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Salmonella Typhimurium Vaccine (Live Oral) for Chickens

General Notices

(Ph. Eur. monograph 2521)

Ph Eur

1 DEFINITION

Salmonella Typhimurium vaccine (live, oral) for chickens is a preparation of a suitable strain of live *Salmonella enterica* Typhimurium. This monograph applies to vaccines intended for the active immunisation of chickens against colonisation by and faecal excretion of *S. enterica* Typhimurium.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The vaccine strain is cultured in a suitable medium. During production, various parameters such as growth rate are monitored by suitable methods; the values are within the limits approved for the particular vaccine. Purity and identity of the cultures are verified on the harvest using a combination of methods such as morphological, serological and biochemical methods and culture on appropriate selective media. Suitable test(s) are conducted to confirm the presence of relevant marker(s). The harvests are shown to be pure and the results obtained from the tests for identity are in accordance with the documented characteristics of the strain.

2-2 CHOICE OF VACCINE STRAIN

The vaccine strain is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the chickens for which it is intended. During development, the safety of the vaccine for the persons handling the vaccine or vaccinated chickens has to be addressed as well as, in accordance with the requirements of general chapter 5.2.6, the safety of the spread of the vaccine to other susceptible species. The strain has a stable marker or markers to distinguish it from wild-type strains.

The following tests described under General safety (section 2-2-1-1), Excretion, duration of excretion and survival in the environment (section 2-2-1-2), Spread of the vaccine strain (section 2-2-1-3), Dissemination and survival of the vaccine strain in vaccinated chickens after each vaccination (section 2-2-1-4), Increase in virulence (section 2-2-1-5), Field trials (section 2-2-1-6), Immunogenicity (sections 2-2-2-1 and 2-2-2-2) may be used during the demonstration of safety and efficacy.

For vaccines intended to prevent and/or reduce colonisation by and faecal excretion of *S. enterica* Typhimurium, the test for immunogenicity (section 2-2-2-1) is suitable to demonstrate that the vaccine is suitably immunogenic.

When the vaccine is recommended for use in laying chickens, the continuing immunogenicity of the vaccine until the end of the laying period has to be demonstrated and the test for immunogenicity at the end of the laying period (section 2-2-2-2) is suitable.

2-2-1 Safety

Unless otherwise indicated below, carry out each test by the oral route of administration, using chickens from a flock free from specified pathogens (SPF) (5.2.2) not older than the minimum age to be recommended for vaccination and that are free from antibodies against *Salmonella* spp. Where the vaccine is recommended for administration to 1-day-old chickens, the vaccine is administered before food is provided. Use vaccine bacteria at the least attenuated passage level that will be present in a batch of vaccine.

Measures taken to ensure absence of contamination by *Salmonella* spp. from the environment before the start of the test and on a regular ongoing basis are described and justified.

Whenever possible, items taken into the facilities are sterilised.

For re-isolation of the vaccine strain, suitably sensitive validated methods that are optimal for the vaccine strain concerned are used. The presence of relevant markers is confirmed to demonstrate that the organisms isolated are vaccine-derived and not wild-type contaminants.

2-2-1-1 General safety. For each test performed in chickens younger than 3 weeks of age, use not fewer than 10 chickens that are free from antibodies against *Salmonella* spp. Administer orally to each chicken a quantity of the vaccine strain equivalent to not less than 10 times the maximum titre(s) likely to be contained in 1 dose of the vaccine. Observe the chickens at least daily for at least 14 days.

The test is not valid if more than 10 per cent of the chickens younger than 3 weeks of age show abnormal signs or die from causes not attributable to the vaccine.

The vaccine complies with the test if no chicken shows abnormal signs of disease or dies from causes attributable to the vaccine.

2-2-1-2 Excretion, duration of excretion and survival in the environment. The same animals can be used for the test for spread of the vaccine strain (section 2-2-1-3) provided they are of the minimum age to be recommended for vaccination. Use for the test not fewer than 10 chickens. Administer orally to each chicken a quantity of the vaccine equivalent to not less than the maximum titre of the strain under study likely to be contained in 1 dose of the vaccine. Samples are collected for reisolation of vaccine from cloacal swabs from each chicken and floor faeces on days 3, 7, 10 and 14 after vaccination and then weekly until 3 consecutive negative weekly samples are obtained from all vaccinated chickens. Samples are collected for re-isolation of the vaccine strain from the caeca of vaccinates at the end of the test.

The test is not valid if more than 10 per cent of vaccinated chickens show abnormal clinical signs or die from causes not attributable to the vaccine. The results are noted and used to formulate the label statement on the length of time of excretion of the vaccine strain.

2-2-1-3 Spread of the vaccine strain. The same animals can be used for the test for excretion, duration of excretion and survival in the environment (section 2-2-1-2). Use for the test not fewer than 10 chickens of the minimum age to be recommended for vaccination. Use 10 chickens as controls. Administer orally to each chicken a quantity of the vaccine strain equivalent to not less than the maximum titre of the strain likely to be contained in 1 dose of the vaccine. 1 day after vaccination, mix the 10 vaccinates with at least 10 non-vaccinated chickens of the same age and source. Samples are collected for the re-isolation of the vaccine strain from cloacal swabs from each chicken and floor faeces on days 3, 7, 10 and 14 after vaccination and then weekly until 3 consecutive negative weekly samples are obtained from all chickens. Collect samples of the caeca and spleens for re-isolation of the vaccine strain from 10 in-contact control chickens at the end of the test. The results are noted and used to formulate the label statement on the extent to which the vaccine spreads to in-contact non-vaccinated chickens.

2-2-1-4 Dissemination and survival of the vaccine strain in vaccinated chickens after each vaccination. Conduct the test after each vaccination as prescribed by the vaccination schedule to be recommended in chickens of each category for which the vaccine is intended with oral administration of the test preparation. Use a sufficient number of chickens to conduct the sampling described below, the number of chickens required being dependent on the number of vaccinations recommended, the interval between vaccinations and the length of time chickens are maintained after the last vaccination. Administer to each chicken a quantity of the vaccine strain equivalent to not less than the maximum titre of the strain under study likely to be contained in 1 dose of the vaccine. Collect cloacal swabs from each chicken for re-isolation of vaccinal bacteria on days 7 and 14 after each vaccination and at later appropriate stages and with sufficient frequency to determine the duration of dissemination.

For example, for broilers, samples are collected from 5 chickens for re-isolation of the vaccine strain on days 7 and 14 after each vaccination and weekly until 8 weeks of age. At the 7- and 14-day sampling points, samples are taken from the liver, caecum and spleen of 5 chickens.

In the case of chickens intended for laying, samples are collected from 5 chickens on days 7 and 14 after each vaccination and weekly until 3 consecutive negative weekly samples are obtained or the time of the next vaccination is reached, whichever is the sooner. At the 7- and 14- day sampling points, samples are taken from the liver, caecum and spleen of 5 chickens. In addition, samples are collected from ovaries and oviducts where dissemination in vaccinated future layers is being investigated.

The test is not valid if more than 10 per cent of vaccinated chickens in any group show abnormal clinical signs or die from causes not attributable to the vaccine. The results are noted and used to formulate the label statement on the length of time the vaccine strain survives in the body and to define a suitable withdrawal period.

2-2-1-5 Increase in virulence. Carry out the test according to general chapter 5.2.6 using SPF chickens (5.2.2) not older than the minimum age to be recommended for vaccination.

Administer orally to each chicken of the 1st group a quantity of the vaccine strain under study that will allow recovery of bacteria for the passages described below. 4 to 7 days after administration of the vaccine strain, prepare a suspension from the liver, spleen and caecum of chickens and pool these samples. Administer pooled samples orally to each chicken of the next group. Carry out this passage operation not fewer than 4 times; verify the presence of the bacteria at each passage. If the bacteria are not found at a passage level, repeat the passage by administration to a group of 10 chickens. Any mortalities are investigated for the presence of the vaccine strain and the properties of any re-isolated vaccine strain determined.

Carry out the test for excretion, duration of excretion and survival in the environment (section 2-2-1-2) and, if the last group of birds from which the bacteria was recovered shows evidence of an increase in virulence indicative of reversion during the observation period, carry out the test for general safety (section 2-2-1-1), using the material used for the 1st passage and the bacteria at the last passage level where it was recovered. Test the bacteria recovered for the final passage for the presence and stability of the marker(s).

The vaccine strain complies with the test if no indication of increased virulence of the bacteria recovered for the final passage compared with the material used for the 1st passage is observed and the presence of the marker(s) is confirmed in the bacteria recovered for the final passage and remains identical to the material used for the 1st passage. If the bacteria are not recovered after an initial passage in 5 animals and a subsequent repeat passage in 10 animals, the vaccine also complies with the test.

2-2-1-6 Field trials. The chickens used for field trials are also used to evaluate safety. A trial is carried out in each category of chickens for which the vaccine strain is intended, in not fewer than 2 sets of premises. Samples are taken from a significant number of chickens for re-isolation of bacteria to provide information on the persistence, dissemination and spread of the bacteria, which can be used, with the data from the laboratory studies, to formulate the statements on the label. The samples include cloacal swabs, floor faeces, spleen and liver and, in laying chickens, samples of ovaries and oviducts. Environmental samples are also tested at regular intervals.

2-2-2 Immunogenicity

The tests described in section 2-2-2-1 and, if appropriate, in section 2-2-2-2, are carried out using chickens not older than the minimum age to be recommended for vaccination and that are free from antibodies against *Salmonella* spp. The quantity of the vaccine strain to be administered orally to each chicken is not greater than the minimum titre to be stated on the label.

Measures taken to ensure absence of contamination by *Salmonella* spp. from the environment before the start of the test and on a regular ongoing basis are described and justified.

Suitably sensitive validated methods are used for re-isolation of bacteria derived from the challenge and for distinguishing these from the vaccine strain.

2-2-2-1 Immunogenicity. Use for the test not fewer than 40 chickens of the same origin and from an SPF flock (5.2.2). Vaccinate according to the schedule to be recommended not fewer than 20 chickens with a single dose of vaccine. Maintain not fewer than 20 chickens as controls. Challenge each chicken after 14 days by a suitable route with a sufficient quantity of a virulent strain of *S. enterica* Typhimurium to give a valid test. Collect cloacal swabs from vaccinates and controls on days 3, 5, 7, 10 and 14 post-challenge. Samples of caecum, liver and spleen are collected from 10 chickens of each group on days 7 and 14 post-challenge for re-isolation of challenge bacteria. Collect the same samples of internal organs from any chicken that dies. Examine the samples for the presence of the challenge organisms using a suitable sensitive culture medium and compare results for vaccinates and controls.

The test is not valid if, during the observation period, fewer than 80 per cent of the control chickens die or challenge organisms are re-isolated from fewer than 80 per cent of the control chickens.

The vaccine complies with the test if there is a significant reduction in the number of cloacal swabs from vaccinates containing challenge organisms compared with the number from the controls and there is a significant reduction in the number of samples from internal organs from vaccinates containing challenge bacteria compared with the number from the controls.

2-2-2-2 Immunogenicity at the end of the laying period. Use for the test not fewer than 40 chickens of the same origin and from an SPF flock (5.2.2). Vaccinate not fewer than 20 chickens according to the schedule to be recommended. Maintain not fewer than 20 chickens as controls. At the end of the laying period, take serum samples and cloacal swabs

from the chickens and environmental samples from the housing area. Test each serum sample individually for the presence of antibodies to *S. enterica* Typhimurium and each cloacal swab and fresh environmental sample for the presence of *Salmonella* spp. Challenge each chicken by a suitable route with a sufficient quantity of a virulent strain of *S. enterica* Typhimurium to give a valid test. Collect cloacal swabs from vaccinates and controls on days 3, 5, 7, 10 and 14 post-challenge. Samples of caecum, liver, spleen, ovaries and oviducts are collected from 10 chickens of each group for re-isolation of challenge bacteria on days 7 and 14 post-challenge. Collect the same samples of internal organs from any chicken that dies during the observation period. Examine the samples for the presence of the challenge organisms with growth in a suitable medium and compare results for vaccinates and controls.

The test is not valid if, before the challenge, antibodies to *Salmonella* spp. are found in the serum of the controls or *Salmonella* spp. bacteria are isolated from any of the chickens. The test is also not valid if the challenge organisms are re-isolated from fewer than 80 per cent of the control chickens.

The vaccine complies with the test if there is a significant reduction in the number of cloacal swabs from vaccinates containing challenge organisms compared with the number from the controls and there is a significant decrease in the number of samples of internal organs from vaccinates containing challenge bacteria compared with the number from the controls.

3 BATCH TESTS

3-1 Identification

The strain present in the vaccine is identified by a combination of methods such as morphological, serological and biochemical methods and culture on appropriate selective media. Suitable test(s) are conducted to confirm the presence of the relevant marker(s).

3-2 Bacteria and fungi

Carry out the test by microscopic examination and by inoculation of suitable media, or verify the absence of micro-organisms other than the vaccine strain present in the vaccine as described in the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*. The vaccine complies with the test if it does not contain extraneous micro-organisms.

Any diluent supplied for reconstitution of the vaccine complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3 Live bacteria

Titrate the vaccine strain using a suitable medium for the culture of the strain. The vaccine complies with the test if it contains not less than the titre stated on the label.

3-4 Potency

The vaccine complies with the requirements of the test prescribed under Immunogenicity (section 2-2-2-1) when administered by a recommended route and method. It is not necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a representative batch using a vaccinating dose containing not more than the minimum titre stated on the label.

4 LABELLING

The label states:

— the nature of the markers allowing the vaccine to be distinguished from wild-type strains;

— the extent to which the vaccine spreads and is transmitted to non-vaccinated chickens and the time over which this could occur;

— the time that the vaccine survives in the body;

— the length of time of excretion and the time that the vaccine survives in the environment;

— the potential for spread to other susceptible species including humans;

— the withdrawal period.

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