FURTHER INVESTIGATIONS UPON THE DISTRIBUTION OF GAERTNER GROUP BACILLI IN DOMESTIC AND OTHER ANIMALS.

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In another paper I have set out in some detail the reasons which suggest that the sources of infection in food poisoning outbreaks must be sought primarily in infections of some of the domestic animals used for food and possibly in part from rats and mice which gain access to food. In the present report the results of a series of investigations in this direction are described, using the above hypothesis as a basis of inquiry. Much difficulty has arisen from inability to obtain sufficient suitable material for examination.

The investigations have been along the following lines:

I. Examination of internal organs of domestic animals slaughtered for food and presumably healthy.

II. Serological examination of the blood of healthy domestic animals for the presence of Gaertner group agglutinins.

III. Bacteriological and serological examinations of rats.

IV. Bacteriological examinations of domestic animals suffering from certain diseases.

I am much indebted to Dr Howarth, Medical Officer of Health, City of London, and to Mr T. Jones, Sanitary Inspector, Weston-super-Mare, for specimens from healthy animals killed in slaughter houses, to Dr R. A. O'Brien of the Wellcome Physiological Research Laboratories for sera from healthy animals, to Dr Walker Hall, Professor of Pathology, Bristol University, for specimens from rats, and to Sir Stewart Stockman for the organs from several diseased animals.

The investigations are to a considerable extent inter-dependent but are most clearly explained by separate presentation.

I. EXAMINATION OF INTERNAL ORGANS OF HEALTHY DOMESTIC ANIMALS.

Theoretically all the chief internal organs should be examined, but practical experience shows that if any infection results from members of the Gaertner group of bacilli, and such bacilli are present in the internal organs (apart from the intestines), they will always be found in the spleen although not necessarily in the other internal organs. I know of no recorded cases in which such bacilli have been found in liver, kidney, marrow, etc. and have been absent from the spleen. It is therefore not of material importance to examine other organs in addition to the spleen, and for the most part this was not done.

The specimens were all obtained at Weston-super-Mare from animals killed in the Public Abattoir, passed as healthy and subsequently used for human food. The usual methods to avoid outside contamination were taken. The primary inoculations were direct upon two salicin lactose bile salt agar plates while also one agar plate was brushed. In addition a little of the tissue from the interior of the organ was added to mannite malachite green broth and this was brushed over lactose bile salt agar plates, if any growth occurred after 20 hours' incubation at 37° C.

Great care was taken to examine the organs in a fresh condition. The interval between slaughtering and examination was rarely over three to four hours and in no case was over six hours.

The spleens examined were 24 from healthy pigs (except that one was tuberculous) and 10 from healthy calves. All except one appeared perfectly healthy to the naked eye. The remaining spleen showed scattered through it a number of small nodules about $\frac{1}{8}$ inch across. They were hard and not caseous and further examination proved them to be tuberculous. They were associated with tuberculosis of the intestines.

Although *B. coli* and other organisms were found (see below) no Gaertner group bacilli were present in any of the samples. In only one case (Calf No. 16) were para-Gaertner bacilli found. With the cultural definitions for the Gaertner group frequently employed on the Continent this organism might easily be included as a member of that group and it is therefore of some interest.

Its essential characters are as follows: a short bacillus with rounded ends, very actively motile. Stains with ordinary stains but gram-negative. In litmus milk produces first a little acid but after 6—9 days at

37° C. becomes markedly alkaline. No indol formed in peptone water. Bluish translucent growth on gelatine slope, no liquefaction. Ferments, with gas production, glucose and mannite but not salicin, saccharose or lactose. In dulcite produces acid but no gas.

This organism agrees therefore in all its cultural characters with the true Gaertner group bacilli except that it fails to produce gas in dulcite, although acid is produced. Tested on several occasions and after different intervals it still failed to produce gas in dulcite broth.

It was non-pathogenic to a mouse when injected subcutaneously. That it was a para-gaertner organism is also shown by the fact that it failed to be agglutinated in one per cent. dilution by *B. enteritidis*, *B. suipestifer* or *B. paratyphosus* B sera each with a titre of over 1 : 1000. The spleen of this calf was examined $2\frac{3}{4}$ hours after slaughter and was quite healthy and free from all necrotic areas to the naked eye. The serum of this calf failed to agglutinate any of the three sub-group organisms (*B. enteritidis*, *B. suipestifer*, *B. paratyphosus* B) even in a dilution of 1 to 20; while it also failed to agglutinate in the same dilution the para-Gaertner bacillus isolated from the spleen of this animal.

In a second series the gall bladder and bile and a piece of the small intestine were examined very carefully from eight pigs while in four further pigs a piece of large intestine was also examined. All were examined within a few hours of death. In no instance could either Gaertner or para-Gaertner group bacilli be isolated. In several of these cases the serum of the pigs showed well marked agglutination with one or other of the Gaertner strains (see section II).

Bacterial content of the spleens apart from Gaertner group organisms. Although not germane to the objects of these investigations the results are of sufficient interest to be briefly recorded.

Of the 24 spleens from pigs examined, 12 were sterile as regards aerobic organisms (no anaerobic cultivations were made). No special incubation of the whole organ was practised, as in Conradi's method, the inoculations being made from the interior of the spleen by rubbing a sterile glass rod into the pulp and then using this to inoculate the plates, while a small fragment was added to the malachite green broth.

In the 12 sterile cases no growth took place in the malachite green broth or upon the one agar and two lactose bile salt agar plates. The remaining 12 all showed bacteria. *B. coli* group organisms were present in nine, but in no instance in pure culture, being accompanied usually by non-lactose fermenting bacilli while streptococci were present in several and staphylococci in one or two other cases. Two of the remaining three

showed streptococci in pure culture and from the remaining spleen only non-lactose fermenting bacilli were obtained which slowly fermented glucose.

It is of interest to note that the interval between slaughter and bacteriological examination was not materially longer for the sterile spleens than for those containing bacteria. Stated in hours, this interval was for the sterile cases respectively 3, $1\frac{1}{2}$, $1\frac{1}{2}$, 6, 2, 2, 3, 4, 3, 4, 4, 4, hours:—an average of 3·17 hours. For the bacteria-holding spleens the interval was $2\frac{1}{2}$, $3\frac{1}{2}$, 6, 6, $4\frac{1}{2}$, 2, 3, 3, 3, 4, 4, $4\frac{1}{2}$ hours:—an average of 3·83 hours.

No evidence was available to show that the age of the animal had any relationship to the bacterial content of the spleens. It was difficult to ascertain the exact ages of the animals but in 17 cases this was fairly reliably obtainable. The approximate ages of seven of the pigs with sterile spleens were: 4, 8, 5, 5, 8, 10, 10 months—average 7 months and of eight non-sterile cases, 6, 4, 4, 12, 12, 12, 8, 5 months—average ages 7·9 months.

The spleens from the 10 calves were examined in exactly the same way. Of these five were sterile, five non-sterile. The organisms found in the five cases were *B. coli* group bacilli, white staphylococci, a few streptococci, a glucose fermenting, lactose non-fermenting bacillus and the para-Gaertner bacillus (in pure culture). The bacilli were in smaller numbers than in pig spleens while in most cases only one kind of bacterium was present in each specimen.

The interval between slaughter and examination was for the five sterile cases 6, 3, 2, 7, 4 hours respectively, an average of 4·4 hours and for the non-sterile 2, 3, 2, $2\frac{1}{2}$, $6\frac{1}{2}$ hours respectively, an average of 3·2 hours.

All the calves were under three months old. The greatest care was taken to avoid outside contamination and the inoculations were all made from the interior of the spleens.

The results show definitely that in 50 per cent. of the animals selected (pigs and calves) spleens from perfectly healthy animals examined within a few hours of slaughter showed bacilli. The want of correspondence between bacterial content and interval since death and the general shortness of the interval between slaughter and examination makes it a reliable deduction that such bacilli were present at the time of death.

II. SEROLOGICAL EXAMINATION OF THE BLOOD OF HEALTHY DOMESTIC ANIMALS FOR THE PRESENCE OF GAERTNER GROUP AGGLUTININS.

Systematic examination of the sera of domestic animals seemed a useful line of inquiry and one which might throw light upon the problem of Gaertner infections amongst these animals.

Apart from the horses the samples were all obtained from animals actually passed in public slaughter houses (Weston-super-Mare and London) as healthy and fit for human food. The method of examination was throughout the same, all agglutination determinations being microscopic with young broth cultures with a time interval of one hour for dilutions under 1 to 50 and one of two hours for all dilutions of 1 : 50 and higher. During this period the hanging drop preparations were kept at room temperature. Controls were of course made in the usual way. The horses were usually older animals most being 8—10 years old. For the most part they had been used for preparing diphtheria antitoxin and a few for other antitoxins. None had been given typhoid or Gaertner group inoculations.

The results obtained are shown in Table I.

TABLE I.

— signifies no reaction with a dilution of 1 to 20 in one hour.

Animal	No.	Approximate age	Positive agglutination limits		
			B. enteritidis	*B. suipestifer*	*B. paratyphosus* B
Calf	1	6–9 weeks	—	—	—
,,	3	,,	—	—	—
,,	4	,,	—	—	—
,,	10	7–8 ,,	—	—	—
,,	11	,,	—	—	—
,,	16	—	—	—	—
,,	24	—	—	—	—
,,	25	—	—	—	—
,,	26	—	—	—	—
,,	27	—	—	—	—
,,	40	3 weeks	—	—	—
,,	55	,,	—	—	—
,,	56	1 month	—	—	—
,,	117	4 weeks	—	—	—
Cow	42	3 years	200	20	20
,,	49	1 year	20	20	50
Ox	37	3–3½ years	50	100	20
,,	38	,, ,,	50	50	20
,,	39	3 ,,	200	100	20

* Para-Gaertner bacillus isolated from the spleen.

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TABLE I—*continued.*

Animal	No.	Approximate age	Positive agglutination limits		
			B. enteritidis	*B. suipestifer*	*B. paratyphosus* B
Ox	41	3 years	50	50	50
,,	43	3½ ,,	20	—	—
,,	44	4 ,,	50	50	20
,,	45	4 ,,	50	20	—
,,	46	3 ,,	100	—	20*
,,	47	3 ,,	50	20	20
,,	48	4 ,,	100	50	50†
,,	50	4 ,,	100	20	20
,,	51	1½ ,,	100	20	20
,,	52	4 ,,	100	50	50
,,	57	3½–4 ,,	100	50	50
,,	58	4 ,,	50	20	50
,,	77	3¼ ,,	300	50	50
,,	78	3 ,,	100	20	50
,,	79	3½ ,,	100	20	50
,,	80	2½ ,,	20	—	—
,,	81	3 ,,	100	50	100
,,	82	1¾ ,,	—	50	20
,,	116	2 ,,	20	20	20
,,	118	1½ ,,	100	100	100
,,	119	2 ,,	50	20	50
Sheep	53	8 months	20	20	20
,,	54	8 ,,	—	—	—
,,	59	7–8 ,,	20	20	20
,,	60	7–8 ,,	50	—	50
,,	71	12 ,,	20	—	—
,,	72	12 ,,	20	—	—
,,	73	2 years	100	50	100
,,	74	2 ,,	20	—	—
,,	75	2 ,,	20	—	—
,,	76	1½ ,,	20	—	20
,,	114	1 year	20	—	—
,,	115	1 ,,	50	50	50
,,	120	1 ,,	—	—	—
,,	121	1 ,,	—	—	—
,,	122	1 ,,	100	—	—
,,	123	1 ,,	20	—	—
,,	124	1 ,,	20	—	—
,,	125	1 ,,	—	—	—
Pig	5	—	250	20	100
,,	6	—	100	—	50
,,	7	—	100	100	100
,,	8	—	100	—	—
,,	9	6 months	—	—	—

* Abscess in liver: not bacteriologically examined.

† Actinomycosis; in retro-pharyngeal glands only.

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TABLE I—*continued.*

Animal	No.	Approximate age	Positive agglutination limits		
			B. enteritidis	*B. suipestifer*	*B. paratyphosus* B
Pig	12	4 months	20	20	20
,,	13	4 ,,	20	—	50
,,	14	4 ,,	20	—	20
,,	15	12 ,,	20	—	—
,,	17	—	—	—	—
,,	18	—	20	—	100
,,	19	—	100	—	50
,,	20	12 months	20	—	20
,,	21	12 ,,	100	—	100
,,	22	8 ,,	—	—	—
,,	23	8 ,,	20	—	—
,,	28	5 ,,	—	—	20
,,	29	5 ,,	—	—	—
,,	30	5 ,,	50	—	—
,,	31	5 ,,	20	—	20
,,	33	8 ,,	—	—	—
,,	34	8 ,,	250	20	50
,,	35	10 ,,	—	—	—
,,	36	10 ,,	250	—	20
,,	67	—	200	50	100
,,	68	—	100	20	20
,,	69	—	50	50	50
,,	70	—	50	20	50
,,	83	—	50	50	100
,,	84	—	50	20	20
,,	85	—	50	50	50
,,	86	—	50	20	20
,,	87	—	200	50	100
,,	88	—	200	20	50
,,	89	—	50	20	200
,,	90	—	—	—	—
Horse	61	—	—	—	—
,,	62	—	50	100	100
,,	63	—	100	100	20
,,	64	—	50	50	50
,,	65	—	—	—	—
,,	91	"aged"	—	20	20
,,	92	,,	20	—	—
,,	93	,,	20	—	20
,,	94	,,	50	—	20
,,	95	,,	20	—	—
,,	96	,,	20	—	20
,,	97	,,	20	—	20
,,	98	,,	100	20	—
,,	99	,,	100	50	50
,,	100	,,	20	20	20

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TABLE I—*continued.*

Animal	No.	Approximate age	Positive agglutination limits		
			B. enteritidis	*B. suipestifer*	*B. paratyphosus* B
Horse	102	"aged"	100	50	20
,,	103	,,	50	—	—
,,	104	,,	20	—	—
,,	105	,,	—	—	—
,,	106	,,	100	20	50
,,	107	,,	20	—	20
,,	108	,,	—	—	—
,,	109	,,	50	20	50
,,	110	,,	50	20	20
,,	111	,,	—	—	—
,,	112	,,	50	—	20
,,	113	,,	50	50	50
,,	126	,,	50	—	50
,,	127	,,	100	20	20
,,	128	,,	—	50	50
,,	129	,,	100	50	50
,,	130	—	—	—	—
,,	131	—	50	50	20
,,	132	—	50	100	100
,,	133	—	—	—	50
,,	134	—	50	50	50
,,	135	—	50	20	20
,,	136	—	20	—	—
,,	137	—	100	50	50

My own investigations, and those of others in this country, show that while reacting sera may, and usually will, agglutinate differently with different members of the Gaertner group, they all agglutinate one or other of the three chief sub-group organisms and it is only necessary to test unknown sera with these three strains, *B. enteritidis*, *B. suipestifer*, and *B. paratyphosus* B. The actual strains used were for *B. enteritidis* the organism isolated by McWeeney from the Limerick outbreak, for *B. suipestifer* the strain I isolated from the Murrow outbreak or sometimes the strain isolated from the 1911 Chesterfield outbreak and for *B. paratyphosus* B a strain which I isolated from a para-typhoid fever case. The serological reactions and position in the group of all four strains had been very thoroughly worked out.

For convenience in considering the results they have been grouped as shown in Table II.

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TABLE II.

Animal	No. examined	Sera classified into groups A	B	C	D	E	Groups: percentages A	B	C	D	E
Calf	14	14	0	0	0	0	100	—	—	—	—
Cow	2	0	0	1	0	1	—	—	50	—	50
Ox	24	0	3	8	11	2	0	12	33	47	8
Sheep	18	4	10	2	2	0	22	56	11	11	0
Pig	36	7	7	7	8	7	19	19	19	22	19
Horse	39	6	10	13	10	0	15	26	33	26	0
Totals	133	31	30	31	31	10	23·3	22·6	23·3	23·3	7·5

A = No trace of reaction.

B = trace of reaction, i.e. + in dilution of 1 : 20 with any one of the three organisms.

C = Slight reaction, i.e. + in dilution of 1 : 50 with any one of the three organisms.

D = Well marked reaction, i.e. + in dilution of 1 : 100 with any one of the three organisms

E = Very marked reaction, i.e. + in dilutions above 1 : 100 with any one of the three organisms.

If a positive reaction of 1 in 20 is disregarded as being of no significance and included with the negative reactions the following summary is obtained:

Animal	Percentages: No reaction (— and A)	Slight reaction (B and C)	Marked reaction (D and E)
Calf	100	—	—
Cow and ox	11	35	54
Sheep	78	11	11
Pig	38	19	41
Horse	41	33	26

Table I shows that the serum of a considerable proportion of the animals examined gave a positive reaction with Gaertner group strains. I only know of three possible explanations to account for the results.

(*a*) Due to the presence of "natural agglutinins" in the serum.

(*b*) Due to an old infection with Gaertner group organism and resulting in the formation of Gaertner group agglutinins in the blood.

(*c*) Due to infection with some unknown bacillus, or bacilli, not of the Gaertner group but sufficiently allied to this group to cause the serum to have some action on Gaertner group strains.

In the latter case the agglutination action would be of the nature of associated agglutinins. There is really no evidence in favour of this last hypothesis while it is inherently improbable. It cannot be entirely ruled out of consideration but is unlikely.

As regards the first hypothesis it is a recognized fact that perfectly normal serum may agglutinate certain bacteria to a limited extent but there is no satisfactory explanation as to the cause of the phenomenon. As far as I am aware "normal" agglutinins are not present in the new-

born animal and are therefore acquired during life. Such "normal" agglutinins are specific in the same way as ordinary agglutinins acquired as the result of infection and it is, therefore, a possible and not unreasonable supposition that their presence is due either to slight unrecognized infection with the specific bacilli or to the absorption of specific bacterial toxins from the intestinal canal.

In the present series the blood of all the 14 calves failed to agglutinate, even in a dilution of 1:20, any of the Gaertner strains, while 35 per cent. of the cows and oxen showed a slight reaction and 54 per cent. a marked reaction. It is a quite possible hypothesis that the older animals react because of Gaertner agglutinins produced either through toxic Gaertner products absorbed from the intestinal canal or because of slight infection of the animals with Gaertner bacilli, perhaps so slight in character that no appreciable symptoms were caused.

It would be of decided interest to take young calves and examine monthly their blood and note if the presence of these agglutinins coincided with the onset of slight illness or with the presence of Gaertner group bacilli in their excreta. Unfortunately material for this purpose has not been available to me.

The comparative absence of agglutinins in the sheep, an animal which extremely rarely causes food poisoning of Gaertner group origin, is a point of interest, but this may possibly be due to the fact that the sheep examined were not very old.

A number of the sera were also tested against *B. typhosus* and a certain proportion of them gave a positive agglutination reaction. In as much as the typhoid bacillus is not responsible for disease in animals and is never found in the animal intestine this might be taken as evidence of "natural" agglutination power and unassociated with any infection with pathogenic Gaertner or other group of bacilli. Two sets of observations however tend to show that this hypothesis cannot be maintained. The first is that in no case have I found that agglutination takes place with *B. typhosus* when failure to agglutinate the Gaertner strains was met with. The following are some sera which agglutinated *B. typhosus* in considerable dilution.

No.	Animal	Highest possible dilution with *B. suipestifer*	*B. enteritidis*	*B. paratyphosus* B	*B. typhosus*
87	Pig	50	200	100	50
89	„	20	50	200	100
98	Horse	20	100	—	50
99	„	50	100	50	100
102	„	50	100	20	200
118	Ox	100	100	100	100 (200 partial)

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In only a very few cases was the serum capable of agglutinating *B. typhosus* at a higher dilution than for the other bacilli used.

The matter was further tested by a few absorption tests. The results obtained are shown in the following Table.

TABLE III.

	Bacillus	Serum no. 99 1:20	Serum no. 99 1:50	Serum no. 118 1:20	Serum no. 118 1:50	Serum no. 89 1:20	Serum no. 89 1:50	Serum no. 102 1:20	Serum no. 102 1:50
After absorption by *B. enteritidis*	Enteritidis	—	—	—	—	—	—	—	—
	Paratyphosus	—	—	—	—	+	+	—	—
	Typhosus	—	—	—	—	+	—	—	—
After absorption by *B. typhosus*	Enteritidis	+	+	+	+	+	+	—	—
	Paratyphosus	+	+	+	+	—	—	—	—
	Typhosus	—	—	—	—	—	—	—	—
After absorption by *B. paratyphosus* B	Enteritidis	not examined		+	+	—	—	not examined	
	Paratyphosus			—	—	—	—		
	Typhosus			+	+	+	—		

Note. The agglutination reactions of these sera before absorption are shown above.

The results are not absolutely uniform and this could hardly be anticipated in view of the very low titre of the sera used. They show no evidence of the presence of separate natural typhoid agglutinins.

In my paper upon "The Sources of Infection in Food poisoning Outbreaks" three papers are mentioned which may be mentioned here, as they throw definite light upon this question. In O'Brien's paper (1910) in a naturally occurring outbreak due to *B. suipestifer* (*aertrycke*) amongst laboratory guinea-pigs, of which nine survived the epidemic and were bacteriologically examined, five became carriers of the bacillus. The serum of four of these animals agglutinated this bacillus in dilutions of 1 : 50 and 1 : 100. As a control the sera of six stock guinea-pigs were tested and only one gave a reaction with a 1 in 20 dilution and none in higher dilution. The length of time during which these agglutinins persisted was not worked out. Petrie and O'Brien found that guinea-pigs fed with cultures may excrete the bacillus in the faeces for some time subsequently while remaining apparently healthy, and that the blood of some of these animals agglutinated the bacillus.

Reinhardt and Seibold fed a goat with massive doses of a Gaertner group strain. There was some slight rise of temperature but no definite illness. No agglutinins before feeding or four days after, but nine days after feeding the serum reacted in dilution of 1 : 40 and 16 days after the start of the experiment gave a positive reaction 1 in 2800. The animal was then killed. No pathological lesions were present and all the organs were sterile.

The available data is obviously insufficient to determine whether the positive reactions with Gaertner strains recorded above can be accepted as evidence of previous infection with these bacilli but they are suggestive of this.

Reaction of spleen pulp. Some continental workers have suggested that the serum reaction of the animal juices, particularly that of the muscles or spleen, might be of use as a rapid method for the diagnosis of infections from Gaertner group organisms.

In seven cases this possibility was tested by the examination of spleen pulp juice as well as the serum. All were from healthy pigs. Six of the sera samples gave positive agglutination reactions varying from 1 : 20 to 1 : 100 but in all seven cases no trace of agglutination was present when the filtered spleen pulp was used, even in dilutions of 1 : 20 and 1 : 10.

III. BACTERIOLOGICAL AND SEROLOGICAL EXAMINATION OF RATS.

In an investigation reported in 1913 Read and I studied a series of rats to ascertain how far they were infected with organisms of the Gaertner group. In all 41 rats were examined, the organs selected for examination being the liver, spleen, heart blood and intestinal contents. True Gaertner group organisms (all *B. enteritidis*) were isolated from five of them. All five were from Weston-super-Mare. Twelve days before two of these rats were examined, Danysz virus (a living virus which has been identified with *B. enteritidis*) had been distributed in different parts of the town, while in November, 1909, about $2\frac{1}{2}$ years before the first rats were examined the refuse tips and slaughter houses had been extensively dosed with this virus. The positive rats were probably old cases of infection with this virus. Only a very few rat sera were examined but several agglutinated Gaertner strains to a considerable extent, i.e. 1 : 2000, 1 : 200, 1 : 500 in three cases.

In the present series this line of inquiry was followed up and further rats were examined.

This series comprised 48 rats all collected from Bristol or Avonmouth. The rats were being collected and examined by Professor Walker Hall for *B. pestis* in connecting with plague infection in the city. They were all selected as rats which showed no obvious naked eye disease lesions and therefore serve well to study the extent to which Gaertner bacilli and agglutinins are present in apparently healthy animals. The spleen and heart blood were used for cultural examination and the latter also for

serological tests. These organs were removed and sent to me in sterile bottles. Cultural examination of the other organs was not considered necessary. The method of examination consisted of direct brushing from the interior of the organs on to a series of lactose bile salt neutral red agar plates.

Cultural findings. It is not necessary to give these in detail for each rat. In many cases the plates showed no growth but in a considerable number red colonies, presumably *B. coli*, were present. All white colonies were fully investigated. In a number of cases non-lactose fermenting white colonies were met with which failed to ferment glucose but as they fermented saccharose or salicin they were not further studied. In no instance was any true Gaertner organism isolated. On the other hand strains of para-Gaertner bacilli were isolated from five different rats.

These bacilli all possessed similar cultural characters. They were identical with true Gaertner bacilli in morphology, staining properties, characters of growth upon agar, gelatine and in broth, they fermented glucose but not lactose, saccharose or salicin. None produced indol, all five after a little acid production in litmus milk produced in 6—10 days marked alkalinity. Culturally they only differed from true Gaertner strains, as regards their action upon mannite and dulcite. Four out of the five fermented mannite with gas production like true Gaertner strains but the gas produced was only slight in two. The fifth produced acid but no gas in mannite media. All five were sharply separated from true Gaertner strains by their failure to ferment dulcite.

Their agglutination reactions also separated them, as none of the five were agglutinated, even in dilutions of 1 : 100, by *B. enteritidis*, *B. paratyphosus* B or *B. suipestifer* sera of fairly high titre (1 : 1000 to 1 : 4000).

The pathogenicity of only one strain was tested. The intraperitoneal inoculation into a young rabbit of as much as half an agar slope culture mixed with 1 c.c. of a 24 hours' broth culture failed to kill the animal or elicit any symptoms.

These organisms are interesting as culturally apart from the dulcite test (except the one mannite acid producer) they are indistinguishable from the true Gaertner strains and where this test has not been employed have probably been taken for that organism.

Serological findings. In every case each serum was tested against the three organisms (*B. enteritidis*, *B. paratyphosus* B, *B. suipestifer*) of the group. The lowest dilution used was 1 : 50; all microscopic and two hours at room temperature.

Rather to my surprise no less than 45 of the 48 samples showed *no* reaction in 1 : 50 dilution with any of the above organisms. The only positive agglutination reactions were the following:

Rat laboratory no.	Limits of reaction: *B. enteritidis*	*B. suipestifer*	*B. paratyphosus* B	Source
522	—	—	100	Obtained from a ship.
378	50	50 (partial)	50	Bristol food shop.
380	50	50	50 (partial)	Slaughter house.

It will be seen that in no case had any marked agglutination power developed.

IV. BACTERIOLOGICAL EXAMINATIONS OF DOMESTIC ANIMALS SUFFERING FROM CERTAIN DISEASES.

As explained elsewhere there is a number of papers by continental investigators which demonstrate that infections with Gaertner group bacilli may occur amongst domestic animals. It is a singular fact that there are no reports, as far as I have been able to ascertain, of cases of this nature in Great Britain, apart from disease in animals associated with food poisoning outbreaks. The occurrence of food poisoning outbreaks in this country traced to diseased animals makes it probable that such occur and it is most desirable that careful bacteriological examinations into the cause of all deaths of this nature should be carried out. This would appear to be done but rarely. Unfortunately I have had great difficulty in obtaining such material and only the following have been examined.

No. 1. Pig found dead; cause of death not known. Putrefaction had set in when internal organ samples received. No Gaertner group bacilli could be isolated. The agglutination properties of the serum were not tested.

No. 2. Cow died from septic poisoning owing to rupture of womb during calving. A short chain streptococcus was isolated from the spleen. No Gaertner group bacilli could be isolated. The serum showed agglutinins, the limits of reaction being with *B. enteritidis* 1 : 500, *B. paratyphosus* B 1 : 100, *B. suipestifer* 1 : 50.

Evidently the animal did not die of a Gaertner infection. In view of the positive reactions with normal sera it is not possible to say if the agglutination reactions are evidence of old infection.

No. 3. For particulars of this case and for cultures from this case and No. 4 and No. 5 I am indebted to Sir Stuart Stockman. A ram ill with general cachexia for several days died and the post-mortem showed an acute catarrhal area in the urethra accompanied by cystitis and a chronic abscess in an inguinal gland. Bacteriological examination of the abscess showed a bacillus which on investigation was a variety of *B. coli*. No Gaertner organisms found.

No. 4. A lamb born dead practically at full time. The liver was discoloured and blood-stained oedematous fluid was present in both abdominal and thoracic cavities. The only organism which could be isolated was a typical strain of *B. coli*.

No. 5. Pig artificially infected with swine fever June 14th and slaughtered June 23rd. The liver and spleen were bacteriologically investigated but were not examined until over 24 hours after slaughter and removal. No true Gaertner group bacilli were isolated but it is of interest that two pseudo-Gaertner strains were isolated, both from the spleen. One of these differed culturally from true Gaertner organisms only in the fact that it failed to ferment dulcite and definitely produced indol, while also it produced very little acid or gas in glucose media. The other differed only in that it failed to ferment dulcite, produced indol, fermented salicin and only gave slight alkalinity in milk. Both also were motile but much less so than true Gaertner strains. They were not agglutinated by the serum of this pig even in dilutions of 1 : 20.

The serum of this pig failed in dilutions of 1 : 50 and 1 : 100 to exert any agglutination power upon the three Gaertner group organisms.

No. 6. Serum and particulars of the case kindly sent me by Dr McGowan of Edinburgh.

The blood was obtained from the heart of a ten weeks old pig. It was one of a number of animals being killed out for swine fever. Post-mortem the animal showed extensive pneumonic consolidation of the middle lobe right side and upper lobe left side together with acute congestion of the rest of the lungs. Pleurisy was also present. The heart was healthy. Enlargement of the lymphatic glands generally. In abdomen nothing to note except acute inflammation of the small intestine, "so-called typical swine fever ulcers." No other inflammation. This serum showed no trace of agglutination in dilutions of 1: 50 and 1: 100 with the three Gaertner types or with *B. typhosus*.

SUMMARY AND CONCLUSIONS.

The examination of the spleens of 24 pigs and 10 calves and internal organs from 12 other pigs, all passed as healthy and fit for human consumption, failed to show the presence of any organisms belonging to the Gaertner group. One para-Gaertner bacillus was isolated in pure culture from the spleen of one of the calves.

The spleens of the 24 pigs and 10 calves were also examined for the presence of aerobic organisms generally. Exactly half of the pig and half of the calf spleens were sterile, the remaining 50 per cent. containing bacilli which were not completely identified but were mostly *B. coli*, non-lactose fermenters allied to *B. coli*, streptococci and staphylococci. All the organs were examined within a few hours of death. There was no correlation between interval since death and bacterial content nor any relationship to the age of the animal. The bacteria were evidently present in these organs at the time of death.

An extensive series of examinations of sera of animals passed as healthy showed that in a considerable proportion of them specific agglutinins were present against one or other member of the Gaertner group. These agglutinins were absent in all the calves, were mostly absent from sheep, but were fairly well developed in 40 to 50 per cent. of the pigs, cows and oxen examined. They were also present in a considerable proportion of the horses tested. The failure to demonstrate these agglutinins in the calf and their definite development in many of the cows and oxen sera suggest that they are not present in the new born animal but develop later in life. The available data is insufficient to enable a definite opinion to be given as to whether these positive reactions with Gaertner strains are to be ascribed to an old infection with these bacilli but they suggest this possibility.

As a practical point these results show that the demonstration of the presence of agglutinins, in moderate amount, against this group of bacilli in a suspected animal cannot be accepted in itself, and without further corroborative evidence, as proof of an existing infection with these organisms or as conclusive evidence connecting the animal with an outbreak of food poisoning. Much less so can the presence of such agglutinins be accepted as evidence warranting the condemnation of the carcase as unfit for food.

Amongst the animals examined suffering from definite disease one cow which died of septic poisoning showed the presence of a considerable amount of specific agglutinin in its serum, but the other bacteriological data was sufficient to say that the cause of death was not a Gaertner group infection.

The 48 rats examined were selected as showing no macroscopic evidence of Gaertner group infection and from none of them was a member of this group isolated, although five para-Gaertner strains were found which very closely resembled these bacteria.

In nearly every case the sera of these rats failed to show the presence of any Gaertner group agglutinins. This fact is of particular interest in view of the rather different findings which Read and I obtained with another series of rats from a different source and where the possibility of infection with Danysz virus (a Gaertner group organism) was likely. They add weight to the view that in these (mostly young) animals the presence of Gaertner group agglutinins to any considerable extent is evidence of an old infection with a member of this group, and is confirmatory evidence in favour of the agglutinin results with animals used for food being of a similar origin.

The absence of any correlation between the presence of the six para-Gaertner strains and the presence of agglutinins against true Gaertner bacilli is in favour of the view I have expressed from my earlier work, that there is no relationship between these two groups of organisms and that the para-strains cannot be considered as modified Gaertner organisms which under favourable conditions can revert to that type.

The examinations of specimens from diseased animals are too few to enable any deductions to be drawn, but I remain strongly of opinion that work along these lines is likely to throw valuable light upon the etiology of food poisoning and should be systematically undertaken by those who are in a position to obtain and examine such material.