MULTIPLE TYPING OF *SALMONELLA LIVINGSTONE* BY BIOTYPING, ANTIMICROBIAL SUSCEPTIBILITY TESTING AND PLASMID PROFILE ANALYSIS

by

MAHACLA OMUNG'ALA ODONGO, B.V.M.

This dissertation was presented in partial fulfilment of the requirements for the award of the degree of Master of Science in Veterinary Microbiology.

Department of Microbiology

University of Surrey, U.K.

September, 1987.

A C K N O W L E D G E M E N T S

I would like to thank Dr C. Wray, Department of Bacteriology, Central Veterinary Laboratory, Weybridge for supplying me with the isolates of Salmonella Livingstone used in this study and for the valuable guidance during the project.

I am also indebted to Professor J. E. Smith, Department of Microbiology, University of Surrey, whose guidance was most invaluable; Dr Jagdish Vaid, a PhD student, in the same Department for his help in especially the computer work; Mr Ian McLaren of the Department of Bacteriology, Central Veterinary Laboratory Weybridge, UK, for his help in plasmid profile analysis and other laboratory work.

I also wish to thank the British Council without whose funds this project would have been impossible; The Directorate of personnel management and the Office of the President, Kenya, for granting me study leave and allowing me to proceed with these studies; and all my colleagues with whom we attended the course of Veterinary Microbiology.

Lastly, I wish to thank Mrs Walker for her personal sacrifice in making sure that this dissertation could be read by all.

D E D I C A T I O N

To my father Mr LIVINGSTONE ODONGO for his uncompromising stand on education, and to my darling wife, Mary and daughter, HARRIET for their patience and moral support during the entire period of my study.

SUMMARY

Seventy one isolates of Salmonella livingstone were analysed for homogenuity using biotyping, antimicrobial susceptibility testing and plasmid profile analysis.

Biotyping distinguished Salmonella livingstone isolates into 4 biogroups. Biogroup 1 (45%) and 2 (36.6%) were the most prevalent whereas biogroups 3 (9.9%) and 4 (8.5%) were rare.

Antimicrobial susceptibility testing revealed 6 antimicrobial resistant patterns with resistance to both streptomycin 10 ug and compound sulphonomide 50 ug (18.6%) and, to compound sulphonamide alone (18.6%) being the most common, followed by multiple resistance to streptomycin, compound sulphonamide and sulphafurazole (17.1%) and those resistance to none of the above drugs were 27.1 percent of the total.

All isolates were susceptible to ampicillin 10 ug (AMP), tetracycline 10 ug (TE), neomycin 10 ug (H), chloramphenicol 10 ug (C), co-trimoxazole 25 ug (SXT), furazolidone 15 ug (FR), cephalorodine 25 ug (CR), kanamycin 30 ug (K), nalidixic acid 30 ug (NA), gentamicin 10 ug (CN), colistin sulphate 10 ug (CT), and carbenicillin 100 ug (CAR).

All the 71 isolates were resistant to cloxacillin 10 ug (OB), fusidic acid 10 ug (FD), lincomycin 2 ug (MY), novobiocin 5 ug (NV), penicillin G 1.5 ug (P), methicillin 10 ug (MET), erythromycin 10 ug (E) and sulphadiazine 50 ug (SD).

Only 46.8 percent of the Salmonella livingstone isolates had plasmids. Of these, 82.8 percent had light plasmids of between 3.3 and 9.1 Mega daltons, 13.8 percent had heavy plasmids of between 33 and 120 Mega daltons (Mdal), and 3.4 percent had both light and heavy plasmids. There was no correlation between possession of plasmids and antimicrobial resistance overall, but within biogroups, there was some correlation.

Biotyping was even superior to antimicrobial susceptibility testing in correlating isolates to source, and plasmid profile analysis was more specific and superior to the other two methods in correlating isolates to source even within the same biogroup.

The three methods used proved useful for tracing the sources of Salmonella livingstone and are recommended in future epidemiological studies ofthis serotype.

C O N T E N T S

INTRODUCTION

Salmonellosis is a term used to describe the disease in man and all species of animals caused by any of the numerous members of the genus Salmonella (Robertson, 1976). Salmonellosis is one of the most common and widespread zoonosis of worldwide economic importance in both humans and animals (Gunnersson et al, 1974; Baker, 1975; Levy et al, 1975; Robertson, 1976 and Williams, 1986). The disease is however most prevalent in areas of intensive animal husbandry, especially poultry and pigs. For example, the increased incidence of calf salmonellosis in Great Britain from 1965 onwards was attributable to a change in husbandry practices involving the rearing of large numbers of calves in units for the production of baby beef (Sojka and Field, 1970).

Salmonella infection takes a variety of forms including remarkable host specificity, inapparent infections, recovered carriers, enteritis, septicemia, abortion and combinations of disease syndromes. Salmonella infection in animals always constitutes a vast reservoir for the disease in humans (Edel et al, 1973; Christopher et al, 1974; Fox, 1974; Guinee and Valkenburg, 1975; Sojka et al, 1975 and Clarke and Gyles, 1986).

As a rule, the mouth is the port of entry for Salmonella infections and the small intestine is the site of pathological action (Prost and Riemann, 1967). Salmonellae are readily transferred from animal-to-animal, animal-to-man, man-to-man and environment-to-animal or man by direct or indirect pathways (Robertson, 1976;

Williams, 1975 and Clarke and Gyles, 1986). Direct transmission occurs when one host ingests the faeces of an infected animal. On the other hand, indirect transmission occurs when an animal ingests material contaminated by an infected animal either domestic or wild. Infection may also be introduced in feeding-stuffs and fertilizers as well as polluted water, especially sewage contaminated (Robertson, 1976; Williams, 1975, 1977). Human Salmonellosis is almost always foodborne (Prost and Riemann, 1967).

Salmonella infections once acquired can either be confined to the intestine as an enteritis, or spread to the entire body of the host with septicemia and allied pathological changes. The mesenteric lymph nodes, the liver and the gall bladder are often sites of localization and Salmonella can remain in these sites for a very long period of time (Robertson, 1976). The climax of Salmonella infection is bacteremia which results in the infection spreading throughout the host and produces patholcgical changes in tissues, the most susceptible being the rapidly proliferating ones especially the reproductive system (Prost and Riemann, 1967).

Salmonellosis is caused by members of the genus Salmonella which belongs to the family Enterobacteriaceae (Cruickshank et al, 1969; Ewing, 1986). Typically, Salmonellae are non-lactose fermenters or slow lactose fermenters and most schemes for their detection are based on this property. However, some strains of Salmonella arizonae and occasional strains of other serotypes ferment lactose rapidly (Clarke and Gyles, 1986).

Ewing (1986) has given the revised definition of the genus Salmonella as gram-negative motile bacteria that conform to the definitions of the family Enterobacteriaceae and the tribe Salmonelleae. Hydrogen sulphide is produced, the methyl red reaction is positive, and lysine, arginine, and ornithine are decarboxylated. Indole is not formed, and urea is not hydrolyzed. The Voges-Proskauer test is negative, and neither phenylalanine nor tryptophan is decarboxylated Lactose is not fermented by most strains belonging to subspecies 1, 2, 4 or 5. Acid is not produced from sucrose, adonitol, raffinose, nor alpha-methylglucoside. Dulcitol is utilized by strains of subspecies 1, 2, and 5; not by those of 3a, 3b, or 4. Inositol is not fermented by cultures that belong to subspecies 3a, 3b, 4, or 5. The type species is Salmonella enterica subspecies enterica.

The Kauffmann-White Schema subdivided the genus Salmonella into four sub genera, I, II, III and IV (Kauffmann, 1966). The modern scheme (Ewing, 1986) subdivides Salmonella species into five subspecies, 1, 2, 3a, 3b, 4 and 5. Members of subgenus I (Subspecies 1) typically are associated with warm-blooded animals, whereas members of other subgenera are found in cold-blooded animals and the environment (Clarke and Gyles, 1986). Subgenus III is Salmonella arizonae (Ewing, 1986).

Identification of an isolate as a member of the genus Salmonella or as a member of one of the four subgenera is based on a battery of biochemical tests (Clarke and Gyles, 1986). Serotyping is used to identify the organism beyond the genus level, thus serotype or

serovar is equivalent to species (Le Minor, 1984). Approximately 2000 serotypes of Salmonella have been described on the basis of somatic (O), flagella (H), and capsular-like (Vi) antigens, but less than fifty of these are encountered in significant frequency in disease (Clarke and Gyles, 1986).

Phage-typing, biotyping, drug resistance and plasmid profile analysis have all been used in the identification of isolates beyond the level of serovar and are useful for epidemiologic studies. However, phage-typing is limited to a few serovars for which schemes have been developed (Clarke and Gyles, 1986).

1.1 HISTORICAL BACKGROUND OF SALMONELLA

The first serotype of the genus Salmonella to be described was Salmonella typhi (Eberth, 1880; Gaffky, 1884). Soon after, Salmon and Smith (1885) isolated Salmonella Choleraesuis, and this discovery was followed by the isolation and description of a number of other microorganisms belonging to the genus, for example, Salmonella enteritidis (Gaertner, 1888).

The genus Salmonella was first proposed by Lignieres(1900) with the organism isolated by Salmon and Smith (1885) as the type species (Lignieres, 1901). Smith and Stewart (1897) stated that the already isolated organisms (Eberth 1880; Gaffky 1884; Salmon and Smith 1885 and Gaertner 1888) belonged to one great group or species by virtue of the identity of their morphologic and biologic characters. Schuetze (1921) published a short paper which gave an insight into

the numerous serotypes within the genus and the possibility of distinguishing them by using absorbed antisera. Later on White (1925, 1926) established a firm foundation upon which serological classification is based. Kauffmann (1930, 1941a, b, 1966) modified, systematized and greatly expanded White's work to form the basis for the present serological classification within the genus.

In 1934, only 44 serotypes, Variants and biotypes of Salmonella were recognized (Ewing, 1986). In 1941, Kauffmann listed 104 serotypes and biotypes. In 1951, 211 serotypes and biotypes were recorded; in 1954, 309, in 1961, 695, and in 1966, 962 serotypes were listed in the Kauffmann-White Schema (Kauffmann, 1941, 1951, 1954, 1961, 1966 and 1972). Since then, the number of delineated serotypes of Salmonella has continued to grow even though a very high percentage of cultures isolated in daily practice belong to a small number of serotypes (Kelterborn, 1967). To date, about 2000 serotypes of Salmonella are recognized (Clarke and Gyles, 1986) and the year 1986 marked the centenary since the type species, Salmonella Choleraesius was isolated.

2.0 CHARACTERISATION OF THE GENUS SALMONELLA

2.1 ISOLATION AND IDENTIFICATION

2.1:1 Introduction

Isolation and identification of Salmonella organisms in specimens is a very important pre-requisite to the containment of Salmonella infection spread as well as the control of any future occurrences of the disease in both man and animals. The development of a rapid technique for isolation and identification of these organisms would be very important. This goal has not yet been achieved but there has been significant contributions in last couple of years (Muller, 1923; Kaufmann, 1930; Leifson, 1936; Smith,1952, 1959; Banwart and Ayres, 1953; Cook et al, 1954; Rappaport et al, 1956a and b; Silliker et al, 1964; Mc Call et al, 1966; Taylor and Schelhart, 1967, 1973 and Ewing, 1986).

A large variety of cultivation media and identification techniques for Salmonella have been developed since this organism was first isolated and recognised by Salmon and Smith in 1885. However, most methods are based on the same general principles, namely, inoculation into a selective or non-selective medium followed by cultivations in various selective and diagnostic media. Identification is done by culture techniques and serological tests (Prost and Riemann, 1967; Williams, 1980 and Ewing, 1986).

2.1:2 Culture Techniques

Sample Collection and Handling

Any attempt to isolate Salmonella from clinical material or specimens must take into account the way in which the sample is collected and subsequently transported to the laboratory (Ewing, 1986). Experience has shown that the technique for recovery of Salmonella must be adapted to the material which is being examined (Prost and Riemann, 1967), and that the effectiveness of laboratory isolation is determined by the adequacy and condition of the material received for examination. Thus, if specimens or materials are not properly collected and handled, or are not representative, the laboratory can contribute little or nothing to any investigation. This applies to specimens of all sorts (Ewing, 1986). Methods for proper sample collection have been reviewed extensively; (Ten Broeck and Norburg, 1916; Dold and Ketterer, 1944; Bailey and Bynoe, 1953; Floyd, 1954; Stuart, 1956, 1959; Cooper, 1957; Lie, 1960; Blair, 1964; Cary *et al*, 1965; Amies and Douglas, 1965; Ewing *et al*, 1966; Ewing, 1968, 1986; and Dunn and Martin, 1971).

2.1:3 Enrichment Methods

The major method for isolation of Salmonellae from man, animals, feedstuffs, food, or the environment involves the placing of the sample in an enrichment medium, incubating overnight or longer, and streaking a small portion of it onto selective plating media (Williams, 1980; Ewing, 1986). Others streak the sample directly onto a

selective media whereas others first inoculate the sample into an enrichment broth and subsequently isolate the organisms on a selective solid medium (Barnwart and Ayres, 1953).

Enrichment methods have proved invaluable for isolating Salmonellae when present in small numbers in faeces, as in convalescent cases, or in the detection of carriers (Cruickshank et al, 1969; Barnwart and Ayres, 1953; Collard and Unwin, 1958; Iveson et al, 1964; Hooper and Jenkins, 1965; Iveson and Kovacs, 1967; Taylor and Schelbert, 1968 and Ewing, 1986). The purpose of using enrichment broth is to increase the numbers of Salmonella present and at the same time, to prevent the growth of other organisms in order that the probability of isolating Salmonella will be increased (Barnwart and Ayres, 1953; Carlton, 1945; Galton and Hardy, 1948 and Ewing, 1986).

The enrichment broths generally used include Selenite F or Leifson (1936) and Tetrathionate of Muller (1923). Modifications of Tetrathionate broth with brilliant green added (1:100,000 w/v) is useful only for Salmonellae other than bioser typhi. The same media as well as Selenite F cannot be used for isolation of Salmonella Cholerae-suis and Salmonella abortus-ovis as they are both toxic to the two organisms (Smith, 1952). Instead, Rappaport's enrichment broth (Rappaport et al, 1956a, b) is recommended for the isolation of these two serovars (Ewing, 1986).

2.1:4 Plating Media and Identification of Isolates

The enrichment media in 8-to-10 ml amounts are usually inoculated with approximately one gram of faeces or with 1 ml of liquid specimen. The inocula is then emulsified thoroughly in the media. This is incubated at 35° C to 37° C (Selenite, 12-16 hr; tetrathionate, 18-24 hr) and cultures in enrichment media are streaked on plates containing solid media of the same sort used for primary inoculation of specimens (Ewing, 1986).

There are four types of solid media onto which enrichment broth cultures can be streaked onto. These include: non-inhibitory, non-selective media such as blood agar and nutrient agar; differential media with little selectivity such as MacConkey (MacConkey 1905, 1908) and Desoxycholate (Leifson, 1936); differential, moderately selective media like Shigella-Salmonella Agar (SSA) and desoxycholate citrate agar (Leifson, 1935) and highly selective media which include bismuth sulfite (Wilson-Blair, 1931) and Brilliant green (Kristensen *et al*, 1925, modified by Kaufmann, 1935, 1966 and others).

After isolation of colonies conforming to the description of the genus *Salmonella*, further identification and confirmation is done by biochemical tests and serotyping. For those serotypes like *Salmonella typhimirium* where phage typing schemes have been developed, this method is also used for further characterisation beyond the serovar level (Clarke and Gyles, 1986). For details of

the methods used in identification and typing Salmonellae the reader is referred to section 9.0 of this chapter.

3.0 CLINICAL FEATURES OF SALMONELLA INFECTION IN ANIMALS

3.1 Introduction

Infection of animals with Salmonella may result into gastroenteritis, a specific disease syndrome or asymptomatic carriage, the latter being the most common result of infection (Williams, 1980). Five clinical patterns have been described. They include:

1. Primary Salmonellosis in which an organism is the only aeriological factor and cause of serious pathological conditions, for example, abortion in mares and ewes produced by distinctly specific serotypes, Salmonella abortus-ovis, and Salmonella abortus-equi, and Salmonellosis of young animals produced by non-specific Salmonella, a typical example being Salmonella typhimurium (Prost and Riemann, 1967).
2. Secondary Salmonellosis which is associated with another disease, physiological state, or other stress situation. The clinical symptoms and pathological lesions found in these infections are not typical, and are produced almost exclusively by non-specific Salmonellae (Prost and Riemann, 1967: Williams, 1980).

3. Chronic carrier state usually (convalescent), and clinically asymptomatic, but excretes Salmonella for weeks or occasionally for months.

4. A temporary carrier state, often observed in slaughter animals (Williams, 1980).

5. Latent infection which can only be demonstrated by isolation of Salmonellae from mesenteric lymph nodes at the time of slaughter (Prost and Riemann, 1967; Williams, 1980). This form may be part of the carrier-state syndrome resulting from repeated ingestion of contaminated feed (Newell and Williams, 1971). The contribution of such carrier animals to the epidemiology has been extensively reviewed (Prost and Riemann, 1967; Wray and Sojka, 1977; Aitken et al 1983; Le Minor, 1984, and Clarke and Gyles, 1986).

3.2 Salmonellosis in Cattle

In adults, the acute disease is characterized by fever, dullness, anorexia and loss of milk. This is quickly followed by acute diarrhoea, with offensive watery-faeces that may contain blood, mucus and later, shreds or casts of necrotic bowel lining. Pregnant animals may abort during the course of illness. If left untreated about half of the affected animals are likely to die within three to four days of the illness. Those that recover may take several weeks or months to return to normal health (Robertson, 1976).

Subacute Salmonellosis is less dramatic. Fever is variable or absent, and a gradation can be traced from cases showing the

typical signs outlined above to those in which infection is inapparent. The variation in clinical signs may arise from the fact that the illness is not due to a recently acquired infection but to the activation by some other disease or stress of a longer standing infection previously latent in the tissues. Some pregnant animals may abort without showing any other clinical signs (Robertson, 1976).

Calves are particularly susceptible to Salmonella infection during the first three weeks of life but the disease is commonly seen at 2-6 weeks of age (Prost and Riemann, 1967; Robertson, 1976). Typical cases are characterized by fever, dullness, anorexia followed by a"brown scour" with offensive fluidy faeces which frequently contain a little blood or mucus. Affected calves quickly lose condition, become dehydrated, week and emaciated. Pneumonic signs may also be seen and chronic cases may develop arthritis. Some cases may be so mild as to pass unnoticed and others may be acute septicemia without signs of diarrhoea (Robertson, 1976).

In calves, morbidity rates of eighty percent or more are not uncommon and mortality rates of between ten and twenty percent are common but may on occasion be higher (Prost and Riemann, 1967; Robertson, 1976). The serotypes of Salmonella isolated from both diseased and healthy cattle are most frequently Salmonella dublin and Salmonella typhimurium, but a few dozens of other Salmonellae have been found sporadically (Prost and Riemann, 1967).

typical signs outlined above to those in which infection is inapparent. The variation in clinical signs may arise from the fact that the illness is not due to a recently acquired infection but to the activation by some other disease or stress of a longer standing infection previously latent in the tissues. Some pregnant animals may abort without showing any other clinical signs (Robertson, 1976).

Calves are particularly susceptible to Salmonella infection during the first three weeks of life but the disease is commonly seen at 2-6 weeks of age (Prost and Riemann, 1967; Robertson, 1976). Typical cases are characterized by fever, dullness, anorexia followed by a"brown scour" with offensive fluidy faeces which frequently contain a little blood or mucus. Affected calves quickly lose condition, become dehydrated, week and emaciated. Pneumonic signs may also be seen and chronic cases may develop arthritis. Some cases may be so mild as to pass unnoticed and others may be acute septicemia without signs of diarrhoea (Robertson, 1976).

In calves, morbidity rates of eighty percent or more are not uncommon and mortality rates of between ten and twenty percent are common but may on occasion be higher (Prost and Riemann, 1967; Robertson, 1976). The serotypes of Salmonella isolated from both diseased and healthy cattle are most frequently Salmonella dublin and Salmonella typhimurium, but a few dozens of other Salmonellae have been found sporadically (Prost and Riemann, 1967).

3.3 Salmonellosis in Sheep and Goats

Symptoms vary widely from sudden death in the acutely affected animals to the symptomless carrier which excretes Salmonella in the faeces. The young animal is more susceptible and more likely to show acute signs that consist of diarrhoea, dysentery and collapse. In less acute cases, diarrhoea may be intermittent, there is apparent loss of weight and large patches of fleece may fall out. Pregnant animals abort and this may be the only clinical manifestation (Robertson, 1976). Salmonellosis with symptoms of gastroenteritis in sheep and goats is produced most frequently by Salmonella typhimurium, Salmonella dublin, Salmonella oranienburg, and Salmonella java. Salmonella abortus-ovis is a specific cause of of abortion in these animals (Prost and Riemann, 1967).

3.4 Salmonellosis in Pigs

Pigs of all ages are susceptible, but younger pigs up to four months of age are more susceptible. Acute and subacute or chronic syndromes of Salmonella choleraesius infection have been described. The acute form is characterized by fever, inappetance which may be accompanied by diarrhoea, and purple discoloration on the ears and abdominal wall is frequently seen. Death usually occurs after 2-4 days (Robertson, 1976).

The subacute or chronic form starts with a moderate rise in temperature accompanied by a depressed appetite and diarrhoea. The

diarrhoea may persist for several weeks and the affected pigs become emaciated. In both acute and chronic forms, pulmonary complications may be present (Robertson, 1976).

3.5 Salmonellosis in Poultry

Salmonellosis produced by the poultry-specific Salmonella gallinarum and Salmonella pullorum can assume a very acute form with high mortality which can reach 100 percent (Prost and Riemann, 1967). Pullorum disease produced by Salmonella pullorum is primarily a disease of baby chicks, and affected birds may die without showing distinctive signs. Mortality is uncommon after the first 2-3 weeks of life (Robertson, 1976).

Fowl typhoid caused by Salmonella gallinarum is principally a disease of growing and adult birds, in which it causes heavy losses. In severe outbreaks no symptoms may be seen before death. Usually, however, birds show listlessness, loss of appetite, greenish diarrhoea and dark-red discoloration of the comb (Robertson, 1976). Diseases caused by Salmonellae other than Salmonella gallinarum and Salmonella pullorum and Salmonella typhimurium are rare but when they occur, the symptoms displayed in young chicks are indistinguishable from Pullorum disease. Adult birds infected by these other serotypes are symptomless but may remain carriers and shed the organisms in faeces for many months (Robertson, 1976; Prost and Riemann, 1967).

3.6 Salmonellosis in other species of birds

Other species of birds can also suffer from serious disease produced by specific Salmonella gallinarum and Salmonella pullorum, and other Salmonella serotypes (Prost and Riemann, 1967). Publications from numerous countries report a growing number of carriers of Salmonella, mainly Salmonella typhimurium among apparently healthy birds. The carrier state is particularly frequent among ducks, pigeons (Prost and Riemann, 1967) and turkeys (Williams, 1980). Salmonella infected faeces have been found in geese, pheasants, seagulls, canary birds, parrots, cranes and shoe-bills (as quoted by Prost and Riemann, 1967).

3.7 Salmonellosis in Horses

Salmonellosis in horses is caused by the specific Salmonella abortus-equi which is responsible for sporadic cases of abortion in Mares during late gestation, and polyarthritis or novel ill and even septicemia in young foals (Prost and Riemann, 1967; Robertson, 1976). However, Salmonella typhimurium appears to be the most common serotype encountered as a cause of septicemia with rapid death in foals and enteritis in older animals. Other Salmonella serotypes may cause symptoms of colic and gastroenteritis (Robertson, 1976).

3.8 Salmonellosis in other Animal species

Sporadic cases of disease, but most often symptomless carriers have been found in many animal species. Numerous authors have reported the exceptional frequency of Salmonella isolations from dogs, cats, buffaloes, camels, elephants, fur animals, rats and mice, guinea-pigs, rabbits, turtles, snakes, lizards, fish and many other wild animals (Prost and Riemann, 1967; Williams, 1980).

3.9 Pathological Findings in Salmonellosis

A review of pathological findings in animal salmonellosis has been presented by Robertson (1976). A notable feature of the Salmonella pathology is a gastroenteritis which may or may not be haemorrhagic and necrotic. Most other lesions are non-specific. In birds, however, lesions produced by Salmonella pullorum and Salmonella gallinarum are pathognomonic. Other serotypes of Salmonella produce non-pathognomonic or no lesions at all in the affected birds.(Robertson, 1976).

4.0 DIAGNOSIS OF SALMONELLOSIS

In the live animal, confirmation of clinical Salmonellosis is obtained by cultural examination of the faeces or rectal swabs. In dead animals, the heart, liver, spleen and mesenteric lymph nodes are cultured using enrichment broths and selective media, the procedure having been reviewed by Ewing (1986). In food-borne Salmonellosis, it is important that the organisms are isolated from the suspect food material (Robertson, 1976).

After cultural isolation in the above mentioned media, characteristic Salmonella-like colonies are picked and placed in various biochemical media, confirmed as Salmonella, and then serotyped (Williams, 1980).

Fluorescent antibody technique has been used to detect Salmonellae in foods, feeds, condiments and environmental samples with varying success (Insalata et al., 1972, 1973; Hilker and Solberg, 1973; Smyser & Snoeyenbos, 1973 and Karlsson and Thal, 1974).

Serological diagnosis can be used retrospectively as an indication of whether an animal has been in contact with infection. However, it is of limited value because it may not detect latent carriers nor determine whether the animal is still infected. Somatic titres of 1/40 and above, and H-titres of 1/320 may be considered diagnostic. In poultry, a whole-blood rapid plate test using a stained pullorum antigen is used for screening against pullorum disease in eradication programmes (Robertson, 1976).

5.0 TREATMENT OF SALMONELLOSIS

Many antibiotics have been used with varying degrees of success but in general chloramphenicol and nitrofurazones have been found to be most successful in augmenting recovery from clinical disease. Antibiotic therapy should be combined with rehydration therapy, intestinal protectants , vitamin A, and careful nursing and hygiene (Robertson, 1976 and Clarke and Gyles, 1986).

6.0 EPIDEMIOLOGY OF SALMONELLA ORGANISMS

6.1 Host Specificity

Salmonellae are divided into three groups on the basis of their host adaptability (Smith and Halls, 1968; Wray and Sojka, 1977; Turnbull, 1979 and LeMinor, 1984). The first group is characterized by specificity for human host, for example, Salmonella typhi, Salmonella paratyphi, Salmonella chotlmuelleri, Salmonella hirschfeldii (agents of typhoid and paratyphoid fevers) and Salmonella sendai (Clarke and Gyles, 1986). The second group of Salmonella consists of organisms that are more or less adapted to specific animal hosts, for example, Salmonella dublin, Salmonella choleraesius, Salmonella abortus-ovis, Salmonella abortus-equi, Salmonella pullorum and Salmonella gallinarum are adapted to cattle, pig, sheep, horse and poultry respectively. The third group consists of unadapted Salmonellae that cause disease in humans and a variety of animals. Most Salmonellae fall into this group, but Salmonella typhimurium is the most frequent cause of disease (Clarke and Gyles, 1986).

6.2 Sources of Salmonellae

The principal Salmonella reservoir is the vertebrate intestinal tract (Prost and Riemann, 1967; Williams, 1980; Clarke and Gyles, 1986). Excretion results into the spread of the organisms to the environment, food, feed, soil and water (Wray & Sojka, 1977; Turnbull, 1979; LeMinor, 1984) from where they may invade their other hosts (Prost and Riemann, 1967).

The ability of Salmonella to survive and multiply in the environment is an important factor in the transmission and spread of the disease. Salmonella has simple growth requirements and can multiply in a wide variety of media provided that the PH, temperature, water activity, **etcetera** are within the ranges of growth. (Prost and Riemann, 1967).

Salmonellae have been recovered from the intestine of a wide range of animals including fish, reptiles, birds and mammals (Williams, 1980; Clarke and Gyles, 1986). The organisms have also been isolated from insects (Robertson, 1976).

Salmonellosis affects all species of domestic animals, and young animals and parturient ones are the most susceptible. However, poultry are considered to be the most important reservoir of Salmonella infection (Robertson, 1976). Fertilizers and feeds containing animal products are sometimes a source of infection for animals (Clarke and Gyles, 1986). Contaminated milk and milk-

products are other sources of Salmonella, particularly for calves (Wray and Sojka, 1977).

Animals and their products constitute the most important source of Salmonella for humans (Turnbull, 1979). Meats, particularly poultry, are frequently incriminated sources of human infection (Prost and Riemann, 1967; Williams, 1980; Clarke and Gyles, 1986). The importance of the asymptomatic carrier in the epidemiology of Salmonellosis needs no emphasis (McCall et al., 1966). Although Salmonella may survive for long periods in the environment (Prost and Riemann, 1967), it is the carrier state that provides the major source of infection for humans and animals (Wray and Sojka, 1977, and LeMinor, 1984).

6.3 Carrier rates of Salmonella in animals

The importance of the asymptomatic carrier in the epidemiology of salmonellosis is undisputable (McCall et al., 1966). Domestic animals exposed to small numbers of Salmonellae develop a carrier state, often with infection of mesenteric lymph nodes. Excretion from such animals remain low unless the animals are stressed by another disease, severe weather, very high protein ration, overcrowding or transport. (Williams, 1980).

Stress increases excretion, and salmonellae-bearing faeces contaminate pens, watering troughs, hides and fleece, and feeds. These then act as a source of infection for other animals entering the

contaminated environment (Williams, 1980). Contaminated fleece and hides as well as the intestinal contents become sources of contamination for carcasses and edible offal that enter the marketing or food-processing chain, and thus join the Salmonella transmission cycle when man and animals are infected (Williams, 1980).

The carrier rates of Salmonellae in animals from different countries has been reviewed by Williams (1980). The carrier rates vary from one animal species to another as well as from one country to another. Salmonella carriers include pigs, cattle, poultry , sheep, goats, horses, dogs, cats and other pets, wild animals, wild birds, rodents, lizards, snakes and insects (Williams, 1980).

Among livestock, the highest carrier rate has been found in slaughtered pigs. Thus 36% and 30% of pigs slaughtered in Belgium and Netherlands respectively, were found to be carrying Salmonella. In poultry, turkeys have the highest carrier rate followed by ducks and then the domestic fowls (Table 1). The turtles have the highest carrier rate among the pets (Table 1). The carrier rates of Salmonella in different animal species are given in table 1.

The serotypes of Salmonella isolated from animals are numerous and have been reviewed by Buxton (1957), Sojka and Fields(1970), Sojka and Hudson (1974) and Sojka et al. (1977).

TABLE 1.

Carrier-rates of Salmonellae in Animals

Animal Species	Carrier-rate (%)
1. Poultry	
Turkeys	2.5 (UK) to 68 (US)
Ducks	3 - 55 (Germany)
Domestic fowl	0.4 (UK) to 42 (US)
2. Swine	
	3 (Denmark) to 36 (Belgium)
	7 (UK)
	8.4 (Australia)
	30 (Netherlands)
3. Cattle and Calves	
Adult cattle	13 - 55
Calves	1 - 7 (Netherlands) 3.2 (UK)
4. Sheep and Goats	
Sheep	3.1
Goats	3.8
5. Dogs, cats and other pets	
Dogs	0.6 - 25.7
Cats	0.7
Turtles	45 - 70 (US)

From Williams, L.P, Jr in the CRC Handbook Series Section A: Bacterial, Rickettsial and Mycotic diseases. Volume II CRC Press. Page 13-14

6.4 Transmission of Salmonella infections

Salmonellosis is primarily an enteric disease and is subsequently transmitted principally by the faecal-oral route (Robertson, 1976) either directly when one host ingests the faeces of another, or indirectly when an animal ingests material contaminated by an infected animal which could either be domestic or wild. Infection may also be introduced in feeding stuffs and fertilizers and by polluted water, especially when contaminated with sewage (Edel et al., 1973; Gangarosa et al., 1973; Christie, 1974; Hugh-Jones et al., 1975; Robertson, 1976 and Williams, 1975 and 1977).

The epidemiology cycle of Salmonellosis is shown in Figure 2. It consists of the following:

1. Contaminated animal protein meals being fed to domestic animals.
2. Excretor animals infecting other animals directly or via the environment.
3. These healthy animals going to slaughter and contaminating the abbatoir (including pens).
4. The infected viscera being used to make animal protein meals that are contaminated after cooking.
5. These meals being fed to other domestic livestock to begin the major cycle over again.

Two subcycles naturally evolve from the aforementioned. One involves Salmonellae from these excretor animals directly infecting

other animals in the marketing chain or man. The second cycle is the one in which man is most directly involved, especially in developed countries. Foods of animal origin that are contaminated with Salmonellae enter the kitchen and can contaminate this area, or they can enter food-processing plants, markets, or mass-feeding facilities where they contaminate other foods or are recontaminated after cooking (Williams, 1980).

The cycle of Salmonella transmission can be summed up as; animal-to-animal, animal-to-man by direct or indirect pathways (Robertson, 1976; Williams, 1980, and Clarke andGyles, 1986).

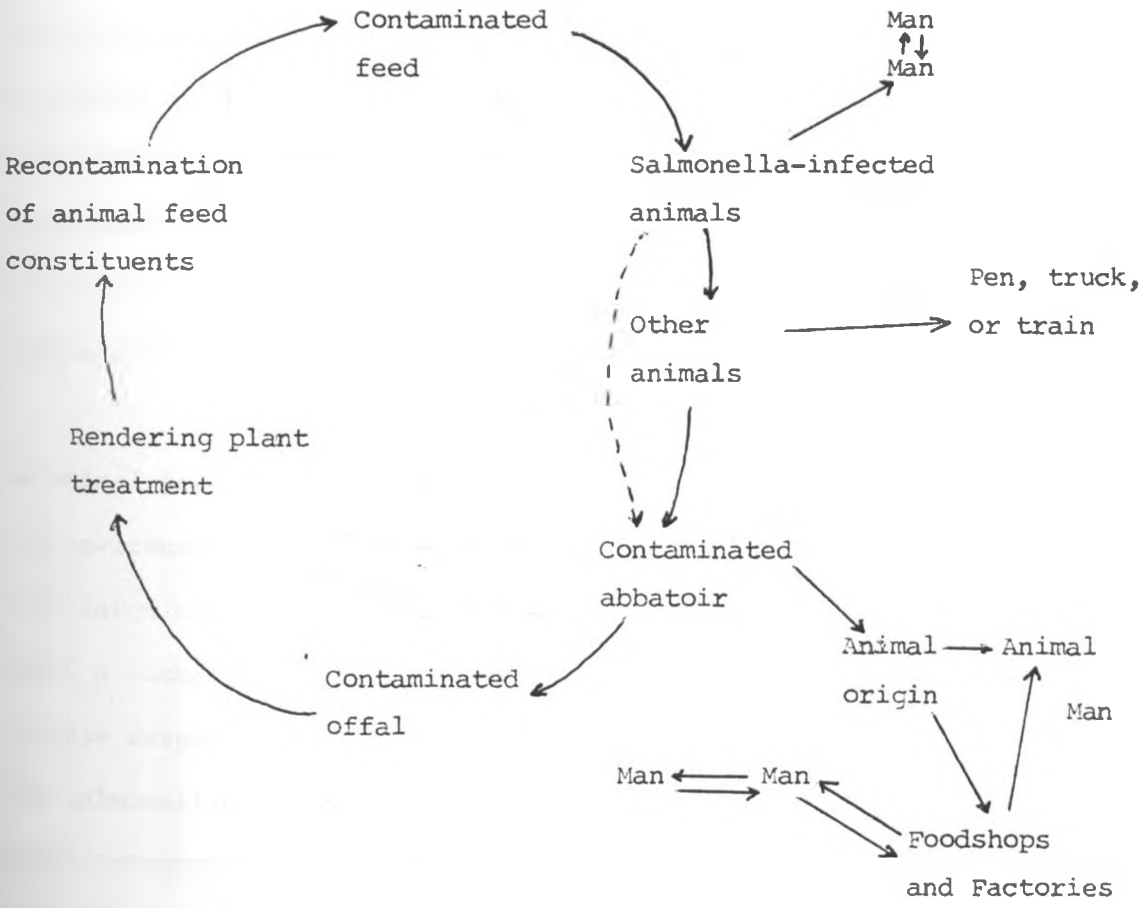


FIGURE 1.

Salmonella cycle

(From Williams L.P Jr, 1980)

7.0 SALMONELLOSIS AND PUBLIC HEALTH

Salmonellosis is one of the most common and widespread zoonosis in the temperate zones (Gibson, 1965). There is no doubt that animals constitute a large and important reservoir of Salmonella infection for man; and cattle, poultry and pigs, are generally regarded as the most important sources, even though household pets cannot be ignored (Kaufmann, 1966; Williams, 1980).

Taylor (1965) lucidly expounded on the major differences between human and animal Salmonellosis. She conceded that the most important factor in human Salmonellosis is infected food, which accounts for nearly all sporadic cases, family outbreaks, and other outbreaks of food poisoning. Because the general population lives under reasonably hygienic conditions, contact spread is of minor importance. In hospitals and other institutions the situation is different, there being close contact between patients so that spread can occur from infected patients, dust and the environment.

In animal population however, the most important factor is contact and environmental spread; for the animals are herded in age-groups with large numbers of the young and susceptible ones in close contact, a situation similar to the human institution. (Taylor, 1965). In this respect, the animal feedstuffs are a minor problem, for the Salmonellae found in them are rarely isolated from, and rarely cause outbreaks in animals; in addition, the serotypes are rarely isolated from man. Taylor (1965) concluded that Salmonella infection cannot be eliminated but can be very materially reduced, for

many of the conditions which favour the spread of Salmonella from animal-to-animal, animal-to-man and man-to-man are known and can be controlled (Sojka and Field, 1970). Williams (1975) stresses the importance of Salmonellosis as an occupational hazard with livestock handlers, Veterinarians and laboratory workers being among the people at risk.

8.0 PREVENTION AND CONTROL OF SALMONELLOSIS

8.1 Hygiene

Much of the Salmonella infection in animals and man could be prevented by an interruption in the faecal-oral-cycle of transmission. This can be done by elimination of the organism from animal meals and subsequent clean up of confinement facilities and animal rearing areas (Williams, 1980).

Prevention of Salmonella infection in animals depends very much on the maintenance of high standards of hygiene and prevention of introduction of infection into Salmonella-free herds (Robertson, 1976). Under range conditions, control may be difficult and attention should be paid to the prevention of precipitating factors such as parasitic infestation. In more intensive systems of animal husbandry, control measures should include isolation and segregation of clinically affected animals, and recovered animals should be retained in isolation for at least two weeks after the cessation of clinical signs (Robertson, 1976). All manure and effluent from isolation

premises should be safely disposed or treated in such a way as to minimize the danger of contaminating the environment in which the animals live (Robertson, 1976; and Williams, 1980).

The protection of water supplies from all manure and effluent should take first priority. General sanitation of barns, pens and isolation and treatment areas, and disinfection whenever possible are also highly recommended (Williams, 1980).

8.2 Vaccination

The next type of defence against Salmonella infection is that of increasing resistance of the animals to infection. In this respect, vaccination of calves and lambs has been used with mixed results (Williams and Hobbs, 1975).

Prevention of salmonellosis by vaccination has been attempted since the late nineteenth century when Wright (1896) described the use of killed *Salmonella typhi* cells in humans (Jones, 1986). The early history of vaccine development was reviewed by Harvey (1929) and White (1929a). Subsequently live attenuated and dead cells have been used as vaccines (Jones, 1986).

The role of vaccination in the prevention of salmonellosis in cattle has been reviewed by Wray and Sojka (1977), and Jones (1986). In these reviews, the use of both live and dead vaccines has been reported. In the United Kingdom, both live and inactivated vaccines are available (Aitken, 1984b) while in United State of America, only dead cell preparations are available (Jacks, 1978).

Others (Cameron and Fuls, 1976; [illegible] Bairey, 1978) have used formalinised preparations in parenteral vaccination of calves but these have thus been discredited by Hardy et al., (1980) as they found them infective (Jones, 1986). Heat-killed cells have also been used for the vaccination of calves against Salmonella dublin and found to be effective (Aitken et al., 1982).

There has been much debate as to the role of vaccines in protection of calves against Salmonella infection. Most investigators have, however, believed that cell-mediated immunity is necessary to provide solid protection and the majority of recent attempts at vaccination have utilised living, attenuated cells. These have either been naturally occurring avirulent strains, laboratory derived or mutated strains, or more recently genetically engineered strains(Jones, 1986).

Botes (1964, 1965) and Cameron and Fuls (1976) found that rough Salmonella typhimurium strains were more useful than killed cells. Smith (1965) tested a rough Salmonella dublin strain which although avirulent in mice, was of reduced virulence for calves. This strain (S 51) which proved successful in protecting calves against Salmodublin and Salmonella typhimurium (Rankin et al., 1967) was made available for commercial vaccine "Mellarax" which is still in use (Jones, 1986). This vaccine has performed well in field trials but may not protect against heavy challenge dose unless vaccine dose is increased to a level which is potentially virulent. The vaccine may also be more virulent in stressed animals (Aitken, 1984b).

may aggravate pre-existing enteric and respiratory disease and may, rarely, result in anaphylatoid reactions following vaccinations (Jones, 1986).

In United States of America, one other vaccine strain has been developed. This is a non-virulent and non-reverting Streptomycin-dependant strain of Salmonella dublin which is an oral vaccine, and has been found to be protective against both Salmonella dublin and Salmonella typhimurium infections (Jones, 1986). Another vaccine under the product name "Bovisaloral" (Dessau) is used commercially in the Germany Democratic Republic (Meyer, 1980). However, this vaccine may not protect calves once the challenge organisms become bacteremic (Jones, 1986).

Other vaccines used are the genetically-engineered strains. These are deficient in the synthesis of certain aromatic compounds, and are generally known as Aro A-strains (Hoiseth and Stocker, 1981). These vaccines are orally administered and are protective against both Salmonella typhimurium and Salmonella dublin but, they suffer from one disadvantage in that, not all constructs are protective (Jones, 1986).

Live and inactivated vaccines have also been used to vaccinate pregnant cows in an attempt to transfer immunity via colostrum (Henning, 1953c; and Royal et al., 1968); however, these vaccines suffer from one big disadvantage in that, the presence of maternal antibodies may interfere with response to active vaccination (Henning, 1953c; Ingram et al., 1970).

Vaccines have also been used in prevention of salmonellosis in pigs. Avirulent strain of Salmonella cholerae-suis (Strain 6) has been developed and used for the active immunisation of young store and fattening pigs against necrotic enteritis and septicaemia caused by Salmonella cholerae-suis (Anon, 1986). The vaccine is now available for commercial use under the proprietory name of Suscovax* and should be administered subcutaneously to pigs at the age of two to four weeks or as early as possible. (Anon, 1986).

* = Coopers animal health Limited

9.0 LABORATORY METHODS USED IN THE STUDY OF SALMONELLA EPIDEMIOLOGY

9.1 Serotyping

All the members of the genus Salmonella have an antigenic structure by which they can be recognized. By international agreement, serologically related species are considered to belong to the genus Salmonella even if their behaviour differs in a few biochemical properties of the genus (Kaufmann, 1966). No organism possessing aberrant cultural or biochemical properties is to be included in the genus Salmonella unless it contains somatic (O), flagella (H) and capsular-like (Vi) antigens typical of the genus Salmonella (Kaufmann, 1966).

The current serological classification of Salmonella is based on the Kaufmann-White Schema presented to the Salmonella Subcommittee by Kaufmann in 1931 (Kaufmann, 1966). Based on this scheme, approximately 2000 serotypes of Salmonella have been described (Clarke and Gyles, 1986; Ewing, 1986). This in itself bears testimony to the very great practical services serology has rendered as a taxonomic tool, for it has turned a field full of uncertainties and pitfalls into one in which identification has become easy, certain and closely correlated with pathology and epidemiology (Kaufmann, 1966).

In the genus Salmonella, the serological differences are so definite as to be valid in erecting species (Kaufmann, 1966) hence

serotyping is used to identify the organism beyond the genus level, so that serovar or serotype the equivalent of species (LeMinor, 1984).

Serotyping alone should not be used for epidemiological studies because it does not identify the isolates beyond the serovar level and yet out of the 2000 known serovars of Salmonella, only 50 or less are encountered at significant frequency in disease (Clarke and gyles, 1986). Other methods, namely, phage-typing, biotyping, drug-resistance and plasmid profile analysis have been used to identify isolates beyond the serovar level and are useful for epidemiological studies (Clarke and Gyles, 1986).

9.2 Phagetyping

Bacteriophages are viruses which infect bacteria. These phages are exceedingly specific for the host bacteria in which they can multiply (Cruickshank et al., 1969). Their specificity allows a finer differentiation between types of species of a bacterium which can be obtained by other methods. This phenomenon is therefore often used to subdivide single bacterial species into 'phage types' according to the number and nature of phages capable of causing their lysis, and has provided much valuable information on the spread of strains of Salmonella and other bacteria (Cruickshank et al.. 1969).

In the genus Salmonella, only a few serotypes have been phagetyped. These include Salmonella typhimurium (Andersson, 1964;

Andersson et al., 1977a), Salmonella typhi and Salmonella paratyphi (Cruicksahnk et al., 1969), Salmonella agona (Tyc, 1980; Barker and Old, 1982), Salmonella hadar (Rowe et al., 1980) and Salmonella dublin (Jones, 1986).

Phage-typing method as developed by Andersson et al. (1977a) and used internationally has proved invaluable as a single typing method which can give an indication as to the likely source of Salmonella infection and its spread. Thus when isolates of a Salmonella serotype come from an outbreak circumscribed in time and place, and are found to be of the same phage type, it is likely that they originated from a common source (Andersson, 1971). However, due to continuing process of type conversion by a change in lysogeny, by gain or loss of a plasmid or by chromosomal mutation (Threlfall et al., 1980), dependance on a single typing method may prove misleading when searching for a common source of isolates retrieved from sporadic and widely dispersed case of infection (Barker, 1986).

9.3 Biotyping

Biotyping is any systematic method which will demonstrate phenotypic similarities and dissimilarities between two or more isolates that have previously been identified as belonging to the same genus and species. In some instances, biotyping strengthens the validity of previous identification (Balows and Isenberg, 1978).

Requirements of a valid biotyping system include: a well developed well-described technology, the reagents, apparatus, media and methods of which must be standardized and appropriate controls must be included in the tests. The results obtained with a given biotyping system performed in another laboratory must be reproducable in another laboratory; however, this can only happen when the exactness of the method used in the two laboratories has been established (Balows and Isenberg, 1978).

The epidemiological value of different methods of distinguishing types within a bacterial species depends on their discriminating ability, reliability and ease of performance. Discriminating ability depends on **reproducibility** of each isolate's results in repeated tests and on the stability of the typing characters among different isolates from the same epidemic episode (Duguid et al., 1975).

A biotyping method based on fermentation tests in nine substrases developed by Kristensen et al. (1937), extended by Hansen (1942) and Harhoff (1948) was found to be discriminatory and able to distinguish different biotypes within individual phage-types of Salmonella typhimurium (Kallings and Laurell, 1957; Kallings et al., 1959; Rische and Kretzscmar, 1962, and Lewis and Stocker, 1971), and that the combination of biotyping with phage-typing gives greater precision in defining relationships between strains (Duguid et al., 1975). Alfredsson et al. (1972) found the use of the organic acids, d-tartaric acid, l-tartaric acid and m-tartaric acid, in the turbidity tests for stimulation of growth and plate inhibition of

growth respectively, in the biotyping of Salmonella typhimurium, to be much more reliable and discriminating than those previously used.

An automated biotyping technique for Salmonella typhimurium was described by Guinee et al. (1972). In this technique, disposable plastic trays instead of glass tubes and agar instead of liquid media are used, and a comparative study revealed no differences in results between conventional biotyping in tubes with liquid media and the newly developed technique.

Duguid et al. (1975) developed a new conventional biotyping scheme for Salmonella typhimurium, which is a two-tier system with "primary types" allocated numbers 1 - 32 in accordance with 32 possible combinations of results in five most discriminating tests, called "primary tests". The subtypes are distinguished within the primary types by the results of the remaining or "secondary tests". Full biotypes are designated by the primary types numbers followed by small letters indicating subtyping reactions. This new scheme was found to give a finer and more reliable differentiation of strains of Salmonella typhimurium than the Kristensen Scheme and is capable of future extension by the addition of new types and new tests.

Biotyping has been used as an epidemiologic tool in tracing infections due to Salmonella typhimurium (Alfredsson et al., 1972; Duguid et al., 1975, and Barker, 1986); Salmonella agona (Barker,

1982) and Salmonella montevideo (Reilly et al., 1985) with some encouraging results but the most outstanding revelation was that biotyping alone is not very good and it requires its use in conjunction with other subtyping methods such as phage typing and plasmid profile analysis in order for it to be reliable and of value in epidemiologic studies of Salmonella infections (Barker and Old, 1982).

9.4 Resistance to Antimicrobial agents

Antimicrobial resistance patterns in Salmonella strains from animals and humans in different countries of the world have been reported (Cohen and Tauxe, 1986). The patterns of drug-resistant Salmonellae vary with the pattern of drug use in animal production and in human medicine (Clarke and Gyles, 1986; Cohen and Tauxe, 1986), as well as from different countries and farms (Sojka et al., 1972, 1984 and 1985; Chung and Frost, 1969 and Murray et al., 1986).

Generally, Salmonella isolates from farm animals are resistant to Streptomycin, tetracycline and sulphonamides; a moderately high percentage are resistant to ampicillin, Kanamycin, neomycin and chloramphenicol, and only a low percentage are resistant to gentamicin and trimethoprim-sulfamethazole. Multiple drug resistance is common in Salmonella isolates (Murray et al., 1986; Cohen and Tauxe, 1986) but is particularly more common in Salmonella typhimurium and is plasmid mediated (Clarke and Gyles).

There is enough evidence (Cohen and Tauxe, 1986; Rantala and Nurmi, 1974) that the use of antimicrobial drugs in animal feeds has contributed to selection for drug-resistant Salmonellae (Clarke and Gyles, 1986). Cohen and Tauxe (1986) contends that the use of subtherapeutic doses of antimicrobial agents in animal feeds both as a means of preventing diseases or as growth promoters have helped in the development of resistant organisms, and that Salmonella strains isolated from healthy food animals show considerable antimicrobial resistance, and the spectrum of the resistance is similar to that in humans and to the spectrum of agents used in food animals.

Epidemiological and laboratory investigations of Salmonella infection outbreaks using subtyping techniques as well as antimicrobial sensitivity testing has provided a considerable insight into the complexity of Salmonella transmission. For example, hamburger contaminated with antimicrobial-resistant Salmonella was traced from well beef cattle, which had been fed subtherapeutic doses of antimicrobials, through meat processing, to supermarkets in another state where ill persons, who were part of the outbreak, had shopped. In another example, approximately 1000 persons were infected with multiple-resistant Salmonella newport in California in 1985 and the strain was characterized by an unusual marker-chloramphenicol resistance (Cohen and Tauxe, 1986).

Holmberg et al. (1984) also found antimicrobial susceptibility testing to be a reliable method in epidemiologic tracing of

Salmonella infections. However, this method should not be used alone as the same author found it to be less specific in identifying Salmonella typhimurium isolates than phage typing and plasmid profile analysis.

9.5 Plasmid Profile analysis

Plasmids are covalently, circular double stranded deoxyribonucleic acid (DNA) molecules that are found extrachromosomally and replicate independently from the bacterial chromosome (Ewing, 1986). Plasmids are classified into large and small according to size as determined by molecular weight which varies between 1.5 and 300 kilo base pairs. The larger plasmids usually are self transmissible or conjugative whereas the smaller plasmids are non-conjugative (Ewing, 1986). Most large plasmids are present at one copy per cell, whereas small plasmids often are present at ten or more copies per cell. Two or more plasmids can exist in the same cell; however, only one of a group of closely related plasmids, usually the same incompatibility group, ordinarily is found in a cell. Plasmids in the same incompatibility group have the same repressor that regulates their replication, and usually have a high degree of DNA relatedness (Ewing, 1986).

Plasmids are transferred rapidly to recipient cells. Once infected, most recipient cells then become donors (Ozeki and Smith, 1962; Stocker et al., 1963). Plasmids in the Enterobacteriaceae mediate the transfer of a variety of genetic determinants, including those for drug-resistance, haemolysin and enterotoxin synthesis

(virulence), resistance to ultra-violet irradiation, heavy metal tolerance, carbohydrate fermentation, hydrogen sulphide synthesis and other metabolic characters (Ewing, 1986). The drug resistances transferred include those to ampicillin, carbenicillin, cephalosporins, chloramphenicol, neomycin, kanamycin, streptomycin, sulphonamides, tetracycles, gentamicin and trimethoprim (Andersson and Threlfall, 1974).

9.5:2 Methods used in isolation and Characterization of Plasmids

9.5:2.1 Isolation methods

There are usually three major steps in isolating plasmids. The first step involves bacterial cell lysis which is done using methods which do not break the plasmid DNA molecules (Hardy, 1981). Lysis is achieved in two steps. First spheroplasts are made by digestion with the enzyme lysozyme in the presence of sucrose, which protects the spheroplasts from bursting by providing a high external osmotic pressure. The spheroplasts are then lysed with detergent, so that the DNA of both the chromosome and the plasmid are released gently (Broda, 1979).

A number of methods can be used to separate plasmid DNA from chromosomal DNA and other cell material (Clewell and Helinski, 1970; Sharp et al., 1972; Guevry et al., 1973; Thompson et al., 1974; Humphreys et al., 1975; Meyers et al., 1976 and Hughes and Meynell, 1977). Commonly a given procedure, developed for one plasmid, gives

poor yields with another plasmid or strain. Hence, in investigating a new system, a number of procedures may have to be tested before a satisfactory one is found (Broda, 1979).

Many procedures invove a relatively slow and short centrifugation. Such a "clearing spin" pellets most of the cell debris and chromosomal DNA, which remains intact or is only fragmented into a few large pieces; the smaller plasmid molecules, usually contaminated with small pieces of chromosomal DNA, remain in the supernatant, the"cleared lysate" (Broda, 1979).

Another widely used method is velocity gradient centrifugation (Hardy, 1981). Here, the molecules move downwards through a preformed gradient of sucrose solution at rates that depend upon their mass and conformation (Broda, 1979).

Purification of plasmid DNA can be achieved in many ways. Cohen and Miller (1969) used the alkaline denaturation and neutralization of DNA in cell extracts method with success, whereas Radloff et al, (1967) used the dye-buoyant density centrifugation method. In the latter method, ethidium bromide was used (Broda, 1979).

Simplified methods for plasmid isolation have been developed. These methods use fewer cells than the previous methods, and can be used to screen large numbers of clones. The same methods are fast, relatively inexpensive and safe (Broda, 1979). These simpler methods of plasmid isolation have been reviewed extensively

(Meyer et al, 1976; Barnes, 1977; Telford et al., 1977; Hansen and Olsen, 1978a; Eckhart, 1978).

9.5:2.2 Plasmid fingerprinting Methods

Plasmid "finger printing" by agarose gel electrophoresis and restriction endonuclease analysis to identify epidemic strains of bacteria is one of the most simple and straightforward technique ever developed by molecular biologists for epidemiologic investigations (Farrar, 1983). Both gram-negative bacilli (Schaberg et al., 1981; John and McNeill, 1981; Markowitz et al., 1983 and John et al., 1983) and Staphylococci (McGowan et al., 1979 and Archer et al., 1982) have been studied by these techniques (Farrar, 1983).

Since most bacteria seem to contain plasmids differing in size and number, the plasmid content or pattern of a bacterial strain may be specific during a limited interval and in a limited area. This property, together with the ease with which the plasmid pattern can be determined by agarose gel electrophoresis, has rendered the determination of such patterns a potentially powerful tool for epidemiologic studies (Brunner et al., 1983).

Chabbert et al., (1979) contended that, since plasmids can spread from one bacterial species to another by conjugation, sometimes it is the plasmid rather than the bacterial strain which is epidemic. A study conducted by O'Brien et al. (1982) on three different

Salmonella serotypes; Salmonella typhimurium variant Copenhagen, Salmonella newport and Salmonella dublin collected from different laboratories in United States found identical or nearly identical antibiotic-resistant plasmids in strains of Salmonella serotypes isolated from animals and humans in widely separated parts of the country. The clustering in time of geographically dispersed cases suggested contaminated food products as sources of infection (Farrar, 1983).

Several authors (Brunner et al., 1983; Riley and Cohen, 1982; Riley et al., 1983 and Holmberg et al., 1984) have reported the effectiveness of plasmid profile analysis as a tool for studying the epidemiology of Salmonella infections. Brunner et al. (1983) and Holmberg et al. (1984) found this method to be as specific as phage typing, and superior to biotyping and drug-resistance testing, in tracing the source of Salmonella typhimurium epidemics.

Farrar (1983) concludes that plasmid profile analysis using techniques such as agarose gel electrophoresis and restriction endonuclease analysis have proved successful in the investigation of hospital associated infections as well as outbreaks of Salmonellosis in community setting. Unlike other methods such as phage typing, serotyping and biotyping, this approach can be used to identify epidemic plasmids that have spread through several different bacterial species. These techniques are rapid, straightforward, and can be applied to drug-sensitive as well as drug-resistant strains provided the organisms contain plasmids, and that this approach

is especially useful for investigation of organisms for which no standard typing system is available.

10.0 CHARACTERIZATION OF SALMONELLA LIVINGSTONE

10.1 Cultural and Biochemical characteristics

Salmonella livingstone belongs to subgenus I of Kaufmann (1966). The organism is gram-negative, motile, and produces on MacConkey agar, colonies typical of the Salmonella group of organisms. The organism ferments glucose, maltose, mannitol, dulcitol, sorbitol, arabinose, rhamnose., xylose and trehalose with production of acid and gas. Lactose, sucrose, salicin, adonitol, raffinose and inositol are not fermented. The citrate utilisation test is positive, Hydrogen sulphide is produced, but indole is not formed and urea is not hydrolysed. The Voges-Proskauer test is negative, the methyl red test is positive and gelatin is not liquefied at 22° C or 37° C (Picton and Stirrup, 1953).

10.2 Serologic classification

Salmonella livingstone belongs to the serologic group C (Kaufmann, 1966). The somatic (O) antigenic formula is 6,7 whereas the flagella (H) antigenic formula is d, d_2, d_5 (phase I) and l, w (phase II) (Picton and Stirrup, 1953). The recognized antigenic structure of the organism is 6,7 = d = l, w (Ewing, 1986).

10.3 Diseases caused by Salmonella Livingstone

10.3:1 Animals

The organism has not been known to cause any major problem in domestic animals even though it has been commonly isolated from them (MAFF Annual Summaries, 1976-1984). It could possibly be responsible for mild gastroenteritis in these animals or the latter could merely be acting as sources of infection for humans, as evidenced by a high prevalence of the organism isolated from poultry and other game birds in England and Wales (MAFF Annual Summaries, 1976-1984).

10.3:2 Man

The organism has been reported to cause severe enteritis in humans (Picton and Stirrup, 1953; Andersson, 1964). The first ever reported cases of infection with Salmonella livingstone were in 1951, in two members of the B.O.A.C. staff who had severe attacks of diarrhoea while staying at a hotel in Livingstone town on Victoria falls. These patients were able to fly the next morning after medical attention in a hospital. Later on, a waiter at the hotel volunteered information that such cases were very common among guests (Picton and Stirrup, 1953) suggesting a common source of infection, food being the most likely.

10.4 Epidemiology of Salmonella livingstone

Salmonella livingstone is non-specific as far as host adaptibility is concerned (Clarke and Gyles, 1986). The organism has thus been isolated from a variety of sources which include environment, feed, poultry, wild birds, pigs, cattle and other animals (MAFF Annual Summaries, 1976-1984).

According to the Ministry of Agriculture, Fisheries and Food (MAFF) annual summaries of 1976-1984, the major serotypes isolated from animals and birds in England and Wales, were Salmonella typhimurium, Salmonella dublin, Salmonella seftenberg, Salmonella hadar, Salmonella montevideo, Salmonella agona, Salmonella Virckow, Salmonella heidelberg, Salmonella derby and Salmonella Indiana. Salmonella livingstone and other serotypes are infrequently isolated.

11.0 OBJECTIVES OF THE STUDY

The objectives of the study were:

1. To determine whether the serotype Salmonella livingstone is a homogenous group when examined by methods other than serological methods for example, biotyping, antimicrobial susceptibility testing and plasmid profile analysis.
2. To find out whether observed minority types by any one method used in the study are:
   a) Closely related to the majority types when all methods are combined,
   b) are derived from the majority types by invivo variation

in typing characters

or

c) are distinct.

3. To find out which one of the methods used in the study best correlates epidemic strains and source.

12.0 PLAN OF STUDY

The study was carried out in two main phases as follows:

12.1 Phase I

This consisted of the following:

1) Biotyping

Biotyping using the method of Duguid et al (1975) on Salmonella typhimurium as a model. This was carried out in two steps:

a) preliminary biotyping of a few isolates of Salmonella livingstone using as many characters as possible so as to determine the most discriminating.

b) secondary biotyping of all the available isolates of Salmonella livingstone using the most discriminating characters as determined above.

2) Antimicrobial Susceptibility testing

This was also carried out in two steps as follows:

a) Preliminary antimicrobial susceptibility testing of a few isolates of Salmonella livingstone using as

many antimicrobial agents as possible.

b) Antimicrobial susceptibility testing of all Salmonella livingstone isolates using the most discriminating antimicrobial agents as determined by (a) above.

12.2 Phase II

Plasmid profile analysis of all isolates of Salmonella livingstone.

Deviations from main plan of study

1) An experiment to find out whether antimicrobial resistance in Salmonella livingstone is plasmid mediated and transferrable.

2) An experiment to find out whether certain strains of Salmonella livingstone survive longer in the environment than others.

II MATERIALS AND METHODS

1.0 SAFETY PRECAUTIONS

1.1 *Salmonella livingstone* belongs to the category of the partially restricted organisms, according to the Code of practice for the prevention of laboratory acquired infection of the Department of Microbiology, University of Surrey. The general safety precautions of the department regarding the handling and disposal of infected material were observed.

2.0 SOURCE AND PRESERVATION OF CULTURES

2.1 SOURCE

Seventy one isolates of *Salmonella livingstone* from 15 veterinary investigation centres in England and one centre in the Kingdom of Saudi Arabia, collected during the period 1984 to 1986, were used in the study. The sources of the cultures included 52 from feed, 10 from poultry, 5 from the environment, 3 from pigs and one from a calf(Tables2 and3, Figure2)

2.2 PRESERVATION

The isolates had been subcultured several times and stored on Dorset egg slopes at room temperature and in the dark when I received them. I accepted the identification of the strain as Salmonella livingstone made by the supplier, but confirmed them as Salmonella by serotyping using both polyvalent "O" and polyvalent "H" (phase 1 and 2) antisera.

TABLE 2 LIST OF ISOLATES OF SALMONELLA LIVINGSTONE USED IN THE STUDY

Serial No.	Reference No.	V.I Centre	Animal Species or Source
1	39/85	Liverpool	Poultry
2	339/85	Lincoln	Pig
3	331/87	PFN	Poultry
4	565/86	Thirsk	Poultry
5	703/86	Sutton B.	Feed
6	1932/85	Langford	Feed
7	338/86	Chester	Feed
8	2161/86	Moulton	Pig
9	5385/86	Reading	Pig
10	285/85	Cambridge	Feed
11	97/85	Penrith	Poultry
12	4593/86	Thirsk	Poultry
13	12/85	Winchester	Bovine
14	506/86	Cambridge	Poultry
15	1917/86	Norwich	Feed
16	177/86	Leeds	Environment
17	613/85	Worcester	Poultry
18	547/86	Norwich	Feed
19	3276/84	Cambridge	Feed
20	3277/84	"	"
21	3278/84	"	"
22	4372/84	"	"

23	4374/84	Cambridge	Feed
24	4375/84	"	"
25	4645/84	"	"
26	4649/84	"	"
27	1374/85	Cambridge	Feed
28	1375/85	"	"
29	2625/85	"	"
30	2626/85	"	"
31	505/86	"	Poultry
32	506/86	"	"
33	507/86	"	"
34	2712/85	Norwich	Feed
35	2713/85	"	"
36	2714/85	"	"
37	3229/85	"	"
38	3230/85	"	"
39	3235/85	"	"
40	3239/85	"	"
41	3240/85	"	"
42	3241/85	"	"
43	3242/85	"	"
44	3243/86	"	"

45	549/86	Norwich	Feed
46	551/86	"	"
47	552/86	"	"
48	554/86	"	"
49	3009/86	Preston	"
50	3010/86	"	"
51	3011/86	"	"
52	3066/86	"	"
53	3067/86	"	"
54	3226/86	"	"
55	3227/86	"	"
56	4098/86	Preston	Feed
57	4099/86	"	"
58	4100/86	"	"
59	410186	"	"
60	4102/86	"	"
61	4103/86	"	"
62	4104/86	"	"
63	1123/86	Barton Hole	"
64	1124/86	"	"
65	1126/86	"	"
66	1128/86	"	"
67	1131/86	"	"
68	2271/85	Leeds	Environment
69	3019/85	"	"
70	3186/85	"	"
71	4702/85	"	"

TABLE 3

VETERINARY INVESTIGATION CENTRES FROM WHICH THE SALMONELLA LIVINGSTONE ISOLATES WERE OBTAINED

V I Centre	No. of Isolates
Barton Hole	5
Cambridge	20
Chester	1
Langford	1
Leeds	5
Lincoln	1
Liverpool	1
Moulton	1
Norwich	14
Penrith	1
PFN	1
Preston	14
Reading	1
Sutton Bonington	1
Thirsk	2
Winchester	1
Worcester	1
Total No. of isolates	71

Figure 2 Map of Great Britain showing the Veterinary Investigations centres from which the 71 isolates of Salmonella livingstone came from.

Footnote

1. Penrith (1)*
2. Thirsk (2)
3. Preston (14)
4. Leeds (5)
5. Liverpool (1)
6. Chester (1)
7. Lincoln (1)
8. Worcester (1)
9. Sutton-Bomington (1)
10. Norwich (14)
11. Cambridge (20)
12. Langford (1)
13. Reading (1)
14. Winchester (1)

A = SCOTLAND; B = NORTHERN REGION; C = THE LONDON MIDLANDS
D = EASTERN REGION; E = WESTERN REGION; F = GREATER LONDON
G = SOUTHERN REGION

* = No of isolates

The cultures received were plated onto desoxycholate citrate agar (DCA) and a single colony of morphology conforming to the genus Salmonella was picked and subcultured onto Nutrient agar plate. A single colony was picked and subcultured on nutrient agar slabs to serve as the stock for preparation of inocula for the subtyping tests. Cultures on the nutrient agar slabs were stored at room temperature and in the dark.

3.0 STERILIZATION

Except where stated, culture and characterisation media were sterilized by autoclaving. The following recommended total holding periods in steam at 121° C and 15 lb pressure were observed; 12 minutes, for 10 ml volumes in tubes tightly packed in wine baskets; 15 minutes for volumes up to 100 ml in bottles or flasks; 20-25 minutes for 500 ml volumes; 25-30 minutes for 1000 ml volumes and 35-45 minutes for 2000 ml volumes (Cruickshank et al., 1969).

4.0 CULTURE MEDIA

4.1 BIOTYPING MEDIA

4.1:2 Nutrient broth (NB)

Oxoid nutrient broth (pH 7-4) was prepared and dispensed into 3 ml volumes in bijoux bottles and then sterilized by autoclaving.

4.1:3 Nutrient agar (NA)

This was Oxoid nutrient agar (pH 7-4) prepared and sterilized according to Cowan and Steel's Manual (Cowan, 1986).

4.1:4 Semi-solid agar (SSA)

This was Oxoid nutrient broth and Oxoid nutrient agar mixed in 3 : 1 v/v proportions, dispensed in 10 ml volumes in universal bottles and then sterilized by autoclaving.

4.1:5 Sugar peptone Waters

These were prepared by dissolving into one litre of distilled water, 1.5% Oxoid peptone water (peptone, Oxoid L37, 0.85% NaCl in deionised water, pH 7-2) and 0.002% bromocresol purple (purple at PH 6-8 and yellow at PH 5-2) indicator. These were dispensed in 3 ml volumes in bijoux bottles and sterilized by autoclaving.

4.1:6 Test Sugars

These were prepared as 10% (w/v) solutions in sterile distilled water and steamed at 100° C for 1 h. The solutions were then added to separate volumes of autoclaved peptone waters with bromocresol purple and the mixtures dispensed in 3 ml volumes into screw-capped bijoux bottles, and then steamed at 100 C for 30 minutes. The glucose bottles contained Durham tubes for detection of gas production. (Duguid et al., 1975).

4.1:7 Bitter medium (Bitter et al., 1926)

This medium contained in one litre of distilled water, Bacto-peptone 0.05 g, Na_2HPO_4 $2H_2O$-0.5 g, $(NH4)_2SO_4$-1.0 g, Sodium citrate

2.0 g, and bromocresol purple 0.02 g; The pH was 7-1. The test sugars, Xylose and Rhamnose prepared and sterilized separately were added to a concentration of 0.5% and the complete medium dispensed in 3 ml amounts in screw-capped bijoux bottles and steamed at 100 C for 30 minutes (Duguid et al., 1975).

4.1:8 Stern's (1916) glycerol medium (Cruickshank et al., 1969, page 823)

This medium contained in 1 litre of distilled water, Lab-Lemco meat extract 10 g and Dofco Bacto-Peptone 20 g (pH 8.0) to which was added 2 ml of a 10% solution of basic fuchsin in ethanol, 16.6 ml of a fresh 10% solution of Na_2SO_3 in water and 10 ml of glycerol. It was dispensed in 3 ml volumes in screw-capped biloux bottles and sterilized by autoclaving.

4.1:9 Tartrate peptone waters

These were for turbidity tests for the utilisation of dextro (d, i.e,+) and levo (l, i.e,-) tartaric acids and were prepared as by Alfredsson et al., (1972). The tartaric acids d-tartaric acid and l-tartaric acid were from BDH chemicals. The acids were made as - 10% solutions in distilled water, neutralised with 5N NaOH and autoclaved. These were then added to sterile peptone water so that the completed medium contained tartaric acid 10 g, Oxoid L37, peptone 6-7 g and NaCl 3.3 g to 1 litre of distilled water, pH 7-2. This mixture was dispensed in 10 ml volumes into plastic-capped test-tubes (1.5 x 15 cm). For growth of control cultures, part of the batch of peptone water was brought to the same final concentration by the addition of distilled water instead of tartrate solution.

4.2 Desoxycholate citrate agar (DCA) (Cruickshank, 1969 p. 762)

This was Oxoid desoxycholate citrate agar (CM227) of Hynes (1942). 5.2 g of this medium were suspended in 1 litre of distilled water which was then brought to the boil to dissolve completely. The medium was then allowed to cool to 56° C in a water-bath and then dispensed in single vent petri-dishes in 14 ml amounts, and allowed to dry.

4.2:1 Simmon's citrate (Cruickshank, 1969 p. 832)

This medium was prepared by adding to each litre of Koser's medium (Oxoid, CM65) 20 g of washed agar and 40 ml of 1 in 500 aqueous solution of bromothymol blue.

4.2:2 Triple Sugar Iron agar (TSI) (Report, 1958)

Oxoid triple sugar iron agar (CM277) was used. 65 g of this medium was suspended in 1 litre of distilled water and brought to the boil to dissolve completely. The medium was dispensed in 10 ml amounts into universal bottles and autoclaved. The bottles were then set in sloped form with a butt about 1 in long.

4.3 BIOTYPING METHODS

4.3:1 Standardisation of the inocula

The inocula was standardised by plating out the stock culture on Nutrient agar (Oxoid) and after growth for 18-24 h at 37 C, one isolated colony was subcultured onto 1 cm by 1 cm area on a Nutrient agar plate incubated for growth for 18-24 h at 37 C. The growth on the 1 cm x 1 cm area was harvested using a sterile wire

loop and suspended in saline (0.85% NaCl) solution to a density of approximately 1.9 x 10^3 bacteria per ml which was equivalent to an optical density of 0.5 as determined by a turbidimetric method using a spectrophotemeter (spectronic 20, Bausch and Lomb) at a wavelength of 500 mm (Grehardt et al., 1981).

A similar cell suspension with an optical density of 0.5 was prepared and preserved in 2.5% formalin and stored at 4 C to serve as a standard for subsequent preparations of inocula. Subsequent suspensions were thus compared with this standard and 0.25 ml of these was inoculated into each of the typing medium using a sterile Pasteur pipette.

4.3:2 Methods of tests

4.3:3 Preliminary biotyping

Eighteen stock cultures on Nutrient agar slabs were plated for single colonies on Desoxycholate citrate agar (DCA) and incubated for 18-24 h at 37 C. A single colony of each isolate was picked from the DCA cultures and subcultured onto an area of 1 cm x 1 cm on a Nutrient agar plate and grown for 18-24 h at 37 C. The growth was harvested and suspended in saline (0.85%10NaCl) and standardised using the formalinised stock cell suspension.

The cell suspensions were inoculated into the biotyping media as follows:

a) a Nutrient agar slab using a sterile wire loop to serve as a stock culture for subsequent tests;

b) a test-tube of semi-solid agar using a sterile straight wire;

c) using a sterile Pasteur pipette, 0.25 ml of each cell suspension were inoculated into the following:

i) Glucose in peptone water

ii) Lactose in peptone water

iii) Dulcitol in peptone water

iv) Arabinose in peptone water

v) Trehalose in peptone water

vi) xylose in peptone water

vii) Sorbitol in peptone water

viii) Raffinose in peptone water

ix) meso-Inositol in peptone water

x) Simmons citrate

xi) Mucate

xii) Triple sugar iron (TSI)

xiii) Bitter rhamnose

xiv) Bitter xylose

xv) d-tartrate

xvi) l-tartrate

xvii) Stern's glycerol

xviii) peptone water to serve as control for turbidity tests.

xix) Nutrient broth

d) using a sterile Pasteur pipette, one drop of each cell suspension was transferred onto:

i) plate of minimum agar medium supplemented with glucose,

ii) plate of minimum agar medium supplemented with gly-

cerol

iii) plate of minimum agar medium supplemented with glycerol and m-tartrate, and,

iv) Nutrient agar plate to serve as control for growth.

4.3:4 Incubation of tests

All tests were incubated aerobically at 37 C. The tests were kept static and caps were loosely applied to screw-capped bottles and test-tubes to ensure free access of air. Tests for fermentation of m-Inositol were incubated in parallel at both 37 C and 25 C (waterbath). All tests were incubated for 18-24 h except Simmon's citrate and Stern's glycerol which were incubated for 96 h and 48 h respectively. The plates for growth on minimum agar medium were enclosed in plastic bags to minimize drying before being incubated aerobically at 37 C for 24 h and 48 h.

4.4 Reading of tests (Duguid et al, 1975)

4.4:1 Fermentation of Sugars in peptone water

These tests were observed for acid and gas production. The indicator used was bromocresol purple which is purple in colour at pH 6.8 and yellow at pH 5.2. Therefore, a change in colour of the test media from purple to yellow within 18-24 h was taken to be positive for acid production. Only bottles containing glucose had durham tubes and these were observed for both acid and gas production. The m-Inositol test in a waterbath at 25 C was read after 48 h incubation.

4.4:2 Fermentation of Sugars in Bitter media

These tests were observed for acid production which was indicated by a colour change of the indicator used (bromocresol purple) from purple to yellow within 18-24 h.

4.4:3 Tartrate utilisation (Turbidity) tests

The tests were observed for turbidity and the difference between tartrate-containing and peptone water cultures (control) compared with the naked eye. The test culture was read as positive if it was more turbid than the control culture (Alfredsson et al., 1972).

4.4:5 Test for growth on minimum agar media

The minimum agar media cultures were observed for the presence and amount of growth of each inoculation after 24 h and 48 h incubation. The growth was compared between all the three culture plates and to the nutrient agar plate culture which served as a control for viability of the isolate. The test was interpreted as positive for utilisation of m-tartrate when there was more growth on the plate of minimum medium agar supplemented with glycerol and m-tartrate than on the plates of minimum media supplemented with either glucose or glycerol (Alfredsson et al., 1972).

4.4:6 Stern's glycerol test

This test was observed for utilisation of Stern's glycerol which is indicated by a colour change of test media from yellow to deep red. The reaction depends on the ability of an organism to convert glycerol into an aldehyde product which recolorises fuchsin-sulphite (Cruickshank et al., 1969).

4.4:7 Mucate test

This test was observed for the utilisation of mucic acid which was indicated by a colour change in the indicator used (bromothymol blue) which is blue at pH 7-4 and yellow at an acid pH. Therefore, a change in the colour of the test media from blue to yellow was interpreted as positive for utilisation of mucic acid (Cowan, 1985).

4.4:8 Simmon's citrate

This was done to test the ability of the isolates to utilise citrate as the sole source of carbon and energy for growth. A positive reaction was indicated by a change in the colour of the indicator (bromothymol blue) from the original green colour to blue, and a streak of growth in the slab (Cruickshank et al., 1969).

4.4:9 Hydrogen Sulphide (H_2S) test

The Triple Sugar Iron (TSI) agar (Sulkin and Willet, 1940) was used to test for the ability of an organism to produce Hydrogen sulphide gas. A positive reaction was indicated by blackening of the test media after incubation for 18-24 h at 37 C. (Ewing, 1986).

4.5 Secondary biotyping

4.5:1 Phase I

All the 71 isolates of Salmonella livingstone were biotyped using tests which had been found to be discriminating in the preliminary biotyping. The tests carried out included those for utilisation of Stern's glycerol, d-tartrate and l-tartrate and mucate. The

methods used were the same as those used in the preliminary biotyping. These tests were repeated three times in order to check for their reliability, stability and reproducibility.

4.5:2 Phase II

All the 71 isolates of Salmonella livingstone were biotyped using the most discriminating, reliable, stable and reproducible tests as determined by phase I of secondary biotyping. The methods used were the same as above.

5.0 ANTIMICROBIAL SUSCEPTIBILITY TESTS

5.1 MATERIALS AND METHODS

5.1:1 Culture media

The culture media used was Diagnostic sensitivity test (DST) agar (Oxoid, CM261). The media was prepared by suspending 40 g in 1 litre of distilled water, boiling to dissolve completely and then sterilising by autoclaving. The media was then poured onto plastic petri dishes (single vent) in 15 ml amounts, after cooling to 56 C in a waterbath. The plates were kept at 4 C overnight when not in use.

5.1:2 Antimicrobial agents used

The following antimicrobial agents were used:

Chloramphenicol (C_{10})	10 ug/ml
Streptomycin (S_{10})	10 ug/ml

Neomycin (N_{10})	10 ug/ml
Tetracycline (TE_{10})	10 ug/ml
Ampicillin (AMP_{10})	10 ug/ml
Co-trimoxazole (SXT_{25})	25 ug/ml
Furazolidone (FR_{15})	15 ug/ml
Compound Sulphonamide ($S3_{50}$)	50 ug/ml
Cephaloridine (CR_{25})	25 ug/ml
Colistin Sulphate (CT_{10})	10 ug/ml
Kanamycin (K_{30})	30 ug/ml
Nalidixic acid (NA_{30})	30 ug/ml
Carbenecillin (CAR_{100})	100 ug/ml
Penicillin G ($P_{1.5}$)	1.5 ug/ml
Erythromycin (E_{10})	10 ug/ml
Methicillin (MET_{10})	10 ug/ml
Sulphadiazine (SD_{50})	50 ug/ml
Novobiocin (NV_5)	5 ug/ml
Lincomycin (MY_2)	2 ug/ml
Cloxacillin (OB_5)	5 ug/ml
Fusidic acid (FD_{10})	10 ug/ml
Sulphafurazole (SF_{500})	500 ug/ml

The above antimicrobial agents were contained in the standard multodisks (Oxoid) with the following codes: U2, S2, U4, 2023E and 8725E. The multodisks were kept at - 20 C when not in use and were allowed to thaw for 1-2 h at room temperature before use.

5.2 METHODS

5.2:1 Preliminary antimicrobial susceptibility test

Eighteen Isolates of Salmonella livingstone used in the preliminary biotyping were tested for susceptibility against all the 22 listed antimicrobial agents above. Cruickshank et al (1969, p.900) method was used. The stock cultures on nutrient agar slabs were plated for single colonies on DCA agar and grown for 18-24 h at 37 C. A single colony was subcultured onto a nutrient agar plate and grown for 18-24 h at 37 C. Three isolated colonies from the nutrient agar plate culture were inoculated into 3 ml of nutrient broth (Oxoid) using a sterile wire loop. The nutrient broth cultures were incubated for 2 h at 37 C.

The broth cultures were then inoculated onto DST agar plates using a sterile Pasteur pipette. The entire surface of the DST agar plate was irrigated with the broth culture and the excess was drained and pipetted off. The inoculated DST agar plates were then left on the bench in an upright position for 1 h to dry. After drying, the multodisks (after thawing) were applied onto the surface of the plates using a sterile pair of thumb forceps. The plates were incubated for 18-24 h at 37 C. The NCTC strain of E. coli which is susceptible to all the antimicrobial agents on multodisk 8725E was used as a control. This was repeated three times.

5.2:2 Secondary antimicrobial susceptibility test

70 isolates of Salmonella livingstone were tested for susceptibility against all the antimicrobial agents found to be discriminating in the preliminary susceptibility test. The method used was the same as above. These tests were also repeated three times for each antimicrobial agent to check for reliability, stability and reproducibility.

5.3 Reading of antimicrobial susceptibility tests

The cut-off point between susceptibility and resistance to any of the drugs used was determined arbitrarily as used by Jones (1986), by taking the diameter of zone of growth inhibition that gave the best discrimination between the isolates tested. A zone of inhibition of diameter 13 mm or less was taken to indicate resistance whereas a zone of 14 mm or more in diameter indicated susceptibility to that particular drug.

6.0 PLASMID PROFILE ANALYSIS

6.1 Materials and Methods

6.1:2 Lysing Solution
SDS/TRIS

6.06 g TRIS (BDH GPR 227119)

30.0 g SDS (BDH. Spec. pure. 44215)

The above was made up to 1 litre by adding 1.5 ml. 2M NaOH to 98.5 ml SDS/TRIS just before use. The lysing solution

was prepared freshly each day.

6.1:3 E buffer 10X

10.9 g TRIS

55.6 g Boric acid (FSA,A.R. B3800)

9.3 EDTA (Koch, A.R 2534.00)

The above were dissolved in 990 ml of distilled water and then made to 1 litre by adding 10 ml of concentrated hydrochloric acid (pH 8.0). This buffer was diluted 1 : 10 in distilled water before use because the stock solution was prepared to 10 times strength.

6.1:4 Ethidium bromide 20X (Sigma E 8751)

The stock solution was made by dissolving 20 mg of ethidium bromide in 1 litre of distilled water. For use, this solution was diluted 1 : 20 (1 mg/1 ml) with tap water.

6.1:5 Phenol-chloroform solution

Frozen neat phenol (BRL redistilled 5509) from - 20 C freezer (in 2.5 ml volumes in bijoux) was removed and placed in 37 C incubator for 15 minutes to thaw. An equal amount (2.5 ml) of chloroform was added and mixed till dissolved.

6.1:6 Equipment

Centrifuge

Mini bench top centrifuge Eppendorf 5414.

6.1:7 Electrophoresis cell

Horizontal submerged type BRL H_4

6.1:8 Photographic equipment

Camera	Polaroid CUS
Filters	2B23A
Film	Poleroid 667
Speed	1 sec
Aperture	f8/f11

6.1:9 Transilluminator

UVP	TM 20

6.2 Agarose

Agarose Type II (Sigma A 6877)

6.3 Methods (Modified Kado and Li.u method, 1981)

6.3:1 Preparation of cells for plasmid analysis

Isolates were plated onto nutrient agar for single colonies and incubated overnight at 37 C. A single colony was picked from each plate using a sterile wire loop and inoculated into 3 ml of Luria-Bertani broth (Bacto-tryptone 10 g; Bacto-yeast extract 5 g and NaCl 5 g in 1 litre of distilled water) in bijoux and incubated overnight at 37 C.

6.3:2 Cell lysis and plasmid purification

The overnight broth cultures were decanted into micro-centrifuge cups (until nearly full - approximately 1.5 ml), and centrifuged for 2½ minutes. The supernatant was poured off in a biohazard cabinet immediately after the centrifuge had stopped. The cell pellets were mixed by flicking centrifuge cups over rack and then 200 ml of lysing solution added. These were then heated in a waterbath at 56 C for 35 minutes.

The samples were cooled in a +4C fridge for 5-10 minutes. 200 ml of freshly prepared phenol-chloroform solution which had been in a fridge for 5 minutes were added to the samples and the mixture emulsified by shaking for approximately 10 seconds. About 100 ml of the upper aqueous phase were carefully pipetted into a fresh tube using a pipette tip cut to give a larger bore (this reduces risk of aspirating interface).

6.3:3 Electrophoresis, staining and photographing

1.75 g of agarose were dissolved into 250 ml of E buffer and heated gently over bunsen flame until boiling. This was heated further for 5 minutes at low heat until the agarose had completely dissolved. It was then placed in a waterbath at 56 C for 10 minutes then cooled for 5-10 minutes to approximately 45-50 C on bench before being poured into a warmed gel former (37 C) on a levelling tray and allowed to set at room temperature.

6.3:3.1 Agarose gel electrophoresis

The prepared samples were loaded into wells (about 30 ml) after which the gel was set into the electrophoresis tank which was carefully filled, each half being done at a time, with E buffer until the ends of the gel slab (but not the surface) were covered. The gel was electrophoresed at 150 V for 5 minutes and then stopped. The surface of the slab was flooded with a minimum volume of E buffer and electrophoresed at 136 V for 3 hours.

6.3:3.2 Staining of the gel slab

The gel slab was removed from the electrophoresis tank and placed into ethidium bromide stain (1 mg/ml) in a basin for 30 minutes. The gel slab was then washed in a basin of tap water for 10 minutes.

6.3:3.3 Photographing of the gel slab

The gel slab was placed onto a U/V transilluminator and photographed using a polaroid camera. U/V safety goggles were worn to protect the eyes from U/V light.

7.0 DEVIATIONS FROM PLAN OF STUDY

7.1 AN EXPERIMENT TO FIND OUT WHETHER ANTIMICROBIAL RESISTANCE IN SALMONELLA LIVINGSTONE IS PLASMID MEDIATED AND TRANSFERRABLE

7.1:1 Materials and Methods

An isolate previously found to be susceptible to streptomycin, compound sulphonamide and sulphafurazole, and another one known to be resistant to the three aforementioned drugs were each plated on nutrient agar for single colonies and incubated at 37 C for 18-24 h. A single colony from each plate was picked and inoculated into the same 3 ml of nutrient broth and incubated for 18-24 h at 37 C.

The broth culture was diluted (1 : 100,000) and plated out for single colonies. 41 isolated colonies were both biotyped and tested for susceptibility to the three antimicrobial agents mentioned above.

As a control, the original two isolates were tested in parallel to the 41 mixed isolates. The susceptible isolate was also inoculated, grown in nutrient broth, diluted as above and plated out for single colonies. 31 isolated colonies were tested for susceptibility against the antimicrobial agent that was discriminating between the 41 isolates of the cocktail broth culture.

7.2 EXPERIMENTS TO FIND OUT WHETHER THERE IS A DIFFERENCE IN THE SURVIVAL RATES BETWEEN THE DIFFERENT BIOGROUPS OF SALMONELLA LIVINGSTONE

7.2:1 Heat tolerance

7.2:1.1 Materials and Methods

Four cultures of Salmonella livingstone, one from each of the 4 biogroups were plated out for single colonies on nutrient agar plate. Isolated colonies were inoculated into nutrient broth in duplicates and incubated overnight at 42 C and 44 C respectively. They were observed for growth and subcultured onto nutrient agar plates. Those showing growth were reinoculated into nutrient broth and incubated in a waterbath at 46 C.. Those still growing were subcultured and inoculated into nutrient broth which was incubated overnight at 50 C.

7.2:2 Survival in sterile tap water at room temperature

7.2:2.1 Materials and Methods

Four isolates, each from a different biogroup were plated on nutrient agar for single colonies. Each isolated colony was inoculated into 3 ml of sterile tap water which was left on the bench at room temperature for 2 weeks. Meanwhile the cultures were being subcultured onto nutrient agar plates every 3 days.

7.2:3 Reading of tests

7.2:3.1 Heat tolerance

Growth of subcultures on nutrient agar was interpreted as tolerance to that particular temperature of exposure. Lack of growth on nutrient agar plate was interpreted as intolerance.

7.2:3.2 Survival in sterile tap water

The duration of survival in sterile tap water was determined by the No of positive nutrient agar subcultures obtained in two weeks. The higher the number, the ~~lower~~ higher the survival rate of an isolate.

III RESULTS

1.0 BIOTYPING

1.1 Preliminary biotyping

Eighteen isolates of Salmonella livingstone were biotyped using 21 biological characters or tests (Table 4). All the 18 isolates were positive in all tests except for lactose, raffinose and meso-inositol fermentation, and tests for the utilisation of mucic acid, tartaric acid isomers (l-and d-) and Stern's glycerol which discriminated between the isolates. On repeated testing, the test for the utilisation of mucic acid was found not to be reproducible hence the designation variable (V) in Table 4, which represents those isolates which gave variable results in this test.

Tests for the utilisation of d-tartaric acid, l-tartaric acid and Stern's glycerol gave consistent and reliable results when repeated three times on different occasions. Similar results have been found with the tartaric acid isomers on isolates of Salmonella typhimurium (Alfredsson et al., 1972; Duguid et al., 1975).

The turbidity test for the utilisation of d-tartaric acid divided the 18 isolates of Salmonella livingstone tested into 11 (61%) positives and 7 (38.9%) negatives; the l-tartaric acid test gave 9 (50%) positives and 9 (50%) positives, whereas, Stern's glycerol test gave 12 (66.7%) positives and 6 (33.3%) negatives (Table 4).

TABLE 4 PRELIMINARY BIOTYPING OF SALMONELLA LIVINGSTONE

SERIAL No.	MOT.	GLU.	LAC.	CIT.	DUL.	ARAB.	TRE.	XYL.	SORB.	RAFF.
1	+	+	-	+	+	+	+	+	+	-
2	+	+	-	+	+	+	+	+	+	-
3	+	+	-	+	+	+	+	+	+	-
4	+	+	-	+	+	+	+	+	+	-
5	+	+	-	+	+	+	+	+	+	-
6	+	+	-	+	+	+	+	+	+	-
7	+	+	-	+	+	+	+	+	+	-
8	+	+	-	+	+	+	+	+	+	-
9	+	+	-	+	+	+	+	+	+	-
10	+	+	-	+	+	+	+	+	+	-
11	+	+	-	+	+	+	+	+	+	-
12	+	+	-	+	+	+	+	+	+	-
13	+	+	-	+	+	+	+	+	+	-
14	+	+	-	+	+	+	+	+	+	-
15	+	+	-	+	+	+	+	+	+	-
16	+	+	-	+	+	+	+	+	+	-
17	+	+	-	+	+	+	+	+	+	-
18	+	+	-	+	+	+	+	+	+	-

continued;

TABLE 4 PRELIMINARY BIOTYPING OF SALMONELLA LIVINGSTONE

SERIAL No.	m-INO	B.XYL	B.RHA	MU	H S	d-T	l-T	S.GLY	mMG	mGLY	mGLY+m-T
1	-	+	+	-	+	-	-	-	+	+	+
2	-	+	+	v	+	+	+	+	+	+	+
3	-	+	+	-	+	-	-	-	+	+	+
4	-	+	+	-	+	-	-	-	+	+	+
5	-	+	+	v	+	+	+	+	+	+	+
6	-	+	+	v	+	-	+	+	+	+	+
7	-	+	+	v	+	-	-	-	+	+	+
8	-	+	+	v	+	+	-	+	+	+	+
9	-	+	+	v	+	+	+	+	+	+	+
10	-	+	+	-	+	+	+	+	+	+	+
11	-	+	+	v	+	-	-	-	+	+	+
12	-	+	+	v	+	+	-	+	+	+	+
13	-	+	+	v	+	+	+	+	+	+	+
14	-	+	+	+	+	+	+	+	+	+	+
15	-	+	+	v	+	-	-	-	+	+	+
16	-	+	+	-	+	+	-	+	+	+	+
17	-	+	+	v	+	+	+	+	+	+	+
18	-	+	+	+	+	+	+	+	+	+	+

1.2 Biotyping of the full collection of the 71 isolates of Salmonella livingstone

All the 71 isolates of Salmonella livingstone were biotyped using the three tests found to be not only discriminating but also reproducible during the preliminary biotyping. These were tests for utilisation of l-tartaric and d-tartaric acids, and the Stern's test for aldehyde production from glycerol. Table 5 shows the results obtained.

The turbidity test for the utilisation of d-tartaric acid divided the 71 isolates of Salmonella livingstone into 39 (54.9%) positives and 32 (45.1%) negatives, whereas l-tartaric acid had 38 (53.5%) positives and 33 (46.5%) negatives, and Stern's glycerol test had 45 (63.4%) of the isolates positive and 26 (36.6%) negative (Table 5).

The results were coded in a test and results (n x t) matrix (Goodfellow, 1977) according to the possible combinations of the results obtained in the three discriminating tests (Table 6). Of these eight possible combinations of results, Salmonella livingstone isolates could only fit into 4 of them and these were taken to represent biogroups (Table 6).

Isolates of biogroup 1 were positive in tests for utilisation of d-tartaric, l-tartaric acids and Stern's glycerol, biogroup 2 were negative in all the three tests.

TABLE 5 BIOTYPING OF SALMONELLA LIVINGSTONE USING THE THREE DISCRIMINATING SUBSTRATES

SERIAL No.	REFERENCE No.	d-TART.	l-TART.	S.GLY.
1	39/85	-	-	-
2	339/85	+	+	+
3	331/87	-	-	-
4	565/86	-	-	-
5	703/86	+	+	+
6	1932/85	-	+	+
7	338/86	-	-	-
8	2161/86	+	-	+
9	5385/86	+	+	+
10	285/85	+	+	+
11	97/85	-	-	-
12	4393/86	+	-	+
13	12/85	+	+	+
14	506/86	+	+	+
15	1917/86	-	-	-
16	177/86	+	-	+
17	613/85	+	+	+
18	547/87	+	+	+
19	3276/84	-	-	-
20	3277/84	-	-	-

21	3278/84	-	-	-
22	4372/84	+	+	+
23	4374/84	+	+	+

SERIAL No.	REFERENCE No.	d-TART.	l-TART.	S.GLY.
24	4375/84	+	+	+
25	4645/84	+	+	+
26	4649/84	-	-	-
27	1374/85	-	-	-
28	1375/85	-	-	-
29	2625/85	-	-	-
30	2626/85	-	-	-
31	505/86	+	+	+
32	506/86	+	+	+
33	507/86	+	+	+
34	2712/85	-	-	-
35	2713/85	-	-	-
36	2714/85	-	-	-
37	3229/85	-	-	-
38	3230/85	-	-	-
39	3235/85	-	-	-
40	3239/85	-	-	-
41	3240/85	-	-	-
42	3241/85	-	-	-
43	3242/85	-	-	-
44	3243/85	-	-	-
45	549/86	-	-	-
46	551/86	+	+	+
47	552/86	+	+	+

48	554/86	+	+	+
49	3009/86	+	+	+
50	3010/86	+	+	+

SERIAL No.	REFERENCE No.	d-TART.	l-TART.	S.GLY.
51	3011/86	+	+	+
52	3066/86	+	+	+
53	3067/86	+	+	+
54	3226/86	+	+	+
55	3227/86	+	+	+
56	4098/86	+	+	+
57	4099/86	+	+	+
58	4100/86	-	+	+
59	4101/86	-	+	+
60	4102/86	-	+	+
61	4103/86	-	+	+
62	4104/86	-	+	+
63	1123/86	+	+	+
64	1124/86	+	+	+
65	1126/86	+	+	+
66	1128/86	+	+	+
67	1131/86	+	+	+
68	2271/85	+	-	+
69	3019/85	+	-	+
70	3186/85	+	-	+
71	4702/85	+	-	+

TABLE 6

POSSIBLE COMBINATIONS OF TEST AND RESULTS ,AND BIOGROUPS

CHARACTER STATE	TAXA(OTUs') A	B	C	No. OF ISOLATES
1	+	+	+	32
2	-	-	-	26
3	+	+	-	0
4	+	-	+	7
5	+	-	-	0
6	-	+	+	6
7	-	+	-	0
8	-	-	+	0
			TOTAL	71

Footnote: A= d-tartrate, B= l-tartrate and,C=Sten's glycerol

TABLE 7 BIOGROUPS OF SALMONELLA LIVINGSTONE FROM THE THREE DISCRIMINATING SUBSTRATES

BIOGROUP	SERIAL Nos. OF ISOLATES	TOTAL	% OF TOTAL
1	2,5,9,10,13,14,17,18,22, 23,24,25,31,32,33,46,48, 49,50,51,52,53,54,55,56, 57,63,64,65,66 and 67.	32	45.0
2	1,3,4,7,11,15,19,20,21, 26,27,28,29,30,34,35,36, 37,38,39,40,41,42,43,44, and 45.	26	36.6
3	8,12,16,68,69,70 and 71.	7	9.9
4	6,58,60,61 and 62.	6	8.5.
	Total	71	

Footnote

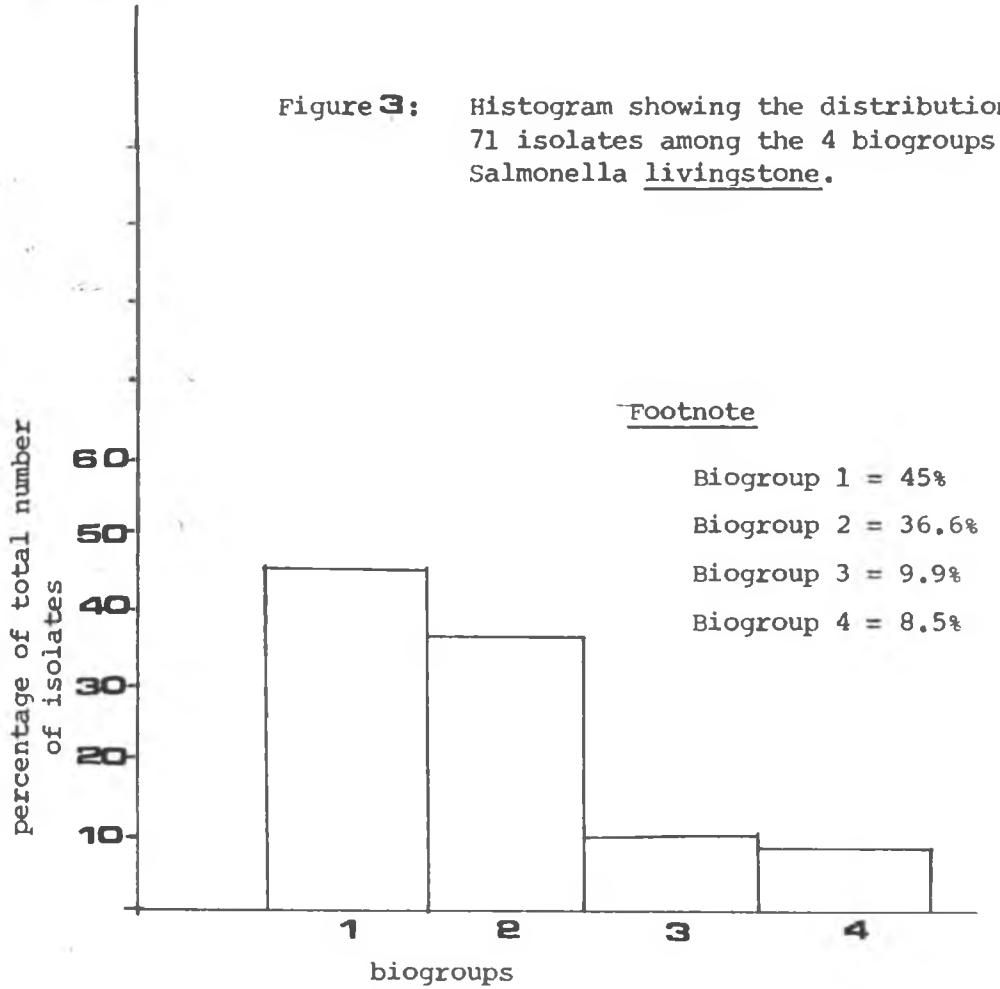
Biogroup 1 = + in all three tests

Biogroup 2 = - in all three tests

Biogroup 3 = - only in l-tartrate test

biogroup 4 = - only in d-tartrate test

Figure 3: Histogram showing the distribution of the 71 isolates among the 4 biogroups of Salmonella livingstone.



Footnote

Biogroup 1 = 45%

Biogroup 2 = 36.6%

Biogroup 3 = 9.9%

Biogroup 4 = 8.5%

Biogroup 3 were positive in d-tartaric acid and Stern's glycerol tests but negative in l-tartaric acid test, and biogroup 4 isolates were positive in all the tests above except d-tartaric acid test. (Table 6).

Out of the 71 isolates of Salmonella livingstone tested by biotyping, 32 (45%) were biogroup 1; 26 (36.6%) were biogroup 2; 7 (9.9%) were biogroup 3, and 6 (8.5%) were biogroup 4 (Table 7). There was no significant difference ($p < 0.05$) between biogroups 1 and 2, and between biogroups 3 and 4 in terms of the total number of isolates per each biogroup. However, the differences between biogroup 1 or 2 and either biogroup 3 or 4 were quite significant ($p > 0.05$). The distribution of the 71 isolates among the 4 biogroups of Salmonella livingstone then recognized is shown in Figure 3.

1.3 Relationship of biogroup to Veterinary Investigation Centres

The centres from which the isolates of Salmonella livingstone were used in the study are shown in figure 3. The distribution of the biogroups within the geographical regions of England and Wales is shown in Table 8b. Most isolates in the study came from the Eastern region, 37 (52%). The Midlands contributed 17 (25.4%) and the Northern region 13 (18.3%). The Western and the Southern regions provided only 3 (4.2%) of the isolates used in the study (Table 8b).

The distribution of the different biogroups varied from one region to another. However, there was no significant difference ($p < 0.05$) in the distribution of biogroup 1 between the Eastern region and the Midlands (Table 8b). On the other hand there was quite a significant difference ($p > 0.05$) in the distribution of biogroup 2 isolates between these two regions (Table 8b). Biogroup 3 isolates came exclusively from the Northern region whereas 5 (83.5%) of the biogroup 4 isolates came not only from the Midlands but from only one centre, Preston, within this region (Table 8b).

There were also notable differences in the distribution of the biogroups within regions. For example, there was a significant difference ($p > 0.05$) in the distribution of biogroup 1 isolates between Norwich (6.7%) and Cambridge (80%) even though they are both in the same Eastern region (Table 8b). Similarly, there was a significant difference ($p > 0.05$) in the distribution of biogroup 3 isolates between Leeds (71.4%) and the other centres (28.6%) within the same Northern region of England (Table 8b).

1.4 Relationship of biogroup to animal species or source

The sources of the isolates of Salmonella livingstone used in the study are shown in Table 9. Most of these isolates (73.2%) came from feed samples (Table 9). Of the 52 isolates from feed samples, 24 (46.2%) were of biogroup 1; 22 (42.3%) were of biogroup 2, and 6 (11.5%) were of biogroup 4. None of the isolates

TABLE 8a VETERINARY INVESTIGATION CENTRES AND BIOGROUPS

VI-CENTRE	BIOGROUPS				
	1	2	3	4	TOTAL
Barton Hole	5 (15.5)	0 (0.0)	0 (0.0)	0 (0.0)	5 (7.0)
Cambridge	12 (37.5)	8 (30.8)	0 (0.0)	0 (0.0)	20 (28.2)
Chester	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	1 (1.4)
Langford	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (1.4)
Leeds	0 (0.0)	0 (0.0)	5 (71.4)	0 (0.0)	5 (7.0)
Lincoln	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Liverpool	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	1 (1.4)
Moulton	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	1 (1.4)
Norwich	1 (3.1)	13 (50.0)	0 (0.0)	0 (0.0)	14 (19.7)
Penrith	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	1 (1.4)
PFN	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	1 (1.4)
Preston	9 (28.1)	0 (0.00	0 (0.0)	5 (83.3)	14 (19.7)
Reading	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Sutton Bonington	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Thirsk	0	1	1	0	2

	(0.0)	(3.8)	(14.3)	(0.0)	(2.8)
Winchester	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)
Worcester	1(3.1)	0(0.0)	0(0.0)	0(0.0)	1(1.4)

TABLE 8b REGIONAL DISTRIBUTION OF BIOGROUPS OF SALMONELLA LIVINGSTONE IN ENGLAND

REGIONS	BIOGROUPS				TOTAL
	1	2	3	4	
Northern	5	1	7	[redacted]	13
London-					
Midlands	9	3	0	5	17
Eastern	15	21	0	1	37
Western	1	0	0	0	1
Southern	2	0	0	0	2
	32	25	7	6	70

Footnote : one isolate not indicated in the table came from PFN(Saudi Arabia) and was of biogroup 2

was of biogroup 3 (Table 9). On the other hand, the 10 isolates from poultry were mainly of biogroups 1 (50%) and 2 (40%) with only a single isolate (10%) of biogroup 3 and none of biogroup 4 (Table 9).

The most interesting observation was that of isolates from the environment which were exclusively of biogroup 3.

1.5 Relationship of biogroup to year of collection

Table 10 shows the distribution of biogroups between the year 1984 and 1987. Thus, the isolates of Salmonella livingstone collected during the year 1984 were of biogroup 1 (50%) and biogroup 2 (50%). In 1985, however, isolates of all the 4 biogroups were collected but biogroup 2 (65.4%) was the most common (Table 10).

In 1986, there was a significant difference ($p > 0.05$) between the number of isolates of biogroup 1 and 2; thus, biogroup 1 isolates increased in number from 4 (12.5%) in 1985 to 24 (75%) in 1986, whereas those of biogroup 2 decreased from 17 (65.4%) in 1985 to 4 (15.4%) in 1986 (Table 10).

TABLE 9 ANIMAL SPECIES OR SOURCE AND BIOGROUPS

ANIMAL SPECIES OR SOURCE	BIOGROUPS 1	2	3	4	TOTAL
Bovine	1	0	0	0	1
Pig	2	0	1	0	3
Environment	0	0	5	0	5
Poultry	5	4	1	0	10
Feed	24	22	0	6	52
Total	32	26	7	6	71

TABLE 10 YEAR OF COLLECTION AND BIOGROUPS

YEAR	BIOGROUPS 1	2	3	4	TOTAL
1984	4 (12.5)	4 (15.4)	0 (0.0)	0 (0.0)	8 (11.3)
1985	4 (12.5)	17 (65.4)	4 (57.1)	1 (16.7)	26 (36.6)
1986	24 (75.0)	4 (15.4)	3 (42.9)	5 (71.4)	36 (50.7)
1987	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	1 (1.4)
TOTAL	32	26	7	6	71

2.0 ANTIMICROBIAL SUSCEPTIBILITY TESTING RESULTS

2.1 Preliminary antimicrobial susceptibility testing

Eighteen isolates of Salmonella livingstone which had been biotyped in the preliminary biotyping scheme (See section 1.1 of this chapter) were tested for susceptibility against 20 different antimicrobial agents (Table 11). Escherichia coli (NCTC) strain which is susceptible to 15 of the antimicrobial agents used was tested in parallel with the isolates of Salmonella livingstone and thus acted as the control for susceptibility.

The diameter of zone of growth inhibition were measured in millimetres (mm) using a ruler. Susceptibility and resistance of an isolate to any of the antimicrobial agents used was determined arbitrarily (Jones, 1986) by taking a diameter of zone of inhibition that gave the most discrimination between the resistant and susceptible isolates. In my case, a diameter of 13 mm or less was interpreted as resistance whereas that of 14 mm or more, was the mark for susceptibility.

The results of preliminary antimicrobial susceptibility testing are shown in Table 11. All the 18 isolates of Salmonella livingstone tested were susceptible to ampicillin (AMP), tetracycline (TE), neomycin (N), chloramphenicol (C), co-trimoxazole (SXT), furazolidone (FR), cephaloradine (CR), kanamycin (K), nalidixic acid (NA), gentamicin (CN), colistin sulphate (CT) and carbenicillin (CAR),

(Table 11) and resistant to cloxacillin (OB), fusidic acid (FD), lincomycin (MY), novobiocin (NV) and penicillin G (P). In the latter 5 antimicrobial agents, there were no zones of growth inhibition beyond the antimicrobial disks, hence it was not necessary to include them in Table 11.

Streptomycin (S), compound sulphanamide (S3) and sulphafurazole (SF) divided the 18 isolates of Salmonella livingstone tested into susceptible and resistant ones. Thus, 12 (66.7%) of the isolates were susceptible to streptomycin, and 6 (33.3%) resistant, 9 (50%) were susceptible and 9 (50%) resistant to compound sulphonamide, and 16 (88.9%) susceptible and 2 (11.1%) resistant to sulphafurazole respectively. See Table 11.

The 18 isolates were divided into 6 groups according to antimicrobial resistance patterns (Table 12). On the basis of these resistant patterns, two isolates were resistant to streptomycin only, 3 were resistant to both streptomycin (10 ug) and compound sulphonamide (50 ug); one isolate was resistant to streptomycin, compound sulphonamide and sulphafurozole (500 ug); one was resistant to both compound sulphonamide and sulphafurozole; 3 isolates were resistant to compound sulphonamide only, and 8 isolates were not resistant to any of the three discriminating drugs (Table 12).

TABLE 11 PRELIMINARY ANTIMICROBIAL SUSCEPTIBILITY TESTING RESULTS

SERIAL No.	DIAMETER(mm) OF ZONE OF INHIBITION														
	AMP	TE	N	S	C	SXT	FR	CR	S3	K	NA	SF	CN	CT	CAR
1	26	18	20	14	20	30	24	22	26	18	23	24	22	16	28
2	26	20	20	18	24	32	30	26	22	16	20	30	24	18	28
3	26	18	18	13	20	30	26	24	12	17	21	20	22	15	26
4	26	16	20	12	20	30	24	23	12	17	21	20	22	15	26
5	26	16	18	15	18	25	20	25	16	16	22	18	22	16	28
6	26	16	18	12	20	30	28	23	13	18	22	16	22	26	29
7	25	17	18	12	19	30	24	26	13	19	22	20	25	18	29
8	28	18	20	-	20	22	25	25	-	17	21	-	23	17	30
9	26	18	22	18	22	28	25	27	18	16	19	24	25	16	28
10	27	18	20	18	22	30	25	24	13	17	22	27	23	16	30
11	27	18	20	14	20	28	26	24	12	16	20	18	22	16	30
12	28	18	23	18	16	29	28	26	-	19	21	27	28	18	34
13	26	19	20	18	21	31	26	27	15	22	24	24	23	27	29
14	29	18	22	17	22	30	27	27	14	17	22	22	25	17	31
15	27	18	19	12	19	29	26	24	15	18	23	20	23	25	28
16	18	16	22	18	20	24	27	21	-	18	20	-	25	17	29
17	26	18	20	18	22	29	26	23	15	19	23	22	23	17	30
18	28	18	20	18	20	32	25	25	20	18	22	26	24	15	31
E.coli (NCTC)	20	22	18	17	26	25	18	18	28	28	32	36	18	15	26

TABLE 12 RESISTANCE PATTERNS OF 18 STRAINS AFTER PREL8IMINARY ANTIMICROBIAL SUSCEPTIBILIRY TESTING

RESISTANCE GROUP	ANTIMICROBIAL AGENT RESISTED	REFERENCE Nos' OF RESISTANT ISOLATES	TOTAL
1	streptomycin only	3 and 15	2
2	both streptomycin and compd. sulphonamide	4,6 and 7	3
3	streptomycin, compd. sulphonamide and sulphafurazole	8	1
4	compd.sulphonamide and sulphafurazole	16	1
5	compd. sulphonamide only	10,11 and 12	3
6	no resitance to any of the three drugs above	1,2,5,9,13,14, 17 and 18	8
		TOTAL	18

2.2 Antimicrobial susceptibility testing of all the 71 isolates of Salmonella livingstone

Seventy one isolates of Salmonella livingstone were tested for susceptibility against the three drugs found to be discriminating between isolates, reliable and reproducible in the preliminary susceptibility testing. The results are shown in Table 13.

The 70 isolates were divided into 6 resistant patterns as shown in Table 14. Three (4.3%) isolates showed resistance to streptomycin only; 13 (18.6%) to both streptomycin and compound sulphonamide; 12 (17.1%) to all the three drugs; 10 (14.2%) to both compound sulphonamide and sulphafurazole; 13 (18.6%) to compound sulphonamide only; and 19 (27.1%) showed no resistance to any of the three discriminating drugs above. There was a significant difference ($p > 0.05$) between the number of resistant (72.9%) and non-resistant isolates (27.1%) and so was that between isolates showing multiple resistance (68.6%) and those resistant to only one drug (31.4%) Table 14.

2.3 Relationship between antimicrobial resistance patterns and biogroups.

The antimicrobial resistant patterns of the Salmonella livingstone isolates varied between the 4 biogroups. Table 15 shows distribution of biogroups within the different drug resistance

TABLE 13 ANTIMICROBIAL SUSCEPTIBILITY TESTING RESULTS USING THE THREE MOST DISCRIMINATING DRUGS

SERIAL No.	DIAMETER(mm) OF ZONE OF INHIBITION		
	streptomycin	compd. sulphonamide	sulphafurazle
1	14	26	24
2	18	22	30
3	13	16	24
4	12	12	20
5	15	16	18
6	12	13	16
7	12	13	20
8	-	-	-
9	18	18	24
10	18	13	27
11	14	12	18
12	18	-	27
13	18	15	24
14	17	14	22
15	12	15	20
16	18	-	-
17	18	15	22
18	18	20	26
19	ND	ND	ND
20	17	22	30
21	14	14	26

22	16	-	-
23	13	10	-
24	18	18	30

continued;

25	13	-	18
26	10	-	-
27	13	12	28
28	12	20	28
29	15	14	31
30	12	12	24
31	18	14	16
32	14	-	-
33	14	-	-
34	14	-	-
35	18	22	34
36	16	22	30
37	11	-	-
38	12	-	22
39	15	24	30
40	14	12	26
41	14	13	32
42	12	-	-
43	14	12	26
44	-	-	20
45	11	10	28
46	15	12	24
47	15	-	26
48	15	14	28
49	16	-	20
50	13	-	22

51	19	16	30
52	16	13	26
53	15	-	26

continued;

54	14	-	22
55	14	-	22
56	15	18	26
57	12	-	-
58	10	10	-
59	13	-	22
60	11	-	18
61	-	-	18
62	12	10	-
63	14	-	-
64	14	-	-
65	16	-	-
66	13	-	-
67	13	-	-
68	15	-	-
69	13	-	-
70	14	-	-
71	13	-	-
E.coli (NCTC)	18	28	36

patterns of Salmonella livingstone.

All the three isolates resistant to streptomycin (10 ug) only were of biogroup 2. Biogroups 3 and 4 isolates were all resistant to the three drugs used. 20 (62.5%) of isolates of biogroup 1 showed multiple resistance whereas 12 (37.5%) showed no resistance at all (Table 15). On the other hand, 18 (72%) of biogroup 2 isolates showed resistance showed resistance to either of the three drugs or their combination, and only 7 (28%) isolates showed no resistance to any of the three drugs (Table 15).

Within the same biogroup there was a significant difference ($p > 0.05$) between the number of resistant isolates and non-resistant isolates. However, there was no significant difference ($p < 0.05$) between the 4 biogroups as far as multiple resistance to all the three drugs is concerned (Table 15). The difference between resistant isolates of biogroups 1 and 2 was not significant ($p < 0.05$). However, there was a significant difference ($p > 0.05$) between biogroups 1 and 2 in the number of isolates resistant to streptomycin only (Table 15).

2.4 Plasmid Profile Analysis of 62 isolates of Salmonella livingstone

The results of plasmid profile analysis using agar gel electrophoretic method are shown in Table 16. A total of 62 isolates of Salmonella livingstone were analysed for the presence or absence

TABLE 14 RESISTANCE PATTERN GROUPS AFTER ANTIMICROBIAL SUSCEPTIBILITY TESTING OF ALL THE ISOLATES OF S.LIVINGSTONE

RESISTANCE GROUP	ANTIMICROBIAL AGENTS RESISTED	REFERENCE Nos' OF RESISTANT ISOLATES	TOTAL
1	streptomycin only	3,15 and 28	3(4.3)
2	both streptomycin and compd.sulphonamide	4,6,7,25,27,30, 38,44,45,50,60, and 61	13(18.6)
3	streptomycin,compd.-sulphonamide and sulphafurazole	8,23,26,37,42, 57,58,62,66,67, 69 and 71	12(17.1)
4	compd.sulphonamide and sulphafurazole	16,22,32,33,34, 63,64,65,68 and 70	10(14.3)
5	compd.-sulphonamide only	10,11,12,40,41,43, 46,47,49,52,53,54, and 55	13
6	no resistance to any the three drugs above	1,2,5,9,13,14,17, 18,21,22,29,31,35, 36,39,48,51 and 56	19 (27.1)
		TOTAL	70

TABLE 15 RELATIONSHIP BETWEEN ANTIMICROBIAL RESISTANCE PATTERNS AND BIOGROUPS

RESISTANCE PATTERNS	BIOGROUPS 1	2	3	4	TOTAL
S only	0	3	0	0	3
S and S3	2	7	0	4	13
S,S3 and SF	4	3	3	2	12
S3 and SF	6	1	3	0	13
S3 only	8	4	1	0	13
No. resistance to the above drugs	12	7	0	0	19
TOTAL	32	25	7	6	70

Footnote: S = streptomycin

S3 = compound sulphonamide

SF = sulphafurazole

of plasmids. Of these 29 (46.8%) had plasmids whereas 33 (53.2%) had no plasmids (Table 17). There was no significant difference ($p < 0.05$) between the number of isolates of Salmonella livingstone with plasmids and those without plasmids (Table 16).

The 62 isolates analysed for plasmid content were grouped on the basis of plasmid patterns or profiles. Table 17 shows the 4 groups. Of the 29 isolates with plasmids, 24 (82.8%) had light plasmids only, 4 (13.8%) had heavy plasmids only, and 1 (3.4%) had both light and heavy plasmids (Table 17).

The light plasmids had an approximate weight of between 3.3 Mega daltons(Mdal.) and 9.1 Mdal. Whereas the heavy plasmids had an approximate weight of between 33 Mdal. and 120 Mdal. See Table 16. The weights of these plasmids were determined using known weights of Salmonella typhimurium strain containing both light and heavy plasmids of 120, 60, 33 and 4.2 Mdal. See Figures 4a to 4c.

2.5 Relationship between plasmid profiles and antimicrobial resistance patterns

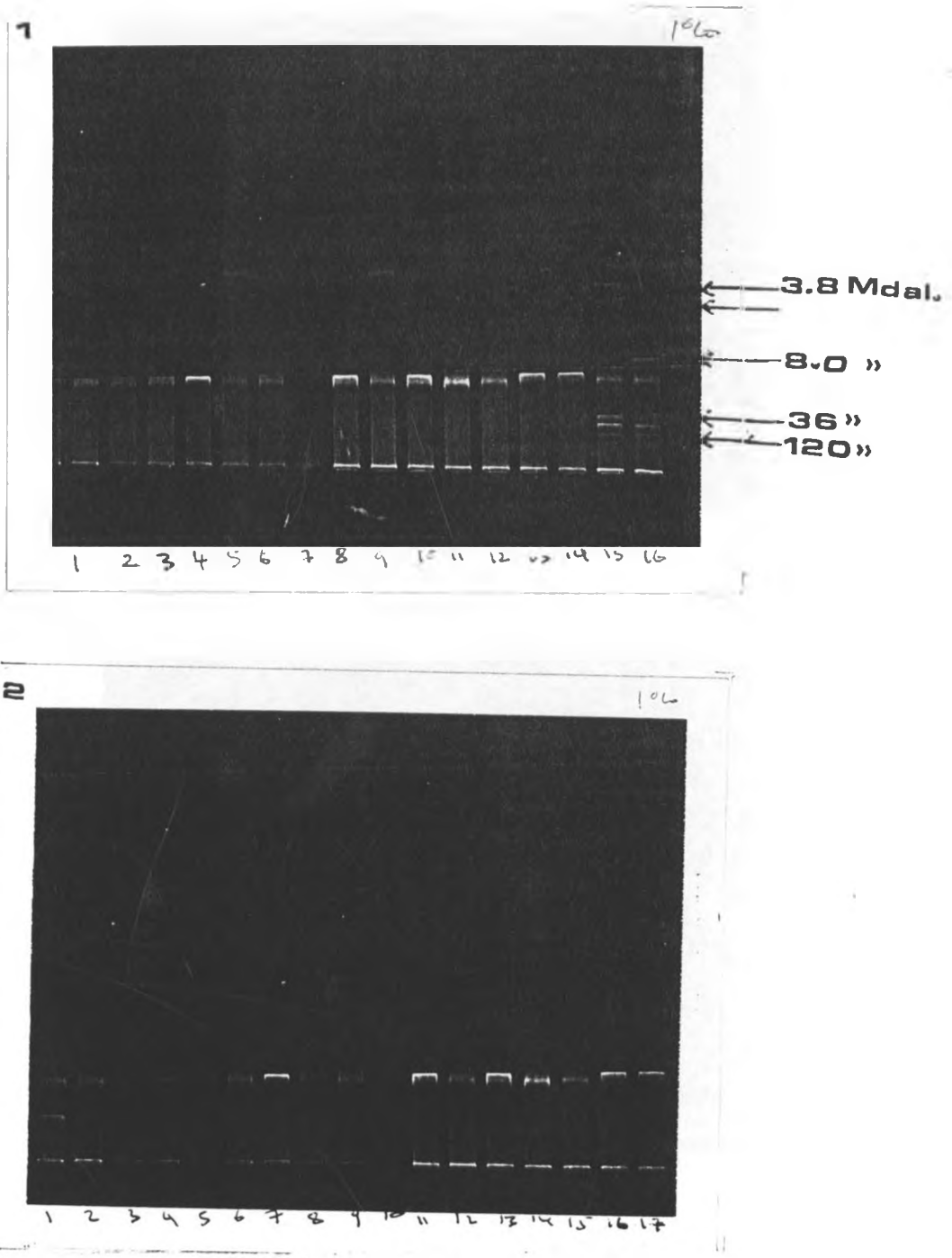
Table 18 shows the relationship between plasmid content and antimicrobial resistance patterns. 45 isolates showing resitance to either streptomycin, compound sulphonamide or sulphafurazole or combinations of the three drugs, were analysed for plasmid content and, 21 (46.6%) found to contain plasmids. 24 (53.3%) of the resistant isolates had no plasmids. There was no significant

difference ($p < 0.05$) between the number of plasmid containing isolates and those without plasmids within the drug-resistant group.

14 isolates which were not resistant to any of the three drugs above, were analysed for plasmid content and, 9 (64.3%) had plasmids whereas 5 (35.7%) had no plasmids. The difference between the number of plasmid-containing isolates in the non-drug resistant group was significant ($p > 0.05$).

None of the non-drug resistant isolates contained heavy plasmids (Table 18). There was not much correlation between drug-resistance patterns and plasmid profiles except for multiple resistance to streptomycin, compound sulphonamide and sulphafurazole which was a feature of those isolates containing only light plasmids (Table 18).

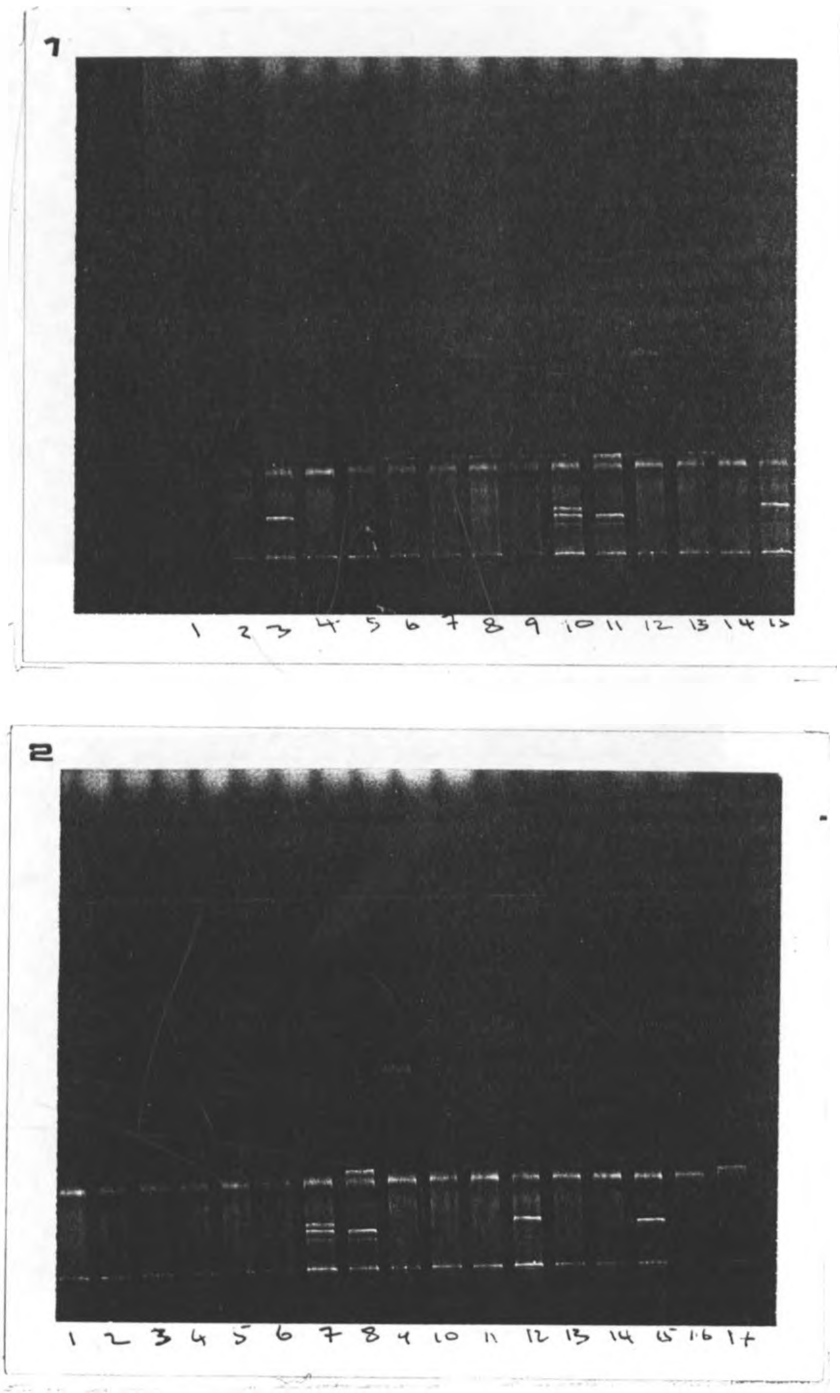
FIGURE 4a : PLASMID PROFILES OF S.LIVINGSTONE IN 1% AGAROSE GEL ELECTROPHORESIS



Footnote: tracks 15 and 16 in photograph 1 were the control strains of S.typhimurium.

tracks 5 and 9(photo 1),and tracks 8 and12(photo2) have similar plasmid profiles.

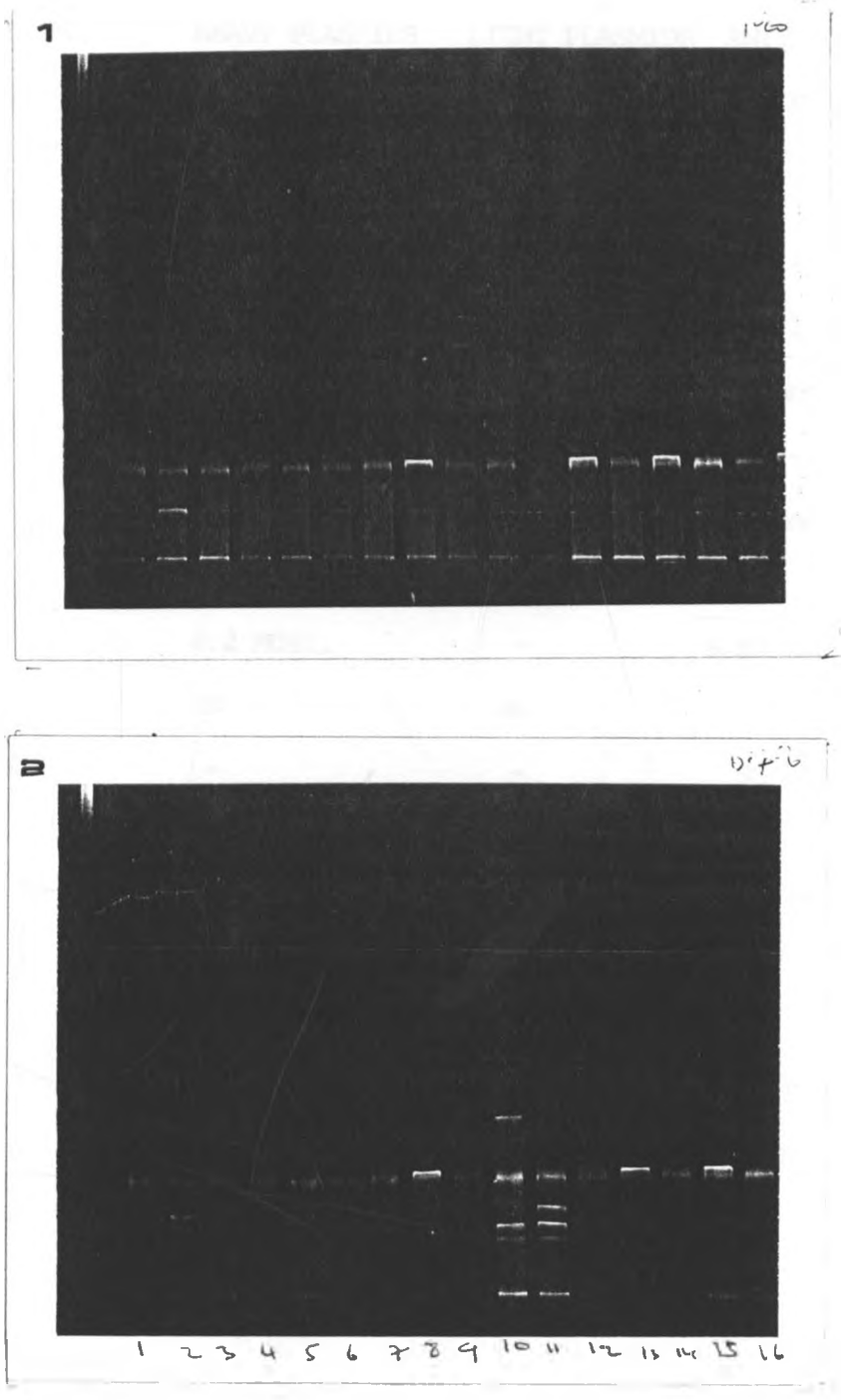
FIGURE 4b : PLASMID PROFILES OF S.LIVINGSTONE IN 1% AGAROSE GEL ELECTROPHORESIS



Footnote: tracks 10,11(photo 1),and 7 and 8(photo2) are the control strains of S.typhimurium. tracks 4,6,8 (photo1) and 1,3,5,9,13 and 14(photo2) have similar profiles.

FIGURE 4c: PLASMID PROFILES OF S.LIVINGSTONE IN 1%

AGAROSE GEL ELECTROPHORESIS



Footnote: tracks 10 and 11(photo2) are for the control strains of S.typhimurium.

tracks 8,12,14(photo1) and 8,13 and 15(photo2) have similar profiles.

TABLE 16 PLASMID PROFILE ANALYSIS

SERIAL No.	HEAVY PLASMIDS	LIGHT PLASMIDS	ANTIMICROBIAL RESISTANCE
1	-	-	-
2	-	-	-
3	-	-	S only
4	-	-	S and S3
5	ND	ND	-
6	-	-	S and S3
7	ND	ND	"
8	4.2 Mdal.	-	S,S3 and SF
9	ND	ND	-
10	-	-	S3 only
11	-	-	"
12	3.3 Mdal.	-	"
13	ND	ND	-
14	-	-	-
15	ND	ND	S only
16	3.3 Mdal.	120 and 36 Mdal.	S3 and SF
17	ND	ND	-
18	-	-	-
19	3.8 Mdal.	-	ND
20	"	-	-
21	"	-	-
22	-	36 Mdal.	S3 and SF
23	4.2 Mdal.	-	S,S3 and SF

24	-	-	-
25	-	60 Mdal.	S and S3

continued;

26	3.8 Mdal.	-	S,S3 and SF
27	-	-	S and S3
28	3.8 Mdal.	-	S only
29	-	-	-
30	-	-	S and S3
31	-	-	-
32	-	-	S3 and Su
33	-	-	"
34	-	-	"
35	3.8 Mdal.	-	-
36	-	-	-
37	-	-	S,S3 and SF
38	3.8 Mdal.	-	S and S3
39	ND	ND	-
40	-	-	S3 only
41	3.8 Mdal.	-	"
42	-	-	S,S3 and SF
43	-	-	S3 only
44	-	-	S and S3
45	3.3 Mdal.	-	"
46	-	-	S3 only
47	-	-	"
48	-	-	-
49	9.1 Mdal.	-	S3 only
50	"	-	S and S3
51	"	-	-

(115)

52	"	-	S3 only
53	"	-	"
54	"	-	"

continued;

55	9.1 Mdal.	-	S3 only
56	9.1 Mdal.	-	-
57	"	-	S,S3 and SF
58	-	-	"
59	-	-	S and S3
60	-	-	"
61	-	60 Mdal.	"
62	-	-	S,S3 and SF
63	9.1 Mdal.	-	S3 and SF
64	"	-	"
65	"	-	"
66	ND	ND	S,S3 and SF
67	ND	ND	"
68	-	-	S3 and SF
69	-	-	S,S3 and SF
70	-	120 Mdal.	S3 and SF
71	-	-	S,S3 and SF

TABLE 17 GROUPING OF S.LIVINGSTONE ON THE BASIS OF PLASMID PROFILES

GROUP	TYPE OF PLASMID	TOTAL No. OF ISOLATES
1	Light plasmids only	24(38.7)
2	Heavy plasmids only	4(65)
3	Both heavy and light plasmids	1(1.6)
4	No plasmids	33(53.2)
	TOTAL	62

TABLE 18 RELATIONSHIP BETWEEN PLASMID PROFILES AND ANTIMICROBIAL RESISTANCE PATTERNS

PLASMID PROFILE	No. OF RESISTANCE ISOLATES S	S+S3	S,S3+SF	S3+SF	S3	No. OF NONE RESIST-
Light plasmids	1	3	4	3	5	5
Heavy plasmids	0	2	0	1	0	0
Heavy and light	0	0	0	1	0	0
No.plasmids	1	7	6	4	6	9
TOTAL	2	12	10	10	11	14

Footnote: total no of isolates with light plasmids=21

" heavy " = 4

" light and heavy " = 1

" without any plasmid =33

2.6 Relationship between plasmid profiles, antimicrobial resistance patterns and biogroups

2.6:1 a) Resistance to streptomycin only

Only biogroup 2 isolates showed resistance to streptomycin (Table 19a). There was no correlation between plasmid content and streptomycin resistance as the difference in the number of isolates between plasmid-containing and non-plasmid containing in this drug resistance group was not significant ($p < 0.05$). See Table 19a.

2.6:2 b) Resistance to both streptomycin (S) and compound sulphonamide (S3)

Biogroups 2 (45.5%) and 4 (36.4%) had the most number of isolates that were resistant to both streptomycin and compound sulphonamide. There was no significant difference ($p < 0.05$) between these two biogroups in total plasmid content but biogroup 2 had one isolate with a light plasmid whereas biogroup 4 had one isolate with a heavy plasmid (Table 19b). The difference between the number of plasmid-containing isolates and those without plasmids within the same biogroup was not significant ($p < 0.05$). Thus, in biogroup 2, only 1 (20%) of the isolates had plasmids, compared to 4 (80%) without plasmids. Similarly, only 1 (25%) of biogroup 4 isolates had plasmids compared to 3 (75%) isolates without plasmids. None of the biogroup 3 isolates in this drug resistance pattern group had plasmids. All the isolates of biogroup 1 had plasmids (Table 19b).

2.6:3 c) Resistance to streptomycin (S), compound sulphonamide (S3) and sulphafurazole (SF)

All the isolates of biogroups resistant to the three drugs had light plasmids except isolates of biogroup 4 which had no plasmids. In biogroup 1, none of the isolates without plasmids showed resistance to the three drugs, whereas in biogroups 2 and 3 there was no significant difference ($p < 0.05$) in numbers between plasmid-containing isolates and those without (Table 19c). Overall, there was no correlation between plasmid content and resistance to the three drugs in all the isolates of the biogroups except biogroup 1 (Table 19c).

2.6:4 d) Resistance to compound sulphonamide (S3) and sulphafurazole (SF)

Biogroup 3 isolates with heavy plasmids only and those with both light and heavy plasmids were resistant to both drugs. (Table 19d). However, there was no significant difference ($p < 0.05$) in number of isolates between plasmid-containing and those without plasmids (Table 19d).

None of the biogroups 2 isolates resistant to both drugs had plasmids (Table 19d). On the other hand, there was significant difference ($p > 0.05$) in number of isolates between plasmids containing 4(66.7%) and those without plasmids 2(33.3%) within biogroup 1 (Table 19d). Overall, there was positive correlation between plasmid content and resistance to both drugs as 60% of isolates in all the 4 biogroups with plasmids

TABLE 19 RELATIONSHIP BETWEEN PLASMID PROFILES , ANTI-MICROBIAL RESISTANCE PATTERNS AND BIOGROUPS

a) resistance to streptomycin only

Plasmid profile	biogroups				total
	1	2	3	4	
Light plasmids	0	1	0	0	1
Heavy "	0	0	0	0	0
Heavy and light	0	0	0	0	0
No. plasmids	0	1	0	0	1
TOTAL	0	2	0	0	2

b) resistance to streptomycin and compound sulphonamide

Plasmid profile	biogroups				total
	1	2	3	4	
Light plasmids	1	1	0	0	2
Heavy "	1	0	0	1	1
Heavy and light	0	0	0	0	0
No. plasmids	0	4	0	3	7
total	2	5	0	4	11

TABLE 19, continued;

c) resistance to streptomycin, compound sulphonamide and sulphafurazole

Plasmid profile	biogroups				total
	1	2	3	4	
Light plasmids	2	1	1	0	4
Heavy "	0	0	0	0	0
Heavy and light	0	0	0	0	0
No. plasmids	0	2	2	1	5
total	2	3	3	1	9

d) resistance to compound sulphonamide and sulphafurazole

Plasmid profile	biogroups				total
	1	2	3	4	
Light plasmids	3	0	0	0	3
Heavy "	1	0	1	0	2
Heavy and light	0	0	1	0	1
No. plasmids	2	1	1	0	4
total	6	1	3	0	10

TABLE 19, continued;

e) resistance to compound sulphonamide only

Plasmid profile	biogroups				total
	1	2	3	4	
Light plasmids	2	1	0	0	4
Heavy "	0	0	0	0	0
Heavy and light	0	0	0	0	0
No. plasmids	2	2	0	0	4
total	4	3	0	0	7

were resistant when compared to 40% of isolates without plasmids. None of biogroup 4 isolates were resistant to both drugs (Table 19d).

2.6:5 e) Resistance to compound sulphonamide only (S3)

None of biogroups 3 and 4 isolates showed resistance to compound sulphonamide only (Table 19e). The resistant isolates in biogroups 1 and 2 were both plasmid-containing and those without plasmids and the difference between the two was not significant ($p < 0.05$), hence there was no correlation between plasmid content and resistance to compound sulphonamide only (Table 19e).

3.0 SURVIVAL AND HEAT RESISTANCE OF SALMONELLA LIVINGSTONE

Table 20 summarizes the result of two experiments conducted to study some of the epidemiological aspects of Salmonella livingstone. From these simple experiments it can be seen (Table 20) that it is possible to group isolates of Salmonella livingstone according to duration of survival in tap water and heat resistance. In my study, two isolates of Salmonella livingstone, one from biogroup 1 and the other from biogroup 3 which had shown resistance to both compound sulphonamide and sulphafurazole, were found to survive exposure to a temperature of 50° C overnight and were able to remain viable in sterile tap water at ambient temperature for two weeks (Table 20). The two isolates though of similar character as far as drug-resistance, heat resistance patterns and survival in tap water is concerned, were different in plasmid content since only biogroup 3 had a light plasmid (Table 20).

Isolates of biogroup 2 and 4 were only able to grow at a maximum temperature of 46° C but were killed by 50° C. They also died when left in sterile water at room temperature for two weeks (Table 20).

The two isolates of biogroups 2 and 4 had shown multiple resistance to streptomycin, compound sulphonamide and sulphafurazole (biogroup 2) and to both streptomycin and compound sulphonamide (biogroup 4) and were found to contain no plasmids (Table 20).

The most interesting observation from my simple study was that biogroups 1 and 3 isolates were both from animal sources whereas biogroup 2 and 4 isolates were from feed samples (Table 20).

4.0 DRUG-RESISTANCE AND ITS TRANSFER

Two isolates of Salmonella livingstone, one of biogroup 1 which was not resistant to streptomycin, compound sulphonamide and sulphofurazole, and one of biogroup 2, which had shown multiple resistance to all the three drugs above (Table 21a) were grown in a mixed culture in nutrient broth. Forty one isolated colonies were biotyped and tested for antimicrobial susceptibility against all the three drugs above. Out of 8 colonies of biotype 1, 1 (12.5%) was found to have acquired streptomycin resistance whereas 7 (87.5%) were still susceptible to all the three drugs (Table 20b). All the 8 colonies of biogroup 1 had also acquired resistance to compound sulphonamide and sulphafurazole (Table 20b).

Biogroup 1 isolate was plated on nutrient agar for isolated colonies. 32 isolated colonies were tested for susceptibility against streptomycin, compound sulphonamide and sulphafurazole. The results are shown in Table 20c. Out of 32 colonies of biogroup 1 tested, 4 (12.5%) were resistant to streptomycin whereas 28 (87.5%) were not resistant to the same drug. However, all the 32 colonies were susceptible to compound sulphonamide and sulphafurazole (Table 21c). These results show that streptomycin resitance which was thought to have been transferred (see Table 21b) from biogroup 2 isolate was found to be occurring at a rate of 12.5% among the strains (colonies) of biogroup 1 isolate (Table 21c). Resistance to compound sulphonamide and sulphafurazole was however transferable as shown by the results in Tables 21b and 21c.

TABLE 20 SURVIVAL AND HEAT RESISTANCE OF SALMONELLA LIVINGSTONE

Biogroup	Source	Antimicrobial resistance patterns	Plasmid content	survival at temps.			survival in tap-water
				42C	44C	46C	50C	
1	poultry	S3+SF	-	+	+	+	+	-
2	feed	S,S3+SF	-	+	+	+	-	+
3	pig	S3+SF	+	+	+	+	+	-
4	feed	S+S3	-	+	4	+	-	+

TABLE 21 DRUG-RESISTANCE AND ITS TRANFER IN S. LIVINGSTONE

a) biogroup and drug-resistance patterns before mixed growth in broth culture

biogroup	S	S3	SF
1	+	+	+
2	-	-	-

b) biogroups and drug-resistance patterns of 41 isolated colonies from mixed broth culture

biogroup	no. of colonies	S	S3	SF
1	8	1	8	8
2	33	33	33	33

c) drug-resistance patterns of 32 isolated colonies of biogroup 1 above

no. of isolated colonies	S	S3	SF
32	4	0	0

IV DISCUSSION

1.0 Biotyping

Biotyping of 71 isolates of Salmonella livingstone was able to distinguish 4 biogroups. There were two major distinct biogroups 1 (45%) and 2 (36.6%) and two minor biogroups 3 (9.9%) and 4 (8.5%) which could have been derived from the two major biogroups by the typing characters. The three tests used, namely, turbidity tests for utilisation of tartaric acid isomers (l and d) and Stern's glycerol test were found to be stable, reliable and reproducible as consistent results were obtained when the tests were repeated on three different occasions. Duguid et al (1975) had found similar results with isolates of Salmonella typhimurium. The three tests above are therefore recommended for use in future biotyping schemes of Salmonella livingstone.

1.1 Relationship between biogroups and veterinary investigation centres

Biogroup 1 isolates were the most diverse as they were isolated in equal proportions in the Midlands and Eastern region of England, the regions from which most isolates of Salmonella livingstone used in the study came from (See Figure 3). Biogroup 2 isolates were mainly from two centres in the Eastern region of England, whereas biogroup 3 isolates were exclusively from Northern England. Biogroup 4 isolates were almost exclusively from the Midlands.

The distribution of Salmonella livingstone biogroups within the geographical regions of England shows how biotyping can serve as a tool in tracing epidemiologically related isolates. Thus, it appears as if biogroup 3 derived from biogroup 1 through mutation during its spread from the Eastern region through the Midlands into the Northern region (Table 8b).

Biogroups 3 and 4 are variant biotypes of 1 and 2 and their relatively small size suggests that Salmonella livingstone is a serotype of recent origin, a conclusion in keeping with its recognition in only the past 34 years, when compared to an ancient serotype like Salmonella typhimurium which has a considerable serotype diversification (Duguid et al, 1975; Barker et al, 1982).

1.2 Relationship between biogroups and animal species or source

The fact that biogroup 3 isolates were almost exclusively from the environment (Table 9) shows how biotyping can be used to trace the source of Salmonella livingstone strains, should such an isolate appear in epidemic proportions. The most likely reason for the association of biogroup 3 isolates and environmental sources is that, maybe these isolates changed from either of the major two biogroups through adaptation to the environment.

Another possible explanation is that the environmental sources have a minimal role to play as a source in the epidemiology of

Salmonella livingstone since biogroup 3 isolates were rare when compared to biogroups 1 and 2. Feed samples had 46.2% biogroup 1, and 43.3% of biogroup 2 isolates and 11.5% were biogroup 4 isolates. The fact that all the biogroups were isolated from feed samples shows the significance of animal feeds in the epidemiology of Salmonella livingstone. This is in agreement with the incidence of this serotype in animal feeds in England (MAFF. Reports 1976-1983).

1.3 Biogroup and year of collection

In 1984, biogroups 1 and 2 were isolated from the various sources in England, in equal proportions (Table 10). In 1985, biogroup 2 was the most prevalent (65.4%). In 1986, biogroup 2 decreased from a prevalent rate of 65.4% to 15.4%, whereas biogroup 1 increased in prevalence from 12.5% (1985) to 75%. These observations show how biotyping can be used to monitor the prevalence rates of Salmonella livingstone strains in a circumscribed area within a specified period of time.

2.0 ANTIMICROBIAL SUSCEPTIBILITY TESTING

All the isolates of Salmonella livingstone tested were resistant to claxacillin 5 ug (OB), fusidic acid 10 ug (FD), lincomycin 2 ug (MY), novobiocin 5 ug (NV), penicillin G 1.5 ug (P), methicillin 10 ug (MET), erythromycin 10 ug (E) and sulphadiazine 50 ug (SD). Resistance of Salmonella livingstone to these

drugs cannot be adequately explained because it has not been reported before among the other serotypes of Salmonella. However the concentrations of these drugs in the multodisks used may be below the minimum inhibitory concentration (MIC) for Salmonella livingstone, since experience with other serotypes of salmonellae have shown that drugs like the sulphonamides have given different drug susceptibility patterns when used in different concentrations (Sojka et al, 1977, 1986).

All the isolates were susceptible to ampicillin 10 ug (AMP), tetracycline 10 ug (TE), neomycin 10 ug (N), chloramphenicol 10 ug (C), co-trimoxazole 25 ug (CR), kanamycin 30 ug (K), nalidixic acid 30 ug (NA), gentamicin 10 ug (CN), colistin sulphate 10 ug (CT) and carbonicillin 100 ug (CAR). These findings are contrary to what has previously been reported with other serotypes of Salmonella. Thus, in those reports (Sojka et al., 1977 and 1986; Murray et al., 1986) resistance rather than susceptibility to ampicillin, gentamicin, tetracycline and co-trimoxazole were found especially in isolates of Salmonella typhimurium.

The isolates of Salmonella livingstone showed resistance to streptomycin, compound sulphonamide and sulphafurazole in varying proportions. Thus, these three drugs were found to distinguish the isolates of Salmonella livingstone into 6 antimicrobial resistance patterns as follows: 4.3 percent were resistant to streptomycin only; 18.6 percent to both streptomycin, compound sulphafurazole and sulphonamide; 17.1 percent to streptomycin, compound sulphonomide and sulphafurozole; 14.2 per cent to both compound

sulphonamide and sulphafurazole; 18.6% to compound sulphafurazole only, and 27.1 percent were not not resistant to any of the three discriminating drugs. Resistance to both streptomycin and compound sulphonamide and to either streptomycin or compound sulphonamide alone has been reported among other serotypes of Salmonella isolated from animals and environment in England and Wales (Sojka and Field, 1970; Sojka et al., 1972, 1975, 1984 and 1986).

In this study, resistance to sulphonamides was the most common followed by multiple resistance to streptomycin and compound sulphonamide, or to compound sulphonamide and sulphafurazole.

2.1 Relationship between drug-resistance patterns and biogroups

The fact that biogroup 2 isolates showed resistance to streptomycin alone, and that all biogroup 3 and 4 isolates showed multiple resistance to three drugs, namely streptomycin, compound sulphonamide and sulphafurazole shows how antimicrobial susceptibility testing can be used in distinguishing strains of Salmonella livingstone according to source. Thus biogroup 3 isolates were from environmental sources in the Northern region of England whereas most biogroup 2 isolates were from the Eastern region of England. Drug resistance has been used before as an epidemiologic marker, when a multiple resistant strain of Salmonella newport involved in an outbreak involving 1000 persons was characterized by an unusual marker of chloramphenicol resistance (Cohen and Tauxe 1986).

3.0 PLASMID PROFILE ANALYSIS

Plasmid profiles as determined using agarose gel electrophoresis, and a modified method of Kado and Liu (1981) was found to be very reliable and reproducible within those isolates with plasmids. Only 46.8 percent of the isolates had plasmids, 53.2 per cent were without plasmids. This is not an unusual finding since not all isolates of any species of bacteria have plasmids and, the latter can either be lost or acquired depending on the circumstances (Broda, 1979; Hardy, 1986).

There were three types of plasmid profiles observed. Isolates with light plasmids were the most prevalent (82.8%). The isolates with heavy plasmids were 13.8 percent, whereas those with both light and heavy plasmids were only 3.4 percent.

3.1 Plasmid profiles and antimicrobial resistance patterns

Of the isolates found to be resistant to streptomicin, compound sulphanomide and sulphafurazole, 46.6 percent were found to contain plasmids, and 53.3 percent had no plasmids. On the other hand, 64.3 percent of the non-resistant isolates of Salmonella livingstone had plasmids whereas only 35.7 percent had no plasmids. These findings suggest other roles played by plasmids beside resistance to antimicrobial agents. Thus, plasmids do code for so many other characteristics of a bacterial species (Hardy, 1986; Ewing, 1986).

Among the non-drug resistant isolates of Salmonella livingstone, none contained heavy plasmids almost suggesting a link between heavy plasmids and antimicrobial resistance. Resistance to Streptomycin was not plasmid dependant so was resistance to both streptomycin and compound sulphonamide. There was positive correlation between plasmid content and resistance to both compound sulphonamide and sulphafurazole.

3.2 Correlation between plasmid profiles, antimicrobial resistance patterns and biogroups

In all the biogroups except biogroup 1, there was no correlation between plasmid content and antimicrobial resistance. All the biogroup 3 isolates showing multiple resistance to streptomycin and compound sulphonamide had no plasmids suggesting that in this biogroup, drug resistance was not plasmid mediated, or if it was, then the isolates must have lost their plasmids. 75 per cent of biogroup 3 isolates showing multiple resistance to the afore mentioned drugs, had no plasmids. This might explain why these two biogroups were very rare among the isolates of Salmonella livingstone.

In biogroup 1, all the isolates resistant to streptomycin, compound sulphonamide and sulphafurazole, had plasmids. In this biogroup therefore, multiple resistance was plasmid dependent.

The results of biotyping, antimicrobial susceptibility testing and plasmid profile analysis show that all these methods can be useful in distinguishing strains within any serotype of Salmonella. Thus, biotyping alone using a new biotyping scheme of Duguid et al (1975), distinguished 4 biogroups, antimicrobial resistance was able to subdivide the 4 biogroups further into 6 subgroups according to drug-resistance patterns and plasmid profile analysis help to identify closely related isolates regardless of biotype or drug-resistant pattern.

The results of the study have shown that Salmonella livingstone is not a homogenous group as previously thought, but rather, it is composed of 2 major biogroups with 2 variants which have been derived from the main groups by either adaptation to new environment or through loss or acquisition of plasmids. It has also shown that resistance to streptomycin and compound sulphonamide is not plasmid dependant. This could be chromosomally determined as it has been found to be common even among other serotypes of Salmonella (Sojka et al., 1977, 1986). Multiple resistance is plasmid mediated as those isolates with heavy plasmids were all resistant.

Biotyping was able to distinguish the 71 isolates of Salmonella livingstone into 2 major biogroups (1 and 2) and 2 variants or subgroups (3 and 4). There was positive correlation between biogroup prevalence and veterinary centre from which they came from. Thus, for example, biogroup 3 isolates came exclusively from Leeds Veterinary Investigation Centre in the Northern region of England whereas biogroup 4 came from Preston Centre in the Midlands.

Similarly, biotyping was able to distinguish the biogroups according to animal species or source. Thus, biogroup 3 isolates were exclusively from the environmental sources. On the other hand, feed which provided the bulk of the samples had all isolates of all biogroups except biogroup 4.

Biotyping was also able to show how the prevalence of the 4 biogroups of Salmonella livingstone varied with time. Thus, in 1984 only biogroup 1 (50%) and 2 (50%) isolates were isolated in England. In 1985, biogroup 2 isolates were the most prevalent. However, in 1986 biogroup 2 isolates decreased in number whereas those of biogroup 1 were increased. These findings add credit to biotyping as an epidemiological tool as this study has shown that it is possible to use this method in monitoring the prevalence rates of Salmonella livingstone and other serotypes of Salmonella in animals and environment sources within a given geographical region.

Antimicrobial susceptibility testing has however not been able to correlate to veterinary investigation centres or animal species or sources except for resistance to streptomycin alone which was only shown by a few isolates from biogroup 2,and biogroup 3, which did not show any resistance to compound sulphonamide alone. However, other drug-resistance patterns were distributed equally in the 2 most diverse biogroups (1 and 2). Other workers (Old et al., 1980; Holmberg et al., 1984) have found antimicrobial resistance not to be a reliable method in epidemiological investigations. However, antimicrobial resistance when compared to biogroup was positively correlated only as far as streptomycin and compound sulphonamide resistance was concerned.

Plasmid profile analysis was more specific for those isolates having plasmids. Thus, it was able to differentiate Salmonella livingstone from Salmonella typhimurium which was used as a control strain. However, there was very little correlation between plasmid content and source or similarly, there was very little correlation between plasmid content and antimicrobial resistance; this is a bit strange because plasmids are known to code for antimicrobial resistance (Broda, 1979). The most probable explanation is that most likely these plasmids were lost during subcultures.

There was however, good correlation to source, when plasmid content antimicrobial resistance patterns and biogroups were compared. Thus, all isolates of biogroup 3 from environmental sources in Northern region (Leeds) of England had no plasmids even though resistant to compound sulphonamide.

To conclude, biotyping, antimicrobial susceptibility testing and plasmid profile analysis as used in combination to trace the sources of isolates of Salmonella livingstone have proved invaluable and recommemded for use on other serotypes of Salmonella.

This study has also found that a single biotyping scheme is not suitable for all serotypes of Salmonella. Thus, a simple study of heat tolerance and survival in sterile tap water was able to discrimainate between biogroups. Those properties of Salmonella livingstone should further be examined in future studies as there was no time to come up with any conclusive remarks.

The study has also shown that Salmonella livingstone is not a homogenous serotype, but rather, is a mixture of serotypically related strains as shown by the biotyping results. It is therefore, safe to conclude that Salmonella livingstone is a fairly recent biotype since it has only 2 major biotypes and 2 variants.

B I B L I O G R A P H Y

AITKEN, M. M.; Jones, P.W; and Brown, G.T.H. (1982). Protection of cattle against experimentally induced salmonellosis by intradermal injection of heat-killed Salmonella dublin. Americal Journal of Veterinary Research 41, 1947-1951.

AITKEN, M.M.; Brown, G.T.H.; Jones, P.W.; and Collins, P. (1983) Salmonella Saintpaul infection in calves. Journal of Hygiene 91, 259-265.

AITKEN, M.M. (1984b). Methods of achieving protective immunity to salmonellosis in cattle in "Priority Aspects of Salmonellosis Research". Ed. Errebo Larsen, H. and Linton, A., CEC Workshop, Brussels, 1983, 239-247, Brussels, Belgium.

ALFREDSSON, G.A.; Baker, R.M; Old, D.C.; and Duguid, J.P. (1972). Use of tartaric acid isomers and citric acid in the biotyping of Salmonella typhimurium. Journal of Hygiene (Camb.) 70, 651-666.

ANDERSON, E.S. (1964). The phage typing of salmonellae other than Salmonella typhi in "The World Problems of Salmonellosis" (ed. E. Van Oye) pp. 89-110. The Hague: Dr W. Junk.

ANDERSON, E.S.; and Lewis, M.J. (1965). Drug resistance and its transfer in Salmonella typhimurium. Nature 206, 579-583.

ANDERSON, E.S. (1971). The modern ecological study of Salmonella typhimurium infection in "Recent Advances in Microbiology" (ed Perez-Miravete and D. Pelaez), p. 381. Xth International Congress for Microbiology, Mexico City, 1970.

ANDERSON, E.S.; and Threlfall, E.J. (1974). The characterization of plasmids in the enterobacteria. Journal of Hygiene (Camb.) 72, 471-486.

ANDERSON, E.S.; Ward, L.R.; De Saxe, M.J.; and Desa, J.H. (1977a). Bacteriophage typing designations of Salmonella typhimurium. Journal of Hygiene, 78, 297-300.

ANON, (1985). Suscovax* Pig Salmonella cholerae-suis Vaccine, living BVET 1970. In the ABPI Compendium of Data Sheets for Veterinary Products 1985-86, p. 179.

ARCHER, G.L.; Vishniavsky, H and Stiver, H.G. (1982). Plasmid pattern analysis of Staphylococcus epidermidis [illegible] isolates from patients with prosthetic valve endocarditis. Infectious Immunology, 35, 627-632.

BAILEY, W.R. and Bynoe, E.T. (1953). Cited by Ewing (1986).

BAIREY, M.H. (1978). Immunization of calves against salmonellosis. Journal of the American Veterinary Medical Association 173, 610-613.

* = Coopers Animal Health Limited

BALOWS, A. and Isenberg, H.D. (1978). Biotyping in the Clinical Microbiology Laboratory. Charles C. Thomas publisher, p. 12-32.

BANWART, G.J. and Ayres, J.C. (1953). The effect of various enrichment broths and selective agars upon the growth of several species of salmonella. Applied Microbiology, 1, 296-301.

BARKER, R.M. and Old, D.C. (1982). Differential typing of Salmonella agona: type divergence in a new serotype. Journal of Hygiene (Camb.) 88, 413-423.

BARKER, R.M. (1986). Special article: Tracing of Salmonella tymurium infections. Journal of Hygiene (Camb) 96, 1-4.

BOTES, H.J.W. (1964). Calf paratyphoid immunity. Evaluation of formol-killed and live attenuated vaccines. Bulletin Office International des Epizooties, 62, 581-605.

BOTES, H.J.W. (1965). Live vaccines in the control of salmonellosis. Journal of the South African Veterinary Medical Association, 36, 461-474.

BRODA, P. (1979). Plasmids. W.H. Freeman and Company Limited. Oxford and San Francisco, p. 1-22.

BRUNNER, F.; Margadant, A.; Peduzzi, R. and Piffaretti, J. (1983). The Plasmid Pattern as an Epidemiologic Tool for Salmonella typhimurium Epidemics: Comparison with Lysotype. The Journal of Infectious Diseases 148, 7-11.

BUXTON, A. (1957). "Salmonellosis in animals. A review". Review Series No 5, Commonwealth Bureau of Animal Health, Farnham Royal, UK.

CAMERON, C.M. and Fuls, W.J.P. (1976). Immunization of mice and calves against Salmonella dublin with attenuated live and inactivated vaccines. Onderstepoort Journal of Veterinary Research, 43, 31-37.

CARY, S.G. and Blair, E.B. (1964). New transport medium for shipment of clinical specimens. I. Fecal specimens. Journal of Bacteriology. 88, 96-98.

CARY, S.G.; Fusillo, M.H.; and Harkins, C. (1965). Survival of Shigella and Salmonella in a new transport medium. American Journal of Clinical Pathology 43, 294-296.

CHABBERT, Y.A.; El Solh, N.; Le Pors, M.J; Roussel, A. and Witchitz, J.L. (1979). Plasmid epidemics. In Grassi C, Ostino, G, eds. Antibiotics and Hospitals. New York: Alan R. Liss, 27-32.

CHRISTIE, A.B. (1974). Infectious Diseases, Churchill, Livingstone, Edinburg, 3.

CHRISTOPHER, P.J.; Claxton, P.D.; Dorman, D.C.; O'Connor, B.F.; Proudford, R.W. and Sutton, R.G.A. Salmonellosis: An increasing health hazard. Medical Journal of Australia, 1, 337.

CHUNG, G.T. and Frost, A.J. (1969). Sensitivity of Salmonella organisms to various chemotherapeutic agents. Australian Veterinary Journal 45, 354-359.

CLARKE, R.C. and Gyles, C.L. (1986). In the Pathogenesis of Bacterial Infections in Animals. Iowa State University Press/ Ames. p.96-107.

CLEWELL, D.B.; and Helinski, D.R. (1969). Supercoiled circular DNA-protein complex in Escherichia coli: purification and induced conversion to an open circular DNA form. Proceedings of National Academy of Science, USA. 62, 1159-1166.

COHEN, M.L. and Tauxe, R.V. (1986): Drug Resistant Salmonella in the United States: An Epidemiologic perspective. Science, 234, 964-969.

COLLARD, P. and Unwin, M. (1958). A trial of Rappaport's medium. Journal of Clinical Pathology II, 426-427.

COOPER, G.N. (1957). The prolonged survival of upper respiratory tract and intestinal pathogens on swabs. Journal of Clinical Pathology 10, 226.

COWAN, S.T. (1985). Cowan and Steel's Manual for the Identification of Medical Bacteria. 2nd ed. Cambridge University Press, p.113-114.

CRUICKSHANK, R.; Duguid, J.P. and Swain, R.H.A. (1969). Medical Microbiology: A Guide to The Laboratory Diagnosis and Control of Infection. 11th edition. E and S. Livingstone Limited. Edinburgh and London.

DOLD, H. and M. Ketterer (1944). Z. Hygiene, 125, 444 (As quoted by Ewing, 1986).

DUGUID, J.P.; Anderson, E.S.; Alfredsson, G.A.; Ruth Barker and Old, D.C. (1975). A new biotyping Scheme for Salmonella typhimurium and its phylogenetic significance. Journal of Medical Microbiology, 8, 149-166.

DUNN, C. and Martin, W.J. (1971). Comparison of media for isolation of salmonellae and shigellae from faecal specimens. Applied microbiology, 22, 17-22.

EBERTH, C.J. (1880). Die Organismen in Der Organen bei Typhus abdominalis. Archiv fur pathologische Anatomie und Physiologie, 81, 58-74.

EDEL, W.; Guinee, P.A.M.; Van Schothorst, M. and Kampelmacher, E. H. (1973). Salmonella cycles in feeds with special reference to the effects of environmental factors, including feeds. Canadian Institute of Food-Science and Technology Journal 6, 64.

EWING, W.H.; A.C. McWhorter and Montague, T.S. (1966). Transport media in the detection of Salmonella typhi in carriers. Journal of the Conference of Public Health Laboratory Directors, 24, 63-65.

EWING, W.H. (1968). Transport methods for Enterobacteriaceae and allied bacteria. Formulas, summaries and references. CDC publication.

EWING, W.H. (1986). Edward's and Ewing's Identification of Enterobacteriaceae. 4th edition. Elsewier Publishing Co. Inc. New York.

FARRAR, W.E.Jr (1983). Medical Perspective: Molecular Analysis of Plasmids in Epidemiologic investigation. Journal of Infectious diseases, 148, 1-6.

FLOYD, T.M. (1954). American Journal of Tropical Medicine and Hygiene, 3, 294. (as quoted by Ewing, 1986).

FOX, M.D. (1974). Recent trends in salmonellosis epidemiology. Journal of American Veterinary Medical Association, 165, 990.

GAERTNER, A. (1888). As quoted by Ewing (1986).

GAFFKY, G. (1884). Cited by Harvey (1929).

GANGAROSA, E.J.; Baker, W.H. Jr; Baine, W.B.; Morris, G.K. and Rice, P.A. (1973). Man vs Animal feeds as the source of human salmonellosis (letter to the editor). Lancet I, 878.

GALTON, M.M. and Hardy, A.V. (1948). Studies of acute diarrhoeal diseases. XXI. Salmonellosis in Florida. Public Health Reports. 63, 847-851.

GOODFELLOW, M. (1977). Numerical Taxonomy in the CRC Handbook of Microbiology. Laskin, A.I. and Lechevalier, H.A. Editors. 2nd edition. Volume 1: Bacteria, CRC Press. p. 579-596.

GUERRY, P.; LeBlanc, D.J. and Falkow, S. (1973). General Method for the isolation of plasmid deoxyribonucleic acid. Journal of Bacteriology, 116, 1064-6.

GUINEE, P.A.M.; Van Leeuwen, W.J. and Jahsen, W.H. (1972). New Technique for Biotyping. Applied Microbiology, 23, 1172-1174.

GUINEE, P.A.M. and Valkenburg, J. (1975). Salmonella isolations in the Netherlands, 1966-1973. Zentralbl. Bacteriol. Parasitenkd. Infektionakr. Hyg. Abt. 1: Orig. Reihe A. 231, 97.

GUNNARSSON, A.; Hurvell, B.; Nordb;om, B., Rutqvist, L. and Thal, E. (1974). Salmonella isolated from animals and feedstuffs in Sweden over the period 1968-72. Nord. Veterinaermed. 26, 499.

HARDY, K. (1986). Aspects of Microbiology 4. Bacterial Plasmids. 2nd edition. Van Nostrand Reinhold (UK) Co. Ltd.

HARVEY, D. (1929). Bacillus typhosa in "A System of Bacteriology". Volume IV. p. 17-85, HMSO, London.

HANSEN, J.B. and Olsen, R.H. (1978). Isolation of Large Bacterial Plasmids and Characterisation of the P2 incompatibility group plasmids PMG1 and PMG5. Journal of Bacteriology, 135, 227-238.

HELGASON, S. and Old, D.C. (1981). Comparison of four methods of differential typing of isolates of Shigella sonnei. Journal of Hygiene (Camb.) 87, 339-355.

HENNING, M.W. (1953c). Calf paratyphoid III. The transmission of antibodies to newly born calved. Odersteport Journal Of Veterinary Research, 26, 45-59.

HILKER, J.S. and Solberg, M. Evaluation of a fluorescent-antibody enrichment serology combination procedure for the detection of Salmonellae in condiments, food products, food by-products, and animal feeds. Applied Microbiology 26, 751.

HOISETH, S.K. and Stocker, B.A.D. (1981). Aromatic-dependant Salmonella typhimurium are non-virulent and effective as live vaccines. Nature, 291, 238-239.

HOOPER, W.L. and Jenkins, H.R. (1965). An evaluation of Rappaport's Magnesium chloride, Malachite green medium in the routine examination of faeces. Journal of Hygiene, 63, 491-495.

HOLMBERG, S.D.; Wachsmuth, I.K.; Hickman-Brenner, F.W. and Cohen, M.L. (1984). Comparison of Plasmid Profile analysis, phage-typing, and antimicrobial susceptibility testing in characterizing Salmonella typhimurium isolates from outbreaks. Journal of Clinical Microbiology, 19(2), 100-104.

HUGH-JONES, M.E.; Harvey, R.W.S, and McCoy, J.H.A. (1975). Salmonella california contamination of turkey feed concentrate. Bristol Veterinary Journal, 131, 673.

HUGHES, C. and Meynell, G.G. (1977). Rapid screening for plasmid DNA. Molecular and General Genetics 151, 175.9.

HUMPHREYS, G.O.; Willshaw, G.A and Anderson, E.S. (1975); A simple method for the preparation of large quantities of pure plasmid DNA. Biochim. Biophys. Acta 383, 457-463.

INGRAM, D.G. and Malcomson, M.E. (1970). Antibodies to E. coli in young calves: O antigens. American Journal of Veterinary Research, 31, 61-69.

INSALATA, N.F.; Mahnke, C.W. and Dunlap, W.G. (1972). Rapid direct fluorescent-antibody method for the detection of Salmonellae in foods and feeds. Applied Microbiology, 24, 645.

INSALATA, N.F.; Mahnke, C.W. and Dunlap, W.G. (1973). Direct fluorescent-antibody technique for the microbiological examination of food and environmental swab samples for Salmonellae. Applied Microbiology, 26, 268.

IVESON, J.B.; Kovacs, H. and Laurie, W. (1964). An improved method of isolating Salmonellae from contaminated dessicated coconut. Journal of Clinical Pathology, 17, 75-78.

IVESON, J.B. and Kovacs, H. (1967). A comparative trial of Rappaport's enrichment medium for the isolation of salmonellae from faeces. Journal of Clinical Pathology, 20, 290-293.

JACKS, T.M. (1978). Comments on Immunisation of calves against salmonellosis. Journal of the American Veterinary Medical Association, 173, 613-614.

JOHN, J.F. Jr.; McKee, K.T.Jr.; Twitty, J.A. and Schaffner, W. (1983). Molecular epidemiology of sequential nursery epidemics caused by multi-resistant Klebriella pneumoniae. Journal of Pediatrics (as cited by Farrar, 1983).

JONES, P.W. (1986). Characterisation of strains of Salmonella dublin with particular reference to pathogenicity. PhD. Thesis.

KADO, C.I. and Liu, S.T. (1981). Rapid procedure for detection and isolation of large and small plasmids. Journal of Bacteriology, 145, 1365-73.

KALLINGS, L.O. and Laurell, A.B. (1957). Relation between phage types and fermentation types of Salmonella typhimurium. Acta Pathologica et microbiologica Scandinavica, 40, 328-42

KALLINGS, L.O. Laurell, A.B. and Zetterberg (1959). An outbreak due to Salmonella typhimurium in veal with special reference to phage and fermentation typing. Acta pathologica et microbiologica scandinavia, 45, 347-56.

KARLSSON, K.A. and Thal, E. (1974). Studies on the detection of Salmonellae in fishmeal by the direct and indirect immunofluorescent method. Nord. Veterinaermed, 26, 492.

KAUFMANN, F. (1930). Zentralbl. Bakteriol. I. Orig. 119, 152.

KAUFMANN, F. (1941a). Die Bakteriologie der Salmonella-Gruppe. Copenhagen. Munksgaard.

KAUFMANN, F. (1951). Enterobacteriaceae Copenhagen: Munksgaard.

KAUFMANN, F. (1954). Enterobacteriaceae. 2nd edition. Copenhagen Munksgaard.

KAUFMANN, F. (1961). Die Bakteriologie der Salmonella species. Copenhagen: Munksgaard.

KAUFMANN, F.(1966). The Bacteriology of Enterobacteriaceae. Copenhagen: Munksgaard.

KAUFMANN, F. (1972). Serological diagnosis of Salmonella species. Copenhagen: Munksgaard.

KELTERBORN, E. (1967). Salmonella species. The Hague: Dr W. Junk.

KRISTENSEN, M.; Lester, V. and Jurgens, A. (1925). On the use of trypsinized casein, bromothymol blue, bromo-cresol purple phenol red and brilliant green for bacteriological nutrient media. British Journal of Experimental pathology, 6, 291-299.

LEIFSON, E. (1936). New selenite enrichment media for isolation of typhoid and paratyphoid (Salmonella) bacilli. American Journal of Hygiene, 24, 423-432.

LEMINOR, L. (1984). Genus Salmonella. In Bergey's manual of Systematic Bacteriology. Volume 1. ed. N.R. Krieg and J.G. Holt. Baltimore: Williams and Wilkins.

LEVY, B.S.; McIntire, W.; Damsky, L.; Lashbrook, R.; Hawk, J.; Jacobsen, G.S. and Newton, B. (1975). The Middleton Outbreak: 125 cases of foodborne Salmonellosis resulting from cross contaminated food items served at picnic and a smorgasbord. American Journal of Epidemiology, 101, 502.

LEWIS, M.J. and Stocker, B.A.D. (1971). A biochemical subdivision of one phage type of Salmonella typhimurium. Journal of Hygiene, 69, 683-91.

LIGNIERES, J. (1900). Cited by Jones (1986).

LIGNIERES, J. (1901). Cited by Jones (1986).

MARKOWITZ, S.M.; Smith, S.M. and Williams, D.S. (1983). Retrospective analysis of Plasmid patterns in a study of burn unit outbreaks of infection due to Enterobacter choacae. Journal of Infectious Diseases, 148, 18-23.

McCALL, C.E.; Martin, W.T. and Boring, J.R. (1966). Efficency of cultures of rectal swabs and faecal specimens in detecting salmonella carriers: correlation with numbers of Salmonella excreted. Journal of Hygiene (London). 64, 261-269

McGOWAN, J.E. Jr; Terry, P.M.; Huang, T.S.R.; Honk, C.L. and Davies, J. (1979). Nosocomial infections with gentamicin-resistant staphylococcus autius: Plasmid analysis as an epidemiologic tool. Journal of Infectious Diseases, 140, 864-872.

MEYERS, J.A.; Sanchez, D.; Elwell, L.P. and Falkow, S. (1976). Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. Journal of Bacteriology, 127, 1529-37.

MEYER, H. (1980). Experiences of Salmonella dublin live vaccine for oral use in calves. Archiv fur Experimentalle Veterinarmedizin, 34, 99-104.

MINISTRY OF AGRICULTURE FOOD AND FISHERIES. (MAFF). REPORTS. Zoonosis order: Annual Summaries. 1976-1984.

MULLER, L. (1923). Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C.R. Soc. Biology, 89, 434-437.

MURRAY, C.J.; Ratcliff, R.M.; Cameron, P.A. and Dixon, S,F. (1986). The resistance of antimicrobial agents in Salmonella from veterinary sources in Australia from 1975-1982. Australian Veterinary Journal, 63(a), 286-292.

RANKIN, J.D.; Taylor, R.J. and Newman, G. (1967). The protection of calves against infection with Salmonella typhimurium by means of a vaccine prepared from Salmonella dublin (Strain 51). Veterinary Record, 80, 720-726.

RANTALA, M. and Nurmi, E. (1974). Hazards involved in the use of furazolidone for the prevention of salmonellosis in broiler chicken. Journal of Hygiene (Camb.) 72, 349-354.

RAPPAPORT, F.; Stark, G.J. and Konforti, N. (1956a). UMAGIS medium. A medium for single tube differentiation of Enteric bacteria. Applied Microbiology, 4, 157-161.

RAPPAPORT, F.; Konforti, N. and Navon, B. (1956b). A new enrichment medium for certain Salmonellae. Journal of Clinical Pathology, 9, 261-266.

REILLY, W.J.; Old, D.C.; Munro, D.S. and Sharp, J.C.M. (1985). An epidemiologic study of Salmonella montevideo by biotyping. Journal of Hygiene (Camb,). 95, 23-28.

RILEY, L.W.; and Cohen, M.L. (1982). Plasmid profiles and Salmonella epidemiology. Lancet, p. 573.

RILEY, L.W.; George, T.D. Ferdinando, Jr.; Thomas, M. Demelfi and Cohen, M.L (1983). Evaluation of isolated cases of Salmonellosis by Plasmid Profile Analysis: Introduction and

NEWELL, K.W. and Williams, L.P. Jr. (1971). The control of Salmonellae affecting swine and man. Journal of American Veterinary Medical Association, 158, 89.

O'BRIEN, T.F.; Hopkins, J.D.; Gilleece, E.S.; Medeiros, A.A.; Kent, R.L.; Blackburn, B.O.; Holmes, M.B.; Reardon, J.P. Vergeront, J.M.; Schell, W.L.; Christenson, E.; Bisett, M.L. and Morse, E.V. (1982). Molecular epidemiology of antibiotic resistance in Salmonella from animals and human beings in the United States. New England Journal of Medicine, 307, 1-6.

OLD, D.C.; Helgason, S. and Scott, A.C. (1981). Discrimination by multiple typing of isolates of Shigella sonnei in Dundee. (1971-6). Journal of Hygiene (Camb.) 87, 357-367.

PICTON, W.H.A.; Stirrup, W.; Price, A. and Joan Taylor (1953). Journal of Pathology and Bacteriology, 66, 310.

PROST, E. and Riemann, H. (1967). Foodborne salmonellosis. Annual Review of Microbiology, 21, 495-517.

RADLOFF, R.; Bauer, W; and Vinograd, S. (1967). A dye-buoyant density method for the detection and isolation of closed circular duplex DNA: the closed circular DNA in Hela cells. Proceedings of the National Academy of Sciences, Washington 57, 1514-20.

ROBERTSON, A. (1976) (ed). Handbook on animals Diseases in the tropics, 3rd edition. British Veterinary Association p. 151-155.

Transmission of a Bacterial Clone by precooked roast beef. Journal of Infectious Diseases, 148, 12-17.

ROWE, B.; Hall, M.L.M.; Ward, L.R and Desa, J.D.H. (1980). Epidemic spread of Salmonella hadar in England and Wales. British Medical Journal, 280, 1065-1066.

SALMON, D.E and Smith, T. (1885). The bacterium of swine plague. 2nd Annual Report. Bureau of Animal Industry, Department of Agriculture, Washington DC, 184-244

SCHABERG, D.R.; Tompkins, L.S. and Falkow, S. (1981). Use of agarose gel electrophoresis of plasmid deoxyribonucleic acid to fingerprint gram-negative bacilli. Journal of Clinical Microbiology, 13, 1105-1108.

SCHUETZE, H. (1921). Cited by Ewing (1986).

SILLIKER, J.H.; Deibel, R.H. and Fagan, P.T. (1964). Enhancing effect of faeces on isolation of Salmonellae from selenite broth. Applied Microbiology, 12, 100-105.

SMITH, H.W. (1952). The evaluation of culture media for the isolation of Salmonellae from faeces. Journal of Hygiene (London), 50, 21-32.

SMITH, H.W. (1959). Cited by Ewing (1986).

SOJKA, W.J. and Field, H.I. (1970). Salmonellosis in England and Wales 1958-1967. Veterinary Bulletin, 40, (7), 515-531.

SOJKA, W.J.; Slavin, G.; Brand, T.F. and Davies, G. (1972). A survey of drug resistance in salmonellae isolated from animals in England and Wales. British Veterinary Journal 128, 189-197.

SOJKA, W.J.; Wray, C.; Hudson, E.B. and Benson, J.A. (1975). Incidence of salmonella infection in animals in England and Wales, 1968-73. Veterinary Record, 96, 280.

SOJKA, W.J.;Wray,C. and Hudson, E. (1977). A survey of drug resistance in salmonellae isolated from animals in England and Wales during 1973 and 1974. British Veterinary Journal, 133, 292-311.

SOJKA, W,J.; Wray, C. and McLaren, I (1984). A survey of drug resistance in salmonellae isolated from animals in England and Wales in 1979 and 1981. British Veterinary Journal, 140, 576-591.

SOJKA, W.J.; Wray, C. and McLaren, I. (1986). A survey of drug resistance in salmonellae isolated from animals in England and Wales in 1982 and 1983. British Veterinary Journal, 142, 371-380.

TAYLOR, W.I and Schelhart, D. (1973). Effect of temperature on performance of media in detection of enteric pathogens. Applied Microbiology, 25, 940-944.

TEMBROECK,C. and Norbur, F.G. (1916). Cited by Ewing (1986).

THRELFALL, E.J.; Ward, L.R.; Ashley, A.S and Rowe, B. (1980). Plasmid encoded trimethoprim resistance in multi-resistant epidemic of Salmonella typhimurium phage types 204 and 193 in Britain. British Medical Journal i, 1210-1211.

WHITE, P.B. (1925). Cited by Ewing (1986).

WHITE, P.B. (1926). Cited by Ewing (1986).

WILLIAMS, L.P. Jr and Hobbs, B.C. (1975). Enterobacterioceae infections, in"Diseases transmitted from Animals to Man" 6th edition, Hubbert, W.T., Mc Culloch, W.E. and Schnurrenberger, P.R.; Eds., Charles, C Thomas, Springfield III. 33.

WILLIAMS, L. P. Jr. (1980). Salmonellosis CRC Handbook Series in Zoonosis. James, H. Steele; Herbert Stoenner, William Kaplan and Michael Torfen Editors, Section A: Bacterial Rickettsial and Mycotic diseases. Volume II CRC Press. p. 11-34.

WRAY, C.; Hedges, R.W.; Shannon, K.P. and Bradley, D.E. (1986). Apramycin and gentomicin resistance in Escherichia coli and Salmonellae isolated from farm animals. Journal of Hygiene (Camb.) 97, 445-456.