Salmonella in Natural Animal Casings

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Destruction of salmonellae in inoculated and naturally contaminated natural animal casings was studied. Salmonellae were effectively destroyed (99.999%) in inoculated hog casings after exposure for 24 h to saturated brine at pH 4.0 and 10.0 adjusted with acetic acid and sodium hydroxide, respectively. Treatment of inoculated hog and sheep casings in saturated brine or saturated brine with citric acid was not nearly as effective as brine containing acetic acid or sodium hydroxide. Salmonellae in naturally contaminated hog casings were virtually eliminated after 21 days of storage in crystalline sodium chloride. Salmonellae in sheep and hog casings were eliminated after 7 days of storage in crystalline salt. Treatment of naturally contaminated hog casings with glycerin-salt or sorbitol-salt solutions was not as effective in destroying salmonellae as treatment with crystalline salt.

Natural casings are considered as processed meat products, and as such must be free of Salmonella. Therefore, the processing applied to natural casings must effectively destroy this organism. The raw natural casings are the intestinal tracts of animals and, therefore, would be that part of the carcass most likely to be contaminated with Salmonella.

The basic processing of natural casings involves the removal of fecal material and washing. The casings may then be wet packed in saturated brine or packed dry with crystalline salt. Natural casings are decontaminated in two of the process steps. First is the physical removal of bacteria through washing, and second is the destruction of bacteria by high concentrations of salt. The natural level of contamination and efficiency of the washing procedure as well as the temperature of storage in salt will determine the length of storage time required to rid the casings of Salmonella. Sanitation in handling of unsalted casings also has an effect on the numbers of salmonellae that contaminate the unsalted material.

Investigators at the University of Zurich (3) found that salmonellae survived in inoculated salted casings after 27 days of storage. The casings were not studied beyond 27 days. Zabel (D. V. M. thesis, Free Univ. of Berlin, 1959) reported that salmonellae in inoculated casings treated with dry salt and soda were destroyed after 11 days of storage.

Several attempts have been made to develop processes which destroy salmonellae more rapidly than occurs with salting alone. Paddock (U.S. Patent 2,673,804) patented a process involving a combination of acetic acid and hydrogen peroxide to improve the texture and appearance of tripe. This process may also serve to destroy salmonellae. Bickel (U.S. Patent 2,735,776, 1956) preserved sausage casings in a solution of 2 to 10% tartaric acid and sodium hexametaphosphate. Swift and Co. (C. A. Rinehart and L. B. Jensen, U.S. Patent 2,966,415, 1960) obtained a patent for the use of peracetic acid for bleaching casings. Unfortunately, none of these processes was evaluated for the ability to destroy salmonellae even though the chemical agents used are known to have bactericidal effects.

The Danish Meat Research Institute (2) described a procedure involving the salting of casings in 4% lactic or 4% tartaric acid for 8 h followed by a cold-water rinse and then immersion in 1% sodium tripolyphosphate for 12 h. The procedure was effective in killing bacteria and has been approved for use in Denmark. The casings apparently were not weakened by the process.

In our studies, the effect of pH-adjusted brine solutions on the survival of salmonellae inoculated into natural casings was evaluated. In addition, the destruction by salt and brine solutions of naturally occurring salmonellae in casings was examined.

MATERIALS AND METHODS

Sources of casings. All casings used in these studies were cleaned, finished, and unsalted. Hog and sheep casings for inoculation with salmonellae were
obtained from two processors in the Midwest. Naturally contaminated hog and sheep casings were obtained from three processors, one each in the East, Midwest, and West. Naturally contaminated beef casings were obtained from a single Midwestern processor.

Preparation of inoculum. Five Salmonella strains of the serotypes C, (O) b (H), G (O) zsp (H), E, (O) 1 complex (H), C, (O) G complex (H), and B (O) G complex (H) were used. The salmonellae were freshly isolated from low-moisture food samples. The cultures were grown separately for three transfers at 24-h intervals with incubation at 35 C. Equal volumes of each culture were mixed and then added at the rate of 1 to 1,000 ml of sterile phosphate-buffered distilled water at pH 7.2. Each casing for inoculation was attached to a sterile 50-ml funnel outlet and filled with the inoculum, which then was allowed to drain through the casing. After inoculation of the lumen, the casings were dipped in the inoculum to contaminate the outer surface.

Test I: treatment of inoculated casings with pH-adjusted brine solutions. Saturated sodium chloride solutions were prepared, and acetic acid or sodium hydroxide was added so that the initial pH of the brine solutions was 3.0 and 12.4, respectively.

Hog casings were treated with regular saturated brine as a control and with pH-adjusted brine solutions. The inoculated hog casings were cut into about 20 segments (5 cm) and introduced into 500 ml of each of the pH-adjusted solutions. The ratio of casings to brine was 1:1 (wt/vol). The pH values of the casing-brine mixtures were 4.0 and 10.0 for the acetic acid and sodium hydroxide-containing brine solutions after 15 h at 25 C.

Test II: treatment of inoculated casings with acidified salt. Citric acid was dry blended with salt so that addition of water to obtain a saturated solution provided a pH of 4.0. A control of regular salt was used for comparison. The dry citric acid-salt blend and regular salt were supplied by the Morton Salt Co., Chicago, Ill.

Inoculated hog and sheep casings were passed through acidified or regular salt crystals. Excess salt was allowed to drop off. The inoculated and salted casings were then introduced at the rate of 30 segments (5 cm) into 500 ml of the corresponding saturated salt solution. Three inoculated casings of each type (hog and sheep) were not exposed to salt to determine the initial level of contamination.

Sampling of inoculated casings. The hog casings from test I were sampled immediately after inoculation and after 15 and 48 h exposure to the brine solutions. In addition, the brine solutions were sampled 15 and 48 h after the casings had been added.

The hog and sheep casings from test II were held at 25 C and sampled after 1, 2, 4, 8, 12, 24, 36, 48, and 74 h of exposure to the brine. One control and two-acid treated samples (sheep and hog) were removed at each time period.

Test III: treatment of naturally contaminated casings. Thirty samples each of hog and sheep casings and 10 beef casings were used. The samples were drawn from the processing lines in early morning, noon, and late afternoon for 5 days. Each casing sample was packaged individually and frozen immediately by the processors. Each sample weighed approximately 100 g. Prior to testing, the casings were thawed overnight at 6 C. After thawing, 11 g of each casing was weighed into a sterile Waring blender jar by cutting through the mass of casing with sterile scissors. These samples were used to determine the natural level of salmonellae prior to treatment.

The remaining portion of each hog and sheep casing sample was then divided into three equal smaller portions for treatment. One portion was packed in a sterile plastic bag with sodium chloride at a ratio of 30 g of casing to 200 g of salt. The second portion was packed with 250 ml of glycercin-salt solution (25% glycercin, 22.7% salt) and the third portion was packed with 250 ml of sorbitol-salt solution (20.9% sorbitol, 19.3% salt). Beef casings were stored in salt only. Immediately after packing, the casings were placed at 6 C for the entire test period.

Samples were removed from storage for analysis after 7, 14, and 21 days. Each treated casing was sampled by weighing 11 g into a sterile Waring blender jar.

Microbiological testing. Bacteriological media used in these studies were obtained from Difco, Detroit, Mich. Casings from tests I and II were analyzed by blending an 11-g sample of casing with 99 ml of sterile buffer in a sterile Waring blender for 1 min. From this 1:10 dilution, three 10-ml portions of the sample were introduced into three tubes of single-strength nutrient broth. Further dilutions were prepared to analyze the samples for Salmonella by the most-probable-number technique.

Salmonellae were also determined by using direct plating on brilliant green agar. Total coliforms were determined by direct plating of appropriate dilutions with deoxycholate agar. Total aerobic plate counts were determined with standard plate count agar. The colonies on these plates were counted after 24 or 48 h of incubation at 35 C.

The naturally contaminated casings from test III were analyzed for salmonellae only. The 11-g sample weighed into the sterile blender jar was blended with 99 ml of lactose broth containing 1% tertgal anionic-7 (Union Carbide, Chicago, Ill.) for 1 min at high speed. The blender contents were then removed to a sterile bottle, and serial dilutions were made in lactose broth with tertgal to yield single subsamples of 1, 0.1, and 0.01 g. A single 10-g sample of each casing was weighed directly into 90 ml of lactose broth.

For all samples, inoculated and naturally contaminated, Salmonella analysis was completed by inoculation of the pre-enrichment broth to tetradionate broth containing brilliant green dye. The tetradionate broth cultures were incubated for 24 h at 35 C and then streaked onto differential agars. Salmonella-suspect colonies were identified by appropriate biochemical and serological techniques (4). For the inoculated casings, however, most salmonellae numbers were determined by direct plating on brilliant green agar with biochemical and serological confirmation of suspect colonies.
RESULTS

Results from test I using inoculated hog casings treated in saturated brine at pH 4.0 and 10.0 indicated that these treatments effectively reduce the level of *Salmonella* in the casings by greater than 99.999% after 15 h at 25 C (Table 1). After 48 h, no salmonellae were detectable in either the casings or brine solutions at either pH. This represents a reduction of greater than 99.9999%.

Results from test II show that the treatment of hog and sheep casings in regular saturated brine or saturated brine with citric acid was not nearly as effective as that observed with the acetic acid- or sodium hydroxide-containing brines solutions (Tables 2, 3). In the citric acid-adjusted brine, salmonellae were isolated after treatment for 72 h. The results indicate that citric acid-treated salt was no more effective in destroying salmonellae than saturated brine.

**Table 1. Effect of saturated salt with acetic acid or sodium hydroxide* survival of bacteria in inoculated hog casings**

| Sample          | Exposure time (h) | Salmonella/g | Total count/g | Coliforms/g |
|-----------------|------------------|--------------|---------------|-------------|
| Control casings | 0                | 4,600,000    | 430           | 3           |
| Acid casings    | 0                | 2,400,000    | 10            | <1          |
| Alkaline casings| 0                | 240,000      | 40            | <1          |
| Acid casings    | 15               | <10          | <10           | <1          |
| Acid brine      | 15               | 9.3          | <10           | <1          |
| Alkaline casings| 15               | 11.0         | <10           | <1          |
| Alkaline brine  | 15               | 0.39         | <10           | <1          |
| Acid casings    | 48               | <0.3         | <10           | <1          |
| Acid brine      | 48               | <0.3         | <10           | <1          |
| Alkaline casings| 48               | <0.3         | <10           | <1          |
| Alkaline brine  | 48               | <0.3         | <10           | <1          |

*Brine-casing mixtures were 4.0 and 10.0, respectively, after 15 h.

**Table 2. Effect of saturated salt with and without citric acid on the survival of bacteria in inoculated hog casings**

| Time (h) | Salmonella/g | Total count/g | Coliforms/g |
|----------|--------------|---------------|-------------|
|          | Brine control | Acid brine treatment | Brine control | Acid brine treatment |
| 0a       | 310,000      | 2,790,000     | 17,100      |
|          | 50,000       | 3,270,000     | 18,000      |
|          | 5,400        | 5,620,000     | 25,800      |
| 1        | 13,500       | 270,000       | 1,500       |
|          | 12,400       | 1,100,000     | 1,680       |
|          | 30,000       | 2,280         | 600         |
| 2        | 290,000      | 160,000       | 1,930       |
|          | 20,000       | 850,000       | 1,700       |
|          | 200,000      | 1,930         | 380         |
| 4        | 2,700        | 240,000       | 980         |
|          | 40,000       | 1,100,000     | 1,680       |
|          | >30,000      | 1,180         | 380         |
| 8        | 420,000      | 1,270,000     | 2,000       |
|          | 10,000       | 890,000       | 1,510       |
|          | 5,700        | 2,280         | 1,180       |
| 12       | 7,400        | 290,000       | 780         |
|          | 40,000       | 670           | 2,170       |
|          | 210,000      | 670           | 2,170       |
| 24       | >30,000      | 1,200         | 600         |
|          | 1,200        | 800,000       | 300         |
|          | 10,000       | 800,000       | 300         |
| 36       | 6,700        | 290,000       | <10         |
|          | 6,700        | 21,000        | <10         |
|          | 110          | 43,700        | <10         |
| 48       | 200          | 7,700         | <10         |
|          | 110          | 360,000       | <10         |
|          | 46           | 360,000       | <10         |
| 72       | 50,000       | 9,100         | <10         |
|          | 1,700        | 12,000        | <10         |
|          | 4,900        | 12,000        | <10         |

* All untreated, no salt.
TABLE 3. Effect of saturated salt with and without citric acid on the survival of bacteria in inoculated sheep casings

| Time (h) | Salmonella/g | Total count/g | Coliforms/g |
|----------|--------------|---------------|-------------|
|          | Brine control | Acid brine treatment | Brine control | Acid brine treatment | Brine control | Acid brine treatment |
| 0*       | 1,000,000     | >30,000,000    | >30,000      | >30,000,000          | >30,000       | >30,000,000          |
| 1        | 20,000        | 18,000,000     | 18,000,000   | >3,000               | >3,000        | >3,000               |
| 2        | 150,000       | 48,000,000     | 51,000,000   | >3,000               | 64,000        | >3,000               |
| 4        | 70,000        | 56,000,000     | 41,000,000   | >3,000               | 20,100        | >3,000               |
| 8        | >30,000       | 69,000,000     | 7,000,000    | >3,000               | >3,000        | >3,000               |
| 12       | 1,340,000     | 10,000,000     | 6,410,000    | >3,000               | >3,000        | >3,000               |
| 24       | 10,000        | 16,000,000     | 19,000,000   | 33,200               | 36,100        | 1,700                |
| 36       | 9,900         | 13,000,000     | 4,220,000    | 6,700                | 36,000        | 370                  |
| 48       | 100           | 4,000,000      | 1,000,000    | 90                   | 200           | 390                  |
| 72       | 130,000       | 3,140,000      | 3,510,000    | 350                  | 1,280,000     | 1,670                |

*All untreated, no salt.

The data from test III for casings naturally contaminated with salmonellae and treated with crystalline salt are presented in Table 4. Hog casings were the most heavily contaminated of the types tested. Salmonellae were virtually eliminated from the hog casings after 21 days of storage at 6 C. Salmonellae in the sheep and beef casings were eliminated after 7 days in salt packs.

The bactericidal effect of glycerin-salt or sorbitol-salt solutions was not as great as that of salt alone for the hog casings (Tables 5, 6). After 21 days of storage, salmonellae were detected in 1 of the 30 10-g samples in both solutions. No salmonellae were detected after 7 days in the sheep casings stored in the solutions.

DISCUSSION

The bactericidal properties of acetic acid, beyond its influence as an acidulant, are obvious when the destruction of salmonellae by acetic acid- and citric acid-adjusted brine solutions are compared. The buffering capacity of the casings was considered in the initial adjustment of the brine pH with acetic acid, but was not taken into account with citric acid since the commercial source of citric acid-adjusted salt was used as supplied.

It has been shown previously that acetic acid more effectively inhibits salmonellae than other commonly used acidulants such as hydrochloric, citric, tartaric, gluconic, malic, lactic, and succinic acids (1). Obviously, this information implies that the cidal effects are due to more than pH alone. The effect of the sodium hydroxide at the initial sampling period appeared greater than acetic acid, but this greater kill was due to the lag time in analyzing the alkaline-treated casings. The discrepancy between the total count results and Salmonella
numbers for untreated casings at the initial sampling period in Table 1 was the result of holding the samples overnight before analyzing for total bacteria. This illustrates the destructive capacity of brine alone.

The treatments involving pH adjustment of the brine were performed on artificially contaminated casings. Information pertaining to the natural levels of Salmonella contamination must be used in evaluating bactericidal processing steps. The segment of the study involving naturally contaminated samples revealed that the levels of salmonellae are relatively low. If casings are treated with acidic brine adjusted with acetic acid or packed in salt, the food poisoning significance of natural casings would be minimal as compared to other raw meat and poultry products.

For rapid treatment of casings, acetic acid brine would render naturally contaminated casing virtually free of salmonellae within 24 h. Storage of casings thus treated in salt would contribute to further reduction in the numbers of salmonellae. If a processor chooses to use the more traditional approach of salting alone, casings could be expected to be Salmonella negative after 2 to 3 weeks. The use of glycerin-salt or sorbitol-salt solutions would have nearly the same effect as dry salting, but the manipulations involved in handling the casings would be considerably greater.

There would be essentially no Salmonella risk with natural casings moving in international trade because of the lengthy exposure of the casings to saturated brine solutions. There may be some risk entailed in using casings fresh from the cleaning and finishing process, since no salting or other kill treatment would be employed.

In conclusion, the appropriate treatment of natural casings contaminated by salmonellae with acetic acid or sodium hydroxide pH-adjusted brine solution or crystalline salt would effectively destroy salmonellae. Such treated

| Casing type | Total no. samples | Day 0 (unsalted) | Day 7 (salted) | Day 14 (salted) | Day 21 (salted) |
|-------------|-------------------|-----------------|----------------|----------------|----------------|
| Hog         | 30                | 10^1 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 |
| Sheep       | 30                | 22 12 5 1      | 3 0 0 0       | 1 0 0 0       | 0 0 0 0       |
| Beef        | 10                | 4 0 0 0       | 0 0 0 0       | 0 0 0 0       | 0 0 0 0       |

* Grams.

| Casing type | Total no. samples | Day 0 (untreated) | Day 7 (treated) | Day 14 (treated) | Day 21 (treated) |
|-------------|-------------------|-----------------|----------------|----------------|----------------|
| Hog         | 30                | 10^1 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 |
| Sheep       | 30                | 22 12 5 1      | 3 0 0 0       | 3 0 0 0       | 1 0 0 0       |

* Grams.

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|-------------|-------------------|-----------------|----------------|----------------|----------------|
| Hog         | 30                | 10^1 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 |
| Sheep       | 30                | 22 12 5 1      | 1 0 0 0       | 4 0 0 0       | 1 0 0 0       |

* Grams.

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| Sheep       | 30                | 22 12 5 1      | 3 0 0 0       | 1 0 0 0       | 0 0 0 0       |
| Beef        | 10                | 4 0 0 0       | 0 0 0 0       | 0 0 0 0       | 0 0 0 0       |

* Grams.

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|-------------|-------------------|-----------------|----------------|----------------|----------------|
| Hog         | 30                | 10^1 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 |
| Sheep       | 30                | 22 12 5 1      | 3 0 0 0       | 3 0 0 0       | 1 0 0 0       |

* Grams.

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|-------------|-------------------|-----------------|----------------|----------------|----------------|
| Hog         | 30                | 10^1 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 | 10 1 0.1 0.01 |
| Sheep       | 30                | 22 12 5 1      | 1 0 0 0       | 4 0 0 0       | 1 0 0 0       |

* Grams.
casings would be insignificant in terms of a Salmonella infection risk to the public.

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