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Canine Leptospirosis Vaccine (Inactivated)

General Notices

(Ph. Eur. monograph 0447)

Ph Eur

1 DEFINITION

Canine 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 interrogans* serovar canicola, serovar icterohaemorrhagiae or any other epidemiologically appropriate serovar, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of dogs 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 harvests are collected separately and 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 dogs 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

Carry out the test for each route and method of administration to be recommended for vaccination and in dogs of each category for which the vaccine is to be intended, using in each case dogs not older than the minimum age to be recommended for vaccination. 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 dogs that do not have antibodies against the principal *L. interrogans* serovars (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Administer to each dog 1 dose of the vaccine. If the schedule to be recommended requires a 2nd dose, administer 1 dose after the recommended interval. Observe the dogs 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 dog shows abnormal local or systemic reactions, signs of disease or dies from causes attributable to the vaccine.

2-2-2 Immunogenicity

For each type of the serovars against which protective immunity is claimed on the label, carry out a separate test with a challenge strain representative of that serovar.

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

Use for the test not fewer than 12 dogs that do not have antibodies against the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis). Vaccinate not fewer than 6 dogs, according to the schedule to be recommended. Maintain not fewer than 6 dogs as controls. Challenge each dog after 25-28 days by the conjunctival and/or intraperitoneal route with a sufficient quantity of a suspension of the relevant pathogenic *L. interrogans* serovar. Observe the dogs at least daily for 28 days after challenge.

Examine the dogs daily and record and score clinical signs observed post-challenge and any deaths that occur. If a dog shows marked signs of disease, it is euthanised. Monitor body temperatures each day for the first week after challenge. Collect blood samples from each dog on days 0, 2, 3, 4, 5, 8 and 11 post challenge. Collect urine samples from each dog on days 0, 3, 5, 8, 11, 14, 21 and 28 post challenge. Euthanise surviving dogs at the end of the observation period. Carry out post-mortem examination on any dog that dies during the observation period and on the remainder when euthanised at the end of the observation period. In particular, examine the liver and kidneys for macroscopic and microscopic signs of leptospira infection. Take a sample of each kidney and test each blood, urine and kidney sample for the presence of challenge organisms by re-isolation or by another suitable method. Analyse blood samples to detect biochemical and haematological changes indicative of infection and score these.

The test is not valid if: samples give positive results on day 0; *L. interrogans* serovar challenge strain is re-isolated from or demonstrated by another suitable method to be present in fewer than 2 samples on fewer than 2 different days, to show infection has been established in fewer than 80 per cent of the control dogs.

The vaccine complies with the test if: at least 80 per cent of the vaccinates show no more than mild signs of disease (for example, transient hyperthermia) and, depending on the *L. interrogans* serovar used for the challenge, one or more of the following is also shown:

— where the vaccine is intended to have a beneficial effect against signs of disease, the clinical scores and haematological and biochemical scores are statistically lower for the vaccinates than for the controls,

— where the vaccine is intended to have a beneficial effect against infection, the number of days that the organisms are detected in the blood is statistically lower for the vaccinates than for the controls,

— where the vaccine is intended to have a beneficial effect against urinary tract infection and excretion, the number of days that the organisms are detected in the urine and the number of kidney samples in which the organisms are detected is statistically lower for the vaccinates than for the controls.

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 A suitable validated sero-response test may be carried out. Vaccinate each animal in a group of experimental animals with a suitable dose. Collect blood samples after a suitable, fixed time after vaccination. For each of the serovars present in the vaccine, an *in vitro* test is carried out on individual blood samples to determine the antibody response to 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.

2-3-2-3 If leptospira from more than one serovar (for example *L. interrogans* serovar canicola and serovar icterohaemorrhagiae) has been used to prepare the vaccine, carry out a batch potency test for each serovar against which protective immunity is claimed on the label. Use for the test 10 healthy hamsters not more than 3 months old, that do not have antibodies against the principal serovars of *L. interrogans* (icterohaemorrhagiae, canicola, grippotyphosa, sejroe, hardjo, hebdomonadis, pomona, australis and autumnalis) and which have been obtained from a regularly tested and certified leptospira-free source. Administer 1/40 of the dose for dogs by the subcutaneous route to 5 hamsters. Maintain 5 hamsters as controls. Challenge each hamster after 15-20 days by the intraperitoneal route with a sufficient quantity of a virulent culture of leptospirae of the serovar against which protective immunity is claimed on the label. The vaccine complies with the test if not fewer than 4 of the 5 control hamsters die showing typical signs of leptospira infection within 14 days of receiving the challenge suspension and if not fewer than 4 of the 5 vaccinated hamsters remain in good health for 14 days after the death of 4 control hamsters.

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.