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Field Evaluation of Prophylactic and Therapeutic Effects of a Vaccine against (Papillomatous) Digital Dermatitis in Dairy Cattle on Two California Dairies

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Abstract

Objectives of this study were 1) to determine prophylactic and therapeutic effects of a *Treponema* bacterin against papillomatous digital dermatitis ((P)DD) in lactating dairy cows on two California dairies, and 2) to determine and compare serologic response to the *Treponema* bacterin between vaccinate and placebo groups of cows. A total of 1160 Holstein cows were enrolled. Cows were grouped according to treatment (vaccinated or placebo) and visible (P)DD lesion status prior to treatment. One hundred and twenty cows with no visible lesions prior to treatment were bled for serologic evaluation. Monthly visual and serologic evaluations were performed before and after the treatment for six months.

Comparison of monthly proportion of cows between groups of vaccinates and placebos showed no significant prophylactic or therapeutic effects due to the *Treponema* bacterin. Results were also not significant when monthly proportions of cows were stratified by lactation groups or for only those cows present at every monthly observation and stratified by lactation groups. ELISA titers of the 120 cows bled prior to treatment revealed that 43% of the cows had positive titers, indicating prior exposure to *Treponema* spp. Comparisons between monthly proportions of vaccinates and placebo cows that had no visible (P)DD lesions, and which had negative serologic titers to (P)DD-associated *Treponema* spp antigens prior to treatment, showed that a significantly higher proportion of vaccinates developed a positive serologic state during the first, second and third months after treatment.

Résumé

Les objectifs de cette étude étaient d'une part de déterminer les effets thérapeutiques et prophylactiques d'une bactérine de *Treponema* pour lutter contre la dermatite digitale papillomateuse ((P)DD) chez des vaches laitières en lactation dans deux fermes de la Californie et d'autre part de déterminer et de comparer la réponse sérologique à la bactérine de *Treponema* entre des groupes de vaches vaccinées et témoins. Un total de 1160 vaches Holstein ont été sélectionnées. Les vaches ont été regroupées en fonction du traitement (avec vaccin ou sans vaccin) et selon la présence de lésions (P)DD visibles avant le traitement. Des prises de sang ont été faites pour évaluation sérologique chez 120 vaches ne montrant aucunes lésions visibles avant le traitement. Des évaluations visuelles et sérologiques ont été faites à tous les mois avant et après le traitement pendant six mois.

La comparaison des groupes de vaches vaccinées et témoins avec l'aide des résultats mensuels n'a pas dévoilé d'effet significatif de l'administration de la bactérine de *Treponema* tant au niveau prophylactique que thérapeutique. Les résultats n'étaient guère plus significatifs lorsque l'analyse était stratifiée selon le groupe de lactation dans l'ensemble de toutes les vaches ou selon le groupe de lactation dans le sous-ensemble de vaches présentes à chacune des évaluations mensuelles. Le titrage ELISA des sérums prétraitements provenant des 120 vaches indiquait que 43% des vaches avaient des titres positifs et avaient donc été exposées à *Treponema* spp. La comparaison des groupes vaccinés et témoins, restreinte au sous-ensemble de vaches qui n'avaient pas de lésions visibles de (P)DD et qui avaient des titres sérologiques prétraitement négatifs aux antigènes associés à *Treponema* spp, a montré qu'une réponse sérologique positive était plus fréquente lors du premier, second et troisième mois suivant le traitement dans le groupe des vaches vaccinées.

Introduction

(Papillomatous) digital dermatitis ((P)DD), also known as digital dermatitis, foot warts, or hairy heel warts, is a worldwide, superficial, painful and contagious disease causing ulceropro-liferative lesions of the skin, most commonly at the plantar surface of rear feet near the interdigital space and heels of cattle.[2,24] Today, (P)DD accounts for 40-70% of all skin lesions associated with lameness in dairy cattle around the world.[11] Papillomatous digital dermatitis was first reported in Italy in 1974[7] and in the US in 1980.[25] Since 1980, (P)DD has been reported from many countries.[11,28]

California epidemiological surveys found that 90 to 97% of southern, 75% of central and 30% of northern herds were affected by (P)DD, resulting in great financial losses.[23,26] Economic loss to dairy producers results from premature culling, lameness, decreased milk yield, poor reproductive performance, weight loss, milk discard due to treatment with antibiotics, treatment and labor costs.[13] Papillomatous digital dermatitis is also an animal welfare concern, as well as a source of human health and environmental hazards when using and disposing of hazardous chemicals in footbaths used for treatment and control.[1,6,14,19]

The precise etiology of (P)DD is unknown, but it is a multifactorial disease involving environmental, management and microbial factors.[16,19,24,27,28] Experimental morphopathogenesis studies have suggested that invasive spirochetes play a major role in disease pathogenesis.[20] Microbes identified most consistently and predominantly from active (P)DD lesions around the world are spirochetes of the genus *Treponema*.[5,8-10,12,17,34]

Although treatment and control measures, such as antibiotic and non-antibiotic topical sprays, foot wraps and footbaths, have been found effective, they are labor intensive and costly. Footbaths are potentially hazardous to human health and the environment, and are problematic when outbreaks involve a large number of cattle in a herd in which (P)DD incidence and recurrence are high.[1,2,6,14,19,23,30-32,35] Thus, development of an efficacious and cost-effective vaccine to prevent (P)DD would be highly advantageous.

The objective of this field (clinical) trial was to determine whether a *Treponema* bacterin[a] provided prophylactic or therapeutic effects for controlling (P)DD in cattle. A total of 740 and 420 Holstein cows were enrolled from two commercial California dairies with pre-vaccination (P)DD prevalence of 29 and 27%, respectively.

Materials and Methods

Study population — The study was conducted on two commercial Holstein dairies located in the central valley of California. Dairy 1 had approximately 1200 lactating cows with a pre-treatment (P)DD prevalence of 29% measured on July 2, 2002. Dairy 2 had approximately 850 lactating cows with a pre-treatment (P)DD prevalence of 27% measured on July 3, 2002. Both dairies housed lactating cows in similar freestall barns, fed total mixed rations (TMR), milked cows twice per day and used footbaths for cows exiting the parlor. Both dairies used footbaths during one milking per day, Monday through Friday. Dairy 1 used a weekly rotation of 3.6% $CuSO_4$, 1.7% poultry litter compound and 3.6% $ZnSO_4$. Dairy 2 used 1.5% $CuSO_4$. Footbaths were cleaned and recharged after 150-200 cow passes on both dairies. Both dairies used private hoof trimmers to trim all dry and lame cows. Dairy 1 had the hoof trimmer on the dairy four days per month, and dairy 2 twice per month. Cows were held for the hoof trimmer on both dairies by dairy personnel who identified cows that were clinically lame and/or had visible (P)DD lesions. Treatment of (P)DD lesions consisted of cleaning the lesion and applying powdered oxytetracycline or lincomycin under a light bandage.

Enrollment of subjects and treatment — All lactating cows were administered a 4 ml subcutaneous injection of either *Treponema* bacterin[a] or placebo. A total of 740 and 420 Holstein lactating cows were included in the study from dairies 1 and 2, respectively. Study enrollment criteria were lactating cows receiving all three injections, consisting of three doses three weeks apart (according to label directions) of either vaccine[a] or placebo, and were evaluated before the study began and at least once during the study. The placebo had the same constituents as the vaccine, but lacked killed *Treponema* spp. A total of 60 lactating cows (30 vaccinates and 30 placebos) from each dairy without visible (P)DD lesions prior to treatment were bled for serologic evaluation for serum antibodies to (P)DD-associated *Treponema* spp by ELISA.

Treatment groups — Study cows were grouped according to disease status prior to treatment (visible lesion, no visible lesion) and treatment received (vaccine[a], placebo). Treatment criterion was based on odd (vaccine) or even (placebo) ear tag ID numbers, and consisted of 378 vaccinates and 362 placebos for dairy 1, and 213 vaccinates and 207 placebos for dairy 2. For each dairy, study cows were assigned to four groups: 1) pre-treatment lesion absent and vaccine, 2) pre-treatment lesion absent and placebo, 3) pre-treatment lesion present and vaccine and 4) pre-treatment lesion present and placebo. Based on these criteria, Groups 1 and 2 (n= 415 and 408, respectively) were evaluated for prophylactic effects, while Groups 3 and 4 (n= 176 and 161, respectively) were evaluated for therapeutic effects. To

account for selection bias, differences in lactation groups, milk yield and days-in-milk among all groups were assessed for each dairy and both dairies combined prior to treatment.

Treatment methods — Three 4 ml doses of vaccine[a] or placebo were administered subcutaneously in the neck at three-week intervals. For both dairies, all lactating cows were locked in stanchions for routine management procedures and received the first treatment on July 18 and July 19, 2002, respectively; the second treatment on August 08 and August 09, 2002, respectively; and the last treatment on August 29 and August 28, 2002, respectively.

Data collection and records — Cows' feet were sprayed with water from drop hoses in the parlor (waterjet test or WJT) as a sensitive screening test to detect cows that had hard-to-see (P)DD lesions and assess pain response.[28] Diagnosis of all lactating cows was made by gross visual examination using a bright light in the milking parlor to determine presence or absence, as well as location, of active (P)DD lesions (LR, RR, LF or RF foot; Figure 1).[4,24] Ear tag number (ID) was recorded for all evaluated cows. Pre-treatment (P)DD prevalence was determined by this evaluation method on July 02 and July 03, 2002 for dairies 1 and 2, respectively. Monthly evaluations using the same method began on October 04 and October 03, 2002 for dairies 1 and 2, respectively, (four weeks after third treatment) and monthly over the next six months. Visual observation records were matched and cross-checked with hoof trimmers' records for the same cows during the same month of observation.

The first 60 cows treated (30 vaccinates and 30 placebos) from each dairy without gross visible (P)DD lesions were leg-banded for identification, and blood samples from the tail were collected at the stanchions. Alleys had been cleaned (flushed or scraped) immediately prior to this to facilitate visual examination. Additional blood samples were collected from the same cows at the time of the third treatment, and at each of the following six post-treatment monthly evaluations. Ear tag ID was recorded for all cows that were bled. Whole blood was collected by venipuncture from the coccygeal vein of cows using vacuum tubes (gel serum separator red/gray). Blood samples were allowed to clot at ambient temperature, stored in an ice chest or refrigerator and later centrifuged at an angular velocity of 3000 rev/min for 15 minutes. Serum samples were shipped to the California Animal Health and Food Safety Laboratory (CAHFS)-San Bernardino where they were processed and stored in aliquots at -4°F (-20°C). Finally, boxes of cryovials containing samples of approximately 1 ml of serum from each cow from each dairy were shipped on icepacks in polystyrene boxes to Novartis Animal Health[a] (NAH), where they were serologically analyzed.

Representative skin biopsies of gross lesions from three cows were evaluated by one of the authors (DHR) by histopathology, and results confirmed that the diagnostic criteria were accurate.[22]

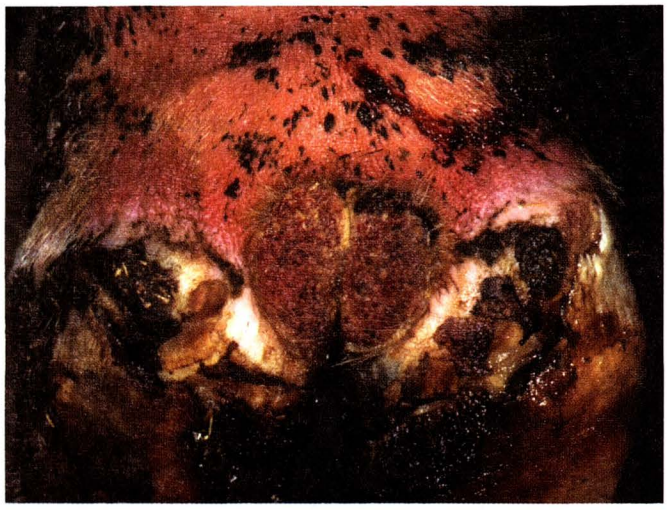

Figure 1. Photograph of a representative (papillomatous) digital dermatitis lesion.

Serologic analysis — Papillomatous digital dermatitis-associated *Treponema* spp antibody levels in serum samples were measured by NAH personnel using an enzyme linked immunosorbent assay (ELISA).[33] The positive and negative control sera for the assay were prepared by NAH personnel. Cattle used in the study to develop the control sera were received by NAH from an outside vendor on August 27, 2002. The cattle were 13 to 15-month-old Holstein steers. The assay was capable of measuring titers ranging from 1:800 to 1:25600. A negative serologic state was considered as ≤1:1600. A positive response was presumed to occur when titers were >1:1600 (DJ Keil).[b]

Statistical analysis of visible lesions — Differences in proportions of cows with visible (P)DD lesions between vaccinate and placebo groups were statistically analyzed for significance by 2-proportional z-test analyses with 95% CI[c] in order to determine prophylactic or therapeutic effects of *Treponema* bacterin[a] on both dairies. Proportions were analyzed by month (to account for missing values and variable sample sizes due to attrition) for three different lactation groups: namely 1) all lactations; 2) lactation 1; and 3) lactation 2 and higher (according to individual records from Dairy Comp 305[d] at the time of enrollment). Comparison of proportions of cows with visible (P)DD lesions between vaccinate and placebo groups by month was done in two

ways: 1) all cows examined on a particular date, and 2) only cows present at all six observations. Proportions of vaccinates and placebos with visible (P)DD lesions during the six months post-treatment period were compared for significant differences. This was done for the total enrolled cows and the total enrolled first-lactation cows, as well as for each separate dairy and for both dairies combined.

In order to account for misclassification bias, Win Episcope 2.0[e] software was used to calculate the percentage of agreement between diagnoses made by the hoof trimmer and by the author. The hoof trimmer used a hydraulic upright trimming chute, while author diagnoses encompassed visual examination with WJT prior to treatment and at any time during the six month post-treatment evaluation period.

Statistical analysis of serologic titer — Pre-treatment serum ELISA values from the 120 cows sampled pre-treatment from both dairies was performed by NAH. Only 63 of the 120 bled cows from both dairies had pre-treatment titers of ≤1:1600. A sample group of 14 vaccinates and 17 placebos from the 63 cows with pre-treatment titers of ≤1:1600 were followed serologically over the post-treatment period, and statistically analyzed both as dichotomized measurements in terms of positive or negative serologic titer by month and by $\log_2$ transformation of actual titer by month. Titers ≤1:1600 were considered to represent negative serologic states, while titers >1:1600 were considered to represent positive serologic states (DJ Keil).[b] These titers indicated either pre-trial non-exposure or exposure to (P)DD-associated *Treponema* spp. Differences in percentage of cows with positive titers were compared using a chi-squared test.[f] Differences in $\log_2$ transformations were analyzed by ANOVA.[f]

Results

One-way ANOVA showed no significant differences ($P>0.05$) in lactation group, milk yield and days-in-milk between Groups 1, 2, 3 and 4 at pre-treatment evaluation. Records from gross visual examinations of active (P)DD lesions were compared with hoof trimmer's records for the same cows during the same month of observation. Percent agreement between diagnosis by hoof trimmers using a hydraulic upright trimming chute and by author using in-parlor visual examination with WJT from pre-treatment to the end of the six-month post-treatment evaluation period was 93, 98 and 94% for dairy 1, dairy 2 and both dairies combined, respectively.

Comparison of monthly proportion of cows from each dairy with post-treatment visible (P)DD lesions between Groups 1 (pre-treatment lesion absent and vaccine) and 2 (pre-treatment lesion absent and placebo) showed no significant prophylactic effect ($P>0.11$), and comparison between Groups 3 (pre-treatment lesion present and vaccine) and 4 (pre-treatment lesion present and placebo) also showed no significant therapeutic effect ($P>0.12$) due to the *Treponema* bacterin.[a] Monthly (P)DD proportions were also analyzed by lactation groups, and no significant prophylactic effects due to the *Treponema* bacterin[a] were observed in first-lactation cows ($P>0.06$) or in second-or-later-lactation cows ($P>0.23$) from each dairy. Similarly, no consistently significant therapeutic effects due to the *Treponema* bacterin[a] were observed in first-lactation cows or in second-or-later lactation cows ($P>0.30$) from each dairy, except at month six in dairy 2 where the proportion of (P)DD in first-lactation vaccinates was significantly lower than in placebos ($P=0.02$).

Data were further analyzed for lactation groups for those cows present at every monthly observation from each dairy. No consistent significant prophylactic or therapeutic effects due to the *Treponema* bacterin[a] were observed in cows from all lactation groups ($P>0.09$), first-lactation cows or second-or-later- lactation cows ($P>0.31$) on dairy 1. However, on dairy 2 the proportion of (P)DD lesions was significantly lower in the first-lactation vaccinate group than in the placebo group, in the prophylactic group at month three ($P=0.03$) and in the therapeutic group at month six ($P=0.04$).

Pre-treatment ELISA titers of serum antibodies to (P)DD-associated *Treponema* spp from 60 cows per dairy with no gross visual (P)DD lesions prior to treatment revealed that 43% had pre-existing exposure to *Treponema* spp antigens (Table 1). To evaluate whether the *Treponema* bacterin[a] generated a positive serologic titer to (P)DD-associated *Treponema* spp over the six months of evaluation, a sample group of 14 vaccinates and 17 placebos from both dairies combined was selected. Twenty-nine of the cows had pre-treatment titers of ≤1:1600 and no visible (P)DD lesions, and two cows (placebo) had titers of ≤1:1600 and visible (P)DD lesions prior to treatment. Comparisons between monthly proportions of vaccinates and placebos that had positive serologic titers from pre-treatment to the end of the six months of post-treatment evaluation showed that a significantly higher proportion of vaccinates developed a positive serologic titer in the first and third month after the pre-treatment examination ($P=0.001$ and $P=0.044$, respectively), compared to placebos. Further analyses by ANOVA were made on $\log_2$ based transformations of actual titers, which showed that vaccinates had significantly lower titers pre-examination ($P=0.014$) and significantly higher titers at periods 1 ($P=0.000$), 2 ($P=0.004$) and 3 ($P=0.008$) after treatment (Figure 2).

Table 1. Proportions of cows with no gross lesions of (P)DD prior to treatment with titers compatible with non-exposure (≤1:1600) or exposure (> 1:1600).

Dairy ID	Blood samples	Titers compatible with no exposure (≤1:1600)		Titers compatible with exposure (> 1:1600)	
	n	n	%	n	%
1	48	19	40	29	60
2	53	39	74	14	26
Both	101	58	57	43	43

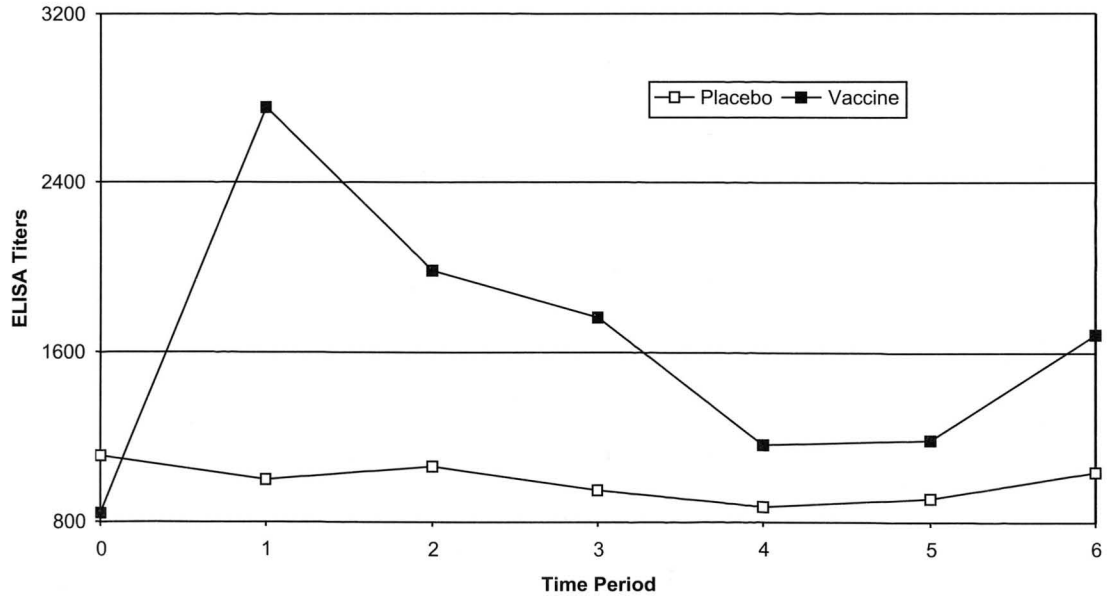

Figure 2. Comparison of ELISA titers (1:800 to 1:3200) of 14 vaccinates and 17 placebos from both dairies from pre-treatment (period 0) until six months after treatment (period 6). A positive titer was considered to be >1:1600. Differences between vaccinates and placebos were significant at pre-treatment (P=0.01) and for periods 1-3 (P<0.01).

Discussion

The non-significant difference in lactation group, milk yield and days-in-milk between Groups 1, 2, 3 and 4 from both dairies at pre-treatment evaluation indicated the method of treatment allocation probably did not result in confounding in this study. Overall, results from this study show a trend for *Treponema* bacterin[a] to provide some prophylactic effects only to first-lactation vaccinates, and no effects to second-or-later-lactation vaccinates. Inconsistency of results throughout the six months of evaluation may be due to variability in hoof trimmer treatment frequency (four times per month for dairy 1 and two times per month for dairy 2) and to the length of time an individual animal may develop and maintain a humoral response after completion of vaccination. Other studies have found that first-lactation cows appear to have higher frequencies of (P)DD lesions than older cows, who may become immune as they age.[29,35] If this is true, a change in humoral response due to the vaccine would be hard to detect in older cows that may have been exposed prior to treatment. Therefore, the vaccine might be more efficacious in cows in their first lactation because they may be more susceptible to the disease.

Although Laven *et al*[15] suggested there is no protective effect of exposing heifers to (P)DD prior to calving, results from our study support findings from a recent controlled study using a *Treponema* spp bacterin in a Nebraska dairy, where statistical difference in (P)DD incidence was found for heifers vaccinated prior to calving, but not for cows vaccinated during their dry period.[3] Thus, a whole-herd vaccina-

tion protocol with a *Treponema* bacterin may not provide the most efficient results, as suggested by another study where no significant vaccine effect was observed.[18]

In our study, pre-vaccination ELISA titers for serum antibodies to (P)DD-associated *Treponema* spp from 120 bled cows from both dairies revealed that 43% of the cows considered naive, based on gross visual examination of their feet prior to treatment, actually had prior exposure to (P)DD. A possible explanation is that the ELISA measures a response to exposure to *Treponema* spp antigens, whereas visual observation with WJT measures clinical manifestation of infection based on presence of a gross visual (P)DD lesion and pain. Thus, an animal with serologic response to *Treponema* spp antigen may not necessarily be clinically affected or have a visible (P)DD lesion. Presence of IgG_2 antibodies to *Treponema* spp detected by ELISA may not necessarily indicate an active immune protective response by affected cows, but reflects prior infection or repeated exposure to treponemes.[18]

Visual examination with the aid of WJT has been shown to be a useful screening test for (P)DD diagnosis with a high sensitivity and specificity.[28] However, findings from this study suggest that using visual observation with WJT to define pre-treatment disease status of a cow in order to study seroconversion over time was not effective. The finding that 43% of the cows in this study without visible (P)DD lesions pre-treatment were apparently not naïve may be a reason why this study did not demonstrate prophylactic efficacy of a *Treponema* bacterin.[a]

Monthly ELISA titer status from a sample group of 31 out of 120 cows from both dairies combined showed an interesting trend of greater serologic response in the 14 vaccinates compared to the 17 placebos, particularly during the first three months post-treatment (Figure 2). These results indicate that *Treponema* bacterin[a] provided vaccinates from both dairies with serologic increases of twofold or greater than the titer of 1:1600. According to Murray *et al*,[18] presence of *Treponema* spp antibodies detected by ELISA may not necessarily indicate an active protective immune response by affected cows. Note that pre-treatment screening for this sample group of 31 cows was done by visual observation and WJT at the stanchions rather than at the milking parlor, and diagnosis was probably less accurate for hard-to-see lesions. For this reason, two of these 31 cows that we believed did not have visible lesions actually had visible lesions in the first parlor examination after treatment.

We speculate that *Treponema* bacterin[a] may be more efficacious if used on heifers prior to exposure rather than on the whole lactating herd. Although serology results from this study suggest that serum antibodies are produced in response to the vaccine, the results from visual evaluations did not show significant prophylactic effects. Both dairies had a high (P)DD prevalence prior to treatment, and it is possible that the immune system of the cows may have been overwhelmed.

Despite efforts to produce effective treatments for (P)DD, recurrence remains high.[21,31] This suggests that natural infection does not establish a long-lasting immunity, and that the development of a vaccine may be challenging. Also, since precise etiology is still unknown, development of an efficacious vaccine is further compromised. Efficacious control of (P)DD will likely include appropriate housing, environment and management considerations designed to attenuate infection, along with appropriate vaccination protocols.[31]

Conclusions

Vaccinating the entire lactating herd with a *Treponema* bacterin[a] did not provide significant prophylactic or therapeutic effects in vaccinated cows studied during a six-month post-vaccination period on two California dairies with high (P)DD prevalence. We speculate that *Treponema* bacterin[a] might prove more efficacious if used on heifers prior to exposure to high infection pressure, i.e., before joining the breeding or milking herd. Management must also be considered important in the prevention and control of (P)DD.

Further research is needed to discover the precise etiologic organism(s) of (P)DD, to improve vaccine efficacy and to determine more advantageous vaccination protocols.

Acknowledgments

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Footnotes

[a] *Treponema* bacterin, 2002 prototype, Novartis Animal Health, Inc, Bucyrus, Kansas (ImmTech Biologics® U.S. Vet. License No. 480).

[b] Dr. Dan Kiel, Novartis Animal Health, Inc, Bucyrus, Kansas (unpublished data).

[c] Minitab Statistical Software 13.32, MINITAB, INC, +1-814-238-3280, www.minitab.com

[d] Dairy Comp 305, Valley Agricultural Software, Tulare, California.

[e] Win Episcope 2.0 Software, Facultad de Veterinaria Zaragoza, (EPIDECON) Aragon, Spain.
[f] SAS, version 8.02 for Windows, SAS Institute, Inc, Cary, NC

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Baytril® 100

(enrofloxacin)

100 mg/mL Antimicrobial Injectable Solution
For Subcutaneous Use In Cattle Only

Not For Use In Cattle Intended For Dairy Production Or In Calves To Be Processed For Veal

BRIEF SUMMARY:
Before using Baytril® 100, please consult the product insert, a summary of which follows:

CAUTION:
Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.
Federal (U.S.A.) law prohibits the extra-label use of this drug in food producing animals.

INDICATIONS:
Baytril® 100 (enrofloxacin) injectable solution is indicated for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica, Pasteurella multocida* and *Haemophilus somnus.*

ADVERSE REACTIONS:
No adverse reactions were observed during clinical trials. For medical emergencies or to report adverse reactions, call 1-800-422-9874.

ANIMAL SAFETY:
Safety studies were conducted in feeder calves using single doses of 5, 15, and 25 mg/kg for 15 consecutive days and 50 mg/kg for 5 consecutive days. No clinical signs of toxicity were observed when a dose of 5 mg/kg was administered for 15 days. Clinical signs of depression, incoordination, and muscle fasciculation were observed in calves when doses of 15 or 25 mg/kg were administered for 10 to 15 days. Clinical signs of depression, inappetance, and incoordination were observed when a dose of 50 mg/kg had been administered for 3 days. No drug-related abnormalities in clinical pathology parameters were identified. No articular cartilage lesions were observed after examination of stifle joints from animals administered 25 mg/kg for 15 days.

A safety study was conducted in 23-day-old calves using doses of 5, 15, and 25 mg/kg for 15 consecutive days. No clinical signs of toxicity or changes in clinical pathology parameters were observed. No articular cartilage lesions were observed in the stifle joints at any dose level at 2 days and 9 days following 15 days of drug administration.

An injection site study conducted in feeder calves demonstrated that the formulation may induce transient reaction in the subcutaneous tissue and underlying muscle. No painful responses to administration were observed.

WARNING:
Animals intended for human consumption must not be slaughtered within 28 days from the last treatment. Do not use in cattle intended for dairy production.
A withdrawal period has not been established for this product in pre-ruminating calves. Do not use in calves to be processed for veal.

HUMAN WARNINGS:
For use in animals only. Keep out of the reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight. For customer service or to obtain product information, including a Material Safety Data Sheet, call 1-800-633-3796. For medical emergencies or to report adverse reactions, call 1-800-422-9874.

PRECAUTIONS:
The effects of enrofloxacin on bovine reproductive performance, pregnancy, and lactation have not been adequately determined.
Subcutaneous injection can cause a transient local tissue reaction that may result in trim loss of edible tissue at slaughter.

Baytril® 100 contains different excipients than other Baytril® products. The safety and efficacy of this formulation in species other than cattle have not been determined.

Quinolone-class drugs should be used with caution in animals with known or suspected Central Nervous System (CNS) disorders. In such animals, quinolones have, in rare instances, been associated with CNS stimulation which may lead to convulsive seizures.

Quinolone-class drugs have been shown to produce erosions of cartilage of weight-bearing joints and other signs of arthropathy in immature animals of various species. No articular cartilage lesions were observed in the stifle joints of 23-day-old calves at 2 days and 9 days following treatment with enrofloxacin at doses up to 25 mg/kg for 15 consecutive days.

NADA # 141-068, Approved by FDA

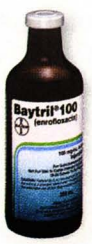
Bayer HealthCare LLC
Animal Health Division
Shawnee Mission, Kansas 66201 U.S.A.

Bayer

 12635 August, 2004

If one of your cowboys took seven days to complete a one-day job, you'd fire him.

It should be no different with your BRD antibiotic. Why waste a week playing wait-and-see with a long-lasting therapy that may increase his chances of dying or becoming a chronic?* Single-dose Baytril® 100 (enrofloxacin) rapidly attacks and kills the three major bacteria that cause BRD, helping calves look and feel better in hours, and get back to work in a day. You know the drill; you turn to the one that gets the job done and done right. Baytril 100. Right the first time.® Extra-label use of this product in food-producing animals is prohibited.



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*Bayer Study BL3260

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