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The effect of feeding pigs on food naturally contaminated with salmonellae

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INTRODUCTION

The finding that organic supplements of animal food such as bone meal, meat meal, meat and bone meal and fish meal of non-European origin are frequently contaminated with salmonellae (Muller, 1952, 1957; Richter, 1956; Rohde & Bischoff, 1956; Thal, Rutqvist & Holmqvist, 1957; Walker, 1957; Report, 1959; Kovacs, 1959) has awakened interest in the possibility that they may be an important source of salmonella infection in animals and, indirectly, in man. Most of this interest has centred around pigs because they are commonly fed on diets containing such supplements, and because pig meat is the meat most commonly incriminated in outbreaks of food poisoning in man.

The salmonella serotypes found in the mesenteric lymph nodes of pigs in Britain (Smith, 1959) are similar to those found in food supplements in this country (Report, 1959). In Northern Ireland it has been shown that, in some cases, the salmonella serotypes found in the faeces of pigs were similar to those found in their food (Newell, McClarin, Murdock, MacDonald & Hutchinson, 1959). Therefore, the course of events following the feeding of salmonella-free pigs with food naturally contaminated with salmonellae has been studied. The results are reported in this paper.

MATERIALS, METHODS AND PLAN OF EXPERIMENT

The examination of food supplements for salmonellae

In order to obtain suitable naturally contaminated foods, a variety of methods were used in examining material. These included incubation of varying amounts of supplements in varying amounts of selenite broth (Hobbs & Allison, 1945) for periods of 1–4 days at temperatures of 37° to 43° C. Tetrathionate broth (Oxoid C.M. 29) and brilliant green MacConkey broth (Smith, 1959) were also used as an enrichment media, the brilliant green broth being included because selenite and tetrathionate broths are unsuitable for cultivating *Salm. cholerae-suis* (Smith, 1952, 1959). Sub-cultures were made from the enrichment media on to desoxycholate-citrate agar (Hynes, 1942) containing 1% each of sucrose and salicin, and on to bismuth sulphite agar (Oxoid C.M. 201).

In addition, thin suspensions of supplements were made in distilled water, agitated in a shaking machine for 5–10 min., the coarser particles allowed to settle and the supernatant fluid filtered through Whatman No. 1 filter-paper. The filtrate

was centrifuged at 3000 r.p.m. for 30 min. in an M.S.E. Major centrifuge and the deposit suspended in 0·5–1·0 ml. of distilled water. This material was cultured on desoxycholate-citrate agar and bismuth sulphite agar both directly and after enrichment in selenite broth and tetrathionate broth. Several hundred grams of supplement could be conveniently examined at any one time by this method.

Colonies suspected of being salmonellae were submitted to slide-agglutination tests with Polyvalent O antiserum (Standards Laboratory, London, N.W. 9) either directly from the plates of solid media or after sub-culturing. Those that agglutinated were tested against other appropriate antisera and their fermentation reactions determined. All those classified as salmonellae were submitted to the Salmonella Reference Laboratory, London, N.W. 9 for final identification.

As a result of these examinations, it was decided to use a consignment of Angola fish meal and of Pakistan bone meal in the feeding experiments.

Salmonella-free pigs

For the supply of salmonella-free pigs a self-contained herd was sought in which the food was home-mixed and in which the only supplement of animal origin was British white fish meal, which is much less likely to be contaminated with salmonellae than imported varieties. A herd in this category was discovered and over a period of 3 months the mesenteric lymph nodes from 40 pigs coming from this herd were collected at a bacon factory and examined for salmonellae with negative results. Four litters of 8-week-old pigs were then selected from this farm and one pig from each was killed and its lymph nodes examined, again with negative results. Twenty-four pigs from the four litters referred to above were transferred to the laboratory premises; of these, twenty were housed in accommodation in which no pigs had been kept for 9 months and which had been thoroughly disinfected. The remaining four pigs, used as controls, were housed in a separate building approximately 50 yards away and were looked after by a different attendant. The housing and methods of feeding and cleaning were comparable to those used in the commercial production of pigs.

The twenty experimental pigs were fed on the following diet *ad lib.*

British barley meal 58 %
British weatings 30 %
Angola fish meal 10 %
Pakistan bone meal 2 %
Vitamin A and D additive 1 lb./ton

The amounts of fish meal and bone meal in the ration approximated those employed in commercial pig production. The control pigs were fed on this diet but without the addition of the fish and bone meals.

Before the experiment was commenced, and at intervals of 2–6 days afterwards, the faeces of all the pigs were examined for salmonellae by inoculation of rectal swabs containing a liberal portion of faeces on to desoxycholate-citrate agar before and after incubation in selenite broth.

At appropriate intervals, fourteen of the experimental pigs were killed with a

humane killer and, with full precautions to avoid contamination, portions of the internal organs, alimentary contents, skin and peritoneal swabs were collected and examined for salmonellae. A serum sample was also examined for salmonella agglutinins.

At 50 days after the commencement of the experiment, the feeding of the infected diet was discontinued and the six surviving experimental pigs were given the same diet as the controls, the pens cleaned out and three of the pigs moved to fresh accommodation. These six pigs were killed at appropriate intervals and the usual examinations for salmonellae performed. The four control pigs were killed at 50 days and examined for salmonellae in the same manner as the experimental pigs.

The following materials were collected from the pigs for examination for salmonellae, the approximate amount examined, where applicable, being shown in brackets:

Skin swab
Peritoneal swab
Liver (20 g.)
Bile (8 ml.)
Spleen (20 g.)
Lung (10 g.)
Kidney (20 g.)
Submaxillary and retropharyngeal lymph nodes (all)
Mesenteric lymph nodes (60 g.)
Hepatic lymph nodes (all)
Small intestinal contents (8 ml.)
Large intestinal contents (8 ml.)
Caecal contents (8 ml.)
Rectal contents (8 ml.)
Fore-limb muscle (20 g.)
Hind-limb muscle (20 g.)

Examination of material from pigs for salmonellae

Organs such as mesenteric lymph nodes and liver were dipped in ethyl alcohol, a fresh pot of alcohol being used for each organ, flamed, and the process repeated. Previous studies (Smith, 1959) had shown that this treatment was adequate to deal with any possible surface contamination with salmonellae. Finally, the organs were ground in a sterile mortar with sterile sand. A loopful of this material was inoculated on to desoxycholate-citrate agar and the remainder incubated in 120 ml. of selenite broth for 24 hr. at 37° C. and sub-cultured on to desoxycholate-citrate agar. Fluid material such as bile and faeces received no treatment before culture.

Only half of the suspension of mesenteric lymph nodes was examined in the first place; the other half was retained for further examination to gain an impression of the degree of infection should the first half prove to be positive for salmonellae.

Examination of sera for antibodies

Various salmonella O-suspensions were prepared from strains so selected that these agglutinable suspensions contained, between them, the principal O-antigens of all the salmonellae isolated from the infected food materials. Slide agglutination tests were then performed using these suspensions and the sera obtained from the pigs.

RESULTS

Salmonella content of the fish meal and bone meal

Two salmonella serotypes were found in the Angola fish meal, *Salm. orion* and *Salm. blockley*. Cultivating graded amounts of this material in selenite broth indicated that the probable number of salmonella organisms present was 50 per 100 g., the ratio of *Salm. orion* to *Salm. blockley* being 10:1.

The Pakistan bone meal was cultured in enrichment media on numerous occasions and was found to contain three serotypes, *Salm. adelaide*, *montevideo* and *anatum*, the probable number of organisms present being 700 per 100 g. However, by the filtration method, an additional thirteen serotypes were found, *Salm. stanley*, *reading*, *derby*, *typhi-murium*, *richmond*, *tennessee*, *newport*, *blockley*, *dublin*, *meleagridis*, *portsmouth*, *poona* and *cubana*. Some serotypes were only found by enrichment of the centrifuged deposit, others by enrichment and direct culture and one, *Salm. dublin*, by direct culture only. Bismuth sulphite agar was better than desoxycholate-citrate agar for direct culture and tetrathionate broth better than selenite broth for enrichment.

Salmonellae were not found in the barley meal, the weatings or the vitamin supplement.

The effect of feeding pigs on the contaminated food

During the 50 days they were fed on the contaminated food, all pigs appeared well and made normal weight gains. At post-mortem examination no macroscopic lesions were found in the alimentary tract and other organs.

The results of bacteriological examination of the organs of the pigs killed at different times after the commencement of the experiment are shown in Table 1. Salmonellae were found in the mesenteric lymph nodes only; none was isolated from examinations of the sub-maxillary, retropharyngeal and hepatic lymph nodes, the liver, bile, spleen, kidney, lung and muscle. No salmonellae were isolated from the skin and peritoneal swabs but they were found in the alimentary contents, caecal or rectal, of three pigs.

The chances of salmonella infection of the mesenteric lymph nodes increased with the duration of feeding the contaminated food. For example, those taken from the six pigs killed between the 2nd and the 12th day after the commencement of the experiment contained no salmonellae but two of the four killed between the 18th and the 37th days and all four killed between the 39th and the 50th day were infected. Only one of the six pigs killed after the feeding of the contaminated food was discontinued was found to be harbouring salmonellae in the mesenteric lymph nodes. This pig, killed 8 days after the discontinuation of the contaminated diet, was one of the three pigs moved to fresh accommodation.

The numbers of salmonella organisms in the mesenteric lymph nodes and alimentary contents of the seven infected pigs were very small and were isolated by indirect culture and not by direct culture. In four of these pigs it was necessary to culture approximately 10 g. of ground-up mesenteric lymph nodes in selenite

broth before a positive isolation was obtained. In another one, 2·5 g. was required and in the remaining two, 1·0 g.

No salmonellae were found in the four control pigs killed on the 50th day.

The result of fourteen examinations of the faeces of the experimental group of pigs during the 50-day period in which the contaminated diet was fed, and of examinations carried out on six occasions afterwards are shown in Table 2. Salmonellae were not found in the faeces of any of those pigs until the third examination, 14 days after the commencement of the experiment. Positive isolations were recorded from 19 of the 134 faecal specimens examined (14 %) from this time until

Table 1. *The isolation of salmonellae from pigs fed on contaminated food*

Pig no.	Days fed on contaminated food	Materials in which salmonellae were found
1	2	None
2	3	None
3	4	Rectum (*Salm. reading*)
4	6	None
5	9	None
6	12	None
7	18	M.L.N.* (*Salm. dublin*)
8	24	None
9	30	M.L.N. (*Salm. typhi-murium*)
10	37	None
11	39	M.L.N., rectum (*Salm. typhi-murium*)
12	44	M.L.N. (*Salm. typhi-murium*)
13	46	M.L.N. (*Salm. reading*)
14	50	M.L.N., caecum, rectum (*Salm. give*)
	Days after discontinuation	
15	8	M.L.N. (*Salm. typhi-murium* and *anatum*)
16	8	None
17	15	None
18	15	None
19	20	None
20	20	None

* M.L.N. = mesenteric lymph nodes.

the 50th day but not from any of the twenty specimens examined afterwards; no positive isolation was obtained by direct culture. Salmonellae were isolated on more than one occasion from four pigs but the type found on consecutive occasions was often not the same. For example, in pig No. 14, *Salm. anatum* was found in the faeces on the 19th day, no salmonellae on the 22nd and 25th, *Salm. poona* on the 29th, no salmonellae on the 32nd, 36th, 39th and 43rd day, *Salm. anatum* on the 46th and *Salm. give* on the 50th. *Salm. derby* was found in the faeces of pigs no. 19 on the 16th day, none on the 19th, 22nd, 25th and 29th, *Salm. dublin* on the 32nd and 36th, none on the 39th and 43rd, *Salm. stanley* on the 46th and *Salm. senftenberg* on the 50th day; no salmonellae were found either in the faeces of

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this pig 6, 10, 14, 15, 17 and 20 days after the use of the contaminated food was discontinued or in its mesenteric lymph nodes when it was killed.

The faeces of all four control pigs were examined on sixteen occasions during the 50-day period; no salmonellae were isolated.

No salmonella antibody was demonstrable in the sera of either the experimental pigs or the control pigs.

Table 2. *The isolation of salmonellae from the faeces of pigs fed contaminated food*

Days after commence-ment	No. of pigs examined	No. positive for salmonellae	*Salmonella* sp. isolated
0	20	0	—
5	17	0	—
10	15	0	—
14	14	3	Not typed
16	14	1	*Salm. derby* (19)
19	13	2	*Salm. amager* (11) *Salm. anatum* (14)
22	13	1	Not typed (8)
25	12	0	—
29	12	2	*Salm. tennessee* (20) Salm. poona (14)
32	11	1	*Salm. dublin* (19)
36	11	2	*Salm. dublin* (19) *Salm. enteritidis* (17)
39	10	2	*Salm. reading* (12) *Salm. typhi-murium* (11)
43	9	1	*Salm. derby* (12)
46	8	2	*Salm. stanley* (19) *Salm. anatum* (14)
50	7	2	*Salm. senftenberg* (19) *Salm. give* (14)
Days after discontinuation			
6	6	0	—
10	4	0	—
14	4	0	—
15	2	0	—
17	2	0	—
20	2	0	—

The figures in brackets are the identification nos. of pigs from which salmonellae were isolated.

The types of salmonellae found in the contaminated food and in the pigs

The different salmonella serotypes isolated from the fish and bone meals and from the faeces and mesenteric lymph nodes of the pigs are shown in Table 3. Eighteen different serotypes were found in the fish and bone meals, twelve in the faeces but only five in the mesenteric lymph nodes. Of these five, *Salm. typhi-murium* was most commonly found being present in four of the seven sets of positive

lymph nodes despite the fact that cultural studies indicated that it was probably present in the meals in much smaller numbers than most of the other serotypes.

Four of the five serotypes found in the mesenteric lymph nodes and ten of the twelve found in the faeces were also found in the meals.

Table 3. *The different salmonellae serotypes found in the food and in the pigs*

Serotype	Found in: Bone meal	Fish meal	M.L.N.*	Faeces
Salm. typhi-murium	+	−	+	+
Salm. reading	+	−	+	+
Salm. stanley	+	−	−	+
Salm. derby	+	−	−	+
Salm. montevideo	+	−	−	−
Salm. richmond	+	−	−	−
Salm. tennessee	+	−	−	+
Salm. newport	+	−	−	−
Salm. blockley	+	+	−	−
Salm. enteritidis	−	−	−	+
Salm. dublin	+	−	+	+
Salm. anatum	+	−	+	+
Salm. meleagridis	+	−	−	−
Salm. give	−	−	+	+
Salm. amager	−	−	−	+
Salm. orion	−	+	−	−
Salm. portsmouth	+	−	−	−
Salm. senftenberg	+	−	−	+
Salm. poona	+	−	−	+
Salm. cubana	+	−	−	−
Salm. adelaide	+	−	−	−

* M.L.N. = mesenteric lymph nodes.

DISCUSSION

Under normal farm conditions it is unlikely that many pigs would receive foodstuffs so heavily infected with salmonellae as those used in the present experiment. The degree of contamination of the bone meal used was higher than usual (Report, 1959) and the diet contained at least eighteen serotypes including *Salm. typhi-murium*, the most common serotype in food poisoning in man and also a cause of clinical disease in the pig. There are technical difficulties in isolating individual serotypes from a number of other serotypes in materials such as fish and bone meal and it is considered likely that the three additional serotypes isolated from the experimental pigs originated from these supplements; thus the number of serotypes to which the pigs were exposed was at least twenty-one.

No harmful effect from feeding this infected diet was noted in the experimental pigs. The principal salmonella pathogen in pigs in Britain is *Salm. cholerae-suis* (Field, 1958) but in spite of the fact that suitable methods were used for isolating this serotype (Smith, 1952, 1959) none was found. Even if *Salm. cholerae-suis* occurred as a dietary contaminant, this does not necessarily imply that clinical

infection would occur in pigs receiving it in the diet; *Salm. typhi-murium* caused no clinical disease in the present experiment. Also, in a separate observation, two 4-month-old calves were fed 1 oz. daily for a month of the bone meal containing *Salm. dublin* and *Salm. typhi-murium*, the two principal salmonella pathogens of calves, mixed in the food. The calves remained healthy and neither serotype was found in their faeces; nor were they isolated from any internal organ including the mesenteric lymph nodes at slaughter; no antibody to either serotype was demonstrated in their serum.

The present experiments indicate that little or no clinical disease appears in pigs fed on these salmonella-contaminated foodstuffs and this finding is supported by the results obtained from a survey (Smith, 1959) where a 12% isolation rate was achieved from healthy pigs at slaughter. The high rates of isolation obtained in the present work and the survey probably results from the relatively large amounts of tissue examined from each pig for in many cases the numbers of salmonella organisms present in the infected mesenteric lymph nodes was surprisingly low. However, the fact that salmonella organisms including *Salm. dublin* and *Salm. typhi-murium* may be introduced on to farm premises and might become established in pigs or other stock is a serious implication. Nevertheless, it would appear that the main danger of feeding salmonella contaminated foods to pigs lies in the risk of meat becoming contaminated by alimentary contents at slaughter and resulting in infection in man. There was no evidence to indicate that the meat of any of the edible organs became infected with salmonellae during life and there should be little risk from infected mesenteric lymph nodes because they are normally discarded at slaughter.

The dangers arising from the use of salmonella-contaminated food for pig feeding might be reduced or eliminated either by prohibiting the importation of supplements likely to be infected, or by their adequate bactericidal treatment. It is not within the scope of this paper to discuss such control measures but the results here indicate that the risk of spread to human beings could be reduced by withdrawing for a short period before slaughter supplements likely to be contaminated. This suggestion is made because none of the experimental pigs became *permanent* faecal excreters of salmonellae and after discontinuing the use of the contaminated food, no salmonellae were found in their faeces. In addition, only one of the six pigs killed after this period of time was harbouring salmonellae in its mesenteric lymph nodes. Since the last four pigs killed before the contaminated food had been withdrawn were found to be harbouring salmonellae in their mesenteric lymph nodes it is probable that these six pigs in question had also been infected, but that all except one of them had overcome the infection and remained salmonella-free in the absence of a source of re-infection.

SUMMARY

1. The course of events following the feeding of salmonella-free pigs on food naturally contaminated with salmonellae has been followed. The pigs were killed at varying times after the commencement of the experiment and their organs examined for salmonellae.

2. None of the pigs showed any signs of ill-health and no pathological lesions were observed in them when they were killed. Salmonellae were found, however, in very small numbers in the mesenteric lymph nodes of some of them but not in any of their other internal organs or in their muscular tissue. The longer the pigs were fed on the contaminated food the more likely were their mesenteric lymph nodes to be infected.

3. Salmonellae were isolated from time to time from the faeces of the pigs but there was no suggestion of any of the pigs becoming permanent faecal excreters of these organisms.

4. Six pigs were retained for a short time after the use of the contaminated food was discontinued. Salmonellae were never found in their faeces and when they were killed the mesenteric lymph nodes of only one of them was found to be infected.

5. The results are discussed from the agricultural and public health viewpoints.

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