OBSERVATIONS CONCERNING BACILLARY FOOD INFECTION IN DUNDEE DURING THE PERIOD 1923–38

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I. Introduction

During the period 1923–38 several outbreaks of food infection due to *Salmonella* bacilli have occurred in the City of Dundee.

Of these, three have been major outbreaks involving large numbers of people, while the remainder have been sporadic, or familial, causing illness in only a few persons. Representative strains of the bacilli isolated from cases (both epidemic and sporadic) have been maintained in culture since their isolation.

With the introduction of the Kauffmann-White classification of the *Salmonellas* it became necessary, for teaching purposes, to investigate these various strains which had, when collected, been designated according to the information then available.

The majority of the sporadic cases and familial outbreaks have been due to *B. aertrycke*, and one major epidemic, caused by that micro-organism, occurred in 1924. This was by far the most widespread outbreak of bacillary food infection in this area during the period under consideration, and it has been fully described by W. L. Burgess, Medical Officer of Health, City of Dundee (1925).

The micro-organism which caused this epidemic was recently reinvestigated and proved to be a typical *B. aertrycke* possessed of somatic antigens IV and V and with flagellar antigen "*i*".

II. Sporadic cases

Apart from sporadic and familial cases in which the causal agent was also *B. aertrycke* there have, in addition, been limited outbreaks in which, on subsequent investigation, the responsible micro-organism proved to be one or other of the less frequently encountered members of the *Salmonella* group.

In each instance the micro-organism, when isolated, exhibited the typical morphological, tinctorial and fermentative characters of the group in question, but in some instances difficulty was experienced in definitely placing it by the

serological procedures then available. The cases due to these less frequently encountered varieties were as follows:

(*a*) Two cases of typical acute gastro-enteritis, an adult and a child in one family, occurred in 1930. The organism when first isolated was provisionally designated an "aberrant *aertrycke*". On later investigation it proved to be diphasic and in the specific phase possessed somatic antigens IV and V and flagellar antigen "*d*". It was, therefore, a *stanley* bacillus.

(*b*) A case in which a *Salmonella* was obtained in pus from an appendix abscess (1925). As this bacillus when isolated reacted with anti-*newport* serum it was provisionally regarded as a *newport* strain. Subsequent investigation showed that its somatic antigen corresponded to subgroup VI and VII (predominantly VII), while it possessed flagellar antigens "*enlv*". Clearly it was a "*potsdam*" strain or one closely related thereto.

(*c*) A single case (1938) in which the causal organism possessed somatic antigens I and III, and reacted with a specific antiserum to flagellar substance "*s*", was encountered. This was, therefore, a *senftenberg* infection.

(*d*) Two related cases (1938) were encountered in which the only, presumably, pathogenic organism recovered had the morphological, cultural and fermentative characters of the "paratyphoid-food infection" group.

On serological investigation these strains reacted, but not to full titre, when deflagellate (alcoholized) suspensions were tested with antiserum to *Salmonella aberdeen*. Although motile they failed to give floccular agglutination either with anti-*aertrycke* serum or anti-*aberdeen* serum. A more complete antigenic analysis would be required to determine whether the organism belongs to a known species or not.

As antibodies of low titre (1/50) developed in the serum of the patients during convalescence there was at least some evidence that the organism had been responsible for illness.

These two cases are, therefore, mentioned solely to draw attention to the fact that the group in question may be even larger than it is at present assumed to be.

In none of these four minor outbreaks was the source, or vehicle, of infection established.

III. TWO MAJOR EPIDEMICS DUE TO THE *DUBLIN* VARIETY OF THE *GAERTNER* BACILLUS

While the sporadic cases dealt with in the previous section of this communication are of academic interest as indicating the wide distribution of the less frequently encountered *Salmonella* bacilli, they are of minor significance from the standpoint of public health.

What may, on the other hand, be of considerable importance is that two relatively large outbreaks of bacillary food infection in Dundee—August 1926 and October 1927—were proved to be due to the *dublin* variety of the *gaertner*

bacillus. Both were at first regarded as instances of true (classical) *gaertner* infection as, at the time when they occurred, the *dublin* variety had not been differentiated therefrom.

This differentiation was achieved by Bruce White (1929) and it subsequently transpired that the same antigen complex characterized a strain isolated by Pesch (1926) from a case of meningitis. The strain described by Pesch proved to be the same as that designated by Jensen as the "paracolon bacillus"; and it is interesting to note that Jensen regarded it as a causal agent of "calf dysentery".

In 1933, Smith described a circumscribed outbreak of gastro-enteritis caused by this micro-organism and in the following year (1934) two fatal cases of septicaemia due thereto. Montgomery (1938) recovered the same strain of bacillus from sporadic cases of meningitis in infants in Glasgow, and in the same year Conybeare & Thornton (1938) issued a report on "An outbreak of food poisoning due to *Salmonella*, type '*dublin*', and conveyed by raw milk".

The observations of Jensen, of Bosworth & Lovell (1931) and of Conybeare & Thornton draw attention to the pathogenicity of the *dublin* type for bovines, while those of Souper *et al.* (1930), of Smith (1933) and of Montgomery (1938) indicate that infection of human beings with that micro-organism is widespread.

So far as the writer is aware there have not so far been reported any extensive outbreaks of "food poisoning" due to the *dublin* bacillus.

The two outbreaks described herein may therefore be of some interest.

A. *Outbreak No.* 1, "*dublin*", 1926

(*a*) *Outbreak of epidemic.*

On Friday, 20 August 1926, the first cases of this epidemic were reported to Dr Burgess, Medical Officer of Health, Dundee, and he at once traced the source of infection to milk. Actually the earliest cases had declared themselves late on Wednesday, 18 and early on Thursday, 19 August.

These very early cases did not occur in Dundee itself but all were persons employed at farm "X" situated close to the City. From this farm approximately 142 gallons of milk were produced daily and the greater part of this was distributed by a dealer in whose "milk round" the majority of cases occurred, the remainder being workers on farm "X". It was among these workers that the first cases declared themselves, while most of those within the City developed on the following day.

Enquiry at the farm revealed that a cow had been ill for some days and, on the advice of a veterinary practitioner, was isolated. Until Thursday, 19 August, however, it had been milked and the product mixed with that of the remainder of the herd.

This animal died on Sunday, 22 August, and the carcase was so disposed of that further spread of infection was precluded.

(*b*) *Extent of outbreak.*

There were 373 known cases in 192 households, all of which obtained milk either directly or indirectly from farm "X". Of these 269, in 134 households, were fully investigated epidemiologically.

The age distribution of the cases was:

Under 5 years	59
5–15 ,,	42
Over 15 ,,	272
	373

Fortunately, none of the cases proved fatal and in all convalescence was quickly established.

(*c*) *Bacteriological observations.*

The bacteriological examinations conducted in the investigation of this outbreak were as follows:

(1) Examination of suspected milk.

(*a*) Sample collected 20 August 1926, produced 18 August 1926 and supplied to family "R" (*vide infra*).

(*b*) Sample from suspected cow taken 20 August 1926.

(*c*) Sample from suspected cow taken 21 August 1926.

(*d*) Sample of alvine discharge from suspected cow.

(*e*) Slough from rectum of this animal taken post-mortem.

(*f*) Blood taken post-mortem.

From (*a*), (*b*), (*c*), (*d*) and (*e*) "*B. enteritidis*" was recovered, while from the dejecta of those members of family "R" who had consumed milk (*a*) the same micro-organism was isolated.

It was also shown to be present in fourteen further specimens of stools derived from persons suffering from acute gastro-enteritis who had obtained their milk supply from farm "X".

In addition specific serological response was shown to occur as convalescence was established in those who had been infected. Specimens of blood were collected from nineteen convalescents taken at random and these were tested against suspensions of

(i) *B. aertrycke.*

(ii) *B. newport.*

(iii) *B. gaertner* (two strains).

(iv) The bacillus isolated from the cases.

The result obtained was clear-cut in that the serum of these convalescents contained antibodies to *B. gaertner* and to strains isolated either from the suspected milk or from the stools of patients involved in the outbreak.

Of nineteen specimens of blood taken 24 August 1926—the peccant food having been consumed 18 August 1926—fifteen failed to show the presence of antibodies while four reacted to titres of 1/25, 1/30, 1/50 and 1/100.

Antibodies became demonstrable, however, in some of the cases between the sixth and fifteenth day following infection as shown in the following table which indicates the results of duplicate tests performed with serum derived from convalescents; the antigen was a suspension of stock *B. gaertner*.

Peccant food consumed	Serum first tested	Second test of serum and result
18. viii. 1926	24. viii. 1926	31. viii. 1926
,,	—	Positive 1/200
,,	—	,, 1/200
,,	—	,, 1/200
,,	—	,, 1/100
,,	—	,, 1/50
,,	—	,, 1/50

(*d*) *Characters of the bacillus isolated.*

The bacillus isolated from the milk, from the material obtained from the cow post-mortem and from the dejecta of cases which occurred during the outbreak, exhibited the morphological, tinctorial, cultural and fermentative characters of the "paratyphoid-food infection" subgroup of the *Salmonella* organisms.

As it reacted with anti-*gaertner* serum to full titre and as broth cultures proved to be pathogenic for the guinea-pig, it was regarded as a true *B. enteritidis* of the *gaertner* type.

Representative strains both of bovine and human origin were retained for future reference. When, therefore, the Kauffmann-White classification of the *Salmonella* group was introduced it was possible to reinvestigate these with a view to determining accurately their precise serological relationships.

Two strains of bovine origin and two isolated from human dejecta have been fully examined by modern serological procedures: the findings were as follows:

(1) Tested as alcoholized suspensions these strains were found to possess somatic antigen IX.

(2) Tested as formalinized suspensions, all reacted with sera containing antibodies to the "*g*" and "*p*" flagellar components.

(3) Tested as formalinized suspensions, they failed to react with sera whose antiflagellar quality corresponded to (flagellar) antigen "*o*" and "*m*".

These results leave no doubt that the causal agent of the outbreak under consideration was *B. enteritidis* of the *dublin* type.

B. *Outbreak No.* 2, "*dublin*", *October* 1927

(1) *Outbreak of epidemic.*

The first notification of this outbreak was received by the Medical Officer of Health on the afternoon of Saturday, 29 October, but subsequent enquiry revealed that the majority of the cases had first shown symptoms of gastro-enteritis during the night of 27 October and the earliest time of onset that could be definitely determined was 3 p.m. 27 October 1927.

The distribution of the cases in the City was found to coincide with the "rounds" of two milk purveyors "A" and "B", and it subsequently transpired

that both obtained their supplies from farm "X" which had been proved to be the source of the outbreak in August 1926.

In October 1927 this farm was producing 120–140 gallons of milk per day and the bulk of this was purveyed by dealers "A" and "B".

On the occurrence of the outbreak the farmer of "X" was interviewed. He stated that the herd was healthy apart from one cow which became ill on 26 October 1927 with "pneumonia". Seen by a veterinary surgeon on 27 October 1927 it was isolated and on his advice was sent for slaughter 28 October 1927. There was, however, some doubt as to whether the milk of this animal had or had not been mixed with that from the remainder of the herd between 25 and 27 October 1927.

The following are the notes of the post-mortem examination of the cow in question. These have been supplied by Mr Anderson, Superintendent of the Slaughter House, Dundee, and to him the writer is most indebted for permission to use them.

"Gastro-enteritis was present in the third stomach, the spleen was enlarged and congested, the liver flukey and cirrhotic, while the kidneys and lungs were slightly congested. The heart was normal. There was no visible septic condition of the udder. The left retro-pharyngeal lymph gland showed an abscess about the size of a hen's egg, the right being caseous and slightly enlarged. This process strongly suggested tuberculosis, although no further evidence of that disease was found in the carcase.

"The carcase was well set, the flesh firm and clear with a complete absence of external petechiae, effusion of serous fluid, or haemorrhages in the muscles. The right side of the peritoneum was slightly bile stained and the deep lymph glands of the carcase, although deeper in colour than normal, were free from pronounced enlargement or oedema."

The feature of this report then is that nothing of a very definite nature was revealed by autopsy.

Fortunately, the organs of the animal were not destroyed and they were secured for bacteriological investigation.

(2) *Extent of outbreak.*

Thirty-four families were involved and all obtained milk produced at farm "X", which milk was retailed either directly or through "A" and "B".

The age and sex incidence of the cases were:

Age (years)	Males	Females	Total
Under 5	3	3	6
5–10	4	5	9
10–15	1	4	5
15–25	15	7	22
25–35	8	17	25
35–45	5	3	8
Over 45	12	13	25
	48	52	100

These hundred cases do not include all that were affected but comprise only those in which complete epidemiological, and in several instances bacteriological, evidence was available. It is estimated that at least 280 cases occurred but fortunately again none proved fatal.

(3) *Bacteriological observations.*

The following were made the subject of complete bacteriological investigation:

(*a*) Portion of udder from cow slaughtered 28 October 1927.
(*b*) Portion of spleen from cow slaughtered 28 October 1927.
(*c*) Portion of liver of cow slaughtered 28 October 1927.
(*d*) Specimen of faeces from case in household (i).
(*e*) Specimen of faeces from case in household (ii).
(*f*) Specimen of faeces from case in household (iii).
(*g*) Specimen of faeces from case in household (iv).

From specimens (*a*), (*b*) and (*c*) derived from the cow, and from the human stools (*e*), (*f*) and (*g*), there was isolated a bacillus having all the characters of one of the "paratyphoid-food infection" group of the *Salmonellas*.

Although the bacillus was not recovered from (*d*), later investigation showed the development of specific antibodies in the serum of this patient which agglutinated both stock *gaertner* suspensions and suspensions of bacilli isolated from the organs of the cow and from the human stools.

As in the case of the strain isolated during the outbreak of the previous year, the organism in this instance was regarded as a true *B. enteritidis* of Gaertner, but its investigation by more exact serological procedures showed unequivocally that it too conformed to the characters of the *dublin* type of that bacillus.

IV. Summary

1. The most common cause of bacillary food infection in this area is the *aertrycke* bacillus which has been responsible for most of the sporadic and familial outbreaks of so-called food poisoning. It was also responsible for one, the largest, outbreak of this condition in Dundee during the period under consideration.

2. The other sporadic cases were caused by different members of the group of *Salmonella* organisms—*stanley*, *potsdam*, *senftenberg*, while two cases were possibly due to a micro-organism having somatic relationship with the *aberdeen* bacillus although not possessed of the "*i*" flagellar antigen. It is probable, therefore, that these less frequently encountered *Salmonellas* are of wide distribution.

3. Two extensive outbreaks of milk-borne bacillary food infection due to the *dublin* type of the *gaertner* bacillus are described. In both, the source of infection was determined and proved to be a cow which had indubitably suffered from a septicaemic infection due to that micro-organism.

These two *dublin* outbreaks are of special interest in view of the findings of Conybeare & Thornton (1938) concerning the presence of antibodies to this bacillus in the blood of bovines and its presence in dejecta of a cow which, in view of the absence of evidence of illness, could only be regarded as a "carrier".

If there be an appreciable number of carriers among bovines such observations as those of Montgomery (1938) concerning sporadic infantile meningitis due to the *dublin* bacillus are comprehensible though, of course, no explanation can be offered concerning the peculiar tendency to invade the meninges exhibited by this particular *Salmonella*.

APPENDIX

Technical notes

Two minor technical points are worthy of note.

(*a*) The use of brilliant green, and brilliant green telluric acid enrichment for cultural investigation of material.

In all cultural investigations of faeces, foodstuffs, etc., both direct culture on MacConkey's neutral red bile salt lactose agar and also subcultures on that medium after enrichment have been used.

The procedure in most instances was to inoculate a series of tubes of peptone water containing various suitable concentrations of brilliant green and, after 24 hr. incubation, those which showed minimal growth were plated out on neutral red agar.

The value of the method is shown by the fact that in a series of sixteen specimens of stools from milder cases in the 1926 outbreak only two positive results were obtained by direct plating while all were positive after enrichment.

(*b*) A technique which assists in obtaining specific phase strains of organisms showing diphasic variation.

The following technique was based on that used by Dr James Craigie to obtain exceptionally motile strains of the *Salmonella* bacilli.

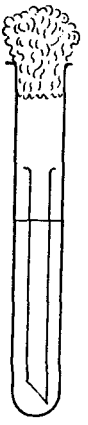
As the usual procedure advised—serial broth cultures of the mixed group and specific phases in presence of group antiserum—is rather laborious, owing to the number of colonies that must be examined at each transference from tube to tube of the "group-serum broth" medium, the following modification was attempted:

(1) *Group sera*. These were prepared by immunization with group-phase *newport*, and *kunzendorf*.

Only when the titre was 1/10,000 or more was the animal bled out. The serum obtained was dried from the frozen state (Craigie, 1931) so that no "preservative" was required for storage.

Such sera can be reconstituted when required by redissolving 0·1 g. of the dried material in 1·6 c.c. of sterile water or saline. Actually a 1 in 5 dilution is prepared by dissolving 0·1 g. in 8 c.c. of saline, this is then filtered through a small Berkefeld candle and is ready for addition to the culture medium.

(2) *Culture medium.* Ordinary nutrient agar in which the content of agar is 0·75 % is put in 6 × ½ in. tubes each of which contains a small inner tube open at both ends.



These culture tubes, containing approximately 5 c.c. of medium and provided with inner tubes, which must project well above the agar as 0·5–1 c.c. of the reconstituted group antisera have to be added after sterilization, are sterilized in the usual way by steaming. They are cooled to 50° C. and 1 or 0·5 c.c. of the filtered 1 in 5 group serum added, giving a final concentration of 1 in 25 and 50, they are then heated to 56° C. for 20 min. and are finally allowed to solidify in the upright position.

The culture from which isolation of the specific phase is required is inoculated by stab into the agar *within the inner tube* and after incubation cultures are made from the upper surface of the agar *outside the inner tube.*

If tubes of this kind are warmed at 37° C. and inoculated at 9–10 a.m. the more motile organisms have escaped from the central to the outer column of agar by evening and naturally it is these which travel most rapidly that are in the specific phase. If one waits too long to subcultivate from the outer column, group-phase organisms may appear.

If it be inconvenient to proceed thus, then it is well to inoculate last thing at night and examine for travel to the outer column first thing in the morning.

The procedure is equally applicable to the differentiation of *B. pseudo-tuberculosis rodentium* from *B. pestis.* The suspected strain is inoculated into the inner tube (no addition of serum or other reagent to the medium is required) and incubation carried out at 22° C. Often within 36 hr., and nearly always after 48 hr., *B. pseudo-tuberculosis* can be recovered from the condensation water floating on the outer column; *B. pestis*, on the other hand, remains within the small inner tube. To my old colleague, Dr Craigie, I feel that an apology is due in that so free a use has been made of a method for which he alone is responsible, and I regret that neither he nor I appreciated its potentialities when he first used it in this laboratory.

The writer desires to record a debt of gratitude to Dr Burgess, Medical Officer of Health, City of Dundee, and his staff, also to Mr Anderson, Superintendent of the Slaughterhouse, Dundee, for their willing co-operation and access to epidemiological information.

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