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Porcine Actinobacillosis Vaccine, Inactivated

General Notices

(Porcine Actinobacillosis Vaccine (Inactivated), Ph. Eur. monograph 1360)

Ph Eur

1 DEFINITION

Porcine actinobacillosis vaccine (inactivated) is a preparation which has one or more of the following components: inactivated *Actinobacillus pleuropneumoniae* of a suitable strain or strains; toxins, proteins or polysaccharides derived from suitable strains of *A. pleuropneumoniae*, and treated to render them harmless while maintaining adequate immunogenic properties; fractions of toxins derived from suitable strains of *A. pleuropneumoniae* and treated if necessary to render them harmless while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of pigs against actinobacillosis.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The seed material is cultured in a suitable medium; each strain is cultivated separately. During production, various parameters such as growth rate, protein content and quantity of relevant antigens are monitored by suitable methods; the values are within the limits approved for the particular product. Purity and identity are verified on the harvest using suitable methods. After cultivation, the bacterial harvests are collected separately and inactivated by a suitable method. They may be detoxified, purified and concentrated. The vaccine may be adjuvanted.

2-2 CHOICE OF VACCINE COMPOSITION

The choice of strains is based on epidemiological data. The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the pigs for which it is intended.

The following tests for safety (section 2-2-1) and immunogenicity (section 2-2-2) may be used during the demonstration of safety and efficacy.

2-2-1 Safety

2-2-1-1 Laboratory tests Carry out the test for each route and method of administration to be recommended for vaccination and where applicable, in pigs of each category for which the vaccine is intended, using in each case pigs not older than the minimum age to be recommended for vaccination. Use a batch containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test, use not fewer than 8 pigs that do not have antibodies against the serotypes of *A. pleuropneumoniae* or its toxins present in the vaccine. Administer to each pig 1 dose of the vaccine. If the schedule to be recommended requires a 2nd dose, administer another dose after an interval of at least 14 days. Observe the pigs at least daily until 14 days after

the last administration. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 4 days; note the maximum temperature increase for each pig.

The vaccine complies with the test if no pig shows abnormal local or systemic reactions or dies from causes attributable to the vaccine, and if the average temperature increase for all pigs does not exceed 1.5 °C and no pig shows a rise greater than 2.0 °C.

2-2-1-2 Field studies The pigs used for field trials are also used to evaluate safety. Carry out a test in each category of pigs for which the vaccine is intended. Use not fewer than 3 groups each of not fewer than 20 pigs with corresponding groups of not fewer than 10 controls. Examine the injection site for local reactions after vaccination. Record body temperature the day before vaccination, at vaccination, at the time interval after which a rise in temperature, if any, was seen in test 2-2-1-1, and daily during the 2 days following vaccination; note the maximum temperature increase for each pig.

The vaccine complies with the test if no pig shows abnormal local or systemic reactions or dies from causes attributable to the vaccine, and if the average temperature increase for all pigs does not exceed 1.5 °C and no pig shows a rise greater than 2.0 °C.

2-2-2 Immunogenicity

The challenge strain for the following test is chosen to ensure challenge with each Ap toxin[1] produced by the serotypes to be stated on the label; it may be necessary to carry out more than one test using a different challenge strain for each test.

Each test is carried out for each route and method of administration to be recommended. The vaccine administered to each pig is of minimum potency.

For each test, use not fewer than 14 pigs that do not have antibodies against *A. pleuropneumoniae* and Ap toxins. Vaccinate not fewer than 7 pigs according to the schedule to be recommended. Maintain not fewer than 7 pigs as controls. 3 weeks after the last vaccination, challenge all the pigs by the intranasal or intratracheal route or by aerosol with a sufficient quantity of a virulent serotype of *A. pleuropneumoniae*. Observe the pigs at least daily for 7 days; to avoid unnecessary suffering, severely ill control pigs are euthanised and are then considered to have died from the disease. Euthanise all surviving pigs at the end of the observation period. Carry out a post-mortem examination on all pigs. Examine the lungs, the tracheobronchial lymph nodes and the tonsils for the presence of *A. pleuropneumoniae*. Evaluate the extent of lung lesions at post-mortem examination. Each of the 7 lobes of the lungs is allotted a maximum possible lesion score[2] of 5. The area showing pneumonia and/or pleuritis of each lobe is assessed and expressed on a scale of 0 to 5 to give the pneumonic score per lobe (the maximum total score possible for each complete lung is 35). Calculate separately for the vaccinated and the control pigs the total score (the maximum score per group is 245, if 7 pigs are used per group).

The vaccine complies with the test if the vaccinated pigs, when compared with controls, show lower incidence of: mortality; typical signs (dyspnoea, coughing and vomiting); typical lung lesions; re-isolation of *A. pleuropneumoniae* from the lungs, the tracheobronchial lymph nodes and the tonsils. Where possible, the incidence is analysed statistically and shown to be significantly lower for vaccinates.

2-3 MANUFACTURER'S TESTS

2-3-1 Batch potency test

It is not necessary to carry out the potency test (section 3-4) for each batch of vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. The following test may be used.

Use 5 mice weighing 18-20 g and that do not have antibodies against the serotypes of *A. pleuropneumoniae* or its toxins present in the vaccine. Vaccinate each mouse by the subcutaneous route with a suitable dose. Where the recommended schedule requires a booster injection to be given, a booster vaccination may also be given in this test provided it has been demonstrated that this will still provide a suitably sensitive test system. Before the vaccination and at a given interval within the range of 14-21 days after the last injection, collect blood from each mouse and prepare serum samples. Determine individually for each serum the titre of specific antibodies against each antigenic component stated on the label, using a suitable validated test such as enzyme-linked immunosorbent assay (*2.7.1*). The vaccine complies with the test if the antibody levels are not significantly lower than those obtained for a batch that has given satisfactory results in the test described under Potency.

2-3-2 Bacterial endotoxins

A test for bacterial endotoxins (*2.6.14*) is carried out on the final bulk or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory in safety test 2-2-1-1 described under Choice of vaccine composition or in the residual toxicity test described under Batch tests, carried out using 10 pigs. Where the latter test is used, note the maximum temperature increase for each animal; the vaccine complies with the test if the average temperature increase for all animals does not exceed 1.5 °C. The method chosen for determining the amount of bacterial endotoxin present in the vaccine batch used in the safety test for determining the maximum acceptable level of endotoxin is used subsequently for batch testing.

3 BATCH TESTS

3-1 Identification

When injected into healthy animals that do not have specific antibodies against the antigenic components of *A. pleuropneumoniae* stated on the label, the vaccine stimulates the production of such antibodies.

3-2 Bacteria and fungi

The vaccine, including where applicable the diluent supplied for reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3 Residual toxicity

Use 2 pigs of the minimum age recommended for vaccination and that do not have antibodies against the serotypes of *A. pleuropneumoniae* or its toxins present in the vaccine. Administer to each pig by a recommended route a double dose of the vaccine. Observe the pigs at least daily for 14 days. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 2 days.

It is recommended to use the mean temperature of the days before administration of the vaccine (e.g. day -3 to day 0) as the baseline temperature to have clear guidance for evaluation of the test.

The vaccine complies with the test if no pig shows notable signs of disease or dies from causes attributable to the vaccine; a transient temperature increase not exceeding 2.0 °C may occur.

3-4 Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-2) when administered by a recommended route and method.

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1 The nomenclature of the toxins of *A. pleuropneumoniae* is described by J. Frey *et al*., *Journal of General Microbiology*, 1993, 139, 1723-1728.

2 The system of lung scores is described in detail by P.C.T. Hannan, B.S. Bhogal, J.P. Fish, *Research in Veterinary Science*, 1982, 33, 76-88.