SALMONELLA RESERVOIRS IN ANIMALS AS SOURCES OF HUMAN INFECTION

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D E C L A R A T I O N

This thesis is my original work and has not been presented for a degree in any other University

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This thesis has been submitted for examination with our approval as University Supervisors.

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SUMMARY

Salmonellosis is a cosmopolitan zoonotic disease of considerable importance. In Kenya, salmonellosis may be more significant in terms of number of infections and mortality than typhoid fever and cholera. Animals and animal products have been regularly incriminated as sources of human infections for most salmonella serotypes except the anthropophilic ones. These reservoirs also serve as sources of Salmonella infections in domestic animals. An attempt has been made to define these reservoirs through a survey of domestic animals and rodents in the Nairobi area. Forty nine salmonella strains were isolated from a variety of sources, comprising 32 serotypes. The highest carrier rate (7.3%) was found in wild rodents, mainly Rattus rattus. The high carrier rate and the tendency of this species to invade houses indicate that these rodents play an important role in the epidemiology of salmonellosis, both in man and animals. The serotype most commonly recovered in this species was S. enteritidis which was also the second most prevalent serotype from human patients at Kenyatta National Hospital during the period 1974 - 1979. This observation further supports the view that wild rodents are of major importance as sources of infection.

In addition to the standard slide agglutination

method for typing Salmonella strains, the Staphylococcus coagglutination method according to Kronvall was used in parallel. Complete agreement was found between the two methods, but the coagglutination method was more rapid and far more economical in terms of reagents than the slide test.

1. INTRODUCTION

Salmonellosis is an infectious disease caused by one of the approximately 2000 serotypes of bacteria included in the genus Salmonella.

Members of the genus Salmonella are ubiquitous in nature, and are able to survive for prolonged periods in the inanimate environment.

It is expected therefore, that the many and varied conditions under which the human population comes into contact with animals and animal products may present opportunities suitable for the transfer of infection from one host to another.

As enteric pathogens, the salmonellae cause a variety of conditions in man and animals. The infection may be asymptomatic, the so-called carrier state, which can be regarded as a mobile source of infection and contamination.

Clinically sick animals excreting large numbers of salmonellae present a grave risk of infection from one animal to another or to man. Conversely, infection in animals or man, may originate from human carriers. Contaminated food and drinking water are among the most important vehicles of Salmonella. Foodstuffs of animal origin, such as meat and bone meal, fish meal, hoof and horn meal, and others are also important

sources of infection.

Waste water especially that from slaughterhouses represents another hazard to farm animals. It has been shown that some sewage treatment plants reduce the number of Salmonella organisms only slightly; thus large numbers of the organisms enter water courses with the effluent from such plants and contaminate sources of drinking water. Recreation areas used for fishing and water sports are also contaminated, creating an additional public health danger.

Lack of host specificity commonly found in most Salmonella has led to the realization that the human-animal salmonellosis is one disease entity and its control is an arduous task.

Increased attention to salmonellosis is being given by health workers concerned with human and animal health. It is considered by the Joint FAO/WHO Expert Committee on Zoonoses to be a problem of major importance (Wld. Hlth. Org. tech. Rep. Ser. 169-1959). This is due to its high incidence, the often serious nature of the infection involving septicaemias, meningitis and metastatic abscesses, as well as the common gastroenteritis normally associated with Salmonella organisms (Mirza and Nsanzumuhire, 1979; Wamola and Mirza, 1981).

In addition, as a communicable disease,

its spread has changed from local to national and then international by the turn of the century. This has been as a result of the widespread national and international distribution of foodstuffs and other materials and the greatly increased movement of human populations.

Salmonellosis is also of considerable economic importance because of the associated costs of medical care, lost working time and the damaging effect on trade. It was recognised that a local public health service alone was inadequate for the control of nationally and internationally distributed diseases. This led to the creation in some developed countries of Communicable Disease Centres, Public Health Laboratories in-charge of Surveillance Programmes.

The WHO Salmonella Surveillance Programme receives laboratory data from about 30 countries but the reliability of the information varies considerably, and regular surveillance information is available from only a small number of countries (Bull. Wld. Hlth. Org. 1980).

The real incidence of salmonellosis in many countries is not well known and comparison of the number of isolations may be misleading owing to variations in population characteristics, under-reporting, and differences in epidemiological and laboratory techniques. For example, in the USA

the number of reported isolations is estimated to be only about 1% of the number of cases (Wld. Hlth. Org. Tech. Rep. Ser. 598-1976). In the developing countries, the lack of surveillance data makes it particularly difficult to evaluate the importance of salmonellosis in the family unit or community, although a few extensive common-source food or water-borne outbreaks and hospital outbreaks have been described. A high mortality rate, possibly related to coexistent malnutrition has been reported (Bull. Wld. Hlth. Org. 1980).

Occasional surveys and studies such as those carried out by the WHO Diarrhoeal Diseases Advisory Team in seven developing countries in 1960-1965 have confirmed that salmonellosis has a worldwide distribution and is a problem of considerable magnitude (Bull. Wld. Hlth Org. 1966). The prevalence of salmonellae in man, animals, foods, and the environment has been studied extensively in many countries. The factors that influence survival and spread of salmonellae in the environment, the effect of different systems of animal husbandry, and faults in food preparation that may lead to salmonellosis in man are now rather well clarified. It has been shown that the main reservoir of Salmonella is the intestinal tract of man and animals, and cycles of transmission occur between animals, man and the environment through foods, feeds, and insects (Wld. Hlth. Org. Tech. Rep. Ser. 598-1976).

Salmonellosis is usually food-borne and generally results from the consumption of contaminated foods that have been improperly handled, allowing the organisms to grow. Person to person spread may occur, especially in acute care hospitals, paediatric wards, nurseries and nursing homes.

The relative frequency of Salmonella as a cause of food-borne disease varies from country to country and depends on such factors as dietary habits, personal hygiene and standards in food production and service establishments, as well as animal husbandry practices such as intensive-rearing systems. In the USA, about 40% of reported food poisoning cases are due to Salmonella, whilst in England and Wales the comparable figure is 80% (Bull. Wld. Hlth. Org. 1980).

In developed countries, salmonellosis is a zoonosis and the overall epidemiological pattern is related to the predominant source of animal protein in the diet. Thus, in the USA, food of bovine origin is the main source of Salmonella infection, while in England and Wales poultry accounts for about 50% of outbreaks and beef for only 2% (Bull. Wld. Hlth. Org. 1980). In developing countries where animal protein does not constitute a major part of the diet, it appears less likely that salmonellosis is a food-borne disease. In these situations, intensive rearing of food animals is uncommon and its associated

problems insignificant. However, hygienic standards in food production and catering are likely to be lower and thus Salmonella carriers are probably a more important source of food infection than they are in developed countries (Bull. Wld. Hlth. Org. 1980).

In addition, in those developing areas where water is often obtained from local sources and thus is not purified or protected, water-borne salmonellosis is probably more common. The relative importance of of various sources of infection and modes of transmission has not been adequately elucidated in developing countries. Reliance upon data collected under entirely different conditions of life may lead to erroneous conclusions which may result in wrong measures to control salmonellosis in the developing world.

Kampelmacher (1980) made the following statement: "in the past 25 to 30 years, salmonellosis research has been limited chiefly to stocktaking and far less work has been done on the epidemiological evaluation of the information obtained. This would give a better insight into the course of infection and contamination and suggestions could then be made as how to avoid or even solve the various problems." Salmonellosis is a public health problem in Kenya but the extent of the problem in the community is not known

(Wamola and Mirza, 1981). Similarly, the carrier rate of Salmonella in domestic and wild animals has not yet been sufficiently documented. The spectrum of serotypes occurring in animals and their significance to human health have not yet been subjected to detailed studies.

The work presented in this thesis deals with the isolation and identification of Salmonella serotypes from apparently healthy animals, in an attempt to elucidate the role of animals as sources of human infections in this area.

The Staphylococcus coagglutination method of Kronvall (1973) has been compared with the standard slide agglutination method in the serotyping of Salmonella strains.

2. LITERATURE REVIEW

The first Salmonella was isolated from a human source by Eberth in 1880. In 1885 Salmon and Smith reported for the first time isolation of a Salmonella organism from an animal source. Studies then continued on the identification and classification of Salmonella, culminating in the adoption of the terminology and antigenic schematization of Kauffmann and White (1966), (Steele, 1969).

The worldwide distribution of salmonellosis in animals and the attention which it has received in all countries, has resulted in the publication of an enormous amount of scientific information during recent years. The emphasis of this review will be on aspects which are directly relevant to this study.

Various Salmonella serotypes have been isolated from numerous sources: domestic animals, their products and by-products; wild animals including rodents and reptiles; domestic and wild birds; water and effluents of any kind. Many authors have amply documented the epidemiology of Salmonella, methods of isolation and identification of the bacteria, the zoonotic aspects of the disease and its control.

For the sake of clarity, the present literature review is divided into two chapters.

CHAPTER ONE - deals with literature concerning the distribution of Salmonella serotypes in man, animals and their environment, the epidemiology of Salmonella infections and their public health aspects.

CHAPTER TWO - is a review of the literature on methods of isolation and identification of Salmonella, including the antigenic structure of the latter.

The year 1955 was set as the starting point for the review. A quarter of a century was deemed to be enough time for the review. At the same time the period was though not to be too long, taking into account the huge volume of literature on the subject. There are, however, two exceptions where literature before 1955 was reviewed: any research work done on Salmonella in Kenya and the same in other countries but only for rodents. This was found appropriate in order to include all the few authors who wrote on the subject in Kenya and as regards rodents, it is due to their importance in the present study.

2.1. Epidemiology, distribution, serotypes and public health significance of Salmonella.

Daubney (1927) investigated the mortality among dairy calves in Kenya and S. dublin was found to be the main cause, though he considered the infection of calves with certain tick-borne diseases to be an important predisposing factor.

Kerrin (1928) attempted to estimate the percentage of wild rats in Aberdeen (U.K.) which were harbouring organisms of Salmonella group. Out of 100 rats, 11 were carriers of S. enteritidis.

Khalil (1938) isolated organisms of the Salmonella group from 35 out of 750 wild rats trapped in Liverpool (7.3%). About 70% of rats were of the large brown variety, probably Rattus norvegicus and 30% of the small black variety, probably Rattus rattus. The organisms isolated were S. typhimurium, S. enteritidis, S. newport and S. thompson.

Ghosal (1941) working in Calcutta (India), found 49 out of 364 rats (13.4%) positive of Salmonella. S. typhimurium and S. enteritidis were isolated. The danger of contamination of food, especially as the rodents live in intimate association with human being, was emphasized.

Ludlam and H., D.L.O. (1954) investigated Salmonella in rats, with special reference to findings in a butcher's by-products factory.

Salmonella organisms were isolated from 4.4% of 518 rats killed in various types of premises in

the Nottingham area 1949-1954. Up to September, 1953 salmonellae were isolated from 6.4% of 94 rats from a butcher's by-products factory. In the last three months of 1953 the incidence rose to 40% of the 60 rats examined, and in the first four months of 1954 the incidence was still high (27.6%) of 29 rats.

S. enteritidis var. danysz and S. newport were the commonest serotypes outside the factory and S. enteritidis var. danysz and S. typhimurium were the most prevalent serotypes in the factory.

Mackey (1955) investigated salmonellosis in Dar-es-Salaam and a record of Salmonella isolations from human, lizards and other sources made during the period 1948-1953 in the Central Medical Laboratory was presented. Out of 586 human isolates of which S. typhi was the most prevalent (204 isolates), small proportion (3%) were thought to be from carriers. One hundred and forty-four isolations were made from lizards, S.mission was the most prevalent serotype (20 isolates), followed closely by S.dar-es-salaam and S.abony (17 isolates each). From cockroaches, 12 isolations were made; S.nairobi and S. heidelberg were predominant (3 isolates each). Salmonella isolations from lizards, lizard droppings and cockroaches were of public health significance since 21 out of 33 different Salmonella serotypes found in lizard droppings were also isolated from human cases and S. typhi was isolated from one

cockroach. Lee (1955) reported the isolation of 24 strains of Salmonella from 842 rats collected from various parts of Brisbane (Australia) over a period of 4 years, an average carrier rate of 3%. The isolates respresented eight serotypes: S.typhimurium (the most prevalent), S.oranienburg, S.anatum, S.bovis-morbificans, S.chester, S.paratyphi C, S.meleagridis and S.adelaide.

Van Oye (1955) in his third report on Salmonella in the then Belgian Congo, mentioned 26 seroptypes found during the years 1953 and 1954. Among those, 7 were new strains. Up to 1954, there were 594 serotypes of human origin: S.typhimurium (125), S.dublin (58), S.paratyphi C (45), S.kisangani (41), S.enteritidis (36).

Collard and Sen (1956) examined mesenteric lymph nodes and faeces from 200 Zebu cattle from slaughter slabs in Ibadan. The carrier rate was 5.5% and the Salmonella types isolated were S.typhimurium, S.dublin, S.elizabethville, S.rubislaw, S.oranienburg, S.monschaui and S.johannesburg

The public health significance of S.dublin S.elizabethville and S.rubislaw, was noticed since those serotypes were also isolated from the faeces of hospital patients in Ibadan.

Buxton (1957a) made a review of salmonellosis in animals which is,as he puts it "an attempt to orientate data accumulated by many workers over the course of few decades, on a biological problem

which has been largely shaped and continues to develop, under the influence of the changing phases of life in man and animals".

The author gives a detailed account of general characters of Salmonella, its classification and the distribution of Salmonella serotypes among animals. On the latter aspect, it can be seen that there is a range of host animals for Salmonella: domestic and wild animals, rodents, reptiles, insects and ticks.

The following is a summary of the review of the above-mentioned author.

In cattle, S.typhimurium and S. enteritidis are mentioned in earlier references as the causal organisms in the majority of cases. But later, S.dublin was found to be the commonest serotype affecting cattle and it is widely distributed throughout the world. Although in many districts S.typhimurium was not isolated from cattle as frequently as S.dublin, the global distribution of the former organism has resulted in bovine infections in widely separated countries all over the world. The serotypes most frequently associated with salmonellosis in sheep and goats are S.abortus ovis and S.typhimurium. Pigs are frequently carriers of Salmonella and form, with poultry, the main animal reservoir for these organisms. The relatively high carrier rate in pigs is given for several countries. The mesenteric lymph nodes are a common site for latent Salmonella infections, especially in clinically normal pigs. In dogs and

cats the prevalence of salmonellosis may vary from approximately 1 to 36%, according to the conditions under which the animals are kept - S. typhimurium is the organism most frequently isolated.

Regarding poultry, Buxton (1957a) mentions that apart from S. gallinarum and S. pullorum, S.typhimurium is the most common serotype encountered in various parts of the world in the different avian species, particularly ducks, of which as many as 10% may be faecal excretors in some countries.

In rodents, S. typhimurium has been isolated frequently from symptomless carriers. S. enteritidis is also not uncommon in these species. Numerous surveys of infection among wild rats and mice in different countries have been carried out. The carrier rate ranges from 1.5 to 13.6%.

In reptiles, a variety of serotypes has been isolated from tortoises, lizards and snakes. From time to time salmonellae have been isolated from various insects, including flies, fleas and cockroaches.

Ticks may occasionally become infected with Salmonella, usually after close contact with an infected host.

Buxton (1957a) also stated that host specificity has become increasingly unimportant as it has been shown that practically all serotypes are potentially pathogenic for man and animals alike. He quoted Schofield (1945) who wrote: "To-day we recognise no boundary line

separating animal from human Salmonella, but rather is there an ever-growing recognition of the great importance of the so-called animal strains in human infections". Concurrent with the increased infection rate among food producing animals, there has been a similar increase in the incidence of Salmonella food poisoning in the U.K. and S. typhimurium was the serotype most frequently isolated.

In 1941 Kauffmann(quoted by Buxton, 1957a) estimated that S. typhimurium was responsible for 55 to 70 percent of all human Salmonella infections in England, Germany, and Denmark. During 1954, no less than 81.5 per cent of outbreaks of food poisoning and 98.3 percent of sporadic cases were attributed to Salmonella infections. Of these, approximately 90 percent were caused by S. typhimurium. Thus, in both man and animals there has been an apparent increase in the incidence of Salmonella infections and in both groups S. typhimurium is the organism most frequently isolated (Buxton, 1957a).

Muller (1957) examined the problem of animal salmonellosis in Denmark. The incidence of Salmonella infections in Denmark was not considered high: 2.21% of 8816 dead calves during the period 1948-1956. S. dublin was found in 1.71%, S. typhimurium in 0.33%, and other Salmonella types in 0.18% of cases. Imported animal feeds were incriminated for the introduction of several Salmonella serotypes in the country.

The observed increase in the number of S.typhimurium infections in Denmark seems to be connected with the increasing industrialization of food production, especially the production of salad dressings (mayonnaise) and scrambled eggs.

Plowright (1957) found an approximate 1% Salmonella carrier rate in adult cattle and that S.dublin infection could be an important cause of disease and mortality in adult cattle in West Africa. Earlier, Hughes (1954) quoted by Plowright (1957), had found an overall 2.7% Salmonella carrier rate from the gall bladder of 150 cattle slaughtered at Accra (Ghana). He described two human cases of generalised S.dublin infection and stated that this infection among cattle was high in comparison with Western Europe. Other workers (Wiktor and Van Oye, 1955), isolated 2 S.dublin out of 12 strains of salmonellae from the mesenteric lymph nodes of 205 cattle slaughtered at Stanleyville. They also recorded the isolation of S.dublin from 8 of 110 cases of human salmonellosis in Stanleyville.

Stewart (1957) reported the isolation of S.typhimurium from one healthy animal following collection and examination of 924 samples of faeces, bile and lymph nodes from healthy cattle passing through Dalkeith abattoir. However, S. dublin was not isolated from any of the samples. Sen and Collard (1957) examined 119 healthy pigs in Ibadan for salmonellae and found a faecal rate of 16 percent. Three

Salmonella types were isolated. The commonest type was found to be S. poona. All the three types of salmonellae found in healthy pigs were also isolated from patients suffering from gastroenteritis.

Collard et al., (1957) reported the isolation of Salmonella from rats in Ibadan (Nigeria). Ten out of 253 rats examined were found to be carriers of salmonellae (3.9%). Six Salmonella types were isolated and S. agama, which bears a close antigenic relationship to S. typhimurium was found to be the commonest type. S. typhimurium and S. enteritidis were not isolated. The authors discussed the important role played by rats in the spread of food poisoning organisms. They mentioned Bainbridge (1912) who considered rats to be an important reservoir of S. enteritidis and, since then, this has been demonstrated by several workers. They quoted the works of several investigators who had recorded the following carrier rates in rats or rodents.

In the U.S.A. 1.2% (Welch et al., 1941), 4.2% (Li and Davis, 1952), 5.2% (Verder, 1938) and 7.3% (Meyer and Matsumura, 1927). Collard and his co-workers further quoted the data of Hatta (1939) who found a very low rate (0.4%) in Japan, Varela et al., (1948) who reported a 1.7% carrier rate in Mexico city, and McGaughey et al.,(1954) who confirmed that S. enteritidis is a very common serotype in rats in Ceylon. Salmonella serotypes found in rats in various

countries compiled from the literature available to Collard et al., (1957)are shown in Appendix 4 & 5.

Brown et al.,(1957) carried out a survey of Salmonella in the spleen and gut of rodents in the city and port of Manchester. The survey showed that 4.4% of brown rats were infected, 2.4% of them with S. enteritidis, var danysz.

Walker (1957) found that 40% of the samples of organic fertilizers purchased mainly from retail shops in U.K., particularly bone meal, were positive of salmonellae. The three types most often isolated from the fertilizers, S. senftenberg, taksony and anatum are high on the list of salmonellae isolated from human cases and practically all the other types have been reported from man and animals within the past few years.

Ganguli (1958) reported 15 serotypes isolated from human infections (in addition to S. typhi and S. paratyphi A) and 7 serotypes from animals identified in India during 1950-1957. S. typhimurium is the most prevalent followed by S. enteritidis and S. chester in humans. In animals S. enteritidis is prevalent followed by S. typhimurium and S. dublin.

Earlier literature on the subject has been reviewed and the total number of serotypes so far encountered in India has been found to be 32. The importance of estalishing a National Salmonella Centre for working out the epidemiology of salmonellosis

in India has been stressed. Hughes (1958) gave a list of the Salmonella serotypes encountered in Accra (Ghana) between 1952 and 1955. Forty-five identified and three unidentified types were isolated from human sources. The author remarked that information about the importance of animal reservoirs was scanty but referred to Mackey (1955) who showed that lizards harboured 21 of 77 Salmonella isolated from man in Tanganyika, and that cockroaches also carried salmonellae. He suggested that "the same state of affairs probably existed in Ghana (Hughes, 1955c), a suggestion since confirmed by Vella in Accra." (quoted by Hughes, 1958).

Johnson (1958) presented data on salmonellosis of various animal species in West Africa and described three new serotypes: S.bukuru, S. jos and S. vom.

In 300 sheep and goats examined no carriers were found. In cattle, S.dublin was commonly found in systemic salmonellosis, S. stanleyville was found in rectal mucus of animals showing diarrhoea and S.vom in the gall bladder.

In poultry 147 specimens with 2 S.typhimurium, two S.derby and one S. gallinarum were recorded.

Salmonellae were found in rabbits and guinea pigs, S.typhimurium being predominant. S. dublin was found in one dead dog while one with diarrhoea had S. typhimurium. In cats there were 2 with S.saint paul and one S. jos.

Van Oye (1958), in his 6th Report on Salmonella in the then Belgian Congo stated that 5 more serotypes were isolated in addition to the 133 reported previously. A complete list of all salmonellae isolated from poultry was given and the epidemiological importance of those birds was stressed.

McDonagh and Smith (1958) stated that of the common domestic animals slaughtered for human consumption, pigs are much more frequently found to be infected with Salmonella organisms than sheep or cattle. Rectal swabs of 171 pigs taken by these workers immediately after arrival of the animals at the lairage, showed that 2.9% of them were excreting Salmonella organisms, whereas bacteriological examination of 371 animals, after 1 - 7 days or even longer in the lairage, showed that 13.5% were harbouring salmonellae, 36% of which were S. typhimurium and 64% S. derby.

In 1959, research work on Salmonella continued unabated (Field, 1959; Newell, 1959; Smith, 1959; Soliman and Quddus, 1959; Bokkenheuser and Richardson 1959). A Joint WHO/FAO Expert Committee on Zoonoses issued its Second Report. In the latter, the typhoid and paratyphoid bacilli are not discussed. It is stated that salmonellosis is most common in chicken, ducks and turkeys; is frequent in rodents, less frequent in swine, not uncommon in cattle, sporadic in sheep, and occasional in various wild

animals.

Salmonella typhimurium is in most countries the commonest Salmonella isolated from man and is also a frequent pathogen in animals. Other types of Salmonella wax and wane in incidence in man from time to time and from place to place. The role of the human excretor as a source of salmonellosis is difficult to assess. Man is himself a reservoir of Salmonella and at any given time there must be many food handlers who are symptomless excretors. In Britain and USA, the carrier rate in the general population has been estimated at about 2 per 1000 (Wld. Hlth. Org. Tech. Rep. Ser. 169-1959). This report further stated the following: Cattle may harbour many different types of Salmonella. S.dublin is specially adapted to cattle, and causes disease in calves as well as in adult animals in many parts of the world. To a lesser extent than S.dublin, S.typhimurium may cause clinical disease in cattle. Carriers of S.typhimurium are encountered, though the carrier state probably does not last as long as in S. dublin infection. In pigs S.choleraesuis is common, but in addition to acting as host to that pathogen, pigs rival fowls in the frequency with which they become infected with other types of Salmonella.

Infections of sheep with S.abortus ovis and horses with S. abortus equi may be of considerable economic importance under certain conditions.

Human infections with these pathogens have never been clearly established. S. typhimurium infections are not infrequent in sheep in some countries and infections with S. choleraesuis and S. bovis-morbificans have also been reported. Outbreaks in sheep caused by these types have sometimes resulted in spread to human beings.

As far as other species are concerned, salmonellae have been isolated from practically every animal species. Surveys carried out in dogs in recent years have shown an incidence from 1% to over 30% in different areas and over 50 types have already been isolated from them, including the ubiquitous S. typhimurium. Surveys on cats have uncovered more than 20 types, the incidence reaching up to 12% according to area and conditions under which the animals lived (Wld. Hlth. Org. Tech. Rep. Ser. 169- 1959).

Shirlaw (1959) carried out a survey on 33 farms in Kenya and reported the isolation of 90 strains of Salmonella from cases of calf paratyphoid. Eighty three of those strains were typed as S. dublin and the remainder as S. typhimurium.

Smith (1959) isolated salmonellae from mesenteric lymph nodes of 60 (12%) of 500 pigs, 9 (4.5%) of 200 dogs, 5 (2.5%) of 200 cats and none of 200 cattle and 100 sheep. None was found in the chickens. Of the 17 serotypes found in pigs, S. typhimurium,

S. anatum and S. choleraesuis occurred most frequently.

From the public health point of view, it is significant that the serotypes found in the mesenteric lymph nodes of animals are those commonly associated with outbreaks of food poisoning in human beings. It is also significant, in view of the fact that the mesenteric lymph nodes of pigs are more commonly infected with salmonellae than are those of any of the other species of domestic animals, that pig meat is the most commonly incriminated in these outbreaks.

Taylor (1960) discussed the diarrhoeal diseases of man in England and Wales and put the carrier rate in human at 2.1 per 1000 in children under 5 years and the general carrier rate at 0.25 per cent.

As already stated S. typhimurium has been, and has remained, the commonest serotype since 1923,when records of serotypes started to be kept. The second most common types have been either S. thompson S. newport or S. enteritidis. S. typhimurium accounts for 75-80%, and the second group for about 12%, of human infections, the remaining cases, about 10-15%, have been due to a variety of Salmonella types.

Survey of Salmonella in animals and their environment continued and yielded interesting results. Public health significance of Salmonella was emphasized by several workers (Galbraith, 1961,

Gibson, 1961; Harvey and Phillips, 1961; Hobbs,1961; Mortelmans et al., 1961). Methods of Salmonella isolations were discussed by some authors and others isolated salmonellae from pet food and garden fertilizers (Galbraith et al., 1962; McCoy, 1962).

Hobbs (1961) in U.K. discussed the public health significance of Salmonella carriers in livestock and birds. She stated that the carriage of Salmonella by cattle and other domestic animals has frequently been described in the literature, but for some years S. dublin was regarded as the only significant serotype in cattle. Recently,combined veterinary and public health investigations have shown that S.typhimurium is far commoner among farm animals, both as carriers and as victims of salmonellosis, than was hitherto known.

Harvey and Phillips (1961) found that all 111 out of 274 (40.5%) swabs from gullies in abattoirs were positive for Salmonella; 21 different serotypes were isolated, of which S. typhimurium was the commonest and S. dublin the next in frequency. Of 30 phage typed S. typhimurium from human infections (mostly sporadic) in the area, 23 belonged to phage types found in the abattoirs and on local farms at approximately the same time. There was a similar correspondence between human cases of salmonellosis due to other serotypes and the isolation of these serotypes from abattoir swabs.

The same workers found that 31 out of 111 (27.9%) swabs taken from bakehouse floor drains were positive for Salmonella , including S.typhimurium, S.aberdeen, S.thompson and S.paratyphi B, and all except S.thompson were isolated from 15 out of 93 swabs from the staff department sewage.

Sojka and Gitter (1961) gave the incidence of salmonellosis in pigs in England and Wales. S.choleraesuis was the most prevalent serotype in all the incidents reported by the Veterinary Investigation (V.I) Centres: 71% from 1953-1957, 87.7% in 1958 and 90.9% in 1959, followed by S.typhimurium with 12, 7.5 and 6.7% ; S. dublin and S. enteritidis occupy the 3rd and 4th places. They further stated that in apparently healthy pigs, it has been shown by workers in many countries that they commonly carry Salmonella. From the public health point of view, they observed that it is highly significant that Salmonella are isolated frequently from mesenteric lymph nodes of apparently healthy slaughtered pigs and that many serotypes recovered from pigs, appear also on the list of isolates from food poisoning outbreaks in man.

Galbraith et al., (1962) found that 27% of raw horse meat, 16% of other raw meat, 12% of prepared pet meat and 13% of garden fertilizers were contaminated with Salmonella . The authors stated that in

recent years many materials sold for pet foods or garden fertilizers have been shown to be contaminated with Salmonella organisms.

Khera (1962) reported that in India, as compared with the magnitude of the problem, the published information with regard to the incidence of Salmonella infection, both in man and animals, is rather meagre. He noted, however, that from the information which has accumulated incidental to the general disease investigation work in animals, it is fairly clear that the incidence of salmonellosis is quite high among domestic animals and that a large number of serotypes are involved.

Karlsson et al.,(1963) reported on salmonellae isolated from animals and animal feeds in Sweden during 1958-1962.

Forty Salmonella serotypes were isolated from 593 outbreaks in various species of animals. Nearly half the outbreaks represented infection by S.typhimurium. The next common types in that country in order of frequency were S. montevideo, S.choleraesuis, S.gallinarum/pullorum, S.oranienburg S.dublin and S.enteritidis. In comparison with previous surveys there was a great increase in the number of serotypes isolated during the last five years. Cattle, pigs and chickens accounted for about 70 per cent of the Salmonella outbreaks.

Kampelmacher et al., (1963) studied Salmonella in

meat, organs, lymph nodes and faeces from normal slaughter pigs. From 600 normal slaughter pigs (3 different slaughterhouses in Netherlands), 181 (30.1%) of them were carriers of Salmonella. The site of isolation was the diaphragmatic crura in 5.5%, the spleen in 3.1%, the liver in 3.9%, the gallbladder in 3.6%, the portal lymph nodes in 8%, the mesenteric lymph nodes in 15% and the faeces in 11%. Of the strains isolated, 37% were S.typhimurium. They concluded that there was a serious problem of "internal contamination" with Salmonella.

Guinée et al., (1963) studied the incidence of Salmonella in brown rats (Rattus norvegicus) caught in and near slaughterhouses, farms and mink farms. The carrier rate was found to be 30.8% for rats caught in slaughterhouses, 4.0% for those on unselected farms and 17.4% for the ones caught in mink farms. S.typhimurium was found in 31.3% of the total number of positive rats.

Richardson and Bokkenheuser (1963) found that 29.3% of peri-urban South African Bantu school children were infected with Salmonella. In most cases the infections were asymptomatic. A few of the children showed evidence of being Salmonella carriers of long standing. Eighteen different Salmonella types were recovered. The most prevalent serotypes were S.labadi, S.typhimurium, S.chester, and S.anatum. The investigators observed that the drinking water was of poor quality and may well have been implicated in the

transmission of the infections. In a report of the working party of the Public Health Laboratory, U.K., thirty two abattoirs were studied (Dixon, 1964). Salmonellae were isolated from 930 (21%) of 4496 swabs of abattoir drains. There was great variation between different abattoirs, but in general, Salmonella were found most frequently in those which slaughtered a high proportion of cattle and a low proportion of sheep; more serotypes were isolated from bacon factories than from abattoirs which slaughtered cattle and sheep. Of 11,347 tissue specimens collected at abattoirs, 218 (1.93%) yielded Salmonella . Drain swabs from butchers' shops were examined and 73 (6.5%) of 117 swabs were positive. Meat and meat products were less commonly contaminated, but 0.8% of 4127 samples yielded salmonellae.

S.typhimurium was the serotype isolated most frequently from all sources. It was often shown that the same serotypes or phage types were occurring in abattoir and human cases in an area at the same time.

Wilson and Miles (1964) in Topley and Wilson's Principles of Bacteriology and Immunity, reviewed, among other topics on Salmonella, the animal reservoirs. They observed that cattle are probably less important as a source of human infection than poultry and pigs. In pigs, S.choleraesuis was found to be practically the only type which cause clinical illness but many others give rise to symptomless infections and can be isolated from the faeces and from the mesenteric

lymph nodes. The authors further stated that Salmonella infection has a rather localized distribution in sheep and goats. They found that both rats and mice suffer naturally from infections with S.typhimurium, S.enteritidis and other salmonellae.

Hansen et al., (1964) reported the incidence of Salmonella in pigs as affected by handling practices prior to slaughter. In pigs slaughtered after arrival at the plant, the incidence of Salmonella was 10% but in the 2nd group held in the holding pens for 2 to 3 days prior to slaughter, the incidence was 35%.

Guinée et al.,(1964) found that the prevalence of Salmonella infections in healthy cows is very low (0.8%). In normal slaughter calves, the incidence of Salmonella was in the range of 4.1 to 15%. S. dublin, although the commonest cause of salmonellosis in cattle in England and Wales, plays little part in human infection. S.typhimurium, in contrast, causes by far the greatest number of infections in man. It was, for example, responsible for 73% of the incidents of Salmonella infection in man in the period 1954-1959 in the U.K. The contrast between serotypes commonly found in feeding stuffs and those found in bovine infections is marked.

In another report of the Joint Working Party of the Veterinary Laboratory Services of the Ministry of Agriculture, Fisheries and Food, and the Public Health Laboratory Service (1965) the incidence of salmonellosis in cattle in England and Wales was given. S.dublin

and S.typhimurium were much the commonest serotypes encountered. In a study of 10 incidents of salmonellosis in cattle, it was shown that the same phage-type or serotype was commonly found in man and in other farm animals and occasionally in feeding-stuffs.

Dennis (1965) reported the incidence of salmonellosis in domestic animals and poultry in Western Australia. Thirteen Salmonella spp.were isolated from animals. S.typhimurium was the most ubiquitous serotype and accounted for just over half the isolates other than those due to S. pullorum.

The Council of the American Veterinary Medical Association on Public Health and Regulatory Medicine (1966) reported on the tremendous increase of cases of Salmonella infections in man. Within five years, reported Salmonella infections in man, exclusive of typhoid had increased from 1733 cases in 1951 to 20,865 bacteriologically proved infections in 1965. It has been estimated that more than 1% of the population of the U.S.A. becomes infected with Salmonella each year and only 1% of these infections are reported to public authorities. The Council stated that salmonellosis was considered to be the most important of the zoonotic diseases, since it affected more people than any other disease.

Pateraki et al., (1966) investigated Salmonella in the mesenteric lymph nodes of pigs and cattle slaughtered in Athens. Several serotypes were isolated.

Daleel and Frost (1967) reported the isolation of Salmonella from cattle at Brisbane (Australia) abattoirs. A total of 11.6% of cattle were infected with 32 serotypes. Salmonellae were isolated from 18% of 300 rumen samples, 9% of 100 samples from the large intestines and from 7-8% of 671 samples from the small intestines. Only one isolation was made from 150 samples of bile. The most common serotypes were S.anatum, S.adelaide and S.typhimurium.

Edwards and Galton (1967) gave an account of recent developments in Salmonella research and possible future trends were pointed out. They gave the definition of the genus Salmonella supported by detailed observations. Genetic changes that affect serotyping and epidemiology, sampling and isolation procedures were discussed. Concerning the occurrence in man and type distribution the authors recognised the increase of salmonellosis in man and animals. It was noticed that S.typhimurium far out-numbered other serotypes from man and it likewise ranked first in fowls and in other domestic animals.

The reported frequency of salmonellae isolated from fowl indicates that domestic poultry is the largest single reservoir of these organisms among animals (Edwards, 1958; quoted by Edwards and Galton,1967; Hobbs, 1961). S.typhimurium is now the most common type but a greater number of Salmonella serotypes have been isolated from fowls than from any other species except man. Although S.typhimurium and S.dublin are

the most common serotypes encountered in cattle, more than 75 other serotypes have been identified.

In swine, S.choleraesuis is the serotype most commonly associated with enteritis, but the serotypes found in normal animals at slaughter are more frequently similar to those obtained from the human population in an area, suggesting that they are spread from one to the other or they are derived from the same source. In horses there is an increase in the number of unusual types which may be associated with contaminated feed fed to the animals. Present knowledge of the wide distribution of salmonellae in domestic and wild animals, as well as reptiles and wild birds, indicates that rodents now must be considered only as one of many sources of a variety of serotypes.

Rislakki (1967) investigated Salmonella in a Pretoria slaughterhouse and butcher shops with interesting results. Only a few of the samples collected during the process of slaughtering contained salmonellae. In samples taken after slaughtering, salmonellae were found relatively abundantly (14.8%). In the butcher shops salmonellae were found mostly from blocks, minced meats and offals. The number of the isolated salmonellae from 458 samples was 34 (7.4%) belonging to 21 serogroups.

During 1967 and the following year, several workers continued the investigation of Salmonella in animals and their environment, including sewage effluents. Meat, offal and other possible sources

of human enteric infections were investigated (Sharma and Singh, 1967; Van Schothorst and Kampelmacher, 1967; Taylor, 1967; Thuang and Lo, 1967 and Stevens et al., 1967).

Richardson et al., (1968) made a bacteriological assessment of meat, offal and other possible sources of human enteric infections in a Bantu township in South Africa. Offal, consisting mainly of tripe and intestines, is eaten in large quantities by the Bantu population and is both nutritious and economical. The incidence of Salmonella isolations was 48% in the tripe and 29% in the intestines. Dog faeces collected from the township pavements yielded 21% salmonellae and faeces from fowls sold live to the shopkeepers yielded 14%.

From the offal specimens, S.typhimurium (23%) and S.london (18%) were the Salmonella types most frequently isolated. The prevalence of salmonellae in the various meat samples tested in this survey supplements the findings of various workers in other countries who have indicated the close correlation between animal and human infections.

In U.S.A., Williams and Newell (1968), found that a commercial swine fattening ration containing animal origin ingredients was shown to be related to the Salmonella excretion of market pigs being sent to slaughter from a well managed farm. They stated that their findings support the view that the building up of Salmonella in pigs is by contact with

contaminated environment, but they also indicated that the primary source of the contamination is most probably the Salmonella-excreting pig which has consumed contaminated feed ingredients on its farm of origin.

Robinson and Daniel (1968) found 38% carrier rate of Salmonella in rats (Rattus norvegicus) from farms in New Zealand, where salmonellosis in sheep was enzootic.

Schnurrenberger et al., (1968) working in Illinois (USA), isolated S.give and S.derby from two Norway rats out of 181 (1.1%).

In 1969, many investigators reported on Salmonella in its various aspects. Many serotypes were isolated from various sources: animals and birds, reptiles and terrapins, sewage and cattle slurry, food, food products and animal feeds. Epidemiology and isolation procedures were discussed (Heard et al., 1969; Hobbs, 1969; Hugh-Jones, 1969; Jephcott et al., 1969; Jack and Hepper, 1969; Rankin and Taylor, 1969; Riemann, 1969; Smith, 1969; Velaudapillai et al., 1969; Weissmann and Carpenter, 1969; Williams et al., 1969).

Gitter and Brand (1969) investigated Salmonella in wildlife in the Nairobi National Park. Salmonellae were isolated from 5 out of 492 faecal samples representing 22 species of animals and birds from the Nairobi National Park but none from 251 samples involving 42 species from the Animal Orphanage at the Park. S.bovis-morbificans was isolated from 3 out of 5 positive samples.

Martin and Ewing (1969) gave the distribution of species and serotypes of Salmonella isolated in the U.S.A. between October 1966 and September, 30,1967 in addition to other data obtained from the Salmonella surveillance summaries for the past 5 years. S.typhimurium heads the list of human isolates followed by S.typhi, S.thompson and S.newport respectively.

Steele (1969) reviewed salmonellosis as a major zoonosis. He stated that the main reservoir of salmonellae is in animals and that the organisms are transmitted to man either directly or through contaminated products of animal origin. He further stated that surveys of rat populations have indicated incidences from 0.7% to 13%. The variety of serotypes isolated from rodents indicates that their Salmonella flora reflects their environment.

Heard et al.,(1969) surveyed the incidence of salmonellosis in pig herds in U.K. Of 11,953 pigs included in this survey, 173 symptomless carriers of Salmonella were found, an incidence of 1.47%.

Chung and Frost (1969) examined one thousand pigs at slaughter for Salmonella. Eighty seven strains belonging to 26 serotypes were isolated from 84 (8.4%) pigs. S.anatum, S.derby, S.muenchen, S.give and S.typhimurium were the main serotypes. Ten serotypes not previously recorded from pigs in Australia were isolated. Faeces and mesenteric lymph nodes were the best sites for isolation, and no salmonellae were isolated from bile or spleen and only one from liver. The significance of the

pig in human salmonellosis was discussed. In 1970, many workers discovered new serotypes of Salmonella and emphasized the sources of infection especially animal carriers and their environment (Edel and Kampelmacher, 1970; Haddock, 1970; Harvey and Price, 1970; Riley, 1970; Timoney, 1970; Linton et al., 1970; Goyal and Singh, 1970).

Gitter and Brand (1970) made a survey of Salmonella in Kenya abattoirs. Salmonellae were isolated from 17 out of 550 pigs (3%) and 3 out of 299 calves (1%) killed in the Uplands (Kenya) slaughter-house but none from 455 steers slaughtered at Nakuru. Follow-up visits to the farms of origin revealed no Salmonella problems. Out of 20 salmonellae isolated 5 were S.typhimurium, 4 S.enteritidis var.jena, 2 were S.kiambu and 2 were S.london. No Salmonella was isolated from steers slaughtered at Nakuru but 2 salmonellae, S.schwarzengrund and S.enteritidis var. jena were isolated from the 25 drain swabs.

Khan (1970) examined 810 apparently healthy cattle in the Sudan and from 13 (1.6%) of them isolated salmonellae. He observed that mesenteric lymph nodes yielded a bigger number of salmonellae than intestinal contents or bile. Eleven Salmonella serotypes were recovered during this investigation, 10 of them for the first time from cattle in the Sudan. He pointed out that S.typhimurium which is common in certain other countries has not yet been reported from cattle in the Sudan. The same author reported Salmonella

infections in dogs, cats and in wild animals. He also investigated Salmonella infections in healthy sheep and goats, reporting a carrier rate of 3.77% in sheep and 1% in goats. The most prevalent serotype in sheep was S.london and in goats S.reading.

Vassiliadis (1970) studied the prevalence of salmonellae in drain swabs from three abattoirs in Athens. The frequency of Salmonella isolations was higher in samples from abattoirs killing only pigs and lower in samples from abattoirs killing only cattle or only sheep. The predominant serotype in abattoirs dealing with cattle was S.tennessee, while abattoirs dealing with sheep had a predominance of S.typhimurium. No predominant serotype was found in samples from abattoirs dealing mostly with pigs.

Woodward (1970) reporting on food borne disease surveillance in the U.S.A., observed that although data were incomplete and, therefore, not truly representative, various trends were apparent. More states participated in 1967 than in 1966. The total number of individuals affected and the number of outbreaks increased in 1967 as compared with 1966, but the larger numbers were primarily due to better reporting. Bacterial contamination accounted for the largest number of outbreaks. Salmonella was the cause of most cases, and the responsible vehicles were beef, turkey, eggs , egg products and milk. S.typhimurium was the predominant serotype.

Aserkoff et al., (1970) presented a 5 year review of salmonellosis in the United States. The results of the 5 years of Surveillance (1963-1967) showed that among the most frequently reported serotypes both in human and non-human isolations, S.typhimurium topped the list. Close correlation between human salmonellosis and isolations from non-human sources was documented. Poultry and poultry products which were responsible for nearly half of the common-vehicle epidemics were also associated with nearly half of the non-human isolations. In contrast to non-typhoid salmonellosis, the annual number of cases of typhoid fever has dropped markedly during the past 25 years.

Sojka and Field (1970) presented and discussed data on the incidence of Salmonella infection in cattle, sheep, pigs, birds (including poultry) and other species of animals in England and Wales during the 10 year period 1958 to 1967.

In all 19,371 incidents of Salmonella infection were diagnosed at the Ministry's Laboratories, of which 8,968 were reported in cattle, 8,575 in poultry and other birds, 895 in pigs, 768 in sheep and 165 in other species of animals.

Ninety-four different serotypes were isolated but relatively few were frequently involved. S.typhimurium was commonly encountered in all species of animals.

The most salient feature during this period was the striking increase of Salmonella infection in

cattle and equally striking decrease in the incidence in poultry. During the years 1971-1975 many reports on the problem of Salmonella appeared. Some of these deserve mentioning.

Chambron et al., (1971) isolated 28 Salmonella strains from the mesenteric lymph nodes of healthy pigs slaughtered in Dakar; 19% of them were carriers. The presence of 2 new serotypes was demonstrated.

Groves et al., (1971) studied the occurrence of Salmonella infection in market swine, in abattoirs and on selected swine farms in Ontario. Salmonella were isolated from 20.3% of the mesenteric lymph nodes of market swine examined in 5 abattoirs over a 9 month period.

S. typhimurium was isolated with the highest frequency. From the farm investigations, salmonellae were detected in pigs on 33.3% of the farms and the same percentage in feed samples.

Miller (1971) found a carrier rate of 6.7% on testing faecal samples from 89 apparently healthy cattle in Botswana. A total of 35 Salmonella serotypes were isolated from specimens of bovine origin. S.typhimurium and S.anatum were the most prevalent serotypes with equal number of isolates.

Skovgaard and Nielsen (1972), on behalf of a Public Health Laboratory Service Working Group, reported on a comparative study on the incidence of Salmonella in pigs and feeding-stuffs in England and Wales and in Denmark. The percentage isolation

rate from faecal samples was 7% in England and 3% in Denmark, and from mesenteric lymph nodes 6% in England and 4% in Denmark. Forty-three serotypes were isolated in England and 7 in Denmark. S. typhimurium accounted for 60% of all isolations in Denmark but only 15% in England. In Denmark, 0.3% of resterilized imported meat and bone meal was contaminated with salmonellae. This compared with 23% of meat and bone meal in England and Wales and 20-27% of other ingredients of animal origin.

Lee et al., (1972) reported 18% Salmonella infection in slaughter pigs from one farm and only 3% in pigs from another farm. They found that the high carrier rate in pigs from the first farm was due to contaminated fish meal.

Bruner (1973) in U.S.A., reported that a total of 3,945 Salmonella cultures were classified within a 22 year period (1950-1971). Exclusive of man, there were 30 species of animals involved. Antigenic analysis of the cultures revealed 75 serotypes. S.typhimurium was found in 46% of the isolations, S.pullorum in 20%, S.infantis in 3% and S.anatum in 3%. The remaining types occurred less frequently.

Forty-three of the serotypes encountered occurred in turkeys, 40 in chickens, 27 in water, 24 in feed, 17 in ducks, 13 in dogs, 12 in horses, 11 in cattle and 11 in swine. Other animal species furnished from 1 to 7 types.

Carpenter et al., (1973) examined 420 pork carcasses from 4 abattoirs for the presence of salmonellae by the use of swabbing-enrichment techniques. and contact plate methods. Carcasses from only one abattoir were found to be contaminated by swabbing-enrichment (23.3%) and contact plate (17.9%) methods. The most frequently isolated species in this study were S.derby, S.anatum, S.typhimurium and S.indiana.

Baine et al., (1973) discussed the problem of institutional salmonellosis. They observed that between 1963 and 1972, 112 (28%) of 395 outbreaks that were reported occurred in institutions (hospitals, mental institutions and nursing homes); 3,496 cases were associated with these 112 outbreaks and institutional salmonellosis represented 13% of all cases associated with reported outbreaks. Institutions ranked second to the homes in frequency of place of occurrence of reported outbreaks.

Thus, the investigation and control of cross-infection outbreaks should include consideration of the role of contaminated objects in the environment as well as careful search for symptomatic and asymptomatic excretors of salmonellae among patients and hospital staff.

Kumar, et al., (1973) investigated the carrier rate of Salmonella in sheep and goats, slaughtered for food, at Mhow, Central India. Five thousand

nine hundred and eighty samples comprising faeces, mesenteric lymph nodes, liver and spleen were collected from 812 sheep and 683 goats. Twenty five sheep (3.1%) and 26 goats (3.8%) were found to be Salmonella carriers. The total number of strains isolated from all four sources, was 72, which represented 22 different Salmonella serotypes. S.typhimurium was the commonest of all the Salmonella serotypes recorded in the present study; its frequency of isolation was higher in goats than in sheep.

Meara (1973) reviewed salmonellosis in slaughter animals in South Africa as a source of human food poisoning. Beef, veal, pork and poultry are responsible for sporadic cases and outbreaks of human disease. Twenty-one per cent of abattoir drain swabs were positive, and salmonellae were found more frequently where a high proportion of cattle was slaughtered. Apparently healthy active carriers and latent carriers therefore present an insidious hazard necessitating satisfactory slaughterhouse hygiene if contamination of their carcasses and of others is to be avoided. The author reviewed the different carrier rates of Salmonella found by some workers in various species of slaughter stock and animal feedstuffs in several countries. In 1959, 4.3% of prospective or employed food handlers in South Africa were found to be infected with Salmonella or Shigella organisms. A carrier rate of Salmonella ranging from 29.3% to 72% was found in rural Bantu

school children. Notwithstanding negative bile and faecal test results for approximately 50,000 sheep and 8,000 cattle slaughtered, a variety of Salmonella serotypes were found in different sites at the new Port Elizabeth abattoir. A survey of the municipal abattoir and retail shops in Pretoria, revealed 7.4% of positive samples.

Ola Ojo (1974) carried out a survey of Salmonella in goats and dogs in Nigeria. Salmonellae were isolated from 10 of the 125 dogs attending the Veterinary Clinic in Ibadan. All the animals were free from diarrhoea. The most prevalent serotypes were S.agama and S.patience. No Salmonella was isolated from 375 specimens obtained from goats at the slaughter slab in Kano.

The author mentioned that Johnson (1958) did not isolate salmonellae from systemic organs of over 300 goats and Macadan (cited by Johnson, 1958) also failed to isolate salmonellae from the bowel of 40 sheep and 90 goats.

Hummel (1974) isolated 17 Salmonella serotypes from cattle in Dar-es-Salaam (Tanzania). The total number of isolations was 44 from 714 animals (an infection rate of 6.16%) and 3 from 770 animals (a carrier rate of 0.38%). The most prevalent serotypes were S.dublin, followed by S.typhimurium. The findings suggest that cattle may play a part in the epidemiology of Salmonella infections in Tanzania.

Rosted et al., (1975) examined 2,184 meal samples of animal origin for Salmonella between July, 1968 and June 1973. All samples were from three slaughterhouses in Kenya. There were 95 positive samples representing 4.31% of the total meals examined. A total of 20 various serotypes were diagnosed of which none was S.typhimurium. S. montevideo was found to be most prevalent and S.senftenberg was isolated seven times.

Basu et al.,(1975) carried out a 16 year study on the prevalence of Salmonella serotypes in India. During the period 1958-1973; 8,027 strains of Salmonella were tested: 3,834 strains from man, 3,018 from animals, 839 from sewage and water sources and 336 of unknown origin. A total of 99 serotypes were identified: 47 from man, 83 from animals, and 35 from sewage and water sources. S.typhi was the commonest serotype in man while S.typhimurium was the commonest isolate from animals. In sewage and other water sources S.weltevreden was the commonest serotype identified, followed by S.typhimurium.

Muhammed and Morrison (1975) investigated water quality in Kiambu District, Kenya. Although 42 sites of Nairobi River were examined only one sample from one site yielded Salmonella organisms. The serotype was S.typhimurium.

From 1976 up to 1981, new Salmonella serotypes were discovered, methods of isolation and identification were improved and of course more light was shed

on its epidemiology (Nazer and Osborne, 1976; Zhvaniya,1977; Williams et al., 1978; Bebora,1979; Garg and Sharma, 1979; Martel et al., 1979; Mirza and Nsanzumuhire, 1979; Al-Hindani and Taha, 1979; Ayanwale et al., 1980; Pöhn, 1980; Pramanik and Khanna, 1980, Smeltzer et al., 1980, Counter and Gibson, 1980, Le Minor, 1980; Offor, 1980).

Doutre (1976) carried out a survey on "reservoirs" of Salmonella in small ruminants slaughtered in Dakar abattoir. The infection rate was 4.7% in sheep and 3.6% in goats. The authors observed that those results were closely related to those obtained in other African countries. The influence of healthy carriers of Salmonella among small ruminants on public health was discussed.

Wld. Hlth. Org. Expert Committee (1976) stated among other topics that some 1700 serotypes of Salmonella are known but in most countries only some 40 to 50 serotypes are isolated regularly from humans, animals and foods. Of these serotypes, only about 10 are endemic in any country at any one time. In most countries S.typhimurium is the predominant serotype isolated from man, but the frequency of isolation of different phage types within this serotype varies widely among different countries, in much the same way as variation in serotype frequency.

Abd El-Ghani (1977) reported the occurrence and significance of Salmonella isolates from wild rats

in Egypt. The carrier rate was 19.71% and four different serotypes were isolated. S.typhimurium and S. enteritidis were the most prevalent types isolated with the percentage of 46.42 and 28.58 respectively; while the incidence of S.bovis-morbificans and S.dublin were low.

Chambers (1977) gave the sources and serotypes of some salmonellae isolated from abattoirs, domestic animals, birds and man in Rhodesia. A wide range of Salmonella serotypes was isolated from sources in abattoirs. Drains and effluent material yielded the highest incidence of positive abattoir samples. From specimens submitted from domestic animals the most frequently isolated serotypes were S. dublin, S.typhimurium, S.enteritidis, S.heidelberg, S.virchow and S.bovis morbificans. No S. dublin were isolated from fresh faeces taken from cattle awaiting slaughter, it was isolated only once from abattoir samples. On the other hand, S.typhimurium has been frequently isolated from several sources, including abattoirs and food.

Gospar and Hrabeta (1977) carried out a survey of salmonellosis in domestic animals in Zambia. Sixty-one Salmonella cultures consisting of 11 different serotypes were isolated. S.dublin and S.typhimurium were the most prevalent in domestic animals.

Wray and Sojka (1977) reviewed bovine salmonellosis and stated that the incidence of salmonellosis due to serotypes other than S.dublin and S.typhimurium, the

two commonest isolates in cattle, had increased in recent years. Although there have been many surveys of the incidence of Salmonella in healthy cattle at abattoirs, there has been no uniformity of the material examined, of the sampling techniques and consequently the results are often not comparable. Surveys in various countries have shown that in adult cattle the incidence of Salmonella infection varied from 0.3-11.6% and in calves 4.3 - 14.3%.

Trudov (1977) reported on Salmonella species occurring in the Georgia, USSR. The 83 strains obtained from cattle, swine and buffaloes included 46 of S.enteritidis, 14 of S. dublin, 11 of S.typhimurium, 4 of S.choleraesuis, 1 of S.heidelberg and 1 of S.ngozi.

Andreani et al., (1978) investigated Salmonella carriers among cattle, sheep, goats and dromadaries in the Somali Democratic Republic. The carrier rate was 5.6% in cattle, 2% in goats, 5% in sheep and 6% in dromadaries. The most common species were S.dublin, S.bredeney, S.saint paul, S.newport and S.schwarzengrund.

Blackburn and Harrington (1978) reported serotyping of Salmonella and Arizona cultures from animal disease cases and epidemiologically related sources from October 1976 to September, 1977. The commonest Salmonella serotypes were S.typhimurium, S.choleraesuis var-Kunzendorf, S.typhimurium var. Copenhagen, S.anatum and S.heidelberg. The commonest Arizona serotype was 7a, 7b: 1, 7, 8. The most frequent sources of

cultures were turkeys, followed by chickens, swine and cattle.

Itotia et al., (1978) carried out bacteriological and parasitological investigations on faeces from diarrhoeal cases and apparently healthy persons with reference to food handlers in Kenya. Four thousand and nine foecal samples from food handlers, newly appointed personnel, cases of diarrhoea with abdominal pain, cases of diarrhoea suspected to be cholera, and cases of food poisoning were examined. Pathogenic bacteria were isolated from 253 cases, of wich 144 isolates were shigellae, 79 salmonellae and 12 E. coli. The infection rate of Salmonella was then 1.97%. S.typhimurium and Shigella flexneri were the most commonly isolated organisms.

Joseph et al., (1978) reported a total of 860 Salmonella isolations made in Peninsular Malaysia from 15 animal species (domestic and wild), eggs, molluscs, flies and animal feed. The authors observed that according to Bruner (1973), S.typhimurium was the leading serotype in incidence and zoological distribution, but in Peninsular Malaysia the most common serotype isolated was S.pullorum, followed by S.choleraesuis and S.infantis. S. typhimurium had the widest zoological distribution.

Pietzch (1978) studied the prevalence of Salmonella infections in animals, foods of animal origin and feedstuffs in the German Federal Republic in 1975 and 1976. S.typhimurium including var. Copenhagen was

isolated most frequently (60% of isolation in 1975 and 49% in 1976), followed by S.dublin (11.8% and 17%). Noteworthy was an increase in S.dublin infections, predominantly in calves, with simultaneous decrease of S.typhimurium infections; increase in S.hadar infections, particularly in turkeys.

Linton, (1979) noticed that the majority of Salmonella serotypes found in the pigs are non-host adapted. S.typhimurium has been found most frequently but 26 other serotypes have also been reported.

Doutre and Cartel (1979) carried out a survey on "reservoirs" of Salmonella in cattle and horses in Senegal. In cattle the infection rate was 4.8%, while in horses it was found to be 7.4%. S.typhimurium was the most frequently isolated serotype in cattle and S.albany in horses. Two new serotypes were discovered and several serotypes isolated from cattle were commonly encountered in man.

Samuel et al., (1979) studied the distribution of Salmonella in the carcasses of normal cattle at slaughter. Lymph nodes and other tissues from a number of sites in the viscera, carcass and heads were collected from 28 cattle which had been held for at least 4 days before slaughter. Salmonella was isolated from 21 of these cattle, but in all except three of them it was confined to the gastrointestinal tract and the mesenteric lymph nodes. Individual mesenteric nodes were collected from a further 85 cattle and Salmonella was isolated from

61 of the animals by direct plating. The predominant serotype in the mesenteric lymph nodes was S.typhimurium

Søgaard and Nielsen (1979) reported on the occurrence of Salmonella in waste water from Danish slaughterhouses. Effluent from 11 slaughterhouses was examined continually during one week and 58% of 66 samples were positive. Only four serotypes were isolated: S.agona, S.indiana, S.senftenberg, and S.typhimurium.

This distribution of types was incompatible with findings in human beings, animals and feedstuffs.

Singh et al., (1979) studied the prevalence of Salmonella in sheep and goats in India. The examination of intestinal/rectal content samples from 101 sheep and 618 goats resulted in the isolation of 2 strains of S.anatum from sheep and 12 isolates of Salmonella, viz. S.typhimurium (4), S.typhimurium var. Copenhagen (1), S.infantis (2), S.saint-paul (1) and S.anatum (1), from apparently healthy goats and those having diarrhoea.

Del Baglivi et al., (1979) investigated Salmonella in cattle in Uruguay and found that mesenteric lymph nodes or faeces from 21 of 500 slaughtered were positive, but no salmonellae were recovered from bile. Thirty-eight of 74 strains were S.typhimurium.

Kane (1979) studied the prevalence of Salmonella infections in apparently healthy sheep slaughtered in New Zealand. The crude prevalence rate was 4.7%

(96/2027) and S.typhimurium was found in 78% of the infected sheep. Other serotypes isolated were S.bovis-morbificans, S.derby, S.newington, S.saint-paul, S.anatum and S.enteritidis.

Wld. Hlth. Org. (1979) on Surveillance of Salmonella in U.K., mentions an increase in S.dublin incidents in cattle from 525 in 1977 to 560 in 1978. S.typhimurium was the commonest serotype in calves. In sheep the most common serotypes were S.dublin, S.typhimurium and S.montevideo. In pigs the most common serotypes were S.derby, S.typhimurium and S.choleraesuis.

Cortesi and Catellani (1980) reported that from 1976 to 1979 about 5,000 samples were examined to evaluate the environmental distribution of salmonellae and their presence in man and animals in a small province in central Italy. Salmonella organisms were more frequently isolated from the rectal swabs and from the faeces of the staff and the patients of the hospital and from those of shopmen than from animal faeces, minced meat and waterways. The results confirm that the carrier rate of Salmonella is higher in man than in animals. The most prevalent serotypes were S. enteritidis, S.typhimurium and S.wien.

The very limited distribution of Salmonella in the environment and among animals compared with the higher percentage of human carriers and of human salmonellosis confirm that animals, slaughterhouses and meat and meat products are not always the link to human salmonellosis.

El-Nawawi et al., (1980) investigated samples of intestinal contents and mesenteric lymph nodes of 190 animals slaughtered at a Cairo abattoir. The results obtained indicated that camels had the highest Salmonella carrier rate (33.3%), followed by pigs (26.2%) while buffaloes and sheep were rarely affected. The most frequently isolated serotypes were S.derby, S.kottbus and S.muenster. Meanwhile, these serotypes are listed among the 10 most commonly identified from human and non-human sources in Egypt during the last 2 years.

Gangarosa (1980) emphasized why Salmonella Surveillance should be established. The author argued that the usefulness and cost-effectiveness of Salmonella Surveillance Programmes (S.S.P.) in directing control efforts have been persuasively demonstrated. S.S.P. have been important in identifying outbreaks, high risk foods and the mechanisms by which transmission occurs. Investigations of outbreaks have revealed risk factors in production, distribution and preparation of foods. The important role of animal and animal foods in transmission and the relatively minor role of human carriers are well documented. S.S.P. have provided administrative guidance in the assignment of priorities for optimum food protection, institution of better training programmes and more rational utilization of resources. Unfortunately, even the most efficient S.S.P. have not been able to eliminate the problem.

Surveillance can help control and prevent epidemics and identify sources, but control of the root issues of the international Salmonella problem has not yet been seriously addressed to with some notable exceptions. The problem merits the full attention of all countries and a close collaboration of international and national health authorities, industry and academics.

Gledel (1980) studied Salmonella and bovine pathology in France and reported that during the years 1976-1977-1978, 1,270 strains of Salmonella isolated from sick calves and cattle represented 25% of total strains from all animal species. Thirty one serotypes have been found, but two among them contain 94% of all strains (S.typhimurium (59%) and S.dublin (35.2%).

Jones et al., (1980) investigated sewage and sewage sludge for the presence of Salmonella. A total of 882 samples of settled sewage, sewage sludges and final effluents from eight sewage treatment plants were examined. Of these, 68% were positive and S.oranienburg, S.typhimurium, S.newport, S.give, respectively were the most frequent isolates. The authors found that samples usually contained less than 200 salmonellae/100 ml and arguments were presented that such concentrations should not lead to disease in animals if suitable grazing restrictions are followed.

Mered et al., (1980) carried out a survey of Salmonella in animals in Algeria. Of 1795 domestic and wild animals examined for Salmonella during 1972-1974, 118 were shown to be carriers. All of ten horses

and 282 slaughter cattle were negative, and only one of 405 young goats, six of 268 slaughter sheep and one of 134 broilers were positive. All six positive sheep yielded S.arizonae.

Papadakis, et al., (1980) investigated the presence of salmonellae in fresh vegetables and compared the efficiency of three enrichment media. From the total of 423 samples examined, only three (0.7%) samples of lettuce yielded salmonellae. These belonged to serotype S.montevideo. It is worth noting that all three isolations were obtained through the Rapapport's enrichment medium at 43°C.

Reilly et al., (1980) reported that during the period 1973-1979 in Scotland, there were 28 outbreaks of salmonellosis resulting from environmental contamination which affected animals and/or humans. These outbreaks, the authors observed, clearly illustrated the direct or indirect sewage animal man sewage cycle. The various characteristics of these outbreaks are presented as are the economic consequences of inadequate sewage disposal facilities.

Wld. Hlth. Org. (1980a) on Salmonella Surveillance in Peru, reported that during a seven-year period (1972-1978) 7,902 strains of Salmonella were received and studied at the National Enteric Reference Laboratory. S.newport accounted for 43% of isolates, S.typhi for 24%, S.typhimurium for 11%, S.oranienburg for 9% and S.derby for 4%.

Wld. Hlth. Org. (1980b) on Salmonella Surveillance

in U.S.A., reported that in 1979, 31,132 isolations of salmonellae (including S.typhi) from humans were reported to the Centre for Disease Control (CDC)-an increase of 8.3% over 1978. S.enteritidis alone accounted for over one fourth of 8.3% increase. S.enteritidis, S.heidelberg, S.saint-paul and S.infantis accounted for almost two-thirds of the increase.

Wld. Hlth. Org. (1980c) on Salmonella Surveillance in Canada, stated that in 1979 there were 8704 Salmonella isolates reported, an increase of 3% over 1978 figure of 8474. This increase observed in 1979 was well above that expected as a result of population increase (annual population increase in the last few years has been slightly over 1%). S.typhimurium was isolated the most frequently (39%).

McKinley et al., (1980) investigated the incidence of salmonellae in faecal samples of production swine at slaughter plants in the U.S.A. in 1978. The survey indicated a high incidence of salmonellae in faeces of swine held for slaughter. S. derby and S. agona were the most common (37.5 and 31.3%), followed by S.adelaide, S.bredeney, S.anatum, S.infantis, S.manhattan and S.newington.

Salmonellae were recovered from 5 (13%) of 38 production swine samples (19 units). The five positive samples yielded five different serotypes: S.london S.montevideo, S.thomasville, S.typhimurium and S.livingstone.

Singh et al., (1980) investigated the occurrence

of salmonellae in rodents, shrews, cockroaches and ants. Salmonellae were isolated from 16 of 254 rats, 11 of 109 house mice, 11 of 104 shrews, 3 of 270 cockroaches and 2 of 30 ants. The serotypes isolated included S.saint-paul, S.bareilly, S.newport, S.weltevreden, S.enteritidis, S.typhimurium, S.hvittingfoss, S.anatum, S.matopeni, S.waycross, S.paratyphi B. One shrew yielded three different serotypes, while dual infection was detected in three shrews and a rat.

Sandstedt et al., (1980) reported on Salmonella isolated from animals and feedstuffs in Sweden during 1973-1977. Data are presented on isolated serotypes from animals, outbreak in cattle and swine from 1963-1977, phage typing of S.typhimurium strains from animals and Salmonella isolated from foodstuffs, meat meal, fish meal, bone meal, blood meal and liver meal.

Pohl et al., (1981) studied three groups of Salmonella isolated in Belgium in 1980. Most of the strains from diseased animals were S.typhimurium and in cattle in addition S.dublin and S.typhimurium var. copenhagen. Salmonella from meat belong to various serotypes: about 30% are S.typhimurium. Salmonella from feed ingredients (fish-meal, meat-meal....) belong to numerous serotypes but seldom to S.typhimurium and never S.dublin.

Wld. Hlth. Org. (1981a) on Salmonella infections in U.K., stated that the number of outbreaks caused

by S.typhimurium fell by 28% in the five years 1975-1979, but those caused by other serotypes increased. The overall result was that the number of outbreaks remained relatively constant throughout the ten-year period. There has been an apparent changing prevalence, with ascending and descending curve, of the following commonest serotypes over the last ten years, S.typhimurium, S.enteritidis, S.panama, S.agona, S.heidelberg, S.virchow, S. indiana, S. anatum and S. hadar but S.typhimurium remained the commonest serotype isolated in 1976-1979.

Wamola and Mirza (1981) emphasized the magnitude of the problem of salmonellosis which may be more significant in number of infections and mortality than the dreaded typhoid fever in Kenya. They pointed out that there has been a marked increase in the past 10 years of infection due to S.typhimurium and this figure is especially striking compared with S.typhi. They observed that the problem of salmonellae in the hospital reflects a worldwide increase in non-enteric fever Salmonella infections attributed to S.typhimurium. In addition, Salmonella species other than S. typhi are common in a wide range of animal hosts as well as in asymptomatic human carriers and both these sources are known to occur in Kenya. Also noted is the more alarming fact that S.typhimurium was very resistant to the currently available antibiotics compared to

S.typhi which is relatively sensitive. They drew the attention to the fact that infections by S.typhimurium are of a serious nature involving septicaemias and meningitis as well as the common gastroenteritis normally associated with this organism. Infants and children are particularly at risk with resultant high mortality.

Wld.Hlth.Org. (1981b) on Salmonella Surveillance in Australia noted that between January and March, 1980, 1740 Salmonella isolations from humans were reported, involving 86 serotypes. S.typhimurium predominated. The editorial note mentioned that the serotypes sampled from the environmental sources in 1979 followed a similar isolation pattern to that seen with the human infections. S.typhimurium was the most prevalent serotype (4832 of which 1017 were from human sources), followed by S.anatum (2376, 71 from human sources), S.havana (998, 77 from human sources), S.saint-paul (950, 90 from human sources). Poultry and beef were the major environmental sources of the isolates, but this is influenced by the surveillance programmes active in these industries.

2.2. Isolation and Identification of Salmonella

Several authors have made numerous contributions to our knowledge of the methods of isolation and identification of Salmonella and great strides have been made towards a more thorough understanding of the antigenic structure of the latter.

Some of the literature on the subject is reviewed here below:

Smith (1959) compared the results of culturing the pig mesenteric lymph nodes in selenite broth and in brilliant green McConkey broth and found selenite broth unsuitable for isolating S.choleraesuis. All strains of S.choleraesuis were isolated in brilliant green McConkey broth, since tetrathionate was also found unsuitable for isolating this serotype.

In his previous study in 1952, the author investigated the sensitivity of different culture media for isolating salmonellae from the faeces of man, dog, horse, cow, sheep, pig, chicken, duck and turkey. Suitable dilutions of tissue fluids of animals that had died from Salmonella infection, and in which the numbers of viable salmonellae could be accurately estimated, had previously been added to the faecal specimens. Selenite and tetrathionate media were greatly superior to liquid desoxycholate-citrate medium, liquid Wilson and Blair medium, Cacotheline broth and brilliant green peptone water. By the use of either selenite or tetrathionate media it was usually possible to recover salmonellae from faecal specimens to which less than ten salmonellae had been added. They could nearly always be recovered from specimens containing 100 salmonellae.

Selenite medium was preferable to tetrathionate for examining cow and chicken faeces but the reverse was true in the case of dog faeces; slight differences

only were noted in other species. Taken as a whole, selenite was slightly superior to tetrathionate, but best results were obtained by the use of both media.

With lightly infected specimens an incubation period of 24-30 hrs was optimum for selenite and tetrathionate media. A longer period was detrimental with tetrathionate but not with selenite medium.

Hormaeche and Peluffo (1959) discussed laboratory diagnosis of Shigella and Salmonella infections. For Salmonella isolation, they stated that better results are obtained by enrichment than by direct plating; in some cases however, enrichment fails when direct plating is positive, and that is one of the reasons why in routine work both methods are used simultaneously. The authors prefer Muller-Kauffman's tetrathionate (MKT) to Leifson's selenite (SF) for Salmonella isolation as in their hands it has given more positive results. SF is better than MKT for isolation of S.typhi, but for this purpose Wilson and Blair plates are to be preferred. No selective medium, the author said, is completely satisfactory, as none of them fulfils entirely the bateriologist's ideal of eliminating all bacteria save the ones looked for.

McCoy (1962) discussed the isolation of salmonellae from foodstuffs and other materials. He emphasized the necessity of enrichment before plating on a slective medium. He stressed the importance of the size of the inoculum, stating that within limits, the

larger the quantity of sample examined the higher is the percentage of positive samples.

A series of comparisons of two enrichment media, involving strength of the medium, selective temperature and three varieties of secondary enrichment, were carried out. Tetrathionate broth incubated at 43°C was not included because a preliminary series of tests had shown that at this temperature tetrathionate broth was lethal to salmonellae and to most organisms.

No difference in efficiency was shown between single strength and double strength tetrathionate broth, or between tetrathionate broth at 37°C and selenite broth at 37°C. However, isolations from selenite broth incubated at 43°C were significantly fewer. Although no difference was found in the yield of salmonellae from portions of the same sample enriched in selenite and tetrathionate B broths, it is recommended that both broths be used for the routine examination of samples. The broths are complementary, selenite broth is superior to tetrathionate B broth for the isolation of S.typhi, but the tetrathionate B broth supports the growth of a wider range of serotypes than does selenite broth.

Many times and combinations of times for subculture from enrichment broths are recommended in the literature; 24 and 48 hr, and 24 and 72 hrs are the combinations most frequently recommended by the author. Several selective media are recommended but Harvey (1956) compared bile salt lactose media with

bismuth-sulphite agar and with brilliant green - McConkey's agar and recommended the latter.

Jameson (1963) recorded the importance of the ratio of the volume of inoculum introduced into a Salmonella enrichment broth to the volume of enrichment medium receiving the inoculum. The term inoculum ratio was used to describe this variable. The ratios used previously by Jameson (1961) varied between 1:25 and 1:100 in a secondary enrichment technique. Provided the inoculum to the secondary enrichment medium contained at least one viable Salmonella, the greater the ratio between the volume of the medium and the volume of the inoculum, the greater, normally, was the opportunity for secondary enrichment to succeed. Jameson (1963) also suggested that an isolation technique involving primary enrichment in a relatively small volume of nutrient broth followed by secondary enrichment in a relatively larger volume of enrichment medium might be advantageous.

This is akin to pre-enrichment with subsequent enrichment, a technique which has later proved valuable in recovery of salmonellae from naturally contaminated water supplies (Harvey and Price, 1977).

Harvey and Price (1967) examined six methods for isolation of Salmonella from samples with multiple serotypes: (1) use of colonial character to isolate certain serotypes; (2) picking of large numbers of suspicious colonies from a selective agar; (3) use of multiple subculture from selenite F broth (Harvey 1957);

(4) splitting the sample into several equal parts and selectively culturing each part in selenite F broth; (5) use of agglutinating sera to remove serotypes from a mixture in an orderly and premeditated manner (Harvey and Price, 1962) and (6) use of a physical or chemical method to encourage the growth of one serotype rather than another.

The authors recognize that it is difficult to put forward an optimum technique for examination of samples containing multiple serotypes, that to some extent the time available for the examination is the deciding factor. Where this is short, method 4 is the most convenient. Where the full range of serotypes in a material is being investigated and time is not important, a combination of methods 4 and 5 is best. Where less complex material is examined, method 5 is the one of choice. Methods 2 and 3, though effective, are rather tidious.

The various methods recorded by the authors have been found valuable over a period of almost twenty years.

Harvey and Price (1968) discussed elevated temperature incubation of enrichment media for the isolation of salmonellae from heavily contaminated materials.

It was in 1953 that Harvey and Thomson described a technique using selenite F broth incubated at 43°C for the isolation of Salmonella. This paper mentioned three temperatures: 42, 43 and 44°C (Harvey and Price 1968).

For the conditions and media in use, it was suggested that 43°C was the optimum for incubation with the proviso that 42°C might be safer as 43°C possibly represented the upper end of the useful temperature range. The authors showed for three different materials, that incubation of selenite F broth at 43°C had advantages over incubation at 37°C. The modification of tetrathionate broth for incubation at 43°C and the adjustment of the incubation temperature to suit more inhibitory enrichment broths is discussed. Spino (1966) for example, used a temperature of 41.5°C with selenite brilliant green broth and Kauffmann's tetrathionate broth.

Edel and Kampelmacher (1968), on behalf of the members of a Working Group under the initiative of Common Market Scientific Veterinary Group on salmonellosis and financial support of the World Health Organization, produced a useful report on Comparative Studies on Salmonella isolation in eight European Laboratories.

The participating laboratories used standard materials and their own methods of examination. The results were then compared to consider whether standardization of techniques was necessary. Edel and Kampelmacher (1969) again on behalf of the above mentioned Working Group reported on Salmonella isolation in nine European Laboratories using a standardized technique. It uses tetrathionate-bile-brilliant-green broth as enrichment medium.

Just after putting the samples in the enrichment medium, the jars or flasks are put immediately in a 45°C water-bath for 10 minutes. They are then removed without drying them and transferred to an incubator at 43°C. First subcultures are made after 18-24 hours; second subcultures after 48 hrs.

The selective medium is brilliant-green-phenol-red agar (BGA) incubated at 37°C for 18-24 hrs. Suspicious colonies are inoculated into lysine decarboxylase medium (LDC) and then into triple sugar iron (TSI) which are incubated for 18-24 hrs at 37°C. Slide agglutination is done from a pure culture on the TSI when LDC and TSI are positive.

Edel and Kampelmacher (1969) once again on behalf of the above Working Group produced a report on comparative studies on the isolation of "sublethally injured" salmonellae in nine European laboratories. An enrichment procedure (pre-enrichment) was introduced for the reactivation of salmonellae. Samples were placed in a non selective medium (buffered peptone water) for 18-20 hrs at 37°C and then 10 ml of the culture were transferred into 100 ml of the tetrathionate liquid enrichment medium. The standardized method described earlier was then applied.

Edwards and Ewing (1972) published the third Edition of "Identification of Enterobacteriaceae" which is a valuable contribution to the isolation and identification of the members of the genus Salmonella. Tests and media that are helpful in preliminary

examination of isolates are given. For example, the use of triple sugar iron agar (TSI) and lysine agar media are discussed and the reactions given by the majority of Enterobacteriaceae are tabulated. Serological identification of Salmonella including its antigenic structure and the production of antisera are discussed in details.

Nabbut (1973) in Beirut (Lebanon), studied the comparative efficiency of the elevated, 41°C and the conventional, 37°C temperatures of incubation of selenite-enrichement cultures on the isolation of salmonellae from sewage and human faeces. The enrichment of swab cultures at 41°C was found to be appropriate for the isolation of salmonellae from sewage. This is in agreement with reports by other workers (Spino,1966; Harvey and Price, 1968; Morahan and Hawksworth, 1969).

Moreover, results of the recovery rates of S. paratyphi B, S.typhi, and S.typhimurium indicate that an incubation temperature of 37°C is more appropriate for recovering salmonellae from artificially infected faecal samples than an incubation temperature of 41°C.

Carlson and Snoeyenbos (1974) carried out a comparative study on the efficiency of selenite and tetrathionate enrichment broths for the isolation of Salmonella serotypes. They found that the tetrathionates were generally better than the selenites regardless of the sample cultured with the salmonellae. The tetrathionate brilliant green (TBG) enrichment medium

was slightly better than the tetrathionate medium, and both were equal to, or better than selenite brilliant green-sulfa; the latter was distinctly unsatisfactory for some strains and was more likely to allow major die off of salmonellae between the 24th and the 48th hour of incubation, particularly at 43°C. Incubation of the enrichment at an increased temperature (41.5 to 43°C) has been reported to facilitate Salmonella isolation when large numbers of competing organisms are present.

The Salmonella test populations were recovered in relatively pure culture from the tetrathionate enrichment medium held at 43°C when cultured with the faecal microflora and to a lesser degree with the chick starter mash and used poultry litter.

Cowan and Steel (1974) described methods for the isolation and identification of Salmonella in the Manual for the identification of medical bacteria.

Ewing and Martin (1974) in the Manual of Clinical Microbiology, described techniques for processing of specimens, enrichment and plating media. They also described biochemical reactions of members of different groups and serological examination of cultures of Salmonella and Shigella.

Edel and Kampelmacher (1974) once again on behalf of the Working Group, on salmonellosis under the sponsorship of WHO, made comparative studies on Salmonella isolations from feeds in ten laboratories. It was concluded that, in general, a pre-enrichment

method using a non-selective medium, should be used for the isolation of salmonellae, especially when they are injured or small numbers of them are expected in the material to be investigated. Extending the duration of incubation of the enrichment medium to 48 hrs followed by a second subculture improved the isolation rates. Ewing (1975) described methods for differentiation of enterobacteriaceae by biochemical reactions.

The Committee on Salmonellosis and Arizonosis of The American Association of Veterinary Laboratory Diagnosticians (1976) in a Manual of Culture Methods for the detection of animal salmonellosis and arizonosis, described the isolation and identification techniques for Salmonella and Arizona. The manual is a compilation of necessary information for isolation methods used when dealing with the specimens from swine, cattle, sheep, goats and poultry. In addition, methods of culturing unincubated hatching eggs, incubated hatching eggs and embryos, hatcheries and general environmental samples are discussed.

Culture identification including serological examination is also discussed.

Ewing (1976) supplied detailed information on isolation and identification of Salmonella and Shigella. The isolation and preliminary examination of Salmonella comprising the collection and processing of specimens, the selection and isolation of colonies leading to the primary differentiation were thoroughly

discussed. Ewing and Ball (1977) gave a detailed account of the biochemical reactions of members of the genus Salmonella. Ewing and Davis (1977) also described in detail methods for isolation and differentiation of Enterobacteriaceae.

Smith (1977) in U.K., described a standard technique for the isolation of Salmonella from animal feeds. This technique consists of pre-enrichment with buffered peptone water (BPW) incubated at 37°C for 18 hrs. Then inoculation of selenite broth (SB) and Muller Kauffmann tetrathionate broth (MKTB) incubated at 43°C for 24 hrs and 48 hrs. Subculture is done on brilliant green, bismuth sulphite and desoxycholate citrate lactose sucrose agar (DCLS). Suspect colonies are inoculated onto triple sugar iron agar (TSI) and urea. Finally sero-agglutination is carried out.

Van Schothorst et al.,(1977) studied the multiplication of salmonellae in various enrichment media at different incubation temperatures. The author found that several selected Salmonella strains did not multiply in tetrathionate brilliant green bile medium when the inoculum was small and the medium was incubated at 43°C. Gradual heating from 20°C to 43°C and dilution of the medium with buffered peptone water (1:10) containing egg yolk did not decrease its inhibitory properties. It became less inhibitory, however, after the growth of other enterobacteriaceae. These findings stress the

advantage of using non-selective pre-enrichment since in that case the Muller-Kauffmann tetrathionate will be inoculated with a large number of salmonellae and other enterobacteriaceae, thus facilitating the isolation of the former.

Thomason et al., (1977) investigated the incidence and persistence of salmonellae in weather pools on the top of Stone Mountain in U.S.A., using lactose and buffered peptone water as pre-enrichment broths. The use of buffered peptone water increased the recovery of salmonellae by approximately 25%. Pre-enrichment in lactose broth yielded one less isolate than did direct enrichment in tetrathionate broth. The combined use of direct enrichment in tetrathionate broth containing brilliant green dye and pre-enrichment in buffered peptone water followed by enrichment in tetrathionate broth made it possible to detect all 37 of the contaminated samples. The lactose and peptone water cultures were incubated at 35^{o}C for 24 hrs. All tetrathionate broth cultures with the exception of those inoculated with faecal samples, were incubated at 41.5^{o}C for 48 hrs. The faecal samples were incubated at 35^{o}C for 24 hrs.

Ewing and Davis (1978) published a report on the media and tests for differentiation of Enterobacterioaceae. This compilation of formulas and methods serves a useful purpose for better taxonomy. The authors stated that the media and tests presented have been proved; they are known to

have given excellent results in the hands of many investigators, and they are recommended for use in any laboratory of bacteriology, large or small.

van Schothorst et al., (1978) made comparative studies on the isolation of Salmonella from minced meat. Those studies were carried out in four laboratories and revealed that in the examination of frozen or fresh minced meat samples, tryptone soya broth and glucose mineral salts medium used for pre-enrichment gave the same results as buffered peptone water. The three tetrathionate media (Cardiff formula Difco and Oxoid) used for enrichment after pre-enrichment, yielded approximately the same number of positive samples and about the same number of frozen and coded samples was found positive when data of all four laboratories were considered. Between laboratories considerable variation in results was found. This indicates the necessity to conduct comparative trials in more than one laboratory to validate the use of a specific method for regulatory purposes.

van Schothorst (1979) discussed the sources of variation in Salmonella isolation procedures. Useful observations were made on sample treatment, inoculation, pre-enrichment, enrichment and plating. He drew the attention of other workers on the pH of the media during inocubation when using buffered peptone water (BPw) for pre-enrichment. When using tetrathionate broth as an enrichment medium, the temperature during incubation (43^{o}C) is also very critical. When this

temperature is too low many competitive organisms may grow and when it is too high salmonellae may not multiply sufficiently or may even die.

Harvey and Price (1979) reviewed the principles of Salmonella isolation. They stated that it would be hazardous and arrogant to describe an optimum method but evidence is increasing that a standardized technique can be evolved allowing results obtained in one country to be compared with those obtained in another. Many bacteriologists are in agreement on the main principles of a suitable cultural technique, although their methods differ in detail. For instance, there is a wide acceptance of the value of pre-enrichment, elevated temperature incubation and the use of some form of brilliant green agar as a selective medium.

Several workers studied the advantages and disadvantages of the method developed at an earlier date using incubation temperatures above 37°C for enrichment media. Some commercial media are too inhibitory for use at 43°C but can function well at slightly lower temperatures. The success of elevated temperature incubation is also influenced by the nature of the material examined (i.e. the quality of the inoculum). Harvey et al., (1975) noted the importance of the inoculum in quality control tests of enrichment media and showed that pure culture techniques could not always differentiate between

good and bad enrichment broths, while inocula of sewage polluted water readily demonstrated differences. Elevated temperature incubation has been effectively used by Pateraki et al., (1975) for pre-enrichment in heart infusion broth before enrichment in a modification of the medium described by Rappaport et al., (1956). The temperature used for pre-enrichment was 43°C, and 37°C, for enrichment, as difficulties have been encountered in using Rappaport's enrichment broth at temperatures above 37°C. Chau and Huang (1976) also used incubation temperatures above 37°C to increase the specificity of their selective motility technique for rapid diagnosis of salmonellosis in clinical specimens. They found a temperature of 41°C to be optimum.

The consensus of opinion appears to favour elevated temperature enrichment as long as the temperature selected is matched with the cultural system used by the microbiologist. If the system is in any way inhibitory to multiplication of salmonellae the technique will not work. One of the outstanding advantages of the method is the greater purity of Salmonella growth obtained on plating media.

The success of elevated temperature incubation for selective media is rather dubious. According to the experience of the authors of the literature under review, incubating selective agars at temperatures above 37°C using brilliant agreen MacConkey agar (Wilson and Darling 1918; Wilson and Blair 1931;

Harvey, 1956 quoted by the authors) indicated that developing Salmonella colonies were reduced in size and atypical in their appearance. This interfered with colony differentiation and screening by slide agglutination.

As regards subculturing from enrichment broth, the authors have studied multiple subculture at a single time after incubation and after different times of incubation. Salmonellae are not invariably distributed uniformly in enrichment cultures and that several subcultures, all made at the same time, may be necessary to demonstrate them.

Loeffler (1906, quoted by Harvey and Price 1979) calculated that at least 18 hrs incubation of a fluid medium inoculated with 1-2 cells of S.typhi was necessary to ensure that a loopful of the culture contained the organism. Since then, the importance of subculture timing from fluid media has been increasingly recognized. McCoy (1962, also quoted by Harvey and Price, 1979) demonstrated that the time of appearance of salmonellae in platings from enrichment media was related to the numbers initially present at incubation. If the number of salmonellae were small in the original inoculum, subculture at a time later than 24 hrs might be required to demonstrate them. The inhibitory nature of the enrichment medium is also relevant. The method of multiple subculture has been found valuable by many other workers. The authors quoted Grunnet (1975) who found that there

was no advantage in using enrichment periods shorter than 24 hrs. At 24 hrs 60% of his samples were positive. Subculture at 48, 72, and 96 hrs increased the isolations by 23%, 11% and 6%. The gain obtained in extending the period of enrichment beyond 4 days proved to be minimal (Ca.2%).

Harvey and Price (1979) further discussed the quality and size of inoculum and stated that the quality and size of an inoculum containing salmonellae involves both the Salmonella content and the vehicle in which the organisms are suspended. If the organisms have recently left the human or animal intestine, they should be easily recovered whatever method is used. If, on the other hand, the organisms are sublethally injured by heat treatment, drying, irradiation, survival in sewage-polluted fresh water or salt water or by exposure to other adverse circumstances, they may fail to multiply if inoculated directly into the relatively hostile environment of an enrichment medium. This is particularly true if the enrichment medium is incubated at a temperature above 37^{o}C.

In general, increasing the size of the inoculum introduced into an enrichment medium increases the number of positive isolations. There is,however, a danger in this practice: overloading an enrichment broth with large inocula of material can decrease the selectivity of the medium and the authors also believe it can alter the time of a successful culture.

The authors further noted that in practice, isolation is probably best made by a combination of unselective culture and enrichment culture. This is called two-stage enrichment (Grunnet 1975) or pre-enrichment. Material is inoculated into quarter-strength Ringer's solution, buffered peptone water, nutrient broth or lactose broth and incubated at a suitable temperature (usually 37^{o}C) for 2-18 hrs and finally subcultured into an enrichment medium. This is incubated at 37 or 43^{o}C, according to preference, and subcultured at 24 and 48 hrs on to a selective agar.

Concerning enrichment media, the authors accepted that enrichment broths are a necessary part of Salmonella isolation from all types of samples and discussed the main types of enrichment media available. The authors observations on clinical specimens indicate that selenite is as good as Muller-Kauffmann tetrathionate for Salmonella diagnosis if the faecal samples are fresh. If, however, the samples are stored the tetrathionate broth is significantly better than selenite. This means that an enrichment medium used in food and water bacteriology is also very suitable for medical diagnosis if the isolation of S.typhi and Shigella sonnei is of secondary importance. As the incidence of the former is very low and the latter is adequately cultured by direct plating the choice of tetrathionate for clinical diagnosis may be sound.

For selective plating media, the authors stated that the choice of an optimum selective agar is made on common sense grounds.

If it is important to have a medium capable of growing Shigella,desoxycholate citrate, Salmonella-Shigella,Hektoen enteric agar or Xylose lysine desoxycholate (XLD) agar must be used. If the bacteriologist is only interested in non-fastidious salmonellae, a brilliant green agar would be suitable. If isolation of S.typhi or sub-genus III salmonellae is required, a bismuth sulphite agar would be mandatory. Obviously the geographical area in which the microbiologist is working must govern the choice.

Lastly, the authors discussed the quality control of methods. They observed that successful Salmonella isolation involves a combination of subjective and objective qualities. The former is concerned with recognizing the Salmonella colony. Surface appearance, translucency, ease of removal from a selective agar and ease of emulsification in saline determine selection of a suspicious colony for slide agglutination. Those criteria can only be learnt by experience and knowledge can easily be lost by lack of bench practice.

Objective qualities of media and method can be controlled to some extent. The authors test their media weekly by ensuring that they are able to grow small numbers of salmonellae.

Harvey et al., (1979) compared selenite F, Muller-Kauffmann tetrathionate and Rappaport's medium for the isolation of salmonellae from sewage polluted natural water using a pre-enrichment technique. The three media, prepared from single ingredients in the laboratory, were compared with their commercial equivalents. Laboratory prepared media were more efficient for isolating salmonellae from sewage polluted natural water samples. A pre-enrichment stage using buffered peptone water was employed throughout the investigation. The size of inoculum from the pre-enrichment medium was relevant to successful Salmonella isolation. Inocula studied were 1 ml and one loopful (3mm. diameter loop). The smaller inoculum gave better results with Rappaport, the larger with selenite and tetrathionate. Using the optimal inocula, Rappaport was the most efficient enrichment broth of the three fluid media in this study.

Andrews et al., (1979) in U.S.A., studied the relative productivity of brilliant green (BG), bismuth sulphite (BS), Salmonella-Shigella (SS), Hektoen enteric (HE) and xylose lysine desoxycholate (XLD) agars for recovering Salmonella from 9 food types. Following pre-enrichment, selective enrichment of food samples in tetrathionate broth followed by streaking to BS agar was the single most productive selective enrichment broth-agar combination for recovery of Salmonella from 5 of these food types.

A study of the performance of these 5 agars used individually and in various combinations showed that none of the 5 agars used individually nor any of the possible paired combinations of these agars could be used to satisfactorily detect Salmonella in the 9 food types. The use of all 5 agars was not necessary because one combination of 4 agars (BG, BS, HE and XLD) recovered 100% of the Salmonella isolates, as compared with the number of Salmonella isolates recovered by the 5 agar combination, in each food category. Finally, the relative cost of using these agars, singly and in various combinations, were determined.

Rigby and Pettit (1980) in Ontario, Canada, studied a delayed secondary enrichment for the isolation of salmonellae from broiler chickens and their environment. Specimens collected from six broiler flocks were cultured for salmonellae by three methods (i) for direct enrichment, the specimen was homogenized and 1 ml of homogenate was inoculated into tetrathionate brilliant green broth. (ii) for pre-enrichment liquid specimens and homogenates were incubated at 37°C, and on the next day 1 ml was inoculated into tetrathionate brilliant green broth; and (iii) for delayed secondary enrichment, incubated pre-enrichment cultures were held at room temperature for 7 to 10 days and then subcultured into fresh tetrathionate brilliant green broth. All tetrathionate brilliant green were incubated at 42°C for 24 to 48 hrs before plating.

The use of delayed secondary enrichment significantly improved the isolation of salmonellae from these specimens.

2.3 Serological identification of *Salmonella*

The serotyping of *Salmonella* is based on the identification of the antigenic determinants (factors) of the 3 main antigen groups, the H-antigens (flagellar), the O-antigens (somatic) and the Vi-antigens, as delineated in the Kauffmann-White scheme (see also Edwards and Ewing (1972).

The chemical basis for antigenic specificity in the Enterobacteriaceae has largely been clarified by the work of Westphal and his associates (Lüdenitz *et al*., 1973).

The most commonly used technique for serotyping *Salmonella* strains is the rapid slide agglutination test. A battery of antisera specific for single antigenic (factors) determinants is needed and the method is rather wasteful on antisera which are expensive and sometimes difficult to obtain. Recently, attempts have been made to serotype members of the Enterobacteriaceae using the staphylococcal coagglutination method of Kronvall (1973). The method is based on a unique characteristic of protein A of certain strains of *Staph.aureus* to combine strongly with the Fc part of immunoglobulin G (IgG). When specific antiserum is added to stabilized staphylococci the specific antibodies are adsorbed and become

oriented with their antigen-combining sites directed outwards. The co-agglutination method according to Kronvall (1973) will be discussed in detail later.

Christensen et al., (1973) described a new method for serological grouping of streptococci with specific antibodies adsorbed to protein A-containing staphylococci. The stabilization and coating staphylococci to make specific coagglutination reagent followed procedures described previously by Kronvall (1973).

Edwards and Hilderbrand (1976) described a method for identifying Salmonella and Shigella directly from the primary isolation plate by coagglutination of protein A - containing staphylococci sensitized with specific antibody. The preparation of protein A-containing staphylococci was carried out according to the method described by Kronvall (1973).

Svenungsson and Lindberg (1978) in Sweden, studied the identification of Salmonella bacteria by coagglutination, using antibodies against synthetic disaccharide protein antigens O_2, O_4 and O_9 and adsorbed to protein A containing staphylococci. The method was also compared with the standard slide agglutination test (SSA) and its sensitivity compared with that of the indirect immunofluorescence test (IFL). Preparation of protein A-containing staphylococci was performed essentially as described by Kronvall (1973). Salmonella O factor sera for SSA tests were prepared according to Kauffmann (1966).

As compared to standard slide agglutination with conventional anti-Salmonella O factor, the coagglutination method was favourable in that the reactions were stronger, although the concentration of antiserum used was from 20 to 200 times lower. The applicability of the coagglutination method for typing Salmonella bacteria was subsequently investigated by testing unknown enteric bacteria from faecal samples. The results were excellent and no false positive reactions were observed.

Danielsson et al.,(1979) described the rapid typing E.coli K1 bacteria using the coagglutination technique. Protein A-containing staphylococci were produced and stabilized as described by Kronvall (1973).

The coagglutination technique was more rapid and gave more clearcut reactions than direct bacterial agglutination. It was also much more economical than all the other techniques since 10 ml reagent staphylococci, sufficient for 150 tests, could be made up from 0.1 ml adsorbed antiserum.

Sanborn et al., (1980) studied an enrichment culture coagglutination test for rapid, low-cost diagnosis of salmonellosis in U.S.A. Specific diagnosis of salmonellosis by conventional culture and identification methods usually requires 2 to 4 days. Since Salmonella may be disseminated from infected individuals during this period, the time required for diagnosis may be too long to aid in epidemic control. To obtain earlier diagnosis of

salmonellosis, a coagglutination test was used for rapid, simplified detection of S. oranienburg antigens in enrichment broth cultures of faecal specimens from infants involved in a nursery outbreak.

The coagglutination (COAG) reagents were prepared according to the method described by Kronvall (1973). The authors found that enrichment COAG yielded a diagnosis in one-third of the time required for conventional culture. Estimated costs for tests by selective enrichment-COAG test were much lower than by conventional methods. The enrichment-COAG method described appeared to have potential as a useful clinical and public health diagnostic tool.

3. MATERIALS AND METHODS

3.1. Collection of Materials:

Information on the number and serotypes of Salmonella isolated in Kenyatta National Hospital during the period 1974 - 1979 was obtained through the courtesy of Professor H. Nsanze, Chairman of the Department of Microbiology, Faculty of Medicine.

Permission to get additional information from the National Public Health Laboratory Service was granted by the acting Director, Dr. G.L. Timms.

Similar data on Salmonella isolations were also collected from the Veterinary Research Laboratories, Kabete with the kind permission of the Director of Veterinary Services.

3.1.1. Cattle samples

3.1.1.1. Faecal samples

One hundred and thirty-three faecal samples were collected from cattle slaughtered at the Kenya Meat Commission (KMC), Athi River plant and the abattoirs at Ongata Rongai and Banana Hill. An incision was made in the caecal area and approximately 10gm. of faeces were scooped into specimen cups.

Faecal samples from live cattle were obtained

from the Veterinary Investigation Laboratory (V.I.L.) Karatina.

Our study coincided with a project on parasites of cattle conducted by the V.I.L.

Two hundred and sixty seven faecal samples were collected from individual animals as they arrived at the dip. The specimens were kept at $4^{o}C$ until they were processed for Salmonella isolation. Generally, the samples were processed the same or the following day.

3.1.1.2. Lymph node samples

Mesenteric lymph nodes were collected from 175 bovine carcasses at three slaughterhouses: KMC Athi River, Ongata Rongai and Banana Hill.

3.1.1.3 Bile samples

Twenty bile samples were collected from cattle slaughtered at KMC abattoir, Athi River.

3.1.1.4 Carcass swabs

Ten bovine carcasses were swabbed at different areas and samples taken into sterile universal bottles. The swabs were taken at Dagoretti and Nyonjoro slaughterhouses.

3.1.2. Small stock samples

3.1.2.1. Faecal samples

Twenty-seven faecal samples from sheep and 19 from goat intestines were collected (coecal contents)

at the time of evisceration at Ongata Rongai slaughterhouse.

3.1.2.2. Lymph node samples

Mesenteric lymph nodes were collected in specimen cups from 18 sheep and 5 goats at Ongata Rongai and Banana Hill slaughterhouses.

3.1.2.3. Bile samples

Bile samples were collected from 16 sheep and 14 goats slaughtered at Ongata Rongai abattoir.

3.1.3. Pig samples

3.1.3.1. Faecal samples

Sixty-one faecal samples were collected from Uplands Bacon Factory. Of these 22 were from live pigs in the factory pig unit while 39 were from the slaughter pigs. Ten faecal samples were obtained from Kenya Super Butchery at the Ruaraka Pig slaughterhouse. The samples from slaughtered animals were obtained from the caecum. Seventeen faecal samples were collected from live pigs in different pens at Bahati Duck Farm.

3.1.3.2 Lymph node samples

Mesenteric lymph nodes were collected in sterile plastic cups from 30 slaughter pigs at Uplands Bacon Factory and Kenya Super Butchery, Ruaraka.

3.1.3.3. Bile samples

Ninety-five samples were collected from slaughter pigs at Uplands Bacon Factory. Sterile universal bottles were used for collection.

3.1.4 Rodent samples

Seventy-nine samples of rat faeces were collected from different parts of the building of the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine. Samples consisting of intestinal contents were collected from 370 rodents caught mainly by the Nairobi City Council rodent eradication team in different areas of the City. A small number of those rodents were trapped in the buildings at Kabete Campus and brought by workers in our Department. A small reward was given for every rodent trapped and brought alive to our laboratory. The predominant rodent species from which the samples were obtained was Rattus rattus but few Arvicanthus and Otomys were also included. Sterile universal bottles containing nutrient broth were used for the collection of rodent samples.

3.1.5. Duck samples

Twenty-one faecal samples were collected from ducks of different ages at Bahati Duck Farm.

3.1.6. Abattoir effluents

One hundred and seven samples of abattoir effluents were collected into sterile universal

bottles. The samples were from drains of the following slaughterhouses: KMC - Athi River, Ongata Rongai, Banana Hill and Bahati Duck Farm.

3.1.7. Nairobi Sewage

Twenty sewage samples were taken from Kariobangi Sewage Treatment Plant at the inlet point. Sterile half-litre bottles were used and sewage was collected in each of them hourly during day-time. Samples were kept at 4°C up to the time of processing which was generally done after two days.

3.2 Media and Reagents

Several media were used: Tetrathionate, Selenite and Nutrient broths, Dextrose and Tryptone soya broths, Nutrient agar, Triple Sugar Iron, Urea agar base, Desoxycholate citrate and Brilliant green agars. In addition, phosphate buffered saline (PBS) and physiological saline were prepared for use in agglutination and coagglutination processes. The following tests were performed: decarboxylase reactions: Lysine and Ornithine, Beta galactosidase (ONPG); utilization of Malonate; fermentation of Glucose, Dulcitol, Lactose, Mannitol, Salicin, Sucrose and production of Indole. A set of Salmonella antisera from Hoechst Aktiengesellschaft Frankfurt, Germany, were used for both direct slide agglutination test and the coagglutination method.

3.3. Stock cultures

Stock cultures of Staphylococcus aureus, strain

Cowan I on nutrient agar slopes were used for the preparation of the typing reagent for coagglutination technique (COA).

Staph. aureus strain Cowan I was grown overnight at 37°C in nutrient broth.

The broth culture was inoculated on nutrient agar in Roux bottles to have a larger surface area for growth. Part of the broth culture was inoculated into Tryptone Soya broth (Ox.CM 129) in one litre screw-capped bottles. Both Roux and screw-capped bottles were incubated overnight at 37°C.

Staphylococcus cultures were harvested from the Roux bottles using sterile glass beads and sterile PBS, and poured into sterile centrifuge tubes. Broth cultures in screw-capped bottles were directly placed in centrifuge tubes.

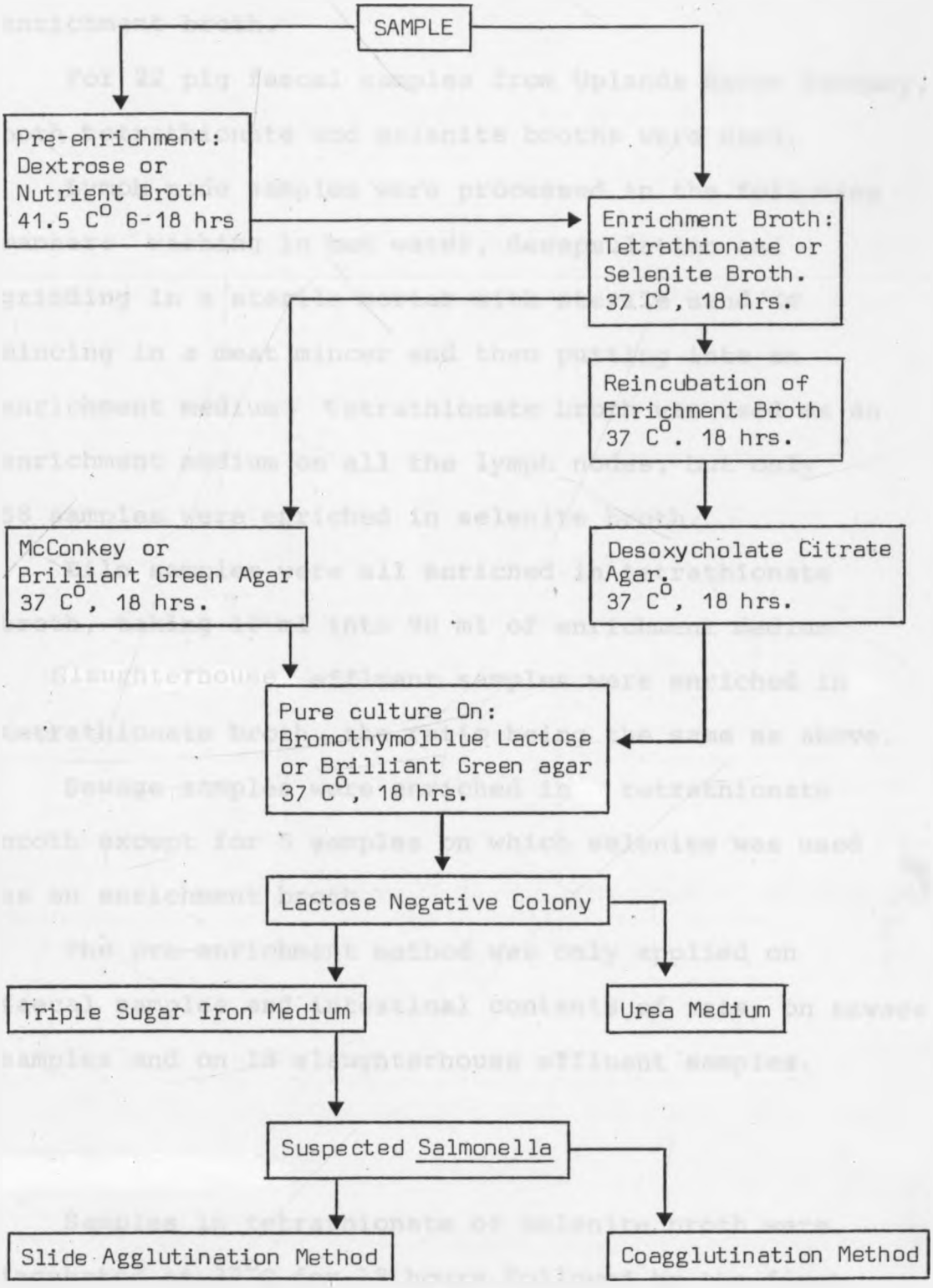
The culture suspension was centrifuged in refrigerated centrifuge at 3000xg at 4°C for 15 min. The supernatant was discarded and the pellet resuspended in PBS. The washing procedure was repeated three times. The pellet obtained after the last wash was resuspended in a 0.5% formaldehyde and the solution was kept at room temperature for 3 hours. The suspension was centrifuged in a

refrigerated centrifuge at 3000xg at 4°C. The supernatant was discarded and the washing procedure repeated three times. The pellet obtained after the last wash was resuspended to 10% (v/v) in PBS and the suspension heated to +80°C for 10 min. The washing procedure was repeated twice, and finally the bacteria were resuspended to a 10% (v/v) suspension in PBS - Sodium azide was added to a final concentration of 0.1% and the stabilized suspension stored at +4°C up to the time it was used.

3.4 Processing of specimens

In the first phase of the investigation, samples were processed only through enrichment in tetrathionate or selenite broth before plating. A third of the samples, however, were pre-enriched in nutrient or dextrose broth at 41.5°C for several hours before enrichment in tetrathionate or selenite broth. After enrichment, the specimens were plated on selective differential media, McConkey, desoxycholate citrate and brilliant green agars. Lactose-negative colonies were streaked on triple sugar iron medium (TSI) and urea medium. Cultures giving reactions compatible with *Salmonella* on TSI and urea medium were typed serologically using both the standard slide agglutination method as well as the staphylococcus coagglutination method. A composite scheme for the isolation and identification of *Salmonella* is given in Fig. 1.

Fig. 1 SCHEME FOR THE ISOLATION AND IDENTIFICATION OF SALMONELLA.



Most of the samples were enriched in tetrathionate broth. Only 103 were enriched in selenite broth. The ratio of enrichment was 10 gm of faeces to 90 ml of enrichment broth.

For 22 pig faecal samples from Uplands Bacon Factory, both tetrathionate and selenite broths were used.

Lymph node samples were processed in the following manner: washing in hot water, decapsulation and grinding in a sterile mortar with sterile sand or mincing in a meat mincer and then putting into an enrichment medium, tetrathionate broth was used as an enrichment medium on all the lymph nodes, but only 58 samples were enriched in selenite broth.

Bile samples were all enriched in tetrathionate broth, taking 10 ml into 90 ml of enrichment medium.

Slaughterhouse effluent samples were enriched in tetrathionate broth, the ratio being the same as above.

Sewage samples were enriched in tetrathionate broth except for 5 samples on which selenite was used as an enrichment broth.

The pre-enrichment method was only applied on faecal samples and intestinal contents of rats, on sewage samples and on 18 slaughterhouse effluent samples.

Samples in tetrathionate or selenite broth were incubated at 37°C for 18 hours followed by the first plating on solid medium and then reincubated for another 18 hrs at 37°C before the last plating.

Samples in the pre-enrichment media were incubated at 41.5°C for 6 to 18 hrs before putting them into tetrathionate broth as an enrichment medium and the latter incubated at 37°C for 18 hrs.

After 18 hours incubation of the sample in tetrathionate or selenite broths at 37°C, the first streaking was done on McConkey agar or in a few cases, on Brilliant green agar and incubated overnight at 37°C.

Colonies resembling Salmonella were subcultured on bromothymol blue lactose agar or brilliant green to obtain pure cultures. The plates were incubated overnight at 37°C.

At the same time the enrichment broth was reincubated for another 18 hours at 37°C.

Colonies on brilliant green or bromothymol blue lactose agar that resembled Salmonella were inoculated onto triple sugar iron (TSI) and urea slant.

The second plating from the reincubated enrichment broth was carried out on desoxycholate citrate agar and incubated overnight at 37°C. Colonies with Salmonella appearance were streaked on brilliant green or bromothymol blue lactose agar and from there onto triple sugar iron and urea slant.

Colonies with reactions compatible with Salmonella on triple sugar iron and which did not split urea were typed serologically.

Direct slide agglutination was carried out in parallel with the coagglutination method. The slide agglutination method followed the procedure for preliminary and final identification of Salmonella as described by Edwards and Ewing (1972). The coagglutination test was performed according to the method described by Kronvall (1973). The method is briefly as follows:

To each 1 ml of 10% Staph. aureus suspension 0.1ml of Salmonella antiserum was added.

It was allowed to react at room temperature for 2 hours under continuous gentle agitation. Thereafter, it was washed twice with PBS in a refrigerated centrifuge and resuspended in PBS to 1%.

The coagglutination test was performed by placing a drop of the above reagent on a slide near a drop of the culture suspension to be tested and mixing the two with a wire loop.

Isolates showing biochemical reactions compatible with Salmonella, but giving negative reactions with "O" polyvalent antisera were suspended in sterile physiological saline and heated in a boiling water bath for 20 min to destroy K antigens which might inhibit the reaction with "O" antisera.

Organisms agglutinating with Salmonella "O"

antiserum were analysed further for their antigens using the appropriate Salmonella "H" antisera.

For some the reversal of phase was necessary in typing and it was done according to the Craigie tube method (Baker et al., 1980). It consists of melting 0.5% nutrient agar in a Craigie tube and cooling at 45°C. A few drops of composite phase 1 and 2 serum according to which of the two is to be reversed were added to the medium, both inside and outside the tube. After mixing well and allowing to set, the culture was inoculated overnight at 37°C.

The organisms growing on the surface of the outer medium were then subcultured onto nutrient agar plate and incubated overnight at 37°C. The slide agglutination test and the coagglutination method were then applied as described earlier.

The isolates showing Salmonella characteristics on urea and triple sugar iron were not typed immediately from the latter medium. They were stored on nutrient agar slant and kept at 4°C until all the antisera were obtained. In addition each isolate was kept in cooked meat medium.

For serotyping, the isolates were returned to nutrient agar plates or triple sugar iron tubes. It was, in some cases, necessary to passage the strains through nutrient broth and then back to a solid medium, nutrient agar or triple sugar iron, before proceeding with serotyping.

4. RESULTS

Table 1 shows the most prevalent serotypes isolated from stools of patients at Kenyatta National Hospital during a 6-year period. The most prevalent serotype was S.typhimurium, followed by S.enteritidis and S.typhi.

The other Salmonella species comprise a wide range of serotypes with S.oranienburg coming fourth on the list and S. havana which appeared in 1974 and 1975 with a high number of isolates but was not isolated during 1976-1979. Other serotypes appeared rather infrequently. These include S.senftenberg, S. agona, S.infantis, S.paratyphi A, B, and C, S.agbeni, S.braenderup, S.eastbourne, S.heidelberg, S.alagbon, S.gloucester, S.wil, S.virchow, S.adelaide, S. shubra, S.chailey, S. nigeria, S.entebbe, S.schwarzengrund, S.amager, S. montevideo, S.pomona, S.mission S.richmond, S.larochelle, S.mendoza, S.concord, S.colindale, S.miami, S.salmonella, S.derby, S.kiel, S.duisburg, S.worthington, S.cerro, S.birkenhead, S.garoli, S.thomasville, S.gombe, S.bristol, S.blockley, S.tshiongwe, S.essen, S.muenchen, S.tamale, S.saint paul, S.chailey, S.corvallis, and S.manchester.

Salmonella has also been isolated from blood and cerebro-spinal fluid (CSF) of patients at Kenyatta

National Hospital. The main serotypes involved were S.typhimurium, S.typhi, S.enteritidis, S.oranienburg, S.paratyphi C, S.senftenberg and S.braenderup.

At the National Public Health Laboratory, the records showed few Salmonella isolations during the period 1974-1979. This is explained by the fact that the laboratory deals mainly with specimens sent by institutions or individuals in case of outbreaks or samples of water or food occasionally sent for bacteriological investigations. The few Salmonella serotypes isolated comprise S.typhi, S.paratyphi C., S.enteritidis, S.amherstiana and S.group D.

At the Veterinary Research Laboratories Kabete some isolations of Salmonella serotypes from various types of materials received for diagnosis were recorded as follows: During the period 1974-1979, 10 strains of S.typhimurium were isolated from the following sources: a buffalo calf, a colobus monkey, a bush baby, rabbit and mice. Four isolates of S.enteritidis were recovered from cultures from cattle and cooked meat origin. In addition S.heidelberg was isolated from calf blood, S.group C from mesenteric lymph nodes of a dik-dik.

S. dublin from culture of bovine origin and S.derby from culture of unknown origin.

Table 2 shows the results of all samples analysed in this study. Forty nine strains of Salmonella were isolated from a total of 1515 samples, giving an average carrier rate of 3.23%. The highest number of

samples (606) were from cattle. Seven of these were positive for Salmonella, giving a carrier rate of 1.1% Rodent samples (449) gave a carrier rate of 7.3%. Out of 213 samples from pigs, 4 (1.9%) were positive. The 61 samples from sheep and 38 samples from goats did not yield any Salmonella.

About 14.3% of 21 faecal samples from ducks were positive. Of the 107 abattoir effluent samples examined, two (1.9%) were positive, while 20 samples from raw sewage were negative.

Table 3 lists the 32 serotypes isolated according to sources. S. enteritidis was the predominant species with 11 isolates. Nine of these were from faeces or intestinal contents of rodents. The only two strains of S. typhimurium were isolated from rodents. The other strains with two isolates each were: S. agona, S. amersfoort, S. kapemba, S. ridge, S. rhodesiense and S. tarshyne. The remaining strains had one isolate each.

The coagglutination method of Kronvall gave results which were in complete agreement with the rapid slide agglutination test.

5. DISCUSSION

The carrier rates found in animals in this study: 7.3% in rodents, 1.9% in pigs and 1.1% in cattle, are fairly similar to those recorded worldwide as summarized in the literature review.

The presence of Salmonella in rodents has attracted the attention of several investigators who

have found a wide range of carrier rates. Buxton (1957) in his review of salmonellosis in animals, pointed out that numerous surveys in wild rats and mice carried out in different countries have shown a carrier rate ranging from 1.5 to 13.6%. Wilson and Miles, in Topley and Wilson (1964), observed that in unselected rats the carrier rates varied between 4 and 7%.

Steele (1969) stated that surveys of rat populations have indicated incidences from 0.7% to 13%. Some workers have found carrier rates similar to those in this study (Meyer and Matsumura 1927, quoted by Collard et al., 1957; Khalil 1938, Zwart 1962 and Singh, 1980).

While a carrier rate as low as 0.4% was found in rats in Japan (Hatta, 1939 cited by Collard et al., 1957), most investigators have recorded rates ranging from 1.2 to 13.6% (Kerrin, 1928, Khalil, 1938, Verder (1927), quoted by Khalil, 1938; Ghosal, 1941; Welch et al., 1941 and Li and Davis, 1952; both quoted by Collard et al., 1957; Ludlam, 1954; Lee, 1955; Brown et al., 1957, Collard et al., 1957, and Schnurrenberger et al., 1968).

However, some investigators have recorded an unusually high carrier rate ranging from 20-40% in rats trapped in slaughterhouses, around meat factories and near poultry or mink farms (Ludlam, 1954; Guinée et al., 1963; Robinson and Daniel 1968; Goyal and Singh 1970).

Abd El Ghani (1977) who found a carrier rate of 19.71% in wild rats trapped from different localities near Cairo (Egypt), observed that the conditions and climate prevailing in the places where these wild rats were trapped may have affected the incidence of Salmonella among rodents. He found that S.enteritidis followed S.typhimurium as the most prevalent serotype isolated from wild rats in Egypt.

It appears, therefore, that the Salmonella carrier rate in rats is influenced by the environment in which those species live and obtain their food.

Slaughterhouse environs seem to be highly contaminated with Salmonella organisms, hence the high carrier rate in rodents trapped in those areas.

In the present study, S.enteritidis appears to be a common serotype in rats. This has been demonstrated earlier by several workers (Bainbridge, 1912, quoted by Collard et al., 1957, Savage and Read, 1913-14 and Savage and White, 1922 both quoted by Khalil, 1938; Kerrin, 1928; Khalil, 1938; Brown et al., 1957). All these workers agree that S.enteritidis is commonly found in wild rats. Wilson and Miles (1964) observed that British rats seem to be endemically infected with S.enteritidis var danysz, and possibly also with S.typhimurium and S.newport but may pick up sporadic infections with many other serotypes from their food.

In U.S.A., Verder (1927) quoted by Khalil (1938) isolated 5 strains of S.enteritidis and Meyer and Matsumura (1927) quoted by Collard et al., (1957) isolated an almost equal number of S.typhimurium and S.enteritidis from rats.

In Ceylon, McGaughey et al., (1954) quoted by Collard et al., (1957), and Ghosal (1941) in India both confirmed that S.enteritidis is a very common serotype in rats. However, some investigators did not find S.enteritidis or S.typhimurium in rats but found other serotypes more prevalent than these two (Collard et al., 1957. Atkinson et al., 1953 quoted by Collard et al., 1957; Lee, 1955, Zwart, 1962, Schnurrenberger, 1968; Goyal and Singh, 1970).

It appears that there are few recent references on Salmonella infection in rats. We have noticed that after 1970 the literature on Salmonella investigation in rats is especially scarce.

The carrier rate of 1.1% found in cattle in the present study is similar to that found by Plowright (1957) in adult cattle (1%) in West Africa and also to that found by Gitter and Brand (1970) in calves (1%) at the Uplands (Kenya) slaughterhouse. It is slightly lower than that found by Khan (1970) in cattle (1.6%) in the Sudan and also that found by Dixon (1964) in thirty two abattoirs in U.K. (1.9%).

Other investigators have reported higher carrier rates in cattle, namely; Botswana: 6.7% (Miller, 1971), Senegal: 4.89% (Doutre and Cartel, 1979), Tanzania: 6.16% (Hummel, 1974), Somali Democratic Republic: 5.6% (Andreani et al., 1978) and Del Baglivi et al., 1979), Uruguay: 4.2% (Collard and Sen, 1966), Ibadan - Nigeria: 5.5% and Daleel and Frost (1967) in Australia: 11.6%.

On the other hand, Guinée et al. (1964) observed that the prevalence of Salmonella infections in healthy cows was very low (0.8%) in the Netherlands.

Stewart (1967) found a carrier rate as low as 0.1% in slaughter cattle in U.K. Hummel (1974) found a carrier rate of 0.38% in cattle in Tanzania and Richardson (1968) mentions a carrier rate of 0.4% found in cattle and sheep in South Africa.

Gitter and Brand (1970) failed to isolate Salmonella organisms from 455 steers slaughtered at Nakuru abattoir in Kenya.

Mahlan (1965) quoted by Hummel (1974), investigated Salmonella in cattle in Tanzania and the 1271 heads he examined showed no evidence of either Salmonella infections or carriers.

Meara (1973) mentions in his review of salmonellosis

in slaughter animals, 8000 cattle slaughtered at the New Port Elizabeth abattoir - South Africa, which were negative for Salmonella. Gospar and Hrabeta (1977) working in Zambia, failed to isolate Salmonella from 147 rectal swabs from adult cattle and Mered et al., (1980) in Algeria, were not able to isolate Salmonella from 282 slaughter cattle. Smith (1959) failed to isolate salmonellae from 200 cattle in Essex - England.

Morgan (1969) quoted by Hummel (1974), examined 675 faecal samples from 5 Costa Rican abattoirs with negative results. Wray and Sojka (1977) reviewed bovine salmonellosis and stated that surveys in various countries have shown that in adult cattle the incidence of Salmonella infection varied from 0.3 to 11.6% and in calves 4.3 - 14.3%.

Stress due to transportation and cross-infection due to the stay in the lairages may influence the carrier rates. Anderson et al., (1961) found an increase in Salmonella infection in calves corresponding to an increase in holding time in the lairages.

It is noteworthy that no isolations were made from specimens from sheep and goats in this study. This is, however, in conformity with findings by other investigators. Ojo (1974) found no Salmonella in 375 specimens obtained from goats at the slaughter slab in Kano - Nigeria. He quoted Johnson (1958) who did not isolate Salmonella from organs of over 300 goats and Macadan (1958) cited by Johnson (1958) who also failed to isolate Salmonella from the bowel contents of 40 sheep and 90

goats. Smith (1959) did not isolate any Salmonella from 100 sheep in Essex, England.

Meara (1973) stated that no Salmonella was isolated from 50,000 sheep slaughtered at the new Port Elizabeth abattoir in South Africa.

Chambers (1977) held the view that sheep do not serve as important reservoirs of Salmonella in Rhodesia.

Those investigators who have obtained isolates of Salmonella from sheep and goats agree that the carrier rate is generally low. Khan (1970) found 3.77% in sheep and 1% in goats in the Sudan. Doutre and Boche (1976), working in Sénégal, found carrier rates of 4.7% in sheep and 3.6% in goats. Kumar et al (1973) in India, found carrier rates of 3.1% in sheep and 3.8% in goats. Andreani et al., (1978) found 5% sheep and 2% goats positive in Somali Democratic Republic. Kane (1979) found 4.7% carrier rate in sheep in New-Zealand while Singh et al., (1979) found 2% in sheep and 1.9% in goats in India. Mered et al., (1980) in Algeria, found a carrier rates of 2.2% in sheep and 0.25% in goats. It is interesting to note that El-Nawawi et al. (1980), working in Egypt, obtained results indicating that camels had the highest Salmonella carrier rate (33.3%), followed by pigs (26.2%) while buffaloes and sheep were rarely affected.

The carrier rate of 1.87% found in pigs in the present investigation appears to be below the average found in other countries which may be around 7%.

However, the carrier rate we found is somewhat higher than that found by Chambers (1977) in Rhodesia (0.8%), but fairly similar to that found in Britain (1.47%) by Heard et al., (1969). It is also close to that found in Uruguay (2.2%) (Hormaeche and Salsamendi, 1936) quoted by Buxton (1957) and not far from that found in Kenya (3%) by Gitter and Brand (1970) and 3% found in Denmark by Skovgaard and Nielsen (1972).

A carrier rate in pigs above the average referred to above has been found in some countries.

In Indonesia, for example, 16.7% of pigs were found positive (Kraneveld et al., 1951 quoted by Buxton 1957). Sen and Collard (1957) found a 16% carrier rate in Nigeria. In Australia, a carrier rate of 27% was found by Riley (1970) in slaughter pigs.

In Netherlands, the range of carrier rates reported in pigs has been quite wide. Clarenburg et al., (1949) quoted by Buxton (1957), found 2.8% of pigs positive but Kampelmacher (1963) found a carrier rate of 30.1%.

In Canada, Groves et al., (1971) found a carrier rate of 20.3% in market swine examined in 5 abattoirs over a 9 month period. Chambron et al., (1971), found a carrier rate of 19% from healthy pigs slaughtered in Dakar, Sénégal. El-Nawawi et al., (1980) recorded a carrier rate of 26% in pigs slaughtered in Cairo, Egypt.

In the U.S.A. several workers reported the incidence of Salmonella infection in pigs as being in the range of 5 and 13% (Hansen et al. 1964; Williams and Newell, 1970;

McKinley et al., 1980). Salmonellae were found in 12% mesenteric lymph nodes of pigs slaughtered in Athens (Pateraki et al., 1966). Smith (1959) reported a carrier rate of 12% in pigs in U.K., but Lee et al., (1972) found 3% and 18% from two farms they investigated.

Timoney (1970) reported a 10.9% incidence of Salmonella infection in Irish pigs at slaughter.

In Australia, Chung and Frost (1969) reported 8.4% carrier rate of Salmonella in pigs at slaughter but Riley (1970) found that 27% of the pigs examined yielded Salmonella.

The occurrence of Salmonella infection in pigs at slaughter was found to be 20.3% in Ontario, Canada (Groves et al., 1971).

These investigations have illustrated the variable but relatively high incidence of Salmonella in pigs throughout the world. This wide range of carrier rates is attributed by some authors to the environment in which the pigs were kept before slaughter. Sojka and Gitter (1961) stated that it is well known that such an environment is often heavily contaminated with a wide range of Salmonella and that for reliable figures on carrier rates it would be necessary to examine pigs slaughtered away from a contaminated environment.

Other investigators have found that the stress of transport was important in the conversion of non-shedding carrier pigs into contaminators of the environment (Williams and Newell 1970; Kampelmacher

et al., 1963). The importance of cross-infection during transport or stay in lairages has been stressed by several other workers (McDonagh and Smith 1958 and Hansen et al., 1964). Chung and Frost (1969) observed that although cross-infection is the conventional explanation, investigations of longitudinal design (Williams and Newell, 1967) suggested that differences in Salmonella recovery rates on the farm and at slaughter described as "build-up" may be the result of physiological change as a result of stress.

In their study of the sources of Salmonella in market swine, Williams and Newell (1968) stated that, while their findings support the view that the build-up of salmonellae in pigs is by contact with contaminated environment, they indicate that the primary source of the contamination is most probably the Salmonella-excreting pig which has consumed contaminated feed ingredients on its farm of origin. If the great majority of pigs went to slaughter Salmonella-free they would not serve as a source of infection to other pigs being sent to slaughter.

The carrier rate in ducks in this study is strikingly high but since only few samples were collected from only one farm, little significance can be attached to the 14.3% carrier rate found in ducks.

Similarly the small number of samples from Nairobi sewage could most likely account for the absence of Salmonella isolates.

The two isolates of S.enteritidis and S.agona obtained from abattoir effluents compare favourably with the findings of Gitter and Brand (1970) who isolated two serotypes S.enteritidis var. jena and S. schwarzengrund from abattoir drains at Nakuru.

A wide range of Salmonella serotypes exists in the animal population in the Nairobi area and the vicinity. Although there is some overlap between serotypes found in animals and those in human patients (i.e. S.enteritidis, S.typhimurium, S.agona, S. heidelberg, S.eastbourne and S.alagbon), no definite conclusions can be drawn with regard to the epidemiological pattern or the zoonotic aspect of human salmonellosis, except perhaps in the case of S. enteritidis. This serotype was the second most common type isolated from stools of patients, and was the most frequent isolate from wild rodents.

The high carrier rate in these rodents and their tendency to invade houses, with subsequent contamination of foods or feeds, support the idea that wild rodents may play a major role in the epidemiology of both human and animal salmonellosis.

It is surprising to note the few isolations of S.typhimurium from animals, but interesting to note that the only two isolates were from rodents.

Although S.typhimurium is the commonest species worldwide, several investigators have failed to detect this serotype in animals Hughes (1954) quoted by Plowright (1957) isolated only S.dublin

from cattle in Accra, Ghana. Wiktor and van Oye (1955) failed to isolate S.typhimurium from slaughter cattle in Stanleyville (former Belgian Congo) but isolated one from pigs. Plowright (1957) and Johnson (1958),both working in Nigeria, could not isolate S.typhimurium from cattle. Khan (1970) was not able to isolate S.typhimurium from cattle, sheep and goats in the Sudan. The rare occurrence of S.typhimurium carriers in animals has been confirmed in this study.

In view of the frequent isolations of S.typhimurium from human patients and infrequent occurrences in the animal population in this study, one might suggest that human salmonellosis due to S.typhimurium is contracted,to a large extent, from human carriers. This concept is also supported by other observations in Kenya (Itotia et al., 1978; Wamola and Mirza, 1981), and elsewhere (Bokkenheuser and Richardson, 1959 and 1960; Richardson et al., 1968; Baine et al., 1973, Bull. Wld. Hlth. Org. 1980; Cortesi and Catellani, 1980).

A comparison was made between the rapid slide agglutination test for typing Salmonella and an adaptation of the coagglutination method developed by Kronvall (1973). There was complete agreement between the results of the two tests. However, the coagglutination method was more economical by a factor of about 100 due to the small amount of

reagents required. The experiences with the coagglutination in this study agree with those of Sanborn et al., (1980) who concluded that the coagglutination method appeared to have a potential as a useful clinical and public health diagnostic tool. The reagents prepared in our laboratory were found to be stable for at least 4 months when stored at 4°C.

6. CONCLUSION

Salmonella is a public health problem of considerable importance in Kenya, as elsewhere in the world. A wide range of Salmonella serotypes have been isolated from animals, particularly rodents, in the Nairobi area. Some of those serotypes are also found in human patients with clinical disease. S.enteritidis, in particular, was found to be the most prevalent serotype in rodents and it is also one of the commonest isolates in human patients. The findings indicate that rodents may serve as an important source of human salmonellosis due to S.enteritidis. Although it is too early at this stage to draw firm conclusions about the connection between Salmonella infections in animals and man in Kenya, it is clear from our findings that animals, especially rodents are a potential source of infection. Their exact role in the epidemiology of salmonellosis in this country remains to be elucidated.

Although salmonellosis has been known for almost a century, gaps in our knowledge still exist. Development and evaluation of control measures require baseline data showing the incidence of Salmonella and its main reservoirs. Studies should be conducted to increase understanding of the nature and occurrence of Salmonella infections in man and animals, the modes of transmission, the relative pathogenicity of different serotypes, including strain differences,

and to define more clearly the infective dose and factors that affect host susceptibility. A surveillance programme is an essential adjunct to any control programme designed to prevent or minimize salmonellosis. Surveillance is necessary to know the magnitude of the problem to indicate areas where investigation is necessary, and to measure the effectiveness of corrective measures.

To control salmonellosis in domestic animals will require radical and very expensive changes in management practices. It is therefore unreasonable to expect complete elimination of all Salmonella infections in the foreseeable future. However, a great deal of improvement could be made simply by adherence to well-known principles of disease control. Rodents and vermin control programmes, such as the one conducted by Nairobi City Council, should be encouraged in view of the high carrier rate generally found in rodents and also confirmed in this study.

To what extent human carriers may contribute to salmonellosis in animals is not entirely clear. They appear to be an important factor in the spread of Salmonella in general, and S.typhimurium in particular here in Kenya. Important corrective steps in attempt to reduce the spread of salmonellosis in a population must consist of nationwide education and training programmes with particular emphasis on an increased understanding of the disease and the

importance of proper environmental sanitation and personal hygiene.

In view of the many facets of the Salmonella problem it is unlikely that salmonellosis can ever be eradicated, but it is nevertheless mandatory to control the disease as far as possible within the framework of health priorities and financial constraints of the health services.

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APPENDIX I

TABLE 1:

ISOLATION OF SALMONELLA FROM STOOLS OF PATIENTS AT KENYATTA NATIONAL HOSPITAL

SEROTYPES	YEAR						TOTAL
	1974	1975	1976	1977	1978	1979	
S.typhi	11	8	9	19	10	6	63
S.typhimurium	75	101	118	106	49	70	519
S.enteritidis	12	8	5	29	5	11	70
Others	56	20	23	21	60	37	217
TOTAL	154	137	155	175	124	124	869

TABLE 2:

SALMONELLA POSITIVE SAMPLES, THEIR SOURCES AND CARRIER RATES

Source of Sample	Type of Sample	Number of Samples	Number positive for *Salmonella*	Percentage
Rodents	Faeces	79	8	10.0
	Intestinal contents	370	25	6.8
Cattle	Faeces	401	6	1.50
	Lymph Nodes	175	--	0
	Bile	20	1	5
	Carcase swabs	10	--	0
Pigs	Faeces	88	1	1.1
	Lymph Nodes	30	--	0
	Bile	95	3	3.2
Sheep	Faeces	27	--	0
	Lymph Nodes	18	--	0
	Bile	16	--	0
Goats	Faeces	19	--	0
	Lymph Nodes	5	--	0
	Bile	14	--	0
Ducks	Faeces	21	3	14.3
Abattoir	Effluent	107	2	1.9
Sewage Plant	Sewage (Nairobi)	20	--	0
	TOTAL	1515	49	av.3.2 %

SEROTYPES	TOTAL NO. OF ISOLATES	CATTLE			SHEEP & GOATS			PIGS			RODENTS		DUCKS	ABATTOIR	SEWAGE
		FAECES	LYMPH NODES	BILE	FAECES	LYMPH NODES	BILE	FAECES	LYMPH NODES	BILE	FAECES	INTESTINAL CONTENTS	FAECES	EFFLUENTS	NAIROBI PLANT
S.enteritidis	11	1										9			
S.typhimurium	2											2			
S.agona	2										1				
S. amersfoort	2											2			
S. kapemba	2											2			
S. ridge	2											2			
S. rhodesiense	2	1									1				
S. tarshyne	2	1										1			
S. koumra	1	1													
S. dublin	1									1					
S. heidelberg	1									1					
S. holcomb	1									1					
S. panama	1							1							
S. kuilsrivier	1			1											
S. dar-Es-Salaam	1												1		
S. gallinarum	1												1		
S. eastbourne	1												1		
S. reading	1										1				
S. caledon	1										1				
S. alagbon	1										1				
S. ruki	1										1				
S. hato	1											1			
S. limete	1											1			
S. bournemouth	1											1			
S. clairbornei	1											1			
S. daytona	1											1			
S. sarajane	1											1			
S. ljubljana	1	1													
S. othmarschen	1	1													
S. shamba	1										1				
S. ayinde	1											1			
S. blegdam	1										1				
POSITIVE/NO.EXAMINED	49/1515	6/401	0/175	1/20	0/46	0/23	0/30	1/88	0/30	3/95	8/79	25/370	3/21	2/1[illegible]7	0/20
PERCENTAGE POSITIVE	3.23	1.5		5				1.13		3.15	10	6.75	14.3	1.[illegible]6	/

TABLE 4:

SALMONELLA CARRIER RATE IN RATS FROM SEVERAL COUNTRIES

COUNTRY	AUTHOR	TOTAL NO. EXAMINED	CARRIER RATE
Australia	Lee (1955)	842	3.0%
Ceylon	McGaughey et al (1954)	254	3.5%
Ghana	* Zwart (1962)	-	8.6%
India	Ghosal (1941)	364	13.4%
	* Kaura & Singh (1968)	100	1%
	* Goyal & Singh (1970)	34	25.8%
	* Singh et al (1980)	254	6.3%
Japan	Hatta (1939)	1075	0.4%
Mexico City	Varela et al (1948)	1927	1.7%
Netherlands	*Guinée et al (1963)	237(slaughterhouses)	30.8%
		429 (Farms)	4.0%
		69 (Mink Farms)	17.4%
New Zealand	*Robinson & Daniel (1968)	42	35.7%
Nigeria	Collard et al (1957)	253	3.9%
U.K.	Kerrin (1928)	100	11.0%
	Khalil (1938)	750	7.3%
	Ludlam (1954)	518	4.4%
U.S.A.	Verder (quoted by Khalil, 1938)	114	5.2%
	Meyer & Matsumura (1927)	775	7.3%
	Welch et al., (1941)	420	1.2%
	Li and Davis (1952)	1382	4.2%
Kenya	Present investigation	449	7.3%

* Information obtained from sources other than Collard et al (1957).

TABLE 5:

SALMONELLA SEROTYPES IN RATS FROM SEVERAL COUNTRIES

COUNTRY	AUTHOR	PREVALENT SEROTYPES
Australia	Lee (1955)	S. adelaide, S.anatum, S. bovis morbificans, S.chester, S.meleagridis, S. oranienburg.
Ceylon	McGaughey et al. (1954)	S.enteritidis, S.typhi, S.virchow, S.weltevreden.
Ghana	Zwart (1962)	S. duisburg, S.poona, S.enteritidis.
India	Ghosal(1941)	S.enteritidis, S.typhimurium.
	Kaura & Singh (1968)	S. anatum.
	Goyal & Singh (1970)	S.anatum, S.stanley, S.virginia.
	Singh et al (1980)	S. saint paul, S.bareilly, S.newport, S.weltevreden, S.enteritidis.
Japan	Hatta (1939)	S.bareilly var. mikawasima, S.enteritidis, S.enteritidis var. danysz.
Mexico City	Varela et al (1948)	S.newport, S.pensacola, S.pensacola var anahuac.
Netherlands	Guinee et al (1963).	S.typhimurium, S.dublin, S.worthington S.livingstone, S.bredeney, S.heidelberg, S.muenchen, S.bovis morbificans, S.infantis, S.taksony.
New-Zealand	Robinson& Daniel (1968)	S.typhimurium, S.bovis morbificans.
U.K.	Kerrin (1928) Khalil(1938) Ludlam(1954)	S.bovis morbificans, S.enteritidis var. danysz, S.enteritidis var, Jena, S.newport, S.thompson, S.typhimurium.
U.S.A.	Verder(quoted by Khalil,1938), Meyer & Matsumura(1927), Welch et al (1941), Li & Davis(1952)	S.anatum, S.derby, S.enteritidis, S.newington, S.typhimurium, S.typhimurium var. copenhagen.
Kenya	Present investigation	S.enteritidis, S.typhimurium, S.amersfoort, S.kapemba, S.ridge, S.agona.