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The incidence and level of contamination of British fresh sausages and ingredients with salmonellas

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SUMMARY

A five-tube most probable number (MPN) method, with pre-enrichment and enrichment stages, was used in a study of the incidence of salmonella contamination of British fresh sausages and the ingredients used in their manufacture. All samples were taken from a large factory in the course of routine production. There was an incidence of 65% contamination of pork ($n = 20$) and 55% ($n = 20$) in pork and beef sausages. The incidences of contamination of uncooked ingredients varied from 95% for mechanically recovered meat ($n = 20$) to 10% for another type of meat. Cooked and/or dried ingredients were rarely contaminated with these organisms and, when contamination occurred, coliforms were also important.

The numbers of salmonellas isolated ranged from 7–40 for pork sausages, from 8–24 for beef and pork sausages and from 0·8–378 organisms/g for ingredients.

The following salmonella serotypes were isolated (ranked in descending order of incidence): *S. derby*, *S. dublin*, *S. newport*, *S. stanley*, *S. typhimurium*, *S. heidelberg*, *S. infantis* and *S. agona*.

INTRODUCTION

Although many surveys have shown that various serotypes of *Salmonella* can occur in abattoirs and meat processing plants (Anon, 1964; Barrell, 1982; Chau, Shortridge & Huang, 1977; Kampelmacher *et al.* 1961; Weissman & Carpenter, 1969), there is little information on the level of contamination of meat products with these organisms. Indeed it has been suggested (Kilsby & Pugh, 1981) that undue emphasis has been given to the results of surveys based on qualitative rather than quantitative methods. The British fresh sausage has been cited as a case in point (Anon, 1975). Thus the survey of 3309 samples of sausages and sausage meat by Roberts *et al.* (1975) established an average incidence of contamination of *ca.* 30% (range, 1·7–60%) and demonstrated the occurrence of a variety of salmonella serotypes in these products; it provided only vague clues about the levels of contamination. The same situation obtained in two recent surveys of fewer samples; Turnbull & Rose (1982) reported an incidence of contamination of 2·9% and Barrell (1982) one of 16·4%. Although this evidence suggests that sausages have the potential to disseminate salmonellas among the human population, they are rarely implicated in cases of food poisoning (Jones *et al.* 1964). Indeed only ten outbreaks of food poisoning were attributed (Anon, 1975) to sausages in the

period, 1967–72, immediately preceding the extensive survey by Roberts *et al.* (1975).

The good public health record of sausages has been associated with the widespread use of sulphite (Dyett & Shelley, 1962, 1966), a preservative that is permitted at a concentration of 450 μg/g of fresh sausage. The effectiveness of sulphite diminishes during storage of sausages because some is oxidized and some is rendered ineffective through binding by as yet uncharacterized products of meat and microbial origin (Banks & Board, 1982*a*). Moreover it has been demonstrated recently that sulphite acts as a bacteriostatic rather than a bactericidal agent towards salmonellas (Banks & Board, 1982*b*). Thus the available evidence does not support fully the opinion of Dyett & Shelley (1962, 1966) and there is a need to establish not only the incidence but also the levels of contamination of sausages with salmonellas because it is well established (Bryan, 1979) that these organisms need to occur at numbers of 10^5 or more before they are likely to cause illness in humans who are not otherwise debilitated.

This communication presents the results of a survey of the incidence and level of contamination of ingredients and sausages with salmonellas. It needs to be stressed that all samples were taken in a large factory under normal conditions of manufacture. Thus the results are novel in that they cover a stage that is commonly omitted in surveys of abattoirs and meat produced therein and ignored by those who obtain their samples from shops.

MATERIALS AND METHODS

Sampling and isolation of salmonellas

Samples of pork sausage, pork and beef sausages and all the ingredients used in their manufacture were obtained from a large factory during normal production in the period 2 March 1980 to 5 April 1981. One kg of pork meats were taken from material awaiting processing. The meat came from pigs slaughtered at the factory; butchered meat was stored at 4 °C overnight. Cattle were neither slaughtered nor butchered on the site and 1 kg samples were taken from blocks of frozen, de-boned beef. Samples of rusks, seasoning, polyphosphate (Fibrisol V/10), rinds and linked sausages were examined also. All samples were stored at 4 °C and examined within 3 h of collection. To minimize sampling errors, all the meats were comminuted with a sterile mincer (Kenwood A901, England). Of each sample 60 g and 540 ml of buffered peptone water containing 1 % w/v peptone (BPW) were blended in a Colworth Stomacher 400 (Seward, London) for 60 s. Five subsamples (each of 100 ml) of the homogenate were distributed in bottles. Five sub-samples (each of 10 ml) were added to 90 ml of sterile BPW in bottles and shaken for 10 s, and 5 sub-samples of 1 ml of homogenate were added to 99 ml BPW and shaken for 10 s. These dilutions were used for pre-enrichment. They were incubated at 37 °C for 24 h and, after all bottles had been shaken vigorously, 1 ml samples were transferred to tetrathionate (Difco) broth (9 ml amounts) and these subcultures incubated at 43 °C for 48 h. At 24 and 48 h, loopfuls of the enrichment cultures were streaked out on dried surfaces of Brilliant Green Agar (BGA; Oxoid CM329), Bismuth Sulphite Agar (BSA; Oxoid) and Desoxycholate Citrate Agar (DCA;

Difco according to the methods of Edel & Kampelmacher (1969). These plates were incubated at 37 °C for 48 h, with colonies of presumptive salmonellas being transferred to Plate Count Agar (PCA; Oxoid) after 24 and 48 h.

Characterization of salmonellas

Pure cultures on PCA were inoculated into Kohns I and II media (Oxoid) and the API 20E series (API, Andover, Hants). Isolates presumptively identified as salmonella were characterized further by the biochemical tests of Edwards & Ewing (1972) and analysis of somatic and flagellar antigens using slide and tube agglutinations with sera from Burroughs Wellcome (Beckenham, Kent). The most probable number (MPN) of salmonella/g sample was established, with McCrady's (1915) tables, only after the complete characterization of all isolates.

Isolation of Enterobacteriaceae

Samples of minced meat weighing 20 g, other ingredients and sausages were homogenized (Colworth Stomacher) in 180 ml of quarter-strength Ringers solution and decimal dilutions made in this solution. Appropriate dilutions, 1 ml, were mixed with 15 ml of Violet Red Bile Agar (VRB; Oxoid) cooled to 45 °C. When set, the surface of the agar was overlaid with 10 ml of VRB and incubated at 37 °C for 24 h. Colonies of lactose-fermenting organisms (deep red colonies surrounded by a halo of precipitated bile salts) were presumptively identified as coliforms. Samples of 1 ml were added also to 15 ml of Violet Red Bile Glucose Agar (VRBG; Oxoid) cooled to 45 °C. When the agar had set, its surface was overlaid with 10 ml of VRBG and incubated at 30 °C for 24 h. Deep red colonies with a halo of precipitated bile salts caused by acid from glucose breakdown were presumed to be members of the Enterobacteriaceae. All counts were done in triplicate. Colonies with the characteristics noted above were selected randomly from Petri dishes containing the lowest countable dilution and purified by plating on PCA. Gram-negative, oxidase-negative, fermentative bacilli were tentatively identified with Enterobacteriaceae. They were characterized futher by the methods of Edwards & Ewing (1972) and the API 20E and API 50 CHE systems (API, Andover, Hants).

Statistical analyses

A Hewlett–Packard 97 calculator and appropriate programs were used to do simple linear regressions and Spearman's rank correlation.

RESULTS

The MPN system given in the Materials and Methods section was adopted following extensive preliminary work in which comparison was made with 0·1 % (w/v) peptone water and buffered 1 % peptone water for pre-enrichment, selenite-cystine and tetrathionate broths for enrichment with incubation at 37 or 43 °C.

Although salmonellas were isolated frequently (Table 1) from all the meat ingredients other than back-fat of pork sausages as well as the finished product, the actual levels of contamination were low (3–40 salmonellas/g ingredient). Thirty

Table 1. *Contamination of ingredients and pork sausage with salmonella*

Samples	Sausage	Lean pork	Belly meat	Head meat	Semi-lean meat	Rinds
Number tested	20	15	20	20	20	20
Number positive	13	6	7	2	7	6
% positive	65	40	35	10	35	30
Mean MPN/g	20	24	21	7	20	11
MPN range for positive samples	7–40	11–40	7–23	3–11	11–37	6–24

Table 2. *Contamination of ingredients and pork and beef sausage with salmonella*

Samples	Sausage	Beef flank	MRM*	Head meat	Rinds†
Number tested	20	20	20	20	10
Number positive	11	4	19	6	1
% positive	55	20	95	30	10
Mean MPN/g	17	11	265	23	0·8
MPN range for positive samples	8–24	8–17	60–378	8–40	—

* Mechanically-recovered meat.
† Heat-processed, dried rinds.

Table 3. *Separate isolations of salmonella from ingredients and pork sausage*

Organism	Lean pork	Belly meat	Head meat	Semi-lean meat	Rinds	Sausage
S. derby	4	3	1	4	—	12
S. dublin	4	3	1	2	—	9
S. newport	2	2	—	1	1	5
S. heidelberg	3	—	—	—	2	5
S. stanley	2	2	—	1	—	5
S. infantis	—	—	—	—	4	4
S. typhimurium	2	—	—	—	—	2
S. agona	—	1	1	—	—	2
	17	11	3	8	7	44

Table 4. *Separate isolations of salmonella from ingredients and pork and beef sausage*

Organism	MRM*	Head meat	Beef flank	Rinds†	Sausage
S. derby	8	—	1	—	9
S. newport	5	1	1	—	7
S. stanley	4	2	1	—	6
S. dublin	5	2	—	—	6
S. typhimurium	3	—	1	1	4
S. heidelberg	3	1	—	—	4
S. infantis	2	—	—	—	2
	30	6	4	1	38

* Mechanically recovered meat.
† Heat-processed, dried rinds.

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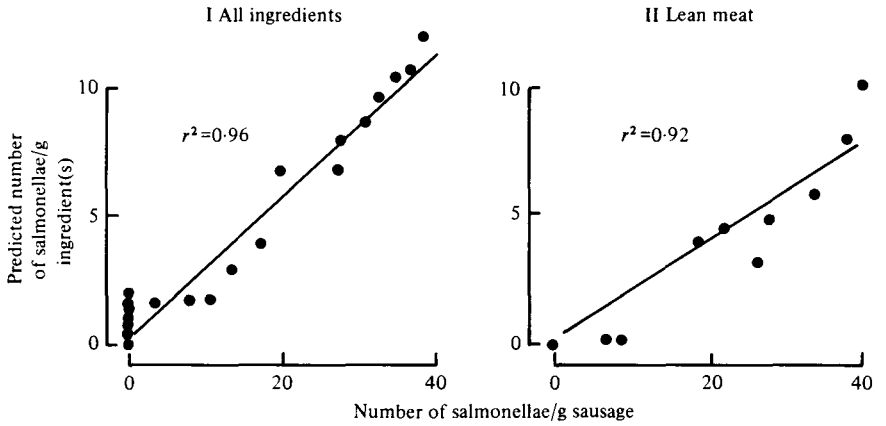

Fig. 1. Linear regression of predicted number of salmonellas from ingredients with number of salmonellas recovered from pork sausage. Regression coefficients (data points not shown): Semi-lean meat, $r^2 = 0{\cdot}44$; rinds, $r^2 = 0{\cdot}07$; head meat, $r^2 = 0{\cdot}03$ and belly meat, $r^2 = 0{\cdot}15$.

percent of the cooked and comminuted rinds contained salmonellas but these organisms were not isolated from the non-meat ingredients (rusk, spices, polyphosphates) of pork sausage. Salmonellas were isolated from all the meat ingredients other than pork back-fat of pork and beef sausages (Table 2) but in low numbers only, viz 8–40/g of beef flank and pigs' head meat. In contrast, 95% of the mechanically recovered meat (MRM) contained 60–378 salmonellas/g. One sample of dried rinds but no samples of the non-meat ingredients of pork and beef sausages yielded salmonellas.

Of the eight salmonella serotypes isolated from ingredients and sausages, *S. derby* and *S. dublin* (Tables 3 and 4) occurred most frequently. *S. agona* was isolated on four occasions only and *S. infantis* was the only serotype recovered from the cooked rinds. The incidence of serotypes (7 out of the 8 isolated) was the highest with the mechanically recovered meat.

On no occasion was a serotype isolated from sausages without it being recovered also from one or more of the ingredients used to produce that particular batch. On several occasions, however, serotypes were isolated from ingredients but not from sausages containing the ingredients. In such instances, the level of contamination was low or the ingredient was used in small amounts only.

As the level of salmonella contamination of ingredients and the latter's contribution to the sausage were known, it was possible to compare the predicted and the observed levels of contamination of pork sausages providing there were at least two of these organisms/g of ingredient. With this type of sausage, the predicted contribution by all ingredients and the observed levels of contamination were in accord (Fig. 1); a linear regression line described the data with a coefficient of determination, $r^2 = 0{\cdot}96$. In addition there was a good correlation ($r^2 = 0{\cdot}92$) of the predicted contribution by lean pork alone and the observed levels of salmonella contamination of sausages. As all the other meat ingredients gave low r^2 values (footnote to Fig. 1), it was concluded that their contribution to salmonella contamination of pork sausages were subordinate to that of lean pork, the principal

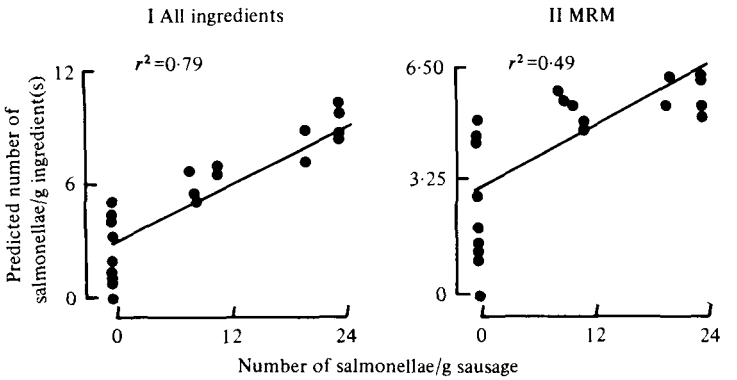

Fig. 2. Linear regression of predicted number of salmonellas from ingredients with number of salmonellas recovered from pork and beef sausage. Regression coefficients (data points not shown): Beef flank, $r^2 = 0{\cdot}20$ and head meat, $r^2 = 0{\cdot}42$.

Table 5. *Extent of contamination of ingredients and pork sausage with enterobacteria and coliforms*

Sample	Number of samples tested	Enterobacteria ($\log_{10}$ c.f.u./g) $\bar{x}$	Enterobacteria ($\log_{10}$ c.f.u./g) σ	Coliforms ($\log_{10}$ c.f.u./g) $\bar{x}$	Coliforms ($\log_{10}$ c.f.u./g) σ
Sausage	20	3·26	0·10	2·92	0·10
Lean pork	15	4·01	0·51	2·96	0·33
Semi-lean meat	20	3·82	0·36	3·16	0·29
Head meat	20	3·64	0·37	3·31	0·32
Belly meat	20	3·42	0·27	2·81	0·36
Rinds	20	3·17	0·45	2·49	0·42
Fat	20	2·58	0·38	1·94	0·37

$\bar{x}$, mean of three replicates; σ, standard deviation of three replicates.

meat ingredient. An acceptable correlation ($r^2 = 0{\cdot}79$) of the predicted and observed contamination of ingredients and pork and beef sausages respectively was also noted (Fig. 2). In this instance, the threshold level of contamination of an ingredient was 5 salmonellas/g (*cf.* 2/g for pork sausages). Possible reasons for this higher value are considered in the Discussion. The observations summarized in Figs. 1 and 2 direct attention at the role of dilution in diminishing the level of contamination of a product by an ingredient that is relatively heavily contaminated. This was particularly notable with MRM, the ingredient of pork and beef sausages with the highest level of contamination with salmonellas (Table 2) and the greatest variety of serotypes (Table 4). In practice, the extent of dilution of this material was such that there was a poor correlation ($r^2 = 0{\cdot}49$) of the predicted and observed levels of contamination of a product.

Enterobacteriaceae and coliform organisms were present in all the samples of meat and sausages but none of the non-meat ingredients of pork sausages (Table 5). There was little variation in the levels of contamination of sausages with these organisms but a pronounced variation with ingredients, especially whole pieces of meat (Figs. 3 and 4). It was concluded that the former reflected ran-

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Table 6. *Extent of contamination of ingredients and pork and beef sausage with enterobacteria and coliforms*

Sample	Number of samples tested	Enterobacteria ($\log_{10}$ c.f.u./g) $\bar{x}$	Enterobacteria ($\log_{10}$ c.f.u./g) σ	Coliforms ($\log_{10}$ c.f.u./g) $\bar{x}$	Coliforms ($\log_{10}$ c.f.u./g) σ
Sausage	20	1·73	0·24	1·44	0·12
Mechanically recovered meat	20	3·89	0·19	3·35	0·25
Head meat	20	3·80	0·25	3·26	0·35
Beef flank	20	3·27	0·52	2·81	0·59
Fat	20	1·99	0·48	1·69	0·41
Rinds	10	0·60*	—	0·48*	—

* One sample only positive.
$\bar{x}$, mean of three replicates; σ, standard deviation of three replicates.

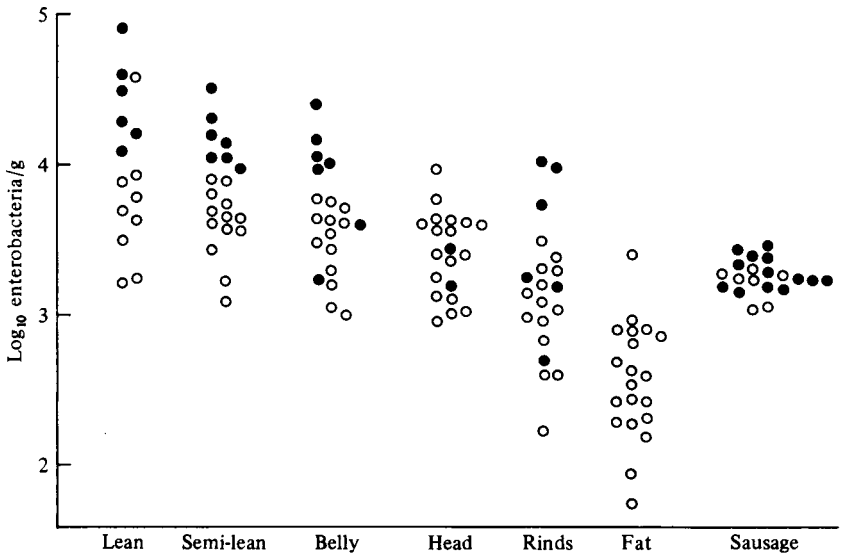

Fig. 3. Relationship between numbers of Enterobacteriaceae and presence of salmonella in ingredients of pork sausage. ○, 60 g sample negative for salmonella, ●, 60 g sample positive for salmonella.

dom contamination resulting from the vigorous mixing of ingredients in sausage production and the latter contagious contamination resulting from contact of meat with dirty equipment, etc. In addition, dilution of contaminants was again noted. Thus MRM, the ingredient containing the largest number of coliform organisms, was used only in pork and beef sausages which contained fewer of these organisms than pork sausages. Only one sample of a dry ingredient, rinds, of pork and beef sausages contained coliforms and this was the sample from which *S. typhimurium* was isolated (Table 6).

In general, the Enterobacteriaceae count (VRBG medium) appeared to be a more reliable index of possible salmonella contamination of sausages and ingredients

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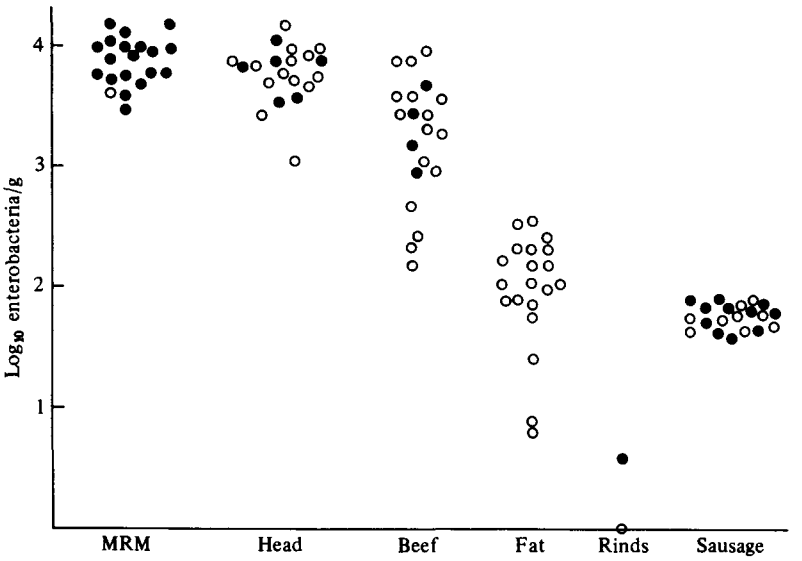

Fig. 4. Relationship between numbers of Enterobacteriaceae and presence of salmonella in ingredients of pork and beef sausage. ○, 60 g sample negative for *Salmonella*, ●, 60 g sample positive for *Salmonella*.

than was the coliform count (VRB medium). This is evident in the results obtained with the lean, semi-lean pork and belly meat used in pork sausages (Fig. 3). There was no apparent association between the level of contamination of head meat, cooked rinds and pork and beef sausages with Enterobacteriaceae (Fig. 4). As would be expected, Enterobacteriaceae in a heat-processed, dry material such as the rinds used in beef and pork sausages provides evidence not only of post-processing contamination but, judging from our observations (Table 2), a warning that food poisoning organisms such as salmonellas may have gained access to the product.

DISCUSSION

In order to achieve an overall perspective of salmonella contamination of a commodity such as British fresh sausage, the results of surveys of the finished product must be considered together with observations on the precision of quantitative methods, variations due to season and causes of contamination of ingredients. Finally, of course, the behaviour of the food poisoning organism in a product post-manufacture has to be considered also. As we had to use the MPN method to enumerate salmonellas, the number of replicates per dilution and the precision of the methods have to be considered in the context of the work involved. Indeed the number of samples that had to be examined dictated the use of 5 tubes per dilution in the work discussed in this report. As the standard error of the logarithm of the MPN is inversely proportional to the square root of the number of tubes at a single dilution (Cochran, 1950), it might be anticipated that a higher incidence, and maybe a higher level, of salmonella contamination of sausages would be demonstrated by doubling the number of tubes per dilution. In practice, there was no appreciable difference in the results presented above and

those obtained in a limited survey (Pain, 1981) using 10 tubes/dilution. Indeed, as the latter survey was done in the late autumn/winter, it showed a diminishing incidence of contamination of ingredients and sausages with salmonellas and thus confirmed the many observations (e.g. Roberts *et al.* 1975) that ambient temperature influences salmonella contamination of meat and meat products. Additional confidence in the MPN method was given by our observations that a serotype recovered from sausages was invariably isolated also from one or more of the ingredients.

Apart from dissemination of salmonellas of gut origin through a pig's tissues at slaughter (Kampelmacher *et al.* 1961), puncturing of the gut at evisceration can be an important cause of contamination of a particular carcass (Kampelmacher *et al.* 1961) and a source for cross contamination in subsequent processing (Kampelmacher *et al.* 1961; Chau *et al.* 1977). Indeed, it has been shown that a 'comet tail' type of spread of salmonellas follows the processing of a contaminated carcass until the organisms are eventually diluted out from the system. In this instance, the distribution of micro-organisms will exhibit greater clustering (contagious contamination) than would be expected to exist in a truly random one. In other instances, a process such as the washing and de-hairing of pigs' carcasses or the mincing of material obtained therefrom, can provide not only a longer term source of infection but also opportunities for the contaminants to be randomly rather than contagiously spread in or on meat products. Indeed the influence of mincing or some other form of comminution on the distribution of contaminants has been demonstrated by Kilsby & Pugh (1981). Moreover, the variation in the viable counts of organisms, be they salmonellas or coliforms, decreases as the contaminants become more randomly distributed. Conversely the apparent mean of the counts will increase with a more random spread of organisms. These influences were noted in this study also (Tables 5 and 6) and account for the differences between the observed levels of contamination of the major sources of salmonellas and the predicted level in the final product (Figs. 1 and 2).

The present study has shown that the influence of dilution must be taken into account also when considering the relative importance of ingredients as causes of contamination of a product. Thus although MRM contained the largest numbers and greatest variety of salmonellas, the use of small amounts (e.g. 1·7 % of sausages) meant that its contribution to the contamination of the product was negligible when compared with belly meat (cf. Tables 1 and 2). Indeed, the latter would appear to be the ingredient that ought to be monitored routinely in sausage manufacture when the objective was to minimize contamination of the product with salmonella. The viable counts of Enterobacteriaceae appeared to be poor indices of salmonella contamination of the majority of uncooked ingredients included in this study (head meat and backfat in pork sausage and MRM, head meat, beef flank and backfat in pork and beef sausage). From the view point of management in a factory, enterobacteria are of particular value as would be expected in the routine examination of processed ingredients. Thus there was a good correlation of Enterobacteriaceae contamination of rinds and dried rinds with the recovery of salmonella. Moreover, the isolation of *S. infantis* from one ingredient only, the cooked rinds, may indicate that a human carrier rather than pigs was the source of this serotype.

Although the survey of Roberts *et al.* (1975) included both sulphited and

unsulphited sausages, the small number of samples (312) of the latter did not permit an assessment of the possible influence of the preservative on salmonellas in the commodity mainly because the survey was qualitative in nature. Our observations that the actual was invariably greater than the predicted level of contamination of sausages with salmonellas can be taken in part as evidence that sulphite had little if any bactericidal action on these organisms during the manufacturing stage. Moreover the low and unpredictable levels of contamination of sausages with salmonellas means that samples taken from a factory cannot be used in studies of the fate of these organisms during storage. It was for these reasons that we (Banks & Board, 1982*b*) deliberately inoculated sausage meat with small numbers of salmonellas resistant to rifampicin – this property allowed the quantitative recovery of the organisms without recourse to the MPN method. In the context of the results of the survey given in the present paper, two important observations were made: (1) the size of populations of rifampicin-resistant *S. virchow* did not change in either sulphited or unsulphited sausage meat stored at or below 9 °C whereas extensive growth occurred in unpreserved material at 15, 20 and 25 °C, and (2) at least 20 μg free SO_2/g was required to maintain salmonellas in the quiescent state when sausage meat was stored at room temperature.

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