AN OUTBREAK OF GASTRO-ENTERITIS DUE TO *SALMONELLA DERBY*

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In 1946 an outbreak of gastro-enteritis occurred among infants in a hospital population. The outbreak lasted eight calendar months commencing in late autumn, continuing throughout winter and spring and subsiding at the beginning of summer. Sixty-eight children were involved in this outbreak and ten died, but as in most of them gastro-enteritis was complicated by other conditions, it was impossible to determine mortality.

The causative organism of this interesting outbreak was *Salmonella derby*, a bacterium not commonly found up till now in gastro-enteritis in Australia. Amongst the various accounts on salmonellosis in this country, two papers (Atkinson & Woodroofe, 1944; Atkinson, Woodroofe & Macbeth, 1947) describe the isolation of *Salm. derby* from man, seven sporadic cases being reported, while only one reference was found (Gorrie, 1946) on isolation of this bacterium from an animal source. For this and other reasons it was considered appropriate to describe our present findings. This communication is mainly concerned with the isolation of the causative organism from a variety of sources, its identification, pathogenicity and epidemiological aspects.

EPIDEMIOLOGY

Between May and the middle of July, five cases which were admitted to the hospital with the primary diagnosis of gastro-enteritis were found on bacteriological examination to be due to *Salm. derby* infection. However, the majority of patients contracting infection subsequent to these initial cases were infants admitted for causes other than gastro-enteritis. Although separate foci of infection might have existed outside the hospital at the time of the outbreak, no correlation of contact could be detected in the initial phase, and the evidence presented separately (Rubbo, 1948) strongly suggests that most of the children developed gastro-enteritis while patients in the hospital.

It was of importance to determine what factors contributed to the spread of infection. The patients involved in the outbreak were mostly babies on a milk diet and the general picture of the distribution of the infection, which was confined mainly to two wards, did not indicate the central food-room to be the initial source. In order to confirm this supposition, several kinds of foods like lactogen, lactose, glucose, and cane sugar were bacteriologically examined. In no instance was a *Salmonella* bacilli detected.

Bacteriological investigation was then conducted in the wards on the assumption that food was contaminated here directly or indirectly. Eleven specimens of dust from ward floors and linen annexes were examined bacteriologically and five of them yielded *Salm. derby*. Five mice were caught in the wards and on autopsy *Salm. derby* was recovered from the spleen and small intestine of one animal. Another important result of the investigation was the fact that an examination of two roller towels used by nurses resulted in the isolation of *Salm. derby* from one towel, and *Pseudomonas pyocyanea* and coliform organisms from the other, indicating gross contamination with faecal types of bacteria. Thus it was demonstrated that the infective agent, *Salmonella derby*, was present in ward dust, in a mouse caught in the ward and on a ward towel. The significance of this distribution in regard to the direct or indirect contamination of food in the wards is discussed separately (Rubbo, 1948).

It became important to determine whether, in addition to the factors described, the outbreak was maintained by carriers, either nurses or children. Bacteriological examination of stools from patients with no history or symptoms of diarrhoea showed that a number of children harboured *Salm. derby*. In some cases, however, the symptoms of gastro-enteritis appeared after the bacteriological diagnosis had been made. Further search for carriers amongst nurses handling food and caring for the patients could not be done for reasons outside our control. Only a few faecal and blood specimens of the staff personnel were examined but these gave negative results.

To present a more complete picture of the outbreak, it can be stated that of the sixty-eight patients involved, ten were admitted with a primary diagnosis of gastro-enteritis, thirty-seven apparently contracted the infection while in hospital and twenty-one were found to be carriers.

CLINICAL FEATURES

The incidence of infection was concentrated mainly among children under 1 year of age, this group comprising fifty-six patients, whereas *Salm. derby*

was not detected in children above 3 years of age. It should be noted, however, that patients in the affected wards were mostly infants and so this group of children was exposed to infection more than others.

Age	Under 1 year	1–2 years	2–3 years
Number of patients with *Salm. derby* infection	56	8	4

The clinical picture presented three types of infection: (1) no symptoms of gastro-enteritis (carrier cases), (2) mild diarrhoea with loose motions, often mucus present, and (3) severe gastro-enteritis with fever, dehydration, vomiting, diarrhoea and frequent offensive stools containing mucus and blood.

symptoms of diarrhoea but examined for the purpose of detecting carriers; in some instances, several specimens were taken from the same child. The results of the examination are shown in Table 1, in which it will be noted that of the ninety-nine stools containing *Salmonella* bacilli as determined by tetrathionate broth →'SS' agar method of isolation, 87 or 88 % had *Salm. derby*. It is worth noting that the best results were obtained with the aid of tetrathionate broth enrichment medium and subsequent cultivation on 'SS' agar.

Comparison of direct plating on plain desoxycholate and 'SS' agar media showed the superiority of the latter in selectivity of *Salmonella* bacilli, but in four instances *Salm. derby* was detected on desoxycholate agar while 'SS' agar gave negative results. This was probably due to the abundance of *Proteus* bacilli, the growth of which is favoured by 'SS' agar.

Table 1. *Frequency of isolation of* Salmonella *bacilli and* Salm. derby *from faeces with the use of various media*

Medium	No. of specimens	No. of isolations of *Salm.* bacilli	No. of isolations of *Salm. derby*	No. of *Salm.* bacilli other than *Salm. derby*	Efficiency of medium
Plain desoxycholate agar	399	31	28	3	Poor
'SS' agar	399	60	51	9	Fair
Tetrathionate broth→'SS' agar	382	99	87	12	Good

BACTERIOLOGICAL INVESTIGATION

Methods of isolation

The routine method adopted in the examination of faeces was to place a representative sample of the stool in a preservative solution (Sachs, 1939), consisting of a buffered 30 % glycerin saline. Whenever present in the specimen blood-stained mucus washed in normal saline was used as a source of inoculum. At the beginning of the outbreak the method of cultivation consisted in the use of the following media: plain desoxycholate agar (Leifson, 1935), 'SS' agar ('Difco' product) and Wilson & Blair's (1941) tellurite-rosolic acid medium, Gregory's (1944) modification, the last one being employed for the detection of members of the *Shigella* group. However, when *Salmonella derby* was found to be the causative organism of a number of cases the use of Wilson & Blair's medium was discontinued and tetrathionate broth enrichment medium with subsequent subcultures to 'SS' agar plates was added to the routine investigation.

Results

In all, 399 faecal specimens were submitted for bacteriological investigation from a variety of cases of gastro-enteritis and from patients showing no

Similarly, on plating out from tetrathionate broth to 'SS' agar in two instances *Salmonella* bacilli were not recovered, while on direct plating from the same specimen to 'SS' agar the organism was detected. However, the impression obtained strongly supports the view (Ferris & Hertzberg, 1945) that the optimal method of isolation of *Salmonella* bacilli from stools or from other sources consists in tetrathionate broth →'SS' agar technique in spite of the fact that tetrathionate favours the multiplication of *Proteus* bacilli.

Due to the abundance of *Proteus* bacilli on the 'SS' plates it proved often of advantage to make preliminary subcultures of colonies to 1 ml. quantity of mannite peptone water; cultures positive for *Salmonella* strains were fermented after a few hours of incubation at 37° C. and could be further examined.

Identification of Salmonella derby

Non-lactose fermenting colonies, found on desoxycholate agar or on 'SS' agar plates, were identified by biochemical reactions on Hajna's (1945) triple-sugar iron agar medium and by serological means.

Slide and serial agglutination tests placed the causal organism of the outbreak in group B of Kauffmann-White schema; the organism was

further identified as *Salm. derby* with absorbed serum H specific for factor '*f*' (Edwards & Bruner, 1942).

Cultural and biochemical characters of Salmonella derby

On 'SS' agar plates after 24 hr. incubation at 37° C. the colonies were pale or with a brown or black centre due to the production of hydrogen sulphide. Mucoid variants were not encountered. Fermentation reactions showed that mannite, glucose, maltose, galactose, dulcite, trehalose, sorbitol, arabinose, rhamnose, xylose, dextrose and inosite were acidified and gas produced after 18 hr. incubation at 37° C. whereas lactose, saccharose and salicin were not fermented. Other features were fermentation of *d*-tartrate, abundant production of H_2S in triple-sugar iron agar medium, growth in citrate, gelatin not liquefied, indole negative, *MR* positive, *VP* negative, litmus milk alkaline.

Antigenic structure of Salmonella derby

As two types of *Salm. derby* are recognized (Edwards & Bruner, 1942; Kauffmann, 1937) one with the O antigenic pattern I, IV, XII, and the other IV, XII, the cultures were tested for the presence of *l* factor, using *Salm. paratyphi A* antiserum (I, II, XII) absorbed with *Salm. paratyphi A* var. *durazzo* (II, XII). All cultures proved to contain factor *l*.

Mirror agglutination-absorption tests were then performed. Absorption of an antiserum from a rabbit immunized with a mixed suspension of three cultures of *Salm. derby* isolated from different sources (patient, mouse, dust) with a standard *Salm. derby* culture 15145 (I, IV, XII) from the 'National Collection of Type Cultures' removed all the homologous O agglutinins. Similarly, absorption of *Salm. derby* O antiserum, prepared with the standard culture (I, IV, XII), by a number of freshly isolated strains of this organism removed completely the homologous agglutinins.

Mirror tests were then performed for the H antigen. A freshly isolated strain of *Salm. derby* failed to absorb some minor H antigen factors from antiserum prepared with the standard culture of *Salm. derby* containing factors *f*, *g*, ... It seemed of interest to compare several of our cultures of *Salm. derby* from different sources, including cultures isolated from the first six patients and a child carrier, from a mouse, towel and dust. All behaved similarly, removing completely the homologous agglutinins for each other but not absorbing fully agglutinins for the standard culture. Absorption of the antiserum prepared with a mixed suspension of the freshly isolated organisms by the standard culture of *Salm. derby*, and by a number of the freshly isolated cultures removed completely the homologous agglutinins.

The above tests show the identical antigenic structure of the freshly isolated strains and a slightly different flagellar pattern from a standard culture of *Salm. derby*.

Serological tests

Agglutination tests were performed with sixty-nine sera, nine being from patients infected with *Salm. derby*, four from babies with no history of gastro-enteritis, six from members of the hospital staff, and fifty sera from adults not connected with the hospital. Among the patients were eight cases of cross-infection and one carrier. The sera of these patients were obtained between 1 and 3 weeks from the apparent onset of illness.

To prepare O antigen, an agar culture of *Salm. derby* was suspended in normal saline, boiled for 2 hr., standardized to 1000×10^6/ml. opacity and preserved with 0·2% formalin. For H antigen formalin (0·2%) was added to a 6 hr. old broth culture of the organism whose motility had been previously enhanced by passage through semi-solid agar.

	Titre of H agglutination				
	1/20	1/40	1/80	1/160	1/320
Number of patients	1	1	5	1	1
Age of patients (in months)	3	9	1–5	3	4

As shown above, the sera of patients were found to have a titre of 1/20 to 1/320 for *Salm. derby* H antigen, titre of 1/80 being encountered in five out of nine patients. O agglutinins were not detected. As the carrier state was often encountered it is difficult to assess at what stage of the infection agglutinins H were circulating in blood.

Both H and O agglutinins were absent from the sera of the normal babies and of the staff personnel. Of the fifty sera of adults, serving as controls, two showed 1/10 agglutination with O antigen but none with H.

On the basis of the above findings, although limited in number of tests, it seems that serological examination could be a valuable aid in diagnosis of *Salm. derby* infection by detecting H agglutinins.

PATHOGENICITY TESTS

It will be remembered that in the course of the epidemiological investigation a mouse was found harbouring *Salm. derby* (see p. 151). In view of this, and in view of the undoubted human pathogenicity of *Salm. derby*, it was considered of importance to determine the virulence of the organism under controlled conditions.

To this end the following experiments were carried out. *Salm. derby* was administered to mice by oral or intraperitoneal routes. Feeding was done by delivering various doses of 4 hr. and 18 hr. old broth

cultures by means of a calibrated Pasteur pipette into the mouth of a mouse, or by giving infected intestinal organs from experimental mice which had died of *Salm. derby* septicaemia. Intraperitoneal injections were carried out with a number of *Salm. derby* strains of different origin, namely, isolated from a clinical case, carrier, mouse, dust and towel. In addition, *Salm. typhi-murium*, which is a natural pathogen of mice (Topley & Ayrton, 1923), was tested for virulence to obtain comparative data. In general, groups of mice, comprising ten to twenty animals, were used in these experiments; the mice were kept under observation for periods up to 6 weeks, and a few were retained for several months to determine the duration of carrier state.

The results of the pathogenicity experiments were as follows:

(1) *Salm. derby* was of low virulence to mice when administered *per os*. The organism was not fatal when drops of broth cultures containing up to 200 million bacteria, as determined by opacity standards, were administered. However, a carrier state was induced when doses starting with 25 million bacteria were given, the mice excreting *Salm. derby* a few days after feeding and continuing to do so during the observation period of 6 weeks. Feeding with *Salm. derby* infected intestinal organs induced a carrier state in normal mice but proved to be fatal to young mice, whose resistance was lowered by starvation.

(2) *Salm. derby* is more virulent to mice when administered intraperitoneally. When intraperitoneal injections of 5 million bacteria were given the average mortality within 1 week from the time of injection was shown to be 40%, the figure ranging from 20 to 60% for various strains. In one case delayed death was observed 4 weeks after the injection. When larger doses of 25 million bacteria were administered intraperitoneally, septicaemia occurred within 24–48 hr.; with smaller doses ranging from 40,000 to 1 million bacteria no fatal cases were recorded but a carrier state was induced.

(3) *Salm. derby* was recovered at post-mortem examinations carried out on infected mice which died as a result of the infection, or were killed for the purpose of bacteriological investigation. The organism was found in various organs, in the heart in septicaemic cases, in the spleen, the small intestine or the bladder. Mice harboured *Salm. derby* 3 months after the bacterium was administered, but not after 5 months.

(4) Comparison of results of pathogenicity tests carried out on mice with *Salm. typhi-murium* and *Salm. derby* strains showed the latter to be a less virulent organism for these rodents, while comparison of results on duration of carrier state indicated that *Salm. typhi-murium* was harboured for a longer period of time.

On the evidence of the above experiments it is apparent that *Salm. derby* is of low virulence to mice *per os*, but is fatal when an appropriate dose is administered intraperitoneally, various strains differing in virulence. Animals which survive the infection can become faecal and urinary carriers.

DISCUSSION

It seemed of interest to ascertain the frequency of occurrence and distribution of *Salm. derby* in Australia and in other countries.

The organism was first isolated by Peckham in an outbreak of pork-pie poisoning at Derby in 1923. The incriminating organism was found in a pie and in a butcher's tank. Agglutination tests were performed with the sera of patients but faecal specimens were not examined. The bacterium was found to be related to *Bacillus gaertner* and was originally named *B. enteritidis*. Savage & White (1925) isolated the same type from swine-fever material and from human cases and referred to this organism as *Salm. derby*. Altogether, between 1923 and 1944, *Salm. derby* was listed eleven times in cases and outbreaks of food poisoning in England and Wales (Wilson & Miles, 1946). It is worth noting that three strains of this organism were isolated in Great Britain from spray-dried eggs brought from America (Med. Res. Coun. 1947), but the import of this product started toward the end of 1941 while up till then *Salm. derby* was recorded already six times. On the other hand, Gordon & Buxton (1946), in a survey of avian salmonellosis in Great Britain covering the period 1933–44, did not list the above strain. Edwards & Bruner (1943), in a survey of *Salmonella* occurrence in the U.S.A. between 1934 and 1941, encountered *Salm. derby* in forty-four outbreaks mostly in swine and fowls, once with ruminants and rarely in man, the organism being recovered from human sources four times only, twice from gastro-enteritis cases and twice from carriers. Hinshaw, McNeil & Taylor (1944) isolated the bacterium from cases of salmonellosis in turkeys and chickens. Seligman, Saphra & Wassermann (1943), of the New York Salmonella Center, in their analysis of *Salmonella* infections in man, covering the period 1939–43, gave a higher incidence of *Salm. derby*, twenty-seven isolations, involving twenty-six outbreaks, being made from human stools. In their list of carriers five food handlers were detected harbouring this organism. In general, *Salm. derby* was found to be widely distributed in America: thus, to quote only a few workers, Hajna & Perry (1945) reported this organism in Maryland; Galton & Quan (1943, 1944) encountered it in Florida; Rubinstein, Femster & Smith (1944) in Massachusetts; Morris, Brim & Sellers in Georgia (1944); Hormaeche, Suracco, Peluffo & Aleppo (1943), in their research on causes of summer diarrhoea in Uruguay between

1936 and 1942, isolated eighteen strains; Varela, Zozaya & Olarte (1943) listed it in Mexico and Stone (1943) in the Panama Canal zone in asymptomatic food handlers.

During investigations in America on the occurrence of *Salmonella* with regard to problems of public health, *Salm. derby* was often encountered in apparently healthy or slaughtered animals. For example, Rubin, Scherago & Weaver (1942) and Varela & Zozaya (1941) found this organism in the lymph glands of normal hogs, and Cherry, Scherago & Weaver (1943) obtained cultures from retail meat products.

In Australia, Atkinson & Woodroofe in 1944 identified three cultures of *Salm. derby*, two of them isolated from children with gastro-enteritis and one from an adult with ulcerative colitis. These cultures contained factor *l*. The organism was not encountered in a later survey (Atkinson *et al.* 1944), but was recently again reported (Atkinson *et al.* 1947), four cases of gastro-enteritis due to this bacterium being listed, involving three children and one adult. The seven reported strains of *Salm. derby* form approximately 3 % of a collection of 243 *Salmonella* cultures identified at Adelaide during a period of 3 years. This organism was not included in the list of *Salmonella* isolated from animals in Australia (Atkinson & Woodroofe, 1944), and Gray (1946) in a review of veterinary aspects of bacterial food poisoning, did not report its occurrence in this country. However, in a survey of enteric diseases of swine (Hayston, 1946; Gorrie, 1946), Gorrie mentioned the isolation of one strain of *Salm. derby*.

Figures on the occurrence of *Salmonella* in some other countries are quoted in Kauffmann's work of 1941. *Salm. derby* was not isolated in Denmark, Norway or India, but was found in Germany and the Dutch Indies. Rauss (1941) encountered it in Hungary. In more recent surveys of salmonellosis problems in various countries this bacterium was not recorded in India (Hayes & Freeman, 1945) or Palestine (Olitzki, 1944). *Salm. derby* was listed amongst enteric bacteria reported from various areas of military operations, e.g. the Mediterranean theatre (Bruner, 1945), the Pacific area (Lindberg & Bayliss, 1946). It is apparent, however, that the organism was isolated from sporadic cases of gastro-enteritis and from carriers but not in association with any outbreaks.

It is interesting to note that *Salmonella* bacilli, including *Salm. derby*, were isolated from children's tonsils (Varela & Olarte, 1942), a fact of epidemiological significance. *Salmonella* organisms have been also found in lymphatic nodes (Varela & Olarte, 1944) in post-mortem examinations of people who died of various maladies, but *Salm. derby* was not cultivated from this source.

This review of the literature, indicates that *Salm. derby* is often encountered in America in infections of man and animals but is apparently not frequent in Great Britain. In Australia *Salm. derby* is not a common organism and up till now the bacterium has been isolated only from sporadic cases of gastro-enteritis. In most other countries surveys on the occurrence of salmonellosis are not up to date.

The outbreak of *Salm. derby* described in this paper is the only one reported in Australia and the largest one found on record in the available literature.

All the strains of *Salm. derby* isolated in this outbreak had a similar antigenic pattern, the characteristic feature being the presence of factor *l* and the absence of a minor flagellar antigen which was encountered in two of the standard cultures. In reference to factor *l* Kauffmann (1937), examining thirteen strains of *Salm. derby*, observed that cultures without factor *l* were isolated from pigs, while those possessing factor *l* were of human origin. Atkinson & Woodroofe's (1944) three cultures from human sources also contained factor *l*. The results of our investigation confirm Kauffmann's suggestion that strains possessing factor *l* are of human origin but this observation should be supported by still more evidence.

In this outbreak sixty-eight children were found to harbour *Salm. derby*, but the actual incidence was probably higher as the search for carriers began in the later stages of the bacteriological investigation. Most of the patients were submitted to one bacteriological examination only and *Salmonella* bacilli, which may be excreted in faeces in an intermittent manner, might have been absent or not detected in some stools.

The investigation of this outbreak revealed the fact that transmission of the causal organism was possible by a number of channels. Clinical cases, carriers, dust in wards, mouse droppings, and an infected hand towel all represented potential sources for further spread of the bacteria (Rubbo, 1948). It is worth noting that in this outbreak a large number of human carriers of *Salm. derby* was encountered and that occasional carriers of this organism had been previously reported (Edwards & Bruner, 1943; Seligman *et al.* 1943; Stone, 1943). In addition, in the course of this investigation it was found that a clinically normal child was passing this organism in stools for a period of 2 months; as there was no opportunity for a further bacteriological examination, the duration of the excretion of the bacterium could not be determined. However, this evidence and the results of our experiments on mice, which were shown to be faecal and urinary excreters of *Salm. derby*, point to the menace of both human and animal carriers.

Children are more susceptible to *Salmonella* infection than adults, and the picture of this epidemic also suggests increased resistance in older

children. Outbreaks of gastro-enteritis as a rule are more prevalent in summer, but in this case the sickness which started in late autumn continued during winter and spring. However, a few outbreaks of epidemic diarrhoea of the newborn reported lately in this city (Campbell, 1945) occurred in late autumn and winter.

Although only a small number of tests was made, the presence of antibodies of *Salm. derby* was demonstrated in sera of patients. This suggests the advisability of serological investigation during an outbreak in a hospital in addition to bacteriological examination of faecal specimens.

SUMMARY

1. An outbreak of *Salmonella derby*, involving sixty-eight patients, is described.

2. The organism was isolated from clinical cases, from carriers and also from dust in the wards, from a mouse and from a hand towel used by nurses.

3. The value of tetrathionate broth with subsequent cultivation on 'SS' agar as an aid to isolation of *Salmonella* bacilli was confirmed.

4. Cultural and biochemical characters of *Salm. derby* were recorded.

5. The antigenic structure of the isolated strains of *Salm. derby* was demonstrated, showing the presence of factor *l* and the absence of a minor flagellar antigen.

6. A serological response to *Salm. derby* infection was shown in patients, their sera containing H antibodies of a titre 1/20 to 1/320, whereas O agglutinins were absent at the time the tests were made.

7. Pathogenicity tests were performed on mice indicating the possibility of infection *per os* or by intraperitoneal injections. Mice were found to be potential faecal and urinary carriers.

Thanks are expressed to Prof. S. D. Rubbo for his help and for facilities provided in his Department, to the Superintendent and Staff of the hospital for co-operation and to Miss N. Atkinson, Miss G. M. Woodroofe and Miss A. M. Macbeth of the Institute for Medical and Veterinary Research, Adelaide, for typing the first few cultures of *Salm. derby* isolated in this work.

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(*MS. received for publication* 13. II. 48.—Ed.)