Serological Cross-reactivity Between a Porcine *Actinobacillus* Strain and *Haemophilus pleuropneumoniae*

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ABSTRACT

During serological screening of a closed SPF-herd free of pleuropneumonia, more than half of the pigs were positive for complement-fixing antibodies to *Haemophilus pleuropneumoniae*. *Actinobacillus* bacteria closely related to *A. suis* were isolated from tonsillar tissue of 14 out of 20 slaughtered pigs submitted for pathological and bacteriological evaluation. None of the pigs had evidence of respiratory disease. Two pigs inoculated endobronchially with a selected *Actinobacillus* strain developed mild focal pneumonia and complement-fixing antibodies cross-reacting with *H. pleuropneumoniae*. Five pigs exposed and vaccinated with the *Actinobacillus* strain and five pigs spontaneously infected with the strain also developed complement-fixing antibodies against *H. pleuropneumoniae* and appeared to be less susceptible to experimental *Haemophilus* pleuropneumonia than pigs not exposed to the *Actinobacillus* infection. The agglutination test applied on serum treated with 2-mercaptoethanol detected antibodies against *H. pleuropneumoniae* serotype 5 but not against serotype 1 in pigs exposed to the *Actinobacillus* strain. Antibodies reactive with the *Actinobacillus* strain were also found in pigs hyperimmunized against *H. pleuropneumoniae* serotypes 1-5 in 2-mercaptoethanol tube agglutination test and rabbits hyperimmunized against serotypes 1, 2 and 7, and strain 73567 in the immunodiffusion test. Conversely rabbits immunized against the *Actinobacillus* strain had antibodies against *H. pleuropneumoniae* serotypes 1, 3, 4, 5 and 6. It is concluded that pigs infected with *Actinobacillus* organisms may become false positive reactors against *H. pleuropneumoniae*.

Key words: *Haemophilus pleuropneumoniae, Actinobacillus*, serology, cross-reactivity, complement-fixation, pneumonia, swine.

RÉSUMÉ

Lors du tamisage sérologique d'un troupeau de porcs fermé, exempt d'agents pathogènes spécifiques et de pleuro-pneumonie, l'épreuve de la déviation du complément révéla la présence d'anticorps contre *Haemophilus pleuropneumoniae*, chez au delà de 50% des sujets. On isola aussi des bactéries du genre *Actinobacillus*, étroitement reliées à *A. suis*, à partir de 14 des 20 échantillons d'amygdales prélevés au moment de l'abattage. Aucun de ces porcs n'avait manifesté de signes d'une maladie respiratoire. Deux porcs dans les bronches desquels on avait introduit une souche choisie d'*Actinobacillus* développèrent une légère pneumonie focale et des anticorps qui réagissaient aussi avec *H. pleuropneumoniae*, comme le démontra l'épreuve de la déviation du complément. Cinq porcs exposés à la souche précitée d'*Actinobacillus* et ultérieurement vaccinés contre elle, de même que cinq autres porcs infectés de façon spontanée avec cette souche, développèrent des anticorps fixateurs du complément contre *H. pleuropneumoniae* et semblèrent moins susceptibles à la pleuro-pneumonie expérimentale à *Haemophilus* que les porcs non exposés à l'infection par *Actinobacillus*. L'épreuve d'agglutination effectuée avec les échantillons de sérum traités au 2-mercaptoéthanol démontra la présence d'anticorps contre le sérotype #5 d'*H. pleuropneumoniae*, mais non contre son sérotype #1, chez les porcs exposés à la souche précitée d'*Actinobacillus*. Cette épreuve permit aussi de déceler des anticorps qui réagissaient avec la même souche d'*Actinobacillus*, chez les porcs hyperimmuns à l'endroit des sérotypes #1-#5 d'*H. pleuropneumoniae*, ainsi que chez les lapins hyperimmuns à l'endroit de ses sérotypes #1, #2 et #7. L'épreuve de la précipitation en milieu gélifié permit par ailleurs de détecter de tels anticorps, chez les lapins hyperimmuns à l'endroit de la souche #73567 d'*H. pleuropneumoniae*. D'un autre côté, les lapins immunisés contre la souche d'*Actinobacillus* possédaient des anticorps contre les sérotypes #1, #3, #4, #5 et #6 d'*H. pleuropneumoniae*. Les auteurs conclurent que les porcs infectés avec des bactéries du genre *Actinobacillus* pourraient donner de fausses réactions positives, lorsqu'on tente de déterminer s'ils possèdent des anticorps contre *H. pleuropneumoniae*.

Mots clés: *Haemophilus pleuropneumoniae, Actinobacillus* spp., sérologie, immunité croisée, épreuve de la déviation du complément, agglutination, pneumonie, porcs.

INTRODUCTION

Subclinical infection of pigs with *Haemophilus pleuropneumoniae* is best diagnosed serologically. The complement-fixation (CF) test seems to be the preferred test (1,2,3,4), although others, such as the enzyme-linked immunosorbent assay (ELISA)

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(5) and direct or indirect agglutination tests (6,7), have been adapted for this infection as well. The diagnostic sensitivity and specificity of the CF test was evaluated recently (3). At a serum dilution of 1:2 the specificity (the proportion of test negative animals out of the noninfected animals) was 78.4%. As expected, the specificity increased with increased serum dilution and reached 100% at 1:128 dilution. False positive seroreactions may be due to infections with microorganisms sharing antigens with *H. pleuropneumoniae*. Since *Actinobacillus lignieresii* was recently shown to be genetically closely related to *H. pleuropneumoniae*, such antigenic cross-reactivity may be sought among other bacteria of the *Actinobacillus* genus (8).

Sera from 30 feeder pigs from herd "A" were recently submitted for *H. pleuropneumoniae* serological screening. The herd was closed, had SPF status, and no history or clinical evidence of pleuropneumonia. Sixteen animals were positive for CF antibodies (titers ranged from 1:8 to 1:128) to *H. pleuropneumoniae*, using a pooled antigen of serotypes 1, 2, 5 and 7. Of 15 serum samples submitted two months later, only one was positive (1:8) in the CF test. However, using tube agglutination test after 2-mercaptoethanol (2-ME) treatment (6), two pigs were positive against *H. pleuropneumoniae* serotype 1, one pig against serotype 2, two pigs against serotype 4, and all against the *Actinobacillus* isolate described in this report.

The purpose of this report is to describe the isolation of *Actinobacillus* from tonsillar tissue of pigs from herd "A" and describe antigenic cross-reactivity between the *Actinobacillus* isolate and *H. pleuropneumoniae* which may account for the seroreaction in the herd.

MATERIALS AND METHODS

EXAMINATION OF PIGS WITH NATURAL ACTINOBACILLUS INFECTION (HERD "A")

The heads and lungs of 20 marketweight pigs slaughtered at a local abattoir were submitted for culture and necropsy examination. An incision was made with a sterile scalpel into the tonsils. A dry swab rubbed on the cut surface was immediately streaked onto selective blood agar containing 0.1% nicotinamide adenine dinucleotide (NAD) (9). Culture plates were incubated under normal atmospheric conditions. Haemolytic colonies developing within 48 hours were subcultured and examined morphologically and biochemically (10).

EXPERIMENTAL INFECTION OF SPF PIGS WITH AN ACTINOBACILLUS STRAIN

Experiment 1 — Two SPF pigs, three months of age, from the Arkell Swine Research Station Centre of the Ontario Ministry of Agriculture and Food, were kept in isolation and ascertained to be free of *Actinobacillus* or *Haemophilus* infection by culturing swab specimens taken from the nares and pharynx, using the selective NAD enriched blood agar (9). Both pigs were inoculated endobronchially with 7 x 10^9 CFU of *Actinobacillus*, strain S04, suspended in 10 mL of phosphate buffered saline. Strain S04 was isolated from the tonsil of one of the slaughtered pigs from herd "A". The pigs were observed for 18 days, euthanized and necropsied. Tissues from the lung, pulmonary lymph nodes, spleen, carpal and hock joints were cultured for bacteria and fixed in buffered formaldehyde and processed for histological examination. Serum derived from blood samples taken before inoculation and at necropsy was titrated in the modified direct CF test for antibodies against *Haemophilus pleuropneumoniae* using an antigen-pool of strains Shope 4074, S1536, K17 and WF83 representing serotypes 1, 2, 5 and 7 respectively (3). The same sera were also titrated by the tube agglutination technique after treatment with 2-ME (6). The antigens for the tube agglutination tests were formalin-treated suspensions of bacteria of the following strains: *H. pleuropneumoniae* serotype 1, Shope 4074; serotype 2, S1536; serotype 3, S1421; serotype 4, M62; and *Actinobacillus*, strain S04.

Experiment 2 — Two groups of two month old "Arkell" SPF pigs were used. Each group had five pigs and was housed in two separate isolation rooms in the same building. The pigs were ascertained to be free of *Actinobacillus* or *Haemophilus* infection by culture of nares and pharynx, before experimentation. Before exposure blood samples for serology were taken from all pigs. Group 1 pigs were exposed to 2 mL of a saline suspension of *Actinobacillus*, strain S04, containing 10^9 CFU/mL, instilled into the nostrils and 10 mL given orally. In addition, these pigs were injected subcutaneously with 4 mL of aluminum hydroxide adjuvanted (2 + 2 mL) *Actinobacillus* suspension. Group 2 pigs were given placebo treatment. Each day during the week following the initial exposure, the pigs in group 1 were fed five blood agar cultures with confluent growth of *Actinobacillus*, strain S04.

One week following initial exposure, all pigs were bled and nostrils and pharynx swabs cultured to determine presence of *Actinobacillus*. Also at this time the pigs in group 1 were revaccinated subcutaneously and the pigs in group 2 were given placebo treatment.

Three weeks after the initial *Actinobacillus* exposure, the pigs in groups 1 and 2, together with four additional three month old pigs (group 3) obtained from the "Arkell" SPF-herd were bled for serology and examined for *Actinobacillus* and *Haemophilus* infection by culture of nostrils and pharynx. Because nose and pharynx cultures of the pigs in group 2 became positive for *Actinobacillus* bacteria, it was necessary to add the group 3 pigs to the experiment. All 14 animals were subsequently challenged with an aerosol of *H. pleuropneumoniae*, strain CM5, serotype 1, containing 1.2 x 10^7 CFU/mL (11). From the time of challenge all pigs were housed in the same room. Pigs dying as a result of the challenge were necropsied immediately and the following tissues were cultured for bacteria: tonsils, lung, spleen and one hock joint. Surviving pigs were bled and euthanized and necropsied eight days after *Haemophilus* challenge. Serum was tested for CF antibodies to the pooled antigen of *H. pleuropneumoniae* serotypes 1, 2, 5 and 7 and 2-ME agglutinating antibodies to *H. pleuropneumoniae* serotypes 1 and 5 and *Actinobacillus*, strain S04.

REACTIVITY OF SERUM FROM HYPERIMMUNIZED PIGS AND RABBITS

Experiment 3 — An experiment was carried out in which sera from pigs hyperimmunized against *H. pleuropneumoniae*, serotypes 1-5 and 7, were titrated for antibodies by tube agglutination test after 2-ME treatment against the homologous strain and *Actinobacillus*, strain S04 before and after absorption with strain S04.

Experiment 4 — In another experiment, sera from two rabbits hyperimmunized with *Actinobacillus* strain S04 were titrated for antibodies against the homologous strain and reference strains of serotypes 1-7 of *H. pleuropneumoniae* employing tube agglutination test before and after treatment with 2-ME.

Experiment 5 — The immunodiffusion test (10) was used to detect antigens in a sonicated suspension of *Actinobacillus*, strain S04 reacting with hyperimmune rabbit sera against the homologous strain and strains of *H. pleuropneumoniae* serotypes 1 through 7; *H. parasuis*, C5; Taxon C, J94; "Minor group", strain 202; and *H. pleuropneumoniae*, strain 73567. The latter serum was provided by Dr. Nicolet, Bern, Switzerland.

RESULTS

HERD "A"

Haemolytic colonies, resembling *H. pleuropneumoniae*, grew on plates inoculated with tonsillar swabs from 14 out of 20 pigs submitted for cultural and morphological evaluation of heads and lungs. None of these animals had lesions in the respiratory tracts. The haemolytic colonies from each animal were subcultured onto noninhibitory media in order to obtain pure cultures. All haemolytic isolates were found to be Gram-negative coccobacilli, cytochrome oxydase positive, weakly catalase positive, urease positive and able to grow on MacConkey agar with small red-purplish colonies. Detailed biochemical reactions of a selected strain S04 have been published (11).

EXPERIMENT 1

The two experimental pigs were anorexic for two days following inoculation and appeared reluctant to rise. No clinical signs of disease were observed later. At necropsy one pig had a firm nodule 15 mm in diameter dorsally in the right lung over the cardiac lobe. There was a fibrotic adhesion between the visceral and parietal pleura over the nodule. Histologically the nodule was composed mainly of scar tissue, collapsed bronchioles and islands of mononuclear inflammatory cells, mainly lymphocytes. The other pig had an abscess approximately 25 mm in diameter with fibrous adhesions to the parietal pleura in the same area of the lung as the first pig. No macroscopic or microscopic changes were found in pulmonary lymph nodes, spleen or joints. *Actinobacillus* bacteria of the same morphology and biochemical characteristics as the inoculation strain were isolated from the abscess, but not from the lung of the first pig or other tissues of either pig. The antibody titres in the pigs to *H. pleuropneumoniae* and *Actinobacillus* are given in Table I and it shows that cross-reacting antibodies to *H. pleuropneumoniae* were demonstrated by the CF test in both the pigs after experimental infection with *Actinobacillus* strain S04. However, the 2-ME resistant agglutinins did not increase significantly in the sera of the two pigs against either *H. pleuropneumoniae* serotype 1, 2, 3 and 4 or *Actinobacillus* strain S04.

TABLE I. Antibody Titers in Two Pigs Before and After Experimental Infection with *Actinobacillus* Strain S04

Pig #	CF antigen pool of *H. pl.* serotype 1, 2, 5, 7	2-ME Resistant Agglutinins *H. pl.* serotype 1	2	3	4	Act. S04
1 preinf	< 2	10	< 10	ND	10	10
postinf	16	20	< 10	10	10	10
2 preinf	< 2	10	< 10	ND	10	10
postinf	16	10	< 10	< 10	< 10	20

EXPERIMENT 2

Actinobacillus or *Haemophilus* organisms were not recovered from pigs in groups 1 and 2 before experimentation. *Actinobacillus* bacteria culturally and biochemically similar to strain S04 were isolated from the nostrils and pharynx of all pigs in groups 1 and 2 one week after the initial exposure of group 1. Two pigs from group 1 and four from group 2 had *Actinobacillus* bacteria in nostrils and pharynx three weeks after exposure (before *Haemophilus* challenge). *Actinobacillus* or *Haemophilus* bacteria were not cultured from the four pigs in group 3 brought in from Arkell.

Two of the animals from group 3 died 24 hours after challenge with *H. pleuropneumoniae*, strain CM5. The two remaining in group 3 were dyspneic, anorexic and in sternal recumbency and one died 60 hours after challenge. The last one recovered from the acute signs, but remained unthrifty until it was euthanized eight days after challenge. None of the pigs in group 1 or 2 developed clinical signs of respiratory disease. A summary of the necropsy and cultural findings is given in Table II. The spleen and hock joint were negative for bacteria except in the cases indicated in the table.

Four of the pigs in group 1 developed CF antibodies reacting with *H. pleuropneumoniae* after exposure to *Actinobacillus*, strain S04 (Table III). The titers had declined eight days after *Haemophilus* challenge. In group 2 only one animal had a low titer one week after simulated *Actinobacillus* exposure, but before *Haemophilus* challenge all five had titers. None of the pigs in group 3 had antibody titers. Using the tube agglutination test after 2-ME treatment only antibodies against *H. pleuropneumoniae* serotype 5 and *Actinobacillus* strain S04 seemed to be produced as a result of *Actinobacillus* exposure, while antibodies against *H. pleuropneumoniae*, serotype 1 were not demonstrable (Table III).

EXPERIMENT 3

Pigs hyperimmunized against reference strains of *H. pleuropneumoniae*

TABLE II. Summary of Necropsy and Cultural Findings in Pigs Exposed to *Actinobacillus* sp (Groups 1 and 2) and Subsequently Challenged with *H. pleuropneumoniae* Strain CM5 Serotype 1

		Necropsy Findings			Cultural Findings			
Grp	Pig #	Lungs	% changed lung tissue	Injection site	Tonsil	Lung	Pulmonary lymph node	Injection site
1	38	2 areas of necrosis with local pleuritis	10	Calcified scar (2 x 3 cm)	*Act.*	*H. pl.*	NG	NG
	39	2 areas of necrosis and 1 abscess w. local pleuritis	10	No lesion	*Act.*	*H. pl.*	*H. pl.*	NG
	40	No lesions	0	Calcified scar	*Act.*	NG	NG	NG
	41	No lesions	0	Scar (2 x 3 cm)	*Act.*	NG	NG	NG
	42	No lesions	0	Calcified scar	*Act.*	NG	NG	*Act.*
2	33	No lesions	0		*Act.*	NG	NG	
	34	No lesions	0		*Act.*	NG	NG	
	35	Many small abscesses and necrotic foci w. local pleuritis	15		*Act.*	*H. pl.*	NG	
	36	Diffuse pleuritis over rt diaphragmatic lobe	0		*Act.*	NG	NG	
	37	1 abscess w. local pleuritis	5		*Act.*	*H. pl.*	NG	
3	43	Bilat. fibrinohemorrhagic pleuropneumonia	100		*H. pl.*	*H. pl.*	*H. pl.*[a]	
	44	Large abscesses and necrotic tissue w. extensive pleuritis	65		*Act.*	*H. pl.*	*H. pl.*	
	45	Bilat. fibrinohemorrhagic pleuropneumonia	100		*H. pl.*	*H. pl.*	*H. pl.*	
	46	Bilat. fibrinohemorrhagic pleuropneumonia	100		*H. pl.*	*H. pl.*	*H. pl.*[a]	

[a]Few *H. pl.* isolated from joint and spleen as well
H. pl. = *Haemophilus pleuropneumoniae*
NG = No growth
Act. = *Actinobacillus* sp.

serotypes 1-5 and 7 had antibody titers against the homologous strain as well as *Actinobacillus* strain S04 except for serotype 7 pig serum which had only the homologous antibody titer (Table IV) when tested by tube agglutination after 2-ME treatment. No reduction in titers against the homologous *Haemophilus* strain was found when antibodies against strain S04 were removed by absorption.

EXPERIMENT 4

Rabbits immunized against *Actinobacillus* strain S04 developed antibodies against *H. pleuropneumoniae* serotypes 1, 3, 4, 5 and 6, but not 2 and 7 (Table V). Preimmunization titers were found against serotypes 3, 4, 6 and strain S04; but these reactions were eliminated after 2-ME treatment. Postimmunization titers were generally reduced after 2-ME treatment but only eliminated completely in one serum each against *H. pleuropneumoniae* serotypes 4 and 5 (Table V).

EXPERIMENT 5

In initial tests *Actinobacillus* strain S04, gave lines of precipitation in immunodiffusion with serum against strain S04, strain BC97 (*H. pleuropneumoniae*, serotype 1), S1536 (serotype 2), WF83 (serotype 7) and 73567 identified as *H. pleuropneumoniae*, but of uncertain serotypic affiliation. In subsequent tests in which reactive heterologous sera were placed next to the S04 serum, lines of identity were found between strain S04 and S1536 and S04 and 73567.

DISCUSSION

Highly virulent bacteria identical with *Haemophilus pleuropneumoniae* serotype 2, except for the NAD requirement, have been isolated in Switzerland (13), East Germany (14) and Italy (15). The NAD-independent strains and *H. pleuropneumoniae* are genetically related to *Actinobacillus lignieresii* which is the type species of the genus *Actinobacillus*. Pohl *et al* (8) suggested on this basis to rename *Haemophilus pleuropneumoniae: Actinobacillus pleuropneumoniae*. *Actinobacillus* bacteria identified as *A. suis* are occasionally isolated from young, usually preweaned, pigs with septicemia (16). Other *Actinobacillus* organisms of uncertain species affiliation have been isolated from the genital tract of older pigs (17). To what extent these *Actinobacillus* bacteria share antigens with *H. pleuropneumoniae* had not been investigated until we isolated *Actinobacillus* bacteria from the tonsils of pigs with CF antibody titers. The *Actinobacillus* bacteria were isolated in surprisingly large numbers from some of the pigs submitted from herd "A" for slaughtercheck. Whether this reflects a recent infection is not known. The *Actinobacillus* isolates were examined culturally, morphologically and biochemically and compared with *Haemophilus*

TABLE III. Antibody Titers in Pigs Before and After Exposure to *Actinobacillus* Strain S04 and Challenge with *H. pleuropneumoniae*, Strain CM5, Serotype 1

			2-ME Resistant Agglutinins		
	CF-antigen pool of *H. pl.* serotype 1, 2, 5, 7		*H. pl.* serotype 1	5	*Act.* S04
Group 1 (5 pigs)	Preexposure to *Act.* S04	< 2 (5)[a]	< 10 (5)	< 10 (3) 10 20	< 10 (5)
	1 week after S04 exposure	< 2 128 (4)	< 10 (5)	10 (2) 10 20 40	< 10 (3) 10 (2)
	Before *H. pl.* CM5 chall. (3 weeks after exposure)	< 2 128 (4)	< 10 (5)	10 10 20 40 (2)	40 (3) 80 (2)
	8 days after challenge	< 2 8 16 32 (2)	< 10 (3) 10 20	< 10 10 20 40 (2)	40 (3) 80 (2)
Group 2 (5 pigs)	Preexposure to *Act.* S04	< 2 (5)	< 10 (5)	< 10 (4) 10	< 10 (5)
	1 week after S04 exposure	< 2 (4) 4	< 10 (5)	< 10 (4) 10	< 10 (5)
	Before *H. pl.* CM5 chall. (3 weeks after exposure)	 8 16 128 (3)	ND[b]	< 10 10 (3) 40	10 20 (2) 40 80
	8 days after challenge	16 (3) 32 128	< 10 (2) 10 (2) 20	< 10 10 (3) 20	40 (3) 80 (2)
Group 3 (4 pigs)	Before *H. pl.* CM5 chall.	< 2 (4)	< 10 (4)	< 10 (4)	< 10 (4)
	8 days after CM5 chall.	< 2	< 10	< 10	< 10

[a]Number in brackets indicates number of pigs with the titer
[b]ND = Not done

TABLE IV. Cross-reactivity Between *H. pleuropneumoniae* and *Actinobacillus* in Hyperimmune Porcine Sera Raised Against *H. pleuropneumoniae* Reference Strains

Porcine hyperimmune serum against *H. pleuropneumoniae*	Absorption with *Actinobacillus* strain S04	2-ME resistant agglutinin titers against *H. pleuropneumoniae* homologous antigen	2-ME resistant agglutinin titers against *Actinobacillus* strain S04
Serotype 1 Shope 4074	Before	320	20
	After	320	< 10
Serotype 2 S1536	Before	40	80
	After	40	< 10
Serotype 3 S1421	Before	320	20
	After	320	< 10
Serotype 4 M62	Before	10	80
	After	10	< 10
Serotype 5 K17	Before	160	20
	After	160	< 10
Serotype 7 WF83	Before	160	< 10
	After	160	< 10

organisms isolated from pigs (11). They are closely related to organisms usually referred to as *A. suis* (18).

Strain S04, selected among the isolates, was of low virulence causing a focal pneumonia only in two pigs inoculated endobronchially with a relatively large number of organisms. It is likely that the lesions were caused by the endotoxin carried in the bacterial suspension and perhaps not more severe than a lesion induced by any other Gram-negative bacterium inoculated under similar circumstances.

Two-ME-sensitive agglutinins, presumably of IgM class, against various serotypes of *H. pleuropneumoniae* are commonly found in pigs. Serotype specific antibodies, however, appear to be 2-ME-resistant (6).

The suggestion that the seroreaction in pigs in herd "A" was due to the *Actinobacillus* infection was substantiated in experiment 1 in which the two experimentally-exposed pigs seroconverted in the CF test to *H. pleuropneumoniae*. When the same sera were tested in tube agglutination after 2-ME treatment, this seroconversion was not detected, probably because the antibodies were predominantly of the IgM class 18 days after infection. The same trend was observed in experiment 2 in which CF antibodies against *H. pleuropneumoniae* appeared before antibodies detectable with the 2-ME agglutination test. It is possible that serodiagnostic specificity of the CF test can be improved by 2-ME treatment of serum before testing. It was unfortunately not possible to test the sera by CF using strain S04 as antigen because all antigen preparations turned out to be strongly anticomplementary.

In experiment 2 the pigs experimentally exposed to the *Actinobacillus* strain did not show clinical signs and the infection seemed to establish readily. It is surprising, however, that the pigs in group 2 became infected with the *Actinobacillus* strain during the week when group 1 was exposed, even though the groups were kept in separate isolation rooms and the animal attendants exercised great care, using iodine disinfectants, to avoid transmission on clothing and fomites. Because of the cross-infection it was necessary to add a third group of animals to serve as environmental controls in experiment 2. These environ-

TABLE V. Demonstration of Antibodies Against *H. pleuropneumoniae* Antigens in Rabbits Immunized with Formalinized Whole Cells of *Actinobacillus* Strain S04

Rabbit No.	Days after immunization	Treatment of serum with 2-ME	Antibody titers against *H. pleuropneumoniae* serotype 1	2	3	4	5	6	7	*Actinobacillus* strain S04
45	0 day	No	0[a]	0	20	10	0	20	0	20
45	0 day	Yes	0	0	0	0	0	0	0	0
45	4 weeks	No	40	0	80	20	20	160	0	1280
45	4 weeks	Yes	20	0	10	0	0	20	0	640
148	0 day	No	0	0	40	10	0	20	0	40
148	0 day	Yes	0	0	0	0	0	0	0	0
148	4 weeks	No	40	0	160	40	20	160	0	1280
148	4 weeks	Yes	20	0	20	10	20	10	0	640

[a]0 = <1:10

mental control pigs (group 3) were highly susceptible to pleuropneumonia when challenged with a virulent strain of *H. pleuropneumoniae* serotype 1. The animals in groups 1 and 2, on the other hand, did not become clinically affected and only 50% of them had lesions at necropsy. It appears, therefore, that preceding *Actinobacillus* infection provides some cross-protection against *H. pleuropneumoniae* serotype 1. This cross-protection may be due to antibodies induced by the *Actinobacillus* strain, since all pigs exposed to the *Actinobacillus* strain had CF antibodies to both *Actinobacillus* strain S04 and *H. pleuropneumoniae* in serum taken before challenge. Unfortunately antibodies against *H. pleuropneumoniae* serotype 1 were not determined before challenge. However, it must be emphasized that although the pigs in group 3 originated from the same herd as groups 1 and 2 and were of the approximate same age, the stress imposed by the transfer to the isolation building just two days before the challenge might have increased their susceptibility.

The hyperimmune sera raised against *H. pleuropneumoniae* serotypes reacted, except for serotype 7, against *Actinobacillus* strain S04. In the serotype 2 and 4 sera the titer against strain S04 was even higher than the homologous titer. It appears, however, that these antibodies differ in specificity since absorption of the sera with strain S04 did not reduce the titers against *H. pleuropneumoniae*. Thus, the absorption experiment did not provide evidence for common antigenic determinants.

Actinobacillus strain S04 appeared to cross-react with all serotypes of *H. pleuropneumoniae* judging from all the experiments. Thus, the 2-ME test using rabbit sera against strain S04 revealed cross-reactivity with serotypes 1, 3, 4, 5 and 6 and the immunodiffusion test detected antigens revealed by antisera against serotypes 1, 2, 7 and strain 73567. However, lines of identity were seen only between strain S04 and serotype 2 and strain S04 and strain 73567. It appeared from experiment 2 that *Actinobacillus*-exposed pigs had the highest titers against serotype 5, whereas pigs hyperimmunized against *H. pleuropneumoniae* serotype 1-5 all had antibodies against S04. The different results in these experiments are probably related to differences in immune response as a result of differences in exposure and differences between animals. It is also likely a result of differences in antigen content of antigen preparation used for serological testing. Besides common antigenic determinants the cross-reactivity between *Actinobacillus* and *H. pleuropneumoniae* may be explained on the basis of polyclonal B-cell stimulation induced by either organism. This could lead to an antibody production reactive with the heterologous organism. Further research is necessary to resolve this question.

In conclusion, haemolytic *Actinobacillus* bacteria isolated from pigs cross-react serologically with strains of *H. pleuropneumoniae*. Pigs infected with *Actinobacillus* may become false positive reactors against *H. pleuropneumoniae*. Preliminary evidence suggests that pigs exposed to *Actinobacillus* bacteria may be less susceptible to pleuropneumonia caused by *H. pleuropneumoniae*.

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