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Case Report – Isolation of *Serratia liquefaciens* from Sodium Chlorite Lactic Acid Teat Dip Stored on a Dairy Farm

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Abstract

Two apparently healthy cows on a commercial dairy farm died within 48 hours following intramammary (IMM) infusion of dry-cow treatment at the end of lactation. Teat dip containing sodium chlorite lactic acid had been stored for two years on the farm, and was used only to dip teats following IMM infusion with dry-cow treatment. The sodium chlorite lactic acid teat dip, stored in the original container that was visibly soiled externally, was culture-positive for *Serratia liquefaciens*. This is the first reported isolation of a contaminant from sodium chlorite lactic acid teat dip. Soiled containers of teat dip or those opened and stored for long periods of time should be cultured to monitor for contamination with bacteria, or preferably discarded.

Keywords: bovine, teat dip, contamination, *Serratia*, dairy, microbiology

Résumé

Deux vaches apparemment en santé d'une ferme laitière commerciale sont mortes en moins de 48 heures suite à l'infusion intra-mammaire en fin de lactation d'un traitement au tarissement. Une solution de trempage de trayons contenant du chlorite de sodium et de l'acide lactique avait été entreposée pendant deux ans à la ferme et n'était utilisée que pour tremper les trayons suivant l'infusion intra-mammaire du traitement au tarissement. La solution de trempage de chlorite de sodium et d'acide lactique, entreposée dans son contenant original qui était visiblement souillé à l'extérieur, s'est révélée positive pour *Serratia liquefaciens*. Ceci représente le premier cas d'isolation d'un contaminant d'une solution de trempage avec du chlorite de sodium et de l'acide lactique. Les contenants souillés de solution de trempage ou ceux qui sont ouverts et entreposés pendant de longues périodes de temps, devraient avoir des cultures afin de surveiller la contamination bactérienne ou devraient tout simplement être jetés.

Introduction

A commercial dairy producer from Idaho submitted diagnostic samples to the Utah Veterinary Diagnostic Laboratory (UVDL) following the death of a cow, with the suspicion that products could be contaminated with a causative infectious agent. There were 50 lactating cows in the herd. Bacteriological culture was requested on two unused and unopened commercial tubes of cephapirin intramammary (IMM) dry-cow treatment (antibiotic for IMM infusion at the beginning of the non-lactating period approximately 60 days before the next calving date), and on a partially used, open container of sodium chlorite lactic acid (SCLA) teat dip. The teat dip container was visibly soiled and had been in use for some time (Figure 1).

Figure 1. Original teat dip container stored on farm for two years.

Materials and Methods

A sample of the contents from within the commercial tubes of cephapirin (0.1 mL) was streaked onto Columbia blood agar (CBA), MacConkey agar and chocolate agar. Additionally, 0.1 mL of this sample was placed in 10 mL of brain heart infusion broth (BHI), incubated for 24 hours, and streaked onto CBA. All culture samples were incubated aerobically in a non-$C0_2$ incubator at 98.6°F (37°C).

From the original container of SCLA teat dip (opened), a 10 mL sample of the teat dip was aspirated with a sterile Pasteur pipette. Then 0.1 mL of this sample was streaked onto CBA, MacConkey agar, and chocolate agar. Additionally, 0.1 mL of the teat dip sample was placed in 10 mL of BHI and incubated for 24 hours. All culture samples were incubated aerobically in a non-$C0_2$ incubator at 98.6°F. Then 0.1 mL of the 1% teat dip in BHI broth mixture was plated every 24 hours on CBA for 14 consecutive days. Plates were evaluated for growth every 24 hours for five days after they were inoculated.

The SCLA teat dip (approximately 250 mL) remaining in the container was transferred to a sterile Erlenmeyer flask. The dip was grossly abnormal with green-to-gray, viscous cloudiness and light precipitates visible (Figure 2). The inside of the original container was lined by a green-to-gray film. BHI broth (40 mL) was added to the original container and lightly agitated. The BHI broth rinse was then transferred to a sterile, clear 50 mL conical tube. The 50 mL conical tube containing the BHI container rinse was incubated at 98.6°F for five days. After five days, patches of opaque, white strongly adherent material were visible on the internal lower portions of the conical tube. The initial broth rinse was removed, the conical tube was lightly rinsed with 20 mL of new BHI broth, and a loop of the material from the surface of the conical tube was streaked onto CBA. After 24 hours, heavy growth of pure culture of large opaque, mucoid colonies were observed. Following subculture, identification was performed utilizing commercial test strips.[a]

Results

From the samples of the commercial tubes of cephapirin and the 10 mL sample of teat dip, no bacterial growth was observed following standard plating or enrichment following five days of observation. The large opaque, mucoid colonies from the BHI broth rinse of the teat dip container were tested with commercial test strips as described earlier. Isolates were identified as *Serratia liquefaciens*.

A dairy cooperative extension agent in Idaho familiar with the source farm was contacted. After initial discussions between the extension agent and the dairy producer, the producer was contacted directly six weeks after the samples had been submitted to the UVDL. The producer revealed that the visibly soiled container of SCLA teat dip had been used for approximately two years, and was used to dip teats following IMM infusion with dry-cow treatment. The teat dip was not used on lactating cows, but only on cows that had just been dry-treated. Just before samples were submitted to the UVDL, two apparently healthy cows had died within 48 hours following IMM infusion of dry-cow treatment at the end of lactation. The producer was advised that the isolation of *Serratia liquefaciens* from the teat dip indicated a possible source of spread of mastitis to cows. The producer had already decided to dispose of the teat dip, and had submitted the entire container with remaining contents to the laboratory. In the six weeks following disposal of the contaminated teat dip, no cows had died following dry-cow treatment.

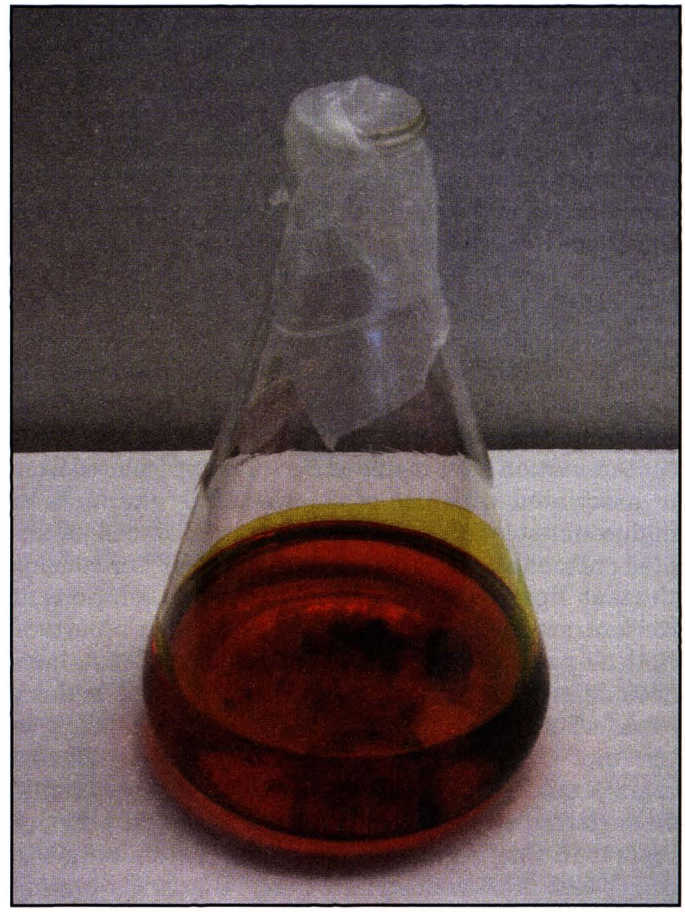

Figure 2. Teat dip showing visible contamination.

Culture of aseptically collected milk samples from cows dipped with the contaminated teat dip after dry-cow treatment, after those remaining dry cows calved again, was recommended. The producer was interested in collecting the milk samples for culture, and planned to

discuss this with the regular herd health veterinarian. When the producer was contacted after several weeks, he repeated his intentions to discuss culturing milk from the cows, but had not yet talked with his veterinarian. However, no samples were received and the herd history was lost to follow up. No information is available regarding whether any cows in the herd had intramammary infection (IMI) caused by *Serratia liquefaciens*.

Discussion

Contamination of commercial germicidal teat dip for prevention of bovine IMI with bacterial contaminants has been reported only infrequently. Most reports of contamination have included *Serratia* spp isolated from or associated with use of chlorhexidine gluconate or digluconate teat dips. These have also included associated outbreaks of *Serratia* spp IMI in dairy herds using the teat dips.[2,6] Growth of *Serratia* spp in solutions as concentrated as 2% chlorhexidine has been reported.[5] Both *Serratia marcescens* and *Serratia liquefaciens* have been described as causative agents of mastitis in dairy cows, whether or not the bacteria were isolated from teat dip.[1,3,4,7] Resultant effects have included elevated somatic cell counts (SCC) in subclinical cases, and mild or moderate clinical mastitis. There are no previous reports in the literature of bacterial contamination of SCLA teat dips as described here. Bacteria were not isolated from the unopened, commercial antibiotic infusion tubes.

This is our first experience with *Serratia liquefaciens*, or any type of bacterial contamination, being isolated from SCLA teat dip. The dip was stored for over two years in the original container, which was grossly soiled. Cows dry-cow treated and then teat dipped with the contaminated dip were lost to follow up. Therefore the extent, if any, of *Serratia* IMI in the exposed dairy herd could not be determined.

Standard culture and enrichment methods did not detect *Serratia liquefaciens* in the teat dip. Only when BHI rinse of the original teat dip container was incubated, and then streaked for culture, was the pathogen isolated from the film on the inside of the container.

When teat dip is opened and used for long periods of time, especially when the container becomes visibly soiled, there is risk of contamination with potential mastitis pathogens, especially *Serratia* spp. In the experience of one of the authors (DW), pumps on teat dip containers, teat dip cups, or teat dip sprayers can also become contaminated, therefore a clean reservoir of dip can be subsequently contaminated when applied with them. Soiled containers of teat dip should be cultured to monitor for contamination with bacteria using methods described above, or preferably discarded. Possible fomites, such as teat dip applicators, sprayers or pumps, should be regularly cleaned, and if contaminated teat dip is suspected, it should be tested for bacterial contamination as well.

Conclusion

This case demonstrates risk of contamination of SCLA teat dip, as has been reported with other types of teat dips.

Endnotes

[a]API 20E strips, bioMerieux, Inc., Durham, NC

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