STAPHYLOCOCCAL FOOD INTOXICATION DUE TO CHEDDAR CHEESE II. LABORATORY EVALUATION*

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Introduction

As indicated in a previous paper (13), an outbreak of food-poisoning occurred in an Iowa Institution in August 1958, and a thorough epidemiological investigation incriminated cheddar cheese as the vehicle of transmission. Approximately 900 persons ate the meal in question and 200 of these became ill 3 to 5 hours later. All who became sick had eaten cheese, but not all who ate cheese were affected. The illnesses were characterized by sudden onset, nausea, abdominal pain, vomiting, diarrhea, exhaustion and prompt recovery. The laboratory performed several important functions and aided the field investigators considerably in their efforts to uncover the probable source of contamination.

Since the literature has been reviewed rather completely in the first paper concerning staphylococcal food-poisonings of this type, literature sources will be cited only as they pertain to the laboratory findings.

Outbreaks of food-poisoning definitely related to staphylococci in milk or milk products have been reported on a number of occasions. While raw milk has been the main cause, pasteurized milk and dairy products made from pasteurized milk have been implicated in a few instances. Recently cheddar cheese has played a more important role as the contaminated agent in food-poisoning outbreaks. Most of the outbreaks have been due to the raw milk (natural) variety of cheddar cheese (13).

Bacteriological Analysis of Specimens

Sterile instruments were used to expose an internal portion of the cheese specimen, and a quantity about the size of a small olive pit was removed and placed in a sterile mortar and pestle. After the addition of sterile alundum, the specimen was ground to a paste. Approximately 2 ml of sterile saline was added, and the paste emulsified. A drop of the saline emulsion was then placed on each of two blood agar plates, and the plates streaked with a bacteriological loop. One plate was incubated aerobically and the other anaerobically. The next day the plates were examined and colonies exhibiting *beta* hemolysis were picked to rabbit plasma (Difco) for the coagulase test.

Cheese specimens exhibiting no hemolytic colonies were examined a second time before they were entered as negative.

Bacteriophage Typing

Cultures were typed by the Public Health Laboratories of the Kansas State Board of Health with the human staphylococcal bacteriophages and by Dr. E. H. Coles, Department of Pathology, Kansas State College with the bovine staphylococcal bacteriophages.

Phosphatase Test and Bacterial Density

These tests were performed as outlined in Standard Methods for the examination of Dairy Products, 10th Edition.

Kitten Feeding Test

Six kittens were obtained, vaccinated for distemper, and maintained under observation for four weeks prior to testing.

Two days before the actual feeding experiment the test culture was transferred to tryptone broth and incubated under 10 per cent CO_2 at 37°C for 24 hours. The next day one pound of lean ground beef was divided into two equal parts, placed in covered beakers, and sterilized in the autoclave at 121°C for 15 minutes. After cooling, one aliquot was inoculated with the broth culture, and then both were incubated at 37°C for 18 hours.

The kittens were divided into a test and control group of three each and fed the respective materials.

Results

A total of 85 wheels of cheddar cheese were examined for the presence of *beta* hemolytic coagulase positive *Staphylococcus aureus*. The results of this survey are presented in Table 1.

As will be seen from these results approximately 87 per cent of the wheels examined contained *Staph. aureus*. Commercial cheese was obtained by the field investigators, coded and placed with other

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TABLE 1—SUMMARY OF AMERICAN CHEDDAR CHEESE WHEELS EXAMINED

Month of manufacture	No. of wheels sampled	No. containing Staph. aureus
April 1957	2	0
January 1958	5	5
February 1958	8	8
March 1958	19	17
April 1958	23	19
May 1958	8	5
June 1958	9	9
July 1958	8	8
August 1958	3	3
Total	85	74
Commercial Cheese (control)	5	0

TABLE 2—STANDARD PLATE COUNTS ON FIVE SELECTED CHEESE SAMPLES

Identity	Date of manufacture	Colonies/gm (x 10 million)
FM-6	3-17-58	9
FM-8	4-14-58	2.1
FM-11	4-21-58	2.3
FM-13	3-17-58	17
FM-22	4- 7-58	7.4

samples to be examined in the laboratory. This served as a control for the laboratory. Only after the results were obtained did the laboratory know the exact identity of each specimen.

It is entirely possible that the 11 wheels that were negative on our tests actually did contain the suspect organism. Core specimens were taken with a cheese trier from each wheel. The small portions of the cores from 11 wheels were negative, but tests on entirely different core specimens from the same wheel might have proved positive.

Total bacterial counts on each specimen were not done. It was, however, considered advisable to obtain some information as to the bacterial density. Table II presents the data from duplicate standard plate counts on five selected cheese samples. The density of *Staph. aureus* per gram of cheese was not determined.

Early in the investigation of this outbreak, field investigators were led to believe that the cheese was made from pasteurized milk. It was suspected, however, that the heating process was not sufficient to properly pasteurize the milk, so chemical tests for the enzyme phosphatase were performed on the cheese. Of 18 specimens selected at random and tested, 14 gave very strongly positive results, and four were negative. Further checking revealed that the four negative specimens were from the five commercial cheese specimens included as controls for the bacteriological analysis. All eighteen specimens were negative when examined for inhibitive characteristics (5).

Barber, *et al*, (2) observed that a large number of coagulase positive staphylococci possessed the ability to hydroyze phosphoric esters by a phosphatase enzyme. In order to eliminate the possibility that the phosphatase activity in the cheese was due to the presence of *Staph. aureus*, several aliquots of sterile milk were inoculated with a pure culture of *Staph. aureus* isolated from one of the cheese specimens. After incubation at 37°C for 24 hours, the aliquots were examined for the presence of phosphatase. No detectable phosphatase was present.

In view of the phosphatase test results it was decided to return to the cheese factory and make a more extensive survey of the operational methods and to obtain samples of raw milk from the institution herd and the seven commercial herds from which milk for the cheese had been obtained. The results of the bacteriological analysis of these two groups of specimens are presented in Table 3.

Conditions in the institution farm milk house and methods of handling milk were not in accordance with present accepted practices. The milk house doors were without screens, and the result was an extremely high fly density. The milk house rooms were dirty and disorderly. Milking was done by machine. After each use teat cups were dipped in a germicidal solution (made at the institution) very briefly and then allowed to touch the floor of the milking barn, workmen's shoes, wheels of the cart that carried the germicidal solution, or the floor of the stall before being applied to the next cow. The milk was poured from the milking machine bucket into an open bucket in the barn and carried to the milk house for weighing and straining. The milk was cooled in 10 gallon cans in a concrete tank with flowing well water. As each 10 gallon can of milk was filled, a 20 ml specimen was removed using a sterile 10 ml pipette. Earlier in the day the local milk sanitarian had collected a pooled herd sample from each of the commercial herds which had supplied milk to the cheese factory.

From the data in Table 3 it will be observed that 1 out of 7 specimens from the commercial herds contained *Staph. aureus* while 7 out of 13 specimens or 53.8 per cent from the institution contained the same organism. These milk specimens were also subjected to standard plate count, thermoduric count,

TABLE 3—ANALYSIS OF RAW MILK SAMPLES FROM COMMERCIAL HERDS AND INSTITUTION HERD

Specimen Identify	Location	Microscopic Examination	Standard Plate Count, per ml (x 1000)	Thermoduric Count, per ml (x 10)	Inhibitive Character	Coagulase	*Staph. aureus*	Culture No.
8309	CH	EHCC; strep.	8.3	5	–		–	
8310	CH	EHCC; strep.	140	1	–		–	
8311	CH	EHCC; sporing rods	6.3	17	–		–	
8312	CH	EC; sporing rods	32	1	–	+	+	2027
8313	CH	EC	41	none	–		–	
8314	CH	EC	8.3	3	–		–	
8315	CH	EHCC; strep. sporing rods	32	780	–		–	
8316	I	EC; sporing rods	31	30	–	+	+	2011
8317	I	EHCC; strep.	55	6	+		–	
8318	I	EC	4.9	none	–	+	+	2013
8319	I	EHCC	12	none	–	+	+	2014
8320	I	EHCC	6.4	1	–	+	+	2015
8321	I	EHCC	9.6	none	–		–	
8322	I	EHCC	7.6	none	–		–	
8323	I	EHCC	6.7	none	–	+	+	2018
8324	I	EHCC	5.8	none	–	+	+	2019
8325	I	EHCC	4.7	1	–		–	
8326	I	EHCC	8.2	none	–		–	
8327	I	EHCC	19	1	–	+	+	2022
8328	I	EHCC	7.5	none	–		–	

Key: CH = commercial herd
I = institution
EC = excessive cells, 1 to 1.5 x 10^6/ml.
EHCC = extremely high, >1.5 x 10^6/ml. cell count

analysis of inhibitive characteristics and microscopic examination.

It was decided to select several of the cultures of *Staph. aureus* isolated from the cheese specimens and type them using both the human staphylococcal bacteriophages and the bovine staphylococcal bacteriophages. Since typing facilities were not available locally, and because of the large number of cultures, it was considered not feasible to have all the isolates typed. Table 4 presents the human and bovine staphylococcal bacteriophage patterns obtained on 21 cultures isolated from various lots and/or wheels of cheddar cheese. Bacteriophage patterns on four of the eight cultures isolated from the raw milk mentioned previously are presented in Table 5.

A kitten feeding test was also performed using one of the cultures (#1310) isolated from a specimen of cheddar cheese. Two out of three kittens which were fed the material prepared as described previously became ill with explosive vomiting and diarrhea.

Nose and throat cultures were obtained on all persons working in the cheese factory at the time of inspection as well as samples of coloring, rennet extract, and swab specimens of the agitator shaft, cover of the agitator and milling machine motor cord. All specimens were negative with the exception of one workman who harbored *Staph. aureus* in the nose and throat; however he was only recently assigned to work in the cheese factory and was not employed during the time the cheese in question was made. Bacteriophage typing of these two cultures revealed that the culture from the nose had a pattern of 42E/70/75/47C/VA4 and the culture from the throat was type 7.

DISCUSSION

As was indicated in the first paper of this series, the clinical manifestations of the illnesses, and epidemiological findings were indicative of staphylococcal food-poisoning. The finding of *Staph. aureus* in the cheddar cheese and not in any of the other foods served at this one meal led the authors to reasonably conclude that the cheese was the contaminated vehicle. The observation that the cheddar cheese was manufactured at another state institution prompted the laboratory and field investigators to make a complete survey of the cheese manufacturing process.

The entire investigative procedure was an attempt to discover the actual point and source of contamination of the product. While the outbreak involved cheese made during the period January - April, this part of the investigation also included cheese made during the period April through August 18. No cheese was made after August 18 as all the milk was used as fluid milk.

Core samples were taken from all wheels of cheddar cheese sent to the institution at which the outbreak occurred, as well as from some scraps (unidentifiable as at lot) that remained from the wheels served at the incriminated meal. Every lot of cheese, representing one day's production, in storage at the cheese factory was sampled at least once. It was estimated that approximately 7½ tons of cheese was involved. As was indicated earlier, 87 per cent of the specimens examined contained *beta* hemolytic coagulase positive *Staph. aureus*.

According to Hammer (12) the microbial density of cheddar cheese is highest early in the ripening process and then decreases gradually. The peak count is somewhere in the vicinity of 1,400 million organisms per gram. The mean standard plate count on five determinations presented in Table 2 was 75.6 million organisms per gram. The mean value does not appear to be too different from normal cheddar cheese.

The analysis of raw milk samples from the commercial herds and the institution herd presented in Table 3 did not demonstrate abnormal standard plate counts, and the thermoduric counts appeared normal with the exception of one specimen which had a count of 7800 per ml. Extremely high cell counts were observed in 57 per cent of the commercial herd samples and in 84.6 per cent of the institution herd samples. One specimen from the institution herd demonstrated inhibitive characteristics.

The isolation of *Staph. aureus* from 53.8 per cent of the institution herd raw milk samples would seem to indicate that several cows in this herd were harboring and shedding this organism.

Typing of 21 strains of *Staph. aureus* isolated from as many different wheels of cheddar cheese with human staphylococcal bacteriophages presented 14 different patterns plus a group of non-typables

TABLE 4—BACTERIOPHAGE TYPING OF TWENTY-ONE CULTURES OF *Staph. aureus* ISOLATED FROM CHEDDAR CHEESE SPECIMENS

Date of cheese manufacture	Culture No.	Pattern	
		Human staphylococcal bacteriophages	Bovine staphylococcal bacteriophages
1-13-58	1743	52/52A	NT*
2- 3-58	1536	79/80/3A/3B/3C/ 6/7/42E/47/53/54/ 73/75/77/42B/47C/ VA4/42D/81	A8/A10/A13/87/S2
3- 3-58	1533	NT	A8/S2
3- 6-58	1544	6/VA4/42D	A8/A10/A13/87/S2
3-17-58	1362	7/42E/47/42B/VA4	A8/A10/A13/87/S2
	1363	6/42E/47/54/75/ 47C/VA4	A8/A10/A13/S2
	1365	6/42E/47C/VA4	A8/A10/A13/87/S2
	1538	NT	A8/S2
3-21-58	1546	NT	NT
3-31-58	1310	6/42E/47/54/75/ 47C/VA4/42D	A8/A10/A13/S2
4-14-58	1369	47C/VA4/42D	A8/A10/A13/87
	1374	79	A8/A13
	1532	79	A8/A13
4-21-58	1350	42D	A8/A10/A13/87
4-24-58	1744	7/42E	A8/A10/A13
4-28-48	1535	29/79/3B/6/42E/47/ 54/73/75/42B/47C/VA4	A8/A13
	1539	29/79/3B/6/42E/47/ 54/75/42B/47C/VA4	NT
5- 8-58	1733	42D	A8/A10/A13/87/S2
5-12-58	1721	VA4	A8/A10/A13/87/S2
6-26-58	1704	NT	A8/S2
7- 3-58	1708	VA4	A8/A10/A13/87/S2

*non-typable

TABLE 5—BACTERIOPHAGE TYPING PATTERNS ON FOUR ORGANISMS ISOLATED FROM RAW MILK (SEE TABLE III ALSO)

Source	Culture No.	staphylococcal Human bacteriophages	Bovine staphylococcal bacteriophages
IH	2011	54	A8/A10/A13
IH	2015	54	A8/A10/A13
IH	2019	NT*	NT
CH	2027	6/7/42E/77	A8/A10/A13/S2

*non-typable
IH = institution herd
CH = commercial herd

(Table 4). When these same strains were typed with bovine bacteriophages they could be grouped into 7 different patterns plus the group of non-typables. Only one of the cultures isolated (#1546) was non-typable by both groups of phages.

Allison (1) has reported that in 47 enterotoxin food-poisoning outbreaks in various parts of the world approximately 81 per cent of the strains belonged to phage type 6/47 or 42D. He further indicated that 42D is commonly found in raw cow's milk and is a frequent cause of bovine mastitis. Martyn (14), Saint-Martin, *et al.* (17) and Drysdale (9) also implicated staphylococci of phage type 6/47 in food poisoning outbreaks. In the 21 strains typed 11 were lysed by one, two or all three of these bacteriophages. Two strains were typable only by the 42D phage. MacDonald (15) reported that of 150 strains of *Staph. aureus* isolated from milk, 123 were type 42D and that of 34 strains isolated from cases of bovine mastitis all were type 42D.

Blair (4) has reported that there is a considerable degree of group specificity: for example cultures that are lysed by the phages of Group II are not susceptible to lysis by phages of Group I or III. He states that occasionally strains are encountered that are lysed by phages of both Groups I and III but represent only a small proportion of the typable staphylococci. In this study at least 5 of the 21 strains typed were lysed by phages from 2 or more of the groups established by Blair.

When the results of Tables 4 and 5 are compared it is readily observed that there is no correlation between the human staphylococcal bacteriophage and bovine staphylococcal bacteriophage patterns; however it is the opinion of the authors that the patterns presented by the bovine bacteriophages are of greater epidemiological significance in this poisoning outbreak.

Chapman (6), Feldman (11), and Evans *et al.* (10) have indicated that there is now general agreement that only coagulase positive staphylococci are enerotoxigenic; however, Dolman (8) and Dack (7) have stated that not all coagulase positive staphylococci are capable of producing enerotoxin. One of the cultures (#1310) was selected for use in a kitten feeding experiment. This culture was isolated from a remaining portion of one of the wheels (not identifiable by lot) of cheese that was used for serving the Sunday evening meal. No experiments were performed by intraperitoneal injections of culture filtrates. As indicated previously 2 out of 3 kittens developed diarrhea and explosive vomiting approximately five hours after feeding. No human volunteers were fed either culture filtrates or cheddar cheese suspected of containing enterotoxin.

Bell and Veliz (3) reported that 67% of 37 cultures of staphylococci from 37 quarters of 27 cows were enterotoxigenic as demonstrated by the kitten test. Minett (16) tested 38 strains of *Staph. aureus* from bovine udders. Of 15 strains from udders with mastitis and 23 strains from milk of 17 normal cows, 9 and 7 strains respectively produced enterotoxin.

SUMMARY

A food-poisoning outbreak involving two hundred cases was traced to the consumption of contaminated cheddar chese. One or more samples from each lot of the unused cheese (from about 7½ tons) was examined in the laboratory. Eighty-seven per cent of the cheese in storage was found to contain *beta* hemolytic coagulase-positive *Staphylococcus aureus*. One strain tested for enterotoxin production caused vomiting and diarrhea when fed to kittens.

Culture isolates were typed by both human and bovine staphylococcal bacteriophages. The comparison of these patterns is discussed.

Staphylococcus aureus was found in raw milk from herds serving as a source of milk from the cheese factory.

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COMMENTS ON A NEW TEST FOR PENICILLIN IN MILK

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In connection with the current campaign for the elimination of penicillin from milk supplies, Arret and Kirschbaum (2) have described "a simplified and rapid method for detecting the presence of penicillin in milk in concentrations as low as 0.05 units per ml." Their method differs from the modified Difco method (*1*) principally in holding poured plates of seeded agar at approximately 15°C. (59°F.) for not less than three, or more than five, days. This modification is said to enable detection of penicillin in 2½ hours at 37°C.

To compare the new method with the "standard" procedure, several trials were made using both the Bacto B453 Standardized Spore Suspension of *Bacillus subtilis*, and of a spore suspension of *B. subtilis* ATCC #6633. Unfortunately, in each trial, after 3 days at 15°C., growth of the test organism was so extensive that no zones of inhibition could be expected when discs saturated with milk containing penicillin were "spotted" thereon and the plates incubated at 37°C. With two-day-old plates growth was not evident at the start, but no zone of inhibition appeared even with milk containing 0.1 unit of penicillin per ml. *Thus there is a danger that negative results will be reported from milk containing amounts of penicillin detectable by other methods.*

In the writer's experience, the method described by Arret and Kirschbaum is also less simple, less reliable and less sensitive than the modified Difco method available for years. *It is less simple* in requiring (*a*) preparation of a spore suspension, when one is commercially available, (*b*) a low temperature incubator set at 15°C, and (*c*) an incubator at 37°C. (Neither of these two temperatures is usually available in dairy plant laboratories).

It is less reliable in that (*a*) in our hands the method, *as described*, failed to detect penicillin, (*b*) there is more likelihood of variation in sensitivity to penicillin with "home-made" spore suspension than with the commercially available ones, and (*c*) there is no warning that special flat-bottomed petri plates (Corning #3162) should be used, and special care taken to harden the agar layer on a perfectly level surface. *It is less sensitive* in that (*a*) it specifies the use of a 0.25″ disc, whereas the 0.5″ disc absorbs six times the volume of milk and will detect roughly one-fifth the concentration of antibiotic detectable by the smaller disc (*4*); the larger disc is also much easier to load uniformly by capillary absorption of 0.1 ml from a graduated 1 ml pipette held horizontally, and (*b*) it calls for the use of 10 ml of agar medium per plate, when the greater sensitivity of a thinner layer has been shown (*4*) and is generally recognized.

Speed in obtaining results in testing milk for antibiotics is desirable. However, no laboratory test will ever be as useful as a "marker" dye incorporated into the antibiotic preparation (*3*, *5*) which would