2002

Serologic Response of Gnotobiotic Pigs Challenged with Actinobacillus Pleuropneumoniae Serotype 5 or Actinobacillus Suis Field Isolates

C.C.L. Chase
South Dakota State University

K. Wright
South Dakota State University

J. Torrison
South Dakota State University

L.J. Braun
South Dakota State University

Serologic response of gnotobiotic pigs challenged with actinobacillus pleuropneumoniae serotype 5 or actinobacillus suis field isolates

C.C.L. Chase[1], K. Wright[1], J. Torrison[2] and L.J. Braun[1]
Department of Veterinary Science

SWINE 2001 - 26

Summary

Three studies, a pilot study with conventional early-weaned pigs and two studies with gnotobiotic pigs were completed. The pilot study indicated that conventional pigs could be challenged with at least 10^7 colony forming units (cfu) or *Actinobacillus pleuropneumonia* (*APP*) or *Actinobacillus suis* (*A. suis*) without developing clinical signs. No serological response was detected in these pigs. In the first gnotobiotic study, nine pigs were used: 3 control, 3 *APP* or 3 *A. suis*. The two groups of challenged pigs failed to respond clinically or serologically to the initial challenge of 10^6 cfu of either *APP* or *A. suis* but the *APP* pigs did respond clinically and serologically to a second challenge of 10^7 cfu. A second study with twenty gnotobiotic pigs was completed. Eight pigs were assigned to the *Actinobacillus pleuropneumoniae* (APP) serotype 5 group; eight pigs were assigned to the *Actinobacillus suis* (A. suis) group and 4 pigs were assigned to the control group. Each group of gnotobiotic pigs were challenged with 10^7 colony forming units (cfu) of either *APP* or *A. suis*. In both gnotobiotic studies, serological tests indicated that the hemolysin neutralization test (HNT) specificity was poor as it was unable to discriminate between *APP* or *A. suis* infections. The HNT test detected more *APP* positive animals than any other test and detected APP infected animals one-month post challenge. In the first gnotobiotic study, APP infected pigs were detected at one-two weeks post challenge with the *APP* 5 ELISA developed by the University of Montreal (ELISA-M). ELISA-M was a more sensitive test than the *APP* 5 ELISA developed by Oxford Laboratories (ELISA-O). In the second gnotobiotic study, the ELISA-M and ELISA-O failed to detect any APP 5 infected animals. In both gnotobiotic studies, the complement fixation test failed to detect any animals and was insensitive to *APP* infections.

Introduction

Serology is the tool of choice for determining exposure of animals to *Actinobacillus pleuropneumonia* (*APP*)[1]. A number of different tests are available including the hemolysin neutralization test (HNT), enzyme labeled immunosorbent assays (ELISA) and the complement fixation (CF) test. The cross reactivity between *APP* and *Actinobacillus suis* (*A. suis*) has been thought to be confounding factor resulting in *A. suis* animals being identified as *APP* infected. The hemolysin RTX toxin of *A. suis* and *APP* serotype 5 have almost identical genetic, molecular and antigenic structure[2,3,4] and previous infections with *A. suis* can protect pigs against subsequent *APP* infections[5]. The detection of *A. suis* and *APP* has been based on higher hemolysin neutralization antibodies with *APP* infected pigs than with *A. suis* infected pigs. This study was designed to follow the serological response as measured by HNT, ELISA developed by Dr. M. Gottschalk, University of Montreal (ELISA-M), ELISA developed by Oxford Laboratories (ELISA-O) and the CF test following infection with either *APP* serotype 5 or *A. suis* in gnotobiotic pigs.

Experimental Approach

Bacteria: An *APP* Type 5 culture and seven *A. suis* isolates (#5, 7, 9, 11, 12, 20 and 24) were received from Dr. James Collins, University of Minnesota.

Animals: *Pilot Study*-Eight, 14 day old high health barrows were received from a PIC based genetics herd in western Minnesota. The pigs were divided into two groups of 4 and place in two separate rooms. One pig in the *APP* group died following the initial blood collection. The

[1]Department of Veterinary Science
[2]PIC Franklin, KY.

pigs were tagged and their temperatures recorded each day. Three pigs were the *APP* serotype 5 group and four pigs were the *A. suis* pigs.

Gnotobiotic Studies: In the first gnotobiotic study, gnotobiotic surgery was performed on one sow. The gnotobiotic pigs were moved out of the isolettes. Three pigs in an isolette in APP Room, three pigs in an isolette in *A. suis* Room and three pigs in Control Room.

In the second gnotobiotic experiment, gnotobiotic surgeries were performed on three sows. The gnotobiotic pigs were moved out of the isolettes at 23 days of age. Twenty pigs were then moved to three separate areas. Eight pigs in two isolettes in APP room, eight pigs in two isolettes in *A. suis* room and four pigs in one isolette in control room.

In both gnotobiotic studies, the pigs were exposed to unfiltered air when the isolettes were opened in each room at 23 days of age. The gnotobiotic pigs were then fed a starter pellet containing Apralan™ and treated with oral chlortetracycline at a rate of 10 mg/lbs for 5 days. The pigs were tagged and their temperatures recorded each day. After 4 days, the pigs were removed from the isolettes and placed on the floor. The pigs were kept on a pelleted diet for 2 weeks and then phased unto a non-medicated meal diet.

Challenge: *Pilot Study-* Three pigs were inoculated intranasally with 1 ml of *APP* serotype 5 using 1/2 ml/nostril: one pig with 1 ml of 10^5 cfm/ml, one pig with 1 ml of 10^6 cfm/ml, and one pig with 1 ml of 10^7 cfm/ml. Four pigs in another isolation room were inoculated intranasally with *A. suis*: one pig with 1 ml of 10^4 cfm/ml, one with 1 ml of 10^5 cfm/ml, one with 1 ml of 10^6 cfm/ml, and one with 1 ml of 10^7 cfm/ml. The pigs were monitored for clinical signs twice daily for 14 days.

Gnotobiotic Studies The gnotobiotic animals were challenged at 36-43 days of age. Prior to challenge, serum samples were collected from each pig. In Gnotobiotic Study 1, the three APP challenged pigs were each inoculated intranasally with ½ ml/nostril (1 ml total) of 10^6 cfu/ml of *APP* serotype 5. The three *A. suis* pigs were each inoculated with 1 ml of 10^6 cfu/ml of a mixture of *A. suis* isolates #9, #12, and #24. The two control pigs were inoculated with media. The pigs were monitored for clinical signs twice daily for 14 days. No antibody titer was detected in these animals on weekly bleedings following challenge. On Day 42 post challenge, the pigs had their nasal passages re-swabbed for bacterial isolation, serum collected and pigs were re-inoculated using a 1 in. nasal cannula. The APP pigs were each inoculated intranasally with 1 ml of 10^7 cfu/ml of *APP* serotype 5 using 1/2 ml/nostril. The *A. suis* pigs were each inoculated with 1 ml of 10^7 cfu/ml of a mixture of *A. suis* isolates #9, #12, and #24 using 1/2 ml/nostril. The two control pigs were inoculated with media. The pigs were monitored for clinical signs twice daily for 7 days.

For gnotobiotic study 2, eight pigs were individually inoculated intranasally with 1/2 ml per nares (1 ml total) of 10^7 cfu/ml of *APP* serotype 5. Eight pigs were each inoculated with 1 ml of 10^7 cfu/ml of a mixture of *A. suis* isolates #9, #12, and #24. The four control pigs were inoculated with media. The pigs were monitored for clinical signs for 14 days (three times daily for 9 days, twice a day for 4 days and once a day for one day). The pigs were bled weekly for APP antibody titers. On day 54 after challenge, the pigs were re-inoculated as described above. The pigs were monitored for clinical signs twice daily for 7 days. The pigs were moved off-site on day 97 where the pigs were placed in separate nursery rooms and fed a commercial grow-finish diet and bled weekly to day 135 post-challenge.

Clinical signs: In all three studies, clinical observations were made prior to challenge and for 7-14 days post challenge.

Necropsy: In the pilot and gnotobiotic study 1, all animals were euthanized, necropsied and tonsils and bronchial lymph nodes were cultured. In gnotobiotic study 2, only animals that died were necropsied and cultured.

Sample Collection: Blood samples were collected bi-weekly for the pilot study and weekly for the gnotobiotic studies by venipuncture. Nasal swabs were collected by restraining the pigs and swabbing each nostril with a cotton tipped applicator. These swabs were immediately submitted to the SDSU Diagnostic Laboratory Bacteriology Section for analysis.

Serological Testing: At the end of each study 2 ml of serum from each sampling was aliquoted into four 0.5 ml aliquots. One aliquot was then sent to Kansas State University for *APP* HNT. The cutoff for the HNT test were <3000 negative,

3001-6000 suspect and >6000 were positive. A second aliquot was sent to Oxford Laboratories for *APP* serotype 5 ELISA titers (ELISA-O). The cutoff for the ELISA-O was < 0.3 negative and >0.3 positive. A third aliquot was sent to University of Montreal for *APP* serotype 5 ELISA titers (ELISA-M). The cutoff for ELISA-M was <0.29 negative, 0.30-0.39 suspect and .0.40 positive. The last sample was sent to Iowa State University for *APP* CF titers.

Results

Bacterial cultures: Identification of the challenge cultures submitted to the SDSU Bacteriology Section indicated the cultures were *APP* and *A. suis*. Pre-challenge nasal swabs from all the studies had normal bacterial flora.

Clinical signs and treatment: *Pilot study* None of the animals developed clinical signs.

Gnotobiotic Study 1

Control Pigs: There were no elevated temperatures or clinical scores throughout the study in this group.

***A. suis* Group:** There were no clinical signs in the *A. suis* group following either challenge.

APP Group: Challenge 1: One pig had a temperature of 104.5 on day 4, and was treated once a day for three days with Naxcel at 2 mg/lbs. On day 7, this same pig developed a swollen left hock and was treated with Procaine Penicillin G at 10,000 units/lbs sid for 4 days. On day 9, the synovial space on the left hock was tapped for culture. Culture results found only a few fecal *Streps*.

Challenge 2: On day 45 (3 days following the second challenge), three APP pigs developed a fever and were treated with Naxcel (ceftiofur hydrochloride, Pharmacia-Upjohn) at 2 mg/lbs. On day 46, a fourth pig, who was an unthrifty pig, 50% smaller than his cohorts, developed a fever and died. On day 56 one pig's head was swollen on the left side. The pig was treated with Naxcel at 2 mg/lbs. The pig returned to normal by the next day.

Gnotobiotic Study 2.

Control Group: Challenge 1: These pigs had slightly elevated body temperatures on day 5 and 6 that returned to normal without treatment. All pigs developed moderate diarrhea on days 7 or 8.

Challenge 2: There were no elevated temperatures or clinical scores throughout the one-week observation period. One pig developed a severe lameness and respiratory distress on day 118 and was treated with Procaine Penicillin G for three days (10,000 units/lbs).

***A. suis* Group**: Challenge 1: One pig had an elevated body temperature on day 5 and day 6 and two pigs had elevated body temperatures on days 7 and 13. One pig was slightly depressed and panting on day 2 and had moderate depression on day 13. This pig was treated on day 2 and two pigs were treated on day 13 with a single dose of Excenel (ceftiofur hydrochloride, Pharmacia-Upjohn) at 2 mg/lbs intramuscularly. One pig died on day 23.

Challenge 2: There were no elevated temperatures or clinical scores throughout the one-week observation period.

APP Group: Challenge 1: One pig had an elevated body temperature on days 4 and 6 and two pigs had an elevated body temperature on day 6. Six of the APP group exhibited moderate to severe depression on day 5. One pig died on day 6 and one pig died on day 7. The remaining 6 pigs were treated for 4 days with Excenel at 2 mg/lbs intramuscularly on days 7-10.

Challenge 2: There were no elevated temperatures throughout the one week observation. One pig developed moderate depression on days 60-62 (10-12 days following second challenge).

Necropsy results: *Pilot study*: There were no gross lesions in any of the pigs. Bacterial cultures from the tonsil and bronchial lymph nodes contained normal bacterial flora with the exception of one pig that had a *Streptoccus suis* isolation (data not shown).

Gnotobiotic Study 1

All pigs were necropsied at day 90 unless otherwise noted. There were no gross lesions or significant bacteria in the control pigs.

***A. suis* group**: One pig had adhesive pericarditis but no bacteria was isolated.

Bacterial cultures of the tonsil and bronchial lymph node of the *A. suis* group were negative.

APP group: The necropsy of the pig that died on day 46 indicated that the animal was emaciated and only hemolytic E.*coli* was isolated from the intestine and no bacteria were isolated from the lung or pericardium. One pig had a focal abscess in the left lung. The right apical lobe of the lung was adhered to the pericardial sac. The heart had chronic adhesive pericarditis. No bacteria were isolated from these lesions. Bacterial cultures of the tonsil and bronchial lymph node were negative.

Gnotobiotic Study 2

Only pigs that died were necropsied.

***A. suis* group**: One pig that died on day 23 was necropsied. This pig had a reddening of the left-middle lung lobe. *A. suis* was isolated in heavy growth from lung and liver. No other pathogens were isolated.

APP 5 Group: Two pigs were necropsied. Both pigs had pleuritis, peritonitis and pericarditis. This was characterized by fibrinopurulent exudate. Pure cultures of APP were isolated from both pigs. No other pathogens were isolated.

Serology

Pilot study: No serological titers developed in any pig at any time point.

Gnotobiotic Study 1:

Control pigs: The control pigs developed no *APP* serological titers (data not shown).

***A. suis* pigs**: No APP titers were detected with any test until following the second challenge. All three pigs developed HNT following the second challenge. Two pigs developed HNT 3 weeks following re-infection (Table 1). These titers increased in both pigs in subsequent weeks and the final titer in the pigs was comparable to titers seen with the *APP* infected pigs. One pig developed a HNT one month after re-infection and this titer increased in the last week of the experiment (Table 1 and 3). The *A. suis* pigs developed no titers detected by either ELISA test (ELISA-M or ELISA-O) or complement fixation (CF) (Table 1).

APP: No App titers were detected with any test until following the second challenge. The *APP* HNT test detected all three *APP*-infected pigs (Table 2). The first pig was detected by two weeks post challenge and by one month for all three pigs (Table 2). Two of *APP* pigs, developed ELISA titers (Table 2). One pig's *APP* titer was detected by both ELISA tests. The average HNT titers for the APP group were higher than those for the *A. suis* group (Table 3). The ELISA-M test detected *APP* titers one week after infection compared to two weeks with the ELISA-O. The ELISA-M detected one more pig than the ELISA-O. This was detected two weeks post challenge. One pig was negative by both *APP* ELISA tests. This pig approached the ELISA-M cutoff of 0.30 but was always below it. The CF test did not detect any serological positive animals (Table 2).

Gnotobiotic Study 2

Control pigs: One pig developed positive HNT titers at day 126. This was 10 days after this pig developed severe respiratory signs and lameness. At day 135, all four control pigs were HNT positive with an average HNT of 9908. The other three APP serology tests were negative at all time points for all pigs (data not shown).

A. *suis* Group: HNT test detected all of *A. suis* challenged animals. The first detection was on day 21 in 1 of 6 animals. At day 28, 4 of 7 animals were positive;at days 35and 42, 6 of 7 were positive; and at day 49, 7 of 7 animals were positive (Table 4). These titers continued to increase in most animals at levels higher than that seen with APP (Table 6). The HNT levels were still high at the end of the study (Table 6). ELISA-M did not detect any positive animals using the cutoff of 0.3 (Table 3). Titers were consistently at background throughout the study. The ELISA-O did not detect any suspect or positive animals using their S/P cutoff of 0.3 (Table 4). All of the pigs were at background levels. APP 5 CF test did not detect any positive pigs throughout the study period (Table 4).

APP Group: HNT test detected APP 5 seropositive animals. The first detection was made on day 42 in 1 of 6 animals (Table 5). By day 49, 2 of 6 animals were positive and by day 56, 6 of 6 animals were positive (Table 5). These titers continued to increase in most animals and then they began to decline by the end of the study (Table 6). All animals were still seropositive at the end of the study (Table 5).

APP 5 ELISA-M did not detect any suspect or positive animals using the cutoff of 0.3 (Table 5). The APP 5 ELISA-O did not detect any positive animals (Table 5). APP 5 CF test did not detect any pigs throughout the study period (Table 5).

Discussion

Three major conclusion were reached with the two gnotobiotic studies: 1) the specificity of the HNT test is poor; 2) the sensitivity of the tests in rank from highest to lowest is: HNT>ELISA-M>ELISA-O; and 3) the CF test was totally insensitive in this experiment. In these experiments it is clear that the HNT can not discriminate between *A. suis* or APP infected pigs. This is not suprising considering how closely the RTX toxin is related between the two species of bacteria[2,3,4]. In the first experiment, the highest HNT titers in either group were in the animals that developed the most severe pathological lesions. This is also true for the ELISA-M test where the highest values were seen in the APP animal with severe lesions and this was the only animal detected by the ELISA-O. The use of therapeutic antibiotics in one of the APP animals probably prevented this pig from developing a high immune response. In the second experiment, the *A. suis* pigs had higher HNT titers than the APP challenged pigs (Table 6).

A problem in this first experiment was developing a consistent challenge model. Because of the unknown pathogenesis of *APP* and *A. suis* in gnotobiotic pigs, a very conservative approach was taken. It is clear that at least 10^7 cfu of *APP* are needed in gnotobiotic pigs to cause disease. The use of a intranasal cannula for inoculation also maybe important. A third consideration is minimizing the use of antibiotics in the feed or water as these could have hampered the first challenge in the gnotobiotic pigs. From the knowledge gained from this experiment, future studies can be done in high health pigs of known health status to minimize expense and increase the numbers of animals in each group to make statistical analysis of serological results possible.

The results of the second experiment were different than the previous experiment. Clinically, fewer pigs developed elevated body temperatures in this trial but sudden death was seen with this trial while no sudden deaths were observed in the previous experiment. The serology results were similar with regard to the poor specificity of the HNT test, the higher sensitivity of the HNT test and the total insensitivity of the CF test. The ELISA test results were different between studies. In the first study, the ELISA-M detected 2 of 3 APP 5 infected animals and the negative animal was 0.27. The levels of 3 of the 6 pigs in this study were at 0.24-0.29. The ELISA-O detected 1 of 3 in the first test but in this experiment all animals were negative.

What factors could have accounted for these differences? The two challenge doses used in this experiment were the same as the second challenge dose used in the previous experiment (10^7 colony forming units). The same seed bacterial cultures were used. The environment and rooms used for the first 12 weeks were exactly the same. The major difference was in the genetics of the pigs. In the first study, the gnotobiotic pigs were Babcock genetics. In this study, the pigs were predominately Yorkshire.

From the knowledge gained from this experiment, future studies can be done in high health pigs of known health status to minimize expense and increase the numbers of animals in each group to make statistical analysis of serological results possible.

References

1) Fenwick, B. and S. Henry. 1994. Porcine pleuropneumonia. JAVMA 204:1334-1340.
2) Devenish, J., S. Rosendal, R. Johnson and S. Hubler. 1989. Immunoserological comparison of 104-kilodalton proteins associated with hemolysis and cytolysis in *Actinobacillus pleuropneumoniae, Actinobacillus suis, Pasteurella haemolytica*, and *Escherichia coli*. Infect. Immun. 57:3210-3213.
3) Burrows, L.L. and R.Y.C. Lo. 1992. Molecular characterization of an RTX toxin determinant from *Actinobacillus suis*. Infect. Immun. 60:2166-2173.
4) Kamp, E.M., T.M.M. Vermeulen, M.A. Smits, and J. Haagsma. 1994. Production of Apx toxins by field strains of *Actinobacillus pleuropneumoniae* and *Actinobacillus suis*. Infect. Immun. 62:4063-4065.
5) Fenwick, B. 1995. Protection against Porcine Pleuropneumonia (APP) provided by previous infection with *Actinobacillus suis*. Proceedings of the 1995 AASP Meeting, pp. 365-366.

TABLE 1 SEROLOGY GNOTOBIOTIC PIGS TRIAL 1-*A. SUIS**

Day	HNT[1]	ELISA-M[2]	ELISA-O[3]	CF-ISU[4]
0 Challenge 1	0/3	0/3	0/3	0/3
7	0/3	0/3	0/3	0/3
14	0/3	0/3	0/3	0/3
21	0/3	0/3	0/3	0/3
28	0/3	0/3	0/3	0/3
35	0/3	0/3	0/3	0/3
42 Challenge 2	0/3	0/3	0/3	0/3
49	0/3	0/3	0/3	0/3
56	0/2#	0/3	0/3	0/3
63	2/3	0/3	0/3	0/3
71	3/3	0/3	0/3	0/3
78	3/3	0/3	0/3	0/3

*number of pigs positive/total number of pigs.
[1]Hemolysin Neutralization Titer-Kansas State University
[2]APP 5 ELISA-University of Montreal
[3]APP 5 ELISA-Oxford Laboratories
[4]APP 5 Complement Fixation-Iowa State University
one sample was not tested

TABLE 2 SEROLOGY GNOTOBIOTIC PIGS TRIAL 1-*APP**

Day	HNT[1]	ELISA-M[2]	ELISA-O[3]	CF-ISU[4]
0 Challenge 1	0/3	0/3	0/3	0/3
7	0/3	0/3	0/3	0/3
14	0/3	0/3	0/3	0/3
21	0/3	0/3	0/3	0/3
28	0/3	0/3	0/3	0/3
35	0/3	0/3	0/3	0/3
42 Challenge 2	0/3	0/3	0/3	0/3
49	0/3	1/3	0/3	0/3
56	1/3	2/3	1/3	0/3
63	1/3	2/3	1/3	0/3
71	3/3	2/3	1/3	0/3
78	3/3	2/3	1/3	0/3

*Number of pigs positive/total number of pigs.
[1]Hemolysin Neutralization Titer-Kansas State University.
[2]APP 5 ELISA-University of Montreal.
[3]APP 5 ELISA-Oxford Laboratories.
[4]APP 5 Complement Fixation-Iowa State University.

TABLE 3 COMPARISON OF HNT BETWEEN *A. SUIS* AND *APP* IN GNOTOBIOTIC TRIAL 1

Day	No. positive *A suis* HNT*	Average HNT-*A suis*	No. Positive APP-HNT*	Average HNT-APP
0 Challenge 1	0/3	1030	0/3	2088
7	0/3	2104	0/3	1589
14	0/3	1938	0/3	2039
21	0/3	1666	0/3	1508
28	0/3	1857	0/3	1576
35	0/3	1742	0/3	1417
42 Challenge 2	0/3	1134	0/3	1037
49	0/3	1190	0/3	1291
56	0/2#	2777	1/3	3495
63	2/3	5930	1/3	8427
71	3/3	10397	3/3	25164
78	3/3	17163	3/3	20012

*Number of pigs positive/total number of pigs.
One sample was not tested.

TABLE 4 SEROLOGY GNOTOBIOTIC PIGS TRIAL 2-*A. SUIS**

Day	HNT[1]	ELISA-M[2]	ELISA-O[3]	CF-ISU[4]
0 Challenge 1	0/8	0/8	0/8	0/8
7	0/8	0/8	0/8	0/8
14	0/8	0/8	0/8	0/8
21	1/6*#	0/7+	0/7+	0/7+
28	4/7	0/7	0/7	0/5#
35	6/7	0/7	0/7	0/7
42	6/7	0/7	0/7	0/7
49	7/7	0/7	0/7	0/7
56 Challenge 2	7/7	0/7	0/7	0/7
63	7/7	0/7	0/7	0/7
70	6/6#	0/7	0/7	0/7
77	7/7	0/7	0/7	0/7
84	7/7	0/7	0/7	0/7
91	7/7	0/7	0/7	0/7
98	7/7	0/7	0/7	0/7
105	7/7	0/7	0/7	0/7
112	7/7	0/7	0/7	0/7
119	7/7	0/7	0/7	0/7
126	7/7	0/7	0/7	0/7
134	7/7	0/7	0/7	0/7

*Number of pigs positive/total number of pigs.
[1]Hemolysin Neutralization Titer-Kansas State University.
[2]APP 5 ELISA-University of Montreal.
[3]APP 5 ELISA-Oxford Laboratories.
[4]APP 5 Complement Fixation-Iowa State University.
+One animal died.
#Animal'(s) sample was not tested.

TABLE 5 SEROLOGY GNOTOBIOTIC PIGS TRIAL 2-*APP**

Day	HNT[1]	ELISA-M[2]	ELISA-O[3]	CF-ISU[4]
0 Challenge 1	0/8	0/8	0/8	0/8
7	0/6[+]	0/6[+]	0/6[+]	0/6[+]
14	0/6	0/6	0/6	0/6
21	0/6	0/6	0/6	0/6
28	0/6	0/6	0/6	0/6
35	0/6	0/6	0/6	0/6
42	1/6	0/6	0/6	0/6
49	2/6	0/6	0/6	0/6
56 Challenge 2	6/6	0/6	0/6	0/6
63	6/6	0/6	0/6	0/6
70	6/6	0/6	0/6	0/6
77	6/6	0/6	0/6	0/6
84	6/6	0/6	0/6	0/6
91	6/6	0/6	0/6	0/6
98	6/6	0/6	0/6	0/6
105	6/6	0/6	0/6	0/6
112	6/6	0/6	0/6	0/6
119	6/6	0/6	0/6	0/6
126	6/6	0/6	0/6	0/6
134	6/6	0/6	0/6	0/6

*Number of pigs positive/total number of pigs.
[1]Hemolysin Neutralization Titer-Kansas State University.
[2]APP 5 ELISA-University of Montreal.
[3]APP 5 ELISA-Oxford Laboratories.
[4]APP 5 Complement Fixation-Iowa State University.
[+]Two animals died.

TABLE 6 COMPARISON OF HNT BETWEEN A. *SUIS* AND *APP* IN GNOTOBIOTIC TRIAL 2

Day	No. Positive *A suis* HNT*	Average HNT-*A. suis*	No. Positive APP-HNT*	Average HNT-APP
0 Challenge 1	0/8	4958	0/8	4893
7	0/8	5528	0/6++	1482
14	0/8	5229	0/6	1569
21	1/6+#	9154	0/6	1494
28	4/7	11266	0/6	1555
35	6/7	17756	0/6	1989
42	6/7	17123	1/6	4047
49	7/7	14262	2/6	5797
56 Challenge 2	7/7	20177	6/6	25012
63	7/7	22699	6/6	21366
70	6/6#	25515	6/6	22287
77	7/7	28454	6/6	19094
84	7/7	38699	6/6	22686
91	7/7	30195	6/6	28364
98	7/7	35400	6/6	18344
105	7/7	33200	6/6	21443
112	7/7	24782	6/6	15618
119	7/7	19983	6/6	16205
126	7/7	30198	6/6	17842
134	7/7	34355	6/6	16094

*Number of pigs positive/total number of pigs.
+One animal died.
++Two animals died.
#One sample was not tested.