Effect on milk production of vaccination with a bovine herpesvirus 1 gene-deleted vaccine

J. C. Bosch, K. Frankena, J. T. van Oirschot

A field trial was conducted to determine the effect of vaccination with an inactivated bovine herpesvirus 1 (BHV1) gE-negative marker vaccine on the milk production of dairy cows. The daily milk yield of 455 cows in six herds was measured electronically from six days before vaccination until 14 days after vaccination. The treatment consisted of two injections with either vaccine or placebo, both at an interval of four weeks. There was a small, but significant (P<0·05), decrease of about 1·4 litres per cow in milk production after a double vaccination, the negative effect being slightly greater after the second vaccination.

BEFORE a new vaccine can be registered and released, it has to fulfil the requirements of official documents, such as the European Pharmacopoeia, which stipulate standards for the safety and efficacy of medicinal products.

According to the directives of the European Union, an investigation of safety aspects has to demonstrate the possible adverse effects of a medicinal product under practical conditions of use (Commission Directive 92/18/EEC). In safety investigations, not only local and systemic reactions are measured but also the animal's performance. The daily milk production of lactating cows is a good indicator of performance, because potential decreases in milk production after vaccination can have an economic impact.

Bovine herpesvirus 1 (BHV1) is an economically important pathogen for cattle, causing infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). Attenuated and inactivated virus vaccines are used to control BHV1 infection. There have been few investigations of the adverse effects of these BHV1 vaccines. One investigation of the intramuscular injection of an attenuated BHV1 combination vaccine (Cortese 1991) showed no significant differ-ences in milk production between small groups of vaccinated and control animals during a short period. On the other hand, the animal industry has great interest in good field trials (Baars and Egger 1993).

Recently, several BHV1 marker vaccines, which make it possible to differentiate between vaccinated and field virus-infected animals serologically, have been developed (Drunen Littel van-Hurk van den and others 1993, 1994, Kaashoek and others 1994, 1995). These vaccines lack at least one protein that is present in the corresponding wild-type virus and they could play an important role in BHV1 eradication programmes (Lemaire and others 1994, Strube and others 1995). This paper describes a field trial to determine the effect, on milk production, of vaccination with a novel inactivated BHV1 glycoprotein E-negative marker vaccine.

Materials and methods

Farms

The study was carried out on five farms belonging to Dutch cattle research centres, with standardised management. One farm had two separate herds. Holstein-Friesian breeds dominated five of the herds, and the other herd was of the Meuse-Rhine-Yssel breed. The smallest herd had 49 cows.

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Animals

All 455 lactating cows in the six herds were used. On two farms all the cows were seropositive to BHV1 in a commercially available ELISA (Svanova). The other four herds were BHV1-positive in a bulk milk sample (Svanova). Some animals in three herds were known to have been vaccinated as yearlings with a modified live non-marker vaccine.

Allocation of treatment

In each herd, the cows were listed according to their most recent calving date and pairs of consecutively listed animals were formed. From each pair, one animal was randomly allotted (by the drawing of lots) to either group 1 or group 2, and the other was then assigned to the other group. Next, the treatment with vaccine (V) or placebo (P) was randomly assigned to group 1 or 2. In each herd both treatment groups were mixed.

Treatment

Group 1 received the vaccine Bayovac (Bayer). Each dose contained log $10^{8·8}$ TCID50 inactivated gE-negative killed BHV1, strain Za, and 2 ml of adjuvant consisting of aluminium hydroxide and Quil A. Group 2 received 2 ml phosphate buffered saline (PBS) as placebo (Kaashoek and others 1995). The vaccine and the placebo were injected subcutaneously. The first injection was given in June 1993 and the second was given four weeks later.

Measurements

Daily milk production was measured electronically, in millilitres, from six days before vaccination until 14 days after vaccination. These daily milk productions are abbreviated as MP–6 to MP14; MP–6 to MP–1 being the pre-vaccination yields. Vaccination was carried out on day 0. Owing to failures in the automatic milk recording, it was decided not to use MP12 to MP14. Body temperature was measured electronically from two days before until 10 days after vaccination in 63 cows in two of the herds; the measurements were made daily at 08.15.

Statistical analysis

To measure the effect of vaccination, the mean post treatment yield was subtracted from the mean pre-treatment yield, the difference being abbreviated as δMP (delta milk production). To calculate the mean pre- and post treatment yield, restrictions were placed on the number of missing values. When in the pre-treatment period three or more values for the daily milk production were missing, the observation was deleted. This procedure was thought necessary to stabilise the variance of the mean pre-treatment yield. The post treatment period was from one to 11 days, resulting in 11 dependent variables, δMP1 to δMP11. For δMP1 and δMP2, both MP1 and MP2 had to be available. For δMP3 to δMP8 one missing value in MP1 to MP8 was allowed and for δMP9 to δMP11 no more than two missing values were allowed in MP1 to MP11.

A repeated measures model was used in order to take the cow effect into account (each cow had two observations). A balanced data set was created by using only those cows having a valid observation for both the first and second injection.

TABLE 1: Numbers of animals and mean milk productions of each group before each treatment

Injection	Treatment	Number of cows	Daily milk production before treatment (litres)
1	Placebo	182	25·0
1	Vaccine	190	24·0
2	Placebo	176	22·9
2	Vaccine	181	21·8

The main effect model was:

$$\delta MP x_{ijkl} = \text{constant} + TR_i + HERD_j + e_{ijk} + INJ_l + \text{interactions} + e_{2ijkl}$$

δMP_x : difference in milk production before and after treatment (x = 1 to 11)

TR_i : effect of treatment (i = 1 and 2 for groups V and P, respectively)

$HERD_j$: effect of herd (j = 1 to 6)

e_{ijk} : error term 1, which represents the random effect of cow nested within treatment i and herd j (k = cow number)

INJ_l : effect of first or second injection (l = 1 or 2)

e_{2ijkl} : error term 2

Treatment and herd effects and their interaction were tested against error term 1. The effects of injection and other interactions were tested against error term 2. If they were not significant, any interaction effects were omitted stepwise from the model, starting with the three-way interaction TR*HERD*INJ. Main effects were always included, irrespective of the significance. The analysis used PROC GLM of SAS (SAS 1990). The statistical significance level was chosen as P≤0·05.

Results

Data editing

The initial data set included production data from 455 animals. The 21 cows which received a first injection only were deleted from the data set as well as the 18 cows that entered the herds between the first and second injection. Records from the 31 cows which had been lactating for less than 21 days on the first day of measurement were also deleted in order to exclude major effects of parturition. The 16 lactations exceeding 335 days were also removed.

As a result of the restrictions on the number of missing values for daily milk production before treatment, 75 cows were deleted. Varying numbers of cows were removed from the data set owing to restrictions on the number of missing values in daily milk production after treatment. Depending on the latter and the prerequisite for balance, the minimum number of cows in the analysis was 236 (472 injections) and the maximum 283 (566 injections).

TABLE 2: Differences in the estimated daily milk production per cow between the vaccinated and placebo-treated groups and its significance, measured at various periods after treatment

Length of post treatment period (days)	Number of observations	Difference in mean milk production (kg/day)	P
1	542	0·54–0·09 = 0·45	<0·01
2	522	0·62–0·22 = 0·40	<0·01
3	566	0·64–0·28 = 0·36	<0·01
4	558	0·66–0·36 = 0·30	0·01
5	520	0·74–0·48 = 0·26	0·03
6	494	0·81–0·58 = 0·23	0·05
7	480	0·81–0·60 = 0·21	0·10
8	472	0·86–0·66 = 0·20	0·12
9	560	0·89–0·72 = 0·17	0·28
10	552	0·90–0·77 = 0·13	0·47
11	534	0·92–0·80 = 0·12	0·33

Data analysis

Table 1 shows the numbers of animals and the daily milk production per cow before the treatment of the placebo and vaccine groups. Despite the random allocation of pair members to groups, the placebo group gave on average 1 litre/day more milk than the vaccine group before the first treatment and the difference was about the same at the second treatment. The decrease of about 2 litres in the daily milk production before treatment between the first and the second injection can be explained by the fact that most of the animals had passed the top of the lactation curve.

The resulting statistical model was equal for $\delta MP1$ to $\delta MP11$. The three-way interaction TR*INJ*HERD and the two-way interactions TR*INJ and HERD*TR were not significant, but the interaction HERD*INJ was included in all the models. There was a significant effect of vaccination (P≤0·05) on mean milk production during the first six days after treatment ($R^2 = 0·63$). The effect was estimated as 0·23 litres/day. Therefore, the initial estimate of the effect of vaccination on total milk production in the first six days after treatment was about 0·23 × 6 = 1·4 litres per animal per vaccination. Furthermore, Table 2 shows that the difference in milk production levelled out after day 6. To check whether this difference already existed before the treatment was given, the six days before treatment were divided into two periods of three days each and these periods were analysed as pre- and post treatment periods. The difference between the daily milk production during these new pre- and post treatment periods was 0·14 litres (based on 307 cows, P<0·15), which is about equal to the level of the difference in mean milk production after day six post treatment. The actual reduction in milk yield due to vaccination was thus 6 × (0·23-0·14) = 0·54 litres per animal per vaccination. However, this does not mean that the milk production per day was negatively affected during those six days. By the first day after vaccination the effect was estimated as (0·45-0·14) = 0·3 litres (P<0·05). A separate analysis using only MP2 showed that the effect on that day was (0·35-0·14) = 0·2 litres (P<0·05). The (adjusted) effect at day 3 was 0·2 (P<0·05) but it was not significant on the subsequent days. Based on the daily milk yield, the
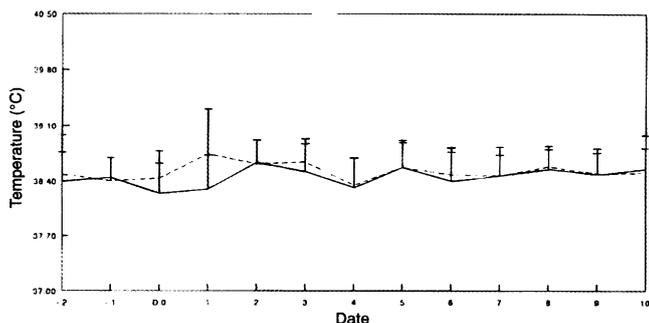
FIG 1: Mean (sd) body temperatures of the placebo group (—) and the vaccinated group (----) after the first vaccination
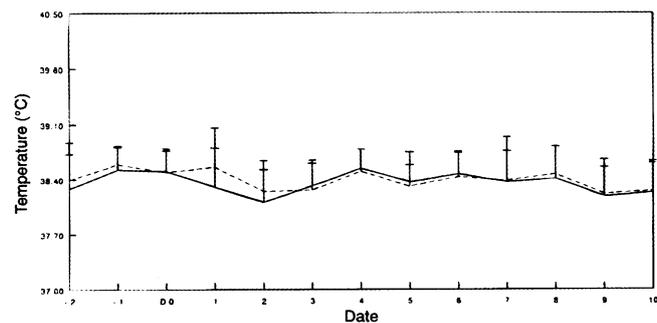
FIG 2: Mean (sd) body temperatures of the placebo group (—) and the vaccinated group (----) after the second vaccination

total loss in milk production was about $(0·3+0·2+0·2) = 0·7$ litres per cow per injection which is about equal to the calculated loss using δMP3, $3 × (0·36-0·14) = 0·66$. The influence of the rank number of vaccination during the first six days after treatment was 0·38 and 1·00 litre for the first and second injection, respectively.

The mean daily temperatures of the placebo and vaccine groups after the first and second treatment are shown in Figs 1 and 2. The difference in temperature between the placebo and vaccine group one day after the first treatment was 0·45°C ($P=0·02$). One day after the second treatment the difference was 0·26°C ($P=0·06$).

Discussion

Under normal conditions, the daily milk yield is highly variable and, even in well-managed herds, a variation of 10 per cent has been reported (Michel and others 1982). Thus in order to detect smaller effects on milk yield large numbers of animals must be studied (Michel and Mulholland 1981).

Several methods had been described for measuring the influence of a medicinal product on milk production. Some investigators have compared the adjusted milk productions of treated and untreated groups (Spence and others 1992, Barkema and others 1994), whereas others have compared the actual milk production with a predicted standard lactation curve (Michel and others 1982, Ploeger and others 1990, Barlett and others 1991). To predict such a lactation curve, several mathematical models have been developed (Masselin and others 1987).

Because the lactation curve of Wood (1967) is one of the most frequently used deterministic models (Masselin and others 1987), an attempt was made to use this model on the present data. An average lactation curve was estimated for the unvaccinated animals. Deviations from this curve, before and after vaccination, were calculated for the vaccinated animals, assuming that they would have the same curve as the unvaccinated animals. The difference between the deviations before and after treatment was then used as the outcome variable.

However, the estimated coefficients of the lactation curves varied considerably between herds and between individual animals within herds. However, only 20 daily measurements of milk production per cow were available. As a result, a direct comparison between the milk production before and after treatment was deemed more appropriate, using the placebo group to indicate possible trends in the population.

A very mild adverse effect of vaccination on mean milk production was observed during the first six days after injection (Table 2). The effect became significant because of the large number of animals. The decrease in milk yield was slightly larger after the second injection. The total production loss of about 1·4 litres for a double vaccination had occurred within the first three days after injection. As a result, if the period after injection is relatively long, even large short-term effects would not be detected by using the mean milk production during that period.

A slight difference was observed between the calculated effect of vaccination using the mean milk production ($2 × 0·54$ litres) and the total loss in milk production based on daily milk yield ($2 × 0·7$ litres). This difference was due to the fact that different numbers of animals were used for the different calculations (Table 2). The power of this study (1-type 2 error), indicating the probability that the observed small treatment difference was a real difference, was computed to be almost 0·9.

After six days, the difference in the mean milk production was due to the systematic difference between the vaccine and placebo group. This large difference in milk production between the two groups had been observed before the treatment and was larger than expected, because the pair-members were allotted to the groups at random (Table 1). This systematic difference was estimated as 0·14 litres/day.

Little has been published about the influence of vaccination on milk yield because the effect should be only mildly adverse. Cortese (1991) investigated the effect of a modified live BHV1 combination vaccine on the milk yield of 939 cows in 18 herds. Milk production was measured for six milkings, three before and

three after vaccination. The results were tabulated in several ways, including comparisons in individual herds of vaccinated cows with unvaccinated controls. No significant differences between the controls and the vaccinated cows were found, nor did significant interactions occur between treatment and pregnancy, or various ages or stages of lactation. Unfortunately, the report did not describe the statistical method used.

Kahrs and others (1973), in a study of the effects of a modified live IBR-parainfluenza (BHV1/PI3) vaccine, detected a slight decrease in milk production in one of 12 herds, on the basis of superficial inspections of daily milk weights.

An investigation on the adverse reactions of a capripox vaccination in cattle was conducted by Yeruham and others (1994). After the initial post vaccination reactions, which lasted for six days, 442 cows in two dairy herds were closely monitored. Milk yields before and after vaccination were compared and a decrease in milk production of 3·5 per cent over a period of 12 days was recorded. No placebo group was used. The initial post vaccination reactions were not described.

In this study, an inactivated BHV1 vaccine with adjuvant was used. Since adjuvants frequently cause adverse reactions (Vomand and Sumano 1990), the adjuvant used in the BHV1 marker vaccine might have been responsible for the slight decrease in milk production. However, there were no indications of severe adverse system reactions in two of the herds after the first and second treatment (Figs 1 and 2). The mild, but significant, temperature reaction after the first vaccination was accompanied by a small decrease in milk production. In contrast, after the second vaccination a negligible increase in temperature was accompanied by a larger decrease in milk production.

The goal of the trial was to investigate the possible adverse effects on milk production of the total vaccine, which is now used in practice as such. Therefore, it was decided not to use the adjuvant as the placebo. Few, if any, of the vaccines on the market have been subjected to such a thorough study as this BHV1 gE-negative marker vaccine. The results of this trial show that vaccination had a significant but negligible negative effect on mean milk production for six days. The inactivated BHV1 gE-negative marker vaccine is therefore safe with regard to its effects on milk production. The results of this trial confirm that in order to detect the possible small effects of vaccination on milk production, any trials must be large scale and use sensitive and appropriate statistical analyses to detect them.

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Prevention of surgical infections in dogs with a single intravenous injection of marbofloxacin: an experimental model

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Eighteen healthy beagle dogs of both sexes were each given 0, 2 or 4 mg/kg marbofloxacin intravenously before the subcutaneous implantation of a silicon tissue cage. Two millilitres of a suspension containing $1 \cdot 3 \times 10^4$ colony forming units (CFU)/ml of *Staphylococcus intermedius* were then injected into the cage 15 minutes after the intravenous injection. The dogs were clinically assessed immediately, and then two, four, eight and 24 hours after the challenge. Samples of inflammatory fluid were harvested at the same times in order to count staphylococci and to assay marbofloxacin concentrations. Blood samples were taken in order to assay plasma marbofloxacin levels. The staphylococcal counts were lower in both treated groups than in untreated dogs (P<0·01). All the clinical criteria were similar in the three groups. The concentration of marbofloxacin was similar in plasma and inflammatory fluid. Both doses were well tolerated and no adverse reactions were observed.

PREVENTION of infection in surgical wounds is a challenge to all surgeons because true surgical asepsis can never be achieved. Many efforts have been directed at reducing contamination at the operation site and preventive techniques are now well described (Romatowski 1989) and quite effective. Recorded infection rates are low and comparable in veterinary and human surgery, with overall rates of 5·1 per cent (Vasseur and others 1988) and 4·7 per cent (Cruse and Foord 1980), respectively, for all types of surgery. Numerous papers have been published reporting clinical results of antimicrobial prophylaxis of surgical infections, both in the veterinary and human fields, but with controversial conclusions. The best way to provide accurate and well-controlled results is through experimental models, because of the very low infection rates recorded in practice. Several models have been described (Burke 1961, Bowers and others 1973, Scher and Jones 1985, Rosin and others 1989, Kaiser and others 1992, Rosin and others 1993), but most of them require the sacrifice of the animals concerned. Ethical and practical considerations led the authors to develop a more conservative approach to test a new antibacterial agent in the prevention of surgical wound infections.

Marbofloxacin is a bactericidal antimicrobial agent belonging to the family of fluoroquinolones that has been specifically developed for veterinary medicine. It has recently been registered in several European countries. Marbofloxacin has a broad spectrum of activity including the major pathogens encountered in surgical infec-

tions, in particular Enterobacteriaceae and staphylococci (Van Der Bogaard and Weidema 1985, Moissonnier 1990, Spreng and others 1995). Moreover, marbofloxacin has pharmacokinetic properties of particular interest in the prevention of surgical infection, it has a very long elimination half-life, close to 14 hours in dogs (Schneider and others 1996), and its tissue diffusion is excellent: the ratio between tissue and plasma concentrations is 1·6 in muscle and skin and even higher in liver (2·5) and kidney (2·3). These properties are of particular importance and explain the good results obtained in field trials involving canine skin infections (Carlotti and others 1995) and urinary tract infections (Cotard and others 1995). In addition, marbofloxacin has been shown to be very safe in dogs, with no articular damage when administered to juvenile dogs at a dose of 6 mg/kg per day for three months (unpublished observations). In this study, marbofloxacin was used in the form of a 2 per cent injectable solution. An intravenous injection of 2 or 4 mg/kg marbofloxacin maintains plasma concentrations above the minimum inhibitory concentrations (MICs) of the major pathogens (Enterobacteriaceae and staphylococci) for 12 to 24 hours.

The aim of this study, performed according to the standards of Good Laboratory Practice, was to select an effective and safe dose of marbofloxacin to prevent surgical infections in dogs.

Materials and methods

Experimental design and test system

Eighteen healthy beagle dogs were selected and randomly assigned to three groups of six animals and two periods of time (three animals per group per period of time), in a complete-block design. The blocks were constituted to ensure homogeneity between groups. All the animals were tattooed and recorded on the 'Société Centrale Canine' file. They were fed a standardised diet and water was available ad libitum.

Experimental model

All the dogs were clipped at the incision site before the study began.

Tissue cages. – The tissue cages consisted of 60 mm long Silastic tubes (515-019; Dow Corning) of 12·7 mm external diameter and 9·5 mm internal diameter. Both ends were sealed with silicon glue and holes were opened along the tube to let inflammatory fluids in. The cages were sterilised by autoclaving.

Bacterial strain used for the experimental infection. – A *Staphylococcus intermedius* strain isolated from a naturally occur-

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