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Porcine E. Coli Vaccine, Inactivated

General Notices

Porcine Escherichia Coli Vaccine, Inactivated

(Neonatal Piglet Colibacillosis Vaccine (Inactivated), Ph. Eur. monograph 0962)

Ph Eur

1 DEFINITION

Neonatal piglet colibacillosis vaccine (inactivated) is a preparation from cultures of one or more suitable strains of *Escherichia coli*, carrying one or more adhesins or enterotoxins. This monograph applies to vaccines intended for the active immunisation of sows and gilts for passive protection of their newborn progeny against enteric forms of colibacillosis, administered by injection.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The *E. coli* strains used for production are cultured separately in a suitable medium. The cells or toxins are processed to render them safe while maintaining adequate immunogenic properties and are blended. The vaccine may be adjuvanted.

2-2 CHOICE OF VACCINE COMPOSITION

The *E. coli* strains used in the production of the vaccine are shown to be satisfactory with respect to expression of antigens and the vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the sows and gilts for which it is intended.

The following tests for expression of antigens (section 2-2-1), safety (section 2-2-2) and immunogenicity (section 2-2-3) may be used during the demonstration of safety and efficacy.

2-2-1 Expression of antigens

The expression of antigens that stimulate a protective immune response is verified by a suitable immunochemical method (*2.7.1*) carried out on the antigen obtained from each of the vaccine strains under the conditions used for the production of the vaccine.

2-2-2 Safety

2-2-2-1 Safety in pregnant sows. Carry out the test for each route and method of administration to be recommended for vaccination and in pregnant sows. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test, use not fewer than 8 pregnant sows per group that have not been vaccinated against colibacillosis, at the relevant stages of pregnancy in accordance with the schedule to be recommended or at different stages of pregnancy. Administer to each sow 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 sows at least daily until farrowing. 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 sow.

The vaccine complies with the test if:

— no sow shows abnormal local or systemic reactions or dies from causes attributable to the vaccine;

— the average temperature increase for all sows does not exceed 1.5 °C and no sow shows a rise greater than 2.0 °C; and

— no adverse effects on gestation or the offspring are noted.

2-2-2-2 Field studies. The pigs used for field trials are also used to evaluate safety. 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 2-2-2-1, 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, the average temperature increase for all pigs does not exceed 1.5 °C, and no pig shows a rise greater than 2.0 °C.

2-2-3 Immunogenicity

Carry out the test with a challenge strain representing each type of antigen against which the vaccine is intended to protect: if a single strain with all the necessary antigens is not available, repeat the test using different challenge strains.

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

Use not fewer than 8 gilts susceptible to *E. coli* infections and that do not have antibodies against the antigens to be stated on the label. Take not fewer than 4 at random and vaccinate these at the stage of pregnancy and according to the schedule to be recommended. Maintain not fewer than 4 gilts as controls. Within 12 h of their giving birth, take not fewer than 15 healthy piglets from the vaccinated gilts and 15 healthy piglets from the controls, taking at least 3 from each litter. Challenge each piglet by the oral route with a sufficient quantity of a virulent strain of *E. coli* before or after colostrum feeding and using the same conditions for vaccinated piglets and controls. The strain used must not be one used in the manufacture of the vaccine. Return the piglets to their dam and observe at least daily for 8 days.

On each day, note signs in each piglet and score using the following scale.

0	no signs
1	slight diarrhoea
2	marked diarrhoea (watery faeces)
3	dead

Calculate total scores for each piglet over 8 days.

The test is not valid if fewer than 40 per cent of the piglets from the control gilts die and more than 15 per cent of the piglets from the control gilts show no signs of illness. The vaccine complies with the test if there is a significant reduction in score in the group of piglets from the vaccinated gilts compared with the group from the unvaccinated controls.

For some adhesins (for example, F5 and F41), there is published evidence that high mortality cannot be achieved under experimental conditions. If challenge has to be carried out with a strain having such adhesins: the test is not valid if fewer than 70 per cent of the control piglets show signs expected with the challenge strain; the vaccine complies with the test if there is a significant reduction in score in the group of piglets from the vaccinated gilts compared with the group from the unvaccinated controls.

2-3 MANUFACTURER'S TESTS

2-3-1 Batch potency test

It is not necessary to carry out the potency test (section 3-3) 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 7 pigs not less than 3 weeks old and that do not have antibodies against the antigens stated on the label. Vaccinate each of 5 pigs by the recommended route and according to the recommended schedule. Maintain 2 pigs as controls. Alternatively, if the nature of the antigens allows reproducible results to be obtained, a test in laboratory animals (for example, guinea-pigs, mice, rabbits or rats) may be carried out. To obtain a valid assay, it may be necessary to carry out a test using several groups of animals, each receiving a different dose. For each dose, carry out the test as follows. Vaccinate not fewer than 5 animals with a single injection of a suitable dose. Maintain not fewer than 2 animals as controls. 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. At a given interval within the range of 14-21 days after the last injection, collect blood from each animal and prepare serum samples. Use a suitable validated test such as an enzyme-linked immunosorbent assay (*2.7.1*) to measure the antibody response to each of the antigens stated on the label. The vaccine complies with the test if the antibody levels in the vaccinates are not significantly less than those obtained with a batch that has given satisfactory results in the test described under Potency and there is no significant increase in antibody titre in the controls.

Where animals that do not have antibodies against the antigens stated on the label are not available, seropositive animals may be used in the above test. During the development of a test with seropositive animals, particular care will be required during the validation of the test system to establish that the test is suitably sensitive and to specify acceptable pass, fail and retest criteria. It will be necessary to take into account the range of possible prevaccination titres and establish the acceptable minimum titre rise after vaccination in relation to these.

2-3-2 Bacterial endotoxins

A test for bacterial endotoxins (*2.6.14*) is carried out on the final lot or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or the 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 2-2-2-1 given under Choice of vaccine composition. 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 testing of each batch.

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigen or antigens stated under Definition.

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 Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-3) when administered by a recommended route and method.