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Survival of *Salmonella dublin* and *Salmonella typhimurium* in Lebanon Bologna

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ABSTRACT

Survival and destruction of *Salmonella dublin* and *S. typhimurium* added to Lebanon bologna was studied during manufacture of this sausage. During the aging period of salted cubed beef at 5 C, viable cell counts of *S. dublin* did not change over a 10-day period. Cell counts of *S. Dublin* were reduced 3 to 4 log cycles during the 4-day fermentation at 35 C; further reduction in the viable *Salmonella* count occurred during mellowing of the bolognas at 5 C. The number of *S. typhimurium* was usually reduced to an undetectible level before the end of the fermentation. *Salmonella typhimurium* was consistently more sensitive to the acid conditions of Lebanon bologna than was *S. dublin*. Introduction of an optional cooking step indicated that heating of bolognas to 51.7 C or above led to destruction of salmonellae. Unaged beef which was not inoculated with starter culture did not ferment and there was very little reduction in numbers of added salmonellae. Salmonellae were destroyed more rapidly in Lebanon bologna made from unaged beef with starter culture than in bologna made from aged beef (natural flora fermentation). Smoking also appeared to contribute to destruction of salmonellae. Four commercial Lebanon bolognas were tested for the presence of salmonellae but none were detected.

Occurrence of salmonellae in a wide variety of fresh meats has been well documented (*3, 5, 14, 16, 18*) but little information is available regarding the presence of these organisms in fermented and dried sausages. Only rarely have outbreaks of salmonellosis been attributed to these sausages; for example, the presence of *Salmonella choleraesuis* in salami was reported by Marazza and Crespi (*8*) to be responsible for a food poisoning outbreak. Behavior of *Salmonella* in artificially contaminated fermented sausages has been investigated in a few studies. Ostlund and Regner (*9*) reported that *Salmonella typhimurium* survived in artificially contaminated "Isterband," a Swedish fermented sausage; however, the influence of storage on continued survival of the organisms was not studied. Numbers of salmonellae in artificially contaminated thuringer declined during fermentation and subsequent refrigerated storage of the finished sausage (*4*). However, the rate of decline was too slow to ensure complete destruction of salmonellae even at low levels of contamination. When Takacs and Simonffy (*15*) inoculated dry sausage with low levels of salmonellae before ripening, the rate of decline of the organisms was a function of the pH, salt, and water content. If the initial count of salmonellae was approximately 10^4/g or more, the sausages remained contaminated up to the time of consumption. Therefore, it would appear that salmonellae are able to survive in dry and fermented sausages. The purpose of this investigation was to study the fate of salmonellae in artificially contaminated Lebanon bologna during the manufacturing process.

MATERIALS AND METHODS

Preparation of Lebanon Bologna

Beef chuck (canner and cutter grade) was ground through a ¾-inch plate and NaCl was added to obtain a final concentration of 3%. Fresh or frozen beef was used; if frozen, the meat (previously ground through a ¾inch plate) was thawed for 24 h at 12 C, reground through a ¾inch plate and then salted. Salted meat was placed in plastic bags (3-4 kg per bag) and aged at 5 C for approximately 10 days. At the end of the aging period, sugars, spices, and potassium nitrate were added to the salted meat according to the formulation of Palumbo et al. (*10*) and the mixture ground through a 3/32-inch plate. The material was then stuffed into fibrous casings 55 mm in diameter. Sausages were covered with paraffin (mp 52 C) and allowed to ferment in an incubator at 35 C and 80-85% RH for up to 4 days. Paraffin was added to prevent drying of sausages and to prevent growth of mold. When the effect of smoke was studied, bolognas (without the paraffin coating) were placed, immediately after stuffing, into the smoke house at 35 C and about 90% RH. At the end of the fermentation period, bolognas were either allowed to mellow at 5 C or were heat processed. For more complete details concerning the processing of Lebanon bologna see (*10*). In certain experiments, unaged beef, fresh or frozen, was used to make bolognas. Salt, sugars, nitrate, and spices were added to the meat (thawed if frozen beef) and the mixture was ground through a 3/32-inch plate and stuffed into casings.

Inoculation of bolognas with salmonellae or starter culture

Twenty-four hour cultures of *Salmonella dublin* or *S. typhimurium* grown in trptic soy broth (Difco)[2] at 37 C dere diluted in 0.1% peptone water to give the appropriate concentration of cells and aseptically mixed into the meat by hand kneading before aging or stuffing. Lactacel MC starter culture (Merck and Co. Rahway, N.J.; a mixture of *Lactobacillus plantarum* and *Pediococcus cerevisiae*) was utilized in certain experiments; unaged beef was used and the starter was added before stuffing. Handling and addition of the starter culture was according to recommendations on the manufacturer's label. However, in contrast to their general directions, a straight nitrate cure was employed.

Acid tolerant S. dublin and S. typhimurium

Gradient agar plates (bottom layer, Tryptic Soy Agar (TSA, Difco); upper layer, TSA + 0.34% lactic acid in slanted square petri dishes) were used to isolate acid-tolerant *S. dublin* and *S. typhimurium*. By

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[2]*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

continued replating of isolated colonies from the most acid part of the gradient plates, acid-tolerant salmonellae were obtained. Acid-tolerant strains of both species had a minimum pH for growth of 4.6; wild-types had a minimum of 5.2.

Determintion of the viable count of salmonellae

Three separate 112-g portions (one from each end and one from the middle) of an individual salmonellae-contaminated bologna were removed from the casing and added to 370 ml of Selenite-Cystine broth (Difco) and blended for 3 min at high speed in a Waring Blendor. Both the direct count and the Most Probable Number (MPN) counts were made from the blended sample. For the direct count of viable salmonellae, appropriate dilutions were spread on the surface of Brilliant Green Agar (BGA, Difco) plates and typical magenta colonies were counted after 24 h at 35 C. For the MPN count, graded volumes of 430, 43, 4.3, 0.43, and 0.043 ml of the blended mixtures (equivalent to 100, 10, 1.0, 0.1, and 0.01 grams of the original bologna) were placed in glass bottles or test tubes. The first three (430, 43, 4.3 ml) were added directly to the container; for the 0.43 ml sample, 0.5 ml of the blend was diluted with 7.5 ml Selenite-Cystine broth (0.4 ml was discarded); and for the 0.043 ml level, 0.7 ml of the preceding dilution was added to 4.9 ml of Selenite-Cystine (0.1 ml was discarded). Incubation was at 35 C for 24 h. Portions of these enrichments (0.01 ml) were streaked onto BGA and incubated 24 h at 35 C. Typical magenta colonies were picked and inoculated into Lysine Iron Agar (Difco); lysine decarboxylase positive slants were confirmed as salmonellae by use of somatic "O" antisera. The MPN/g was calculated by the use of a table.

The possibility existed that the above procedures for quantitating salmonellae may not recover cells that were injured by acid or heat. A limited number of samples were first preenriched in lactose broth (Difco nutrient broth + 1% lactose) and then treated by the above isolation techniques. Samples preenriched in lactose broth gave no higher counts than did samples processed in the usual way. Therefore, injury was not considered to be a problem and techniques utilized were adequate to quantitate the salmonellae present in the Lebanon bolognas.

Determination of the pH of meat and bolognas

The pH of bolognas or meat was determined by placing the electrode of the pH meter directly into the meat mass or into a slurry containing 1/5 dilution of meat in distilled water.

Heat processing of contaminated Lebanon bolognas

Thermal distruction of salmonellae in Lebanon bologna was studied by adjusting the smoke house temperature and humidity settings to 76.7 C (dry bulb) and 65.5 C (wet bulb). A thermocouple was inserted into a noncontaminated bologna and at predetermined internal temperatures, bolognas were removed, quickly quartered with a sterile knife, placed in platic bags, and cooled in an ice bath. Quartered bolognas were kept refrigerated until counts were made.

EXPERIMENTAL AND RESULTS

Lebanon bologna is a fermented, highly spiced and smoked all beef sausage. Its manufacture consists of three steps: (*a*) aging salted beef cubes for about 10 days at 5 C; (*b*) smoking the sausage 4 days at 35 C; and (*c*) mellowing the smoked bolognas at 5 C for at least 3 days. Details of Lebanon bologna processing and microbiology are given elsewhere (*10, 13*). Data showing the fate of added salmonellae during the three steps are presented below.

When *S. dublin* was added to salted beef at 5 C, there was no growth of the organism over a 10-day period. At zero time, the number of viable *S. dublin* was 1.6×10^4/g meat; at 10 days, it was 1.9×10^4/g. Thus, *S. dublin* survived the aging process in salted beef but showed no growth.

The fate of salmonellae during the fermentation and mellowing periods in artificially contaminated bolognas is summarized in Table 1. At the end of 4 days of fermentation, the pH of the sausages dropped from 5.7 to 4.3-4.4. The acid-tolerant strains of *S. dublin* and *S. typhimurium* survived the fermentation period better than did the wild-type strains. All strains of *S. dublin* showed better survival during fermentation than *S. typhimurium*. After fermentation was complete, the Lebanon bolognas were mellowed at 5 C for several days. In Table 1, it can be seen that the wild-type of *S. dublin* survived for at least 11 days of mellowing at 5 C and the acid-tolerant type was still present at 24 days even though the environment of the bolognas was quite acid. *S. typhimurium* appeared to be more sensitive to the acid than *S. dublin* because after 4 days of fermentation, neither the wild-type nor the acid-tolerant types of *S. typhimurium* were present. *S. typhimurium* was detected in small numbers during mellowing, indicating that the acid environment may not be consistent in killing salmonellae.

Although not typically part of the Lebanon bologna process, a cooking (heating) step was investigated to determine the heat sensitivity of salmonellae in the acid environment of the bolognas (Table 2). A shortened fer-

TABLE 1. *Survival of wild-type and acid-tolerant strains of* Salmonella dublin *and* Salmonella typhimurium *during fermentation and mellowing of Labanon bologna made from aged beef*

		Salmonella dublin				*Salmonella typhimurium*			
		Wild-type		Acid-tolerant		Wild-type		Acid-tolerant	
Total days	Days-fermentation	Number/g	pH	Number/g	pH	Number/g	pH	Number/g	pH
0	0	5.7×10^4	5.7	1.4×10^4	5.7	4.5×10^4	5.7	8.5×10^3	5.7
2	2	9.4×10^2	4.5	2.1×10^2	4.5	1.4×10^1	4.6	8.6×10^1	4.6
3	3	1.1	4.4	positive[a]	4.4	0[b]	4.4	0.007	4.4
4	4	0.075	4.4	0.21	4.3	0	4.3	0	4.3
	Days-mellowing								
7	3	positive	4.4	0.46	4.4	0	4.3	0.02	4.3
15	11	0.21	4.4	0.15	4.4	0.007	4.4	0	4.4
28	24	0	4.3	0.007	4.3	0	4.3	0	4.3

[a]All MPN tubes were positive.
[b]The lowest number of salmonellae that could be detected was 0.003 cells/g; any number less than 0.003 was considered to be zero.

TABLE 2. *Effect of fermentation and cooking on the survival of acid-tolerant* Salmonella dublin *and* Salmonella typhimurium *in Lebanon bologna*

Days-fermentation	*Salmonella dublin*		*Salmonella typhimurium*	
	Number/g	pH	Number/g	pH
0	1.2×10^5	5.6	3.1×10^3	5.6
1	1.7×10^4	5.0	5.7×10^1	5.0
2	1.7×10^4	4.7	1.1×10^1	4.7

After 2 days fermentation, the bolognas were placed in the smoke house at 76.7 C (dry bulb) and 65.6 C (wet bulb) without smoking.

Internal temperature of bologna (C)	Time to reach internal temperature (min)	*Salmonella dublin*	*Salmonella typhimurium*
		Number/g	Number/g
27.2	0	1.7×10^4	1.1×10^1
35.0	18	1.1×10^4	3.4
43.3	27	0.015	2.1
48.9	36	0[a]	0.15
51.7	42	0	0
54.4	52	0	0

[a]The lowest number of salmonellae that could be detected was 0.003 cells/g; any number less than 0.003 was considered to be zero.

mentation period (2 days in contrast to the usual 4) was utilized to ensure that viable salmonellae would be present before the heating step. Data in Table 2 indicate that at internal temperatures of 51.7 C or above, viable salmonellae could not be detected.

Survival of *S. typhimurium* in Lebanon bologna prepared from unaged beef, aged beef, and unaged beef plus stater culture is shown in Table 3. The characteristic flavor of Lebanon bologna is brought about by a fermentation mediated by lactic acid bacteria which develop during the aging of salted beef at low temperature. However, the aging step can be eliminated by using unaged beef plus a lactic acid starter culture. Acid production does not occur in bolognas made from unaged beef in the absence of starter. In Lebanon bologna made from unaged beef, there was no decrease in the pH and an initial slight increase in numbers of salmonellae was followed by a decrease to about the original starting level. Bolognas made from aged beef showed a rapid decrease in pH but *S. typhimurium* was not completely killed in the 4-day fermentation period. The natural fermentation process with aged beef varies with each batch of meat and this could influence salmonellae survival. When started culture was used, no viable *S. typhimurium* could be detected by the third day of fermentation.

Survival of *Salmonella* shown in Tables 1, 2, and 3 is for bolognas fermented in a constant temperature-constant humidity incubator. Since Lebanon bologna is typically given a long smoke, the effect of smoking and nonsmoking (incubator) conditions on survival of salmonellae was studied. Use of starter culture led to a more effective destruction of *S. dublin* and *S. typhimurium* when compared to the natural fermentation involved in aged beef (Table 4). With both the starter culture and aged beef systems, there were fewer salmonellae surviving under smoked conditions in contrast to nonsmoked conditions. In Lebanon bolognas made from aged beef, regardless of whether they were smoked or not, *S. dublin* was more resistant than *S. typhimurium*.

Data in Tables 1, 2, 3, and 4 strongly suggest that commercial Lebanon bologna should contain few if any viable salmonellae after processing. Duplicate 100-g samples of Lebanon varieties from three companies (one

TABLE 3. *The survival of* Salmonella typhimurium *in Lebanon bologna made from unaged beef, unaged beef plus starter culture, and aged beef*

Days-fermentation	Unaged beef		Aged beef		Unaged beef plus starter culture	
	Number/g	pH	Number/g	pH	Number/g	pH
0	1.4×10^4	5.3	1.3×10^4	5.3	8.4×10^3	5.3
1	4.5×10^5	5.4	3.0×10^3	4.5	3.5×10^4	4.8
2	4.5×10^5	5.4	1.1×10^3	4.3	2.4×10^1	4.1
3	5.4×10^4	5.3	2.4×10^2	4.1	0[a]	4.0
4	2.2×10^4	5.3	3.6	4.1	0	4.0

[a]The smallest number of salmonellae that could be detected was 0.003 cells/g; any number less than 0.003 was considered to be zero.

TABLE 4. *Effect of nonsmoking and smoking on the survival of* Salmonella dublin *and* Salmonella typhimurium *during fermentation of Lebanon bologna*

Days-fermentation	*Salmonella dublin*				*Salmonella typhimurium*			
	Nonsmoked		Smoked		Nonsmoked		Smoked	
	Number/g	pH	Number/g	pH	Number/g	pH	Number/g	pH
Unaged beef plus starter culture								
0	4.0×10^4	5.2	4.0×10^4	5.2	3.0×10^4	5.2	3.0×10^4	5.2
1	7.8×10^3	4.5	2.7×10^3	4.5	2.2×10^3	4.5	5.0×10^2	4.5
2	0[a]	4.0	0	4.0	0	4.0	0	4.0
4	0	4.2	0	4.2	0	4.2	0	4.2
Aged beef								
0	2.5×10^4	5.3	2.5×10^4	5.3	6.1×10^4	5.3	6.1×10^4	5.3
1	1.6×10^6	5.2	5.5×10^4	5.2	8.2×10^4	5.2	2.8×10^4	5.2
2	1.5×10^6	4.5	1.5×10^6	4.5	4.1×10^3	4.5	5.6×10^2	4.6
3	6.9×10^4	4.4	3.4×10^4	4.4	4.6×10^1	4.4	0	4.4
4	8.2×10^3	4.4	0	4.4	0	4.4	0	4.4

[a]The lowerst number of salmonellae that could be detected was 0.03 cells/g; any number less than 0.03 was considered to be zero.

regular and one sweet Lebanon bologna from one company and one regular variety from each of the other two companies) were tested for the presence of salmonellae; all were negative.

DISCUSSION

During the aging step in the Lebanon bologna process, ¾-inch cubes of salted beef (3% NaCl) are held for approximately 10 days at 5 C. *S. dublin*, added to aging beef, survived but did not grow. This is not surprising because it has been shown in a variety of foods that salmonellae are able to survive but do not multiply at 5 C (*2, 6*).

Fermentation of the stuffed bolognas at 35 C is the second stage of Lebanon bologna processing. The lactic population which had developed during the aging step grows out rapidly due to the increase in temperature during fermentation, and ferments the sugars to lactic acid with a concomitant lowering of the pH (*13*). Most of the salmonella population was inactivated during the fermentation period. In the absence of fermentation (Table 3, unaged beef), there was some growth of salmonellae during the first 24 h with a slow reduction in numbers during the remaining 3 days. It would appear then, that the acid conditions of Lebanon bologna contribute greatly to destruction of both *S. dublin* and *S. typhimurium*. Goepfert and Chung (*4*), studying survival of salmonellae during the processing of thuringer, concluded that both the presence of salt and acid were the prime factors contributing to destruction of salmonellae. Salmonellae were not killed in thuringer sausage that lacked a fermentable sugar. Work with cottage cheese whey and fermented skimmilk (*11, 17*) suggested that the major factors responsible for the loss of viability in salmonellae in these fermented milk products was the low pH and acid produced by the lactic acid starter culture.

At the end of the fermentation period, Lebanon bolognas are stored at 5 C for several days; this third step of processing is called mellowing. Mellowing appears to be necessary to allow full development of the typical flavor (*10*). Data in Table 1 indicate that there were decreases in *Salmonella* numbers during the mellowing period; however, the acid tolerant strain of *S. Dublin* was still present in small numbers at 24 days of storage. In thuringer, *S. typhimurium* survived storage at 5 C for 28 days (*4*). The pH of thuringer is generally higher than that of Lebanon bologna and this fact may explain the longer survival of *S. typhimurium* in thuringer.

Smoking of Lebanon bolognas appears to have some destructive effects on *Salmonella* populations (Table 4). Anderton (*1*) suggested that smoking exerts an inhibitory action on salmonellae near the surface of the meat product but does not necessarily inhibit organisms in the deeper layers. Smoking had no effect on survival of salmonellae in thuringer (*4*). However, thuringer is generally smoked for less than 24 h in comparison to the 96 h smoke given Lebanon bologna.

Cooking of the bolognas to a temperature above 51.7 C at a pH of 4.7 is necessary to ensure complete destruction of salmonellae in Lebanon bologna. Goepfert and Chung (*4*) showed that *S. typhimurium* was not detectable in thuringer (pH 4.8) that had been heated for 1 h at 52 C. A cooking temperature above 48.9 C was recommended to ensure complete destruction of salmonellae during the manufacture of cottage cheese (*7*).

Use of a starter culture resulted in more efficient and quicker killing of salmonellae than with the natural flora fermentation. Destruction of salmonellae under natural fermentation conditions was not uniform. For example, in Table 1, *S. typhimurium* could not be detected by the third day of fermentation. However, data in Table 3 indicate that viable *S. typhimurium* could be detected at 4 days even though the pH of bolognas was quite similar as shown in Tables 1 and 3 (aged beef data). Park and Marth (*12*) noted that in cultured skimmilks contaminated with *S. typhimurium*, the survival of the pathogen differed markedly depending on the type of lactic acid starter culture used even though the pH of the milk products produced by these starters were often similar. Strain differences were noted also. It may be possible that in the natural fermentation of Lebanon bologna different batches may have completely different lactic populations which could explain the variability in the destruction of *S. typhimurium* in natural flora fermentations.

A high level of salmonellae contamination was used in the experimental Lebanon bolognas to facilitate enumeration of these organisms. Large numbers should provide a model demonstrating the effects of normal processing conditions on survival of any size population of salmonellae. It is probable that salmonellae contamination in commercial Lebanon bologna is of a very low order of magnitude, if present. Such a low level of contamination by *Salmonella* species has been demonstrated in fresh pork sausage (*14*) and similar levels should be expected in fresh beef.

While an exhaustive search of the literature was not made, only one paper was found which discussed an outbreak of salmonellosis resulting from Italian dry sausage (*8*). Thus, the lack of documentation suggests that fermented and dry sausages may rarely be involved in salmonellae food poisoning outbreaks.

To ensure control of *Salmonella* during sausage manufacture, our data suggest that the processor who wishes to use a natural flora fermentation can effectively reduce or eliminate salmonellae from his product by heating the sausage to an internal temperature of 52 C at a pH of approximately 4.7; if a lower pH is achieved, a short period of mellowing should be effective and a heating step is unnecessary. Alternately, the processor who properly uses a known starter culture with proven acid producing ability can be quite confident that the sausage will be free of *Salmonella*.

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Conference on Mechanized Microbiology

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