Salmonella infantis Isolated From Ham in Food Poisoning Incident

ROBERT ANGELOTTI, Ph.D., GEORGE C. BAILEY, M.S., MILTON J. FOTER, Ph.D., and KEITH H. LEWIS, Ph.D.

AN OUTBREAK of gastroenteritis occurred among eight members of a family in Cincinnati, Ohio, in June 1960. Epidemiologic investigation by the Hamilton County Board of Health revealed that a 5½ pound portion of smoked ham was purchased from a supermarket about 12 noon, June 17. The ham was taken home and refrigerated at approximately 60° F. Between 1 p.m. and 6 p.m. of the same afternoon, the eight persons ate either raw or fried slices of ham. Peas, cream-style corn, bread, coffee, and milk were available, but the only food eaten in common was ham. Within 8 to 23 hours after ingestion, the members of the family suffered some symptoms of gastroenteritis characterized by vomiting, diarrhea, fever, and prostration. All eight members experienced acute diarrhea, but hospitalization was not required. A physician was called and the Hamilton County Board of Health was notified of the outbreak.

The uneaten portion of the ham was obtained by the board of health for bacteriological analysis. A sanitarian was sent to the supermarket to report on its general sanitation level and to gather any additional epidemiologic information related to the outbreak. He reported that the meat storage box in the supermarket was dirty. It was operating above 50° F. and was overloaded. A few of the hams were in contact with hot electric light bulbs, causing them to drip melted fat.

Although food-poisoning outbreaks are not investigated routinely by the milk and food research laboratory of the Public Health Service's Sanitary Engineering Center, the epidemiologic evidence in this instance presented an interesting research opportunity; therefore, the invitation to participate was accepted.

We have observed that the routine laboratory examination of foods incriminated in outbreaks of gastroenteritis is often inadequate. Generally, these examinations are perfunctory because the laboratory workers may be unfamiliar with the necessary procedures for performing a critical microbiological analysis or they may be unaware of the significance of the numbers as well as the types of organisms present. Therefore, the following report of our investigation is presented in detail.

Bacteriological Analysis

A portion of the raw ham was examined in the laboratory 2 days after the outbreak occurred. Thirty grams of the ham was placed in 270 ml. of sterile, phosphate buffered dilution water (*1*) and blended for 2 minutes at slow speed in a Waring Blendor cup. The resulting 1:10 dilution was examined microscopically by Gram's stain and the blend analyzed as follows.

Total aerobic count. Appropriate tenfold dilutions were prepared and plated in duplicate in Bacto-plate count agar, and the plates were incubated 48 hours at 35° C.

Dr. Angelotti is chief of the Food Microbiology Section, Dr. Foter is assistant chief, and Dr. Lewis is chief, Milk and Food Research, Robert A. Taft Sanitary Engineering Center, Public Health Service. Mr. Bailey is sanitarian supervisor, Hamilton County Board of Health, Cincinnati, Ohio.

Coliform count. Decimal dilutions were plated in duplicate in Bacto-violet red bile agar and incubated 24 hours at 35° C. Dark red colonies, at least 0.5 mm. in diameter, were counted and confirmed by inoculating into tubes of brilliant green lactose bile broth which were incubated 24 hours at 35° C.

Fecal streptococci. Decimal dilutions were plated in duplicate in Bacto–KF streptococcus agar (*2*) and incubated 48 hours at 35° C. In addition to the plate-count method, an MPN determination was performed by transferring 1.0-ml. portions from each selected dilution of sample into five tubes of azide dextrose broth and incubating 24 and 48 hours at 35° C. Confirmation was made by transferring a loopful of broth from each positive tube in the last three positive dilutions to a tube of ethyl violet azide broth and incubating at 35° C. for 24 and 48 hours and observing for turbidity (*3*).

Staphylococcal count. The surfaces of duplicate plates of Bacto-staphylococcus 110 agar were inoculated with 0.1-ml. aliquots of each decimal dilution. The aliquots were spread evenly over the surface with a sterile, bent, glass rod. The plates were incubated 48 hours at 35° C. The ratio of pigmented to nonpigmented colonies was determined for each of the countable plates, and representative colonies of each were picked and tested for coagulase production in the same ratio.

Salmonellae count. One-tenth-milliliter aliquots of each of the decimal dilutions were spread evenly over the surface of duplicate Bacto-brilliant green agar plates containing 0.06 mg. of sulfadiazine per milliliter to suppress the growth of *Proteus*, *Pseudomonadaceae*, and coliform organisms (*4* and personal communication from M. M. Galton, 1958). The plates were incubated 24 hours at 35° C., and the number of pink colonies was recorded. In addition to the plating procedure, 1.0 gm. of ham was placed in 9.0 ml. of Hajna's tetrathionate broth (*5*) and incubated for 24 hours at 35° C. as a pre-enrichment technique. Growth from the tetrathionate broth was streaked onto duplicate plates of the brilliant green agar described above and incubated 24 hours at 35° C. Typical *Salmonella* colonies were picked from both sets of brilliant green agar plates, and the isolates were identified according to the biochemical and serologic schema of Edwards and Ewing (*6*).

Clostridia. One milliliter of the original 1:10 blend was inoculated into duplicate tubes containing 15 ml. each of Bacto-fluid thioglycollate broth which was steamed 10 minutes just prior to inoculation to drive off dissolved oxygen. The broth was incubated for 24 hours at 35° C. A loopful of growth from the thioglycollate broth was streaked onto duplicate plates of 5 percent rabbit blood agar and McClung-Toabe egg yolk agar (*7*). Both sets of plates were incubated 24 hours at 35° C. in a Case Anaero Jar (*A*), under an atmosphere of 90 percent nitrogen and 10 percent carbon dioxide (*B*). Following incubation, the blood plates were observed for hemolytic colonies (both incomplete and complete hemolysis) and the egg plates for lecithinase-producing colonies.

Production and Assay of Enterotoxin

This laboratory has evolved over the years a fairly reliable assay for testing the enterotoxicity of staphylococcal culture filtrates based on the cat test. This assay requires the injection of 5.0 ml. of culture filtrate into the saphenous veins of four cats (4 to 5 pounds each). Cultures are considered positive for enterotoxin when they elicit vomiting in at least three of the four cats, between 20 minutes and 3 hours after injection. This procedure consistently yields positive results with filtrates of *Staphylococcus aureus*, 196E and S6, known as enterotoxigenic strains. A modification of Casman's (*8*) medium is used to produce toxic filtrates because the injection of unaltered medium sometimes results in the death of cats. The modified medium is composed of K_2HPO_4, 0.05 percent; KH_2PO_4, 0.05 percent; $MgSO_4 \cdot 7H_2O$, 0.01 percent; l-cystine, 0.0025 percent; sodium acetate, 0.35 percent; l-tryptophane, 0.0075 percent; casamino acids (Bacto), 1.0 percent, and the following concentrations per liter of Ca pantothenate, 500 micrograms; thiamin HCl, 40 micrograms; nicotinic acid, 1,200 micrograms. The medium is adjusted to pH 7.2 to 7.4 with 0.1 N NaOH and sterilized at 121° C. for 15 minutes.

Toxic filtrates produced from the modified

Types and numbers of bacteria isolated from raw ham incriminated in an outbreak of gastroenteritis

Type of determination	Medium	Number of organisms per gram of ham
Total aerobic plate count.	Plate count agar	268, 000, 000
Coliform bacteria	Violet red bile agar. Brilliant green lactose bile broth.	15, 000
Fecal streptococci	KF streptococcus medium.	31, 000, 000
Fecal streptococci MPN.	Azide dextrose broth. Ethyl violet azide broth.	31, 000, 000
Staphylococci [1]	Staphylococcus 110 agar.	200, 000, 000
Salmonellae [2]	Brilliant green agar. Tetrathionate broth.	23, 000
Clostridia	Blood agar plates. Egg yolk plates.	0

[1] An eight-to-one ratio of white to yellow colonies observed; neither type was coagulase-positive.
[2] Identified serologically as *Salmonella infantis*.

medium are obtained from shake cultures grown in an atmosphere in which 30 percent of the air is replaced with CO_2 as follows: 2 ml. of a 24-hour staphylococcal nutrient broth culture is added to 25 ml. of medium in a 125-ml. Erlenmeyer flask. This flask is partially evacuated, and 30 percent of the original volume of air is replaced with CO_2. The flask is then incubated for 24 hours at 35° C. on a reciprocating shaker operating at 120 strokes per minute. After incubation, 5.0 ml. of this culture is used to inoculate a second 125-ml. Erlenmeyer flask containing 25 ml. of medium, which is gassed as described above and incubated for 6 hours at 35° C. on the shaker. After 6 hours' incubation, 3.0 ml. of culture from the second flask is added to each 100 ml. of medium in a 500-ml. Erlenmeyer flask. The final flasks are similarly gassed and incubated at 35° C. on a reciprocating shaker (100 strokes per minute) for 4 days. It is possible to produce 1,200 ml. of toxic culture broth in a 6-liter Erlenmeyer flask.

Following the final incubation, the cultures are centrifuged at 3,000 rpm for 20 minutes to remove the cells. The supernate is passed through a Seitz filter, collected, and heated for 30 minutes in a boiling water bath. After cooling, the filtrates are injected into cats as previously described. The staphylococci recovered from the ham were tested for enterotoxin production by the above procedures.

Results

The results shown in the table summarize the bacteriological data obtained from the sample of ham. Though the total bacteria count was exceedingly high it was composed mostly of staphylococci and fecal streptococci. The isolated staphylococci, both pigmented and nonpigmented, were tested for coagulase production with negative results. Both types of colonies were cultured in modified Casman's medium and tested for enterotoxin production by the cat test with similarly negative results.

Only one streptococcal species, *Streptococcus faecalis*, and only one *Salmonella*, *Salmonella infantis*, were isolated from the ham.

These data indicated that the organisms present in significant quantities in the ham were the staphylococci, enterococci, and salmonellae. Little significance was attached to the staphylococci because none of 18 colonies tested (8 white and 1 yellow per countable plate) yielded positive coagulase reactions. In addition, the separate testing of mixtures of yellow colonies and mixtures of white colonies for enterotoxin yielded negative results. Because of the large number of enterococci present, and the well-known pathogenicity of the *Salmonella*, these organisms were viewed with suspicion. Stool specimens were requested for examination, to help clarify the situation, but they were not available until 10 days after the outbreak. Of the eight persons affected, specimens were obtained only from the mother and father and two sons of grade school age living at home. The stools were examined for salmonellae and enterococci, employing the procedure described. Only one streptococcal species, *S. faecalis*, was isolated from the stools of both parents and one son. *S. faecalis* var. *liquefaciens* was the only one of the streptococci isolated from the stools of the other son. The number of enterococci in the feces ranged from 7 million to 35 million per

gram. Salmonellae were not recovered from the stools of the two boys; however, *S. infantis* was isolated from the feces of both parents.

Discussion

Though available evidence indicates that *S. faecalis* and its related varieties are associated with food poisoning, their role as etiological agents of gastroenteritis is not universally accepted. However, when they have been incriminated they have usually represented the predominant flora, with other types of food-poisoning organisms present in small numbers or undetected. In view of the inconclusive results obtained from feeding experiments conducted by various investigators (*9–13*), their real role is unknown. Nevertheless, until additional information is provided which unequivocally demonstrates whether this group of organisms is pathogenic or not, the presence of large numbers of alpha-type streptococci in foods incriminated in gastroenteritis outbreaks cannot be ignored.

The occurrence of salmonellae in processed poultry has been established by Galton and others (*14*), and surveys conducted by members of the Communicable Disease Center, Public Health Service (*15*), have shown the association of *S. infantis* with processed poultry. However, the occurrence of salmonellae in other meats is low (*4*, *16*, *17*), and in cured meats practically nonexistent. In a recent survey conducted at the Sanitary Engineering Center, none of 314 cured or prepared meat products yielded salmonellae (*18*). Of the salmonellosis outbreaks reported in the United States from 1956 through 1959, none was shown to be transmitted through ham (*19*). Thus, the occurrence of salmonellae in cured meats, particularly ham, apparently is a rarity in this country. For this reason, the isolation of *S. infantis* from the ham in this outbreak is noteworthy.

Though the oral infective dose for humans is unknown for *S. infantis*, an estimate is obtained from the data of McCullough and Eisele (*20*). They found that the oral infective dose for several species of salmonellae ranged from a low of 125,000 for *S. bareilly* to a high of more than 1 billion for *S. pullorum*. The *S. infantis* concentration in the ham in this outbreak was estimated as 23,000 per gram. Assuming a 50- to 100-gram weight per slice of ham, the ingested number of salmonellae approach 1 to 2 million per slice. Two persons consumed three ham sandwiches each. Such concentrations conceivably approach oral infective dosages. The number of *S. infantis* recovered from the ham, the prolonged incubation period, and the isolation of this organism from the stools of some of the patients is evidence for establishing a *Salmonella* etiology in this instance. Because the ham was in the home for less than 1 hour before a portion of it was eaten and because of the significant number of *Salmonella* it contained, the ham was assumed to be the original source of infection to the family. However, no attempt was made to establish the existence of a carrier in the family or to establish the possible origin of contamination for the ham.

The presence of enterococci and salmonellae together in the ham presents the possibility of a mixed infection. Though the weight of evidence incriminates the *Salmonella*, the possibility of a mixed infection or an enterococcal etiology cannot be excluded.

This outbreak lends further support to our theory, derived from observation of previous reported outbreaks in which implicated foods contained large numbers of enterococci and relatively small numbers of other pathogenic bacteria, that associative growth between certain enterococci and other organisms may possibly contribute to the development of typical food-poisoning symptoms. For example, investigation of an outbreak aboard an interstate common carrier revealed large numbers of enterococci and some *Clostridium perfringens* in the implicated turkey dressing (*21*).

Evidence is not currently available to weigh the significance of associative action in enterococcal outbreaks. However, if such a condition is necessary, it may account for the failure of many pure cultures of enterococci to cause illness when fed to volunteers in past investigations.

Often, in the performance of a laboratory analysis of foods incriminated in food-poisoning outbreaks, only the staphylococci are sought and merely the presence or absence of coagulase-positive types are reported. We should

like to stress again the importance of the number of organisms present, as well as the type, in a suspected food. Coagulase-positive staphylococci are not uncommon in perishable foods, and the reporting of their presence with no reference to numbers per gram is insufficient evidence to establish the etiology of an outbreak. The incubation periods of staphylococcal, enterococcal, and clostridial types of food poisoning are so similar and overlapping that their use for determining the type of organism to be sought in the food may lead to erroneous conclusions.

The latest figures from the National Office of Vital Statistics of the Public Health Service reveal that approximately half of all the reported food-poisoning outbreaks are classified as "unknown etiology." This situation may be remedied by more intensive analysis of the foods involved. Examination of foods implicated in outbreaks of gastroenteritis should include detection and enumeration for enterococci and clostridia. However, the presence of these organisms in food is not uncommon, and, as with the staphylococci, an estimate of the number present is necessary before their role can be assessed. Instances in which large numbers of these organisms are found, or in which they constitute the predominant flora, may be considered significant. Though salmonellae normally are present in small numbers in some raw foods, their presence in cooked foods or foods which receive heat treatment sufficient to destroy them is particularly noteworthy. However, here again an attempt should be made to enumerate the salmonellae in order to determine the numbers which are associated with cases.

Summary

Investigation of an outbreak of gastroenteritis affecting eight members of a family revealed that the only food eaten in common was ham. Bacteriological examination of the ham disclosed that the organisms of significance present in large numbers were *Streptococcus faecalis* and *Salmonella infantis*.

Stool specimens of the persons affected contained large numbers of *S. faecalis*. Stools of two of the patients were positive for *S. infantis*.

The contamination of ham with *S. infantis* is noted because of its rarity, and the data available indicate the outbreak was due to either a mixed infection of enterococci and salmonellae or to *S. infantis* alone.

EQUIPMENT REFERENCES

(*A*) Bacteriological culture media. Case Laboratories, Inc., 515 N. Halstead Street, Chicago 22, Ill.

(*B*) Gasses in mixture form. Matheson Company, Inc., P.O. Box 966, Joliet, Ill.

REFERENCES

(*1*) American Public Health Association: Standard methods for the examination of dairy products. Ed. 10. New York City, 1960, p. 67.

(*2*) Kenner, B. A., Clark, H. F., and Kabler, P. W.: Fecal streptococci. II. Quantitation of streptococci in feces. Am. J. Pub. Health 50: 1553–1559 (1960).

(*3*) American Public Health Association: Recommended methods for the microbiological examination of foods. New York City, 1958, p. 139.

(*4*) Galton, M. M., Lowery, W. D., and Hardy, A. V.: *Salmonella* in fresh and smoked pork sausage. J. Infect. Dis. 95: 232–235 (1954).

(*5*) Hajna, A. A., and Damon, S. R.: New enrichment and plating media for the isolation of *Salmonella* and *Shigella* organisms. Applied Microbiol. 4: 341–345 (1956).

(*6*) Edwards, P. R., and Ewing, W. H.: Identification of the *Enterobacteriaceae*. Minneapolis, Burgess Publishing Co., 1955.

(*7*) McClung, L. S., and Toabe, R.: The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. J. Bact. 53: 139–147 (1947).

(*8*) Casman, E. P.: Serologic studies of staphylococcal enterotoxin. Pub. Health Rep. 73: 599–609, July 1958.

(*9*) Cary, W. E., Dack, G. M., and Myers, E.: An institutional outbreak of food poisoning possibly due to a streptococcus. Proc. Soc. Exper. Biol. & Med. 29: 214 (1931).

(*10*) Cary, W. E., Dack, G. M., and Davison, E.: Alpha type streptococci in food poisoning. J. Infect. Dis. 62: 88 (1938).

(*11*) Dolman, C. E.: Bacterial food poisoning. Canad. J. Pub. Health 34: 205 (1943).

(*12*) Osler, A. G., Buchbinder, L., and Steffen, G. I.: Experimental enterococcal food poisoning in man. Proc. Soc. Exper. Biol. & Med. 67: 456 (1948).

(*13*) Dack, G. M., Niven, C. F., Kirsner, J. B., and Marshall, H.: Feeding tests on human volunteers with enterococci and tyramine. J. Infect. Dis. 85: 131 (1949).

(*14*) Galton, M. M., et al.: Salmonellosis in poultry and poultry processing plants in Florida. Am. J. Vet. Res. 16: 132–137 (1955).
(*15*) U.S. Communicable Disease Center: Veterinary Public Health Newsletter, August 1960, pp. 5–6.
(*16*) Cherry, W. B., Scherago, M., and Weaver, R. H.: The occurrence of *Salmonella* in retail meat products. Am. J. Hyg. 37: 211–215 (1943).
(*17*) Felsenfeld, O., Young, V. M., and Yashimura, T.: A survey of *Salmonella* organisms in market meat, eggs, and milk. J. Am. Vet. M. A. 116: 17–21 (1950).
(*18*) Wilson, E., Paffenbarger, R. S., Jr., Foter, M. J., and Lewis, K. H.: Prevalence of salmonellae in meat and poultry products. J. Infect. Dis. To be published.
(*19*) U.S. National Office of Vital Statistics: Morbidity and Mortality Weekly Report, January 1956 through December 1959.
(*20*) McCullough, N. B., and Eisele, C. W.: Experimental human salmonellosis. I. Pathogenicity of strains of *Salmonella meleagridis* and *Salmonella anatum* obtained from spray-dried whole egg. J. Infect. Dis. 88: 278–289 (1951); III. Pathogenicity of strains of *Salmonella newport*, *Salmonella derby*, and *Salmonella bareilly* obtained from spray-dried whole egg. J. Infect. Dis. 89: 209–213 (1951); IV. Pathogenicity of strains of *Salmonella pullorum* obtained from spray-dried whole egg. J. Infect. Dis. 89: 259–265 (1951).
(*21*) Hart, C. J., Sherwood, W. W., and Wilson, E.: A food poisoning outbreak aboard a common carrier. Pub. Health Rep. 75: 527–531, June 1960.

British Editor Prescribes Vigorous Writing

American physicians should write in the same vigorous language they use in speaking, advises Dr. T. F. Fox, editor of *The Lancet*, in an interview reported in *Medical World News*, March 31, 1961. "What they say aloud is usually concise and effective . . . yet when the American takes pen in hand, he all too often seems constrained to use a sort of scientific literary language which conceals sense instead of displaying it," says Dr. Fox.

"In my opinion, the writer is not writing as himself—as a human being, as a person—but in the pretentious way he thinks fitting for an author. . . .

"I believe that real harm has been done to medical practice by teaching doctors to think in terms detached from people. It is this that leads to Mrs. Smith becoming, in the doctor's mind, the case of splenomegaly in bed 61—at which point the human relationship is broken.

"In *The Lancet* we prefer that the facts not be disguised—that patients be called patients, not subjects, or material, or a series. And if people have to die, I'm against efforts to escape reality with phrases such as 'proceeded to a fatal termination'. . . .

"We should base our writing on our speech. Language is primarily something spoken, and only the highly expert should venture far from the straightforward terms in which we instruct our wives, reprove our children, and tell the baker how many loaves to deliver."