Field vaccination of sheep with bivalent modified-live vaccine against bluetongue virus serotypes 2 and 9: effect on milk production

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Summary

In response to complaints of the potential side-effects of the bivalent live-modified vaccine used to control the spread of bluetongue (BT) virus (BTV) serotypes 2 and 9 in Italy, a study was conducted to determine the effects of immunisation on milk production. Thirty-four Comisana cross-bred sheep were vaccinated with the bivalent BTV-2/BTV-9 modified-live vaccine produced by Onderstepoort Biological Products in South Africa; six animals served as unvaccinated controls. All animals were bled twice a week for two months and the presence and titres of BTV in the blood determined. The somatic cell count, pH, fat, protein and lactose content of the milk, as well as the quantity of the milk produced, were also measured. Vaccine virus was isolated from vaccinated animals between day 3 and day 20 post vaccination (pv) with peak titres observed on days 3 and 6 pv for BTV-2 and BTV-9, respectively. Milk production declined in the vaccinated group between days 8 and 14 pv, with the greatest decrease on day 9 pv. No differences were observed in the somatic cell count and pH, or in the milk fat, protein and lactose content.

Keywords

Bluetongue – Italy – Lactose – Milk fat – Milk pH – Milk production – Milk protein – Sheep – Somatic cell count – Vaccine – Virus – Viraemia.

Introduction

Since the first isolation of bluetongue (BT) virus (BTV) in Italy in 2000, four serotypes (BTV-2, BTV-4, BTV-9 and BTV-16) have been reported to occur in the country; of these, BTV-2 and BTV-9 were the most widespread (Fig. 1). To control the spread of these two serotypes and to ease the pressure created by livestock movement restrictions, the Italian government implemented a compulsory vaccination campaign in May 2001. In those areas where both viruses were circulating, the bivalent modified-live vaccine against BTV-2 and BTV-9 was administered and in 2001 and 2002, almost all domestic ruminants were vaccinated. It was the first time that a vaccine with this combination of BTV serotypes had been used in the field and therefore no data were available on its potential side-effects. This study reports on the effects this bivalent modified-live vaccine has on the milk production of dairy sheep.

Material and methods

Vaccine

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

Animals

This study was conducted between May and July 2002 in the province of L'Aquila (at an altitude of 1 100 m) in a flock comprising nearly 1 000 Comisana cross-bred sheep raised for both milk and meat. A group of 34 BT seronegative sheep was selected and vaccinated subcutaneously with a single dose of the vaccine. Another group comprising six seronegative sheep served as unvaccinated controls. To avoid any potential

management bias, both groups were maintained under similar conditions. Temperatures were recorded daily and the animals observed for clinical signs. Total morning milk production per group was recorded daily and individual milk samples were collected four days before commencement of vaccination. The total farm milk production was recorded for twenty days. Ethylene-diaminetetra-acetic acid (EDTA) and plain blood samples were collected twice a week from each animal for the following two months.

Figure 1
Regions of Italy in which monovalent bluetongue virus serotype-2 (BTV-2) and bivalent BTV-2/BTV-9 modified-live vaccines were used

Virological and serological tests

EDTA blood samples were screened for the presence of BTVs and their titres measured. The competitive enzyme-linked immunosorbent assay(c-ELISA) (3) and the serum-neutralisation (SN) test (2) 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.* (5). The serotype of each virus isolated from the blood of viraemic animals (6), and the viral titres, were determined.

Milk production

Milk samples were analysed for protein and lactose content using the Milkoscan system 4 000 and for somatic cell count (SCC) the Fossomatic 400. The pH was measured using a Crison Micro TT250 electrode probe.

Statistical analysis

Differences between the mean milk production per week of the vaccinated and unvaccinated groups of sheep were analysed using the nonparametric Mann-Whitney test for independent groups. Similarly, milk quality data were grouped and for each group the mean weekly value calculated. Statistical differences between weekly data of vaccinated and unvaccinated groups were determined also using the Mann-Whitney test.

Results

The vaccinated animals showed an increase in temperature and exhibited facial oedema between days 7 and 10 post vaccination (pv). Vaccine virus was isolated from the blood of vaccinated animals from day 3 to day 20 pv, with peak titres observed on days 3 and 6 pv for BTV-2 and BTV-9, respectively. BTV-9 viraemia titres were much higher than those for BTV-2 (Fig. 2). No significant differences were observed between the somatic cell count, pH, milk fat, protein and lactose content of the vaccinated and unvaccinated groups. However, milk production in the vaccinated group dropped significantly between days 8 to 14 pv ($p<0.05$); the lowest production was recorded on day 9 pv (Fig. 3).

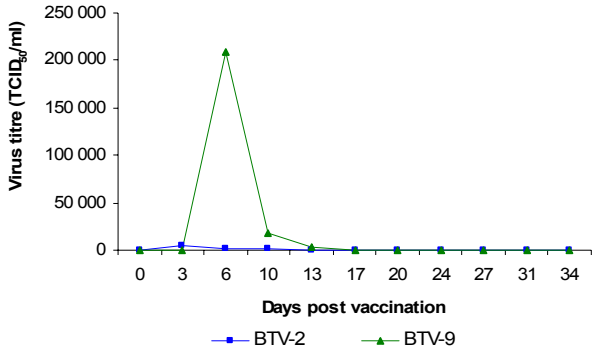

Figure 2
Bluetongue virus titres in sheep following vaccination with a bivalent BTV-2/BTV-9 modified-live vaccine, central Italy

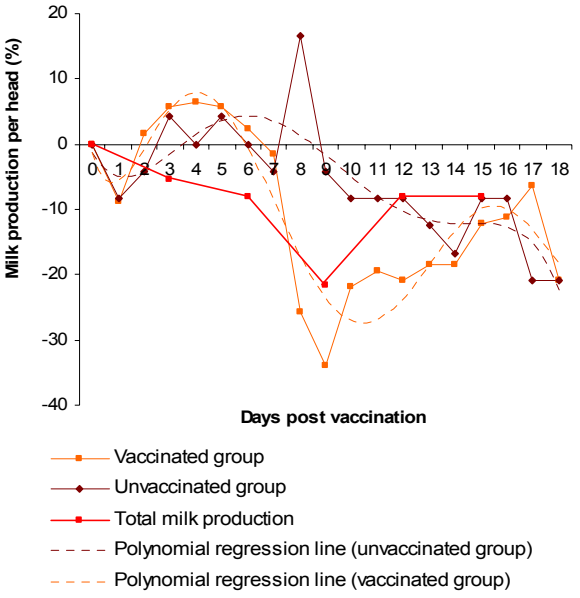

Figure 3
Milk production in sheep vaccinated against bluetongue with a bivalent (BTV-2/BTV-9) modified-live vaccine, central Italy

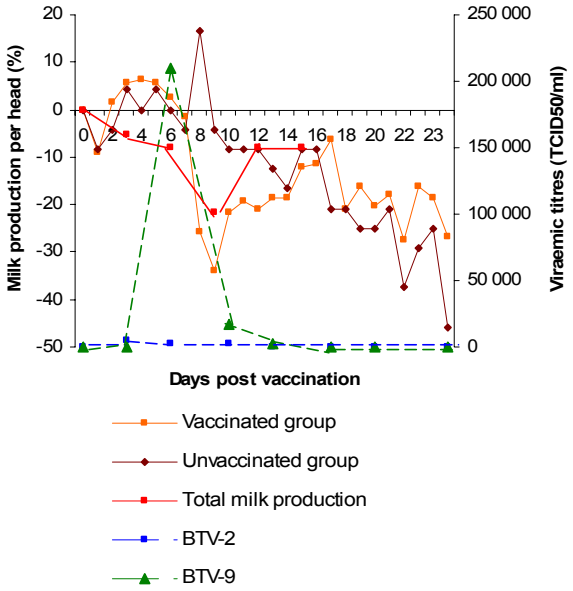

Figure 4
Relationship between bluetongue virus serotype 2 (BTV-2) and BTV-9 viraemia and milk production in sheep vaccinated with the homologous bivalent modified-live vaccine, central Italy

Discussion and conclusions

This study demonstrated that vaccination with a bivalent BTV-2/BTV-9 modified-live vaccine had an impact on total milk production but not on the milk quality. Losses commenced two weeks following vaccination and lasted for one week. On day 9 pv, milk production decreased to less than 30% of normal production levels. This drop was also seen in the total milk production of the farm with a 21% decline between days 9 and 12 pv. The decrease was significant and occurred just after peak viraemia of BTV-9. The relationship between the two events was clearly demonstrated (Fig. 4). BTV-9 showed the highest viraemia levels and probably accounted for the drop in milk production. This argument is supported by results obtained in an earlier study on dairy sheep where the use of monovalent BTV-2 vaccine did not affect either milk quantity or quality (1). In another study, no effect on milk yield or on total production was observed when cows were injected with the same BTV-2/BTV-9 vaccine combination; similarly, a higher BTV-9 viraemia titre was also recorded in cattle (4). In sheep, peak viraemia coincided with or occurred just before the appearance of clinical symptoms; in cattle, no clinical signs were observed after vaccination. Thus, it would appear that BTV did not interfere directly with the mammary tissue and that the decrease in milk production was due to BT disease that the vaccine had initiated in the sheep but not in the cattle; the fact that there were no changes in the quality of the various components of the milk would tend to confirm this.

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