Salmonellae From Animal Byproducts

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FROM THE STANDPOINT of public health, salmonellae have long been considered as comprising one of the most significant groups of micro-organisms. All of the more than 800 known serotypes are potential pathogens. Salmonellosis is one of the most widespread problems confronting today's public health worker. Because of inadequate reporting methods, we have no real way of evaluating the problem of salmonellosis. Conservative estimates of the Public Health Service place the probable number of cases at 20 times the reported number (1).

Investigations of salmonellosis outbreaks frequently result in the incrimination of poultry, poultry products, or foods containing eggs. In fact, domestic poultry is considered to be one of the largest reservoirs of Salmonella organisms. Large animals, such as cattle and swine, are also commonly found to be infected. Control of salmonellae in these animal reservoirs and the breaking of the routes of transmission to human beings is challenging.

Modern methods of poultry and large-animal production would appear to tend to reduce the prevalence of infection. However, the increasing number of isolations from such animal sources indicates that this is not occurring.

The possibility of large animals and poultry coming in contact with salmonellae in nature is greatly reduced by the confining methods used in modern meat and egg production. In many instances, poultry maintained for egg production is encaged, and the birds have access to only that which is provided for them. Under such conditions, where the problem of salmonellae exists, the possibility of infection from contaminated feeds seems worthy of consideration.

It is common practice to process the offal, blood, feathers, and hair from poultry and large-animal slaughtering operations and to reclaim the protein in these materials for use as feed supplement. Reclaiming is done by heating the offal to facilitate removal of the grease and moisture and milling the solid residue. The product is a common source of protein supplement for both large-animal and poultry feeds. Since Salmonella organisms can normally be found in offal, the potential hazard in such a procedure is evident.

This study was made to evaluate the effectiveness of the procedures followed in the processing and use of offal. It was also an effort to demonstrate the significance of the use of animal byproducts in the spread of salmonellae.

When offal is processed, two finished products result. The first is a meal derived from the rendering and milling of the solid offal. This completed product is commonly referred to as byproducts meal. Its protein content is approximately 50 percent. The second product is made from the blood and feathers of poultry or the blood and hair from large animals. This is commonly referred to as blood meal or blood and feather meal, and its protein content averages 85 percent.

The reduction process begins with the cooking of the raw offal. The scrap is cooked under steam pressure for periods ranging from 6 to 10 hours. Moisture is removed from the material either by allowing it to escape to the atmosphere or by the use of vacuum. The material is then dumped into open vats and allowed to drain and cool.

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Grease is removed and salvaged by passing the cooked offal through a press or expeller. At this point the moisture content of the material ranges from 3 to 7 percent. The solid residue from the press is in the form of meat cracklings. These cracklings are milled, screened, and bagged for storage or shipping.

The procedures followed in the processing of blood, hair, and feathers are similar to those used in processing offal, except that since grease removal is not necessary, the dried material is conveyed directly from the cooker to a storage area for cooling and milling.

Materials and Methods

This study was initiated by investigating the facilities and handling practices followed in 11 byproduct plants in the State of Maryland over a period of approximately 18 months.

In order to demonstrate the significance of the use of contaminated byproducts in poultry feed, feeds containing contaminated supplement were fed to *Salmonella*-free laying hens.

Samples of byproducts, droppings from the hens, eggs, and waste water from byproduct processing were examined for salmonellae using the following materials and methods.

Byproduct materials and feeds. One 50-gram specimen of byproduct material or feed was transferred to a sterile Waring Blendor jar fitted with a threaded screwcap top and 120 ml. of sterile phosphate buffer with a pH of 7.2 was added. After blending for 1–3 minutes at approximately 8,000 rpm, 100 ml. of the mixture of sample and diluent was transferred to a sterile 250–ml. Erlenmeyer flask fitted with a sterile cotton and gauze stopper and incubated for 2 hours at 35° C. After 2 hours' incubation, 100 ml. of double-strength selenite F enrichment medium (*2*) containing 10 mg. per liter of cystine, as recommended by North and Bartram (*3*), was added to the flask and shaken until a homogenous suspension was obtained. This was then reincubated for an additional 18–24 hours at 35° C.

Chicken droppings. One 5-gram specimen of chicken droppings was added to a sterile 16-oz. widemouth glass bottle, with frosted ground glass stopper, containing approximately 40 glass beads 6 mm. in diameter and 100 ml. of

single strength selenite F. The mixture was shaken vigorously for 2–3 minutes and incubated at 35° C. for 18–24 hours.

Waste water. Approximately 200 ml. of processing plant waste water was added to a 250-ml. sterile centrifuge bottle and centrifuged for 15 minutes at approximately 3,000 rpm. The water was decanted and the sediment added to selenite F enrichment broth. The mixture was incubated for 18–24 hours at 35° C.

Eggshells. In an attempt to isolate salmonellae from the outside of the eggs of laying hens, proved by culture to be excreting *Salmonella* organisms, approximately one-half of the 624 available eggs were swabbed with a sterile cotton-tipped swab wetted in nutrient broth. This broth was then added to an equal quantity of selenite F enrichment medium and incubated 18–24 hours at 35° C.

Egg slurries. All of the 624 eggs used in this study were scrubbed with soap and water and placed in 70 percent ethyl alcohol for 3 minutes. They were removed to a wire rack and flamed. Eight to ten eggs, depending on size, were broken into a sterile Waring Blendor jar and blended for 2 minutes at approximately 8,000 rpm. A total of 52 slurry specimens were prepared, each containing from 8 to 16 eggs. In some instances it was necessary to combine the eggs from two Waring Blendor jars to make one specimen.

A 100- to 500-ml. aliquot of each blended egg specimen was added to an equal quantity of double-strength selenite F-cystine, shaken until thoroughly mixed, and incubated for 18–24 hours at 35° C.

The plating media employed for the isolation of *Salmonella* organisms from the byproduct materials, chicken droppings, eggs, and processing plant waste water were bismuth sulfite (BiS) agar, brilliant green (BG) agar, *Salmonella-Shigella* (SS) agar, and brilliant green agar containing 80 mg. per liter of sodium sulfadiazine (BGS). All were prepared from dehydrated media in accordance with the manufacturer's directions, with the exception of BGS. The required amount of sodium sulfadiazine was added to the dehydrated brilliant green agar when prepared.

After incubation for 18–24 hours, each specimen of byproduct material, chicken droppings,

eggs, and waste water was streaked to BiS, BG, SS, and BGS plates.

The SS and BG plates were incubated for 18–24 hours and the BiS and BGS for 36–48 hours. Colonies on the BGS were small and atypical after 18–24 hours, and the BiS showed little growth of salmonellae in 18–24 hours.

All plates were streaked with a 6- to 8-mm. wire loop. The first one-third of the plate was streaked heavily with strokes of the loop close together. The strokes were then gradually widened until the entire surface was covered. In positive specimens, well-isolated colonies of *Salmonella* usually resulted, regardless of the number of organisms present. Dilutions of the enrichment medium at 1 : 100 and 1 : 1,000 were prepared in sterile phosphate buffer on the majority of specimens and streaked to the four isolation media in the same manner as the undiluted specimen. *Salmonella* organisms were isolated from these dilutions on one occasion when not isolated from the undiluted specimen.

The *Salmonella-Shigella* and brilliant green plates were examined for suspicious *Salmonella*-like colonies after 18–24 hours, the brilliant green agar with sodium sulfadiazine and bismuth sulfite plates after 48 hours. If colonies were present, 15 to 20 were picked to triple sugar iron agar slants and incubated for 18–24 hours.

The triple sugar iron agar slants showing typical *Salmonella* reactions after 18–24 hours' incubation were subjected to complete antigenic analysis in accordance with the procedure recommended by Edwards and Ewing (4). Complete antigenic analysis was done to insure valid identification and to obtain epidemiologic information. A representative number of biochemical studies were performed to confirm serologic findings. The number of biochemical tests depended upon the particular serotype under study.

Too few samples of contaminated water were examined to justify any conclusions. The number of salmonellae isolated from the broth of the swabbed contaminated eggs and the egg slurries was quite small, and further investigation is necessary to determine the efficacy of this procedure.

One or more *Salmonella* serotypes were isolated from 43 of the 71 offal specimens; 6 of the

34 specimens of chicken droppings; 1 of the processing plant waste water specimens; and 2 of the 52 specimens of egg slurries.

Each of the four isolation media used in this study had certain advantages and disadvantages. Bismuth sulfite agar was perhaps the most reliable and specific for the *Salmonella* group of organisms. Only rarely does a typical colony picked from this medium fail to confirm. Brilliant green agar was quite satisfactory for isolation of salmonellae provided the microbiological flora was relatively free of coliforms, *Proteus*, and non-lactose fermenting enteric organisms. It was of little value with specimens that were grossly contaminated with other enteric organisms. Brilliant green agar with sodium sulfadiazine was valuable in grossly contaminated specimens, as it limited the growth of a number of the non-lactose fermenting, non-pathogenic enteric organisms. *Salmonella-Shigella* agar was perhaps the least inhibitory of the four media used, but the differentiation of *Salmonella* colonies from those of *Proteus* and other non-lactose fermenting enteric organisms was difficult. By using all four media, the isolation of salmonellae from byproducts and similar products is far less difficult than to rely on one or two plating media.

To determine if the egg slurries would support the growth and multiplication of *Salmonella* organisms, the following procedure was carried out.

Serial dilutions of an 18-hour broth culture of *S. binza*, which had been isolated from offal, were prepared in standard phosphate buffer so that 1 ml. contained 1–5 organisms, as determined by plating on Standard Methods agar (SMA). One ml. of the dilution containing 1 to 5 *Salmonella* organisms was inoculated into 100 ml. of egg slurry. After 2 hours' incubation at 35° C., 1 ml. of the inoculated slurry was plated to Standard Methods agar and incubated for 18 hours. The plates were examined, but no colonies were present. After the inoculated egg slurry had been incubated for a total of 18 hours at 35° C., 1 ml. was plated to SMA, and, after 18 hours' incubation at 35° C., the *Salmonella* count was 1.6×10^7 organisms.

One ml. of the dilution just described containing *S. binza* was inoculated into a mixture

of 50 ml. of egg slurry and 50 ml. of double-strength selenite F-cystine enrichment. The mixture was incubated for 2 hours at 35° C. and 1 ml. plated to Standard Methods agar. No colonies developed. After 18 hours' incubation at 35° C., 1 ml. was plated to SMA, after serial dilution, and the *Salmonella* count was 7.5 x 10^7 organisms. A representative number of the colonies from the SMA were confirmed as salmonellae.

An attempt was made to isolate salmonellae from 10 of the 43 positive specimens of byproducts by streaking directly to isolation media before incubation. No growth was obtained on any specimen. In addition, these specimens were incubated 3 hours in selenite F-cystine, pre-enriched in lactose broth for 3–5 hours, and then streaked to isolation media. All specimens gave negative results.

Ten specimens of byproducts found to be negative for *Salmonella* organisms by the regular procedure were re-examined after pre-enrichment in lactose broth for 5 hours. After this period of incubation selenite F-cystine was added to equal quantities of lactose broth and byproducts and reincubated for an additional 16–18 hours. No salmonellae were isolated from any specimens. Two ml. of the 5-hour incubated lactose broth and byproducts was added to approximately 15 ml. of selenite F and incubated for 18–24 hours at 35° C. and streaked to the four isolation media. No salmonellae were isolated.

Results

In every byproducts plant included in this study, the temperature of the processed material was in excess of 180° F. when removed from the cooker. At this point, because of the high temperatures, samples of the material were consistently negative when examined for salmonellae. However, 8 of the 11 plants examined were found to be producing byproducts meal or blood meal containing viable *Salmonella* organisms.

This report reflects primarily activities in one plant where our efforts were concentrated, because it was easily accessible and large enough to operate continuously.

Samples of the material collected at various points in the reduction process indicated that the first point where *Salmonella* organisms could be recovered from the byproduct materials in this plant was at the crackling storage areas. (See diagram.) Samples scraped from the storage area floors were found to be positive. From this point on in the plant in question, every piece of equipment along the processing line contained viable *Salmonella* organisms.

Since the meat scrap material was essentially sterile after leaving the cooker but contaminated with salmonellae following additional processing, the problem was to determine how the material was being contaminated. Airborne contamination could hardly be a major contributing factor because of the large numbers of organisms observed. Since the moisture content of the material was as low as 7 percent, growth of salmonellae within the material itself hardly seemed possible. The metal equipment could not be suspected as incubation sites because of lack of moisture. This left only the storage area surfaces to suspect. In the plant in question, investigation of one storage area revealed that, on occasion, water dripped from the floor above, providing the moisture necessary for bacterial growth. This condition was corrected by repairing the leaks in the ceiling. Since the volume of material stored in this area was small, metal containers were provided for the cracklings.

Another crackling storage area on the bottom floor of the building caused greater difficulties. The reduction plant was operated in conjunction with a large slaughtering operation. The waste water from the eviscerating floor, consisting of scrub-up water which contains ponch contents and other materials unfit for rendering, is treated in holding basins where the solids are settled and the grease floated to the top and removed. The treatment operation is carried out in a subbasement in a room adjoining the crackling storage area. The effluent from the water treatment is discharged into the public sewer. The discharge line passes beneath the floor of the room housing the crackling storage area. Occasionally the effluent line to the public sewer became clogged, and water backed up through a floor drain onto the storage floor.

A retaining wall was built to separate the stored material from the floor drain, and the

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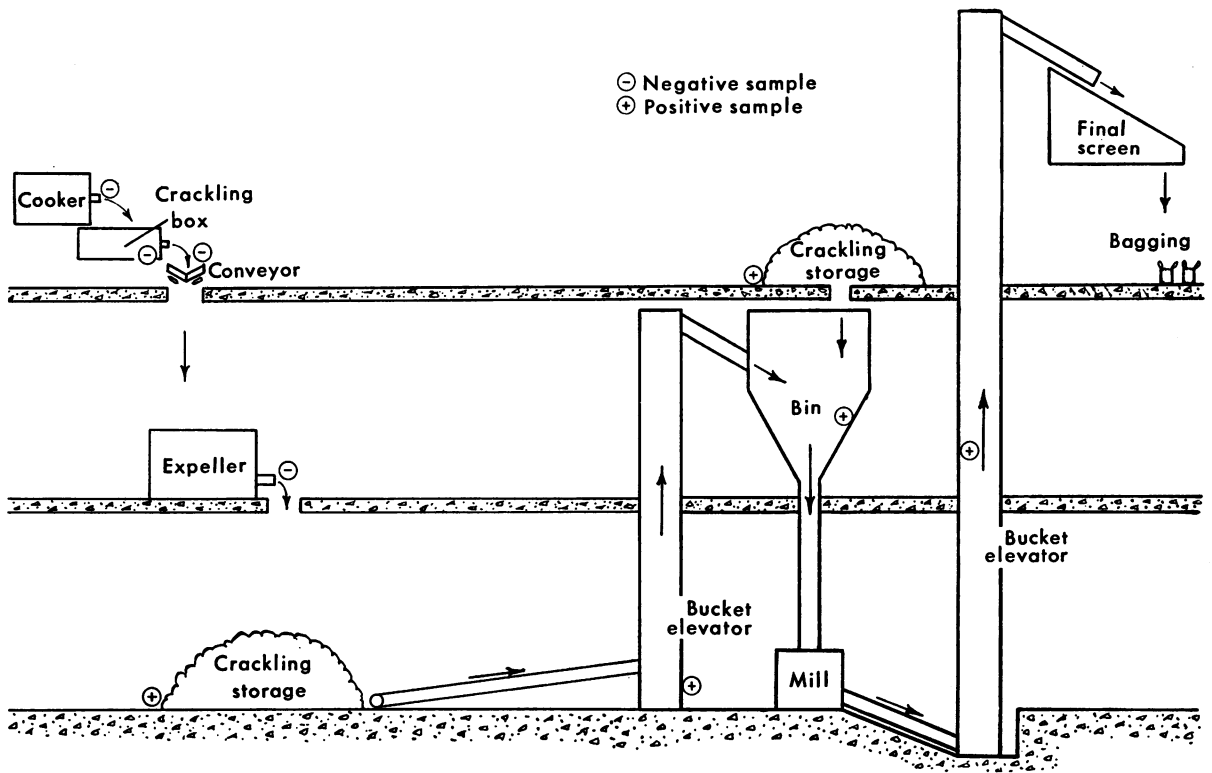
Flow diagram of offal reduction plant indicating sampling points for Salmonella organisms

drainage system corrected to reduce the possibility of flooding. The floor and walls of the storage area were thoroughly cleaned and heavily chlorinated. Samples taken following this sanitizing showed the materials throughout the processing line to be free of salmonellae. This floor, however, remained damp owing to its low elevation, and examination of samples taken 2 weeks later showed that floor scrapings from beneath the stored cracklings and the finished products again contained salmonellae.

Following the isolation of salmonellae from byproduct protein supplement, a bank of laying cages was prepared with individual trays to catch the droppings from each compartment. Twelve leghorn pullets were purchased and placed in the cages. Droppings from the pullets were examined over a period of 2 weeks to ascertain if they were free of salmonellae. During this period and for sometime thereafter, the pullets were fed a high protein-mash which contained vegetable-base protein instead of meat byproduct meal. They were kept on this feed until they came into full egg production; at

that time four of the pullets were removed, isolated, and kept on the uncontaminated feed. The remaining eight pullets were fed laying mash containing contaminated meat scrap supplement.

After being fed commercially prepared mash containing contaminated supplement for a period of 1 week, the caged layers were found to be excreting *Salmonella* organisms. After 2 weeks two hens had diarrhea and were excreting salmonellae freely. Several hens without diarrhea were found to be excreting salmonellae sporadically.

Both the shells and the yolks of the caged layers' eggs remained free of salmonellae. Droppings from the hens being fed uncontaminated feed remained free of salmonellae.

Eight other hens, free of salmonellae, were housed loose in a small enclosure and forced to use a common laying nest. These hens were fed laying mash containing contaminated byproduct meal originating in a poultry offal reduction plant.

The shells of eggs from the hens which were

housed loose were found to contain *S. worthington* after the hens had been fed contaminated feed for 1 week. Eggs laid by these hens during the second week were held unrefrigerated for 1 additional week before being examined. *S. worthington* was then isolated from both shells and yolks.

During the investigation, a total of 71 byproduct samples were examined. From these, 94 *Salmonella* isolations were made involving 27 serotypes, as shown in the table.

Thirty-four specimens of droppings from the caged layers were examined for *Salmonella* organisms. *S. anatum* was isolated twice, and *S. binza*, *S. cerro*, *S. derby*, *S. heidelberg*, and *S. indiana* one time each. Two specimens of water from the processing plant were examined for salmonellae, and *S. minnesota* was isolated from one specimen.

Fifty-two specimens of egg slurries were examined, and from these *S. worthington* was isolated twice.

It was not the primary purpose of this study to isolate multiple types from a single specimen; therefore, only a limited number of colonies were picked and completely identified. If the number of colonies had been increased from 20 to 100 per specimen, the increase in the number of serotypes would probably have been greater.

Conclusions

A complete evaluation of the significance of such a commercial source of salmonellae is beyond the scope of this limited study. The number and sizes of samples were restricted because of the shortage of laboratory time available for research, which is usual in an agency charged with routine responsibilities. In addition, only one aspect—that of direct contamination of eggs through the use of contaminated feed—was dealt with.

This protein source also is used in feeds for other animals, and there is created, through the use of contaminated supplements, a vicious circle where offal from infected animals is being reclaimed and fed to uninfected animals. The very handling of material containing salmonellae by the employees of byproduct plants, by persons engaged in feed blending, and by farm-

Salmonella serotypes isolated from 71 samples of byproduct materials

Serotype	Number times isolated	Serotype	Number times isolated
alachua	1	minnesota	2
anatum	1	montevideo	1
bareilly	1	newington	1
binza	2	oranienburg	2
bredeney	2	ruiru	1
cerro	9	saint-paul	2
chester	1	schwarzengrund	4
derby	14	senftenberg	1
give	1	tennessee	3
heidelberg	1	thomasville	2
illinois	15	thompson	2
indiana	5	worthington	3
johannesburg	5		
kentucky	1	Total	94
livingston	11		

ers using such feeds is hazardous. Since many byproduct plants are operated in conjunction with slaughtering and processing operations, the possibility of airborne contamination of food in close proximity may also be significant.

In the past interest in the preparation and use of such materials has appeared to be lacking. Little, if any, control has been exercised over the processing of offal, and little effort has been made to assure the absence of salmonellae in such products.

It is evident that protective storage of rendered materials prior to milling is imperative. It is also apparent that, when adequate protection is provided, it is possible to produce *Salmonella*-free protein supplement.

A change in attitude seems to be needed. Once offal is heat processed and salvaged, it ceases to be offal and becomes a food. From that point on, it must be handled as such. Among many processors of animal byproducts, such discernment is lacking.

Summary

Plants which reduce offal to reclaim protein for use as a feed supplement were investigated because salmonellae are normally found in offal. Eight of 11 offal reduction plants in Maryland were found to be producing supplements containing salmonellae. Unprotected storage of

the offal following cooking and rendering was responsible for contamination in one plant.

Byproduct samples, droppings from hens, eggs, and waste water used in byproduct processing were examined. The laboratory methods for isolation of salmonellae varied in accordance with the needs for the particular material being examined. A total of 94 isolations (27 *Salmonella* serotypes) were made from 71 samples of byproducts examined. One or more *Salmonella* serotypes were also isolated from 6 of 34 specimens of chicken droppings, 1 sample of plant waste water, and 2 of 52 specimens of egg slurries.

To demonstrate the significance of the use of poultry feeds containing salmonellae, hens found to be salmonellae-free were placed on feeds containing contaminated supplements. After 1 week, hens confined in laying cages were diarrheal, droppings were found to contain salmonellae, but eggs were free of salmonel-lae. Other hens, housed loose, were also fed on contaminated feed. Salmonellae were isolated from the shells of their eggs, and after 1 week of unrefrigerated storage, from the egg yolks.

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Recommends Influenza Vaccination

The Surgeon General's Advisory Committee on Immunization Practices has noted that the U.S. influenza experience during 1964–65 was relatively limited. The last major epidemic of type A influenza occurred in 1962–63 in most of the country and in 1963–64 on the west coast.

In view of the 2- and 3-year periodicity of the disease, the committee anticipates a greater incidence of influenza in the coming season. Areas which experienced fairly heavy outbreaks last winter may expect to be less affected in the coming year.

The committee reiterated previous general recommendations for vaccination of persons in the following groups, which have experienced high mortality from epidemic influenza.

• Persons with chronic debilitating diseases, including diseases of the heart and circulatory systems, the lungs, and the metabolic system.

• Older age groups, based on three successive recent epidemics in which mortality increased moderately among persons over 45 years of age and markedly among those over 65.

• Pregnant women, among whom mortality increased during the 1957–58 epidemic, although similar increases have not been noted in subsequent epidemics.

• Patients residing in nursing homes, chronic disease hospitals, and other environments in which crowded living arrangements may lead to more rapid spread of the disease.

The committee urged that vaccination begin in September and be completed by mid-December for maximum effectiveness during the winter season. Persons who have not received influenza vaccination since July 1963, when the last major change was made in vaccine composition, should receive two doses with an interval of approximately 2 months between them. They noted, however, that even a single dose gives significant protection.