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A SURVEY OF *Erysipelothrix insidiosa* AGGLUTINATING ANTIBODY TITERS IN VACCINATED PORPOISES *(Tursiops truncatus)*

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Abstract: Antibody activity against *Erysipelothrix insidiosa* was measured in 15 porpoises which had undergone a series of vaccinations, at 6 month intervals, with erysipelas bacterin. Relatively high levels of agglutinating antibodies were found to persist in the serum for at least 6 months following booster inoculations of the vaccine.

INTRODUCTION

Erysipelothrix insidiosa, frequently referred to as *Erysipelothrix rhusiopathiae*, is the cause of swine erysipelas and is associated with disease in other animals, including man.[5] *Erysipelothrix* infections have been reported in several species of small cetaceans, in which the disease is manifested as either an acute septicemia [1,2,7] or as sharply-delineated, elevated plaques on the skin.[9] Because of the public health significance of the disease, as well as the need to protect captive animals against infection, immunization against *E. insidiosa* has been recommended for all porpoises held in captivity.[2,6] Several commercial vaccines are available, and agglutinating antibody responses to both live and killed preparations have been measured in the Atlantic bottlenose porpoise, *Tursiops truncatus*.[3] Immunologic responsiveness to *E. insidiosa* antigens has also been demonstrated in the porpoise by indirect immunofluorescence.[4]

Porpoises maintained by the Naval Undersea Center, Hawaii Laboratory, are routinely vaccinated with erysipelas bacterin when acquired, and thereafter receive booster injections of the bacterin every 6 months. This study was undertaken to evaluate this vaccination schedule, in terms of the levels of *E. insidiosa* agglutinating antibodies which persist in the serums of animals between vaccinations.

METHODS AND MATERIALS

Fifteen adult Atlantic bottlenose porpoises, eight males and seven females, were used. All animals had received at least two intramuscular injections of erysipelas bacterin[1] at intervals of approximately 6 months. The standard vaccine dose was 3 ml. The number of immunizations given prior to sampling varied from two to eight, depending on the time each individual animal had been held in captivity. Half the animals surveyed had received four or more vaccinations. Serum samples used in this survey were obtained 6 to 7 months after the last vaccination.

A commercial *E. insidiosa* antigen preparation[2] was used, in accordance with manufacturer's instructions, to test for agglutinating antibody activity. Serum samples were serially diluted from 1:50 to 1:1280 in 0.85% saline, then mixed with equal volumes of the antigen. Duplicate titrations were performed on each

[1] Fort Dodge Laboratories, Fort Dodge, Iowa.
[2] Difco Laboratories, Detroit, Mich.

serum specimen tested. The serum-antigen mixtures were first incubated for 4 h at 42C, then held at 37C overnight. Prior to reading, a drop of methylene blue (0.5%) was added to each tube to stain the cells. The reaction was read under a microscope (45X objective), and the endpoint was taken as the highest dilution of serum showing a minimum of 2+ agglutination (50% of cells clumped). Controls incorporated in the test included *E. insidiosa* antiserum[2] (positive control) and a saline-antigen mixture (negative control).

RESULTS AND DISCUSSION

All animals tested had measurable antibody activity against *E. insidiosa* 6 to 7 months after receiving booster injections of erysipelas bacterin. Agglutinating antibody titers ranged from 1:100 to 1:1600 (Table 1), and most of the animals (73%) had titers of 1:400 or greater. The lowest titer was found in a single animal which had received only two vaccinations, while the highest titer occurred in an animal which had been vaccinated 6 times. However, a direct correlation between serum antibody titers and the number of vaccinations a porpoise had received was not indicated by the data.

These results indicate that relatively high levels of agglutinating antibodies persist in the serums of porpoises for at least 6 months following booster inoculations of erysipelas bacterin, when booster injections are given at 6 month intervals. The advisability of revaccinating at such frequent intervals is indicated by the fact that porpoises vaccinated with bacterins every 12 months had little or no demonstrable antibody when it came time for their booster.[5] In the same study, live avirulent cultures of *E. insidiosa* were found to stimulate antibody production better than the killed vaccine. However, primary immunization with the living product has been implicated in porpoise deaths, and the authors

TABLE 1. Anti-**Erysipelothrix insidiosa** agglutinating antibody titers in 15 vaccinated porpoises (**Tursiops truncatus**).

Animal Identification	Number of Vaccinations	Antibody Titer*
45	2	100
35	3	400
36	3	400
37	3	400
42	3	200
43	3	400
44	3	200
34	4	400
38	4	200
29	5	800
12	6	800
18	6	1600
32	6	400
8	8	800
9	8	800

* Reciprocal, serum dilution endpoints.

therefore proposed an immunization schedule utilizing an initial exposure to the bacterin with subsequent exposures, at 6 month intervals, to the live vaccine.

Cross-agglutination tests have shown that all strains of *E. insidiosa* have at least one common antigen, and that separate serologic types of the organism may be due either to type-specific antigens or to quantitative differences in the antigens common to all strains.[5] The relationship between agglutinating antibody titres and protective immunity to *E. insidiosa* infection in porpoises is not known. However, titers of 1:160 and above have been correlated with resistance to challenge infection in swine.[8]

The indirect immunofluorescent staining technique has also been used to detect circulating antibody to *E. insidiosa* in porpoises.[4] In the same study, passive protection tests were conducted in mice, using porpoise serum dilutions and an LD_{50} dose of the organism. *T. truncatus* serum having a 1:1024 immunofluorescent titer prevented the development of clinical signs and bacteremia produced by *E. insidiosa,* when surum-bacteria mixtures were inoculated in mice.

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