SEROLOGIC EVIDENCE OF SEROTYPE SPECIFICITY IN IMMUNITY OF VACCINATED SWINE TO ERYSIPELOTHRIX RHUSIOPATHIAE

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Serotypes 1 and 2 of Erysipelothrix rhusiopathiae are the only types proven to cause epizootics of swine erysipelas; however, at least 20 other relatively rare serotypes have been reported and some of them have been shown to be pathogenic for swine. In previous experiments, swine vaccinated with erysipelas adsorbate bacterins were immune to challenge with virulent strains of serotypes 1 and 2, but susceptible to challenge with a strain of serotype 10. Further studies in mice provided statistical evidence that other strains of serotype 10 are also refractory to immunity induced by standard erysipelas bacterin. The objective of the present experiment was to determine whether serotype specificity in immunity of vaccinated swine can be detected serologically by using test antigens representing specific serotypes of E. rhusiopathiae.

Standard Reference Erysipelas Bacterin (SRB) was used for vaccination of swine. The bacterin was prepared from 4 immunogenic strains of serotype 2 and adsorbed on aluminum hydroxide gel according to a method prescribed by the U.S. Department of Agriculture.

For control, a blank preparation (BP) was made, consisting of sterile incubated production medium adsorbed on aluminum hydroxide gel in the same manner as the SRB.

Sera from 64 white cross-bred swine, 4 months old, were used. The swine had been derived by hysterectomy, deprived of colostrum, and raised in isolation. The SRB and BP were each given subcutaneously to 32 swine in 2 doses of 5 ml, 3 weeks apart. Sera were taken from all swine before inoculation (week 0), at the time of the second inoculation (week 3), and immediately before challenge exposure (week 6). The sera were filter-sterilized and stored at -70°C without preservatives. At week 6, all swine were challenge-exposed by intramuscular or intravenous injection with virulent cultures of E. rhusiopathiae representing serotypes 1, 2, or 10.

All sera were heat-treated (56°C for 30 min) and tested with a microtitration agglutination test (MAT) and a growth agglutination test (GAT), using test antigens prepared from E. rhusiopathiae strains HC-585 (serotype 1), NF-4E1 (serotype 2), and 2179 (serotype 10).

Antigens for the MAT consisted of cells grown 48 hr. at 37°C in beef infusion broth, washed once in 0.85% NaCl solution containing 0.05% thimerosal, and adjusted to 40% T at 600 nm. The MAT was conducted in round-bottom microtitration plates, using doubling dilutions of serum in 0.8% NaCl solution, with the first well containing a serum dilution of 1:5. The plates were covered and incubated at 37°C and reactions were read at 48 hrs.

Antigens for the GAT consisted of 24-hour beef infusion broth cultures. The test was conducted in 11 x 100 mm tubes containing 1 ml of sterile clear beef infusion broth supplemented with 1% calf serum, 400 µg/ml kanamycin, 50 µg/ml neomycin, and 25 µg/ml vancomycin. Doubling dilutions of test sera were made, with the first tube in the series containing a serum dilution of 1:5. To each tube was added 25 µl of antigen culture. The tubes were covered and incubated 24 hrs. at 37°C. The highest dilution containing evidence of clumping at the bottom of the undisturbed tube and also upon shaking was read as the end point.

Detailed clinical data from most of the swine used in this experiment have been reported elsewhere. All controls (given BP) were susceptible to challenge exposure. All vaccinates (given SRB) challenged with strains of serotypes 1 or 2 were immune, and all vaccinates challenged with a strain of serotype 10 were susceptible.

Results of serologic testing, expressed as geometric mean titers, are given in Tables 1 and 2. The GSK method of statistical analysis was used for comparison of mean titers of sera obtained at week 6. In sera of vaccinates, mean titers obtained with test antigens of serotypes 1 and 2 were significantly higher ($P < 0.05$) at week 6 in both the MAT and GAT than the mean titers obtained with serotype 10 antigens, but were not different from each other. In sera of controls, mean titers obtained with the different test antigens were not significantly different.

Vaccination did not stimulate a pronounced serologic response, even when the test antigen (NF-4E1) was of a serotype homologous to that of the vaccine. The response nevertheless was detectable with test antigens of type 1 or 2, but not with antigen of type 10. There is a parallel between these serologic responses and acquired immunity to challenge with the specific serotype strains. The specificity in immunity probably involves surface antigens, which may also be specific serotype determinants.

Table 1. Geometric Mean Microtitration Agglutination Titers of Sera from Vaccinates (n=32) and Controls (n=32)

Test antigen	Serotype	Week 0	Week 3	Week 6
Vaccinates:				
HC-585	1	10.00	20.00	32.92
NF-4E1	2	11.89	27.68	43.62
2179	10	10.67	20.89	19.57
Controls:				
HC-585	1	9.79	17.56	15.76
NF-4E1	2	11.39	20.44	20.44
2179	10	11.39	19.57	19.15

Table 2. Geometric Mean Growth Agglutination Titers of Sera from Vaccinates (n=32) and Controls (n=32)

Test antigen	Serotype	Week 0	Week 3	Week 6
Vaccinates:				
HC-585	1	10.67	40.88	55.36
NF-4E1	2	8.59	35.89	57.81
2179	10	9.37	13.84	36.68
Controls:				
HC-585	1	10.44	12.42	10.44
NF-4E1	2	9.17	12.79	10.44
2179	10	8.59	13.54	13.25

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