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Porcine Progressive Atrophic Rhinitis Vaccine, Inactivated

General Notices

(Porcine Progressive Atrophic Rhinitis Vaccine (Inactivated), Ph. Eur. monograph 1361)

Ph Eur

1 DEFINITION

Porcine progressive atrophic rhinitis vaccine (inactivated) is a preparation containing either the dermonecrotic exotoxin of *Pasteurella multocida*, treated to render it harmless while maintaining adequate immunogenic properties, or a genetically modified form of the exotoxin that has adequate immunogenic properties and that is free from toxic properties; the vaccine may also contain cells and/or antigenic components of one or more suitable strains of *P. multocida* and/or *Bordetella bronchiseptica*. This monograph applies to vaccines intended for the active immunisation of sows and gilts for passive protection of their progeny against porcine progressive atrophic rhinitis.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The bacterial strains used for production are cultured separately in suitable media. The toxins and/or cells are treated to render them safe. The vaccine may be adjuvanted.

2-2 DETOXIFICATION

A test for detoxification of the dermonecrotic exotoxin of *P. multocida* is carried out immediately after detoxification. The concentration of detoxified exotoxin used in the test is not less than that in the vaccine. The suspension complies with the test if no toxic dermonecrotic exotoxin is detected. The test for detoxification is not required where the vaccine is prepared using a toxin-like protein free from toxic properties, produced by expression of a modified form of the corresponding gene.

2-3 ANTIGEN CONTENT

The content of the dermonecrotic exotoxin of *P. multocida* in the detoxified suspension or the toxin-like protein in the harvest is determined by a suitable immunochemical method (*2.7.1*), such as an enzyme-linked immunosorbent assay, and the value found is used in the formulation of the vaccine. The content of other antigens stated on the label is also determined (*2.7.1*).

2-4 CHOICE OF VACCINE COMPOSITION

The strains used for the preparation of the vaccine are shown to be satisfactory with respect to the production of the dermonecrotic exotoxin and the other antigens claimed to be protective. 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 production of antigens (section 2-4-1), safety (section 2-4-2) and immunogenicity (section 2-4-3) may be used during the demonstration of safety and efficacy.

2-4-1 Production of antigens

The production of antigens claimed to be protective is verified by a suitable bioassay or immunochemical method (*2.7.1*), carried out on the antigens obtained from each of the vaccine strains under the conditions to be used for the production of the vaccine.

2-4-2 Safety

2-4-2-1 Safety in pregnant sows. Carry out the test for each route and method of administration to be recommended for vaccination using in each case pregnant sows or gilts that do not have antibodies against the components of the vaccine, from a herd or herds where there are no signs of atrophic rhinitis and that have not been vaccinated against atrophic rhinitis. Use a batch containing not less than the maximum potency that may be expected in a batch of vaccine.

Use not fewer than 8 pregnant sows or gilts per group, at the stage or at different stages of pregnancy according to the schedule to be recommended. Administer to each pregnant sow or gilt 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 pregnant sows or gilts 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 pregnant sow or gilt.

The vaccine complies with the test if no pregnant sow or gilt shows abnormal local or systemic reactions or dies from causes attributable to the vaccine, if the average temperature increase for all pregnant sows or gilts does not exceed 1.5 °C and no pregnant sow or gilt shows a rise greater than 2.0 °C, and if no adverse effects on gestation and offspring are noted.

2-4-2-2 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 2-4-2-1, 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 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.

2-4-3 Immunogenicity

Each test is carried out for each route and method of administration to be recommended, using in each case pigs that do not have antibodies against the components of the vaccine, that are from a herd or herds where there are no signs of atrophic rhinitis and that have not been vaccinated against atrophic rhinitis. The vaccine administered to each pig is of minimum potency.

2-4-3-1 Vaccines containing dermonecrotic exotoxin of P. multocida *(with or without cells of* P. multocida*)*. Use not fewer than 12 breeder pigs. Vaccinate not fewer than 6 randomly chosen pigs at the stage of pregnancy or non-pregnancy and according to the schedule to be recommended. Maintain not fewer than 6 pigs as controls. From birth allow all the piglets from the vaccinated and unvaccinated breeder pigs to feed from their own dam. Constitute from the progeny 2 challenge groups each of not fewer than 30 piglets chosen randomly, taking not fewer than 3 piglets from each litter. On the 2 consecutive days preceding challenge, the mucosa of the nasal cavity of the piglets may be treated by instillation of 0.5 mL of a solution of acetic acid (10 g/L $C_2H_4O_2$) in isotonic buffered saline pH 7.2.

Challenge each piglet at 10 days of age by the intranasal route with a sufficient quantity of a toxigenic strain of *P. multocida*. At the age of 42 days, euthanise the piglets of the 2 groups and dissect the nose of each of them transversally at premolar-1. Examine the ventral and dorsal turbinates and the nasal septum for evidence of atrophy or distortion and grade the observations on the following scales.

Turbinates

0	no atrophy
1	slight atrophy
2	moderate atrophy
3	severe atrophy

4	very severe atrophy with almost complete disappearance of the turbinate

The maximum score is 4 for each turbinate and 16 for the sum of the 2 dorsal and 2 ventral turbinates.

Nasal septum

0	no deviation
1	very slight deviation
2	deviation of the septum

The maximum total score for the turbinates and the nasal septum is 18.

The test is not valid if fewer than 80 per cent of the progeny of each litter of the unvaccinated breeder pigs have a total score of at least 10. The vaccine complies with the test if a significant reduction in the total score has been demonstrated in the group from the vaccinated breeder pigs compared to that from the unvaccinated breeder pigs.

2-4-3-2 Vaccines containing P. multocida *dermonecrotic exotoxin (with or without cells of* P. multocida*) and cells and/or antigenic components of* B. bronchiseptica. Use not fewer than 24 breeder pigs. Vaccinate not fewer than 12 randomly chosen pigs at the stage of pregnancy or non-pregnancy and according to the schedule to be recommended. Maintain not fewer than 12 pigs as controls. From birth allow all the piglets from the vaccinated and unvaccinated breeder pigs to feed from their own dam. Using groups of not fewer than 6 pigs, constitute from their progeny 2 challenge groups from vaccinated pigs and 2 groups from control pigs each group consisting of not fewer than 30 piglets chosen randomly, taking not fewer than 3 piglets from each litter. On the 2 consecutive days preceding challenge, the mucosa of the nasal cavity of the piglets may be treated by instillation of 0.5 mL of a solution of acetic acid (10 g/L $C_2H_4O_2$) in isotonic buffered saline pH 7.2. For a group of piglets from not fewer than 6 vaccinated pigs and a group from not fewer than 6 controls, challenge each piglet by the intranasal route at 10 days of age with a sufficient quantity of a toxigenic strain of *P. multocida*. For the other group of piglets from not fewer than 6 vaccinated pigs and the other group from not fewer than 6 controls, challenge each piglet at 7 days of age by the intranasal route with a sufficient quantity of *B. bronchiseptica*. In addition, challenge each piglet at 10 days of age by the intranasal route with a sufficient quantity of a toxigenic strain of *P. multocida*. At the age of 42 days, euthanise the piglets of the 4 groups and dissect the nose of each of them transversally at premolar-1. Examine the ventral and dorsal turbinates and the nasal septum for evidence of atrophy or distortion and grade the observations on the scale described above.

The test is not valid if fewer than 80 per cent of the progeny of each litter of the unvaccinated breeder pigs have a total score of at least 10. The vaccine complies with the test if a significant reduction in the total score has been demonstrated in the groups from the vaccinated breeder pigs compared to the corresponding group from the unvaccinated breeder pigs.

2-5 MANUFACTURER'S TESTS

2-5-1 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 not fewer than 7 pigs not less than 3 weeks old and that do not have antibodies against the components of the vaccine. Vaccinate not fewer than 5 pigs by a recommended route and according to the recommended schedule. Maintain not fewer than 2 pigs of the same origin as controls under the same conditions. Alternatively, if the nature of the antigens allows reproducible results to be obtained, a test in laboratory animals that do not have antibodies against the components of the vaccine 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 quantity of vaccine. For each quantity of vaccine, carry out the test as follows: vaccinate not fewer than 5 animals with a suitable quantity of vaccine. Maintain not fewer than 2 animals of the same species and origin 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 administration, collect blood from each animal and prepare serum samples. Use a validated test such as an enzyme-linked immunosorbent assay to measure the antibody response to each of the antigens stated on the label.

The test is not valid if there is a significant antibody titre in the controls. The vaccine complies with the test if the antibody responses of the vaccinated animals are not significantly less than those obtained with a batch of vaccine that has given satisfactory results in the test or tests (as applicable) described under Potency.

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 prevaccination antibody titres and to establish the acceptable minimum antibody titre rise after vaccination in relation to these.

2-5-2 Bacterial endotoxins

A test for bacterial endotoxins (*2.6.14*) is carried out on the batch 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 shown satisfactory in safety test 2-4-2-1 given 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 pig; the vaccine complies with the test if the average temperature increase for all pigs 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 testing of each batch.

3 BATCH TESTS

3-1 Identification

In animals that do not have specific antibodies against the antigens 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 not fewer than 2 pigs that do not have antibodies against *P. multocida* and that preferably do not have antibodies against *B. bronchiseptica*. 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 tests mentioned under Immunogenicity (section 2-4-3) when administered by a recommended route and method.