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Mycoplasma Gallisepticum Vaccine for Chickens, Living

General Notices

(Mycoplasma Gallisepticum Vaccine (Live) for Chickens, Ph. Eur. monograph 3133)

Ph Eur

1 DEFINITION

Mycoplasma gallisepticum Vaccine (live) is a preparation of one or more suitable strains of *Mycoplasma gallisepticum*. This monograph applies to vaccines intended for the active immunisation of chickens intended for laying and breeding.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

The vaccine strain is grown in a suitable solid or liquid medium that ensures optimal growth under the chosen incubation conditions. Each strain is cultivated separately and identity is verified using a suitable method.

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 chickens 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

Unless otherwise indicated below, each test is carried out for each route of administration to be recommended for vaccination and using chickens from a flock free from specified pathogens (SPF) (*5.2.2*) not older than the minimum age to be recommended for vaccination. Use a batch of vaccine containing the least attenuated passage level that will be present in a batch of vaccine and with not less than the maximum titre that may be expected in a batch of vaccine. For re-isolation of the vaccine strain, suitably sensitive validated methods are used.

2-2-1-1 General safety. Carry out the test for each route and method of administration to be recommended for vaccination.

For each test performed in chickens younger than 3 weeks of age, use not fewer than 10 chickens. For each test performed in chickens older than 3 weeks of age, use not fewer than 8 chickens. Administer to each chicken by a route and method to be recommended, a quantity of the vaccine strain equivalent to not less than 10 times the maximum titre likely to be contained in 1 dose of the vaccine. Observe the birds 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 of disease or die from causes not attributable to the vaccine. For chickens older than 3 weeks of age, the test is not valid if non-specific mortality occurs.

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

2-2-1-2 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. If the properties of the vaccine strain allow sequential passage through 5 groups via natural spreading, this method may be used, otherwise passage as described below is carried out.

Administer by the route and method to be recommended for vaccination, to each chicken of the 1st group, a quantity up to the maximum titre of the strain under study likely to be contained in 1 dose of the vaccine, at the least attenuated passage level that will be present in a batch of vaccine, to provide greatest opportunity for recovery of the bacteria for the passages described below. On the day of highest excretion after administration of the vaccine strain, introduce a fully susceptible youngest age group of birds and allow comingling for not less than 24 hours so that natural spread can occur. 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 birds. Any mortalities are investigated for the presence of the vaccine strain and the properties of any re-isolated vaccine strain are determined.

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), as appropriate.

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) (where appropriate) is confirmed in the bacteria recovered for the final passage and remains identical to the material used for the 1st passage. If 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.

2-2-1-3 Examination of reproductive performance. Transmission of field strains of *M. gallisepticum* to eggs has been shown in breeder flocks and can result in infertile eggs, embryo death and reduced egg production, as well as vertical transmission to progeny flocks. Studies of reproductive performance, including relevant parameters such as egg production, must be performed by laboratory studies and/or by a combined safety and efficacy field study.

2-2-2 Immunogenicity

A test is carried out for each route and method of administration to be recommended for vaccination. The quantity of vaccine strain to be administered to each chicken is not greater than the minimum mycoplasma titre to be stated on the label and the strain is at the most attenuated passage level that will be present in a batch of vaccine.

Use for each test not fewer than 20 SPF chickens (5.2.2) not older than the minimum age to be recommended for vaccination. Vaccinate not fewer than 15 chickens according to the schedule to be recommended. Maintain not fewer than 5 chickens as controls. Challenge each bird from both groups not more than 28 days after the last administration of vaccine by a suitable route with a sufficient quantity of virulent *M. gallisepticum* (R-strain or equivalent). Observe the chickens at least daily for 14 days after challenge. Record the deaths and the number of surviving chickens that show clinical signs of disease (e.g. respiratory distress, nasal discharge), and record air sac lesions.

The test is not valid if:

— during the observation period after challenge, fewer than 50 per cent of the controls die or show lesions or clinical signs of disease; and/or

— during the period between vaccination and challenge, more than 10 per cent of the chickens from the control group or from the vaccinated group show abnormal clinical signs of disease or die from causes not attributable to the vaccine.

Thoracic and abdominal air sacs are evaluated individually on each side of the animal. The scoring system presented below may be used.

The vaccine complies with the test if the score for the vaccinated birds is significantly lower than that for the controls and if the reduction is not less than 30 per cent.

0	no air sac lesions
1	in a limited area of 1 or 2 air sacs: cloudiness with slight thickening of the air sac membrane or flecks of yellowish exudate
2	in 1 air sac or portions of 2 air sacs: greyish or yellow, sometimes foamy exudate, with thickening of the air sac membrane

3	in 3 air sacs: extensive exudate, with clear thickening of most air sacs
4	severe air-sacculitis with considerable exudate and thickening of most air sacs.

3 BATCH TESTS

3-1 Identification

The vaccine strains are identified by a combination of suitable methods. Suitable tests are conducted to confirm the presence of the relevant markers, where applicable. Compliance with the test is demonstrated if only the presence of the vaccine strain is detected.

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 *M. gallisepticum* 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 Freedom from contaminating mycoplasmas

The vaccine is free from extraneous mycoplasmas.

3-4 Live mycoplasma

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 minimum mycoplasma titre stated on the label.

3-5 Potency

The vaccine complies with the requirements of the test prescribed under Immunogenicity (section 2-2-2) 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 mycoplasma titre stated on the label.