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Salmonella typhimurium Phage-Type 10 from Cheddar Cheese Implicated in a Major Canadian Foodborne Outbreak

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ABSTRACT

Levels of *Salmonella typhimurium* phage-type 10 in Cheddar cheese implicated in a major Canadian foodborne outbreak ranged from 0.36 to 9.3 salmonellae/100 g. Such a low level contamination likely accounted for the uneven distribution of the organism among subsamples of individual lots. Coliform and *Escherichia coli* counts were within acceptable limits, whereas three of the 11 lots tested contained $\geq 10^5$ *Staphylococcus aureus* per gram but no staphylococcal enterotoxins. *Campylobacter* and *Yersinia spp.* were not detected in any of the 12 lots examined. Ability of *S. typhimurium* to survive up to 8 months in Cheddar cheese stored at refrigerator temperature (5°C) underlines the inadequacy of current regulations requiring a 60-d storage of cheese manufactured from heat-treated (unpasteurized) milk before sale. Results underlined the greater sensitivity of selective enrichment in tetrathionate brilliant green (43°C) than in selenite cystine (35°C) for detection of *Salmonella* in cheese.

Although milk and milk products are not generally recognized as a major source of human foodborne salmonellosis, recent events underline the need for continued, if not increased, vigilance on the bacteriological quality of these foods. Consumption of raw, unpasteurized fluid milk in the United Kingdom and United States has for many years contributed significantly to the national statistics on foodborne salmonellosis and campylobacteriosis (*7,17,29,31*); milk-borne outbreaks have also been reported with some frequency in Canada (*4,13,34*). Recent introduction of regulations prohibiting the sale of unpasteurized milk in Scotland produced a dramatic reduction of human salmonellosis from fluid milk (*30*). A similar regulatory approach is currently envisaged for England, Wales and Northern Ireland (*24*). Restrictive regulations in the U.S. would likely bring the current debate on the safety of certified raw milk to a close (*2*).

Although pasteurization effectively protects consumers against pathogenic microorganisms in raw milk, pasteurized milk has been identified on rare occasions as the vehicle of infection. In 1983, distribution of whole and 2% pasteurized milk contaminated with *Listeria monocytogenes* resulted in 49 cases of illness and 14 deaths in Massachusetts (*15*). A recent outbreak from *Salmonella typhimurium* in pasteurized fluid milk involved more than 14,000 cases of illness and five deaths in Illinois (*33*). Although the cause of these two outbreaks remains obscure, seepage of raw milk into pasteurized milk lines or holding tanks as a result of faulty connections, defective pasteurizer or poor plant operations can lead to post-process contamination of products.

Production of cheese under intensive manufacturing conditions and the use of heat-treated (non-pasteurized) milk in cheese manufacture has led to several important outbreaks of salmonellosis in recent years. In 1976, seven lots of pasteurized Cheddar cheese contaminated with *Salmonella heidelberg* were incriminated in an outbreak of 339 confirmed and an estimated 28,000 to 36,000 cases of illness (*16*). Another outbreak of salmonellosis associated with non-pasteurized Cheddar cheese contaminated with *Salmonella muenster* was recognized in Canada in 1982 (*1,36*). The present report deals with the distribution and survival of *S. typhimurium* phage-type 10 in Cheddar cheese responsible for a major Canadian outbreak of more than 1500 confirmed cases of salmonellosis between January and July 1984.

MATERIALS AND METHODS

Cheese samples

Cheese from 21 lots previously found to be contaminated with *S. typhimurium* phage-type 10 was collected from the incriminated cheese plant in eastern Canada and from warehouses of two Ontario distributors. Thirty to 60 subsamples were obtained from each of the 21 contaminated lots involved in the present study. Such stringency in sampling is required by Health Protection Branch methods for the investigational analysis of suspect foods (*20*). The pH of selected lots was determined as the mean of duplicate samples using a flat bottom electrode. Survival of *Salmonella* during refrigerated storage was monitored at 4-wk intervals until two consecutive negative results were obtained. For the quantitative analysis of refrigerated cheese, 20-g portions from each of the 30 subsamples in a lot were dry blended together and salmonellae in the composite sample were enumerated by the most probable number

(MPN) technique using triplicate 100-, 10- and 1.0-g samples. Distribution of *Salmonella* within each lot was determined by analysis of 25 g of cheese from each of the 30 subsamples taken from each lot. Although cheese samples were stored under refrigeration (5°C), heavy mold growth was evident at the conclusion of the study.

Bacteriologic analyses

For detection of *Salmonella,* cheese samples were preenriched for 16 to 18 h at 35°C in nine volumes of nutrient broth. Duplicate 1-ml portions from each preenrichment culture were selectively enriched for 16 to 18 h in tetrathionate brilliant green (TBG) and selenite cystine (SC) broths incubated at 43°C and 35°C, respectively. Each enrichment culture was then streaked on bismuth sulfite (BSA) and brilliant green sulfa (BGS) agars and incubated for 16 to 18 h at 35°C. If necessary, BSA plates were reincubated for an additional period of up to 24 h. Suspect colonies were screened biochemically on triple sugar iron (TSI) and lysine iron (LI) agars, and confirmed serologically with polyvalent and single grouping antisera.

A representative sample of cheese (11 g) from each lot was diluted tenfold in a 2% aqueous solution of sodium citrate and subjected to coliform, fecal coliform, *Escherichia coli* and *Staphylococcus aureus* determinations. Enteric microorganisms were enumerated by the 5-tube most probable number (MPN) procedure based on gas production in lauryl sulfate tryptone (35°C), brilliant green bile lactose (35°C) or EC (45°C) broths (*22*). *E. coli* was isolated on eosin methylene blue (EMB) plates of gas-positive EC broths and confirmed using the IMViC reactions. For the quantitative determination of *S. aureus,* serial dilutions of the cheese homogenate prepared for coliform analysis were plated on Baird-Parker (BP) agar and incubated for 48 h at 35°C. Brain heart infusion (BHI) broth cultures of suspect colonies on BP agar were confirmed using the coagulase (*22*) and thermonuclease (*21*) tests. Staphylococcal enterotoxins A, B and C were detected in cheese extracts by solid-phase radioimmunoassay (RIA) using ^{125}I-labelled toxin (*14*). In this procedure, cheese extracts were added to antibody-coated plastic tubes and incubated for 20 h at 4°C. The cheese extract was removed and labelled toxin was added to the plastic tube and incubated for 2 h at room temperature; unbound toxin was removed by aspiration.

Twelve lots of cheese, including seven lots found to contain salmonellae in our laboratory, were tested for the presence of *Campylobacter* spp. and *Yersinia* spp. In the former assay, replicate 25-g samples of cheese were enriched in Preston broth (*5*) and in brucella broth supplemented with three antibiotics (vancomycin, polymyxin and trimethoprim) and $FeSO_4$, sodium metabisulfite and sodium pyruvate (*27*). Enrichment broths were incubated with agitation for 48 h at 42°C under microaerobic conditions using a gas mixture of 5% O_2, 10% CO_2 and 85% N_2 (*5,27*). Enrichment cultures were streaked on Skirrow's agar (*32*) and plates were incubated for 48 h at 42°C under microaerobic conditions. Determinant biochemical tests facilitated identification of *Campylobacter* spp. (*27*). For the isolation of *Yersinia* spp., 25 g of cheese was enriched in sorbitol bile salts broth for 4 d at 22°C (*23*). Enrichment cultures were adjusted to an alkaline pH (*3*) and streaked on MacConkey agar; plates were incubated for 36 to 48 h at 22 to 25°C. Suspect colonies were screened biochemically on Kligler's iron agar, LI and other biochemical media (*23*). All commercially available bacteriological media were obtained from Difco Laboratories.

RESULTS AND DISCUSSION

S. typhimurium PT 10 was first isolated in 1967 in Saskatchewan and has since become the most common phage type of *S. typhimurium* in Canada. In 1979, it accounted for 68% of all reported human and 31% of non-human strains of *S. typhimurium (26).* Retesting of the 21 lots of cheese originally found to contain *S. typhimurium* PT 10 found eight lots to be contaminated at levels ranging from 0.36 to 9.3 salmonellae per 100 g (Table 1). Similar levels of 0.36 to 1.8/100 g were reported for pasteurized Cheddar cheese implicated in a major U.S. outbreak of *S. heidelberg (16).* These findings clearly indicate that high numbers of *Salmonella* are not a prerequisite to infection and that few salmonellae in cheese can lead to serious epidemiological consequences. Data from the outbreak of *S. typhimurium* in Canada suggested that even a single cell could cause illness (*11*). Foods such as cheese and chocolate (*12,18*) are sensitive products because they are not usually subjected to bactericidal heat treatments before consumption, and contain an appreciable amount of fatty substances that may protect the infective organisms against the acidic pH of the stomach.

The pH of cheese, which was determined in parallel with MPN enumeration of *Salmonella,* ranged from pH 4.97 to 5.40. These values are well within the pH 5.0 to 5.5 range normally encountered in Cheddar cheese (*6,35*). Information on the phosphatase reaction in incriminated cheese is generally lacking. Three of six household samples of cheese associated with human illness in Ontario were phosphatase positive (Styliadis, personal communication). Further analyses of cheese by this group also showed the presence of the enzyme in vats 299, 314, 475, 495 and 530 but not in vat 89. These results, with the exception of vat 475, concur with the applied thermal treatment of the milk used in cheesemaking (Table 1).

Distribution of *S. typhimurium* within the 30 subsamples taken from each of the six lots tested varied between 0 and 50% (Table 1). Such a recovery pattern likely resulted from the random distribution of low numbers of *Salmonella* rather than a pocketing of infectious organisms owing to the extensive mixing involved in cheese manufacture. These findings further indicate that several lots would have gone undetected if a stringent sampling plan had not been used in the investigation of this outbreak. Levels of coliforms, fecal coliforms and *E. coli* in *Salmonella*-contaminated lots were well within acceptable limits (*8*) and were slightly higher than homologous counts in *Salmonella*-negative lots (Table 2). There was no apparent relationship between the number of indicator organisms and presence of salmonellae. Enumeration of $\geq 10^5$ *S. aureus* per g in three of the 11 lots tested led to further screening for staphylococcal enterotoxins A, B and C by the RIA procedure; no toxin was detected in any of the samples. Testing for *Campylobacter* spp. or *Yersinia* spp. also yielded negative results.

TABLE 1. *Enumeration of* S. typhimurium *PT 10 in Cheddar cheese.*

Type of cheese	Thermal treatment[a]	Vat No.	pH	Date of: Manufacture	Date of: MPN testing	Most probable number (Salmonellae/100 g)	No. of subsamples: Tested[b]	No. of subsamples: Positive (%)
Salmonella positive								
Mild Cheddar	P	89	5.22-5.40	30.1.84	30.7.84	0.91	30	3 (10)
Mild Cheddar	HT	299	5.18	23.4.84	24.7.84	9.3	30	15 (50)
Mild Cheddar	HT	314	5.18	27.4.84	24.7.84	9.3	30	7 (23)
Mild Cheddar	P	383	NT[c]	14.5.84	24.7.84	4.3	ND[d]	ND
Mild Cheddar	P	384	NT	14.5.84	24.7.84	0.36	ND	ND
Mild Cheddar	P	475	5.09	1.6.84	30.7.84	0.91	30	2 (6.6)
Mild Cheddar	HT	495	5.11-5.14	5.6.84	31.7.84	2.3	30	8 (27)
Mild Cheddar	HT	530	5.11-5.18	13.6.84	31.7.84	0.36	30	0 (0)
Salmonella negative								
Mild Cheddar	P	217	5.40-5.42	17.3.84	30.7.84	<0.3	ND	ND
Mild Cheddar	P	359	5.10-5.13	9.5.84	30.7.84	<0.3	ND	ND
Mild Cheddar	P	385	NT	14.5.84	16.7.84	<0.3	ND	ND
Mild Cheddar	P	427+428	4.97-4.98	23.5.84	30.7.84	<0.3	ND	ND
Mild Cheddar	P	535	5.10-5.18	15.6.84	31.7.84	<0.3	ND	ND
Mild Cheddar	P	585	5.09-5.10	29.6.84	31.7.84	<0.3	ND	ND
Skim milk cheese	P	773	NT	11.7.84	23.7.84	<0.3	ND	ND
Skim milk cheese	P	774	NT	11.7.84	23.7.84	<0.3	ND	ND
Skim milk cheese	P	776	NT	11.7.84	24.7.84	<0.3	ND	ND
Skim milk cheese	P	786	NT	13.7.84	24.7.84	<0.3	ND	ND
Skim milk cheese	P	787	NT	13.7.84	24.7.84	<0.3	ND	ND
Skim milk cheese	P	789	NT	13.7.84	24.7.84	<0.3	ND	ND

[a]P, pasteurized for 16 s at 73.8°C; HT, heat treated for 16 s at 66.7°C.
[b]Subsamples were tested between 14 August - 20 September, 1984.
[c]NT, not tested.
[d]ND, not done because of insufficient sample or found to be free of *Salmonella* upon retesting.

TABLE 2. *Bacterial profile of Cheddar cheese.*

Vat No.	Cell counts (per g): Coliform	Fecal coliforms	*E. coli*	*S. aureus*
Salmonella positive				
89	54.0	54.0	54.0	2.4×10^6
299	2.3	2.3	2.3	2.5×10^5
314	54.0	54.0	54.0	9.0×10^4
475	54.0	54.0	4.5	Neg
495	3.3	3.3	1.1	NT[a]
530	4.9	4.9	4.1	Neg
Salmonella negative				
773	3.3	3.3	3.3	Neg
774	13.0	7.9	7.9	3.8×10^3
776	7.9	7.9	7.9	2.5×10^5
786	13.0	0.45	0.2	Neg
787	1.1	<0.18	<0.18	Neg
789	24.0	0.20	0.2	5.1×10^3

[a]NT, not tested.

TABLE 3. *Survival of* S. typhimurium *PT 10 in stored Cheddar cheese.*

Vat No.	Age of cheese (months): 2	3	4	5	6	7	8	9	10
89	NT[a]	NT	NT	NT	0.91[b]	Neg	Neg	NT	NT
299	NT	4.3	0.9	0.3	4.3	1.5	0.7	Neg	Neg
314	NT	0.9	4.3	Neg	0.9	0.7	Neg	Neg	NT
475	0.9	Neg	0.3	Neg	Neg	NT	NT	NT	NT
495	2.3	0.3	0.3	0.3	Neg	Neg	NT	NT	NT
530	0.3	Neg	Neg	NT	NT	NT	NT	NT	NT

[a]NT, not tested.
[b]Number of salmonellae/100 g cheese determined by the most probable number (MPN) technique.

TABLE 4. *Productivity of enrichment-plating conditons.*

Sample size (g)	No. of samples		*Salmonella*-positive samples (%)			
			TBG_{43}		SC_{35}	
	Tested	Positive (%)	BSA	BGS	BSA	BGS
100	167	41 (25)	41	41	35	32
25	180	35 (19)	35	35	30	28
10	156	18 (12)	18	18	16	17
1.0	168	1 (0.6)	1	1	1	1
TOTAL	671	95 (14)	95 (100)	95 (100)	82 (86)	78 (82)

The present study showed that *S. typhimurium* PT 10 in Cheddar cheese survived up to 8 months of refrigerated storage (Table 3). These data support earlier reports on the ability of *S. typhimurium (28)* and other serovars (*35*) to survive 9 to 10 months in Cheddar cheese stored at 4.5 to 13.0°C. Selective enrichment in TBG incubated at 43°C was more productive than homologous enrichment in SC incubated at 35°C (Table 4). These findings reiterate earlier conclusions on increased method sensitivity through synergism between the selectivity of TBG and repressed growth of competing microorganisms at elevated temperature (*9,10*). Low level recovery of *Salmonella* with SC_{35}-BGS is well-established for high moisture foods (*9*).

Recent outbreaks of salmonellosis have shed new light on the importance of cheese as a vehicle of human infection. Although pasteurization of milk used in cheesemaking increases the safety of the finished product, use of heat-treated (unpasteurized) milk in the manufacture of medium and old Cheddar cheese and survival of *Salmonella* during prolonged periods of refrigerated storage (Table 3) raises legitimate doubts on the safety of current manufacturing practices. Our results (Table 3) and those of others (*19,25,28,35,36*) suggest that regulations requiring that raw milk cheese be stored for no less than 60 d at refrigerator temperatures are inadequate with respect to *Salmonella*. The growing problem of bacterial pathogens in fluid milk and milk products must be confronted if its potential impact on human health and on the economic viability of sectors in the food industry is to be minimized.

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