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VACCINATION OF PORPOISES (*Tursiops truncatus*) AGAINST *Erysipelothrix rhusiopathiae* INFECTION

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Abstract: Agglutinating antibody responses of several Atlantic bottlenose dolphins were measured after exposure to live and killed *Erysipelothrix rhusiopathiae* vaccines. The live product was found to stimulate antibody production better than the killed bacterin. An immunization schedule utilizing an initial exposure to the bacterin with subsequent exposures with the live vaccine product is proposed.

INTRODUCTION

Wild as well as captive cetaceans are susceptible to infections by *Erysipelothrix rhusiopathiae* and deaths due to this infection have occurred at several aquaria (Prescott, J. H., J. White, personal communication).^{3,5}

Both killed and live vaccines are available for immunization of captive animals and most public aquaria and research facilities vaccinate their cetaceans regularly. While live vaccines have been used frequently without adverse reactions in porpoises, at least two cases exist, with confirmation at autopsy, in which the injected culture has been implicated. After two such porpoise deaths at the Naval Undersea R & D Center's Point Mugu Facility, live culture vaccinating was discontinued. However, after serum antibody testing was begun it was found that animals being vaccinated with bacterins at 12 month intervals had little or no antibody remaining when it came time for their booster.

Although parallel data are not available for porpoises, agglutination titers of 1:40 and less against *E. rhusiopathiae* in swine have been shown not to inhibit challenge infections.⁴ In the same experiment pigs with agglutinating antibody titers of 1:160 and 1:320 had no apparent reaction other than a slight increase in body temperature for one or more days after challenge with live cells.

This study was undertaken to determine average levels attained and persis-

tence of agglutinating antibodies to this organism in porpoises, using both formalin inactivated and live avirulent *erysipelas* vaccines. A method for promptly inducing a high antibody titer with little risk of disease is presented.

MATERIAL AND METHODS

Ten Atlantic bottlenose dolphins (*Tursiops truncatus*) were used. Injections of vaccine were made with a 21 gauge 1½" needle inserted to the hub into the left side of each porpoise 6" below the anterior insertion of the dorsal fin. This location was chosen only as a standard. It has no known unique capabilities or characteristics relative to this work.

The vaccine injections were made as follows:

1. Group A (2 porpoises: 154 lb & 169 lb): given Erysipelas Bacterin, Cutter Laboratories, 2 ml, 6.0×10^9 cells/ml.
2. Group B (2 porpoises: 342 lb & 325 lb): given Erysipelas Bacterin, Ft. Dodge Laboratories, 5 ml, 7.5×10^9 cells/ml.
3. Group C (2 porpoises: 420 lb & 334 lb): given EVA (live), Norden Laboratories, 2 ml, 2.0×10^8 cells/ml.
4. Group D (3 porpoises: 245 lb, 225 lb, 195 lb): given Erysipelas Bacterin, Ft. Dodge Laboratories, 5 ml, followed at 7 weeks by EVA (live), Norden Laboratories, 2 ml.

5. Animal E (1 porpoise: 246 lb): given Erysipelas Bacterin, Cutter Laboratories, 2 ml at 2 week intervals for 4 weeks (3 doses), blood sample for titer analysis taken 2 weeks after the third dose.

Serum samples required for monitoring antibody titers were taken as frequently as possible. Training schedules and tidal influence on the holding tanks did not permit a fixed sampling schedule.

These serum specimens were tested for agglutinating antibodies to *E. rhusiopathiae* using the method of Rice, et al² as modified by Shuman.⁴ Initially, *Erysipelothrix* strain M3LP3 (obtained from Dr. Richard L. Wood, U.S.D.A., National Animal Disease Laboratory, Ames, Iowa) was grown in large quantity, killed, suspended in saline, and standardized to be used as the antigen.

Late in the study, a commercially available Difco antigen (Cat. #2583) was tested and found to yield results comparable to *E. rhusiopathiae* strain M3LP3 antigen. All specimens taken for this report were then checked with the same lot of the Difco antigen product. The antibody titers reported here are those read after serum reaction with this commercial antigen. The endpoint was taken as the highest test serum dilution showing a minimum of 2+ agglutination (4+ maximum).

A 6 month period was chosen as the interval between booster inoculations for all groups except group A. With the exception of group D, all revaccinations were of the same type administered initially. Only the animals of group A were observed until agglutinating antibodies disappeared.

RESULTS

Agglutinating antibody titers to *E. rhusiopathiae* from all five groups are plotted against time in Figure 1. The lines on the graph represent the mean titer of the animals in the respective groups at the various sampling times. All animals in the study were without antibody at the beginning of the study.

DISCUSSION

A positive reaction in an agglutination test demonstrates the presence of antibodies to the test organism's cell wall and/or capsular antigens. Antibodies to bacterial metabolites, however, are not detected with this type of whole cell test. It should be noted, therefore, that a live *E. rhusiopathiae* vaccine product will stimulate production of certain antibodies that this agglutination test will not detect.

Gledhill¹ investigated the antigenic structure of strains of *Erysipelothrix* and found them to be "qualitatively homogeneous as regards to their antigens" (agglutinating cell wall and/or capsular antigens) and divisible into strains or serologic types based on quantitative differences of these antigens. Therefore, immunization of an animal with a given *E. rhusiopathiae* vaccine will stimulate production of antibodies against *E. rhusiopathiae* cell antigens, the proportion of which is dependent on the particular strain of the immunizing organism. The *E. rhusiopathiae* strain used in the agglutination reaction is testing qualitatively for the same antibodies and when this technique is used under standardized conditions with the same cell antigen lot, reliable comparative data can be collected.

The decision to give the booster vaccinations at 6 month intervals was based on the aforementioned negative titers in animals vaccinated with bacterin 12 months prior to testing.

Comparison of the antibody response of group A (2 ml) to group B (5 ml) in Figure 1 can be explained as being mainly a result of the cell dosage difference, and to a lesser degree variations in production techniques between the two products. Higher levels as well as more persistent antibodies were obtained in the group B animals given the larger dose. The manufacturer's 2 ml dose recommended for swine given to group A was not sufficient to cause agglutinating antibody production at a measurable level beyond 4 months. However, the 5 ml dose given to group B (mean weight of animals in group B approximately twice group A mean weight) caused the agglutinating antibody level to be main-

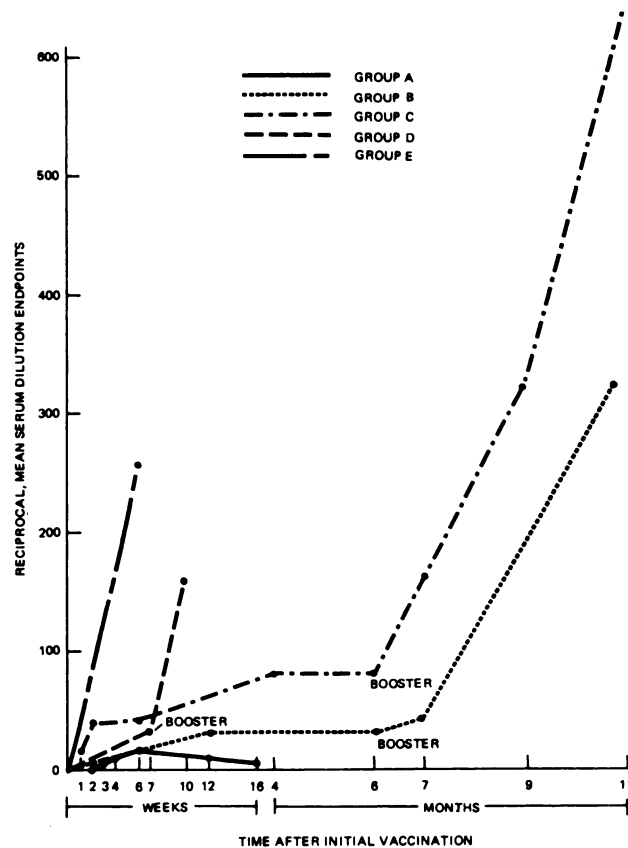


FIGURE 1. Anti-*Erysipelothrix rhusiopathiae* agglutinating antibody titers of Groups A through E vs. time.

tained until the booster vaccination at 6 months initiated a secondary response.

The response of animal E which received three times the group A dosage and a dose equal to that given animals in group B illustrates the pronounced effect of increased frequency of antigen administration. Six weeks after the initial vaccination, porpoise E had a titer of 1:256 while group A and group B animals had an average titer of 1:15.

Comparison of agglutinating antibody response curves of groups A, B, and C,

demonstrate the greater efficacy of a live culture vaccine (C) rather than a killed suspension (A & B) of *E. rhusiopathiae*. These data demonstrate that when using the manufacturer's recommended bacterin dosages, or up to 2½ times this dosage, and giving booster vaccinations at 6 month intervals, the response to the live vaccine was consistently at least twice that of the killed product.

The three animals of group D were initially exposed to a bacterin to stimulate antibody production. Seven weeks

later they were dosed with the live culture vaccine. Anti-*E. rhusiopathiae* antibody production already in progress was greatly enhanced in a secondary response (Figure 1). It appears that subsequent booster vaccination with the live product at 6 month intervals will result in a substantial antibody level similar to that of group C.

Attempts were not made to challenge any of these porpoises with a known

virulent *E. rhusiopathiae* strain, so it is not known if the highest levels attained here are in fact "protective" antibody levels. The technique used on group D has eliminated much of the risk of accidental *E. rhusiopathiae* infection, however, by having specific antibody production already in progress when the live culture is injected. The animal can then be maintained on live culture immunization, which was found to best stimulate anti-*E. rhusiopathiae* agglutinating antibodies.

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