Journal of Food Protection, Vol. 52, No. 1, Pages 51-54 (January 1989)

An International Outbreak of *Salmonella Nima* from Imported Chocolate

J. C. HOCKIN[1], J.-Y. D'AOUST[2*], D. BOWERING[1], J. H. JESSOP[3], B. KHANNA[3], H. LIOR[1], and M. E. MILLING[4]

[1]Laboratory Centre for Disease Control, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2; [2]Food Directorate, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, Canada K1A OL2; [3]Division of Laboratories, British Columbia Department of Health, Vancouver, B.C. V6J 4M3; [4]Field Operations Directorate, Health Protection Branch, Health and Welfare Canada, Burnaby, B.C. V5G 4P2

(Received for publication July 15, 1988)

ABSTRACT

Twenty-nine confirmed cases of *Salmonella nima* enterocolitis in Canada and four cases in the United States were traced to gold-foil wrapped chocolate coins from Belgium. Illness in Canadian cases occurred between September 1985 and October 1986 where the primary case in each of 24 affected families was a child ≤ 14 years of age. A product recall was issued on October 3, 1986 in Canada. Quantitative analysis of four composite samples of suspect chocolate by the most probable number (MPN) technique showed levels of 4.3 to 24.0 *S. nima* per 100 g product. These levels of contamination and consumption of approximately 25 g of chocolate by primary cases suggest that small numbers of *S. nima* precipitated clinical symptoms.

Salmonellae are prominent human pathogens that are widely distributed in the food chain, notably raw meats and derived products (4,16,21). The microorganism was recently involved in major foodborne outbreaks linked to improperly pasteurized fluid milk *(17)*, Cheddar cheese *(9)* and contaminated chocolate bars *(11)*. Although the propensity for foodborne disease is commonly associated with improper handling of food in the home and in food-service establishments *(3,19)*, several reports have shown that faulty processing in commercial food plants can lead to defective lots with serious socioeconomic repercussions *(8,9,17,22)*.

The first human isolate of *S. nima* ever recorded in Canada was isolated from a toddler by the British Columbia Public Health Laboratory on December 16, 1985 *(14)*. Cheddar cheese emerged as the presumptive vehicle of infection following a preliminary investigation of six families affected by *S. nima*. However, further interview of these and control families failed to associate illness with cheese consumption *(15)*. By mid-January 1986, fourteen additional cases of *S. nima* infection had been reported in four Canadian provinces.

This report summarizes the findings of an investigation of an international outbreak of *S. nima* that primarily affected children.

MATERIALS AND METHODS

Epidemiological investigation

A case was defined as any person from whom *S. nima* was isolated. A standard questionnaire was administered to a parent in each affected family by an inspector from the local or federal health departments. Questions were asked about family illness, travel, pets and pet food, infant foods and toys. Brand names of a variety of foods including milk, milk products, spices, chocolates and other confectioneries were also requested. For each affected family, an attempt was made to interview two neighborhood households who had not recently suffered from diarrhea.

Affected families were contacted again by telephone following identification of foil-wrapped chocolate coins as the vehicle of infection. This follow-up enquiry was meant to ascertain consumption of chocolate coins by family members, to obtain details of purchase and verify the availability of incriminated product in the home. These enquiries were conducted prior to the public announcement of a product recall on October 3, 1986.

Bacterial Analyses

Stool specimens were examined by several laboratories using similar analytical procedures and presumptive *Salmonella* cultures were confirmed serologically by the appropriate provincial serotyping centres.

Chocolate coins were examined for *Salmonella* by the regional (Vancouver) laboratory of Health and Welfare Canada using a standard cultural method *(12)*. Samples were preenriched at 35°C for 24 h in nine volumes of reconstituted nonfat dry milk solution (10% w/v) with added brilliant green. Portions (1 ml) of preenrichment cultures were selectively enriched in selenite cystine (35°C) and tetrathionate brilliant green (43°C) for 24 h. Each enrichment culture was then streaked on bismuth sulfite and brilliant green sulfa agar plates and incubated at 35°C for 24 h.

[1]Laboratory Centre for Disease Control, Ontario, Canada.
*[*2]Food Directorate, Health and Welfare Canada, Ontario, Canada*
[3]Division of Laboratories, Vancouver, B.C.
[4]Field Operations Directorate, Health and Welfare Canada, Burnaby, B.C.

Suspect colonies were screened biochemically and serologically with polyvalent and single grouping somatic antisera. *Salmonella* isolates were serotyped by the National Enteric Reference Center (Health Protection Branch). Similar cultural methods of isolation were used by the provincial (British Columbia) laboratory.

Quantitation of *S. nima* in chocolate coins was obtained by the most probable number (MPN) technique using triplicate 100, 10 and 1.0 g samples. Portions (1 ml) of each preenrichment culture were analyzed as described above except that only tetrathionate enrichment medium was used.

RESULTS

Of 29 symptomatic cases (diarrhea with or without other symptoms, and a positive stool culture), 18 (62%) were between 1 and 4 years of age and another 6 cases (21%) between 5 and 17 years. The primary case in each of the 24 affected families was a child $\leq$ 14 years of age. There were 5 confirmed and 3 non-confirmed secondary cases for a secondary attack rate of 25%. The onset of diarrhea in 17 (71%) of 24 primary case was in December 1985 or January 1986 with the earliest onset of illness in September 1985 (Fig. 1). Most cases resided in western provinces of Canada including 8 cases in British Columbia, 2 in Alberta, 4 in Saskatchewan, 7 in Manitoba and 2 in Ontario.

On August 31, 1986, a 4-year-old Vancouver area girl became ill with diarrhea and fever but recovered fully within 7 days. *S. nima* was isolated from her stools and investigation revealed that she had eaten 7 to 9 small gold-wrapped imported chocolate coins labelled "Albert 1er". Two chocolate coins recovered from the home refrigerator were found positive for *Salmonella nima* by the provincial (British Columbia) health laboratory. Results of follow-up enquiries within 19 families with onset of illness prior to August 1986 were revealing. Parents in 17 of the 19 families recalled child consumption of the incriminated product when specifically asked about home availability of gold-foil-wrapped coins sold in red, orange, or white net bags. The success of this probing investigative approach contrasts with the failure of earlier epidemiological investigations to identify chocolate as the vehicle of infection.

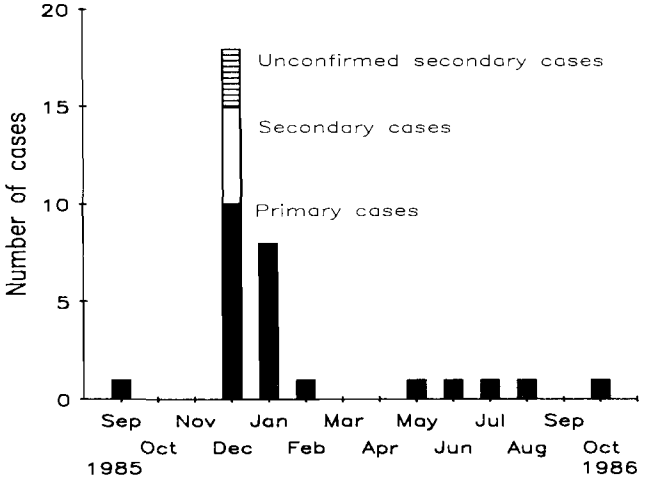

Figure 1. *Month of onset of cases of* S. nima *in Canada, 1985-1986.*

Information on the manufacture of the contaminated chocolate coins was provided by the Belgian embassy in Ottawa. The implicated chocolate molding plant purchased 5-tonne lots of molten chocolate held at 40°C to 42°C from only two suppliers. The latter firms routinely monitored the bacterial quality of their products but did not provide for *Salmonella* testing in their quality control program. In the manufacturing process, molten chocolate (40°C to 42°C) was filtered and then tempered to 26.5°C prior to injection molding. The molds were cooled for approximately 17 min to solidify the chocolate. Molded chocolates were then carried in covered plastic pails to a packaging machine which wrapped the coins in distinctive foil wrappers. The finished product was hand-packed in colored net bags.

Two Canadian importers with restricted areas of distribution East and West of Lake Superior were identified. The western distributor received 1500 kg of chocolate coins in April 1985 and an undetermined amount in April 1986. The eastern distributor imported undetermined quantities of product in December 1985. Products were not identifiable by lot number nor by date of manufacture. The Canadian shipments represented 0.6% of the total product molded by the Belgian chocolaterie in 1985. Four sizes of chocolate coins were imported: 14 g medallions with a diameter of 6.4 cm and smaller coins of 4.8, 2.9 and 2.0 g with diameters of 3.6, 3.0 and 2.3 cm, respectively. Medallions were packaged in pairs whereas mixtures of the smaller coins were sold 9 or 11 to a package with a total weight of 25 to 30 g. The foil wrappers were imprinted to resemble foreign currency.

Various chocolate products from the western distributor contained *S. nima* (Table 1). Of 44 samples of medallions tested, 13 (31%) were found to be contaminated. The three test bags of small coins imported in April 1985 and 1 (1%) of 89 bags imported in April 1986 were also positive. No contaminated samples were found on extensive testing of chocolate held by the eastern distributor. Quantitative recovery of *S. nima* from four composite samples of chocolate coins showed levels of 4.3, 7.5, 24.0 and 24.0 organisms per 100 g product.

Following the isolation of *S. nima* from products obtained at retail outlets, a voluntary product recall was announced on October 3 and completed by October 12, 1986. Identification of chocolate coins as the vehicle of infection ended a 9-month epidemiological investigation.

DISCUSSION

The single isolation of *S. nima* in Canada prior to this outbreak was obtained from a snake in 1970 *(13)*. Recovery of this microorganism in chocolate recalls earlier episodes of human salmonellosis from cocoa products contaminated

TABLE 1. Salmonella nima *from chocolate coins.*

Year imported	Importer	Product	Number of Samples Tested	Positive (%)
1985/86[1]	Western	Medallions	44	13 (31%)
1985	Western	11-coin bags	3	3 (100%)
1986	Western	9-coin bags	24	1 (4%)
1986	Western	11-coin bags	65	0
1985	Eastern	Medallions	60	0
1985	Eastern	11-coin bags	60	0

[1]The year of importation is uncertain.

with *S. napoli (11), S. eastbourne (5,8)* and *S. durham (10).* It had been established that contamination of finished chocolate products can result from the lack of separation of raw bean rooms from other processing areas, and from the non-bactericidal roasting of beans resulting in contaminated cocoa nibs and liquor *(6,8).* The propensity for *Salmonella* survival in foods of low water activity (a_w) is well documented *(6).* Ability of salmonellae to persist for periods of up to 13 years in chocolate stored at ambient temperature appears to be strain-specific (1,18,20, D'Aoust, unpublished data). It was therefore not unusual in this episode that chocolate coins manufactured prior to April 1985 yielded viable *S. nima* in November 1986.

The international distribution of the incriminated chocolate coins prompted retrieval of epidemiological data from a limited number of importing countries. One case of *S. nima* infection in an eight-month child with no documented exposure to chocolate was reported in Belgium in 1986. In the same year, three infected children was registered by the Communicable Disease Surveillance Centre (London, England) with no association with chocolates. In the United States, four of eight cases of *S. nima* reported in 1986 to the Centers for Disease Control (Atlanta) gave a history of eating chocolate coins purchased in the States of Pennsylvania and Nevada similar to those described by Canadian cases. No secondary cases of infection were reported in these incidents. Further epidemiological data from the 20 other countries who imported coins from the 244 tonnes of chocolate molded by the Belgian chocolaterie in 1985 were not available. Report of a large outbreak of *S. nima* in Canada only may coincide with shipment of coins manufactured from a single contaminated tank of molten chocolate to one country. The absence of date codes on finished products complicated the investigation of the outbreak through failure to rapidly identify the number and distribution of suspect products. Our inability to distinguish dates of manufacture for the April 1985 and 1986 shipments also raises the possibility that the single positive sample from the 1986 shipment (Table 1) was misidentified by the western distributor and actually represented a product molded in 1985.

The occurrence of primary cases almost exclusively in young children not only underscores the popularity of chocolate within this segment of the population, but also suggests a greater susceptibility of this age group to infection. Low levels of *S. nima* in imported chocolate coins (4.3 to 24.0 cells/100 g) and consumption of approximately 25 g of product by primary cases are consistent with evidence that small numbers of *Salmonella* can precipitate clinical symptoms *(2,7).*

The importance of thorough questionnaires in epidemiological investigations was clearly demonstrated in the present episode. Although chocolate figured as a potential vehicle of infection in the questionnaire originally administered to parents of primary cases, it is noteworthy that mention of chocolate coins in a second interview of the same families triggered a vivid recollection of the product. The second interview also revealed that two households originally held remnants of chocolate coins which were subsequently discarded. Such test material would have been invaluable in the estimation of an infective dose. A direct investigation approach of the type used in the present outbreak should always be considered when common food associations are not apparent from histories of food consumption.

Investigation of a major outbreak of *S. nima* that primarily affected children identified foil-wrapped chocolate coins imported from Belgium as the vehicle of infection. These conclusions were based on the isolation of *S. nima* from chocolate coins obtained from the home of the index case and from a variety of retail products distributed in western Canada.

ACKNOWLEDGMENT

We wish to thank L. Shipman of the Centers for Disease Control (Atlanta U.S.A.), P.N. Sockett of the Public Health Laboratory Service (London, England) and G. Ghysels of the Salmonella Centre of the Institute of Hygiene and Epidemiology (Brussels, Belgium) for sharing information on cases of *S. nima* in their countries.

Although many individuals participated in the case investigations, the following are acknowledged for their important contributions: L. Poffenroth, R. Burke, D.M. Burgener, G. Eng, R. Wong and A. Sewell.

REFERENCES

1. Barrile, J. C., J. F. Cone, and P. G. Keeney. 1970. A study of salmonellae survival in milk chocolate. Manuf. Confectioner 50:34-39.
2. Blaser, M. J., and L. S. Newman. 1982. A review of human salmonellosis; I. Infective dose. Rev. Infect. Dis. 4:1096-1106.
3. Bryan, F. L. 1978. Factors that contribute to outbreaks of foodborne disease. J Food Prot. 41:816-827.
4. Bryan, F. L. 1980. Foodborne diseases in the United States associated with meat and poultry. J. Food Prot. 43:140-150.
5. Craven, P. C., D. C. Mackel, W. B. Baine, W. H. Barker, E. J. Gangarosa, M. Goldfield, H. Rosenfeld, R. Altman, G. Lachapelle, J. W. Davies and R. C. Swanson. 1975. International outbreak of *Salmonella eastbourne* infection traced to contaminated chocolate. Lancet 1:788-793.
6. D'Aoust, J.-Y. 1977. *Salmonella* and the chocolate industry. A Review. J. Food Prot. 40:718-727.

7. D'Aoust, J.-Y. 1985. Infective dose of *Salmonella typhimurium* in Cheddar cheese. Am. J. Epidemiol. 122: 717-720.
8. D'Aoust, J.-Y., B. J. Aris, P. Thisdele, A. Durante, N. Brisson, D. Dragon, G. Lachapelle, M. Johnston and R. Laidley. 1975. *Salmonella easthourne* outbreak associated with chocolate. Can. Inst. Food Sci. Technol. 8:181-184.
9. D'Aoust, J.-Y., D. W. Warburton and A. M. Sewell. 1985. *Salmonella typhimurium* phage-type 10 from Cheddar cheese implicated in a major Canadian foodborne outbreak. J. Food Prot. 48:1062-1066.
10. Gastrin, B., A. Kampe, K. G. Nystrom, B. Oden-Johanson, G. Wessel and B. Zetterberg. 1972. An epidemic of *Salmonella durham* caused by contaminated cocoa. Lakartidninger 69:5335-5338.
11. Gill, O. N., P. M. Sockett, C. L. R. Bartlett, M. S. B. Vaile, B. Rowe, R. J. Gilbert, C. Dulake, H. C. Murrell and S. Salmaso. 1983. Outbreak of *Salmonella napoli* infection caused by contaminated chocolate bars. Lancet 1:574-577.
12. Health Protection Branch. 1981. Microbiological examination of cocoa and chocolate. MFO-11. Health and Welfare Canada, Ottawa.
13. Kennedy, M. E. 1973. *Salmonella* isolation from snakes and other reptiles. Can. J. Comp. Med. 37:325-326.
14. Laboratory Centre for Disease Control. 1986. *Salmonella nima* 28:Y:1,5 Can. Dis. Wkly Rep. 12:9.
15. Laboratory Centre for Disease Control. 1986. *Salmonella nima* in Canada. Can. Dis. Wkly. Rep. 12:97-98.
16. Lammerding, A. M., M. M. Garcia, E. D. Mann, Y. Robinson, W. J. Forward, R. B. Truscott and F. Tittiger. 1988. Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. J. Food Prot. 51:47-52.
17. Lecos, C. 1986. Of microbes and milk: probing America's worst *Salmonella* outbreak. Dairy Food Sanit. 6:136-140.
18. Rieschel, H. and J. Schenkel. 1971. Das verbalten von mikroorganismen, speziell Salmonellen in schokoladenwaren. Alimenta 10:57-66.
19. Roberts, D. 1982. Factors contributing to outbreaks of food poisoning in England and Wales 1970-1979. J. Hyg. 89:491-498.
20. Tamminga, S. K., R. R. Beumer, E. H. Kampelmacher and F. M. van Leusden. 1977. Survival of *Salmonella easthourne* and *Salmonella typhimurium* in milk chocolate prepared with artificially contaminated milk powder. J. Hyg. 79:333-337.
21. Todd, E. C. D. 1983. Foodborne disease in Canada - a 5-year summary. J. Food. Prot. 46:650-657.
22. Todd, E. C. D. 1985. Economic loss from foodborne disease and non-illness related recalls because of mishandling by food processors. J. Food Prot. 48:621-633.

Llabrés and Rose, *con't. from p. 50*

often insufficient to remove the inhibitory agents from the sponges. Therefore, before the initiation of a microbiological surface sampling procedure, sponges should be tested for inhibitory properties.

The bacteria that were most susceptible to the inhibitory properties of the sponges are all of interest to food microbiologists and the pathogens, at least, might well be sought in environmental sampling to trace sources of contamination.

The only disadvantage encountered with polyurethane sponges was the lower capacity of absorbing liquids when compared with cellulose sponges. The polyurethane sponges as used in this study were not able to absorb more than 5 ml of buffered saline. The purchase cost of the only non-inhibitory cellulose variety was five times higher than the polyurethane brand we tested.

REFERENCES

1. Favero, M. S., D. A. Gabis, and D. Vesley. 1984. Environmental monitoring procedures. p. 47-61. *In* M.L. Speck (ed.), Compendium of methods for the microbiological examination of foods. Second edition. American Public Health Association, Washington, D.C.
2. National Research Council. 1985. An evaluation of the role of microbiological criteria for foods and food ingredients. Subcommittee on Microbiological Criteria on Food Protection, Food and Nutrition Board. National Academy Press, Washington, D.C.
3. Quevedo, F., J. A. Lasta, and J. A. Dinelli. 1977. Control microbiológico de superficies con esponjas de poliuretano. Rev. lat-amer. Microbiol. 19:79-82.
4. Silliker, J. H., and D. A. Gabis. 1975. A cellulose sponge sampling tecbnique for surfaces. J. Milk Food Technol. 38:504.