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Comparison of Killed and Live Vaccines Against Teschen Disease Virus (TDV) Using Different Routes of Inoculation

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An immunological response depends on many endegenous and extraneous factors. Good preservation of antigenicity and the composition of vaccine are the most important factors in active immunization with killed vaccine, on the other hand the route of inoculation and the amount of virus used are decisive factors when working with live vaccines.

The aime of our work was to determine for practical use the most effective method of vaccination against TDV ensuring long-lasting immunity. We wish to present several selected findings from our experiments conducted over many years.

1. The Inactivated Vaccines

Two identical samples of TDV strain Praha were inactivated, 1 with formalin diluted 1:4000 at pH 7,4 for 12 days at 37 C, and the other with 1% beta-propiolactone aqueous solution for 2 h under identical conditions. Both types of vaccines were mixed with lipoid adjuvants and injected subcutaneously into piglets weighing 15–20 kg, in 5 ml volumes. One dose contained approximately $2.10^{6.5}$ $TCID_{50}$ of virus. The immunizing effect was evaluated according to the neutralizing antibody titers obtained before—and 6 weeks after vaccination, and according to resistance to challenge by intracerebral inoculation of 100 $TCID_{50}$ of virulent virus 6 weeks after vaccination. The results showed that:

1. Both types of vaccines enhance neutralizing antibody titers, the beta-propiolactone-inactivated vaccine being more potent as can be seen from the antibody response and resistance to challenge.

2. The amount of virus used in our experiments was sufficient for effective immunization with beta-propiolactone-inactivated vaccine. This amount, according to Mayr [2] is slightly below the limit of effectivity in formalized vaccines.

3. Our comparison again demonstrated that beta-propiolactone inactivation is less deleterious to the antigenic structure of TDV than formalization under conditions of our experiments.

4. The highest antibody titers (1:256 to 1:512) attained in our experiments persisted for at least 6 weeks.

5. In some animals immunized with formol inactivated vaccine, antibodies present in titers 1:128 did not prevent paralytic disease after challenge.

2. The Live Vaccine

A non-virulent strain of TDV was obtained in our laboratory by serial passage of TDV in cell cultures and subsequent cloning by the plaque technique. In all our experiments we observed 2 characteristic types of antibody response in pigs after *oral* administration of this non-virulent strain of TDV. Fasting animals were fed approximately 5.10^7 $TCID_{50}$ of virus suspended in 250 ml of milk and their serum titers were then determined at regular intervals. Forty-three days after vaccination the animals were challenged intranasally with 200 $TCID_{50}$ of virulent TDV. The results of this experiment show that the oral administration of non-virulent virus in the amount employed leads to an irregular antibody response, which in some instances is not only of short duration but sometimes does not even give protection against challenge. In other cases increase of antibody level starts only long after vaccination. Our results are in agreement with HECKE's findings [1] where successful immunization required rather higher doses of non-virulent virus given orally.

Excellent results were obtained with *intranasal* immunization with our non-virulent clone A3b. Piglets weighing 15–20 kg received 1 ml of virus suspension into each nostril, *i.e.* 6.10^7 $TCID_{50}$ of virus per animal. All immunized animals developed neutralizing antibodies in a detectable quantity towards the end of the first week after immunization, the highest levels were reached in 12 days and persisted at least up to day 43 when the animals were challenged intranasally with 200 $TCID_{50}$ of virulent TDV. These results indicate that:

1. Intranasal instillation of non-virulent clone A3b of TDV induces antibody formation which becomes apparent within 5 days and attains maximum levels in 12 days after immunization.

2. These antibody titers persist for at least 42 days.

3. The induced immunity protected all animals against intranasal challenge which led to paralytic disease in control animals.

Summarizing, we conclude that intranasal vaccination with non-virulent clone A3b of TDV in comparison with oral, or subcutaneous vaccination [1, 3] has its definite merits. These merits lie in the long-persisting high titers of neutralizing antibodies which can be elicited by only one dose of vaccine and in the high resistance to challenge with the virulent virus which follows. In our opinion the administration of antigen by intranasal vaccination corresponds to the natural pathway of infection and therefore seems to fulfil the conditions required for the optimal rate of vaccination. This may be supported by the finding that apparently non-virulent virus, unable to induce biological and histological changes in the

CNS, still readily multiplies at least in the nasal mucosa. The production of new virus ensures an ample supply of viral antigen which finally induces resistance the vaccinated animals, mediated either by neutralizing antibodies or by another mechanism.

Summary

Comparison of Teschen disease virus live vaccine administered orally or intranasally with formalin, or beta-propiolactone inactivated vaccine injected with adjuvants subcutaneously, showed the marked superiority of the live vaccine, but only after intranasal instillation. Beta-propiolactone inactivated vaccine gave better results than the formalized one. Both were more effective than the live vaccine administered orally, as measured by antibody response, persistence of antibodies and resistance tests.

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