Mouse Potency Assay for *Bordetella bronchiseptica* Bacterins

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A potency assay for *Bordetella bronchiseptica* bacterins has been developed using mice. The immunogenicities of three bacterins, B, C, and D, were evaluated for ability to prevent death in mice as compared with a reference standard bacterin (RSB-A). Bacterins RSB-A, B, and C were evaluated for ability to prevent death in mice as compared with a reference standard bacterin (RSB-A). Bacterins RSB-A, B, and C were evaluated in swine for efficacy against nasal turbinate atrophy. Swine immunized with RSB-A demonstrated 25% gross nasal turbinate atrophy (GNTA), whereas nonimmunized swine had 85% GNTA. Swine vaccinated with bacterins B and C demonstrated 0 and 100% GNTA, respectively, whereas the nonimmunized groups had 64 and 75% GNTA, respectively. RSB-A and bacterins B, C, and D provided average mouse survivals of 94, 88, 49, and 32%, respectively when the mice were given 1/10,000 of a recommended swine-immunizing dose, whereas an average of 88% of the unvaccinated mice died.

Infectious atrophic rhinitis is a transmissible disease of swine characterized by atrophy of the nasal turbinates. The primary cause of turbinate atrophy has been established to be a chronic infection of the nasal cavity of the young pig by *Bordetella bronchiseptica* (1, 6, 8, 11).

The efficacies of various experimental *B. bronchiseptica* bacterins have been studied by a laboratory host animal immunization-challenge test and in swine clinical field trials (5, 6).

Inactivated *B. bronchiseptica* bacterins have been used to protect mice challenged intraperitoneally, intracerebrally, or intranasally (9; K. Sekiya, T. Kasuga, M. Kawahira, R. Sugimoto, and Y. Nakase, Proc. Meet. Jpn. Soc. Vet. Sci. 74th, Tokyo, Japan, p. 7, 1972).

Cross-protective ability of the *Bordetella* species in mice was demonstrated by Eldering (2) by immunization with either *B. bronchiseptica*, *B. pertussis*, or *B. paraptussis* and then challenge with *B. bronchiseptica*. Ganaway et al. (4) prevented acute *B. bronchiseptica* pneumonia in guinea pigs by immunization with an emulsified *B. bronchiseptica* vaccine.

The present study was undertaken to develop a rapid and sensitive potency assay test where bacterins can be compared to a reference standard bacterin (RSB-A) that had previously been proved efficacious against atrophic rhinitis in swine.

MATERIALS AND METHODS

Swine. *B. bronchiseptica* culture-negative specific-pathogen-free Yorkshire sows were used. Nasal swab specimens were obtained from each sow just before

farrowing and from each newborn pig at 3 days of age to determine if test swine were susceptible. The techniques for nasal swab specimen collection and the isolation and identification of *B. bronchiseptica* have been previously described (3, 7, 11).

Mice. CF-1 SASCO strain mice (SASCO, Inc., Omaha, Neb.) were used. Mice of the same sex were used whenever possible. If both sexes were used, mice of each sex were equally distributed throughout the test. The weights of mice ranged between 18 and 22 g.

Bacterins. RSB-A and bacterin B were prepared from Formalin-inactivated *B. bronchiseptica* whole cell cultures with aluminum hydroxide added as adjuvant. Bacterin C (atrophic rhinitis bacterin; Grand Laboratories, Inc., Crofton, Neb.) was prepared from whole cell bacterial cultures of *B. bronchiseptica*, *Pasteurella multocida*, and *Pseudomonas* serotypes causing atrophic rhinitis and pneumonia in swine with aluminum hydroxide added as adjuvant. Bacterin D (Dr. Mayfield Special Bacterin; Dr. Mayfield Laboratories, Charles City, Iowa) is a whole broth suspension of chemically inactivated bacterin, consisting of *Bordetella* sp. (14%), *Streptococcus* sp. (14%), *Pasteurella* sp. (28%), *Salmonella* sp. (14%), and *Corynebacterium* sp. (28%), containing no adjuvant.

Experimental design. (i) Swine. Thirty-nine pigs from five litters were designated randomly into vaccinated and nonvaccinated groups. Two groups each of eight and seven pigs were used for testing RSB-A and bacterin B. Groups of five and four pigs were used for evaluation of bacterin C. One-week-old pigs in the vaccinated group were inoculated subcutaneously in the fold of the flank with 2 ml of the test bacterin. The pigs were weaned at 21 days of age and inoculated intranasally with 0.5 ml per nostril of a culture containing approximately 5.0×10^8 *B. bronchiseptica* organisms per ml. At 28 days of age, each vaccinee received a 2-ml booster vaccination. The pigs were necropsied at 9 weeks of age. The nose of each pig

was horizontally sectioned at the level of the second premolar tooth for a gross nasal turbinate atrophy (GNTA) evaluation. Any atrophy of the dorsal or ventral turbinates and/or lack of osseous development of the turbinates was considered positive GNTA.

(ii) **Mice.** Twenty mice were injected intraperitoneally with 0.2 ml each of bacterins B, C, and D, which had been diluted 1/1,000 in normal saline. As positive controls, twenty mice were injected intraperitoneally with 0.2 ml of the RSB-A, which had been diluted 1/1,000 in normal saline. Twenty mice served as unvaccinated controls. All mice in each group were inoculated intraperitoneally 15 days after immunization with a broth culture of virulent *B. bronchiseptica*. The interval between the initiation of inoculation and the inoculation of the last mouse did not exceed 1 h. All mice were observed for 10 days postinoculation.

Test evaluation. At least 90% of all mice had to survive and remain healthy during the 14-day immunizing period before challenge for testing to proceed. A bacterin was considered satisfactory if at least 80% of the test bacterin vaccinates survived. The test was considered valid if 80% or more of the mice vaccinated with RSB-A survived while 20% or less of the nonvaccinated mice survived. Bacterins RSB-A, B, C, and D were tested in triplicate.

Statistics. To test for equality among the variances, Bartlett's test for homogeneity of variance was performed (10).

RESULTS

Evaluation of swine nasal culturing. Diagnostic tests conducted on nasal swabs from test swine proved negative for *B. bronchiseptica*.

Incidence of GNTA in swine. Two of eight pigs vaccinated with RSB-A and six of seven nonimmunized pigs had GNTA. None of eight pigs vaccinated with bacterin B and six of seven nonvaccinated pigs had GNTA. Five of five pigs vaccinated with bacterin C and three of four nonvaccinated pigs demonstrated GNTA.

Effects of *B. bronchiseptica* inoculation in mice. Untreated inoculated mice became generally unhealthy starting day 3 postinoculation, with matted eyes and hair and diarrhea, and death commenced on the same day. Autopsy of mice revealed a greatly inflamed lower gastrointestinal tract and gross hemorrhagic lesions of the lungs. Surviving immunized mice possessed none of these untoward characteristics.

Mouse potency assay. RSB-A and bacterins B, C, and D protected mice at average levels of 94, 88, 49, and 32%, respectively, whereas 88% of the nonvaccinated mice died (Fig. 1).

Statistics. The difference in variance between results of triplicate testing of each bacterin when examined for homogeneity was found to be insignificant ($P > 0.1$).

DISCUSSION

The mouse potency assay for *B. bronchiseptica* bacterins described in this work was de-

FIG. 1. Percentage of survival of mice immunized with various *B. bronchiseptica* bacterins. NC*, Nonvaccinated controls; A, RSB-A.

signed to measure differences between bacterins. Each bacterin was assayed in mice, and the results were compared to a RSB-A, which has been proven efficacious in swine against atrophic rhinitis. Although efficacies of bacterins can be satisfactorily evaluated in swine, the mouse potency assay was needed since swine testing requires a minimum of 10 weeks and four susceptible litters of pigs to provide statistically significant data. This mouse assay test can be conducted in 25 days and readily provides statistically significant data. Bacterins RSB-A and B provided 75 and 100% protection, respectively, in swine against virulent *B. bronchiseptica*, whereas bacterin C provided no significant protection to swine under similar conditions.

Bacterin RSB-A protected mice at 94%, whereas bacterins B, C, and D provided 88, 49, and 32% protection, respectively.

The homogeneity of variance from triplicate testing indicates that the differences between trials conducted on the various bacterins are due to experimental procedure and not due to test inconsistency.

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