IMMUNOLOGICAL STUDIES IN PIGS USING EDEMA DISEASE PRINCIPLE (E.COLI NEUROTOXIN).

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E.coli neurotoxin has been shown to be responsible for Edema Disease in pigs (1, 2). Our attention was focussed on the antigenicity of this toxin with a view toward the possible prophylactic value of immunisation against post-weaning Edema Disease. Previous studies have shown that active immunisation against neurotoxin confers a significant degree of protection in laboratory animals (3) and in pigs (unpublished data). However, the immunogenicity of a neurotoxoid has not yet been demonstrated. Indeed, formaldehyde inactivation of the neurotoxin resulted in the loss of its antigenicity (4).

Current problems in the development of a neurotoxoid vaccine include the production of an antigenic toxoid and the design of an immunisation regime able to protect piglets which have been early weaned. Data concerning both of these two aspects are presented in this paper.

Crude neurotoxin prepared from E.coli serotype O139K82 by a method previously described (3) was used. It was incubated at ambiant temperature with glutaraldehyde of different concentrations. A series of samples obtained after various incubation periods were tested for residual toxicity and antigenicity using the mouse model (5). The results indicate that the use of a glutaraldehyde concentration of .013 M and a reaction time of 1 hour were the optimal conditions for the preparation of an antigenic toxoid. This neurotoxoid was adsorbed on aluminium hydroxide gel and evaluated for its immunizing capacity in pigs in the following manner :

1. an antigenicity study aimed at comparing the neurotoxin and the neurotoxoid was conducted in piglets 5 to 8 weeks of age; the animals were inoculated either with the toxin or the toxoid preparation according to three different vaccination schemes. The vaccine doses are expressed as mice ED_{50}. The anti-neurotoxin antibodies are determined by a seroneutralisation test in Vero cells (submitted for publication). The results of this study indicate that the serological response induced by the neurotoxoid is comparable to the response elicited by the active toxin. The pigs immunised with the toxoid showed an anti-neurotoxin response following the three vaccination schemes similar to that produced by the active toxin. These data are in line with those obtained in mice using the same type of toxoid.

2. The relationship between anti-neurotoxin antibodies and protection against an artificial neurotoxin challenge was investigated. For this purpose pigs were separated into two age groups : 5 to 6 weeks and 1 to 2 weeks old piglets. Pigs were vaccinated twice using different neurotoxin or neurotoxoid batches. Control animals were not inoculated.
One to two weeks after the second vaccination, the animals were challenged by the intravenous route with a standard neurotoxin preparation using $2.2ED_{50}$/kg body weight. All piglets were examined for the appearance of characteristic Edema Disease symptoms. Pigs were bled before vaccination and before challenge inoculation and antitoxin titrations performed by the "in vitro" seroneutralisation test previously described.
The results indicate that there is a strong correlation between the seroconversion rate and the protection rate observed against a neurotoxin challenge, which killed all the control animals. The protection rates are highly significant when compared to the controls ($p < 0.01$).
Evidence also indicates that a modest anti-neurotoxin titer is nevertheless sufficient to protect.

3. Several vaccination schemes were evaluated in young pigs of 7 and 14 days of age. Control and vaccinated animals were challenged by the intravenous route using $2.2ED_{50}$/kg body weight of neurotoxin. The results show that the lower vaccinal dose ($64ED_{50}$) failed to protect both age groups, whereas a larger antigenic mass corresponding to $256ED_{50}$ succeeded in protecting both age groups to a similar degree. This finding was reflected in similar seroconversion rates in both groups. In a comparison of different vaccination intervals (7 and 14 days) conducted in 7 day old piglets, it was shown that the 14 days interval was superior to the 7 days interval with regard to stimulating anti-neurotoxin antibodies. It is also clear that by using a two weeks vaccination interval, significant protection has been achieved by four weeks of age.

In conclusion, the results of our study show that by using a glutaraldehyde neurotoxoid, the immunogenicity of the neurotoxoid has been preserved, with the result that very young piglets can be protected against an overwhelming challenge of Edema Disease Principle.

References :Clugston R.E. and N.O. Nielsen, 1974, Can.J.Comp.Med.38,22(1); Schimmelpfennig H., 1970. Dtsch.Tierärzt.77,263(2);Dobrescu L. and F. Van Wijnendaele, 1979, Zbl.Vet.Med.B,26,239(3);Schimmelpfennig H. and R. Weber, 1979, Adv.Vet.Med.29,25(4); Schimmelpfennig H., 1970, Fortschr.Vet.Med.13,49 (5).