

# **Mastitis-causing bacterial numbers on towels used for udder preparation: comparison of four laundering methods**

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## **INTRODUCTION AND BACKGROUND**

Udder health and hygiene are crucial to control of mastitis on dairy farms. Mastitis can cause decreases in milk production, discarded milk, cow death and culling, and treatment costs. Milking parlor procedures can minimize teat-end exposure to environmental pathogens and prevent transmission of contagious mastitis pathogens. One step utilized to decrease transmission of environmental mastitis pathogens is the rinsing or disinfection and drying of teats prior to milking machine attachment.

Producers can choose disposable one-time use paper towels or washable towels to dry teats. In either case, only one towel is used per cow. Disposable towels are convenient, inexpensive and don't carry the risk of transmitting mastitis pathogens because they are discarded after each use. However, they pose a problem of disposal. Washable cloth towels can be laundered and reused and may be associated with lower cost in the long run. However, because cloth towels are reused, they may act as fomites for transmission of mastitis pathogens.

The laundering methods used on cloth towels may influence the number of mastitis pathogens that remain on clean towels. In human hospitals, the choice of laundering methods has been shown to influence the bacterial load on clean towels. The objective of this experiment was to determine the effect of different laundry treatments (the use of bleach and forced hot air drying) on the recovery of mastitis pathogens from towels used for pre-milking teat preparation.

## **MATERIALS AND METHODS**

A local 1400 cow dairy was chosen for this experiment. A commercial Maytag washer-dryer unit in place on the dairy was used to launder towels. Optimal detergent amount (4 oz Windfresh Concentrated Laundry Detergent), bleach amount (3/4 cup Albertson's Simply Clean Ultra Bleach), and load size (20 pounds), were obtained from the Maytag distributor. Following the normal procedure for udder preparation at the dairy, dirty towels were collected from the parlor by a dairy worker and placed on the floor in front of the washing machine. The investigator weighed out twenty pounds of towels into a clean basket, placed them in the washing machine and added detergent to the load. Each load was randomly assigned to a treatment group which included: (1) bleach and drying, (2) bleach without drying, (3) drying but no bleach, and (4) no bleach and no drying. Ten clean towels were randomly selected from each treatment group, placed in individual bags and stored in a cooler for transport to the laboratory. In addition, ten unlaundered towels were collected and handled in the same manner. During the experiment, water temperature was recorded for each load, the wash cycle was timed and number of towels in each load was counted. Each

load was also given a cleanliness score upon removal from the washing machine.

A 10 x 10 cm section was cut from the center of each towel using cold-sterilized scissors and forceps. The sections were placed in 300 ml of isotonic 0.9% NaCl and agitated in a commercial stomacher (BagMixer 400W, Interscience Laboratories, Inc) for 90 seconds. One milliliter of the resultant suspension was used to perform dilutions. Based on the results of pre-experiment trial runs, samples from clean towels were diluted 1:10 and 1:100. Samples from dirty towels were diluted 1:10,000. Using a colorized 70% isopropyl alcohol to flame glass spreaders between plates, 100 microliters of each of the dilutions was plated on MacConkey agar, sheep blood agar and phenyl ethylene alcohol agar (PEA). One milliliter of each sample was reserved and placed in the refrigerator in case further dilutions were determined to be necessary.

After twenty-four hours of incubation, very little bacterial growth was noted on any of the plates. The plates were placed back in the incubator for another twenty-four hours. At the same time, two undiluted samples from each treatment group, plus two samples from dirty towels were plated on blood agar and MacConkey agar. After twenty-four hours of incubation for the undiluted broth, and after forty-eight total hours of incubation for the diluted broths, the bacterial colonies on each of the plates were identified and counted.

## **RESULTS**

After 48 hours of incubation for the diluted samples, and 24 hours of incubation for the undiluted samples, there was very little bacterial growth on any of the plates. However, in the diluted solutions, there was a slight trend toward greater bacterial growth with treatment four (no bleach and no drying) than with the other treatments. In the undiluted solutions, all the treatments reduced bacterial numbers compared to unlaundered towels, with treatments one and two showing less bacterial growth than treatments three and four ( without bleach treatment).

## **DISCUSSION**

Bacteria were often too numerous to count in our trial runs, so we were puzzled as to why so little growth was seen during the experiment. There were three changes in protocol from the trial runs to the actual experiment. In the trial runs we did not control the load size or the amount of soap and bleach. In addition, we used 0.1M PBS for the agitation and dilutions, while for the experiment, isotonic 0.9% NaCl was used for this purpose. Finally, during the trial runs, clear 70% isopropyl alcohol was used to flame the spreaders used to plate the bacteria, while a colorized 70% isopropyl alcohol was used during the experiment. To determine if either the broth and dilution solution or the alcohol could have contributed to experimental error, two small trials were set up to test the effect of these variables on bacterial growth from dairy towels:

Trial 1) Three clean and two dirty towels were collected from the dairy. One treatment (treatment two) was selected for the clean towels and performed as described in the materials and methods. A side-by-side comparison using 0.1M PBS and 0.9% NaCl for suspension and dilutions was performed. Seventy-percent clear isopropyl alcohol was used for flaming the spreader. Samples were plated on blood agar only. Total bacterial growth for both methods was more consistent with the numerous colonies observed during the trial runs. There did not appear to be a difference in growth with either PBS or NaCl.

Trial 2) Two clean and two dirty towels were collected from the dairy. Load size, detergent amount and bleach amount were not controlled and isotonic 0.9% NaCl was used as the diluent for this trial. A side-by-side comparison using the clear isopropyl alcohol and the colorized isopropyl alcohol for flaming the spreader was performed. Samples were plated on blood agar. Total bacterial growth for both methods was consistent with the decreased amount of bacteria seen during the original experiment. Bacterial growth appeared similar on the clear isopropyl alcohol plates and the colorized isopropyl alcohol plates.

We concluded that: 1) The substitution of 0.1 M PBS for isotonic 0.9% NaCl was not likely to be responsible for the decreased bacterial growth observed during the original experiment versus the growth seen during trial runs and 2) the substitution of clear 70% isopropyl alcohol for colorized 70% isopropyl alcohol was not likely to be responsible for the decreased bacterial growth observed during the original experiment versus the growth seen during trial runs.

Further trial runs could be designed and replicated in order to troubleshoot the problems observed during the experiment. It is possible that our technique of flaming the glass spreaders needs improvement, as this could have led to a small amount of residual alcohol being deposited onto agar plates along with each sample. A third trial comparing the use of disposable, sterile spreaders versus a flamed glass spreader could be performed to determine if this is the source of the problem.