

Effects of vaccination against bluetongue on milk production and quality in cattle vaccinated with live-attenuated monovalent type 2 vaccine

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Summary

The first epidemic of bluetongue (BT) to affect the three regions of Sardinia, Sicily and Calabria (Italy) in 2000 induced high economic losses caused by the disease itself and by the cessation of ruminant movements both within, and out of, the infected areas. In order to reduce virus circulation, and to create a resistant livestock population, the Italian Ministry of Health ruled, in May 2001, that all sheep, cattle, goats and water buffalo, in infected and in neighbouring regions, be vaccinated. The live-attenuated BTV-2 monovalent vaccine produced by Onderstepoort Biological Products in South Africa was to be used. Accordingly, in 2002, 98.6% of the sheep and goats, and 88.1% of the cattle, on Sardinia were vaccinated. Included was the vaccination of >70% of the cattle in the province of Oristano where >18 000 dairy cows in >220 herds are concentrated in the municipality of Arborea (Oristano) and which account for 65-70% of the milk produced in Sardinia. Using data collected at the centralised dairy co-operative since 1999 the quantity and quality of milk produced before vaccination against bluetongue was compared to that produced after vaccination. The following variables were analysed: average milk production/cow/month, monthly average fat content (%), monthly average protein content (%), average monthly somatic cell count and average monthly platelet count. The findings indicate that vaccination against BTV-2 in Sardinian dairy cattle did not impact negatively upon milk quantity nor milk quality.

Keywords

Bluetongue – Cattle – Italy – Milk production – Sardinia – Vaccine.

Introduction

Since 2000, Italy has been affected by the largest bluetongue (BT) epidemic ever to be recorded in Europe. The infection was first reported in Sardinia in August 2000 and then spread to large areas in central and southern Italy (2, 12). In May 2001, the Minister of Health ruled that all domestic ruminants were to be vaccinated not only in the infected regions but also in neighbouring regions to which infection could spread. Selection of the vaccination strategy was based on a risk assessment which indicated that viral circulation within Italy could only

be interrupted by creating a large population of infection-resistant ruminants (11).

The use of a live-attenuated vaccine may induce a rise in temperature ranging from 39.4°C to 39.8°C and lasting one to three days with transient viraemia. It has also been reported that, under experimental conditions, sheep vaccinated during the first four months of pregnancy may show teratogenic effects (10) but this is a phenomenon that has not been observed in the field. However, after the commencement of the vaccination campaign in Italy, vaccine-related problems were recorded in several regions, particularly in the south. In addition to the

problems described in the literature, a decrease in milk production was also reported. Existing studies on the effect of BT vaccination have only been conducted in sheep; no studies have been performed on dairy cattle.

An earlier study on the Sardinian sheep breed examined the effect of the vaccine on milk production under controlled conditions. Two groups of sheep studied, the first was vaccinated with BTV-2 vaccine while the second acted as the non-vaccinated control. No significant differences in the quantity and quality (fat, protein and lactose content, somatic cell count) of the milk produced by the two groups, before and after vaccination, were reported (4). A later study conducted in Sardinia to assess the effects of infection and vaccination on milk production (13) showed that infection significantly decreases milk production but is influenced by poor flock management. No effects on milk quality were reported. In most flocks, vaccination did not have any significant statistical effects on the lactation curve but even when the lactation curve was significantly affected, the effect was negligible from a practical viewpoint (maximum recorded loss: 10.5 g milk/animal/day).

The aim of this study was to establish whether the use of a monovalent BTV-2 significantly affects the quality and quantity of milk of dairy cattle.

Materials and methods

Population involved in the study

Dairy cattle in this study were located in the municipality of Arborea (Oristano Province, Sardinia). Two-thirds of the total bovine milk production of Sardinia is concentrated in the Arborea municipality, which has more than 18 000 cows distributed in about 220 holdings with an average monthly milk production of 12 700 tons. All milk produced in this municipality is collected by the '3A Assegnatari Associati Arborea' Co-operative, where the milk is checked for both quality and quantity.

The dairy cows of the Arborea Municipality were selected for the following reasons:

- they are genetically homogenous, almost all animals being Italian Friesian
- almost all the farms in the municipality apply the same nutritional uni-feed schemes
- the health status of the population is relatively homogenous
- similar management practices are applied throughout the area.

Data collected are standardised and are therefore comparable, as all the milk produced is delivered to a single point.

Vaccination

The first vaccination campaign in Sardinia took place in 2002. In total, 98% of the sheep and goats and 88.1% of the cattle were vaccinated. The first cattle vaccination campaign in the Arborea municipality was conducted from May to September 2002, after the vaccination of sheep and goats had been completed during the first few months of 2002 (Fig. 1). A live-attenuated monovalent BTV-2 vaccine, produced by Onderstepoort Biological Products (OBP) in South Africa, was used.

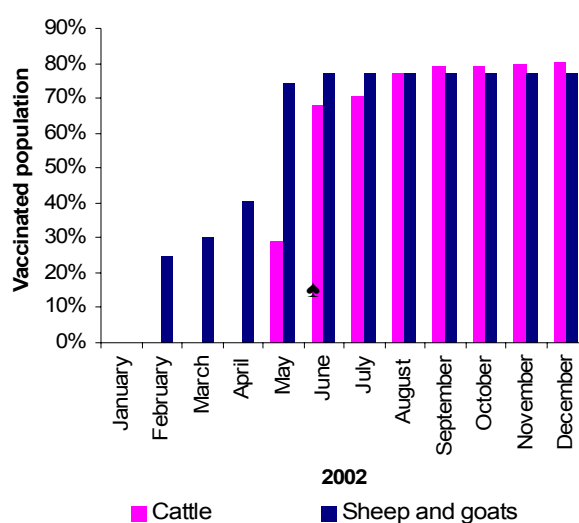


Figure 1
First vaccination campaign against bluetongue in the municipality of Arborea (Province of Oristano), Italy
Percentage of vaccinated population per month and animal species

Variables considered in the study

The qualitative and quantitative data on the milk collected prior to vaccination (April 1999-April 2002) by the Co-operative '3A' and were analysed and compared to the data collected after vaccination (May 2002-April 2003). The following variables were considered:

- number of farms to deliver milk
- average number of cows per farm
- total number of cows on the farms that delivered milk
- total quantity of milk delivered
- average monthly production per animal
- average monthly fat and protein percentage in the milk delivered
- average monthly plate count in the milk delivered

- average monthly somatic cell count in milk delivered.

The following laboratory methods were used:

- fat and protein content: FIL/IDF 141C:2000 standard procedure ‘Determination of milk fat, protein and lactose content (guide for the operation of mid-infrared instruments)’
- somatic cell count: method described in the Italian Ministerial Decree dated 26/03/1992, ‘Transposition of Commission Decision 91/180/EEC laying down certain methods of analysis and testing of raw milk and heat-treated milk’ (Annex II, chapter VII) (9)
- plate count: FIL/IDF 330:1998 and FIL/IDF 128A:1999 standard procedures, ‘Enumeration of mesophilic micro-organisms’.

The average number of animals in lactation for each herd was derived from the annual declaration made by farmers (L1 form), in compliance with the Commission Regulation (EC) 1392/2001 of 9 July 2001, laying down detailed rules for applying Council Regulation (EEC) 3950/92 for establishing an additional levy on milk and milk products (7, 8).

Statistical analysis

To verify whether increasing or decreasing trends existed, linear regressions were used (1, 3). Variables used were: average milk production per animal per month, somatic cell count, plate count, fat and protein content. Time was the independent variable for all regressions. The monthly numbers were transformed into the corresponding serial numbers of the days that had elapsed since 1 January 1900, up to the fifteenth day of every month.

Paired samples Student’s t-test with one tail significance (3) was used to compare the average monthly values of each variable before and after vaccination. The following hypotheses (H_0) were tested:

- individual milk production: average values, May 2002/April 2003 \geq April 1999/April 2002
- somatic cell count: average values, May 2002/April 2003 \leq April 1999/April 2002

Table I

Results of regression between time and average monthly values of production per animal, somatic cell count, plate count, fat and protein content

	Average milk production per animal	Somatic cell count	Plate count	Fat content	Protein content
Adjusted R ²	0.00	0.45	0.40	0.03	0.12
F	0.95	40.48	33.48	2.73	7.41
P	0.33	7.6×10^{-8}	5.7×10^{-7}	0.11	0.009

- plate count: average values, May 2002/April 2003 \leq April 1999/April 2002
- fat content: average values, May 2002/April 2003 \geq April 1999/April 2002
- protein content: average values, May 2002/April 2003 \geq April 1999/April 2002.

With reference to the variables with a statistically significant regression, the Student’s t-test was used to compare the regression residuals in order to remove trend effects.

Results

The production data show that from 1999 to 2003:

- The average monthly production/animal ranged from 590 kg to 760 kg, depending on the season (Fig. 2); the slight increase recorded over time is not statistically significant ($R^2=0$; $p=0.33$) (Table I); the production between May 2002 and April 2003 (during the vaccination campaign) was not significantly lower than that between April 1999 and April 2002 (before vaccination) ($t=5.55$; $p=0.99$) (Table II)

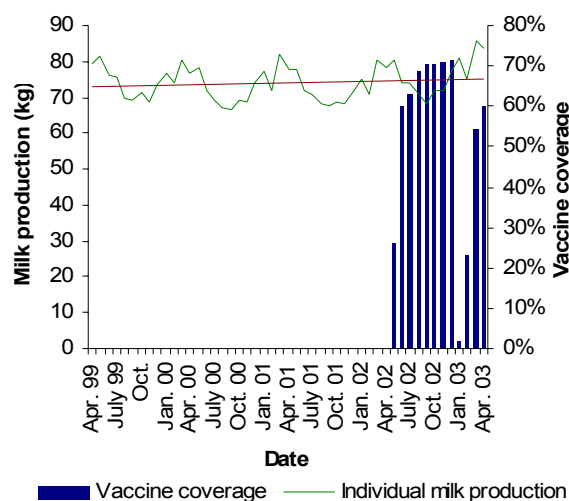


Figure 2

Dairy cattle in Italy: individual milk production per month and vaccine coverage during the first vaccination campaign against bluetongue

Table II

Results of comparison between average milk production per animal per month, somatic cell count, plate count, fat and protein content, April 1999-April 2002 and May 2002-April 2003

	Average milk production per animal	Somatic cell count	Plate count	Fat content	Protein content
Compared periods and tested hypothesis	May 02-Apr. 03 ≥ Apr. 99-Apr. 02	May 02-Apr. 03 ≤ Apr. 99-Apr. 02	May 02-Apr. 03 ≤ Apr. 99-Apr. 02	May 02-Apr. 03 ≥ Apr. 99-Apr. 02	May 02-Apr. 03 ≥ Apr. 99-Apr. 02
Average values	Apr. 99-Apr. 02: 651.2 May 02-Apr. 03: 676.8	Apr. 99-Apr. 02: 299.7 May 02-Apr. 03: 266.5	Apr. 99-Apr. 02: 40.9 May 02-Apr. 03: 26	Apr. 99-Apr. 02: 3.67 May 02-Apr. 03: 3.65	Apr. 99-Apr. 02: 3.32 May 02-Apr. 03: 3.33
Student's t	5.55	10.01	4.44	0.81	0.99
p	0.99	1	0.99	0.22	0.83

- The average monthly somatic cell count improved significantly during the entire study period ($R^2=0.45$; $p=7.6 \times 10^{-8}$) (Table I) from 300 cells/ml in 1999 to about 250 in 2003 (Fig. 3); the cell count between May 2002 and April 2003 (during the vaccination campaign) was not significantly higher than that between April 1999 and April 2002 (before vaccination) ($t=10.01$; $p=1$) (Table II); the same result was obtained even when the trend effect was taken into account ($t=1.01$; $p=0.83$) (Table III)
- The average monthly plate count decreased significantly ($R^2=0.40$; $p=5.7 \times 10^{-7}$) (Table I), from 40-100 cfu/ml in 1999 to less than 40 cfu/ml in 2003 (Fig. 4); the plate count between May 2002 and April 2003 was not significantly higher than that between April 1999 and April 2002 ($t=4.44$; $p=0.99$) (Table II); the same result was obtained even when the trend effect was taken into account ($t=1.77$; $p=0.052$) (Table III)
- The average monthly fat content remained steady and only showed seasonal fluctuations (Fig. 5); the slight increase recorded over time was not statistically significant ($R^2=0.03$; $p=0.11$)

(Table I); the fat content between May 2002 and April 2003 was not significantly lower than that recorded between April 1999 and April 2002 ($t=0.81$; $p=0.22$) (Table II)

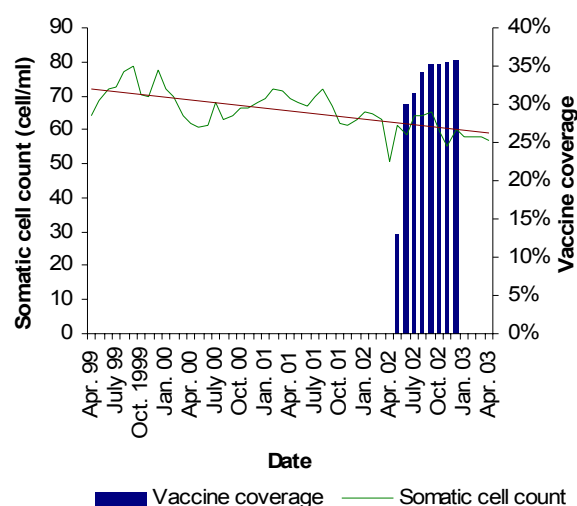


Figure 3
Dairy cattle in Italy: somatic cell count and vaccine coverage during the first vaccination campaign against bluetongue

Table III

Results of comparison between regression residuals of average monthly values of somatic cell count, plate count and protein content, April 1999-April 2002 and May 2002-April 2003

	Somatic cell count	Plate count	Protein content
Compared periods and tested hypothesis	May 02-Apr. 03 ≤ Apr. 99-Apr. 02	May 02-Apr. 03 ≤ Apr. 99-Apr. 02	May 02-Apr. 03 ≥ Apr. 99-Apr. 02
Average values	Apr. 99-Apr. 02: 1.59 May 02-Apr. 03: -1.775	Apr. 99-Apr. 02: -1.01 May 02-Apr. 03: 4.94	Apr. 99-Apr. 02: 0.01 May 02-Apr. 03: -0.03
Student's t	1.01	1.77	3.40
p	0.83	0.052	0.003

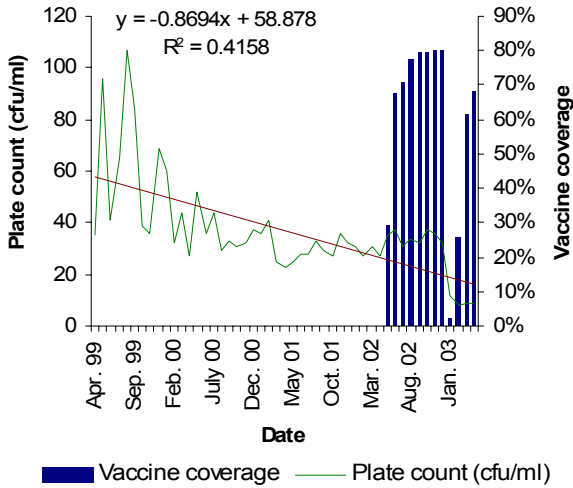


Figure 4
Dairy cattle in Italy: plate count and vaccine coverage during the first vaccination campaign against bluetongue

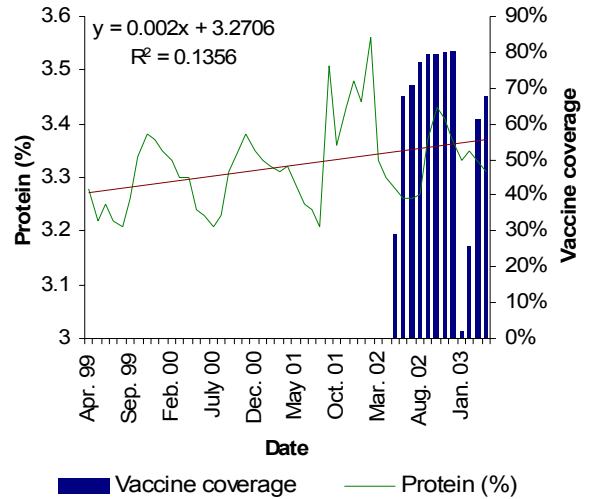


Figure 6
Dairy cattle in Italy: milk protein content and vaccine coverage during the first vaccination campaign against bluetongue

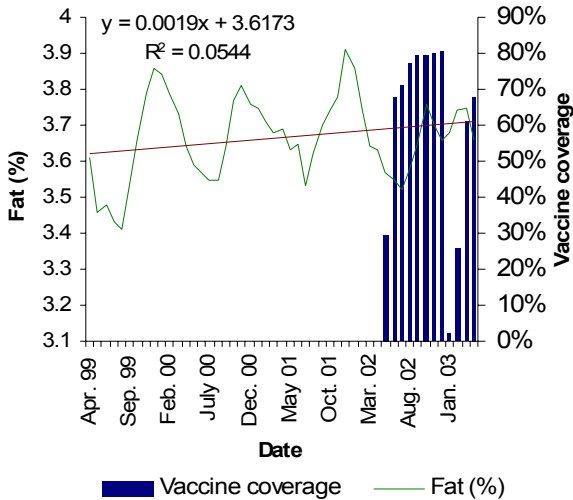


Figure 5
Dairy cattle in Italy: milk fat content and vaccine coverage during the first vaccination campaign against bluetongue

- The average monthly protein content increased significantly ($R^2=0.12$; $p=0.009$) (Table I) from an average 3.28% in 1999 to 3.33% in 2003 (Fig. 6); the protein content between May 2002 and April 2003 was not significantly lower than between April 1999 and April 2002 ($t=0.99$; $p=0.83$) (Table II); since a significant increase in protein content was observed over time, this trend had to be removed; therefore, the residuals of regression during the vaccination period were significantly lower than the residuals before the vaccination campaign ($t=3.40$; $p=0.003$) (Table III), indicating an increase in protein content over time that was lower than expected according to the trend.

Discussion

The goal of the study was to verify, through field data, whether BT vaccination affects the quality and quantity of milk produced by cattle vaccinated with a live-attenuated BTV-2 vaccine. The cattle population selected were characterised by similar genetic, health and management features (especially the type of nutrition) and also single-source standardised data. The data assessment showed that milk production in the Arborea municipality over the past four years has generally improved, especially milk quality (a statistically significant reduction in the average somatic cell and plate counts), probably due to compliance with the Council Directives 92/46/EEC, 92/47/EEC and the national laws on milk quality (5, 6). The comparison of variables between April 1999 and April 2002 and between May 2002 and April 2003, i.e. before and after vaccination, shows clearly that vaccination did not have a negative effect on the quantity and quality of milk produced in the municipality (Fig. 2 and Table II). The only significant difference to be noted was a decreased milk protein content. However, no pathogenic mechanism is known, whereby BT vaccination could decrease milk protein content, especially when no other variables are affected. Since such a difference was recorded only after removal of the trend in the data (compare Table II with Table III), the association detected between vaccination and protein content could be due to non-linearity of the trend itself, characterised by a horizontal asymptote. This trend was not considered in the analysis, due to the data fluctuations observed (Fig. 6). The residuals of regression in the horizontal asymptote area being

deviated towards low values, thus leading to a significant, but artificial, difference between the two study periods.

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