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The Microbiology of Sweet Water and Glycol Cooling Systems Used in HTST Pasteurizers in Fluid Milk Processing Plants in the United States

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ABSTRACT

Refrigerated water and water/glycol mixtures used in high temperature short time (HTST) pasteurizers have been implicated as potential sources of spoilage and pathogenic bacteria in milk. This study was carried out to determine the incidence of bacteria in these cooling systems. Sweet water and glycol coolants from 68 fluid milk plants were sampled to determine the incidence of psychrotrophs, mesophiles, coliforms, and salmonellae. A modified most probable number (MPN) technique was performed using 333 ml of coolant in lactose broth (LB, Difco). Samples were incubated for 7 d at 10°C or for 48 h at 37°C. MPN vessels positive for growth at 37°C were used to inoculate selective enrichment, differential and biochemical confirmation media for salmonellae, and brilliant green bile broth (BGB, Difco) for enumeration of coliforms. In addition, 5 L of each sample were passed through 0.45 μm filters (Millipore). The filters were incubated in LB. The previously described procedure for the detection of salmonellae was used. The procedure of Lovett et al. *(17)* was used to examine each sample for the presence of listeria. The population of organisms that grew at 10 and 37°C varied greatly from plant to plant. MPN values ranged from <0.21 to >240/100 ml of coolant. When present, coliforms were usually at low levels. Eight samples had coliform MPN values >2.2/100 ml of coolant. *Salmonella typhimurium* was isolated from one sweet water sample using the filtration procedure. No *Listeria* were isolated. The use of sanitizer in dairy coolant was not associated with low microbial populations in the coolant samples. This study suggests that coolants used in HTST pasteurizers may serve as a reservoir for bacterial contamination of pasteurized milk.

Coolants used in high temperature short time (HTST) pasteurizers are refrigerated or sweet water, or a mixture of approximately 30% propylene glycol and 70% water. These coolants often contain varying levels of milk solids. rust, sediment, and other debris. Cooling media contaminated with low levels of nutrients could support the growth or survival of undesirable organisms.

The Grade A Pasteurized Milk Ordinance (PMO) (Anon., 1985) requires that recirculated cooling water used in milk processing plants be tested semi-annually. An MPN of <2.2 coliforms/100 ml of cooling water or <1 coliform/100 ml by a membrane filter method is the limit specified in the PMO. When coliform populations exceed this limit, the water supply is to be physically inspected and necessary corrections made until subsequent samples are in compliance. The PMO does not indicate what methods are to be used to obtain satisfactory samples. The PMO also requires that pressure differentials must be maintained in the regenerator section of HTST pasteurizers so that, in the event of leakage, flow will be from the heated milk into the raw milk. There are no federal regulations governing the pressure in the section of pasteurizer where cooling takes place. Three of eight diary plants that responded to a survey by Zottola and Smith *(10)* maintained higher pressure on the coolant than on the milk.

A survey of sweet water and glycol coolants used in Minnesota dairy plants *(4)* reported recovery of high levels of psychrotrophic bacteria from both types of coolants. Although no coliforms were recovered from glycol coolant, approximately one-third of the sweet water samples demonstrated coliform contamination. It was suggested that coolants serve as a source of spoilage organisms in dairy products and that pin holes, cracks, and other defects in the HTST plates could allow contamination of pasteurized milk by sweet water and glycol coolants.

The objectives of the present research were to determine the microbiological quality of dairy cooling systems and to determine if dairy coolants could serve as a reservoir for microbial contamination of pasteurized milk.

MATERIALS AND METHODS

Two surveys were conducted. The first involved grade A fluid milk plants in the North Central states of Minnesota, North Dakota, and South Dakota. The National survey involved 51 plants throughout the United States.

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Sample collection

North Central survey. Approximately 8 L of coolants used in HTST pasteurizers were collected from grade A fluid milk plants by project personnel. Plant personnel provided information on the coolant and sanitizer usage. An attempt was made to obtain coolant both before and after it had passed through the cooling section of the pasteurizer; the design of several dairies precluded collection of both types of samples. Samples were collected aseptically and transported on ice to our laboratory for analyses within 24.

National survey. Sterile sample bottles to collect 8 L of coolant and insulated shipping containers were shipped to 51 dairies in 17 states. Included with each shipment were instructions on sampling and shipping procedures and a sample information sheet which was to be filled out at the time of sample collection. Information was requested on coolant type, sanitizers used in the coolant, and pressures maintained in the HTST. No attempt was made to obtain coolant before and after it had passed through the cooling section of the HTST. Eight L of coolant was collected aseptically by plant personnel and shipped to our laboratory in insulated containers by an overnight express service. Analyses were initiated within 2 h of receipt of the coolant.

Sample analysis

Growth at 37°C. Samples were analyzed using a modified 3-tube most probable number (MPN) technique. A total of 333 ml of sample was used. One hundred ml were aseptically transferred to 3 1L flasks containing sterile triple-strength LB (Difco); 10 ml were used to inoculate three 25 x 15 mm screw-cap test tubes containing double-strength LB; and 1 ml was used to inoculate three 16 x 150 mm test tubes containing single strength LB.

If the coolant was glycol, an appropriate amount of sterile water was added to the sample to decrease the glycol concentration to less than 6%. The amount of water to add was determined from the information on glycol concentration received from the plant supplying the sample. Zottola and Smith *(10)* indicated that high concentrations of glycol may inhibit recovery of microorganisms.

When the National survey samples were analyzed, the need to dilute the glycol was avoided by inoculating the 100 and 10 ml aliquots of sample into the volume of 1.2 strength LB which would result in a final glycol concentration of 6%. MPN vessels were incubated at 37°C for 24 to 48 h.

Enumeration of coliforms. One ml from flasks or tubes showing growth and gas production at 37°C was used to inoculate BGLB. BGLB tubes positive for broth growth and gas after 48 h at 37°C were considered coliform-positive.

Enumeration of salmonellae. One ml of the broth from 37°C MPN vessels indicating growth at 37°C was used to inoculate selenite-cystine (Difco) and tetrathionate broths (Difco) which were incubated at 37°C. Those selective broths showing growth after 24 h were streaked onto bismuth sulfite (Difco), xylose lysine desoxycholate (Difco), and brilliant green agar (Difco) and were incubated at 37°C for 24-48 h. Colonies with reactions typical for salmonellae were used to inoculate triple sugar iron (Difco) and lysine iron agar (Difco) slants. Resultant isolates with *Salmonella* characteristics were serotyped by the University of Minnesota Veterinary Pathobiology Laboratory.

Filtration procedure. Because very low levels of salmonellae were expected, a membrane filtration technique was used. Five liters of sample were passed through 0.45 µm filters (Millipore Corp., Bedford, MA). The filters were incubated in LB at 37°C for 24-48 h. The previously described procedure for detection of salmonellae was used.

Growth at 10°C. The MPN of psychrotrophic organisms in the coolant samples was determined as previously described for growth at 37°C, except MPN vessels were incubated at 10°C for 7 d.

Detection of Listeria monocytogenes. The procedure of Lovett et al. *(7)* was used to examine each coolant sample for the presence of *Listeria monocytogenes.*

Identification of isolates. Isolated colonies were streaked on trypticase soy agar (TSA, Difco) from randomly selected MPN vessels, BGLB tubes, and differential plates. Isolates were Gram-stained and identified using API 20E, Rapid NFT, and Staph Trac identification strips (Analytab Products, Plainview, NY). Organisms not identified with identification strips were classified based on Gram-stain, catalase and oxidase reactions, and carbohydrate fermentation patterns *(6).*

Determination of glycol concentration. A Hewlett-Packard 5840 was used to determine propylene glycol concentration by gas chromatography. The column was a 15 m x 0.2 mm Carbowax 20M. Helium was used as the carrier gas at a head pressure of 15 psi. An injection size of 0.3 µl with a split ratio of 100:1 was used. Quantitation was performed using butyl cellulose and propylene glycol in methanol as internal standards. A flame injection detector was used.

RESULTS

The number of psychrotrophic and mesophilic organisms that were recovered from samples obtained in the North Central survey varied greatly from plant to plant (Table 1). Both sweet water and glycol coolants demonstrated a 10°C and 37°C MPN range from <0.21 to >168 organisms/100 ml of coolant. No coliforms were recovered from most samples, however the coliform population from five coolant samples did exceed the PMO limit (Table 1).

The number of bacteria recovered in the North Central survey was lower than reported by Ginn et al. *(4).* No salmonellae or *Listeria* were isolated from any North Central survey sample. Increased regulatory activity by the Minnesota Department of Agriculture, Dairy Division, which included the sampling of cooling media for microorganisms during routine inspections probably influenced the results.

Glycol concentration of North Central survey samples ranged from 20 to 46% (Table 2). The pH values of most North Central survey coolants were 7.8 or higher. One sample had a pH of 5.8 (Table 2).

Only three North Central survey plants routinely used sanitizer in the recirculated cooling water (Table 2). Each of the three common types of dairy sanitizers, chlorine, iodine, and quanternary ammonium compounds, were used. A comparison of the psychrotrophic standard plate count (PSPC) results of coolant systems indicated that the microbiological quality of dairy coolants was not guaranteed when sanitizers were used (Table 2). Sweet water treated with iodine (sample E1125) had a PSPC of 1200/ml of coolant, while sweet water samples with no added sanitizer (samples F95 and R1031) had PSPC values of 130

TABLE 1. *Summary of MPN results for North Central survey coolant samples.*

MPN/100 ml value	Number of samples with MPN value		
	10°C	37°C	Coliforms
<0.2	6	6	11
0.2-0.6	4	7	2
1.1-1.4	4	2	0
2.2	1	1	2
3.5	1	3	0
4.2-4.62	1	1	0
5.3-9.5	1	3	2
13	1	0	0
18	1	2	1
24	2	0	0
32	1	4	0
46	2	0	0
93	1	1	0
107	1	0	0
122	0	2	0
140	1	1	0
>168	10	5	0
Total	38	38	18*

*Coliform MPN determined on one sample per dairy.

TABLE 2. *Comparison of the effect of added sanitizers on the psychrotrophic standard plate count (PSPC) of North Central survey coolant samples.*

Sample	Coolant medium	Sanitizer used	pH	PSPC/ml
A1118	glycol (22%)	none	7.8	120
B1114	glycol (34%)	none	8.2	240
C1125	glycol (33%)	none	8.2	<1
E1125	sweet water	iodine	8.4	1200
F95	sweet water	none	9.4	130
H1215	glycol (20%)	chlorine	8.4	1
J1016	glycol (33%)	none	8.7	<1
L1013	glycol (39%)	none	8.2	<1
N1017	glycol (46%)	none	8.8	<1
0923	glycol (24%)	quat*	8.3	<1
P125	glycol (29%)	none	8.5	8
Q128	glycol (31%F)	none	5.4	<1
R1031	sweet water	none	9.1	768

*Quaternary ammonium compounds.

and 760/ml of coolant, respectively. This suggested that the addition of santizer to the cold water was not effective in controlling the psychrotrophic population. The same was true when sanitizer was added to the glycol systems. Glycol coolant with added chlorine (sample H1215) had a PSPC of 1/ml of coolant. Glycol sample C1125, with no added sanitizer, had a PSPC value of <1/ml of coolant. The glycol coolant sample that had been treated with quaternary ammonium compounds (sample 0923) also had a PSPC value of <1/ml of coolant.

The most widely used method to control microbial populations in cooling water used in the canning industry is the application of chlorine compounds *(8)*. Maintenance of an adequate chlorine residual may be difficult due to contamination of the coolant by food and fluctuations in pH and temperature *(5,8)*. It is possible that the high pH of the dairy coolants reduces the effectiveness of added sanitizers. Low temperatures, constant agitation caused by continuous pumping, and the organic content will also complicate control of sanitizer levels in dairy coolants.

The population of psychrotrophic bacteria in the National survey samples ranged from <0.3/100 ml of coolant to >240/100 ml of coolant (Table 3). There was no difference between the psychrotrophic populations found in the summer or in the spring. The same range in population of mesophilic bacteria was observed in the MPN/100 ml at 37°C. However, almost 50% of the summer samples had higher 37°C MPN values than those observed in the spring. Six of the over 100 samples analyzed in the National survey exceeded the coliform standard for potable water.

TABLE 3. *Summary of MPN results for National survey coolant samples.*

MPN/100 ml value	Number of samples with MPN value		
	10°C	37°C	Coliforms
<0.3	39	12	82
0.3-0.9	2	10	9
1.5	0	3	0
2.1	0	2	2
2.3	1	7	0
3.0	0	1	0
4.3	2	2	2
7.5	1	0	0
9.3	1	4	1
24	4	20	1
46	3	4	1
110	0	5	0
160	8	0	0
>240	38	29	1
Total	99	99	99

The presence of added sanitizer appeared to have little, if any, effect on the microbial populations of the cooling media (Table 4). Sweet water samples 128 and 129 to which algicide had been added had very high and very low 10°C MPN values, while the 37°C MPN values were similar. The coliform population of sample 128 exceeded the limit established in the PMO. Both the 10°C and 37°C MPN values of untreated sweet water samples ranged from very low levels of recovery to recovery of >160 organisms/100 ml of coolant. One sweet water sample treated with iodine (sample 207) had very low levels of all three types or organisms. Sample 202, which had also been treated with iodine, had a 10°C MPN of >168 organisms/100 ml of coolant. Glycol coolant with no added sanitizer (sample 206) had excessively high levels of psychrotrophic, mesophilic, and coliform organisms. Coliforms were not recovered from two untreated glycol samples (samples 306 and 302). The addition of sanitizers did not appear to control the microflora of the cooling media.

A possible explanation of the ineffectiveness of the

TABLE 4. *Comparison of the effect of added sanitizers on the microflora of National survey coolant samples.*

Sample	Coolant*	Sanitizer*	pH	MPN/100 ml 10°C	MPN/100 ml 37°C	MPN/100 ml Coliform
130	S	Chlorine	9.4	160	24	0.4
131	S	Chlorine	8.3	160	24	<0.3
201	S	Chlorine	7.4	160	0.7	<0.3
203	S	Chlorine	7.8	7.5	0.9	<0.3
205	S	Chlorine	6.9	<0.3	<0.3	<0.3
209	S	Chlorine	8.5	46	24	<0.3
301	S	Chlorine	9.7	24	>240	0.3
308	S	Chlorine	6.9	>240	110	<0.3
309	S	Chlorine	8.0	>240	0.3	<0.3
311	S	Chlorine	7.4	<0.3	>240	2.1
312	S	Chlorine	7.1	<0.3	24	<0.3
202	S	Iodine	7.2	160	24	<0.3
207	S	Iodine	6.5	<0.3	<0.3	<0.3
208	S	Quat	8.2	46	2.3	<0.3
206	G	None	8.3	160	>240	>240
210	S	None	7.3	160	24	0.3
211	S	None	7.7	160	24	24
302	G	None	7.9	>240	110	<0.3
304	S	None	11.6	<0.3	1.5	<0.3
305	S	None	8.4	<0.3	0.4	<0.3
306	G	None	8.5	>240	24	<0.3
307	S	None	7.2	>240	>240	<0.3
310	S	None	8.0	<0.3	2.1	<0.3
128	S	Algicide	9.7	160	4.3	4.3
129	S	Algicide	9.2	<0.3	2.3	<0.3
204	S	Keego	11.1	<0.3	0.4	0.3
303	S	Anti-bact B	8.5	4.3	4.3	<0.3

*S = sweet water, G = glycol.
+Data provided on questionnaires completed by plant personnel.

santizers was revealed in the responses to questions on sanitizer usage in the dairy plants. Information provided on the usage of sanitizers indicated that few of the dairy plants carefully controlled sanitizer levels. Only four plants added chlorine in response to testing of the coolant, by either a standard plate count or determination of residual chlorine levels (Table 5). Generally, sanitizer levels were maintained by addition of set volumes of liquid or dry agents to the coolant at intervals that raged from daily to every 3-6 months. One plant used chlorine as a sanitizer while using quaternary ammonium compounds to adjust the pH of the coolant. The same indiscriminant use of iodine and quaternary ammonium compounds was noted (Table 5).

It is interesting to note that *S. typhimurium* was isolated from only one sample (Table 4, sample 308) using the membrane filtration technique. Chlorine was added monthly to the contaminated sweet water to maintain a level of <5 ppm. The presence of this organism was not detected by coliform analysis or by MPN analysis at 37 or 10°C. The sweet water had a population of <0.30 coliforms/100 ml of coolant, relatively low numbers of mesophiles and a high psychrotrophic population.

The four major genera of bacteria isolated from both sweet water and glycol were *Staphylococcus, Pseudomonas, Bacillus,* and *Enterobacter* (Table 6). Other genera were identified with less frequency (Table 7). Of particular importance was the isolation of *S. typhimurium* from sweet water. Other potential pathogens including *Yersinia enterocolitica, Escherichia coli, Aeromonas, hydrophila, Vibrio* species, *Staphylococcus aureus* and *Klebsiella pneumonia* were also isolated.

L. monocytogenes was not isolated from any coolant samples analyzed. However, research investigating the persistence of *Listeria* is simulated milk cooling systems showed that *L. monocytogenes* was able to grow at 4°C in dilute solutions of nonfat dry milk (NFDM), peptone water, and trypticase soy broth (TSB) *(9)*. As improved methods for the isolation of listeria are developed, it is possible that this organism may be isolated from dairy cooling systems. It has also been reported that *S. typhimurium* had the ability to survive for extended periods at low temperature *(1)*. The strain of *S. typhimurium* that was isolated from an outbreak of salmonellosis associated with the consumption of pasteurized milk survived for more than one year at 7°C in TSB and in 0.1% peptone water.

The data obtained in the two surveys, and the isolation and identification of both potential pathogens and

TABLE 5. *Sanitizer usage in National survey coolant samples*[1].

Sanitizer	Amount used	Frequency of use
Chlorine	1 gallon	Every 3 to 6 months
	1 quart	Weekly
	5-7 ppm*	Weekly if needed after testing
	50 ppm	3 times per year
	30 ppm	Monthly; quat** added to adjust pH
	1 gallon	When needed as per SPC***
	<5 ppm	Monthly
	<1 ppm	Weekly
	200 ppm	As needed every 24 hours
	10 ppm	Automatically monitored
	1 lb	Weekly
	10-20 ppm	Daily
	6 lb/24000 lb water	Weekly if SPC >1000
Quat	100 ppm	Weekly
	Estimated 20 ppm	Monthly
	1 gallon	Monthly
	1 gallon	3 times per week
	2 ppm	1 time per week
Iodine	3 gallons	Every 40 days
	Unknown volume	Weekly
	1 pint	Weekly

[1]Information provided on questionnaires completed by plant personnel when coolant samples were collected.
*Indicates parts per million.
**Indicates quaternary ammonium compounds.
***Indicates standard plate count.

TABLE 6. *Percent of coolant samples from which the most frequently isolated organisms were recovered.*

Genus	% sweet water samples	% glycol samples
Staphylococcus	29.6	4.5
Pseudomonas	15.7	7.0
Bacillus	12.5	34.8
Enterobacter	5.0	20.9

TABLE 7. *Organisms isolated with low frequency from dairy coolant samples.*

Organism	Glycol	Sweet water
Acinetobacter		+
Aeromonas/Vibrio	+	+
Alcaligenes	+	+
Brochothrix		+
Citrobacter		+
Enterococcus	+	
Escherichia		+
Flavobacterium		+
Klebsiella	+	
Kurthia/Caryopanon		+
Micrococcus	+	+
Salmonella		+
Serratia	+	
Sporolactobacillus	+	+
Yersinia		+

TABLE 8. *Pressure differentials maintained in the HTST pasteurizer cooling sections of 14 dairy plants.*

Coolant under lower pressure		Coolant under greater pressure	
Coolant	Milk	Coolant	Milk
5-10	30	44	32
40	54	32	32
24	37	17	13
32	35	60	52
34	35		
60	65		
40	54		
30	>30		
20	32		
30	40		

spoilage organisms, indicate that the cooling media used in the HTST pasteurizer in a fluid milk processing plant could serve as a source of undesirable microorganisms in pasteurized dairy products. Such contamination could occur if the material separating the pasteurized product from the cooling media is cracked or contains pin holes, and the pressure differential permits flow from the cooling media into the milk. Four of 14 National survey dairy plants that responded to questions on pressures maintained in the cooling section of the HTST unit did indicate that coolants were under pressure greater than or equal to the pressure of the milk (Table 8).

To prevent contamination of pasteurized products with cooling media, it is essential to maintain proper pressure differentials. Equipment and instrumentation is available to the industry to monitor pressures in HTST systems.

Cooling systems that use water/glycol mixtures might encounter fewer problems with microbial contamination if the concentration of glycol in the system exceeds 30%. Petran and Zottola *(9)* reported that *L. monocytogenes* was not able to grow in glycol coolant with 0.01% added NFDM solids when the propylene glycol concentration was 20% or greater. A decline in the population of *S. typhimurium* was observed at -1°C in glycol concentrations from 0 to 40%. The higher the glycol concentration, the faster the decline in population *(2)*.

Microorganisms in sweet water can be controlled by the addition of chlorine, iodine or quaternary ammonium sanitizers. However, the addition must be continuous and constantly monitored. Corrosion of cooling systems is likely when high levels of halogen compounds are added to coolants. Indiscriminant addition of sanitizer does not ensure microbial control.

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