POULTRY MEAT AND HUMAN SALMONELLOSIS:

Establishing the Epidemiological Relationship

by

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DEDICATION

This Thesis is dedicated to the everlasting memory of my beloved mother, **Mrs Elizabeth Oboegbulem** who died in Nigeria in 1989, while this research programme was in progress. Her industry, her commitment and sacrifice towards my early education laid the foundation and nurtured the motivation that made the present stage possible.

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DECLARATION

I declare that this thesis has been composed by myself and is a record of research performed by myself. It has not been submitted previously for a higher degree.

This research was carried out in the Department of Community Medicine, University of Glasgow; the Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow; and the Communicable Diseases (Scotland) Unit, Ruchill Hospital, Glasgow from October 1986 to September 1989.

March 1990 S I Oboegbulem

LIST OF PRESENTATIONS AND RELEVANT PUBLICATIONS

1 OBOEGBULEM S I, Reilly W J and Munro D. Poultry meat and Human Salmonella Infection: Paper presented at the 10th International Symposium of the World Association of Veterinary Food Hygienists, Stockholm, Sweden; July 1989.

2 REILLY W J, Oboegbulem S I and Munro D. Human Salmonellosis and Poultry Meat: Clarifying the epidemiological relationship by comparing isolates from the effluent of a static population with those in the poultry meat consumed: Final Report, Scottish Hospital Endowment Research Trust: Research Grant 872.

3 REILLY W J, Forbes G I, Sharp J C M, Oboegbulem S I, Collier P W and Patterson G M. Poultry Meatborne Salmonellosis in Scotland. *Epidemiol & Infect* 1988; **101**: 115-122.

SUMMARY

Extensive literature review confirms that in the industrialized countries, salmonellosis is a major public health problem, causing considerable social and economic losses. Non-typhoid salmonellosis occurs primarily as a foodborne zoonosis. There are different animal sources of human infections, and this raises the question of determining and defining which meat types constitute the major hazards for man. The studies reported in this thesis were designed to establish and clarify any epidemiological relationship between consumption of poultry meat and human salmonellosis. Three epidemiological approaches were employed:

A 20-year **retrospective study** was undertaken to determine the epidemiological characteristics of foodborne salmonella infections and outbreaks. It was hoped that the retrospective analysis would generate some hypotheses on the incidence, risk factors, and trends of human salmonellosis in Scotland. All the 1,791 outbreaks of foodborne infections and intoxications recorded by the Communicable Diseases (Scotland) Unit (CD(S)U) between 1980 and 1987 were computer-analysed. A one in 5 systematic sample (n = 5,776) of approximately 29,000 human salmonella infections (laboratory reported isolations) listed in the Weekly and Annual Reports of the CD(S)U for the period 1968 to 1987 was also computer-analysed.

Eight-five per cent of all foodborne outbreaks recorded from 1980 to 1987 were caused by the salmonellae. Salmonella food poisoning has been increasing in Scotland, as in England and Wales. Laboratory isolations of salmonellae were made from an average of 1,400 persons per year, and the standardized crude incidence rate is approximately 30/100,000 per year. Both the crude incidence and the standardized incidence rate showed a trend of a steady rise. There was a three-fold (300%) increase in the standardized incidence rate from 14/100,000 population per year for the

period 1968-72 to 42/100,000 per year during 1983-87. With a mean age-specific incidence rate of 63/100,000 per year, children 5 years old and below are at highest risk of foodborne salmonellosis. Although highest case fatality rate occurs among the elderly, the age-specific incidence rate among persons over 70 years old is comparatively very low (15.3/100,000 per year). Significantly higher incidence rates of salmonella infections were recorded in males than in females. There is a consistent seasonal trend in salmonella infections and outbreaks; more than half of all the outbreaks occurred during the months of July, August and September.

S.typhimurium, *S.enteritidis* and *S.virchow* are the three major causes of foodborne salmonella infections and outbreaks. Up to 1985, *S.typhimurium* remained the primary cause of salmonellosis; but since 1986, *S.enteritidis* has assumed the primary place. Between 1968 and 1987, there was a four-fold (400 per cent) increase in the incidence of *S.enteritidis*; the upsurge in *S.enteritidis* seems to be due to an unprecedented increase in the incidence of phage type 4 in poultry products.

Poultry meat was the primary vehicle of foodborne salmonella outbreaks, accounting for 69 per cent of all meatborne episodes. Between 1975 and 1987, there has been 400 per cent increase in the proportions of outbreaks in which poultry was implicated; and there has been no change in the primary place of poultry during the past 10 years. From the retrospective analysis, the hypothesis is that poultry is the major risk factor; consumption of poultry meat is significantly associated with salmonella infections.

In most reported incidents of salmonellosis, the evidence which incriminates poultry meat is only circumstantial. In many outbreaks, the causative salmonella types are isolated from both clinically infected and symptomless excretors. It seems important to be able to demonstrate an epidemiological association between consumption of poultry meat and salmonella excretion without relying on investigation of

clinical incidents. Poultry meat used in a catering establishment can be screened to identify salmonella types to which the consumers are exposed. Salmonella excretion (an indicator of salmonella infection or transient carriage) in the consuming population can be investigated by parallel monitoring of the sewers draining the defined population area. By comparing the salmonella types isolated from the poultry meat and the sewers, and the frequency of isolation of identical types, an epidemiological association between the poultry meat and human infection can be assessed. This was the overall objective of the second epidemiological approach employed - the **bacteriological surveys.**

Over a period of 40 weeks in 1988, batches of raw chicken carcases supplied to the kitchen of a long-stay psychiatric hospital were sampled for the presence of salmonellae. Parallel weekly survey of the sewers draining the residential accommodation of the patients was carried out, using the Moore's swab technique. Following a change of policy in the hospital kitchen, from raw chicken carcases to precooked chicken, samples of frozen packs of deboned whole chicken were examined for a further 5 weeks, alongwith the weekly sewer survey. Two swabs were left in place in the sewer flow for 7 days. Contaminated swabs collected in a given week were expected to monitor the presence of the same salmonella types recovered from the chicken carcases during the preceding week. Samples were examined from both chicken and sewers during 35 corresponding or matching weeks. Technical microbiological aspects of the study were carried out by the author at the Scottish Salmonella Reference Laboratory (SSRL). Salmonella isolation and identification were carried out in accordance with standard biochemical and serological protocol, as adapted by the SSRL.

Two hundred and fourteen (45%) of the 477 fresh and frozen chicken carcases sampled were positive for salmonella. The proportions of weekly samples of carcases contaminated with salmonellae ranged from 27.5 - 67 per cent. Every weekly batch of carcases sampled during 38 weeks contained individually contaminated carcases. The public health

implication is that the salmonellae are continually getting into the hospital kitchen through contaminated carcases, and by inference, into most domestic and commercial kitchens. A total of 19 salmonella serotypes were isolated from the carcases. No salmonellae were recovered from 102 packs of precooked chicken examined. Salmonellae were detected in 30 (38%) of the 79 sewer swabs examined in 28 of the 40 weeks when raw chicken carcases were used in the hospital kitchen. Only one of the 20 swabs examined during the 5 weeks that cooked chicken was used, yielded salmonella. Eleven of the 16 salmonella types (different serotypes and phage types) detected in the sewer were also recovered from the chicken carcases; seven of the 11 salmonella types were isolated from both carcases and sewer during corresponding or matching weeks. The same salmonella types were isolated from both sources in 13 of the 35 matching weeks. The detection of the same salmonella type from chicken carcase and sewer in corresponding weeks (+, +) occurred more frequently than would be expected to happen by chance (X^2 = 15.08; $p < 0.005$).

S.enteritidis PT4, *S.typhimurium* PT49 and 104, and *S.virchow* were the most common salmonella types isolated from the chicken carcases and from the sewer. The 3 serotypes were the most predominant poultry salmonellae reported in the United Kingdom in 1988 under the Zoonoses Order; the same serotypes were topmost in the ranking order of serotypes responsible for outbreaks analyzed in the retrospective study, and were also major causes of poultry-implicated salmonella outbreaks recorded in Scotland during the survey period (1988). In view of the predominance of the three serotypes during the period, their detection in chicken carcases and sewer in matching weeks should be expected to occur by mere chance in a number of times. However, chi-square analysis of the observed and expected frequencies showed that *S.enteritidis* (PT4), *S.typhimurium* PT49 and 104 and *S.virchow* were detected from both sources in excess of the frequency that would be expected by chance (x^2 = 9.19, $p < 0.005$).

It is arguable that red meat (beef, pork/ham, lamb) might be the source of the major salmonella serotypes detected in the sewer. However, in 1987, no isolations of *S.enteritidis* were recorded in cattle in Scotland; and in 1988 only 16 incidents of *S.enteritidis* were reported for cattle in the whole UK. Although *S.typhimurium* predominated also in livestock other than poultry, the ranking order of the phage types recorded in cattle were not similar or related to the order of phage types responsible for foodborne incidents during 1980-88. These data provided valid and plausible evidence against red meat being the primary source of the major salmonellae excreted in the sewer.

The change from raw to precooked chicken coincided with or resulted in statistically significant reduction in the incidence of salmonella types detected in the sewer ($p = 0.046$, Fisher's Exact Test). The removal of the presumed risk factor (raw chicken carcases) was thus followed by significant reduction of the unwanted outcome (excretion of salmonellae). This seems to provide **plausible** evidence of causal association.

All the analyzed data provided evidence to reject a null hypothesis of no association; and suggest a significant association between poultry meat and human salmonellosis.

A third epidemiological approach, a Matched Neighbourhood Case-Control Study, was employed to further test the null hypothesis of no epidemiological relationship between poultry meat and human salmonella infections. There is need to further clarify any evidence of an association between salmonella infection and

(i) the **type of meat** (beef, pork/ham, lamb) consumed in the 48 hours prior to illness;

(ii) the **form of poultry meat** (precooked, fresh, frozen) eaten 48 hours before onset of illness;

(iii) the **method of cooking** poultry meat (boiling, roasting, frying, grilling);

(iv) the **number of times** in a typical week that poultry meat is consumed.

Sporadic and primary household cases of foodborne infections occurring in the Glasgow district between April 1988 and March 1989 were accessed through the assistance of environmental health officers (EHOs). Relevant data on food histories were obtained by a self-completing questionnaire from 125 responding cases. A variable number of controls (one, two or three) per case, matched for neighbourhood (maximum of post code area), sex and age were obtained for 118 cases. Controls were randomly selected and accessed by post on a weekly basis, as the reported and investigated cases responded. Controls were asked the same questions as the cases. Mantel-Haenszel Analysis of Odds Ratio with one, two or variable number of controls per case was employed to test if the odds of exposure to a specified variable differed significantly between cases and controls.

Salmonella infection was significantly associated with consumption of poultry meat (Mantel-Haenszel Odds Ratio = 4.2). Cases were significantly more likely to have eaten poultry meat in the 48 hours before onset of illness than were matched controls (X^2_{mh} = 19.25, $p < 0.005$). Eating poultry meat increased the risks of salmonellosis by 320 per cent. In contrast, consumption of red meat did not increase, but rather decreased the risk of salmonella illness (Odds $Ratio_{mh}$ = 0.38). Highest risk of salmonella infection was associated with consumption of frozen poultry meat (Odds $Ratio_{mh}$ = 4.0, $p < 0.005$); frozen poultry meat (chicken or turkey) increased the risk of infection by 300 per cent. Eating fresh poultry meat was less significantly associated with salmonella illness (Odds $Ratio_{mh}$ = 2.58). Precooked poultry meat was not significantly associated with salmonellosis (Odds $Ratio_{mh}$ = 1.21). Consumption of roasted poultry meat was significantly associated with salmonella illness (Odds $Ratio_{mh}$ = 4.0, $p < 0.005$). In contrast,

eating boiled chicken significantly decreased the risk of salmonella infection (Odds $Ratio_{mh}$ = 0.56). Consumption of poultry meat 3 or 4 days in a typical week increased the risk of salmonellosis by 126 per cent (odds $Ratio_{mh}$ = 2.26; $p < 0.005$).

From the retrospective analysis, the bacteriological surveys, and the case-control study, the epidemiological criteria of **strength of association, temporal association (time sequence), consistency** and **biological plausibility** were sufficiently satisfied to accept an hypothesis of significant association between poultry meat and human salmonellosis. Poultry meat is the primary vehicle of sporadic and outbreak incidents of salmonella infection. Lapses in kitchen hygiene and kitchen practices create the opportunities for cross contamination of kitchen environment and cooked foods, and make it likely for the salmonellae to get to consumers.

Veterinary public health preventive measures to reduce poultry infection and cross-contamination of carcases; intensified efforts by the industry towards safe production, slaughter and processing of poultry products; strict enforcement of recent legislations; some form of safe treatment of poultry carcases such as irradiation; and public health education of domestic and institutional (hospital) kitchen staff, and commercial caterers must be intensified to reduce the incidence of human salmonella infections.

CHAPTER ONE

REVIEW OF LITERATURE

CHAPTER ONE

REVIEW OF LITERATURE

1.1 SUMMARY:

Three epidemiological approaches were employed in this thesis in an attempt to establish and clarify the association between consumption of poultry meat and human salmonella infections. Before the objectives, specific aims, hypotheses, methodology and results of the various approaches are presented, available literature relating to relevant aspects of poultry and human salmonellosis are reviewed. The detailed literature review highlights the public health and economic importance, as well as the nature and magnitude of the salmonellosis problem. By way of background, the review establishes the basis and the justification for the present study.

Non-typhoid salmonella infections occur primarily as foodborne zoonosis. As a public health hazard, the illness has serious social and economic effects on the patient and the health institutions, in addition to its economic impact on agriculture and the food industry. Studies have established that salmonellosis imposes on the society, identifiable **tangible** and **intangible** costs. The costs of prevention and control of poultry salmonellosis have been calculated on the basis of losses arising during **production** and **slaughtering** processes. Such costs include economic effects of clinical salmonellosis on the poultry production industry, as well as the public health consequences of asymptomatic poultry carriers and contaminated end products. The direct and indirect costs arise from the morbidity and mortality in poultry, culling rate, and varying levels of condemnation at processing. Other categories of costs relate to enforcement of legislations on the safe production, processing, distribution, retailing, and

preparation of poultry and poultry products. Thus, in addition to the Zoonoses Order 1989, sixteen other Orders have been promulgated by the Agriculture Ministry to control the various aspects of the salmonellosis problem; and between January and April 1989, nearly 195,000 chickens from 16 flocks were destroyed, and 14 broiler breeding flocks were placed under restriction orders. By the end of 1989, some one million birds in 87 flocks had been destroyed!

Studies in Scotland and other countries have sought to evaluate the total costs of outbreaks of foodborne salmonellosis and the most economic methods for prevention and control of human salmonellosis. In the USA, it is estimated that human salmonellosis might be responsible for a loss amounting to 1.2 billion dollars each year! In the Federal Republic of Germany, the cost of human salmonellosis in 1977 was estimated to be 120 million German Marks for sickness and death. In Scotland, the cost per reported case of poultry-borne salmonellosis in 1985 was estimated to range from £900 to £3,655, and the estimated total costs of reported and unreported cases were in excess of £10,000,000 each year, based on the maximum upper bound predictions of costs of unreported cases.

Clinical salmonellosis in poultry has been known to be caused by two avian host-specific *Salmonella* serotypes: *S.gallinarum* and *S.pullorum*. These serotypes are not considered significant public health hazards, as infection in man is quite rare. With virtual eradication of these host-specific serotypes in the United Kingdom, clinical salmonellosis in poultry was no longer common. However, there have been recent reports of clinical disease in broilers, broiler breeders and laying flocks caused by *S.enteritidis* phage type 4. A growing list of other salmonella serotypes has been isolated from poultry farms and processed products. The frequencies of the isolations have provided evidence of the endemicity of non-host-specific salmonellae in poultry flocks and eviscerated carcases. Some surveys have shown a correlation between serotypes occurring in carcases and those isolated from the

flocks of origin. Some salmonella serotypes like *S.typhimurium* occur regularly in poultry; some exotic serotypes appear for a short time and then disappear; while other serotypes like *S.agona*, *S.virchow* and *S.enteritidis* have become established and produce episodes of poultry-associated foodborne outbreaks. Cited references show that the contamination or incidence rates of the salmonellae in poultry carcases range between 20 per cent and 80%.

There are many possible sources of salmonellae in poultry. The most important source is said to be poultry feeds - specifically, the animal protein components of the feed (meat and bone meal, blood meal, poultry offal, etc). There is a demonstrable relationship between contaminated feed, the incidence of particular salmonella serotypes in poultry, and the appearance of the same serotypes in outbreaks of human salmonellosis.

Since the mid 1950s, when the broiler industry began in the UK, broiler production has become so expansive that in 1985, the total production in Scotland was around 91,000 tons. Poultry slaughter has become highly automated, with modern plants processing over 10,000 birds per hour. At such processing rates, it has become impossible to prevent cross-contamination from carcase to carcase in the multi-stage operation. The various stages of the broiler production and processing, and the critical points for salmonella cross-contamination are reviewed. General control measures relating to husbandry practices; specific and non-specific veterinary public health preventive measures, as well as critical control points during production, slaughter and processing are also reviewed in detail.

Human salmonellosis usually occurs after an incubation period of 6 to 48 hours, and manifest as **enteric infections** caused by any of the numerous non-typhoid salmonellae. Clinical symptoms experienced by most patients include diarrhoea, cramps, nausea, vomiting and fever. The infection is an enterocolitis rather than a gastroenteritis. Mortality is usually very low. Development and severity of

clinical syndrome are influenced by age, undercurrent infection, and immune status. More serious symptoms and most deaths occur in neonates, infants, the elderly, in immuno-depressed individuals, and persons whose resistance is otherwise compromised. In the industrialized countries, the salmonella infection rate has been reported to range from 10 to 70 per 100,000 persons per year.

The salmonellae can induce a carrier-phase in convalescent and apparently recovered persons, which is marked by intermittent excretion of the organism. In untreated convalescent patients, duration of excretion is 4 to 5 weeks. Antibiotic therapy has been shown to prolong carriage and duration of excretion. However, long-term or permanent carriage of non-typhoid salmonellae is unusual. Because salmonella infection can be latent and asymptomatic in many individuals, there are healthy and symptomless excreters. In the UK, one study found 2.5 per 1000 children and adults to be excreting salmonella. Based on an extensive review of studies in the developed countries, another study in the USA found the **median** carriage rate to be 1.5 per 1000 persons. For the developing countries, the median carriage rate was 18 per 1000 persons or 12-fold higher than in the developed nations. In one of the epidemiological approaches in the present study, the design of the salmonella survey was based on the probability of latent infection, transient carriage, and symptomless excretion in a "closed" population.

Statutory notification of food poisoning has been instituted in Scotland since 1956, and a more structured national surveillance system has remained effective since January 1980, when Scotland became the first country to participate formally in the WHO Surveillance Programme for the Control of Foodborne Infections and Intoxications in Europe. Under the Scottish Surveillance System, a person suffering from suspected foodborne illness presents to the General Practitioner (GP) or the hospital, who would have appropriate specimens submitted to the diagnostic laboratory. Through the Community Medicine Specialist

(CMS), the local environmental health officer (EHO) is contacted who carries out routine epidemiological investigation. Routine notifications are made by the CMS/EHO and the diagnostic laboratories to the Communicable Diseases (Scotland) Unit (CD(S)U) which serves as the national coordinating centre for the WHO Surveillance Programme. In spite of the well coordinated, sequential system, salmonellosis surveillance is essentially **passive.** The result is that in Scotland, as in other developed countries, vast proportions of the infections are not reported, and the true magnitude of the salmonellosis problem remains undetermined. By extensive literature review and by applying independent methods - determination of **median** carriage rate and duration of excretion, and calculation of "sequential artifacts" within the national surveillance system workers in the USA recently established a mean estimate of 1.4 million cases per year. This is in contrast to an average of 40,000 reported cases each year.

There are several sources of human foodborne infection, but over the years, the foods most commonly incriminated have been milk, meat (poultry meat and red meat), eggs, and their products. Prior to 1983, milk was the primary vehicle associated with foodborne salmonellosis in the UK; and the problem was particularly serious in Scotland. Since the introduction of compulsory pasteurization of cows milk in Scotland, poultry meat appears to have replaced milk as the primary source of human salmonella infections. Reviewed literature suggest a steady rise in poultryborne salmonellosis. Epidemiological studies on the role of meat, and specifically, the establishment of evidence of an epidemiological association between poultry meat and human salmonella infections constitute the central objective of this thesis.

The problem of clarifying the epidemiological link between foodborne salmonellosis and food animal sources may be resolved by detailed differentiation or discriminating typing schemes for **salmonella** strains isolated from outbreak incidents and the suspected food sources. The various

discriminating schemes for the bacterial strains based on genetic or biochemical properties have been described as "epidemiological markers". Such markers include **serotyping, phage typing, biotyping, antimicrobial resistance, restriction enzyme fingerprinting,** and **DNA hybridization** of the salmonellae isolated from human and from the food sources. Those epidemiological markers considered absolutely necessary (the serotypes and phage types) are in routine use world-wide; the others are employed only in specialized laboratories. In the following studies of the epidemiological association between poultry meat and human salmonella infections, the serotypes, phage types and antimicrobial resistance were employed. Biotyping and plasmid profile analysis could not be undertaken within the scope of time and resources available.

In point source outbreaks, it is the practice to determine the food source usually by application of a number of epidemiological markers. Thus, isolation of the same salmonella serotype, phage type, biotype or with identical plasmid profile from both the outbreak cases and the suspected common food provides **bacteriological evidence** of association. Examples of point-source outbreaks in which poultry was linked by bacteriological evidence are reviewed. In many outbreaks, it may not be possible to identify the food vehicle, because of delays in notification or difficulties in obtaining the food remnants. In such cases, the incriminated food may be determined by **epidemiological evidence**: analysis of attack rates; biological plausibility; case-control studies; isolation of identical salmonella types from other animals in the same farm or flock, or from other meat samples in the same batch; isolation of salmonella types concurrently recovered from and generally associated with the particular animal species; or a history of the suspected common food having been consumed 2 or 3 days prior to onset of the illness. However, only varying proportions of persons affected in outbreaks manifest typical clinical symptoms. Besides, the vast majority of foodborne salmonella infections occur as **sporadic** cases rather than as outbreak incidents. For these outbreaks of

uncertain food vehicles; for the latent, asymptomatic infections, or transient carriage; and for the predominant sporadic episodes, other epidemiological approaches would have to be employed to establish the link with specific food sources such as poultry meat. It is the recognition of, and the need to provide an answer to, such situations, that forms the basis for applying some of these other epidemiological approaches for my thesis.

1.2 INTRODUCTION:

The fundamental objective of this thesis is to establish and clarify the epidemiological relationship between poultry meat and human salmonellosis. Three epidemiological approaches will be employed to try to achieve the objective. Before the specific aims, hypotheses and methodology of the different approaches are presented, it is appropriate, by way of background to review the literature relating to relevant aspects of the salmonellosis problem. In this Chapter, therefore, available literature on the following areas is reviewed in detail:

(1) Public Health and Economic Importance of Salmonellosis;

(2) The Nature of the Infection and the Endemicity of Salmonellae in poultry flocks and carcases;

(3) The Poultry Industry in the United Kingdom, including poultry processing and the critical points for (salmonella) cross-contamination;

(4) Control of Poultry Salmonellosis;

(5) Sources and Manifestations of Human Salmonellosis;

(6) The Magnitude of Foodborne Salmonellosis in Industrialized Countries;

(7) Foodborne Salmonellosis in Developing African Countries;

(8) Epidemiological Markers applied in Identification of the Salmonella and in tracing Infection Pathway;

(9) Selected Outbreaks of Foodborne Salmonellosis: Implication of poultrymeat by bacteriological and epidemiological evidence;

(10) Organization of Surveillance Programme for Foodborne Salmonella and other Infections in Scotland.

1.3 PUBLIC HEALTH AND ECONOMIC IMPORTANCE OF SALMONELLOSIS:

Epidemiological studies provide one reliable means of assessing the risks of zoonotic diseases to human health. Accurate epidemiological data depend largely on the level of awareness of the public health hazard of the disease, effective and available methods of diagnosis, and an efficient, properly organized surveillance system. The willingness of the poultry industry and government agricultural or health agencies to commit resources to the control of salmonellosis will depend on the perceived public health and economic importance of the disease. Producers of poultry and poultry products would be unwilling or reluctant to spend more on aspects of salmonellosis control, unless they are convinced that this disease is costing them money or that the risk of salmonellosis to public health poses a serious economic threat to the industry.

One approach to economic classification of disease is incidence probability (1). Under this approach, salmonellosis belongs to the category or class of infections which are sure to continue to cause frequent economic and public health losses, if no effective preventive and control measures are adopted. This is because, in the industrialized countries, salmonellosis has become an endemic zoonosis, showing a "regular level of incidence" and

a high degree of certainty of occurrence. In recent years, there has been substantial increase in the incidence of human salmonella infections. Part of the increase may be apparent - reflecting the combined effects of improved diagnostic methods, an organized routine surveillance and better reporting. However there may be evidence that the increased incidence is real - the result of actual increase in the numbers of infections. Accompanying the increased incidence is a greater public awareness and greater concern about the public health hazard and economic damage caused by salmonellosis.

Non-typhoid salmonella infections occur essentially as a foodborne zoonosis. Thus, the disease has social and economic impact on agriculture and the food industry, in addition to the serious socioeconomic effects on the patients and the health institutions. The costs of sickness or death of humans and animals such as poultry may be estimated by applying principles of "economic appraisal" in identifying **relevant** costs **(2, 3)**. Such relevant costs include (a) **opportunity** costs, representing the value of benefits foregone because resources were channelled or diverted from other uses as a consequence of the outbreak; (b) **marginal** or extra costs imposed by the episode; (c) costs to the society, both **tangible** and **intangible.** Costs are said to be tangible or intangible according to whether or not they are easily measured in money terms. The overall costs of an outbreak are derived by multiplication of the costs per case with the determined incidence of cases. In order to appraise the total costs, the percentage of unreported cases should be determined, in addition to the officially notified or registered cases. Studies in different countries have estimated that true incidence of human salmonella infections is 10 to 12 times higher than the officially reported cases **(3, 4, 5)**.

1.3.1 Economic Costs of Poultry Salmonellosis:

The costs of poultry salmonellosis may be calculated on the basis of the losses which arise during production and

slaughtering processes. It is difficult to assess with accuracy the total economic effects of clinical salmonellosis on the poultry production industry - as different and separate from the public health consequences of symptomless poultry carriers and contaminated processed products. Two avian host-specific salmonella serotypes (*S.gallinarum* and *S.pullorum*) remained for decades the established causes of serious economic losses in commercial production of poultry and poultry products. With the virtual elimination of these salmonella serotypes, salmonellosis appeared no longer a disease of major economic importance or a significant cause of mortality in poultry (6, 7, 8). However, the indication is that salmonellosis still causes losses of broilers from infected flocks. Thus, *S.typhimurium* was reported to have caused 16% loss among infected fowls (9). There are reports of clinical infection of *S.enteritidis* phage type 4 in young chickens and in laying flocks, resulting in high morbidity and mortality, increased culling rate, and varying levels of condemnation at processing (10-14). One study in the Federal Republic of Germany estimated the cost of poultry salmonellosis to be 12.8 million German marks. Losses incurred during feeding (production) accounted for two thirds of these cost (3).

The prevention and control of poultry salmonellosis imposes several other categories of costs both to the Poultry Industry and to Food Hygiene related authorities (15). For Food Hygiene, the costs relate to the enforcement of legislation or Orders on the safe production, processing, distribution, retailing and preparation of poultry and poultry products. To the Poultry Industry, such costs result from the presumed hazard to public health and they include:

(a) compliance costs - incurred by the producer, processor and retailer to comply with an enforced Order;

(b) consumer costs - relate to proportions of the compliance or adherence costs which are passed on the consumer;

(c) loss of condemned products - live birds (broilers, breeders, layers etc), eggs, carcases, feeds;

(d) cost of withdrawal of condemned products from the market;

(e) loss of anticipated profits during period of closure or restriction on the poultry establishment;

(f) losses incurred by cessation of production and destruction of infected flocks or infected eggs;

(g) losses due to cessation or decrease in sales because of loss of confidence of consumers (even if there has been no official ban);

(h) costs of advertising and lobbying to recover the confidence of consumers;

(i) loss or reduction of international market.

These categories of losses are typified by the recent "Salmonella-in-Eggs" crisis in the United Kingdom. A major public health, economic and political row erupted during the period December 1988 to February 1989, following a comment by the junior minister in the Ministry of Health that "most egg production in the country are infected with salmonella" **(16, 17)**. Apart from the political fallout, the Minister's statement and subsequent conflicting and confusing infection incidence data produced by interested parties succeeded in creating much public scare and loss of public confidence in the egg industry. Most consumers simply stopped eating any eggs! Within weeks, the poultry industry in general and the major egg producers in particular, lost tens of millions of pounds, destroying tons of unwanted eggs and thousands of layers. In the heat of the crisis, the junior minister

resigned and the Agriculture Committee of the House of Commons embarked on a public inquiry (16). The Minister of Agriculture replaced the Zoonoses Order 1975 with an extended, all-embracing Zoonoses Order 1989 (see 1.6.4). Sixteen other Orders were promulgated by the Agriculture Ministry to fight the various aspects of the Salmonella and Salmonellosis problem. By April 1989, nearly 195,000 chickens from 16 flocks had been destroyed; a ban on egg sales from 13 other flocks was in force; and 14 broiler breeder flocks were placed under restriction orders (18). By the end of 1989, some one million birds in numerous flocks throughout Britain have been destroyed.

1.3.2 Socioeconomic Costs of Human Salmonellosis:

A number of studies in different countries have sought to evaluate the total costs to the society of outbreaks of foodborne salmonellosis and the most economic methods for the prevention and control of human salmonella infections. In the United States of America, such studies have established that the economic consequences of salmonellosis both in medical expenses and in lost productivity can be considerable (19, 20). In one outbreak caused by *S.typhimurium*, 89 members of staff of a hospital were affected. The victims required hospitalization and bed rest totalling more than 500 days, an average of 6 days per staff member. The total days off duty amounted to about 900, or an average of 10.3 days per patient (15). In 1982, a major outbreak of salmonellosis occurred among participants of a Eurotop Conference. Contaminated cheese was the vehicle in the outbreak caused by *S.indiana*. A cost analysis produced the following average costs to the 280 individuals involved: to those who did not consult a physician - 125 dollars; to those who consulted a physician but were not admitted to a hospital - 222 dollars; and to those who were on hospital admission - 1,750 dollars (15). Of 117 employed persons affected by the outbreak, 102 (87%) lost an average of 12 days from work and 39 family members missed an average of 3 work days caring for a patient. On the basis of the number of reported cases, the distribution of the contaminated

batch of cheese, the number of persons exposed and the attack ratios (attack rates?), the total costs of the outbreak were estimated at four million dollars. This did not include the intangible cost in the form of the loss of reputation to the firm supplying the product and the tangible costs of recall of the contaminated batch of cheese. With an estimated annual incidence of salmonellosis in the United States of about 2 million cases **(4, 5)**, it has been suggested that on a national scale, salmonellosis might be responsible for a loss amounting to 1.2 billion dollars each year **(15, 20-23)**.

In the Federal Republic of Germany, the social costs from salmonella infections has been estimated by Krug **(3)**. On the basis of total reported and calculated unreported cases, the total costs of human salmonellosis in 1977 were estimated to be 108 million German Marks for sickness and 12 million Marks for deaths. The amounts covered such tangible and intangible costs as loss of leisure during illness; welfare or productivity losses; losses in patients' consumption during the period of illness; treatment costs; examination costs; and other costs.

In the Netherlands, the estimated medical costs of one salmonellosis outbreak in 1981 was £200,000 to £500,000 and the economic loss to the food caterer was estimated to be £500,000 **(24)**. The outbreak kept health officers occupied for 150 working days. An economic study of the costs of salmonella poisoning and control measures has also been carried out in Canada **(25)**.

In Scotland, the socioeconomic costs of salmonella food poisoning have been studied in recent years. In 1982, Neilson **(26)** analyzed the cost-significance of a community outbreak of salmonellosis in which the implicated food vehicle was not specified. Total tangible costs were estimated to be £6,514, while the intangible costs were put at £1,850. Total average cost per confirmed case was £270. Cohen and others **(27)** carried out a thorough investigation to appraise the benefits which would result from the newly

introduced ban on the sale of non-pasteurized milk. The study was carried out at a time when raw milk was the primary vehicle for foodborne salmonellosis in the United Kingdom. The benefits were viewed as reductions in the incidence of milkborne salmonellosis. Detailed estimation of the benefits was based on the costing of a community outbreak of milkborne salmonellosis in the Grampian Region during October/November of 1981. During the outbreak, caused by *S.typhimurium* phage type 204, there were 654 reported cases, 448 of which were laboratory confirmed. Twenty-three victims were admitted to hospital with an average in-patient stay of 12 days. Two deaths were associated with the outbreak. Tangible direct costs appraised were those incurred in the investigation, control and treatment aspects of the outbreak and included medical costs (hospitalization, general practitioners, nursing staff etc), laboratory examinations of both the patients and the milk, veterinary and environmental health surveillance. Tangible indirect costs covered travel costs to visit hospital patients, and lost productivity (total work days' lost). The total tangible costs (direct and indirect) amounted to £84,000. Intangible costs which could be accurately assessed, included loss of housewives output, "pain, grief and suffering", loss of times, and loss of lives. Estimates of the total intangible costs ranged from £151,912 to £3,137,958, depending on the method of economic assessment used. The total costs of the milkborne outbreak thus varied from £236,000 to £3,222,000. With 654 reported cases involved in the outbreaks, the total estimated cost per case ranged from £360 to £4,900. The cost of banning the sale of non-pasteurised milk in Scotland and the benefits of the anticipated reduction in milkborne infections were also estimated. The total cost of the ban (estimated to be £91,880) did not exceed the total annual benefits (£92,777) when the mid-values were attached to the intangible benefits. Thus, even the minimum estimate of the benefit of reduced milkborne salmonellosis justified the ban.

Since the statutory ban on the sale of non-pasteurized milk and the introduction of compulsory pasteurization of cows milk in 1983, poultry meat seemed to have replaced milk as the most important source of foodborne salmonellosis. It soon became necessary to determine the cost imposed on society by poultryborne salmonellosis and assess the economic efficiency of specific control measures. Utilizing the results of the previous analysis of the tangible and intangible costs of community outbreaks of salmonellas (27), Yule and others (28, 29) estimated the costs of an institutional outbreak of poultryborne salmonellosis, and evaluated the costs and benefits of irradiation as an alternative control measure. The outbreak occurred at a long-stay geriatric hospital in Edinburgh, in December 1985. Hospital patients, hospital staff and Blood Transfusion Service (BTS) staff were affected. The source of the outbreak was identified epidemiologically as turkey consumed by patients and staff during an annual Christmas lunch. *S.thompson* and *S.infantis* were isolated from affected persons; there were 161 bacteriologically positive cases. These comprised of 60 hospital patients, 88 hospital staff, 9 BTS staff and another 4 persons who were home contacts of index cases. In addition, 50 patients and 31 staff had symptoms but were bacteriologically negative. The total number of persons affected was 242. Three deaths were associated with the outbreak. The total tangible costs of the outbreak ranged from £113,509 to £114,194, while the estimated intangible costs were put at £86,090 to £770,550. The total estimated costs of the poultry-borne salmonellosis outbreak ranged from £199,579 to £884,744. The total costs per reported case varied from £825 to £3,655. By extrapolating the costs of the two community outbreaks (milkborne and poultryborne) to the national level, the estimated total cost of reported and unreported cases of poultryborne salmonellosis in Scotland is in excess of £10,000,000 each year, based on the maximum upper bound predictions of costs of unreported cases.

The above analyses would suggest that, for public health and economic considerations, research on epidemiological inter-

relationships of poultry and poultryborne salmonellosis is worthwhile, relevant and timely.

1.4 SALMONELLA INFECTIONS IN POULTRY:

1.4.1 Clinical Salmonellosis:

In poultry, there are two kinds of salmonella infections: **systemic** (generalized) and **enteric** (usually confined to the gut). Systemic salmonellosis in poultry flocks had been known to be caused exclusively by two host-specific salmonella serotypes - *S.gallinarum* and *S.pullorum*. Epidemiologically, *S.gallinarum* affects mainly adult birds and young chickens that are past 10 weeks of age. It produces the disease known as fowl typhoid which is marked by greenish-yellow watery discharges and pasting of the vent (30, 31). *S.pullorum* affects much younger chickens, producing the clinical condition called pullorum disease characterized by white pasty or chalky faeces. In poultry flocks, morbidity and mortality caused by fowl typhoid - *pullorum* are very high, and may be up to 100%. At post mortem, *S.gallinarum - pullorum* produce marked enlargement of the liver and spleen, and septicaemia indicated by the presence of pin-point haemorrhages (petechiae) in the breast muscle, the heart muscle, lungs and other visceral organs (30, 31, 32). The ovaries and oviduct may be affected, often resulting in distorted, misshapen, and ruptured ova. This ensures transovarian transmission of the salmonella organisms from the infected hen to the chicks, through the yolk.

The clinico-pathologic features of fowl typhoid-pullorum disease are not specific or pathognomonic, but may sometimes resemble those produced by other poultry conditions such as Newcastle disease, fowl cholera and acute colibacillosis (30, 31). For this reason, positive serological findings are of special value in detecting infected birds, especially in screening test of adult flocks and in control programmes; but serological tests alone are not adequate for a definitive diagnosis. Confirmatory diagnosis of fowl

typhoid-pullorum disease invariably requires laboratory isolation and identification of *S.gallinarum* and *S.pullorum*. As poultry-specific serotypes, *S.gallinarum* and *S.pullorum* are not considered significant public health hazards. Clinical infections in man, although they have been documented, are quite uncommon, if not rare.

With the virtual eradication of *S.gallinarum* and *S.pullorum* in the United Kingdom **(6, 7, 33)**, clinical salmonellosis was no longer common in poultry flocks. Infection by the other salmonellae often produced no symptoms in poultry. However, there are very recent reports of clinical disease in broilers, broiler breeders, and laying flocks caused by *S.enteritidis* phage type 4 (PT4).

Prior to 1987, *S.enteritidis* had only been rarely found in poultry in Britain. Notification of salmonella incidents compiled by the Ministry of Agriculture, Fisheries and Food (MAFF) shows that incidents of infection due to *S.enteritidis* have increased from 8 in 1981 to 111 in 1987 **(33)**. In young chicks, the main feature of clinical infection by *S.enteritidis* PT4 is pericarditis, which is occasionally accompanied by septicaemic lesions such as necrotic foci and petechiae in the liver **(10, 11)**. Mortality at 1 to 5 weeks of age was 20%. In mature broilers, a mucopurulent inflammation of the pericardium is seen; the pericardium is thickened and leathery, and grossly distended with up to 15 millilitre of exudate. In one outbreak of *S.enteritidis* PT4 infection, 12000 out of 54000 5-week old broilers (22%) became ill and 1600 birds died **(10)**. The clinical signs included weakness, pericarditis, and septicaemia which resulted in high culling rates, and high levels of condemnation at processing. In August 1988, the MAFF instructed that any broilers with such pericarditis should be declared unfit for human consumption **(10)**. Ovarian infections have been reported in a broiler breeding flock and also in the ovaries and oviduct of hens from a laying flock in which *S.enteritidis* PT4 was prevalent **(12, 13)**. Infected ovaries are misshapen, shrunken, discoloured and congested; the lesions appear similar to those described for

S.pullorum, infection of laying flocks. Transovarian transmission would appear to occur with *S.enteritidis*. This has the combined effects of making control more difficult, and constituting a risk of eggborne salmonellosis.

1.4.2 Endemicity of Salmonellae in Poultry Flocks:

Over the years, a growing list of salmonella serotypes have been isolated from poultry. The frequency of isolations and the variety of serotypes involved provide evidence of the endemicity of the non-host-specific salmonellae in many poultry flocks. These organisms produce symptomless infections in healthy birds. Many reviews of salmonella infections in poultry in the United Kingdom confirm the presence of many serotypes and variations in the predominance of particular serotypes (7, 8, 34-37). Some serotypes like *S.typhimurium* occur regularly; some exotic serotypes appear for a short time and then disappear, while other exotic types become established and enzootic. Following its introduction into the UK in imported fish meal, *S.agona* was established in poultry, becoming a common cause of salmonella food poisoning (35). *S.virchow* was another exotic serotype which attracted little attention when it was first reported, until it too became established in poultry, causing a serious outbreak of food poisoning in the north of England in 1967-68 (35). *S.virchow* is now one of the major causes of poultry-associated salmonella outbreaks in man.

Dougherty (38) followed the salmonella infection rate in a poultry flock from growth period until processing at 8-9 weeks. The infection rate was as high as 37.5% initially, although it dropped significantly by the third to fifth week. The incidence of salmonellae in a broiler flock raised in litter in 32 pens was determined by Rigby and others (39). Salmonellae were recovered from the litter of 7 pens and from the intestines of dead or culled chickens from 2 other pens. Eighty-seven per cent of plastic crates used for transportation of the chickens to the processing plant were contaminated with salmonellae; 29 of 31 chickens

(93.3%) sampled when the birds were unloaded were external carriers of salmonellae; and 11 of 25 carcases (44%) from the flock yielded salmonellae. Bhargava and co-workers (40) assessed the level of salmonella contamination in broiler chickens. Before the initial placement of chicks, the broiling equipment, feed, water and fresh litter were all found to be free of salmonellae. After the placement of the chicks, salmonellae were found in 6 of 10 flocks surveyed. Contaminated carcases at the consumer outlets were associated with infected broilers which introduced the salmonellae into the processing plant. The salmonella serotypes isolated from eviscerated dressed carcases were the same as those isolated from the flocks and from the litter samples.

1.4.3 Incidence of Salmonellae in Eviscerated Carcases:

The number of broiler chickens entering a processing plant that harbour salmonellae is probably low. The relatively low incidence in birds that are alive and apparently healthy contrasts with the incidence found in birds that have been processed in a packing plant. In the processing operation, mechanical spread and cross-contamination readily occur, so that as much as 90% of the final product may be contaminated with the salmonella organism (41). A number of surveys of the incidence and of the serotypes occurring in processed poultry have been carried out in the USA, Canada, UK and other developed countries (42-51). Morris and Ayres (42) recovered salmonellae from one-third of eviscerated carcases in two surveys, and in a study of retail products, Wilson and others (43) found that 24% of whole carcases and 13 to 21% of chicken parts contained salmonellae. Woodburn (44) isolated salmonellae from 72 of 264 (27%) broiler-fryer chickens purchases in retail stores; 13 salmonella serotypes were recovered. Wilder and MacCready (45) noted that an average of 50% of 237 market broiler fryer chickens from 9 different poultry processing plants were contaminated with salmonellae. In Britain, one survey of frozen chicken from 4 packing stations showed contamination rates of 3 to 62%; the mean was 35% (46). English frozen chicken sampled from

poultry retail outlets in 3 different surveys showed that salmonella contamination was 24.4%, 13.0% and 14.8% respectively **(47)**. In Canada, the results of a survey conducted in 1979 to determine the incidence of salmonellae and salmonella serotypes in processed chicken in 15 plants were compared with the results obtained in 1967 in an identical survey of the same plants. In 1967, salmonellae were isolated from 171 of 597 whole carcases (28.6%); in 1979, 222 of 601 of similarly analyzed chicken carcases carried salmonella (36.9%). Positive isolations from identical plants ranged from 7.5% to 73.7% in 1967, and from 2.5% to 87.5% in 1979 **(48)**. D'Aoust, Stotland and Boville **(49)** detected salmonellae in 70 of 100 naturally contaminated broiler carcases.

Rigby **(50)**, using the most probable number (MPN) method, assessed quantitatively the numbers of salmonellae in fluids collected by rinsing contaminated broiler carcases. MPN values of 80 or less were obtained from all carcases in 3 of 8 lots tested, and 2 lots contained carcases which yielded more than 1000 salmonellae. Similar numbers of salmonellae were recovered in repeated rinses of the same carcases, suggesting that only a small proportion of the total population was recoverable in each rinse.

Gilbert and Roberts **(51)** reported that 79 of 100 frozen chickens examined in the Food Hygiene laboratory, Central Public Health Laboratory, Colindale during the period 1979-80 were contaminated with salmonellae; giving an incidence of 79%. Eighteen salmonella serotypes were isolated.

Under the 1975 Zoonoses Order (see **1.6.4**), statutory notifications of incidents of salmonella in poultry and other animals (live animal, carcases, products etc) throughout Great Britain are made to the MAFF Central Veterinary Laboratory, Weybridge which compiles and publishes annual summaries **(33)**. In Scotland, routine notifications of salmonella infections are, in addition, made by veterinary and medical laboratories to the Communicable Diseases (Scotland) Unit (CD(S)U) as an

integral part of a national surveillance under the WHO Surveillance Programme for the Control of Foodborne Infections and Intoxications in Europe. Recent analysis of the reports recorded at the CD(S)U showed that during the period 1976-87, there were 421 reported incidents of salmonellae in poultry in Scotland; 319 isolations were made from chickens (fowls), while 102 were from turkey **(Oboegbulem and Reilly, 1989; Unpublished Observations).** A total of 45 salmonella serotypes were isolated from both chickens and turkeys - 42 in chicken and 18 in turkey **(Tables 1.4.1 & 1.4.2A & B).** The 10 most frequently isolated serotypes in chicken were *S.typhimurium* (31.7%), *S.virchow* (11.3%), *S.livingstone* (9.1%), *S.infantis* (8.5%), *S.enteritidis* (5.0%), *S.worthington* (5.0%), *S.newport* (3.4%), *S.bredeney* (2.8%), *S.agona* (2.5%) and *S.ohio* (2.2%). While *S.typhimurium* was fairly common every year, there was a variation in predominance of the other serotypes during different time periods. During 1976-80, *S.infantis*, *S.agona* and *S.worthington* predominated; during 1978-82, *S.virchow* and *S.newport* dominated; and in 1985-87, *S.enteritidis* prevailed. The predominance of *S.enteritidis* continued in 1988. Reports by the MAFF showed that up to the first week of September 1988, 63% of incidents in poultry were due to *S.enteritidis* PT4 **(33).**

1.4.4 Salmonella in Eggs:

Salmonella contamination of egg contents may occur in two ways. One is by direct transmission from infected ovary, before the egg is laid; this is more likely to occur when the bird has a systemic infection. Duck eggs were particularly regarded as being more commonly infected by ovarian transmission with such serotypes as *S.typhimurium* and *S.enteritidis* **(35).** The second and more common way of egg contamination occurs when the shell of the egg is contaminated with the faeces of a bird that has had only an enteric infection but which is a salmonella excreter. The salmonella penetrates through the pores of intact eggshell as the egg cools. Alternatively, faecal matter adherent to the shell may contaminate the contents when the eggs are

broken out in the manufacture of egg products. A survey carried out in Britain in 1955 and 1956 showed that 27.5% of samples of English whole egg products examined were contaminated with *S.typhimurium* (52). Another survey of English egg products in 1959 showed that 1.2% of samples from small packing stations contained salmonellae, while 2.6% of samples from large-scale English frozen egg plants, and 7% of imported eggs were salmonella positive (53). Extracts from the Public Health Laboratory Service (PHLS) Weekly Summary showed that during the period 1966-67, a total of 205 salmonella isolates were made from 1,148 eggs and egg-products examined; the egg products comprised of liquid egg, dried whole egg, egg albumen and egg pasta (54). A total of 20 salmonella serotypes were isolated.

The proportion of eggs that are infected internally is low, and it may be difficult to establish the true incidence. The examination of shell eggs sampled at random from retail shops and other outlets is not a "rewarding approach" to the estimation of the risk; this is because incidence is low and infection among eggs is unlikely to be evenly distributed (55).

A more fruitful approach is to try to trace the flocks of hens that have laid eggs associated with human salmonellosis or are otherwise known to be infected, and then to test the eggs (55). In practice, it is not easy to trace eggs to source flocks: by the time human infection had been diagnosed, all implicated eggs are likely to have been eaten or discarded. Even when any eggs are left, it will still be difficult to trace them to the precise source. Investigation of flocks linked with episodes of egg-associated salmonellosis, along with veterinary studies to estimate and establish the proportion of laying flocks in the country infected with salmonellae, would give a rough indication of the magnitude of salmonella contamination of eggs generally and the level of national risk.

In 1988, the PHLS tested about 2000 eggs from sources suspected of being associated with human infection.

S.enteritidis PT4 was obtained from the shell only of 7 eggs, from both the shell and contents of 2, and from the contents only of 2 eggs (55). In another report, *S.enteritidis* PT4 was isolated from a small domestic flock associated with a family outbreak of salmonellosis (55). In 1981, the Division of Enteric Pathogens, PHLS isolated 75 salmonella strains from raw bulk liquid egg sampled before pasteurization; none of the isolates was *S.enteritidis* phage type 4. In 1987, however, 103 of 256 salmonella isolates from raw bulk liquid egg were *S.enteritidis* phage type 4. The isolates came almost exclusively from two processing plants in one area; 46 of 87 (53%) of samples of bulk liquid egg entering one of the plants in January and February 1988 contained *S.enteritidis* phage type 4 (55).

1.4.5 Source of Poultry Infection:

There are many possible sources of salmonella infection in poultry, some being more important than others. The most important source is poultry feed (imported and local). As early as the 1950s, animal foods had become recognized as one of the main sources of exotic salmonellae in domestic animals (56). A wide range of ingredients of animal and plant origin are incorporated in the preparation of poultry feedstuffs. Most of these ingredients have at one time or the other been shown to contain salmonellae; but the organisms occur most frequently in the animal protein component (meat and bone meal, blood meal, poultry offal, feather meal and fish meal). Meat meals are made from animal remains from butcheries and abattoirs, from offal at poultry processing plants, and from poultry waste. The presence of salmonellae results from either faulty processing or more commonly post processing recontamination. Ingredients broken down, mixed and prepared as meals or mash are more likely to contain salmonellae. Pelleting greatly reduces the numbers of salmonellae present in feedstuffs (35, 56).

There is a demonstrable relationship between contaminated feed, the incidence of particular salmonella serotypes in

poultry, and the appearance of the same serotype in outbreaks of human salmonellosis (57). During a survey in a large poultry organisation, MacKenzie and Bain (58) observed that a significant correlation existed between salmonella serotypes isolated from raw feed ingredients and those from dressed carcases. Of 17 serotypes isolated from chicken carcases, 14 serotypes or 82% were identical to those in the original isolations in meals or grains. Sampling of protein products in England and Wales from 1982-85 resulted in salmonella isolations at 55 of the 102 registered premises examined, and contamination rates for the different products varied from 0.7% for white fish meal to 25% for feather meal (59). The sampling of imported animal protein over the same period showed that 16.4% of 2020 samples were salmonella positive, by comparison with 9.5% for local or domestic production. Despite a high rate of contamination of poultry feed in most countries, this rarely gives rise to clinical infection or outbreaks of salmonellosis in poultry flocks. However, in large, integrated operations, there may be perpetuating cycle of infection in which waste offals processed and included in feed, transmit the same serotype in subsequent batches of birds. The result is an enzootic symptomless infection which leads to a constant re-cycle of salmonella serotypes into the various sections of the poultry industry. All reputable feed manufacturers have a system of monitoring feed materials for salmonellae. Although serious efforts are made to produce salmonella-free feeds, complete elimination of salmonella contamination of feed has not been possible.

In some large integrated poultry organisations, vertical transmission of salmonellae from infected breeding stock to the table birds, via the hatchery, is one main source of flock infection (60). Imported birds and wild birds have also been known to introduce exotic salmonella serotypes into the poultry flocks.

The rearing environment, involving rodents (rats and mice), insects, domestic pets, constitutes another possible source of salmonella infection in poultry. Heavy grain

contamination by rodents was found to have transmitted infection throughout an integrated poultry establishment in Australia (35). Droppings of rodents may also cause re-contamination of feeds after processing. One survey in Britain suggested that 2% of rodents in agriculture areas could be carriers (35).

1.5 BROILER INDUSTRY IN THE UNITED KINGDOM:

1.5.1 Introduction:

Before the early 1950s, the broiler industry did not exist in the United Kingdom; broiler chicken production only began in 1953, following the end of feedstuff rationing. The broiler production has become so expansive that in the 1980s, the national production has grown to about 450 million broilers annually (61, 62). The poultry meat industry is now organised in to a small number of integrated companies; half of the national production is grown by less than 5% of producers with units in excess of 50,000 birds. Ten companies share about 75% of the market, with one company effectively controlling 30% (Steel W. Unpublished seminar paper). In 1985, the total poultry production in Scotland was around 91,000 tons, most of which was chicken (28). Seventy-five to 80% of Scottish poultry is produced by a single firm in central Scotland (28).

Although production costs have increased dramatically, that had been offset to some extent by the improved performance of broilers and breeding stock as well as the improved efficiency of the integrated system (from breeders, through hatchery, to growers). One parameter by which the improvement in production performance can be measured is growing time and food conversion. At the start of the broiler industry, an average broiler had 4 lb (1.8 kg) live weight at 12 weeks. By 1987 there was performance of 5.1 lbs (2.3 kg) live weight at 8 weeks for males and 4.1 lbs (1.8 kg) live weight at 8 weeks for females (Steel W. Unpublished Seminar Paper 1989). Production data show that although previously the killing age of broilers was 8 weeks;

the time has been reducing and most broilers would now be slaughtered at 6 or 7 weeks of age. The killing weight of the average broiler is 1.95 kg.

Today's increased broiler production levels have been brought about by an ever-increasing consumption of chicken. The annual consumption in pounds per person rose steadily from about 5 lbs (2.27 kg) during the period of feed rationing to 18 lbs (8.18 kg) in 1968, 26 lbs (11.8 kg) in 1972, and 22.7 lbs (10.3 kg) in 1984 **(63)**. The astronomical development of the broiler industry, brought about by modern intensive production systems and application of modern technology in feed production, meat processing, and freezing for carcases, has had the effect of making poultry meat the cheapest animal protein available. By 1980, the prices of chicken and turkey were at 44 pence per pound and 55 pence/lb respectively, while beef and lamb stood at £1.80/lb and £1.30/lb **(Steel W. Unpublished Seminar Paper)**.

The local market demands are principally met by probably one or two broiler breeds in England and Wales, and by about one breed in Scotland **(Steel W. Unpublished Seminar Paper)**. In the 1960s the United Kingdom was a net importer of poultry meat; there has now been an increasing volume of poultry meat exported over the years. In the early years, the big wholesale markets were very important retail outlets. With the growth of the multiple grocers (including the Co-operatives), the oven-ready processed birds, which are more convenient to the retailer and the consumer, have taken over from the rough plucked. By 1971, the types of poultry meat in the retail outlets were: oven-ready (74%), other uncooked whole-chicken (4%); chicken portions and further processed (22%) **(Steel W. Unpublished Seminar Paper)**.

The increase in the production of fresh and frozen poultry meat, in distribution and retail outlets, and in the consumption rate have resulted in increased opportunities for cross-contamination with the salmonellae.

1.5.2 Poultry Processing and Critical Points for Salmonella Contamination:

Poultry slaughter has become an automated multi-stage operation, and modern plants can process more than 10,000 birds per hour. Most broilers are slaughtered at an age (6 or 7 weeks) when they will not have naturally eliminated any salmonella infections. At the above processing rate, it is difficult, if not impossible, to prevent contamination from carcase to carcase. The various stages of broiler production and processing, and the critical points for cross-contamination are illustrated in **Figure 1.1.**

The excretion of salmonellae in healthy poultry is usually intermittent and of a low level, until the bird experiences some kind of stress **(15).** The stress of catching, caging and transporting broilers to the processing plant contributes to greater excretion and contamination of the outside of the bird. During loading, transportation and unloading, crates are contaminated with faecal material and if some of the birds are salmonella-carriers, the skin and feathers of the salmonella-free birds also become contaminated. Most critical at this stage is the dissemination via the crates from salmonella-infected flock to salmonella-free flocks.

After removing from the crates, the birds are **hung** on the line before electrical **stunning** and then **exsanguination.** During hanging, birds very often flap their wings and in the process create aerosols which may contain salmonellae. It has been shown that air samples can be positive for salmonella in the hanging area, whilst they are negative in the other areas in the slaughter house **(15).** After the birds are bled, the carcases are **scalded** in a tank of hot water for up to 4 minutes. Scalding loosens the feathers and facilitates **plucking** or defeathering. There are two scalding regimes: "Soft or low scalding" at 50-52^{o}C, primarily for fresh birds which are increasingly more popular than the frozen chicken; and "hard or high scalding" at 58^{o}C or above 60^{o}C. The temperature of soft scalding is

lower - to avoid damage to the final appearance of the carcase. This means that the temperature will not kill salmonellae attached to the chicken skin and thus dry contamination on the birds is converted to wet contamination which spreads more easily. Scalding is one of the important critical points in salmonella cross-contamination of carcases; organisms that survive scalding are more difficult to remove during subsequent stages of the slaughter process **(15, 60)**.

Plucking or defeathering takes place by a series of machines inside which rubber flails remove the feathers. Considerable aerosol formation in the pluck area and surroundings constitutes a risk of salmonella spread; significant contamination of the carcase occurs at this point.

The next stage is **evisceration** or the process of removing the intestinal contents. In most plants, the process is now carried out mechanically, with one machine cutting around the vent and another scooping out the viscera for veterinary inspection. Intestinal contents can be heavily infected with the salmonellae. Evisceration is, therefore, one of the most critical points for salmonella contamination. It has been shown that one infected carcase can contaminate up to 100 others in the process **(15, 60, 64)**. The rather frequent accidental cutting and rupture of the intestines during evisceration, followed by smearing of faecal materials on the outer and inner parts of the carcase increase the number of salmonella-contaminated carcases. After evisceration the hearts and livers are harvested and this constitutes another likely source of salmonella spread when offal from different birds is mixed together before packing. In the European Economic Community (EEC), the carcase must be washed after evisceration.

Following evisceration, the carcases are **chilled** before storage. Chilling can be carried out either by blasting with cold air (for the fresh carcase trade) or by immersion in chilled water bath (for frozen birds). Immersion or spin

chilling permits cross-contamination (65). It is likely that the blast chiller produces less cross-contamination than the immersion chilling, and so air chilling is becoming more popular. This may also be because market demand is changing from frozen to fresh carcase. However, surface microbial counts on air-chilled birds can be higher than on those that have been chilled in the immersion bath (66).

Other operations which are carried out after chilling include freezing, cutting-up, and further processing of poultry portions and parts. Some of these operations constitute other critical points for salmonella contamination. The intensive, fast-line nature of poultry slaughter renders difficult all attempts to control or minimize carcase contamination. The result is that as many as 65% to 79% of frozen and 55% of fresh broiler carcases may carry salmonellae (51, 67).

1.6 CONTROL OF POULTRY SALMONELLOSIS:

1.6.1 Introduction:

Eradication programmes incorporating serological tests of *S.gallinarum* and *S.pullorum* have been successful in eliminating these host-specific serotypes from the poultry industry. No significant effects have been apparent, however, with all the efforts that have been made in several countries to eliminate infection of live birds and to prevent contamination of the end product, by the other non-host specific salmonellae. Control measures have been unsuccessful largely due to the several sources of poultry infection, the complex infection cycle and transmission pathways. The main objectives in poultry salmonellosis control are: clean breeding stock, clean environment, clean feed, safe slaughtering and processing, and clean meat products. Poultry breeders cannot eliminate salmonella from their breeding flocks unless salmonella-free feed is available. Feed manufacturers and suppliers, on the other hand, claim that control on their own part is valueless where contaminated poultry houses and environment cannot be

efficiently cleaned (15). Overall control of poultry salmonellosis aimed at interrupting the infection cycles involve the following aspects of production and processing operations: (i) husbandry practices; (ii) critical control points in the slaughter and processing line; and (iii) veterinary public health activities.

1.6.2 Control Measures Relating to Husbandry Practices:

Strict hygienic procedures on the farm, including prevention of feed and water contamination by rodents, insects and wild birds reduce salmonella infections of poultry farms (15). Prompt removal of dead and sick birds, proper and early disposal of droppings and litter, regular cleaning of houses and equipment, and routine bacteriological monitoring of flock and environment by veterinary or other diagnostic laboratories have been shown to reduce the level of salmonella infection in the farm (15). Heat-processing in feed production, pelleting and rodent-free silos are thought to be sufficient to destroy salmonella or reduce the incidence below the level required to infect poultry (60). However, these measures may fail when opportunities exist for re-contamination of the finished product.

In Great Britain, a number of legislative measures, aimed at reducing salmonella infection in poultry production are in force. In 1982, two specific measures to control contamination of animal feed were introduced; these are the Diseases of Animals (Protein Processing) Order 1981 and the Importation of Processed Animal Protein Order 1981. In effect, animal protein intended for incorporation into feedstuffs is required to be free from salmonellae, and feed production plants are subjected to spot checks. The Importation Order required imported protein material to be made available for sampling on arrival at port of entry. In spite of the legislations, existing measures seemed, in practice, to have done little to prevent potentially contaminated feed from entering the food chain. This is partly because, prior to 1989, there was no attempt to prevent the use of contaminated materials in feed

production. The Protein Processing Order which was designed to stop the distribution of infected animal feed has not been of much success either, because of lack of inspectors and the reluctance of local authorities to take action (60).

In Britain, prior to 1969, antibiotics such as penicillin and the tetracyclines were incorporated in animal feed both to reduce bacterial infection of the animals and as growth promoters. This practice resulted in the emergence of antibiotic resistant bacterial strains and the possible transfer of the resistance to human enteric pathogens (60). Consequently, in 1971, legislation was introduced prohibiting the use in animal feeds of those antibiotics that are used in therapy. Several new antibiotics were then developed by the pharmaceutical industry specifically to be used in feeds as animal growth promoters. The routine use of antibiotic additives led to the discovery that some of the antimicrobials may actually prolong salmonella carrier-state in poultry. This is due to the fact that such antimicrobials disturbed the ecological balance of the gut and inhibited important organisms (commensal flora) responsible for limiting intestinal population of salmonella by the principle of "competitive exclusion" (60).

1.6.3 Critical Control Points during Slaughter and Processing:

In groups of animals subjected to stress during loading, transport and holding prior to slaughter: more animals shed salmonella; more salmonella are shed per animal; and more animal carriers have salmonella in their mesenteric lymph nodes (68). The greater the transport distance and the longer the period between delivery and slaughter, the greater is the proportion of animals which become infected and the greater the number of salmonellae excreted (15). In order to minimize cross-infection, transportation should produce as little stress as possible to the poultry. Regular cleaning and disinfection of cages and boxes used in transporting birds as well as provision of sufficient ventilation are important preventive measures.

For the purposes of prevention and control of salmonella cross-contamination during poultry slaughter and processing, the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO) jointly produced a document, the Recommended International Code of Hygienic Practice for Poultry Processing (69). In addition, the **hazard analysis critical control points** (HACCP) concept is recommended for monitoring hygienic requirements for minimizing salmonella cross-contamination in the slaughter and processing plant (69). A critical control point is a step in the slaughter and processing operation at which a preventive or control measure can be exercised; the aim is to prevent or minimize any cross-contamination that has occurred prior to that point or stage. One critical control point is **hanging** and **stunning** during which salmonella-laden aerosols may be created by hung birds flapping their wings. Physical separation between the area where live birds are hung, stunned, bled, scalded and plucked, from the area where evisceration and chilling take place, is the recommended control measure (15). **Scalding** is one of the more important critical control points. To prevent salmonella cross-contamination during the scalding operation, the following measures have been recommended; continuous supply of fresh hot water for the scald tank; continuous monitoring of the water temperature; and cleaning and disinfection of the scald tank at least once a day. The hazard of cross-contamination during **plucking** or defeathering is reduced by physical separation of plucking machine from the rest of the slaughter line, by continuous removal of feathers from the pluckers, and daily disinfection of the pluckers.

Evisceration is another critical control point in the processing operation. In most plants, the equipment used is calibrated for use on birds of different sizes and weights. However, natural variations in birds sometimes lead to damage and rupture of the viscera, and consequent contamination of other carcases with gut contents. In the EEC, carcases must be washed after evisceration. Because contamination with salmonellae is mostly on the surface, efficient spray-washing may significantly reduce the numbers

of the organisms. In countries of the EEC the regulation requires that 1.5 litres of water should be used for spraying carcases weighing not more than 2.5 kg (15, 68). Super-chlorination of the washing water has been recommended to reduce the bacterial contamination on the carcase (15, 60). However, the effectiveness of chlorination in removing salmonellae attached to the surface is not certain; 50 parts per million chlorine was demonstrated to be insufficient to decontaminate salmonella-contaminated carcases (15).

Chilling of poultry carcases immediately after evisceration, and to a temperature below $4^{o}C$ is a control measure aimed at inhibiting the multiplication of any salmonellae on the carcase. However, the current methods of chilling - by air blaster and immersion chiller - are not perfect and do not often remove salmonellae from the carcase. The increased risk of cross-contamination in the immersion or spin chiller is reduced by a **counter-current** immersion chilling system: in the system, the flow of the water is in the opposite direction to the flow of the carcase. In the EEC, this is the only permitted system for wet chilling of poultry (68).

1.6.4 Veterinary Public Health Control and Preventive Measures:

A number of veterinary public health measures are available and are utilized for the prevention and control of salmonellae in the food-chain of poultry production and processing. At the level of the poultry farm, veterinary prevention measures relate to specific methods of prophylaxis as well as non-specific control activities. Prophylactic vaccination programmes have been successful in eradication or marked reduction in incidence of the host-specific *S.gallinarum* and *S.pullorum*. Immunization and other specific methods of prophylaxis against the zoonotic salmonellae are in the experimental or research stage. In the *Nurmi culture* technique, caecal content (flora) of adult birds are fed to very young chicks, to prevent the establishment of zoonotic salmonellae by the principle of "competitive exclusion". The Nurmi culture has been used in

Canada to prevent salmonellae in chicks (70). Non-specific veterinary measures, taken in collaboration with animal husbandry and the poultry industry, for the prevention and control of cross-infection in poultry production include: hygienic production of poultry feed, disinfection of farms, encouraging the production of salmonella-free feeds, clean egg production, hygienic collection of eggs and fumigation of eggs, hygienic disposal and rendering of dead birds etc.

For the purpose of evaluating whether poultry meat is fit for human consumption, birds are inspected before and after slaughter, and diseased birds are condemned as unfit for human consumption. In most countries, ante-mortem inspection and post-mortem examination of all poultry carcases going through slaughter are carried out by officially appointed veterinary inspectors. However, ante-mortem inspection can only detect clinically or otherwise obviously diseased birds, but cannot detect apparently healthy salmonella carriers. Similarly, post-mortem examination will not detect birds carrying most zoonotic salmonellae which do not produce discernable pathologic lesions in birds. Those salmonella serotypes that cause clinical avian salmonellosis in the very young chicks e.g. *S.enteritidis* and *S.typhimurium* do often produce pathologic changes, even in symptom-less carriers; this will result in the rejection of such infected carcases at post-mortem. It is recognised, though, that many salmonella carriers will not be detected even by a most careful veterinary inspection (15). Indeed, it can be argued that the presentation of viscera for inspection may lead to increased carcase contamination.

The Zoonoses Order 1975 (71) is one important aspect of veterinary public health measure aimed at reducing salmonella infection in poultry and other animals, through surveillance. The 1975 Order stipulates compulsory notifications to be made to the Veterinary Division when, as a result of laboratory isolation of salmonella from samples, the organism is known or suspected to be present in animals or birds of certain species, or in the products, carcase, or

environment of the animal. The Order also provides for cases of animal salmonellosis to be investigated and controlled by the application of restriction on the movement of animals, their carcases, products or feedstuffs. The species of birds specified include domestic fowls, turkeys, geese, ducks, guinea fowls, pheasants, partridges and quails. Under the Zoonoses Order 1975, the obligation to report was with the owner of the animal or bird, his veterinary surgeon, or with a meat inspector. The 1975 Zoonoses Order was replaced by the Zoonoses Order 1989 (72) which is much more inclusive and wide ranging, and which plugs some of the loopholes in the former Order. Under the new Order, the requirement to report becomes the responsibility of "**the person in charge of the laboratory**", making the isolation. This means that the isolating laboratory must report every isolation of the salmonella from the statutory sources, to the Divisional Veterinary Officer (DVO) of the Animal Health Division in which the laboratory is situated.

1.7 HUMAN SALMONELLOSIS:

1.7.1 Manifestations of Infection:

With respect to symptoms, source, mode of spread, and pathogenesis, salmonella infections in man may be divided into two forms. These are (1) **typhoid** and **paratyphoid fevers** which are caused by *S.typhi* and *S.paratyphi* A, B or C. These serotypes are host-specific and practically occur only in man (and other primates); (2) **enteric infections**, caused by the numerous other salmonella serotypes. Most but by no means all non-typhoid salmonellae are potentially pathogenic in man. Epidemiologically, **salmonellosis** refers to "clinical disease of man and animals resulting from infection by salmonellae other than *S.typhi* and *S.paratyphi* A, B or C" (15). On the basis of clinico-pathologic manifestations, salmonellosis in man may conveniently be divided into **intestinal** and **extra-intestinal** infections, although both forms may occur in the same patient.

Intestinal salmonellosis usually occurs after an incubation period of 6-48 hours, with an average of 24 hours. With the extra-intestinal salmonellosis, the incubation is longer, up to a maximum of 3 weeks (15). The majority of human salmonella infections involve the gastrointestinal tract alone. Because the pathological manifestations in intestinal salmonellosis involve only the small bowel and colon, the disease is actually an *enterocolitis* rather than a gastroenteritis (15). Clinical symptoms experienced by most patients include diarrhoea, cramps, nausea, vomiting and fever. Vomiting is not a very regular feature, but occasionally high fever and prolonged diarrhoea occur. Mortality from enterocolitis is very low, if not rare. Development and severity of clinical syndrome as well as death are influenced by such factors as age, undercurrent infections, and immune status. Although persons of all ages are affected, most serious symptoms and most deaths occur in neonates, infants, the elderly, persons with underlying disease and in immuno-depressed individuals or persons whose resistance is otherwise compromised.

The salmonellae exhibit a tendency for wide dissemination throughout the body, as well as the phenomenon of relapse and poor response to antimicrobials. This is due to the capacity of the salmonellae to survive in host cells, being protected from the inhibitory effects of natural substances and antimicrobial agents. As a consequence of the dissemination, the salmonellae tend to localize in, and are frequently isolated from, the blood and other extra intestinal sites, where they cause extra intestinal infections. Such infections include bacteriaemia and septicaemia, focal infections, especially in the heart, gall bladder, peritoneum, lungs, urinary tract, bone marrow, brain, meninges, spleen, and even skin, testicles, joints etc (15).

1.7.2 Duration of Excretion of Salmonellae:

The salmonellae can induce a carrier-phase in convalescent, and apparently recovered persons. Prolonged carriage with

intermittent excretions may follow intestinal infections. However, longterm or permanent carriage of non-typhoid salmonellae is unusual (15, 73, 74). Antibiotic therapy for intestinal salmonellosis has been shown to prolong carriage (15, 74). Individual studies in different countries indicate that the time taken for clinically infected and convalescent "shedders" to be free of the organisms varies considerably. One study in Great Britain confirmed that during the acute stage, large numbers of salmonella organisms are excreted in the faeces. As recovery takes place, the numbers of salmonellae excreted gradually decrease, until eventually the faeces are free of the organism. The majority of patients were free of salmonella after 4 weeks; others remained positive for longer periods (73). In a review of 6 episodes of infection caused by non-typhi salmonellae, Sharp (74) noted that 57% of clinical patients treated with antibiotics were excreters of the organisms. During the first month after treatment, 57.7% were excreting salmonellae. By the third month after treatment, 12% were shedders; but by the 5th month, the carriage rate was less than 4%. The study found that one per cent of the treated patients were still excreting salmonellae after 12 months. Studies in the USA indicate that the **median** duration of convalescent excretion of non-typhi salmonellae after acute infection is approximately 5 weeks (75).

Apart from the clinically infected, convalescent shedders, there may be healthy and symptomless carriers of salmonellae. These are infected persons with no history of illness who, nevertheless, are found to be excreting salmonellae. In an unpublished study by the Salmonella Sub-Committee of the Public Health Laboratory Service, cited by Taylor (54), 2.5 per 1000 children and adults investigated were found to be excreting salmonellae. Chalker and Blaser (5) reviewed 25 studies on salmonella carriage rates among 4,086,753 healthy, asymptomatic persons (all ages) in developed countries, and found that the overall **median** carriage rate was 0.150% or 1.5 per 1000. For adults, the median carriage rate was about 1.2 per 1000, whereas for

children, the median carriage rate was about twice as high. From 20 studies involving 102,311 asymptomatic persons in the developing countries, the authors found an overall median carriage rate to be 1.8% or 18 per 1000. This median carriage rate is **twelve fold** greater than that observed among healthy persons in the developed countries.

1.7.3 Sources of Human Infection:

Although incidents of direct transmission from person-to-person have been recorded, especially in acute-care hospitals, paediatric wards, nurseries, and geriatric homes, non-typhoid human salmonellosis is practically a foodborne zoonosis, acquired by eating contaminated food of animal origin. There are several animal sources of human infection, but over the years, the foods most commonly incriminated have been milk, meat (red meat and poultry meat), eggs and their products.

(a) **Milk:** Prior to 1983, milk was the primary vehicle associated with foodborne salmonellosis in the United Kingdom. The problem of milkborne salmonellosis seemed particularly serious in Scotland, where between 1973 and 1977, milk accounted for 33% of 66 general outbreaks of salmonella food poisoning for which the food vehicle was identified (77). Most milkborne outbreaks resulted from the consumption of raw, unpasteurized milk, mainly on farms producing milk. But extensive general outbreaks also occurred amongst consumers supplied by producer-retailers of raw milk(77).

Between 1970 and 1982, there were a total of 50 reported outbreaks of milkborne salmonellosis in Scotland, affecting 3,518 persons, with 12 deaths (77). The annual infection rate was 5.3 persons per 100,000 population. This contrasted with the incidence of milkborne salmonellosis in England and Wales during the same period, with an infection rate of 0.2 persons per 100,000. Eighteen of the 50 outbreaks in Scotland occurred in dairy farming communities. In August 1983 legislation was implemented in Scotland

introducing compulsory heat-treatment of all cows' milk intended for sale to the public. During the first 3 years **after** the compulsory pasteurization, there were no general community outbreaks of milkborne salmonellosis. This was in marked contrast to the 3 years **before** the legislation when 14 general community outbreaks had occurred **(78)**. The 1983 legislation did not include dairy farm workers who are given milk by their employers, free or in part payment of wages. As a result, outbreaks of milkborne salmonellosis persisted and continued to be reported in the farming communities. Thus, in the 3 years after the ban (1983-85), 15 outbreaks affecting 101 persons occurred in the farming communities. During this period there were 8,000 farm workers, including their families who received raw milk in Scotland. This meant an annual incidence rate of milkborne salmonellosis in the farming sub-population of 107 per 100,000 persons during 1980-85. In contrast, the overall incidence rate in the general population was 5.2 per 100,000 during the years 1970-82 **(78)**.

(b) **Meat:** The first case of human salmonella infection in which meat was incriminated was recorded by Gaertner in 1888 **(79)**. In England and Wales, during the period 1949-63, meat (including poultry meat) accounted for 47% of 733 general and family outbreaks of salmonella infection in which the food vehicle was identified **(80)**. In 1984 in England and Wales, meats (all types) were responsible for 64 of 109 or 60% of outbreaks of salmonellosis for which the suspected food was identified **(81)**. Trends in incidence of meat-borne salmonellosis in different countries have been associated with differences in relative proportion of meat from different animal species consumed **(15, 76)**. In the USA, beef was the main source of foodborne salmonella infections until recently. In the UK, beef accounts for about only 2%, while poultry meat has become increasingly incriminated as the source of most outbreak and sporadic incidents in which the food vehicles are identified **(15, 28, 76, 81-84)**. In most Western European countries, poultry and pork are the commonest sources **(15)**. These variations are attributable mainly to differences in the predominant source of animal

protein in the diet, and especially to the consumption rate of poultry meat (15). In the United Kingdom, the increase in consumption rate of poultry meat has been accompanied by a steady rise in poultry meat-associated salmonella incidents. Thus, in England and Wales during the period 1956-63, poultry meat accounted for only 8% of outbreaks for which the food was identified; in 1964-68, poultry meat was associated with 33% of the outbreaks, and in 1969-72, the figure was 52% (76). During the period 1980-85, poultry meat accounted for 45% of 524 general and household outbreaks of salmonella food poisoning in England and Wales (60). In Scotland from 1973 to 1977, poultry was incriminated in 50% of 66 general outbreaks of salmonella food poisoning in which the food was known (77). During 1980-85, 224 of 413 (54%) foodborne outbreaks where a food vehicle was identified were associated with poultry (83).

Factory and home-made red meat (beef, pork/ham, and mutton) have been incriminated in significant but far smaller proportion of foodborne salmonella outbreaks in the UK. During the period 1973-77, red meat was incriminated in 14% of the 66 general outbreaks in Scotland (77). Processed uncooked meat, minced meat, boned meat, sausages etc which are easily contaminated during preparation, constitute another important source of human infection. The importance of home-produced meat as a source of human infection had been demonstrated in a study of abattoirs, and butchers' shops which was carried out in many parts of the United Kingdom as early as 1964 (54, 85).

Epidemiological studies on the role of meat and specifically the association between poultry meat and human salmonellosis constitute the central work in this thesis. The studies are presented in subsequent Chapters.

(c) **Egg:** The first evidence about the danger of infection from inadequately cooked duck eggs was given about 57 years ago (86). For so many years duck egg was incriminated in most egg-borne salmonellosis; hen egg was far less frequently a source of salmonella infection. It is

possible, though difficult and rare, to trace human infection to individual shell eggs; egg products seemed to be more important. Human infection has been traced to bulk liquid egg, frozen eggs, dried eggs and other egg products. In England and Wales between 1949-63, egg and egg products accounted for 25% of the 733 general and family outbreaks of salmonella infection in which the food was known **(80)**. In January 1964 the Liquid Egg (Pasteurization) Regulations, 1963 came into force, making it obligatory that no liquid or frozen whole egg, home-produced or imported, be used in manufacture of egg products, unless it has been pasteurized. In Scotland, egg and egg products were **not** incriminated in any of the 66 general outbreaks of salmonella food poisoning between 1973 and 1977; in England and Wales, only 2 reports of egg-borne incidents over the same period were attributed to hen eggs **(77)**.

Ten years later (by 1988), hen eggs have become important source of salmonella food poisoning, particularly where *S.enteritidis* were involved. Outbreaks are associated with raw eggs consumed either as health food, or used raw in mayonnaise, egg-nog, milk shake, and sandwiches **(87)**. Data gathered from outbreak investigations and case-control studies suggest that scrambled eggs, soft boiled eggs, and scotch eggs can be sources of infection. The temperatures within the yolk of soft boiled eggs have been shown not to reach bactericidal levels **(14)**. Case-control studies have also established significant association between illness and the consumption of raw egg products, including home-made mayonnaise, home-made ice cream, home-made raw egg-containing milk products, and shop-bought egg sandwiches **(55)**.

1.7.4 Factors Contributing to Outbreaks of Foodborne Salmonellosis:

The factors that contribute to or otherwise influence outbreaks of foodborne salmonellosis have been examined in detail **(15, 64, 83, 88, 89)**. Certainly, the endemicity of salmonellae in food animal sources; the speed and complexity

of modern slaughter and processing practices; inadequate and ineffective veterinary inspection are the primary factors that ensure the continued contamination of the end product, and hence contribute to frequent outbreaks of salmonella food poisoning. There have been considerable changes in the eating habits in the industrialized countries, in the last 2 or more decades. "Fast food" outlets and other commercial catering establishments have expanded in number **(15, 83)**. The sale of pre-cooked poultry meat (whole or parts) and other pre-cooked meat portions has become common. The need to reduce or rationalize costs by the catering industry may lead to the development of inadequate kitchen practices which allow cross-contamination to occur **(83)**. The development of the frozen-food chain makes for the distribution of a potentially contaminated product over a very wide geographical area and over prolonged periods of time. The public health consequence, is that the consumer faces a greater risk of being exposed to contaminated food, and a higher probability of foodborne salmonella infection. In a detailed study of 792 general and family outbreaks of salmonella food poisoning in England and Wales during the period 1970-79, Roberts **(89)** noted that the following factors most commonly contributed to the outbreaks: (1) preparation of the food too far (more than half a day) in advance (21.8%); (2) storage of prepared food at ambient temperature (14.5%); (3) use of contaminated processed food such as cooked meat and poultry, take-away meals (13.3%); (4) undercooking (11.5%); (5) inadequate cooling (9%); (6) cross-contamination (7.2%); (7) inadequate re-heating (6%); (8) inadequate thawing (5.3%); (9) consumption of raw food (4.7%) and (10) infected food handler (1.1%).

In Scotland, contributory factors recorded are about similar to those described for England and Wales: the most frequently recorded factors are unsafe sources, inadequate cooking, cooling, thawing and re-heating **(82)**. Frozen poultry meat and particularly turkey, are more likely to be associated with inadequate thawing and under cooking. Large frozen turkey (25 lbs or more), because of their size, create difficulties in thawing, cooking, cooling adequately

to prevent multiplication of surviving or re-contaminating salmonellae (90). Although infected food handlers were recorded in 126 salmonella outbreaks, in only 9 (1.1%) was there evidence to suggest that they were the original source of the contaminating organism. In most instances, food handlers are victims, rather than sources, of foodborne salmonellosis. They become infected either from frequent contact with contaminated raw foods, from tasting during preparation or from eating left over contaminated cooked food.

1.8 MAGNITUDE OF FOODBORNE SALMONELLA INFECTIONS IN THE DEVELOPED COUNTRIES:

In most developed countries where national surveillance programmes had been established, reports of infections due to salmonellae have risen progressively within the past two decades. In the United States, for example, approximately 40,000 cases of salmonella infections have been reported annually to the Centre for Disease Control (CDC), in recent years (90). In the United Kingdom, reports indicate a steady rise in the number of isolations of the salmonellae from foodborne outbreaks (77). In England and Wales, the salmonellae accounted for 38% of 1044 general and household outbreaks that occurred between 1970 and 1979 (89); however, during the period 1982-84, the salmonellae were responsible for 75% of the annual average of 10,429 incidents (81). In Scotland, 80% of 1,381 general and household outbreaks of food poisoning recorded by the CDSU for 1980-85 were caused by salmonella (91). Annual reports published in the WHO Surveillance Programme for Foodborne Infections and Intoxications in Europe (92) indicate that in most European countries the salmonellae constitute the most frequent cause of foodborne infections. Since 1978, salmonella, particularly *S.enteritidis* has been increasingly responsible for outbreaks of foodborne diseases in Spain (93). Thus, in 1977, *S.enteritidis* equalled *S.typhimurium* in accounting for about 8% of the outbreaks; by 1984, *S.enteritidis* accounted for 40%. In Canada, salmonella remains the primary cause of food poisoning; reported cases rose from 4,200 in 1970 to

9,200 in 1982 (94, 95). Taking unreported cases into account, the real incidence in Canada was estimated at well over 500,000 annually (95).

In the industrialized countries generally, the salmonella infection rate has been reported to range from 10 to 70 per 100,000 persons per year (4). However, surveillance of salmonellosis in almost all the countries is primarily **passive** since it depends on reporting of cases by general practitioners (GPs) and reporting of isolations by medical and veterinary diagnostic laboratories. An obvious shortcoming of passive surveillance system is incomplete reporting. Little information is readily available to document accurately the true incidence of salmonellosis in the developed countries. Some estimates have been made through extrapolation from investigation of outbreaks; but the greater proportion of salmonella infections appear to be **sporadic** rather than outbreak-related (5, 82, 92). Passive surveillance tends to be biased towards the investigation, detection, and reporting of outbreak-related incidents. The vast majority of sporadic cases are neither investigated nor reported.

There are a number of reasons for estimating the true magnitude of salmonella infections. Such an estimate allows health policy planners to compare the importance of salmonellosis with that of other health problems; an accurate case-count is essential for cost-benefit analysis of specific measures designed to control salmonellosis; under-reporting makes it impossible to assess accurately the potential benefit of any control programme that may be introduced (5). Some recent studies have estimated that in the developed countries the true incidence of human salmonella infections is 10 to 12 times higher than officially reported cases (3-5). In a more recent study Chalker and Blaser (5), attempted, through a review and synthesis of literature, to assess the true magnitude of salmonella infections in the United States vis-a-vis the developed countries. They employed the following three

independent approaches to estimate the actual number of salmonella infections;

(1) Determination of carriage rates and duration of excretion;

(2) Calculation of sequential artifacts within the national surveillance system;

(3) Estimation of overall surveillance artifact by analysis of outbreak investigations.

The estimates derived from the three methods were used to form a range of values for the annual incidence of salmonella infections.

1 Calculation of Salmonella Incidence based on estimated carriage rates and duration of excretion:

Estimates of the annual incidence of any infection that has a convalescent carrier phase can be calculated from the formula:

$$\text{total number of infections annually} = \frac{\text{carriage rate}}{\text{duration of excretion}} \times \text{Population at risk}$$

The carriage rate of salmonella in the USA population was estimated by a review of 25 studies from developed countries involving 4,086,753 asymptomatic subjects. The overall **median** carriage rate in the studies was 0.15% (0.12% for adults and 0.24% for children). The **median** duration of convalescent salmonella excretion within the USA has been estimated to be approximately 5 weeks or 0.096 years (75). The total population of the US during the study was approximately 236 million. Thus, the total number of salmonella infections per year = (carriage rate)$(236 \times 10^6/0.096)$ or $(2.46 \times 10^9) \times$ carriage rate.

Given the assumed overall median carriage rate of 0.15% the estimated number of infections per year in the US is $2.46 \times 10^9 \times 0.15/100$ or 3.7 million salmonella infections annually.

2 Calculation of Incidence based on Estimation of Sequential Surveillance Artifacts:

Chalker and Blaser (5) listed the sequential steps required for a case of salmonella infection to be reported to the national centre (CDC) under the passive surveillance system in the US. Many cases are not notified as a result of **artifacts** within the various steps. Each step is subject to an artifact and because the steps are sequential, the artifacts tend to compound. An **artifact** within a step is defined as **the percentage of true infections that, having arrived at the step, fail to pass on to the next step.** The inverse of this percentage is a multiplier or factor that corresponds to the proportion of infections lost to the national epidemiology centre at that step. This approach provides an estimated under-reporting rate due to the sequential artifacts. Multiplying this estimated rate by the number of cases actually reported gives an approximate estimate of true annual incidence. The sequential steps in salmonella surveillance are as follows:

Step 1: The patient must be infected with salmonella. By definition and by case validity, 100% of the subjects (cases) are considered to be infected and pass on to the next step. Therefore, the multiplier is 100/100 = 1.0.

Step 2: The patient must be ill, and illness is defined as symptoms of acute gastro-enteritis or enterocolitis. In most outbreak and sporadic incidents, varying proportions of culture-proven salmonella carriers show any symptoms. For example, in a restaurant outbreak of salmonellosis, only 5 of 9 (55%) culture-positive employees had symptoms (5); this gives a multiplier (factor) of 100/55 or 1.8. A review of literature on 12 investigations provided a range of

multipliers from 1.25 to 15.0, with the **median multiplier** of 2.2.

Step 3: The patient must consult a doctor. The affected persons must be ill enough to consult or report to a physician, a general practitioner, a hospital or other health care officer. It is known that for most enteric illnesses patients do not consult the doctor; under-reporting of enteric illness in general is a common occurrence. Furthermore, most salmonella infections are sporadic rather than outbreak incidents, and are not investigated. In a salmonella outbreak on a cruise ship (5), only 32 or 8% of 386 passengers who became ill consulted the ship physician during the cruise. This provides a multiplier of 100/8 or 12.0. In determining the rate at which persons with symptoms consult a doctor, the authors reviewed a number of investigations and obtained a **median** multiplier of 2.2.

Step 4: The doctor or hospital must obtain a culture from the patient. This is necessary for case validity. Taking the example of the 32 persons who reported ill to the cruise ship's physician, 22 or 66% provided specimens for microbiological identification of salmonella. This proportion gives a multiplier of 100/66 or 1.5. By a review of other salmonellosis outbreak investigations, a **median** multiplier of 2.4 was obtained for the Step 4 artifact.

Step 5: The culture must be positive. In other words, the laboratory receiving the culture must be able to isolate and identify a salmonella organism. Failure to isolate may be due to several reasons. Firstly, the patient may not be excreting sufficient numbers of salmonella at the time the specimen is taken. It has been determined that 10^6 organisms per gram of faeces usually will permit detection of greater than 90% of all positives (96). Secondly, with a median duration for excretion of 5 weeks (75), cultures obtained more than five weeks after onset of illness would be negative 50% of the time (5). Also laboratory techniques may be different and inadequate; an indication of **observer**

variation. In a quality evaluation programme in USA, 84% of 4,374 laboratories sent one of 20 common bacterial pathogens were able to identify it correctly; this gives a multiplier of 100/84 or 1.2. An evaluation of about 800 microbiology laboratories in the USA in 1975 revealed that 83% were able to correctly isolate and identify salmonella (5), giving a multiplier of 1.2. National salmonella surveillance programmes are based on validated reports from accredited laboratories. However, in consideration of obvious observer variations in laboratory results and by a review of studies on laboratory performance, the authors obtained a median multiplier of 1.4 for Step 5 (laboratory failure).

Step 6: The laboratory must report the isolation to State or local health department. A study of a number of hospital microbiology laboratories showed that between 42% and 78% of cases with a discharge diagnosis of salmonellosis were reported to the State or local health department. The data produced a range of multipliers from 1.3 to 2.4, with a median multiplier of 2.0 for this step.

Step 7: The local health department must report the isolation to the national co-ordinating centre (in the case of USA, the CDC). Reviewed records and data on proportion of isolations notified, produced a multiplier of 1.2

By successive multiplication of multiplier factors from each step, salmonella surveillance total multiplier of 39.0 was obtained. Multiplication of this factor by the average number of cases reported each year within the salmonella surveillance system (approximately 40,000 per year), gave an estimated 1.6 million cases of human salmonella infections in the USA during 1984 (5).

3 Calculation of salmonella incidence based on estimation of overall surveillance artifact:

A third approach used by Chalker and Blaser (5) for estimating the artifact within the national salmonella surveillance system is through the study of outbreaks in

which the total number of persons ill and the number of positive salmonella isolations are known. Assuming that an actually ill person during a salmonella outbreak represents a true case, whether or not a culture was obtained, the ratio of total number ill to number of positive isolations reported would serve as a measure of the ratio of true cases of salmonellosis not reported to the national surveillance co-ordinating centre. For an example, a survey of data from a study of a college outbreak revealed that only 8 positive cultures were obtained and reported from 232 ill students; this produced a multiplier of 232/8 or 29.0. By using a modification of this approach, Aserkoff et al (4) estimated that less than one per cent (1 in 100) of symptomatic infections in the USA were reported. In a review of 8 studies of salmonella outbreaks, Chalker and Blaser (5) obtained a median multiplier of 19.2. Multiplying this factor by the total number of reported cases (40,000) yields approximately 800,000 cases of salmonellosis per year in the United States of America.

On the basis of the three independent methods of analysis, Chalker and Blaser (5) derived true estimates of the number of salmonella infections in the USA ranging from 800,000 to 3,700,000 per year! The mean of the estimates by the three approaches is approximately 900,000 and the median is approximately 1.4 million. Although each of the three separate methods has certain specific limitations, it seemed clear that the number of cases of human salmonellosis (40,000) reported to the national co-ordinating centre (the CDC) each year represents between 1% and 5% of the actual yearly incidence in the USA. This implies that greater than 95% of all salmonella infections are not being reported. Improved physician awareness of the necessity for obtaining cultures from symptomatic patients, continued improvements in laboratory proficiency, active surveillance by local health departments, statutory rather than passive notification of salmonella infections by relevant laboratories to appropriate health authorities - these measures would help eliminate potential sources of artifacts

and make national salmonella surveillance systems more complete and comprehensive.

The magnitude of human salmonella infections in most other developed countries may be estimated by appropriate application of the methods and extrapolation of the data and calculations obtained for USA. In spite of the well-organized sequential surveillance system operating in the United Kingdom, notification of human infections remains passive. It is obvious that only a small proportion of the actual number of salmonella infections are reported each year. It is desirable and timely in Scotland to determine the true annual incidence of salmonella infection and the full magnitude of the salmonellosis problem, by the application of a combination of all three methods described by Chalker and Blaser (5). Accurate assessment of the true number of infections per year will allow Health and Agriculture authorities to appreciate and compare the full impact and significance of salmonellosis as a health hazard. Such an awareness of the real magnitude of the problem will lend pressure for initiating, and evaluating the success of, alternative control programmes.

1.9 THE PROBLEM OF FOODBORNE SALMONELLOSIS IN DEVELOPING AFRICAN COUNTRIES:

Elements of food hygiene can be found in very traditional societies in most developing countries. The peoples of these cultures, had developed their own simple food hygiene practices and protective food habits, and had learnt to live with risks of their environment. Problems developed in these countries when food habits were changed or were otherwise modified through the influence of technology and the introduction of new types of food, especially processed, pre-cooked, and ready-to-eat foods. The consequence is an increase in incidents of foodborne infections, since the necessary technology for control is hardly available and low priority is often placed on food surveillance.

In developing African countries, information on the role of animals and foods of animal origin in the epidemiology of salmonellosis is quite scanty. The very limited scope of studies and the lack of a well-organized surveillance network for foodborne infections make it difficult to assess the actual magnitude of salmonella infections. Comparatively few outbreaks of foodborne salmonellosis are documented or reported. This has been brought about by a number of factors:

(a) Foodborne infections are not (statutorily) notifiable in many of the countries;

(b) In most countries where food poisoning is notifiable, there are very few public health laboratory services designed to investigate exhaustively suspected foodborne infections and intoxications. For most reported outbreaks, therefore, the causative agents are not conclusively investigated or established.

(c) Bulk preparation of foods in advance and large scale commercial catering with their potentials for general outbreaks are not yet part of accepted pattern of living in many parts of Africa.

(d) A few of the traditional food habits are rather protective. For example, with the exception of some local dishes and delicacies, thorough cooking of home-prepared foods is the custom. This ensures the destruction of bacterial organisms in the foods. Ironically, this traditional food habit as well as the small numbers of reported incidents have tended to create the false notion that foodborne infections constitute a low public health hazard in Africa.

Many socio-cultural, economic, and ecological conditions existing in the African continent, create opportunities for high rate of livestock infections, cross-contamination, and for human infections from foods of animal origin.

(1) There has been a tremendous growth of the poultry industry in most of the developing African countries in recent years (70).

(2) Developing countries have imported increasing numbers of breeding and commercial chicks from the developed countries, where poultry is already a major source of human salmonellosis. The increased production and slaughtering of poultry by the application of modern technology could hardly avoid the problem of salmonellosis. The growth of poultry and other meat production without proper and adequate veterinary inspection is an important factor in the epidemiology of zoonotic salmonella.

(3) Large scale, poorly controlled movement of animals and animal products across international borders is a major factor for the spread of salmonellosis.

(4) In urban and even in rural areas of many African countries, economic and job consideration has resulted in increased consumption of meals outside the home. These meals are taken at restaurants and in an increasing number of "fast food" and "take-away" places. Food hawkers and street vendors satisfy a significant proportion of this need. Many of the foods are prepared well in advance and are left for several hours at ambient temperatures before sale or consumption.

(5) In most meat-eating rural communities, private home slaughter is every day practice. Generally, such animals never undergo normal veterinary inspection.

(6) Where food animals are slaughtered in official abattoirs and slaughterhouses, the condition could sometimes be very unhygienic and unsatisfactory, with inadequate sanitation, unskilled staff, use of dirty knives by butchers and mixing of carcases and offal.

(7) The sale of much of the hygienically slaughtered and inspected meat in very unhygienic conditions in fly-swamped open-air markets and butchers shops promote cross-contamination and spread of infection.

(8) The objective of proper slaughter and official veterinary inspection is often at times defeated when inspected and passed carcases are loaded in trucks, boots of taxi cabs, or carried in open wheel-barrows!

(9) In most areas, poultry is not slaughtered in official slaughter places, but privately by the owner or buyer. At roadsides, bus stops and motor parks, pre-cooked poultry meat (whole and portions) is sold in an unhygienic way.

(10) The tropical high ambient temperatures and relative humidity reaching 99^{o} promote the multiplication of salmonella and other organisms to infective dose in foods. The situation is worsened by the scarcity of refrigerators and cold-storage facilities.

(11) Certain traditional dishes and delicacies prepared with raw or insufficiently cooked meat, such as "suya" in West Africa, "kebab" in North Africa, and "samosa" in East Africa, have been known to be potential and real sources of salmonella and other zoonotic organisms **(70, 97)**.

(12) Among pastoral and nomadic communities throughout Africa, drinking of raw milk is common, while sour and unpasteurized milk is used to prepare local products such as cheese, yoghurt and butter. One main source of non-pasteurized milk is the small-holder producer-retailer dairy herds owned by the pastoral tribes who hawk the output of their herds directly or sell through small scale retailer outlets.

In spite of the absence of a surveillance system, published studies on the carrier rates of salmonellae in food animals, the contamination rate of animal products, and the carriage rates in the human populations, confirm the seriousness of the salmonellosis problem in Africa. In their study to estimate the magnitude of salmonella infections, Chalker and Blaser (5) made a comparison of carriage rate from studies in developed and developing countries. Among 20 studies involving 102,311 asymptomatic subjects from the developing nations, the overall median carriage rate was 1.8%. This rate was about **twelve times** greater than the figure among asymptomatic persons in the developed countries. The difference in carriage rates suggested that the incidence of human salmonellosis is higher in developing than in the developed countries.

In different research conducted in Egypt, it was shown that salmonellae were responsible for about 3% of cases of infantile diarrhoea in rural areas and about 4% in urban areas (70). Another study of infantile diarrhoea in Egypt showed that salmonella was detected in 14.8% of cases and ranked second after enterotoxigenic *E.coli* (70). Other surveys in Egypt revealed that salmonellae were isolated from 75% of dead chickens; 10% of table eggs; 6% of milk products (cheese, butter, ice-cream); and from raw meat and minced meat in commercial food service establishments (70). In Accra, Ghana, 42 salmonella isolates were obtained from poultry at slaughter; nine serotypes were identified, including *S.typhimurium*, *S.bredeney*, *S.infantis*, *S.poona* and *S.birkenhead* (98). In Ethiopia, 27 salmonella serotypes were isolated from food animals (chicken, cattle and camels) and their by-products (79, 99). In Botswana an unspecified number of salmonella serotypes were recovered from meat and meat-products (100). In Kenya, salmonella infections have been studied in a limited degree, mainly in connection with infantile diarrhoea. One study showed that in 1974 the salmonellae accounted for 0.8% of all admissions and 1.2% of all deaths at the Kenyan National Hospital (101). *S.typhimuirum* accounted for 78% of all the salmonella isolations.

In Nigeria, a review of available records of food poisoning showed that between 1974 and 1983, there were 29,598 reported cases (102). Several salmonella serotypes have been identified in surveys of livestock and poultry (103-109) as well as raw meat, dressed carcases (108, 110) and pre-cooked meat (111). One survey of mesenteric nodes from 200 slaughtered cattle showed salmonella carrier rate of 5.5%; three of the 7 salmonella serotypes recovered from cattle were also isolated from hospital patients (103). Collard and Sen (104) isolated *S.dublin* from trade cattle, market meat, and from human beings. In another survey of trade cattle at a local abattoir in eastern Nigeria, *S.typhimurium* and *S.dublin* were isolated from the gall bladder; an overall carrier rate of 3% was recorded (105).

Although evidence of direct epidemiological association was not established between specific animal sources and human infections, many of the salmonella serotypes recovered from food animals and their products were also isolated from human patients suffering from gastroenteritis (104, 106, 107).

1.10 EPIDEMIOLOGICAL MARKERS FOR STRAIN IDENTIFICATION OF THE SALMONELLAE AND FOR TRACING INFECTION:

The problem of establishing and clarifying the epidemiological association between human salmonella infection and foods of animal origin may be resolved by detailed **strain identification** based on discriminating typing schemes (112, 113). All discriminating typing schemes depend on the demonstration of one or more **epidemiological markers**; that is, genetic and biochemical characteristics which hopefully permit an increasing degree of differentiation of salmonellae isolated from human outbreaks and from the incriminated food (112-117). The development of new techniques particularly in the field of taxonomy has resulted in methods becoming available which allow a much finer degree of discrimination than had hitherto been possible. In his recent review, Le Minor (115) classified the epidemiological markers available for

the typing of the salmonellae as **major** and **minor**. The major epidemiological markers are those determined by the chromosomal genes and are not affected by the extra-chromosomal or plasmid elements. The minor markers, according to Le Minor, are considered to be either affected by extra chromosomal (plasmid) deoxy ribonucleic acid (DNA) or do not have sufficiently proven genetic stability. Le Minor's review and classification, whilst commanding a degree of respect in some European countries, have not found general international favour. This is largely as a result of his adopting some highly contentious views which may have an element of value from a purely taxonomic stand point, but which may not be of much aid to the laboratory and field investigators. The concept of major and minor epidemiological markers must be taken with caution, as time and environmental considerations are inevitably involved.

Differentiation and discrimination within the salmonellae is carried out by the application of one or more typing schemes that include not only more traditional methods such as serotyping, phage typing and biotyping that are based on phenotypic markers, but also more modern methods such as plasmid profile analysis, restriction enzyme-fragment profiles of plasmid or chromosomal DNA (fingerprinting), and DNA/DNA hybridization - which are based on genotypic markers. The choice of technique for the study of the epidemiology of a particular infection or outbreak depends on the scope of the episode or event, in terms of time and locality.

1.10.1 Genus and Subspecies of Salmonella:

The Salmonellae normally conform to the general definition of the family Enterobacteriaceae **(118, 119)**; although, for practical purposes, the other characteristics have been retained, as they are valid for epidemiological purposes. According to the International Code of Nomenclature of Bacteria, the genus *Salmonella* consists of only one species; the species name should be *S.cholerasuis* which was the first name given to the organism. The salmonella species

comprises of six subspecies or subgenera that can be readily identified by some biochemical property. The first four subspecies of the species correspond to the members formerly called sub-genera I - IV Kauffmann **(120)**. Salmonellae of subspecies I are the most frequently found; about 99.7% of salmonella strains isolated from humans and warm-blooded animals belong to the subspecies I.

1.10.2 Serotypes:

As a rule, salmonella subspecies or subgenera are divided into **serotypes** (serovars) according to the O (somatic) and H (flagella) antigen specifications of the strain or isolate. However, with some serotypes, final designations can only be made, based on biochemical characteristics. Over 2000 salmonella serotypes have been recognized and their distribution among the subspecies has been documented **(116,121)**. The general method of serotyping of salmonella was first described by Kauffmann and White **(122)**. The genetic determinants of the antigenic factors are now known to be stable and this epidemiological marker has been found to be of great value in tracing infection pathways. This is especially true for the major factors defining O groups, example O4 and O9. However, the ubiquity of many serotypes is such that the value of serotyping as an epidemiological marker is better demonstrated in those uncommon serotypes which become obvious by virtue of their rarity.

Progress in modern knowledge of the antigenic structures of **Salmonella** and their determinants has enhanced the application of this marker to epidemiology. Serological typing has been useful in providing evidence of an epidemiological link between consumption of a particular food item and the outbreak of salmonellosis within a particular time and defined area **(123)**. However, serotyping has its limitations as an epidemiological tool, because the majority of outbreaks of foodborne salmonellosis are caused by a few predominant serotypes. Therefore, further discriminating characterization of salmonella serotypes by

other typing schemes or epidemiological markers becomes necessary.

1.10.3 Phage Types:

Strains of a salmonella serotype may be subdivided or further discriminated by their sensitivity or resistance to bacteriophages, giving rise to **phage-types** or lysovars. The phage type is subject to change by lysogeny, by bacteriophages, or by acquisition of plasmids. It is determined by genes in the bacterial chromosomes, prophages and plasmids, as well as by receptors on the bacterial surface **(115)**. Many combinations of the determining genes are possible. This marker is sufficiently stable during an outbreak to be of epidemiological value. Different phages show different host ranges, that is, they will attack only certain bacteria, and those they attack are often restricted to certain strains of the same or related serotypes. For example, strains derived from one particular serotype may be incorporated into a typing scheme designed to discriminate between strains of another serotype. It follows that strains of bacterial serotype are sensitive to a defined range of phages **(124)**. These properties provide the foundation or the basis of phage-typing as an epidemiological marker. If it can be shown in a single bacterial serotype, such as *S.typhimurium*:

(i) that different strains (isolates) are sensitive to different phages or different combination of phages; and

(ii) that in many strains of *S.typhimurium* from various food sources, a reasonable number (say ten or more) of different phage sensitivity spectra are demonstrable;

(iii) that the sensitive spectrum of strains epidemiologically related or connected with each other is the same; and

(iv) that the sensitivity spectra are stable;

then a usable phage-typing scheme is achieved (124). Anderson *et al* (125) have reviewed the value of phage-typing as an epidemiological marker in investigation of foodborne salmonellosis. Some salmonella serotypes such as *S.typhimurium* are so widespread that the epidemiological investigation of outbreaks caused by these serotypes requires discrimination of "subtypes" within the serotype. Such discrimination requires the identification of genetic markers by which individual strains can be recognized when isolated from different hosts and different vehicles of infection. Phage-typing scheme is probably the best method of sub-division of the serotype. When salmonella serotypes are isolated from an outbreak circumscribed by time and place, and are found to be the same phage-types, it would seem safe to assume that the salmonella strains originated from the same source. However, some phage-types have themselves become so prevalent and predominant that the above view may not be tenable. In such cases, additional discriminating scheme, or a combination of phage-typing and another typing scheme may have to be employed.

The experience of many investigators (bacteriologists and epidemiologists) is that, when it can be applied, bacteriophage typing remains one of the most satisfactory and routine epidemiological markers for sub-dividing the bacteria that cause infectious diseases and foodborne outbreaks (124), the reasons being consideration for speed, cost and reliability. For initial epidemiological purposes, phage typing which now discriminates and distinguishes 229 phage types of *S.typhimurium*, will suffice to indicate likely sources of infection and to highlight the major epidemic strains present in a community, a country, or in a given animal species. Phage typing has helped to clarify the epidemiological connections between human and animal infections; it has been used to demonstrate that certain phage types are predominant in and are associated with particular animal hosts (124, 125, 126).

1.10.4 Biotypes:

Another of the other methods for further subdivision of salmonella serotypes or differentiation of strains is **biotyping**, which can be used in conjunction with phage-typing to sharpen the precision of strain identification **(116, 126)**. Le Minor in his review **(115)** included biotyping in the category of "minor epidemiological markers". The studies of Barker and Old **(112, 116, 117, 126, 127, 128)** have demonstrated however, that the markers on which biotyping is based are remarkably stable and generally not determined by plasmids. The results of biotyping tests are reproducible and afford excellent strain discrimination; that is, different types of strains within any named serotype or phage type can be recognized **(128)**. The basic scheme has been applied successfully not only for the differentiation of epidemic strains of *S.typhimurium*, but also of strains of salmonellae of other serotypes with the serogroup 04 and a number of other serogroups **(112, 114, 126,127, 128, 129)**.

A scheme of biotyping of fermentation characters developed by Kristensen *et al* **(130)** for the typing of *S.typhimurium* distinguished 21 biotypes of the serotype. Different biotypes of this scheme have been found in the same phage type, so that combination of biotyping with phage-typing has improved the identification of epidemic strains **(126)**. A new biotyping scheme by Duguid *et al* **(114)** introduced additional methods and identified a larger number of biotypes of *S.typhimurium*. The two-tier system recognized 32 potential primary biotypes by possible combinations of positive and negative reactions in five primary tests giving most discrimination of strains of *S.typhimurium*. Subtypes within the primary biotypes were recognized by the reactions in ten additional biotype tests.

The value of biotyping is greatly enhanced when used in conjunction with phage typing. A good example has been described for strains of *S.typhimurium* phage type 141, an uncommon phage type in the UK before 1972 **(116, 117,126)**.

Biotyping of this phage type identified three independent phage type - biotype groups: 141/1f, 141/9f and 141/31bde. Biotyping may be used alone in epidemiological investigation to identify strains within a serotype for which no other methods of strain identification (such as phage typing or plasmid profile analysis) are available. During the period 1977-1983 when infections caused by *S.montevideo* were common among sheep in Scotland and in the human population in England and Wales, the only method reported in the USA for discriminating strains of this serotype was phage typing, but this technique was not available then in the UK **(116)**. Biotyping was successfully used to recognize 27 biotypes of *S.montevideo* belonging to two major biogroups 2d and I0di **(129)**. The biotyping also revealed differences in the epidemiological distribution of the two biogroups in Scotland, and in England and Wales. Biotype 10di was predominant in all animals in Scotland but only in sheep in England and Wales. Biotype 2d was responsible for almost all human, cattle and poultry infections in England and Wales, but accounted for only 24% of human infections in Scotland **(129)**. This suggested that human infections in Scotland were caused by *S.montevideo* biogroup 10di derived from various animals, while in England and Wales human infections were caused by biogroup 2d derived from cattle and poultry. Different biogroups of the same serotype accounted for human infections in Scotland, and in England and Wales.

The application of biotyping technique to salmonellae assists not only in interpreting the epidemiology of particular outbreaks, but also in following the emergence and disappearance of particular epidemic clones. Biotyping is thus considered by Barker and Old **(116)** as an epidemiological marker of major importance.

1.10.5 Plasmid Profile Analysis:

In recent years the application of molecular techniques has provided a level of discrimination within the salmonellae

which had hitherto been unavailable, and has provided essential data on the epidemiology of outbreak and sporadic incidents. One technique which has been particularly useful and most widely employed is the plasmid profile analysis. Plasmids are extrachromosomal DNA elements that are self-replicating. They can specify or confer a variety of different properties, such as antimicrobial resistance, production of exotoxins and the ability to utilize certain biochemical substrates, and they can mediate a variety of virulence factors. Most wild-type salmonellae seem to contain plasmids **(131, 132, 133)** although, it is difficult to assess carriage with any real degree of accuracy, Electrophoresis of plasmids in an agarose gel provides a fairly accurate estimate of the size or mass of a plasmid, and the ease with which the plasmid profile can be determined, has rendered the determination of such profiles or patterns a potentially useful tool for epidemiological studies **(131, 132, 133, 134, 135, 136).**

Plasmid profile techniques have been shown to be an effective tool for the investigation of the epidemiology of salmonellosis, provided the salmonella strains responsible contain plasmids. In July-August 1981, the number of sporadic cases of salmonellosis in two north eastern states of USA increased markedly. During the same period, two outbreaks in these states caused by the same **salmonella** serotypes were traced to a brand of precooked roast beef. Riley *et al* **(134)** determined the plasmid profile(s) of salmonella isolates from the incriminated roast beef and cases in the two outbreaks. They also examined the plasmid profiles of the sporadic strains, the authors could determine whether the contaminated roast beef accounted for many of the sporadic cases of salmonellosis in the two states. *S.newport* isolates from the implicated pre-cooked beef and from the outbreak cases were identified by a unique profile, which was found to be also present in 45% of reported strains from sporadic cases in the area during the same period. Analysis of food histories in the sporadic cases demonstrated association between this plasmid profile and consumption of precooked beef. Plasmid profile analysis

was thus useful in studying the epidemiology of sporadic or isolated cases of salmonellosis.

Plasmid profile analysis was shown to be helpful in investigating salmonella outbreaks caused by relatively uncommon serotypes (137, 138). In 1981, the technique was used to establish the likelihood that marijuana was the vehicle of 85 cases of *S.muenchen* in four states in the USA, for which no common food source could be found (137). Plasmid profile analysis was also used in scattered *S.drypool* outbreaks in the USA when the source was traced to a Mexican dish prepared from steamed cow heads (138).

In England and Wales, plasmid profiling was used in the investigation of salmonella outbreaks in which 131 cases of *S.goldcoast* infection in 1984 were epidemiologically associated with one brand of pate imported from France. The plasmid patterns of salmonella strains from humans in England and Wales and from the imported pate were similar to those in human strains from France (139).

1.10.6 Resistance to Antimicrobial Agents:

Strains of serologically defined salmonellae and of known phage types may be further subdivided and differentiated by sensitivity or resistance to specified antimicrobial agents. The property of drug-resistance can be plasmid-mediated and phage type-related (115,140,141). Conjugation procedures have demonstrated that the antibiotic resistance determinants in a number of *S.typhimurium* phage type 204 strains in Britain are located in the R-plasmids (140, 141). Drug resistance plasmids carried by epidemic strains of *S.typhimurium* PT204 and 193 from Britain have been characterized by genetic and molecular studies (140). The role of some of the plasmids in determination of these two phage types have been described in detail. Resistance to streptomycin and sulphonamide has been the predominant resistance pattern in cultures of *S.typhimurium* PT204 isolated in Britain from humans and bovines (140). When first isolated in 1974, phage type 204 was resistant to

sulphonamides and tetracycline, and was probably derived from a strain of *S.typhimurium* PT49 resistant to sulphonamide, which was converted to PT204 by the addition (acquisition) of a plasmid coding for tetracycline resistance. After 1976, PT204 strains resistant to chloramphenicol and streptomycin, in addition to sulphonamide and tetracycline were identified. Genetic studies showed that these new strains carried an additional (second) plasmid. Later in 1977 this PT204 strain gained a third plasmid coding for resistance to ampicillin, kanamycin and streptomycin. The presence of this third plasmid resulted in change of resistance pattern from sulphonamide-tetracycline-chloramphenicol-streptomycin to ampicillin-kanamycin-streptomycin and consequently a change of phage-type from 204 to 193 **(140).** Examination of agarose gel electrophoresis of the plasmid (plasmid profile) confirmed that PT204 and 193 were related.

Comparative studies with biotyping, phage typing and plasmid profile analysis, have shown that the pattern of antimicrobial resistance is of less epidemiological value than any of the above. Most sporadic cases (up to 75%) of salmonella infection are caused by common serotypes which lack unusual antimicrobial resistance patterns, that would enable distinguishing among them **(115, 135).** Thus, as a useful epidemiological marker, antibiogram is of more limited value.

1.10.7 Plasmid Restriction Enzyme Fingerprinting:

The analysis of plasmid pattern can be made more accurate and more useful information of epidemiological value may be obtained by application of a more detailed indirect molecular technique. The indirect technique involves restriction endonuclease analysis, also called restriction enzyme **fingerprinting.** Restriction endonucleases are bacterial enzymes that cleave double stranded DNA at specific recognition sites to generate a series of plasmid fragments **(132).** The number and sizes of these fragments depend upon the number and location of specific recognition

sites present on the plasmid DNA. Plasmids differ considerably in the number of restriction sites they possess for a given enzyme, and various workers have employed a variety of restriction enzymes to generate "finger prints" from the plasmids harboured by different bacterial genera and species (142).

Ideally any plasmid should be cleaved by two restriction enzymes, each of which generates a sufficient number of fragments to ensure specificity. To be applicable to routine use in epidemiology a minimum number of restriction enzymes should cleave a maximum number of plasmids to yield an optimum number of fragments (142). If two plasmids from two separate salmonella strains are of the same size and yield the same size and identical patterns of fragments on restriction enzyme analysis, especially if two or more restriction enzymes are used, then the probability is that the plasmids are identical. Two plasmids of the same size may have entirely different base sequences; this can be recognized by the different plasmid patterns generated by a simple restriction endonuclease. Plasmid fingerprinting techniques have been used to investigate the epidemiology of salmonellosis. The technique showed that plasmids from human and animal salmonella isolates were identical, thus providing evidence of a relationship such that human infection might have been acquired from a possible animal source (134, 142). The converse possibility must also be considered, with the decision being based on the relevant epidemiological data. During sporadic and outbreak incidents in north eastern states of USA, Riley *et al* (134) used restriction enzyme fingerprinting in conjunction with plasmid profile analysis to identify a specific and unique plasmid in a clone of *S.newport* transmitted via a contaminated precooked roast beef.

1.10.8 DNA Hybridization:

Direct techniques of plasmid analysis involve hybridization of DNA strands from different sources and may permit a

quantitative assessment of base sequence homology (142). DNA-DNA hybridization may be used to determine the extent of homology between two fragments of plasmid DNA (132). In the technique the DNA of the plasmid is radio labelled, the double strands of the DNA of both plasmids are separated into single strands; the four strands are mixed together and allowed to reanneal. After reannealing, the degree of homology is calculated on the basis of amount of radioactivity present in precipitated double-stranded DNA (142).

Progress in recombinant DNA technology offers possibilities of its application as a tool in the epidemiology of foodborne infections. The method has been used to detect salmonellae from different food sources (143). The technique has been used to demonstrate differences among strains of *S.dublin* with identical plasmid profiles and restriction enzyme fingerprints, as well as to show differences among strains of *S.enteritidis* belonging to the same phage type (115). Epidemiologists in the USA used recombinant DNA technology to trace chloramphenicol-resistant *S.newport* which had caused a large outbreak of disease through hamburgers and back to the dairy where the beef had originated(144).

1.10.9 Summary:

In conclusion, there is a hierarchy of epidemiological markers for *salmonella* which may be employed for strains belonging to the same *salmonella* serotype. Those epidemiological markers considered absolutely necessary (that is, serotyping and phage typing) are in routine use worldwide; the others (biotyping, plasmid profile analysis, restriction enzyme fingerprinting and DNA hybridization) are employed mainly in specialized laboratories and are still the subject of research. Some markers are simpler, more rapid, and relatively less expensive to determine; others are more difficult, more time-consuming, and more expensive to apply. The choice of which discrimination scheme to be employed will depend on the incident being investigated or

the project being undertaken, as well as the difficulties involved. In general, a large degree of co-operation between the epidemiologist and the microbiologist is essential. In most cases, the question to be answered and the ultimate objective is to establish or clarify any epidemiological link between human and animal infections, or between human infection and suspected food sources.

In the present study of the epidemiological association between poultry meat and human salmonella infections, serotypes, antimicrobial sensitivity, and phage types of salmonellae have been employed within the scope of time and resources available; biotyping and plasmid fingerprinting could not be undertaken, but have been strongly suggested.

1.11 SELECTED FOODBORNE SALMONELLOSIS OUTBREAKS: IMPLICATION OF POULTRY BY BACTERIOLOGICAL AND EPIDEMIOLOGICAL EVIDENCE:

The implication of an animal source such as poultry in point epidemics relies essentially on **bacteriological evidence**, usually by application of the various epidemiological markers. It has been the practice to determine the food source of many general and household outbreaks by isolation of the same salmonella serotype, phage type or biotypes from both the human cases and the suspected common (food) vehicle. In point source outbreaks such as in a household, a hospital, or a restaurant, food remnants or supplies from the same batch may more often be available for bacteriological examination. In a few outbreaks it is also possible to demonstrate the presence of the same organism in the flocks or farm of origin of the incriminated animal or animal product. In many outbreaks however, it may not be possible to identify with any degree of certainty the responsible food vehicle due to delays in notification and the difficulties in obtaining samples of the suspected food. Consequently, it will not be practicable to isolate bacterial types identical to those from a human case. In such episodes, the food vehicle maybe determined on circumstantial **epidemiological evidence** by the (i) analysis

of attack rates (attack ratio) of individual food items; (ii) isolation of identical bacterial types from animals in the same farm or flock, or from meat or eggs in the same consignment or batch; (iii) isolation of a bacterial serotype/phage type consistently recovered from and associated with the particular animal species; and (iv) a history of the suspected food item having been consumed and/or prepared within the same kitchen premises 2 or 3 days prior to onset of illness (83).

Below is presented a review of some selected published reports of foodborne salmonellosis outbreaks in which poultry products are incriminated by bacteriological and epidemiological evidence. The reviews illustrate procedures for investigation of foodborne point-source epidemics and for establishment of epidemiological association between human infections and poultry sources:

1.11.1 Implication of Poultry by Bacteriological Evidence:

Outbreak One (General Community Outbreak): On 6th December 1980, a lunch was given in a Community Centre for old age pensioners living within a housing scheme in Kilmarnock (145). Of the 219 pensioners attending the function, 99 developed symptoms of food poisoning. In addition 23 out of 61 guests, helpers and committee members also experienced some symptoms, giving a total of 122 persons affected out of 280 attending the function. Faecal specimens were obtained from 17 of the persons present and positive isolations of *S.muenchen* were made in respect of 14.

Nine turkeys were supplied for the function, each having an average weight of 11.5 lbs. The turkeys were delivered frozen to a small bakery in an adjacent village, where they were allowed to defrost on the evening of 2nd December. Six of the turkeys were cooked on the 4th December and the remaining three on the morning of the 5th December. The turkeys were cooked in tinfoil simply in domestic size ovens. After cooking, the stock was run off and the turkeys kept in the tinfoil until taken to the Community Centre on

the morning of 6th December. The turkey was served cold at the function with a hot gravy prepared from the turkey stock.

Samples were obtained of left-over turkey stock at the bakery and also of turkey portions from a committee member who had taken it home. Both samples of turkey and turkey stock proved to be positive for *S.muenchen*. The isolation of identical **salmonella serotypes** from both the human cases and the turkey specimens provided sufficiently incriminating bacteriological evidence that the original source of the infection was the turkey. The most important factor seemed to be that, in the absence of a refrigerator, the cooked turkeys were held in the warm and humid conditions of the bakery house for up to 48 hours after cooking. Indications are that defrosting from 2nd to 4th December was adequate, but that the cooling may not have been sufficient to destroy all the bacteria present.

Outbreak Two (Institutional Outbreak): An outbreak of foodborne salmonellosis affecting patients, neonates, staff and family contacts occurred at a maternity hospital in Lanarkshire during late August and early September, 1985 **(146)**. Forty three individuals comprising 24 patients, 2 babies, 15 members of staff and 2 family contacts developed symptoms of gastroenteritis during the first week of the outbreak. Positive salmonella isolations were made from a total of 75 cases and 5 asymptomatic excreters. During the subsequent three weeks a further 27 salmonella isolates were reported by the laboratories; these involved 9 patients, 3 babies, 12 members of staff, and 2 community contacts.

The initial cases occurred on 21st August 1985. On 23rd August the laboratory reported that *Salmonella* had been isolated from 10 individuals. Consequently, rectal swabs were taken from all patients, babies and members of staff. By this time it was thought that a point-source foodborne outbreak had occurred and that brisket of beef was the probable food vehicle. That afternoon, a preliminary inspection of the hospital kitchen, the taking of swabs and

the submission of food samples for bacteriological analysis were undertaken by Environmental Health Officers. Subsequent interviews suggested that the initial infection had occurred prior to the brisket appearing on the menu. In addition, there was no obvious common factor in respect of a single food item having been eaten by those affected. In fact, the brisket was later reported salmonella negative by the laboratory. Nevertheless, further cases were reported on 24th and 25th August.

On 27th August, following the elimination of the brisket as the source of the outbreak, a more detailed inspection of the hospital kitchen was carried out, and this revealed a number of unsatisfactory practices regarding preparation and cooling of foodstuffs: the thermostat on a large double-door refrigerator was malfunctioning; raw and cooked foods were kept within the refrigerator; the lid of the adjacent cabinet-freezer was broken; and the cellophane wrapper surrounding a frozen, raw chicken was seen to be punctured. Swabs of the debris from the freezer and from the frozen chicken were submitted for full laboratory examination. On 3rd September, the laboratory reported the isolation of salmonella from the freezer debris and the chicken. The isolates were both subsequently identified as *S.enteritidis* phage type 8; all the 75 isolates from patients, babies, staff and community contacts were also identified to be *S.enteritidis* phage type 8.

It was not possible to determine the vehicle of infection because none of the food eaten prior to the onset of symptoms was available for sampling. Enquiries revealed, however, that on the morning of 20th August (one day before the initial case), frozen chickens for roasting had been delivered to the hospital and stored in the cabinet freezer. Carcases were defrosted in the main kitchen that day and subsequently cooked for use as chicken dishes which were served at a presentation ceremony for the staff on the afternoon of 21st August. Thirty-two members of staff attended and 15 of this group later yielded *Salmonella sp.*

Although samples of the actual food consumed were not available for sampling and in spite of the false trail provided initially by the brisket of beef, it is plausible that cross-contamination of other foodstuffs occurred within the kitchen from the chicken as it was defrosting. *S.enteritidis* phage type 8 is more commonly isolated from chicken and is frequently associated with poultryborne infections in Britain. This satisfies the criterion of **consistency** for establishing causal association between the defrosted chicken and the present outbreak. Furthermore, there was conclusive bacteriological evidence that uncooked, frozen chicken delivered to the hospitals harboured *S.enteritidis* phage type 8, and that contamination of a freezer had occurred. It is reasonable to suppose (assume) that other chicken carcases from the same delivery (batch) were also infected with *S.enteritidis*. The lack of a proper cooling area in the kitchen and the malfunctioning of a refrigerator may have been contributory factors in the dissemination of infection.

Outbreak Three (Household Outbreak): During the weekend of 7th/8th November 1987, nine members of a family who had gathered at a house in Fife for a reunion and birthday celebration, became ill with symptoms of diarrhoea, vomiting, fever and abdominal pain (147). The onset of symptoms of seven who were hospitalized, occurred between 13 and 19 hours after eating a common meal on the evening of 6th November; and about 40 hours for two less severely affected. The main course of the meal, consisting of fried haddock, baked beans, bread and butter, had been taken by all nine. Eight of the group had eaten the sweet; the ninth who was one of those not hospitalized, had merely tasted it during preparation.

The sweet "leche creme" had been prepared at about 12.30 pm on 6th November and consisted of margarine, flour and pasteurised milk heated together to boiling point to form a white sauce, which was then left to cool for half an hour. The sauce was then enriched by beating in two *raw egg yolks*, and flavoured with vanilla essence. The sweet was placed in

a refrigerator where it remained until served that evening.

By the time investigation commenced none of the food from the evening meal remained. However, investigation revealed that the eggs used for the sweet had been produced by the household's own hens. No eggs were available at the time of the initial inquiry because the hens had apparently gone off the lay; subsequently, however, the hens came back on lay and 3 were submitted for microbiological examination.

S.enteritidis phage type 4 was isolated from faecal specimens of all nine persons affected, from the hen droppings, and from the inside of two eggs which had been collected. All other samples proved to be negative for salmonella. The "leche creme" was almost certainly the food vehicle of infection, having been contaminated by the raw egg yolk incorporated therein. The isolation of identical salmonella types from the clinical cases and from the eggs provided bacteriological evidence for implicating the household's poultry source in the outbreak.

1.11.2 Implication of Poultry Source by Epidemiological Evidence:

Outbreak One (Community Outbreak): Seven persons out of a total of 17 from five households who had consumed meals at a Chinese restaurant in West Lothian over a 2-month period were affected by salmonella food poisoning **(148).** The meals were taken between the 23rd June and 31st August 1981, and involved chicken and chicken curry. Five clinical cases were found to be excreting salmonella, while 2 were symptomless excretors. Six samples of foodstuffs from the restaurant were examined, but with negative results. Six of the seven patients excreted *S.typhimurium* phage type 110, and the other was excreting *S.virchow.* Both salmonella serotypes had been isolated from samples of raw chicken from a poultry producer who had a problem at that time. Although two brands of frozen chicken were supplied prepacked for the Chinese restaurant from an intermediate wholesaler, both brands originated from this same producer.

Although identical salmonella types were not isolated directly from the meals consumed by the families, the following circumstantial epidemiological factors had emerged:

(1) All the cases ate chicken at the suspect restaurant.

(2) Two brands of birds (chicken) supplied to the restaurant came from the same source of supply (same producing farm).

(3) Salmonella serotype/phage types identical to the ones involved in the food poisoning had been isolated from chicken from the producing farm, during the same period.

(4) Cases of poultry-associated food poisoning due to *S.typhimurium* phage type 110 were investigated by two other health authorities elsewhere in Scotland around the same time.

(5) From these factors the assumption that the salmonella types isolated from the cases were poultryborne is **consistent** with established knowledge.

(6) Investigation of this food poisoning incident revealed that the 5 to 6 lb birds used in the curries were defrosted overnight for 9 1/2 hours and cooked for one hour only; the 3 to 4 lb birds used for roasting were similarly defrosted and cooked for 1 hour. The duration of cooking is considered inadequate particularly for the larger birds.

Evidence for implication of chicken in the food poisoning appear to have been provided by epidemiological criteria of **plausibility** and **consistency**.

Outbreak Two: Thirty-seven out of a group of 59 miners on a day bus outing from Fife to Edinburgh and Stirling on Sunday 3rd June 1984, developed typical salmonella food poisoning

symptoms (149). Initial investigation indicated that the source of infection was a meal eaten in a Stirling Club on 3rd June. The incubation period varied from 2 to 96 hours, with the median being 36 hours. *S.stanley* was isolated from 29 of those affected and from 6 other symptomless persons who had attended the meal. In addition *S.stanley* was obtained from four family contacts who became secondarily affected.

Detailed investigation revealed that the club's kitchen was being used as a "Soup Kitchen" during a recent miners strike and food was being prepared and cooked by miners and miners' wives. However, on 3rd June, the meal for the bus trip had been prepared by an employee of the club. The meal was a cold buffet with salad and a choice of chicken, ham or both. The ham of an unknown canned brand had been purchased from a local "cash and carry". The chickens were supplied in cooked portions from a local retailer and delivered to the club at about 1.30 pm on Saturday 2nd June, in a cardboard box. On arrival at the club, these portions were individually wrapped in tinfoil and refrigerated until 12.30 pm the following day, when the meals for the bus group were plated and then left at an ambient temperature until they were consumed at 6.00 pm. The local retailer who supplied the chicken received deliveries of frozen birds from a major producer (Plant A). His practice was to defrost the chickens overnight in the boxes in which they were delivered and cook them in batches of nine for 2¼ hours at 190°C. The chickens eaten by the bus group were cooked on the night of Friday 1st June, left in the cabinet overnight and portioned the following morning. Then they were stored in a refrigerated display cabinet until transported to the club in a cardboard box which had previously been used for the delivery of raw chickens.

Inquiries indicated that no isolation of *S.stanley* had been recorded since past two years from the products of the producer (Plant A) of the chickens served at the meal. The retailer was revisited and subsequently admitted purchasing each week a supply of fresh uneviscerated chickens from the

Glasgow Fish Market. These fresh chickens were produced by another plant (Plant B) at which *S.stanley* had been isolated in April 1984.

Numerous swabs taken at the club and in the retailer's shop proved negative for salmonella organisms. On the basis of circumstantial epidemiological evidence, it would appear most likely that the infection of the cooked chicken portions delivered to the club, was *via* cross-contamination by the fresh chickens from Plant "B".

The establishment of an epidemiological link between food poisoning episodes and poultry by bacteriological evidence is the classic procedure in point source outbreaks. This is particularly applicable where remnants of the incriminated food items are available for microbiological analysis. However, as pointed out earlier, in many general and household outbreaks the responsible food vehicle cannot be identified with certainty and sometimes, leftovers of the suspected food item are no longer available. Besides, only a proportion of individuals affected in an outbreak manifest typical clinical symptoms of salmonellosis.

The vast majority of foodborne salmonella infections occur as **sporadic** cases rather than as outbreak incidents. More difficult to establish and clarify is the source of scattered sporadic incidents and the epidemiological link with specific food items such as poultry meat. For these outbreaks of undeterminable or uncertain food vehicles and for the predominant sporadic episodes, other epidemiological approaches will have to be employed to establish the causal relationship with specific food vehicles such as poultry. Application of some of these approaches forms the basis of the present study and they are presented in subsequent chapters.

1.12 WHO SURVEILLANCE PROGRAMME FOR FOODBORNE INFECTIONS AND INTOXICATIONS IN SCOTLAND:

Collation of routine data has been widely used in the control of communicable diseases. Policies on intervention strategies are based on accumulated epidemiological data. An indication of increasing incidence of outbreaks of foodborne salmonellosis in the industralized nations led to the establishment, in most of these countries of National Surveillance Programmes for Salmonellosis and other foodborne infections usually as an integral part of overall National Disease Surveillance (4, 15, 82, 95, 151). Because of the potential international dissemination of foodborne diseases, there is also need for surveillance at an international level and in particular for rapid analysis of available data to make possible the swift application of measures to prevent spread (151). To meet this need the World Health Organisation has established a worldwide surveillance programme for the control of foodborne infections and intoxications, starting with Europe (92, 151). Surveillance in this context means "the collection and intepretation of data on epidemiology, including causation and incidence of foodborne diseases, to enable responsible authorities to concentrate on appropriate prevention and control measures" (141). Surveillance has also been defined as "the systematic monitoring of infection in the population, to identify single unexpected (sporadic) occurrences or outbreaks, changes in incidence over time and in the groups of persons infected, or risk factors implicated (152). The principal components of a surveillance programme are comprehensive documentation and systematic reporting of the occurrence of specified diseases including: (1) the presence of corresponding pathogens in human beings, animals, food and the environment; (2) investigation of outbreaks and sporadic cases; (3) collation, analysis and interpretation of data gathered; and (4) dissemination of information to relevant institutions and agencies to initiate speedy and efficient directive action (151). Epidemiological investigation of episodes of foodborne illness as well as monitoring of actual and

potential sources of causative agents requires the participation of staff in wide range of disciplines and working in different government agencies and administrations; including the veterinary and food hygiene services, food control administrations, agricultural services and environmental health services. In a number of countries, one of the methods of ensuring coordination, collaboration, and cooperation is the creation of veterinary public health units in health administrations or the appointment of public health veterinarians (Veterinary Advisers) in Communicable Diseases units of the health ministry (153).

In Scotland, statutory notification of foodborne infections had been instituted since 1956. With the development in 1967 of an informal surveillance programme coordinated by the Communicable Diseases (Scotland) Unit (CD(S)U), more comprehensive epidemiological information became available. The epidemiological data were based primarily on reports and notifications from the medical and veterinary diagnostic laboratories. A much more structured reporting mechanism became effective in January, 1980 when Scotland became the first country to participate formally in the WHO Surveillance Programme for the Control of Foodborne Infections and Intoxications in Europe (82,92). Under the Scottish surveillance system, persons suffering from suspected foodborne infections present to their general practitioners (GP) or to the hospitals, who would have appropriate clinical specimens submitted to a diagnostic laboratory for examination. The GP or the hospital is also required to notify the Area Community physician, otherwise known as Community Medicine Specialist (CMS) who in turn informs the appropriate (local) environmental health officer (EHO). The EHO undertakes visits of investigation to the persons and locations affected. Screening of contacts and tracing of infection source is also carried out by the EHO. As has been indicated elsewhere (1.8) the actual number of non-presenting and unreported cases may be as large as 100 times that reported (4,5). Incidents of foodborne infections are recorded as General Outbreak, Household or

Family Outbreak, or as Sporadic Case. A case of salmonellosis is defined as a person with symptoms of acute gastro-enteritis or enterocolitis characterized by fever, abdominal pain, diarrhoea and sometimes vomiting and from whom the salmonella organism has been isolated. By the definition of the WHO Surveillance Programme for Foodborne Infections and Intoxications in Europe (92), a General Outbreak is an incident in which two or more persons experience a similar illness after consumption of the same food, and where bacteriological and other epidemiological evidence implicates the food as the source of the illness. A Household or Family Outbreak is an outbreak affecting one or more persons in the same private household not apparently connected with another case or outbreak. A Sporadic case is one case which, as far as can be ascertained, is unrelated in time and place to other cases in respect to consumption of food. An Incident is any outbreak or any single case.

For general and household outbreaks, under the Scottish Surveillance System, more detailed epidemiological data are recorded on a standard Outbreak Investigation Form (Appendix I). Copies of duly completed forms are sent by the EHO to the appropriate CMS. The information contained in the Investigation Forms are abstracted and summarized on a separate Outbreak Report Form (Appendix II) by the Area or District CMS. Copies of the Summary Report Forms are forwarded by the CMS to the CD(S)U which serves as the WHO Coordinating Centre for Scotland. The diagnostic laboratories also forward weekly reports of isolations to the CD(S)U. The EHOs and the labratories, in addition, send reports of outbreaks and isolations to the appropriate Area Health Board, which processes its own records.

At the end of each year, accepted data relating to General and Household outbreaks are collated into a comprehensive report by the Scottish Coordinating Centre (the CD(S)U). This annual return is forwarded to the WHO Collaborating Centre in West Berlin for inclusion in the Annual Reports of the WHO Surveillance Programme for the Control of Foodborne Infections and Intoxications in Europe (92). The CD(S)U

also forwards reports to the Information and Statistics Division of the Scottish Health Service, Edinburgh for national analysis. The CD(S)U publishes a WEEKLY REPORT (Communicable Diseases Scotland) and, in collaboration with the Information and Statistics Division, also publishes an ANNUAL REPORT (the Surveillance Programme for Foodborne Infections and Intoxications Scotland) which are distributed to all Area Health Boards and to other interested persons in the UK and elsewhere.

The Flow-Chart of the sequential steps in the Scottish Surveillance Programme from the point the index case presents to the GP or hospital to the stage the CD(S)U submits Annual returns to the WHO/FAO Collaborating Centre in Berlin - is presented in **Figure 1.2.**

CHAPTER TWO

OBJECTIVES AND HYPOTHESIS

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2.1 INTRODUCTION:

The increased awareness of the problems of foodborne infections over the years has resulted in a large volume of publications from various parts of the world. Many of these reports indicate that **salmonellae** have become the most frequent cause of foodborne infections in the industrialized nations. The reviewed literature suggests a steady rise in incidents of foodborne salmonellosis in most developed countries. Epidemiological relationships between human salmonellosis and isolations from meat and other food animal products have been demonstrated **(4, 76, 145-152)**. There are many animal sources of human salmonella infections, and this raises the question of defining which meat types constitute the major hazards for man. In the United Kingdom where poultry is the primary source of animal protein, poultry meat has become increasingly incriminated as the source of most outbreak and sporadic incidents in which the food vehicles were identified **(60, 76, 77, 83)**. But, as reviewed in Chapter One, the incrimination of poultry meat is very often based on circumstantial evidence. There is the need to establish and clarify the epidemiological relationship between poultry meat and human salmonella infections. Three epidemiological approaches were employed in the present study in an attempt to achieve this overall objective:

(1) A 20-year Retrospective Study of human salmonellosis in Scotland;

(2) A 12-month Bacteriological Survey in a "closed" long-stay institution of chicken carcases and sewer drains, and an epidemiological analysis of salmonella types from the chickens and the sewer;

(3) A Matched Case-Control Study of poultry meatborne salmonella infections.

The specific aims, objectives and hypotheses (where appropriate) of each epidemiological approach are presented in the following sections.

2.2 FOODBORNE SALMONELLOSIS IN SCOTLAND, 1968-87: RETROSPECTIVE STUDY:

Validation of Data: The establishment of well-organised surveillance programme in Scotland, with the existence of routine records makes feasible a retrospective analysis of foodborne salmonellosis and enables trends to be assessed. In order to avoid the problems of **observer variation** inherent in solely clinical diagnosis and to ensure that the study was based on accurately documented notified incidents, it was necessary to use **validated** records and data. Validity has been defined as "an expression of whether a measure, a response, or an entry actually represents what it purports to; essentially, a measure of 'truth' within the terms of references" **(155)**. In accordance with the method for retrospective validation of registers described by Stone **(155)**, two methods of validation were employed; namely, ascertainment of cases diagnosed as salmonellosis (**case validity**) and the accuracy, completeness and concordance of recorded data (**item validity**). The details of the procedures for validation of cases and recorded items are presented in Chapter Three (Materials and Methods). The aims of the data/record validation are: (1) to ensure that all foodborne infections and outbreaks recorded as salmonellosis have been based on confirmatory laboratory diagnosis; (2) to assess the extent to which the data compiled and collated at the WHO Co-ordinating Centre (the CD(S)U) compare or agree with items recorded and submitted by the diagnostic laboratories or by the area health boards; (3) to determine the extent to which omission, errors in transcription and recording of individual items of data had occurred at the Co-ordinating Centre. Consequently, only

reported salmonella infections and outbreaks with laboratory ascertainment will be included in the study; and only if a random sample of the routine data at the WHO Co-ordinating Centre attains at least 95 per cent level of accuracy and completeness, would the records serve as the sampling frame for the retrospective survey.

The Retrospective Study: Most published reports and analysis of foodborne infections in Scotland cover relatively shorter periods at varying intervals, and tended to relate to specific and limited aspects of foodborne salmonellosis **(77, 78, 82, 83, 84, 156, 157, 158).** The present study is intended to be a more comprehensive analysis for a continuous period of twenty years, beginning from 1968 - one year after a formally co-ordinated national surveillance was introduced. This extensive period would provide a clearer picture of the magnitude of the salmonella problem, of the changes and any trends in incidence, risk factors, and other relevant epidemiological parameters. Reports indicate a steady rise in incidents of foodborne salmonellosis in the developed countries. In Scotland, such reports suggest a variation in the relative role of different animal products as the primary cause of human salmonella infections **(75-78, 83).** The relative importance of certain salmonella serotypes/phage types as the cause of foodborne infections has been reported to fluctuate from year to year or after a number of years **(72, 83, 157).** The incidence of foodborne salmonellosis is reported to follow some seasonal trend; marked increases in outbreaks tend to occur during the summer months **(82-84).** The specific aims of the retrospective study are to provide answers to the following research questions:

(1) What proportions of foodborne infections over the years are caused by **salmonella**?

(2) What changes in frequency or incidence rates of salmonellosis have occurred since 1968? Is there any

trend in incidence? The aim is to be able to detect, with greater than 80% confidence, at least 5 per cent changes in incidence over 5 year intervals.

(3) What are the main types of food implicated in salmonellosis outbreaks, and in what proportions of foodborne salmonellosis had poultry meat been incriminated? Is there any trend in poultry meatborne salmonella outbreaks over time?

(4) What sub-groups of the population (such as sex and age groups, hospital patients and staff, farm workers) are at high risk. What are the standardized sex and age-specific incidence rates?

(5) Is there any variation or trend in the salmonella serotypes and phage types most frequently isolated in foodborne salmonellosis over 5-year periods?

(6) Are there any variations in the proportions or frequency distribution of General Outbreaks and Household (Family) Outbreaks of foodborne salmonellosis?

(7) What places, venues or institutions of food consumption (such as restaurants, and hotels, schools, hospitals, picnics) constitute the main risk factors in general community outbreaks?

(8) What proportions of salmonella outbreaks are imported and what regions of the world are the major sources of salmonellosis imported into Scotland?

(9) Is there any observable and consistent seasonal pattern in the incidence of foodborne salmonellosis?

It is hoped that the twenty-year retrospective study would generate some hypotheses on the incidence, risk factors and trends of foodborne salmonellosis in Scotland.

2.3 BACTERIOLOGICAL SURVEY: EPIDEMIOLOGICAL RELATIONSHIP OF SALMONELLAE ISOLATED FROM CHICKEN CARCASES AND SEWER DRAINS:

Published reports of foodborne salmonellosis indicate that reasonable proportions of salmonella-positive persons involved in outbreaks do not manifest any overt clinical symptoms (5, 67, 75, 79); often times, many such symptomless infected persons remain salmonella excreters for varying periods of time (73-75). In most incidents of clinical salmonellosis, the evidence which implicates poultry meat is only circumstantial, How can infection source be established for scattered sporadic cases and even for outbreak incidents in which the common food vehicles are not readily identifiable? It is important to be able to demonstrate an epidemiological association between consumption of poultry meat and human salmonella infections - without relying on investigation of clinical incidents. Isolations, from foodborne infections, of salmonella types known consistently and concurrently to be associated with poultry may provide an epidemiological link between consumption of poultry meat and human salmonella infection. The problem of establishing an epidemiological association between human infections and, a food animal source can be solved by an approach which requires detailed salmonella strain identification, based on the determination of discriminating epidemiological markers or typing schemes for the salmonellae isolated from human patients and from the suspected food (112). Poultry meat used in a catering establishment can be screened to identify salmonella types to which the consumers are exposed. Salmonella infection and salmonella excretion in the consuming population can be investigated by parallel monitoring of the sewers draining the defined population area. By comparing the salmonella types isolated from the poultry meat and the sewers, the epidemiological association between the poultry and human infections can be clarified. This was the overall objective of the bacteriological survey. The hypothesis is that there is significant association between poultry meat and human salmonella infections; that contaminated poultry meat is

a significant risk factor. The specific aims are to find answers to the questions:

(1) What proportion of chicken carcases are contaminated by the salmonellae?

(2) What proportion of sewer swabs will detect salmonella types excreted by the consuming population?

(3) Based on the number of identical salmonella types isolated weekly from chicken and from the sewers, and based also on the number of corresponding weeks during which the same salmonella types were isolated from chicken carcases and sewers, is there any evidence of an association between salmonella types recovered from chicken and those detected in the sewers?

(4) Are the salmonella serotypes/phage types obtained from chicken or the sewer the same as those reported in concurrent poultry-associated food poisoning incidents in Scotland?

(5) What criteria for establishing an epidemiological association between poultry meat and human salmonella infections (as determined by sewer swabs) have been satisfied by the survey?

(6) Do the proportion of contamination of chicken carcases and sewage by salmonella serotypes vary by season? If so, does this relate or correspond to the seasonal variation reported for salmonellosis outbreaks?

2.4 A MATCHED CASE-CONTROL STUDY OF POULTRY MEATBORNE SALMONELLA INFECTIONS:

In many household (family) outbreaks of salmonellosis in which the food vehicles are not readily identifiable, and for sporadic cases which constitute the majority of recorded

incidents (81,82, 84), little epidemiological investigation is carried out to trace and establish the food source. It has been observed that a necessary move in the control of foodborne salmonellosis should be to define and clarify the source of sporadic cases (113). The variations in the incidence of meat-borne salmonella infections in different countries have been attributed, in part, to differences and changes in the relative proportions of meat from different animals consumed (15, 76). There have been considerable changes in the eating habits over the years. "Fast food" outlets have expanded; the sale of pre-cooked poultry meat (whole or portions) is common place; while the advent of frozen chicken has led to widespread distribution of a contaminated product (83). Often at times, however, the implication of poultry meat in salmonellosis incidents is based on circumstantial epidemiological evidence. In most outbreak and sporadic incidents in which a poultry source is incriminated or established, insufficient epidemiological information is available on the form of the meat (that is, pre-cooked, fresh or frozen) or the methods of preparation (boiling, roasting, grilling) which may be important contributory factors. Reports and studies by Bryan (88), Collier *et al* (82), and Roberts (89) suggest that inadequate thawing of frozen chicken, undercooking, and cross-contamination of pre-cooked meat are among the major contributory factors.

Epidemiological information on the source of infection and on the food practices is based on food histories obtained from affected persons. When laboratory examination of the implicated food is inadequate, unavailable, or impossible, the analysis of food history questionnaires remains the only data with which to link a food item to an illness (159). One epidemiological approach to examine and clarify the association between poultry meat and human salmonella infections is a Matched Neighbourhood Case-Control Study of household and sporadic cases, based on a food history questionnaire. There is need to show if there is any evidence of an association between the type of meat (chicken, beef, pork, lamb) consumed within 48 hours prior

to illness and the development of salmonellosis. Is there a significant relationship or association between the form of chicken (pre-cooked, fresh, frozen) eaten 48 hours prior to illness and the risk of salmonella infection? Does the method of cooking poultry meat make any significant difference in the risk of poultry meat-borne salmonellosis? Is there an association between the frequency of poultry consumption in a week and the risk of salmonella infection?

The specific aims of the Case-Control Study are to test the following null hypotheses:

(1) There is no relationship between the type of meat (chicken, beef, pork or lamb) consumed 48 hours prior to illness and the development of salmonellosis;

(2) there is no association between the consumption of poultry meat within 48 hours prior to illness and salmonella infection. Consumption of poultry meat is not a significant risk factor.

(3) there is no association between the **form** of chicken (pre-cooked, fresh or frozen) eaten 48 hours prior to illness and the risk of salmonella infection;

(4) there is no significant difference in the risk of poultry meat-borne salmonellosis by four methods of cooking chicken (that is, boiling, roasting, grilling and frying);

(5) there is no association between the number of times in an average week that chicken is consumed and the risk of poultry meat-borne salmonellosis.

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

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3.1 RETROSPECTIVE STUDY:

3.1.1 Sources of Data:

Sporadic and outbreak incidents of foodborne infections reported to the Scottish National Coordinating Centre (the CD(S)U) from January 1968 to December 1987 and extracted from routine records were reviewed and analysed for the relevant epidemiological data. Only infections and outbreaks accepted by the CD(S)U to be foodborne were used in the analysis. Episodes of non-foodborne infections, such as those recorded as direct person-to-person spread, or following direct contact with infected pets or farm livestock when included in the study were only to enable the calculation of proportions of foodborne incidents.

The two main sources of data which routinely contribute to the national surveillance programme in Scotland were used. These are

(i) records of all reports from the diagnostic laboratories; that is, hospital, veterinary, university and reference laboratories which send routine notifications of salmonella isolations to the national coordinating centre. The data on laboratory isolations, available in official Weekly and Annual publications **(160)** were sampled for the retrospective epidemiological analysis of **salmonella infections.**

(ii) records of **foodborne outbreaks** reported from the Health Boards (specifically, the CMS or the EHOs) and available at the CD(S)U as Outbreak Investigation and Outbreak (Summary) Report Forms (Appendix I and II).

Formal documentation of comprehensive data on foodborne outbreaks recorded as General and Household (Family) Outbreaks under the WHO Surveillance Programme became available from 1980. Relevant data on outbreaks were abstracted personally from the existing records for the retrospective study of salmonella outbreaks.

3.1.2 Validation of Records/Data:

Two approaches were employed for the validation of routine records and data collated at the CD(S)U under the WHO Surveillance Programme for foodborne salmonellosis. These were in accordance with the methods for retrospective validation of health registers described by Stone **(155)**.

(i) Case Validity:

Validation of cases of human salmonella infections was based on ascertainment by the various diagnostic laboratories and confirmation by the Scottish Salmonella Reference Laboratory (SSRL).

Diagnostic laboratories routinely make weekly reports of salmonella isolations to the CD(S)U. The laboratories also routinely submit isolated cultures to the SSRL for confirmatory serotyping and phage-typing. Since the SSRL in turn submits routine weekly reports to the CD(S)U, the SSRL can serve as an authoritative "external source of data" on salmonella infections.

The "screening method" was employed in case validity analysis. This method assumes that for analytical purposes, the "external data source" represents the "truth" **(155)**. Thus, if the Weekly/Annual Reports of the CD(S)U may be regarded as bearing the same designation as a "screening test", the official Register of isolations at the SSRL may serve as the "reference test". The validity of CD(S)U records may be assessed in the same way as a screening test by calculating the sensitivity (a/(a + c)) **(155)**. That is,

the proportion of salmonella infections (SSRL isolations) that are correctly recorded by the CD(S)U.

The sampling method for "cases" (confirmed salmonella infections) was as follows: A simple random sample (without replacement) of the years 1970 to 1988 was made until 10 years were selected. From the selected years, a 1 in 10 systematic sample of human cases was made from the SSRL Register of salmonella isolations until a sample size of 1500 was selected. The case validity (specified salmonella serotype) of the random sample was tested by the screening method.

(ii) Item Validity: For validation of recorded items, the following sampling methods were employed:

(a) Using the SSRL as the reliable external source, the systematic sample selected from the SSRL Register for case validity was also used for item validity. The appropriate Laboratory Report Forms submitted by the SSRL to the CD(S)U were traced from the files and for each selected case, information on the Form on the following items were examined: reference code of the original laboratory/hospital, name, address of patient, sex and age. These items were then compared with entries in the SSRL Register. Proportions of entries traceable were determined and the concurrent validity (or concordance) of the two records was calculated. The same item from the SSRL Register (with the exception of name and address) were also compared with the information recorded in the CD(S)U Weekly Report.

(b) A one in 5 systematic sample of salmonella infections listed in the CD(S)U Weekly Reports **(160)** between 1968 and 1987 inclusive was selected. For each case, an attempt was made to use the specified week/year and the reference code of the diagnostic laboratory to trace and examine the Laboratory Report Form from which the following items were abstracted: diagnostic

laboratory, sex, age, and salmonella serotype. The items in the Forms were compared with the same items in the Weekly Reports. Proportions of cases traced were assessed and the concurrent validity of items in the two sources was calculated.

(c) For the validation of the accuracy and completeness of data on foodborne outbreaks, no random samples were taken. Instead, all the General and Household Outbreaks listed in CD(S)U records (files) between 1980 and 1987 were included. For each outbreak, the Outbreak (Summary) Form sent from the health boards (the CMS), and, where available, the Outbreak Investigation Form (from EHOs) were examined for the following epidemiological data: date, health board area, local government district, type of outbreak, number of persons affected, number ill, number hospitalized, the suspected food item, place of consumption of suspected food, and the causative agent. These items were compared with the same data on the CD(S)U records of outbreaks. The proportion of foodborne outbreaks traced was determined and the validity or concordance of the two records was again established.

3.1.3 The Retrospective Study: Sampling Procedures:

Salmonella Infections: The national co-ordinating centre of the WHO Surveillance Programme for Foodborne Infections (the CD(S)U) has recorded nearly 30,000 notified cases of human salmonella infections in Scotland between 1968 and 1987 (160). Owing to the large volume of cases and in consideration of the limited time available for this aspect of the thesis, it was not possible to include all the recorded cases in the retrospective epidemiological study. Only a random sample of the human cases could be analysed. A 1 in 5 systematic sample of all human salmonella infections listed in the CD(S)U Weekly Reports (160) for the 20-year period was selected and validated as described. The

statistical justification for choosing a one in 5 sample size is arguable on the basis of:

(i) 95% Confidence Interval or level of significance. The critical value for detecting statistically significant smallest difference in mean incidence of salmonella infections between two consecutive 5-year periods is denoted by α , and is specified as α = 0.05. If calculated probability (P), of detecting 5 per cent differences in the mean crude cumulative incidence or standardized age-specific incidence between two periods, is equal or less than ($p < 0.05$), the null hypothesis (no difference in salmonella incidence) would be rejected.

(ii) 80% Power. The probability of accepting the null hypothesis when in fact it is not true; that is, the probability of not detecting a significant difference in salmonella incidence between two time periods is denoted by B and is arbitrarily specified at 0.2 (β = 0.2). Thus, the power of the test sample is one minus the probability of a Type II error ($1-\beta$); that is 1-0.2 or 80 per cent.

From the systematic sample, the numbers and proportions of salmonella infections during consecutive 5-year periods (1968-72, 1973-77 etc) were determined and the differences in incidence rates were calculated. The aim was to be able to estimate, from the sample, the true difference (d_0) between the true incidence rate (πa) of salmonella infections during a 5-year period, and the true incidence rate during the preceding or succeeding 5-year period (πc). Any observed difference was an estimate of the true difference in infection rate between periods. The probability of detecting an observed difference in incidence of 5% from the random sample was calculated on 80% Power and at 95% level of significance. If the difference actually observed has a very small probability ($p < 0.05$) of occurring by chance, then the null hypothesis is rejected. Thus from the one in 5 random sample, I would need to be

able to determine that the incidence of human salmonella infections was increasing or decreasing at 5-year periods.

Salmonella Outbreaks: Formal records and more comprehensive epidemiological data on **outbreaks** of foodborne infections and intoxications in Scotland have been available at the CD(S)U only since 1980. In view of the smaller number of outbreaks as compared to the number of salmonella cases (infections), no samples were selected for the retrospective study of foodborne outbreaks. Rather, all the General and Household Outbreaks confirmed and recorded on a yearly basis at CD(S)U Outbreak Files were included in the study.

3.1.4 Analysis and Presentation of Data:

Descriptive epidemiological data on foodborne salmonellosis in Scotland are first presented. Abstracted data were computer-analysed (SPSS X package), summarized on annual basis and for the overall 20-year period. The summaries are presented in appropriate tables, graphs, histograms, or pie charts. The numbers and proportions of foodborne outbreaks due to **salmonella** organisms were calculated for each year, and changes or differences in the incidence of salmonella outbreaks from 1980 to 1987 were determined. Crude incidence rates of salmonella infections per 100,000 persons were next calculated at 5-year periods from 1968 to 1987. The 1961, 1971 and 1981 national population Censuses for Scotland as well as the sex and age-group distributions of the population in the censuses, were used as **population standards.** Standardization of the annual populations from 1968 to 1970 and from 1972 to 1980 was made by interpolations in-between the 1961 and 1971 or 1971 and 1981 censuses. For 1982 to 1987, the official estimates of the population for each year served as the standards. Changes in incidence rates of salmonella infections at the 5-year periods were calculated; this enabled any trends in incidence rates to be assessed.

The incidence rates of salmonella infections were standardized for sex and age (5-year bands). Changes in sex- and age-specific incidence rates between 5-year periods

were analysed for any trends. Evidence of significant difference (at least 5%) in age-specific incidence and of any association between age-specific incidence and time periods was tested by the standard chi-square analysis. These analyses provided answers to objectives 1, 2 and 4.

Numbers of salmonella outbreaks associated with poultry and other types of meat (red meat) were calculated for each year from 1980 to 1987. Relative percentage annual changes in meat-specific salmonella outbreaks were determined. Evidence of significant difference in the distribution of meat-specific incidence of salmonella outbreaks, was determined by the t-test method. This calculation provided an answer to Objective 3.

For the overall 20-year span and for each of the 5-year periods, the 10 most commonly isolated salmonella serotypes were set in a ranking order of frequency. The incidence trends of each of the 5 most common salmonella serotypes at the 5-year periods were determined, and any trends were tested for evidence of significant difference by the chi-square analysis. This enabled the achievement of Objective 5.

The relative frequencies and proportions of General Outbreaks and Household Outbreaks between 1980 and 1987 were calculated; so also were the relative frequencies and proportions of salmonella outbreaks associated with health institutions, educational institutions, commercial catering establishments (restaurants, hotels), work place, farm house, social outing, or private household. These calculations provided the answers to objectives 6 and 7. The proportions of salmonella outbreaks reported from each of the Health Boards in Scotland over the entire study period were determined. The mean proportion of salmonella outbreaks reported by the various health board areas over the 8-year period, and the ranges of the means from the national averages were determined.

Annual seasonal incidence of infections and outbreaks were determined by pooling the numbers and proportions per quarter (Jan-March, April-June etc) per year. Seasonal trend was assessed by calculating the number of infections per quarter for each of the 5-year periods. Statistical evidence of seasonal trend was tested by the chi-square analysis. This analysis provided the answer to Objective 9.

3.2 BACTERIOLOGICAL SURVEY: EPIDEMIOLOGICAL RELATIONSHIP OF SALMONELLAE ISOLATED FROM CHICKEN CARCASES AND SEWER SWABS:

3.2.1 The Study Institution:

A large long-stay psychiatric hospital in the Greater Glasgow Health Board area was selected for the study. Weekly batches of chicken carcases supplied to the kitchen of the hospital were sampled for salmonellae. Salmonella excretion among the patients was monitored by parallel survey of the sewers draining the residential wards of the patients. A "closed" long-stay residential institution was selected in order to exclude the possibility of salmonellae from effluents of industries and retail shops, or from sewers of other residential areas. The patients in the hospital (approximately 800) constituted a cohort whose latent infection or transient carriage and excretion of salmonellae was monitored by the Moore's sewer swab method (161, 162).

3.2.2 Chicken Carcase Survey:

The swabbing of the surface area of dressed chicken carcases and the collection of pericloacal skin were, for some time, the traditional methods for the detection of salmonella carriers (163-166). However, the uneven distribution and the low numbers of salmonella usually present on the chicken carcase led to the introduction of the whole-carcase rinsing as a more sensitive alternative sampling procedure for the detection of salmonella from the raw product (166-169). The recovery of contaminating salmonella from whole carcase

rinse fluids rather than from thaw or defrosted fluid has also been demonstrated to result in a significantly improved isolation rate (166). The whole carcase rinse technique has become the standard method employed by workers in North America, Australia, and the United Kingdom (164-173). Among the various workers, there is a range of the type and volume of rinse fluids used. Cox, Thomas and Bailey (168) used 100 ml of distilled water; D'Aoust, Stotland and Boville (166) used 1 litre of nutrient broth; while Mann and McNabb (171) used 1 litre of peptone water. The International Commission on Microbiological Specifications for Food (174) recommended rinsing a chicken carcase with 300 ml of lactose broth in a plastic bag, and adding an additional 300 ml of double-strength lactose to promote growth of salmonella.

In the present survey, batches of fresh and frozen eviscerated chicken delivered on contract to the hospital kitchen, (by a commercial supplier) were sampled at weekly intervals. By the EEC labels on some of the batches, it was determined that the chickens originated from different poultry producers in Scotland and England. A one-in-ten systematic sample (n = 10 to 17 carcases) were examined from each weekly batch, by the whole carcase rinse method. The survey was designed to last for 52 weeks. However, after 43 weeks, the supply of raw dressed chicken carcases to the hospital was terminated; pre-cooked de-boned whole-chickens vacuum-packed in plastic packets were substituted. The change in policy coincided with the general media publicity given to the problem of salmonella in poultry products. Over the 43-week period (2nd February to 5th December 1988) samples were actually taken for 38 weeks. For the other five weeks no chickens were cooked in the hospital either because of public holidays or an inability to supply the product. A total of four hundred and seventy-seven (477) fresh and frozen chicken carcases were examined during the 38 weeks that samples were taken.

Each carcase was placed in a polythene bag and 300 ml of sterile buffered peptone water (pre-enrichment broth) in a plastic container was carefully poured onto the carcase.

About a third of the fluid was allowed into the cavity of the eviscerated carcase. The bag was sealed and shaken back and forth in such a manner as to make the rinse fluid pass over and through the carcase, for one minute. Whilst still sealed in the bag, the carcase was rubbed by hand over the bag to thoroughly rinse the external surface. The carcase was lifted from the fluid in the bag, drained for 15 to 20 seconds, then removed. The rinse fluid was carefully transferred back into the plastic container. A separate pair of disposable gloves was worn for each carcase rinsing. The specimens were transported to the Scottish Salmonella Reference Laboratory within two hours of sampling.

3.2.3 Pre-Cooked Chicken Samples:

In order to compare the incidence of salmonella in the pre-cooked chicken with the incidence in raw whole carcases and also with that of the sewers during both periods, the newly introduced cooked meat was equally sampled. The pre-cooked whole carcase de-boned chickens were supplied in frozen vacuum-packs, containing one de-boned chicken. A one-in-ten sample of the packs was examined each week. About one gram of meat was aseptically collected from each pack, using sterile scissor and forceps. The piece of meat was aseptically cut into small portions and placed into 10 ml of sterile buffered peptone water. Random samples were taken for five weeks and a total of 102 packs were examined before the project was terminated.

3.2.4 Sewer Survey:

The use of the Moore's gauze swabs **(161)** in drains or sewers, to identify the route by which salmonellae are transferred, from source to the human host, has been reviewed **(162, 175)**. The principle of the sewer swab method is that continuous sampling of sewage passing through in a particular sewer should be a more sensitive index of the passage of enteric organisms than the examination of bulk samples of sewage taken at times that could not always be related to the dietary habits of the potential excretors

(162). The length of exposure of the Moore's swab to sewage may vary from mere wiping the swab along the sewer surface, to leaving the swab in the flow for 48 hours or up to 7 days (175-178). Sewer swabbing has been described as a useful means of salmonella surveillance (162). Correlation in time and place has been demonstrated, by the sewer swab technique, between occurrence of salmonella serotypes in effluents of abattoirs, meat processing plants, butcher's premises and incidents of human salmonella infections (175-178). Sewer swabbing has been used to demonstrate the frequent entry of salmonella serotypes into households in a residential area (175), as well as to monitor the salmonellae excreted in sewage, in latent, non-clinical infections in a restricted residential area (179).

In the present survey, Moore's swabs were laid at two man-holes (A & B) draining the residential wards of the hospital patients. The two man-holes were located **proximal** to the entry of the kitchen effluent into the hospital sewer (**Figure 2.1**). This eliminated the possibility of salmonellae from the kitchen effluents entering and compounding the results. Each sewer swab was left in place for 7 days; a replacement swab was placed every week, while a contaminated swab was collected into 300 ml of sterile buffered peptone water. Moore's swabs were laid over a 45 week period. A total of 79 swabs were examined for the 40 **weeks** during which chicken carcases were also sampled. One of the swabs was lost in the sewer flow. Following the introduction of pre-cooked chicken, sewer swabs were taken for a further 5 weeks, during which a total of 10 swabs were examined.

3.2.5 Isolation and Identification of Salmonellae:

For the chicken carcase rinse fluids, the pre-cooked meat, and the sewer swabs, the buffered peptone water pre-enrichment was incubated at 37^{o}C for up to 48 hours. The whole sewer swab was incubated in the pre-enrichment, as the large inoculum has been shown to enhance salmonella isolation (180). Subcultures were made from the pre-enriched

peptone water into enrichment media - after 18-24 hours, and again after 42-48 hours. The enrichment procedure was based on standard methods, and was carried out in Selenite F broth and modified Rappaport-Vassiliadis (RV) medium. The RV enrichment had been shown to be superior in the isolation of salmonellae from naturally contaminated meat products (181, 182). Both Selenite F broth and RV were used in the present study for comparative purposes and to maximize the recovery of salmonellae from the various sources. One-fifth millilitre (0.2 ml) of pre-enriched peptone water was sub-cultured into 10 ml Selenite F broth which was then incubated at 37°C. One-tenth millilitre (0.1 ml) of the same pre-enriched peptone water was transferred into 10 ml of the RV medium and the RV was incubated at 43°C. Prolonged incubation of the two enrichment media was allowed in order to increase the rate of salmonella isolation (15). Subcultures from each enrichment were made at 24 hours and 48 hours, onto three selective and differential solid media - desoxycholate citrate agar (DCA), xylose-lysine-desoxycholate agar (XLD), and brilliant green agar (BG). The above protocol was based on work carried out previously at the Scottish Salmonella Reference laboratory (183). The selective agar plates, incubated at 37°C, were examined at 24 and 48 hours, and colonies typical of salmonella were carefully picked and subcultured onto MacConkey agar for purity. Purified isolates were then characterized, using the SSRL standard biochemical protocol. This included reactions in urea agar, glucose, lactose, sucrose, mannitol, tryptone water (for indole test), triple sugar iron agar, dulcitol, lysine and citrate media (118, 119, 183). Each isolate was then serotyped by standard methods (118, 121, 122) as modified by SSRL. Since each carcase rinse fluid and sewer swab may be potentially contaminated with more than one salmonella serotype (168, 172, 175), the search for multiple serotypes was achieved by picking five typical colonies from each selective agar plate. Cultures of all salmonella serotypes isolated and identified by the author each week were confirmed by SSRL using an automated modification of the scheme cited.

Routine antimicrobial sensitivity tests of all the salmonella strains were also carried out by the SSRL. Phage-typing of *S.typhimurium* and *S.enteritidis* isolates was performed by the SSRL, according to standard methods; however, early in the course of the study isolates of *S.enteritidis* were sent to the Division of Enteric Pathogens, Public Health Laboratory Services, Colindale, London for phage typing. Computer-based records of all the salmonella isolates are maintained at the SSRL and all the isolates have been taken into the culture bank at the SSRL.

3.2.6 Analysis of Data:

Proportions of positive salmonella isolations from the chicken carcases and the sewer swabs were calculated. The frequencies of salmonella types recovered from both sources were determined. Salmonella types recovered from chicken carcases and from the sewer during corresponding or matching weeks are presented in appropriate tables. The corresponding weeks during which the same salmonella types were recovered from both chicken carcases and sewer swabs are also listed in appropriate tables. This gave an indication of the frequency of detecting in the sewage, the same salmonella types isolated from chicken during the preceding week. Using the epidemiological markers of serotypes and phage types, an attempt was made to establish the similarity of salmonellae from chicken carcases and those from the sewer - and hence, the association between contaminated poultry meat and human salmonella infection (Objective 3). Statistical tests for evidence and strength of this association were based on (i) the observed and expected numbers of identical salmonella serotypes/phage types detected in chicken and in sewers; and (ii) the observed and expected numbers of corresponding weeks during which the same salmonella types were isolated from chicken and sewer. The standard chi-square analysis was performed.

Assessment of the epidemiological association between poultry meat and human salmonellosis was also made using, as an epidemiological marker, the antimicrobial sensitivity

patterns of the salmonella serotypes obtained from chicken carcases and the sewer.

The frequencies or ranking order of 10 most commonly isolated salmonella types from chicken and from sewer were compared with those of salmonellae reported to CD(S)U under the WHO Surveillance Programme. The ranking orders were also compared with notifications in 1988 of Poultry Salmonelllae, under the Zoonoses Order. The comparison enabled the establishment of epidemiological association based on the criteria of consistency and plausibility.

3.3 MATCHED CASE-CONTROL STUDY OF POULTRY MEAT-BORNE SALMONELLA INFECTIONS:

3.3.1 Problems Encountered with Earlier Approaches for Case-Control Questionnaire Study of Foodborne Salmonellosis:

Different approaches were designed for the conduct of a matched case-control study to test the hypothesis that sporadic and household (family) incidents of human salmonella infections are associated with consumption of poultry meat. Before a feasible approach was finally designed and successfully carried out, three alternative methods were attempted but abandoned, because serious problems in identifying significant numbers of cases as well as insurmountable difficulties in accessing matched controls were encountered.

(a) Case-Control Study with Matched Neighbourhood Controls Nominated by Cases:

After a standard self-completing questionnaire had been developed, a pilot study was conducted in two local districts in Scotland (City of Glasgow and Lothian districts) through the assistance of the respective Department of Environmental Health. Questionnaires were administered to Cases by the EHOs, during their routine investigation of reported food poisoning incidents. Each

case was asked in the questionnaire to nominate three persons of the same sex and approximately the same age who live in their neighbourhoods, and who had not suffered known diarrhoeal illness during the past month, for use as matched neighbourhood controls. In more than 80 per cent of the 30 questionnaires returned, the question on nominated controls was left blank. It was apparent, from some of the comments made, that the respondents were unable, unwilling, or reluctant to volunteer names. It would appear that the "case-nominated controls" approach raised ethical, legal and social problems. Many respondents who saw the question as an invasion of privacy, suggested they needed first to obtain the consent of their neighbours before they could nominate them! It became obvious from the pilot study, that it would be unproductive, if not impossible, to access case-nominated matched controls. Consequently, this approach to the Case-Control Study was abandoned. It must be pointed out, though, that the nominated neighbourhood control method was said to have been used in a recent unpublished study of national outbreaks of salmonellosis in England (185). However, Coyle et al (186) encountered "insuperable difficulties" in identifying and accessing matched controls when this approach was used in a household matched case-control study of egg-borne salmonellosis in England; they abandoned the approach.

(b) Hospital-based Case-Control Study:

An hospital-based case-control study was suggested as an alternative approach. The study was designed as follows: all cases of hospital-diagnosed foodborne salmonellosis admitted to a network of hospitals in the Glasgow district were to be ascertained during a period of 12 months. Both in-patient and out-patient services were to be selected for the study from the hospital Admissions and Discharge records. At least two controls matched for sex, age and hospital of admission were to be selected per case, from patients admitted for conditions other than foodborne illness.

It soon became obvious that this approach to the case-control study was prone to serious statistical, practicality and logistic difficulties. The definition and ascertainment of many hospital based foodborne incidents are uncertain, because in many instances, the diagnosis recorded is "clinical". By the time the laboratory results of some "clinical cases" are returned to the hospitals, the patients, particularly the outpatients, had left the hospital. For such patients who are no longer hospital-based, accessing the cases and identifying appropriate hospital-based matched controls can present impossible logistic problems.

A significant proportion of victims of foodborne infections present to the GPs rather than at hospitals, and many such patients are never referred to the hospitals, if the illness is not serious enough. Certainly, all such cases would be missing or omitted in any hospital-based case-control study. The consequence is that the number of laboratory-ascertained, hospital-based cases, cannot be a representative sample of all cases of foodborne salmonellosis in the target population. To check this assumption, information on the numbers of salmonella cases based in a network of hospitals in the Glasgow district was requested and obtained for a 3-month period in 1988. A computer print-out of notified salmonella infections for the same period was also obtained at the CD(S)U. By comparison, the hospital-based cases represented less than 25 per cent of the notified cases! One retrospective analysis of poultry-associated salmonella outbreaks in Scotland from 1980 to 1985 also showed that out of 2245 persons affected in 224 outbreaks, only 472 or 21% were hospitalized (83). From statistical stand point, therefore, hospital-based cases do not seem to constitute a significant sample size for a case-control study.

There is also an imputed lesser validity of hospital controls as compared with population-based controls. The use of hospital-based controls leaves room for doubt whether the procedure for the selection process is as representative

as a random selection from all potentially eligible controls in the target population (187). Thus, persons on admission in hospitals are served about the same hospital meals. In a case-control study of foodborne illness, where the primary exposure variable under study is the consumption of poultry meat, a series of hospital-based controls seem unlikely to produce reasonable categories of controls exposed and unexposed to the study exposure. This could lead to a biased estimate of the relative risk and odds ratio.

There are serious logistic problems in identifying and accessing sufficient numbers of non-household matched controls. Coordination of the entire protocol of administering questionnaires, identifying and accessing matched controls, retrieval of completed questionnaires - all simultaneously from the network of hospitals in the district - would prove practically difficult, given the time available to the author for both the case-control study and the bacteriologic survey.

In consideration of all the above factors, the idea of an hospital-based case-control study was abandoned.

(c) Neighbourhood Matched Controls Selected from Voluntary Population Survey:

An alternative population-based matched case-control study was next designed, to cover the entire Strathclyde Region. Under this approach, matched controls would be selected weekly as the cases occurred and as case-completed questionnaires are returned. Controls would be matched for sex, age, and neighbourhood with cases. Ascertained neighbourhood control series would be selected by using the Voluntary Population Survey conducted by the Strathclyde Regional Council. From the survey registers, appropriate council officials would produce lists of persons matched for sex, age and neighbourhood. From these lists, controls would be randomly selected. The method of selection within a neighbourhood would be by a simple one-in-n random sample from the generated lists of matched controls; that is, every

nth name on the list would be picked until the specified number of controls per case have been selected. Letters would be sent seeking the consent of each of the selected controls to participate in the study. The standard questionnaire would then be mailed to consenting control subjects, with the hope of getting at least **two** matched controls per case.

The cooperation and assistance of certain officials in the Chief Executive's Department, Strathclyde Regional Council, in the selection and accessing of the control series, was solicited and was promised. However, final approval for participation by the Council and for the use of the Voluntary Population Survey, was being expected from most senior officials in the Chief Executive's Department. Regular contacts were maintained with the Regional Council official concerned, both by the telephone and by personal visits. All along, indications were given that the arrangement for the case-control study would be approved.

As it turned out, after several months of waiting, the Voluntary Population Survey could not be used for the study; approval could not be granted, and assistance from the Council official could no longer be expected! Something about confidentiality of the survey records was mooted. Thus, while the use of the Voluntary Population Survey seemed a most appropriate sample frame for identifying and accessing matched control groups, this approach too had to be abandoned - after considerable loss of time. The following case-control study design was eventually successfully carried out.

3.3.2 Case-Control Study with Household (Neighbourhood) Matched Controls Accessed from Electoral Registers:

A Introduction:

The Case-Control approach allows an estimation of the effects (odds) of exposure to poultry meat on the risk of salmonella infection. Individuals who suffered foodborne

salmonellosis (cases) were selected for comparison with a series of individuals in which the illness was absent (controls). Cases and controls were compared with respect to past exposures (consumption of poultry meat) believed to be associated with the development of the illness under study. This would provide an answer to the question whether the proportion or ratio of salmonella cases who ate poultry meat is in excess or higher than would be expected. The control group provided an estimate of the frequency or proportion of exposure expected among individuals free of the illness.

B Concept of Matching:

Matching in the sense used for case-control studies refers to the pairing of one or more controls to each case on the basis of their "similarity" with respect to specified variables **(189)**. If it can be shown that the cases and the controls are similar on the matching variables (age, sex, location of household etc), their differences with respect to development of the illness (salmonella infection) can be attributed to some other risk factors or exposure variables (for example poultry meat). A significant difference between cases and controls with respect to the exposure variable under study (consumption of poultry meat), would suggest an association of the variable with the illness. This association cannot, therefore, be explained by or attributed to case-control difference on any of the matching variables. In other words, differences of age, sex, location of household etc cannot be said to account for, or be the underlying factor for, the observed association of the study exposure and the illness. The purpose and value of matching, therefore, is the elimination of biased comparisons of cases and controls.

C Definition of Exposure:

The overall exposure variable analysed between cases and controls is the **type** of meat consumed 48 hours prior to

onset of the illness. In the context of the case-control study, therefore, **exposure** is defined specifically as consumption of poultry meat (chicken or turkey) within 48 hours prior to the onset of foodborne salmonellosis, or during the previous 48 hours (for controls). A number of related variables are believed to modify, influence or actually account for the apparent effect of an exposure to poultry meat. These **contributory risk factors** (or are they "confounding variables"?) include the nature or **form** of the poultry meat purchased (that is, whether pre-cooked, fresh or frozen), and the **method of cooking** the poultry meat (boiling, roasting, grilling, or frying). Inadequate thawing of frozen chicken/turkey, undercooking, and cross-contamination of pre-cooked meat have been suggested as the major factors contributing to foodborne salmonella infection **(82, 88, 89)**. These other variables are intrinsically associated with the primary exposure under study; they exist as a consequence or part of the study exposure. To the extent that the association of these potential risk factors with the illness cannot occur in the **absence of exposure** to the poultry meat, these other variables do not fit into the classical definition of a confounder **(187)**. They are treated as contributory risk factors rather than as confounding variables.

An attempt was made to control for the effects of these contributory variables between cases and controls. Consequently, with respect to the **form of meat**, exposure is defined as consumption of pre-cooked, fresh or frozen poultry meat within 48 hours prior to illness. On the **method of cooking**, exposure is defined as consumption of boiled, roasted, grilled or fried poultry meat 48 hours prior to salmonella infection. Exposures to pre-cooked, fresh and frozen poultry meat were analysed separately to determine the effect of each form of meat on the odds of illness in both cases and controls. Similarly, exposures to boiled, roasted, grilled and fried poultry meat were analysed separately. So also, were the frequencies or number of days (0, 1-2, 3-4, etc) in an average week that poultry meat is consumed.

D Design, Validation/Standardization of Study Questionnaires:

A questionnaire designed to elicit epidemiological information on food history - specifically on meat consumption practices, was developed. The first section of the questionnaire related to information on the personal data of the respondent - residential address, postcode, sex, age, whether the respondent worked in a meat-related establishment (restaurant, abattoir, meat processing plant, meat market, or meat retail shop); whether the respondent is a vegetarian, and any type of meat not eaten on account of religion, culture or other personal reasons; whether the respondent returned within the week from travel outside the United Kingdom. The main part of the questionnaire contained specific questions pertaining to consumption of meat:

(i) whether any meat was consumed during the 48 hours before the onset of symptoms of food poisoning (or during the past 48 hours, for the Controls);

(ii) the type(s) of meat consumed during the 48 hours; respondents were asked to tick from a list of beef, chicken, lamb, pork, and none of the above;

(iii) the form of the meat at the time it was purchased (whether **precooked**, **fresh** or **frozen**);

(iv) the method of cooking the meat consumed (boiling, roasting, grilling or frying);

(v) the number of times in an average week that poultry meat is consumed; respondents were requested to tick one from a list of four frequencies.

Another section of the questionnaire sought information on the respondents' history of foodborne illness. Respondents were requested to tick "yes" or "no" to each of the following questions: if during the past **one week**, they had

symptoms of diarrhoea (watery faeces), stomach pain or cramp, fever and vomiting which the respondent thought could be due to food poisoning; whether the symptoms were serious enough to report to the GP or the hospital; whether it was confirmed that the foodborne infection was caused by **salmonella**; and whether any members of the family (household) or other known persons in the neighbourhood were affected by the same episode of food poisoning. In general, the questionnaire was designed to contain no "open-ended" questions; this was to avoid responses or comments that would be difficult to quantify or compare.

The questionnaire was validated in a series of pilot studies. The purpose was to amend, delete, modify or otherwise clarify any questions that respondents could not supply the desired responses, because they found such questions to be vague, illogical, ambiguous, too personal, or an invasion of privacy.

(a) The draft questionnaire was first of all given to individuals randomly picked from among students, lecturers, office workers, shop keepers, housewives and a few hospital patients. A total of thirty questionnaires were completed and handed back by these classes of individuals.

(b) The questionnaire was then corrected and more formal pilot studies were carried out. Through the assistance of some officials of the Department of Environmental Health in Lothian and Glasgow Districts, the questionnaire was administered (given and collected) by EHOs during their routine investigation of reported food poisoning incidents. The questionnaire was administered to the cases in the two districts for a trial period of two months. It was after this particular pilot study that the approach of selecting case-nominated controls was abandoned.

(c) A random sample (n = 50) of "controls" was selected from the Glasgow district Electoral Register, to whom the questionnaire was sent by post. Thirty-eight or 76% of the "controls" duly completed and returned the questionnaire.

From the pilot studies, a standard questionnaire was finally developed (Appendix III) and this was used in the substantive case-control study.

E Definition and Accessing of Cases:

All primary household cases of foodborne salmonellosis and sporadic cases investigated by the EHOs or otherwise reported and occurring within the City of Glasgow local district between April 1988 and March 1989 constituted the cases. Clinical salmonellosis is an acute gastroenteritis or enterocolitis with an incubation period of 6 to 48 hours and characterized by fever, abdominal pain, diarrhoea, and vomiting (15). In the context of the population-based case-control study, a primary household case is a patient who was the first or the only person in a household (family) outbreak to have had (reported) gastrointestinal symptoms following the consumption of food considered contaminated on the basis of epidemiological evidence or laboratory analysis, who was not part of a point source general community outbreak, and from whose faecal culture, salmonella serotype was isolated. A sporadic case is one case which, as far as was ascertained, was unrelated to any other cases in respect to consumption of the incriminated food, and from whom salmonella was isolated. Household and most sporadic incidents of food poisoning reported to the GPs, hospitals, or laboratories were routinely investigated by the EHOs, under the WHO Surveillance Programme for Foodborne Infections and Intoxications in Scotland. Cases were followed up immediately as they occurred and were reported and they were accessed through the assistance and cooperation of the EHOs.

By an official arrangement with the Department of Environmental Health, batches of the study questionnaire were issued to the officers in the seven geographical Divisions of the Glasgow District. The self-completing questionnaires were administered to the primary cases by the EHOs in course of their routine investigations of reported incidents of foodborne infections.

A brief letter of explanation accompanying each questionnaire solicited the consent of the primary cases to participate in the study, and requested the respondents to return completed questionnaires to the Department of Community Medicine, University of Glasgow in the enclosed postage-paid self-addressed envelopes. For primary cases who were below 11 years, the questionnaires were administered to surrogate respondents (usually parents or guardians), who were requested to complete and return them on behalf of their wards. The EHOs were requested to encourage the respondents to complete and return the questionnaire as soon as possible. Each EHO was also requested to maintain a list of cases to whom questionnaires were given and to submit weekly or monthly returns to the office of the Deputy Director of the Department of Environmental Health, from whom the list could be retrieved periodically. This extra workload proved rather difficult to sustain uniformly and had to be abandoned. Since routine weekly returns of outbreak incidents and laboratory-ascertained sporadic cases are submitted to the CD(S)U under the WHO Surveillance Programme, the computer-based records at the CD(S)U constituted the base-population of cases within the District, and proved very effective for cross-checking and establishing the cases who had not returned completed questionnaires. At the end of each month, reminder letters were sent to those cases for the month, who had not responded.

For purposes of analysis of responses, a primary household or sporadic case was NOT included if:

(i) there was no evidence from the CD(S)U or SSRL records that the particular foodborne incident was laboratory-ascertained as salmonella;

(ii) there was plausible evidence that the case was part of a general community point source outbreak;

(iii) the respondent returned from foreign travel outside the United Kingdom, and may probably have acquired the infection outwith the UK;

(iv) the respondent is a vegetarian, and, naturally the odds of exposure to meatborne salmonellosis is for practical purposes nil (zero)!;

(v) there was NO matched control for the case; that is, none of the corresponding controls returned the questionnaire or none of the questionnaires returned were satisfactorily matching for age of the case; or no other sex and age matched controls within the same postcode could be found; and

(vi) the respondent worked in a meat-related establishment and might have acquired the infection by occupational contact.

F Selection and Accessing of Matched Controls:

Household (neighbourhood) matched controls were selected on weekly basis as the cases occurred; that is, as duly completed questionnaires returned by the cases during the week were ascertained and accepted for the study. Using the residential (street) address, the postcode and the sex of the primary case, matched controls were selected from the revised national Electoral Registers for the City of Glasgow district. The case-control study was designed for a fixed number of controls, specifically **two** matched controls per case. A systematic 1 in 10 random sample was used to select persons of the same sex as the case, listed in the same street or the next street within the same postcode area.

six matched controls were selected per case. The reason was to increase the probability of getting the two matched households per case from the control respondents. One of the questions in the questionnaires requested the respondent to list the sex and ages of every member of the household. This was with a view to finding or selecting a household in which at least one member is matched in sex and age with the primary case. This process or system was particularly essential for those cases who were below the voting age of 18 years.

The same questionnaires as for the cases were sent to selected controls by post. An accompanying letter explaining the purpose of the study, solicited the consent of the control respondents to participate in the study, and requested them to return completed questionnaires in pre-paid envelope addressed to the Department of Community Medicine, University of Glasgow. All questionnaires were administered (mailed) to the controls within a maximum of two weeks of the reporting and accessing of the primary case. Only respondent controls who were matched for age within a 10-year band (+ 5 years), sex (if over 10 years old), and neighbourhood (to a maximum of post-code area); who were without a recent history of gastro intestinal illness; and who were not vegetarians, were selected or included in the analysis.

G Statistical Analysis of Strength of Association and Test of Significance:

An association would be assumed to exist between the illness and the exposure, if the odds of exposure (that is, proportion of exposed) differed significantly between the cases and the controls. This means that the odds ratio differs significantly from unity (one), being either increased or decreased. A test of this significance was calculated. The Mantel-Haenszel chi-square test (X^2mh) for a 2 x 2 contigency table (187) was used to test the significance of the difference, and hence of the association. If the observed odds of exposure falls beyond

reasonable limits of chance variation under the null hypothesis of no association, then the null hypothesis would be rejected, and the data from the case-control study would have provided evidence of (strong) association between poultry meat and the risk of developing salmonellosis.

The statistical analysis of the data took account of the matching procedures used. In selecting six controls per case, it was aimed to have a fixed case : control ratio of 1 : 2. However, the problems of postal questionnaires and the absence of age in the electoral register, made it difficult to obtain a full complement of two controls for all the cases. Thus, the responses produced a variable matching ratio, so that the matched controls ranged from one to three per case. Consequently, three Mantel-Haenszel methods for matched controls were employed in the statistical analysis of the strength of association (187).

(i) Mantel-Haenszel method for one matched control per case. The purpose of using this method was to ensure that all the 118 cases, though not all matched controls, were included in the analysis. For each of the eighty-one cases with more than one control per case, a simple random sample was made to select just one matched control. For the data layout for analysis, the four possible outcomes of the presence or absence of exposure in 118 pairs of cases and controls were presented in an appropriate 2 x 2 table for 1 : 1 matched controls. By a comparison of the proportion (odds) of exposed cases (A + B) versus the proportion (odds) of exposed controls (A + C) the case control difference in proportion was calculated. The Mantel-Haenszel estimate of the odds ratio was computed from the equation

$$\text{Odds Ratio } (\Upsilon_{mh}) = B/C \quad (187)$$

A test of the level of significance of the odds of exposure (probability of Type 1 error) was

determined by using the McNemar's Chi=Square test (187).

$$X^2_{mh} = (|B - C| - 1)^2/(B + C)$$

(ii) Mantel-Haenszel method of analysis of two matched controls per case (1 : 2). In this approach, only the 81 cases with two or more matched controls were analysed. For each of the nine cases with three matched controls per case, a simple random sample without replacement was taken to select the two controls required. Under the approach of two matched controls per case, the observations were represented as matched triplets (Case, Control 1 and Control 2). With the exposure being dichotomous (+ or -), the frequencies (n_o, n_1, n_2, n_3, etc) with which the eight possible triplet outcomes occurred were determined and the Mantel-Haenszel estimates of the illness-exposure odds ratios were calculated, according to the equation:

$$\text{Odds ratio } (\Upsilon_{mh}) = (n_1 + n_2 + n_3)/(2n_4 + n_5 + n_6)$$ (187).

This calculation again estimated the proportion or percentage by which consumption of poultry meat increased (?) the risk of salmonella infection.

Mantel-Haenszel chi-square test of significance of the null hypothesis (Ho : $\Upsilon = 1$; that is, no difference in odds of exposure between cases and controls) was performed from the equation:

$$X^2_{mh} = (|Ni| - \tfrac{1}{2})^2/N_2 \quad (187)$$

where $N_1 = [n_1 + n_2 + 2(n_3 - n_4) - (n_5 + n_6]/3$

and $N_2 = 2\,[n_1 + n_2 + n_3 + n_4 + n_5 + n_6\,]/9$

This test was used to provide any evidence that the estimated odds ratio is significantly increased. An approximately 95% $(1 - \alpha)$ Confidence Interval

for the odds ratio was also estimated from the equation

$$\exp\left[\left(1 + Z\alpha/\sqrt{X^2mh}\right) \text{ (natural log odds ratio)}\right] \quad (187)$$

(iii) Mantel-Haenszel point estimate of the odds ratio for three matched controls per case (1:3) was calculated by using the equation

$$\text{Odds Ratio (mh)} = \sum_{j=1}^{3} (c-j)\, n_j\,(+) \;/\; \sum_{j=1}^{3} j n_j\,(-) \quad (187)$$

Each case and its corresponding set of matched controls was regarded as a separate subgroup with a 2 x 2 indicating the frequencies of exposed $^{(+)}$ and unexposed $^{(-)}$ cases and controls.

nj (+) = number of matched sets in which the case is (+) and j controls are (+).

nj (-) = the number of matched sets in which the case is (-) and j controls are (+).

(iv) Mantel-Haenszel analysis for variable number of matched controls (1 matched control, 2 matched controls, and 3 matched controls). With this approach, all the 118 cases and all the respective matched controls were included in the analysis. Each matched set was regarded as a separate subgroup with a corresponding 2 x 2 table indicating the numbers of exposed and unexposed cases and controls. The Mantel-Haenszel estimate of the Odds Ratio, adjusted for the effects of stratification, was calculated using the equation:

$$\text{Odds Ratio (Ymh)} = \sum_{}^{118} (a_i d_i/N_i) \;/\; \sum_{}^{118} (b_i c_i/N_i) \quad (187)$$

CHAPTER FOUR

RESULTS

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RESULTS

4.1 RETROSPECTIVE STUDY:

4.1.1 Validity of Cases/Items of Data:

All the 1500 cases (salmonella isolations) selected from the Register of the Scottish Salmonella Reference Laboratory (SSRL) by systematic sampling were traced in the CD(S)U records, through the Laboratory Report Forms. There was a 100 percent concordance or agreement on the specified aetiologic salmonella serotype recorded by the SSRL and the CD(S)U. That is, the salmonella serotypes identified by the SSRL for each of the 1500 sampled cases were accurately recorded by the CD(S)U. The following items of data on each of the sampled cases were compared with entries on the Laboratory Report Forms submitted to the CD(S)U by the Reference Laboratory: primary laboratory of cases, name, address and age of the patient. All the items transcribed on to the Laboratory Report Forms at the SSRL were found to be complete and accurate.

Similarly, the entries in the CD(S)U Weekly Report on the age, sex, and primary laboratory of each of the cases were compared with information on the SSRL sample and also with entries in the SSRL Laboratory Report Forms. In all cases, the records in the SSRL Report Forms and the CD(S)U Weekly Reports were complete and in agreement.

Approximately 4,330 (76%) of 5770 salmonella infections (laboratory isolations) selected from the CD(S)U Weekly Reports, by systematic sampling, were traced in the Laboratory Report Forms from the diagnostic laboratories. In all the traced cases, information on sex, age and causative salmonella serotype listed in the Weekly Reports were in

concordance with the records and information on the Laboratory Report Forms. Thus, the validity or sensitivity of the CD(S)U records was 100 percent.

Records on the items in respect of the 1422 salmonella outbreaks documented during 1980-87 were also 100 percent valid. There was only one apparent omission in 1982. One Household Outbreak caused by *S.stanley* which occurred on 9th March 1982 in the Greater Glasgow Health Board area was accepted by the CD(S)U and assigned Outbreak Reference Number 82/0060. However, there was no evidence that this particular outbreak was recorded in the final list of outbreaks for that year.

On the whole, the items and data on salmonella infections and outbreaks recorded by the CD(S)U attained a 100 percent level of accuracy, completeness, and concordance. The diagnoses of all the infections and outbreaks were not simply "clinical"; they were based on laboratory-ascertainment. The items on data transcribed and recorded at the coordinating centre (the CD(S)U) were comparable to and in concordance with information submitted by the diagnostic laboratories, the SSRL, and the CMS. The Weekly and Annual Reports of the CD(S)U are thus valid to be used as the sampling frame for the retrospective study.

4.1.2 Salmonella Outbreaks:

A Cumulative and Annual Incidence:

Between 1980 and 1987 a total of 1,797 outbreaks of foodborne infections were reported in Scotland, In 1,683 (approximately 94%) of this total the causative agents were laboratory-ascertained, while for the remaining 114 outbreaks the agents were not known. Of the 1,683 outbreaks in which the agents were established, a total of 1,422 or 84.5% were caused by the salmonellae. The other 260 outbreaks were caused by *Campylobacter* species, *Clostridium perfringens*, *Staphylococcus aureus*, *Bacillus cereus*, other bacterial agents, viruses and other biological agents.

The annual frequencies of salmonella outbreaks during the period 1980-87, and the relative proportions of the 1422 cumulative incidence are summarized in **Table 4.1.1A** and **Figure 4.1.** The lowest number of salmonella outbreaks (121 or 8.5%) were reported in 1980, while the highest number (238 or 16.7%) was recorded in 1983. The annual mean of salmonella outbreaks was 178. Statistical analysis confirms that the incidence of salmonella outbreaks differed significantly from year to year ($t = 11.25$, $p < 0.01$). Analysis of the incidence of salmonella outbreaks at 2-year intervals revealed that during the period 1982-83, the incidence of foodborne salmonella outbreaks increased by 50 per cent over the 1980-81 incidence, from 319 to 473 **(Table 4.1.1B).** During the next two-year period (1984-85) the incidence decreased again by 50 per cent to the 1980-81 level. Although **Table 4.1.1A** shows approximately 3% rise in 1987 over the 1986 incidence, the cumulative incidence for the period 1986-87 was virtually the same as for 1980-81 and 1984-85 **(Table 4.1.1B).** Thus, apart from the period 1982-83 when there was 50% rise, the two-year cumulative incidence of salmonella outbreaks seem to have been maintained at a steady level.

B Persons Affected in the Outbreaks:

The numbers of persons affected by the salmonella outbreaks each year and for the 8-year period, the number of persons clinically ill, numbers laboratory-confirmed as cases of salmonellosis, the numbers hospitalized, or dead are presented in **Table 4.1.2.** A total of 8,051 persons in Scotland were affected in the 1,422 outbreaks that were reported between 1980 and 1987. An average of 1006 or approximately 1000 persons were affected in foodborne salmonella outbreaks each year. In 1982 and 1983 as many as 1451 and 1360 persons respectively were affected. The number of persons involved rose from 521 in 1986 to 1,021 in 1987; an increase of 100 per cent!

Cumulatively, 5949 or 74% of all persons affected had their salmonella infections confirmed by the laboratories. The

percentage of affected persons who were laboratory-ascertained ranged from 56% in 1980 to 87.5% in 1982. Seven thousand and fifty-two or approximately 89% of the affected persons were clinically ill; that is, they manifested some typical symptoms of foodborne salmonellosis. The other 898 persons affected were symptomless victims; while they were not clinically ill, they were salmonella positive. On the other hand, about 10 per cent of the ill persons were salmonella negative. Only in 425 (30%) of the 1,422 salmonella outbreaks were any of the affected individuals hospitalized; in the majority of the outbreaks (70%), none of the persons affected was ill enough to require hospitalization. Altogether, 817 or only 11.6% of the ill persons were hospitalized. Forty-two of those affected between 1980 and 1987 died; this gives an average annual death figure between 5 and 6. Highest mortality (17) occurred in 1982. Only one affected person died in 1986 and 1987. From the data, the case fatality rate of foodborne salmonella outbreaks is approximately 6 per 1000 affected persons per year.

C Types of Outbreak:

One thousand one hundred and thirty-eight (80%) of the 1422 salmonella outbreaks occurred as Family (Household) Outbreaks; that is, only persons in single unrelated households were affected. Two hundred and eighty-four (20%) outbreaks occurred as General Outbreaks; that is, a number of individuals from two or more households were affected in each outbreak. In a General Outbreak, the affected persons (or households) either purchased the incriminated food item such as contaminated milk and poultry meat from the same supplier, or they were exposed simultaneously (or during the same week) to the same common source such as restaurant or flight meals. There was little variation in the proportions of Household (Family) and General Outbreaks over the years.

D Premises/Venues or Places of Food Consumption:

Table 4.1.3 summarizes, in ranking order, the premises, venues or places where the foods incriminated in the salmonella outbreaks were consumed. In all, the premises of food consumption were determined or specified in 931 (65.5%) outbreaks; in the other 491 outbreaks the locations of food consumption were unknown. In approximately 61 per cent of the outbreaks for which the premises were specified, the incriminated food was consumed in private households. One hundred and sixty-four (17.6%) outbreaks occurred in the general community; that is, the same food source was consumed in more than one private household. Foods consumed in public catering establishments including hotels, restaurants, "fast food" and "take-away" shops accounted for 108 (11.6%) outbreaks. The data show that private households, general community and public catering enterprises constitute the major risk venues for foodborne salmonella outbreaks; these three locations were responsible for 90% of outbreaks for which the place of food consumption is established. The next set of significant risk factors are foods consumed in work places, social gatherings (picnics, parties, wedding receptions, camping), farm houses, and health institutions (hospitals, maternities, old peoples homes etc). These venues accounted for salmonella outbreaks ranging from 1.5% to 2.6% **(Table 4.1.3)**. Six of the outbreaks were associated with meals consumed in transit during Air Flights, while meals consumed in educational institutions (universities, colleges, schools, nurseries) were incriminated in only two salmonella outbreaks during the 8-year period.

E Food Vehicles (Food Items) Associated with Outbreaks:

In five hundred and eight (36%) of the 1422 outbreaks, the food vehicles associated with the outbreaks were identified; in 914 other outbreaks the incriminated food items were not established. The annual frequencies and cumulative incidence of outbreaks associated with the various food vehicles are presented in **Table 4.1.4**. Four hundred and six

(80%) of the 508 outbreaks were meat-borne, while 48 outbreaks or 9.4% were milkborne. Poultry meat was incriminated by bacteriological and epidemiological evidence in 281 or 55.3% of outbreaks where a food vehicle was identified, and in approximately 20% of all the outbreaks. Chicken was associated with 251 of the outbreaks, while turkey accounted for 30 outbreaks. Red meat (beef, pork ham and lamb) was incriminated in 95 or 18.7% of outbreaks in which the food vehicles were known. Eggs were implicated in only six outbreaks over the 8-year period. The pie chart of frequencies of salmonella outbreaks associated with poultry (chicken, turkey and eggs), red meat, milk and other foods is presented in **Figure 4.2.**

Of the outbreaks associated with red meat, 62 were related to beef; 24 were associated with pork ham; and lamb meat accounted for 9 outbreaks. Statistical analysis confirmed a significant difference in the cumulative incidence of meat-specific salmonella outbreaks ($t = 2$, $df = 3$, $p < 0.05$). The incidence of poultry meat-borne outbreaks is threefold or 300% that of outbreaks associated with the other types of meat put together. Analysis of the data at two-year periods reveals that the proportion of outbreaks associated with poultry meat remained about steady at 53-55% from 1980-81, through 1982-83, to 1984/85 **(Table 4.1.5).** However, during 1986-87, there was a sharp increase in the proportion of poultry meat-borne outbreaks to 62%, a rise of 7-9% over the proportions for 1980-85!

F Outbreak Reports from Health Board Areas:

The ranking order of the Scottish Health Boards on the basis of the **crude cumulative** incidence of salmonella outbreaks reported between 1980 and 1987 is presented in **Table 4.1.6A.** The largest number of outbreaks (316 or 22%) was reported from the Lothian Health Board. In all, seven Health Board areas reported an annual average of at least 10 salmonella outbreaks. These areas are Lothian (40), Grampian (27), Lanarkshire (23), Fife (17), Tayside (17), Argyll and Clyde (12) and Greater Glasgow (10). The seven Health Board

collectively accounted for approximately 77% of the total outbreaks recorded. Dumfries and Galloway, Highland, Ayrshire and Arran and Borders reported an annual average ranging from 3 to 7. Shetland and Orkney reported an annual average less than one.

When the mean human populations of the various Health Board areas are taken into account, the standardized cumulative incidence rate yielded a completely different ranking order **(Table 4.1.6B)**. Grampian Health Board had the highest standardized incidence rate of 4.4/10,000 persons, while the Greater Glasgow Health Board recorded the lowest incidence rate of 0.8/10,000. Six Health Boards recorded standardized incidence rates above the national average of 2.75/10,000 persons; the incidence rates of three Health Boards fall reasonably within the national average. The standardized incidence rates for Grampian and Lothian are 1.5 times above the national average, while the rate for Greater Glasgow is 3.5 times below the national average. Although the mean population of the Greater Glasgow area is exactly twice or double the population of the Grampian area, yet the Greater Glasgow Health Board recorded a standardized incidence rate that is 5.5 times below that of the Grampian Health Board!

G Imported Salmonella Outbreaks:

Three hundred and fourteen (22%) of the salmonella outbreaks recorded between 1980 and 1987 were imported; that is, the incriminated food was consumed outside Scotland. Forty outbreaks were acquired in England and Wales; while 274 were aquired outside the United Kingdom. Two hundred and twenty-four (82%) of the outbreaks imported from outside the United Kingdom, were acquired in eleven European countries **(Table 4.1.7)**. One hundred and sixty nine (75%) of the ex-Europe outbreaks were imported from Spain and Spanish territories (mainly Majorca, Ibiza and Tenerife). Thirty-one (11.3%) outbreaks were imported from 10 African countries (mainly Tunisia, Morocco, Nigeria and Gambia). Twelve (4.4%) outbreaks were imported from the Mediterranean region, mainly from Malta.

H Major Salmonella Serotypes/Phage Types:

Table 4.1.8 summarizes, in ranking order of frequency, salmonella serotypes isolated in the 1422 outbreaks. A total of 1,489 salmonella isolations were recorded; more than one salmonella serotype were isolated from persons affected in 67 outbreaks. *Salmonella typhimurium* was isolated in a total of 602 (40.2%) outbreaks; *S.enteritidis* was recovered in 336 (22.6%) outbreaks; while *S.virchow* was isolated in 197 (13.2%) outbreaks. Altogether, these three serotypes were responsible for 1,135 (76%) of the outbreaks. *S.typhimurium* ranked first in the order of frequency from 1980 to 1985; however, from 1986 *S.enteritidis* has assumed the first position in the ranking order. Other major salmonella serotypes which caused at least 10 outbreaks during the 8-year period include, in order of frequency, *S.stanley*, *S.heidelberg*, *S.agona*, *S.saint-paul*, *S.infantis*, *S.bredeney*, *S.hadar*, *S.panama*, *S.montevideo* and *S.anatum*. The first four serotypes predominated mainly during 1981 and 1982.

Several *S.typhimurium* phage types were identified in the outbreaks, including in order of frequency, 110, 10, 204, 49, 12, 104, 193, 170 and 66 **(Table 4.1.9)**. In some *S.typhimurium* outbreaks, the phage types were not specified. The most commonly identified phage types of *S.enteritidis* are 4 and 8; both phage types occurred in equal frequency **(Table 4.1.9)** and accounted for 90% of *S.enteritidis* outbreaks in which the phage types were specified. Phage type 8 predominated during 1980 to 1985, but since 1986 phage type 4 has been most commonly identified.

I Seasonal Trend:

The cumulative monthly incidence of salmonella outbreaks during 1980-87 is described in **Figure 4.3**. There was a steady rise of monthly incidence from January to August, followed by a steep drop from October to December. When the incidence data were analyzed on quarterly basis, a consistent seasonal trend for both the cumulative quarterly

incidence and annual mean incidence per quarter became more evident **(Table 4.1.10 and Figure 4.4)**. There was an indication of a fixed difference in the cumulative quarterly incidence of outbreaks; the cumulative mean incidence (quarter) increased by a geometrical progression from January/March, through April/June, to July/September. Thus, the mean incidence in January/March is 22. In April/June, the incidence rose to 40, that is, a two-fold increase. By July/September, the incidence has jumped to 87 - another two-fold increase or a 4-fold rise over the January/March incidence. In October/December, the quarterly incidence dropped sharply to 28; this is a three-fold decrease, or a drop of 68 per cent. The highest incidence of salmonella outbreaks occurred in the third quarter of the year, particularly during the summer months of July and August **(Figure 4.3 and Figure 4.4)**. Nearly 50% of all the outbreaks occurred during the third quarter; that is during the summer.

4.1.3 Salmonella Infections (Laboratory Isolations):

With respect to salmonella infections (confirmed laboratory isolations) during 1968 to 1987, the mean incidence at 5-year periods was determined, using 1 in 5 systematic sampling **(Table 4.1.11)**. For the periods 1968-72 and 1973-7, the sample sizes required to detect at least 5% difference in the mean incidence of infection between the two periods were calculated to be 250 and 1,886 respectively **(Figure 4.5)**. The sample sizes actually obtained for the two periods are 727 and 2,128 **(Table 4.1.11)**. The calculated probability of detecting 5% difference in incidence, using the sample size, was $p = 0.01$ **(Figure 4.6)**. The calculated probability is less than the specified critical value ($p = 0.05$) for detecting statistically significant difference in incidence. From both calculations, therefore, the sample sizes obtained with the 1 in 5 systematic sampling were adequate for detecting statistically significant differences at 5-year periods.

A total of 28,881 human salmonella infections reported by the diagnostic laboratories were recorded by the CD(S)U during 1968 to 1987. An overall sample size of 5,776 was obtained for the 20-year period. From 1968/72 to 1973/77, the mean incidence of salmonella infections, based on the systematic samples, rose from 145 per year to 197.8 per year - a rise of 36 per cent. During the next 5-year period (1978/82), the mean incidence had risen to 386 per year or a rise of 95 per cent! For the period 1983/87, the mean incidence was 425.6 per year, a rise of 10 per cent over the preceding period **(Table 4.1.11)**. Altogether, there was a 293 per cent increase in the mean annual incidence of infections between 1968-82 and 1983-87. The absolute numbers of salmonella infections actually recorded for the 5-year periods are as follows: 3635 in 1968/72; 4945 in 1973/77; 9660 in 1978/82; and 10.641 in 1983/87 **(Figure 4.7)**. The data presented a trend of a steady increase in crude incidence of salmonella infections during the 20-year period. Between 1968-72 and 1983-87, there has been a three-fold increase in the crude incidence of salmonella infections.

The standardized incidence rates based on the mean human population during the four 5-year periods are presented in **Table 4.1.12**. There was a steady increase in the standardized incidence rate of foodborne salmonellosis: 14 per 100,000 population per year for 1968-72; 19/100,000 per year (1973-77); 37/100,000 (1978-82); and 41.5/100,000 during 1983-87. The standardized incidence rate increased three-fold between 1968-72 and 1983-87.

The number of salmonella infections recorded for the various age-groups, the mean populations of the respective age-groups during the 5-year periods, and the standardized age-specific incidence rates are presented in **Tables 4.1.13A-D**. With a mean standardized age-specific incidence rate of 63.8 per 100,000 per year, children 5 years old and below seemed to be at the highest risk of developing salmonellosis. Persons 21 to 30 years are the next age-groups most frequently affected in human salmonellosis. There was a

steady rise in incidence rate in children (0-5 year olds) during consecutive 5-year periods. For persons in the 11-15 years age-group, there was a three-fold increase in the incidence rate from 10 during the first 10 years (1968-77) to 28 during the second 10-year period (1978-87). There was no significant variation in the standardized incidence rates among the different age-groups, during the 20-year period. Although the case fatality rate is much higher in the elderly people, the standardized age-specific incidence rate of salmonella infection among persons over 70 years was relatively very low (mean = 15.6/100,000 per year).

Table 4.1.14 shows the number of infections and standardized incidence rates in males and females. Higher proportions of infections alternated between males and females during the 5-year periods. However, there was significant (7.5%) difference in the standardized incidence rate between the sexes; the incidence rate of salmonella infection was higher in males than in females.

Tables 4.1.15A and B show the proportions (percentages) of the major salmonella serotypes isolated during 5-year intervals. The ranking order of top 10 serotypes during the 20-year period and at 5-year intervals is presented in **Table 4.1.16.** *S.typhimurium*, *S.enteritidis* and *S.virchow* were the three most prevalent serotypes over the 20-year period. *S.typhimurium* was the single most prevalent serotype throughout the four 5-year periods. *S.enteritidis* was the second or third most prevalent; the proportion of *S.enteritidis* increased from 7% during 1968-82 to 27% of all serotypes isolated in 1983-87 - a 285% increase! *S.virchow* accounted for less than 0.5% of serotypes isolated during the first 10 years (1968-77); however, during the second 10 years, the proportion increased to 8-13% with the serotype assuming the second place in the ranking order of frequency in 1978-82.

Before 1978, only a very small proportion of *S.typhimurium* and *S.enteritidis* were fully phage-typed. During 1978-82, *S.enteritidis* PT8 accounted for 65.6% of *S.enteritidis*

isolates that were phage typed, while PT4 accounted for 28%. By 1983-87, PT4 accounted for 55.7%, while PT8 constituted 28 per cent.

The seasonal trend in the number of laboratory isolations of salmonella from human foodborne infections is clearly demonstrated in **Figure 4.8**, and **4.9**). Highest incidence of salmonella infections were recorded during the months of July, August and September. There was consistency in the seasonal incidence of infections throughout the four 5-year periods (**Figure 4.9**).

4.2 BACTERIOLOGICAL SURVEY: EPIDEMIOLOGICAL RELATIONSHIP OF SALMONELLAE ISOLATED FROM CHICKEN CARCASES AND SEWER DRAINS:

4.2.1 Sampling Schedule:

Between February 1988 and March 1989, raw chicken carcases and pre-cooked chicken in the hospital kitchen, as well as sewer drains of patients' residential accommodation were sampled for the presence of salmonellae. The periods during which raw chicken and cooked chicken were prepared in the kitchen, and the duration of the respective sampling are represented diagramatically in **Figure 4.10**.

4.2.2 Raw Chicken Carcases:

Positive salmonella isolations were made from two hundred and fourteen of the 477 fresh and frozen chicken carcases sampled. This gives an overall contamination or incidence rate of approximately 45% (**Table 4.2.1**). Salmonellae were isolated in each of the 38 weeks during which raw chicken carcases were sampled. This means that every single batch of the chicken carcases examined contained individually contaminated carcases. The proportions of contaminated carcases for the 38 weekly batches ranged from 27% to 67%; the median incidence rate is 50%. The 214 salmonella-positive carcases yielded a total of two hundred and thirty-one salmonella isolates, comprising of 19 different

salmonella serotypes (**Table 4.2.1**). All the salmonella isolates belonged to the Subspecies 1 of the genus Salmonella. The frequencies of isolation of the different salmonella serotypes and phage types are summarized in **Table 4.2.2**. The 15 most frequently occurring serotypes are *S.enteritidis* (51 isolates), *S.typhimurium* (41), *S.virchow* (21), *S.hadar* (19), Salmonella rough type, Rough :gm (14), *S.bredeney* (13), *S.binza* (11), Monophasic salmonella serotype 6,7:-:1,5 (9), *S.eimsbuettel* (7), *S.schwarzengrund* (7), *S.minnesota* (7), *S.mbandaka* (7), *S.senftenberg* (6), *S.montevideo* (6) and a non-motile salmonella serotype 6,7,14:-:- (4).

S.enteritidis phage type 4 (PT4) constituted 94% of the 51 strains of *enteritidis* isolated from the chicken carcases. *S.enteritidis* PT4 also accounted for 21% of the 231 salmonella isolates. Three *S.typhimurium* phage types (PT49, 104 and 141) accounted for 90% of the *typhimurium* strains and 16% of the overall salmonella isolates recovered from the raw poultry meat.

Multiple salmonella serotypes or phage types were isolated from each of 16 individual carcases **(Table 4.2.3)**. Two different serotypes were isolated from each of 15 carcases, while three serotypes (*enteritidis*, *binza* and *hadar*) were recovered from a single carcase. Two phage types of *S.enteritidis* (PT4 & 7 and PT4 & 11) were isolated from two individual carcases.

The salmonella serotypes isolated from the chicken carcases were very similar to those poultry salmonellae notified in England, Wales and Scotland in 1988 under the Zoonoses Order **(Table 4.2.4)**. The raw carcase serotypes also related closely to those reported to the CD(S)U during the same period, under the WHO Surveillance Programme for Foodborne Infections and Intoxications **(Table 4.2.4)**. Eleven (58%) of the 19 serotypes obtained from the chicken carcases were also notified under the Zoonoses Order; four of the 8 fully serotyped poultry salmonellae reported to the CD(S)U from veterinary laboratories were also among the major serotypes

detected in the chicken carcases. Ten of the salmonella serotypes isolated from the carcases are among the 12 most common serotypes detected in 1988 from poultry processing plants in Scotland **(Reilly, Unpublished Observations).** In all the instances of salmonella isolations and notifications (chicken carcases, Zoonoses order, CD(S)U reports, and poultry plants) *S.enteritidis* and *S.typhimurium* ranked first and second.

4.2.3 Pre-Cooked Chicken:

Over a period of 5 weeks, samples were taken of packs of pre-cooked whole chicken introduced as a substitute for the raw carcases. Not one of the 102 packs examined yielded any salmonellae **(Table 4.2.1).** All the samples were also negative for **Listeria** organisms. However, coagulase-positive, DNase-positive *Staphylococcus aureus* was isolated from twenty-nine (28.4%) of the 102 packs. Investigation into the production of enterotoxin by the staphylococci isolates is continuing. Staphylococcal contamination of the cooked poultry meat is outside the scope of this thesis.

4.2.4 Sewer Drains:

A total of 89 sewer swabs were examined over the period of 45 weeks during which raw chicken carcases or pre-cooked chicken were also sampled. Salmonellae were detected from thirty (38%) of the 79 swabs examined when raw chicken carcases were used in the hospital kitchen **(Table 4.2.5A).** Only one (10%) of the 10 swabs examined after pre-cooked chicken had been introduced, was positive for salmonella. At least one of the pairs of swabs yielded salmonella during 28 (70%) of the 40 weeks when raw chicken carcases were used in the kitchen, whereas a positive swab was obtained only in one (20%) of the 5 weeks after the change to pre-cooked chicken **(Table 4.2.5A).**

A total of thirty-three isolates comprising of 13 salmonella serotypes were detected from the sewer drains, during the period that raw chicken was being used. The frequencies of

the salmonella serotypes and phage types are presented in **Table 4.2.2.** The most frequent serotypes are *S.enteritidis* (7 isolates), *S.virchow* (6), *S.typhimurium* (4), *S.clichy* (4), *S.thompson* (3), and *S.montevideo* (2). All but one of the seven *S.enteritidis* isolates are phage type 4. Multiple salmonella serotypes were detected from three individual sewer swabs: one swab yielded *S.typhimurium* PT104 and *S.enteritidis* PT4; another swab yielded *S.virchow* and *S.clichy*; while *S.hadar* and *S.thompson* were recovered from one swab. A single isolate of *S.enteritidis* PT4 was obtained from the 10 swabs taken when pre-cooked chicken was being supplied **(Table 4.2.5A).**

4.2.5 Comparison of Chicken and Sewer Drain Salmonella Isolates:

Eleven (69%) of 16 different salmonella types (serotypes and phage types) isolated from drain swabs were also recovered from chicken carcases **(Table 4.2.2).** The exceptions are *S.clichy* (4 isolates), *S.thompson* (3) and single isolates of *S.enteritidis* PT8, *S.typhimurium* PT10 and *S.heidelberg*. Of the eleven salmonella types occurring in both chicken and sewers, seven were recovered from both sources in corresponding or matching weeks. Specifically, the following salmonella types were detected in the sewer drain one week after each type had been isolated from the chicken carcases: *S.enteritidis* PT4, *S.typhimurium* PT49 & 104, *S.virchow*, *S.minnesota*, *S.eimsbuettel* and *S.montevideo* **(Table 4.2.6).** *S.enteritidis* PT4 was observed in both chicken and sewer during 5 matching weeks; *S.virchow* was observed from both sources during 3 matching weeks; while *S.typhimurium* PT49 was observed during 2 matching weeks. Of the 38 weeks during which raw chickens were sampled, sewer drains were monitored in 35 matching weeks. This provided 35 matching week-pairs for the chicken:sewer sources. In 13 of these 35 week-pairs, the same salmonella type was detected in both chicken and sewers. A total of 30 salmonella types (serotypes and phage types) were recovered from chicken or sewer. The observed number of matching weeks in which **each** of the 30 salmonella types was isolated

from both chicken and sewer (chicken +, sewer +) was determined. The expected frequencies of (+, +) was then calculated for each of the 30 salmonella types (Tables 4.2.7A & B). The observed and expected frequencies respectively were aggregated, and the The standard chi-square analysis was applied to test the null hypothesis of no association between salmonella types isolated from chicken and sewer during the matching weeks. The result shows that the isolation of the same salmonella serotype/phage types from both chicken and sewer during matching weeks (+, +) occurred in excess of the frequency that would be expected to happen by chance (X^2 = 15.08, $p < 0.005$). Thus, the data provided evidence to reject a null hypothesis of no association.

In both chicken carcases and sewer drains, *S.enteritidis*, *S,typhimurium* and *S.virchow* were the three most frequent serotypes detected. In chicken, these three serotypes constituted 50% of the 231 salmonella types isolated; while in the sewers, the three serotypes accounted for 52% of the 33 isolates. The three serotypes were observed in both chicken and sewer in 11 matching week-pairs. Using the same method as above the calculated expected frequency of (+, +) was 4.54 (Table 4.2.7B). Thus, for the three most common serotypes, the observed frequency as of (+, +) was again in excess of the frequency expected to occur by chance; the X^2 value is 9.19, with $p < 0.005$.

While salmonellae were isolated from raw chicken during each of 38 weeks that samples were taken, no salmonellae were recovered from pre-cooked chicken during the 5 weeks that cooked meat was examined. The change from raw to cooked chicken correlated in time with a marked drop in the recovery of salmonellae from the sewer. Thus, salmonellae isolations were made from 38% of sewer swabs examined in 28 (70%) of 40 weeks when raw chicken was prepared in the hospital kitchen. After the change from raw to cooked chicken, salmonella isolation was made from only one (10%) of 10 swabs examined and only in one (20%) of the 5 weeks that samples were taken (Table 4.2.5A). This observed

difference was statistically significant at 5% level ($p = 0.0468$, Fisher's Exact Test; **Table 4.2.5B**).

4.2.6 Antimicrobial Sensitivity of Salmonellae Isolated from Chicken Carcases and Sewers in Corresponding Weeks; Epidemiological Relatedness:

Taken as an epidemiological marker, the antimicrobial susceptibility patterns of the identical salmonella types (serotypes/phage types) isolated from chicken and from sewer drains during corresponding or matching weeks were compared, to determine the epidemiological relatedness of the isolates (**Table 4.2.8**).

All the 19 isolates of *S.enteritidis* PT4 recovered from chicken carcases in five corresponding weeks were sensitive to the following eight antimicrobial agents routinely tested at the Scottish Salmonella Reference Laboratory: Ampicillin, Chloramphenicol, Gentamicin, Kanamycin, Streptomycin, Tetracycline, Sulphonamide, and Trimethoprim.

Similarly all 6 isolates of *S.enteritidis* PT4 detected in the sewer during five matching weeks were sensitive to all the eight antimicrobial agents.

Nine strains of *S.typhimurium* PT49 obtained from chicken and the 4 strains detected in the sewer in matching weeks were all sensitive to the eight antimicrobials (**Table 4.2.8**). The single *S.typhimurium* PT104 isolated from chicken in the fifth week and one strain obtained from sewer in the corresponding week were also sensitive to the agents tested.

Two isolates of *S.virchow* recovered from chicken in the 42nd week and two isolates detected from the sewer in the corresponding week had identical antibiogram: all four were resistant to Sulphonamide and Trimethoprim, but sensitive to other six agents. However, the strains of *S.virchow* detected from chicken and sewer during corresponding 16th/17th and 17th/18th weeks exhibited differing antimicrobial resistance patterns. All six *S.virchow*

isolates from chicken were resistant to Sulphonamide and Trimethoprim, but sensitive to the other agents. In contrast, all 3 isolates detected in the sewers in the two consecutive weeks were resistant to Chloramphenicol and Tetracycline in addition to Sulphonamide and Trimethoprim. But then, nine strains of *S.virchow* isolated from chicken carcases (in the 21st, 22nd and 33rd weeks) were resistant to Chloramphenicol, Tetracycline, Sulphonamide, and Trimethoprim. This antibiogram is identical to those of the strains detected in the sewer.

The isolates of *S.minnesota*, *S.eimsbuettel* and *S.montevideo* obtained from both chicken and sewer in matching weeks, exhibited identical sensitivity patterns. All the isolates were sensitive to the eight antimicrobial agents.

Thus, there was little or no variability in the antibiogram of the 68 salmonella isolates from chicken and sewer, representing 30 different salmonella types (serotypes and phage types). This suggests some form of epidemiological relatedness of identical salmonella serotypes/phages detected from both chicken and sewer in corresponding weeks.

4.2.7 Comparisons with Salmonellae Isolated in Concurrent Poultry-Associated Foodborne Salmonella Incidents:.

Eleven of the 19 salmonella serotypes isolated from the chicken carcases and 8 of the 13 serotypes detected in the sewers were also recorded from poultry in 1988, under the Zoonoses Order **(Table 4.2.2** and **Table 4.2.4)**. In all three sources, *S.enteritidis* PT4 ranked first, while *S.typhimurium* ranked second or third **(Table 4.2.9)**.

During the period of the survey (1988) there were, in Scotland, 39 outbreaks of foodborne salmonella infections associated with chicken. In 31 (80%) of the 39 outbreaks, the causative salmonella types (serotypes and phage types) were the same as those isolated from chicken or sewers in the present survey **(Table 4.2.2)**. The only exceptions are *S.typhimurium* PT 66, 110 and 204 and *S.saint-paul* which

collectively accounted for 8 of the outbreaks. In chicken carcases, sewer drains and in poultry-associated foodborne outbreaks, *S.enteritidis* PT4 ranked first in frequency. This phage type accounted for 94%, 86% and 92% respectively of *S.enteritidis* isolates obtained from the three sources.

S.enteritidis, *S.typhimurium* and *S.virchow* are the three most frequent serotypes detected in chicken carcases and in the sewer drains. These same serotypes were the top three in the ranking order of serotypes isolated in 224 poultry-associated foodborne salmonella outbreaks in Scotland during 1980-85 **(83)**. As shown in the Retrospective Study, the three serotypes are also the topmost in the ranking order of serotypes responsible for 1422 foodborne salmonella outbreaks recorded in Scotland between 1980 and 1987; the three accounted for 76 per cent of all serotypes isolated and 80% of the 1422 outbreaks **(Table 4.1.8)**.

4.2.8 Seasonal Trend:

The change in policy, from raw to cooked chicken, meant that the salmonella survey of chicken carcases could not be carried out for full 52 weeks. Although weekly samples were taken on 38 occasions over a 43-week period, and although pre-cooked chicken were sampled for further 5 weeks, the data were incomplete and incompatible for full assessment of seasonal trend. However, during the 43 weeks that the carcase survey was carried out, there was detectable seasonal variation in the mean proportions of chicken carcases contaminated with salmonella. The mean contamination rates for February/March, April/June, July/September, and October/December were 36%, 45%, 42% and 51% respectively. This observed seasonal (quarterly) difference in proportions of salmonella contamination was statistically significant ($t = 12$, $p < 0.01$). The highest proportion of carcases contaminated by salmonellae was recorded during October-December. When the data for the winter months (February/March and October/December) are pooled, the average proportion of contamination in winter becomes approximately 44%. This rate is about the same as

for Spring (April/June) and Summer (July/September). Thus, it would seem, there is in actuality, no significant seasonal variation in the proportions of contaminated or infected chicken carcases.

4.3 MATCHED CASE-CONTROL STUDY OF POULTRY MEAT-BORNE SALMONELLA INFECTIONS:

4.3.1 Proportions of Case Respondents:

During the period of the case-control study, one hundred and sixty-two reported sporadic and primary household cases of salmonella infections in the City of Glagsow district were listed in the computer-based records at CD(S)U. Cases occurring within the district were determined from the code of Diagnostic Laboratory and the residential address of the patient. Household outbreaks occurring in the district were also established by a review of the Outbreak Investigation and Summary Report Forms. Altogether, one hundred and thirty-six cases returned duly completed questionnaires. However, 125 cases considered eligible for the study were used for the selection of matched controls. Of the eleven cases for whom no controls were selected, 2 were vegetarians; 3 returned recently from travels outside the United Kingdom; and 2 were found to be part of General Community Outbreaks. The names of the other 4 respondents were not listed in the CD(S)U records for the period - suggesting that their foodborne infections may not be salmonella; that these cases were not officially notified, or that reports on them were among official CD(S)U "rejects". In any case, the 4 case respondents were not included in the study. The 132 respondent cases whose names are listed (validated) constituted 81% of the 162 cases formally reported. Of the 125 cases used for selection of controls, no questionnaires were returned from the controls in respect of 3 cases. For 4 other cases, no matched controls could be selected from the questionnaires returned. Thus, the effective and substantive number of cases actually used in the Case-Control analysis is one hundred and

eighteen. This number constituted approximately 73% of the base-population of notified and recorded cases occurring in the district between April 1988 and March 1989.

4.3.2 Proportions of Control Respondents:

With six controls selected for each of the 125 eligible cases, a total of 750 questionnaires were mailed to controls. The numbers of control questionnaires returned per case, varied from four to nil. The frequency distribution of matched control responses is summarized in **Table 4.3.1.** A total of 208 matched controls were selected from the control responses. Matched controls could not be selected in respect of seven (5.6%) of the eligible cases - either because no corresponding questionnaires were returned or that there were no matching controls from the questionnaires returned. One, two or three matched controls were obtained for each of the other 118 cases (94.4%). Thirty-seven (31.4%) of the 118 cases had case:control ratio of 1:1; seventy-two (61%) had case:control ratio of 1:2; while nine (7.6%) cases had a ratio of 1:3 **(Table 4.3.2).**

4.3.3 Validation of Age-Distribution of Controls with Cases

The distribution of age-groups among the 118 cases and 118 matched controls (the 1:1 ratio) is presented in **Table 4.3.3.** To ascertain that the numbers and proportions of cases and controls in the various age-groups do not significantly differ, a validation of age distribution among the controls was performed, both by the significance testing and by the interval estimation approaches. There was no difference in age distribution between Cases and Controls both by the 2-sided t-test ($t = 0$, $p < 0.01$) and the 95% Confidence Interval estimate (- 0.22, 0.22). Thus, in addition to matching in sex and neighbourhood (postcode area), the Controls were similar to the Cases with respect to age variable.

4.3.4 Consumption of Poultry Meat among Cases and Controls:

Tables 4.3.4, 4.3.5 and 4.3.6 show three independent associations that were observed between salmonella infection and consumption of poultry meat within 48 hours before onset of illness. The consumption of the poultry meat and the association observed were without regard to and irrespective of the form of the poultry meat at the time of purchase and the method of cooking the poultry meat. Mantel-Haenszel matched analysis with one control per case (1:1 test) shows that 79 of the 118 cases ate poultry meat, while 40 of the 118 controls also ate poultry meat. However, only in 22 matched case:control pairs did both case and controls indicate eating poultry meat **(Table 4.3.4)**. In 57 matched pairs, the cases ate poultry meat but the controls did not; and in 18 matched pairs, the controls consumed poultry meat, while the cases did not. Thus, the ratios of odds of exposure to poultry meat among the cases, relative to the controls, the **Odds Ratio** is 3.2 **(Table 4.3.4)**. This implies that the odds of exposure among the cases is three-fold relative to the controls. In other words, the consumption of poultry meat is estimated to increase the risk (the odds) of salmonella infection by 220 per cent. By the application of the McNemar's chi-square test of a null hypothesis that the Odds Ratio is unity (H_o : Odds Ratio = 1), the level of significance of the calculated odds of exposure was determined (X^2 = 19.25, $p < 0.005$) **(Figure 4.11)**. Thus, the data provided evidence to reject the null hypothesis; the odds ratio differed significantly from unity. There is no evidence to accept the null hypothesis that consumption of poultry meat prior to salmonella infection was no more likely in Cases than in Controls. The chi-square test establishes that consumption of poultry meat is more likely in cases than in controls; hence, there is evidence of an association between consumption of poultry meat and the risk of salmonella infection (Objectives 1 and 2).

By the approach of Mantel-Haenszel matched analysis with two controls per case (1:2), the calculated Odds Ratio is 4.2 **(Table 4.3.5)**. This approach establishes that the odds of exposure to poultry meat among the Cases was four-fold; the consumption of poultry is estimated to increase the risk of salmonella infection by 320 per cent. The level of significance of this Odds ratio was determined by the Mantel-Haenszel two-sided chi-square test of a null hypothesis that the odds ratio is equal to one (unity). The calculated chi-square again provided evidence that the Odds Ratio is significantly elevated (X^2 = 26.7, $p < 0.005$) **(Figure 4.12)**. Therefore, salmonella infection is significantly associated with consumption of poultry meat. A 95% Confidence Interval estimation for the odds ratio yielded 2.44 for the lower limit and 7.66 for the upper limit (2,44, 7.66; **Figure 4.13**). The data provided evidence to reject a null hypothesis that the odds ratio is unity. This re-affirms that the established association of salmonella infection and consumption of poultry meat is statistically significant.

Using the method of Mantel-Haenszel analysis with variable number of matched controls per case, the calculated odds ratio is 3.57 **(Table 4.3.6)**. This approach confirms that consumption of poultry meat is more likely in Cases than in Controls; the consumption of poultry meat is here estimated to increase the risk of salmonella infection 3.5 times and by more than 250 per cent.

With respect to consumption of red meat (beef, pork or lamb) the Mantel-Haenszel analysis with variable number of matched controls per case yielded an odds ratio value of 0.38 **(Table 4.3.7)**. The calculated odds ratio differed significantly from unity, indicating that the consumption of red meat did not elevate the risk (odds) of salmonella infection. Rather, there was a negative association between salmonella infection and consumption of red meat: red meats seem to reduce the risk of infection. The conclusion is that, contrary to the null hypothesis, there is an association between the type of meat consumed 48 hours prior to illness

and the risk of salmonella infection; poultry meat is postively and significantly associated with the development of salmonella infection (Objectives 1 and 2).

4.3.5 The Form of Poultry Meat Purchased and the Risk of Salmonella Infection:

A total of 36 out of the 118 Cases and only 21 of 208 Controls indicated that they purchased and consumed frozen chicken 48 hours before onset of illness. But by the approach of Mantel-Haenszel analysis with variable number of matched controls, the ratio of odds of exposure to frozen poultry meat among Cases relative to Controls (the Odds Ratio) is 4.0 **(Table 4.3.8)**. This implies that the odds of exposure to frozen poultry meat is 4-fold among the Cases relative to the Controls. The data provide evidence to reject a null hypothesis of no association (Objective 3). Calculated Odds Ratio indicates that consumption of frozen poultry meat is significantly associated with salmonella infection; consumption of frozen poultry meat is estimated to increase the risk of infection by 300 per cent!

With respect to fresh poultry meat, the Mantel-Haenszel Odds Ratio is 2.58 **(Table 4.3.9)**. This indicates that foodborne salmonella infection is associated with consumption of fresh poultry meat; and implies that consumption of fresh chicken elevated the risk of infection by approximately 160 per cent. Similar, the ratio of oods of exposure to pre-cooked poultry meat in Cases relative to Controls (Mantel-Haenszel Odds Ratio) is 1.21 **(Table 4.3.10)**. In other words, the odds of exposure among the Cases relative to Controls is increased by 21 per cent. This indicates a positive but less significant association between the consumption of pre-cooked poultry meat and the risk of salmonella infection; and means a rejection of the null hypothesis (Objective 2).

4.3.6 Methods of Cooking Poultry Meat and the Risk of Salmonella Infection:

Table 4.3.11 shows that with respect to consumption of roasted poultry meat, the Mantel-Haenszel Odds Ratio is 4.00. This means that the odds of exposure to roasted poultry meat among Cases relative to Controls is four-fold! The data provide evidence to reject the null hypothesis of no association between foodborne salmonella infection and the method of cooking poultry meat. There is evidence of significant association between consumption of roasted poultry meat and foodborne salmonella infection; consumption of roasted chicken or turkey elevated the risk of infection by 300 per cent!

On the other hand, the Mantel-Haenszel Odds Ratio with respect to exposure to boiled chicken is 0.56 **(Table 3.4.12)**. This odds ratio is significantly less than unity and therefore, the data provide evidence to reject a null hypothesis of no association. Thus, the odds ratio suggests there is negative association between the consumption of boiled poultry meat and the risk of foodborne salmonellosis; boiled chicken is not a significant risk factor; boiling reduces the risk of salmonella infection.

Only 5 of the 118 cases indicated they consumed grilled poultry meat, and only 7 of the 208 matched controls stated they ate grilled chicken. Thus, the responses do not provide sufficient data to enable calculation of the odds of exposure to grilled meat, among the cases and controls. Similarly only two of the cases and three controls indicated they ate fried chicken. Therefore, the Mantel-Haenszel odds ratio could not be calculated for fried poultry meat. The indication, or rather the implication is that grilling and frying of poultry meat are not common methods of cooking poultry meat, and therefore, may not be significantly related to development of foodborne salmonella infections.

The data on roasted and boiled poultry meat provide sufficient evidence to reject the null hypothesis that there

is no significant difference in the risk of poultry meatborne salmonellosis associated with the various methods of cooking (Objective 4).

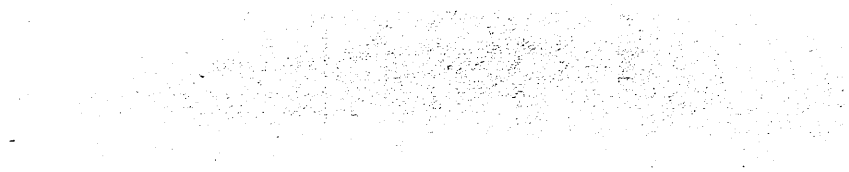
4.3.7 Frequency of Consumption of Poultry meat and the Risk of Salmonella Infection:

Among the cases and the controls, the relative odds (odds ratio) of consumption of poultry meat 1 or 2 days in an average is 0.71 **(Table 4.3.13)**. Thus, the Mantel-Haenszel odds ratio is not significantly less than unity (<1) and implies there is evidence to reject the null hypothesis of no association. The suggestion is that consumption of poultry meat in one day or two days in the week is not significantly associated with the risk of salmonella infection. On the other hand, the relative odds of exposure to poultry meat is 3 days or 4 days of an average week, among the cases and controls is 2.26 **(Table 4.3.14)**. That is, the odds of consumption of poultry meat 3-4 days in a week among the cases relative to controls is more than 2-fold. Cases are statistically more likely to eat poultry meat for 3 to 4 days in the week than did the controls. The data thus provide evidence of a significant association between foodborne salmonella infection and consumption of poultry meat 3 or 4 days in an average week. Consumption of poultry meat 3 or 4 days in the week increased the risk of salmonella infection by 200 per cent.

The numbers of Cases and Controls who indicated they ate poultry meat 5-6 days, or 7 days in an average week were too few to enable reasonable calculations of the respective odds ratio.

Available data presented in **Table 4.3.13** and **Table 4.3.14** show that while there was no positive association between infection and consumption of poultry meat 1-2 days in the week, there was significant association with consumption of poultry meat 3-4 days in the week. However, since there were insufficient and incomplete data to calculate for 5-6 days and for 7 days, the strength of association could not

be clearly estimated (Objective 5): Any evidence of dose-effect response with respect to frequency of consumption of poultry meat and the strength of association could not be determined.



CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

Although Scotland has a well-developed system for investigating and reporting incidents of foodborne infections and intoxications, probably most cases of foodborne illness never come to the notice of general practitioners, hospitals, environmental health departments and the diagnostic laboratories. Not all cases, in particular the sporadic cases, that come to the notice of the authorities are conclusively investigated and, probably reported to the CD(S)U which serves as the national co-ordinating centre of the WHO Surveillance Programme for Foodborne Infections and Intoxications in Europe. This means that the infections (laboratory isolations) and outbreaks recorded by the CD(S)U may not reflect the true magnitude of the salmonellosis problem in Scotland. Nevertheless, retrospective analysis and correct interpretation of the published figures based on the existing surveillance network, does provide an indication of the trends and changes in incidence rates, the types of agents involved, the range of foods implicated, and the location of salmonella infections and outbreaks.

Between 1980 and 1987, a total of 1,797 outbreaks of food poisoning or an annual average of 225 outbreaks were recorded in Scotland. The lowest incidence of foodborne outbreaks was recorded in 1980, the year Scotland became the first country to formally participate in the WHO Surveillance Programme. In 1,687 (94%) of the total outbreaks, the causative agents were laboratory-ascertained. The salmonellae were the single most important cause of food poisoning, accounting for 1,422 (85%) of the laboratory-confirmed outbreaks. Thus, as a public health problem, human salmonellosis has become established as a major foodborne zoonosis in Scotland. The salmonellae have also

been shown to lead the list of reported foodborne pathogens in England and Wales **(81, 152, 154)**, in other countries of Europe **(91, 152, 154)** and in North America **(4, 5, 90, 92-95, 189)**. The mean annual incidence of foodborne salmonellosis in Scotland and in England and Wales seem particularly prominent and by far exceed the incidence of outbreaks associated with *Clostridium perfringens*, the second most prevalent aetiologic agent in Europe and North America **(189, 190)**.

While the levels remained generally high, there has been no definite trend in the annual incidence of salmonella outbreaks since 1980. During the period 1982-83, there was as much as a 50 per cent rise over the 1980-82 incidence; however, in 1984-85, the incidence fell back by 50% to the 1980-81 level. A comparable incidence was recorded in 1986-87. Thus, apart from the dramatic rise in 1982-83, the cumulative incidence of salmonella outbreaks at two-year intervals maintained a steady but high level **(Table 4.1.1B)**.

Over the 8-year period, foodborne salmonella outbreaks affected an average of approximately 1000 persons per year, 5 of whom died from the incidents. The mean number of persons affected (cases) per outbreak was 5.6. Less than 5 persons (cases) per outbreak were affected in 1220 (86%) of the 1422 salmonella outbreaks. Only in fifteen outbreaks were there more than 50 persons per outbreak, and only eight outbreaks affected more than 100 persons per outbreak. A previous report for the period 1980-85 had the mean number of cases per salmonella outbreak at 5.8 **(84)**. In England and Wales the mean number of cases per outbreak during 1980-84 was 5.3 **(191)**. The mean number of cases in Scotland, England and Wales is far below those recorded in most of Europe and in North America where the mean numbers ranged from 28 to 69 per outbreak **(189, 192-194)**. It is likely, perhaps, that in Europe and North America only large outbreaks are investigated. In general, the mean number of cases associated with outbreaks of salmonellosis tended to be lower than the figures for outbreaks caused by *Clostridium perfringens* **(189)**. The lower number of cases

per outbreak of salmonellosis in Scotland and in England and Wales is thought to reflect the widespread occurrence of family outbreaks (189). Eighty per cent of the 1422 salmonella outbreaks recorded occurred as family outbreaks; that is, the outbreak was confined to a single household and affected members of the same family. The remaining 20% occurred as general outbreaks in which a cluster or sometimes widely scattered households were simultaneously affected. These households either purchased the incriminated food vehicle (milk, poultry meat, etc) from the same supplier or they had simultaneous exposure to a common meal or otherwise contaminated common food vehicle. There was very little variation in the proportions of family and household outbreaks over the years.

In 78% of the 931 outbreaks for which premises were specified, the incriminated food was consumed in the home; that is, in single or multiple **private homes.** The implication is that meals prepared and eaten in private households constitute the primary risk factor in foodborne salmonella outbreaks. Meals prepared or served in public catering establishments (hotels, restaurants, fast-food canteens), work places, social gatherings (wedding reception), farm houses and health institutions (hospitals, maternity, old peoples' homes) accounted for 53% of general outbreaks; persons exposed to meals served in these locations constitute the next set of reasonably high risk individuals. From the data, meals eaten at educational institutions constituted the least risk factor for foodborne salmonella outbreaks. The relative importance of places of consumption observed in Scotland is very similar to that recorded for England and Wales (81). Foods consumed in private homes accounted for 74% of 1061 outbreaks reported in England and Wales during 1983-84. This was followed by outbreaks associated with foods consumed in hotels, restaurants, receptions and canteens (13%); while meals served in hospitals and similar welfare/health institutions were responsible for 5.6% of the outbreaks (81).

In only 508 (36%) of salmonella outbreaks was a suspected food vehicle identified by bacteriological or epidemiological evidence. Eighty per cent of the 508 outbreaks were meatborne. Poultry meat was the primary food vehicle incriminated, accounting for 69% of the meatborne episodes and 55.3% of the 508 outbreaks. The number of poultry meatborne outbreaks was three times that of episodes associated with red meat (beef, pork/ham, lamb). During 1980-85, the proportions of outbreaks associated with poultry meat, in 2-year periods, remained steady at 53 to 55 per cent. However, during 1986-87, there was a rise to 62 per cent. An average of 35 episodes per year were associated with poultry meat; this figure excludes the fact that many of the 914 (64%) outbreaks in which the food vehicle was not determined, may well be associated with poultry meat. The report of Reilly and others **(195)** shows that between 1975 and 1984, the annual mean of episodes in which poultry meat was incriminated is 11. The food vehicle was identified in far greater proportion of the outbreaks during 1975-84 than in the present analysis (60% versus 36%); the proportions of episodes that were poultry meatborne during the two periods are comparable (58% versus 55.3%); and the proportions of meatborne outbreaks that were poultry-specific are similar (81% versus 80%). The implication, therefore, is that between 1975 and 1987, there has been a three-fold increase in the annual incidence of salmonella outbreaks associated with poultry! In England and Wales, food poisoning reports of the Public Health Laboratory Service (PHLS) showed that in 1959-62, only 9 (12.9%) of 70 episodes of known vehicles were associated with poultry. During 1969-72, the number was 66 (52%) out of 127 outbreaks; in 1981-83, 179 (51.3%) of 347 episodes; and in 1984-85, it was 57 (32%) of 177 outbreaks. Although the proportion of poultry-associated episodes in 1984-85, fell below the 1981-83 figure, there was a 3-fold rise over the proportion in 1959-62 **(60)**. The pre-eminence of poultry in foodborne salmonellosis has been recorded also in most of Europe and North America **(189)**. In all the countries, the primary place of poultry has remained unchanged in the past decade.

The finding that eggs were incriminated in only 6 salmonella outbreaks during the entire 8-year period is interesting. The possibility that hens eggs might be a significant source of salmonella infection was not generally recognised by the public or the EHOs before 1988. Therefore, routine epidemiological investigation by the EHOs and the recall of food histories by cases seemed less likely to implicate eggs, while bacteriological examination of eggs was uncommon. A follow-up analysis of CD(S)U reports for the period 1988-89 shows an increase in egg-associated episodes to 14 within the past 2 years alone. The publicity now given to the potential dangers of eating raw eggs and egg products would seem to influence the current incidence. However, there is evidence from reports in England and Wales, and a number of other West European countries of a similar increase in the number of foodborne salmonellosis associated with eggs, particularly where *S.enteritidis* PT4 were involved **(55, 87, 186, 196, 197)**. In England and Wales, only 2 incidents of egg-borne salmonellosis were recorded between 1973 and 1977 **(77)**; however, during 1988-89, a total of 76 egg-associated outbreaks were reported by the PHLS, about 65% of which were due to *S.enteritidis* PT4 **(55, 87, 197)**. In recent times, *S.enteritidis* PT4 has been cultured from bulk liquid eggs, dead laying hens, from ovaries and oviducts of dead hens, and from liquid yolk of inadequately cooked shell eggs **(10-13, 198, 199)**; and salmonella outbreaks have been associated with eggs used raw in mayonnaise, egg-nog, milk shakes, sandwiches, and home-made ice cream **(55, 80, 87, 186)**.

It has been mentioned that prior to 1983, milk was the most important food vehicle associated with salmonella outbreaks in Scotland **(83, 200)**. The results obtained in the present retrospective study show that since 1980, poultry has remained the primary food vehicle in salmonella episodes. Certainly, the legislation on compulsory heat-treatment of cows milk introduced in Scotland in 1983 was prompted by the high incidence of milkborne salmonella outbreaks and the relatively large number of persons affected **(77, 78, 84, 91, 200)**.

Based on the crude cumulative incidence of salmonella outbreaks reported, the Lothian Health Board area recorded the highest annual average of episodes. Grampian, Lanarkshire, Fife, Tayside, Argyll and Clyde and Greater Glasgow health boards followed in ranking order of frequency **(Table 4.1.6A)**. However, population-based standardized incidence rates, ranged from 4.4 per 10,000 persons for Grampian to 0.8/10,000 for the Greater Glasgow Health Board area. In all, six health boards recorded standardized incidence rates above the national average of 2.75/10,000 persons **(Table 4.1.6B)**. The standardized incidence rates for Grampian and Lothian are 1.5 times above the national average, while the rate for Greater Glasgow is 3.5 times **below** the national average. Although the mean human population of the Greater Glasgow area is twice the population of the Grampian area, yet the Greater Glasgow health board recorded a standardized incidence rate that is 5.5 times **below** that of the Grampian area! Even the least populated areas recorded standardized incidence rates higher than that of the Greater Glasgow health board area. The reason for the wider disparity in standardized incidence rates and, in particular, for the very low rate for Greater Glasgow health board is not readily discernible. One is tempted to disregard and discount an hypothesis that there is a real difference in the incidence of salmonella outbreaks; that there is an association between the incidence of outbreaks and the health board areas; or that the number of outbreaks varies significantly according to health board area. It seems unlikely that such factors as food habits and kitchen practices, consumption rates of poultry and red meat, the sources of meat in retail outlets, salmonella contamination or carrier rates in poultry carcases, environmental cross-contamination, and laboratory diagnostic protocol are sufficiently varied between Scottish Health Board areas, to produce difference in incidence of salmonella outbreaks of the magnitude revealed by the retrospective analysis. One plausible explanation, therefore, is that the variation in the number of notified incidents most probably reflects differences in interest and intensity of epidemiological investigation and surveillance

of foodborne outbreaks, and in the notification of investigated incidents. For one thing, notification of foodborne infections and intoxications is passive rather than statutory; the CMS, the EHOs or other appropriate authorities in the various health board areas would tend to report incidents according to the priority accorded salmonellosis as a notifiable pubic health problem. Even so, why the Greater Glasgow health board would seem less keen to investigate or notify investigated incidents is another matter. The alternative inference, is that, for reasons not so obvious, residents of Greater Glasgow health board area are at much less risk of foodborne salmonellosis.

Nine hundred and forty-one persons affected in two hundred and seventy-four (19.3%) of the salmonella outbreaks were reported to have acquired their infection abroad. These outbreaks were considered to be imported because the suspected foods were consumed outside the United Kingdom. One hundred and twenty-seven (48%) of the imported outbreaks, affecting 491 people (52%) were due to *S.enteritidis*, while *S.typhimurium* accounted for 51 (19%) outbreaks involving 182 people. Two hundred and twenty-four (73%) of the imported outbreaks were acquired by 815 persons who visited eleven European countries. One hundred and sixty-nine (75%) of the ex-Europe outbreaks involved people who recently returned from Spain and Spanish territories (mainly Majorca, Ibiza, Benidorm and Tenerife). This reflects the popularity of these tourist resorts, but also underscores the high risk potential of certain meals consumed in those places. Thirty-one outbreaks (11.3%) affected visitors to 10 African countries, primarily North African countries of Tunisia and Morocco. One hundred and ninety-two (72.5%) of the 274 imported outbreaks, affecting 658 (70%) of the visitors were recorded during the months of June, July, August and September. This seasonal trend in the incidence of imported outbreaks is consistent with large scale movement of tourists and holidaymakers during the summer.

Salmonella typhimurium was isolated in a total of 602 (40%) of the 1422 outbreaks recorded between 1980 and 1987. *S.enteritidis* was responsible for 336 (22.6%) of the outbreaks; while *S.virchow* caused 197 (13%) of the outbreaks. Altogether, these top three serotypes accounted for 1,135 (76%) outbreaks. Ten other serotypes which caused at least 10 outbreaks over the 8-year period are, in ranking order, *S.stanley*, *S.heidelberg*, *S.agona*, *S.saint-paul*, *S.infantis*, *S.bredeney*, *S.hadar*, *S.panama*, *S.montevideo* and *S.anatum*. *S.typhimurium* ranked first in the order of frequency from 1980 to 1985; but in 1986 and 1987, *S.enteritidis* assumed the first position in the ranking order. A follow-up analysis of salmonella outbreaks recorded after 1987 showed that *S.enteritidis* continued to rank first during 1988 and 1989, accounting for 56 per cent of 281 salmonella outbreaks reported in the two years. *S.stanley*, *S.heidelberg* and *S.agona* were prominent only during 1981 and 1982.

The nine commonest phage types of *S.typhimurium* (110, 10, 204, 49, 12, 104, 193, 170 and 66, in ranking order) accounted for 84 per cent of the total incidence of this serotype. The two most commonly identified phage types of *S.enteritidis* (PT 4 & 8) accounted for 90 per cent of *S.enteritidis* outbreaks in which the phage types were specified. Phage type 8 predominated during 1980-85, while PT4 was most commonly identified in 1986 and 1987. Subsequent analysis revealed that PT4 continued to predominate in 1988 and 1989. The ranking order of salmonella serotypes and phage types identified in the cumulative outbreaks, corresponded remarkably with the order of salmonella types recorded specifically for poultry-associated outbreaks (83). There was also a marked similarity between the serotypes and phage types recorded in the outbreaks and those isolated from poultry and poultry meat reported to the CD(S)U (83).

An analysis of data on salmonella infections (laboratory isolations) showed a trend of steady increase both in the absolute numbers of infections recorded during 5-year

periods, and in the standardized incidence rates. The incidence rate rose from 14/100,000 population in 1968-72 to 37.5/100,000 in 1978-82, and 41.5/100,000 during 1983-87. Thus, between 1968 and 1987 there has been a three-fold increase in the incidence rate of human salmonella infections in Scotland. In the USA, there was also an increase of the incidence rate from 3/100,000 in 1955 to 17.4/100,000 in 1982 **(189)**. This implies that by 1982, the incidence rate of salmonellosis in Scotland was 2 times the rate in the USA. The incidence rate of salmonella infections was highest in the 0-5 years age-group (an average of 63.8/100,000 per year). Children 5 years old and below may not necessarily experience higher risk of exposure. Rather, because of their tender age and reduced resistance, persons in this age group tend to more easily succumb to infection and manifest severe symptoms of enterocolitis. Cases involving children are also more likely to present or be reported to the GP or the hospital. Similarly, most severe symptoms and highest case fatality rate are recorded among elderly persons. But as the data over the 20-year period show, the age-specific incidence rate in persons over 70 years is comparatively very low **(Tables 4.1.13A-D)**. There was no significant variation in the distribution of salmonella infections among the various age-groups during the four 5-year periods. Although higher proportions of infection were recorded in males or females during alternating 5 years intervals **(Table 4.1.14)**, there was significant difference (7.5%) in the mean standardized sex-specific incidence rates. The mean standardized incidence rate of salmonella infections was significantly higher in males than in females.

S.typhimurium, *S.enteritidis* and *S.virchow* were the three serotypes most frequently recorded in confirmed laboratory isolations, which represent both outbreak and sporadic incidents. *S.typhimurium* remained dominant throughout the study 20-year period; while *S.virchow* became prominent from 1978, especially during 1978-82.

There was nearly 400 per cent increase in the incidence of *S.enteritidis* from 7% in 1968-72 to 27.4% in 1983-87; most dramatic increases were recorded from 1986. Some serotypes such as *S.bredeney*, *S.reading*, *S.dublin*, *S.agona* and *S.panama* which were prevalent during 1968-72 or 1973-82 had become far less significant during 1983-87.

The annual and cumulative quarterly mean incidence of salmonella outbreaks demonstrated clear evidence of seasonal trend. The cumulative mean incidence increased by a geometric progression during the first three quarters of the year **(Table 4.1.10)**. The number of outbreaks during July - September was two times the incidence during April - June, and four times the number of outbreaks in January - March. During the last quarter (October - December) the incidence dropped sharply to the January - March level. Nearly fifty per cent of all the outbreaks occurred during the summer months of July, August and September. The cumulative incidence of salmonella infections (laboratory reported isolations) during the twenty-year period (1968-87) as well as the mean incidence at 5-year periods also followed a consistent seasonal trend **(Figures 4.8 & 4.9)**. More than 42 per cent of the cumulative infection were reported during the summer months. No significant seasonal variation was detected in the proportions of weekly batches of chicken carcases sampled during the bacteriological survey. This suggests there was no evidence of seasonal trend in natural infection of poultry by salmonella or in contamination of chicken carcases in nature. Therefore, the highest incidence of human infection and outbreaks observed during the months of July - September more or less reflect the life style and social activities of the human population during the summer months. Social factors such as eating practices, increased human mobility, increase scale of tourism and holiday-making, increased consumption of meals in commercial catering establishments, and large-scale purchase of items from "fast-food" canteens play a greater role in increasing the risk of human exposures and actual infection during the summer. In addition, environmental factors such as high temperatures and inadequate temperature

control of foods allow the background level of contamination to become a hazard.

From the 20 year retrospective analysis, the following hypotheses on the incidence, risk factors and trends of foodborne infections and intoxications may be drawn:

(1) Salmonellosis is the single most important reported foodborne zoonosis in Scotland. Over 80% of all foodborne outbreaks are caused by the salmonellae.

(2) An average of 1000 persons are affected in foodborne salmonella outbreaks each year; and various salmonella types are isolated from an average of 1500 persons per year.

(3) Foods consumed in the home (private households) and in commercial catering establishments (hotels, restaurants, "fast food" canteens) are the major risk factors for foodborne salmonellosis. Foods prepared and served in health institutions (hospitals, maternities, old folks homes) and in social receptions constitute the next significant set of risk factors.

(4) Highest risk of salmonella infection and salmonella outbreaks is associated with poultry meat. Eggs and egg-products have become important risk factors in recent years. Milk and red meat now constitute much less important risk factors.

(5) *S.typhimurium*, *S.enteritidis* and *S.virchow* are the most significant causes of foodborne salmonellosis in Scotland. *S.typhimurium* had been the primary cause of infections and outbreaks; since 1986, *S.enteritidis* has assumed the primary place. The upsurge in *S.enteritidis* is due to an unprecedented increase in incidence of phage type 4 in poultry products (meat and eggs).

(6) There is a consistent seasonal trend in the incidence of foodborne salmonella infections and outbreaks. More than 50 per cent of all incidents occur during the summer.

(7) Nearly one-fifth of the foodborne salmonella outbreaks are imported into Scotland; that is, acquired outside the UK. Approximately three-quarters of the imported outbreaks are acquired in Europe and 75 per cent of the ex-Europe episodes are following meals consumed in Spain and Spanish territories. About 73% of imported outbreaks were acquired during the summer months.

Two hundred and fourteen or 45% of the 477 raw chicken carcases sampled in the hospital kitchen were positive for one or more salmonella types. The proportions of contaminated carcases for the weekly batches ranged from 27 - 67 per cent. Many surveys of eviscerated chicken in the United Kingdom during the past fifteen years have reported contamination rates that fall within this range **(42, 43, 44, 46, 47)**. Other surveys have recorded levels of carcase contamination much higher than the median 50% observed in the present study **(41, 45, 46, 48, 49)**. In particular, Gilbert and Roberts (51) recorded a contamination level of 79%. In the present survey, a large number of carcases from different commercial sources in Scotland and England were sampled over a longer time scale (38 weeks). Compared with the other surveys, therefore, the level reported here may be a more accurate indication of the general rate of contamination of raw chicken carcases.

Perhaps of more significance and particular concern was the fact that every single batch of carcases sampled during the 38 weeks contained individually contaminated carcases. The range of contamination observed would seem to reflect the degree of salmonella contamination within the poultry industry by 1988. The public health implication is that every single consignment of raw carcases delivered to the hospital kitchen, and by inference to most other private and

public catering kitchens, contained salmonellae. This means that if there is any lapse in kitchen hygiene, cross-contamination of kitchen environment and/or other prepared foods is not only possible but likely. Any lapse in kitchen practices or any improper operation and functioning of kitchen equipment such as an oven or microwave would mean inadequate heat-treatment of salmonella contaminated carcases. The possible consequence is clinical or inapparent (latent) foodborne salmonella infection, or transient carriage and excretion of salmonella.

That the contamination level and the types of salmonellae detected in the carcase survey might reflect the degree of salmonella infection in the poultry industry, is supported by the fact that the salmonella types (serotypes and phage types) isolated from the raw carcases are very similar to those poultry salmonellae notified in England, Wales and Scotland in 1988, under the Zoonoses Order 1975 **(Tables 4.2.2 & 4.2.4).** Eleven of the 19 serotypes detected in the chicken carcases were also notified under the Zoonoses Order. The carcase serotypes were also very similar to poultry salmonellae reported to the CD(S)U by veterinary laboratories in 1988 under the Scottish Surveillance system **(Table 4.2.4).** Ten of the salmonella serotypes isolated from the carcases were again among the 12 most common serotypes detected in 1988 from poultry processing plants in Scotland (Reilly, W J, Unpublished Observation). In all the four sources (chicken carcases, Zoonoses Order, CD(S)U veterinary reports, and poultry plants), *S.enteritidis* and *S.typhimurium* ranked first and second (or first and third) **(Table 4.2.4 & Table 4.2.9).** The logical inference is that the hospital patients as well as other consumers of poultry prepared in kitchens in private homes or mass catering establishments were exposed to similar salmonella types.

The Moore's sewer swab has been known to be a sensitive index of the excretion and passage of enteric organisms, as it allows continuous sampling to be taken for periods which are related to the dietary intake of potential excretors **(161, 162, 175).** Sewer swabs taken in parallel with

sampling of the food sources have been shown to be of value in demonstrating salmonella excretion in hospital areas, including a paediatric ward, a maternity ward, and a hospital kitchen (179, 201, 202). By overcoming the problem of screening and identifying individual excreters in a large population setting, the sewer swab technique was of value in relating salmonella excretion by hospital patients to the infected or contaminated food items. In surveys carried out in a small population in a university hospital Harvey and Price (202) recorded positive swabs ranging from 5% to 31% of total swabs laid during four seasons. In the present survey, the sewer swabs proved a very useful method for monitoring latent salmonella infection and excretion in the "closed" relatively static hospital population. Salmonellae were detected from 30 of the 79 (38%) swabs examined over a 40-week period, when raw chicken carcases were used in the hospital kitchen. At least one of the pair of swabs was positive for salmonellae in 28 of the 40 (70%) weeks. Thirty-three salmonella isolates comprising of 13 serotypes were detected from the sewer drains during the 40-week period. During the period of the survey, six stool samples taken from the hospital patients were submitted to the Stobhill diagnostic laboratory/SSRL. All the six specimens were negative for salmonella and a variety of other pathogens and parasites **(Munro D, SSRL, Personal Communication).**

Investigations are still in progress to determine whether or not there were any incidents of enteric illness in any patients treated symptomatically, without microbiological investigation **(Reilly W J, Personal Communication).** Even in the absence of reported clinical salmonellosis, it is apparent that latent salmonella infection and excretion or transient carriage took place and that this was effectively monitored by using sewer swabs. The sewer survey detected the entry of an exotic salmonella serotype, *S.clichy* into the "closed" hospital population. This serotype, previously not reported in Scotland, was isolated in pure cultures from the two manholes and for two consecutive weeks. It was not possible to establish the source of the *S.clichy* in the

hospital under study, since the serotype was never recovered from the chicken carcases. However, the same serotype was isolated from two apparently unconnected cases in Scotland at approximately the same weeks of its recovery from the sewer drains **(Sharp J C M, Personal Communication)**. As was the case when some exotic serotypes were newly introduced, the epidemiological significance in Scotland of *S.clichy* may unfold in the future.

An hypothesis of an epidemiological relationship between the chicken carcases prepared in the hospital kitchen and salmonella excretion by the patients is based on the following observations:

(a) Identical salmonella types (serotypes and phage types) were isolated from both chicken carcases and the sewer drain, and during corresponding weeks. Eleven of the 16 (69%) different salmonella types detected in the sewer swabs had also been recovered from the carcases **(Table 4.2.2)**. Seven of the 11 salmonella types were isolated from chicken and sewer in corresponding or matching weeks. Each of the following salmonella types was detected in the sewer drain one week after it had been recovered from the chicken carcases: *S.enteritidis* PT4, *S.typhimurium* PT49 and 104, *S.virchow*, *S.minnesota*, *S.eimsbuettel*, *S.montevideo* **(Table 4.2.6)**. *S.enteritidis* PT4 was observed in both chicken and sewer during 5 matching weeks; *S.virchow* was observed in both sources during 3 matching weeks; while *S.typhimurium* was observed during 2 matching weeks. The isolation of these three salmonella types from both sources during corresponding weeks could be expected to occur by chance, considering the high frequency of the three types in chicken and in sewer. However, the isolation of *S.minnesota*, *S.eimsbuettel* and *S.montevideo* from carcases and drain in matching weeks is a different matter; these serotypes were recovered from the chicken only 6 or 7 times during the 38-week period. Altogether, the same salmonella

type was detected in both chicken carcases and sewer drains in 13 of 35 matching weeks (or week pairs). In all cases, the detection of the salmonella type in the sewer was preceded by the recovery of the same salmonella type in the carcases. In other words, the presumed risk factor (consumption of contaminated chicken or other foods cross-contaminated by the raw carcases) predated the observed outcome (salmonella infection and excretion). This means that the criterion of **time sequence** or **temporal association** for establishing causal relationship between a risk factor and infection would seem to have been satisfied. Application of the standard chi-square to test a null hypothesis of no association between salmonella types isolated from chicken and sewer during the 35 matching weeks, showed that the observed frequency of correspondence occurred in excess of what would be expected to happen by chance ($X^2_{mh} = 15.08$, $p < 0.005$). Thus, the data provided evidence to reject the null hypothesis of no association. The **strength of association** provided by the chi-square test satisfies another important criterion for establishing epidemiological association between the contaminated chicken carcases and (latent or transient) salmonella infection in the study population.

In chicken carcases and in sewer drains, the three most frequent serotypes (*S.enteritidis*, *S.typhimurium* and *S.virchow*) accounted for at least 50% of salmonellae detected. Again, in view of the predominance of *S.enteritidis* and *S.typhimurium* in the United Kingdom during the period of the survey, one would naturally expect the detection of these serotypes from chicken and sewer in matching weeks to occur by mere chance in a number of times. However, the three serotypes were observed in chicken and sewer in 11 of the 35 matching weeks, while the calculated expected frequency was 4.4 **(Table 4.2.6)**. These data provided a chi-square value of 9.19, with

$p < 0.005$. Thus, the observed frequency was clearly in excess of the frequency expected to occur by chance, and indicates a statistically significant association between the contaminated chicken and salmonella infection. Again, for establishing a causal association, the epidemiological **criterion of strength** seems to have been satisfied.

(b) The identical salmonella serotypes/phage types recovered from chicken and sewer exhibited somewhat similar antimicrobial sensitivity patterns. Two isolates of *S.virchow* obtained from chicken in the 42nd week and two isolates detected in the sewer in the corresponding (43rd) week showed identical antibiogram: all four isolates of *S.virchow* were resistant to sulphonamide and trimethoprim, but sensitive to the other six antimicrobial agents. However, isolates of *S.virchow* recovered from chicken in the 16th and 17th weeks and the isolates detected in the sewer during corresponding 17th and 18th weeks respectively, exhibited differing sensitivity patterns. All six *S.virchow* isolates from chicken were resistant to sulphonamide and trimethoprim, but sensitive to the other agents; in contrast, all three isolates from the sewers were resistant to chloramphenicol and tetracycline, in addition to sulphomamide and trimethoprim. But then, 9 isolates of *S.virchow* recovered from chicken carcases, in the 21st, 22nd and 23rd weeks, were resistant to chloramphenicol, tetracycline, sulphonamide and trimethoprim - an antibiogram similar to that of the three sewer isolates. This shows that *S.virchow* which contain the genetic factors coding for resistance to the 4 agents do in fact exist in some of the batches of chicken carcases. Therefore, the chances are not totally remote that the chloramphenicol - and tetracycline - resistant strains detected in the sewer were probably acquired from the chicken carcases.

All 19 isolates of *S.enteritidis* PT4 obtained from the raw carcases and all 6 isolates of PT4 detected in the sewer during five matching weeks, were sensitive to all the 8 antimicrobial agents tested. Similarly, 9 strains of *S.typhimurium* PT49 from chicken and 4 strains detected in the sewer in matching weeks, were sensitive to the 8 agents. So also were all the isolates of *S.typhimurium* PT104, *S.minnesota*, *S.eimsbuettel* and *S.montevideo* recovered from both chicken and sewer. Altogether, 68 salmonella isolates from chicken and sewer, representing 30 different serotypes/phage types showed identical antimicrobial sensitivity patterns. However, it is unsafe to draw any inference, on the basis of the antibiogram, that the salmonella types from chicken and sewer are epidemiologically related or not. There was little or no variability in the antibiogram of the most common salmonella serotypes/phage types in the chicken carcases. The sensitivity patterns are not sufficiently discriminating to allow valid epidemiological categorization or differentiation for each of the salmonella types. These observations thus support the findings of authorities cited in the Literature Review **(115, 135)**, that as an epidemiological marker or strain discriminating scheme, antimicrobial resistance is of much limited value.

(c) Towards the latter period of the bacteriological survey, precooked whole chicken became introduced into the hospital kitchen as a substitute for the raw chicken carcases. The change in policy, apparently due to the general media attention to the whole problem of foodborne salmonella infections, meant that raw chicken carcases were no longer permitted for patients. For a period of five weeks that specimens were taken from 10% random samples of the precooked chicken packs, none of the 102 packs examined was positive for salmonella. However, the use of precooked meat was not without risk. As many

as 29 (28.4%) were contaminated with coagulase-positive, DNase-positive, and possibly enterotoxin-producing *Staphylococcus aureus*. The survey of the precooked chicken indicates that adequate cooking is a satisfactory method of eliminating the salmonella organisms, although there is still the inherent risk of cross-contamination in the kitchen. This potential risk, in addition to the method and adequacy of cooking, may explain the observation in the case control study that the ratio of odds of exposure to precooked poultry meat in cases relative to controls (the Odds Ratio) was 2 **(Table 4.3.10)**. This means that the odds of exposure to precooked chicken among the cases was two-fold, and suggests a significant association between the consumption of precooked poultry meat and the risk of salmonella infection. In a recent case-control study of primary sporadic infections with *S.enteritidis* PT4 in England and Wales, Cowden and co-workers **(203)** also found that illness was significantly associated with eating precooked hot chicken (matched $p = 0.006$).

It is important to observe that the change in kitchen policy, that is, from raw to precooked chicken, coincided with or resulted in a marked change in the recovery rate of salmonella from the sewer. Whereas isolations were made from 38% of drain swabs and in 28 of the 40 weeks when raw chicken carcases were used in the kitchen, the rate dropped to 10% of the swabs and in only 1 of the 5 weeks after the change to cooked chicken **(Table 4.2.5)**. The removal of the presumed or associated risk factor (contaminated raw chicken) was followed by a statistically significant reduction in the unwanted outcome (salmonella excretion by the patients). This again suggests that the raw chickens were the important source of salmonellae to the hospital patients.

During the period of the bacteriological survey (1988), there were, in Scotland, 39 outbreaks of foodborne

salmonella infections associated with poultry. In 31 (80%) of the 39 outbreaks, the causative salmonella serotypes/phage types were the same as those isolated from chicken carcases or the sewer drains in the present survey (**Table 4.2.2**). In chicken carcases, sewer drains, and in concurrent poultry-associated outbreaks, *S.enteritidis* PT4 ranked first in frequency. This phage type accounted for 94%, 86% and 92% respectively of *S.enteritidis* recovered from the three sources. Outbreaks of salmonellosis in humans in England and Wales reported in 1988 by the Division of Enteric Pathogens of the Public Health Laboratory Service, show a similar trend. An epidemic increase in human salmonella infections in England and Wales during the period was largely due to a marked increase of *S.enteritidis* PT4. The increased incidence of *S.enteritidis* food poisoning was attributed to poultry, and specifically, to the spread of PT4 in chicken carcases and eggs (**55**).

S.enteritidis, *S.typhimurium* and *S.virchow* were the three most frequent serotypes detected in the chicken carcases and in the sewer. These same serotypes were the top three in the ranking order of serotypes isolated in 224 poultry-associated salmonella outbreaks in Scotland during 1980-85 (**83**); the three serotypes were topmost in the ranking order of serotypes responsible for salmonella outbreaks analysed in the retrospective study; they were also the most frequent causes of outbreaks in England and Wales. These observed similarities would seem to satisfy some of the other criteria widely used to establish the likelihood of causal relationships. The criteria are **consistency** and **biological plausibility**. Fulfillment of these criteria further support the hypothesis that poultry meat is the primary risk factor in the epidemiology of foodborne salmonella infections.

Because of the pre-eminence of *S.typhimurium* in livestock other than poultry, especially in cattle, it can be argued that red meat may as well be the source of the major salmonella serotypes detected in the sewer drains; that beef, and not necessarily poultry meat, is the principal risk factor in human salmonellosis. In 1987 in Scotland

S.typhimurium placed first in the ranking order of serotypes reported to the CD(S)U for cattle from veterinary sources; the serotype accounted for 80.7% of all the salmonella isolates from cattle (204). In 1988, *S.typhimurium* ranked first in the incidents of salmonella infections in cattle, sheep, and pigs in the United Kingdom reported to the State Veterinary Service under the Zoonoses Order 1975; the serotype was responsible for 47% of all serotypes isolated in cattle (33).

However, the ranking order of the phage types recorded in cattle were not similar or related to the order of phage types detected in foodborne incidents during 1980-87. Thus, *S.typhimurium* PT110 and 10 were the top two recorded in human incidents (**Table 4.1.9; 83**) and accounted for 36% of all phage types recorded. In cattle, these two phage types ranked fifth and seventh (83) and accounted for only 2% of *S.typhimurium* phage types isolated (204). While phage types 204c, 204a, and 204 ranked first, second and third in cattle (83, 204), the three phage types ranked 8th, 7th and 5th in humans (83). A more valid evidence against cattle being the primary source of the excreted salmonellae is that in 1987, no isolations of *S.enteritidis* were recorded in cattle in Scotland (204), and in the whole United Kingdom in 1988, only 16 incidents of *S.enteritidis* (all phage types) were reported for cattle under the Zoonoses Order (33). This was in the year that *S.enteritidis*, particularly PT4 was overwhelmingly predominant in human foodborne incidents in Scotland (**Table 4.1.9**) and in England and Wales (55, 60). During the same period, *S.enteritidis*, in particular PT4, predominated in poultry, poultry meat, and poultry-associated outbreaks (55, 60, 81, 83; **Table 4.2.2**). Further, the case-control study established that there was no statistically significant association between salmonella infection and consumption of red meat - beef, pork/ham, lamb (**Table 4.3.7**). With an odds ratio value significantly less than one, exposure to red meat did not increase the risk of salmonella infection.

The association between poultry meat and human salmonellosis was perhaps better established and clarified by the case-control study. Eating chicken was significantly associated with salmonella infection (Mantel Haenszel Odds Ratio = 4.2; **Table 4.3.5**). Cases were significantly more likely to have eaten poultry meat in the 48 hours before onset of illness than were matched household controls (X^2 = 19.25, $p < 0.005$; **Figure 4.11**). In contrast, eating red meat was not associated with salmonella illness (Odds Ratio = 0.38; **Table 4.3.7**). When the form of the poultry meat was assessed, consumption of frozen poultry meat was significantly associated with illness (Odds Ratio = 4.0; **Table 4.3.8**). The odds of exposure to frozen chicken was four-fold among cases relative controls; thus, eating frozen poultry meat was estimated to increase the risk of infection by 300 per cent. Consumption of fresh poultry meat was also significantly associated with illness (Odds Ratio = 2.58), but fresh poultry meat was estimated to increase the risk of infection by 158 per cent. There was also a positive association between illness and eating precooked chicken; but precooked poultry meat elevated the risk of infection by 21 per cent **(Table 4.3.10)**. With respect to the method of cooking, consumption of roast poultry meat was significantly associated with salmonellosis (Mantel Haenszel Odds Ratio = 4.0, **Table 4.3.11**). Eating roast chicken or turkey increased the risk of salmonella infection by 300 per cent! On the other hand, consumption of boiled chicken was negatively associated with illness (Odds Ratio = 0.56; **Table 4.3.12**). Eating boiled chicken did not increase the risk of salmonella infection, suggesting that boiled chicken is not a significant risk factor. From the data, eating boiled chicken reduced the risk of infection. Any association between salmonellosis and the frequency of consumption of poultry meat in a typical week was not clearly and conclusively established. There was no association between illness and eating chicken 1 or 2 days in an average week **(Table 4.3.13)**. However, eating chicken 3 or 4 days in a typical week was significantly associated with salmonella infection (Odds Ratio = 2.26; **Table 4.3.14**). The number of cases and controls who indicated they ate poultry meat 5-6

days, or 7 days in a typical week were too few to enable reasonable assessment of the respective odds ratio.

Certainly, the cases included in the case-control study, although they constituted 73% of the base-population of notified laboratory-confirmed cases, represented only a proportion of all sporadic and household incidents of foodborne salmonellosis in the area of study. Generally, notified cases represent those foodborne illnesses that were serious enough to present to the general practitioner or the hospital. Usually, milder episodes do not present and, therefore, are not often investigated by the environmental health officers. This fact notwithstanding, the numbers of cases and controls used in the study are sufficiently large for statistically acceptable analysis. The respondent cases constituted 81% of cases formally reported. Response rates of 63%, 69%, 72% and 81% have been shown to be sufficient to establish associations in other recent case-control studies of salmonella infections (186, 203, 205, 206).

The case-control study provided sufficient evidence to reject the null hypothesis that there is no association between salmonella infection and:

(1) the type of meat (poultry meat or red meat) consumed;

(2) the consumption of poultry meat in the two days before onset of illness;

(3) the form of poultry-meat purchased (fresh, frozen, precooked);

(4) the method of cooking poultry meat (boiling, roasting etc); and

(5) the number of days in a typical week that poultry meat is eaten.

The study clearly implicated poultry meat, in particular frozen and roasted poultry meat, as the main vehicles of

infection in sporadic and primary household incidents of foodborne salmonellosis. The epidemiologic association established by the case-control study satisfies the **criterion of strength** - which here refers to the ratios of exposure to the hypothesized causal factor among persons with and without the illness (207, 208). The strength of an association is described by an increased risk which is experienced by individuals who are exposed to the risk factor (in this case, poultry meat). In the present study, the strength and significance of the association have been established by both the Odds Ratio analysis and the Chi-square test.

Apart from the statistical evidence, the three epidemiological approaches employed appear to have fulfilled other formal criteria for causal association (207, 208). Schelessman (187) has noted that, in observational studies, one need not be too concerned with the problem of multiple comparisons leading to chance associations; a decision regarding the effect of an exposure variable should never be based solely on a statistical p - value! Biological plausibility and consistency of the evidence within and across studies are also important elements in the interpretation of associations, Schelessman advised. The strong association of salmonellosis with eating poultry established by the case-control study is consistent, and supports the epidemiological evidence provided from

(i) the bacteriological survey of chicken carcases and sewer drains;

(ii) the retrospective analysis of foodborne outbreaks and salmonella infections (laboratory reported infections);

(iii) the reports and studies from public health and veterinary laboratories in Great Britain and abroad (55, 60, 81, 83, 150, 189, 193, 194); and

(iv) other concurrent case-control studies in the United Kingdom.

In a matched case-control study carried out in England and Wales between August and September 1988 to determine the source of indigenous sporadic infections with *S.enteritidis* PT4, Cowden and others (203) reported that illness was significantly associated with consumption of raw shell egg products (home made mayonnaise, ice cream and home-made raw egg-containing milk products). Illness was also significantly associated with eating lightly cooked eggs, but not with boiled eggs and with ready prepared (precooked) hot and take-away chicken. In another case-control study in south-east Wales, focusing on consumption of eggs, Coyle and Co-workers (186) found a statistically significant association between sporadic *S.enteritidis* PT4 infection and eating eggs or egg products in the 3 days before onset of symptoms. However, chicken consumption did not differ significantly between cases and controls in this small study; only 19 cases and 19 controls participated in the study. But in the study by Cowden and others (203), one, two or three matched controls (total 196) were obtained for each of 157 cases. Cowden's case-control study, for the first time in a large national study in England and Wales, confirmed poultry products as significant vehicles in indigenous salmonella infections. In view of concurrent reports that *S.enteritidis* PT4 was the commonest salmonella type in fresh and frozen chicken on retail shelves in England and Wales (55, 60). Cowden and co-workers described as "surprising" their observation that the controls were more likely than cases to have eaten poultry other than ready prepared hot and take-away chicken.

The strong association between salmonella infection and consumption of frozen and roast poultry meat observed in the present case-control study is not spurious nor surprising. An interpretation of a causal nature of this association is plausible and consistent with current knowledge about poultry processing and the natural history of the salmonella organism. In the processing operation, eviscerated carcases

are chilled before storage. In most plants, carcases intended for fresh poultry trade are cooled in a blast chiller, while frozen birds are cooled in a spin chiller. In the latter procedure, the carcases are immersed in a cold water bath. More bacterial contamination tends to build up under this system and all carcases may be exposed to contamination. The result is that as many as 65% of frozen and 55% of fresh broiler carcases may carry salmonellae **(15)**, and contamination rate of up to 79% has been reported **(51)**. The ability of salmonella to survive prolonged periods of time in frozen foods is well established **(189)**. *S.enteritidis* could be detected readily from poultry and minced beef held for 4 months at - 18°C **(209)**. *S.enteritidis* and *S.typhimurium* were isolated from ice cream held at - 23°C for 7 years! **(210)**.

The bacteriological survey carried out in a long stay psychiatric hospital was not specifically intended to be an epidemiological surveillance of the salmonellosis problem in the particular institution or in the National Health Service (NHS) premises. However, it is relevant that between 1973 and 1987, there were 98 hospital-based outbreaks of food poisoning reported in Scotland **(211, 212)**. A total of 3,803 persons (patients, neonates, hospital staff, ancillary personnel, visiting relatives) who consumed hospital-prepared food were affected, 19 of whom died. Between 1973 and 1977, fifty outbreaks occurred in 33 hospitals, 9 of which reported 2 or more episodes. Twenty-two of the 50 outbreaks occurred in hospitals for psychiatric or subnormal patients **(211)**. Between 1978 and 1987, there were 48 outbreaks reported in 34 hospitals, 10 of which experienced 2 or more episodes **(212)**. Eleven (22%) of the 50 outbreaks in 1973-77 were due to foodborne salmonella infection; while in 1978-87, salmonella accounted for 17 (35%) of the 48 episodes. Among the 11 salmonella outbreaks recorded in 1973-77, frozen poultry which appeared to have been inadequately de-frosted, undercooked, or contaminated after cooking were incriminated in 5 outbreaks. In 1978-87 poultry meat accounted for 9 (75%) of 12 salmonella episodes where a food vehicle was identified. An epidemiological

investigation of a major outbreak of foodborne salmonellosis in a Lanarkshire maternity hospital in 1985 found conclusive bacteriological evidence that uncooked frozen chicken delivered to the hospital harboured *S.enteritidis* PT8 and that contamination of a freezer and possible cross-contamination of other food stuffs had occurred **(146).** *S.enteritidis* PT8 was isolated from 75 **clinical cases** and **symptomless excreters.** This finding underscores the phenomenon of inapparent, latent salmonella infection and symptomless excretion or transient carriage in a semi-closed hospital community, and justifies the approach of bacteriological survey of sewer drains in a hospital adopted in the present study.

With the evidence of an epidemiological association between poultry meat and human salmonellosis presently established, some of the reasons for poultry-borne salmonellosis are readily discernible:

(1) Considerable changes in the eating habits in the industrialized countries, in particular the increased consumption rate of poultry meat and other poultry products. Poultry meat had become the cheapest animal protein available; and public awareness of the association between blood cholesterol levels and consumption of red meat meant a shift from beef and pork to poultry meat. Indeed, because of the high incidence of cardio-vascular mortality, people are advised and encouraged to eat more poultry meat and less red meat.

(2) The endemicity of salmonellae in poultry feeds, poultry flocks and poultry farm environment; the speed and complexity of modern slaughter and processing with in-built critical points for cross-contamination; and the high incidence of carcase contamination with a variety of salmonella serotypes and phage types **(see Chapters 1.4 and 1.5).**

(3) The expansion of "fast-food" outlets and other

commercial catering establishments; the ubiquitous sale of precooked hot and cold poultry meat (whole or portions); and the advent of frozen-food chain which allows distribution of potentially contaminated products over a very wide geographical area and over prolonged period of time;

(4) The fact that a number of retailers still sell both cooked and raw meat at the same counter, where both are weighed, cut-up, and wrapped by the same assistant (213).

The natural consequence of the above circumstances, is that salmonella infected and contaminated poultry meat and products are regularly and constantly introduced into the kitchen environment in most private homes and health institutions, as well as in commercial catering establishments. Any lapses in kitchen hygiene and kitchen practices would result in human exposures with consequent clinical or inapparent (latent) salmonella infection or large scale outbreak! Studies in the United Kingdom and other countries (82, 88, 89, 189, 211) have identified such lapses and they include:

(i) Inadequate chilling and refrigeration of fresh food stuffs, often caused by a break down or improper functioning of cold-storage facilities.

(ii) Inadequate thawing or defrosting of frozen poultry carcases and other meat. It is observed that the procedures carried out from thawing a frozen chicken or turkey carcase to cooking the meat offer many opportunities for the survival and multiplication of the salmonella organism inside inadequately thawed and under-cooked portions of the carcase (89).

(iii) Inadequate cooking of raw poultry meat by roasting or even by boiling. Frozen carcases are more likely to be associated with undercooking. Large

frozen turkey (25 lbs or more), because of their size, create difficulties in thawing and cooking adequately to prevent multiplication of surviving salmonellae (90).

(iv) Slow cooling and improper hot storage of cooked food. Use of improper holding temperatures was identified as one of the single most common faults in food handling practices (88, 89).

(v) recontamination of cooked food from raw meat, and inadequate re-heating of potentially contaminated precooked meat.

(vi) cross-contamination of table surfaces, utensils, knives, and hands of kitchen staff through faulty and unhygienic handling of raw poultry meat and other raw ingredients. Although cross-contamination appeared quite low on the list of contributory factors recorded by Roberts (89), it probably plays a much greater part than is indicated (214). Economic pressures on institutional (hospital) and commercial catering establishments (hotels, restaurants, take-away shops) may encourage the development of faulty and unsatisfactory kitchen practices which allow cross-contamination to occur (83). Collier, Sharp and Gilbert (212) observed that it would be interesting to speculate what effect the trend towards privatization of catering services will have on the future incidence of hospital food poisoning. Investigation into a recent outbreak of food poisoning in a psychiatric hospital in south east England showed evidence of poor food handling by the catering staff, and the reasons were attributed to staffing levels and morale of the catering and cleaning department - a situation, the authors claimed, was worsened by the introduction of competitive tendering (215). In a paper titled "Crisis in our hospital

kitchens", the authors reported that morale was low because of staff shortages resulting from a long-term recruitment problem; staff worked double shifts and supervision was inadequate. Interviews with catering staff suggested that this crisis way of working culminated in the events which resulted in the outbreak. The authors cited other reports which indicated that understaffing, inadequate supervision, and falling standards of ancillary services have been implicated in outbreaks of food poisoning in hospitals.

During the salmonella survey of chicken carcases, the following observations were made in the hospital kitchen:

(1) Frozen chicken carcases delivered to the kitchen for the week were laid out in trays to defrost, usually in the evening before the morning of cooking.

(2) Trays of fresh and frozen chicken carcases (both wrapped in cellophane bags and unwrapped) were left overnight or for days in the same cold room with other food stuffs (precooked beef, minced meat, large sausage rolls etc).

(3) In the morning of cooking, the trays of raw chicken carcases were laid out on trolleys either in an adjacent room or sometimes left in the open hall where many kitchen activities take place, with constant movement of kitchen staff who were handling different food stuffs.

(4) On a number of occasions, trays of precooked beef and sausages were left at the opposite end of the same table on which samples were being collected from the raw chicken carcases.

(5) Before cooking, the chicken carcases were often washed in large sinks, which were also used by staff preparing other food items.

(6) On a number of occasions, the liquid soap containers attached to the wash-hand sinks were dry, with no soap available for washing hands.

(7) Chicken was cooked either on the same day it was served to the patients or on the previous day. In at least one week, sampling of carcases was not carried out because no chickens were cooked that week as a result of oven break down! Cooked chicken were stored chilled until served.

Although human salmonellosis causes very low numbers of deaths, as a foodborne zoonosis the disease has considerable social and economic consequences on the patient and the health institutions, in addition to the economic impact on agriculture and the food industry **(Chapter 1.3)**. Estimates for poultry-borne outbreaks have been prepared on the basis of direct and indirect costs **(28, 29, 189)**. Direct costs include expenses associated with epidemiological investigation of incidents, laboratory diagnosis, treatment of patients and loss of income by the affected persons **(189)**. Indirect costs relate to arbitrary monetary compensations for grief, pain and suffering and for loss of life. In the USA, it has been estimated that human salmonellosis might be responsible for losses amounting to 1.2 billion dollars each year (20-23). In the Federal Republic of Germany, the costs of human salmonellosis in 1977 was estimated to be 120 million German Marks for sickness and death (3). In Scotland, the cost per reported case of poultryborne salmonellosis in 1985 was estimated to range from £900 to £3,655, and the estimated total costs of reported and unreported cases were in excess of £10,000,000 each year - based on the maximum upper bound predictions of costs of unreported cases (28, 29). To Agriculture and the poultry industry, the direct and indirect costs arise from morbidity, mortality and culling rate in poultry flocks, condemnation of carcases at processing; epidemiological surveillance of poultry farms and processing plants; laboratory examinations; enforcement of legislation on the safe production, processing, distribution, retailing and

preparation of poultry and poultry products; losses sustained because of negative publicity, re-call of condemned products, and legal settlements **(28, 29, 189)**. Thus, in addition to the amended Zoonoses Order 1989, sixteen other orders were promulgated in 1989 by the Ministry of Agriculture to control the various aspects of the salmonellosis problem. By December 1989, over one million birds in 87 flocks in the United Kingdom had been destroyed.

With poultry and poultry products strongly established as the principal risk factor in foodborne salmonellosis, the prevention and control of sporadic and outbreak incidents requires the elimination of the salmonellae, in particular *S.enteritidis*, *S.typhimurium* and *S.virchow*, from both broiler and layer flocks. The achievement of such a long term goal is primarily dependent upon the work and efforts of the veterinary profession and the poultry industry. The control of salmonellosis has become a most challenging task to veterinarians and the industry, since modern poultry production and processing became highly complex and intensive, with interdependence of various aspects of husbandry, slaughtering and processing. Measures adopted by the industry for the control of poultry salmonellosis as well as veterinary public health activities have been reviewed **(Chapter 1.6)**. Eradication programmes have been successful in elimination of host-specific *S.gallinarum* and *S.pullorum* from the poultry industry in most industrialized countries. However, retrospective analysis of control measures and their impact on the bacterial quality and safety of poultry and other animals products have been disappointing because problems identified decades ago still feature prominently on the prevalence of salmonella in the human food chain **(88)**. Improvements in the farm and in processing plants have not had significant effects in reducing salmonella infection of the flocks and contamination of the final products. Contaminated carcases continue to reach the consumer!

The demonstrable increase in the incidence of foodborne salmonellosis, especially arising from poultry products (meat and eggs), and the growing public concern, have made the need ever more urgent to reduce the levels of flock infection, food contamination, and human incidents. Various lines of approach, beginning at the farm and going right through to the kitchen have been advocated or implemented. In the wake of the "salmonella-in-eggs" crisis in Britain in 1988-89, the Minister of Agriculture, Fisheries and Food in 1989 promulgated a series of legislative Orders aimed at reducing salmonella infection in poultry production, minimizing cross-contamination during processing, and improving the salmonella surveillance network. Some of the legislations are:

(1) The Poultry Breeding Flocks and Hatcheries (Registration and Testing) Order 1989 **(218)**. This Order prohibits a person from keeping a breeding flock on any premises or from using any premises as a hatchery unless his name is entered in the Breeding Flocks Register or in the Hatcheries Register in respect of such premises. The Order also requires a registered person to ensure that samples are taken in respect of the breeding flock or hatchery and are submitted to a laboratory for testing for the presence of salmonella. The person in charge of the laboratory is required to ensure that the result of the test is reported to the person submitting the sample or otherwise to the person who is registered. The registered person is required to keep records of samples taken and, of the results of tests, and also to keep record of the movement of poultry, chicks and eggs onto and off the premises.

(2) The Poultry Laying Flocks (Testing and Registration etc) Order 1989 **(218)**. This requires the owner or a person in charge of a laying flock, that is, a flock of poultry consisting of not less than 25 birds which are kept for the production of eggs for human consumption to ensure that samples are taken in

respect of the flock and are submitted to a laboratory for testing for the presence of salmonella. The order applies also to a person in charge of a flock of less than 25 birds the eggs of which are sold for human consumption. The persons in charge are required to keep records of samples taken, of the results of tests, and of the movement of any poultry onto or off the premises.

(3) The Processed Animal Protein Order 1989 **(219)** which re-enacts the Diseases of Animals (Protein Processing Order 1981), enables authorized officers to take for testing at a laboratory samples of processed animal protein from the premises where it is produced. The Order requires the registration of animal protein processors, imposed the onus on the registered person to ensure the taking of samples and its submission to a laboratory for testing for salmonella; requires the registered person to keep records of the results of tests and to give information to enable the tracing of contaminated feeding stuffs.

(4) The Zoonoses Order 1989 **(72)** which revokes and re-enacts with amendment The Zoonoses Order 1975 **(71)**, provides for the declaration as an infected place, of premises on which there is or has been an animal or any poultry in which salmonella is or was present, and the imposition of movement restrictions. The Order empowers an official inspector to carry out such examinations and tests, and to take such samples as are necessary to ascertain whether or not salmonella is or has been present. Unlike the Zoonoses Order 1975, the 1989 amendment requires the person in charge of the laboratory, rather than the owner of the flock or premises, to report to the State Veterinary Service the presence of salmonella and the identification of salmonella in a sample taken from the animals or birds in the premises.

Since production of salmonella-free poultry and the processing of salmonella-free poultry meat is a remote, if not impossible goal, a more direct approach to the salmonellosis problem is some form of safe treatment of the processed carcases before they get to the retail market. Such a measure of rendering the final product "safe" for human consumption will be similar to measures that have already been accepted and have proven effective for other "high risk" raw foods, such as statutory heat treatment of liquid egg and, in Scotland, the mandatory pasteurization of cows milk (77, 78, 83). Since pasteurization is unsuitable for poultry meat, ionizing radiation has been advocated for poultry carcases. Although this process is at present not generally permissible in the UK and is viewed with some concern by a section of the public, a recent report (the Advisory Committee on Irradiated and Novel Foods, 1986) (220) has recommended its use within limits that are suitable for poultry meat for human consumption. A recent commissioned economic assessment of poultry borne salmonellosis has established the cost-effectiveness of irradiation as an alternative control strategy for poultry salmonellae; the report established that the public health benefits outweigh the cost of irradiating poultry meat (28, 29). Irradiation has been accepted as a satisfactory and safe process by the World Health Organization, the Food and Agriculture Organization, the International Atomic Energy Agency and by some countries (221). The primary role of poultry meat in human salmonellosis is now clarified, the epidemiological association has been abundantly demonstrated, and the enormous social and economic costs of poultry meatborne salmonellosis has been established; it is hoped and expected that these facts will lend significant pressure to introduce irradiation or other effective and safe treatment of poultry carcases.

Until salmonella-free poultry can be produced, until some form of safe treatment of the poultry carcase is introduced, prevention of human infection and outbreaks will depend on sound kitchen hygiene and proper kitchen practices. Indeed, good kitchen hygiene has been described as the final line of

defence against salmonellosis (215). One indirect approach to the problem is to continue to advance and sustain public awareness of food poisoning hazards, especially arising from salmonella contamination of poultry products (meat and eggs). An intensive campaign and education need to be maintained advising on correct procedures for domestic, hospital, and commercial kitchen hygiene and kitchen practices. Thorough cooking of poultry meat and eggs, adequate heat-treatment of bulk liquid eggs, and prevention of re-contamination of cooked meat and other food stuffs remain the ever dependable safe-guards. A recent news report on the inadequacy and ineffectiveness of some brands of microwave oven for thorough cooking of certain foods, including poultry carcases, is relevant. To avoid or keep cross-contamination to a minimum, separate surfaces and equipment; and separate kitchen staff (where applicable); regular hand washing with soap, particularly after handling raw foods like poultry carcases, have been stressed (215).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

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6.1 CONCLUSIONS:

On the basis of the data from the retrospective analysis, the bacteriological surveys, and the case-control study, the following conclusions can be drawn:

(1) Salmonellosis is one of the most important foodborne zoonoses in Scotland. Eighty-five per cent of all foodborne outbreaks are caused by the salmonellae.

(2) Human salmonella food poisoning has been increasing in Scotland, as in England and Wales. Based on laboratory-confirmed isolations, there has been a threefold increase of salmonella infections during the past 20 years - from a crude incidence of 3635 infections in 1968-72 to 10,210 during 1983-87.

(3) An average of 1000 persons are affected in foodborne salmonella outbreak incidents each year. Laboratory isolations of the various salmonella types are made from an average of 1400 persons per year.

(4) The standardized incidence rate of salmonella infections in Scotland is approximately 30 per 100,000 persons per year. Given the indication that only 1 to 10% of foodborne salmonella cases may be reported, the true magnitude of the incidence rate falls probably between 300 to 3000 per 100,000 population per year!

(5) There is a trend towards marked annual increases in the standardized incidence rate of human salmonellosis - from 14 per 100,000 population in

1968-72, to 19/100,000 in 1973-77, and 42/100.000 during 1983-87. The incidence rate of human salmonellosis in Scotland shows a 300 per cent increase between 1968 and 1987!

(6) Standardized age-specific incidence rates confirm that persons within the age-groups, 0-5 years are at highest risk of foodborne salmonellosis. Infants 1 year of age or younger are particularly susceptible.

(7) Although clinical salmonellosis is more severe and the case fatality rate is higher in elderly persons, the standardized age-specific incidence rate of salmonella infections in persons above 70 years is relatively very low. Decreased resistance may account for the severity and higher case fatality in the elderly.

(8) Higher proportions of salmonella infections were recorded for males or females during alternating 5-year periods; however, the standardized incidence rate is significantly higher in males than in females.

(9) The bacteriological survey results and the reports of other studies suggest that between 50 to 80% of raw chicken carcases in retail outlets, or delivered to domestic, institutional and commercial kitchens are contaminated with salmonellae.

(10) Every batch, every single consignment of raw chicken carcases delivered to hospital kitchen, and by inference all such deliveries to private and public catering kitchens contain individually contaminated carcases. The salmonellae are continually getting into domestic and commercial kitchens. The consequence for the consumer is that all raw poultry carcases should be regarded as potentially infected or contaminated with salmonella.

(11) Lapses in kitchen hygiene and kitchen practices create the opportunities for cross-contamination of kitchen environment and other cooked foods.

(12) The bacteriological survey established that *S.enteritidis*, *S.typhimurium* and *S.virchow* are the most prevalent salmonella serotypes in chicken carcases in Scotland. The most common salmonella phage type in chicken is *S.enteritidis* PT4; this salmonella type has predominated since 1986.

(13) Veterinary notifications in Scotland, England and Wales (under the Zoonoses Order) also show that *S.enteritidis*, *S.typhimurium* and *S.virchow* are the most common serotypes in poultry and poultry products. These notifications now reveal a rise in the incidence of *S.enteritidis* over *S.typhimurium*, due mainly to increase in isolation of *S.enteritidis* PT4. Veterinary reports to the CD(S)U under the Scottish Surveillance Programme show a similar trend.

(14) Clinical and inapparent (latent) salmonella infection as well as transient carriage in a population of patients in a "closed" long-stay hospital can be shown to take place, by continuous monitoring of the sewers draining the residential accommodation of the patients.

(15) By overcoming the difficulty of screening and identifying individual excretors, the sewer swab technique seemed valuable and effective, even in the absence of any reported clinical incidents, in associating salmonella excretion by the patients to contaminated raw chicken carcases supplied to the hospital kitchen.

(16) *S.enteritidis* PT4, *S.typhimurium* and *S.virchow* were the three most frequent serotypes detected in sewer drains, as in the raw chicken carcases. The salmonella serotypes and phage types detected in the

sewer were clearly similar to those recovered from the chicken carcases during matching weeks. The isolation of the same salmonella types from chicken and sewer during corresponding weeks occurred more frequently than could be expected to happen by chance.

(17) These salmonella serotypes were isolated in an epidemiological setting circumscribed by time and place, and were shown to be of the same phage types: they were isolated from a semi-static population of patients in a "closed" long-stay hospital, and from raw chicken carcases prepared in the hospital kitchen within 7 days prior to their detection in the sewer!

(18) A change in policy, from raw carcases to precooked chicken resulted in a significant drop in the recovery of salmonellae from the sewer. The removal of the presumed risk factor (contaminated raw chicken) was followed by a significant reduction of the unwanted outcome (salmonella excretion by the hospital food consumers).

(19) The observations from the bacteriological surveys of carcases and drains, and from the veterinary notifications are consistent with the predominance of the three salmonella serotypes, in particular *S.enteritidis* PT4, in concurrent foodborne salmonellosis.

(20) Poultry meat (fresh or frozen) is the primary vehicle of foodborne salmonella infections and outbreaks. Since 1980 at least, poultry meat has remained the most significant risk factor in salmonella food poisoning in which the attributable food was identified.

(21) Between 1975 and 1987, there has been a three-fold increase in the proportion of salmonella outbreaks associated with poultry. This trend is consistent

with a similar three-fold rise in the proportion of poultry-associated episodes recorded in England and Wales between 1955 and 1985.

(22) In Scotland, in England and Wales, in Europe and in North America, the place of poultry as the primary vehicle associated with human salmonellosis has remained unchanged in the past 10 years.

(23) From the case-control data, poultry meat is clearly and significantly associated with sporadic and primary household salmonella infections.

(24) Highest risk of salmonella infection is associated with consumption of frozen poultry meat (chicken or turkey). Eating fresh chicken is less significantly associated with salmonella infection.

(25) Consumption of roasted poultry meat (chicken or turkey) is strongly associated with sporadic and outbreak incidents of foodborne salmonellosis.

(26) Cooking is a satisfactory method of eliminating salmonellae in poultry meat, although there is the potential risk of cross-contamination of kitchen environment from the raw meat before it is cooked. Case control data presented suggest that pre-cooked take-away chicken is less significantly associated with salmonella infection. Inadequate cooking, recontamination and cross-contamination from uncooked meat and from kitchen environment are the likely contributory factors. Consumption of chicken cooked by boiling significantly reduces the risk of associated with salmonellosis.

(27) There is no conclusive evidence of an association between foodborne salmonellosis and the frequency of eating poultry meat. Consumption of poultry meat 1 or 2 days in a typical week is not associated with salmonella infection. However, eating poultry meat 3

or 4 days in an average week is significantly associated with salmonellosis.

(28) *S.enteritidis*, *S.typhimurium* and *S.virchow* are the major causes of foodborne salmonellosis in Scotland.

(29) Prior to 1986, *S.typhimurium* was the primary cause of foodborne infections and outbreaks. Phage types 110, 10, 204, 49, 12, 104, 193 and 66 in ranking order, were most commonly identified in outbreak and sporadic incidents.

(30) Since 1986, *S.enteritidis* has assumed the primary place as the cause of foodborne infections and outbreaks. Between 1968 and 1987, there has been a four-fold increase in the incidence of *S.enteritidis*. The upsurge in *S.enteritidis* is due to unprecedented increase in incidence of phage type 4 in poultry products (meat and eggs). The incidence of this serotype in cattle, sheep and pigs, and in red meat during the same period was comparatively very, very low.

(31) The predominance of *S.enteritidis* (PT4), *S.typhimurium* and *S.virchow* in chicken carcases, in sewer drains, and in sporadic and outbreak incidents of foodborne salmonellosis in Scotland is consistent with reports by the Public Health Laboratory Service for England and Wales.

(32) From the retrospective analysis, the bacteriological surveys, and the case-control study, the epidemiological criteria of **strength of association**, **time sequence** (**temporal association**), **consistency** and **biological plausibility** are sufficiently satisfied to accept an hypothesis of significant association between poultry meat and human salmonellosis.

(33) Red meat (beef, pork/ham, lamb) is a less important vehicle of salmonella infection, as consumption of red meat is not significantly associated with foodborne salmonellosis.

(34) In more recent years, eggs have increasingly become an important risk factor for salmonella infections in Scotland, as in England and wales. *S.enteritidis* PT4 is the organism most frequently implicated in egg-borne sporadic and outbreak incidents. From outbreak and case-control data, illness is clearly associated with consumption of raw and lightly cooked egg and egg products. Softly-boiled eggs, scrambled eggs, scotch eggs, and raw shell egg products such as home made mayonnaise, ice cream, milk shakes are the identified sources of infection.

(35) Milk now constitutes a much less important risk factor in human salmonellosis. Prior to introduction in Scotland, of compulsory pasteurization of cows milk (1983) milk was a major vehicle of outbreak incidents.

(36) Foods consumed in the home (private households) and in hotels, restaurants, "fast-foods" and take-away canteens are the primary risk factors for foodborne salmonellosis. Foods prepared and served in hospitals, maternities or old peoples homes and in social receptions (such as wedding) constitute the next significant set of risk factors.

(37) National data show wide variation in number of salmonella outbreaks in the Scottish Health Board areas. The standardized incidence rates for Grampian and Lothian health boards are 1.5 times above the national average, while the incidence rate for Greater Glasgow Health Board is 3.5 times below the national average. The logical inference is that the workers in Greater Glasgow Health Board area are less enthusiastic to investigate and report outbreak

incidents, or that residents in the Greater Glasgow Health Board area are apparently or actually at much less risk of foodborne salmonellosis - for reasons not clearly understood.

(38) Nearly one-fifth of all foodborne salmonella outbreaks in Scotland are imported; that is, the incriminated meal is consumed outside the United Kingdom. Seventy-five percent of imported outbreaks are acquired in Europe, and 75 per cent of ex-Europe episodes are acquired in Spain and Spanish territories (Majorca, Benidorm and Tenerife).

(39) There is a consistent seasonal trend in the incidence of foodborne salmonella infections and outbreaks. Analysed data showed a geometrical progression in the cumulative incidence of outbreaks during the first three quarters of the year! More than half of all outbreaks occur during the summer. Social factors such as increased human mobility and eating practices during the summer months, as well as environmental factors influence and account for the seasonal trend in foodborne salmonellosis.

6.2 RECOMMENDATIONS:

6.2.1 Retrospective Study:

(1) The 20-year retrospective analysis of salmonella infections was undertaken as one of three epidemiological approaches employed. Within the time available, only a 1 in 5 systematic sampling of the approximately 29,000 salmonella infections recorded, could be selected for analysis. Although the sample size (n = 5776) generated was statistically adequate for epidemiological inferences, a more extensive and comprehensive study which includes all the 29,000 cases in the analysis will certainly yield a more definitive, if similar, set of data and conclusions.

(2) The computer programmed analysis of the 5776 sampled cases demonstrates that it is logistically feasible and practicable to feed into the computer all the 29,000 confirmed laboratory isolations (salmonella infections) and all 1,800 or so outbreak incidents. It is, therefore, recommended that a computer-based record be maintained of all foodborne infections and intoxications - beginning from 1967/68, the year a formal surveillance programme co-ordinated by the CD(S)U was developed in Scotland. The package should contain all relevant and comprehensive epidemiological information for infections and outbreaks. Such computer-based records would immensely facilitate identification of cases and outbreak incidents; it would certainly enable relevant data to be readily accessible, easily and quickly retrievable for purposes of trend and other epidemiological analysis. During the present 20-year retrospective study, very considerable amount of time and energy was expended while the author manually sieved through 1,040 weekly records bound in 40 volumes to meticulously select a 1 in 5 systematic sample. Indeed, it was this extensive workload within the available time that compelled the sampling approach.

The 29,000 confirmed salmonella isolations recorded by CD(S)U since 1968 cannot be said to include all cases of human salmonella infections in Scotland during the 20-year period. Certainly the listed infections represent only those cases presenting to the GP or the hospital, which were investigated by the EHOs, in which specimens were submitted for laboratory examination, and which were officially notified to the co-ordinating centre. In view of the fact that surveillance and notification of foodborne infections is passive, and there is no statutory or legal requirement to investigate sporadic incidents; in view of the finding that only 1 to 10% of annual cases of salmonellosis in industrialized countries

are reported (4, 5); in view of the observed wide differences in standardized incidence rates of outbreaks in the various Health Board areas in Scotland, reflecting primarily the enthusiasm and intensity of epidemiological investigation and notification of incidents; and in view of the enormous public health and economic consequences of foodborne salmonellosis and growing public concern, the following recommendations are made to improve the existing surveillance system and to establish the true magnitude of foodborne salmonellosis:

(3) The EHOs, the CMS, the laboratories and other bodies involved in the surveillance network in some of the local districts and health board areas need to be encouraged and motivated to show more interest in the investigation and reporting of incidents; more general practitioners and hospital clinicians need to be reminded of the need to collect and submit appropriate laboratory specimens in suspected cases of foodborne infections.

(4) A legislation or an Order similar to the Zoonoses Order 1989 may be considered, which provides for statutory notification by laboratories of salmonella isolations from humans. Such a legislation would complement the Zoonoses Order 1989.

(5) A research project to determine or estimate the true magnitude of foodborne salmonellosis in Scotland would need to be considered. The project may be similar to the study already carried out in the USA by Chalker and Blaser (5; Chapter 1.8). The aim would be (i) to identify and calculate the sequential artifacts at the various stages of the national surveillance network - from the GP/hospital, through the laboratory to the CMS and environmental health departments; (ii) to determine or estimate the true magnitude of the salmonellosis problem, on the basis of the calculated sequential artifacts and on the

basis of determination of salmonella carriage rate and duration of excretion. Data revealing magnitude of the problem much greater than presently appreciated, would encourage more intensive information and education campaign as well as motivate more intervention measures for the control of foodborne salmonellosis.

It may be argued that the study being proposed could be counter-productive; that it may succeed only in winning the disfavour, disapproval or perhaps non-co-operation from some laboratories and environmental health departments who might view such a study as a presumption of their deficiencies under the present passive surveillance system. While the existing system has proved very effective, there is still much room for improvement, especially in a situation where probably 90% of infections are never reported!

6.2.2 Bacteriological Surveys:

In trying to establish an epidemiological association between contaminated poultry meat and human salmonella infection, only the **serotypes**, **phage types** and antibiogram of salmonellae isolated from chicken carcases and sewer drains during corresponding weeks, and those isolated in concurrent foodborne outbreaks were compared. One very useful marker for tracing infection pathway and for clarifying the epidemiology of outbreak and sporadic incidents is plasmid profile analysis. This specialized procedure could not be undertaken in the present study. It is, therefore, highly recommended that, as a follow-up study, the plasmid profile of the isolates of *S.typhimurium* and *S.enteritidis*, in particular, as well as that of the other serotypes isolated from the chicken carcases and the sewers be determined and compared. The study should also include plasmid analysis of isolates of the same serotypes obtained from sporadic and outbreak incidents occurring in Scotland during the period of the survey, that is, in 1988. By further discriminating the various salmonella strains, the follow-up study could futher clarify the epidemiological

association established by the current survey. All the salmonella isolates recovered during the survey have been taken into the culture bank at the Scottish Salmonella Reference Laboratory. The follow-up study may be carried out at the SSRL or by investigators in other laboratories to whom the cultures can be made available. Work on plasmid profile analysis has very recently been initiated at the SSRL.

Biotyping is another important and valid discriminating typing scheme that is strongly recommended for the major salmonella phage types detected in the bacteriological surveys. Cultures of *S.enteritidis* PT4 and *S.typhimurium* PT104, 49 and 141 isolated from the chicken carcases and sewer drains could be made available to expert and specialist microbiologists in other laboratories in Scotland, who in collaboration with the SSRL, would establish the biotypes of the salmonella isolates. Identification of reasonable proportions of similar biotypes from both chicken carcases and sewer drains, and their similarity to biotypes commonly associated with poultry or poultry-borne outbreaks may provide additional and confirmatory evidence of the epidemiological relationship between poultry meat and human salmonellosis.

6.2.3 Case-Control Study:

In view of the fact that case-control study is one of the most reliable epidemiological approaches in establishing a causal association between illness and a suspected factor, it is suggested that further case-control studies may be carried out to corroborate and confirm some of the findings of the present study, especially with respect to frozen, roasted, and pre-cooked poultry meat. It is recommended that such studies should aim at not only to establish or confirm the association between human salmonellosis and poultry meat, but also between human salmonella infections and eggs and egg products which now seem to have become an important risk factor.

Within the scope of time and resources available, and particularly for logistic problems stated, the present case-control study was limited to Glasgow district. It is therefore recommended that a more extensive study be designed within the existing surveillance network to cover all the local districts in Scotland. The setting should be the CD(S)U, all the diagnostic laboratories that routinely report to the CD(S)U, and all the environmental health departments in Scotland. The experience gained in the present study shows that it is possible to successfully solicit the co-operation of the environmental health department, and that cases are readily and reasonably accessed through the EHOs. Certainly, more cases should be identified and accessed nationwide through direct collaboration and co-ordinated involvement of the laboratories and the EHOs. Case-control studies of egg-borne salmonellosis covering the entire country were recently carried out successfully in England and Wales, by the Division of Enteric Pathogens, Public Health Laboratory Service, in collaboration with all the PHLS laboratories and all the local authority environmental health departments **(203).**

For selection and accessing of matched household (neighbourhood) controls for the case-control study, it is suggested that the Voluntary Population Survey conducted by the Scottish Regional Councils be utilized. In spite of the problems and difficulties encountered during the present study, the Voluntary Population Survey remains, in my opinion, one of the most feasible and reliable sampling frames for random selection of matched controls. It not only overcomes the difficulty of getting sufficient numbers of case-nominated matched controls, it enables direct random selection of all age-groups, including those below the voting age - an inherent disadvantage of using the Voters Register.

6.2.4 Prevention and Control of Poultry-borne Salmonellosis:

While the production of salmonella-free poultry and poultry products may be a longterm, albeit remote objective, the following measures must be advocated or re-affirmed:

To prevent the spread of salmonellae during the processing of poultry meat, all the poultry processors in the country must be seen to follow the Recommended International Code of Hygienic Practice for Poultry Processing (69).

As an important tool for monitoring hygienic requirements aimed at reducing salmonella spread and cross-contamination in poultry husbandry, slaughter and processing, the hazard analysis critical control point (HACCP) concept needs to be uniformly applied by the poultry industry in Scotland, England and Wales. In particular, effective CCP measures should be stressed at the following stages when major cross-contamination occurs: the breeding flock, poultry feeds, disposal of litter, removal of dead birds, collection of eggs, fumingation of hatcheries, scalding, evisceration and chilling.

Veterinary antemortem examination and postmortem inspection do not detect apparently healthy carriers or carcases contaminated with zoonotic serotypes (sero-vars) that do not produce gross pathologic lesions in birds. Nevertheless, veterinary inspection and other veterinary public health preventive activities need to be maintained, as veterinary presence is essential to ensure strict compliance with standards in both the farm and the processing plant.

Every effort must continue to be exerted to reduce or remove salmonella from the final product entering the retail outlets and kitchen environments.

While this project was still going on, several legislations have been promulgated to control the various aspects of the salmonellosis problem. In the past, part of the problem has

been the inadequacy of veterinary and other ancillary manpower and the reluctance or lack of enthusiasm by government officials to enforce some of the legislations. Poultry breeders and processors, animal feed producers, retailers as well as diagnostic laboratories should be encouraged to comply with relevant Orders, and all the legislations must be strictly enforced.

Some form of safe treatment of the final product needs to be introduced as soon as practicable. In particular, after years of commissioned study and tactical delay of official decision on the report, irradiation of poultry meat, with all necessary safeguards, ought to be formally introduced in the poultry industry. Public enlightenment campaign needs to be embarked upon by the government and the industry to re-assure the consumers of the safety and wholesomeness of irradiated poultry meat. Already, some countries of the EEC have accepted and are producing irradiated foods, including poultry products.

Good kitchen hygiene and proper kitchen practices remain the surest measures to prevent human salmonella infections and outbreaks. No other control activity, not even irradiation, can or should be a substitute for sound kitchen practices. The data from the retrospective study revealed that the vast majority of sporadic and outbreak incidents of salmonellosis occur in the home, that is, in private households. Therefore, intensive campaign and education should be targeted not only at commercial caterers, but perhaps primarily at housewives - advising on correct procedures for kitchen hygiene and kitchen practices. The CD(S)U, in collaboration with the Scottish Health Department, and the Information Services Unit may consider the publication of free simplified leaflets on Salmonella and Kitchen Hygiene!

In the course of the salmonella surveys in the hospital kitchen, there was a change in policy - from raw chicken carcases to precooked chicken. There were reports also of some other hospitals totally abandoning the serving of chicken in the hospital menu; the whole idea being to avoid

the salmonella problem. Certainly, total rejection of chicken cannot be the best solution to the problem, not the least in terms of national economy, good nutrition and public health. Measures to ensure the elimination or reduction of salmonella contamination of fresh or frozen carcases delivered to hospital kitchens or bought for the private home would seem a more productive approach.

Thorough cooking of poultry meat and shell eggs, adequate heat-treatment of liquid eggs, prevention of re-contamination of cooked meat and other foods, must remain the traditional but dependable safeguards. In hospital and other commercial kitchens, separate surfaces for raw and cooked meat, separate equipment and storage, separate kitchen staff, regular hand washing with soap should be stressed and be seen to be practised at all times.

The approval of this research topic by the Department of Community Medicine, the interest and logistic support of the CD(S)U and the financial grant in support of an aspect of the research by the Scottish Hospital Endowment and Research Trust, are evidence of the collective desire to understand and provide some solution to the complex epidemiology and control of poultry-borne salmonellosis in Scotland. More research and more financial support are recommended.

POULTRY MEAT AND HUMAN SALMONELLOSIS:

Establishing the Epidemiological Relationship

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Salmonella serotypes isolated from poultry (chicken and turkey) on 10 or more incidents (1976-87)

Salmonella serotype	76	77	78	79	80	81	82	83	84	85	86	87	Total	%
typhimurium	18	5	5	18	9	9	3	11	5	7	5	15	110	26.1
worthington		26	6	10	3			1		2	2		50	11.9
virchow				12	2	16		5				1	36	8.6
livingstone			1					4	1	17	6	1	30	7.1
infantis	3	1	5	8	2	1							20	4.8
reading			13	3								4	20	4.8
enteritidis			1			2		1		7	3	3	17	4.0
senftenberg	5	3	3	3	1	1	1			1			18	4.0
newport					2	4	1	3			1		11	2.6
bredeney					1	1		3	2	1		2	10	2.4
agona	1		3	2	2	2							10	2.4
ohio		5			1			2	1		1		10	2.4
Total	27	40	37	56	23	36	5	30	9	35	18	26	342	81.0
Other serotypes	9	4	9	7	6	10	7	13	8	2	1	3	79	19.0
All serotypes	36	44	46	63	29	46	12	43	17	37	19	29	421	100

Table 1.4.2A

Top 10 Salmonella Serotypes isolated from chicken and years of their dominance

Salmonella serotype	76	77	78	79	80	81	82	83	84	85	86	87	Total	%
typhimurium	18	5	4	16	5	8	3	10	5	7	5	15	101	31.7
virchow				12	2	16		5				1	36	11.3
livingstone			1					4	1	16	6	1	29	9.1
infantis	3	1	4	8	2	1		8					27	8.5
enteritidis			1			2				7	3	3	16	5.0
worthington		8	3	4							1		16	5.0
newport					2	4	1	3			1		11	3.4
bredeney					1	1		3	2	1		1	9	2.8
agona			3	2	1	2							8	2.5
ohio		5			1				1				7	2.2
														81.5
Other serotypes	9	1	9	3	3	11	4	5	6	3	1	4	59	18.5
All serotypes	30	20	25	45	17	45	8	39	15	34	17	25	319	100

Table 1.4.2B

Top 10 Salmonella Serotypes isolated from Turkey and the periods of their predominance

Salmonella serotype	76	77	78	79	80	81	82	83	84	85	86	87	Total	%
worthington		18	3	6	3			1		2	1		34	33.3
reading			13	3								3	19	18.6
senftenberg	3	2	3	3			1						12	11.8
typhimurium			1	2	4	1		1					9	8.8
panama	2	3											5	4.9
ohio								1	1		1		3	2.9
agona	1				1								2	2.0
anatum				2									2	2.0
heidelberg				1					1				2	2.0
saint-paul					1		1						2	2.0
thompson					2								2	2.0
														90.2
Other serotypes	-	1	1	1	1	-	2	1	1	1	-	1	10	9.8
All serotypes	6	24	21	18	12	1	4	4	3	3	2	4	102	100

Table 4.1.1A

Annual Frequencies of Salmonella Outbreaks, 1980-87

Year	Outbreak	Percentage	% Annual Change
1980	121	8.5	
1981	198	13.9	+ 5.4
1982	235	16.5	+ 2.6
1983	238	16.7	+ 0.2
1984	181	12.7	- 4.0
1985	133	9.4	- 3.3
1986	140	9.8	+ 0.4
1987	176	12.4	+ 2.6
1980-87	1422	100	
Annual Mean	178	---	

Table 4.1.1B

Incidence of Salmonella Outbreaks at Two-Year Periods (1980-87)

Period	Outbreaks	% Change
1980-81	319	
1982-83	473	+ 50%
1984-85	314	- 50%
1986-87	316	
1980-87	1422	

Table 4.1.2

Salmonella Outbreaks 1980-87: Numbers of Persons Affected, Ill, Laboratory-Confirmed, Hospitalised or Dead

Year	Persons Affected	Lab-Confirmed		Ill	Hospitalised		Dead
		Number	%		Number	%	
1980	827	465	56.2	782	114	14.6	5
1981	1451	847	58.4	1339	121	9.0	12
1982	1360	1190	87.5	1098	146	13.3	17
1983	934	764	81.8	870	233	26.8	2
1984	1058	801	75.7	915	59	6.4	0
1985	878	662	75.4	784	50	6.4	4
1986	521	437	83.9	443	34	7.7	1
1987	1021	787	76.9	820	60	7.3	1
1980-87	8051	5950	73.9	7052	817	11.6	42
Annual Mean	1006	744	73.9	882	102	11.6	5

Table 4.1.3

Ranking Order of Premises or Venues of Salmonella Outbreaks (Places where the Foods were Consumed)

Premises (Location)	Outbreaks	Percentage
Private Household	565	60.7
General Community	164	17.6
Public Catering Establishment	108	11.6
Work Place	24	2.6
Social Gathering (Picnic, Party, Camping)	20	2.2
Farm House (Farming families & workmen)	18	1.9
Health Institutions (Hospitals, Old Peoples Homes, Maternity etc)	14	1.5
Flight/Transit Meal	7	0.7
Military Establishments	5	0.5
Prisons/Remand Homes	4	0.4
Educational Institutions (Schools, Universities, Nursery)	2	0.2
Total	931	100
Location Unknown	491	---
Grand Total	1422	

Table 4.1.4

Food Vehicles Associated with Salmonella Outbreaks 1980-87

Food Vehicle	80	81	82	83	84	85	86	87	Total	%
Chicken	28	39	37	44	37	17	17	32	251	55.3
Turkey	4	5	5	7	4	2	1	2	30	
Egg	-	-	2	1	1	-	1	1	6	1.2
Beef	5	8	13	6	9	9	6	6	62	12.2
Pork	4	7	3	5	3	1	-	1	24	4.7
Lamb	2	2	1	1	-	2	1	-	9	1.8
Minced sausage & hamburger	4	10	5	2	1	-	4	4	30	5.9
Milk	5	9	13	5	4	7	3	2	48	9.4
Other Foods	3	8	15	4	9	6	-	3	48	9.4
Total	55	88	94	75	68	44	33	51	508	100
Unknown vehicle	66	110	141	163	113	89	107	125	914	---
Grand Total	121	198	235	238	181	133	140	176	1422	---

Table 4.1.5

Proportions of Outbreaks of Salmonellosis Associated with Poultrymeat, at 2-Year Periods:

Periods	Total Outbreaks*	Poultrymeat-Associated	Percentage (Approx)
1980-81	143	76	53
1982-83	169	93	55
1984-85	112	60	54
1986-87	84	52	62
1980-87	508*	281	55

* The figures relate only to Outbreaks in which the incriminated food vehicles were identified or specified.

Table 4.1.6A

Annual and Cumulative Incidence of Salmonella Outbreaks reported from Health Boards, 1980-87

Health Board	Year								Total	Annual Mean
	80	81	82	83	84	85	86	87		
Lothian	27	41	66	57	36	24	32	33	316	39.5
Grampian	18	34	26	22	18	30	27	43	218	27.25
Lanarkshire	13	20	24	43	24	19	10	30	183	22.9
Fife	5	26	24	20	17	13	18	15	138	17.25
Tayside	19	24	27	21	19	10	7	9	136	17.0
Argyll & Clyde	3	7	16	16	17	5	14	15	93	11.6
Greater Glasgow	7	11	9	12	11	4	13	13	80	10.0
Dumfries & Galloway	6	3	9	16	10	3	7	1	55	6.9
Highland	7	12	8	10	5	9	1	3	55	6.9
Forth Valley	6	9	9	8	5	6	2	8	53	6.6
Ayrshire & Arran	6	6	5	7	6	8	3	4	45	5.6
Borders	2	4	9	1	6	-	1	3	26	3.25
Shetland	-	-	2	1	-	1	1	1	6	<1
Off Shore	1	1	-	1	2	-	-	-	5	<13.25
Orkney	-	-	1	1	-	-	-	-	2	<1
	Total								1411	
	National Average								94.0	11.75

Table 4.1.6B

Ranking Order of Standardized Cumulative Incidence Rate of Salmonella Outbreaks in Scottish Health Board Areas 1980-87

Health Board Area	Mean Population*	Cumulative Incidence of Outbreak	Incidence Rate per 10,000
Grampian	490,269	218	4.4
Lothian	745,698	316	4.2
Fife	342,555	138	4.0
Dumfries & Galloway	145,241	55	3.8
Tayside	395,927	136	3.4
Lanarkshire	568,900	183	3.2
Highland	195,867	55	2.8
Borders	101,007	26	2.6
Shetland	23,321	6	2.6
Argyll & Clyde	450,732	93	2.1
Forth Valley	272,147	53	2.0
Ayrshire & Arran	375,337	45	1.2
Orkney	18,942	2	1.1
Greater Glasgow	985,135	80	0.8
National Average	365,077	100.5	2.75

* Population data supplied by Registrar General for Scotland (82)

Table 4.1.7

Salmonella Outbreaks Imported from Outside United Kingdom

Continent	Outbreaks	Percentage	Principal Countries
Europe	224	82.0	Spain and Spanish Territories
Africa	31	11.3	Tunisa, Morocco
Mediterranean	12	14.4	Malta
Asia	4	1.5	
Oceania	2	0.7	
Central America	1	0.34	
Total	274	100	

* Forty other Outbreaks were imported into Scotland from England and Wales

Table 4.1.8

Ranking Order of Salmonella Serotypes causing Foodborne Outbreaks, 1980-87

Salmonella serotype	Year								Total	%
	80	81	82	83	84	85	86	87		
S.typhimurium	39	71	119	130	96	49	40	58	602	40.4
S.enteritidis	13	29	31	33	38	47	68	77	336	22.6
S.virchow	36	39	28	39	22	9	11	13	197	13.2
S.stanley	-	16	18	8	5	8	1	3	59	4.0
S.heidelberg	1	7	10	3	3	2	1	5	32	2.2
S.agona	6	9	3	1	2	1	1	2	25	1.7
S.saint-paul	2	7	7	6	-	1	-	1	24	1.6
S.infantis	4	-	1	2	7	4	1	2	21	1.4
S.bredeney	2	1	2	4	1	2	5	2	19	1.3
S.hadar	8	5	-	1	1	2	1	1	19	1.3
S.panama	1	4	4	1	-	1	3	3	17	1.1
S.montevideo	2	2	1	4	2	-	-	2	13	0.9
S.anatum	4	3	1	1	-	-	-	1	10	0.7
S.newport	-	-	3	4	-	1	1	-	9	0.6
S.indiana	-	-	1	1	3	-	2	1	8	0.5
S.bovis-morbificans	-	2	1	-	-	1	2	-	6	0.4
S.thompson	-	-	1	-	-	1	-	1	3	0.2
S.derby	-	-	2	-	-	1	-	-	3	0.2
Other serotypes									78	5.2
									1489	100.0

Table 4.1.9

Frequencies of *S.typhimurium* and *S.enteritidis* phage types involved in outbreaks (ranking order) 1980-87

S.typhimurium phage types	Frequency	*S.enteritidis* phage types	Frequency
110	108	*4	89
10	66	*8	88
204	65	6	8
49	63	13	3
12	45	18	2
104	22	11	2
193	17	3	1
170	13	5	1
66	11	24	1
195	4	35	1
Others	76		
Total	490	Total	196

* *S.enteritidis* phage type 4 and 8 accounted for 90% of *S.enteritidis* outbreaks in which the phage type was specified.

Table 4.1.10

Seasonal (Quarterly) Incidence of Foodborne Salmonella Outbreaks, 1980-87

Year	Jan-Mar	Apr-Jun	Jy-Sept	Oct-Dec	Total
1980	14	31	58	18	121
1981	30	40	99	29	198
1982	33	51	109	42	235
1983	29	33	130	46	238
1984	34	45	79	23	181
1985	12	45	54	22	133
1986	13	31	77	19	140
1987	13	45	93	25	176
1980-87	178	321	699	224	1422
Annual Mean	22	40	87	28	178
% Quarterly Change	-	100%	100%	300%	---

Table 4.1.11

Incidence of Salmonella Infections (confirmed laboratory isolations) at 5-Year Periods (1 in 5 samples)

1968-72	1973-77	1978-82	1983-87
1968: 177	1973: 171	1978: 250	1983: 457
1969: 133	1974: 120	1979: 293	1984: 448
1970: 138	1975: 234	1980: 307	1985: 336
1971: 152	1976: 284	1981: 552	1986: 413
1972: 127	1977: 180	1982: 530	1987: 474
Total: 727	989	1932	2128
Mean: 145.4	197.8	386.4	425.6
Std Deviation: 19.93	62.9	142.9	54.81

Table 4.1.12

Standardized Incidence Rates of Salmonella Infections (Confirmed Laboratory Isolations) at 5-Year Periods

Period	Sample Size	Actual Incidence Recorded	Mean Human Population**	Standardized Incidence Rate
1968-72	727	3635	5,218,580	14.0/100,000 per yr
1973-77	989	4945	5,209,000	19.0/100,000 per yr
1978-82	1932	9660	5,163,140	37.5/100,000 per yr
1983-87	2128	10640	5,136,100	41.5/100,000 per yr
1968-87	5776	28880	5,181,705	28/100,000 per year

** Based on Census and Estimated Population figures from Registrar General Scotland: Annual Report: HMSO; 1968-87

Table 4.1.13A

Standardized Age - Specific Incidence Rates of Salmonella Infections, 1968 - 72

Age Group	Mean** Population (x 1000)	Number of Infections	Standardized Incidence Rate/ 100,000 per year
0-5	545.12	1010	37.0
6-10	466.74	290	12.4
11-15	425.20	225	10.6
16-20	393.96	275	14.0
21-25	368.24	290	15.8
26-30	330.65	215	13.0
31-35	297.62	175	12.4
36-40	298.20	130	8.7
41-45	307.78	130	8.4
46-50	312.72	115	7.4
51-55	303.20	110	7.3
56-60	293.08	45	3.1
61-65	282.44	100	7.1
66-70	235.50	105	8.9
71+	353.26	150	8.5

** Registrar General Scotland: Annual Report: HMSO; 1968-87

Table 4.1.13B

Standardized Age-Specific Incidence Rates of Salmonella Infections, 1973 - 77

Age Group	Mean** Population (x 1000)	Number of Infections	Standardized Incidence Rate/ 100,000 per year
0-5	473.29	925	39.0
6-10	452.2	371	16.4
11-15	452.75	234	10.3
16-20	408.35	389	19.1
21-25	368.65	471	25.6
26-30	339.70	428	25.2
31-35	303.40	267	17.6
36-40	296.18	220	14.9
41-45	299.39	170	11.4
46-50	308.15	196	12.7
51-55	308.17	174	11.3
56-60	276.91	189	13.7
61-65	280.07	140	10.0
66-70	243.10	141	11.6
71+	390.40	275	14.1

** Registrar General Scotland: Annual Report: HMSO; 1968-87

Table 4.1.13C

Standardized Age-Specific Incidence Rates of Salmonella Infections, 1978 - 82

Age Group	Mean** Population (x 1000)	Number of Infections	Standardized Incidence Rate/ 100,000 per year
0-5	388.83	1635	84.1
6-10	358.60	534	29.8
11-15	396.13	531	28.8
16-20	450.70	830	36.8
21-25	404.65	921	45.5
26-30	357.71	849	47.5
31-35	351.46	570	32.4
36-40	313.50	589	37.6
41-45	292.81	426	29.1
46-50	291.32	400	27.5
51-55	294.24	435	29.6
56-60	287.43	340	23.7
61-65	295.36	195	13.2
66-70	235.23	230	19.6
71+	445.01	475	21.3

** Registrar General Scotland: Annual Report: HMSO; 1968-87

Table 4.1.13D

Standardized Age-Specific Incidence Rates of Salmonella Infections, 1983 - 87

Age Group	Mean** Population (x 1000)	Number of Infections	Standardized Incidence Rate/ 100,000 per year
0-5	388.65	1854	95.4
6-10	326.17	460	28.2
11-15	380.48	540	28.3
16-20	438.10	768	35.1
21-25	429.03	1129	52.6
26-30	375.85	920	49.0
31-35	344.03	805	46.8
36-40	344.30	719	41.8
41-45	298.15	710	47.6
46-50	288.04	402	27.9
51-55	285.20	380	26.6
56-60	281.79	365	25.9
61-65	268.80	304	22.6
66-70	220.59	156	14.1
71+	466.76	427	18.3

** Registrar General Scotland: Annual Report: HMSO; 1968-87

Table 4.1.14

Standardized Sex-Specific Incidence Rate of Salmonella Infections, 1968 - 87

Period	Mean Population (x 1000)	Number of Salmonella Infections	Standardized Incidence Rate
1968-72			
Male	2514.6	1745 (48%)	13.9/100,000 per year
Female	2704.0	1890 (52%)	14/100,000 per year
1973-77			
Male	2505.0	2510 (51%)	20/100,000 per year
Female	2704.0	2395 (49%)	17.7/100,000 per year
1978-82			
Male	2487.0	4800 (49.84%)	38.6/100,000 per year
Female	2676.4	4830 (50.16%)	36.1/100,000 per year
1983-89			
Male	2479.2	5334 (50.4%)	43/100,000 per year
Female	2656.9	5249 (49.6%)	39.5/100,000 per year
1968-87			
Male	2496.45	14389 (50.04%)	28.8/100,000 per year
Female	2685.325	14369 (49.96%)	26.8/100,000 per year

** Registrar General Scotland: Annual Report: HMSO; 1968-87

Table 4.1.15A

Proportions of Major Salmonella Serotypes Isolated from Human Infections during 1968 - 77

1968 - 72		1973 - 77	
Salmonella serotype	Percentage	Salmonella serotype	Percentage
S.typhimurium	27.4	*S.typhimurium*	43.7
S.enteritidis	7.3	*S.heidelberg*	11.4
S.bredeney	3.6	*S.enteritidis*	7.1
S.panama	2.5	*S.agona*	6.2
S.reading	2.2	*S.dublin*	5.0
S.infantis	1.3	*S.hadar*	3.4
S.heidelberg	1.1	*S.newport*	2.8
S.senftenberg	1.1	*S.panama*	2.7
S.indiana	0.9	*S.infantis*	1.8
S.derby	0.8	*S.worthington*	1.1
Other serotypes	49.0	Other serotypes	15.0

Table 4.1.15B

Proportions of Major Salmonella Serotypes Isolated from Human Infections during 1978-87

1978-82		1983-87	
Salmonella Serotype	Percentage	Salmonella Serotype	Percentage
S.typhimurium	42.5	*S.typhimurium*	40.6
S.virchow	13.3	*S.enteritidis*	27.4
S.enteritidis	7.8	*S.virchow*	8.0
S.agona	5.3	*S.stanley*	2.3
S.heidelberg	4.6	*S.infantis*	2.0
S.hadar	4.0	*S.bredeney*	1.8
S.saint-paul	3.9	*S.heidelberg*	1.4
S.infantis	2.6	*S.thompson*	1.3
S.stanley	1.9	*S.newport*	1.2
S.muenchen	1.6	*S.panama*	1.0
Other serotypes	12.5	Other serotypes	11.2

Table 4.1.16

Ranking Order of Top 10 Salmonella Serotypes isolated in Human Infections:

1968-89	1968-72	1973-77	1978-82	1983-87
S.typhimurium	1	1	1	1
S.enteritidis	2	3	3	2
S.virchow	NA*	NA	2	3
S.heidelberg	7	2	5	7
S.agona	NA	4	4	NA
S.hadar	NA	6	6	NA
S.infantis	6	9	8	5
S.panama	4	8	NA	10
S.bredeney	3	NA	NA	6
S.newport	NA	7	NA	9

*NA = Not Among Top 10 Serotypes

Table 4.2.1

Proportions of Raw Chicken Carcases and Precooked Chicken Contaminated with Salmonellae

	Raw Chicken Carcases	Pre-Cooked Chicken
Number examined	477	102
Number positive for Salmonellae	214 (45%)	Nil
Range of incidence rate	27% - 67%	Nil
Median incidence rate	50%	Nil
Number of salmonella isolates	231	Nil
Number of serotypes	19	Nil

Table 4.2.2

Salmonellae isolated from Chicken Carcases, Sewer Swabs & Poultry Associated Foodborne Outbreaks 1988

Chicken			Sewer			Poultry-Associated Foodborne Outbreaks		
S.enteritidis			*S.enteritidis*			*S.enteritidis*		
pt 4	48}		pt 4	6}		pt 4	12}	
pt 7	1}	51	pt 8	1}	7	pt 8	2}	14
pt 11	2}							
S.typhimurium			*S.virchow*		6			
pt 2	3}							
pt 49	13}		*S.typhimurium*			*S.typhimurium*		
pt 104	15}	41	pt 10	1}		pt 10	1}	
pt 141	9}		pt 49	2}	4	pt 49	4}	
pt RDNC	1}		pt 104	1}		pt 66	4}	12
						pt 110	1}	
						pt 204	2}	
S.virchow		21	*S.clichy*		4			
S.hadar		19	*S.thompson*		3			
S.bredeney		13	*S.montevideo*		2	*S.montevideo*		1
S.binza		11	*S.senftenberg*		1	*S.saint-paul*		1
S.eimsbuettel		7	*S.hadar*		1			
S.schwarzengrund		7	*S.eimsbuettel*		1			
S.minnesota		7	*S.minnesota*		1			
S.mbandaka		7	*S.binza*		1			
S.senftenberg		6	*S.heidelberg*		1			
S.montevideo		6	*S.rough: gm*		1			
S.indiana		3						
S.kinshasa		2						
S.thielallee		2						
S.livingstone		1						
S.rough:gm		14						
6,7:-:1,5 (monophasic)		9						
6,7;14:-:- (Non-motile)		4						
Total		231	Total		33			

Table 4.2.3

Raw Chicken Carcases from which Multiple Salmonella Serotypes/Phage Types were Isolated

S/N of Chicken Carcases	Salmonella serotype/phage type isolated
22	*S.enteritidis* 4 + *S.enteritidis* 11
129	*S.enteritidis* 4 + *S.typhimurium* 49
139	*S.enteritidis* 4 + *S.virchow*
160	*S.enteritidis* 4 + *S.minnesota*
316	*S.enteritidis* 4 + *S.enteritidis* 7
318	*S.enteritidis* 4 + unnamed
365	*S.enteritidis* 4 + *S.binza* + *S.hadar*
400	Monophasic C_1 + *S.enteritidis* 4
152	*S.schwarzengrund* + *S.senftenberg*
252	*S.schwarzengrund* + *S.montevideo*
284	*S.typhimurium* 104 + *S.bredeney*
28	*S.enteritidis* 11 + rough
406	Monophasic C_1 + rough
204	*S.eimsbuettel* + *S.virchow*
208	*S.eimsbuettel* + *S.typhimurium* 104
192	*S.eimsbuettel* + Non motile C4

Table 4.2.4

Notifications of Poultry Salmonellae to MAFF and CD(S)U under the Zoonoses order 1975 and the WHO Surveillance Programme in 1988

Zoonoses Order (England, Wales & Scotland)			CD(S)U Surveillance System (Scotland)		
S.enteritidis	401	(48%)	S.enteritidis	33	(51.6%)
S.tennessee	82	(9.8)	S.typhimurium	10	(15.6)
S.typhimurium	68	(8.1)	S.mbandaka	10	(15.6)
S.senftenberg	48	(5.7)	S.newport	3	(4.7)
S.mbandaka	42	(5.4)	S.binza	2	(3.1)
S.montevideo	35	(4.2)	S.cubana	2	(3.1)
S.livingstone	23	(2.7)	S.taksony	2	(3.1)
S.binza	8	(1)	S.arizonae	1	(1.6)
S.indiana	8	(1)	S.Group C	1	(1.6)
S.hadar	7	(0.8)			
S.kedougou	7	(0.8)			
S.thompson	6	(0.7)			
S.virchow	5	(0.6)			
S.bredeney	5	(0.6)			
S.newport	5	(0.6)			
S.anatum	5	(0.6)			
Others	83	(9.9)			
	838			64	

Table 4.2.5A

Incidence of salmonellae in sewer swabs examined during periods when raw chicken carcases and pre-cooked chicken were prepared in the hospital kitchen

	Period Raw Chicken was used	Period Cooked Chicken was used
Number of swabs examined	79	10
Number of swabs positive for salmonellae	30 (38%)	1 (10%)
Number of weeks examined	40	5
Number of weeks positive	28 (70%)	1 (20%)
Number of serotypes isolated	13	1

Table 4.2.5B

Fisher's Exact Test: Significance Level of the Difference in Number of Weeks Salmonellae were Isolated from Sewers during Periods when Raw or Precooked Chicken was Prepared in the kitchen

Observed Data:

		Salmonella +	Salmonella −	
chicken	raw	28	12	40
	precooked	1	4	5
		29	16	45 weeks

Probability of Observed data:

$$P_i = \frac{40!\ 5!\ 29!\ 16!}{45!\ 28!\ 12!\ 1!\ 4!}$$

$$= 0.0432$$

More Extreme Data Expected:

		+	−	
chicken	raw	29	11	40
	precooked	0	5	5
		29	16	45 weeks

Probability of expected data:

$$P_o = \frac{40!\ 5!\ 29!\ 16!}{45!\ 29!\ 11!\ 0!\ 5!}$$

$$= 0.0036$$

Significance Level (P) = 0.0432 + 0.0036

= 0.0468

This Probability is significant at 5% level

Table 4.2.6

Corresponding (Matching) Weeks during which the same Salmonella type was recovered from chicken and sewer drain

Chicken	Week		Sewer
S.enteritidis 4	3	4	*S.enteritidis* 4
S.enteritidis 4 &} *S.typhimurium* 104}	5	6	{*S.enteritidis* 4 & {*S.typhimurium* 104
S.enteritidis 4	7	8	*S.enteritidis* 4
S.enteritidis 4	9	10	*S.enteritidis* 4
S.typhimurium 49	13	14	*S.typhimurium* 49
S.virchow	16	17	*S.virchow*
S.virchow	17	18	*S.virchow*
S.minnesota	18	19	*S.minnesota*
S.eimsbuettel	20	21	*S.eimsbuettel*
S.montevideo	26	27	*S.montevideo*
S.enteritidis 4	32	33	*S.enteritidis* 4
S.typhimurium 49	33	34	*S.typhimurium* 49
S.virchow	42	43	*S.virchow*

Table 4.2.7A

Examples of Calculations of Numbers of Weeks (+, +) when the Same Salmonella Serotype was Isolated from both Chicken and Sewer

(1) Number of matching weeks when *S.typhimurium* PT49 was isolated from both chicken and sewer:

		Sewer +	Sewer −	
Chicken	+	2 (0.343)	4	6
	−	0	29	29
		2	33	35 weeks

Expected frequency (+, +) = (6/35) x 2/1

= 0.343

(2) For *S.enteritidis* PT4:

		Sewer +	Sewer −	
Chicken	+	5 (2.57)	13	18
	−	0	17	17
		5	30	35

Expected frequency (+, +) = (18/35) x 5/1

= 2.57

Table 4.2.7B

Observed and expected frequencies of number of matching weeks during which the same salmonella serotypes/ phage type was isolated in both chicken and sewer

S/N	Salmonella type	Observed (+,+)	Expected (+,+)
1	*S.enteritidis* PT4	5	2.57
2	*S.enteritidis* PT11	0	0.00
3	*S.enteritidis* PT7	0	0.00
4	*S.typhimurium* PT49	2	0.34
5	*S.typhimurium* PT104	1	0.20
6	*S.typhimurium* PT141	0	0.00
7	*S.typhimurium* PT12	0	0.00
8	*S.typhimurium* PT10	0	0.00
9	*S.typhimurium* RDNC	0	0.00
10	*S.virchow*	3	1.43
11	*S.eimsbuettel*	1	0.09
12	*S.minnesota*	1	0.09
13	*S.montevideo*	1	0.11
14	*S.hadar*	0	0.14
15	*S.binza*	0	0.11
16	*S.senftenberg*	0	0.09
17-30	*S.bredeney* and others	0	0.00
	- Aggregate	14.00*	5.17

[X^2 = 15.08, $p < 0.005$]

* *S.enteritidis* PT4 and *S.typhimurium* PT104 were observed in same matching weeks 5/6 (Table 4.2.6)

Table 4.2.8

Comparison of Antimicrobial Sensitivity Pattern of Salmonella types Isolated from Chicken and Sewer During Corresponding Weeks

Matching Weeks	Salmonella type	Antimicrobial Sensitivity	
		Chicken Isolate	Sewer Isolate
3/4 } 5/6 } 7/8 } 9/10 } 32/33}	*S.enteritidis* PT4	All 19 isolates sensitive to all agents	All 6 isolates sensitive to all agents
13/14} 33/34}	*S.typhimurium* PT 49	All 9 isolates sensitive to all agents	All 4 isolates sensitive to all agents
5/6	*S.typhimurium* PT 104	Single isolate sensitive to agents	Single isolate sensitive to all agents
16/17} 17/18}	*S.virchow*	All 6 isolates resistant to Sul & Tri	All 3 isolates resistant to Chl, Tet, Sul & Tr
42/43	*S.virchow*	The 2 isolates resistant to Sul & Trim; sensitive to others	The 2 isolates resistant to Sul & Trim; sensitive to others
18/19	*S.minnesota*	All 4 isolates sensitive to all	The single isolate sensitive to all
20/21	*S.eimsbuettel*	All 4 isolates sensitive to all	The single isolate sensitive to all
26/27	*S.montevideo*	All 4 isolates sensitive to all	The single isolate sensitive to all

Table 4.2.9

Ranking Order of Salmonella Serotypes isolated from Chicken Carcases, Sewer Swabs, and under the Zoonoses Order in 1988

Chicken	Sewer	Zoonoses Order (England, Wales & Scotland)
S.enteritidis	*S.enteritidis*	*S.enteritidis*
S.typhimurium	*S.virchow*	*S.tennessee*
S.virchow	*S.typhimurium*	*S.typhimurium*
S.hadar	*S.clichy*	*S.senftenberg*
S.bredeney	*S.thompson*	*S.mbandaka*
S.binza	*S.montevideo*	*S.montevideo*
S.eimsbuettel	*S.senftenberg*	*S.livingstone*
S.schwarzengrund	*S.hadar*	*S.binza*
S.mbandaka	*S.eimsbuettel*	*S.indiana*
S.minnesota	*S.minnesota*	*S.hadar*
S.senftenberg	*S.binza*	*S.kedougou*
S.montevideo	*S.heidelberg*	*S.thompson*
S.indiana	*S.rough:gm*	*S.anatum*
S.kinshasa		*S.bredeney*
S.thielallee		*S.newport*
S.livingstone		*S.virchow*
S.rough:gm		
6,7:-:1,5 (Monophasic)		
6,7;14:-:- (non-motile)		

Table 4.3.1

Frequency Distribution of
Matched Control Responses

Number of matched Controls per Case	Number of Cases affected	% Cases	Total Number of Matched Controls	% Controls
0	7	5.6	0	0
1	37*	29.6	37	17.8
2	72*	57.6	144	69.2
3	9*	7.2	27	13.0
4	0	0.0	0	
5	0	0.0	0	
6	0	0.0	0	
Total	125	100.0	208	100.0

* Number of Cases with matched controls = 118

Table 4.3.2

Frequencies of Cases with varying Case : Control Ratios

Case : Control Ratio	Number of Cases	Percentage of Cases
1 : 1	37	31.4
1 : 2	72	61.0
1 : 3	9	7.6
Total	118	100.0

Table 4.3.3

Validation of age distribution among controls with the cases (1 : 1 ratio)

Age-Group	CASES		CONTROLS	
	Number*	%	Number*	%
0- 9	22	18.6	22	18.6
10-19	10	8.5	6	5.1
20-29	25	21.2	26	22.0
30-39	17	14.4	20	17.0
40-49	14	11.9	14	11.9
50-59	11	9.3	13	11.0
60-69	12	10.2	10	8.5
70 and over	7	5.9	7	5.9
Total	118	100.0	118	100.0

* No significant difference in the distribution of age-groups between cases and controls, both by the 2-sided t-test ($t = 0$, $p < 0.01$) and the 95% Confidence Interval Estimate (-0.22, 0.22)

Table 4.3.4

Consumption of Poultry Meat among Cases of Salmonella infection and Controls (Exposure = ±)
Matched Analysis with One Control per Case
(Mantel-Haenszel 1 : 1 Test)

		Controls		
		+	-	
Cases	+	22 (A)	57 (B)	79 (A + B)
	-	18 (C)	21 (D)	39 (C + D)
		40 (A + C)	78 (B + D)	118 (N)

Mantel-Haenszel Odds Ratio (Υ) = B/C

Υ_{mh} - 57/18 = 3.17

Table 4.3.5

Mantel-Haenszel Matched Analysis with TWO Controls per Case (1 : 2): Consumption of Poultry Meat (Exposure ±):

Frequencies of 8 possible outcomes for matched triplets (Case, Control 1, Control 2)

	Exposure			Frequencies
n_0	+	+	+	1
n_1	+	+	−	18
n_2	+	−	+	10
n_3	+	−	−	28
n_4	−	+	+	3
n_5	−	+	−	11
n_6	−	−	+	3
n_7	−	−	−	7

Mantel-Haenszel Odds Ratio (Υ_{mh}) =

$$(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$$

$$= (18 + 10 + (2 \times 28))/((2 \times 3) + 11 + 3)$$

$$= 84/20 = \underline{4.2}$$

Table 4.3.6

Consumption of Poultry Meat: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1
(N_i = 37)

		Controls +	Controls −	
Cases	+	5 (A)	17 (B)	22
	−	7 (C)	8 (D)	15
		12	25	37

$\text{Odds Ratio}_{mh} = B/C$
$= 17/7$

(ii) Case : Control Ratio 1 : 2
(N_{ii} = 72)

	Possible Exposure			Frequency
n_0	+	+	+	1
n_1	+	+	−	17
n_2	+	−	+	8
n_3	+	−	−	24
n_4	−	+	+	2
n_5	−	+	−	10
n_6	−	−	+	4
n_7	−	−	−	6

$$\text{Odds Ratio}_{mh} = (n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$$
$$= (17 + 8 + 2(24))/(2(2) + 10 + 4)$$
$$= 73/18$$

Table 4.3.6 (continued)

(iii) Case : Control Ratio 1 : 3

Possible Exposure Outcome	Frequency	$(c-j)n_j(+)$	$j \times n_j(-)$
Case +; 3 Controls +	0	(3-3)(0) = 0	
Case +; 2 Controls +	2	(3-2)(2) = 2	
Case +; 1 Control +	4	(3-1)(4) = 8	
Case -; 1 Control +	1		1 x 1 = 1
Case -; 2 Controls +	1		2 x 1 = 2
Case -; 3 Controls +	0		3 x 0 = 0
Case +; 3 Controls -	0	-	-
Case -; 3 Controls -	1	-	-
Summation ($\sum^{9}$)	9	10	3

$$\text{Odds Ratio}_{mh} = \sum (c-j)n_j(+) / \sum jn_j(-)$$

$$= 10/3$$

<u>Summation (i + ii + iii)</u>

$$\text{Odds Ratio}_{mh} =$$

$$\text{"B"} + (n_1 + n_2 + 2n_3) + (c-j)n_j + / \text{"C"} + (2n_4 + n_5 + n_6) + jn_j(-)$$

$$= 17 + 73 + 10/7 + 18 + 3$$

$$= 3.57$$

Table 4.3.7

Consumption of Red Meat: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1
(N_i = 37)

		Controls +	Controls −	
Cases	+	13	10	23
	−	10	4	14
		23	14	37

Odds Ratio = B/C
= 10/10

(ii) Case : Control Ratio 1 : 2
(N_{ii} = 72)

Odds Ratio = $(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$

= (7 + 9 + 4)/(40 + 6 + 7)

= 20/53

(iii) Case : Control Ratio 1 : 3
(N_{iii}) = 9)

Odds Ratio = $(c-j)n_j(+)/jn_j(-)$

= 0/17

Summation (i + ii + iii)

Odds $Ratio_{mh}$ = 10 + 20 + 0/10 + 53 + 17

= 30/80

= 0.38

The calculated Odds Ratio differs significantly from Unity (One).

Table 4.3.8

Consumption of Frozen Poultry Meat: Mantel Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1
(N_i = 37)

		Controls +	Controls −	
Cases	+	2	10	12
	−	2	23	25
		4	33	37

Odds Ratio = B/C
= 10/2

(ii) Case : Control Ratio 1 : 2
(N_{ii} = 72)

	Exposure Outcome			Frequency
n_0	+	+	+	0
n_1	+	+	−	3
n_2	+	−	+	1
n_3	+	−	−	19
n_4	−	+	+	1
n_5	−	+	−	4
n_6	−	−	+	3
n_7	−	−	−	41

$$\text{Odds Ratio}_{mh} = (n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$$
$$= (3 + 1 + 38)/(2 + 4 + 3)$$
$$= 42/9$$

Table 4.3.8 (continued)

(iii) Case : Control Ratio 1 : 3
(N_{iii} = 9)

$$\text{Odds Ratio}_{mh} = \lesseqgtr (c-j)n_j(+)/\lesseqgtr jn_j(-)$$

$$= 4/3$$

Summation (i + ii + iii)

$$\text{Odds Ratio} = 10 + 42 + 4/2 + 9 + 3$$

$$= 56/14$$

$$= 4.0$$

Table 4.3.9

Consumption of Fresh Poultry Meat: Mantel Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1

		Controls +	Controls −	
Cases	+	1	8	9
	−	4	24	28
		5	32	37

Odds Ratio = B/C
= 8/4

(ii) Case : Control Ratio 1 : 2

	Exposure Outcome			Frequency
n_0	+	+	+	0
n_1	+	+	−	1
n_2	+	−	+	1
n_3	+	−	−	18
n_4	−	+	+	1
n_5	−	+	−	7
n_6	−	−	+	5
n_7	−	−	−	39

$$\text{Odds Ratio}_{mh} = (n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$$
$$= 38/14$$

(iii) Case : Control Ratio 1 : 3

$$\text{Odds Ratio}_{mh} = \lesssim (c-j)n_j(+)/\lesssim jn_j(-)$$
$$= 3.1$$

Summation (i + ii + iii)

Odds Ratio = 8 + 38 + 3/4 + 14 + 1

= 49/19

= 2.58

Table 4.3.10

Consumption of Precooked Poultry Meat: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1

		Controls +	Controls -	
Cases	+	1	5	6
	-	4	27	31
		5	32	37

Odds Ratio = B/C
= 5/4

(ii) Case : Control Ratio 1 : 2

Odds Ratio = $(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$

= (4 + 1 + 12)/(6 + 6 + 1)

= 17/13

(iii) Case : Control Ratio 1 : 3

Odds Ratio = 1/2

Summation (i + ii + iii)

Odds Ratio_{mh} = 5 + 17 + 1/4 + 13 + 2

= 23/19

= 1.21

The Odds Ratio does not differ significantly from Unity (One).

Table 4.3.11

Consumption of Roasted Poultry Meat: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1

		Controls +	Controls −	
Cases	+	2	15	17
	−	4	16	20
		6	31	37

Odds Ratio = B/C
= 15/4

(ii) Case : Control Ratio 1 : 2

Odds Ratio = $(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$

= 8 + 7 + 2(22)/2(1) + 9 + 4

= 59/15

(iii) Case : Control Ratio 1 : 3

Odds Ratio = $\lesseqgtr (c-j)n_j(+)/ \lesseqgtr jn_j(-)$

= (3-1)(2) + (3-2)(2)/(1)(1)

= 4 + 2/1

= 6/1

Summation (i + ii + iii)

Odds Ratio_{mh} = 15 + 59 + 6/4 + 15 + 1

= 80/20

= 4.0

Table 4.3.12

Consumption of Boiled Poultry Meat: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1

		Controls +	Controls −	
Cases	+	0	6	6
	−	8	23	31
		8	29	37

Odds Ratio = B/C
= 6/8

(ii) Case : Control Ratio 1 : 2

Odds Ratio = $(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$

= 1 + 2 + 2(4)/2(0) + 3 + 14

= 11/17

(iii) Case : Control Ratio 1 : 3

Odds Ratio = 0/(2)(2)

= 0/4

Summation (i + ii + iii)

Odds Ratio = 6 + 11 + 0/8 + 17 + 4

= 17/29

= 0.56

The Odds Ratio differs significantly from Unity (One).

Table 4.3.13

Consumption of Poultry Meat 1 or 2 Days in Average Week: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1

		Controls +	Controls −	
Cases	+	20	5	25
	−	11	1	12
		31	6	37

Odds Ratio = B/C
= 5/11

(ii) Case : Control Ratio 1 : 2

Odds Ratio = $(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$

= 9 + 6 + 2(7)/2(12) + 7 + 4

= 29/35

(iii) Case : Control Ratio 1 : 3

Odds Ratio = (3 - 2)(2)/(2)(2) + (1)(1)

= 2/5

Summation (i + ii + iii)

Odds Ratio = 5 + 29 + 2/11 + 35 + 5

= 36/51

= 0.71

The Odds Ratio does not differ significantly from Unity (One).

Table 4.3.14

Consumption of Poultry Meat 3 or 4 Days in an Average Week: Mantel-Haenszel Analysis with Variable Number of Controls per Case

(i) Case : Control Ratio 1 : 1

		Controls +	Controls −	
Cases	+	1	7	8
	−	3	26	29
		4	33	37

Odds Ratio = B/C
= 7/3

(ii) Case : Control Ratio 1 : 2

Odds Ratio = $(n_1 + n_2 + 2n_3)/(2n_4 + n_5 + n_6)$

= $(2 + 3 + 2(18))/(2(2) + 10 + 5)$

= 41/19

(iii) Case : Control Ratio 1 : 3

Odds Ratio = $(3-1)(2)/(1)(1)$

= 4/1

Summation (i + ii + iii)

$$\text{Odds Ratio} = \frac{\text{"B"} + (n_1 + n_2 + 2n_3) + (c - jn_j(+))}{\text{"C"} + (2n_4 + n_5 + n_6) + (jn_j(-)}$$

= 7 + 41 + 4/3 + 19 + 1

= 52/23

= 2.26

FIGURES

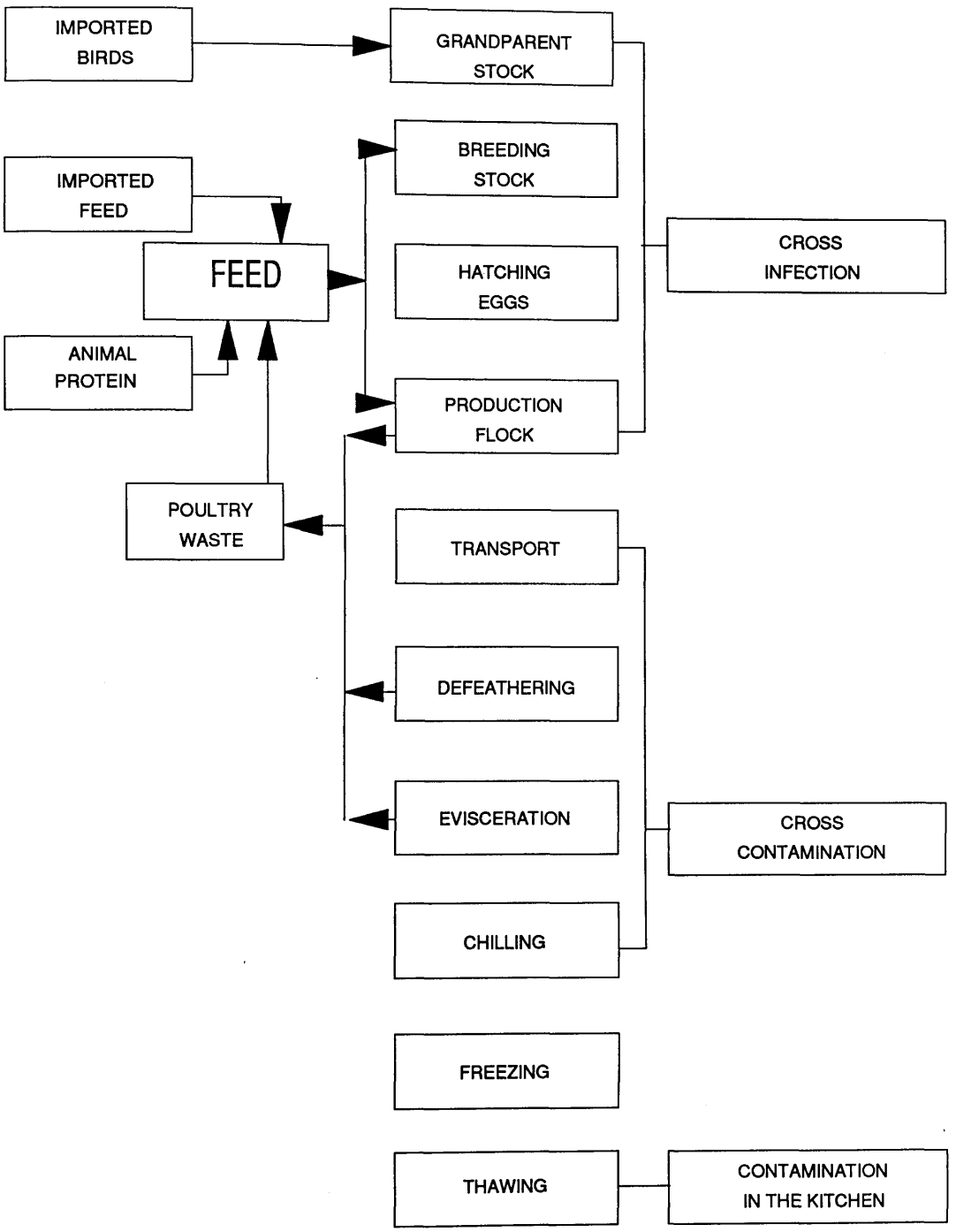


Figure 1.1: Flow Chart: Poultry Production and Processing - Critical Points of Samonella Cross Contamination

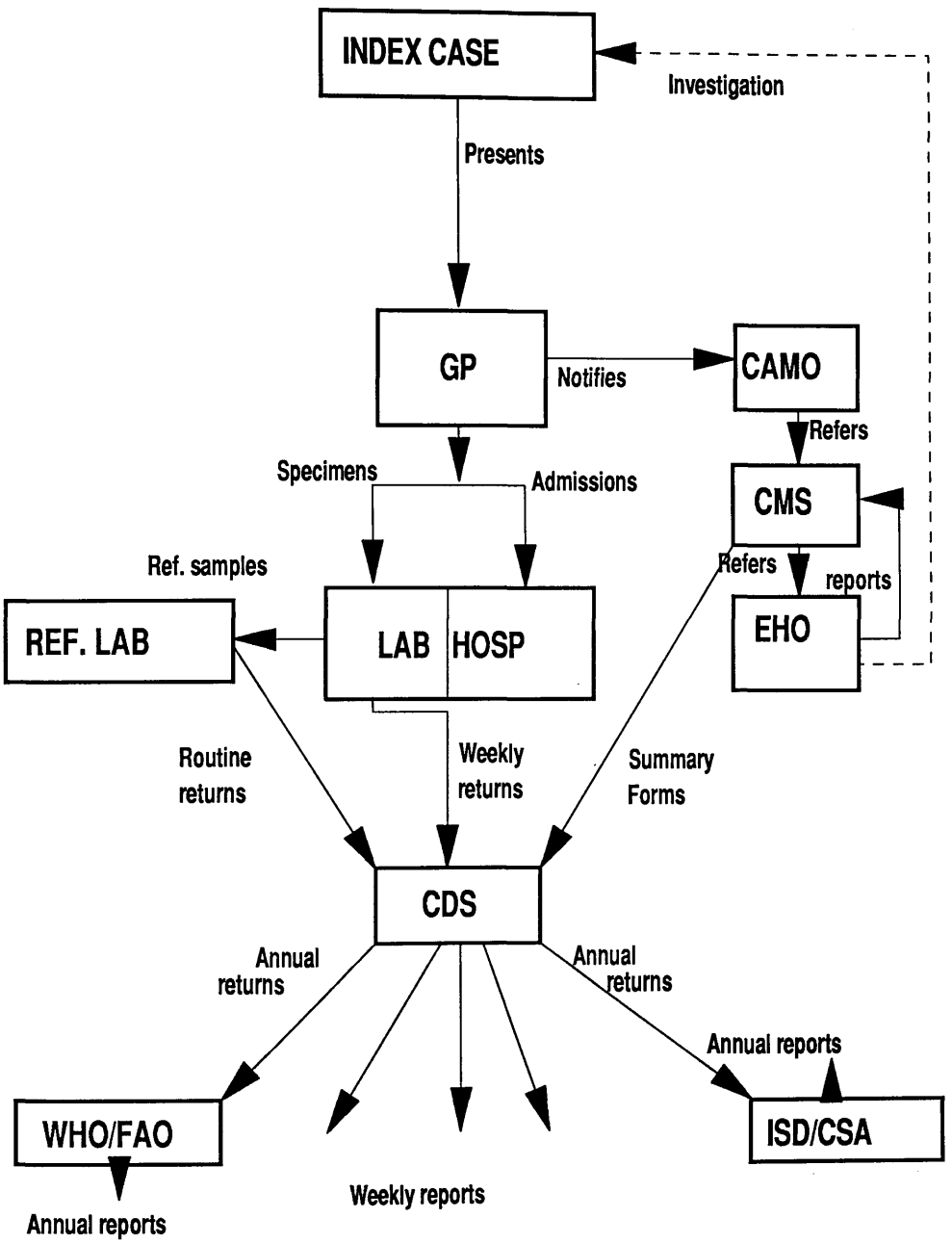


Figure 1.2: Flow Chart: Surveillance Programme for Foodborne Infections and Intoxications in Scotland

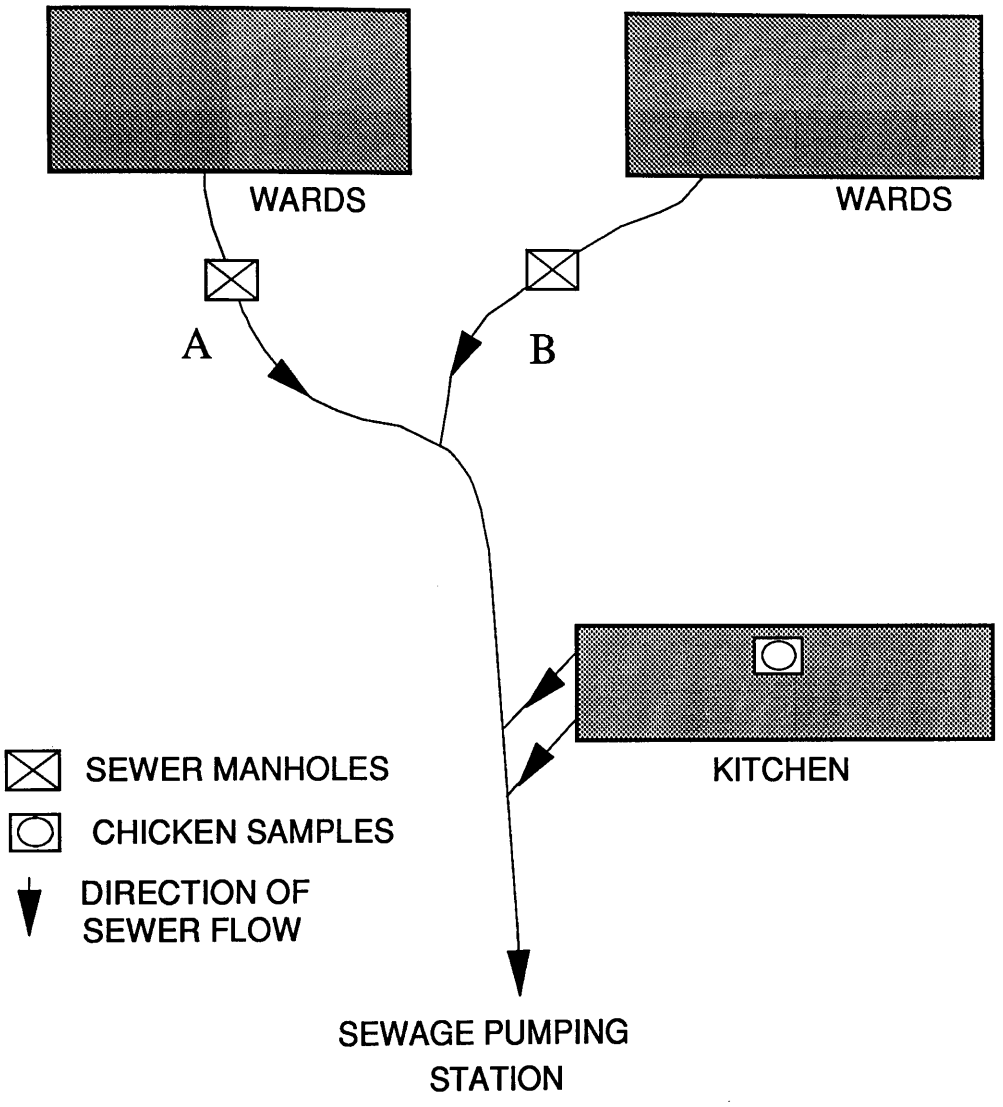


Figure 3.1: Salmonella Survey Sketch Plan of Sample Site (Kitchen and Sewers)

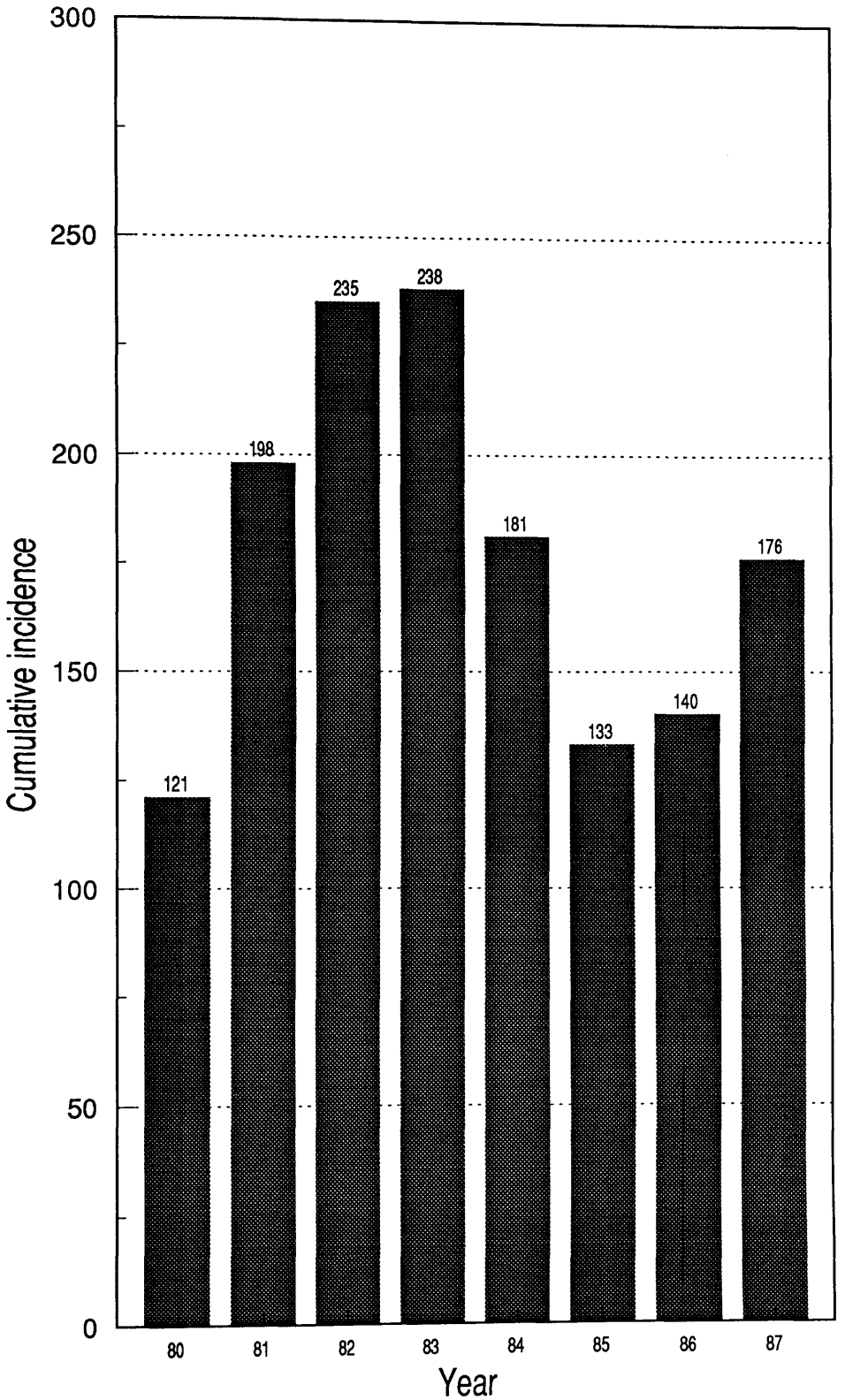
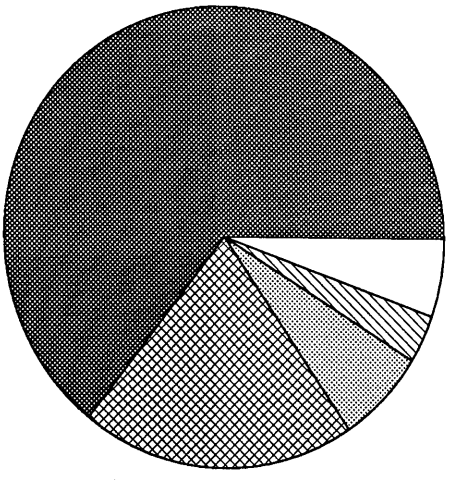
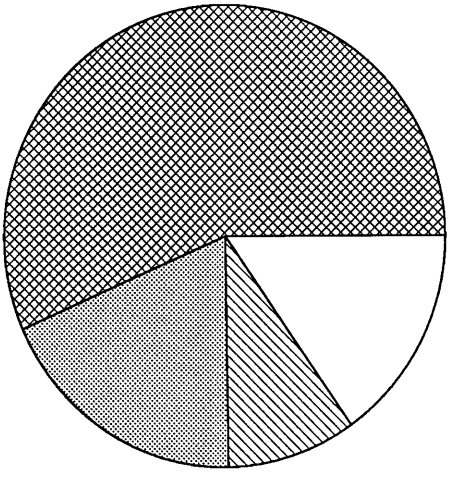


Figure 4.1: Annual Cumulative Incidence of Foodborne Salmonella Outbreaks, 1980-87



Not Known	914
Poultry Product	287
Red Meat	95
Milk	48
Other Food	78
	1422

(a) All Salmonella Outbreaks



Poultry Product	287
Red Meat	95
Milk	48
Other Food	78
	508

(b)Outbreaks in which food vehicle was identified

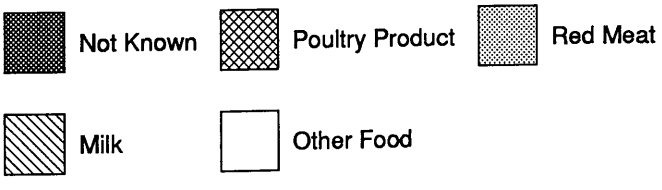


Figure 4.2: Pie Chart of Food-Specific Salmonella Outbreaks, 1980-87

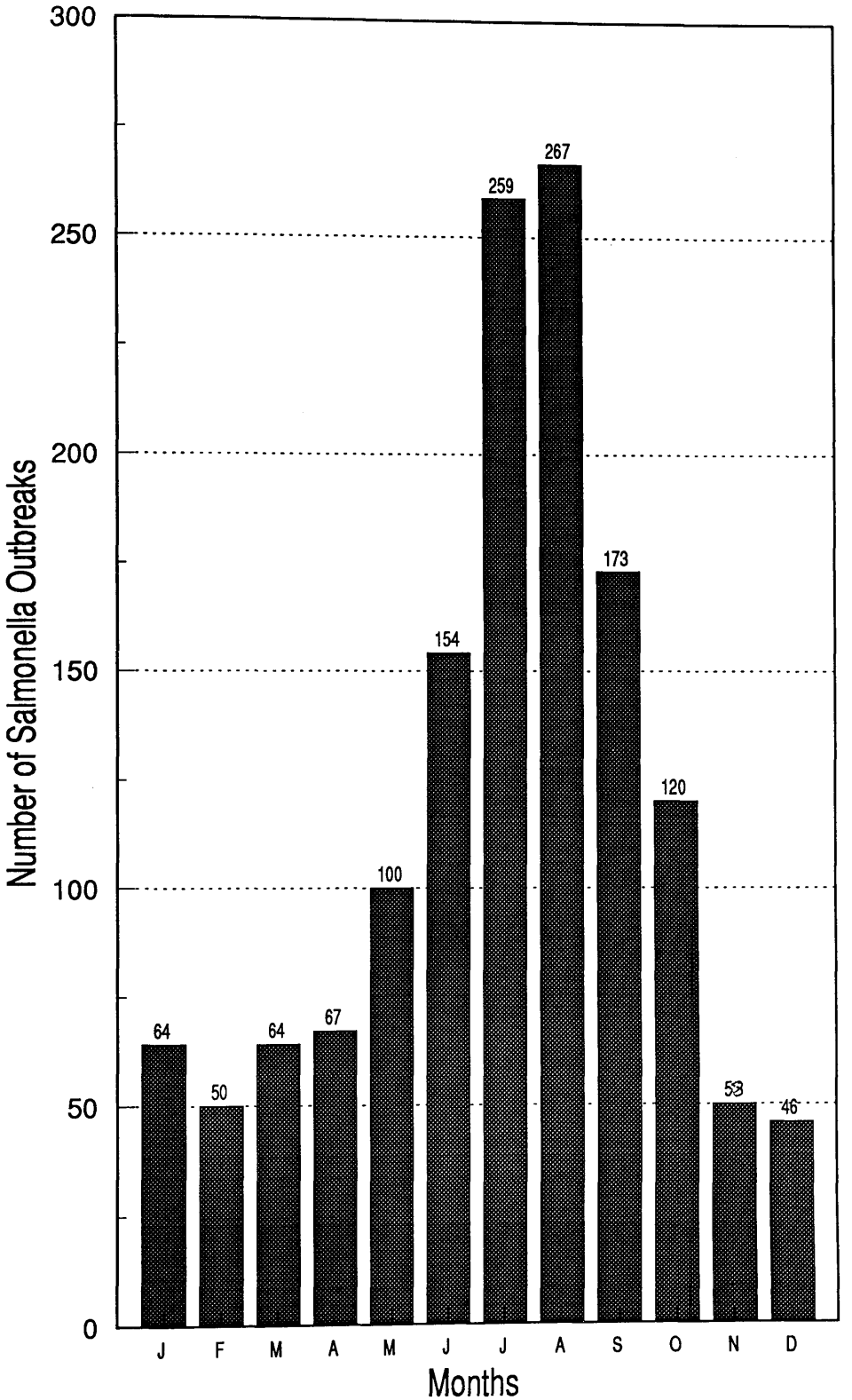


Figure 4.3: Cumulative Monthly Incidence of Salmonella Outbreaks, 1980-87

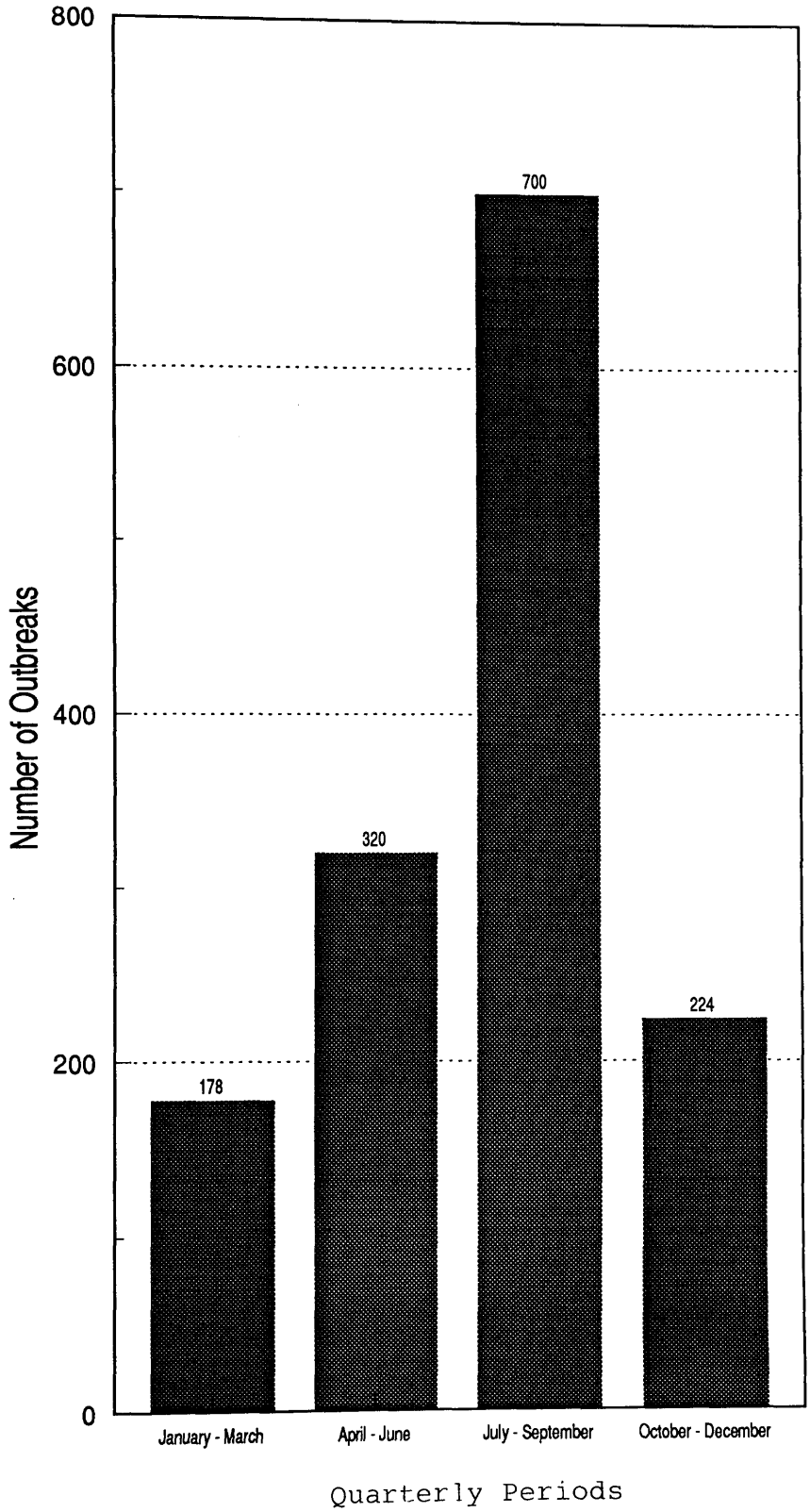


Figure 4.4: Seasonal (Quarterly) Incidence of Salmonella Outbreaks, 1980-87

Figure 4.5

Calculation of samples sizes (n) required to detect 5% difference in mean incidence of Salmonella infection during the periods 1968-72 and 1983-87, using the formula:

$$n = 2\left\{\frac{\left(Z\left(1-\frac{\alpha}{2}\right)+Z(1-\beta)\right)\text{ standard deviation}}{d_o}\right\}^2$$

(a) 1968 - 72:

$$Z\left(1-\frac{\alpha}{2}\right) = Z\left(1-\frac{0.05}{2}\right) = Z(1-0.025)$$

$$= Z(0.975) = 1.96$$

$$Z(1-\beta) = Z(1-0.2) = Z(0.8) = 0.842$$

Standard deviation = 19.93

d_o = 5% difference

$$n = 2\left\{\frac{(1.96+0.842)19.93}{5}\right\}^2$$

$$= 249.5 \text{ or } 250$$

(b) 1983 - 87:

$$Z\left(1-\frac{0.05}{2}\right) = 1.96$$

$$Z(1-\beta) = 0.842$$

Standard deviation = 54.8

$$n = 2\left\{\frac{(1.96+0.842)54.8}{5}\right\}^2$$

$$= 1886$$

Figure 4.6

Calculation of the probability (α) of detecting 5% difference in the mean incidence of Salmonella infections for the period, 1968-82, from the formula:

$$n = 2\left\{\frac{\{(Z(1-\frac{\alpha}{2}) + Z(1-\beta))\ \text{standard deviation}\}}{d_o}\right\}^2$$

$n = 727$; $Z(1-\beta) = Z(0.8) = 0.842$; standard deviation $= 19.93$; and $d_o = 5\%$ difference

$$727 = 2\left\{\frac{(Z(1-\frac{\alpha}{2}) + 0.842)19.93}{5}\right\}^2$$

$$\frac{\sqrt{727}}{2} = \frac{(Z(1-\frac{\alpha}{2}) + 0.842)19.93}{5}$$

$$5\left(\frac{\sqrt{727}}{2}\right) = (Z(1-\tfrac{\alpha}{2}) + 0.842)19.93$$

$$5\left(\frac{\sqrt{727}}{2}\right) \Big/ 19.93 = Z(1-\tfrac{\alpha}{2}) + 0.0842$$

i.e. $3.38 = Z(1-\frac{\alpha}{2}) + 0.0842$

Therefore, $Z(1-\frac{\alpha}{2}) = 3.38 - 0.842 = 2.54$

From Tables, the p value nearest equivalent to 2.54 is 0.994

Thus $\frac{\alpha}{2} = 1 - 0.994 = 0.006$

$\therefore \alpha = 0.012$

The calculated probability is less than $p = 0.05$

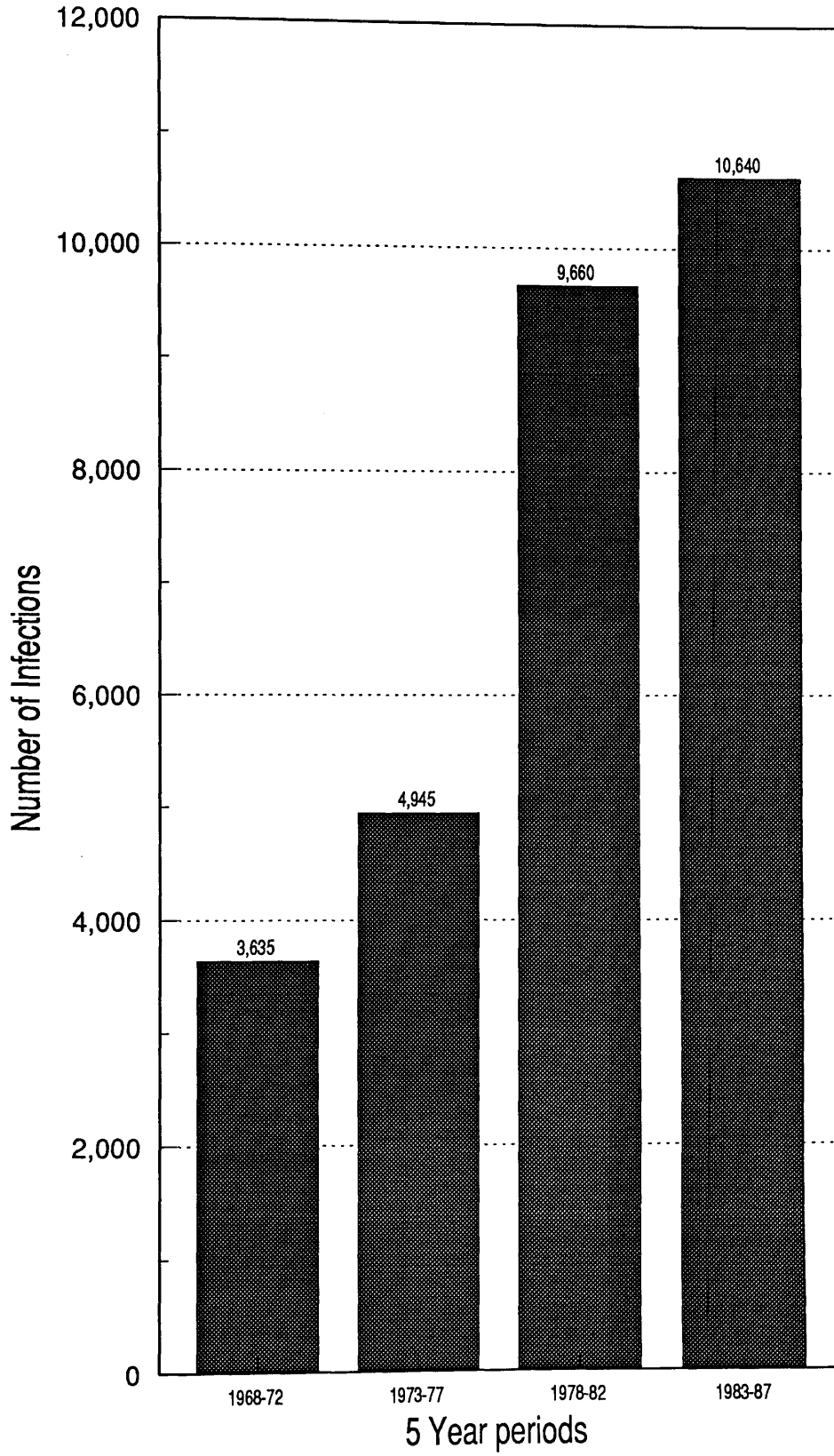


Figure 4.7: Cumulative Incidence of Salmonella Infections (Laboratory Isolations) in 5-year periods

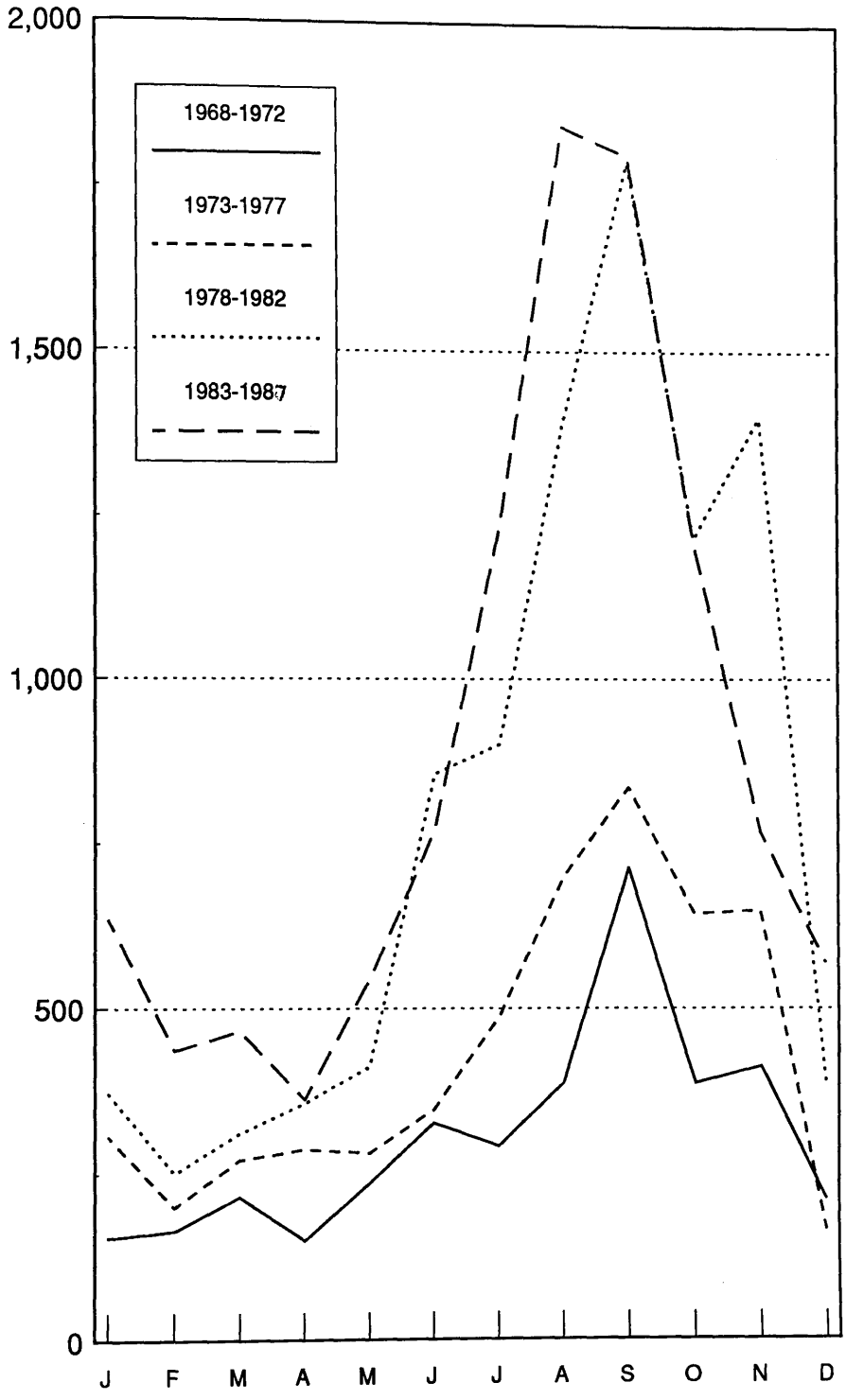


Figure 4.8: Cumulative Monthly Incidence of Salmonella Infections (Laboratory Isolations) at 5-year periods

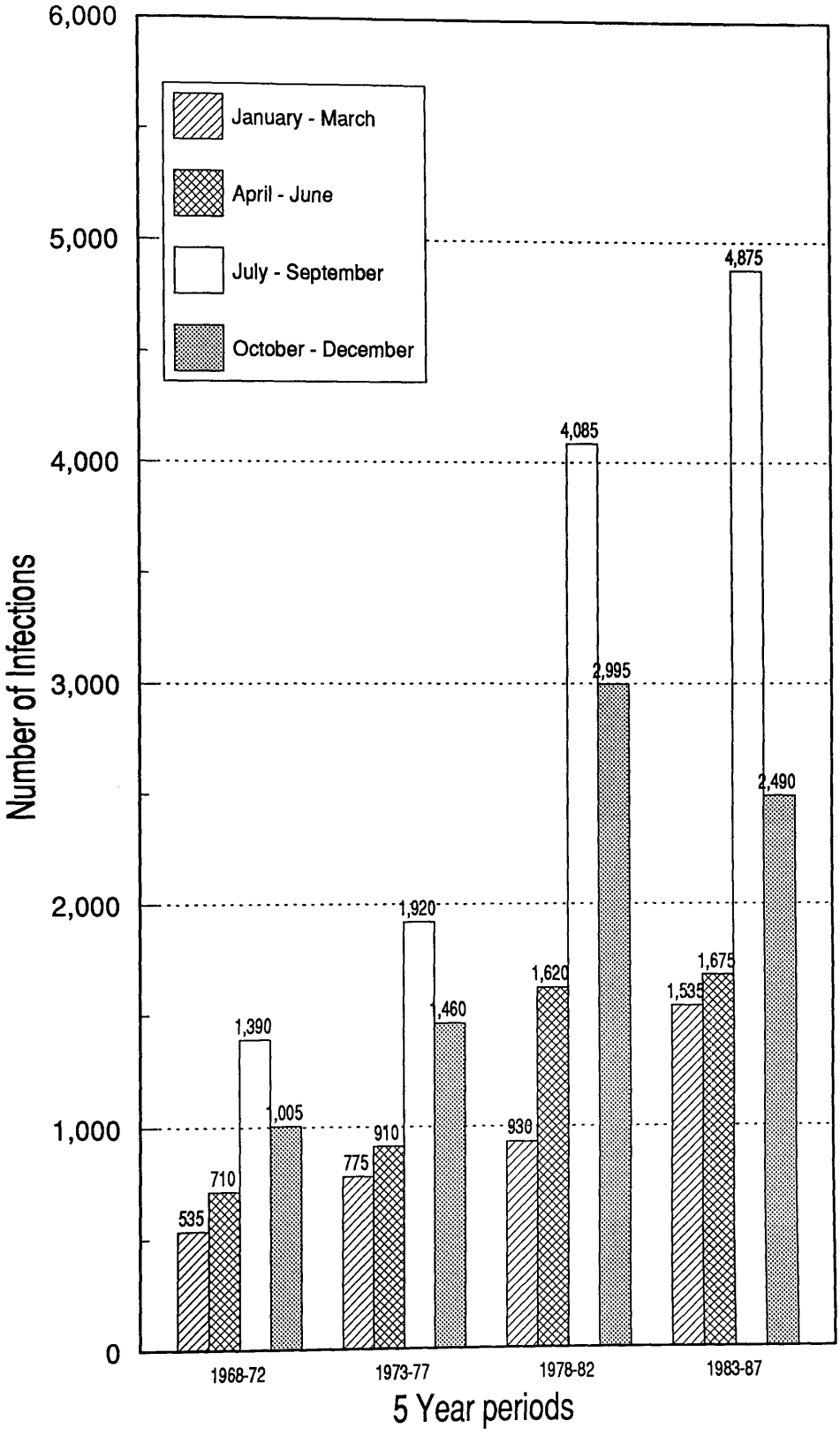


Figure 4.9: Seasonal Trend of Salmonella Infection 1968-87

Figure 4.10: Salmonella Survey Schedule

Figure 4.11

Calculation of the significance Level of the Estimated Odds Ratio : McNeman's Chi-Square Test of Null Hypothesis:

Null Hypothesis : Odds Ratio is Unity (Ho : $\Upsilon = 1$); The Odds of exposure to poultry meat in Cases is not significantly different from the odds of exposure in Controls.

$$X^2_{mh} = (|B - C| - 1)^2 / (B + C)$$

From table 4..3.4, B = 57, C = 18

$$X^2_{mh} = (|57 - 18| - 1)^2 / (57 + 18)$$

$$= (38)^2/75$$

$$= 1444/75$$

$$= 19.25 \; ; \; p < 0.005$$

Figure 4.12

Significance Level of Calculated Odds Ratio : Mantel-Haenszel Two-sided Chi-Square Test of the Null Hypothesis

Null Hypothese : Odds Ratio$_{mh}$ is unity (Ho : $\Upsilon = 1$)

$$X^2_{mh} = (|N_1| - 1/2)^2 / N_2, \text{ where}$$

$$N_1 = [n_1 + n_2 + 2(n_3 - n_4) - (n_5 + n_6)] / 3;$$

$$N_2 = 2[n_1 + n_2 + n_3 + n_4 + n_5 + n_6]/9$$

$$N_1 = [18 + 10 + 2(28-3) - (11 + 3)]/3$$

$$= 64/3 = 21.3$$

$$N_2 = 2[11 + 10 + 28 + 3 + 11 + 3]/9$$

$$= 2(73)/9 = 16.2$$

$$X^2_{mh} = (21.3 - 1/2)^2/16.2$$

$$= (20.8)^2/16.2$$

$$= 432.64/16.2$$

$$= 26.7 \; ; \; p < 0.005$$

Ho is rejected

Figure 4.13

95% Confidence Interval Estimation for the Calculated Mantel-Haenszel Odds Ratio (4.2)

Ho : Odds Ratio = 1

95% Confidence Interval estimated by the equation:

$\exp [(1 + Z\alpha/\sqrt{X^2_{mh}}) \times \ln (\text{Odds Ratio}_{mh})]$

$Z\alpha = 1.96$

$X^2_{mh} = 26.7 = 5.167$

Natural log (ln) of Odds Ratio =

Natural log of 4.2 = 1.435

$\exp [(1 \pm 1.96/5.167) \times 1.435]$

$\exp [1.3793 \times 1.435]$ Upper Limit

$= \exp [1.9793]$

$= 7.24$

$\exp [0.6207 \times 1.435]$ Lower Limit

$= \exp [0.8907]$

$= 2.44$

95% Confidence Interval = (2.44, 7.26)

Ho is rejected

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APPENDICES

APPENDIX I

OUTBREAK INVESTIGATION FORM

FOOD POISONING INVESTIGATION FORM

Case ref. no.	General outbreak		Household outbreak		Sporadic case		Aetiological agent
NAME			G.P.				
Address			Address				Health Board
.....			..... Tel.				
Notification by			Hospital admission date				Local Authority
" date			" discharge date				

DETAILS OF PATIENT(S) AND HOUSEHOLD

+ve or -ve	Name	Age	Occupational details (incl. part-time)	Name and Address of Employer/School	Diarrhoea onset		Vomiting onset		Other symptoms	Duration of -	
					Date	Time	Date	Time		Illness	Incubn

NAMES AND DETAILS OF ANY OTHERS SIMILARLY AFFECTED .....

.....

.....

DETAILS OF RECENT TRAVEL (INCLUDING ABROAD) BY ANY MEMBER OF HOUSEHOLD .....

.....

.....

.....

DETAILS OF FOOD CONSUMED BY INDEX CASE, AND VENUE, IN 48 HOURS PRIOR TO ONSET

Date	Time	Food and Drink (including alcohol)	Where consumed	Remarks

Any type of food(s) NOT EATEN for health, religious or other reasons.....

Food consumed in common by all affected .....

Suspected food(s) .....Brand Name/Batch No.....

Details of purchase, storage, preparation, cooking, re-heating, etc. .....

.....

.....

Details of any raw meats, poultry or pet foods prepared in kitchen during week prior to onset .....

.....

Place(s) where food contaminated or mishandled .....

PERSONS EXCLUDED FROM WORK/SCHOOL

NAME	Exclusion Dates		Clearance Dates		NOTES
	Excluded from Work/School	Letter to Employer/School	Cleared for Work/School	Letter to Employer/School	

NAME	LABORATORY REPORTS											

DETAILS OF FOOD/KITCHEN SAMPLES/SWABS	LABORATORY REPORTS

REGULAR AND CASUAL HOUSEHOLD SUPPLIERS OF FOOD DETAILED OPPOSITE

(include such details as designation, type, brand name/batch no., fresh, frozen etc.)

Milk	
Cream	
Cheese	
Ice Cream	
Bakery produce	
Fish	
Shellfish	
Meat - raw	
" - cooked	
" - tinned	
Poultry	
Eggs	
Fruit	
Vegetables	
Delicatessen foods	
Other	

ENVIRONMENTAL FACTORS

Pets/farm or other animals/vermin .....

Water Supply - specify: Public/private ..... Cistern/mains direct .....

Source ..... Treatment .....

Date visited ..... Signature ..... Designation .....

APPENDIX II

OUTBREAK REPORT FORM

CDS/SF/1

WHO FOODBORNE DISEASE SURVEILLANCE PROGRAMME
OUTBREAK SUMMARY FORM

Household outbreak ☐ General outbreak ☐ CDS Ref. No. _____

Family surname(s) _____

Location of outbreak _____

_____
(please specify town, village, hospital, hotel, farm, etc.)

Aetiological agent
Health Board
Local Authority

Date of onset of - First case _____ Last case _____

No. ill confirmed by laboratory _____ No. ill but unconfirmed by laboratory _____

No. of others confirmed by laboratory, but symptom-free _____

No. at risk (if known) _____ No. hospitalised _____ No. deaths _____

Symptoms (% of these ill) Nausea _____ Vomiting _____ Diarrhoea _____

Abdo. pain _____ Fever _____ Other (specify) _____

Incubation period: Shortest _____ Longest _____ Median _____

Suspected food vehicle (or water source) _____

- confirmed : by Laboratory ☐ Epidemiologically ☐ Unconfirmed ☐

Producer _____ Brand _____ Batch/Code no. _____

Where food prepared/mishandled _____

Methods of processing/preparation _____

_____

Where contaminated (if different from mishandled) _____

_____

Where consumed _____

- (if abroad give brief details) _____

PTO

CDS use only -

ISD form completed ☐ WHO form completed ☐

Factors contributing to foodborne outbreak (tick those appropriate)

Unsafe source	☐	Inadequate thawing	☐	Inadequate cooking	☐
Inadequate cooling	☐	Inadequate reheating	☐	Inadequate refrigeration	☐
Infected handler	☐	Contaminated equipment	☐	Chemical contamination	☐
Other (specify)	_____		☐	Not known	☐

Factors contributing to waterborne outbreak (tick those appropriate)

Sewage pollution	☐	Inadequate treatment	☐	Untreated supply	☐
General flooding	☐	Storage contamination	☐	Not known	☐
Other (specify)	_____		☐	_____	☐

Food/water samples, equipment swabs, etc.	Laboratory results (positive only)

Comments:

Reported by _____ Date _____

THIS FORM TO BE RETURNED TO THE CDS UNIT, RUCHILL HOSPITAL, GLASGOW, G20 9NB.
(Tel. 041-946-7120)

APPENDIX III

QUESTIONNAIRE : SURVEY ON MEAT CONSUMPTION AND ITS ROLE IN FOOD POISONING (A)

SURVEY ON MEAT CONSUMPTION AND ITS ROLE IN FOOD POISONING: FORM A

Please fill OR tick correct answers in the spaces or boxes, as appropriate

Official use:

REF CODE: ☐

1. Your Address :

   (a) Street _____

   (b) Town _____

   (c) Post Code _____

2. Sex : Male ☐ Female ☐

3. Age : _____

4. What is your present occupation?

   _____

5. (a) Does any member of your family work in a meat retail shop, meat processing plant, abattoir or restaurant?

   Yes ☐ No ☐

   (b) If yes, please tick which one of the following applies :

   Meat Retail Shop ☐ Abattoir ☐

   Meat Processing Plant ☐ Restaurant ☐

6. Are you a vegetarian? Yes ☐ No ☐

7. Tick any of the following types of meat which you do NOT eat, because of religious, cultural, health or other personal reason :

Beef ☐ Chicken ☐ None ☐

Pork ☐ Lamb ☐

8. Please try to recall and tick any of the following types of meat you consumed during the past 48 hours.

Pork ☐ Lamb ☐ None ☐

Beef ☐ Chicken ☐

9. Please tick the place(s) where you ate the meat(s) indicated in number 8 above :

	In the home/ household	At work	At a party/ or picnic	Restaurant or hotel	School/ hospital
(i) Pork	☐	☐	☐	☐	☐
(ii) Beef	☐	☐	☐	☐	☐
(iii) Lamb	☐	☐	☐	☐	☐
(iv) Chicken	☐	☐	☐	☐	☐

10. In what form was that meat purchased? Please tick which ones apply :

	Pre-cooked	Fresh	Frozen	Don't Know
Pork	☐	☐	☐	☐
Beef	☐	☐	☐	☐
Lamb	☐	☐	☐	☐
Chicken	☐	☐	☐	☐

11. If the meat you ate was not pre-cooked at the time of purchase, tick the method used in cooking the meat :

	Boiling	Roasting	Grilling	Frying	Don't Know
Pork	☐	☐	☐	☐	☐
Beef	☐	☐	☐	☐	☐
Chicken	☐	☐	☐	☐	☐
Lamb	☐	☐	☐	☐	☐

12. Indicate the source or place of purchase of any chicken you ate during the period indicated in number 11 above (give address or location of retail shop, supermarket, meat shop etc) :

_____

_____

13. If you know the brand of the chicken consumed, please indicate :

_____

_____

14. How many days in a week, on average, would you say you eat each of the following types of meat? Please tick which number of days apply :

	0	1-2	3-4	5-6	7 (days)
Beef	☐	☐	☐	☐	☐
Chicken	☐	☐	☐	☐	☐
Lamb	☐	☐	☐	☐	☐
Pork	☐	☐	☐	☐	☐

15. Did you return from travel outside the United Kingdom in the past week? Yes ☐ No ☐

16. If yes, indicate country visited _____

17. Within the past one year, have you had diarrhoea or vomiting which you thought resulted from food poisoning? Yes ☐ No ☐

18. If yes, was your food poisoning condition serious enough to report to a doctor or hospital? Yes ☐ No ☐

19. Within the past week, have you suffered from diarrhoea or vomiting due to food poisoning? Yes ☐ No ☐

20. If your answer to number 19 is Yes, was it confirmed or were you told that the food poisoning was caused by Salmonella?

Yes ☐ No ☐

21. Were you told that the food poisoning was associated with any meat you ate?

Yes ☐ No ☐

22. If yes, with which type of meat was your food poisoning associated?

Lamb ☐ Pork ☐ Chicken ☐

Beef ☐ Don't know ☐

23. Were any members of your family/household sick from food poisoning within the last 7 days?

Yes ☐ No ☐

24. If yes, please indicate the AGE(S) of the member(s) of the family who suffered food poisoning.

1. Age ☐ 2. Age ☐

3. Age ☐ 4. Age ☐

25. Which meat or other foods was suspected to be the source of the food poisoning?

Beef ☐ Pork ☐ Chicken ☐ Lamb ☐

Other Food ☐

26. At which place did the member(s) of the family eat the suspected meat/food?

In the home/household ☐ School ☐ At work ☐

At a Party/Picnic ☐

Please return the completed questionnaire in the prepaid envelope.