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Bovine Leptospirosis Vaccine (Inactivated)

General Notices

(Ph. Eur. monograph 1939)

Ph Eur

1 DEFINITION

Bovine leptospirosis vaccine (inactivated) is a preparation of inactivated whole organisms and/or antigenic extract(s) of one or more suitable strains of one or more of *Leptospira borgpetersenii* serovar hardjo, *Leptospira interrogans* serovar hardjo or other *L. interrogans* serovars, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of cattle against leptospirosis.

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 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 harvest is inactivated by a suitable method. The antigen may be concentrated. The vaccine may be adjuvanted.

2-2 CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the cattle 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 in cattle of each category for which the vaccine is intended (for example, young calves, pregnant cattle). 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 cattle that do not have antibodies against *L. borgpetersenii* serovar hardjo and the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). 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 cattle at least daily for at least 14 days after the last administration. Record body temperatures the day before each vaccination, at vaccination, 4 h later and daily for 4 days.

The vaccine complies with the test if no animal shows abnormal local or systemic reactions, signs of disease, or dies from causes attributable to the vaccine.

2-2-1-2 Field studies. The cattle used for the field trials are also used to evaluate safety. Use not fewer than 3 groups of 20 cattle with corresponding groups of not fewer than 10 controls in 3 different locations. Examine the injection sites for local reactions after vaccination. Record body temperatures the day before vaccination, at vaccination and on the 2 days following vaccination.

The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine. In addition, if the vaccine is for use in pregnant cattle, no adverse effects on the pregnancy and offspring are noted.

2-2-2 Immunogenicity

Carry out a separate test for each of the serovars for which a claim is made for a beneficial effect on the rates of infection and urinary excretion. If claims are to be made for protection against reproductive or production losses, further specific studies will be required.

Each test is carried out for each route and method of administration to be recommended, using in each case cattle of the minimum age to be recommended for vaccination. The vaccine administered to each animal is of minimum potency.

2-2-2-1 Immunogenicity against L. borgpetersenii serovar hardjo. Use not fewer than 15 cattle that do not have antibodies against *L. borgpetersenii* serovar hardjo and the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Vaccinate not fewer than 10 cattle according to the schedule to be recommended. Maintain not fewer than 5 cattle as controls. 20-22 days after the last vaccination, challenge all the cattle by a suitable mucosal route with a sufficient quantity of a virulent strain of the relevant serovar. Observe the cattle at least daily for a further 35 days. Collect urine samples from each animal on days 0, 14, 21, 28 and 35 post-challenge. Euthanise surviving cattle at the end of the observation period. Carry out post-mortem examination on any animal that dies and on those euthanised at the end of the observation period. In particular, examine the kidneys for macroscopic and microscopic signs of leptospira infection. A sample of each kidney is collected and each urine and kidney sample is tested for the presence of the challenge organisms by re-isolation or by another suitable method.

For the test conducted with *L. borgpetersenii* serovar hardjo, control cattle are regarded as infected if the challenge organisms are re-isolated from at least 2 samples. The test is not valid if infection has been established in fewer than 80 per cent of the control cattle.

The vaccine complies with the test if the challenge organisms are re-isolated from any urine or kidney sample from not more than 20 per cent of the vaccinated cattle.

2-2-2-2 Immunogenicity against other leptospira species. For leptospiral species other than *L. borgpetersenii* serovar hardjo, the test is conducted as described in section 2-2-2-1 but urine samples are collected on appropriate days, determined by the characteristics of the challenge model. In the case of serovars for which there is published evidence that the serovar has a lower tropism for the urinary tract, a lower rate of infection may be justified. Depending on their tissue tropism, for some leptospira serovars, samples from other tissues/body fluids can be used to establish whether the cattle are infected or not by the challenge organism.

2-3 MANUFACTURER'S TESTS

2-3-1 Residual live bacteria

Carry out a test for residual live leptospirae by inoculation of a specific medium. Inoculate 1 mL of the bulk antigen into 100 mL of the medium. Incubate at 30 °C for 14 days, subculture into a further quantity of the medium and incubate both media at 30 °C for 14 days: the bulk antigen complies with the test if no growth occurs in either medium. At the same time, carry out a control test by inoculating a further quantity of the medium with the bulk antigen together with a quantity of a culture containing approximately 100 leptospirae and incubating at 30 °C: the test is not valid if growth of leptospirae does not occur within 14 days.

2-3-2 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 tests may be used. However, in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, an alternative validated method, preferably the 1st option proposed below, should be used for routine testing.

2-3-2-1

For each of the serovars present in the vaccine, a suitable validated *in vitro* test may be carried out to determine the content of one or more antigenic components which are indicators of protection and which are specific for that serovar. The criteria for acceptance are set with reference to a batch of vaccine that has given satisfactory results in the test described under Potency. Methods using lipopolysaccharide (LPS)-based antigen quantification have been shown to be suitable.

2-3-2-2

For each of the serovars for which protection is claimed, the antibody response from vaccinated animals is measured. Use not fewer than 12 guinea-pigs weighing 250-350 g that do not have antibodies against *L. borgpetersenii* serovar hardjo and the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis) and that have been obtained from a regularly tested and certified leptospira-free source. The dose to be administered to the guinea-pigs is that fraction of a cattle dose which has been shown in the validation studies to provide a suitably sensitive test. Vaccinate each of 10 guinea-pigs with the suitable dose. Maintain not fewer than 2 guinea-pigs as controls. At a given interval within the range of 19-23 days after the injection, collect blood from each guinea-pig and prepare serum samples. Use a suitable validated method such as a micro-agglutination test to measure the antibodies in each sample.

The vaccine complies with the test if antibody levels are equal to or greater 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.

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigen or antigens stated under Definition. If test 2-3-2-1 is used for the batch potency test, it also serves to identify the vaccine.

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 live bacteria

This test may be omitted for batch release, as stated in the monograph Vaccines for veterinary use (0062).

Carry out a test for residual live leptospirae by inoculation of a specific medium. Inoculate 1 mL of the vaccine into 100 mL of the medium. Incubate at 30 °C for 14 days, subculture into a further quantity of the medium and incubate both media at 30 °C for 14 days: the vaccine complies with the test if no growth occurs in either medium. At the same time, carry out a control test by inoculating a further quantity of the medium with the vaccine together with a quantity of a culture containing approximately 100 leptospirae and incubating at 30 °C: the test is not valid if growth of leptospirae does not occur within 14 days.

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.