An Outbreak of Gastroenteritis Caused by *Salmonella indiana*

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THE APPARENT INCREASE of salmonellosis can be attributed in part to an increasing awareness of these organisms resulting from more extensive surveillance and the development of improved culture and plating media, biochemical differentiation techniques, and methods of isolating salmonellae from clinical and nonclinical material.

Salmonella indiana is an infrequently occurring serotype, isolated mostly from poultry in recent years, which has occasionally been the etiological agent in sporadic outbreaks of human intestinal disease. The serotype was first described in 1955 when it was isolated from a 9-month-old girl, ill with vomiting, diarrhea, and fever at the St. Vincent's Hospital in Indianapolis, Ind. This serotype was antigenically characterized by Hajna and associates (*1*). With few exceptions, its occurrence in the United States has been in States east of the Mississippi River. The National Animal Disease Laboratory in Ames, Iowa, reported a total of 50 isolations of *S. indiana* from 1957 to June 30, 1963, from 28 turkeys, 20 chickens, 1 duck, and 1 swine (*2*).

S. indiana was isolated five times in Canada in 1960 from poultry which all originated from the same farm in Ontario (*3*). There have been no other reports in the literature of this serotype being isolated as late as the end of 1964. In Germany, where this serotype had not been previously isolated, it was found as a contaminant of chicken feed containing imported fishmeal in 1957 (*4*).

One outbreak of enteric disease caused by *S. indiana* is reported here. The organism was isolated from the stools of patients and from unpasteurized cup cheese which they had eaten.

Cup cheese is a skimmed milk, soft curd cheese product that is produced for a limited population in Pennsylvania. There were two commercial manufacturers of this product in the area where the outbreak occurred. Their plants were located in adjoining counties in southeastern Pennsylvania, and both manufacturers received their raw material, soft curd, from the same dairy farm.

Materials and Methods

All food and environmental specimens were subjected to the lactose pre-enrichment procedures of North (*5*) from which aliquots were taken for selective enrichment in tetrathionate-brilliant green broth and selenite-cystine broth. Six ml. of a 10 percent solution of Tergitol No. 7 (sodium heptadecyl sulfate, Union Carbide and Carbon Corp.) was added to the lactose pre-enrichment broth culture of cup cheese in order to allow for maximal specimen dispersion, emulsification, and media contact.

Plating media of brilliant green-sulfa agar, bismuth sulfite agar, MacConkey agar, and *Salmonella-Shigella* agar were streaked after pre-enrichment and selective enrichment and

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were incubated 37° C. for their optimal periods of incubation—brilliant green sulfa and MacConkey for 24 hours and bismuth sulfite and *Salmonella-Shigella* for 48 hours. Stool specimens were streaked directly onto MacConkey, bismuth sulfite, and *Salmonella-Shigella* plates or enriched with tetrathionate-brilliant green selective broth for 24 hours then streaked into bismuth sulfite and *Salmonella-Shigella* agar plates. The plates were then examined for suspicious colonies and these colonies were transferred to differential tube media (triple sugar iron agar and sulfide-indole-motility) and processed for confirmation according to the biochemical and serologic techniques as outlined by Edwards and Ewing (*6*).

Results

Cultures made from a sample of cup cheese submitted by a nurse were found contaminated with *S. indiana*. Three members of the nurse's family had eaten the dairy product and become ill, and the organism was isolated from their stool specimens. Two members of the family who had not eaten the cup cheese were asymptomatic, and the organism was isolated from their stools 5 weeks after the onset of illness in the other members. It is clear that cross-infection occurred (table 1).

During the previous year 11 patients of one pediatrician, all unrelated, experienced gastrointestinal disease. Cultures of stools taken from these patients during the acute phase of the illness demonstrated the presence of *S. indiana*. The organism persisted in the stools of these patients from 2 to 8 months following onset of disease.

The role of the cup cheese was inapparent at the onset of disease. After the association of the cheese and gastrointestinal disease was established in a much later outbreak, reinvestigation showed that 10 of the 11 patients had been given morsels of cup cheese by adults in the family. It is not known how the remaining child was infected, although it is known that a grandmother brought the product into the home (table 2). This reinvestigation proved that in each case in which cup cheese was consumed, *S. indiana* was obtained from the patient's diarrheal stools. In the other cases of illness, person-to-person contact was the mode of transmission.

In only one instance were two members of the same family culture positive for *S. indiana*. A 24-year-old father of one patient, who had diarrhea previous to the child's illness, had the organism in his stool during his convalescent period. All infected children were the youngest members of their respective families.

The State department of agriculture submitted cultures from 10 butter samples, 4 soft curd samples, 9 milk samples, 13 milk can rinsings, and 5 water samples to the State laboratory for confirmation. A total of 40 fecal specimens from dairy employees and the families of neighboring farmers that supplied the dairy with milk were also submitted. *S. indiana* was isolated from the butter and soft curd produced at the creamery of the dairy farm. Samples of the water supply and stool specimens of the employees were negative for *Salmonella*.

Two local manufacturers purchased the soft curd from the dairy and made cup cheese for the retail market. One manufacturer "flash" pasteurized his product; the other did not. Only in the unpasteurized product were we able to isolate *S. indiana*.

Well water samples from 37 neighboring and adjacent farms were negative for *Salmonella* with one notable exception. *S. indiana* was isolated from one well which had a most probable

Table 1. Family exposed to cup cheese

Patient	Age (years)	Cup cheese ingested	Clinically ill	*S. indiana* isolated	Time organism persisted in stool after onset of illness
Husband	43	Yes	Yes	Yes	6 weeks.
Daughter	16	Yes	Yes	Yes	1 isolation at 6 weeks.
Daughter	14	No	No	Yes	1 isolation at 5 weeks.
Son	10	Yes	Yes	Yes	1 isolation at 6 weeks.
Son	5	No	No	Yes	Isolation beginning at 5th week, continuing for 3 months.

Table 2. *Salmonella indiana* isolated from 10 [1] unrelated patients of a pediatrician

Patient	Age	Ingestion of cup cheese	Clinically ill	Hospitalized	Other family members ill	Duration of time organism persisted in stool (months)
B.B	2 years	Yes	Yes	Yes	No	2.
D.M	3 months	Not known	Yes	No	No	8.
V.L	1 year	Yes	Yes	No	Father	1.
B.S	5 weeks	Yes	Yes	No	No	2.
K.O	2 years	Yes	Yes	Yes	No	2.
I.M	1 year	Yes	Yes	No	No	2.
M.P	2 years	Yes	Yes	No	No	1.
M.S	2 months	Yes	Yes	Yes	No	More than 8.
T.R	1 year	Yes	Yes	No	No	No followup cultures.
D.B	9 years	Yes	Yes	No	No	More than 2.

[1] The 11th patient had not eaten cup cheese; the means of contamination could not be established.

number (MPN) of less than 2.2 coliform organisms per 100 ml. of sample. Of the wells that were negative for *S. indiana*, 22 had an MPN of less than 2.2 per 100 ml. of sample, 2 had an MPN of 8.8, 1 an MPN of 15, 1 an MPN of 27, 3 an MPN of 38, and 7 an MPN of 240 or more.

It is significant that the *Salmonella* organisms were recovered only from the brilliant green-sulfa agar plates streaked from the lactose pre-enrichment broth of this sample, and none were recovered from either pre-enrichment or selective enrichment broth cultures of the other 37 water samples examined.

Discussion

Salmonellosis is a continuing public health problem. It has become apparent to the laboratory worker that the transmission of enteric disease is accomplished not only through person-to-person contact but also by the eating of contaminated food products, ingestion of certain medications and drugs, and handling of fomites. The third method is of particular danger to children. In April 1966, Price reported the isolation of *Salmonella give* from stuffed natural poultry toys (*7*).

In this instance a large segment of the population was exposed to salmonellosis by unpasteurized cup cheese. It is important to note that had not an alert physician requested cultures of stools from his clinically ill patients, the etiological agent would not have been determined. Of equal importance was the submission of cup cheese for bacteriological examination by a nurse whose family had become ill. These seemingly unrelated events led to the resolution of a significant epidemiologic problem. The source of infection was promptly eliminated.

In the bacteriological examination of well water samples from farms adjacent to the producer's creamery, *S. indiana* was isolated from an unchlorinated well. The MPN for coliform was less than 2.2 per 100 ml. of sample. This isolation closely parallels that of the waterborne outbreak of *Salmonella typhimurium* at Riverside, Calif., with approximately 18,000 cases of illness (*8, 9*). In each instance the index of fecal contamination, the MPN, for the water supplies was acceptable according to the present national drinking water standards, and yet *Salmonella* organisms were isolated from both sources.

As is suggested by this outbreak and those reported by other workers, our present system of examining water samples for coliform organisms alone as the index of the sanitary quality of a water specimen is not adequate. We must begin to sample specifically for enteric pathogens as well. This step will necessitate increasing the volume of water sampled from 100 ml. to at least 1,000 ml. (*10*). The method of gauze swab sampling devised by Moore (*11*), or its modification (*12–15*), can be used to greater advantage in sampling even larger volumes of water. At best the present system provides instant small grab samples for study. With the Moore method larger volumes of water come in contact with the gauze swab, and it is possible for larger numbers of micro-organisms to be-

come enmeshed in the swab itself. The efficiency of the method for viral, as well as bacterial, isolations from water has been well established (*16–19*).

Summary

Salmonella indiana was isolated from stools of patients clinically ill with intestinal disease and from unpasteurized cup cheese made by a dairy product manufacturer in southeastern Pennsylvania. Efforts to recover the organism from stools of employees at the dairy farm supplying the manufacturer with soft curd and from the water supply were not successful, although the organism was present in an adjacent water supply.

The study indicates the need for greater surveillance of the preparation of food for public consumption, improved communication between all those concerned with health and potential health problems, and a greater awareness of the lesser known serotypes of *Salmonella* which do, indeed, cause intestinal disease of epidemic proportions.

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