

Production and efficacy of an attenuated live vaccine against contagious ovine ecthyma

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Summary

Contagious ecthyma is caused by the orf virus, a member of the family *Poxviridae*, genus *Parapoxvirus*. Morbidity in affected sheep flocks is approximately 100%, while mortality varies between 1% and 10%. A live attenuated vaccine was produced by the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'*. Quality control was performed in accordance with the *European Pharmacopoeia*. A wild virus strain was attenuated through serial passages on primary chicken embryo fibroblast tissue cultures. The virus suspension was treated according to standard procedures and freeze dried. The immunising dose was 1 ml containing $10^{4.5}$ TCID₅₀, administered intramuscularly. The safety of the vaccine was successfully tested by intramuscular inoculation of 20 susceptible sheep and 20 lambs with the routine dose, 10 times the immunising dose and two normal doses administered at seven-day intervals. The efficacy of the vaccine was tested using three groups of susceptible animals. The first group included 10 lambs and the second 10 adult sheep; the animals were immunised intramuscularly with 1 ml of the reconstituted vaccine. The third group, used as controls, included five sheep and five lambs. Serological reactivity was monitored by indirect enzyme-linked immunosorbent assay (ELISA). The animals were challenged 30 days later with a pathogenic strain administered intradermally along the labial area. Vaccinated animals did not show any clinical signs of disease, whereas all the controls developed

typical signs of contagious ecthyma. To confirm the efficacy of the vaccine, a field trial was conducted in four flocks affected by the disease. The trial showed that the vaccine was able to block the normal course of the disease and induce rapid recovery.

Keywords

Contagious ovine ecthyma, Orf, *Parapoxvirus*, *Poxviridae*, Sheep, Vaccine, Virus.

Introduction

Contagious ecthyma is a highly transmissible disease of small ruminants, especially sheep and goats. It has occasionally been described in large domestic and wild ruminants and can also affect humans. It is present worldwide, with only Madagascar, Japan, Indonesia and islands such as the Seychelles and French Polynesia being unaffected (1, 3). The disease is characterised by lesions of the skin and mucosa, especially of the lips, breasts, gums and oral cavity (8). The aetiological agent is the orf virus, a member of the family *Poxviridae*, genus *Parapoxvirus*.

In areas of high sheep and goat density, the disease tends to become endemic, causing severe economic losses (6). Morbidity can reach 100% on farms (7) while mortality is limited to 1%, although it can reach 10% in groups of stressed or immunodepressed animals (3). Although the disease affects all ages, the most severe clinical forms are observed in younger animals (2) rather than in adults, which have probably acquired some immunity from previous infections. The

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spread of the orf virus and its tendency to become endemic are related to its high resistance in the environment. In favourable conditions the virus, protected within the crust, is capable of surviving for several years. For this reason, protection by prophylaxis in areas with large sheep and goat populations is impracticable and the only possible course of action is vaccination.

The first successful attempts at specific immunisation date back to 1923. The vaccine was obtained by pulverisation and suspension in glycerine of infected crust. Vaccination was performed by scarification (9). This type of vaccination is still used by farmers, but can only be applied after infection; however, it also contributes to environmental contamination (1).

The authors describe the development of an attenuated contagious ecthyma vaccine that was produced and tested in accordance with the *European Pharmacopoeia* to protect the Italian sheep population from this disease which causes severe economic problems for farmers.

Materials and methods

Viral strain

The viral strain used for preparation of the seed, identified as 10177/TE, was isolated from pathological material obtained from sections of tissue from lip lesions in sheep from an infected flock. The tissue samples were suspended in phosphate buffered saline (PBS), homogenised and then filtered through a 0.22 µm membrane. The viral suspension was then used to infect primary fibroblast monolayers of chicken embryos raised from specific pathogen-free (SPF) eggs. Once 70% cytopathic effect (CPE) was reached, the material was collected, aliquoted and frozen at -70°C at +10°C to provide the working stock. One aliquot was taken before freezing for identification, purity testing and confirmation of the absence of bacterial and mycoplasma contaminants. The infectious titre, tested on chicken embryo fibroblasts, was 10^{4.5} TCID₅₀/ml.

Attenuation of viral strain, attenuation level testing and reactivation tests

An aliquot of the working stock was attenuated by 10 serial passages on primary chicken embryo fibroblasts (10). On the tenth passage, a reference stock with a titre of 10^{4.2} TCID₅₀/ml was produced, aliquoted and freeze dried (master seed).

The attenuation level was tested by intradermal inoculation of a number of points of the labial commissure in two ecthyma virus seronegative lambs that were born from ewes raised at the *Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'*.

Samples of erythematous labial tissue from the inoculation sites were harvested by biopsy from the animals under anaesthesia. The harvested tissue was homogenised in PBS and filtered through a 0.22 µm membrane. It was then used for a subsequent passage in two lambs and for virus isolation on a cell substrate. To exclude reactivation and confirm the presence of the aetiological agent, six serial passages were performed using the above method.

A master seed aliquot was amplified on primary chicken embryo fibroblast monolayers and subjected to freeze drying, to make up the working stock (working seed).

Production of experimental vaccine

The working seed was used to produce a batch of experimental vaccine. The virus was amplified on primary chicken embryo fibroblast monolayers. The viral suspension collected at 70% CPE was centrifuged at 3 000 rpm for 30' at +4°C and the supernatant was collected, titrated and subsequently diluted and freeze dried following the 1:1 v/v addition of stabilising solution.

The freeze-dried vaccine, identified as 1/96, had a titre of 10^{4.5} TCID₅₀/dose. The finished product was subjected to the tests specified in the *European Pharmacopoeia* (4) for the production of live attenuated virus vaccines (identification, purity, absence of extraneous viruses, sterility and absence of mycoplasma) and passed all tests.

Safety testing

Safety was evaluated by administering a single dose, an overdose and repeated doses, where a 1 ml single dose had a titre of $10^{4.5}$ TCID₅₀.

A total of 20 lambs aged between 15 and 20 days and 20 gestating ewes in the second term of pregnancy, all seronegative for anti-ecthyma antibodies, were selected. The animals were divided into four groups consisting of five lambs and five gestating ewes.

Per single dose

Each animal in group 1 received one vaccine dose intramuscularly into the right shoulder.

Per overdose

Each animal in group 2 received 10 vaccine doses intramuscularly into the right shoulder. To avoid the administration of 10 ml of vaccine, considered excessive for lambs aged 10 days, the freeze-dried vaccine was reconstituted so as to provide 10 doses in 2 ml of diluent.

Per repeated dose

Each subject in group 3 received one dose of vaccine intramuscularly into the right shoulder at time zero and a second dose one week after the first.

Controls

Group 4 subjects were not treated but were kept in contact with the animals of the other three groups to reveal any transmission of the vaccine virus strain.

Animals in the four groups were examined and their temperature taken every day for 14 days after vaccination to reveal any reactions at the site of inoculation, after-effects or other side-effects that could be attributed to the vaccine.

Samples of saliva, urine, faeces and blood were collected for ecthyma virus testing from two lambs and two ewes in group 1 (single dose) and group 2 (10-fold dose) every 24 h for the three days following treatment.

Lambs in the four groups were weighed at the beginning and end of the trial to evaluate any effect of the vaccination on weight gain.

Immunogenicity testing

A total of 1 ml of vaccine containing $10^{4.5}$ TCID₅₀ of virus was inoculated by intramuscular injection into the right shoulder of a group of 10 sheep and 10 lambs aged between 10 and 15 days and raised in isolation in the barns at the Institute.

At the same time, 1 ml of saline solution was inoculated into a control group of 5 lambs and 5 sheep in the same conditions.

Blood samples were taken from each animal at time zero and 14 and 30 days after vaccination to detect antibody response using the indirect enzyme-linked immunosorbent assay (ELISA). Absorbance values (optical density: OD) above the cut-off, set at 0.225, were considered as the index of antibody reaction.

Thirty days after vaccination, the animals that had been kept in isolation underwent challenge by intradermal labial inoculation of 100 TCID₅₀ of an ecthyma virus strain obtained by homogenisation in PBS of crusts taken from a lamb at the height of the disease.

All subjects were clinically monitored for 45 days to reveal the onset of any clinical signs.

Rectal temperature was taken daily during the first week of the test only.

Field tests

To further confirm the efficacy of the vaccine, field tests were conducted on 300 sheep from four farms where the disease was present. In each infected flock, 90% of the animals were subjected to vaccination and the remaining 10% were left as unvaccinated controls. All animals on the four farms were clinically monitored for two months after the vaccination, daily for the first week and every week thereafter.

The following parameters were considered:

- side-effects in vaccinated animals
- evolution of signs in diseased animals in the vaccinated and unvaccinated groups
- appearance of new clinical cases in the two groups.

Results

Attenuation of viral strain, attenuation level testing and reactivation tests

Areas of erythematous tissue not exceeding 5 mm in diameter and that had not developed into pustules were observed at the site of injection in all lambs inoculated with the virus after 10 passages on chicken embryo fibroblasts (5). Orf virus was isolated from all harvested biopsy samples.

Reactivation tests did not reveal any undesirable effects, enabling the attenuation of strain 10177/TE used for vaccine production to be considered stable.

Safety per single dose, per overdose and per repeated dose

No undesirable, local or general reactions were observed following the administration of the various vaccine doses.

An increase in rectal temperature in animals that received the 10-fold dose was transient and returned to normal within 24-48 h (Fig. 1).

No difference in weight gain was observed between vaccinated and control lambs.

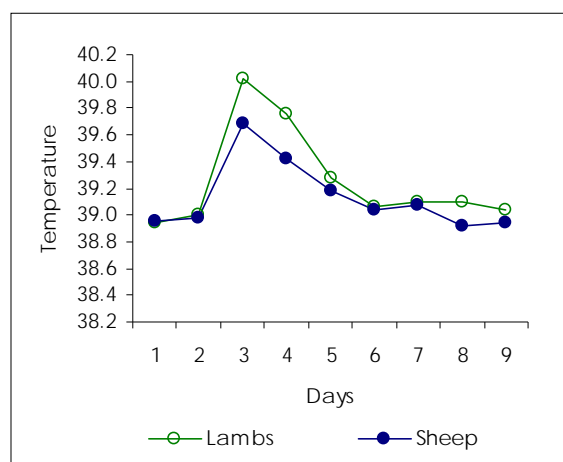


Figure 1 Mean rectal temperatures of animals inoculated with the tenfold vaccine dose

Testing for the aetiological agent of ecthyma in organic products of the sheep and lambs in groups 1 and 2 gave negative results.

From an analysis of the results, it was concluded that the vaccine was harmless for sheep.

Efficacy test

Immunogenicity testing

At time zero, all animals gave negative results for specific antibodies with the ELISA, as were the control group animals throughout the trial. Indirect ELISA results revealed antibody response in vaccinated animals from day 14 (Fig. 2).

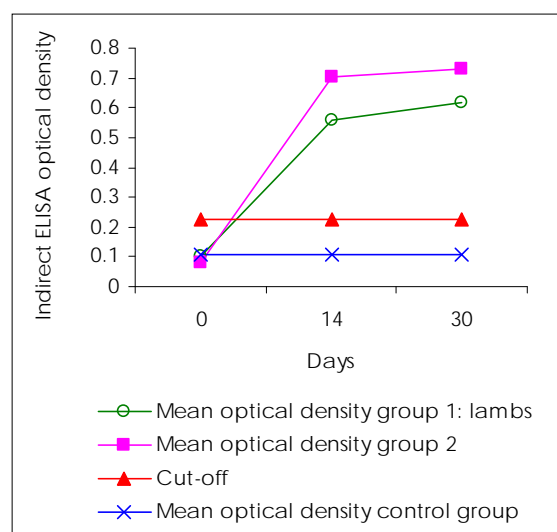


Figure 2 Mean indirect enzyme-linked immunosorbent assay optical density in vaccine immunogenicity tests

Experimental infection test

The virulent virus caused mild inflammation at the site of labial inoculation in immunised animals. This reaction arose between three and four days after infection and diminished over the following 24-48 h, without developing into scabs. Together with the inflammatory reaction, a 1°C rise above physiological temperature was observed; this persisted for 24-48 h.

The five lambs in the control group revealed clinical signs of contagious ecthyma between three and six days after infection, with pustules and papules in the labial area and, in two animals, on the inner thighs. Orf virus was isolated from pathological material taken from

the lesions. The animals survived and were clinically recovered 38 days after infection.

Field tests

A study of the evolution of clinical contagious ecthyma lesions in vaccinated animals revealed the rapid remission of clinical signs and the near absence of new cases from around day 4 post vaccination.

In the control sheep, the disease followed its typical clinical course, with the appearance of new clinical cases.

Conclusions

Preliminary tests demonstrate that following the tenth passage in chicken embryo fibroblasts, inoculation of the contagious ecthyma virus strain 10177/TE into the labial commissures of lambs that were seronegative

for the orf virus produces an erythematous area at the site of inoculation which does not develop into the lesion that is typical of the disease. Reversion to virulence tests in lambs were negative, meaning that the attenuation level could be considered stable.

Innocuity tests conducted in accordance with the *European Pharmacopoeia* with live vaccine prepared with an attenuated viral strain and administered intramuscularly gave favourable results. The vaccine induces antibody production and protects the animals on challenge in both experimental and field conditions.

An innocuous, effective vaccine for the control of contagious ecthyma is in great demand by farmers and this trial has enabled the initiation of procedures for marketing of such a vaccine.

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