THE ISOLATION OF SALMONELLAE FROM THE MESENTERIC LYMPH NODES AND FAECES OF PIGS, CATTLE, SHEEP, DOGS AND CATS AND FROM OTHER ORGANS OF POULTRY

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INTRODUCTION

Scott (1940) isolated salmonellae from the mesenteric lymph nodes of 3·8% of 1000 healthy pigs. Two similar surveys (Report, 1947, 1955), also carried out in Britain, yielded lower isolation rates, 2·5 and 0·4%, respectively. *Salmonella cholerae-suis* was the prevalent serotype found in Scott's survey but was only isolated once in the two later surveys. This difference may be due to the fact that selenite broth, which is unsuitable for the cultivation of *Salm. cholerae-suis* (Smith, 1952), was employed in these two surveys but not in Scott's. Since *Salm. cholerae-suis* is the most common cause of clinical salmonella infection in pigs in Britain (Field, 1958), it seemed worthwhile obtaining information of the extent to which this serotype is now present in our pig population using media suitable for its cultivation. The mesenteric lymph nodes from individual pigs were examined separately in order to gain a more accurate assessment than that obtained in the three previous surveys in which lymph nodes from several pigs were pooled. Additional impetus to carry out this survey was obtained from the observation (Walker, 1957; Report, 1959) that animal protein supplements fed to pigs in Britain are frequently contaminated with salmonellae.

The survey was made more comprehensive by examining mesenteric lymph nodes from healthy cattle and sheep killed at the same abattoir as the pigs and mesenteric lymph nodes from dead cats and dogs brought to a nearby knacker's yard. In the case of chickens the intestinal wall, the spleen and bile were examined; these animals do not possess mesenteric lymph nodes.

MATERIALS AND METHODS

Collection of lymph nodes and other organs

Lymph nodes from healthy pigs, cattle and sheep were collected at an abattoir in Chelmsford, Essex, during a period of 20 weeks. No more than twenty animals were sampled on any one day, the actual number, particularly in the case of cattle, often being much less.

Several mesenteric lymph nodes, together with the surrounding tissue, were removed from the viscera of each animal, placed in a separate sterile screw-capped jar, and kept at 3° C. until they were examined bacteriologically, this examination being performed within 2 days. The pigs and cattle had been reared in Essex but many of the sheep had been brought from other parts of Britain. The majority of

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the pigs were slaughtered within a few hours of arrival at the abattoir; the remainder were killed on the following day. It was not possible to collect the faeces from these pigs; instead, specimens from pigs exposed for sale at two markets near the abattoir were examined.

The mesenteric lymph nodes of dogs and cats were collected in the same manner as were those of the other animals, except that the whole chain of nodes draining the intestine were removed for examination. The cats and dogs had also lived in the Chelmsford area of Essex and their bodies had been brought to the knacker's yard for disposal. The majority of these animals were healthy but a small number had been destroyed because they were suffering from a variety of disease conditions. Because of this, either the liver, spleen or kidney of all cats and dogs included in the survey was cultured on desoxycholate-citrate agar to confirm that they were not suffering from clinical salmonella infection. Rectal swabs were also taken so that the incidence of salmonellae in mesenteric lymph nodes and faeces could be compared.

Material from chickens, all over 11 weeks of age, was collected at a poultry packing station in Essex and at a diagnostic laboratory which received dead chickens from many parts of Britain. The material collected consisted of about 10 in. of the small intestine and either the contents of gall bladder, the spleen or 10 g. of liver. The livers of all the chickens sampled at the diagnostic laboratory were examined in the same manner as those of the dogs to confirm that they had not died from salmonella infection.

Liquid enrichment media

Selenite broth and brilliant green MacConkey broth in 100 ml. quantities were used throughout; the latter was included specifically for the isolation of *Salm. cholerae-suis*.

Selenite broth. A modification of the original medium of Leifson (1936) was used, the lactose being replaced by mannitol (Hobbs & Allison, 1945).

Brilliant green MacConkey broth. This consisted of liquid MacConkey broth with the lactose replaced by mannitol and 1/5000 brilliant green added.

Solid media

Desoxycholate-citrate agar. Hynes's (1942) modification of Leifson's original formula was employed.

Brilliant green MacConkey agar. This medium consisted of MacConkey agar to which was added 1/10,000 brilliant green; apart from its sodium taurocholate content, it was identical to that found to be of value in isolating *Salm. cholerae-suis* from pig faeces (Slavin, 1943).

Technique of examination

After careful removal of the surrounding tissue, the lymph nodes from each animal were washed in running tap water, dipped in ethyl alcohol for 20 sec., flamed, and the process repeated. Finally, they were ground in a sterile mortar with sterile sand and distilled water. A fresh pot of alcohol was used for the glands

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from each animal. Equal amounts of the suspension were poured into selenite broth and brilliant green MacConkey broth. Before adoption, these procedures had been tested with lymph nodes dipped in dilute cultures of salmonellae and found adequate in preventing the survival of these bacteria. Other material examined, except faeces and bile, were treated in exactly the same manner as the lymph nodes. Pig faeces were enriched in both media and also inoculated directly on to brilliant green MacConkey agar; cat and dog faeces and bile were enriched in selenite broth only.

Following inoculation, the enrichment media were incubated at 37° C. for 24 hr. and then subcultured on to plates of desoxycholate-citrate agar which were incubated at 37° C. for 24 hr. and examined. Colonies resembling those of salmonellae were submitted to slide-agglutination tests with Polyvalent Salmonella O antiserum (Standards Laboratory, London, N.W. 9) and those that agglutinated were then tested against other appropriate antisera and their fermentation reactions determined. Those cultures classified as salmonellae were sent to the Salmonella Reference Laboratory for final identification.

RESULTS

The frequency with which salmonellae were found in the mesenteric lymph nodes of 500 pigs, 200 cattle and 100 sheep at the abattoir and of 200 dogs and 200 cats at the nearby knacker's yard is shown in Table 1. The results of examining the intestinal wall of 280 chickens, 200 from the packing station and 80 from the

Table 1. *The frequency with which salmonella were isolated from the mesenteric lymph nodes of healthy animals*

Species of animal	No. examined	Positive for salmonella	
		No.	%
Pigs	500	60	12
Dogs	200	9	4·5
Cats	200	5	2·5
Cattle	200	0	0
Sheep	100	0	0
Chickens*	280	0	0

* Results for examination of intestinal wall.

diagnostic laboratory, are included in the same table. Salmonellae were found most commonly in the pigs (12%) followed by the dogs (4·5%) and then the cats (2·5%); none was found in the cattle, sheep or chickens. The high isolation rate from pigs was confirmed in another survey in which forty-three pigs, all coming direct from different farms in Essex, were examined at this laboratory. None of them was suffering from clinical salmonella infection but salmonellae were found in the mesenteric lymph nodes of five of them, these organisms also being present in the faeces of one of the five. One of the cats but none of the dogs was suffering from generalized salmonella infection, the serotype implicated being *Salm.*

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enteritidis var. *Jena*; this isolation is not included in Table 1. The bile of 120 of the chickens was also cultured but no salmonellae were isolated. Neither were they found in the whole of the spleen of another 200 chickens not referred to in Table 1. Salmonellae were also isolated from the faeces of one (0·5 %) of the dogs and one (0·5 %) of the cats; they were not found in the lymph nodes of these two animals. Salmonellae were isolated from the faeces of 6 (1·2 %) of 500 market pigs in contrast to 12 % in the mesenteric lymph nodes of slaughtered pigs.

The frequency with which the different salmonella serotypes were isolated from lymph nodes or faeces of the different animal species in the studies referred to above is shown in Table 2. Of the seventeen serotypes found in the pigs,

Table 2. *The frequency with which different Salmonella serotypes were isolated from the lymph nodes and faeces of pigs, cats and dogs*

	No. of times isolated from					
	Pigs		Cats		Dogs	
Salmonella serotype	Lymph nodes	Faeces	Lymph nodes	Faeces	Lymph nodes	Faeces
Salm. typhimurium	17	5	2	1	1	.
anatum	16	2	.	.	.	.
cholerae-suis var. *kunzendorf*	11	.	.	.	.	.
enteritidis var. *jena*	2	.	1	.	1	.
enteritidis var. *danysz*	1	.	.	.	.	.
heidelberg	.	.	.	.	3	1
thompson	3	.	.	.	1	.
reading	1	.	2	.	1	.
newington	3	.	.	.	.	.
kiambu	3	.	.	.	.	.
dublin	2	.	.	.	.	.
saint-paul	1	.	.	.	1	.
derby	1	.	.	.	1	.
newport	1	.	.	.	1	.
binza	1	.	.	.	.	.
san diego	1	.	.	.	.	.
bovis morbificans	1	.	.	.	.	.
schwartzengrund	1	.	.	.	.	.

The lymph nodes of one pig and one dog contained two serotypes.

Salm. typhi-murium, *anatum* and *cholerae-suis* were the most prevalent. These three types comprised fifty-one (70·8 %) of the seventy-three cultures isolated from these animals. *Salm. heidelberg* was isolated from the mesenteric glands of three dogs. The lymph nodes of one dog and one pig contained two serotypes.

The comparative efficiency of selenite broth and brilliant green MacConkey broth

All eleven *Salm. cholerae-suis* strains found in the pig mesenteric lymph nodes were isolated in brilliant green MacConkey broth only. Of the remaining fifty-five strains of salmonellae obtained from pig lymph nodes and comprising sixteen

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serotypes, fifty-two were isolated in both media, two in brilliant green MacConkey broth only and one in selenite broth only.

Of the seven strains of salmonellae obtained from pig faeces, none of which was *Salm. cholerae-suis*, one was isolated in both media, five in selenite broth only and one in brilliant green MacConkey broth only. One was also isolated by direct culture on brilliant green MacConkey agar.

The incidence of clinical salmonella infection in pigs

Only four of 168 dead pigs and none of 185 dead piglets, all epidemiologically unrelated and submitted to this laboratory for diagnosis, were considered, as a result of bacteriological examinations, to have been suffering from clinical salmonella infection, the serotype implicated in each case being *Salm. cholerae-suis*. One of these four pigs was affected with necrotic enteritis. However, a careful bacteriological examination of another sixteen epidemiologically unrelated cases of this disease did not result in any salmonellae being isolated from them. In many of the cases this examination included enrichment of pieces of necrotic intestine, mesenteric lymph nodes and bile in brilliant green MacConkey broth.

Faecal specimens from 277 pigs aged from 2 days to 4 months kept on seventy farms in Essex and suffering from diarrhoea were also submitted to this laboratory for routine diagnostic purposes. No salmonellae were isolated from any of them by direct culture on desoxycholate-citrate agar.

DISCUSSION

The salmonella isolation rate from the mesenteric lymph nodes of healthy pigs was higher in this survey than in earlier surveys conducted in this country and is one of the highest rates recorded from any country. Two explanations at least may be offered for this; first, the large amount of lymphatic tissue cultured from each pig and, secondly, the fact that nodes from each pig were examined separately, and were not pooled as in most other surveys. During the collection of lymph nodes at an abattoir, it is probable that several pigs belonging to a salmonella-infected herd may occasionally be sampled. If the lymph nodes of these pigs were pooled before examination, one pig would be recorded as being infected, whereas if they were examined separately they would all be recorded as being infected.

It is known that surface contamination with salmonellae occurs during slaughtering (McDonagh & Smith, 1958) and that the retention of pigs for long periods in lairages before slaughter results in cross-infection with salmonellae (Galton, Smith, McElrath & Hardy, 1954; McDonagh & Smith, 1958). It is unlikely that these two facts accounted for the high isolation rate obtained in the present survey because the pigs were killed soon after they were brought to the abattoir, usually within a few hours, and, before culture, their lymph nodes were subjected to procedures known to kill salmonellae present on their surfaces. It is probable, therefore, that the high figure obtained is a reasonably accurate reflexion of the infection rate in pigs in the area studied. Supporting evidence for this was obtained from the survey on pigs brought to the laboratory, all of which originated from different farms.

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A comparison of the results of culturing the pig mesenteric lymph nodes in selenite broth and in brilliant green MacConkey broth confirmed the observation (Smith, 1952) that selenite broth is unsuitable for isolating *Salm. cholerae-suis*. Since tetrathionate broth was also found to be unsuitable for isolating this serotype (Smith, 1952) it is not surprising that recent surveys in which one or both of these media were employed gave a low isolation rate.

The precise significance of the presence of salmonellae in the mesenteric lymph nodes of animals is unknown and more research work must be carried out before its importance can be assessed both from the agricultural and the public health aspects. Clinical salmonella infection in pigs, when it occurs, is nearly always due to *Salm. cholerae-suis* but the disease is by no means common in Great Britain. The commonly held view that the disease of pigs termed necrotic enteritis or paratyphoid is usually caused by salmonellae is far from true. For example, salmonellae were involved in only one of seventeen cases in the present studies. On the other hand, the high infection rate of mesenteric lymph nodes of pigs would be of considerable agricultural importance if it was shown to be associated, for example, with poor rate of growth.

From the public health point of view, it is significant that the serotypes found in the mesenteric lymph nodes of animals are those commonly associated with outbreaks of food poisoning in human beings. It is also significant, in view of the fact that the mesenteric lymph nodes of pigs are more commonly infected with salmonellae than are those of any of the other species of domestic animals, that pig meat is the meat most commonly incriminated in these outbreaks (see Report, 1958). However, the mesenteric lymph nodes are discarded at slaughter and it is important to know to what extent lymph node infection is indicative of infection of those parts of the carcase that are used for human food. The presence of salmonellae in the intestinal contents, too, is undoubtedly of considerable importance because it is probably this material that accounts for contamination of carcases occurring in the slaughterhouse procedures. The results of the present survey indicate that salmonellae may be isolated more commonly from the mesenteric lymph nodes than from the faeces. Whether this is an expression of the technical difficulties associated with faecal culture and whether salmonellae may occur only intermittently in the faeces of animals with infected mesenteric lymph nodes is not known.

The part played by cats and dogs spreading salmonella infection to man is also not fully understood. However, their relatively high rate of infection with salmonellae, their close association with man, and the fact that they have been shown to have been responsible for some cases of human infection, indicates that they should be considered as possible sources of infection whenever outbreaks in man are being investigated. The isolation of *Salm. heidelberg* from four of 200 dogs is of interest because, since being isolated for the first time in Britain in 1953, it has become the second most important serotype that causes food poisoning in this country (Report, 1958). Added interest derives from the fact that the epidemiology of *Salm. heidelberg* infection in man is obscure.

There appears to be a definite relationship between the serotypes of salmonellae found in the mesenteric lymph nodes of the pigs and those found in fish meal, meat

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and bone meal and other protein supplements used in animal feeding in this country (Walker, 1957; Report, 1959). The conclusion that infected protein supplements are responsible for some of the salmonella infection in pigs is inescapable, although there is an obvious need for experimental work on this subject. Recent studies in Northern Ireland by Newell, McClarin, Murdock, MacDonald & Hutchinson (1959) lend support to this. In some cases, these workers found the same salmonella serotype in pig food, its protein supplement and in the faeces of pigs to which it was being fed. The infection rate found in chickens was very much lower than in pigs, despite the fact that both species are normally fed similar protein supplements often in the same concentration in prepared diet. One reason for this may be that mesenteric lymph nodes, absent in poultry, are the most satisfactory tissue to examine to determine the presence of latent salmonella infection in an animal. An examination of the many types of food they normally consume is necessary before concluding that contaminated food may be playing a part in causing the relatively high infection rate found in dogs and cats. It is noteworthy, however, that Galton, Harless & Hardy (1955) isolated salmonellae from a high proportion of American dog meals.

SUMMARY

1. The mesenteric lymph nodes and faeces of pigs, cattle, sheep, dogs and cats and the intestinal wall and other organs of chickens in Essex, England, have been examined for salmonellae. These animals were either healthy or were not suffering from clinical salmonella infection.

2. Salmonellae were isolated from the mesenteric lymph nodes of sixty (12 %) of 500 pigs, nine (4·5 %) of 200 dogs, five (2·5 %) of 200 cats and none of 200 cattle and 100 sheep. None was found in the chickens.

3. One (0·5 %) of the cats and one (0·5 %) of the dogs and six (1·2 %) of an additional 500 healthy pigs were excreting salmonellae in their faeces.

4. Of the seventeen serotypes found in pigs, *Salm. typhi-murium*, *Salm. anatum* and *Salm. cholerae-suis* occurred most frequently.

5. All strains of *Salm. cholerae-suis* were isolated in brilliant green MacConkey broth; selenite broth was unsuitable for this purpose.

6. Despite the high isolation rate from pigs, clinical salmonella infection was diagnosed relatively infrequently at this laboratory. *Salm. cholerae-suis* was isolated from only one of seventeen epidemiologically unrelated cases of necrotic enteritis in pigs.

7. The results are discussed from the public health and agricultural view points with particular regard to the part infected feedingstuffs may play in causing salmonella infection in animals.

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