JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1985, p. 945–950
0095-1137/85/120945-06$02.00/0

Vol. 22, No. 6

Characterization of *Haemophilus* spp. Isolated from Healthy Swine and Evaluation of Cross-Reactivity of Complement-Fixing Antibodies to *Haemophilus pleuropneumoniae* and *Haemophilus* Taxon "Minor Group"

VICKI J. RAPP, RICHARD F. ROSS,* AND THERESA F. YOUNG

Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011

Received 28 June 1985/Accepted 9 September 1985

Of 30 sows from a herd believed to be free of *Haemophilus pleuropneumoniae* infection, 2 had complement-fixing antibodies to *H. pleuropneumoniae* serotype 5. Necropsy and microbiological examination of the two sows revealed no evidence of *H. pleuropneumoniae* infection; however, *Haemophilus* taxon "minor group" and a urease-negative, indole-positive *Haemophilus* sp. were isolated from numerous respiratory tract sites in both sows. Isolation of these *Haemophilus* spp. was facilitated by serially diluting specimens in two broth media. Pigs from a closed, respiratory disease-free herd were inoculated with four strains of *Haemophilus* taxon "minor group" to determine whether the organism induces antibodies which cross-react with *H. pleuropneumoniae* in the complement fixation test. Antigenic heterogeneity among the taxon "minor group" strains was apparent; however, antibodies cross-reacting between these strains and *H. pleuropneumoniae* serotypes 1 through 5 and 7 were not detected.

Known *Haemophilus* spp. from swine include *Haemophilus pleuropneumoniae*, *Haemophilus parasuis*, *Haemophilus* taxon "minor group" of Kilian et al. (7), and *Haemophilus* taxon C of Kilian (6). *H. pleuropneumoniae* is the cause of swine pleuropneumonia, a disease characterized by a necrotizing, hemorrhagic pneumonia often with fibrinous pleuritis. The clinical course may range from peracute to chronic (15). Although haemophili other than *H. pleuropneumoniae* are also frequently isolated from the porcine respiratory tract, their role in the etiology of swine pneumonia has not been clearly established (15).

The complement fixation (CF) test with a pooled antigen containing the prevalent serotypes of *H. pleuropneumoniae* has been used as an aid in the diagnosis of pleuropneumonia and for the surveillance of herds to determine the prevalence of infection (11, 14, 26; R. Nielsen, Ph.D. thesis, Royal Veterinary and Agricultural University, Copenhagen, Denmark, 1982). Antibodies detected in sera of convalescent swine are generally serotype specific (14, 17, 26), and when used with an antigen reflecting the prevalent serotypes, the CF test has been used to control the disease by allowing for the identification and removal of seropositive animals (19; Nielsen, Ph.D. thesis).

During surveillance of a network of closed, minimal-disease herds for *H. pleuropneumoniae* infection, 799 serum samples from breeding stock from 19 herds were tested for CF antibodies to *H. pleuropneumoniae*. Initially, when the sera were tested against a pooled antigen containing *H. pleuropneumoniae* serotypes 1 through 5, 42 of the 799 samples had titers ≥4 and were considered positive. When the samples were retested with monospecific antigens of serotypes 1 and 5, the two most prevalent serotypes in the United States (23), 29 sera were positive for serotype 5, and 2 sera were positive for serotype 1. One herd, in which 2 of 30 sows had positive CF titers to serotype 5, had never exhibited overt signs of respiratory distress or evidence of pleuropneumonia in animals examined at slaughter. However, because of the possibility that the titers reflected a subclinical or chronic carrier state, the sows were selected for necropsy and examination for lesions or for microbiological evidence indicative of *H. pleuropneumoniae*.

Although *H. pleuropneumoniae* was not isolated from the two sows, the isolation of *Haemophilus* taxon "minor group" and of a urease-negative *Haemophilus* sp. resembling *H. parasuis* prompted us to attempt a better definition of the serological relatedness among swine haemophili. In previous studies, no serological cross-reactions between *H. pleuropneumoniae* and *H. parasuis* have been apparent (5, 14, 16, 17, 26). Possible cross-reactions between *H. pleuropneumoniae* and *Haemophilus* taxon "minor group" have only been examined on a limited basis (5). Thus, we evaluated the possibility that exposure of pigs to taxon "minor group" strains could induce CF antibodies which cross-react with *H. pleuropneumoniae*. Such a cross-reaction could account for occasional positive serological titers detected in pigs believed to be free of *H. pleuropneumoniae* infection.

(A preliminary report of these findings was presented at the 65th annual meeting of the Conference of Research Workers in Animal Disease, Chicago, Ill., 1984.)

MATERIALS AND METHODS

Bacterial strains. The source and designation of reference strains of *H. pleuropneumoniae* serotypes 1 through 5, 7, and *Haemophilus* taxon "minor group" strain 202 were previously described (23). *Haemophilus* taxon "minor group" strains 109 and 215, obtained from C. Pijoan, St. Paul, Minn., were isolated from swine lungs at slaughter (21). *H. parasuis* A9, B26, C5, and D74 and *Haemophilus* taxon C strains CAPM 5111 and CAPM 5113, provided by J. Nicolet, Bern, Switzerland, were used for comparative biochemical testing. The three *H. pleuropneumoniae* cultures used for comparative biochemical testing were field isolates from Iowa and Illinois (23). *H. pleuropneumoniae*, *H. parasuis*, and

* Corresponding author.

Haemophilus taxon C isolates were grown in M96 mycoplasma medium (3) and stored at −70°C. *Haemophilus* taxon "minor group" isolates were grown in the modified brain heart infusion (BHI) broth described below and stored at −70°C after being mixed 1:1 with sterile 18% skim milk.

Swine. Two aged sows were submitted from a closed purebred herd for necropsy and for microscopic and bacteriological examination. Both animals were nursing litters at the time of submission. No previous history of overt disease was reported for either sow.

For production of serotype-specific and hyperimmune antisera, pigs were obtained from the Animal Resource Station, Iowa State University. This herd is known to be free of *Haemophilus* spp. and other swine respiratory tract pathogens.

Media. The following media were used for bacterial isolation: (i) tryptose agar (Difco Laboratories, Detroit, Mich.) containing 5% defibrinated horse blood; (ii) selective medium of Gilbride and Rosendal (4) which contained Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.), 5% defibrinated horse blood, 1 μg of crystal violet (Fisher Scientific Co., Fair Lawn, N.J.) per ml, 1 μg of lincomycin (The Upjohn Co., Kalamazoo, Mich.) per ml, 128 μg of bacitracin (Sigma Chemical Co., St. Louis, Mo.) per ml, and 100 μg of NAD (Sigma) per ml; (iii) BHI broth (Difco) containing 5% horse serum (Pel Freeze Biologicals, Rogers, Ark.), 5% yeast extract (GIBCO Diagnostics, Madison, Wis.), 0.5 μg of lincomycin per ml, 1.5 μg of bacitracin per ml, and 100 μg of NAD per ml, as described by Pijoan et al. (21); and (iv) M96 mycoplasma medium (3) containing 128 μg of bacitracin per ml.

Isolation and culture. The two sows were killed by electrocution and necropsied. Tissue specimens for microscopic examination were fixed in 10% Formalin and stained with hematoxylin and eosin by routine procedures. Swabs for bacterial culture were taken from the nasal passage, nasopharynx, pharyngeal diverticulum, tonsils, trachea, right bronchus, left bronchus, vagina, and cervix. Tissue specimens from tonsils, lungs (a pool of two sites), bronchial lymph nodes and mandibular lymph nodes were collected and approximately 1 cm^3 was homogenized in 5 ml of M96 medium with a tissue grinder. The swabs and homogenized tissue specimens were inoculated directly onto each agar medium. The nonselective blood agar cultures were cross-streaked with a nurse culture of *Staphylococcus epidermidis* to facilitate isolation of *Haemophilus* spp. For primary isolation, agar cultures were incubated at 37°C in a 5% CO_2 atmosphere and observed for growth after 24 and 48 h. Each swab and tissue specimen was also inoculated into the BHI and M96 broth media. As recommended by Pijoan et al. (21), 10-fold dilutions (10^{-1} to 10^{-5}) were prepared, and the entire set was incubated at 37°C. Broth cultures were examined daily, and those with turbidity were inoculated onto each agar medium and incubated at 37°C in a 5% CO_2 atmosphere. After 7 days of incubation, the lowest dilution of each broth culture with no visible growth was inoculated onto the agar media and incubated as above. Colonies suspected to be *Haemophilus* spp., *Pasteurella* spp., or *Actinobacillus* spp. were Gram stained, and a single colony isolate was picked for further evaluation.

Characterization of isolates. For all isolates suspected to be *Haemophilus* spp., a single colony isolate from each site and media was picked for biochemical evaluation. Hemolysin production, dependence on NAD, the Christie-Atkins-Munch-Peterson (CAMP) phenomenon reaction, porphobilinogen production from δ-aminolevulinic acid (requirement for hemin), urease production, and carbohydrate fermentation were evaluated by using procedures described previously (23). Indole production was determined by the addition of Kovacs reagent to 72-h cultures in M96 medium. Oxidase, with the Taxo Disc (BBL), and catalase production were determined by overnight growth on supplemented PPLO agar, prepared as described previously (23). Nitrate reduction was determined by the addition of α-naphthylamine and sulfanilic acid (0.5 and 0.8% in 5 N acetic acid, respectively) to 24-h nitrate broth (Difco) cultures containing 0.01% NAD and an inverted Durham tube to assess gas production. For comparative purposes, biochemical tests were conducted on selected strains of *H. pleuropneumoniae*, *H. parasuis*, *Haemophilus* taxon "minor group," and *Haemophilus* taxon C.

Production of antisera. Type-specific convalescent swine antisera to *H. pleuropneumoniae* serotypes 1 through 5 and 7 were produced by intranasal inoculation of pigs with 6-h M96 broth cultures as described previously (26). Pigs becoming acutely ill were given penicillin-streptomycin (Combiotic; Pfizer Inc., New York, N.Y.) as needed; all pigs were bled 21 to 28 days after inoculation. For production of hyperimmune swine antisera to taxon "minor group" strains, cultures were grown for 5 to 6 h in modified BHI broth, prepared as stated above, except without antibiotics. Swine serum, obtained from the Animal Resource Station herd, was used instead of horse serum in the BHI broth for all cultures except those used for the primary inoculation. Each inoculum contained from 2×10^8 to 2×10^9 CFU/ml. Pigs were initially inoculated intratracheally (1) with 5 or 10 ml of culture. All pigs were reinoculated 30 and 49 days after primary inoculation with 2 or 5 ml of live culture administered intramuscularly. The final inoculation was given on day 65; it consisted of 2 or 5 ml of live culture that was emulsified 1:1 with Freund incomplete adjuvant (Difco) and administered intramuscularly. Pigs were bled after each inoculation, and the sera were tested for CF antibodies to the appropriate homologous antigen. The final bleeding was on day 85, when a serological response had been detected.

CF test. The modified CF test was done as described previously (26). Preparation of test antigens for the taxon "minor group" strains was as described for *H. pleuropneumoniae* (26).

RESULTS

Necropsy of sows. Necropsy of sow 1 revealed small foci of consolidation in the tip of the cardiac lobe of one lung. Necropsy of sow 2 revealed small purple foci suggestive of consolidation in the tips of the cardiac lobes of both lungs. Microscopic examination of lung tissues taken from both sows revealed lesions of chronic suppurative interstitial pneumonia and peribronchiolitis.

Bacterial isolation from sows. Colonies suspected to be of the family *Enterobacteriaceae*, *Proteus* spp., and *Streptococcus* spp. were isolated, occasionally in high numbers, from nasal, nasopharyngeal, pharyngeal diverticulum, and tonsil surface swabs and tonsil tissue samples from both sows. *Pasteurella multocida* was isolated in high numbers from nasopharyngeal and pharyngeal diverticulum swabs and from tonsil tissue samples of sow 2. No *Actinobacillus* spp. were isolated from the respiratory or reproductive tracts of either sow. Of the cultures taken from the respiratory tract, only the tracheal surface, lymph nodes and lung tissue specimens failed to yield *Haemophilus* spp. *Haemophilus* spp. were isolated from four sites in sow 1 and from five sites in sow 2 (Table 1). Only one isolate was obtained

TABLE 1. Isolation of *Haemophilus* spp. from two sows at necropsy

Sow no.	Source of specimen	Medium	Specific dilutions culture positive	Isolate
1	Nasal passage	M96	10^0	Urease-negative *Haemophilus* sp.
	Nasopharynx	M96	10^{-3}	Urease-negative *Haemophilus* sp.
	Pharyngeal diverticulum	M96	10^{-1}, 10^{-4}	Urease-negative *Haemophilus* sp.
	Pharyngeal diverticulum	BHI	10^{-1}–10^{-5}	*Haemophilus* taxon "minor group"
	Tonsil surface	M96	10^{-2}	Urease-negative *Haemophilus* sp.
	Tonsil surface	BHI	10^{-3}	Urease-negative *Haemophilus* sp.
2	Nasal passage	BHI	10^{-3}	Urease-negative *Haemophilus* sp.
	Nasopharynx	M96	10^{-4}, 10^{-5}	Urease-negative *Haemophilus* sp.
	Nasopharynx	BHI	10^{-4}	*Haemophilus* taxon "minor group"
	Pharyngeal diverticulum	Blood agar	Direct	Urease-negative *Haemophilus* sp.
	Tonsil surface	M96	10^0, 10^{-3}, 10^{-4}	Urease-negative *Haemophilus* sp.
	Tonsil surface	BHI	10^{-2}–10^{-5}	*Haemophilus* taxon "minor group"
	Right bronchus	M96	10^0	Urease-negative *Haemophilus* sp.

from primary inoculation onto agar media, whereas the remaining 12 isolations were made from various dilutions of the broth cultures. *Haemophilus* taxon "minor group" was recovered only in BHI broth, whereas the unknown *Haemophilus* sp. was preferentially isolated (7 of 10 times) in M96 medium. In each instance where a taxon "minor group" strain was isolated, the other *Haemophilus* sp. was also isolated, but from M96 medium.

After primary culture in broth media, *Haemophilus* isolates grew equally well on the nonselective and selective blood agar media. On the former, *Haemophilus* colonies were identified by satellitic growth around the *S. epidermidis* nurse culture. *Haemophilus* colonies on the selective medium were very small and translucent, and passage to blood agar with an *S. epidermidis* nurse colony was required to ascertain NAD dependency and to distinguish them from the somewhat larger, semitranslucent colonies of *P. multocida*. Although the isolates were initially isolated in an increased CO_2 atmosphere, no apparent enhancement of growth by CO_2 was detected on subsequent passage.

Biochemical identification of isolates. The biochemical reactions of *H. pleuropneumoniae*, *H. parasuis*, *Haemophilus* taxon "minor group," *Haemophilus* taxon C, and the isolates from the two sows are shown in Table 2. Isolates from the pharyngeal diverticulum of sow 1 and from the nasopharynx and tonsil surface of sow 2 were nonhemolytic, weakly CAMP positive, porphobilinogen test positive, produced urease, were oxidase positive, did not produce catalase, and reduced nitrate to nitrite. These characteristics and the fermentation of eight carbohydrates were identical to those obtained with *Haemophilus* taxon "minor group" (Table 2). Isolates from four different respiratory sites in sow 1 and from five respiratory sites in sow 2 were nonhemolytic, CAMP negative, porphobilinogen test positive, urease negative, oxidase positive, catalase negative, indole positive, and reduced nitrate to nitrite. The negative test for catalase and the positive test for indole and certain differences in carbohydrate fermentation (Table 2) may distinguish this group from *H. parasuis* and from *Haemophilus* taxon C which they resembled morphologically.

Serological testing of sows. Serum taken at necropsy from both sows was tested for CF antibodies to *H. pleuropneumoniae* and *Haemophilus* taxon "minor group" (Table 3). Both sows had antibody titers to *H. pleuropneumoniae* pooled antigen (serotypes 1 through 5) and to monospecific (serotype 5) antigen. CF antibodies to monospecific antigens of *H. pleuropneumoniae* serotypes 1 through 4 or 7 were not detected. Sera was also tested against antigen

TABLE 2. Biochemical characteristics of *Haemophilus* spp. from swine[a]

Test	*H. pleuropneumoniae* (3 strains)	*H. parasuis* (4 strains)	*Haemophilus* taxon "minor group" (4 strains)	Urease-negative *Haemophilus* sp. (10 strains)	*Haemophilus* taxon C (2 strains)
Hemolysis	+	−	+/−	−	−
CAMP	+	−	+/−	−	−
Porphobilinogen production	+	+	+	+	+
Urease	+	−	+	−	−
Oxidase	+	+	+	+	+
Catalase	−	+	−	−	+
Indole	−	−	−	+	−
Nitrate reduction	+	+	+	+	NT
Glucose	A	A	A	A	A
Lactose	0	0	A	V (5/10)	0
Sucrose	A	A	A	A	A
Mannitol	A	0	0	V (2/10)	0
Xylose	A	0	0	V (6/10)	0
Ribose	A	A	V (2/4)	V (2/10)	A
Arabinose	0	0	0	V (2/10)	A
Galactose	A	V (2/4)	A	V (8/10)	A

[a] +, Positive; −, negative; A, acid; 0, no change; V, variable (positive/tested); NT, not tested. Carbohydrate fermentation was determined after 2 days of incubation at 37°C.

TABLE 3. Complement-fixing antibody titers to *H. pleuropneumoniae* and *Haemophilus* taxon "minor group" in sera from two sows

Sow no.	Titer to indicated test antigen[a]					
	H. pleuropneumoniae		Taxon "minor group" strains			
	Pooled serotypes 1 to 5	Monospecific serotype 5	1 NPP	202	109	215
1	32	16	<4	<4	<4	<4
2	8	16	<4	<4	<4	<4

[a] Reciprocal of highest serum dilution resulting in 30% or less hemolysis.

prepared from four strains of *Haemophilus* taxon "minor group," including the homologous isolate, designated 1 NPP, recovered from the pharyngeal diverticulum of sow 1. CF antibodies to the taxon "minor group" strains were not detected in either sow.

Production of antisera to *Haemophilus* taxon "minor group." Pigs developed no detectable CF antibodies to the homologous taxon "minor group" strains after intratracheal inoculation with live cultures. Similarly, no or very low titers were detected after two subsequent intramuscular inoculations with live culture. It was only after a fourth inoculation with live culture emulsified in Freund incomplete adjuvant that titers, ranging from 16 to 128, were obtained. Results obtained when antisera and antigen prepared from *Haemophilus* taxon "minor group" and *H. pleuropneumoniae* were cross-titrated are given in Table 4. No cross-reactions were detected when antisera to the four taxon "minor group" strains were tested against monospecific antigen prepared from *H. pleuropneumoniae* serotypes 1 through 5 and 7. Conversely, testing of antisera to *H. pleuropneumoniae* serotypes 1 through 5 and 7 with antigen prepared from the four taxon "minor group" strains resulted in no detectable cross-reactions, except for a low titer of 8 when serotype 1 antiserum was tested with antigen from taxon "minor group" strain 202 (data not shown). When antisera and antigens for taxon "minor group" strains were compared, serum from a pig immunized with strain 1 NPP reacted with antigens from the other three strains, whereas antisera to strains 202 and 109 reacted only with their homologous antigens. Antiserum to strain 215 reacted with its homologous antigen and gave a one-way cross-reaction with strain 202.

DISCUSSION

As indicated in Table 1, isolation of *Haemophilus* spp. was facilitated by the dilution procedure of Pijoan et al. (21). Both *Haemophilus* spp. were isolated from high dilutions of the broth media, indicating their presence throughout the upper respiratory tract in relatively high numbers. As suggested, the failure to isolate them upon direct inoculation on agar could reflect inhibition or overgrowth by contaminating microorganisms (10, 21). The use of two broth media enhanced our ability to recover *Haemophilus* spp. from swine; the urease-negative isolate grew preferentially in the M96 medium whereas *Haemophilus* taxon "minor group" grew exclusively in the modified BHI broth. This finding is in agreement with previous observations regarding erratic or poor growth of strains of taxon "minor group" in M96 medium, in contrast to the more profuse growth of *H. pleuropneumoniae* and *H. parasuis* in this medium (V. Rapp, unpublished data). Thus, it appears that a dilution technique which uses more than one broth medium may be essential when surveying for swine haemophili.

There was no apparent advantage to subculturing the primary broth dilutions to both selective and nonselective blood agar media, because *Haemophilus* spp. were isolated with equal frequency from either medium. As previously reported (4), the selective medium will inhibit the growth of many gram-positive organisms, but it is less efficient in inhibiting the growth of gram-negative organisms such as the *P. multocida*, *Proteus* spp., and *Enterobacteriaceae* isolated here.

Three of the isolates were identified as *Haemophilus* taxon "minor group" based on biochemical reactions consistent with those obtained with known strains in this and previous reports (2, 7, 23). *Haemophilus* taxon "minor group" has been isolated from swine lungs with lesions of pneumonia (9, 21) and from those with no macroscopic lesions (21) and has been isolated from approximately 18% of lungs examined at slaughter (21). Although a role for this organism in the etiology of swine pneumonia has not been ascertained, its presence has been associated with histological changes, increased severity of pleuritis, and a possible increase in severity of macroscopic pneumonia compared with lungs with no isolations of the organism (13). Inoculation of taxon "minor group" strains directly into the lower airways of pigs produced mild focal necrotic lesions and pleural adhesions but no clinical symptoms, indicating a low virulence potential (24). The organism was isolated only from upper respiratory tract specimens from the two sows examined in the present study. Conceivably, it could have contributed to the chronic pneumonia detected in the sows, but other agents could also have been responsible for those lesions. The lesions were rather nonspecific, possibly resulting from ascarid larval migration.

The 10 urease-negative isolates were indole positive and exhibited a pattern of carbohydrate fermentation which distinguished them from *H. parasuis* and *Haemophilus* taxon C. Differences exist in reported biochemical activity of swine haemophili (2, 6, 7, 12, 20, 23). Although these varying

TABLE 4. Complement-fixing antibody titers of *Haemophilus* taxon "minor group" hyperimmune swine sera to taxon "minor group" strains and *H. pleuropneumoniae*

Antiserum to "minor group" strain	Titer to indicated test antigen[a]									
	"Minor group" strain				*H. pleuropneumoniae* serotype					
	1 NPP	202	109	215	1	2	3	4	5	7
1 NPP	*128*	32	64	64	<4	<4	<4	<4	<4	<4
202	<4	*16*	<4	<4	<4	<4	<4	<4	<4	<4
109	<4	<4	*32*	<4	<4	<4	<4	<4	<4	<4
215	<4	128	<4	*128*	<4	<4	<4	<4	<4	<4

[a] Reciprocal of highest serum dilution resulting in ≤30% hemolysis. Values in italics reflect titers to the homologous strain.

reports may reflect differences in test procedures, it is possible that biotypic differences exist within the species. The 10 isolates we examined originated from different respiratory tract sites in two sows from the same herd; however, the isolates differed in fermentation of lactose, mannitol, xylose, ribose, arabinose, and galactose. Some subjectivity may be involved in reading tests based on changes in the color indicator; however, fermentation of lactose, xylose, and mannitol has not been observed for the *H. parasuis* and taxon C strains tested in our laboratory. Furthermore, a negative indole reaction has been reported for all previously described swine haemophili (6, 12, 20). In addition to biochemical heterogeneity, sodium dodecyl sulfate-polyacrylamide gel electropherograms of outer membrane-enriched fractions from these 10 indole-positive isolates yielded several distinct patterns, indicating the isolates may represent several strains (Rapp, unpublished data). Further studies, such as those recently reported (20; J. Nicolet and T. Morozumi, Proc. 8th Int. Pig Vet. Soc. Congr., p. 105, 1984), are indicated to better define the biochemical, serological, and genetic relatedness of urease-negative swine haemophili and the part, if any, played by these organisms in swine respiratory disease. In the present study, the unknown *Haemophilus* sp. was isolated in relatively high frequency from tissues of the upper respiratory tract, and its presence was not associated with macroscopic lesions.

Sera from both sows had CF antibodies to antigen containing *H. pleuropneumoniae* serotype 5. No titers to the taxon "minor group" strains, including the homologous isolate, were detected, making it unlikely that a cross-reaction produced by the presence of this organism was responsible for the antibodies. We did not examine the possibility of cross-reactions occurring between the urease-negative isolates and *H. pleuropneumoniae*. Biochemical and sodium dodecyl sulfate-polyacrylamide gel characterization indicated heterogeneity among the 10 isolates, and the testing of several strains would have been required in an evaluation of serological cross-reactions with *H. pleuropneumoniae*. The isolates resembled *H. parasuis* biochemically and morphologically, and previous reports indicate that no cross-reactivity occurs between *H. parasuis* and *H. pleuropneumoniae* (5, 14, 16, 17, 26). Recently, however, evidence for considerable serologic heterogeneity among strains of *H. parasuis* has been reported (Nicolet and Morozumi, Proc. 8th Int. Pig Vet. Soc. Congr., p. 105, 1984). It may be that an evaluation of more strains, representing several serotypes, would allow for more valid conclusions regarding possible cross-reactivity between *H. pleuropneumoniae* and *H. parasuis*.

The factor or agent causing development of CF antibodies in these two sows and in other similar herd situations has not been determined. Phenotypic and genotypic relatedness between *H. pleuropneumoniae* and *Actinobacillus* spp. has been demonstrated (12, 22). Rosendal and Mittal (25) recently reported that over 50% of the animals in a closed, specific pathogen-free swine herd had CF antibody titers to *H. pleuropneumoniae* despite having no history or clinical evidence of pneumonia. They isolated an *Actinobacillus* sp. from the tonsils of a majority of pigs examined and demonstrated a strong cross-reactivity between this isolate and *H. pleuropneumoniae*. In our study, no actinobacilli were isolated from the respiratory or reproductive tracts of the two sows.

It has been documented that recovered animals and healthy carriers contribute to the spread of swine pleuropneumonia and that titers may persist after subclinical infection (11, 14, 16, 18, 19). Recently, Japanese workers reported that *H. pleuropneumoniae* was isolated from 47% of the nasal cavities of apparently healthy pigs of all ages (8). Many of the clinically inapparent carriers had CF antibodies to the organism. We did not isolate *H. pleuropneumoniae* from cultures of numerous respiratory tract sites and detected no gross or histopathologic lesions indicative of current or past *H. pleuropneumoniae* infection upon necropsy of the two sows. Such lesions are usually associated with the chronic carrier state (18).

The failure of antisera to four strains of taxon "minor group" to cross-react with antigens from six serotypes of *H. pleuropneumoniae* supports a conclusion that the former organism is not responsible for producing CF antibodies cross-reactive with *H. pleuropneumoniae*. The fact that it took four exposures to live culture to produce appreciable titers to taxon "minor group" strains indicates that they may be rather poor inducers of CF antibodies in swine. Results obtained in this study also indicated that antigenic heterogeneity occurs among taxon "minor group" strains. Only antiserum to strain 1 NPP reacted with antigens prepared from all four taxon "minor group" strains. Antisera to strains 202 and 109 were strain specific, whereas antiserum to strain 215 reacted with only the homologous strain and with strain 202. Strain 202 is generally regarded as the reference strain for *Haemophilus* taxon "minor group" (7). In the present study, antisera to strain 202 reacted only with antigen from the homologous strain, whereas antisera produced against two of three heterologous taxon "minor group" strains contained CF antibodies which were detected with antigen to strain 202. Thus, these results support a previous observation that the strain may not possess sufficient antigenic cross-reactivity for use in serological identification of all taxon "minor group" strains (13).

It has been stated that because titers may persist after subclinical *H. pleuropneumoniae* infection all seropositive animals should be considered potential carriers (18). Although we agree with that conclusion, it is apparent that in some herds, a low percentage of pigs may be seropositive without evidence of past or current infection. This supports the suggestion that, when used for herd surveillance, the CF test is best utilized when evaluation is made on a herd rather than an individual animal basis (11; Nielsen, Ph.D. thesis).

ACKNOWLEDGMENTS

We thank Barbara Zimmermann Erickson, Serge Messier, Douglas Stine, Nancy Upchurch, Steve Penner, and Norma Erickson for technical assistance and Joachim Pohlenz for assistance in microscopic evaluation of the tissue specimens.

This work was supported by the State of Iowa Livestock Health Research Advisory Council and by the Pig Improvement Company, Inc., Franklin, Ky.

LITERATURE CITED

1. **Bentley, O. E., and D. O. Farrington.** 1980. Evaluation of an induced *Pasteurella multocida* swine pneumonia model. Am. J. Vet. Res. **41**:1870–1873.
2. **Biberstein, E. L., A. Gunnarsson, and B. Hurvell.** 1977. Cultural and biochemical criteria for the identification of *Haemophilus* spp. from swine. Am. J. Vet. Res. **38**:7–11.
3. **Frey, M. L., G. B. Thomas, and P. H. Hale.** 1973. Recovery and identification of mycoplasmas from animals. Ann. N.Y. Acad. Sci. **225**:334–346.
4. **Gilbride, K. A., and S. Rosendal.** 1983. Evaluation of a selective medium for isolation of *Haemophilus pleuropneumoniae*. Can. J. Comp. Med. **47**:445–450.

5. **Gunnarsson, A.** 1979. Evaluation of different antigens in the complement-fixation test for diagnosis of *Haemophilus pleuropneumoniae* (*parahaemolyticus*) infections in swine. Am. J. Vet. Res. **40**:1564–1567.
6. **Kilian, M.** 1976. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. J. Gen. Microbiol. **93**:9–62.
7. **Kilian, M., J. Nicolet, and E. L. Biberstein.** 1978. Biochemical and serological characterization of *Haemophilus pleuropneumoniae* (Matthews and Pattison 1961) Shope 1964 and proposal of a neotype strain. Int. J. Syst. Bacteriol. **28**:20–26.
8. **Kume, K., T. Nakai, and A. Sawata.** 1984. Isolation of *Haemophilus pleuropneumoniae* from the nasal cavities of healthy pigs. Jpn. J. Vet. Sci. **46**:641–647.
9. **Little, T. W. A.** 1970. Haemophilus infection in pigs. Vet. Rec. **87**:399–402.
10. **Little, T. W. A., and J. D. J. Harding.** 1980. The interaction of *Haemophilus parahaemolyticus* and *Pasteurella multocida* in the respiratory tract of the pig. Br. Vet. J. **136**:371–383.
11. **Lombin, L. H., S. Rosendal, and W. R. Mitchell.** 1982. Evaluation of the complement fixation test for the diagnosis of pleuropneumonia of swine caused by *Haemophilus pleuropneumoniae*. Can. J. Comp. Med. **46**:109–114.
12. **Mannheim, W., S. Pohl, and R. Holländer.** 1980. Zur Systematik von Actinobacillus, Haemophilus und Pasteurella: Basenzusammensetzung der DNS, Atmungschinone und kulturell-biochemische Eigenschaften repräsentativer Sammulungsstämme. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **246**:512–540.
13. **Morrison, R. B., C. Pijoan, H. D. Hilley, and V. Rapp.** 1985. Microorganisms associated with pneumonia in slaughter weight swine. Can. J. Comp. Med. **49**:129–137.
14. **Nicolet, J., P. A. de Meuron, and P. Bachmann.** 1971. Sur l'hémophilose du porc IV: l'épreuve de déviation du complément, un test de dépistage des infections à *Haemophilus parahaemolyticus*. Schweiz. Arch. Tierheilkd. **113**:191–200.
15. **Nicolet, J., and E. Scholl.** 1981. Haemophilus infections, p. 368–377. *In* A. D. Leman, R. D. Glock, W. L. Mengeling, R. H. C. Penny, E. Scholl, and B. Straw (ed.), Diseases of swine, 5th ed. Iowa State University Press, Ames.
16. **Nielsen, R.** 1974. Serological and immunological studies of pleuropneumonia of swine caused by Haemophilus parahaemolyticus. Acta. Vet. Scand. **15**:80–89.
17. **Nielsen, R.** 1979. Haemophilus parahaemolyticus serotypes serological response. Nord. Veterinaermed. **31**:401–406.
18. **Nielsen, R., and M. Mandrup.** 1977. Pleuropneumonia in swine caused by *Haemophilus parahaemolyticus*. A study of the epidemiology of the infection. Nord. Veterinaermed. **29**:465–473.
19. **Nielsen, R., A. D. Thomsen, and S. Duedahl Vesterlund.** 1976. Pleuropneumonia caused by *Haemophilus parahaemolyticus*. An attempt to control the disease at two progeny testing stations by serological blood testing followed by removal of the seropositive animals and their litter mates. Nord. Veterinaermed. **28**:349–352.
20. **O'Reilly, T., S. Rosendal, and D. F. Niven.** 1984. Porcine haemophili and actinobacilli: characterization by means of API test strips and possible taxonomic implications. Can. J. Microbiol. **30**:1229–1238.
21. **Pijoan, C., R. B. Morrison, and H. D. Hilley.** 1983. Dilution technique for isolation of *Haemophilus* from swine lungs collected at slaughter. J. Clin. Microbiol. **18**:143–145.
22. **Pohl, S., H. U. Bertschinger, W. Frederiksen, and W. Mannheim.** 1983. Transfer of *Haemophilus pleuropneumoniae* and the *Pasteurella haemolytica*-like organism causing porcine necrotic pleuropneumonia to the genus *Actinobacillus* (*Actinobacillus pleuropneumoniae* comb. nov.) on the basis of phenotypic and deoxyribonucleic acid relatedness. Int. J. Syst. Bacteriol. **33**:510–514.
23. **Rapp, V. J., R. F. Ross, and B. Zimmermann Erickson.** 1985. Serotyping of *Haemophilus pleuropneumoniae* by rapid slide agglutination and indirect fluorescent antibody tests in swine. Am. J. Vet. Res. **46**:185–192.
24. **Rosendal, S., D. A. Boyd, and K. A. Gilbride.** 1985. Comparative virulence of porcine *Haemophilus* bacteria. Can. J. Comp. Med. **49**:68–74.
25. **Rosendal, S., and K. R. Mittal.** 1985. Serological cross-reactivity between a porcine *Actinobacillus* strain and *Haemophilus pleuropneumoniae*. Can. J. Comp. Med. **49**:164–170.
26. **Schultz, R. A., T. F. Young, R. F. Ross, and D. R. Jeske.** 1982. Prevalence of antibodies to *Haemophilus pleuropneumoniae* in Iowa swine. Am. J. Vet. Res. **43**:1848–1851.