Monovalent modified-live vaccine against bluetongue virus serotype 2: immunity studies in cows

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Summary

A challenge study was conducted to determine the efficacy of vaccination against bluetongue (BT) virus (BTV) serotype 2 in protecting cattle against infection. A group of 30 cows, vaccinated seven months previously with monovalent BTV-2 modified-live vaccine produced by Onderstepoort Biological Products in South Africa, were challenged subcutaneously with $2 \times 10^5.8$ TCID$_{50}$/ml of BTV-2 field isolate. All cattle originated from the same population in the Sardinian province of Oristano. Eight unvaccinated calves from a BTV-free herd also participated in this study; four were inoculated with BTV-2 and used as positive controls whilst the remaining four were used as negative controls to confirm that no BTV was circulating locally. Blood samples were taken from all animals three times a week for two months. Serum samples were tested for antibody against BTV using the competitive enzyme-linked immunosorbent assay (c-ELISA) and the virus neutralisation (VN) test. Virus isolation was attempted on the blood samples by intravenous egg inoculation followed by two blind passages in Vero cells. Virus titres following challenge were determined also. Of the 30 cows vaccinated, 29 were positive in the c-ELISA and demonstrated neutralising antibodies. At the time of challenge, 11 cows had no virus neutralising antibody while the remainder had low titres ranging from 1:10 (11 cows) to 1:20 (6 cows); two cows showed titres of 1:40 and 1:80, respectively. None of the cows showed signs of disease after challenge and no BTV was isolated from the blood of the 29 cows that had developed antibodies after vaccination. Commencing on day 9 post challenge, BTV-2 was isolated from the blood of the single cow that had not seroconverted following vaccination and from the blood of the unvaccinated controls. Viraemia lasted until day 21 post challenge. Neither BTV nor antibody was detected in the blood samples taken from the negative control group. These observations indicate that the monovalent BTV-2 modified-live vaccine protects most animals when challenged with field virus seven months post vaccination.

Keywords


Introduction

Bluetongue (BT) is an arthropod-borne, viral disease which affects mainly sheep. Due to its ability to spread rapidly under suitable circumstances, BT is classified as a ‘List A’ disease by the Office International des Epizooties (OIE) and occurrence of disease gives rise to trade bans being imposed on susceptible ruminant livestock species in infected areas. Such movement restrictions have had serious socio-economic repercussions on the industry in infected countries. In an attempt to reduce direct losses due to disease and indirect losses due to virus circulation, the Italian Ministry of Health declared vaccination against BT compulsory for all susceptible ruminants in May 2001. During the first year of the campaign, Sardinia and Tuscany vaccinated more than 80% of their cattle populations using a monovalent modified-live vaccine against BT virus (BTV) serotype 2 manufactured by Onderstepoort Biological Products.
Materials and methods

Virus

Challenge virus was prepared from the spleen of a naturally infected sheep that had died in the 2000 BT outbreak in Sardinia. The spleen was disrupted using sterile quartz powder, the product suspended with lactose peptone buffer containing antibiotics (100 IU/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml gentamycin and 50 IU/ml nystatin) and sonicated. A 0.1 ml volume of the spleen suspension was inoculated intravenously into embryonating chicken eggs and subsequently passaged onto confluent Vero cell monolayers as described by Savini et al. (4). At maximal cytopathic effect (CPE), the material was harvested, aliquoted and stored at –80°C. Presence and identity of BTV were determined in the blood of viraemic animals as follows: the blood cells were washed three times in phosphate-buffered saline (PBS) containing antibiotics. After the last washing, the sample was re-suspended in MEM with antibiotics (1/10 v/v) and sonicated. Four tenfold dilutions of each sample suspension (from 1:10 to 1:10,000) were inoculated into 96 flat-bottomed microtitre plate wells, following the method described in the OIE Manual of standards for diagnostic tests and vaccines (3). Four replicates were made for each dilution. Approximately 10⁴ Vero cells were added per well in a volume of 100 µl of minimum essential medium (MEM) containing antibiotics (100 IU/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml gentamycin and 50 IU/ml nystatin) and 3% foetal calf serum (FCS). The test was read after incubating the plates for six days at 37°C under 5% CO₂. The endpoint of the assay was determined using the method of Reed and Muench.

Animals

Between July and August 2002, a group of 1,005 cattle of various breeds and ages were selected at random from 10 herds in the Sardinian province of Oristano. These animals were vaccinated subcutaneously with 1 ml of 10⁵ TCID₅₀ BTV-2 modified-live vaccine. Blood samples from all animals were taken monthly for three months post vaccination (pv) as described by Savini et al. (5). From these, a group of 30 vaccinated cows was selected and divided further into three groups of ten cows each, based on their virus neutralisation (VN) titres. One group included animals which developed VN titres of 1:80 or less, the second group included those with titres ranging between 1:160 and 1:320, whilst the third group included those with VN titres above 1:320. All 30 vaccinated cows were then challenged seven months after immunisation by subcutaneous injection of 1 ml sonicated spleen, containing 10⁴ TCID₅₀/ml of BTV-2 field isolate. Of eight unvaccinated calves from a BTV-free population, four were similarly inoculated with BTV-2 and used as positive controls; the remaining four calves were used as negative controls to detect possible local circulation of wild BTV in the experimental groups. Ethylene-diaminetetra-acetic acid (EDTA) blood and serum samples were taken from all animals three times a week for two months and tested for the presence of BTV-2 antibodies using the c-ELISA and VN test.

Virological tests

Intravenous egg inoculation followed by two blind passages in Vero cells was used to isolate BTV-2 from EDTA blood samples, according to the method described by Savini et al. (4). Virus titres were determined in the blood of viraemic animals as follows: the blood cells were washed three times in phosphate-buffered saline (PBS) containing antibiotics. After the last washing, the sample was re-suspended in MEM with antibiotics (1/10 v/v) and sonicated. Four tenfold dilutions of each sample suspension (from 1:10 to 1:10,000) were inoculated into 96 flat-bottomed microtitre plate wells, following the method described in the OIE Manual of standards for diagnostic tests and vaccines (3). Four replicates were made for each dilution. Approximately 10⁴ cells, in a volume of 100 µl of MEM plus antibiotics and 3% FCS, were added per well and the plates incubated at 37°C under 5% CO₂. The plates were examined after six days and the TCID₅₀ calculated.

Serological tests

The antibody response was monitored using both the c-ELISA (2) and the VN test. The VN was performed as described by Savini et al. (4). Reference virus and serum for BTV-2 were supplied by the OIE Reference Laboratory for bluetongue in Onderstepoort. Those sera neutralising BTV-2 at a dilution of 1:10 were considered positive. The antibody titre of the test serum was the highest dilution capable of neutralising 50% of the virus activity.

Statistical analysis

The BT viraemia data in animals were analysed using the Beta (r+1, n-r+1) distribution where s, the number of successes, is the total number of viraemic animals and n, the number of trials, is the total number of animals tested. The peak of the distribution represents the most probable value of
the percentage of viraemic animals and the extent of deviation gives information about the uncertainty of the estimates due to sample size. However, from an epidemiological point of view, it is far more interesting to know the percentage of vaccinated animals that are protected when challenged using a homologous BTV serotype, 1-beta (s+1, n-s+1), which represents the probability that more than a certain percentage of animals that are protected when challenged, was calculated.

**Results**

Of the 30 vaccinated cows, 29 developed ELISA and virus neutralising antibodies after immunisation; one animal was negative. Of these 29 cows, 8 had VN titres below 1:80, 15 had neutralising antibodies ranging between 1:160 and 1:320 while the remaining 6 had titres above 1:320. At the time of challenge, 11 cows had no VN antibodies, while the rest had low levels of circulating VN antibody titres of 1:10 (11 cows) and 1:20 (6 cows), while one cow had a titre of 1:40 and another a titre of 1:80 (Fig. 1). No animal showed signs of disease either after vaccination or after challenge. In addition, no BTV was isolated from the blood of the 29 cows that had developed antibodies pv. Commencing on day 9 post challenge (pc), BTV-2 was isolated from the blood of the single vaccinated cow which had not seroconverted after immunisation.

Levels and duration of viraemia in this animal were similar to those observed in the positive control group and lasted until day 21 pc (Fig. 2). Neither BTV nor antibody was detected in the blood samples collected from the negative control group. Figure 3 shows the curve of the probability that vaccinated cattle are protected, not showing any detectable viraemia against homologous challenge.

![Figure 1](image1.png)

**Figure 1**
Neutralising antibody titres in cattle vaccinated with monovalent bluetongue virus serotype 2 modified-live vaccine

![Figure 2](image2.png)

**Figure 2**
Circulating virus titres in cattle challenged with bluetongue virus serotype 2 field isolate

![Figure 3](image3.png)

**Figure 3**
Probability that cattle vaccinated with monovalent bluetongue virus serotype 2 modified-live vaccine are protected against homologous challenge

The P value is at least equal to the x-axis percentages

**Discussion**

Subclinical BT infection is common in cattle in infected zones and is characterised by prolonged viraemia, making cattle amplifying hosts and reservoirs for the virus (1). As a consequence, the movement of cattle from infected zones into areas where *Culicoides* and susceptible ruminants co-exist is one of the ways of spreading BT infection. The
Italian strategy to combat BT includes the vaccination of cattle to reduce virus circulation and to eventually ease the ban on cattle movements. This study demonstrated that the monovalent BTV-2 modified-live vaccine has an appropriate balance between attenuation of virulence and ability to replicate in cattle. More importantly, the antigenic stimulus provided by its replication elicits complete protection against challenge using a virulent homologous virus and does not cause any detectable viraemia in serologically positive vaccinated animals. These results show that at least 90.5% of vaccinated cattle will not develop a BT viraemia when challenged with virulent homologous virus seven months after immunisation. Furthermore, it would appear that the risk of spreading BTV-2 infection through the movement of vaccinated cattle will be very low. This study also confirmed that protective immunity against BT is associated with the presence of type-specific neutralising antibodies (6). However, it is worth noting that the level of circulating antibody, at the time of challenge, in animals that had previously seroconverted, does not seem to play an important role in preventing infection. In fact 10 of the 11 animals which had no detectable neutralising antibodies at the time of challenge were protected from homologous BTV challenge; the only animal that showed pc viraemia was the one in which neutralising antibodies had never been observed. According to this observation, it could be hypothesised that circulating antibody is a significant indicator of immunity but is not essential to prevent the development of viraemia in infected animals.

References