PEER REVIEWED

The Efficacy of *Serpens* spp Bacterin Combined with Topical Administration of Lincomycin Hydrochloride for Treatment of Papillomatous Digital Dermatitis (Footwarts) in Cows on a Dairy in California

Steven L. Berry, *DVM, MPVM;* **Thomas W. Graham,** *DVM, PhD;* **Andrea Mongini,** *BS;* **Marit Arana,** *PhD*
Department of Animal Science (Berry), University of California, Davis, CA 95616-8521; Veterinary Consulting Services (Graham), 909 Gregory Place, Davis, CA 95616; School of Veterinary Medicine (Mongini), University of California, Davis, CA 95616; and University of California Cooperative Extension (Arana), 420 S. Wilson Way, Stockton, CA 95205

Abstract

The objective of this prospective, randomized field trial was to determine if a *Serpens* spp bacterin had a therapeutic effect on cows with active lesions of papillomatous digital dermatitis (PDD) before or in combination with treatment with topical lincomycin HCl on a California dairy. Fifty-nine lactating cows with active, painful PDD were randomly assigned to control (not vaccinated) and vaccinated groups. Lesions of the rear feet were evaluated for pain response, color, size, and lesion scores at each of the examinations. Cows in the vaccinated group were vaccinated 3 times with a *Serpens* spp bacterin (days 1, 14, 43). All cows were restrained on a hydraulic tilt table at approximately day 30 and, after examination, all lesions were treated with a slurry containing 3.2 g of lincomycin mixed with 3 ml of deionized water. The slurry was applied to cotton gauze and held in place with an elastic bandage. Bandages were allowed to wear off. Lesions were re-examined while cows were locked in stanchions at approximately days 50 and 70 and while restrained on the hydraulic tilt table on approximately day 110. Lesions improved on all cows during the course of the trial. Lesion and size scores were improved for control and vaccinated cows on days 50, 70, and 110. Pain response and color scores improved on days 50 and 70 but showed evidence of recurrence or recrudescence by day 110. There were no significant differences in evaluated scores between control and vaccinated cows at any of the examination times. When affected cows were re-examined at day 110, recurrence or recrudescence of lesions was evident in 41% of the control cows and 45% of the vaccinated cows. Vaccination of PDD affected cows with *Serpens* spp bacterin had no apparent effect on clinical outcome.

Introduction

Papillomatous digital dermatitis (PDD) is a contagious, painful, wart-like disease of the bovine digit.[5,7,18] Lesions develop most commonly on the plantar surface of the rear feet of dairy cattle near the interdigital space with less common development on the dorsal surface of the foot near the interdigital space or other locations below the level of the dewclaws or on front feet.[18] The disease was first described in Italy in 1974.[8] In the late 1970s, it was diagnosed in the northeastern United States.[21] It was reported in California in the mid 1980s with histologic confirmation in 1988.[16] Papillomatous digital dermatitis is considered to be a multifactorial disease. Two risk factors for herds having a high proportion of cows with the disease are muddy corrals and purchase of replacement animals.[23,28] Currently, cows affected with PDD are most commonly treated with topical administration of antimicrobials (oxytetracycline, lincomycin, or a lincomycin-spectinomycin combination) applied as a footbath, spray, or bandage. The severe lameness caused by PDD in dairy cows has been associated with decreased feed intake, poor reproductive performance, and decreased milk production compared with unaffected cows.[21]

The exact cause of PDD is unknown at this time, although it is believed that an infectious component is involved in the disease process. Results of earlier stud-

ies do not indicate viral, fungal, or parasitic causes.[18-20,25] Bacteria are believed to play an important role in the pathogenesis of PDD because of the observed response to parenteral or topical antimicrobial treatment.[1,2,4-6,13,15,18] The specific bacterial agents involved in PDD are unknown; however, spirochetes have been consistently isolated and identified in the United States, Germany, Canada, and Japan.[9,14,19,22,26,27] Recently, a bacterin[a] has been produced consisting of *Serpens* spp; an organism that is claimed by the manufacturer to be the cause of PDD. The bacterin currently is licensed for sale in California and conditionally in other states as a prevention and treatment for PDD. A vaccine that would enhance regression of PDD lesions and provide a cure would potentially reduce the amount of antimicrobials used and return affected cows to their previous level of performance more quickly than those treated with topical antimicrobials alone.

The objective of this randomized field trial was to evaluate the effect *Serpens* spp Bacterin[a] had on cows with active lesions of PDD before or in combination with topical treatment with lincomycin HCl[b] on a California dairy. Use of lincomycin or any other antimicrobial to treat PDD is extra-label use, and requires a veterinary prescription.

Materials and Methods

Cows—The trial was performed on a 360 cow (306 lactating and 54 nonlactating), commercial, Holstein dairy in central California. Lactating cows were housed in 3 pens according to milk production level. Two of the pens had free-stalls bedded with dried manure and a dirt exercise lot and the third pen had loose housing with shades in a dirt corral. Cows were milked twice per day in an 8-stall carousel parlor. All cows were fed a total-mixed-ration on the basis of milk yield. The estimated prevalence of PDD in the lactating cows was 40% at the time of enrollment.

Experimental protocol—While locked in stanchions on days –6 and 1 (hereafter, day 1), 59 cows with PDD from the 3 lactating cow pens were selected for the trial. Day 1 was the day of the first vaccination. All cows in these pens that had visible lesions on one or both rear feet and had a pain response were enrolled. Pain response was evaluated by spraying the feet with a forceful stream of water from a 16 L hand-pump, backpack sprayer capable of attaining 85 psi (586 kPa). Cows that had visible lesions but no pain response were not enrolled. Randomized treatment data sheets were generated prior to the study and cows were randomly assigned to control (29 cows) or vaccinated (30 cows) groups during enrollment. Prior to the day 30 examination, one of the vaccinated cows died from causes not related to this study. At each examination, the scorer did not have knowledge of which group the cows were in. All evaluations were performed by the same scorer (SLB). During enrollment, cows were examined while locked in stanchions (day 1) and their lesions were photographed with a 100 to 300 mm telephoto lens from a distance of about 1.5 m. Cows assigned to the vaccinated group were vaccinated at days 1, 14, and 43, according to label instructions.

Lesion, pain response, color, and size scores were evaluated at enrollment and at each subsequent examination based on categorical criteria (Appendix). Typical papillomatous digital dermatitis lesions and their corresponding scores are shown in Figure 1. Of cows enrolled in the study, 17 of the 29 control cows and 15 of the 29 vaccinated cows had bilateral lesions on the rear heels. Cows with bilateral lesions had one foot examined for analysis; selection was made on the basis of the most readily observed lesion while the cow was locked in stanchions. The lesion site selected at day 1 was examined for the duration of the study. The size score was made on the basis of an estimate of the widest mediolateral distance across the lesion as viewed from behind the cow.

Lesions were examined and photographed while cows were restrained on a hydraulic tilt table on days 29, 30, 35, and 36 (hereafter, day 30). This examination was approximately 2 weeks after the second administration of the bacterin. Lesions were photographed with a 100 mm macro lens at a magnification of 1:4 (Fig 1). Based on the observation that most of the cows still had active lesions (lesion score > 0; color, red or grey; or, pain response score > 0) at this time, we treated all lesions considered to be active with 3.2 g of lincomycin, topically. The lincomycin was mixed with approximately 3 ml of deionized water to make a slurry that was applied to a gauze pad, placed on the lesion, and held in place with an elastic bandage.[c]

Lesions were examined and photographed with the cows standing in stanchions at days 50 and 54 (hereafter, day 50) and days 71 and 72 (hereafter, day 70). The photographs were taken with a 100 to 300 mm telephoto lens at a distance of about 1.5 m. At days 112, 113, and 117 (hereafter, day 110) the cows were again placed on the tilt table and all lesions were examined, photographed with a 100 mm macro lens, and retreated with 3.2 g of lincomycin mixed with deionized water, if necessary.

Statistical analysis—Baseline data (day 1) for lactation number (1, 2, ≥ 3) and days in milk were compared between control and vaccinated cows using a student's t-test to determine comparability of the 2 groups. Mean lesion, pain response, size, and color scores for control and vaccinated cows were analyzed for each of the examination days using Mann-Whitney rank-sum test, which is the non-parametric equivalent of the student's t-test. Statistics were performed using statistical software.[10] The sample size used in this study

Appendix - Evaluation scores (lesion, pain response, color, and size) for papillomatous digital dermatitis in control cows and cows vaccinated with *Serpens* spp Bacterin

	Evaluation Category			
Score	Lesions Scores	Pain Response	Color	Size*
0	No Lesion or hyperkeratosis	No pain	Flesh, absent or healed lesion	0 cm
1	Flat, raw lesion with distinct margin and erosive appearance involving skin-horn junction between heel bulbs near the interdigital cleft	Moderate pain, cow moves foot when sprayed with stream of water	Black or brown, healing lesion	≤2.5 cm
2	Flat, raw lesion with a granular appearance and hypertrophic true hairs and early hyperkeratosis at the margin	Severe pain, cow moves foot and holds it off the ground or keeps tension on the foot with leg shaking for a few seconds when sprayed with a stream of water	Red or gray, nonhealing lesion	>2.5 cm
3	Raised lesion with early epidermal papillae formation	NA**	NA	NA
4	Mature, raised lesion with advanced epidermal papillae formation	NA	NA	NA

*Size score was an estimate of widest mediolateral distance across the lesion.
**NA is not applicable.

had an expected confidence of 95% (α) and power of 90% (1 - β) for detecting differences between control and vaccinated cows.[12] Results are reported as mean (± SD) values. A P value ≤ 0.05 was considered significant.

Results

There were no significant differences on day 1 in lactation number, number of days in milk, and lesion scores between control and vaccinated cows (lactation number, 2.14 ± 0.69 vs. 1.86 ± 0.58; number of days in milk; 247 ± 146 vs. 230 ± 123; lesion score 2.72 ± 0.65 vs. 2.72 ± 0.84, respectively). By day 30, the lesion score had changed little from day 1 (2.83 ± 0.76 for control cows, 2.69 ± 1.11 for vaccinated cows). Vaccinated cows had received 2 doses of the bacterin by day 30. All active, painful lesions were treated on day 30 based on the scorer's assessment of the lesion, pain response, and color scores (29/29 control, 28/29 vaccinated). The rank-sum test indicated no significant difference in lesion scores between control and vaccinated cows on any day (Fig 2).

Pain response score was 1.28 ± 0.45 for control cows and 1.10 ± 0.31 for vaccinated cows on day 1. Pain response score was similar on day 30 for both groups and then decreased precipitously by day 50. Both control and vaccinated cows had increased pain response scores by day 110. No significant difference was found in pain response scores between control and vaccinated cows on any day (Fig 3).

All lesions were red or gray (color score = 2) on day 1 for both groups. By day 30, color score had decreased slightly to 1.76 ± 0.44 for control cows and 1.66 ± 0.55 for vaccinated cows. Results of evaluations on days 50 and 70 revealed mean color scores of about 1.0, but by day 110 both groups had mean color scores that had increased to about 1.2. No significant difference was found in color scores between control and vaccinated cows on any day (Fig 4).

Size scores were not significantly different on day 1 between control and vaccinated cows (1.79 ± 0.41 and

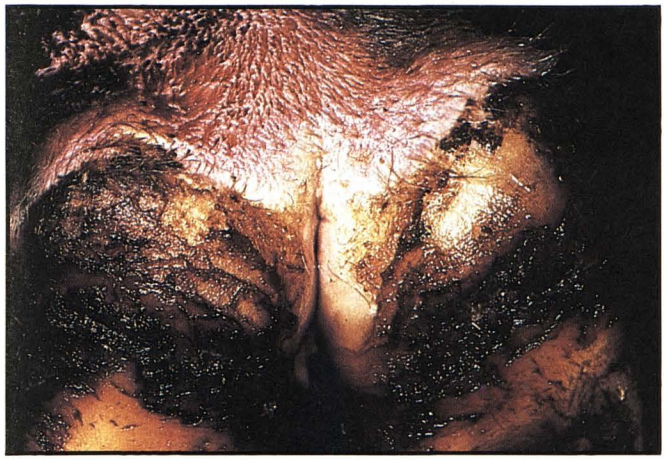
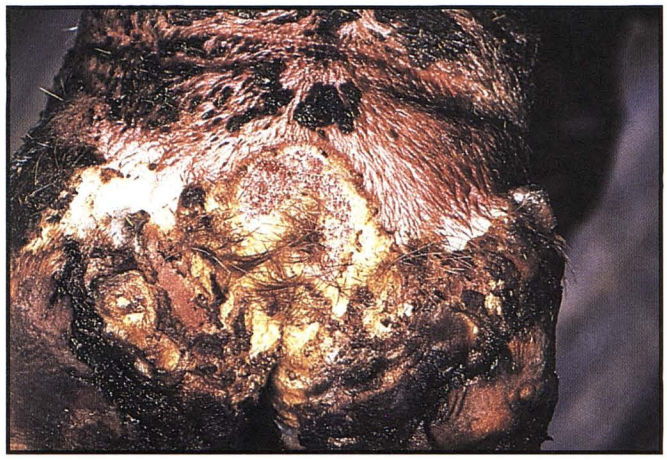
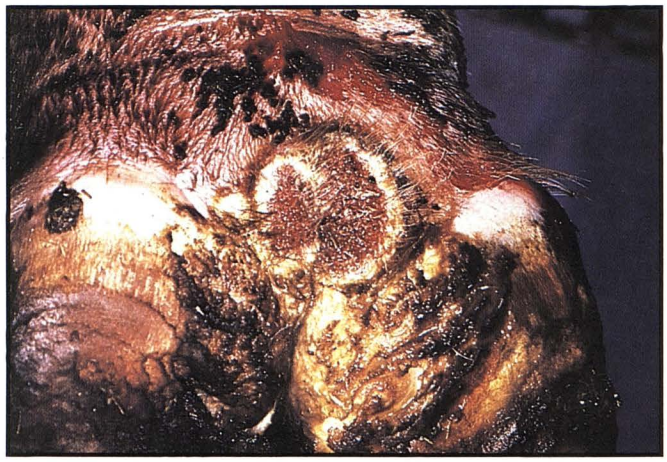


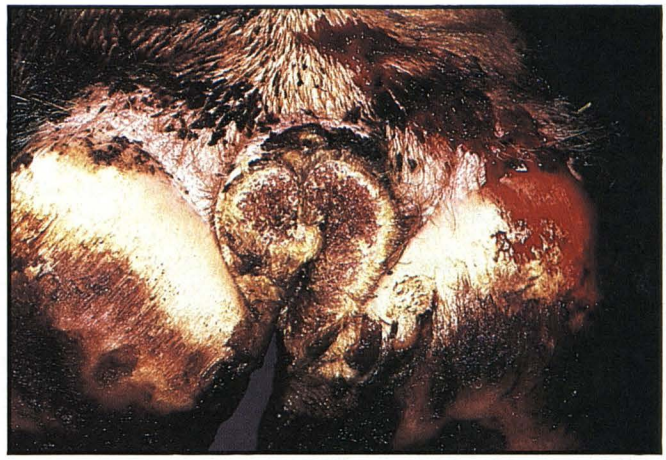
Figure 1. Papillomatous digital dermatitis lesion scores. a) Lesion score = 0. No lesion or hyperkeratosis.



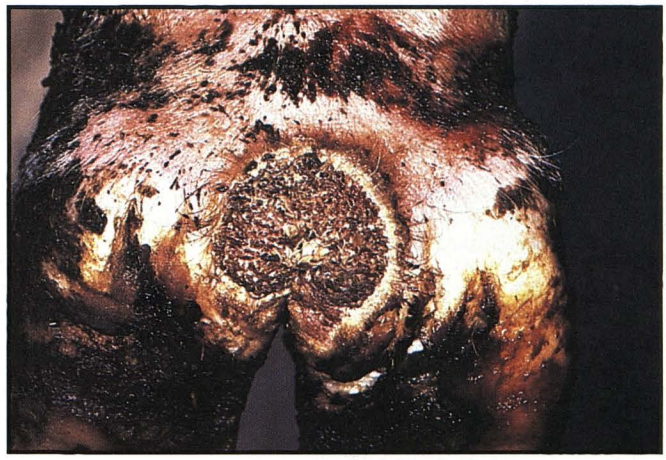
b) Lesion score = 1. Flat, raw lesion with distinct margin and erosive appearance involving skin-horn junction of heel near the interdigital cleft.



c) Lesion score = 2. Flat, raw lesion with a granular appearance and hypertrophic true hairs and early hyperkeratosis at the margin.



d) Lesion score = 3. Raised lesion with early epidermal papillae formation.



e) Lesion score = 4. Mature, raised lesion with advanced epidermal papillae formation.

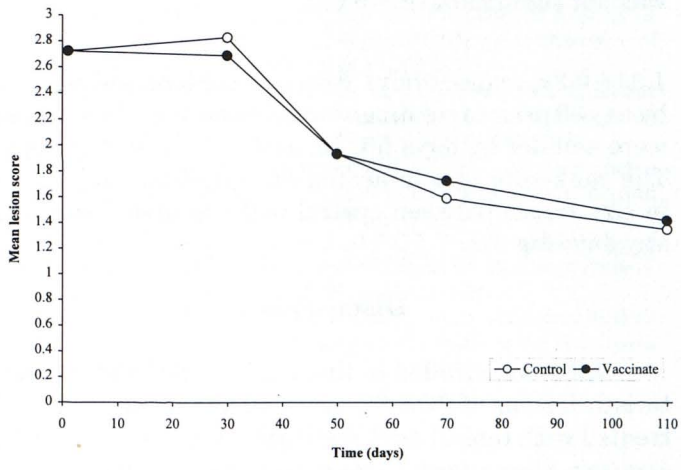


Figure 2. Mean lesion score versus time (days) for control and vaccinated cows. Differences between groups was not significant ($P > 0.49$).

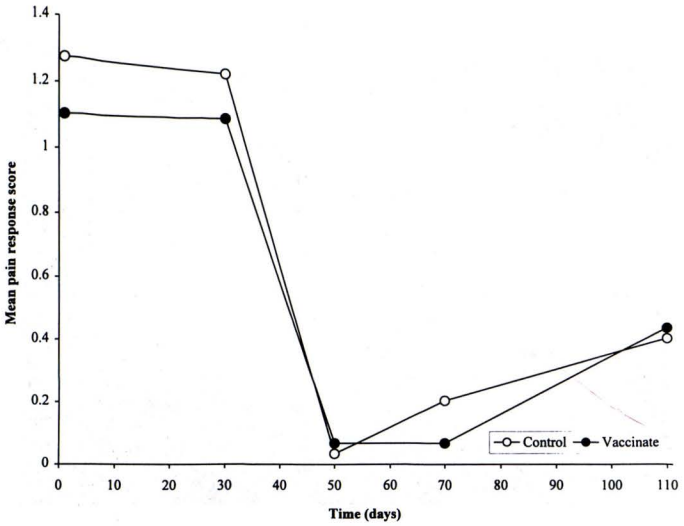


Figure 3. Mean pain response score versus time (days) for control and vaccinated cows. Differences between groups was not significant (P > 0.10).

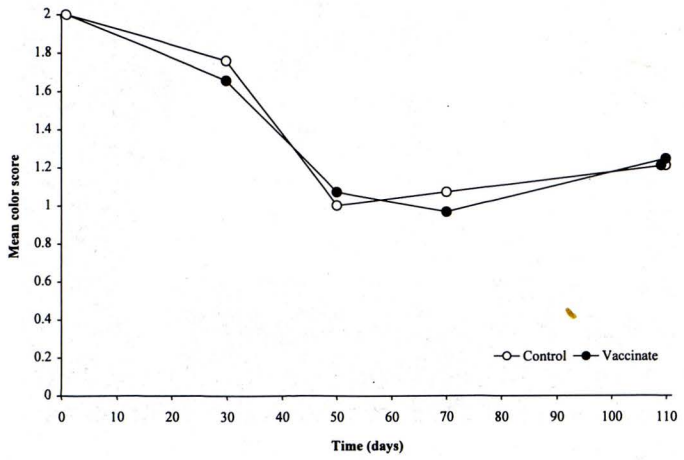


Figure 4. Mean color score versus time (days) for control and vaccinated cows. Differences between groups was not significant (P > 0.51).

1.83 ± 0.38, respectively). Size scores decreased slightly by day 30 prior to treatment with lincomycin. Size scores were smaller by days 50, 70, and 110 for both groups. The rank-sum test indicated no significant difference in size scores between control and vaccinated cows on any day (Fig 5).

Discussion

All cows enrolled in this clinical trial had characteristic lesions of PDD.[5,18] Cows affected with PDD and treated with topical administration of lincomycin had a similar therapeutic response as previously reported.[2,5,6,13,18,20] However, when responses of the vaccinated cows were compared with those of control cows, there was no apparent effect from vaccination with the *Serpens* spp bacterin. In this study, control and vaccinated cows with PDD were comparably distributed by lactation number, stage of lactation, and lesion score, suggesting minimum allocation bias.

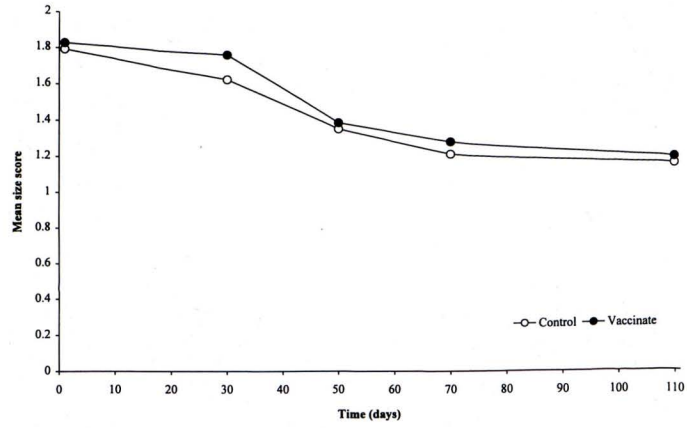


Figure 5. Mean size score versus time (days) for control and vaccinated cows. Differences between groups was not significant (P > 0.18).

The causative agent of PDD remains undetermined. There are no reports of aerobic bacteria or of *Serpens* spp associated with PDD in the peer-reviewed, scientific literature. Current evidence suggests that spirochetes are involved in the etiopathogenesis of PDD.[9,14,19,22,26,27] Other bacteria reported to be associated with PDD are *Borrelia burgdorferi*,[3] *Campylobacter faecalis*,[11] several group-types of anaerobes,[20] large rods,[17] and bacillary and coccobacillary bacteria.[25]

Several laboratories have identified the slender, spiral organisms, consistently associated with PDD, as spirochetes using electron microscopy, isolation techniques, or comparative 16S rRNA sequence analysis.[9,14,19,20,22,26,27] Workers in Germany,[9] England,[22] and the United States[27] have classified these spirochetes as members of the genus *Treponema* based on phenotypic, morphologic, and genotypic characteristics. Spirochetes are organisms that are capable of deeply invading the epidermis.[9,11,17,19,24,25,29] In one study, cows with PDD had significantly greater serum antibody responses to 2 groups of spirochetes compared with cows without PDD from the same dairy and cows from PDD-free dairies.[26] In that study, there was no relationship between serum antibody responses to PDD-associated spirochetes and responses to spirochetes associated with other diseases of cattle.

At the end of the study (day 110), 12/29 (41%) of control and 14/29 (45%) of vaccinated cows experienced recurrence or recrudescence of PDD. In this study, the effect of the *Serpens* spp bacterin on cows without visible lesions of PDD or the effect of the vaccination in the absence of antimicrobial treatment was not evaluated. According to the label instructions, the *Serpens* spp

bacterin is indicated for use in conjunction with other appropriate management practices to help reduce lesions and losses associated with PDD in cattle. We chose to examine the effects of combined topical administration of an antimicrobial and the *Serpens* spp bacterin in cows with active PDD lesions. It was expected that about 90% of vaccinated cows would have been cured (no visible activity and no pain response) with combined antimicrobial and vaccine therapy, and about 50% of the unvaccinated control cows.[1,2] The current sample size had an expected confidence of 95% (α) and power of 90% (β) for detecting differences between vaccinated and control cows, if there was a vaccine effect.[12]

Conclusions

Topical therapy with lincomycin resulted in a clinical response that was in agreement with previous studies.[1,2] Given the results of our study, and an estimated minimum annual cost of $10.50/cow (3.50/dose; 3 doses/cow/year), we concluded that vaccination with the bacterin had no added benefit beyond treatment with lincomycin alone.

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Footnotes

[a] *Serpens* spp Bacterin, Hygieia Biological Laboratories, Woodland, CA

[b] Lincomix Soluble Powder, Upjohn Co., Kalamazoo, MI

[c] 3M Vetrap Elastic Bandage, 3M Products, St. Paul, MN

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