Influence of Addition of Newly Drawn Milk and Fluctuating Temperatures of Farm Bulk Tanks on Growth of Mastitis-Causing Bacteria

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ABSTRACT

Effect of addition of newly drawn fresh milk of consecutive milkings on growth of Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis in milk held at fluctuating temperatures of a farm bulk tank for 48 h was studied. There was shown a significant (p<0.001) growth enhancing effect on S. aureus, S. epidermidis, S. agalactiae and S. uberis but there was a significant (p<0.001) growth enhancing effect on S. dysgalactiae. However, all the bacteria grew significantly (p<0.001) in milk held at fluctuating temperatures of farm bulk tank for 48 h.

Mastitis remains the most costly disease of dairy cattle today. In developing and monitoring a mastitis control program, the first step is to identify which pathogenic organisms are currently present in a herd. This is accomplished by regular monitoring of bulk tank milk due to the high cost of sampling all the cows in a dairy herd in a routine mastitis surveillance program. For this purpose milk samples are taken from the bulk tank by the milk hauler at the time of pick-up and sent to the laboratory for culturing. Culturing a composite sample of 3 to 5 bulk tank milk samples taken at intervals is a widely accepted method of evaluating the herds' mastitis status (4,6,8-10,12,15-17,34,35,39,44-47).

Determination of fluctuating temperatures of farm bulk tank.

Eight farms on alternate-day pick-up, every 48 h, were visited to record the fluctuating temperatures in bulk tank milk. This temperature fluctuation was due to addition of milk of each milking to the milk of previous milkings in the bulk tank which had cooled to 4°C. This fluctuating temperature of farm bulk tank milk was simulated in the laboratory as previously described (30).

Laboratory simulation of fluctuating temperatures

A device to simulate bulk tank conditions was developed which consisted of a 50-gal capacity aquarium and a waterbath. The waterbath was filled with tap water, 20 L, and kept at 37°C (normal temperature of milk in udder). The 50-gal capacity aquarium was used to simulate a farm bulk tank. Both the aquarium and the water-bath were kept in a walk-in cooler set at 2°C. An aquarium heater was placed in the aquarium (simulated bulk tank) and the temperature was set at 4°C (a typical temperature of milk held in farm bulk tanks). To simulate the addition of milk of one milking into a farm bulk tank, the faucet of the water-bath was opened enough to let the appropriate quantity of water enter the aquarium over a 2-h period (simulated milking). The temperature rise and fall after each simulated milking was recorded during a 48-h period were identical to those of recordings of the fluctuating temperatures of farm bulk tank milk. The fluctuating temperatures used in this study were 29, 18, 12, and 9°C at the end of the first, second, third, and fourth milkings, at 0, 12, 24, and 36 h, respectively. (1-3,7,11,13,14,18-31,33,36,37,40-43). However, no information is available on the effect of addition of newly drawn fresh milk of consecutive milkings on the behavior of mastitis-causing organisms in cows' milk during storage at farm bulk tank refrigeration conditions.

This study reports the effect of addition of newly drawn fresh milk of one to three consecutive milkings at 12-h intervals on the growth of Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus dysgalactiae, and Streptococcus uberis in milk held at fluctuating temperatures of the farm bulk tank for 48 h.

MATERIALS AND METHODS

This study was carried out at the Laboratory of Animal Physiology, Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803.
It took 2 h to raise and approximately 6 h for the temperature to return to 4°C after each milking.

**Obtaining milk aseptically**

To select a cow with a mastogenic microorganism-free udder, cows with low somatic cell counts in the dairy herd of the Department of Dairy Science, University of Minnesota, were sampled at six successive milkings and cultured to isolate mastitis causing microorganism before the start of the experiment. A cow with a mastogenic microorganism free-udder was selected for the study. A quarter milker was autoclaved and kept in a sterile package before each milking. The udder of the selected cow was prepared by using separate disposable paper towels saturated with 70% ethyl alcohol to wipe the teat skin and each teat end for 20 sec. As soon as the test skin became dry, the package containing the sterile quarter milker was carefully opened, and the cow was milked to obtain normal aseptically drawn milk. This procedure of obtaining milk from the same cow was repeated each time milk was needed.

Normal aseptically drawn milk was free of mastitis-causing microorganisms as determined by culturing at the beginning of each experiment.

**Experiment**

Normal aseptically drawn milk was dispensed in 100-ml amounts in 250-ml Erlenmeyer flasks and inoculated with portions of 0.1 ml of an 8-h bacterial culture in brain heart infusion broth (Difco) to obtain approximately $2.5 \times 10^3$ CFU/ml of each organism. Microorganisms used were originally cultured from milk of cows with clinical mastitis. Twenty, 10, 7 and 5 ml of this inoculated milk were dispensed into duplicate 50-ml tubes for experiments 1, 2, 3, and 4, respectively, and the tubes were placed in the simulated bulk tank. Experiment 1: After this initial inoculation of the milk at the beginning of the experiment no milk was added at any other time. Experiment 1 served as control to experiments 2, 3, and 4. Experiment 2: Ten ml of newly drawn fresh milk was added to the inoculated milk at the 12th h to simulate the addition of newly drawn fresh milk of one consecutive milking. Experiment 3: Seven ml of newly drawn fresh milk was added to the inoculated milk at the 12th and 24th h to simulate addition of milk of two consecutive milkings at 12-h intervals. Experiment 4: Five ml of newly drawn fresh milk was added to the inoculated milk at the 12th, 24th, and 36th h to simulate addition of milk of three consecutive milkings at 12-h intervals.

Milk samples were taken at 12-h intervals to determine the bacterial number. Samples (1 ml) were taken after mixing the milk samples in the tubes for 20 s by a vortex mixer and fourfold serial dilutions were prepared in 0.1% proteose peptone solution (Difco). Portions (30 µl) from each were plated in duplicate on 5% blood agar plates, incubated aerobically at 37°C for 24 h. Plates with 30 to 300 colony forming unit (CFU) were counted, and the number of CFU in each inoculum was determined. Colonies per ml of milk sample were calculated and the results were analyzed by using the analysis of variance (38). Each experiment was repeated for 4 times for each species of bacterium studied.

**RESULTS**

The initial number of each bacterium inoculated at 0 h was $2.5 \times 10^3$ CFU/ml. The mean numbers ($10^3$ CFU/ml) of *S. aureus*, *S. epidermidis*, *S. agalactiae*, *S. dysgalactiae*, and *S. uberis* at 48 h were 4.45, 4.03, 4.17, 4.35, and 2.44 in experiment 1; 4.59, 4.75, 4.46, 7.20, and 2.36 in experiment 2; 5.41, 4.90, 4.45, 6.21, and 3.28 in experiment 3; and 5.54, 4.08, 5.06, 7.20, and 3.94 in experiment 4. With the exception of *S. dysgalactiae*, the numbers of bacteria were not significantly different in experiments 1, 2, 3, and 4. There was no statistically significant effect of the addition of newly drawn fresh milk of consecutive milkings at 12-h intervals on the growth rate of *S. aureus*, *S. epidermidis*, *S. agalactiae*, and *S. uberis*, but there was a significant (p<0.001) growth-enhancing effect on *S. dysgalactiae*. However, all the bacteria grew significantly (p<0.001) in milk held at fluctuating temperatures of farm bulk tank for 48 h.

**DISCUSSION**

The growth stimulatory and inhibitory effect of newly drawn fresh milk has been previously reported (3,25, 26,36,37). Results of this experiment indicate that the stimulatory effect of newly drawn fresh milk on growth of *S. aureus*, *S. epidermidis*, *S. agalactiae*, and *S. uberis* in milk held at fluctuating temperatures of farm bulk tank was slightly greater than the growth inhibitory effect, but this was not statistically significant. However, growth of all the bacteria in milk held at the fluctuating temperature of farm bulk tank was significant (p<0.001) over a 48-h period.

A single bulk tank count of mastitis-causing bacteria is not favorable because a subclinically infected cow may shed mastitis-causing organisms intermittently or there may be a temporary milk cooling or equipment wash-up problem. Bulk tank milk samples taken at intervals can yield the bacteria shed intermittently by cows or bacteria that are indicative of poor milking procedure, faulty teat dipping or environmental contamination, etc. Therefore, a veterinary practitioner or dairyman is advised to make his conclusion or recommendation on a pattern of count obtained from culturing a composite sample of 3 to 5 bulk tank milk samples taken at intervals rather than one sample (4,6,8,10,12,15,17,34,35,39,44-47). Bulk tank milk samples are usually taken by the milk hauler at the time of pick-up and sent to the laboratory for culturing. The bacteria isolated from such sample(s) represent a mixture of bacteria that have been incubated for 0 to 48 h and exposed to 0 to 4 or more additions of newly drawn fresh milk of consecutive milkings and temperature fluctuations. The number of the mastitis causing organisms isolated from bulk tank milk samples are critical when evaluating the herd's mastitis status or monitoring a mastitis control program. In this experiment, major mastitis-causing bacteria *S. aureus*, *S. epidermidis*, *S. agalactiae*, *S. dysgalactiae*, and *S. uberis* grew significantly in milk held at fluctuating temperatures of bulk tank. Addition of milk of consecutive milkings had a slight but statistically insignificant growth enhancing effect on all the bacteria studied. The number of the bacteria isolated from bulk tank milk samples collected by milk haulers at the time of pick-up is not the same number of bacteria enter-
ing the bulk tank at each milking because of the growth of mastitis-causing microorganisms in milk held in the bulk tank for 48 h. The decision may not be accurate if the number of the organisms isolated from such samples are used to assess the herd’s mastitis status.

As a conclusion, milk samples should be taken from the bulk tank immediately after the first milking following the final pick-up and kept frozen. A composite sample of 3 to 5 of those frozen bulk milk samples taken at intervals should be cultured to assess the actual numbers of the bacteria entering the bulk tank at each milking when monitoring a mastitis control program or evaluating a herd’s mastitis status.

REFERENCES


