SPORADIC SALMONELLA INFECTIONS: A NEW SALMONELLA TYPE

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The intensive work carried out by Savage and White (1925*a*, *b*) has distinctly shown that outbreaks of food poisoning are caused, in this country, mainly by members of the Salmonella group. The papers by White (1926), Kauffmann (1929, 1930), Lovell (1932*a*), and Savage (1932) present the details of the newer knowledge on this group of bacteria. The frequent occurrence, however, of single cases of illness due to Salmonella organisms is not so widely recognised. Since 1929 a study has been made of all such infections, and the results for 1929–32 have been published (Smith, 1933), but during the year 1933 an unusually large number was encountered. The outcome of this investigation is of considerable interest, since a Salmonella organism with hitherto undescribed serological characters has been found, and also one which has not been previously described as occurring in this country.

Methods of identification

The bacteriological and serological classification of the group has become very complicated, but the work of White (1926) and of Kauffmann (1929, 1930) has clearly demonstrated that each strain must be carefully analysed for its "H" and "O" antigenic components before it can be classified. Furthermore, Andrewes (1922) has proved that it is necessary to determine whether such an organism, when diphasic, is in the specific or non-specific phase, and the work of Arkwright (1921) on dysentery organisms clearly indicates that a smooth strain must be employed before "O" antigen can be identified.

The methods employed for isolation and identification of this group of organisms were as follows: All specimens of faeces from cases of acute enteritis and urine from obscure febrile conditions were plated on MacConkey's medium, and more recently, in addition, on Wilson's brilliant green ferrous sulphite glucose agar. This latter medium has been found to be of considerable value for the primary isolation of those organisms, since, on occasion, colonies may be obtained from plates of Wilson and Blair's (1927) medium, and not from the MacConkey medium. On the other hand, however, Wilson's medium entirely inhibits members of the Flexner and Sonne dysentery groups. Non-lactose-fermenting colonies having been obtained on the MacConkey plates, or colonies with the characteristic black sheen on plates containing Wilson's medium, several of each type were picked, and the tubes containing the following media inoculated from each colony: nutrient broth, peptone water,

moist agar, lactose, glucose, mannite, dulcite, and saccharose. If after 24 hours the organism produced acid and gas in glucose, mannite, and usually dulcite, and failed to produce any change in the tubes containing lactose and saccharose, and failed to produce indole in peptone water, it was then reserved for further investigation as to its motility, and to its serological and biochemical characteristics. Whether it was motile or non-motile, the primary stage in the serological classification was proceeded with.

From the National Collection of Type Cultures at the Lister Institute, and Dr Wm. Scott of the Ministry of Health, representative strains of each Salmonella organism, so far described, have been obtained. From these strains, agglutinating antisera have been prepared for the "H" antigens of all monophasic, and for the specific phase of diphasic varieties by the intravenous inoculation of rabbits with killed broth cultures. Agglutinin titres of approximately 1/50,000 have been obtained in practically all instances. To determine whether a diphasic organism was in the specific or non-specific phase, broth cultures from single colonies were tested against a serum prepared from a strain of *S. european suipestifer* isolated locally. When no agglutination was obtained with the organism and this serum in dilutions of 1/100 and upwards, the strain was regarded as being in the specific phase.

As the Salmonella group can be divided into subgroups by identification of the "O" antigen, agglutinating sera were also prepared for the seven "O" groups, using "O" antigens, prepared as described by Gardner, for *S. paratyphosus* A, *S. paratyphosus* B, *S. paratyphosus* C, *S. newport*, *S. typhosus*, *S.* "L," and *S. bombay*. The titres of these sera ranged from 1/3200 to 1/6400.

From the preliminary serological examination of a possible Salmonella, different agglutination sera were used: (1) *S. paratyphosus* B "H" (specific), (2) *S. european suipestifer* "H" (group), (3) polyvalent serum for all monophasic strains, (4) polyvalent serum for all diphasic strains, and (5) polyvalent serum for all seven "O" antigens. The agglutination tests were carried out with the monovalent sera diluted 1/100 and upwards, and with the polyvalent sera diluted 1/20 and upwards, the polyvalent sera being prepared by mixing together equal quantities of the monovalent sera. From the preliminary agglutination test it was possible to say whether the organism was in the specific phase, whether it was possibly a true paratyphoid B, and whether it belonged to the monophasic or diphasic series. From this result, also, a preliminary report could be made as to the nature of the infecting organism. The exact type was next determined.

For this purpose, agglutination tests were carried out with all seven "O" agglutinating sera. Usually the strain under examination agglutinated to the full titre of one serum, and to a less extent with another. Agglutinin absorption tests were then made with the emulsion obtained from the growth of two agar plates in 0·98 c.c. saline and 0·02 c.c. of serum. When the organism under examination absorbed the "O" agglutinins from a particular serum, then the "O" group to which it belonged was identified. If the organism was appa-

rently monophasic, the "H" antigen was prepared by growing in broth at 22° C.; if it was diphasic, and in the group phase, further colonies were picked into broth, the culture obtained being tested against the group *E. european suipestifer* serum. If this method failed to produce the specific phase, then the method described by Scott (1926) was used, that is, subculturing the organism repeatedly in broth containing 10 per cent. group serum, replating, and selecting further colonies for examination. When the specific "H" antigen was obtained, agglutination tests were then carried out with the "H" sera of the organisms to which the "O" group had been found to belong. This usually indicated the type, but to confirm this, further agglutinin absorption tests were made, using an emulsion of the organism—three agar plates—0·98 c.c. of saline and 0·02 c.c. of each specific serum. By these methods the "H" and "O" antigens of an organism were completely identified, and the organism thus classified.

By using the polyvalent "H" monophasic and diphasic sera, and the polyvalent "O" serum, it was practically impossible to miss any organism belonging to the Salmonella group. The "O" polyvalent antigen was often found to be particularly useful, as the organism in the early stage of its cultural life often had only a feebly developed "H" antigen. Furthermore, even for purposes of diagnosing an infection due to *B. paratyphosus* B, it is really essential to identify completely the organism both as regards specific "H" antigen and "O" antigen, otherwise, it may be confused in particular with *S. aertrycke.*

In the meantime, the biochemical characteristics were further examined by inoculating the strain into tubes containing litmus milk, xylose, inosite, arabinose, rhamnose, and lead acetate agar.

RESULTS

Incidence of various Salmonella types

During the years 1929–32, thirty distinct outbreaks involving forty-six cases occurred, whereas during the year 1933 no less than thirty outbreaks involving forty-four cases were encountered. Why there should have been such a high incidence in 1933 as compared with previous years cannot be explained, unless these infections have some definite relationship to a general increase in the daily temperature, for the majority of such infections occur during the summer months. The distribution of the various types in the year periods 1929–32 and 1933 is given in Table I, and is compared with those given by Savage (1932) for England. In the sixty outbreaks, infections due to *S. aertrycke* predominate, followed by those due to *S. thompson*, *S. dublin*, *S. enteritidis*, and *S. european suipestifer*. Again, in twenty-three of the outbreaks during 1933, or in fifty-three instances in all, only one individual appeared to be involved, and the greatest number of cases associated with any one outbreak in 1933 was six.

Biochemical reactions

The biochemical reactions of the various strains isolated during 1933 were investigated by the procedure already described. The *aertrycke* strains, twenty-four in number, all produced acid and gas in dulcite, xylose, arabinose and rhamnose, and only four failed to ferment inosite, while all produced blackening on lead acetate medium. The *gaertner* and *dublin* types failed to produce acid and gas in inosite, but fermented dulcite, xylose, arabinose and rhamnose, while the *thompson* and *potsdam* strains produced acid and gas in all these

Table I

Group		England (Savage) 1919–31	North-east Scotland 1929–32		1933		Total	
		Out-breaks	Out-breaks	Cases	Out-breaks	Cases	Out-breaks	Cases
A	Seftenberg-Newcastle	2	—	—	—	—	—	—
B	Aertrycke-Breslau	76	18	20	17	30	35	50
B	Stanley	1	—	—	—	—	—	—
B	Reading	—	—	—	—	—	—	—
B	Derby	2	—	—	—	—	—	—
B	Heidelberg	—	—	—	—	—	—	—
C	Suipestifer-American	7	1	1	—	—	1	1
C	Suipestifer-European		1	1	3	3	4	4
C	Thompson-Berlin	7	5	17	1	1	6	18
C	Oranienburg	—	—	—	—	—	—	—
C	Potsdam	—	—	—	1	1	1	1
D	Newport	5	—	—	—	—	—	—
D	Morbificans Bovis	3	—	—	—	—	—	—
D	Enteritidis-Gaertner Jena	14	—	—	5	6	5	6
D	Dublin-Gaertner Kiel	1	4	6	2	2	6	8
F	Aberdeen	—	—	—	1	1	1	1
G	Salmonella not fully typed	3	1	1	—	—	1	1
		121	30	46	30	44	60	90

sugars. Contrary to the usual findings, however, the three *suipestifer* strains produced acid and gas in dulcite, as well as in xylose and rhamnose. All strains blackened lead acetate agar medium.

Clinical and epidemiological findings

S. aertrycke *infections*

There were no unusual features associated with the thirty cases of this infection during 1933. In five instances, more than one member of a family was infected (2, 6, 3, 5, 2). All patients had the usual symptoms of a Salmonella infection, and only one case, a man aged 55, died after an illness lasting 24 hours.

S. potsdam *infection*

So far, no case has been reported in this country due to the Salmonella organism of this type. The history of this case was as follows: Case 48, A. P., a male child aged $4\frac{1}{2}$ years, developed vomiting, abdominal pain, and diarrhoea, with slight fever. The illness persisted for 6 days, and then subsided. From

the faeces, a non-lactose-fermenting organism was isolated. Examination of this organism showed it to be motile. It produced acid and gas in media containing glucose, mannite, dulcite, xylose, inosite, arabinose and rhamnose. It failed to produce indole, and produced blackening on lead acetate agar. Investigation of the serological character of the organism showed that it possessed the "O" antigen of the *S. paratyphosum* C group, that it did not possess a group phase, and that its "H" antigen agglutinated to the full titres of the *S. potsdam* and *S. brandenburg* sera, and absorbed the agglutinins from those sera. Similarly, agglutination and agglutinin absorption tests were carried out with sera prepared from the "O" antigen of *S. paratyphosum* A, *S. newport*, *S. typhosum*, *S. "L,"* *S. bombay*, and *S. derby*. No agglutination occurred with the sera, and the organism failed to absorb the agglutinins. To confirm these observations, "H" and "O" sera were prepared from the antigens of the isolated strain, and reciprocal absorption tests showed the "H" antigen to be identical with those of *S. potsdam* and *S. brandenburg*. Similarly, the "O" antigen was found to be identical with that of the paratyphoid C group, and distinct from the "O" antigen of the paratyphoid B group to which *S. brandenburg* belongs.

Infections due to S. dublin

In 1933, two fatal cases of this infection were encountered. The first case (47) was a male infant aged 7 months, whose clinical history was as follows: R. McR. was admitted to hospital on 30. i. 33. The illness was reported to have commenced 2 days prior to his admission with convulsions, cough, sickness and vomiting. The mother stated that the child had been a healthy and thriving baby prior to this acute illness. On admission the temperature was 103° F., pulse rate 148, respiration rate 56, and the child was having definite convulsions. Physical examination revealed no evidence of any exanthem, tongue, mouth and throat were clean, there was no glandular enlargement, and no nasal or aural discharge. Examination of heart and abdomen showed nothing significant, but chest showed evidence of bilateral basal broncho-pneumonia. There was marked hypertonia of all the skeleton muscles, and a moderate degree of neck rigidity. Kernig's sign was positive, but reflexes were not elicited. The pupils were equal, central, and reacted briskly to light; there was no nystagmus or squinting. The spasticity became more marked, and the convulsions more frequent, and physicial examination invariably started off a convulsion which lasted for several hours on end. Definite bilateral basal broncho-pneumonia was proved by radiological examination. Four days after admission there developed classical signs of tetany, with typical carpopedal spasm, while the convulsions became more numerous, and were associated with definite laryngismus stridulus and facial twitchings. There was no evidence, either clinically or radiologically, of rickets, no associated diarrhoea or gastric intestinal disturbance, no albuminuria, and the estimation of the blood calcium showed 6·1 mg. per 100 c.c. There was extreme

rigidity of the muscles of the jaws, which were so tightly clenched that it was absolutely impossible to examine the mouth or throat. Early in the disease there was marked tension of the fontanelles; lumbar punctures showed that the cerebro-spinal fluid was turbid and under pressure. Repeated lumbar punctures, however, showed that by the second week the fluid was almost clear, did not remain under pressure, and could be withdrawn only with difficulty. By this time there was a depression of the fontanelles, and the circumference of the cranium was one inch less as compared with the measurement taken the week previous. In the third week the child became extraordinarily rigid, the legs being extended, with full extension of the ankle joint and drawing up of the heel and flexion of the toes, and the arms being fixed in semi-flexion at the elbow joint. Until this time the child took its food well, and did not lose weight; then, however, there was commencing dehydration, and undoubted mental impairment and blindness. Wasting became marked by the fourth week, the physical signs remaining much the same, with the exception that the convulsions became more severe, and the spasticity and tetany more marked. During the whole of the illness, the child ran a continuously high temperature between 100 and 103° F. Death occurred on the thirty-fourth day after admission to hospital.

The bacteriological findings can be summarised as follows:

Date	Specimen	Protein	Cells per c.mm.	Culture
16. ii. 33	C.S.F.	Increased +	25	Sterile
18. ii. 33	,,	,,	522	S. dublin
21. ii. 33	,,	,,	52	,,
22. ii. 33	,,	,,	83	,,
23. ii. 33	,,	,,	91	,,
22. ii. 33	Urine	—	—	,,
22. ii. 33	Faeces	—	—	Negative
24. ii. 33	Blood	—	—	S. dublin

In addition, an agglutination test showed that the serum on 24. ii. 33 agglutinated:

S. typhosus	"H" antigen	Nil
,,	"O" ,,	1/400
S. enteritidis gaertner	"H" ,,	1/1600
S. dublin	"H" ,,	1/6400
,,	"O" ,,	1/400

When the child died, an autopsy was conducted. The heart showed no abnormality, while the lungs showed basal congestion only; the intestines and kidneys were normal, but the spleen and liver showed slight enlargement. The dura was firmly adherent to the skull; the whole brain was enveloped in a layer of thick, leathery pus, and, on section, the ventricles showed some distension, but throughout the brain substance there was no local abscess formation.

Case 79. A female aged 2 years was admitted to hospital on 12. ix. 33. The throat showed bilateral tonsilar enlargement, with ulceration of left tonsil, and with the posterior pharyngeal wall covered with exudate. On coughing, purulent material was evidently exuding from the trachea. The breath was

fetid, and the cervical glands were much enlarged, and the chest showed signs of an extensive broncho-pneumonia of the left lung. The child died in 3 days.

The bacteriological examinations showed:

12. ix. 33	Throat swab: *S. dublin* and *Str. haemolyticus*
	Blood culture: *S. dublin*
	Serum: Failed to agglutinate any antigen of the typhoid-paratyphoid group
14. ix. 33	Blood culture: *S. dublin* present

Autopsy: The right lung showed basal congestion only. The left pleural cavity was filled with pus, and the left lung showed marked pathological change. The lower lobe was consolidated, showed numerous abscesses, and was actually in a gangrenous condition. The heart showed no definite abnormality. The spleen and liver showed definite enlargement, and the liver and kidneys showed toxic changes. The intestines showed no abnormality. From the pus from the chest, *S. dublin* was again cultivated.

Infection due to S. aberdeen

The organism causing an infection in case 51 was found to have serological characteristics which differed from any Salmonella type already described, and hence has been named *S. aberdeen.*

The history of the case from which this organism was isolated was as follows: A child aged 11 months developed signs of acute enteritis—vomiting, diarrhoea and fever. This continued for 3 days, and then subsided. From the faeces, a non-lactose-fermenting organism was obtained. The organism produced acid and gas in 24 hours in media containing glucose, mannite, dulcite, xylose, arabinose and rhamnose, but failed to ferment inosite, even after an incubation period of 10 days. It failed to produce indole in peptone water, but produced blackening on lead acetate agar, and an acid to alkaline reaction in litmus milk. The organism was apparently in the group phase when first isolated, but after plating and selection of single colonies, no difficulty was found in obtaining a specific phase form, *i.e.* one which would not agglutinate with the group serum for *S. european suipestifer.* The specific phase "H" antigen agglutinated to the full titre of the specific *S. aertrycke* serum, and absorbed the agglutinins without difficulty from that serum. When, however, the organism was tested against the "O" antigen antiserum for the paratyphoid B group, it did not agglutinate with this serum, or absorb the agglutinins from it. Furthermore, the cultural characteristics, salt and thermo-agglutination tests, showed no evidence of any rough variation. Similarly, agglutination tests and agglutinin absorption tests were carried out with all the other "O" sera, but these failed to agglutinate the organism, and the agglutinins were not absorbed by it. Sera were therefore prepared for the specific "H," group "H," and "O" antigens of the organism. Reciprocal agglutination and absorption tests showed that the "H" specific and "H" group antigen were identical with the respective antigens of *S. aertrycke*, but the tests with the "O" antiserum showed that it would neither agglutinate

any of the known "O" antigens, nor would these antigens absorb the agglutinins from it. Also, through the kindness of Dr W. M. Scott, the "O" serum was tested against the "O" emulsions of no less than fifty-five strains of *S. aertrycke* in his possession, with completely negative results. The antigenic structure might, therefore, be formularised by the Kauffmann and White methods as follows:

	Kauffmann			White		
		"H" antigen			"H" antigen	
Type	"O" antigen	Specific	Group	"O" antigen	Specific	Group
Aberdeen	XI	i	1, 2, 3	IX	Aertrycke	G.A.B.C.

Infections due to S. european suipestifer

Three infections due to *S. european suipestifer* were encountered during the year 1933, as compared with only one during the period 1929–32. The first case, No. 50, was a male aged 53, whose main symptoms were those of fever, shivering and sweating, especially at night. The evening temperature varied from 99·8 to 102° F. The tongue was clean, there was no indication of alimentary disturbance, no rose spots, and no splenic enlargement. When first seen by his physician, the urine was found to contain much pus. The blood was cultured, with negative results, and the serum only agglutinated the group Salmonella antigen to a dilution of 1/100. The urine sent was found to contain much pus, and a non-lactose-fermenting organism was obtained in pure culture. The biochemical and serological investigation showed the organism to be *S. european suipestifer*. The illness continued for some 4 weeks, and then subsided, but repeated specimens of urine showed the organism to be still present.

Case 54. The history of this case was as follows: A female aged 28 had an acute attack of follicular tonsilitis. This subsided in a few days, but was later followed by a quinsy. The abscess was evacuated, and the temperature again subsided. Three days later the temperature again rose to 103° F., and the fever was accompanied by headache, vomiting and diarrhoea. After this had continued for several days, the practitioner took a blood culture, which, after an incubation period of 24 hours, showed a non-lactose-fermenting organism, later proved to be *S. european suipestifer*. The symptoms soon subsided, and the patient made an uneventful recovery.

Case 72. A female aged 38 had an acute attack of enteritis, with blood in the faeces. The illness passed off in 3 days. From the faeces, *S. european suipestifer* was isolated.

Infections due to S. thompson *and* S. gaertner

One infection due to *S. thompson*, and six to *S. enteritidis* were found. They were all simple cases of enteritis, without any unusual features.

DISCUSSION

From the foregoing observations, several points emerge for discussion. In the first instance, there is considerable variation in the type of disease produced by certain of the Salmonella organisms. Thus, the *aertrycke, thompson,* and *gaertner* types evidently produce, for the most part, symptoms of acute enteritis, though, on occasion, the *aertrycke* has been found to produce a long-continued septicaemia. On the other hand, *S. dublin* has been associated with septicaemia and meningitis. Thus, in the previous series of cases, three had a blood infection—one developing meningitis—and three had a simple enteritis. In the present series, both cases had septicaemia, and one developed meningitis. Again, no less than four out of the eight cases died. From time to time cases of meningitis due to *S. gaertner* are reported on, and it seems probable that closer investigation of the organism obtained would show that the causal organism was really *S. dublin*, and not *S. gaertner*. The *suipestifer* group also appears to be more capable of tissue invasion. In the previous series, one case of septicaemia due to *S. american suipestifer* and one to *S. european suipestifer* were encountered. In the present series, an infection of the urinary tract, which presumably followed a blood stream infection, a case of septicaemia, and a case of simple enteritis were found to occur. So far, however, these infections have not been found to be associated with the same high mortality rate as the *S. dublin* infections. Recently Sugita (1933), Tsuchimochi and Ran (1933), and Kuttner and Zepp (1933) have reported cases with similar symptoms.

In so far as cases occurring in the immediate vicinity of Aberdeen were concerned, a personal investigation was made of possible sources of infection. In one outbreak due to *S. aertrycke*, in which six cases were involved, it was possible to ascribe the illness to the consumption of an infected meat pie. In the other cases, no definite source of infection could be assigned to them. This is particularly the case when only a single case occurs in a whole family, and it is not possible to incriminate any one article of food. Much more remains to be done yet in the investigation of natural reservoirs of infection for this group of organisms. It seems unlikely that human carriers occur, for, in the examination of thousands of specimens of faeces in this laboratory, Salmonella organisms have been found in cases of acute infection only. The literature on animal and bird Salmonella infections has been reviewed by White (1929) and Lovell (1932*b*), and no doubt it is from these sources that human beings receive their infections.

SUMMARY

1. An account is given of sporadic Salmonella infections. In the period 1929–32, thirty outbreaks occurred, involving forty-six cases, whereas, in the year 1933, thirty outbreaks involving forty-four cases were encountered.

2. The investigation has shown that the most frequent cause of such infections are Salmonella types *aertrycke, thompson, dublin, enteritidis* and *european suipestifer.*

3. It has been found that the *dublin* and *suipestifer* types are definitely more tissue invasive than the other types. Septicaemia and meningitis have been associated with infections due to *S. dublin*, and septicaemia in cases infected with *S. suipestifer.*

4. The biochemical serological characters of a new Salmonella type—*S. aberdeen*—have been described.

5. An infection due to *S. potsdam* has been encountered. This type has not been previously described as occurring in this country.

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