THE PROBLEM OF SALMONELLA INFECTION

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Infection with salmonella organisms has a global distribution and is responsible for a variety of disorders. It still constitutes a major health problem and has been well documented.[1] The recent outbreak of typhoid fever in Aberdeen again focused attention upon its importance.[2] Several reviews of salmonellosis in South Africa have been reported.[3-7] The infection occurs not only in man, but mammals, reptiles, fish and birds are also known to be affected. The group embraces a complex number of organisms, some of which are host specific, e.g. *S. typhi, S. paratyphi A, B* and *C* and *S. sendai*. The latter are found only in man.[8] Others have a certain degree of host specialization, e.g. *S. abortus equi* for the horse and *S. dublin* for the ox. About 700 different strains are recognized, with the number increasing annually and with most, if not all, the organisms pathogenic to humans. Poor sanitation, inadequate surveillance and unrecognized carriers of infection are the hallmarks of salmonella infection, and as sanitation and the public health improves, so does its incidence diminish. Of particular importance is infection occurring in infants and the elderly or debilitated person when it may constitute a serious clinical entity in terms of both morbidity and mortality. In institutions such as boarding houses, hostels, gaols and hospitals, the occupants are particularly at risk. These confined outbreaks are extremely difficult to eradicate. Despite advances in prophylaxis and treatment, salmonella infection among postoperative patients may be a grave source of continued anxiety.[14]

Bacteriology and Antigenic Structure[8,9]

The organisms are slender, Gram-negative bacilli which are actively motile owing to the presence of flagellae. On ordinary media they are alike morphologically and culturally. Preliminary isolation is achieved on special selective media containing bile salts or other substances which inhibit the growth of unwanted organisms. The non-lactose fermenting colonies on these media are then inoculated onto sets of sugars and further differentiation is made on characteristic patterns of acid, gas, and hydrogen sulphide production. As different strains may show some variation in biochemical activity, a more definitive identification is obtained on serological analysis of antigenic constituents. Ultimately bacteriophage typing may be performed to elucidate epidemiological aspects.

Salmonella organisms have a common relationship based on their antigenic structure. These antigens may reside in the body of the bacillus (somatic or 'O' antigens) or in the flagellae (flagellar or 'H' antigens). In addition, some strains, notably *S. typhi* and several others, may have a capsular antigen designated the 'Vi'-antigen. The flagellar antigens may be present in one of 2 forms, namely in a form relatively specific to a particular salmonella type (Phase 1) or with characteristics common to allied types (Phase 2). A monophasic strain may have only Phase 1 while a biphasic strain may be present in either form.

Individual antigens may be shared by many different salmonella types, but the combination of antigens present in a particular type constitutes a formula that is characteristic. Thus *S. paratyphi C* is characterized by the antigenic formula 6,7,Vi:i:1,5. This indicates that somatic antigens '6', '7' and 'Vi' are present together with Phase 1 flagella antigen 'i' and Phase 2 flagellar antigens '1' and '5'.

Antisera can be produced for typing purposes by injecting suitably prepared killed suspensions of salmonella into animals. Thus sera against O, H or Vi-antigens are prepared, which can be made to react even more specifically by absorbing out unwanted antibodies. These absorbed antisera can identify a single antigen in a complex formula or can be combined to produce polyvalent sera. In the Kauffmann-White classification members of the genus that are closely related by their similarities of antigenic formula are placed in arbitrary groups for convenience of serological identification. These groups are designated by capital letters of the alphabet. In each group there is a common shared somatic antigen not found in other groups. This is the 'determining' antigen, and corresponding absorbed sera are used to identify it.

The ultimate serological identification of new salmonella types must be undertaken at highly specialized salmonella reference laboratories.

Transmission of Disease

Many modes of transmission are responsible for salmonella infection. These include direct contact by an infected individual, infected excreta, contaminated foods, particularly powdered egg and milk and egg products, infected animals, house dust, lice and carrier-states. A large variety of foodstuffs has been incriminated as being responsible for widespread dissemination, since the organism may remain viable for considerable periods of time. Food prepared and left standing or refrigerated may be particularly suspect.[85] Among the animals, salmonella has been described in calves, cattle, horses, sheep and pigs. Epizootics in birds are also known to occur.

CARRIER STATES

The carrier-states are particularly important. For the purpose of clarity it is convenient to consider *S. typhi*

separately from the other salmonella infections. Typhoid fever caused by *S. typhi* is a notifiable disease, whereas infections caused by the other salmonella organisms are not, and statistics are therefore readily available for the former disease. It is estimated that about 3% of patients recovering from typhoid fever become carriers.[11] In 1946 Cluver estimated that 2% of the Bantu population were carriers.[12] With the advent of modern chemotherapeutic agents and improved sanitation in many areas, this estimate is probably no longer tenable. Bokkenheuser, in fact, claims that the figure is disputable, since information in regard to the typhoid carrier state is very meagre.[6] In tracing the carrier state in South Africa, reliance has been predominantly placed on the detection of the circulating Vi-agglutinin in the serum and the number of positive reactions would obviously vary according to the titre which is regarded as diagnostic.[4] Until 1959 approximately 20,000 Vi-tests were performed annually at the South African Institute for Medical Research, Johannesburg, and 3 - 5% of these were positive in a dilution of 1 : 10 or more. Bokkenheuser estimated that the proportion of carriers among them was about 5% and this would constitute 0·1% of the Bantu population.[6] However, the shortcomings of the Vi-reaction in the tracing of carriers are well known,[4,39] so that the exact number of affected individuals today probably still remains equivocal.

Since carriers excrete salmonella in their faeces and urine, bacteriological examination of their faeces and urine would obviously be of greater value. Excretion is usually intermittent and irregular, however, so that repeated specimens must be submitted in the attempts to obtain positive results. That subclinical infections with salmonella in food handlers occur, has been shown by an investigation in which positive results were obtained in 4·3% of such individuals attached to the mining industry in the Transvaal.[7] The survey also demonstrated that the submission of urine specimens for the detection of carriers is of limited value and more emphasis should be placed on faecal examinations. It is significant that among the organisms discovered, many are recognized human pathogens.

Asymptomatic Carrier-state

Further analysis into the problem of the asymptomatic carrier-state would indicate that the current approach is far from satisfactory. The treatment of salmonella is still a challenge, and, although the patient may be symptomatically free from infection, there is usually no adequate follow-up examination. The possibility that infrequent and intermittent excretion of organisms over an undetermined period exists, should still be seriously entertained. Post-infective studies of this kind have rarely been carried out in South Africa, and the problem is further aggravated by the migratory Bantu population. Carriers remain an important source of infection. Since the post-symptomatic carrier state is claimed by some to resolve in a short period of time, irrespective of therapy,[13] there is grave danger of underestimating this latent source and its potential hazards. In this connection, serious anxiety has been aroused by reports from the United States that the treatment of acute salmonella gastroenteritis may be adequate without antibiotics and with supportive measures only.[33,34] Yet, in South Africa, where the frequency of salmonellosis as a cause of diarrhoea in Bantu children during the summer months has been emphasized[15,16] it was shown that such infection carries a graver prognosis than that of undetermined aetiology.[17] The situation is also confusing and difficult, since it is usually impossible to decide if infected persons should be considered chronic carriers or merely short-term infected individuals.[5] Its importance in regard to the public health is obvious.

Surveys

Reports of the isolation of salmonella organisms in certain individuals and communities, some apparently free from clinical infection, further complicate the picture. These reports in South Africa embrace a wide cross-section of the population and include the following surveys:

1. 3·9% of White children at the Transvaal Memorial Hospital for Children, Johannesburg. This was a control group in the study of summer diarrhoea in White children in Johannesburg.[18]
2. 44% of apparently healthy Bantu children near Rustenberg.[5]
3. 29·3% of 75 Bantu children at Witkoppen Bantu School. In most cases the infections were asymptomatic.[19]

Types and Virulence

The various features shown in some of the investigations included the multiplicity of types of organisms, the intermittency of their appearance and the lack of particularly predominating strains. It has been suggested that differences in virulence between types may exist[1,10] or feature in host resistance, and this may account for major clinical variations. However, organisms which may cause infection of a subclinical nature in some individuals have been responsible for a fatal outcome in others in the same outbreak.[20,32] Our knowledge of this complex problem is far from complete. It is possible that the organism may undergo occasional highly virulent mutations in different hosts and that these mutations are morphologically undetectable. Obviously we do not have the full answer.

The influence of climate, associated pathogens, commensals, temperature, the reticulo-endothelial system of the host and many other factors must also be evaluated. An interesting hypothesis towards elucidating the problem was suggested in an annotation in *The Lancet*.[21] Acknowledging that resistance is variable and that some other factor besides the host-parasite equation may be implicated in salmonella infection, the suggestion is made that it may be simpler to assume (until we know better) that the dose of salmonella swallowed is critical. It was claimed that the time was opportune to demand quantitative methods of investigation. Because salmonella infection may present many unusual and complicated features in diagnosis and treatment, this new avenue of enquiry merits serious consideration.

EPIDEMIOLOGY

Multiple infections with salmonella organisms are common and are commonly missed.[10,22] This is a practical problem since it is difficult to pick off sufficient colonies from the agar culture plate. Shigella infection is a common bacterial

accompaniment and has also been shown to be present in asymptomatic cases.[5,15,19] There does not appear to be any correlation between viruses and salmonellosis[23-25] but this is not universally accepted.[42]

Seasonal Incidence

Salmonella infection has been encountered throughout the 4 seasons of the year but it has been shown to be twice as common in summer as in winter (12% as compared to 6%).[3] An analysis in 1958 of the positive results obtained in tests performed at the South African Institute for Medical Research, Johannesburg, reflected a probable increase of the incidence in the population in which up to 1958, 132 different salmonella types were encountered.[3] Most of the organisms were recovered from the faeces, some from the urine, and a number from the blood stream. Occasionally an organism was recovered from the cerebrospinal fluid.

Similar findings were noted in the annual report of the South African Institute for Medical Research, Johannesburg, for the year 1962, where out of a total of 474 positive results for the isolation of salmonella organisms other than *S. typhi,* 403 were obtained from faeces and urine, 50 from the blood and 6 from the cerebrospinal fluid.[26] The position in regard to *S. typhi* is somewhat different. Since most patients with typhoid fever present as a pyrexia of undetermined origin, rather than with gastro-intestinal symptoms, a greater number of positive blood cultures are obtained.

At Edenvale Hospital, where approximately 3,500 non-White medical patients are treated annually, about 100 cases of salmonellosis are diagnosed. Of these 50% or more are patients suffering from *S. typhi* and the majority of positive cultures are obtained from the blood.[27] This finding is in agreement with the results obtained at the South African Institute for Medical Research, Johannesburg, during 1962 for the isolation of *S. typhi,* where out of a total of 201 positive results, 151 were obtained from the blood, 42 from the faeces and urine and 1 from the cerebrospinal fluid.[26]

PATHOLOGY AND CLINICAL MANIFESTATIONS

Infants

The pathology of typhoid fever is well known. In infants and children, however, infection with *S. typhi* may present in a very bizarre manner. The condition may present at birth, the organisms having been transmitted through the placenta.[28] The symptoms then are variable and include fever, convulsions, jaundice, diarrhoea and splenomegaly. Infection acquired after birth may present an atypical pattern. It may resemble any form of sepsis or may suggest a mild intestinal disturbance. Splenomegaly, meteorism, diarrhoea, rose spots and irregular pyrexia may be present. The disease may mimic many other conditions. Typhoid encephalopathy has been described with residual neurological sequelae.[29] Whether the condition is due to some form of dehydration or the endotoxin elaborated by the bacillus affecting the brain has not been determined.[30] Diagnosis of typhoid fever may be made by isolation of the organism from the blood, urine, faeces or cerebrospinal fluid. A positive Widal reaction indicates infection of the mother and not necessarily active infection of the offspring.[28]

In the other salmonella infections, pathological changes similar to typhoid fever but less well-marked are encountered. Acute enteritis and superficial necrosis of the lymphoid tissue are the principal changes in the intestinal tract. Deep ulceration and frank haemorrhages are rare and perforation of the bowel in children is practically unknown. The organisms may enter the bloodstream, cause a generalized septicaemia and settle in any part of the body.

Incubation Period

The incubation period of salmonellosis may be short, even a few hours, as in gastroenteritis, or as long as 12 - 14 days, as in the typhoid-like state. Clinically 4 main entities of infection are conveniently recognized. They may occur individually, simultaneously or consecutively in the course of an illness.[1] In order of frequency the infective states are:

(*i*) Gastroenteritis;
(*ii*) The typhoidal or systaemic syndrome;
(*iii*) Focal manifestations; and
(*iv*) The carrier state.

Recently the clinical entity of chronic salmonellosis was described. This is characterized by a protracted onset, prolonged course and an unusual combination of clinical and laboratory features. Particularly noteworthy was the eosinophilia which accompanied the response to therapy.[40]

Symptoms

Infection carries a far graver prognosis in the very young, elderly, debilitated or postoperative patient. The important focal manifestations include meningitis, endocarditis, pericarditis, osteomyelitis, pneumonitis, abscesses and renal involvement. Clinical presentations range from the mildest or asymptomatic, even ambulant form of infection, to the most fulminating septicaemia with fatal termination. Encephalopathy, similar to that caused by *S. typhi* has been observed with infection due to *S. paratyphi B.*[29] In carrier states the stools are apparently normal, but in gastroenteritis they may be watery, exhibit blood and mucus and cause severe dehydration. In some patients considerable drowsiness and disturbance of the sensorium has been described. In paratyphoid A, B and C infection rose spots may be evident. The spleen is not often palpable, but abdominal distension may be present. The blood picture may rarely show a leucocytosis and often a leucopenia and isolation of the organism from the bloodstream may be possible during the first 10 days of the infection.

Suppurative lesions due to salmonella infection are more common with the salmonella organisms other than *S. typhi.* They are particularly characteristic of infection with *S. paratyphi C.* Meningitis is a most important localized lesion and about 4 - 7 cases are recorded annually in Johannesburg. The organisms most commonly responsible for it are *S. typhimurium, S. paratyphi B, S. typhi* and *S. dublin.*[20]

The mortality of systaemic salmonellosis in man due to types other than *S. typhi* was reported by Saphra and Winter to be 4·1%.[1] Since salmonella infection is not a notifiable disease in South Africa, no figures are available. Bokkenheuser reported the mortality of typhoid fever in

South Africa in 1958 to be 0·17 per 100,000 Whites and 1·01 per 100,000 non-Whites.[4]

HOSPITAL SALMONELLOSIS

Epidemics

Of particular importance are the epidemics of salmonella in hospitals where they constitute a special problem. This is illustrated by some of the reports of outbreaks which have appeared in the medical literature. Szanton[20] in 1957 described an epidemic due to *S. oranienburg* which affected 46 newborn infants and 34 other individuals, who were contacts and became carriers. One infant died, 7 were acutely ill, 33 had symptoms of grossly abnormal stools and 5 had asymptomatic infection and were diagnosed by positive stool cultures alone. Three carriers were found and were probably responsible for the infection—a nurse in the maternity ward, a laboratory technician and a food server in the diet kitchen of the hospital. Measures to combat the epidemic included closure of the maternity ward in the hospital as well as the newborn nursery for a 10-day period. A search to determine the source of infection among the adults outside the hospital by investigating the family pets was considered, but not accomplished. Investigations into faulty technique, equipment and other possible sources as a focus of infection in the hospital proved fruitless. Once again the carrier state was emphasized in that it was prolonged to a greater degree in the infant than in the asymptomatic secondarily-infected older individual. Failure of the antibiotic therapy to clear the carrier state confirmed previous findings and indicated the need for a different approach to its eradication.

Keet[32] described a similar outbreak in South Africa in which an epidemic of salmonella gastroenteritis occurred in a newborn nursery. The infection was caused by *S. johannesburg* and affected 23 infants during a trial undertaken to test the efficacy of TBTO (a compound of tributyl tin oxide—Permachem in the United States) as an antistaphylococcal agent. The trial was discontinued following the outbreak of the epidemic in the neonatal unit which acted as the control. It later spread to the other unit where TBTO had already been in use for 1 week. Twenty-three patients were affected including 18 premature babies. Infection was considered to be present when there were signs of gastroenteritis or if *S. johannesburg* was isolated from the stool. The onset of symptoms, and/or signs varied from 4 to 17 days. *S. johannesburg* was isolated in 14 cases including 6 infants in whom rectal swabs had been taken within the first 24 hours of life. In most of the latter patients feeds had not yet been instituted. Four cases exhibited signs of gastroenteritis but no organisms were isolated. In some cases repeated rectal swabs had to be taken before positive results were obtained. *In vitro* the organism was only sensitive to ampicillin, nitrofurantoin and occasionally to streptomycin. Sixteen patients were treated with ampicillin and responded. One patient, after administration of the antibiotic for 10 days, still exhibited a positive culture. The first 5 patients who did not receive ampicillin died. Two patients were not treated. They were asymptomatic and reports of the isolation of the organism from them on routine examination were received only after discharge. One of these infants was seen subsequently at the casualty department with gastroenteritis. The other, when seen at the Child Welfare Clinic, was asymptomatic and a negative stool culture was obtained. Thus it was suggested that infection with *S. johannesburg* in a child could present in an asymptomatic form. The source of the infection could not be determined, but *S. johannesburg* was isolated from patients in other wards during this period and it is possible that infection was transmitted by a mother to her offspring. In assessing the outcome of the epidemic the author emphasized the importance of aseptic technique and care in the management of the newborn baby, the principles of which were listed in the beginning of the report. She also stressed the necessity for identifying the organism in neonatal gastroenteritis and determining its sensitivity.

Watt *et al.*[31] drew attention to salmonellosis in a premature nursery unaccompanied by diarrhoeal disease. Six apparently normal infants, later found to be infected with *S. tennessee*, were placed in a nursery. This led to an epidemic spread of the infection over a 4-month period. No infant was ill at any time. Infection of room air was demonstrated and positive cultures were obtained from bedside tables and recently changed cribs. The hands of one nurse, immediately after washing, produced a positive culture. Evidence showed that an increase in spread of the infection was associated with a decrease in the amount of nursing care exercised as in unsatisfactory hand-washing toilet.

Postoperative Infection

Kohler,[13] in describing an outbreak of hospital salmonellosis due to *S. infantis*, confirmed that morbidity tended to be more severe in patients over 60 years of age, and in those with serious underlying disease. The outbreak of *S. infantis* gastroenteritis involved 17 inpatients and 6 hospital personnel. The source of infection was scrambled egg contaminated after preparation by a food service worker recently recovered from gastroenteritis. Direct person-to-person spread of salmonellosis was indicated by the development of gastroenteritis subsequent to close contact with infected patients. *In vitro* the organism was sensitive to tetracycline and chloramphenicol but the antibiotics had no apparent effect on the clinical course or period of *S. infantis* recovery from the patients' stools. Although a high-risk population was involved, there was no mortality.

Cohen *et al.*[14] drew attention to the problems of diagnosis and therapy in 13 patients where salmonella was first found in the postoperative period. The 3 possible explanations for postoperative salmonella infection were quoted by Black *et al.*[33] as:

1. Activation of a latent infection in a period of postoperative physiology.
2. Spread of infection by surgical manipulation of the gastro-intestinal tract.
3. Unfavourable change in the bacterial flora of the gastro-intestinal tract because of pre-operative antibiotic treatment. Other illnesses which predispose to a Gram-negative bacteraemia among hospital patients were noted and included advanced arteriosclerosis, malignancy, diabetes mellitus and hepatic cirrhosis.

DIAGNOSIS

The diagnosis of salmonellosis is confirmed by isolation of the organism in the stool, urine, blood or cerebrospinal fluid. Culture of the blood clot has been claimed to be a more satisfactory procedure than culture of whole blood.[34] The presence of infection may also be confirmed by a positive Widal test, especially if the titre can be shown to be rising over a definite interval. Watson and Laurie found a 5% false-positive reaction if a dilution of 1 : 250 for the O agglutination is accepted as the minimal diagnostic titre.[34] Difficulties may be encountered with diagnostic titres following TAB vaccination and on occasion by an anamnestic reaction during a non-specific low grade infection. Lack of antibody response on the part of the patient may also give rise to a diagnostic problem.

TREATMENT

In the treatment of salmonellosis, the antibiotic chloramphenicol has been widely employed. This was found to be the drug of choice by Kahn *et al.*,[16] but some strains were found to be resistant to it and to all common antibiotics.[35] Recently ampicillin in high dosage has been used in cases of salmonellosis,[44] particularly in the carrier state, with apparent success[36] but its possible efficacy should be determined over a trial period of several years. Several other agents have been reported to be effective including kanamycin, a nitrofurantoin preparation, polymixin B and neomycin, but further clinical trials are needed.[34] The management and eradication of the carrier state remains a basic problem in the fight against salmonellosis.

PROPHYLAXIS

Prophylaxis against some forms of salmonellosis is available in the form of TAB vaccine made from killed cultured bacilli. This affords protection against typhoid and paratyphoid A and B fever. It is employed particularly in the protection of travellers and was used extensively during wartime. Various types of vaccines have been tested and current trials have shown that acetone-dried vaccine gives greater protection than vaccine prepared from the same strains but inactivated by heat-phenol.[41,42] Recently the value of typhoid vaccine has been questioned,[37] since individuals, despite annual re-inoculation with the current vaccines, suffer attacks of typhoid fever and the severity of these attacks may be as great as those occurring in persons who have never been inoculated. One possible explanation for this may be found in the number of different phage types of *S.typhi* which now number some 6 dozen. The methods of preparation, and the strains of the organisms employed in the manufacture of the vaccine, may also be responsible. It was also suggested that at present we may place what faith we can muster in those vaccines that are available, at the same time recognizing that they do not afford absolute protection in all circumstances.[37] The possibility of producing a multiple-type antigen against salmonellosis seems extremely remote, and the efficacy of such a preparation in counteracting the differences in pathogenicity of an organism in different hosts is open to question. In view of the complex nature of the problem and the multiple factors involved, one cannot escape the conclusion that reduction in the incidence of the malady will probably only be brought about by improved methods of hygiene and vigorous public health campaigns.

SUMMARY

The importance of salmonellosis as a public health problem is emphasized. Some bacteriological and typing characteristics of the organism are presented. Attention is drawn to the importance of the carrier state and examples of epidemics caused thereby are cited. The difficulties in managing the carrier state appear insurmountable. The hazards of hospital salmonellosis are illustrated with reports from the literature and the differences in prognosis in the various age groups are noted. The chemotherapeutic agents used in the treatment of the infection are listed. Further trials are suggested to test the efficacy of those which at present have been shown to be of value. The difficulties in the use of a multiple-type prophylactic vaccine are mentioned. Reliance must be placed on improved personal and public health measures in the fight against salmonellosis.

I wish to express my appreciation to Dr. J. Meyer, senior physician, Edenvale Hospital, for his interest and stimulation in the production of the manuscript. Invaluable assistance was provided by Dr. H. J. Koornhof of the South African Institute for Medical Research by reviewing the paper and providing many of the reprints. I am also indebted to Dr. G. Frank, pathologist, Edenvale Hospital, for his critical review and helpful suggestions.

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