ATROPHIC RHINITIS OF SWINE: EFFECT OF VACCINATION AGAINST *Bordetella bronchiseptica* IN PIGLETS CHALLENGED AT AN EARLY AGE[1]

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Testou-se a eficiência de uma bacterina preparada com *B. bronchiseptica* e adsorvida a hidróxido de alumínio, na prevenção da rinite atrófica dos suínos. Seis porcas foram vacinadas, aos 60 e 100 dias de gestação, e suas leitegadas, aos sete e 28 dias de idade. Cinco leitegadas não vacinadas, nascidas de porcas não vacinadas, serviram de controle. Todos os leitões foram inoculados com *B. bronchiseptica* aos três, quatro e cinco dias de idade. A vacinação contribuiu para reduzir significativamente os sintomas clínicos da doença ($P < 0,001$) e a ocorrência e gravidade das lesões dos cornetos nasais ($P < 0,01$), mas não eliminou a infecção aos setenta dias de idade.

TERMOS DE INDEXAÇÃO: Rinite atrófica dos suínos, *Bordetella bronchiseptica*, vacinação, imunoprofilaxia.

ABSTRACT.- The effectiveness of a bacterin in the prevention of swine atrophic rhinitis (AR) was tested. The bacterin was prepared with *Bordetella bronchiseptica* and adsorved to aluminum hydroxide. Six sows were vaccinated at 60 and 100 days of gestation and their litters at seven and 28 days of age. Five sows and their litters were used as an invaccinated control group. All piglets were challenged with *B. bronchiseptica* at three, four and five days of age. The vaccination significantly reduced the clinical signs ($P < 0.001$), occurrence and severity ($P < 0.01$) of nasal turbinate atrophy, but it did not reduce the rate of infection at 70 days of age.

INDEX TERMS: Swine atrophic rhinitis, *Bordetella bronchiseptica*, vaccination, immunoprophylaxis.

INTRODUCTION

Atrophic rhinitis (AR) of swine has been reported in almost all major swine producing countries as well as in southern Brazil (Guerreiro et al. 1963, Piffer et al. 1978, Williams & Fallavena 1979). In a survey for AR prevalence in the State of Santa Catarina, Brito et al. (1982) found this disease in 113 (75.3%) out of 150 herds. In addition, *Bordetella bronchiseptica* was isolated from 74 (66.7%) out of 111 herds examined. This agent has been incriminated as the etiological agent of AR (Switzer 1956, Cross & Claflin 1962, Duncan & Ramsey 1965, Shimizu et al. 1971, Fetter et al. 1975).

The strategy of AR control has been to use either sulfonamides (Switzer 1963) or immunoprophylaxis (Harris & Switzer 1972, Nakase et al. 1976, Pedersen & Barfod 1977, Farrington & Switzer 1979, Goodnow et al. 1979). The utilization of sulfonamides has led to the selection of strains resistant to this group of drugs, thus reducing its therapeutic effect. In the last decade, several reports appeared showing that vaccination against *B. bronchiseptica* resulted in the reduction of clinical signs and the severity of AR lesions (Harris & Switzer 1972, Nakase et al. 1976, Pedersen & Barfod 1977), Farrington & Switzer 1979, Goodnow et al. 1979).

The objective of this work was to determine whether vaccinating sows and their progenies with a bacterin containing *B. bronchiseptica* would be effective in eliminating carriers, producing high antibody titers and protecting challenged piglets from AR.

MATERIALS AND METHODS

Animals. Eleven commercial sows free of *B. bronchiseptica* and their litters were utilized. The sows were considered *B. bronchiseptica* free after three negative bacteriological examinations. Six of these animals and their offsprings were vaccinated, the other five and their litters were kept as unvaccinated controls.

Bacterin. Phase I *B. bronchiseptica* strains isolated from diseased pigs were grown in tryptic soy broth (TSB, Difco) with 1% fetal bovine serum. The cultures were incubated at 37° C overnight and standardized to contain 3×10^{10} colony forming units (CFU) per ml. The cultures were inactivated with 0.2% formalin and adsorbed to aluminum hydroxide.

Vaccination. Both sows and piglets were vaccinated subcutaneously with 2 ml of the experimental bacterin. Sows were vaccinated in the neck at 60 and 100 days of gestation, and piglets in the fold of the flank at seven and 28 days of age.

Experimental challenge. B. bronchiseptica for challenge was grown in Bordet-Gengou medium with 15% defibrinated sheep blood, at 37° C for 24 hours. It was harvested in TSB and standardized to contain 10^9 CFU/ml. All piglets, vaccinated and unvaccinated, received 0.5 ml of the challenge inoculum which was given through a syringe, in each nostril, at three, four and five days of age.

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Clinical examination and collection of specimens. All piglets were observed for AR symptoms throughout the six month experimental period and nasal secretions were swab-collected from both nostrils at 70 days of age. Blood for serological testing was collected at 15, 35, 55, 75, 95 and 180 days of age.

Isolation and identification of B. bronchiseptica. Nasal swabs were streaked on MacConkey agar with 1% glucose. Non-lactose and non-glucose utilizing colonies were selected and subjected to biochemical analysis for *B. bronchiseptica* identification. A simplified biochemical battery comprised of the urease, citrate, nitrate, oxidase, catalase, and glucose oxidation-fermentation tests was used.

Serum agglutination test. B. bronchiseptica antigen was prepared from the strains used for vaccination, as described by Jenkins (1978) with slight modifications. The microorganisms were cultivated on Bordet-Gengou with 15% defibrinated sheep blood, at 37° C for 24 hours. The cultures were harvested in phosphate buffered saline (PBS, 0.15 M, pH 7.2) and filtered through sterile gauze. Formalin, at a final concentration of 0.2%, was utilized for inactivation. This antigen was kept at 4° C. Before carrying out the test, the antigen was standardized at a transmittance of 60% at a wave-lenght of 625 mm. Thimerosal was added to a concentration of 1:10,000. Two fold serum dilutions from 1:10 to 1:1280 were made in 0.5 ml volumes of PBS and 0.5 ml of the standardized antigen was added to each dilution. The tubes were incubated in a 42° C water bath for four hours and an additional 48 hours at 4° C. The titer was defined by the highest dilution in which there was total agglutination.

Post-mortem examination. Upon reaching market weight (90 – 100 kg), all experimental pigs were slaughtered and the nose of each pig was sectioned at the level of the second premolar tooth (Switzer & Farrington 1975). The nasal turbinate lesions were scored according to Maeda et al. (1969).

Statistical analysis. Both treatment groups were compared by the chi-square test in regard to clinical signs, isolation of *B. bronchiseptica* and occurrence and severity of AR lesions. The Student's t test was utilized to compare the means of the serological response.

RESULTS

Sixty two piglets were born to the six vaccinated sows. Six were born dead, 13 were eliminated because of low birthweight (< 1 kg), crushing or diarrhea, leaving 43 pigs in this group. Fifty piglets were born to the five unvaccinated sows. Two were born dead and 14 were eliminated for the above reasons, leaving 34 pigs in this group. No local or systemic reactions were observed in the vaccinated pigs.

Clinical signs. Twenty-four (70.6%) unvaccinated pigs showed symptoms of AR. Among the vaccinated pigs, only 13 (30.2%) showed clinical signs. Both groups differed statistically ($P < 0.001$).

Bacteriological findings. B. bronchiseptica was isolated from 17 (39.5%) vaccinated and eight (23.5%) unvaccinated pigs. No statistical difference was observed between these groups ($P > 0.05$).

Titer of serum agglutinating antibodies. The agglutinating antibody response is summarized in Table 1. A significantly higher ($P < 0.001$) antibody response was observed in the vaccinated pigs from 15 to 55 days of age. No difference were observed from 75 to 180 days of age.

Turbinate atrophy lesions. The vaccinated pigs showed significantly less ($P < 0.01$) AR lesions than the unvaccinated ones. The results are summarized in Table 2.

DISCUSSION

Data obtained in this work showed that the pigs vaccinated against *B. bronchiseptica* had less clinical symptoms and less gross lesions of AR than the unvaccinated ones. Severe lesions were only observed in unvaccinated pigs, indicating that the vaccination scheme utilized reduced the occurrence and severity of AR. However, vaccination did not eliminate the disease, since 32.6% of the vaccinated piglets had slight to moderate turbinate atrophy. The inability of *B. bronchiseptica* vaccination to completely eliminate AR has also been reported by Nakase et al. (1976), Pedersen and Barford (1977), Farrington and Switzer (1979), and Goodnow et al. (1979).

At 70 days of age, the isolation of *B. bronchiseptica* from nasal secretions of vaccinated and unvaccinated pigs did not differ statistically, although there seemed to be a trend for a higher number of isolations from vaccinated piglets. Thus vaccination did not eliminate *B. bronchiseptica* from the nose of challenged pigs. In contrast, Harris and Switzer (1972, Nakase et al. (1976), Brandenburg (1978), and Farrington and Switzer (1979) reported that vaccination reduced the number of infected pigs. Furthermore, Nakase et al. (1976) found that

Table 1. *Agglutinating antiboby titers in sera from vaccinated and unvaccinated piglets* [(a)] *after challenge with* Bordetella bronchiseptica [(b)]

Experimental group	Age of piglets (days)					
	15	35	55	75	95	180
Vaccinated	4.38 ± 0,74*[(c)] (10 – 320)[(d)]	3.51 ± 1.08* (<10 – 160)	4.77 ± 0,80* (20 – 320)	2.97 ± 1.09* (<10 – 80)	2.04 ± 1.45* (<10 – 40)	2.93 ± 0.91* (<10 – 40)
Unvaccinated	1.62 ± 1.28** (<10 – 40)	1.98 ± 1.29** (<10 – 80)	2.69 ± 1.18** (<10 – 160)	2.77 ± 1.18* (<10 – 160)	1.96 ± 1.73* (<10 – 160)	3.59 ± 1.31* (<10 – 160)

(a) Piglets were vaccinated at seven and 28 days of age and were progeny of sows vaccinated at 60 and 100 days of gestation. Unvaccinated piglets were progeny of unvaccinated sows.

(b) All piglets were challenged intranasally through a syringe, with 10^9 CFU of *B. bronchiseptica* at three, four and five days of age.

(c) Log_2 of the mean of antibody titers ± standard diviation. Figures in a column with a different number of asterisks are significantly different at $P < 0.001$.

(d) Range of antibody titers.

Table 2. *Occurrence and severity of AR lesions in vaccinated and unvaccinated piglets which had been challenged with* Bordetella bronchiseptica[a] *at three, four and five days of age*

Lesions	Vaccinated		Unvaccinated (Control)	
	Number of pigs	%	Number of pigs	%
Normal	29	67.4	9	26.5
Slight	7	16.3	12	35.3
Mild	4	9.3	5	14.7
Moderate	3	7.0	3	8.8
Severe	0	0.0	5	14.7
Total	43	100.0	34	100.0

(a) See legends in Table 1.

B. bronchiseptica recovered from vaccinated pigs were mostly phase II or III which were less pathogenic. In our studies no attempts were made to characterize the strains isolated.

The early higher agglutinating antibody response observed in the vaccinated pigs appears to reflect the effect of vaccination. The antibodies detected in sera of vaccinated piglets may have been from passive or active in origin, but our experimental design did not allow us to identify the source of such antibodies. The low antibody titers obtained after the challenge of unvaccinated pigs and the results of Kemeny (1973) and Brassine et al. (1976) suggest that the serological response observed in vaccinated pigs was not a consequence of challenge, but of vaccination. The first author detected agglutinating serum antibodies in *B. bronchiseptica*-challenged pigs beginning two weeks post inoculations, while Brassine et al. (1976) found them only after 17 days.

These findings are similar to those reported by Harris and Switzer (1972), who found high serum antibody titers in pigs inoculated with sonicated *B. bronchiseptica* and pertussis vaccine. . Brandenburg (1978) also reported that vaccinated pigs exhibited markedly high agglutinin titers. Goodnow et al. (1979) found that increased serum titers to *B. bronchiseptica* correlated significantly with a decrease in the extent of AR lesions.

AR control in Brazil has been based on sulfonamide therapy, although recently this procedure has been shown to be ineffective in several herds (Brito et al. 1982). A probable explanation may be the occurrence of strains resistant to these drugs, since during a period of five years (1977-1981) the rate of susceptibility of *B. bronchiseptica* to sulfonamides went from approximately 96.6% to 37.5% (Brito et al. 1982).

The vaccination of sows and their piglets against *B. bronchiseptica* did not eliminate the infection from the challenged offspring, nor did it result in the eradication of AR. Nevertheless, the vaccinated piglets showed significantly less clinical signs and less turbinate atrophy than the unvaccinated ones.

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