

ABSTRACT

Guelph study of ENDOVAC-BOVI® in Lactating cows

OBJECTIVE: To determine the effect of vaccination with ENDOVAC-BOVI® on lactating dairy cows.

METHOD: Sixty eight multiparous Holstein cows were randomized and divided into two groups. One group received two each, two milliliter doses of ENDOVAC-BOVI® three weeks apart. The other group received a placebo. Milk production, somatic cell counts, progesterone levels for pregnant cows, body temperatures, white cell counts, leucocyte differentials; and feed consumption were determined for either all or subsets of the cows.

CONCLUSION: The majority of the parameters measured in this study showed no significant effects related to immunization with ENDOVAC-BOVI® over the duration of the study. Daily milk production, feed intake, SCS, milk progesterone, rectal temperature, and blood lymphocytes and band cells were not affected by vaccination of the animals. Total WBC counts and the number of mature neutrophils were significantly increased immediately after vaccination which subsided within 12-24 hours.

SUMMARY: Vaccination of lactating cows with two consecutive 2 ml doses of ENDOVAC-BOVI® appeared to have no detrimental effects on milk production and most physiologic parameters. There was, however, a significant increase of neutrophils following vaccination which subsided in 12-24 hours.

FILE: ABSTRACT-Guelph study-ENDOVAC-BOVI in Lactating cows

EFFECTS OF A GRAM NEGATIVE CORE ANTIGEN VACCINE ON PHYSIOLOGIC AND PRODUCTION PARAMETERS IN LATE LACTATION AND DRY PERIOD HOLSTEIN DAIRY COWS

OBJECTIVE

The objective of this study was to evaluate the efficacy of Endovac-Bovi[®], an R17 mutant *Salmonella typhimurium* core antigen vaccine, in reducing the incidence and effects of clinical coliform mastitis, under field conditions on Canadian dairy farms, and for registration of Endovac-Bovi[®] for sale in Canada. The study was completed via direction of IMMIVAC'S scientists and biostatisticians, and under auspices of the Department of Microbiology and Immunology, University of Guelph, Guelph, Ontario, Canada. The results were filed with The Canadian Food Inspection Agency (CFIA), the animal health regulatory agency for Canada.

INTRODUCTION

The incidence rate of clinical mastitis, caused by environmental pathogens such as *E. coli* has become a concern of the dairy industry. It has even been suggested that control of subclinical mastitis and the subsequent reduction in milk Somatic Cell Count (SCC) levels may increase the susceptibility of cows to clinical coliform mastitis. Gram negative core antigen vaccines have been developed to provide cross-protection against a variety of coliform pathogens (4,6). However, some of these products also contain bacterial toxoids which may cause adverse systemic effects in lactating dairy cattle. Systemic changes attributed to endotoxin may be manifested in decreased feed consumption, lower milk production, and increased SCC (1,2,7,9). Adverse reproductive effects may include damaged luteal function, resulting in fetal wastage (1,7). Other systemic and biochemical changes due to endotoxin have been demonstrated in experimental challenge or case series (2,5,9). The current study was conducted to assess the effects of a gram negative core antigen vaccine (Endovac-Bovi[®], Immvac, Columbia, Missouri) on feed consumption, milk yield, SCC, haematologic parameters and milk progesterone in Holstein dairy cows. An additional objective was to observe these parameters in late lactation (non-labelled usage) as well as dry period (labelled usage) dairy cows.

MATERIALS AND METHODS

Sixty eight multiparous Holstein cows from 2 dairy research farms were pair-matched by days in milk and randomized to receive either vaccine or placebo. The vaccine contains a gram negative core antigen (R17 mutant of *Salmonella typhimurium* as bacterin) as well as a proprietary E3 toxoid (4). A placebo of similar consistency and colour (cloudy white) was developed using phosphate buffered saline and very small quantities of mineral oil and Tween (Fisher Scientific). All animals were boosted at 3 weeks. On one research farm only (n=42), daily milk weights and daily TMR feed intakes were recorded. On both research farms (n=65), composite milk SCC were measured once at one week prior and daily for one week following each of initial and booster injections. Milk progesterone levels were assessed for pregnant cows (n=39) at three day intervals starting 6 days before the treatments and continuing until 18 (first treatment) and 9 (second treatment) days respectively after the treatments. At each treatment, and for 2 days after the following parameters (n=68) were measured at 24 hour intervals: 1) rectal temperature, 2) blood leucocyte (WBC) counts, and 3) blood leucocyte differentials (segmented neutrophils,

immature neutrophils and lymphocytes). In addition, the first series of injections were given in the semimembranous / semitendinosus muscles while the second series were given in the gluteal (hip) region. Skin level swelling diameters were measured at one week following injection (n=68).

For the majority of parameters studied, measurements were repeated on animals over time. Treatments were nested within the animals. Analyses were performed on milk and feed weights (one week before and after treatment), SCC converted to linear score ($SCS = (\log_2 SCC/100,000 + 3)$) at one week before and for 9 days after treatment using repeated measures methods (3). In addition, milk progesterone (6 days prior and up to 18 days after), rectal temperature and blood counts (both the morning of treatment and for 2 days after) were analyzed. Statistical analysis for repeated measures was performed using the general linear models procedure; PROC GLM in SAS® (Statistical Analysis System, SAS Institute, Cary, N.C., USA) (10). Each dependant variable was modelled separately at the two treatment periods. Thus, each model contains a time frame before, during and after treatment. The PROC GLM univariate results were adjusted using Greenhouse-Geisser epsilon where the sphericity assumptions of the variance-covariance matrices were in question (2,10). Each model was initially assessed for TREATMENT*PERIOD interactions. Where these were found not to be significant at $p < 0.05$, further examination of treatment and period effects was not considered relevant (2). When TREATMENT*PERIOD interactions were significant at $p < 0.05$, important period effects were noted at individual sampling times. In addition to the repeated measurements, mean differences in injection site swelling at one week post treatment were determined.

RESULTS

The results of the analyses are summarized in Table 1. There were no significant TREATMENT*PERIOD interactions for daily milk weights (see Figures 1 and 2). There is a consistent pre- and

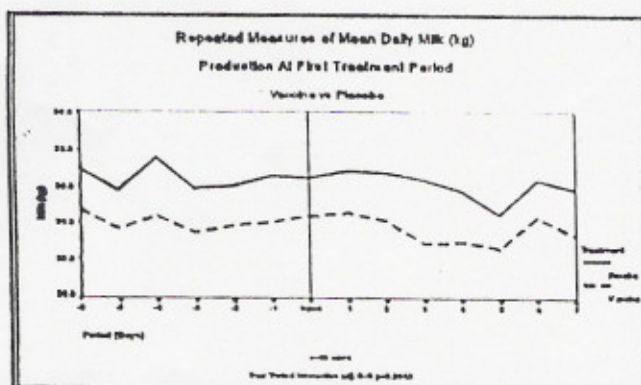


Figure 2

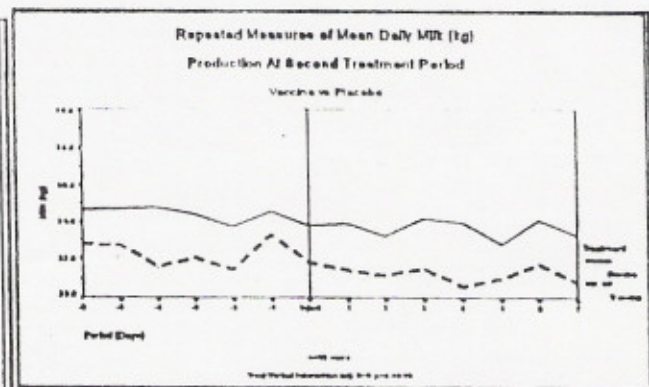


Figure 1

post-treatment difference in mean milk production between vaccine and placebo treated cows. However, this difference does not vary significantly over time at either the first ($p=0.8043$) or second injection times ($p=0.4940$). In particular, there was no visible drop in milk production in the days immediately following injection.

Table 1 (^a= significant at $p < 0.05$, ^b=significant at $p < 0.01$)

Analysis of Treatment Effects (Vaccine vs Placebo) on Physiologic Parameters in Holstein Cows						
Repeated Measures Analysis				*Type III MS Error Term for Treatment is Cow(Treatment)		
Variable	Injection #	n	Treatment*Period Interaction (p)	G-G Adjusted (p)	Period Effect (p)	Treatment Effect (p) ^a
Daily Milk (kg)	1	42	0.8897	0.8043	0.0001	0.1573
	2	42	0.5349	0.4940	0.0001	0.1404
Daily Feed (kg)	1	42	0.5845	0.5337	0.0001	0.0316
	2	42	0.9582	0.9088	0.0001	0.0768
Somatic Cell Score	1	65	0.9754	0.9017	0.0001	0.9275
	2	65	0.9759	0.9166	0.0001	0.8372
Rectal Temp (°C)	1	68	0.2778	0.2778	0.0009	0.0942
	2	68	0.1956	0.1940	0.0559	0.1156
WBC (*1,000/mL)	1	68	0.1114	0.1177	0.0038	0.8993
	2	68	0.0003 ^a	0.0005 ^a	0.0001 ^a	0.4742
Neutrophils (*1,000/mL)	1	68	0.0258 ^a	0.0269 ^a	0.0001 ^a	0.4285
	2	68	0.0001 ^a	0.0001 ^a	0.0003 ^a	0.1618
Immature Neutrophils (*1,000/mL)	1	68	0.4740	0.4365	0.1564	0.2873
	2	68	0.3924	0.3294	0.1983	0.3580
Lymphocytes (*1,000/mL)	1	68	0.5186	0.5562	0.0001	0.4819
	2	68	0.7268	0.7317	0.2529	0.7271
Milk Progesterone (ng/mL)	1	39	0.4641	0.4309	0.0001	0.9071
	2	39	0.2869	0.2911	0.0004	0.9879
Non-Repeated Measures	Location	n	Difference of Means (p)			
Injection Site	Hamstring	68	0.0001 ^a			
Swelling (mm)	Gluteal	68	0.0399 ^a			

Similar to milk production, mean daily feed intakes do not vary over time ($p=0.5337$ and 0.9088 respectively). Mean somatic cell scores (SCS) at each injection showed no TREATMENT*PERIOD interaction ($p=0.9017$, $p=0.9166$ respectively). There were significant period effects ($p=0.0004$ at first treatment) which are evidenced by the fluctuations in the plotted graph (see Figure 3). Note, however, that both treatment groups follow a similar pattern over time and have no significant effects by themselves ($p=0.9275$ at first treatment).

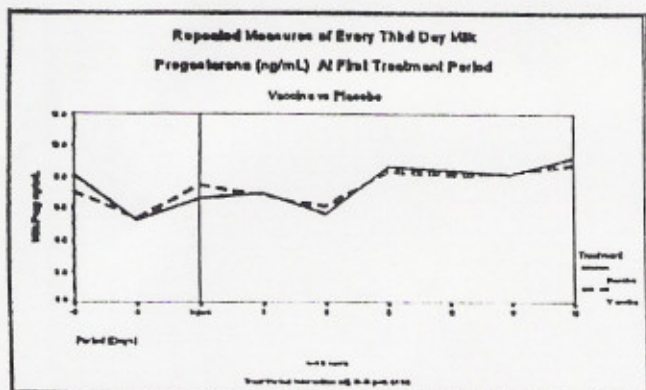


Figure 3

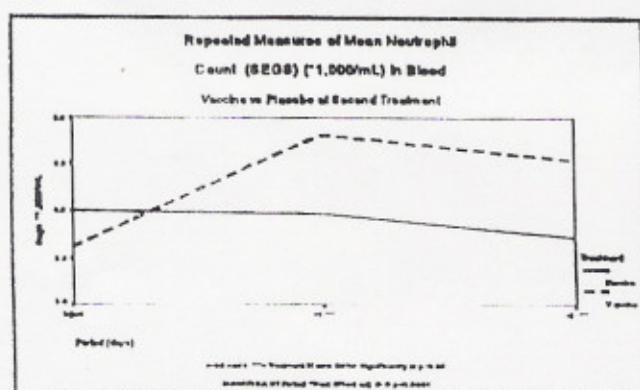


Figure 4

Mean rectal temperature ($^{\circ}\text{C}$), measured at 24 hour intervals, did not change following injection with vaccine or placebo. Milk progesterone levels (only pregnant cows were examined to determine stability of progesterone levels during mid to late gestation) were examined similar to other repeated measures. Neither treatment period resulted in significant effects ($p=0.4309$, 0.2911).

Significant TREATMENT*PERIOD interactions were restricted to complete blood count results. While the first injection leucocyte count showed a trend towards significance ($p=0.1177$), the second injection at 3 weeks showed a demonstrable effect on overall blood leucocyte count ($p=0.0005$). The overall interaction effect is suitably explained by the leucocyte differential profile. Both first and second injection times showed significant TREATMENT*PERIOD effects on blood segmented neutrophil counts (see figure 4). The differences were most marked 24 hours after injection. Immature neutrophil and lymphocyte blood populations do not appear to influence the interaction noted in the blood leucocyte count (WBC).

There were significant differences in injection site swellings between vaccinates and controls. Specifically, there were much more detectable swellings in vaccinates at the semimembranous / semitendinosus sites ($p=0.0001$) than at the gluteal sites ($p=0.0399$).

DISCUSSION

Gram negative core antigen vaccines have been developed as a tool in the prevention of coliform mastitis. Some concern has centered on the safety of these products with respect to the presence of bacterial toxoid in their contents. Current product labelling suggests that dairy producers vaccinate cows at or near dry off. Potential adverse effects on feed consumption, milk production, SCC changes and fetal loss due to luteal dysfunction would be minimized by vaccinating late in gestation and during the dry period. However, interest has also been expressed in applying these products during other periods in the dairy production cycle. Thus it is of interest to observe the physiologic and production parameters of cows treated during mid to late lactation as well as in the dry period.

The majority of the parameters measured in this study showed no significant effects related to treatment over time at injections. Daily milk production, feed intake, SCS, milk progesterone,

rectal temperature and blood lymphocytes and band cells were not affected by the vaccine product in the study animals. There was a significant effect of the vaccine on WBC and segmented neutrophil counts. This result is consistent with the findings of others where endotoxin effect has been monitored in dairy cattle either experimentally or clinically (1,9). Ostensson (9) collected information on white blood cell components during endotoxin induced mastitis on a more rigorous schedule than that presented here. Leukocyte dynamics observed in that study included an immediate rise in total WBC and neutrophils and a somewhat delayed lymphocyte response(9)

The majority of the studies cited here examine effects observed on animals experiencing clinical endotoxemia. One recent paper by Musser and Anderson examined the effects of a different core antigen vaccine (J-5 VAC[®], Sanofi Animal Health, Inc.) on milk production in dairy cattle(8). These authors detected a small but statistically significant ($p < 0.05$) difference in milk production during the second and third milkings post treatment. No similar differences were seen in the present study, although different analytic procedures were applied. Cows in the present study were pair-matched on days in milk alone whereas those in the Musser study were matched on parity and production traits as well. In the present study randomization across multiparous pairs matched by days in milk alone, did not result in equivalent milk production groups for comparison by group means alone. Repeated measures techniques were applied instead.

In summary, this study failed to show a significant effect of the core antigen product on production parameters and most physiologic parameters. There was, however, a significant effect on blood neutrophil counts in the days following treatment.

REFERENCES

1. Cullor, James S. 1992. Journal of the American Veterinary Medical Association, 200:1894.
2. Eades, Susan C. 1993. Journal of Dairy Science, 1993. 76:414.
3. Fleiss, J. L. 1986. The Design and Analysis of Clinical Experiments. New York. John Wiley and Sons. p.223.
4. IMMVAC Inc, Columbia, Missouri, USA Product Bulletin on ENDOVAC-Bovi; Product Information Sheet on ENDOVAC-Bovi and Product Label for ENDOVAC-Bovi.
5. Katholm, J. and Haubro Andersen, P. 1992. The Veterinary Record, 131:513.
6. McClure, A. M. , Christopher, E. E., Wolff, W. A , Fales, W. H., Krause, G. F., and Miramonti, J. 1994. Journal of Dairy Science, 77:2272.
7. Moore, D. A., and O'Connor, M. L. 1993. National Mastitis Council Annual Meeting Proceedings, p.162.
8. Musser, J. M. B., and Anderson, K. L. 1995. National Mastitis Council Annual Meeting Proceedings, p.123.
9. Ostensson, K. 1993. American Journal of Veterinary Research, 54:231.
10. SAS Procedures Guide, Version 6, First Edition. Cary, N.C, USA: SAS Institute, 1993.