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ISOLATION OF SALMONELLAE FROM FAECES OF DOMESTIC ANIMALS

BY

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The importance of salmonellae as causes of disease in man and animals is recognized. Most outbreaks of food-poisoning in man caused by these organisms are thought to follow the consumption of food directly or indirectly associated with infection of animals (Topley and Wilson, 1946). Salmonella infection among domestic animals is also of economic importance to the agricultural industry and is associated with serious disease problems, particularly among young animals.

A considerable amount of evidence has accumulated from studies of clinical salmonella infection to indicate the species most commonly affected and the types of salmonellae that have been isolated from them. Less time has been devoted to the study of animals that are latently infected with salmonellae. The work carried out on this subject has been confined mainly to the examination of such material as mesenteric lymph nodes. Since the bacteria in these nodes may be constantly confined to them, this potential source of food-poisoning would be removed with the viscera after slaughter. Of more importance from the public health and agricultural points of view are animals that are symptomless faecal excreters of salmonellae. During their life such animals may give rise to disease in susceptible animals and may contaminate milk, eggs, and other food products, and after slaughter their carcasses and those of other animals may become contaminated with salmonellae.

The purpose of this paper is to report on the isolation of salmonellae from the faeces of cattle, sheep, horses, pigs, goats, chickens, turkeys, ducks, and geese. Faecal specimens were examined from adult healthy commercial stock, from animals suffering from diseases other than primary salmonella infection, and, where possible, from animals exhibited at agricultural shows. The third group was included in order to determine the effect of hygiene and general management on the incidence of salmonellae in the faeces of healthy animals.

Techniques

Several methods of isolation and preliminary identification of salmonellae from faecal specimens were tested. The method finally adopted was as follows.

A sample of faeces about the size of a hazel nut was inoculated into selenite F medium (Leifson, 1936) and into tetrathionate broth* (Rolfe, 1946). These enrichment media were incubated at 37° C. for 18 to 24 hours and plated on to Hynes's (1942) modification of Leifson's desoxycholate–citrate agar with the addition of 1% sucrose and 1% salicin (D.C.S.S.) as described by Haines, Elliot, and Tomlinson (1947). Tetrathionate broth was always used within three days of its preparation. The inoculated D.C.S.S. plates were incubated at 37° C. for 48 hours. The plates were then inspected and suspicious non-fermenting colonies were marked and tested for indole and urease production by the rapid test described by Williams Smith and Chave (1949).

Colonies negative to this test were then streaked on to a modification of the D.C.S.S. medium, the sucrose, salicin, and lactose being replaced by 1% dulcitol (D.C.D.). The plates were incubated for 24 hours at 37° C. One plate of this medium was sufficient for six to eight strains. The object of this procedure was to obtain pure cultures for further examination and to ascertain whether the cultures fermented dulcitol. Colonies on the D.C.D. medium that either fermented dulcitol and/or agglutinated with polyvalent O serum were transferred into plain Craigie tubes to induce motility and then subcultured on to moist agar slopes. The resulting cultures were studied serologically by means of antisera obtained either from the Standards Laboratory, London, or were prepared by one of us (A. B.). Organisms giving positive slide-agglutination reactions were submitted to a full tube test and their biochemical characteristics were also determined. Where necessary, final identification of cultures were made at the Salmonella Reference Laboratory, Colindale.

In the early part of the work specimens were plated directly on to D.C.S.S. medium as well as being inoculated into enrichment media. The results showed that there was no advantage from direct plating, and, because of the extra labour involved, it was discontinued. When a salmonella was isolated by one or both of the enrichment methods the original faecal specimen was plated direct on to D.C.S.S. medium. Sometimes the initial examination of suspicious colonies for indole and urease production was replaced by slide-agglutination tests with polyvalent O serum. When this was done the

*Medium A with brilliant green omitted.

colonies on the D.C.D. plates were always tested for indole and urease production before proceeding to further identification.

Results

Horses

Healthy Horses.—The total number of faecal specimens examined from healthy horses was 500. These were obtained from horses that were killed for human consumption in a large slaughterhouse in Cambridgeshire which received animals from many parts of the country. Of the 500 specimens examined, one (0.2%) was found to contain salmonellae. This culture was identified as *Salm. thompson* and was isolated after enrichment in selenite medium and in tetrathionate broth. Direct plating of this sample gave a negative result.

Diseased Horses.—Faecal specimens from horses suffering from a variety of diseases other than primary salmonella infection were received from a number of veterinary diagnostic laboratories located in various parts of the country. Two (0.4%) of the 500 faecal specimens that were examined contained salmonellae. The cultures were isolated by both enrichment media, but not by direct plating on D.C.S.S. medium. The two cultures were identified as *Salm. thompson* and *Salm. dublin.*

Cattle

Healthy Cattle.—Most of the 750 specimens from normal cattle were obtained through the help of the veterinary staff of the Animal Health Division of the Ministry of Agriculture. Only one faecal specimen was taken from any one healthy milking cow in each herd. The remaining specimens, 150 in all, were obtained from adult cattle entering a large abattoir in London. Of the 750 specimens that were examined, three (0.4%) were found to contain salmonellae. The organisms were all *Salm. dublin.* All three were isolated after enrichment in selenite. Two were also isolated after tetrathionate enrichment and by direct plating on D.C.S.S. medium.

Diseased Cattle.—Specimens from diseased cattle were received from a number of veterinary diagnostic laboratories. The diseases from which these cattle were suffering, or had died, were considered to be fairly representative of the disease conditions that affect cattle in this country; none of them were suffering from primary salmonella infection. Salmonellae were isolated from three (0.6%) of the 500 specimens examined. The three cultures were identified as *Salm. dublin* and were isolated after enrichment in selenite media. Two of them were also isolated following enrichment in tetrathionate broth. Direct plating on D.C.S.S. medium gave negative results.

Show Cattle.—None of the faecal specimens collected from 430 cows attending the Dairy Show and the Fat Stock Show in London in 1949 were found to contain salmonellae.

Pigs

Healthy Pigs.—Faecal specimens from 600 healthy pigs were obtained through the help of the veterinary staff at two large abattoirs where large numbers of pigs are slaughtered. One abattoir was situated in the East Midlands and the other in the West Midlands. The specimens were collected over a period of a few months, so that they were representative of the pigs entering these abattoirs. Salmonellae were isolated from the faeces of four (0.67%) of the 600 pigs. The organisms were *Salm. meleagridis* (3) and *Salm. typhi-murium.* All four cultures were isolated after enrichment in selenite medium. One strain of *Salm. meleagridis* and the *Salm. typhi-murium* strain were also isolated by means of tetrathionate broth. Direct plating of the four positive specimens on D.C.S.S. medium gave negative results in all cases.

Diseased Pigs.—Owing to unavoidable circumstances it was possible to examine only 33 specimens of faeces from diseased pigs. Very little information could be discovered relating to the diseases with which the pigs were affected. No salmonellae were isolated from these samples.

Sheep

Healthy Sheep.—Specimens of faeces were examined from 500 healthy sheep. These were obtained in batches of approximately 20 specimens a week from a small abattoir receiving animals from many parts of the country. No salmonellae were isolated from these samples.

Diseased Sheep.—Specimens of faeces from 130 diseased sheep were received through the help of several veterinary diagnostic laboratories. These animals were suffering from a variety of diseases, the most common being parasitic gastro-enteritis. No salmonellae were isolated from these samples.

Goats

Diseased Goats.—It was found impossible to collect a sufficient number of faecal samples from healthy goats. Specimens, however, were examined from 100 diseased goats. These specimens were obtained from a laboratory which was responsible for the examination of faeces for intestinal parasites. Very little information was obtained about the diseases affecting these goats, but some of them were suffering from parasitic gastro-enteritis. None of the 100 specimens were shown to contain salmonellae.

Chickens

Healthy Chickens.—The total number of specimens examined from normal apparently healthy adult chickens was 750. Of these, 360 were obtained from Poultry Stock Improvement Plan (P.S.I.P.) flocks—that is, flocks in which *Salm. pullorum* infection is controlled by regular agglutination tests. These specimens were carefully selected so that normally not more than one chicken was sampled from any one farm unless the number of poultry on the farm was very large. Most of the specimens were collected by the veterinary staff of the Ministry of Agriculture and by members of the National Agricultural Advisory Service. The remaining 390 specimens were obtained from chickens in non-designated flocks. Some of these were collected by the method employed in the case of the P.S.I.P. chickens. The majority, however, were obtained from sales yards—a method of collection which was rapid and also ensured a wide distribution, as only one chicken was sampled from any one flock. Salmonellae was isolated from five (0.67%) of the 750 faecal specimens. All the positive specimens came from chickens belonging to non-accredited flocks. The organisms were *Salm. typhi-murium* (2), *Salm. pullorum* (2), and *Salm. anatum.* The five cultures were obtained after selenite enrichment. Tetrathionate cultivation resulted in the isolation of only one strain of *Salm. typhi-murium* and one of *Salm. pullorum.* Only one of the cultures, *Salm. pullorum,* was also isolated by direct plating on D.C.S.S. medium.

Diseased Chickens.—Faecal specimens from 500 adult chickens that were known to have died from causes other than primary salmonella infection were received

from a number of veterinary diagnostic laboratories in Britain. It was not possible to classify the chickens according to whether or not they were from P.S.I.P. flocks. Not more than one chicken from any one flock was examined. The causes of death of the chickens were various, and were considered to be typical of the losses occurring in flocks in this country. Eight (1.6%) of the 500 faecal specimens from diseased chickens were found to contain salmonellae. The organisms were *Salm. typhi-murium* (4), *Salm. pullorum* (2), *Salm. thompson*, and *Salm. senftenberg*. All the cultures except one strain of *Salm. typhi-murium* were isolated after selenite enrichment, and all except two, *Salm. pullorum* and *Salm. senftenberg*, after tetrathionate enrichment. Only one of the cultures, *Salm. thompson*, was also obtainable by direct plating of the eight positive specimens on D.C.S.S. medium.

Show Poultry.—No salmonellae were isolated from specimens collected from 420 chickens exhibited at the National Poultry Show and the Dairy Show in London in 1949.

Ducks

Healthy Ducks.—The total number of faecal specimens examined from healthy adult ducks was 500. The majority were obtained through the co-operation of members of the British Water-fowl Association. Owing to the relatively small number of duck farmers in this country it was not possible to confine examination of faeces to only one duck on any one farm. When, however, more than one specimen was examined from one farm, care was taken to ensure that they originated from ducks kept in different units. The only exception to this rule was when the units comprised large numbers of ducks; more than one specimen was then taken. Salmonellae were isolated from faecal specimens from six (1.2%) of the 500 healthy ducks. The organisms were identified as *Salm. typhi-murium* (5) and *Salm. meleagridis*. Three of the cultures were isolated after both selenite and tetrathionate enrichment; two were isolated after selenite enrichment only and one after tetrathionate only. Only one salmonella culture was isolated by direct plating on D.C.S.S. medium.

Show Ducks.—Salmonellae were not isolated from faecal specimens taken from 155 ducks exhibited at the National Poultry Show and the Dairy Show in London in 1949.

Turkeys

Healthy Turkeys.—With the help of members of the National Agricultural Advisory Service and individual turkey farmers it was possible to examine faeces from 650 adult turkeys. It was found necessary to examine more than one specimen from each turkey farm, but no more than one specimen was taken from each unit or pen. The only exception to this rule was made when large units containing several hundred turkeys were encountered. Salmonellae were isolated from the faeces of 16 (2.5%) of the 650 healthy turkeys. The organisms were *Salm. typhi-murium* (11), *Salm. anatum* (4), and *Salm. tennessee*. Ten of the cultures were obtained by both methods of enrichment, five after selenite enrichment only, and one after tetrathionate enrichment only. Direct plating on D.C.S.S. medium of the 16 positive faecal specimens resulted in the isolation of only two of the salmonella cultures.

Show Turkeys.—No salmonellae were isolated from the faeces of 63 turkeys exhibited at the National Poultry Show and the Dairy Show held in London in 1949.

Geese

Healthy Geese.—Owing to the methods of husbandry, technical difficulties made it impossible to collect a large number of faecal specimens from geese. Of the 100 specimens that were examined, all except 15 were taken from geese from different farms. These were mostly collected at poultry markets.

Salmonellae were isolated from the faeces of two (2%) of the 100 healthy adult geese. The organisms were identified as *Salm. thompson* and *Salm. typhi-murium*. The *Salm. thompson* culture was isolated by means of selenite and tetrathionate enrichment but not by direct plating. The *Salm. typhi-murium* culture was isolated after tetrathionate but not after selenite enrichment. This latter strain was also isolated by direct plating on D.C.S.S. medium.

Analysis of Results

Comparative Frequency of Salmonellae in Faeces of Different Species of Healthy Domestic Animals.—Table I is designed to compare the frequency with which salmonellae were isolated from faecal specimens of the different species of adult domestic animals that were examined. This table shows that turkeys were the most frequent faecal excreters of salmonellae, followed by geese, ducks, pigs, chickens, cows, and horses. As no salmonellae were isolated from the faeces of sheep it has to be concluded that they must be infrequent carriers. It is noteworthy that three species of poultry were the most frequent carriers of salmonellae. Variation between the different species appeared to be quite considerable; judging from the figures, turkeys were approximately four times more frequent excreters of salmonellae than chickens, six times more frequent than cows, and twelve times more frequent than horses.

TABLE I.—*Frequency with which Salmonellae were Isolated from Faeces of Adult Healthy Domestic Animals*

Species of Animal	No. Examined	No. Positive for Salmonellae	% Positive
Turkeys	650	16	2·5
Geese	100	2	2·0
Ducks	500	6	1·2
Pigs	600	4	0·67
Chickens	750	5 (3)*	0·67 (0·4)*
Cattle	750	3	0·4
Horses	500	1	0·2
Sheep	500	0	0

* The figures in brackets are the results of the isolation of salmonellae other than *Salm. pullorum*.

TABLE II.—*Frequency with which Salmonellae were Isolated from Faeces of Adult Animals Suffering from Diseases Other than Primary Salmonella Infection*

Species of Animal	No. Examined	No. Positive	% Positive
Chickens	500	8 (6)*	1·6 (1·2)*
Cattle	500	3	0·6
Horses	500	2	0·4
Sheep	130	0	0
Goats	100	0	0
Pigs	33	0	0

* The figures in brackets are the results of the isolation of salmonellae other than *Salm. pullorum*.

Comparative Frequency of Salmonellae in Faeces of Different Species of Diseased Animals.—Table II compares the frequency with which salmonellae were isolated from adult animals that were suffering, or had died, from diseases other than primary salmonella

infection. Chickens, cattle, horses, and sheep occur in the same order of frequency as was found in healthy animals of these species (Table I). The cattle and chickens were suffering from a variety of diseases; the eight salmonella-positive chickens and the three positive cows were all suffering from different diseases. Although the figures are higher than those for healthy animals, they do not lend much support to the view that salmonellae commonly proliferate in animals suffering from diseases caused by other agents.

TABLE III.—*Showing the Occurrence of Salmonella Types Isolated from Healthy and Diseased Animals*

Types of Salmonella	Total No. Isolated	% of Whole (50)	Species of Animal
Salm. typhi-murium	24	48	Turkey (11), chicken (6), duck (5), pig (1), goose (1)
Salm. dublin ..	7	14	Cow (6), horse (1)
Salm. anatum ..	5	10	Turkey (4), chicken (1)
Salm. thompson ..	4	8	Horse (2), chicken (1), goose (1)
Salm. pullorum ..	4	8	Chicken (4)
Salm. meleagridis ..	4	8	Pig (3), duck (1)
Salm. tennessee ..	1	2	Turkey (1)
Salm. senftenberg ..	1	2	Chicken (1)

Frequency of Isolation of Different Salmonella Types.—Table III illustrates the frequency with which different types of salmonellae were isolated during this work. Nearly half the number of salmonella cultures that were obtained were *Salm. typhi-murium*. This was by far the most common type isolated from turkeys, ducks, and chickens. The next most common types were *Salm. dublin, Salm. anatum, Salm. thompson, Salm. pullorum,* and *Salm. meleagridis.* As expected from data of clinical salmonella infection, *Salm. dublin* was most commonly found in cows, and was, in fact, the only type present in the faecal specimens from these animals. On the other hand, despite the fact that *Salm. pullorum* is by far the most common type causing clinical salmonella infection in chickens, it was not the type most commonly found in the faeces of the adult chicken in this country. It was also surprising that *Salm. thompson,* a common cause of disease in young chickens, was isolated only once from chickens but twice from horses.

Health and Infectivity of Faecal Excreters of Salmonellae

It was possible to examine more critically some of the animals that had been classified as healthy and that had been shown to be excreting salmonellae in their faeces. There was no evidence to indicate that the general health and condition of these animals were not normal.

In some cases it was possible to obtain faecal specimens from other animals on a farm where a faecal carrier of salmonellae had been found, and also to examine these animals on more than one occasion. Specimens from two horses kept in the same stable as a third horse which was a faecal excreter of *Salm. thompson* also contained this organism. Another horse, on different premises, remained a faecal excreter of salmonellae for six months, when examination was discontinued. Investigations following upon the isolation of *Salm. typhi-murium* from the faeces of a turkey showed that 26 of 30 turkeys in the same pen were also excreting this organism. Further examination about a month later revealed that most of the faecal specimens from these turkeys were still positive. A similar type of investigation in the case of another pen of turkeys revealed that 20 of 24 of these turkeys were also excreting *Salm. typhi-murium* in their faeces. The general health of both of these lots of turkeys was considered excellent. Their sera did not contain demonstrable antibodies to *Salm. typhi-murium.*

In contradistinction to this fairly permanent carrier state, examples of a temporary carrier state were also found. The faeces of a pig, examined several times after the isolation of *Salm. typhi-murium,* were consistently negative. Again, following upon the isolation of *Salm. typhi-murium* from the faeces of a duck, faecal specimens from all the ducks in the pen were examined, including, of course, the original duck. *Salm. typhi-murium* was not recovered from any of the faecal samples; two of the ducks were, however, excreting *Salm. give.*

Comment on Bacteriological Findings

Comparison of D.C.A. and D.C.S.S. Media for Faecal Examinations

For the first 150 specimens of bovine faeces Leifson's desoxycholate-citrate agar (D.C.A.) was used, and non-fermenting colonies from 84 specimens required further examination after selenite enrichment and 52 after tetrathionate enrichment. For the next 150 bovine specimens, D.C.A. with the addition of 1% sucrose and 1% salicin (D.C.S.S.) was used, and only 39 specimens required further examination after selenite and 38 after tetrathionate enrichment. The reason for the less noticeable effect of the addition of sucrose and salicin on the number of colonies requiring further examination after tetrathionate than after selenite enrichment was that their addition led to the recognition and elimination mainly of paracolon bacilli which grew more readily in selenite than in tetrathionate medium. *Proteus* strains, growing more commonly in tetrathionate than in selenite, did not ferment these substrates.

Comparison of D.C.S.S. With and Without Selenite and Tetrathionate Enrichment in Isolation of Salmonellae

This survey presented a good opportunity of comparing the value of plating on D.C.S.S. medium directly, and after enrichment in selenite medium and in tetrathionate broth. Tetrathionate broth is probably used more often than selenite medium as a routine procedure in the examination of faeces. Some workers report, however, that selenite is more satisfactory than tetrathionate (Hobbs and Allison, 1945; Cruickshank and Williams Smith, 1949; Preuss, 1949).

Table IV illustrates the comparative frequency with which salmonellae were isolated from the faecal specimens

TABLE IV.—*Showing the Comparative Value of Plating Faeces Direct on to D.C.S.S. Medium, and After Preliminary Enrichment in Tetrathionate and Selenite Media in the Isolation of Salmonellae*

Species	Total No. of Positive Specimens	Positive Selenite, Positive Tetrathionate	Positive Selenite, Negative Tetrathionate	Negative Selenite, Positive Tetrathionate	Positive for D.C.S.S.† (Direct)
Cattle ..	7	6	1	0	3
Horses ..	5	5	0	0	1
Pigs ..	4	2	2	0	0
Chickens	19	12	6	1	3
Turkeys ..	16	8	6	2	2
Ducks ..	6	3	2	1	1
Geese ..	3	2	0	1	2
Total ..	60*	38 (63%)	17 (28%)	5 (8%)	12 (20%)

* Several positive specimens from "in-contact" animals are included in this total.

† Results obtained by plating all positive samples direct on to D.C.S.S. medium.

by means of the different media. A few positive specimens from "in-contact" animals are also included. Of the 60 specimens shown to contain salmonellae, 38 (63%) were identified by both selenite enrichment and tetrathionate enrichment, 17 (28%) by selenite enrichment only, and 5 (8%) by tetrathionate enrichment only. Only 12 (20%) of the 60 specimens were shown to contain salmonellae by direct plating on D.C.S.S. medium. The superiority of selenite over tetrathionate medium was particularly noticeable in the case of the faecal specimens from turkeys, chickens, and pigs. Many of the plates originating from selenite-positive, tetrathionate-negative specimens grew only a small number of salmonellae colonies after selenite enrichment; tetrathionate enrichment of some of these specimens resulted in a prolific culture of *Proteus* colonies.

Types of Non-fermenting Colonies Growing on D.C.S.S. Medium Sown with Faeces of Different Animal Species

The bacterial flora of the different animal species differed from one another. Table V illustrates a few of these differences; the figures are percentages and

TABLE V.—*Showing the Frequency with Which Non-fermenting Colonies were Isolated on D.C.S.S. Medium from the Faeces of Different Healthy Animals*

Animals	Bacterium	% Positive on D.C.S.S.		Total % of Positive Specimens
		Through Selenite	Through Tetrathionate	
Cattle ..	*Proteus*	10	12	16
	Paracolon bacilli	16	11	22
Horses ..	*Proteus*	6	9	12
	Paracolon bacilli	16	14	22
Chickens ..	*Proteus*	12	20	24
	Paracolon bacilli	17	6	20
Turkeys ..	*Proteus*	19	31	40
	Paracolon bacilli	17	12	24
Ducks ..	*Proteus*	25	54	56
	Paracolon bacilli	25	11	28
Pigs ..	*Proteus*	32	70	72
	Paracolon bacilli	30	20	40
Sheep ..	*Proteus*	20	32	38
	Paracolon bacilli	43	45	60

relate to healthy animals. A great variation was noted in the *Proteus* content of the faeces of different animal species. The order of frequency of isolation of *Proteus* sp. from the various species was pigs, ducks, turkeys, sheep, chickens, cows, and horses, the actual percentage being 72, 56, 40, 38, 24, 16, and 12. *Proteus* strains were more commonly found after tetrathionate than after selenite enrichment. The number of faecal specimens containing paracolon bacilli that failed to ferment the sucrose or salicin in the D.C.S.S. medium did not show a great variation between the different species of animals, there being about 20% in all cases except sheep and pigs; the percentages for these two species were 60 and 40 respectively. No significant difference was noted between the numbers of paracolon bacilli and *Proteus* sp. present in the faeces of normal animals and diseased animals.

A Comparison of Screening Methods

Two methods of screening the non-fermenting colonies growing on D.C.S.S. plates were compared. Usually the screening was carried out by rapid testing of suspicious colonies for urease and indole production. Sometimes, however, colonies were submitted to a rapid slide-agglutination test using the polyvalent O serum issued by the Standards Laboratory. First impressions were that this latter test was less time-consuming, but in view of the considerable number of colonies that either partly or completely agglutinated the polyvalent O serum, or were either auto-agglutinable or sticky, the former test was preferred. When using polyvalent O serum for screening it was found necessary to test agglutinating strains for urease and indole production at an early stage to avoid a lot of unprofitable and time-consuming work.

After the preliminary tests, cultures were purified. The D.C.A. plates modified by replacing lactose with dulcitol were extremely useful for this purpose. It was found possible to plate out six cultures on each plate so as to obtain isolated colonies. The salmonella cultures on this medium produced either pink colonies or paler colonies with black centres. Very few indeed of the cultures that were investigated, and which were not salmonellae, produced this reaction. An exception had to be made for *Salm. pullorum*, which does not ferment dulcitol. It must be emphasized that this medium was used mainly to obtain pure cultures, and cultures were not discarded if they did not give a positive reaction.

Discussion

The results of this survey show that the types of salmonellae isolated from the faeces of healthy animals were, with the possible exception of *Salm. pullorum*, those that are commonly incriminated in outbreaks of food-poisoning (M.R.C. Report, 1947). They are also the types that commonly cause clinical disease in domestic animals. It is therefore obvious that symptomless faecal excreters of salmonellae are important from both the public health and the agricultural points of view.

Most of the outbreaks of food-poisoning traced to domestic animals have originated from "emergency-slaughtered" or "casualty" animals. This does not mean that these animals are the most common source of infection, as the vast majority of outbreaks remain untraced (Food Poisoning in England and Wales, 1941–8). The attention of those concerned with the investigation of outbreaks of food-poisoning is naturally directed upon these animals, whereas outbreaks caused by animals that are symptomless faecal carriers would tend to go untraced. Extensive outbreaks, probably caused by symptomless faecal excreters, have, however, been reported (Camps, 1947; Jones and Symons, 1948). That salmonella infection in man is closely related to infection in animals has been shown by Watt and DeCapito (1950) in America. Whenever they discovered a human salmonella excreter they examined faecal specimens from the domestic animals within one block radius. In 50% of cases they were able to isolate from one or more of the animals the same type of salmonella as from the human excreter. Hinshaw, McNeil, and Taylor (1944) also have drawn attention to cases of gastro-enteritis among attendants on poultry farms which have occurred simultaneously with outbreaks of clinical salmonella infection among the poultry. They are of the opinion that in some instances agricultural workers may be the sources of infection for poultry.

The relative importance of different species of animals as reservoirs of salmonella infection depends on the incidence of salmonella carriers in the species, the total population of the species, and, in the case of food-poisoning, the degree of contact they have with human beings and their food. The figures for the total population of some of the species, based on the June,

1950, Livestock Census, are: horses, 500,000; cattle, 8,000,000; pigs, 2,200,000; chickens, 60,000,000; ducks, 2,200,000; turkeys, 750,000; and geese, 800,000. The correlation of these figures with those in Table I shows that merely comparing the frequency of salmonella excreters among different species of animals does not necessarily give a true impression of the importance of the different species as reservoirs of salmonellae. For instance, although the frequency of salmonella excreters among chickens was low, their large population makes them probably the largest reservoirs of infection. Turkeys, found to be the most frequent faecal excreters of salmonellae, do not appear to be so important as a reservoir. They may, however, be of considerable significance as foci of infection and may disseminate it to other species of animals with which they may come in contact. Horses are probably of little importance.

From the food-poisoning point of view, cattle, sheep, and pigs are important, as they are the principal food animals. Symptomless faecal excreters of these species may give rise to infection of human beings after the contamination of meat at slaughter by the accidental rupture of the alimentary tract, or by the contamination of slaughtering instruments, and of the slaughterhouse itself, with faeces containing salmonellae; cloths used for wiping down the carcass may be particularly dangerous in this respect. The excretion of salmonellae in the faeces of milking-cows is an added danger in view of the possibility of the milk becoming infected; many of the outbreaks of food-poisoning caused by *Salm. dublin*, the most common type isolated from cattle, have been traced to contaminated milk. The importance of raw or insufficiently cooked eggs as sources of infection in man has already been indicated, particularly in the case of duck eggs. The contamination of poultry during evisceration may also be of importance, particularly as this process is often carried out by those responsible for the preparation of food.

The difficulties in preventing animal faecal excreters giving rise to salmonella infection in human beings and in other animals are formidable. The detection of carriers by agglutination tests is effective in the control of *Salm. pullorum* infection in chickens, but such methods are much less effective in other salmonella infections. There is no evidence to suggest that vaccination or chemotherapeutic substances would reduce the number of carriers of salmonellae. It may be possible that improvements in general hygiene and management would result in a decrease in the frequency of salmonella carriers. Some support for this is given by the finding that no salmonellae were isolated from the faeces of animals exhibited at the Dairy Show and the Poultry Show in London in 1949, as these animals would have been kept under a better standard of hygiene than ordinary commercial animals. However, another explanation of this fact may be that the general condition of a faecal excreter of salmonellae would not be of the very high standard required for exhibition at these shows. From the food-poisoning point of view it is obvious that more attention will have to be paid to hygiene during and after the slaughter of food animals if the dangers of animal excreters of salmonellae are to be reduced.

Summary

In a survey of healthy adult domestic animals in England and Wales, 16 of 650 turkeys (2.5%), two of 100 geese (2%), six of 500 ducks (1.2%), four of 600 pigs (0.67%), five of 750 chickens (0.67%), three of 750 cows (0.4%), one of 500 horses (0.2%), and none of 500 sheep were found to be excreting salmonellae in their faeces. The organisms from the turkeys were *Salm. typhi-murium* (11), *Salm. anatum* (4), and *Salm. tennessee*; from the geese, *Salm. typhi-murium* and *Salm. thompson*; from the ducks, *Salm. typhi-murium* (5) and *Salm. meleagridis*; from the pigs, *Salm. meleagridis* (3) and *Salm. typhi-murium*; from the chickens, *Salm. typhi-murium* (2), *Salm. pullorum* (2), and *Salm. anatum*; from the cows, *Salm. dublin* (3); from the horse, *Salm. thompson*.

Among animals suffering from diseases other than primary salmonella infection, eight of 500 chickens (1.6%), three of 500 cows (0.6%), two of 500 horses (0.4%), and none of 130 sheep, 100 goats, and 33 pigs were found to be excreting salmonellae in their faeces. Although these figures are higher than those for healthy animals they do not lend much support to the view that salmonellae commonly proliferate in disease processes. The organisms from the diseased chickens were *Salm. typhi-murium* (4), *Salm. pullorum* (2), *Salm. thompson*, and *Salm. senftenberg*; from the diseased cows, *Salm. dublin* (3); and from the diseased horses, *Salm. dublin* and *Salm. thompson*.

No salmonellae were isolated from the faeces of the following species of animals which were exhibited at national shows in this country: cows (430), chickens (420), ducks (155), and turkeys (63).

All the salmonella types that were isolated in this survey, with the possible exception of *Salm. pullorum*, are known to be capable of causing disease in man.

The significance of these findings is discussed with regard to the causation and prevention of food-poisoning in man and the control of disease in animals.

The techniques used in the isolation and identification of the salmonellae are also discussed. Selenite medium was found to be superior to tetrathionate broth as an enrichment medium, but a greater number of positive specimens were found by the use of both media.

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A note in *Nature*, May 5, page 730, draws attention to the unreliability of killing the eggs of worms in faeces with formalin. A faecal specimen from a patient infected with *Ascaris lumbricoides* was preserved in hot 10% formalin in March, and when the specimen was examined in November many eggs were found containing motile Ascaris larvae, although the formalin concentration was still 6%.