Journal of Food Protection Vol. 40, No. 5, Pages 317-321 (May, 1977)
Copyright © 1977, International Association of Milk, Food, and Environmental Sanitarians

Salmonella in Commercially Produced Dried Dog Food: Possible Relationship to a Human Infection Caused by *Salmonella enteritidis* Serotype Havana

PAUL J. PACE, K. J. SILVER, and H. J. WISNIEWSKI

City of Milwaukee Health Department
841 N. Broadway, Milwaukee, Wisconsin 53202

(Received for publication September 16, 1976)

ABSTRACT

Identification of *Salmonella enteritidis* serotype Havana, isolated from a 2½-month old female, engendered efforts to trace the source of infection. The inquiry led to examination of commercially prepared dehydrated dog foods. Twenty-five samples, representing four different manufacturers plus two retail store brands, were examined. Each of 11 samples, produced by one manufacturer, contained one or more *Salmonella* serotypes. Eight of them contained *S. enteritidis* serotype Havana. Isolates of serotype Havana from the index case and her mother had antibiotic susceptibility patterns essentially identical to those of nine of 10 serotype Havana isolates recovered from one sample of dog food. The possibility that the human isolates were related to the dog food isolates could not be eliminated.

Salmonella enteritidis serotype havana had not been encountered by the Milwaukee Health Department laboratory before December, 1975. Therefore, recovery of this serotype from a stool specimen of an infant girl, at a Milwaukee hospital, triggered a series of events which led to presentation of this report.

The child, approximately 2½ months old, had developed a gastrointestinal illness December 6, 1975 and was admitted to a hospital on December 13, 1975. She recovered sufficiently to be returned to her home 2 weeks later. Follow-up cultures, prepared from the child's stooi specimens January 13, 1976 and March 8, 1976, were positive for *S. enteritidis* serotype Havana.

Upon interviewing the child's mother on December 29, 1975, it was learned that a canine household pet had become ill November 28, 1975. The dog had refused food for 6 days and was observed to be suffering from "gagging". He was treated at a veterinary clinic for "digestive disruption", a stool specimen was not cultured at that time. However, a specimen collected from the dog January 13, 1976 was found to be positive for *S. enteritidis* serotype Newington.

The child's mother, although asymptomatic, was found to have a stool culture positive for *S. enteritidis* serotype Havan on February 4, 1976; ssecond specimen was negative one month later. A 3-year-old sibling, and the chnild's father, each had stool cultures negative for *Salmonella*.

The occurrence of *Salmonella* infections among dogs may indeed provide a reservoir for transmission of disease to humans (*1,7*). The magnitude of this reservoir may be considerable; a survey conducted by Galton et al. (*4*) revealed 27.6% of 8,157 rectal swabs, collected from dogs, were positive for *Salmonella*. Dogs, on occasion, have been observed to eat carrion and garbage and to practice coprophagy. Therefore, the mechanism for transmission of *Salmonella* to dogs, and re-infection among dogs, may be present continually.

Dried dog foods were incriminated as the source of *Salmonella* infections among colonies of laboratory animals as early as 1952. (*5*). Results of a survey of commercially produced, dehydrated dog meal were published in 1955; Galton et al. (*3*) reported 26.5% of 98 samples contained *Salmonella*. Thus another source for possible transmission of *Salmonella* to dogs became known some 24 years ago.

Bacteriological examination of a portion, approximately 44 g, of commercial dried dog food, obtained January 24, 1976, from a supply at the home of the index case, yielded isolates of *S. enteriditis* serotypes Infantis and Minnesota.

Isolation of four different serotypes of *Salmonella* (from the index case and her mother, canine pet, and dog food) depressed earlier enthusiasm that identification of a relatively rare serotype (Havana) of *Salmonella* (*10*) presented an unique opportunity to trace the source of infection. However, occurrence of *Salmonella* in commercial dried dog food obtained from the home prompted a question as to whether the food became contaminated in the home or provided a vehicle for entry of *Salmonella* into the home. Therefore, various brands of dried dog food, including the brand which had been samples at the home of the index case, were purchased from retail stores in the Milwaukee area. Bacteriological examination of the retail samples was begun March 1, 1976.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/40/5/317/1649767/0362-028x-40_5_317.pdf by guest on 04 October 2022

MATERIALS AND METHODS

Dehydrated dog foods

Five-pound (2,268 g) bags of dehydrated dog foods, representing four different manufacturers, and two store brands (manufacturer not indentified on bags) were purchased from among 11 supermarkets. Purchases were made between February 26, 1976 and May 21, 1976.

Laboratory analyses

Pre-enrichment, selective enrichment, and isolation procedures were employed essentially as recommended by the International Association of Microbiological Societies (*12*). Biochemical and serological methods were followed as described by Edwards and Ewing (*2*).

Eight samples were subjected to "destructive analysis," i.e., the entire contents of a 5-lb. bag aseptically divided into eight aliquots, approximately 283.5 g, each; each aliquot was transferred to individual 4-liter Erlenmeyer flasks containing 2.5 liters of lactose broth. Flasks were thoroughly shaken to aid in wetting the dry dog food particles. Cultures were incubated at 35 C for approximately 24 h. Incubating flasks were manually shaken periodically during the span of working hours. At completion of pre-enrichment incubation, 1.0-ml inocula from the cultures were transferred into 12.0-ml volumes of selective enrichment media. Both selenite broth and tetrathionate broth were employed. Six tubes, three of each of these media, were prepared for each pre-enrichment culture. Selective enrichment cultures were incubated overnight at 35 C. Selenite broth cultures were subcultured to individual plates of S.S. agar and individual plates of MacConkey agar. Tetrathionate broth cultures were subcultured to individual plates of brilliant green agar. After overnight incubation at 35 C, three suspicious colonies per plate were picked to triple sugar iron agar.

Success in detecting *Salmonella* by the "destructive analysis" procedure led to the question of how many of these organisms might be contained in a 5-lb. sample. A modified most probable (MPN) procedure described by Silliker (appendix C of ref. *8*) was adapted for this purpose. Each of ten 25-g portions, from a 5-lb. (2,268 g) bag, was placed into individual 500-ml Erlenmeyer flasks containing 250 ml of lactose broth. The remaining dog food was divided into seven aliquots, approximately 288 g each, for culture in 4-liter flasks. Fifteen samples were examined by this dual procedure.

In an additional comparative study, a conventional MPN series (*6*), employing five cultures per bank, was set up in conjunction with the Siliiker modified MPN; two 5-lb. bags were cultured to compare the MPN methods. In the first attempt a 60-g portion of dried food was blended with 540 ml of lactose broth. Five 100-ml portions were transferred to individual 16-ounce jars, five 10-ml protions, five 1.0-ml portions and five 0.1-ml portions were transferred to individual tubes containing 10 ml of lactose broth. After overnight incubation at 35 C, 1.0-ml portions of these cultures were transferred to selenite broth and carried on for *Salmonella* detection. Ten portions, 25 g each, of dried dog food were pre-enriched in 500-ml flasks containing 250 ml of lactose broth. In an attempt to determine the effect of abuse, four 60-g portions of dried food were individually combined with 120 ml of sterile distilled water. These portions, which simulated preparation of the food for consumption by pet dogs, were held at room temperature. One portion was sampled after 2 h, one after 4 h, one after 6 h, and the fourth after 24 h. The abuse samples were blended directly with 420 ml of selenite broth. A MPN series was set up including five 100-ml portions for direct culture plus five 10 ml, five 1.0 ml, and five 0.1 ml portions, which were inoculated into tubes containing 10 ml of selenite broth. The remaining 1,718-g portion of the 5-lb. sample was divided into six aliquots for lactose pre-enrichment.

A second 5-lb. bag of dried dog food was also set up for comparison of the conventional MPN with Silliker's modification. In this attempt five 60-g portions were each individually blended with 540 ml of lactose. These five homogenates were combined in a 4-liter flask. Four banks of MPN cultures, five cultures each, were prepared from the composite homogenate as described above. Ten portions, 25 g each, of dried food, were pre-enriched in 500-ml flasks containing 250 ml of lactose broth. Three portions of dried food (300 g each) were distributed into individual beakers of 1 liter capacity. Sterile distilled water, 300 ml, was added to each beaker. These abuse portions were held at room temperature. The first was sampled after 3 h, the second after 6 h, and the third 24 h. A 60-portion of an abuse sample was blended with 540 ml of selenite broth. Four banks of MPN cultures, five cultures each, were prepared in selenite broth. The remaining 818-g portion of dried dog food was divided into three aliquots; each of these was pre-enriched in 2.5 liters of lactose broth contained in 4-liter flasks.

RESULTS

Qualitative detection of salmonellae

Eight packages of dried dog food, representing three different manufacturers, were examined by qualitative destructive analysis. Results are summarized in Table 1.

TABLE 1. Salmonella enteritidis *serotypes isolated from dried dog food by destructive analysis of 5-lb. (2,268 g) samples*[a]

Source	Sample no.	No. flasks pos./ No. cultured	*Salmonella* somatic groups and serotypes isolated
Mfg. #1[b]	0812	4/8	C_1 Infantis
			E_3 Thomasville
			G Havana
			K Siegburg
	0814	1/8	B Schwarzengrund
			C_1 Infantis
	0810	8/8	B Schwarzengrund
			C_1 Infantis
			C_1 Livingstone
			G Havana
			K Siegburg
	1255	1/8	B Agona
	1253	7/8	C_1 Infantis
			E_3 Thomasville
			E_4 Senftenberg
			G Havana
			K Siegburg
			L Minnesota
	0813	8/8	B Schwarzengrund
			C_1 Infantis
			E_1 Lexington
			E_3 Thomasville
			E_4 Senftenberg
			G Havana
			K Siegburg
Mfg. #2[c]	1256	1/8	R Johannesburg
Mfg. #3[c]	1257	0/8	

[a]283.5 g/each of eight 4-liter flasks containing 2,500 ml lactose broth.
[b]Different lot Nos. for each sample except #1255 and #1253 which did not have coded lot numbers. Products produced by alleged manufacturer of product sampled from home of index case.
[c]No lot Nos.

Dog food sampled from the home of the index case was allegedly produced by manufacturer No. 1. It is of interest to note that each of six packages, produced by this manufacturer, contained *Salmonella*. Furthermore, *S. enteritidis* serotype Havana was found in four of these six samples. Isolates of *S. enteritidis* serotype Havana obtained from the index case, her mother, and sample No. 1253, Table 1 were sent to the Enteric Section, Enterobacteriology Branch, Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia. Isolates from the index case, and her mother had antibiotic susceptibility patterns essentially identical to those of nine of 10 isolates recovered from one sample of dried dog food. Although this observation cannot be construed as relating the human isolates to the dog food isolates, the possibiiity was not ruled out.

Eleven different serotypes were recovered from seven

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/40/5/317/1649767/0362-028x-40_5_317.pdf by guest on 04 October 2022

of eight bags. Ten of the serotypes were recovered from among six bags of dog foods produced by manufacturer No. 1. Although 637 individual isolates were serogrouped, and most of these serotyped, data in Table 1 reflect only the *Salmonella* identified by picking three colonies per plate of selective differential medium. Other serogroups and serotypes may have been present among suspicious colonies remaining on the plates. A 12-ounce packet of dog food, produced by a fourth manufacturer, was received as a sample from a store visited by a laboratory staff member. Destructive analysis of this sample, three aliquots of approximately 57 g per 500 ml of lactose broth, failed to yield detectable *Salmonella*.

Experience with the above samples revealed no additional serotypes of *Salmonella* were detected by the tetrathionate broth-brilliant green agar sequence. Use of triplicate cultures of two selective enrichment media likewise failed to yield additional serotypes. Therefore, only one tube of selenite broth was employed per pre-enrichment culture in subsequent studies. Each of these selenite broth cultures was streaked on individual plates of S.S. agar, MacConkey agar, and brilliant green agar.

Comparison of qualitative detection of Salmonellae *with a modified MPN procedure*

Fifteen bags of dried dog food were examined both by destructive analysis and Silliker's modified MPN technique (*8*). Ten serotypes of *S. enteritidis* were detected among three bags of the dried dog food produced by manufacturer No. 1, Table 2. An additional 12 samples (5-lb. bags) of dried dog food, two retail store brands plus products of three different manufacturers, were examined by this dual procedure; none of these yielded isolates of *Salmonella.*

Comparison of modified MPN and conventional MPN procedures with qualitative detection of Salmonellae *in fresh samples and with quantitative detection of* Salmonellae *in abused samples*

Samples No. 1617, and 1616, Table 2, were coded as being from the same production lot as samples Nos. 1712 and 1713, Table 3. Each, of the former pair, was found to yield *Salmonella* MPN levels, by a modified technique, which were remarkably consistent with those determined by both modified and conventional techniques for sample No. 1713, Table 3. Although four serotypes of *S. enteritidis* were isolated from sample No. 1712, (Table 3), MPN values of less than 0.4 per 100 g and less than 2 per 100 g were found by modified and conventional techniques, respectively. The 1,718-g mass of food, which remained after distributing the MPN portions, was divided into six aliquots. Five of these aliquots produced cultures from which *Salmonellae* were isolated. Assuming that one *Salmonella* produced a positive culture (modified technique), one can calculate ($5 \div 1718 = .0029$/g) the MPN value to be 1 per 345 g. Therefore, failure to detect multiplication of *Salmonella* in the abused portions of sample No. 1712, Table 3, is not surprising; each abused portion consisted of only 60-g amounts. Portions employed for a second abuse study were increased to 300-g amounts, sample No. 1713, Table 3. An MPN value of 2 *Salmonella* per 100 g in the original sample did not show an increase after 3 or 6 h

TABLE 2. *Detection of* Salmonella *in 5-lb. (2,268 g) samples of dried dog food by culturing of ten 25-g portions per sample and qualitative destructive analysis of the balance*

Source	Sample no.	288-g Portions: No. flasks pos./no. cultured; Somatic groups isolated	25-g Portions: No. flasks pos./no. flasks cultured; Somatic groups isolated	25-g Portions: Modified M.P.N./100 g[a]	*Salmonella* serotypes identified
Mfg. #1 Identical lot nos.	#1617[b]	7/7 B, C_1, E_1, E_3, E_4, G, K	5/10 C_1, G, K	2.0	Agona Schwarzengrund Infantis Livingstone Lexington Thomasville Senftenberg Havana Siegburg
	#1616[b]	7/7 B, C_1, E_1, E_2, E_4, G, K	6/10 C_1, E_4, G, K	2.4	Schwarzengrund Livingstone Lexington Newington Senftenberg Havana Siegburg
Mfg. #1 Different lot no.	#1254	7/7 B, C_1, E_3, G, K	1/10 G	0.4	Agona Infantis Thomasville Havana Siegburg

[a]Silliker's modification. Ref. *8*. No. flasks positive ÷ total mass cultured × 100.
[b]Lot Nos. identical to those of samples #1712 and #1713, Table 3.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/40/5/317/1649767/0362-028x-40_5_317.pdf by guest on 04 October 2022

TABLE 3. *Detection of* Salmonella enteritidis *in 5-lb. (2,268 g) samples of dried dog food. Comparison of M.P.N. values derived from fresh and abused samples. Balance of sample examined by qualitative destructive analysis.*

Source	Sample no.	Fresh samples: Destructive analysis No. flasks pos./ No. cultured (Somatic Groups Isolated)	Fresh samples: Modified M.P.N./ 100 g[b] No. flasks pos./ No. 25-g portions cultured (Somatic Groups Isolated)	Fresh samples: Conventional M.P.N./100 g (Somatic groups isolated	Abused Samples[a]: Conventional M.P.N./100 g (Somatic groups isolated) Hours held at room temp. 2	3	4	6	24	Serotypes identified
Mfg. #1 Identical Lot Nos.[c]	1712	5/6[d] (C_1, E_4, K)	<0.4 0/10	<2	<2		<2	<2	<2	Infantis Livingstone Senftenberg Siegburg
	1713	3/3[e] (E_1, E_3, G, K)	2.4 6/10 (C_1, E_3, G)	2 (K)		<2		2 (C_1)	>24,000 (E_3, K)	Livingstone Lexington Thomasville Havana Siegburg

[a]Food combined with sterile distilled water.
[b]Silliker's modification. Ref. 8, No. flasks positive ÷ total mass cultured × 100.
[c]Lot No. identical to that of samples 1617 and 1616, Table 2.
[d]286 g/culture.
[e]273 g/culture.

of abuse. However, a significant increase, to greater than 24,000 per 100 g, was observed after 24 h.

DISCUSSION

The modified MPN procedure, described by Silliker (*8*), compared very favorably with a conventional technique as employed in this study. Although it requires almost four times the volume of pre-enrichment medium (2500 ml vs 690 ml), it uses one-half the amount of selective enrichment medium and one-half the amount of selective differential media. It also reduces the number of pre-enrichment cultures from 20 to 10 per sample. Most importantly, it obviates the tedium of preparing homogenates and transferring five decimal dilutions per bank into a set of four banks.

Eight aliquots were prepared from a 2,268-g mass of dried dog food for destructive analysis. Three samples were encountered in which only one of the eight produced positive cultures, Table 1. Using the modified MPN premise of 1 *Salmonella* per positive culture ($1 \div 2{,}268 = 0.00044$/g) one can calculate a MPN of 1 per 2,273 g. However, one of the samples (No. 0814, Table 1) contained two serotypes of *S. enteritidis*. This observation affirms the suspicion that distribution of *Salmonella* within a 5-lb. sample of dried dog food may not be uniform.

Seven of 8 samples, examined by destructive analysis, yielded isolates of *Salmonella*. Three samples were found to have *Salmonella* in one (283.5 g) of eight aliquots, one in four of eight, one in seven of eight, and two in eight of eight, Table 1. With the exception of two of three samples, in which *Salmonella* was detected in only one of eight aliquots, multiple serotypes were isolated from each 283.5-g aliquot. It is reasonable to conclude that quality control and sampling, at the production plant, were inadequate. Sampling and testing plans for *Salmonella*, as employed by the Food and Drug Administration (FDA), have been described (*9*). It would be prudent for the dried dog food industry to consider adopting the FDA sampling recommendation for foods in Category I. That is, random selection of 60 analytical units per production lot. Fifteen analystical units, 25 g each, could then be combined (375 g) for composite cultures. Four composite units (1,500 g) per production lot being negative would provide the manufacturer assurance, at the 95% confidence level, that the lot contained no more that one *Salmonella* per 500 g. The efficacy of compositing multiple analytical units for detection of *Salmonella* in dried foods has been established by Silliker and Gabis (*11*).

Combining of 60 analytical units into four composite units, for product testing, is a less formidable task than the conventional practice of preparing 10 individual cultures of analytical units. Admittedly one does not intend dried dog food to be consumed by infant humans, aged humans, or infirm humans. However, canine pets may also conform with these physiologic states. It is abundantly clear that dogs, infected with *Salmonella*, can provide a link in disease transmission to humans (*7*). Therefore, manufacturers of dried dog food should be interested in adopting more stringent laboratory testing to provide evidence that their products present a low consumer risk.

Eleven 5-lb. bags of dog food, produced by manufacturer No. 1, were examined in the course of this investigation. Multiple serotypes of *S. enteritidis* were recovered from each of 10 bags and a single serotype was recovered from the eleventh. As many as nine serotypes were detected in a single sample. These products were allegedly produced by an expansion extrusion process. The process is briefly described (pg 179, ref. *13*) as one which conditions meal with steam to attain moisture levels of 25 to 30%. Temperatures of 200 to 350 F (93 to 176 C) are reached for 45 to 60 sec. It is reasonable to

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/40/5/317/1649767/0362-028x-40_5_317.pdf by guest on 04 October 2022

consider this a critical control point in production of dried dog food; time, temperature, and moisture parameters lend themselves to continual monitoring for quality control. Methods to eliminate the hazard of post-processing contamination, if it exists, might well be investigated.

Dried dog food products, distributed under labels of four manufacturers and two store brands, were examined in this study. Products of two manufacturers were found to contain *Salmonella*. The possibility that dried dog foods may provide a vehicle to introduce *Salmonella* into the home is not sufficiently recognized by consumers.

This investigation has brought forth the following questions: (*a*) Is it realistic to expect manufacturers to produce *Salmonella*-free dried dog food? (*b*) Should the answer to the first question be negative, what degree of hazard does dried dog food present to pets and to their owners? (*c*) Should pet owners be cautioned about the handling, storage, and potential for abuse of dried dog foods?

ACKNOWLEDGMENTS

The technical assistance of Messrs. Robert Bagley, Richard Pries, and John Sromek is gratefully acknowledged. Antibiotic susceptibility testing of *Salmonella* isolates was done through the kind cooperation of Dr. D. Brenner of the Center for Disease Control, Atlanta, Georgia.

REFERENCES

1. Bowmer, E. J. 1964. The challenge of salmonellosis-major public health problem. Am. J. Med. Sci. 247:467-501.
2. Edwards, P. R., and W. H. Ewing. 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis, Minn.
3. Galton, M. M., M. Harless, and A. V. Hardy. 1955. *Salmonella* isolations from dehydrated dog meals. J. Amer. Vet. Assn. 126:57-58.
4. Galton, M. M., J. E. Scatterday, and A. V. Hardy. 1952. Salmonellosis in dogs. I. Bacteriological, epidemiological, and clinical considerations J. Infect. Dis. 91:1-5.
5. Griffin, C. A. 1952. A study of prepared feeds in relation to *Salmonella* infection in laboratory animals. J. Amer. Vet. Med. Assn. 121-197-200.
6. Lewis, K. H., and R. Angelotti (eds.). 1964. Examination of foods for enteropathogenic and indicator bacteria. U. S. Dept. of Health, Education, and Welfare. Public Health Service Publication No. 1142. U. S. Government Printing Office, Washington, D.C. 20402
7. Morse, E. V., M. A. Duncan, D. A. Estey, W. A. Riggs, and B. O. Blackburn. 1976. Canine salmonellosis: A review and report of dog to child transmission of *Salmonella enteritidis*. Amer. J. Pub. Health 66:82-84.
8. National Academy of Sciences - National Research Council. Committee on *Salmonella*. 1969. An evaluation of the *Salmonella* problem. Pub. 1683, National Academy of Sciences. Washington, D.C.
9. Olson, J. C., Jr. 1975. Development and present status of FDA *Salmonella* sampling and testing plans. J. Milk Food Technol. 38:369-371.
10. Salmonella Surveillance Report; Fourth Quarter. 1974. Report No. 124:7, 1975. Public Health Service, Center for Disease Control, Atlanta, Georgia 30333.
11. Silliker, J. H., and D. A. Gabis. 1973. ICMSF methods studies. I. Comparison of analytical schemes for detection of *Salmonella* in dried foods. Can. J. Microbiol. 19:475-479.
12. Thatcher, F. S., and D. S. Clark (eds.). 1968. Microorganisms in foods: Their significance and methods of enumeration. University of Toronto Press, Toronto, Canada.
13. United States Department of Health, Education, and Welfare. National Conference on *Salmonellosis*. 1965. P.H.S. Publ. No. 1262. U. S. Government Printing Office, Washington, D.C. 20402.

Downloaded from http://meridian.allenpress.com/jfp/article-pdf/40/5/317/1649767/0362-028x-40_5_317.pdf by guest on 04 October 2022