Virological and serological response of sheep following field vaccination with bivalent modified-live vaccine against bluetongue virus serotypes 2 and 9

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Summary

A group of 44 sheep was vaccinated with the bivalent modified-live vaccine against bluetongue virus (BTV) serotype 2 (BTV-2) and BTV-9 to evaluate viraemia and antibody kinetics. Blood samples were taken from the sheep three times a week for two months and screened for the presence of BTV and for antibody using the competitive enzyme-linked immunosorbent assay (c-ELISA) and the virus neutralisation (VN) test. Intravenous egg inoculation, followed by two blind passages in Vero cells, was used to isolate BTV-2 and BTV-9 from the ethylene-diaminetetra-acetic acid (EDTA) blood samples; virus titres were also determined in the viraemic animals. BTV was detected in the blood of 39 sheep between day 3 and day 24 post vaccination (pv). Viraemia peaked on day 7 pv with average titres of $10^{5.3}$TCID$_{50}$/ml. Antibodies were first detected in the c-ELISA on day 6 pv and by day 16, all sheep were seropositive. Only 36 of the 44 inoculated sheep developed virus-neutralising antibodies against both BTV-2 and BTV-9 while 4 were positive to BTV-2 only; neutralising antibodies were not detected in the 4 remaining animals. Antibody titres were very low and unstable and often bordered on the negative/positive threshold.

Keywords

Bluetongue – Competitive enzyme-linked immunosorbent assay – Sheep – Vaccine – Viraemia – Virus – Virus neutralisation.

Introduction

The first isolation of bluetongue (BT) virus (BTV) in Italy led to the implementation of an intensive surveillance and monitoring programme, which eventually revealed the presence of four serotypes (BTV-2, BTV-4, BTV-9 and BTV-16) in the country. To control the spread of BTV-2 and of BTV-9 infection and to ease the pressure caused by livestock movement restrictions, a vaccination campaign, using monovalent BTV-2 or a combination of BTV-2 and BTV-9 attenuated vaccines, was initiated in 2001. The criterion for vaccine selection was based on serotype presence in the area to be vaccinated. Vaccines consisting of attenuated virus strains are highly effective, especially in epidemic situations where only one serotype of BTV is involved. Conversely, in those areas where multiple serotypes exist, the situation is more complicated. The Italian scenario required, at least in some areas, the use of bivalent vaccines despite the potential problems associated with interference between virus strains, differences in immunogenicity and growth rates between various strains, as well as differences in the response of individual animals to the components of such vaccines. Furthermore, although the vaccine viruses are attenuated, they are able to replicate and induce viraemia in the host. The purpose of this study was to evaluate viraemia and antibody kinetics in sheep after immunisation using a bivalent modified-live BTV-2 and BTV-9 vaccine. The duration of viraemia and the virus titres after immunisation were also determined. This may provide information on the importance of vaccinated sheep as a source of BTV vaccine virus.

Materials and methods

Vaccine

A combination of a BTV-2 and a BTV-9 monovalent modified-live vaccine produced by Onderstepoort Biological Products in South Africa was used in this study. Before inoculation, both serotypes were suspended in 100 ml of the appropriate diluent. A single dose of vaccine containing $10^{4.37}TCID_{50}$/ml of BTV-2 and $10^{4.24}TCID_{50}$/ml of BTV-9 was administered.

Animals

This study was conducted between May and August 2002 in the Province of L'Aquila (altitude: 1 100 m) in a flock comprising nearly 1 000 Comisana cross-bred sheep raised for both milk and meat. A group of 44 seronegative sheep were selected and vaccinated subcutaneously with a single dose of vaccine. Ethylene-diaminetetra-acetic acid (EDTA) and plain blood samples were collected from each animal three times a week for the following two months.

Virological and serological studies

EDTA blood samples were screened for the presence of BTVs and their titres measured. The competitive enzyme-linked immunosorbent assay (c-ELISA) (4) and the virus neutralisation test (1) were used to detect BTV antibodies. Intravenous egg inoculation, followed by two blind passages in Vero cells, was used to isolate BTV from EDTA blood samples according to the method described by Savini *et al.* (6). The virus titres of BTV-2 and BTV-9 were determined in viraemic animals by neutralising the samples with specific antisera. Each viraemic sample was divided into two aliquots of 1 ml each; one aliquot was mixed with an equal amount of a 1:10 dilution of BTV-2-specific antisera while the second aliquot was mixed with an equal amount of a 1:10 dilution of BTV-9-specific antisera. The samples were incubated at 37°C for 1 h and the virus titres determined as described previously (6). Virus-typing assays were also employed to verify whether specific virus neutralisation occurred.

Statistical analysis

The extent of viraemia was analysed using the Kaplan-Meier survival technique (Spss® 11.0.1) in which the event is the absence of detectable BTV-2 and/or BTV-9 viraemia. The survival probability at each time interval (i.e. the probability of an animal being viraemic at time t) was interpolated using the exponential function in Spss® 11.0.1, and the date on which the probability of being viraemic decreases below 0.01, calculated.

Results

BTV was detected in the blood of 39 animals (88.6%) from day 3 to day 24 post vaccination (pv). For both serotypes, the highest peak of viraemia was reached on day 6 pv. BTV-9 viraemic titres were much higher than those of BTV-2 (Fig. 1). Antibody was first detected using the c-ELISA on day 6 pv and by day 16 all the animals were positive. Of the 44 inoculated animals, 40 developed virus-neutralising antibodies against BTV-2 (90.9%), while only 36 developed antibody against both BTV-2 and BTV-9 (81.8%) (Figs 2 and 3). Viraemia was not detected in three of the four sheep that did not show neutralising antibodies. Moreover, in those animals with virus-neutralising antibodies, titres were very low and unstable, and were often on the borderline between the negative and positive threshold for serotype 9 (Fig. 4). Figure 5 gives the interpolation of cumulative probability of animals being viraemic after X days following vaccination. The exponential equation is as follows:

$$y = 4.5695 * e^{-0.2203\,x}$$

According to this formula, the probability of finding an animal without viraemia 28 days after immunisation is 0.99.

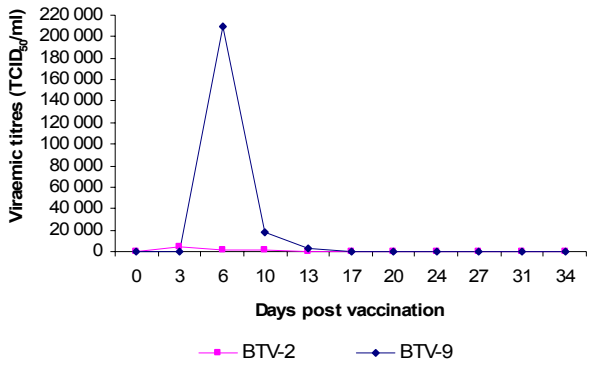

Figure 1
Titres of bluetongue virus serotype 2 (BTV-2) and BTV-9 in sheep following vaccination with a bivalent BTV-2 and BTV-9 modified-live vaccine

Discussion

Modified-live virus vaccines are produced by adapting BTV field isolates *in vitro* through serial passages in tissue culture or in embryonating chicken eggs. This process selects viruses with an increased aptitude to grow *in vitro* but possess a reduced capacity to replicate *in vivo* and to cause disease. Following serial passages, viruses are plaque purified with individually cloned viruses being selected for inoculation into sheep.

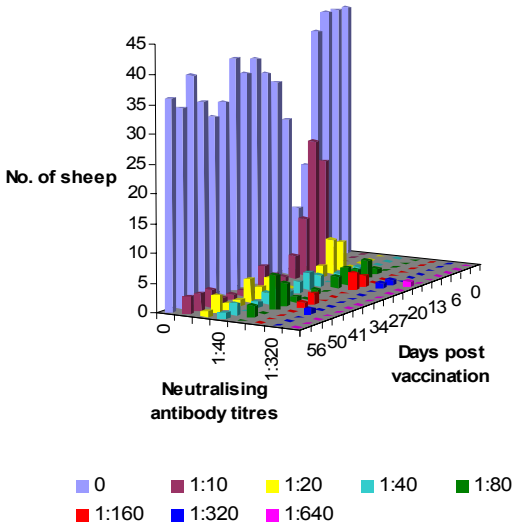

Figure 2
Bluetongue virus serotype 2 (BTV-2) neutralising antibody titres in sheep after immunisation with a bivalent BTV-2 and BTV-9 modified-live vaccine

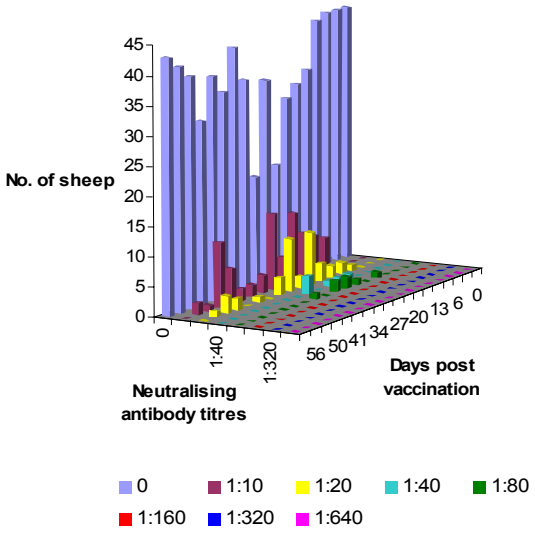

Figure 3
Bluetongue virus serotype 9 (BTV-9) neutralising antibody titres in sheep after immunisation with a bivalent BTV-2 and BTV-9 modified-live vaccine

The virus which elicits only a mild febrile reaction, and which generates titres of less than 10^3 plaque-forming units per ml (pfu/ml) at the height of viraemia, and elicits neutralising antibody, is selected for vaccine production (5). According to the results from this study, the monovalent BTV-2 and the monovalent BTV-9 modified-live vaccines, when combined, do not perform as expected. On average,

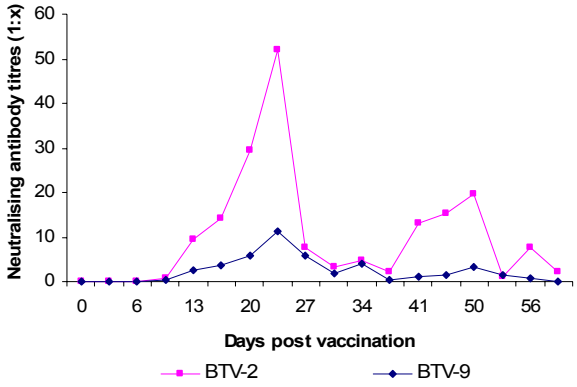

Figure 4
Mean neutralising antibody titres in sheep after immunisation with a bivalent BTV-2 and BTV-9 modified-live vaccine

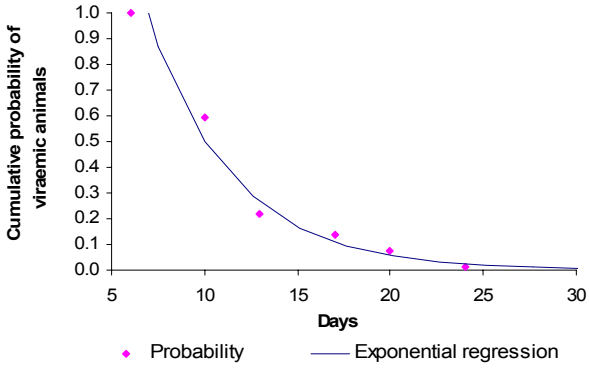

Figure 5
Interpolation of cumulative probability of sheep being viraemic after X days following vaccination with a bivalent BTV-2 and BTV-9 modified-live vaccine

titres higher than 10^5TCID$_{50}$/ml were observed for BTV-9; although lower titres were observed for BTV-2, these were still higher than 10^4TCID$_{50}$/ml. It is not known whether the higher virus titres in vaccinated sheep is a consequence of the two serotypes used in combination, or if it is due to the viruses being insufficiently attenuated during vaccine production. This study marks the first instance in which BTV-2 and BTV-9 monovalent vaccines were combined into a single inoculum for use in sheep. The fact that in a previous report (3) BTV-2 attenuated virus, when administered as a monovalent vaccine, never induced titres higher than 10^3TCID$_{50}$/ml would suggest that the high titres observed in this study might be the result of interference between strains. However, the possibility that BTV-9 had been insufficiently attenuated cannot be excluded. BT disease is caused by an arthropod-borne virus of the genus *Orbivirus* (family: *Reoviridae*) and, in Europe is transmitted by at

least three species of biting midges of the genus *Culicoides* (6). Being an arthropod-borne disease, transmission relies upon the presence of the insect vectors, and of virus circulating in the bloodstream of the animal hosts. It is believed that virus titres lower than 10^3 pfu/ml in vaccinated animals will ensure that the virus is not imbibed by blood-feeding insects (5). The duration of viraemia within the animal host is also crucial to transmit the virus. An understanding of the levels and duration of viraemia in the animal host that are infective to vector *Culicoides* would enable the development of improved low-risk trade policies for BT. In this study, sheep vaccinated with BTV-2 and BTV-9 vaccine showed virus titres higher then 10^3 pfu/ml for 15 days (from 6 to 21 days pv). Previous studies suggest that during this period the elevated virus titres in the vaccinated sheep would have been able to infect the insect vector. The Kaplan-Meier survival technique helped to determine the probability of moving viraemic animals following immunisation. This technique showed that sheep could be moved safely 28 days following immunisation with the BTV-2/BTV-9 bivalent modified-live vaccine. After this interval, the risk of spreading vaccine viruses falls below 0.01%.

Another feature that is characteristic of a good vaccine is its ability to elicit a protective immune response. No challenge studies were conducted in this study and therefore, the levels of immunity had to be calculated on the serological data. Most sheep (90.9%) produced neutralising antibodies following immunisation; protective immunity has been shown to be linked to the levels of neutralising antibody (2). However, in most of the sheep, titres remained low and it appeared that they would not persist for long. Antibody titres to both serotypes peaked 24 days pv and showed a second peak 50 days pv. This study also provided evidence that the strain of BTV-9 used in the vaccine combination was of low immunogenicity, there being a clear discrepancy between viraemia and neutralising antibody titres.

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