 1976, Riising 1980a, Scholl et al. 1978, Wilson 1980, Kislingbury 1981).

In Denmark, Riising (1980) found that vaccination decreased the frequency of chronic pleuritis and the number of feeding days to market in infected herds. Wilson (1980) in Canada found that mortality was reduced from 10% to less than 2% in pigs vaccinated twice. Kislingbury (1981) in the United States, in a trial comparing 2 intra-state bacterins and an autogenous bacterin found that vaccinates required significantly less treatment ($p < .01$) than controls.

At present there are no federally approved vaccines in the United States. Results obtained with autogenous bacterins and intra-state laboratory bacterins have varied from excellent to poor (Schultz 1981). Variability in results may be due to methods used in production of bacterins and to the serotype content in the bacterin being used. The capsule of H. pleuropneumoniae is believed to contain the protective antigen (Nielsen 1976).

Nielsen (1976) found that H. pleuropneumoniae infection may develop in respiratory tracts of vaccinated swine which have been exposed to the organism. Thus, protected animals do not clear the infection completely and may therefore serve as carriers.

As mentioned earlier, Schultz (1981) reported good management, improved environment, absence of stress and vaccination may provide the best course of action in an infected herd.

Immunity

Nielsen (1974) showed that pigs inoculated with H. pleuropneumoniae serotype 2 produced strong immunity to that serotype. Nielsen (1979a) demonstrated that a considerable cross immunity exists between the various serotypes of H. pleuropneumoniae when inoculated intranasally with one serotype and challenged three weeks later with another serotype. The cross immunity between various serotypes of H. pleuropneumoniae was not associated with a corresponding rise in antibody titer to the second challenging serotype.

Nielsen (1974) noted that intranasal challenge did not elicit an anamnestic antibody response in any of the immune pigs. These findings were interpreted to indicate that the protective immunity was mediated by local antibodies in the nasal cavity and other portions of the respiratory tract. Nielsen (1974) found a correlation between CF antibody titers in serum and resistance to reinfection; however, this may only be a reflection of the overall immune response since the CF antibody response is type specific yet protection appears not to be type specific.

Economic significance

Nicolet (1976) concluded that pleuropneumonia of swine caused by H. pleuropneumoniae became of economic importance with advent of modern, intensified commercial production systems. Reduced feed efficiency has been reported by many investigators (Hsu et al. 1976, Nicolet 1976, Byrnes 1979, Henry 1980, Schultz 1980, Wilson 1980). Wilson (1980) noted that some recovered pigs show no clinical signs other than coughing but at slaughter have massive adhesions between the lungs and thoracic wall. These pigs have a reduced growth rate and may,in fact, result in greater economic loss than pigs which die. Henry (1980) suggested that in chronically infected herds, losses in efficiency are much more significant than mortality. In one particular outbreak, feed efficiency dropped (3.25 lbs. of feed per pound of gain to 4.2 lbs. of feed per pound of gain) and rate of gain decreased from 1.8 lbs. per day to 1.2 lbs. per day (Limberg 1980). H. -J. Riising (Northern Drugs and Chemicals Ltd., Copenhagen, Denmark, personal communication) estimated that pigs in infected herds required an average of 7-8 days longer to reach market weight than pigs in non-infected herds.

MATERIALS AND METHODS

Serological Survey for Antibodies to H. pleuropneumoniae

Cultures

Cultures of H. pleuropneumoniae serotypes 1, 2, 3, 4 and 5 were used as antigens in the complement fixation (CF) test, for intranasal inoculation of SPF pigs and for production of hyperimmune rabbit sera.

Serotype 1 was represented by strain 27088 from the American Type Culture Collection. The strain was supplied to the ATCC as strain 4074 by J. Nicolet who obtained it originally from R. E. Shope of New York.

Serotype 2 was represented by strain 27089 from the American Type Culture Collection. The strain was supplied to the ATCC as strain 1536 by J. Nicolet, Switzerland.

Serotype 3 was represented by strain 27090 from the American Type Culture Collection. The strain was supplied to the ATCC as strain 1421 by J. Nicolet, Switzerland.

Serotype 4 was represented by strain M62 from A. Gunnarsson, Uppsala, Sweden. The strain was supplied to A. Gunnarsson as strain M62 from H. Olander, Davis, California.

Serotype 5 was obtained from lungs of pneumonic Iowa swine designated M_1 and serotyped by A. Gunnarsson, Uppsala, Sweden.

H. parasuis strains W5 and KL1 were isolated from nasal secretions of swine with atrophic rhinitis by Gois et al. (1981).

Production of type-specific convalescent swine sera

Yorkshire pigs 3-4 months of age were obtained from the Laboratory Animal Resources farm at Iowa State University and were of second generation, Cesarean-derived, colostrum-deprived (CDCD) origin. They were maintained in isolation units at the Veterinary Medical Research Institute. Prior to use, nasal secretions were collected with cotton tipped swabs for culture to determine that they were free of H. pleuropneumoniae or H. parasuis. In addition, sera were collected and tested for CF antibodies to both organisms. Feed was a 16% corn soy ration with no added antibiotics or growth promotants. Two pigs were housed per unit in strict isolation from the other pigs. Each serotype of H. pleuropneumoniae was grown in 8 ml of M96 (Table 2) mycoplasma medium (Frey et al. 1973) for 6 hours at 37°C. A sample of each culture was titrated and inoculated on chocolate agar (Table 3) to determine the number of colony forming units used. Two pigs were inoculated with 2 ml in each nostril of each respective serotype. The pigs were observed and temperatures were taken at 4 hour intervals for the first 36 hours then once daily. If the temperature reached 106°F, approximately 2 million units of penicillin G was administered. This was to keep the pigs from dying of the peracute H. pleuropneumoinae disease. Blood samples were taken from the anterior vena cava before inoculation and every three days after inoculation for 28-36 days. Sera were tested for presence of homologous and heterologous antibodies to five serotypes of H. pleuropneumoniae (Table 4).

Table 2. M-96 Medium used for growing antigen for swine intranasal inoculation

4.0 gm.	Peptone CS (Albimi-Pfizer)
2.0 gm.	Peptone B (Albimi-Pfizer)
2.0 gm.	Peptone G (Albimi-Pfizer)
2.0 gm.	Yeast Autolysate (Albimi-Pfizer)
2.0 gm.	Yeast Extract (Albimi-Pfizer)
5.0 gm.	NaCl
0.4 gm.	KCl
0.2 gm.	$MgSO_4$ 7 H_2O
0.001 gm.	Catalase (General Biochemicals)

Make 0.025 gm. catalase in 25 ml. H_2O. Add 1 ml. of this to media.

3.5 gm.	Hepes Buffer (0.015 M)
0.02 gm.	DNA
10.0 ml.	MEM Vitamins (Gibco)
2.0 ml.	Cholestrol Emulsion (1 mg/ml)
0.15 ml.	Glycerol
0.06 gm.	L-arginine HCl
0.09 gm.	L-glutamine

Combine next two ingredients. Mix well. Then add to media.

10.0 ml.	DPN 2%
2.0 ml.	Cysteine 1%

QS to 1000 ml. with 3x Deionized H_2O.

Mix at least ½ hr. on magnetic stirrer.

pH to 7.5 to 7.7 with 1% NaOH.

Add swine serum. (250 ml.)

Mix well and filter sterilize.

Tube 5 ml. per 16 x 125 mm. glass screw cap tubes.

Table 3. Number of colony forming units of each serotype of H. pleuropneumoniae inoculated intranasally into pigs

Serotype	Strain	Number of colony forming units per ml[a]
1	27088	1.5×10^9
2	27089	2.5×10^9
3	27090	5.5×10^8
4	M62	2.5×10^9
5	M1	5.5×10^8

[a]Each pig was given 2 ml of undiluted 6 hour culture in each nostril (total of 4 ml).

Complement fixation[1]

The procedure of the Laboratory Branch of the Communicable Disease Center (1965) adapted to microtechnique as described in Public Health Monograph No. 74 was followed with one major exception as described by Slavik and Switzer (1972).

As mentioned earlier, lyophilized guinea pig complement (Difco Lab., Detroit, Mich.) was reconstituted with normal serum from a six to eight week old pig. In this way, the procomplementary effect of the swine serum was accounted for in the titration of the GPC. The addition of normal

[1]Acknowledgement is given to Theresa Young of the Veterinary Medical Research Institute at Iowa State University for her invaluable assistance in adaptation of the complement fixation test to Haemophilus pleuropneumoniae and for coordinating the testing procedures.

Table 4. Complement-fixing antibody titers of convalescent sera from swine infected with various serotypes of H. pleuropneumoniae: single antigen and pooled antigen test. Antigen from an 18 hour culture.

Pig Identification Number	Serotype of H. pleuropneumoniae used for infection	Date Collected	Days Post Inoculation	Antigen[a]					
				Serotypes					Mixture Serotypes
				1	2	3	4	5	1,2,3,4,5,
1451	1 (4074)	5-2-80	18	64[b]	-	-	-	-	NT[c]
		5-5-80	21	64	-	-	-	-	NT
		5-8-80	24	64	-	-	-	-	128
		5-11-80	27	128	-	-	-	-	128
1474	1 (4074)	5-2-80	18	32	-	-	-	-	NT
		5-5-80	21	32	-	-	-	-	NT
		5-8-80	24	8	-	-	-	-	16
1500	2 (1536)	5-23-80	30	-	16	-	-	-	NT
		5-26-80	33	-	64	-	-	±	NT
1711G	2 (1536)	5-23-80	30	0	0	0	0	0	NT
		5-26-80	33	-	8	-	-	-	16
		5-29-80	36	-	8	-	-	-	16
1710	3 (1421)	5-23-80	30	-	-	16	-		NT
		5-26-80	33	-	-	32	-	16	NT

1711B	3 (1421)	5-23-80	30	-	-	16	-	-	NT
		5-26-80	33	-	-	64	-	-	64
		5-29-80	36	-	-	64	-	-	128
1731	4 (M62)	5-14-80	21	-	-	-	32	4	32
		5-17-80	24	-	-	-	32	32	32
		5-20-80	27	-	-	-	64	128	64
		5-26-80	33	8	32	16	128	128	NT
1734	4 (M62)	5-8-80	15	-	-	-	64		32
		5-11-80	18	-	-	-	128	32	128
		5-14-80	21	-	4	-	128	32	64
		5-17-80	24	-	-	-	64	32	64
		5-20-80	27	-	-	-	32		32
		5-26-80	33		16	-	128	128	NT
1462	5 (m1)	4-29-80	15	-	-	-	4	128	128
		5-2-80	18	-	-	-	4	128	128
		5-5-80	21	-	-	-	64	512	32
1472	5 (M1)	4-29-80	15	-	-	-	-	64	NT
		5-2-80	18	-	-	-	-	64	32
		5-5-80	21	-	-	-		64	64

[a]Optimum dilution of antigens were as follows: Serotype 1, 1:128; Serotype 2, 1:64; Serotype 3, 1:64; Serotype 4, 1:64; Serotype 5, 1:128.

[b]Titers are reciprocals of highest dilution of serum with which 30% or less of hemolysis occurred.

[c]No test.

swine serum to GPC also restored the CF activity of heat-inactivated test serum. CF tests were done in microtiter plates (Linbro Chemical Co., Inc., New Haven, Conn.) using five units of GPC (reconstituted with normal swine serum), the optimum dilution of previously titered H. pleuropneumoniae antigen or pooled antigen, and test sera heated at 56°C for 30 minutes. Plates were incubated overnight at 4°C before sensitized sheep RBC's were added. Sheep RBC's were collected in 2% Alsever's solution, washed with Veronal Buffered Diluent (VBD) and sensitized with hemolysin (Bacto-antisheep hemolysin, glycerinated, Difco Lab., Detroit, Mich.). The plates were then incubated at 37°C for 30 minutes, and centrifuged at 1500 RPM for 5 minutes. The reactions were read optically in a convex mirror placed 15 cm under the microplate and the percent hemolysis produced in each well was recorded. Dilutions of serum that resulted in 30% or less hemolysis of the sheep RBC's were considered positive.

Production of antigen for CF test

Mucoid cultures of each of the 5 serotypes of H. pleuropneumoniae were grown for 6 hours on PPLO agar, (Difco, Difco Lab., Detroit, Mich.) with 5% horse serum, 0.1% glucose, 1% NAD, and 5% yeast extract (Nicolet 1971). Following incubation, antigen from each serotype was harvested with 5 ml of 0.85% NaCl per plate. The antigen was washed once and resuspended to a density of 10^{10} organisms per milliliter by reference to a McFarland nephelometer. The antigen was stored in saline with 1:20,000 merthiolate and stored at 4 to 6°C in one ml aliquots until needed.

The optimal dilution of antigen for each serotype was determined by block titration (Table 5).

Antigens of the five serotypes were pooled immediately before use in proportion to the titration of each individual serotype (Nielsen 1979b, Gunnarsson 1979b). The pooled antigen contained serotype 1 (strain 4074), serotype 2 (strain 4226), serotype 3 (strain 1421), serotype 4 (strain M62), and serotype 5 (strain M_1) (Table 5).

An antigen for H. parasuis was prepared in fashion similar to that H. pleuropneumoniae and likewise tested against known convalescent positive H. pleuropneumoniae sera. The H. parasuis antigen was used to evaluate the specificity of CF antibodies to H. pleuropneumoniae in convalescent and reference type-specific sera. The H. pleuropneumoniae serotype specific as well as mixed antigen was tested against hyperimmune sera prepared in swine against H. parasuis strains W5 and KL1. No cross reactions resulted.

Swine serum samples

Swine sera were selected randomly from samples submitted to the Veterinary Diagnostic Laboratory at Iowa State University during the first three months of 1980. The samples represented breeding stock sold during that period as well as those herds being tested for pseudorabies validation. Samples were taken from each of the 9 crop reporting districts[1] of Iowa (9 to 12 counties) in close proportion to the number of swine marketed annually from that area (Appendix IV). There were a total of 61,597 samples submitted during this period of which 7,348 samples from

[1]Iowa Agriculture Statistics 1979. Iowa Crop and Livestock Reporting Service, Des Moines, Iowa.

Table 5. Complement-fixing antibody titers of convalescent sera from swine infected with various serotypes of H. pleuropneumoniae: single antigen and pooled antigen test. Antigen from a 6 hour culture

Pig Identification Number	Serotype of H. pleuropneumoniae used for infection	Date Collected	Days Post Inoculation	Antigen[a]					
				1	2	3	4	5	Mixture Serotypes 1,2,3,4,5
1451	1 (4074)	5-11-80	27	128[b]	-	-	-	-	128
1500	2 (1536)	5-29-80	35	-	64	-	-	-	64
1711B	3 (1421)	5-29-80	35	-	-	64	-	-	32
1734	4 (M62)	5-20-80	26	-	-	-	32	16	32
1462	5 (M1)	4-29-80	16	-	-	-		128	16

[a]Optimum dilutions of antigens were as follows: Serotype 1, 1:128; Serotype 2, 1:64; Serotype 3, 1:64; Serotype 4, 1:32; Serotype 5, 1:128.

[b]Titers are reciprocals of highest dilution of serum with which 30% or less of hemolysis occurred.

597 herds were tested (Appendix V). An average of 12.3 animals per herd were tested with a range of 2 to 20. The sera was stored at -20°C until the day the CF test was conducted. A minimum of 2 and a maximum of 20 samples per herd were selected.

Serotyping of Haemophilus pleuropneumoniae

Serotyping

Fifty isolates of H. pleuropneumoniae were randomly selected for serotyping with a whole cell-agglutination test. The cultures were all from swine with pneumonia. The majority of the cultures were from the Veterinary Diagnostic Laboratory at Iowa State University with one to three submitted for serotyping from each of six other midwestern states.

As mentioned previously, type cultures representing serotypes 1, 2, and 3 were obtained from the American Type Culture Collection and serotypes 4 and 5 from Dr. Anders Gunnarsson, Uppsala, Sweden. The origins of all wild cultures are presented in Table 6.

All cultures were gram-negative rods, satellited Staphylococcus epidermidis 'nurse' culture, dependent on V factor (nicotinamide-adenine-dinucleotide), and were urease and B-galactosidase positive. All cultures grew on medium devoid of X factor or hemin. Biochemical tests using dextrose, nitrate, urea, ONPG, lactose, mannitol, xylose, and indole were carried out in the Minitek^{R} system according to Back and Oberhofer (1978).

Table 6. Identification, origin and date of isolation of wild strains of H. pleuropneumoniae

Isolate No.	Original Source	Date Received	Obtained From
5279	pneumonic swine lung	3-5-80	Iowa Veterinary Diagnostic Laboratory (IVDL)
D. Hansen	"	4-22-80	Schultz, Iowa
12444	"	6-1-80	IVDL, Iowa
Illinois (#2)	"	4-1-80	D. Hoeffling, Illinois
11507	"	5-21-80	IVDL, Iowa
13494	"	6-12-80	IVDL, Iowa
1144	"	1-22-80	IVDL, Iowa
12155	"	6-1-80	IVDL, Iowa
1451	"	5-14-80	Schelkopf, Nebraska
1056	"	1-21-80	IVDL, Iowa
Don Ute	"	1-3-80	Schultz, Iowa
15096	"	6-25-80	IVDL, Iowa
Adolph	"	1-23-80	Schultz, Iowa
B 3345	"	6-1-80	Phillips Roxane, Missouri
2722 - 5	"	6-1-80	Phillips Roxane, Missouri
Schultz 21	"	4-15-80	Schultz, Iowa
Isolate 4	"	4-30-80	Hewitt, South Dakota
Cox	"	4-24-80	S. Henry, Kansas
15155	"	7-2-80	IVDL, Iowa
5352	"	3-8-80	IVDL, Iowa
1273	"	2-23-80	Schelkopf, Nebraska
13380	"	6-10-80	IVDL, Iowa
13136	"	6-11-80	IVDL, Iowa
14448	"	6-25-80	IVDL, Iowa
B & B	"	11-21-79	Schultz, Iowa
10863	"	5-3-80	IVDL, Iowa

11545	"	5-21-80	IVDL, Iowa
Tom Rau	"	4-24-80	Schultz, Iowa
3879	"	6-1-80	Phillips Roxane, Missouri
Green	"	4-4-80	Schultz, Iowa
Isolate 6 (Cornhusker)	"	4-30-80	Hewitt, Nebraska
Poland	"	5-1-80	S. Henry, Kansas
B - 10	"	4-1-80	Burns Biotec, Nebraska
14210	"	6-20-80	IVDL, Iowa
7543	"	4-1-80	IVDL, Iowa
13136	"	6-11-80	IVDL, Iowa
Morr	"	5-1-80	S. Henry, Kansas
Steve Henry	"	11-21-79	S. Henry, Kasnas
M 1	"	6-25-79	Schultz, Iowa
1623	"	11-23-79	IVDL, Iowa
Schelkopf	"	1-3-80	Schelkopf, Nebraska
9742	"	4-24-80	IVDL, Iowa
10758	"	5-3-80	IVDL, Iowa
5235	"	3-5-80	IVDL, Iowa
9431	"	4-22-80	IVDL, Iowa
3261	"	6-1-80	Phillips Roxane, Missouri
10839	"	5-6-80	IVDL, Iowa
10837	"	5-10-80	IVDL, Iowa
19129 T	"	11-21-79	W. Freeze, Minnesota
10758	"	5-3-80	IVDL, Iowa

Antigens

Antigens for immunization of rabbits were prepared according to Gunnarsson et al. (1977) from mucoid cultures obtained from 18 hour cultures grown on PPLO agar plates with 5% horse serum and 10% yeast added. The growth was harvested in 0.15M NaCl, washed three times in the same solution and lyophilized. The concentration of the antigen used was adjusted by weight to 5 mg/ml in 0.15 M NaCl and preserved with 1:10,000 merthiolate. Storage was at $4^{o}C$.

Antigens for the agglutination tests were grown for 6 hours on PPLO agar plates with 5% horse serum, 10% yeast and 1% NAD added.

Antisera

Antisera were prepared as described by Gunnarsson et al. (1977). Rabbits were injected at three day intervals beginning with 0.5 ml subcutaneously. Subsequent injections were given intravenously. The doses were 1 ml intravenously at three day intervals twice, followed by 2 ml intravenously twice at three day intervals, and 3 ml twice at three day intervals. The rabbits were exsanguinated 1 week after the last injection. Two rabbits were immunized with each serotype. The sera were preserved with 1:10,000 merthiolate and stored at $-20^{o}C$.

Preimmunization sera were tested for agglutinating antibodies to the corresponding homologous strain of H. pleuropneumoniae. Sera from rabbits hyperimmunized with each of the serotypes was used undiluted and diluted 1:10 and 1:20 with saline.

Agglutination test

A rapid plate agglutination test was conducted using a Minnesota testing box. A small loopful (2 mm) of a 6-8 hour culture grown on PPLO agar with 5% horse serum, 10% yeast and 1% NAD was mixed with undiluted and diluted 1:10 and 1:20, rabbit antisera against the 5 serotypes of H. pleuropneumoniae using a wooden application stick.

The plate was rotated 5 times. Agglutination was usually noted within thirty seconds but was recorded at three minutes.

To check the accuracy of this method and the consistency between laboratories the isolates were sent to Dr. Anders Gunnarsson and Dr. R. Nielsen for tube agglutination and slide agglutination tests. Results are presented in Table 14.

Antibiotic Sensitivity

The standardized single disc method of Bauer et al. (1966) was used to compare the sensitivity of H. pleuropneumoniae strains to 11 antibacterial agents. The cultures were grown in M96 medium (Table 2) (Frey et al. 1973) for 18 hours then equalized in density to a standard of 0.5 ml of 1% $BaCl_2$ in 99.5 ml of 1% H_2SO_4 (0.36 N) by adding sterile water. (This is tube 1 of the McFarland Nephelometer Scale or an approximate density of 300,000,000 bacterial cells.) For antibiotic sensitivity testing, the standardized suspensions were streaked on freshly prepared, modified Casman's agar (Table 7). For sulfonamide sensitivity testing, freshly prepared Mueller-Hinton agar with 10% yeast and 1% NAD was used. Discs were dispensed and the plates were incubated at 37°C for

18 hours. The diameters of zones of inhibition were read with a Fisher-Lilly Antibiotic Zone reader (Fisher Scientific Co., Chicago, Ill.). Each culture was classified as resistant, intermediate, or sensitive to each drug, according to the criteria established by Bauer et al. (1966) (Table 8).

Table 7. Media used for antibiotic sensitivity testing of H. pleuro-neumoniae

Casman Agar	
Casman Agar Base (BBL)	43.0 g
NaCl (Baker)	5.0 g
Niacinimide (Calbiochem 4813)	0.5 g
Thiamine-HCl (Calbiochem 5871)	0.005 g
Tween-80 (Fisher)	0.1 ml
Adjust pH to 7.5 before heating.	
Autoclave 15 min., 15 psi slow exhaust.	
Cool to 56°C, then add sterile horse serum 10 ml. add egg yolk (6-day egg) 10 ml.	
Separately, dissolve 0.0125 g NADH (Calbiochem) into 10 ml water, filter sterilize with 0.22 um filter and add immediately to the cooled agar before pouring plates.	

Table 8. Zone sizes and their interpretation for nine antibiotics[a] and two sulfonamides

Antibacterials[b]	Disk Potency	Inhibition zone diameter[c]		
		Resistant	Intermediate	Sensitive
Ampicillin	2 ug	11 or less	12 - 13	14 or more
Chloramphenicol	30 ug	12 or less	13 - 17	18 or more
Erythromycin	2 ug	13 or less	14 - 17	18 or more
Gentamycin	10 ug	9 or less	10 - 12	13 or more
Lincomycin	2 ug	9 or less	10 - 14	15 or more
Neomycin	30 ug	12 or less	13 - 16	17 or more
Penicillin-G	2 units	11 or less	12 - 21	22 or more
Penicillin-G	10 units	11 or less	12 - 21	22 or more
Streptomycin	10 ug	11 or less	12 - 14	15 or more
Triple Sulfa	250 ug	12 or less	13 - 16	17 or more
Sulfachlorpyridazine	25 ug	12 or less	13 - 16	17 or more
Sulfonamides	25 ug	12 or less	13 - 16	17 or more
Tetracycline	30 ug	14 or less	15 - 18	19 or more

[a]Classified according to criteria of Bauer et al. 1966.

[b]Baltimore Biological Laboratories, Cockeysville, Md.

[c]Zone diameter in mm.

Statistical Analysis

The Chi Square statistic was used to test for independence of area and incidence with regard to positive or negative herd status.

The Analysis of Variance procedure was used to compare the effect of 2 levels of penicillin to the size of the zone of inhibition produced by 50 isolates of H. pleuropneumoniae. A randomized block complete design was used. Significance was determined by use of the F test (Snedecor and Cochran, 1967).

RESULTS

Serological Survey

Samples tested in the serologic survey were from 7348 swine from 597 herds. The number and percent of sera which were negative, positive at dilutions of 1:4 or higher, suspect and anticomplementary are listed (Table 9). A total of 32.11% of all animals had a CF titer of 1:4 or higher.

The total percentage of herds with at least one animal with a CF antibody titer of 1:16 or higher is shown (Figure 1) for the nine crop reporting districts of Iowa (9-12 counties each). The percentage of all herds tested resulted in 67.34%, having at least one animal with a 1:16 or higher CF antibody titer. The number and percent of swine with various CF antibody titers to H. pleuropneumoniae are presented (Table 10). The number and percent of herds ranked according to the highest titer detected in at least one sample of that herd are listed (Table 11). An average of 46% of the animals in infected herds had a titer of 1:16 or greater.

The Chi square test for independence between district and incidence supports evidence of an interaction between the two ($p<0.05$). Knowledge of whether a herd was positive or negative is enhanced by knowing the district of origin.

The correlation between number of hogs in a crop reporting district and frequency of positive herds was .57 ($p < .05$). The coefficient was not significantly different from zero.

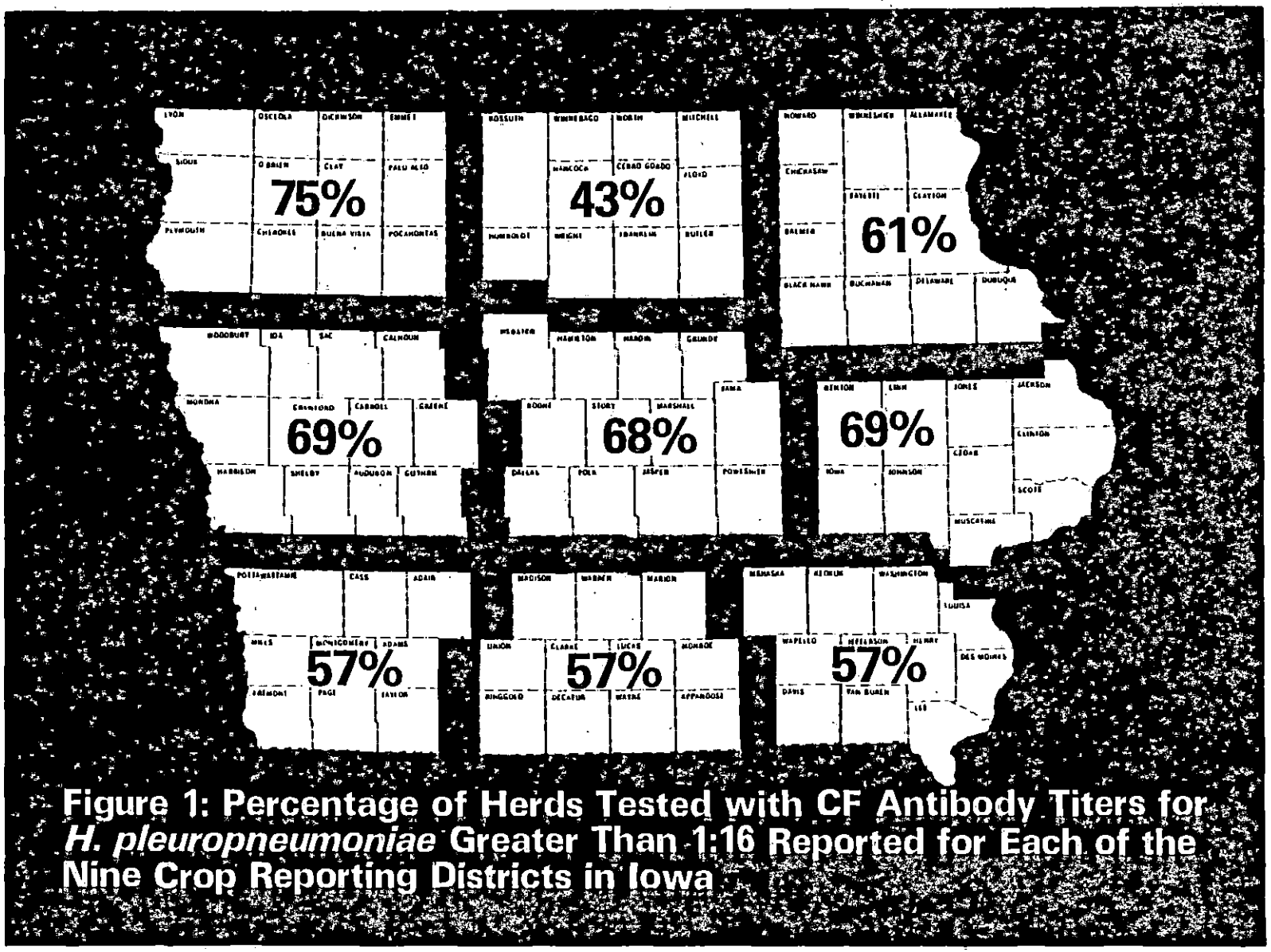

Figure 1: Percentage of Herds Tested with CF Antibody Titers for *H. pleuropneumoniae* Greater Than 1:16 Reported for Each of the Nine Crop Reporting Districts in Iowa

Table 9. Number and percent of 7348 swine negative, positive, suspect and anticomplementary for CF antibodies to H. pleuropneumoniae

Category	Number	Percent
Negative	4237	57.63
Positive	2362	32.11
Suspect	565	7.68
Anticomplementary	184	2.50

Table 10. Number and percent of swine with various CF antibody titers to H. pleuropneumoniae

Titer	Number[a]	Percent	Cumulative per cent of animals
1:0	4241	64.23	64.23
1:4	33	0.50	64.73
1:8	104	1.58	66.31
1:16	310	4.69	71.00
1:32	740	11.21	82.21
1:64	764	11.57	93.78
1:128[b]	411	6.22	100.00

[a]Anticomplementary and suspect reactions excluded from this tabulation.

[b]1:128 highest dilution tested.

Table 11. Number and percent of 597 herds of swine with CF antibody titers ranked by highest titer detected[a,b]

Highest Titer Detected	No. of Herds	Cumulative No. of Herds	Percent Herds	Cumulative Percent of Herds
0	180	180	30.1	30.1
1:4	6	186	1.0	31.2
1:8	9	195	1.5	32.7
1:16	29	24	4.9	37.5
1:32	78	302	13.1	50.6
1:64	130	432	21.8	72.4
1:128[c]	165	597	27.6	100.0

[a]Anticomplementary and suspect reactions excluded from this tabulation.

[b]Average number of samples tested per herd 12.3 (Range 2-20).

[c]1:128 highest dilution tested.

Serotyping[1]

Results obtained in serotyping of fifty isolates of H. pleuropneumoniae with the plate agglutination test are presented in Figure 2. A comparison of results obtained with plate agglutination, slide agglutination, tube agglutination (Gunnarsson) and complement fixation (Neilsen) is presented in Table 12.

[1]Acknowledgement is kindly given to Dr. Anders Gunnarsson, Uppsala, Sweden for the tube agglutination serotyping and Dr. Ragnhild Nielsen, Copenhagen, Denmark for slide agglutination serotyping. Dr. R. Nielsen's further comparison of the antigens in a CF test with known positive serum is deeply appreciated.

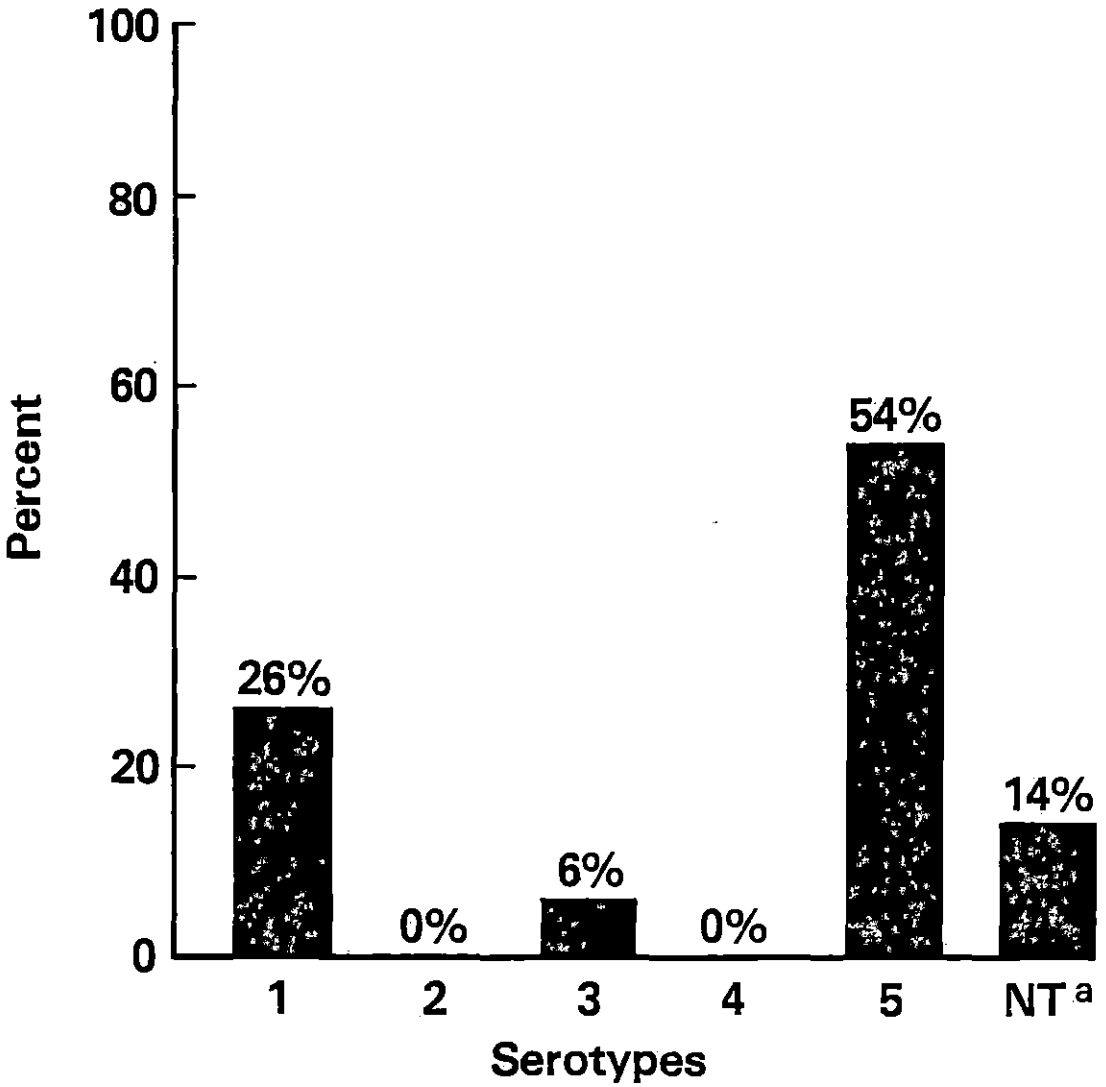

Figure 2: Serotypes of 50 Different Isolates of *H. pleuropneumoniae* from Swine Herds in Iowa and Surrounding States

[a]NT - non-typable; results may be due to organism having lost its capsular antigen or to new unrecognized serotypes.

Table 12. Serotype identification[a] of 50 United States Isolates of H. pleuropneumoniae by tube agglutination, plate agglutination and slide agglutination tests

Isolate Number	Dr. Anders Gunnarsson,[b] Tube Agglutination	Dr. Roy Schultz, Plate Agglutination	Dr. Ragnhild Nielsen,[c] Slide Agglutination	Dr. Ragnhild Nielsen Antigen from Isolate and CF test with known positive serum[d]
5279	NA	NA	1	-
D. Hansen	1	1	1	-
12444	5	5	NA	5
Illinois(#2)	1	1	1	-
11507	5	3	3	5
13493	R	CR,1,3,4,5	Femø,4,5	CR, 3,4
1144	NV	1	1	-
12155	NA	NA	Femø	CR, 3,4
1451	1	1	1	-
1056	1	1	1	-
Don Ute	5	5	NA	5
15096	5	5	NA	5
Adolph	NV	5	5	5
B 3345	5	5	NA	5
2722 - 5	5	5	NA	5
Schultz 21	NA	3	3	5
Isolate 4	NV	5	NV	-

[a]NA - no agglutination; R - rough form; NV - nonviable; CR - cross reaction.

[b]Dr. Anders Gunnarsson, The National Veterinary Institute, Uppsala, Sweden.

[c]Dr. Ragnhild Nielsen, The State Serum Laboratory, Copenhagen, Denmark.

[d]Dr. Ragnhild Nielsen, The State Serum Laboratory, Copenhagen, Denmark.

Table 12 (continued)

Isolate Number	Dr. Anders Gunnarsson,[b] Tube Agglutination	Dr. Roy Schultz, Plate Agglutination	Dr. Ragnhild Nielsen,[c] Slide Agglutination	Dr. Ragnhild Nielsen[d] Antigen from Isolate and CF test with known positive serum
Cox	R	NA	1	-
15105	5	5	NA	5
5352	R	NA	NA	CR, 3,4
1273	NA	NA	NA	CR, 3,4
13380	1	1	1	-
13136	5	5	NA	-
14448	5	5	NA	5
B & B	5	5	5	5
10863	5	5	5	5
11545	NV	1	1	-
Tom Rau	1	1	1	-
3879	NV	5	NA	5
Green	NA	1	1	-
Isolate 6 (Cornhusker)	5	5	5	5
Poland	No Report	1	1	-
B - 10	NA	NA	1	1
14210	5	5	NA	5
7543	1	1	1	-
13136	R	5	NA	5
Morr	5	5	NA	5
Steve Henry	5	5	3	CR, 5,3
M 1	5	5	CR, Femø,4,3	3,4,5, Femø
1623	3	3	CR, Femø,4,3	5
Schelkopf	1	1	1	-
9742	R	5	1	-
10758	5	5	1	-
5235	5	5	5	-

Table 12 (continued)

Isolate Number	Dr. Anders Gunnarsson,[b] Tube Agglutination	Dr. Roy Schultz, Plate Agglutination	Dr. Ragnhild Nielsen,[c] Slide Agglutination	Dr. Ragnhild Nielsen[d] Antigen from Isolate and CF test with known positive serum
9531	5	5	NA	5
3261	5	5	3	5
10839	5	5	NV	-
10837	R	5	CR,Femø,4,3	5
19129 T	NV	NA	NA	CR, 3,4
10758	R	5	NA	-

Antibiotic Sensitivity

The proportions of H. pleuropneumoniae that were resistant, intermediate, or sensitive to nine antibiotics and two sulfonamides are presented in tables 13 and 14 respectively. The classification was made according to criteria for sensitivity described by Bauer et al. (1966).

All strains of H. pleuropneumoniae were resistant to lincomycin and all strains were sensitive to chloramphenicol. When comparing the width of the zone of inhibition of 2 units of penicillin with 10 units of penicillin, a very highly significant difference was found ($P < .001$).

Table 13. Proportion of 50 strains of H. pleuropneumoniae that were resistant, intermediate, or sensitive[a] to nine antibiotics

Antibiotics[b]	No. of strains		
	Resistant	Intermediate	Sensitive
Ampicillin 2 ug	8	2	40
Chloramphenicol 30 ug	0	1	49
Erythromycin 2 ug	46	3	1
Gentamycin 10 ug	1	0	40
Lincomycin 2 ug	50	0	0
Neomycin 30 ug	11	39	0
Penicillin-G 2	8	23	19
Penicillin-G 10	8	4	38
Streptomycin[c] 10 ug	28	1	0
Tetracycline 39 ug	9	18	23

[a]Classified according to criteria of Bauer et al. (1966).

[b]Baltimore Biological Laboratories, Cockeysville, Md.

[c]Only 29 tested.

Table 14. Proportion of H. pleuropneumoniae that were resistant, intermediate, or sensitive[a] to sulfonamides

Sulfonamides[b]	No. of strains		
	Resistant	Intermediate	Sensitive
Triple Sulfa Sulfadiazine Sulfamethazine Sulfamerazine	41	5	4
Sulfachlorpyridazine	3	10	30

[a]Classified according to criteria of Bauer et al. (1966).

[b]Baltimore Biological Laboratories, Cockeysville, Md.

DISCUSSION

In the last five to ten years in Iowa as well as in other hog producing areas of the United States, we have rapidly moved swine into confinement. Donham (1981), in a survey, found 51% of an estimated 55,000 Iowa swine producers have confinement buildings. He found that they had built ninety-one percent of these confinement buildings since 1970, and that these same producers raised 73% of Iowa's swine. The stress caused by unfavorable environmental conditions in many of these confinement units has created an ideal epidemiological situation for the spread of respiratory disease, and especially so for H. pleuropneumoniae.

It is naive of us to think that the rapid rise in the number of reported cases of H. pleuropneumoniae disease at Veterinary Diagnostic Laboratories is all due to increased incidence. Although there is undoubtedly an increase in the manifestation of overt disease, part of the rapid rise is due to our increased awareness of the disease, plus the expanded ability of our laboratories to isolate and recover the causative organism, H. pleuropneumoniae.

The complement fixation test is felt to be specific for H. pleuropneumoniae antibodies. We evaluated specificity by using H. parasuis antigens and by using sera from pigs hyperimmunized with H. parasuis. Nicolet (1971), Nielsen (1974) and Gunnarsson (1975) have found previously that the complement fixation test is specific and Nielsen (1979b) has shown that a pooled antigen of four serotypes of H. pleuropneumoniae may

be used in a screening test. Nicolet et al. (1971) has suggested that titers of 1:10 or more are indicative of previous infection but that lower titers may be non-specific. In the present study, the small proportion of animals with titers of 1:4 and 1:8 (approximately 2%) constitutes a small percentage of the total and contributes little to the incidence of 32% of positive animals.

Evidence obtained in this survey indicated that over 30% of the animals had CF antibody titers of 1:16 or greater to H. pleuropneumoniae. Also indicated, is that over 67% of the 587 herds had one or more animals with 1:16 or greater CF antibody titers to H. pleuropneumoniae.

The 30% of swine with antibodies to H. pleuropneumoniae and the still higher herd incidence of 67% was surprising. The samples were collected in the first three months of 1980. The first reported cases in Iowa occurred in 1976. It does not seem logical that the disease could spread so quickly through 37,000 herds (67% of 55,000).

In retrospect, it does seem logical that the organism has been present for a number of years, waiting only for the stress of confinement to cause infections to become clinically overt.

As mentioned earlier, Nicod (1970) found serological evidence of H. pleuropneumoniae in 64% of Swiss herds using the Waldmann System and Little (1973) found 30% of serum samples from Scotland contained complement-fixing antibodies to the organism. These results are similar to those obtained in the present survey of Iowa swine.

When testing sera, we found an average of 5.7 animals with antibodies to H. pleuropneumoniae in infected herds. The average number of animals

tested per herd was 12.3. This gives a percentage of 46% of animals in infected herds with CF antibody titers 1:16 or greater. In practicality, this suggests that we can obtain with reasonable certainty ($P \geq .95$) an indication of herd status by testing approximately 10.3 animals from a herd of any size. Serology is much more reliable on a herd basis rather than on the individual animal basis.

Testing and removal of animals with antibodies to Haemophilus pleuropneumoniae as a method of control as proposed by Nicolet (1970), Nicolet and Scholl (1981), Nielsen et al. (1976) and E. Scholl (Klinik fur Nutztiere und Pferde, Universitat Bern, personal communication, 1980) has merit; however, Nielsen et al. (1976) was able, only, to reduce the incidence of pleuritis and pneumonia, not eliminate it, when testing incoming swine at a progeny testing station. In this study, the positive animals were not removed until one week after arrival, however. E. Scholl (Klinik für Nutztiere und Pferde, Universität Bern, personal communication, 1980) was working with relatively small herds of 20-30 sows and removing animals with positive titers. Generally two to several complete herd tests were required, and a strict quarantine measure between breeding and fattening swine was required. It does not appear to be a practical approach for the medium-large commercial producers in Iowa, especially since these herds when infected have an average of 46% positive animals. It would be of value, however, to the owner of a known negative herd to test incoming stock to assure that he did not introduce H. pleuropneumoniae with an infected or carrier animal.

Serotyping of 50 isolates of H. pleuropneumoniae from pneumonic swine lungs revealed two serotypes (1 and 3) not previously reported from the United States. Gunnarsson et al. (1977) reported that of 6 isolates they examined from the United States, five were serotype 5 and one belonged to serotype 4.

In the present study, serotyping by the plate agglutination method revealed 3 serotypes. Serotype 5 was the predominant (54%) type in the 50 cultures. Twenty-six percent were serotype 1, six percent were serotype 3, and fourteen percent were not typable. Serotype 2 and 4 were not found in the 50 cultures typed.

Failure of the agglutination test to type strains is not uncommon with H. pleuropneumoniae. Rough and smooth forms of H. pleuropneumoniae which have lost ability to form all or part of their capsular material renders them untypable. However, the non-typable strains may also represent new unrecognized serotypes. This is in agreement with Nicolet (1971), Gunnarsson (1980), and Nielsen (1979b).

The importance of serotype specificity must be recognized. Experience with the complement fixation test has shown that cross reactions between serotypes generally do not occur (Nicolet, 1971, Nielsen, 1979a,b; Gunnarsson 1980). Therefore, a mixture of the five known serotypes must be used for routine serologic diagnosis in countries such as the United States where more than one serotype is present.

Furthermore, since serotype 2 is recognized as the predominant serotype in Europe (Nicolet, 1971) and Scandinavian countries (Gunnarsson, 1980), causing severe economic losses, and since it has not been isolated

in the United States, it seems apparent that regulatory procedures should be established to test incoming swine for evidence of antibodies to this serotype, to assure it is not imported with infected or carrier swine.

An understanding of serotypes present may also be important in the development of control procedures. Research needs to be conducted to determine if vaccination against one serotype will protect against another. Multivalent bacterins would be needed in countries having more than one serotype if little cross protection is found. The need for a cost-effective efficacious bacterin is apparent.

Although penicillin has long been indicated as the drug of choice, we found 16% of the field isolates completely resistant to penicillin. The same isolates were also resistant to ampicillin. Evidence was obtained that the predominant mechanism of resistance was β lactamase production.[1]

In inhibiting the growth of H. pleuropneumoniae, penicillin at the 10 unit level was significantly more effective than the 2 unit level in vitro ($p < 0.0001$). Applying this to the data indicates that therapeutic use of higher dosages of penicillin could be beneficial. Only 46% of the H. pleuropneumoniae isolates were sensitive to tetracycline with 36% being intermediate and 18% being resistant according to the criteria of Bauer et al. (1966).

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[1]Presence of β lactamase as determined by the disc method (Marion Scientific Corp.) and tested by Dr. Arthur E. Girard, Pfizer Central Research, Groton, Conn.

As mentioned earlier, all isolates were completely resistant to lincomycin *in vitro* at the 2 ug level.

In vitro studies revealed that chloramphenicol was the most effective drug on the 50 field isolates studied. While this drug is approved for use in food animals in many parts of the world, it is not in the United States. Its limited use in the United States may play a role in its effectiveness *in vitro*.

It has been theorized that the increased incidence of the clinical manifestations of *H. pleuropneumoniae* may have been due to the decreased use of sulfamethazine being used in feed additives because of drug residue problems at slaughter. This hardly seems likely since over 50% of the *H. pleuropneumoniae* isolates tested were completely resistant to sulfonamides and 82% were resistant using criteria of Bauer et al. (1966). An exception to this is sulfachlorpyridazine. Seventy-two percent of the *H. pleuropneumoniae* isolates tested were sensitive to sulfachlorpridazine. 22% intermediate and only 6% resistant.

All sulfonamides are reported to act by the same mechanism, competitive inhibition of PABA (para amino benzoic acid). It is thought that the increased activity of sulfachlorpyridazine is due to halogenation with chlorine allowing the drug to diffuse through the cell membrane. It then acts like other sulfonamides with its competitive inhibition of PABA.

SUMMARY

In a serological survey of 7348 Iowa swine in 597 herds, 32% of the individuals and 67.3% of the herds had antibodies to H. pleuropneumoniae. While these percentages do not necessarily represent clinical disease, they do indicate that the organism is or has been present in those herds.

Two serotypes (1 and 3) previously unrecognized in the United States were found in serotyping 50 isolates of H. pleuropneumoniae. In this survey of 50 isolates, 54% were found to be serotype 5, 26% serotype 1, 6% serotype 3, and 14% not typable. Serotypes 2 and 4 were not found.

Antibiotic and sulfonamide sensitivity testing of 50 isolates of H. pleuropneumoniae was conducted according to the criteria of Bauer et al. (1966). All isolates were sensitive to chloramphenicol, and all but one sensitive to gentamycin. Eighty-four percent were sensitive or intermediate sensitive to ampicillin and penicillin. Eighty-two percent were sensitive or intermediate sensitive to tetracycline. All isolates were resistant to lincomycin in vitro and all but one resistant to streptomycin. Eighty-two percent of the isolates were resistant to triple sulfa while only 6% were resistant to sulfachlorpyridazine.

The clinical problem with H. pleuropneumoniae has become significant in recent years, but by no means is overt disease as prevalent as animals or herds with complement fixing antibodies. It is clear, however, that H. pleuropneumoniae disease has further complicated the already complex swine respiratory disease picture.

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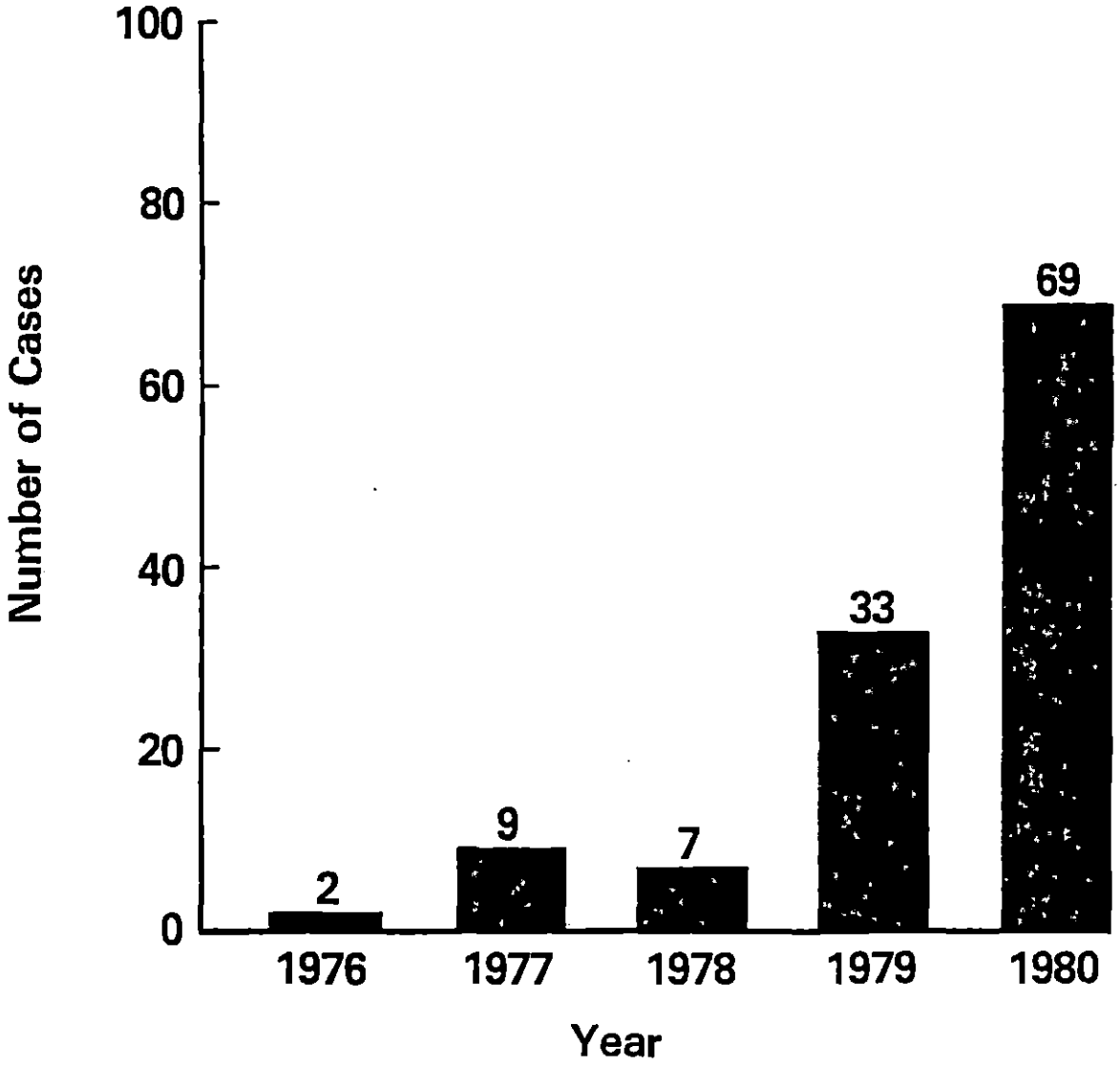
APPENDIX I

Number of cases of H. pleuropneumoniae disease confirmed by culture at the Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa

APPENDIX I: Number of Cases *H. pleuropneumoniae* Disease Confirmed by Culture at the Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.[1]

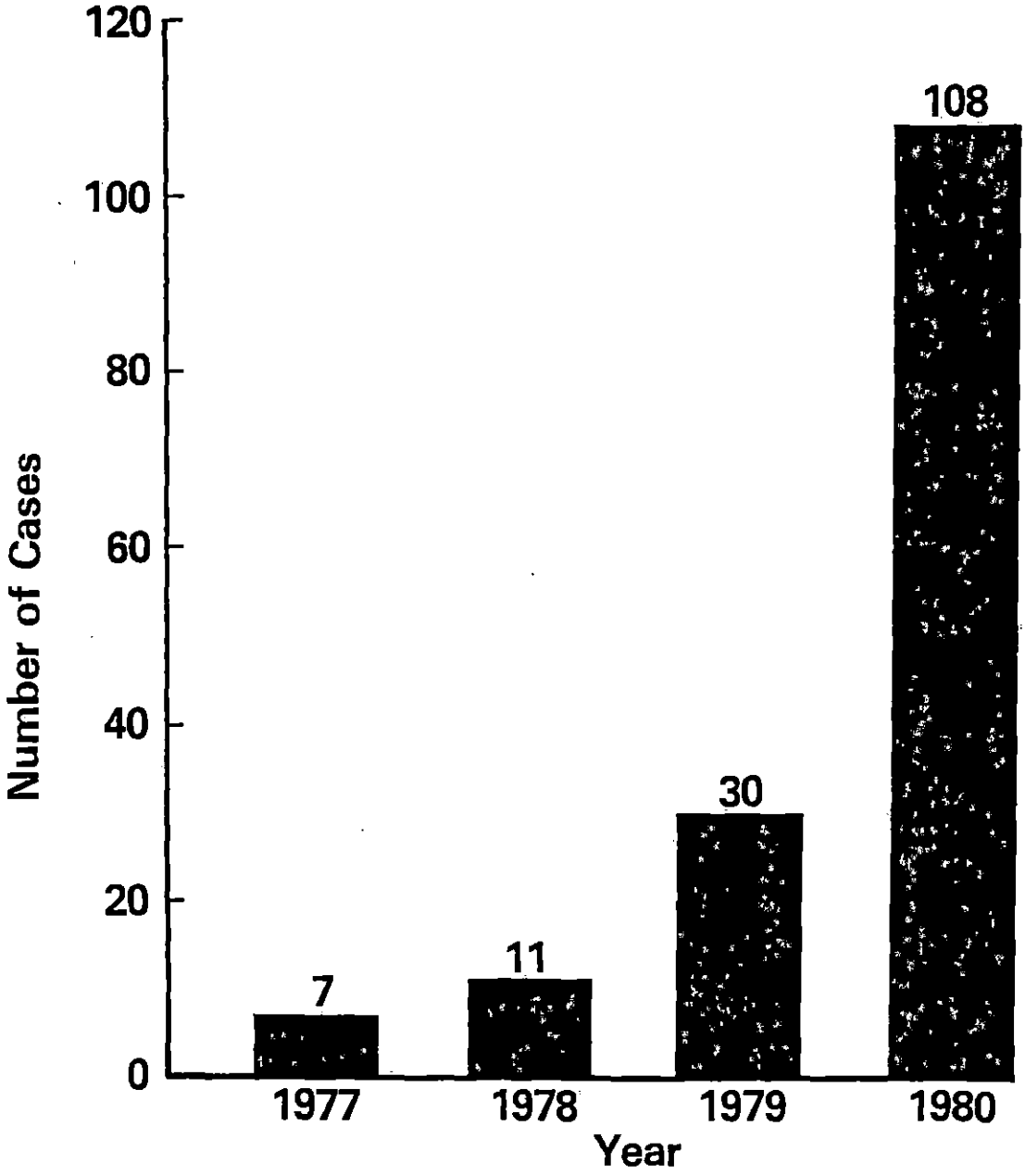


[1]Credit is given to the staff of the Veterinary Diagnostic Laboratory at Iowa State University for collection of the data for this histogram.

APPENDIX II

Number of cases of H. pleuropneumoniae disease confirmed by culture at the South Dakota Veterinary Diagnostic Laboratory, Brookings, South Dakota

APPENDIX II: Number of Cases *H. pleuropneumoniae* Disease Confirmed by Culture at the South Dakota Veterinary Diagnostic Laboratory, South Dakota State University, Brookings, South Dakota [1]

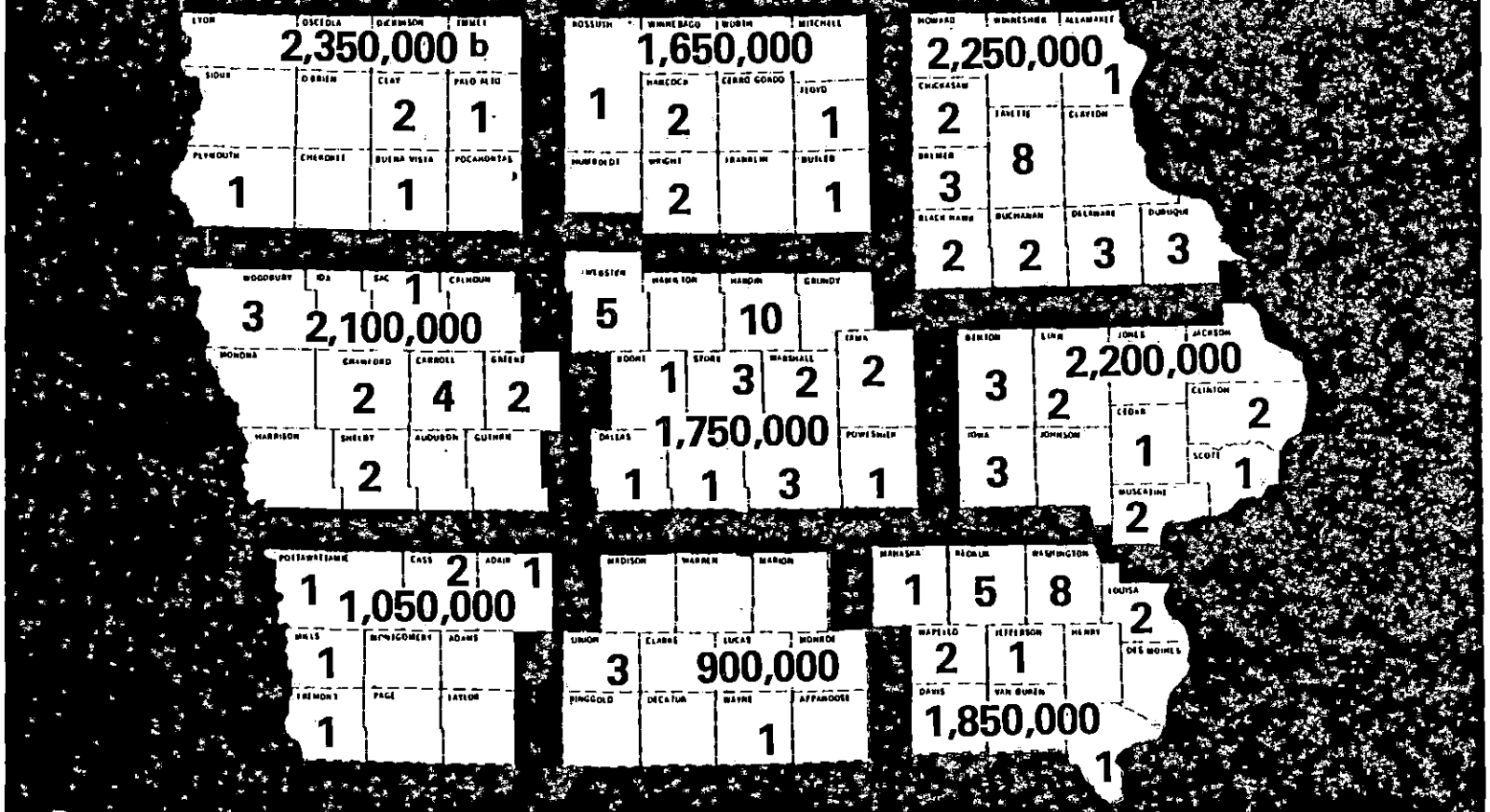


[1]Credit is given to Dr. Martin Bergland of the South Dakota Veterinary Diagnostic Laboratory, South Dakota State University, Brookings, South Dakota for collection of this data.

APPENDIX III

County distribution of *H. pleuropneumoniae* disease outbreaks confirmed by culture at the Iowa State University Veterinary Diagnostic Laboratory from January 1979 - May 1981

APPENDIX III: County distribution of *Hemophilus pleuropneumoniae* herd outbreaks confirmed by culture at the Iowa State University Veterinary Diagnostic Laboratory from January 1979 - May 1981[a]



[a] Credit is given to Dr. Lorraine Hoffman, Veterinary Diagnostic Laoratory, Iowa State University for the collection of this data.

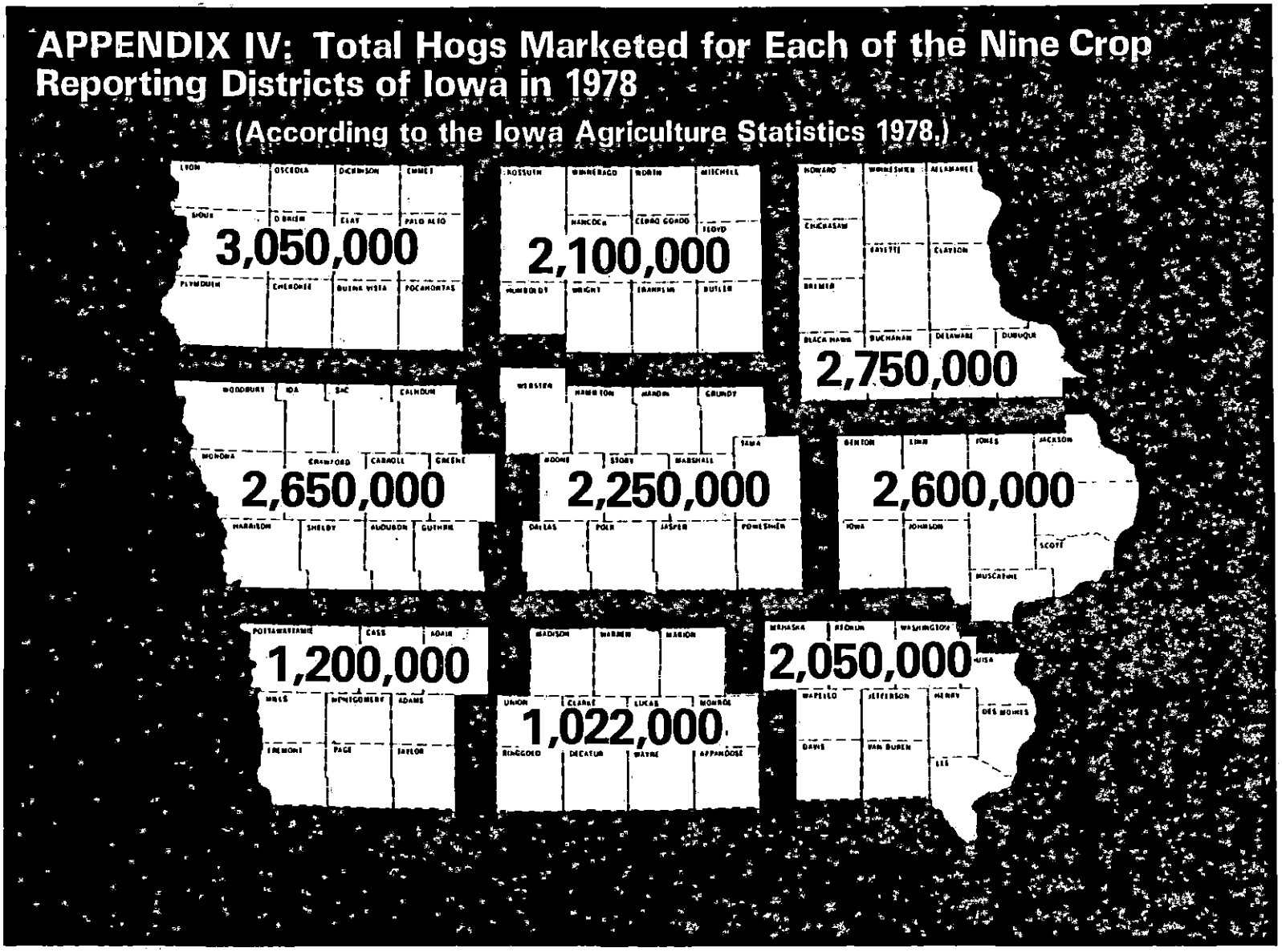
[b] Total number of hogs on farms.

APPENDIX IV

Total hogs marketed for each of the nine crop-reporting districts in Iowa in 1978

APPENDIX IV: Total Hogs Marketed for Each of the Nine Crop Reporting Districts of Iowa in 1978

(According to the Iowa Agriculture Statistics 1978.)



APPENDIX V

Number of herds and sera samples tested for antibodies to *H. pleuropneumoniae* in each crop-reporting district in Iowa

APPENDIX V: Number of Herds and Sera Samples Tested for Antibodies to *H. pleuropneumoniae* in Each of the Nine Crop Reporting Districts in Iowa