Isolation of Salmonellae from Pork Carcasses

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Four hundred and twenty pork carcasses from four abattoirs were examined for the presence of salmonellae by use of swabbing-enrichment techniques and contact plate methods. Carcasses from only one abattoir were found to be contaminated by swabbing-enrichment (23.3%) and contact plate (17.9%) methods. The area of the skin side of the ham, near the anal opening, was determined to be the area to examine for isolating salmonellae from pork carcasses with the greatest frequency. The most frequently isolated species of salmonellae in this study were Salmonella derby, S. anatum, S. typhimurium, and S. indiana.

The isolation of salmonellae from meat animals intended for food has been of interest since the first report on swine plague (2). Several authors have reported on the occurrence of salmonellae in meat products (4, 7-9, 11, 15, 26) and the use of various techniques to recover microorganisms, including salmonellae, from the carcasses of meat animals (11, 16, 18-20, 21, 26).

Methods for assessing the bacterial content on the surface of raw foods are varied. Swabbing is one of the earliest and continues to be one of the most widely used methods because of its versatility. Limitations of swabbing techniques were evaluated by Douglas (5) and Green and Herman (12). Variations of the swabbing techniques are discussed by Green et al. (13) and Walter (25). Dyett (6) proposed using a sharp, sterile knife to scrape the whole, exposed carcass surface and making an estimate of the number of microorganisms in the scrapings by a direct microscopy count or by total viable count.

Direct-surface agar plating was described by Angelotti and Foter (1), and application for sampling meat surfaces was described by L. ten Cate (3). Hall and Harnett (14) described a disposable plastic dish so constructed as to permit direct-contact plating of surfaces.

The purpose of this study was to determine the incidence of salmonellae on pork carcasses in local abattoirs, to find the location on the carcasses most likely to be contaminated with salmonellae, and to report the species of salmonellae found.

MATERIALS AND METHODS

Four hundred and twenty pork carcasses from four different abattoirs were examined, after chilling, for the presence of salmonellae by swabbing an area approximately 25 cm² on the skin surface of the ham near the anus and by placing a contact plate on the analogous area on the other half of the same carcass. Swabbing was accomplished by wetting a cotton swab in sterile, physiological saline, swabbing the designated area thoroughly, and then replacing the swab in the tube containing 10 ml of 0.85% sterile saline for transportation to the laboratory. Samples (1 ml) from the tubes containing swab were transferred into tetrathionate brilliant green (TET) broth (10) and incubated at 37 C for 24 h. After incubation, samples of the TET broth were streaked upon brilliant green agar (BGA, Difco) plates which were then incubated for 24 to 36 h at 37 C. Colonies showing typical growth of salmonellae on BGA plates (11) were inoculated into triple sugar-iron agar (Difco) and incubated at 37 C for 24 h, and those tubes showing positive reactions (10) were sent to the Southeastern Salmonella Serotyping Laboratory, Atlanta, Georgia, for positive species identification.

Contact plates, as described by Hall and Harnett (14), filled with BGA and bismuth sulfite agar (BSA; Difco) were applied to the surface of the carcasses in such a manner as to assure full and complete contact between the medium and the surface being examined. These plates were then transported to the laboratory and incubated at 37 C for 24 to 36 h, and suspected colonies from plates showing positive growth of salmonellae were treated as described above for the swabs in saline tubes.

In a subsequent experiment, 91 pork carcasses from abattoir A were examined at three locations: (i) on the skin surface of the ham near the anus; (ii) on the skin of the jowl; and (iii) on the flesh side of the jowl, for a total of 273 samples. In this experiment, adjacent areas on the same carcass were selected for examination by swabbing and contact plates, thus eliminating variability between halves of carcasses. The isolates obtained were analyzed for salmonellae as described above.
RESULTS AND DISCUSSION

In a survey of pork carcasses from four different abattoirs, salmonellae were recovered from only one establishment (Table 1). None of the abattoirs surveyed obtained hogs from the same farms. Although they did receive hogs from the same general areas or locales on a regular basis, none of these areas overlapped. Galton et al. (9) concluded that there may be true regional variations and attributed these to (i) differences in the incidence of salmonellae infection in hogs, (ii) variation in the nature or control of processing procedures employed, or (iii) climatic factors affecting the viability or multiplication of salmonellae in the environment of abattoirs. Cross-infection of healthy pigs by infected pigs from individual farms may occur in the holding pens (11, 17), leading to a continuing incidence at a particular abattoir. The most probable source of salmonellae is the animal from which the meats were obtained (4).

Carcasses from abattoir A were examined on nine different occasions, or days, throughout a 12-month period, and salmonellae were found on all occasions. The greatest incidence of salmonellae was recovered from carcasses examined on sampling day 4. (Table 1). There was no apparent reason for the large number recovered at this particular sampling time. Total numbers of bacteria on all carcasses examined from abattoir A were in the order of $10^4$ to $10^5$ organisms per 25 cm$^2$ of surface. No differences in total numbers were noted from those carcasses from which salmonellae were isolated.

No salmonellae were recovered from abattoirs B, C, and D where 100, 65, and 34 carcasses, respectively, were examined. This indicates that the isolation of salmonellae from pork carcasses is not a simple, routine matter. Although no actual data were collected, it was noted that abattoirs B, C, and D were receiving hogs from geographic areas different from those of abattoir A.

To determine their relative effectiveness in recovering salmonellae from pork carcass, the techniques of swabbing and the use of contact plates filled with BSA and BGA were compared. Results (Table 1) show that contact plates recovered salmonellae from 40 (17.9%) carcasses examined compared with 52 (23.3%) by swabbing and enrichment techniques. On only 3 sampling days were more salmonellae recovered by contact plates than by swabbing-enrichment techniques. On 5 other sampling days, more salmonellae were recovered by swabbing-enrichment than by contact plates, and on three occasions salmonellae were not recovered at all by contact plates. Salmonellae were recovered by contact plates and swabbing from the same carcass in 12 instances. One important disadvantage of contact plates is that they are not representative of the entire carcass and only reflect the area that they touch. Either method of recovery may be satisfactory when the problem is of a gross nature. Recovery of salmonellae by contact plates seemed to depend upon the medium for recovery. Of 40 contact plates from which salmonellae were isolated, 26 of the isolations came from plates containing BSA and 14 came from plates containing BGA. Many of the plates containing BGA were overgrown. The increased efficiency of BSA or BGA for recovering salmonellae could be due to the greater inhibition of microflora by BSA. Taylor (23) found that, when the ratio of coliforms to salmonellae approached 50:1, the appearance of a typical, well-isolated salmonella colony with its identifying characteristics for a given medium becomes the exception. In these overcrowded areas, the salmonellae are rarely able to disclose their distinguishing characteristics. However, Banwart and Ayres (2), by using pure cultures of salmonellae, found that BGA supported more luxuriant growth of the six species tested, whereas BSA was inhibitory to four. If a processor of pork carcasses desired to monitor the incidence of salmonellae on carcasses by the contact plate method, BSA would appear to be the medium of choice. BSA may require 24 to 48 h to develop characteristic growth of salmonellae.

The incidence of salmonellae from pork carcasses processed by abattoir A, 17.9% by contact plates and 23.3% by swabbing-enrichment technique, is much lower than the figure of 56% reported by Weissman and Carpenter (26) for

| Day | Carcasses examined (no.) | Incidence of salmonellae | | | |
|-----|----------------|-----------------|-----|-----|-----|
|     | | Contact plates | Swabbing | | |
| 1   | 10 | 3 | 0 | | |
| 2   | 20 | 2 | 8 | | |
| 3   | 20 | 0 | 7 | | |
| 4   | 40 | 23 | 19 | | |
| 5   | 40 | 7 | 3 | | |
| 6   | 24 | 1 | 4 | | |
| 7   | 25 | 0 | 3 | | |
| 8   | 17 | 0 | 4 | | |
| 9   | 25 | 4 | 4 | | |
| TOTAL | 221 | 40 (17.9%) | 52 (23.3%) | | |
this same establishment. This decrease probably is due to extensive changes in slaughtering and processing techniques instituted in this particular plant in the interim between the periods of time when the two sets of data were collected.

Recovery of salmonellae from three sampling locations on pork carcases (Table 2) was greater from the area examined on the skin side of the hams, near the anal opening, than from the skin or flesh side of the jowl for both methods of recovery, contact plate and swabbing-enrichment technique. No salmonellae were recovered by contact plates on either the skin or flesh side of the jowl, probably because of the difficulty of obtaining a satisfactory area for application of the plate in the case of the flesh or inside of the jowl. Salmonellae were recovered by swabbing-enrichment technique on both sides of the jowl, but with higher incidence of recovery from the skin side or outside. It might be expected that the jowl or neck area would be subject to greater contamination because of washings from the entire carcass fouling that area during processing. Koelensmid and van Rhee (16) found that water that drips from the skin of pork carcases after singeing is not sterile. They isolated salmonellae from five samples of scrapings taken from 50 carcases after inspection by veterinarians. In a study on beef, Mulcock (18) found the greatest number of bacteria on neck tissues (10^9) compared with sides (10^5) after incubation at 22 C for 5 days. Patterson (20) reported that, in sheep and cattle, contamination acquired during the butchering process is not spread evenly over the carcass. He found greater numbers on the brisket than on the foreleg or rump; yet he concluded that sites of heaviest contamination will vary from one abattoir to the next depending upon methods employed, washing, and other treatments used. Galton et al. (9) examined cultures from anal swabs from living and slaughtered hogs and swabs from sides of pork carcases and found over a third of the samples to be positive for salmonellae. Weissman and Carpenter (26) concluded that no single area of a pork carcass was likely to be more contaminated than another, but suggested examining the area of the ham near the anal opening as the area of choice because of the possibility of fecal contamination. Results of this study (Table 2) also indicate that this would be the most useful area to examine when monitoring pork carcases for salmonellae.

Table 3 lists the species of salmonellae isolated from pork carcases in this study. The three most frequently isolated species, S. derby, S. anatum, and S. typhimurium, are common among isolates of salmonellae found in red meats. The presence of these organisms might be expected because the Center for Disease Control regularly lists these organisms among the 10 most commonly reported from nonhuman sources. However, isolation of S. indiana from red meats has been reported only once since 1967. Six isolations of S. indiana from swine have been reported during the same period. S. indiana is more commonly isolated from poultry and egg products (24).

The serotypes isolated were not uniformly spread over the 9 sampling days. S. typhimurium and S. anatum were more frequently isolated on days 1 to 5. All of the S. indiana serotypes were isolated on day 4, whereas S. derby was isolated on days 5 to 9. Other serotypes listed were isolated on various days throughout the sampling period.

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