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Ruminant E. Coli Vaccine, Inactivated

General Notices

Ruminant Escherichia Coli Vaccine, Inactivated

(Neonatal Ruminant Colibacillosis Vaccine (Inactivated), Ph. Eur. monograph 0961)

Ph Eur

1 DEFINITION

Neonatal ruminant colibacillosis vaccine (inactivated) is a preparation from cultures of one or more suitable strains of *Escherichia coli*, carrying one or more adhesin factors or enterotoxins. This monograph applies to vaccines intended for the active immunisation of dams 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 ruminants 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 animals. Carry out the test for each route and method of administration to be recommended for vaccination and in pregnant animals of each species for which the vaccine is intended. 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 animals per group that have not been vaccinated against colibacillosis. Administer to each animal 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 animals at least daily until parturition. 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 animal.

The vaccine complies with the test if:

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

— the average temperature increase for all animals does not exceed 1.5 °C and no animal 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. Safety is demonstrated in field trials for each species for which the vaccine is intended. Administer the dose to be recommended to not fewer than 60 animals from 3 different stocks by the route and according to the schedule to be recommended. Assign not fewer than 30 animals from the same stocks to control groups. Observe the animals at least daily for 14 days after the last administration.

The vaccine complies with the test if no animal shows abnormal local or systemic reactions or dies from causes attributable to the vaccine and if no rise in temperature of more than 1.5 °C occurs within 2 days of administration of each dose of the vaccine.

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, using in each case animals of each species for which the vaccine is intended. The vaccine administered to each animal is of minimum potency.

For each test, use not fewer than 15 animals that do not have antibodies against the antigens to be stated on the label. Take not fewer than 10 at random and vaccinate these at the stage of pregnancy and according to the schedule to be recommended. Maintain not fewer than 5 animals as controls. Collect colostrum from all animals after parturition and store the samples individually in conditions that maintain antibody levels. Take not fewer than 15 newborn unsuckled animals and house them in an environment ensuring absence of enteric pathogens. Allocate a colostrum sample from not fewer than 10 vaccinated dams and not fewer than 5 controls to the offspring. After birth, feed the animals with the colostrum sample allocated to it. After feeding the colostrum and within 12 h of birth, challenge all the animals by the oral route with a sufficient quantity of a virulent strain of *E. coli* and observe at least daily for 10 days. The strain must not be one used in the manufacture of the vaccine.

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

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

Calculate total scores for each animal over 10 days.

The test is not valid if fewer than 80 per cent of the animals given colostrum from the controls die or show severe signs of disease. The vaccine complies with the test if there is a significant reduction in score in the group of animals given colostrum from vaccinated dams compared with the group given colostrum 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.

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 required, carry out the test as follows. Use not fewer than 7 animals (for example rabbits, guinea-pigs, rats or mice) that do not have antibodies against the antigens stated on the label. Vaccinate not fewer than 5 animals, using 1 injection of a suitable dose. Maintain 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 protective 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 endotoxins 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.