Vaccination of cattle using monovalent modified-live vaccine against bluetongue virus serotype 2: innocuity, immunogenicity and effect on pregnancy

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Summary

The immunogenicity, innocuity and possible teratogenic effects of the monovalent modified-live vaccine against bluetongue (BT) virus (BTV) serotype 2, manufactured by Onderstepoort Biological Products in South Africa, was evaluated in cows. Twenty-one cows, 14 of which were at different stages of gestation, were vaccinated with 2 ml of monovalent vaccine; two served as unvaccinated controls. After immunisation, 16 vaccinated and the 2 unvaccinated controls were kept in the field; the remaining 5 pregnant cows were maintained in an insect-proof stable with a controlled environment. Blood samples were taken from field cattle once a week for two months and from the stable cattle three times a week. All samples were screened for the presence of BTV and for BT 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 from ethylene-diaminetetra-acetic acid (EDTA) blood samples and virus titres in viraemic animals were determined. After immunisation, 9 of the cows developed a viraemia which commenced on day 7 post vaccination (pv) and lasted for three weeks. The virus titres were never higher than $10^{2.8}$TCID$_{50}$/ml with the highest titre observed on day 14 pv. None of the vaccinated animals developed clinical symptoms that could be attributed to BTV; after three weeks all animals showed a serological response to BTV-2. In the c-ELISA, antibodies were detected from day 7 pv while in the VN test, antibodies were observed from day 21 pv. All pregnant cows completed their gestation: 13 gave birth to healthy calves, while one of those in the field group, vaccinated at the six months gestation, delivered a calf with prosencephalic hypoplasia, possibly developed during foetal organogenesis prior to vaccination. Fourteen months after immunisation the stabled cows were challenged subcutaneously by administering $2 \times 10^{6.8}$TCID$_{50}$ BTV-2 Italian isolate. A third group of 4 cows was also inoculated with the BTV-2 Italian field isolate, as described for the second group and was used as the unvaccinated positive control group. Vaccinated cows had a detectable viraemia only on day 14 pv and virus titres were very low. Virus titres never exceeded $10^{2.3}$TCID$_{50}$/ml, while the unvaccinated group developed a long and intense viraemia, peaking on day 14 pv with a titre of 1.18×10^4. It is concluded that the BTV-2 modified-live vaccine used in this study was a harmless and effective immunogen that did not cross the placental membrane.

Keywords

Bluetongue – Cattle – Competitive enzyme-linked immunosorbent assay – Immunogenicity – Innocuity – Italy – Pregnancy – Teratogenic effect – Vaccine – Virus – Virus neutralisation.

Introduction

Bluetongue (BT) is a viral disease of domestic and wild ruminants caused by an *Orbivirus* belonging to the family *Reoviridae* (14). Bluetongue virus (BTV) is transmitted by arthropod vectors of the genus *Culicoides* (17). The disease in sheep can produce severe clinical symptoms (4) while in cattle and wild ruminants few or no clinical signs are observed (2).

BT has been designated by the Office International des Epizooties (OIE) as a 'List A' disease; this has prompted restrictions on trade of susceptible animals from a BT-infected country (15). After the outbreaks of BT in Italy in 2000, the Italian Ministry of Health implemented a campaign whereby all domestic ruminants were to be vaccinated against BT to reduce direct losses due to disease and indirect losses due to virus circulation. The modified-live vaccine against BTV-2 manufactured by Onderstepoort Biological Products (OBP) in South Africa was used; this was the only BTV-2 vaccine commercially available. Use of this vaccine was recommended only in sheep and since it can be teratogenic and can induce abortions, administration was not recommended during the first half of pregnancy. Since little or no data was available on the vaccination of cattle, the present study was conducted to evaluate the immunogenicity, innocuity, efficacy and possible teratogenic effect of monovalent BTV-2 modified-live vaccine in cattle. The virus titre and the duration of viraemia following immunisation were also determined.

Materials and methods

Vaccine

Before use, the modified-live virus (MLV) vaccine was reconstituted in phosphate-buffered saline (PBS) pH 7.2 and titrated and checked for contaminants. Innocuity was tested by inoculating six adult mice and two guinea-pigs intraperitoneally (0.25 ml) and four sheep subcutaneously (1 ml). All animals were monitored daily for two weeks and any clinical signs recorded.

Animals

Eighteen cows virologically and serologically negative for BTV were selected from two different farms located in the provinces of Palermo and Trapani. In both areas, BTV had been reported previously. However, no outbreaks or viral circulation had been observed during the period of the trial (March to July 2001).

Of the 18 animals, 9 were pregnant. A total of 16 were vaccinated in the region of the neck with 2 ml of vaccine and 2 non-pregnant cows were inoculated with a placebo and kept as negative controls.

A second group of 5 pregnant cows was similarly vaccinated and kept in an insect-proof stable with a controlled environment. Fourteen months after vaccination, the 5 cows were challenged by subcutaneously administering a BTV-2 Italian field isolate; the titre of the inoculum was $2 \times 10^{6.8}$TCID$_{50}$. A third group of 4 cows was also inoculated with the BTV-2 Italian field isolate, as described for the second group, and used as unvaccinated positive controls. Table I provides additional details on the cows used in this study.

The temperatures of all cows were recorded daily and clinical signs monitored. All newborn calves were examined for possible malformations and, when possible, blood was taken from each calf before colostrum was consumed. Ethylene-diaminetetra-acetic acid (EDTA) blood and serum samples were taken once a week from all field cows for two months, and three times a week for the stabled cows. The EDTA blood samples were screened for the presence of BTV while sera were examined for c-ELISA and virus neutralising (VN) antibodies. Intravenous egg inoculation, followed by two blind passages in Vero cells, was used to isolate BTV according to the method described by Savini *et al.* (17); virus titres were also determined (17).

Results

The vaccine was found to be free of bacterial and viral contaminants; the virus titre of a single dose was found to be $2 \times 10^{4.2}$TCID$_{50}$ of modified-live BTV-2. No clinical signs were observed in vaccinated or in challenged animals. After immunisation, 14 animals developed viraemia with titres of less than $10^{2.8}$TCID$_{50}$/ml. Virus was first isolated 7 days post vaccination (pv) and viraemia lasted for three weeks reaching a peak on day 14 pv (Fig. 1).

When the vaccinated group was challenged, only two cows developed detectable viraemia ($10^{2.3}$TCID$_{50}$/ml) on day 14 post challenge (pc), whereas in the unvaccinated group, viraemia lasted five weeks and reached higher titres (Fig. 1)

All vaccinated animals developed BTV-2 antibodies; c-ELISA antibodies were detected from day 7 pv while VN antibodies were observed from day 21 pv and peaked four weeks later (Fig. 2). Negative controls remained serologically and virologically negative against BTV throughout the study.

Table I
Gestation status of 27 cows vaccinated using a bluetongue virus serotype 2 live-modified vaccine, Teramo, Palermo and Trapani Provinces, Italy, 2001-2002

No.	Tag No.	Farm	Artificial insemination date	First pregnancy test results	Second pregnancy test results	Pregnancy stage when vaccinated	Delivery date
1	C 279	Farm 1	September	6 months	7 months	6 months	June
2	C 280	Farm 1	February	Doubtful	Negative	–	—
3	C 283	Farm 1	October	5 months	6 months	5 months	July
4	C 270	Farm 1	October	5.5 months	6.5 months	5.5 months	July
5	C 261	Farm 1	September	6.5 months	7.5 months	6.5 months	June
6	C 282	Farm 1	February	Doubtful	Negative	–	—
7	1283	Farm 1	February	Doubtful	Negative	–	—
8	Control	Farm 1	February	Doubtful	Negative	–	—
9	7244	Farm 2	February	Doubtful	Negative	–	—
10	C 001	Farm 2	February	Doubtful	Negative	–	—
11	C 004	Farm 2	February	Doubtful	Negative	–	—
12	C 005	Farm 2	February	1.5 months	2.5 months	1.5 months	November
13	C 012	Farm 2	February	Doubtful	Negative	–	—
14	C 011	Farm 2	March	Doubtful	1 month	<1 month	January
15	2874	Farm 2	October	5.5 months	6.5 months	5.5 months	July
16	2875	Farm 2	October	5 months	6 months	5 months	July
17	C 010	Farm 2	March	Doubtful	1 month	<1 month	January
18	Control	Farm 2	February	Doubtful	Negative	–	—
19	C023	Stable	February	–	Not examined	3 months	November
20	C030	Stable	January	–	Not examined	4 months	October
21	1822	Stable	December	–	Not examined	5 months	September
22	1823	Stable	December	–	Not examined	5 months	September
23	16777	Stable	October	–	Not examined	7 months	July
24	38389	Stable	Non-pregnant	—	—	—	—
24	38386	Stable	Non-pregnant	—	—	—	—
26	C001	Stable	Non-pregnant	—	—	—	—
27	38390	Stable	Non-pregnant	—	—	—	—

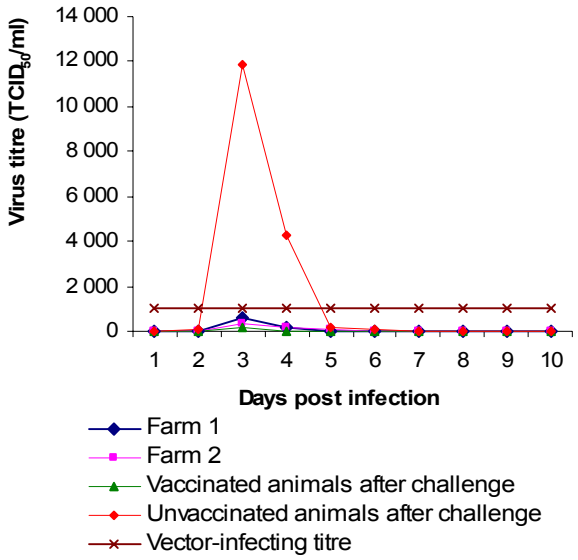

Figure 1
Bluetongue virus serotype 2 titres in cattle following vaccination and/or experimental infection

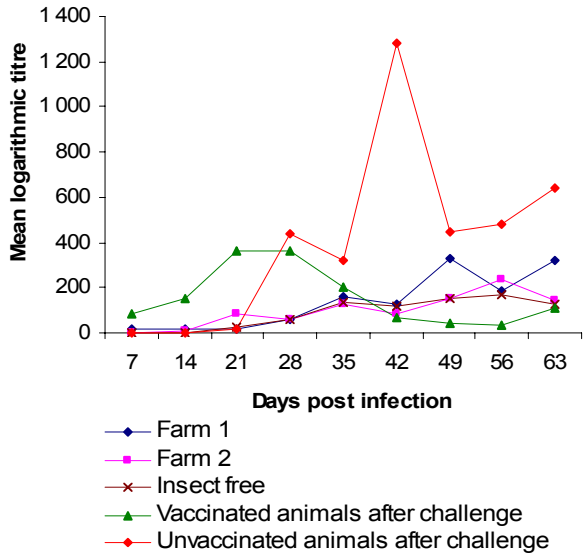

Figure 2
Bluetongue virus serotype 2 mean neutralising antibody titres in cattle following vaccination and/or experimental infection

All pregnant cows completed their gestation: 13 gave birth to healthy calves, while one cow from the field group that had been vaccinated during the sixth month of gestation, delivered a seriously malformed calf. Autopsy revealed an internal deviation in the left foreleg and in the neck. The upper part of the head was flattened and had a hole that opened into the cranial cavity. Post-mortem examination of the skull revealed a thickening of the bone, hyperaemia of the meninx, and hypoplasia of the brain and of the dura madre. A gelatinous subcutaneous oedema and a deviation of the atlas and epistropheus joint were clearly visible in the area of the neck. Heart degeneration and pericardial haemorrhagic petechiae were also detected in the thoracic cavity. Punctiform haemorrhages were observed on the surface of the thymus. The abdominal cavity showed haemoperitoneum, soft kidney, liver degeneration and haemorrhagic foci on the surfaces of the liver and the spleen. Bacterial culture of the organs of the stillborn calf revealed the presence of *Pseudomonas* spp. and of *Escherichia coli*; the polymerase chain reaction (PCR) was negative for bacterial and virological abortigenic agents. Of the 13 calves born to vaccinated dams, four had both VN and ELISA antibodies.

At the time of challenge, animals of the second group were still c-ELISA positive and had average neutralising antibody titres of 1:128. The titres increased rapidly pc and peaked (1:360) at three weeks.

The group of unvaccinated animals developed c-ELISA antibodies from day 14 pv while VN antibodies were observed from day 21 pv, and peaked three weeks later (Fig. 2).

Discussion

The current vaccination strategy to prevent BTV infection in ruminants relies on MLV vaccines such as that used in this trial. The vaccine was developed using field strains isolated from infected animals and then attenuated by serial passage in embryonating chicken eggs and in cell culture. MLV vaccines have to replicate in the vaccinated animal in order to stimulate protective immunity and, in the process, they might induce a mild, or subclinical, illness (6). MLV has several potential drawbacks, including reversion to virulence, insect transmission, impaired reproductive performance and foetal malformations. The latter have been reported in vaccinated ewes, with malformations observed primarily during the first half of gestation (6, 7, 8, 18). In South Africa, the vaccine is used in sheep only and little information is available on its use in cattle. Cases of hydroanencephaly were described in calves born to cows experimentally inoculated by the intrauterine route in the first half of gestation with tissue culture-adapted BTV (1, 10, 11, 20). BT infection (with malformations) was confirmed by the presence of BT antibodies in calves that had not received colostrum (9). It has been demonstrated that field strains of BTV rarely cross the placental barrier; therefore, abortions and foetal malformations should be infrequent under field conditions (11, 12). However, MLVs have been capable of crossing the placental barrier with foetal malformations in sheep (19). In this study, the vaccine appeared to be innocuous despite the administration of a double dose: no animals developed clinical symptoms of BT, and no abortions, or impairment of reproductive function, were observed. The teratogenic malformations reported in the stillborn calf probably commenced prior to vaccination of the dam as the malformation was not typical of BTV-induced hydroanencephaly but was of a prosencephalic hypoplasia, characterised by a small opening in the skull (3). Furthermore, BTV was not isolated from the calf and the haemorrhagic lesions could have been the consequence of a septicaemic form of *E. coli*. Further proof that the MLV did not cross the placental barrier was the absence of BT antibodies in calves that had not received colostrum. Titres were observed in four calves, but blood had been drawn only after they had received colostrum. The vaccine was strongly immunogenic and all vaccinated cattle seroconverted. In line with previous work (16), the c-ELISA proved to be more sensitive than the VN test; c-ELISA antibody was detected during the second week pv. Interestingly, neutralising titres were still present 14 months pv in animals which had no evidence of detectable viraemia after vaccination. Protective immunity against BT has been associated with the presence of type-specific neutralising antibodies (5). In this study, BTV-2 modified-live vaccine was able to protect cattle from infection with the homologous BTV serotype 14 months after immunisation. Vaccinated cows had detectable viraemia only on day 14 pv and the virus titres were very low. Virus titres were never higher than $10^{2.3}TCID_{50}$/ml while the unvaccinated group developed a long and intense viraemia, peaking on day 14 pv with a titre of 1.18×10^4. The duration of viraemia and the high virus titres are of great epidemiological importance as they significantly influence the persistence of BTV in the environment. It has been shown that titres below 10^3TCID_{50}/ml are not able to infect *Culicoides* (13). In this study, the virus titres pv and pc in vaccinated animals were below $10^{2.8}CID_{50}$/ml, indicating that vaccine virus would not have been spread by locally active and competent *Culicoides* vectors (Fig. 1).

In conclusion, these experimental trials demonstrated the BTV-2 MLV vaccine to be both safe and effective, and that it did not cross the placental barrier.

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