THE EFFECT OF FREEZING ON THE STANDARD PLATE COUNT OF MILK

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Milk and cream samples were subjected to (a) slow or (b) rapid freezing, and analysed after 24 and 48 hours storage by the standard plate count method. Bacterial destruction was much less than that reported for liquid egg.

Milk samples are sometimes shipped a considerable distance for analysis. Dry ice is frequently used to refrigerate them in transit, and occasionally samples become frozen. While freezing of liquid whole egg was found to reduce the bacterial count appreciably, no published data were found concerning the effect of bacteria in milk. Consequently, some tests were carried out in this laboratory.

EXPERIMENTAL

Samples of pasteurized milk, cream and chocolate drink were obtained from the Ottawa laboratory of the Ontario Department of Health, and brought to our laboratory. A few raw milk samples were also obtained from a local pasteurizing plant. At the laboratory, after the initial plating, 5-ml. replicate portions of each sample were dispensed into four sterile test tubes (16 x 150 mm.) closed with rubber stoppers. Two of these were laid on the refrigerated shelf in the freezer for quick freezing at 0°F (-17.8°C); the other two were placed at a 20° angle in racks on the floor and subjected to slower freezing at approximately 14°F (-10°C). After 24 hours, one tube of each was removed, defrosted rapidly in water and plate counts made (1). This was done with the remaining tubes after 48 hours. Duplicate plates were poured with tryptose glucose extract milk agar (1) and incubated at 32°C for 48 hours before counting.

RESULTS AND DISCUSSION

For ready comparison the initial count on each sample was given a value of 100, and subsequent counts were expressed on a percentage basis. From the data summarized in Table 1 it is evident that freezing causes much less destruction of bacteria in milk or cream than in liquid whole egg, where the plate count was reduced by almost two thirds after 48 hours freezing at -19°C (-2°F) (2). This probably reflects a difference in the bacterial flora; in pasteurized milk and cream, the bulk of the organisms are Gram-positive species which are more resistant to freezing (3), while in liquid egg Gram-negative species predominate (2). Faster freezing of the much smaller portions of milk may also have been a factor here.

Contrary to expectation too was the frequently greater level of survival after 48 hours than after 24 hours in the freezer. The reason for this is not known. On the other hand, the faster freezing generally resulted in a higher rate of survival than the slower, as might be expected.

The findings in these studies indicate that where milk or cream samples must be shipped long distances to laboratories, partial or complete freezing, before or during shipment, is unlikely to cause an appreciable change in the bacterial content.

### Table 1 — Relative Counts Before and After Freezing

<table>
<thead>
<tr>
<th>Product</th>
<th>No. of Samples</th>
<th>Initial Range of Counts</th>
<th>Median Count</th>
<th>Average Percentage of Initial Count</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pasteurized</strong></td>
<td></td>
<td></td>
<td></td>
<td>Fast Frozen</td>
</tr>
<tr>
<td>Milk — regular</td>
<td>14</td>
<td>9,900 - 100,000</td>
<td>44,000</td>
<td>86.9</td>
</tr>
<tr>
<td>Milk — homogenized</td>
<td>16</td>
<td>2,900 - 78,000</td>
<td>47,000</td>
<td>85.2</td>
</tr>
<tr>
<td>Milk — high test*</td>
<td>7</td>
<td>21,000 - 140,000</td>
<td>36,000</td>
<td>107.0</td>
</tr>
<tr>
<td>Milk — chocolate</td>
<td>3</td>
<td>2,700 - 44,000</td>
<td>36,000</td>
<td>76.1</td>
</tr>
<tr>
<td>Cream — whipping</td>
<td>5</td>
<td>13,000 - 39,000</td>
<td>23,000</td>
<td>93.9</td>
</tr>
<tr>
<td>Cream — table, etc.</td>
<td>9</td>
<td>6,100 - 180,000</td>
<td>46,000</td>
<td>80.1</td>
</tr>
<tr>
<td><strong>Raw</strong></td>
<td></td>
<td></td>
<td></td>
<td>80.1</td>
</tr>
<tr>
<td>Milk</td>
<td>6</td>
<td>10,000 - 1,200,000</td>
<td>24,000</td>
<td>101.6</td>
</tr>
<tr>
<td>Average for all samples</td>
<td>6</td>
<td></td>
<td></td>
<td>90.11</td>
</tr>
</tbody>
</table>

*Jersey and Guernsey milk.

1Contribution No. 397 from the Bacteriology Division, Science Service, Canada Department of Agriculture, Ottawa.

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with the recommendation that all antibiotic therapy be discontinued and that some antifungal agent be administered. Each of the infected quarters was injected with 30 ml. of Capralan**.

Milk from each quarter of the entire herd was taken on February 18, 1954 and in 10 cows a total of 18 quarters were shedding yeasts. Two of the animals which were shedding yeasts on the first sampling were not shedding yeasts at this time.

Development of clinical evidence of the disease followed the milking order and suggested mechanical transmission. The dairyman was questioned about milking procedure and equipment care. He revealed that the local milk sanitarian had recommended dry storage of teat cup inflations. Teat cup washings were taken at random from two of the four teat cups in each respective cluster of the three milking machine units. The washing medium was sterile one per cent peptone broth. The washings were pooled and 0.1 ml. aliquots were cultured on Sabouraud's dextrose agar. After incubation at 37° C. for 48 hours, the surface of the agar plate was completely covered with a confluent growth which was grossly indistinguishable from that obtained from the infected mammary glands. Microscopic examination of suspensions of the growth in lactophenol cotton blue mounting medium revealed yeast-like organisms. The carbohydrate fermentation reactions, the cultural growth on Sabouraud's agar and corn meal agar suggested that these isolates belonged to various species of the genus Candida.

The importance of a laboratory determination of the etiologic agent prior to future therapy was further emphasized to the dairyman and local veterinarian. In addition, it was recommended that teat cup inflations be stored in 0.5 per cent lye solution instead of being stored dry. The presumption that the average dairyman can and will adequately cleanse his equipment is not well-founded. Furthermore, sound precept

and instruction by inspection, extension, and veterinary agencies should be undertaken. Mycotic mastitis and excessively high bacterial counts have been observed by the authors on other farms where dry storage has been practiced. These outbreaks have been overcome following institution of the lye method of storage.

Subsequent investigation revealed that the occurrence of clinical mastitis and abnormal milk had been virtually eliminated. It is recognized that only through adequate laboratory tests can the true prevalence of the infection be determined. Since this herd was located nearly 150 miles from the laboratory, repeated laboratory testing was impracticable.

The findings in this outbreak suggest that the promiscuous use of antibiotic agents in the absence of an etiologic diagnosis may be useless or even detrimental; it is probable that the administration of antibiotics accentuated this bizarre mastitis outbreak. Such a presumption is based on the observation that the prevalence and severity of the disease abated with the cessation of antibiotic therapy.

Everyone is aware of the insurance value of pasteurization and, analogously, that of a lye, or other type disinfectant solution, or heat treatment for teat cup sanitization. It is believed that the practicability of dry storage of teat cups should be carefully scrutinized under experimental field conditions. Limited field experience in Wisconsin has revealed definite shortcomings in this method of storage.

REFERENCES

3. Stuart, F. An Outbreak of Bovine Mastitis from which Yeasts were Isolated, and Attempts to Reproduce the Condition Experimentally. Vet. Record, 63: 314. 1951.

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ADDENDUM

Since this paper was prepared, an abstract has appeared in Dairy Science Abstracts 17 (9), 736 of a paper by E. G. Sarouelson in Svenska Mejeritidn. 47 (5) 59-62. 1955. This abstract states that "the bacteriological state of the milk is practically unaltered by deep-freezing."

REFERENCES