Epidemiological Studies on *Salmonella* Typhimurium DT104 in Scotland

By Jyhmirn Lai MD BVMS

Thesis submitted for the Degree of Doctor of Philosophy in the Faculty of

Veterinary Medicine, University of Glasgow

Division of Animal Production and Public Health

University of Glasgow

May 2007

To my mother, Chin-Chih Lee,

my wife, Pei-Chuan, Hsu,

and my whole family

ABSTRACT

Salmonella Typhimurium definitive type 104 (ST DT104) isolates resistant to antibiotics have been an issue since the multi-resistant clone was identified in 1985. In order to advance understanding of ST DT104 infections in Scotland, 2,796 human and 2,439 animal isolates with their corresponding antibiotic resistance patterns submitted from 1988 to 2004 were used to conduct descriptive, hierarchical, and geographical cluster studies and to construct a time series model. The analyses showed that using the 13 antibiotics used by the Scottish Salmonella Reference Laboratory, isolates could be allocated into two distinct groups. The first group containing the ApClSpStSuTe R-type with its associated resistance patterns, mainly the ApClSpStSuTeTm and the ApClSpStSuTeNa R-types, dominated the trend throughout the study period. The second group, mainly composed of fully sensitive isolates, formed a low proportion except during the period from 1988 to 1990. Descriptive and temporal studies evidenced many similarities between the isolates of human and animal origin. The temporal analyses showed that there was an epidemic from 1993 to 1998 in human ST DT104 and from 1992 to 1999 in animals. The hierarchical cluster analysis also identified many similarities between the isolates from animals and humans although there was a higher proportion of multi-resistance and lower proportion of fully sensitive isolates sourced from animals when compared with humans. Spatial analysis identified the southern part of Scotland as the higher relative risk area for both human and animal infections caused by multi-resistant ST DT104 strains In contrast, the central belt of Scotland was mainly the relative risk lower spatial cluster for the multi-resistant ST DT104 R-types. Of note in the spatio-temporal analysis was the stability and persistence of the chromosomally mediated multiple resistance compared to the more sporadic plasmid mediated resistance types of the second group. Time series analyses were hindered by the quality of the data relating to the animal isolates but there was a suggestion that there may be some underlying fundamental differences in the drivers for patterns from the human and the animal data. When considering the emergence of resistance to individual antimicrobials, although there were many similarities between the infections in humans and animals, there was no consistent temporal association between the emergence of clones in humans compared with animals suggesting that the ecological and epidemiological direction of the relationships is complex. Data quality and uniformity of surveillance systems were identified as important issues for future studies.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

℃: degrees Celsius

AA: Ayrshire & Arran Health Board

AC: Argyll & Clyde Health Board

ACF: the autocorrelation function

Ap: ampicillin

AR: autoregressive

ARIMA: autoregressive integrated moving average

BIC: Bayesian index criteria

BR: Borders Health Board

Cf: cefotaxime

Cl: chloramphenicol

Defra: Department for Environment, Food and Rural Affairs

DG: Dumfries & Galloway Health Board

EC: the European Commission

ED: Euclidean distance

Enter-net: the international surveillance network for human gastrointestinal infections

EU: European Union

FMD: Food and Mouth Disease

FF: Fife Health Board

FV: Forth Valley Health Board

Fz: furazolidone

Ge: gentamicin

GG: Greater Glasgow Health Board

GP: general practitioner

GR: Grampian Health Board

HG: Highland Health Board

HPS: Health Protection Scotland

HRC: higher risk cluster

Ka: kanamycin

Lc: low level ciprofloxacin

LN: Lanarkshire Health Board

LO: Lothian Health Board

LRC: lower risk cluster

MA: moving average

MAPE: the lowest mean absolute percent error

MIC: minimum inhibitory concentration

MR: multi-resistant

Na: nalidixic acid

Ne: netilmicin

OR: Orkney Health Board

PACF: the partial autocorrelation function

PCR: polymerase chain reaction

PFGE: pulsed field gel electrophoresis

Q-Q plot: Quantitle-Quantile plot

RFLP: restriction fragment length polymorphism

RMSE: the lowest root-mean-squared error

R%: resistance percentage

R-type: resistance type

SAC: Scottish Agriculture College

Sal-Net: the international surveillance network for human *Salmonella* infections

SED: square Euclidean distance

SETAR: the self threshold autoregressive model

SGI1: *Salmonella* genomic island 1

SH: Shetland Health Board

SHRC: spatial higher risk cluster

SHRRC: spatial higher relative risk cluster

SLRC: spatial lower risk cluster

SLRRC: spatial lower relative risk cluster

SM: simple matching distance

Sp: spectinomycin

SSRL: Scottish Salmonella Reference Laboratory

ST DT104: *Salmonella enterica* serotype Typhimurium definitive type 104

ST: *Salmonella enterica* serotype Typhimurium

St: streptomycin

Su: sulphonamide

Te: tetracycline

THRC: temporal higher risk cluster

THRRC: temporal higher relative risk cluster

TLRC: temporal lower risk cluster

TLRRC: temporal lower relative risk cluster

Tm: trimethoprim

STHRC: spatio-temporal higher risk cluster

STLRC: spatio-temporal lower risk cluster

TY: Tayside Health Board

UK. United Kingdoms

WI: Western Isles Health Board

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Professor Stuart Reid. Without his teaching and guidance, this work would not have seen the light of day.

I am also deeply grateful for Professor David Taylor's detailed and constructive comments every step of the way. His help with the thesis has been most appreciated.

Without the datasets from Health Protection Scotland and the Scottish Salmonella Reference Laboratory, this study could not have been conducted. I especially appreciate the help of Dr Derek Brown from the Scottish Salmonella Reference Laboratory.

There are many people in the Department of Animal Production and Public Health and the group of Comparative Epidemiology and Informatics at the University of Glasgow who have helped me directly or indirectly. I thank them all, and in particular Dr Giles Innocent for his guidance in statistical methods, Dr. Dominic Mellor for the spatial analysis and Helen Ternent for providing Scottish cultural and other information.

I am indebted to my family for the unconditional support they have given me. Most importantly, my brother, Chih-Cheng Lai and his wife Li-Heng Lin, who by looking after my mother in Taiwan, enabled me to stay away from home for so long to finish my study. My loving thanks to them.

Last, but certainly not least, thank you to the Taiwanese government for their postgraduate scholarship that sustained me during my time in Scotland.

AUTHOR'S DECLARATION

The work presented in this thesis was performed solely by the author except where the assistance of others has been acknowledged. It has not been submitted in any form for another degree or professional qualification.

Some of the work presented in this thesis has been the subject of the following publications and presentations:

Lai JM, Ternent HE, Innocent GT, Mathew L, Reilly WJ, Mellor DJ, Brown D, Coia JE, Reid SWJ. (2007) Using hierarchical cluster analysis to investigate relationships among *Salmonella* Typhimurium DT 104 isolates from humans and animals in Scotland (1990 – 2004). SVEPM. Helsinki, Finland.

Lai JM, Ternent HE, Innocent GT, Mathew L, Reilly WJ, Mellor DJ, Brown D, Reid SWJ. (2006) Antimicrobial resistance; association of trends and clusters of resistant *S.* Typhimurium in contemporaneous animal and human populations. ISVEE XI, Cairns, Australia.

CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

In this chapter, the relevant information about Salmonellae will be described in three sections. The first section contains general information on *Salmonella* and its serotype *Salmonella* Typhimurium definitive type 104 (ST DT104). The second section describes the history of antibiotics and resistance mechanisms found in ST DT104. The third section contains epidemiological information from the UK and other European countries. The definitions used in this thesis are the same as those used by the Veterinary Laboratories Agency (VLA), the Department for Environment Food & Rural Affair (Defra; http://www.defra.gov.uk) and Health Protection Scotland (HPS) (http://www.show.scot.nhs.uk/scieh/).

1.1 INTRODUCTION TO *SALMONELLA*

1.1.1 History of *Salmonella*

Salmonellosis has been causing disease in people and animals for over 100 years. It was first reported by Dr. Daniel Elmer Salmon in 1884 and Theobald Smith isolated the organism two years later. They described an infection caused by hog cholera bacillus which caused "swine plague" and named it as *Baciterium suipestifer*. This organism finally became the typical

species of Salmonella, *S.* Choleraesuis as suggested by Dr. Lingier in 1900 out of respect for Dr. Salmon (Georgala et al. 1999).

In 1888 a German, Dr. Gaeter, isolated *Bacterium* enteritidis during an epidemic. He isolated the micro-organism from both the meat of an emergency-slaughtered cow and the organs of a patient (Bell and Kyriakides 2002). In this epidemic, there were 58 patients who consumed the meat and developed salmonellosis. This was probably the first laboratory confirmed outbreak of salmonellosis (Georgala et al. 1999).

In the following years, some other important species of *Salmonella*, including typhosum (later Typhi), paratyphosum A and B (Paratyphi A and B), Gallinarum and Typhimurium were characterised. These organisms result in a significant range of illnesses including food poisoning (gastroenteritis), typhoid (enteric fever), paratyphoid, bacteraemia and septicaemia in humans and animals. Today, the species of *Salmonella* are very important food-borne and water-borne pathogens and play an important role in public health. To date, more than 2,463 serotypes of *Salmonella* have been identified (Brenner et al. 2000) and they are the most common form of food-borne illness.

Serotypes of *Salmonella* can be grouped into three categories: 1) highly adapted to human hosts, 2) adapted to non-human hosts, and 3) unadapted to specific hosts (Brenner et al. 2000).

The first group includes *S.* Typhi and *S.* Paratyphi A, B (*S. schottmuelleri*), and C (*S. hirschfeldii*), which are pathogenic only in humans and commonly cause enteric fever. The second group causes diseases almost exclusively in animals, although two strains within this group, *S.* Dublin and *S.* Choleraesuis, also cause diseases in humans. The third group, designated *S. enteritidis*, includes 2,000 serotypes that cause gastro-enteritis and account for 85% of all *Salmonella* infections in the USA (Brenner et al. 2000). Usually, when people are talking about *Salmonellae*, they mean *Salmonella enteritidis*.

The epidemiology of the non-typhoidal salmonellosis is similar to, but more complicated than, that of typhoid fever. The main reason is that disease can be spread by direct and indirect contact with infected species of animals, the products derived from these animals, and these animals' excreta. All salmonellae are considered as pathogens and can cause various degrees of salmonella gastro-enteritis (Georgala et al. 1999)

1.1.2 Characteristics of *Salmonellae*

Salmonella is a Gram-negative, facultatively anaerobic, straight, small (0.7-1.5 * 2.0-50 uM), and non-endospore-forming rod. They can move using peritrichous flagellae. Their normal habitat is in the intestinal tracts of animals, especially poultry and cattle. Contamination of food material can occur under insanitary conditions (Georgala et al. 1999). The biochemical characteristics of *Salmonella* are: Catalase (+); Oxidase (-); Acid produced from lactose (-);

Gas produced from glucose (+) – except Typhi; Indole (-); Urease produced (-); Hydrogen sulphide produced from triple-sugar iron agar (+); Citrate utilised as sole carbon source (+), except Typhi; Methyl red (+); Voges-Proskauer (-); Lysin decarboxylase (+); and Ornithine decarboxylase (+) (Georgala et al. 1999).

The genome is about 4.5 Mbp in size. The similarity of the *Salmonella* at the DNA level is between 95% to 99%. *Escherichia coli* is the closest genus to *Salmonella* with between 60% and 70% similarity (Georgala et al. 1999).

Different serotypes of Salmonellae can be distinguished by specific antibodies using a system known as the Kauffmann-White scheme (Brenner et al. 2000; Georgala et al. 1999). This system names an organism by numbers and letters that correspond to specific antigens on the organism's capsule (K), cell wall (O; somatic), and flagella (H). Serotype names designated by antigenic formulae include the following: (i) subspecies designation (subspecies I through VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) (e.g. *S.* serotype IV 45:g,z51:-). Many newly discovered *Salmonella* serotypes are named only by their antigenic formulae. Serovars can be further differentiated by special biochemical or physiological properties into biovars, or biotypes (Brenner et al. 2000).

1.1.3 The nomenclature system of *Salmonella*

The traditional nomenclature system names bacteria by family, genus and then serotype. However, there are too many similar serotypes and there is a high likelihood that this number will continue to increase. In addition and for example, the majority (59%) of the 2,463 *Salmonella* serotypes belong to *S. enterica* subsp. I (*S. enterica* subsp. enterica). Consequently, the Judicial Commission of the International Committee for Systematic of Prokaryotes has decided to adopt the suggestions from Le Minor and Popoff in 1987 to divide the bacterial species *Salmonella enterica* into six subspecies: enterica, salamae, arizonae, diarizonae, houtenae and indica (Judicial Commission of the International Committee on Systematics of Prokaryotes 2005).

The naming system of serovars of subspecies enterica differs from the other five subspecies, in which the familiar serovar names are still assigned to subspecies enterica. However, the other subspecies are designated by antigenic structure only as described in 1.2.2. In this thesis, *S.* Typhimurium DT104 (ST DT104) refers to *S. enterica* subspecies *enterica* serovar Typhimurium definitive type 104.

1.1.4 *Salmonella* as an aetiological agent

From the 1980s onwards, the second most common cause of food poisoning by *Salmonella* species was due to *S.* Typhimurium (ST). As its name suggests, it causes a typhoid-like

disease in mice. In humans, ST does not cause as severe disease as *S.* Enteritidis, and is not normally fatal (Leegaard et al. 2000).

The main route of transmission for all salmonellosis is oral-faecal route, especially via contaminated water or foods. Human multi-resistant (MR) ST DT104 infection has been associated with the consumption of raw meats and their products and/or with contact with infected cattle (Threlfall 2000). The bacteria colonises and then multiplies on the intestinal mucosa. It may pass through the intestinal mucosa to enter the lymphatic and cardiovascular systems and then spread and affect other organs. The infection is more common during the summer and is more associated with some human activities than others (Killalea et al. 1996).

The symptoms of salmonellosis in humans usually begin 12 to 72 hours following ingestion. The symptoms and signs of salmonellosis include gastro-enteritis, enteric fever, focal manifestations, and occasionally bacteraemia. The duration of signs is generally up to 7 days. Patients feel a moderate fever accompanied by nausea, abdominal pain and cramps, and diarrhoea. The patient can shed a billion *Salmonellae* per gram in faeces during the acute phase of the illness and shedding may continue for up to 6 months (Georgala et al. 1999). Persistent shedding of *Salmonella* in faeces longer than 1 year occurs rarely – in only 0.2 to 0.6% of patients with nontyphoidal Salmonella infections (Georgala et al. 1999). Even though bacteraemia is rare, some serotypes of *Salmonella*, e.g. *S.* Typhi, *S.* Paratyphi, *S.* Choleraesuis,

S. Typhimurium, *S.* Dublin, and *S.* Heidelberg, are invasive (Stephen et al. 2003).

The mortality rate is usually lower than 1%. However, the death rate may be higher in infants, in immunocompromised patients, and among the elderly. The main cause of fatality is bacteraemia. The crude mortality associated with bacteriemia may be above 25% due to hospital specific variables (Pessoa-Silva et al. 2001; Pittet and Wenzel 1995). *Salmonella* infection is responsible for 70 to 100 deaths in England and Wales each year, either directly or indirectly (Humphrey et al. 2000).

The reported mortality and morbidity in animals varies because severe diarrhoea and anorexia in production animals will often be treated without specific diagnosis. The infection is generally asymptomatic and may become chronic. Usually, infection in animals is limited to diarrhoea, a common occurrence which generally does not demand extensive medical intervention. The bacteria can be carried in animals for weeks or months, and shedding may continue long after the animal has visibly recovered. Many animals will never show signs, but will serve as reservoirs and passive carriers of the disease (Smith et al. 2007).

1.1.5 Factors affecting growth and survival of *Salmonella*

Temperature is an important factor of direct influence in the growth of Salmonella in different seasons although the precise role of temperature has been an issue for over 100 years. The

normal range of survival temperature for Salmonella is between 5.2℃ to 46℃ but the growth rate is greatly reduced at temperatures below 15℃ (Bell and Kyriakides 2002). *Salmonella* Enteritidis *and* Typhimurium can survive at 27℃ in ice cream for 7 years. (Bell and Kyriakides 2002). It is well documented that the survival in environmental conditions (including heat) rely on the mechanisms regulated by *rpo* genes (Bell and Kyriakides 2002).

Few Salmonella serotypes will grow at temperatures below 7℃ (Bell and Kyriakides 2002). The minimum reported temperature for the growth of *S.* Typhimurium is 6.2℃ (Anon 1992). The resistance to low temperature can vary given other environmental conditions; for example, dry conditions increase heat resistance and low pH reduces heat resistance (Bell and Kyriakides 2002). Examples of the effect of environment on the ability of ST DT104 to survive is presented in Table 1.1 showing varying temperatures and times required for death of the bacteria. The fat content in beef provides protection from heat, thus ST DT104 can survive for longer in mince with a higher content of fat (Juneja and Eblen 2000). The sucrose content of a substrate also affects the ability of Salmonella to survive for longer at 65℃ rather than glucose, sorbitol or fructose (Bell and Kyriakides 2002). The effect of the changing rate of temperature from low temperature to a lethal high temperature can make *Salmonella* produce heat shock proteins (Bell and Kyriakides 2002).

Table 1.1 Death (D) values in minutes for Salmonella in some food substrates at given temperatures (Bell and Kyriakides 2002)

Food substrate/condition	Salmonella serotype	Temperature-℃	D value
Liquid whole egg, pH 8.0	ST	60	0.55 min
Liquid whole egg, pH 5.5	ST	60	2.2 mins
Liquid whole egg	ST DT104	55	6.05 mins
Liquid whole egg	ST (except DT104)	55	8.04 mins
Ground beef	ST	63	0.36 mins
Milk Chocolate	ST	71	6.6 hours
Liquid whole egg + 10% salt	ST DT104	55	4.21 mins
Liquid whole egg + 10% salt	ST (except DT104)	55	4.73 mins
Liquid egg yolk	ST DT104	55	9.40 mins
Liquid egg yolk	ST (except DT104)	55	8.03 mins
Liquid egg yolk + 10% salt	ST DT104	55	9.06 mins
Liquid egg yolk + 10% salt	ST (except DT104)	55	10.85 mins

1.1.6 Identification methods

Methods for detecting the presence of Salmonella in samples have been well established for many decades. Currently, most national and international microbiological standard methods for the detection of Salmonella, and food industry recommended methods, are based on the recommendations of different bodies (Tortora et al. 2001), e.g., European Standard 12824; Association of Official Analytic Chemists; UK Public Health Laboratory Service; Campden and Chorleywood Food Research Association.

1.1.6.1 Bacterial isolation

Bacterial isolation is the first step in identifying the bacteria. The procedures start with

pre-enrichment in media followed by sub-culture into selective enrichment broths and finally selective plating. Once the bacteria have been isolated, identification using a number of biochemical tests and serotyping can be undertaken. The time taken to identify the bacteria ranges from 24 to 72 hours (Bell and Kyriakides 2002).

There are many different methods of isolation that have been developed. The most commonly adopted method of isolation uses buffered peptone water as a pre-enrichment medium with selective enrichment broth Selenite-Cystine (SC) broth (1+9) or Rappaport-Vassiliadis (RV) broth (1+100) and Brilliant Green Agar or Hektoen Enteric Agar, and XLD Agar as the selective agar (Bell and Kyriakides 2002). Once the bacteria has been isolated and identified as *Salmonella* these isolates can be stored for further analysis at a later date.

1.1.6.2 Alternative methods

In order to reduce the identification time, faster and more sensitive methods are continually being developed. Some techniques are based on antibody-antigen response, some are based on the modified biochemical identification, and others are based on the molecular biological processes. Due to concerns about false positives in new techniques, the traditional bacterial culture is still carried out for confirmation (Tortora et al. 2001).

The biochemical identification kits are based on the traditional biochemical reactions (Bell

and Kyriakides 2002). These reactions are prepared in pre-formed chambers and supplied in a disposable unit. After inoculation and incubation of the test chambers, reaction results are read. Usually, the readers just need to describe positive/negative and value the score by specific colour changes in the media. Finally, a profile of the organism is obtained and usually the result is analysed by software.

Enzyme-linked immuno-sorbent assay (ELISA), immuno-chromatography, chemi-luminescent immuno-assays, antibody-coated dipsticks or beads, latex agglutination and other technologies such as electrical conductance methods belong to this field (Tortora et al. 2001). Some kits use prepared Salmonella specific antibodies that attach to Salmonella antigens providing a rapid and useful method of identification, but false positives can occur.

The polymerase chain reaction (PCR) is a very powerful and fast method, which use primers to amplify the number of copies of a pre-selected region of DNA to a sufficient level to test for identification (Georgala et al. 1999). There are many new methods based on PCR, e.g. 16S-23S ribotyping; rep-PCR-(ERIC, REP AND BOX elements)-targets multiple copies of conserved repetitive DNA sequences; RAPD, AP-PCR -targets multiple random chromosomal locations; AFLP-multiple genomic restriction fragments amplified; IS fingerprinting - targets insertion sequences; VNTR, SSR – targets sequence repeats in coding and non-coding sequences; SLST, MLST-sequencing of hyper-variable genes, Pyro-sequencing; and DNA

microarrays (Georgala et al. 1999).

The plasmid profile method presents the plasmid profile on agarose gel after serial procedures including extraction and separation of the target extra-chromosomal circular DNA. Due to the transferable nature of plasmids amongst organisms, and even different species, plasmid profiling is very useful for tracing these epidemiological markers. The method is routinely used in the Salmonella Scottish Reference Laboratory (SSRL) and the results are usually published in HPS (formerly SCIEH) Weekly Reports. (Browning et al. 2005).

Typing of an organism by Pulsed-field gel electrophoresis (PFGE) is also a useful epidemiological tool and is also used in SSRL. It involves the cutting of chromosomal DNA into large fragments by specific enzymes. The chosen enzyme for cutting the DNA of *Salmonella* is *Xba*I. The separation of these fragments by electrophoresis provides a 'fingerprint'. The comparison of fingerprints from different isolates can provide some information such as where the related isolates occurred (Browning et al. 2005). This information helps to find related outbreaks and to trace the source of the infection. Even though the discriminatory power of the PFGE method has been challenged in several reports that show the difficulty of typing DT104 isolates with PFGE (Lindstedt et al. 2003; Liebana et al. 2002), it is still a well-developed and suitable method for epidemiologic analysis. Ridley and Threlfall have reported that sensitive and multidrug-resistant strains of ST DT104 can be

separated by PFGE with *Xba*I, generating a fragment of approximately 10kb which hybridized to the integrons (Baggesen et al. 2000; Ridley and Threlfall 1998). Combining two or more epidemiological methods to trace *Salmonella* is useful in practice to trace the source of an outbreak. A variety of sources exist which may be used for guidance in appropriate method selection. National and International Standard methods published, for example, by the British Standards Institution, the European Committee for Standardisation (CEN), International Organisation for Standardisation (ISO), and the International Dairy Federation (IDF) are available. In addition, methods have been reviewed, practiced and validated by reputable bodies such as the Public Health Laboratory Service (UK), the Association of Official Analytical Chemists (USA) and Campden & Chorleywood Food Research Association (UK).

1.1.7 Sources of salmonellosis

Poultry eggs, and egg products are considered high risk foods due to their likelihood to be contaminated by *Salmonella* (Tortora et al. 2001). Most salmonellosis can be traced back to dairy, poultry and meat products, but *Salmonellae* can grow on many food substrates (Takkinen et al. 2005). Vegetables, such as lettuce, can be a source for spreading salmonellosis (Takkinen et al. 2005). The intestinal tracts of animals are a potential source of the bacteria for the contamination of meat during processing. Another source of *Salmonella* is the faeces of pets, especially those with diarrhoea. Wild birds have been shown to be a vector in the spread of salmonellosis (Hudson et al. 2000),

ST DT104 has been isolated from cattle, pigs, sheep, horses, chicken, pigeons, rabbits, cats, dogs and wild animals (Threlfall 2000). Pets can carry ST DT104 (Carlson and Ferris 2000) and their carrier rate can be as high as 90% (Tortora et al. 2001). One famous epidemic in the 1970s was caused by direct contact with small turtles and, in response, the USA government banned the sale of small turtles (Anon 1986). Wild animals also play a role in dissemination of ST infection among livestock (Hollinger et al. 1998; Liebana et al. 2002). Humans are also a potential source of infection with person to person transmission by the faecal-oral route, principally via contaminated water or food (Parry 2003). In the UK, the main reservoir for ST DT104 and MR ST DT104 is cattle, although they have been isolated in other farm animals (Defra 2003; Browning et al. 2004; Browning et al. 2001a; Browning et al. 2001b; Browning et al. 2005; Browning et al. 1999; Browning et al. 2003).

The are several points throughout the meat production chain, from farm to meat product, which have been associated with the contamination (Nielsen et al. 2001). Chronic infected animals are more likely to shed bacteria during stressful situations, such as transportation, unloading and mixing in the lairage. The slaughterhouse environment can be a source of direct and indirect transmission through contaminated equipment or people (Nielsen et al. 2001). One infected animal could introduce bacteria to the slaughterhouse environment, providing a source of possible contamination for meat being processed.

1.1.8 Control and prevention

The economic cost is huge regardless of whether the infection occurs in humans or animals. The economic impact of dealing with *Salmonella* infection on a farm is very hard to predict and much of the cost depends on management, control and prevention programmes. The total cost of mortality of pigs and increased feed consumption during a *Salmonella* outbreak was £9,608 in a 400 sow farm (Anon 2007d). The costs in industry in theoretical studies conducted by Reading University were estimated as £6 m per year in the poultry industry and between £0.5 m and £2.2 m in the cattle industry (Anon 2007c). For the human infection, the British government estimates costs in excess of £1.7 million (Anon 2007b).

Numerous efforts to reduce the risks and costs of salmonellosis and ST DT104 infections have been made. The implementation of control and prevention methods have been made to cover all aspects of the meat production chain 'from farm to fork'. Two basic standard control protocols are : 1) Herd Biosecurity, which is used at farm level (Anon 2007b), and 2) Hazard Analysis Critical Control Points (Food Standards Agency 2007), which is used to manage the risk of contamination occurring during processing of raw food to prepared food.

Herd Biosecurity recommends that every farm needs to use suitable disinfection against pathogens. Unsuitable disinfection procedures and improper disinfection programmes may

result in competitive pathogens growing and may produce resistant bacteria. "Biosecurity: Publicity campaign at markets 2005" was an initiative launched by Defra (Anon 2007b).

In addition to biosecurity, there are a number of other important methods to prevent salmonellosis spreading within a farm or between farms. Normally, quarantine, buying disease free animals, vaccination, and hygiene are useful and routine activities for farmers. In instances where an infected animal is unlikely to recover, slaughter is the best way to remove the animal and prevent further spread of disease within the farm. Suitable diagnostic methods, appropriate treatments correctly administered, and disease control programs are also important in maintaining disease status on a farm.

The HACCP system is an internationally accepted procedure to manage and prevent food contamination following seven principles. It provides a suitable way to identify and control risks by setting alarm systems at suitable points, thus the food safety hazards can be managed and reduced to an acceptable level. Meat (HACCP) Regulations 200 was replaced by EU Regulation 852/2004 (Article 5). The new regulation requires food business operators in the supply chain to implement and maintain hygiene procedures, which are based on HACCP principles

The implementation of good hygiene is important to prevent human to human spread of

foodborne pathogens. Food and or the environment may be polluted by carriers, especially by those who forget to wash their hands with soap after using the bathroom. Chicken meat is believed to be a factor for widespread dissemination in the kitchen of *Campylobacter* and *Salmonella* (Humphrey et al. 2000). The contaminated food or environment then becomes a possible reservoir of infection for others. Cross-contamination of foods should be avoided during cooking preparation. It is recognised as an important contributory factor in approximately 30% of domestic outbreaks of *Salmonella* infection (Humphrey et al. 2000). Uncooked meats should be kept separate from produce, cooked foods, and ready-to-eat foods. Hands, cutting boards, counters, knives, and other utensils should be washed thoroughly after handling uncooked foods. People who have salmonellosis should not prepare food or pour water for others until they have been shown to no longer be carrying the Salmonella bacterium. Hands should be washed before handling any food, and between handling different food items.

Pets, especially those with diarrhoea are also a potential reservoir of infection for their owners. Reptiles, dogs and cats have been proved as reservoirs of Salmonella (Balotescu et al. 2003). People can become infected if they do not wash their hands after contact with pets' faeces. In 2007, NHS Greater Glasgow and Clyde launched a campaign advocating hands washing (Anon 2007a).

Vaccines are not usually suggested for human adults; however, for children, there are three kinds of vaccine suitable. The traditional vaccine protects efficiently for 4 to 7 year olds and can approach protection rates between 51% to 76%. The live oral recombinant Salmonella vaccine is more efficient, but is only suitable for children over 6 years of age. The polyglycotide vaccine is suitable for children over 2 years of age and its protection efficiency can reach between 64% to 72% (Bell and Kyriakides 2002).

In conclusion, the best way of preventing salmonellosis is good food hygiene and correct cooking. There is a greater risk of infection in the consumption of raw or unpasteurised milk or other diary products. Produce should be thoroughly washed before consuming.

1.1.9 The mechanisms of antimicrobial resistance genes and other resistance mechanisms in ST DT104

1.1.9.1 The emergence of multi-resistant Salmonella serotypes

There is long standing contention that multi-resistant strains spread from animals to humans. In the 1980s, it was asserted that antibiotic resistant bacteria were being transferred from animals to humans directly in meat and milk (Wall et al. 1994). In 1985, An antibiotic-resistant strain of *S.* Typhimurium infected 16,000 people in seven US states and the source of origin was one Illinois dairy farm (Ryan et al. 1987). As a consequence the

emergence of MR ST DT104 strain in animals has been linked by some researchers to the use of antimicrobial agents in some food animals (Angulo et al. 2000)

An increase in resistance of ST to antimicrobial agents has been reported in several countries during the last 10 years (Threlfall 2000). The main reason for this increase is the emergence of a ST DT104 clone, which is resistant to five or more antibiotics: ampicillin (A); chloramphenicol (C); streptomycin (S) (or with spectinomycin); sulphonamides (Su) and tetracycline (T) (Threlfall 2000). This clone is typically known as R-type ACSSuT ST DT104. The clone is famous because these resistance mechanisms are operated by chromosomal-integrated genes. Trimethoprim and ciprofloxacin resistances have recently been observed in ST DT104 isolates (Leegaard et al. 2000). It is suggested that the emergence of the additional trimethoprim resistant strains is caused by use of the drug to treat the ST DT104 ACSSuT R-type infections (Threlfall 2000). It is also suggested that the additional nalidixic acid resistance is related to the licensing of flouroquinolones in veterinary use (Threlfall 2000).

Molecular typing analysis has indicated that ST DT104 isolates from different hosts and geographical origins can be very similar (Malorny et al. 2001; Prager et al. 1999). Conversely, diversity of MR ST DT104 has been demonstrated (Threlfall et al. 2005; Yang et al. 2002). For example, isolates resistant against ApClSpStSuTe and ApClSpStSuTeTm identified in

2000 had different RFLP profiles from previous clones (Threlfall et al. 2005).

1.1.9.2 Where and how does ST DT104 get antimicrobial resistance genes?

Darwin's theory, survival of the fittest, suggests that suitable species can survive under selection pressure. The evolution of antimicrobial resistance bacteria might be considered as a good example of adaptive evolution (Levin et al. 2000). The organisms with resistance genes would be expected to survive and proliferate when the fitness cost associated with resistance is low relative to non-resistant organisms (Dick 2003).

1.1.9.2.1 Mutation: stimulation-response reaction

Mutation may occur in the process of cell proliferation. The mutation can occur through environmental changes that then activate certain genes. The spontaneous rate of mutation in a bacterial population for any particular gene is usually very low – about 1 per 10^6 to 10^8 (Rang and Ritter 1971). However, this is high enough for antibiotic resistance to develop. The surviving bacteria may then have, under pressure, a better chance of continued survival and the potential to propagate the necessary gene to the next generation. Mutation can occur in two phases - the stationary phase and the logarithmic growth phase. The mutation in the former phase has a better chance of leading to adaptive mutation (Loewe et al. 2003;Carlson and Ferris 2000).

The mutation rate is controlled by many mechanisms in *Salmonella* spp.. The *mutS* gene, which is responsible for DNA mis-match repair, can function under stress caused by an environment rich in ampicillin-derivatives (Carlson and Ferris 2000). Presence of bile salts and chloramphenicol can induce the over-expression of *marA* gene, which translate multi-functional efflux pump protein (George and Levy 1983). In addition, the numbers of active efflux pumps on the cell membrane or the numbers of plasmids will be high when ciprofloxacin is present as an inducer (Chu et al. 2005). Environmental response genes, such as the *rpo* gene could be induced and a high mutant rate would be expected (Jorgensen et al. 2000).

1.1.9.2.2 Plasmids, transposons, integron or gene cassette

Transposons can jump out of or into any DNA segment, e.g., plasmid DNA or chromosome DNA. They can also "hitch-hike" on a plasmid to a new species (Hall and Collis 1995). However, transposons cannot replicate themselves but can replicate during transfer. Plasmids can be transferred to another bacteria via conjugation. The conjugation process can occur either in the same species or in different species. This occurs more readily in the gut because there are many gene fragments in the gut contents (Hall and Collis 1995). The antimicrobial resistance genes in plasmids are called *r* genes

Integrons also play a role in the resistance gene transmission and are usually located in a transposon. An integron may contain many different resistance genes. This system – transposon/integron/multi-resistance cassette array – allows particularly rapid and efficient transfer of resistance genes between plasmids, between chromosomes and between plasmids and chromosomes (Hudson et al. 2000).

There are 9 classes of integrons. (Hochhut et al. 2001; Nield et al. 2001). Over 60 distinct antibiotic resistance gene cassettes have been found in a single integron (Naas et al. 2001; Fluit and Schmitz 1999). Many plasmids encoding multi-resistance in the *Enterobacteriaceae* carry a transposon of the Tn21 family, which contains a class 1 integron (Goldstein et al. 2001; Grinsted et al. 1990). In one study, 60% of *Escherichia coli* isolates from poultry possessed a Tn21-like transposon carrying the *aad*A1 streptomycin-spectinomycin resistance gene cassette (Bass et al. 1999).

Class 1 integrons are widely distributed in nature (Hudson et al. 2000). All class 1 integrons possess the following key components: an integrase gene (*intI*1), a recombination site (*attI*1), and a promoter (Pc) for the transcription of cassette-associated genes. These components encompass the basic functions needed for the acquisition and expression of a second type of mobile genetic element known as a gene cassette (Hall and Collis 1995; Stokes and Hall 1989). As a result of multiple insertion and excision events, class 1 integrons can compile and

shuffle arrays of gene cassettes (Hall and Collis 1995; Stokes and Hall 1989). When an integron has hitch hiked into bacterial genome, the bacteria propagate the resistance genes into the next generation. The antibiotic resistance gene cassette, which is called *Salmonella* genomic island 1 (SGI1), is located on a ST DT104 chromosome and is class I integron (Boyd et al. 2001; Boyd et al. 2000). The structure of SGI is shown in Figure 1.1 (Cloeckaert and Schwarz 2001).

1.1.9.3 The structure of SGI 1 in MR ST DT104

It is now believed that SGI1 the original gene cassette of antimicrobial resistance genes inside the ST DT104 chromosome could come from three sources: the bacterial pathogen of fish (Doublet et al. 2003), *Pseudomonas* spp., and from within the *Enterobacteriae* group (Cloeckaert and Schwarz 2001).

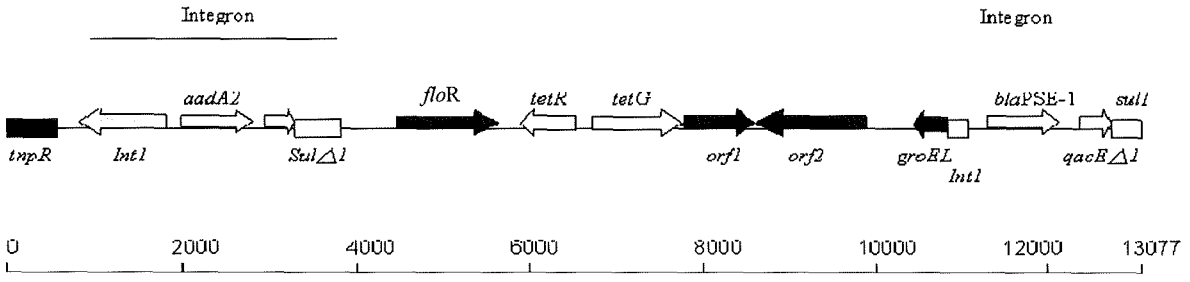


Figure 1.1 Gene organization of SGI1 of ST DT104 according to Cloeckaert and Schwarz (Cloeckaert and Schwarz 2001).

Two different integrons were identified in the ACSSuT R-type ST DT104 clone in 1998

(Ridley and Threlfall 1998;Sandvang et al. 1998). The first integron carries *aad*A2 gene, which confers resistance to streptomycin-spectinomycin and *sul*Δ1 genes, which is a non-functional gene. The second integron contained the β-lactmase gene bla_{PSE-1} (Cloeckaert and Schwarz 2001) and *sul*1 gene, which confers resistance to sulphonamide and disinfectant resistance gene *qac*E1. In 1999, several research groups identified the remaining resistance genes conferring resistance to chloramphenicol and tetracyclines, which are located between the two integrons. As a consequence, all antibiotic resistance genes cluster on a chromosomal locus of about 12.5 kb (Cloeckaert et al. 2000). The gene conferring resistance to chloramphenicol also confers resistance to its fluorinated analogue, florfenicol, and has been named *cml*A-like, *flo*R or *flo*St (Cloeckaert and Schwarz 2001). According to the deduced amino acid sequence homologies and the topology of the assumed protein, this gene encodes an efflux pump, which belongs to the 12-transmembrane segment (TMS) family export proteins of the major facilitator superfamily (MFS) (Paulsen et al. 1996). Downstream of the *floR* gene are the tetracycline resistance genes *tetR* and *tetA*.

This chromosomal antibiotic resistance gene cluster is a part of SGI1, which is about 43 kb (Boyd et al. 2001; Boyd et al. 2000). SGI1 is located in the genome between the *thdf* gene and a prophage CP-4-like integrase gene (int2). It is flanked by an imperfect 18-bp direct repeat (Boyd et al. 2000). A 1.9 kb retron sequence was located in the downstream of SGI1 between genes *int*2 and *yid*Y. This retron sequence may be unique to ST because it has not been

detected in other *S. enterica* serovars (Boyd et al. 2000).

Although it is popularly believed that the distribution of ST DT104 is caused by the use of antimicrobial agents in agriculture, some genes in the cluster, such as *aad*A2, bla_{PSE-1}, or *sul*I, are widely distributed among *Enterobacteriaceae*. The remaining two genes, *flo*R and *tet*A, are most probably not of enterobacterial origin (Cloeckaert and Schwarz 2001). A *flo*R homologue was first identified on a plasmid in a fish pathogen, *Photobacterium damselae* (Cloeckaert and Schwarz 2001). The *tet*G gene has also been detected on plasmids of *Photobacterium damselae*. In addition, the cluster containing *tet*G gene and the *flo*R gene in ST DT104 antibiotic resistance strain was also first identified in a fish pathogen, *Vibrio anguillarum*. They have a similar G+C content (58%) and could be thought to have the same origin. Angulo and Griffin concluded that the resistance determinants of MR ST DT104 emerged among bacteria in aquaculture and then horizontally transferred to ST DT104 (Augulo and Griffin 2000)). Fish have also been identified as the main reservoir of SGI1 (Doublet et al. 2003). The use of antimicrobial agents in animals would then favour the dissemination of the MR ST DT104.

However, another hypothesis by Davis et al. states that the cassette's origin is most likely from *Pseudomonas* spp., which occurs in hospitals (Cloeckaert and Schwarz 2001). Thus, the dissemination of MR ST DT104 could be associated with nosocomial infection and also

associated with usage of antibiotics. The main support for this argument is that *tet*G occurs in bacteria of this genus, and similarly, *flo*R is closely related to the *P. aeruginosa* chloramphenicol-resistance gene *cml*A. Moreover, the bla_{PSE-1} gene is a common feature of hospital *P. aeruginosa* isolates (Cloeckaert and Schwarz 2001).

Nevertheless, identifying the origin of MR ST DT104 only represents the visible part of an iceberg. The fact is that antibiotic resistance genes are chromosomally encoded. The spread of MR ST DT104 might occur even without the selective pressure imposed by the use of antibiotics (Cloeckaert and Schwarz 2001). In 1994, Threlfall said, "Since the resistance now has been shown to be integrated chromosomally, withdrawal of antibiotics is unlikely to have a significant effect" (Threlfall et al. 1994).

Recently, the mobile ST DT104-like antibiotic resistance gene cluster has been found in *Salmonella* Agona. It has been shown that the resistance genes of ST DT104 can be efficiently transduced by P22-like phage ES18 and by phage PDT17, which is released by all DT104 isolates analysed so far (Cloeckaert et al. 2000).

1.1.9.4 The relevant antimicrobial resistance genes and their mechanisms

Antibiotic resistance genes can translate functional proteins to reduce the effect of antibiotics or destroy antimicrobial agents. Usually, the resistance mechanisms are operated by 1) change

in impermeability, 2) enzyme inactivation, 3) enzyme addition, 4) enzyme transfer, 5) active efflux pumps, 6) using an alternative way to synthesize necessary enzymes, and 7) use of an alternative target to bind. There have been more than 100 resistance genes recognised (Dick, 2003). In this section, the resistance genes against the antibiotics used in Scottish Salmonella Reference Laboratory (SSRL) will be introduced.

1.1.9.4.1 Impermeability

Because the cell wall in Gram-negative bacteria is double-layered, antimicrobial agents are a little harder to efflux into Gram-negative bacteria, such as *Salmonella*; this process is called "impermeability" (Gillespie and Bamford 2003). For example, Gram-negative bacteria are impermeable to some beta-lactam antibiotics. Gram-negative bacteria are usually more resistant to antimicrobial agents compared with Gram-positive bacteria.

1.1.9.4.2 Enzyme inactivation – for beta-lactam antibiotics

This is the most common resistance mechanism, especially for Gram-negative organisms (Gillespie and Bamford 2003). It occurs when the bacterium produces beta-lactamase that can break open the beta-lactam ring of penicillins and cephalosporins rendering these agents inactive. The gene $bla_{\text{PSE-1}}$ is the main functional gene in antibiotic resistant ST DT104 (Cloeckaert and Schwarz 2001).

1.1.9.4.3 Enzyme addition

Bacteria may express enzymes that add a chemical group to the antibiotic and the antibiotic function is then reduced or prevented (Gillespie and Bamford 2003). The process of inactivation of aminoglycosides may be via phosphorylation, adenylation or acetylation. The resistance genes are carried on plasmids and several are found on transposons. The *aadA*2 gene can be found in some strains of MR ST DT104 (Cloeckaert and Schwarz 2001). Additionally, *aaa*C(3)IV gene can be found in some R-plasmids (Davies and O'Connor 1978).

1.1.9.4.4 Enzyme transfer

The enzyme acetyltransferase can be produced by gram-positive and gram-negative organisms and it is plasmid-borne (Sands and Shaw 1973). Acetyl-transferase is very important in the inactivation of chloramphenicol. In gram negative bacteria, this enzyme is produced constitutively and consequently the level of resistance in gram-negative bacteria is fivefold higher than that in gram-positive bacteria, in which the enzyme is inducible (Rang and Ritter 1971). The chloramphenicol acetyltransferase (CAT) gene encodes an enzyme which catalyses *O*-acetylation of chloramphenicol by acetyl-coenzyme A (CoA) (Sands and Shaw 1973).

1.1.9.4.5 Active efflux pumps

The ability to be resistant against chloramphenicol in the antibiotic chromosomal integrated ST DT104 strain is operated by the *floR* gene (Threlfall et al. 1997). Such DT104 strains show an eight-fold increase in resistance levels to chloramphenicol and florfenicol when compared to a quinolone-susceptible DT104 strain (Cloeckaert and Schwarz 2001). Another important mechanism is active efflux due to overproduction of the *Acr*AB efflux pump, which is an oxygen response gene (Rychlik and Barrow 2005). It also plays an important role in resistance to bile salts and detergents and in intestinal colonization (Lacroix et al. 1996).

1.1.9.4.6 Using alternative synthesis pathways

Bacteria can avoid attack by enzyme blocks or competitive antibiotics by developing alternative pathways to synthesize necessary enzymes or structural proteins. The *mer*A gene has been found in the hybrid virulence resistance plasmid pUO-StVR2 in some *Salmonella* Typhimurium (Herrero et al. 2006). The *mer*A gene confers resistance to methicillin. It codes an alternative penicillin-binding protein (PBP2') which is not inhibited by methicillin. Although the composition of the cell wall is altered, the organism is still able to multiply (Rang and Ritter 1971).

1.1.9.4.7 Alter the target site or reduce the affinity

When the target site is altered to decrease the affinity to the antibiotic the effectiveness of the antibiotic is reduced. The mechanism of decreased susceptibility to fluoroquinolones in ST DT104 isolates involves point mutations in the quinolone resistance determining region of the target gene *gyr*A (Cloeckaert and Schwarz 2001). The point mutations in gyrA genes also results in low-level ciprofloxacin resistance.

1.1.9.4.8 Environment resistance genes

Many environment resistance genes can cope with environmental challenge and are also involved in the expression of virulence. The virulence factors of *Salmonella* are stress resistance and invasive ability. It is believed that the antibiotic resistant chromosomal integrated ST DT104 has a better environmental resistance ability (Loewen and Hengge-Aronis 1994). The *rpo*S-encodes the delta^{S} subunit of RNA polymerase and is an important regulator of the general stress response. The rpoS gene is also required for virulence in S. Typhimurium in orally infected mice (Loewen and Hengge-Aronis 1994). The *Qac*EΔ1 gene is located between 28,953 and 29,300 bp in SGI1 DNA fragment and can transcribe a quaternary ammonium compound and a disinfectant resistant partial protein. The presence of this gene could be one reason for improved environmental survival ability over other *Salmonella* Typhimurium.

1.1.9.4.9 Change in virulence

Host adaptation is influenced by the distribution of fimbrial and non-fimbrial adhesins among *Salmonellae* (Baumler et al. 1997). Some scientists believe that ST DT104 is not a hyper-virulent strain (Cloeckaert and Schwarz 2001). Some resistant DT104 isolates appear less invasive than non-resistant strains and in a mouse model of infection, MR ST DT104 strains did not reveal evidence of enhanced nor reduced virulence when compared with ATCC *S.* Typhimurium strain 14028s (Cloeckaert and Schwarz 2001).

Conversely, other case control studies have suggested that MR ST DT104 is possibly a hypervirulent strain (Boyd et al. 2001). However, the argument is that the virulence does not appear to be related to a hyperinvasive phenotype as shown in tissue culture assays and the resistant ST DT104 is no more invasive than susceptible *Salmonella* Typhimurium strains with or without exposure to antibiotics (Boyd et al. 2001).

There is evidence to show that ST DT104 containing the SGI1 gene may be more virulent than susceptible strains lacking SGI1 gene in an experimental poultry model (Cloeckaert and Schwarz 2001). The result suggests a potential role for SGI1 in virulence.

1.2 THE HISTORY OF ANTIBIOTIC AGENTS AND THE MECHANISMS OF ACTION OF ANTIBIOTICS

The discovery of penicillin initiated the discovery of numerous antibiotics from fungi.

Antibiotics either kill bacteria or reduce the growth rate of bacteria by different mechanisms. The mechanisms can be classified into twelve groups: 1) beta-lactam antimicrobial agents (penicillins and cephalosporins); 2) amino-glycosides; 3) glycoproteins; 4) quinolones; 5) macrolides; 6) streptogramins; 7) oxazoladinones; 8) metronidazole; 9) tetracyclines; 10) sulphonamides and 11) trimethoprims; 12) chloramphenicols. There is only scope in this thesis to introduce eight categories as discussed below.

1.2.1 Beta-lactam antimicrobial agents - ampicillin and cefotaxime

Ampicillin is the original oral form of penicillins. Cefotaxime is the third generation of cephalosporins. The two groups belong to beta-lactam antimicrobial agents. These agents inhibit peptidoglycan linking with cell wall. The resulting dys-functional cell wall is fatal for the bacteria (Rossi et al. 1995). Gram-negative bacteria are less likely to be affected by these antimicrobials due the differences in structure in the cell wall and hence the target site for the antimicrobial is not present. Some bacteria have the ability to protect themselves against these agents. bla_{PSE-1} gene can encode carpencillinase protein, a beta-lactamase (Boyd et al. 2001), which can denature the beta-lactame ring. Many *Salmonella* strains have the gene in R-plasmid or in genome (Rossi et al. 1995).

Cephalosporins are antimicrobials with both gram-positive and gram-negative activity (Gillespie and Bamford 2003). Because of safety and broad spectrum of activity, they are

frequently chosen for treatment of infection caused by gram-negative bacilli and gram-positive cocci. Compared with penicillins, cephalosporins only have an advantage against gram-negative bacilli.

In the development of cephalosporins there have been five generations (Gillespie and Bamford 2003). The third-generation cephalosporins have much better activity against *Enterobacteriaceae* compared with the first generation. It is believed that the resistant strain of *S.* Typhimurium for cephalosporins in the UK emerged between 1993 to 1995 (Threlfall et al. 1996).

1.2.2 Chloramphenicol

Chloramphenicol belongs to the group of miscellaneous antibiotics. It is primarily a bacteriostatic. It binds to the 50S sub-unit of the ribosome and inhibits the synthesis of bacterial protein. It has a good activity against gram-positive and gram-negative cocci and bacilli. Chloramphenicol is selectively used to treat only serious Salmonella infections.

Chloramphenicol was restricted for use in veterinary practice in Europe from 1994. Florfenicol was introduced and has been used for treatment in veterinary practice since January 1995. However, the chloramphenicol resistant strains still can be seen almost everywhere (Arcangioli et al. 1999). The resistance mechanism can be operated by

chloramphenicol acetyl transferases (CAT), or by non-enzyme mechanism like efflux, impermeability, and ribosomal modification (Arcangioli et al. 1999).

*Flo*R gene, which exists in some *Salmonella,* can transcribe chloramphenicol and florfenicol resistance protein (Boyd et al. 2001). It can be located in R-plasmids or in ST DT104 chromosome (Cloeckaert and Schwarz 2001). From sequence analysis, *flo*R gene belongs to the 12-TMS (trans-membrane segments) multidrug efflux pumps family (Arcangioli et al. 1999). It is established that resistance to chloramphenicol in *S.* Typhimurium isolates is a highly specific marker for ST DT104 (Anon 1997).

1.2.3 Aminoglycosides – gentamicin; kanamycin; streptomycin

The aminoglycosides are bactericidal antibiotics (Gillespie and Bamford 2003). They bind to 30S ribosome and inhibit bacterial protein synthesis. Its target site is inside the nucleus. Aminoglycosides inhibit the function of mRNA restricting the translation of proteins and thus causing bacterial death. The group is only used against aerobic gram-negative bacilli and staphylococci. The disadvantage of aminoglycosides is the toxic effect on the kidney and the eighth cranial nerve. Gentamicin and kanamycin have been widely used in human beings and animals. However, kanamycin has a limited usage because it is more toxic than the other aminoglycosides.

Resistance to gentamicin and kanamycin in *S.* Typhimurium has primarily been identified in phage type 204C (DT204c) (Wray and Davies 1996). Gemtamicin resistance was first reported in *S.* Typhimurium in 1982 (Sojka et al. 1982). The resistance was shown to be transferable and the plasmids responsible for this resistance specified the enzyme 3-N-aminoglycoside acetyl-transferase (AAAC(3)IV) (Davies and O'Connor 1978). The enzyme can use antibiotics with 2-deoxystreptamine rings as acceptors for its acetyl-transferase activity. Aminoglycoside-resistance plasmids have been identified in *E. coli* and some *Salmonella* serotypes including Typhimurium (Threlfall et al. 1983; Threlfall et al. 1985; Wray and Davies 1996; Chaslus-Dancla et al. 1986). The resistance to kanamycin can also be operated by aphA gene, which encodes aminoglycoside 3'-phosphotranferase. In ACSSuT R-type ST DT104, the resistance gene, *Aad*A2, encodes aminoglycoside-(3")(9)-adenylyltransferase, which confers resistance to streptomycin/spectinomycin (Boyd et al. 2001). The gene enhances the activity of the enzyme by one or more point mutations in the gene in Integron C (InC) cassette (Nesvera et al. 1998). The plasmids containing the resistance gene belong to incompatibility group I (InC I) (Threlfall et al. 1985; Threlfall et al. 1986).

1.2.4 Sulphonamide

The sulphonamides are synthetic bacteriostatic antibiotics with a wide spectrum against most

gram-positive and many gram-negative organisms. Sulphonamides inhibit multiplication of bacteria by acting as competitive inhibitors of *p*-aminobenzoic acid in the folic acid metabolism cycle. They inhibit bacterial synthesis of tetrahydrofolate. Bacterial sensitivity is the same for different sulphonamides, because they have the same structure.

The first use of a sulphonamide compound was in 1932. After the introduction of trimethoprim in 1962 in England, they were usually used as a combination after 1968 (Huovinen et al. 1995). They were heavily used in 1980s and 1970s. With awareness of drug resistance growing, the use of sulphonamides declined (Enne et al. 2001). However, the resistance in bacteria remains. The *sul*1 gene encodes the enzyme hydopteroate synthase (Daly and Fanning 2004), which can process an alternative pathway for folic acid metabolism.

1.2.5 Tetracyclines

The tetracyclines interfere with bacterial protein synthesis by locking tRNA to the specific site of mRNA. Tetracyclines are active against many gram-positive and some gram-negative pathogens and are widely used. Because of this widespread use in human and veterinary practice, there are more than 30 different kinds of tetracycline resistance determinants (Furushita et al. 2003). Resistance genes are of two different types; one is operated by a proton-dependent efflux of tetracycline and the other using cytoplasmic ribosomal protecting proteins (Furushita et al. 2003). Class G tetracycline resistance determinant (*Tet*G) has been

found in *Vibrio* spp., *Pseudomonas* spp., and *Salmonella* spp. (Furushita et al. 2003). It has two open reading frames in SGI1: *Tet*R (210 amino acids) and *Tet*A (393 amino acids) in *Salmonella*. *Tet*A encodes proton-dependent tetracycline efflux proteins (Furushita et al. 2003). Based on the analysis of amino acid sequences, the similarity of proteins of *tet* A(G) and tet R(G) is about 60% homology with those of RP1/Tn1721 (class A) and pSC101/pBR322 (class C), and the homology with Tn10 (class B) is about 50% (Zhao and Aoki 1992). The *tet*R gene can transcribe the tetracycline resistance regulator; a repressor protein (Boyd et al. 2001).

1.2.6 Quinolones – nalidixic acid; ciprofloxacin

Quinolones inhibit the function of bacterial DNA gyrase, resulting in DNA synthesis being blocked and rendering the bacteria unable to multiply (Gillespie and Bamford 2003). Nalidixic acid is the original type of quinolone. The fluoro- group was added in order to enhance affinity and efficacy: ciprofloxacin is one of these fluoroquinolones. Quinolones, especially fluoroquinolones, are very efficacious against gram-negative bacteria, including *Pseudomonas* and *Chlamydia*.

The emergence of fluoroquinolone resistance may be related to agricultural use (Threlfall et al. 1996;Chiu et al. 2002). Enrofloxacin was licensed for veterinary use in the UK in 1993 and it has been widely used in cattle and poultry (Threlfall et al. 2000). There was an observed

increased in resistance of human isolates of DT104 to fluoroquinolone following the introduction (Threlfall et al. 1996). The percentage of DT104 isolates that were additionally resistant to quinolones increased from 0.5% in 1994 to 13% in 1997 (Threlfall et al. 1997). This trend was also seen in Finland during the same period, especially in patients who acquired infection abroad (Hakanen et al. 1999). In contrast, the trend was dissimilar in the United States (Herikstad et al. 1997). In the USA in 1998, an outbreak of a fluoroquinolone resistant DT104, occurring in a hospital, resulted in the death of two people (Carnevale et al. 2000).

The quinolone resistance mechanism is associated with the mutation(s) in *gyr*A and with the mutation in *gyr*B to get full resistance to fluroquinolone (Aarestrup et al. 2003). The resistance also can be acquired from the spontaneous gene mutation in R-plasmids (Martinez-Martinez et al. 1998). Travellers provide the perfect vehicle to aid the spread of such a clone from one area to another (Hakanen et al. 2001). The first documented outbreak of fluoroquinolone-resistant salmonellosis occurred in New York in 2001 (Olsen et al. 2001). The index patient is believed to have contracted the disease from the Philippines in 1997 (Olsen et al. 2001).

In 1995, the Food and Drug Administration of the USA agreed that the fluoroquinolone,

sarafloxacin, could be used in the poultry industry for the treatment of *Escherichia coli* infections (Anon 1997). The resistant ST DT104 strain was detected in the USA in 1997 (Anon 1997). Recently, the FDA in USA has proposed the withdrawal of enrofloxacin for use in poultry, but the FDA still allows fluoroquinilones to be used in cattle (Centre for Veterinary Medicine 2003). The development of fluoroquinolone resistance in bacteria may have serious public health implications (Centre for Veterinary Medicine 2003) .

1.2.7 Trimethoprim

Trimethoprim (Tm) has a dihydrofolate reductase that bacteria need to use for folic acid synthesis (Amyes et al. 1989). Tm prevents the reduction of dihydrofolate to tetrahydrofolate in the folic acid metabolism cycle. The resistance mechanism arises when bacteria reduce the dependence on dihydrofolate reductase. The commercial product, trimethoprim-sulfamethoxazole, is a bacteriostat and is a fixed combination of the two antimicrobials which is much more active as a combination of the two than either agent is alone. In the UK, acquisition of trimethoprim resistance may have resulted from use of this agent to treat DT104 R-type ACSSuT infections in cattle (Threlfall et al. 1996).

1.2.8 Nitrofuran – furazolidone

Furazolidone (N-[5-nitro-2-furfurylidene]-3-amino-2-oxazolidone) is a synthetic

antimicrobial drug, which belongs to the nitrofuran family (De Angelis et al. 1999), has good ability against gastrointestinal infections and has been widely used in medical and veterinary practice (De Angelis et al. 1999). The antibacterial ability is activated after the agent is modified to the metabolic form by nitroreductase (Kumar and Jayaraman 1991). The nitrofurans appear to block the initiation of translation and inhibit many microbial enzyme systems (Anon 2006). The resistance mechanism against furazolidone still needs to be clarified but it would be related to either mutations in genome which result in the loss of nitro-reductase activity (Brown et al. 1991; Kumar and Jayaraman 1991) or the development of a permeability barrier (van Duijkeren et al. 2003).

1.3 EPIDEMIOLOGY OF ST DT104

Antibiotic-susceptible isolates of ST DT104 have been obtained from cases of human infection in England and Wales since the early 1960s (Threlfall 2000). In the early 1980s, MR ST DT104 ACSSuT R-type was identified, with the first isolations from cattle and human in the UK (Threlfall 2000). In the following years, MR ST DT104 strains have been isolated from a wide range of animals, and has become the second most common human salmonellosis after *S.* Enteritidis PT4 in the UK and Germany (Baggesen et al. 2000). Outbreaks of MR ST DT104 from origins of poultry, beef, cheese, and swine have also been reported in numerous countries (Boyd et al. 2001). Due to many people not presenting with significant symptoms, it is believed that only one-third of cases were reported in the UK (Wheeler et al. 1999).

The MR ST DT104 strains were initially characterised as having chromosomally located genes for five antibiotics, but in recent years strains with additional resistance susceptibility to gentamicin, trimethoprim, and fluoroquinolones have been observed in many countries (Threlfall et al. 1994; Threlfall et al. 1996; Low et al. 1997). Although some variants have been identified, the majority of MR ST DT104 strains have a distinctive *Xba*I generated macro-restriction fingerprint when studied by PFGE. The ST DT104 strain may be a single clone spreading worldwide, although it shows five different variants by PFGE (Leegaard et al. 2000). The same clone has been isolated in many countries, such as Germany (Almuth et al. 1997), the United States (Besser et al. 1997), Canada (Poppe et al. 1996), Italy (Rubino et al. 1998), Belgium (Imberechts et al. 1998), Israel (Metzer et al. 1998), Irish Republic (Threlfall 2000), Austria (Threlfall 2000), France (Threlfall 2000), the Czech Republic (Threlfall 2000), and Denmark (Baggesen and Aarestrup 1998). Infections in humans have also been recognised in Trinidad, South Africa, The Netherlands, Northern Ireland, the United Arab Emirates, the Philippines and in Israel (Threlfall 2000). In 1996 infections with the MR ST DT104 strain were recognised in cattle and humans in Canada and the USA (Threlfall 2000). In the USA a considerable number of outbreaks have been associated with the consumption of cheese made from raw milk (Threlfall 2000).

It is believed that the spread of ST DT104 strains worldwide is due to international travellers

(Baggesen and Aarestrup 1998; Rodrigue et al. 1990; Rowe et al. 1979; Threlfall et al. 1978). People can travel the breadth of the globe within 24 hours. Thus, the index case of an infection may occur far from the source of contamination. Free movement of people and goods between countries is therefore an effective way of distributing pathogens internationally (Killalea et al. 1996). The MR ST DT104 strain could be considered as an pandemic pathogen. Due to the threat of the MR ST DT104, the SENTRY Antimicrobial Surveillance Program has been established, monitoring the susceptibilities and resistance patterns of key bacterial and fungal pathogens from nosocomial and community-acquired infection, through over 85 sentinel hospitals and laboratories around the world (Pfaller et al. 1998).

1.3.1 Enter-net and Salm-net in Europe

The European Enter-net network is an international surveillance system for human gastrointestinal infections in Europe (Fisher 1999). The network monitors salmonellosis and Verocytotoxin producing *Escherichia coli* (VTEC) O157, including their antimicrobial resistance patterns (Van Pelt 1999; Fisher 1999). When the network began, it involved all 15 countries of the European Union, Switzerland and Norway. The network is funded by the European Commission (EC) and represents a continuation of the Salm-Net surveillance network (1994-97). Salm-Net successfully established a frequently updated international Salmonella database and concentrated on harmonisation of *Salmonella* phage typing.

Salm-Net showed, through the recognition of outbreaks and investigation, that the timely exchange of information between experts in 17 countries in Europe could lead to effective public health action (Fisher 1999; Van Pelt 1999). This network functions to aid experts in achieving excellent public health action in Europe.

1.3.2 United Kingdom

MR ST DT104, with the penta-resistant pattern, became a major cause of salmonellosis in humans in the UK in the late 1980s, and then appeared in several countries during the 1990s (Threlfall et al. 1994). In Scotland, England, Wales, and Northern Ireland, a passive surveillance system set up by the Veterinary Laboratories Agency is now part of Enter-net (Liebana et al. 2002). With the exception of a small outbreak in Scotland in the mid-1980s, there were no isolates of MR ST DT104 from humans until 1989, by which time the strain had begun to be isolated from cattle and became epidemic. In a study done in England and Wales, the incidence of infection with ST DT104 strains alone rose from 259 cases in 1990 to 4006 cases in 1996 (Smith et al. 2007).

In contrast to previous epidemic multiresistant phage types of *S.* Typhimurium, such as DTs 29, 204, 193, and 204c, which were mainly restricted to cattle, MR ST DT104 has also become a dominant strain in many hosts, such as turkeys, pigs and sheep (Threlfall 2000;

Low et al. 1997; Threlfall et al. 1994; Threlfall et al. 1996). In Scotland in 1993 it was the major phage type of *S.* Typhimurium isolated from humans, cattle, pigs, and sheep (Anon 1994).

Twenty-five per cents of pigs were infected with Salmonella at slaughter in (Davies, 2000). The predominant serotype of Salmonella infecting pigs in the UK was S. Typhimurium, which accounted for 68% of those reported (Defra 1999). The Center for Disease Research reported that in 1998, 95% of isolates in human cases were ST DT104; in addition, these isolates also showed resistance to most of the drugs traditionally used to treat salmonellosis, such as aminoglycosides, trimethoprim and nalidixic acid (Smith et al. 2007).

In England and Wales, the incidence of ciprofloxacin resistance in ST DT104 strains increased from 0.6% in 1994 to 13% in 1997 (Threlfall et al. 1999). From 1993 to 1995, trimethoprim-resistant ST DT104 (R-type ACSSuTTm) increased from 1% to 27% of isolates, and ciprofloxacin-resistant ST DT104 (R-type ACSSuTCp) increased from 0 to 6% of isolates (Threlfall et al. 1996). It was believed that only one major clone spread within the UK, but from 2000 onwards, new PFGE profiles of the ACSSuT and the ACSSuTm R-types were identified (Threlfall et al. 2005).

1.3.3 Other European countries

The strains of *S.* Typhimurium in the UK and France are believed to be the same (Baggesen et al. 2000). In France, 69.8% of ST strains of bovine origin have compatible PFGE profiles with the multidrug-resistant strains of ST DT104 in the UK (Baggesen et al. 2000).

In Germany, the rate of fully susceptible ST DT104 strains isolated decreased from 73% in 1987 to 48% in 1996 (Gross et al. 1998). It has been estimated that in Germany 20% of human cases of salmonellosis in 1996-1997 were attributed to Salmonella originating in pigs (Steinbach and Hartung 1999). In Spain, ampicillin resistance in *S.* Typhimurium from humans rose from 10 to 15% in 1986-8 to above 50% in 1989-91 (Ramos et al. 1996). In Italy, a 2002 report mentioned that 52% of *S.* Typhimurium isolated between 1998 and 2000 and 37.7% of other strains of *Salmonella* from animals were multi-resistant (Mammina et al. 2002).

Isolates of *S.* Typhimurium from the Czech Republic have been phage-typed since 1991 (Karpiskova et al. 1999). The first ST DT104 ACSSuT R-type isolates were identified from animals in 1994. The first two human ST DT104 isolates were detected in summer 1996 (Karpiskova et al. 1999). The first outbreak caused by ST DT104 (ACSSuT R-type) was identified in June 1998 (Karpiskova et al. 1999).

In 1998 23% of the human isolates domestically acquired were multi-resistant, and a majority were *S.* Typhimurium DT104. In 2003, *S.* Typhimurium was the only serovar of *Salmonella* that was endemic in Norway (Alvseike et al. 1997; Leegaard et al. 2000; Lindstedt et al. 2003). It is believed the ST DT104 strains have been transmitted within the country, and the trend of multi-resistant *S.* Typhimurium DT104 is going up; however, the majority of the resistant and multi-resistant isolates are imported (Leegaard et al. 2000).

MR ST DT104 might have been present in Denmark for a long time but spread of infection appears to be limited (Baggesen et al. 2000). The identification of MR ST DT104 in Danish food production animals in 1996 resulted in an agreement that the contamination of foodstuffs of Danish origin with MR ST DT104 should be considered unacceptable (Baggesen and Aarestrup 1998). The aim of the Danish Salmonella surveillance system is to eradicate MR ST DT104 from food production animals (Baggesen et al. 2000).

1.4 CONCLUSION

The epidemiology of *Salmonella* Typhimurium DT104 is complex and that of its many resistance manifestations more so. What is clear is that it is a widespread problem that changes over time. The implication of animals and veterinary drug usage in human salmonellosis remains unproven and it is only by careful examination of the appearance of

resistance in space and time that this question will be answered, even in part.

The aims of this thesis are 1) to describe the trends and current situation of antimicrobial resistant *Salmonella* Typhimurium DT104 in human and animals in Scotland; and 2) to describe and explore relationships in time and space of the different antimicrobial resistant ST DT104 in human and animals in Scotland.

CHAPTER II

MATERIALS AND METHODS

CHAPTER II

MATERIALS AND METHODS

2.1 INTRODUCTION

In Chapter 1, the epidemiology of *Salmonella* and its serotype *S.* Typhimurium was discussed. There are two organisations in Scotland which are responsible for the identification and recording of salmonellosis. The Scottish Salmonella Reference Laboratory (SSRL), located within Stobhill Hospital, provides information, advice, and a full complement of techniques, including serotyping, phage typing and a range of biochemical and molecular methods, for identification and typing of *Salmonella* (http://www.show.scot.nhs.uk/ssrl/index.htm). The records of salmonellosis are stored in Health Protection Scotland (HPS; http://www.hps.scot.nhs.uk/). For the purposes of this thesis, the complete datasets were kindly provided by the two above organizations.

A thorough understanding of the biology and epidemiology of *S.* Typhimurium DT104 (ST DT104) should enable appropriate control and prevention policies to be established. In this chapter, the methods used at SSRL for isolation and identification of ST DT104, the means of information storage at HPS and statistical methods employed in this thesis will be discussed.

2.2 LABORATORY METHODS USED IN SSRL

Samples tested in SSRL are from human sources, e.g. faecal samples submitted by general practices or hospitals and from veterinary sources, e.g. routine faecal sample collections, faecal samples from clinically unwell animals, foodstuffs or environment. Samples are tested for the presence of Salmonella using one of a number of different techniques.

2.2.1 Serotyping method

In SSRL, *Salmonella* isolated from humans, animals or feed is biochemically confirmed and serotyped by an agglutination test which was mentioned in Chapter 1. Each culture is tested for the presence of somatic and flagella antigens by mixing with specific Salmonella antiserum. Serovars are derived by reference to the Kauffmann White Scheme (Defra 2003).

2.2.2 Phage typing method

Salmonella Typhimurium, is phage typed according to the Health Protection Agency phage typing schemes (Ward et al. 1987). Different necessary phages are seeded onto LB agar plates covered by the testing strain. After 24 to 48 hours incubation, the degree and pattern of lysis are read and then, according to the Phage Type Scheme for *Salmonella*, a definite type given (Ward et al. 1987). Some phage types are related variants although they are still reported as distinct types, e.g. DT104b of *S.* Typhimurium. Those which cannot be typed are recorded as "untyped".

2.2.3 Antimicrobial susceptibility testing method

The antimicrobial susceptibility testing method was introduced in 2005 (Murray et al. 2005). The most common antimicrobial susceptibility testing method is the Kirby Bauer Disk Diffusion and it is used as the standard method in SSRL. It evaluates the Minimum Inhibitory Concentration (MIC) in a solid agar plate, which is Mueller-Hinton II agar (Becton Dickinson Microbiology Systems) supplemented with 5% bovine blood and contains a predetermined concentration of antimicrobial agents as described in Table 2.1.

Table 2.1 The pre-determined concentration of antibiotics in the MIC test (Murray et al. 2005;Defra 2003) and the years which were introduced.

Antibiotic	MIC*(μg/ml)	Code	Date
Ampicillin	10	Ap	1988
Cefotaxime	30	Cf	1999
Chloramphenicol	12.5 - 25	Cl	1989
Ciprofloxacin	0.5	Cp	1990
Furazolidone	15	Fu	1990
Gentamicin	10	Gm	1988
Kanamycin	30	Ka	1988
Nalidixic acid	40	Na	1989
Netilmicin	12.5	Ne	1989
Spectinomycin	100	Sp	1989
Streptomycin	12.5 - 25	St	1988
Sulphonamide	300	Su	1988
Tetracycline	10 – 30	Te	1988
Trimethoprim	5	Tm	1988
Low level Ciprofloxacin	0.125	Lc	1999

Fourteen antimicrobial agents and fifteen treatments were chosen by SSRL to test the resistance of *Salmonella* spp. The test for the resistance to ciprofloxacin is carried out at two different concentrations to evaluate the trend of resistance to ciprofloxacin (Murray et al. 2005). The low concentration of ciprofloxacin is used to compare the resistance with that for nalidixic acid. In practice, the susceptibility of a bacterial strain to an antibiotic is determined by observing "resistant" or "sensitive" after 24 hours inoculation in a cutting

zone size equal to 13mm. Sometimes, the colony will be categorised "intermediate"; however, for the purposes of this thesis, "intermediate" was considered as "sensitive".

2.2.4 Other methods used in SSRL

There are many other methods used in SSRL, such as polymerase chain reaction and pulse field gel electrophoresis for different purposes. However, the information collected using those methods will not be presented in this thesis.

2.3 COMPILATION OF DATABASES

2.3.1 Data from human sources

The database from human sources was derived from routine collection by the Health Public Scotland (HPS). It is based on reports of isolates from single patients to HPS from the Scottish Salmonella Reference Laboratory (SSRL). The flowchart of the sample transferring system is shown in Figure 2.1. When people become unwell, they can choose to stay at home, they may go to see a general practitioner (GP) or they may present themselves at a hospital. Doctors take a detailed history, examine patients and decide whether antibiotics may be prescribed directly or whether a specimen should be collected and sent to contract laboratories for testing. If the laboratories conclude that there is a *Salmonella* infection, samples are then sent to SSRL for confirmation. The details of the case are recorded at the local hospital and all necessary information is forwarded to SSRL, although no personal identification is logged. SSRL identifies the phage type and antibiotic resistance pattern. If necessary, molecular biological testing is undertaken. The SSRL then forwards a report to HPS.

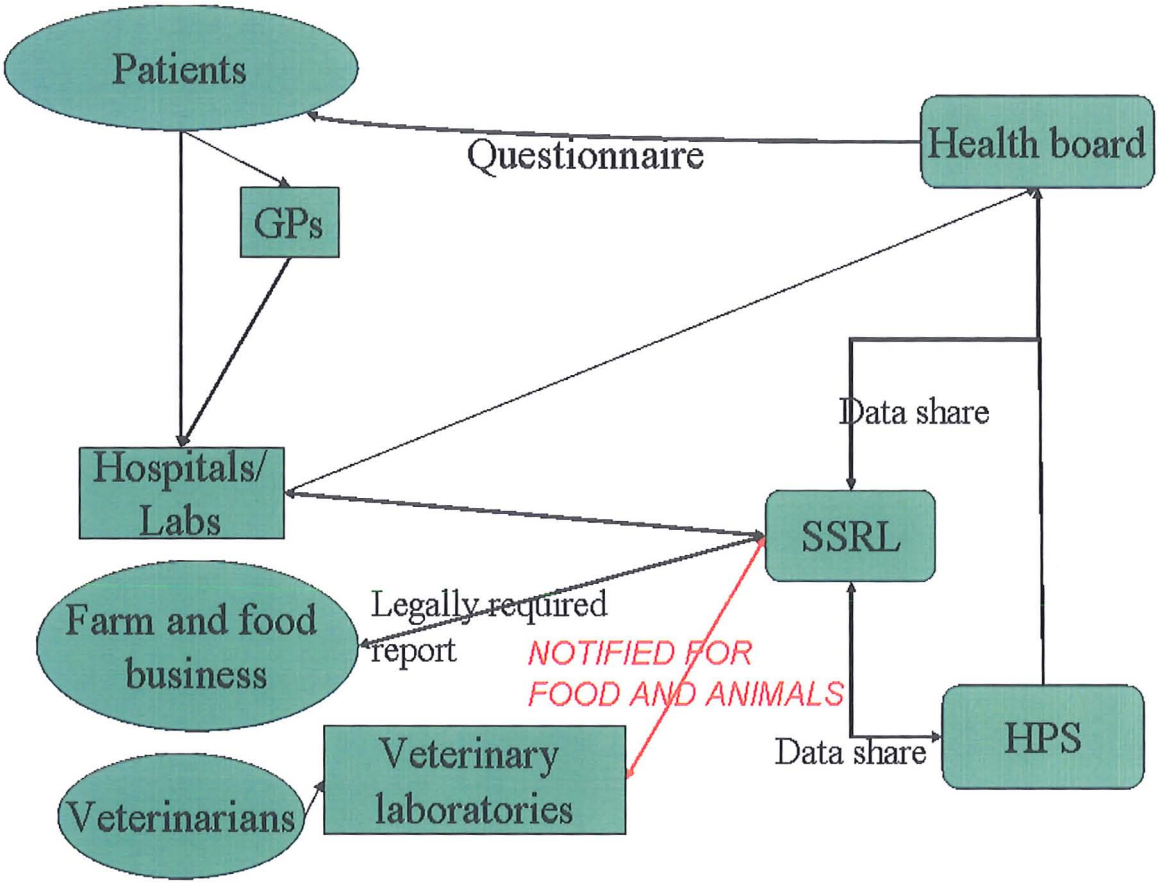


Figure 2.1 Flowchart detailing information transfer among patients, practices and laboratories in cases where infection with *Salmonella* is suspected.

The information system described is a part of Enter-net, which means that the stored data may be shared with other EU countries. The experts in HPS or similar organisations in other countries can use the information to set an alarm or develop suitable policies in advance. SSRL also reports the result back to clients (laboratories) and shares important information with the Health Board where the case occurred. HPS also will send a report to Department for Environment, Food and Rural Affairs (Defra). The corresponding Health Board may formally visit the patient(s). If a visit has been made responses, a formal questionnaire is recorded and all details are be shared among SSRL, HPS and Defra.

2.3.2 Collection of data from veterinary sources

The dataset of samples derived from veterinary sources is maintained by the Veterinary Laboratories Agency (VLA). The Zoonoses Order (1989) of the Animal Health Act (1981) requires that isolation of organisms of the genus *Salmonella* in food-producing animals and birds be reported to a Veterinary Officer of the Department for Environment, Food and Rural Affairs (Defra). Generally, after veterinarians collect samples from owners, or business managers send routinely collected samples, the specimen is sent to any veterinary laboratory, which may be privately owned or inside a veterinary school. The data are recorded on the "*Salmonella* Supplementary Form – General" or "*Salmonella* Supplementary Form –Avian", depending on the source of the sample. In the form, the responsible veterinarian is required to complete Section I with where samples came from and who sent them. In Section 2 (risk assessment) symptoms and environment and business behaviour of the farm are recorded. In Section 3, the veterinary laboratory is required to record what will be done or what situation they have found. For the form relating to avian samples, more details about where the animals are kept are required. After confirmation of identity by the veterinary laboratory, and when the results are known of tests and antibiotic resistance patterns for all *Salmonella*, the forms are distributed to the Scottish Agriculture College (SAC) and SSRL and then to Defra. Defra may arrange a formal visit if necessary.

In contrast to the human data, these statutory animal notifications are based on "incidents"; a term is defined by Defra as "An incident comprises the first isolation and all subsequent isolations of the same serovar or serovar and phage/definitive type combination of a particular *Salmonella* from an animal, group of animals or their environment on a single premises, within a defined time period (usually 30 days)" (Defra 2003). The purpose of

using "incident" is to assess more accurately data in the number of live-stock units. Finally, Defra arranges a visit in order to progress an order if necessary.

2.4 THE STRUCTURE OF DATASETS RECEIVED IN THIS STUDY

The surveillance of *Salmonella* infection in Scotland is based on cooperation between SSRL and HPS. Veterinary laboratories are involved in collecting the data from veterinary sources.

2.4.1 The structure of datasets derived from human sources

Two datasets were kindly supplied by HPS and SSRL: 1) Weekly recordings of all *Salmonella* cases between 1988 and 2002, 2) A different presentation of the data, recording the received dates of ST DT104 only, from 1988 to 2004.

Cases received from different National Health Service (NHS) boards were coded as "report board". In Scotland, there are fifteen Health Boards: Ayrshire & Arran (AA); Argyll & Clyde (AC); Borders (BR); Dumfries & Galloway (DG); Fife (FF); Forth Valley (FV); Greater Glasgow (GG); Grampian (GR); Highland (HG); Lanarkshire (LN); Lothian (LO); Orkney (OR); Shetland (SH); Tayside (TY); and Western Isles (WI) (Figure 1.1). AC has recently been integrated into GG.

The datasets supplied included both Home Cases and Imported Cases, that is, cases in which the patient declared that salmonellosis had been contracted overseas. The subsequent study deals only with Home Cases. The datasets were supplied in excel spreadsheet format, with columns detailing sample identification and further information,

such as phage type, gender of patient and other potentially useful epidemiological details as outlined in Table 2-2.

Table 2.2 The description of variables used in the human databases.

Variables	Dataset1	Dataset2	Description
Pathogen	Yes	Yes	Serotype of an isolate
Type	Yes	Yes	Phage type of an isolate
Year	Yes	Yes	The year of collection
Gender	Yes	Yes	Gender of a patient
Age	Yes	Yes	Age of a patient
Report board	Yes	Yes	Where the cases are from
Imported	Yes	Yes	Developed originally in the UK or not
Isolated site	Yes	Yes	Sampling sites of an isolate
Resistance	Yes	Yes	15 different antibiotic resistances
Received date	No	Yes	The date the case was received
Month	Yes	No	The month the case was received
Week	Yes	No	The week the case was received

'Yes' means the dataset has the variable and 'No' means that the dataset does not have the variable.

2.4.2 The structure of dataset derived from veterinary sources

In the veterinary dataset, samples originated from a wide variety sources - including from animals, foodstuffs and environment. According to the description from a DEFRA publication, since 1993, Salmonella reports from livestock have been separated into "isolations" and "incidents" (Defra 2003). By definition, "Isolations comprise individual reports of Salmonella made from samples and reported to Officers of the Minister" (Defra 2003). Repeat and duplicate samples were removed (Browning et al. 2001a; Defra 2003) Then, incidents and isolations from veterinary sources between July 1990 and December 2004 were added to the national dataset in HPS.

Poultry samples offer a further source of veterinary samples. A poultry monitoring system is operated in the poultry industry and therefore samples from poultry may contain many non-clinical diagnostic samples.

All records were kindly provided by HPS. The datasets were supplied in excel spreadsheet format, with column headings detailed in Table 2.3.

Table 2.3 Details of case information contained within the datasets provided by veterinary laboratories where *Salmonella* infection had been determined.

Variables	Description
Pathogen	Serotype of *Salmonella*
Type	Phage types of *Salmonella* Enteriditis and Typhimurium
Year	The year of collection
NO	The collection number - contains the reported week
Source	Which animal the case is from
True source	The categories of animal
Lab source	The veterinary laboratory of isolation
Resistance pattern	The 15 antibiotic resistance patterns
Week	The week the case was reported to laboratory
Pathogen2	*S.* Typhimurium, *S.* Enteritidis, and Others

2.5 CLEANING THE DATASETS

All data were checked by EXCEL 2000. The two datasets were compared and then the errors were corrected. A unique number was given to each record to trace the original data when necessary. In the human database, the postal district for each record was added using the electronic map enquiry system on the website: http://streetmap.co.uk. The records, which did not have address information, were marked as 'missing'.

In the animal database, the original dataset did not contain the date received. Instead of that, a reference number including the information on the year and week was used. A new variable, week, was generated from the reference numbers. The week ranged from 1 to 53 and the week number was used as the minimum time interval. Thus, a year was divided into 13 four-weekly months. The 53rd week was assigned to the 13^{th} four-weekly month.

Text categories were encoded as nominal variables and "Yes" or "No" answers were changed to binary format. Similarly, antibiotic resistant patterns were re-coded to binary format - 1 referred to resistant and 0 referred to sensitive. "Intermediate" was recoded as "0" – only three cases had intermediate resistance to tetracycline, trimethoprim, kanamycin, respectively. Cases for which there was missing information were considered individually, depending on the analysis being undertaken. Missing data were usually coded as '9'. No information in a variable was coded as '99'. Date format, such as 01/01/2005, was reclassified into two separate fields - "month" and "year".

2.6 EPIDEMIOLOGICAL ANALYSIS METHODS

Descriptive, cluster, spatial and time series modelling were used to conduct different analysis, some details of which will be presented in later chapters. Where appropriate, statistical significance was set at a p-value <0.05; all tests were two-tailed.

2.6.1 Descriptive statistics

Descriptive statistics help to summarise data and identify trends or patterns which can then be further explored using more sophisticated statistical techniques. Functions within the Microsoft Office software package, Excel version 2000, namely pivot table and pivot chart, were used to generate summary tables and charts. To further enhance the readability of figures, PowerPoint version 2000 was employed. The population statistics in the 2001 Census were used to calculate the cumulative incidence in an area or used as the base for other statistical analysis (General Register Office for Scotland 2002) (Table 2.4).

Table 2.4 Mid-year population estimates; Scotland and its Health Boards by single age and sex: 2001

Healthboard	persons	males	females	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59
Argyll & Clyde	425756	204532	221224	23134	26134	27977	26420	23061	24030	31302	34880	32574	28777	30508	25391
Ayrshire & Arran	368149	175486	192663	19186	22761	24614	22492	18961	20656	26096	28423	26942	24745	27194	22813
Borders	106764	51361	55403	5509	6601	6731	5627	4472	5311	7367	8359	8176	7324	8108	6817
Dumfries & Galloway	147765	71303	76462	7467	9001	9523	8247	6192	7344	9670	11254	10563	9973	11199	9893
Fife	349429	167628	181801	19214	21494	23247	22238	20834	19964	25616	26995	25692	23142	25381	20907
Forth Valley	279480	134444	145036	15864	17489	18047	17614	16954	16688	20988	22239	20559	18572	20396	16510
Grampian	525936	259735	266201	28194	31756	33264	33006	34500	34450	40741	42005	40112	36937	37918	28786
Greater Glasgow	855953	404134	451819	47126	51054	53654	56811	61878	59021	66282	69066	64049	54594	51588	43402
Highland	208914	102297	106617	11363	13070	13780	12122	9995	11264	14007	16213	15805	15221	16074	13498
Lanarkshire	558751	268109	290642	32762	35517	37051	37031	32438	35157	44030	45667	42648	37895	37515	31846
Lothian	778367	373045	405322	43221	45572	46007	47330	57846	57709	65390	63396	57968	49838	52196	40544
Orkney	19245	9497	9748	984	1218	1370	1107	796	1007	1356	1483	1404	1383	1428	1330
Shetland	21988	11071	10917	1328	1534	1642	1305	1191	1351	1570	1726	1574	1629	1625	1375
Tayside	389012	186770	202242	20228	22388	24177	24455	24113	21949	25976	29397	27964	25604	27956	23208
Western Isles	26502	13082	13420	1294	1549	1786	1468	1156	1402	1703	1851	1880	1835	2021	1679

Table 2.4 (continued) mid-year population estimates; Scotland and its health board areas by single age and sex: 2001

Healthboard	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100+
Argyll & Clyde	23268	20777	17730	13605	8851	4967	1926	397	47
Ayrshire & Arran	20646	18922	15773	12822	8307	4584	1732	437	43
Borders	6213	5615	5074	4220	2796	1656	613	155	20
Dumfries & Galloway	9108	8302	7528	5832	3642	2020	792	193	22
Fife	17965	16305	14353	12084	7603	4394	1564	382	55
Forth Valley	14846	12898	10879	8799	5587	3107	1160	260	24
Grampian	25360	23390	20078	16136	10323	5851	2482	568	79
Greater Glasgow	42043	40311	35167	27823	17562	9758	3798	859	107
Highland	11827	10459	8972	7046	4337	2577	1001	246	37
Lanarkshire	29034	25645	21411	16022	9723	5030	1829	442	58
Lothian	36338	33181	29153	23824	15571	8841	3536	792	114
Orkney	1159	903	824	648	440	279	96	27	3
Shetland	1053	868	741	667	416	266	97	26	4
Tayside	21240	20077	18002	14858	9129	5466	2218	528	79
Western Isles	1633	1463	1232	1137	702	445	203	54	9

2.6.2 Cluster analysis

The purpose of cluster analysis is to understand the relationships amongst cases and variables, in this situation, the relationship between *Salmonellae* and antibiotics (SPSS Inc 2006). The clustered groups can be further assessed using more advanced statistical methods, such as temporal, spatial or spatio-temporal analyses. There are now a large number of database, spreadsheet and statistical analysis software packages available, and in this study, SPSS, Excel 2000, SatScan and MapInfo 5.5 were used where appropriate for analysis and presentation purposes. Excel 2000 spreadsheets were used for cleaning the datasets and to generate SPSS compatible files. In SPSS, hierarchical cluster analyses, K-means cluster analyses, McNemar and chi-square tests were undertaken. The results were exported to Excel 2000 and modified to fit the Mapinfo 5.5 format to present clusters or other information in the electronic map files, which were all kindly supplied by Dr. Dominic Mellor.

2.6.2.1 The test of independence

The McNemar sign test was used to determine the independence of antibiotic resistances. It is usually used for testing prior and post dichotomous results in the same sample. However, it can be used to test the response for a related pair of variables, e.g. antibiotic resistance patterns. If the null hypothesis is true, it is expected that the frequency table for 'yes, no' and 'no, yes' will be equal. If the p-value is less than 0.05, it means the pair show significantly different responses (SPSS Inc. 2006; Dohoo et al. 2003).

2.6.2.2 Hierarchical cluster analysis

In hierarchical cluster analysis, clusters are not formed in one simple step. It is a long procedure involving many calculations. The results are derived and presented according to the linkage and measure methods chosen by the researcher. Square Euclidean Distance (SED) is the most common means of measuring the distance between a pair of observations. Euclidean distance (ED) and simple matching are both used for calculating the distance between data in binary format (SPSS Inc. 2006). The difference among the measure methods lies in the description of the positions of outliers. Outliers are those results very dissimilar to the major patterns. In the clustering study, SED was chosen as the standard measure method. Simple matching and ED were also used in order to check the positions of outliers.

There are 7 different linkage methods supported by SPSS (SPSS Inc. 2006). However, for binary data, e.g., antibiotic resistant patterns, "between-group-linkage" is a more suitable method. The methods differ in the selection of the first observation (target) in the algorithm. Normally, if the dataset is big enough, different distance measurement or linkage methods will usually generate the same or very similar results. The "between-group-linkage" calculates the distance according to the average of distances between all pairs of objects (isolates). In the "between-group-linkage", $D(r,s)$ is computed as

$$D(r,s) = T_{rs} / (N_r * N_s)$$

Where T_{rs} is the sum of all pairwise distances between cluster r and cluster s. N_r and N_s are the sizes of the clusters r and s respectively

At each stage of hierarchical clustering, the clusters *r* and *s*, for which *D(r,s)* is the minimum, are merged.

The first step in the algorithm is to separate all cases into different clusters containing one case only. Then, the two closest observations are joined. In the next step, a third observation can either join the cluster to form one group or joins another observation to create two different clusters. Each step results in one less cluster than in the previous step. This calculation continues until all cases are combined in one cluster. In this algorithm, missing values are not allowed, and so missing data were removed for the purposes of this particular analysis.

The final results of clustering are usually shown as a dendrogram. The decision maker needs to determine what degree (level) can be thought of as a group. This is known as 'cutting the dendrogram'. This answer can be got from checking the dendrogram or the score in the calculation. The pattern of how these scores change with the clustering steps can help the user to find the final grouping.

The main disadvantage of this type of analysis is that there are no exact rules about which distance measures and linkage methods to use. A sensible approach is to try different linkage methods and use different distance measures to assess the robustness of output; "K-means" analysis can be undertaken to validate conclusions.

2.6.2.3 K-means cluster analysis

Data can be classified by a method with no pre-assumptions, such as hierarchical clustering analysis, or classified by a pre-determined clustering method, such as K-

means clustering. K-means clustering analysis is another classifying package available in SPSS (SPSS Inc. 2006). This method can assist decision-makers in choosing the most appropriate cutting line in hierarchical clustering analysis.

In simple terms, K-means clustering analysis is an algorithm which may be used to classify or to group observations based on a given K number of groups, where K is a positive integer number. The result is given by minimizing the sum of squares of distances between data and the corresponding cluster centroid. The advantages of this method are that it helps users identify homogeneous groups of cases; it can handle large numbers of cases; the centres can be assigned *a priori*, if they are known; and users can select a method for classifying cases. In this method, analysis of variance F statistics will be generated. The weakness of this method is it is not robust with respect to outliers. The centroid may be influenced away from the true centre when some cases are very far from the centroid.

2.6.3 Spatial analysis methods

Spatial analysis is a method used for understanding relationships in a certain time and/or certain zone. It is a powerful, meaningful and useful tool in understanding and predicting the relationships between cases and space. There are many software packages with capabilities in this field, such as MapInfo and SaTScan. In this thesis, MapInfo was used for presenting the clusters on the electronic map. In the thesis, the cumulative incidence in NHS board level and post district level were shown in the thematic map format.

The main analyses were implemented in SaTScan. SaTScan was developed by Kulldorff and colleagues (Kulldorff, 1997). It is able to detect clusters of cases in time, space, and time-space using a random circling scanning method. In the Poisson-based model, the criterion is that the data must follow a Poisson distribution. The model can help users to detect clusters of any size anywhere in the study region. SaTScan uses the random circling method to identify the highest possible cluster(s) in time, space or time-space. The alternative hypothesis is that there is an increased risk of disease in the focused area; the null hypothesis is that the risk is not increased. For each random circle, a likelihood ratio is calculated until the system finds the most likely cluster, which has the largest likelihood ratio. The other possible clusters are listed and the p-value shown under each possible cluster. All random circle finding is obtained by Monte Carlo simulation.

In the Poisson model, census population data over time are necessary. In the thesis, the 2001 Scottish census population data were used. The census data in 1981, 1991, and 2001, did not show a dramatic changes and it was assumed that the population did not change dramatically over the study years. In the animal dataset, this underlying assumption could not be upheld and so the Poisson model was not used.

The second model used in the thesis was a Bernoulli model. It is suitable for binary data and is used as a case-control study model. The null hypothesis is the risk in the case group is the same as that in the control group. This model was more suitable for the animal data. The positions of laboratories were used as specific coordinative position and the assumption was that the proportion of cases at each laboratory was the same under a Poisson distribution.

SaTScan also supports three additional models but they were not used: 1) a space-time permutation model; 2) an ordinal model; or 3) an exponential model.

2.6.4 Time series method

Time series study is a technique used to identify the nature of the phenomenon in a series of observations in a fixed time interval. It was first developed by Yule and Slutsky in the 1920s and used to develop a comprehensive model called autoregressive integrated moving average model (ARIMA) by Box and Jenkins in the 1970s (Box and Jenkins 1976). It has four main purposes: modelling, forecasting, characterisation and classification (Borovkova 1998). The aim of modelling is to build a model which can describe historic data. Then, the generated model can be used for forecasting, providing basic estimates about the future. The fundamental characters of a model can be identified and models can be classified into groups according to the level of similarities (Borovkova 1998). It can focus on the series itself as a uni-variate time series study or take into account other factors as a multivariate study. There are linear and non-linear time series approaches. For example, the ARIMA model is a linear model and the self-threshold auto-regressive model is a nonlinear model (Watier 1995).

The most basic time series model is the simple additive regression form:

$$Z_t = b + S_t + T_t + E_t$$

Where b is the constant. E_t is the white noise, which explains the background information, S_t is the seasonal component and T_t is the trend (level) component.

There is another multiplicative form ($Z_t=b+E_t*S_t*T_t$) which, for disease researchers, is attractive given epidemic increases from one time point to the next. The trend package in SPSS 14 supports two models: the exponential smoothing model and the ARIMA model. The ARIMA model is a group of different forms. It contains: auto-regressive (AR), moving average (MA), auto-regressive and moving average (ARMA) and auto-regressive integrated moving average (ARIMA) models.

The AR model needs a time series in stationary form. In contrast, the MA model can deal with infinite time series. The ARIMA model combines the advantages of both processes and minimises the disadvantages. The general programmatic form is ARIMA(p,d,q), where p is the AR parameters, d is the differencing passes and q is the MA parameters. This thesis focuses on the ARIMA model because it also addresses some of the issues of exponential smoothing models. The ARIMA model is also advantageous because it has a low cost of development and maintenance and finally, it can generate lower root-mean-square-error (RMSE) compared with other models. This means that it has a better descriptive ability and in turn provides a good predictive ability (Abeku et al. 2002; Smelser and Baltes 2001). These issues are discussed further in later chapters.

PAGE

NUMBERING

AS ORIGINAL

2.3.2 Collection of data from veterinary sources

The dataset of samples derived from veterinary sources is maintained by the Veterinary Laboratories Agency (VLA). The Zoonoses Order (1989) of the Animal Health Act (1981) requires that isolation of organisms of the genus *Salmonella* in food-producing animals and birds be reported to a Veterinary Officer of the Department for Environment, Food and Rural Affairs (Defra). Generally, after veterinarians collect samples from owners, or business managers send routinely collected samples, the specimen is sent to any veterinary laboratory, which may be privately owned or inside a veterinary school. The data are recorded on the "*Salmonella* Supplementary Form – General" or "*Salmonella* Supplementary Form –Avian", depending on the source of the sample. In the form, the responsible veterinarian is required to complete Section I with where samples came from and who sent them. In Section 2 (risk assessment) symptoms and environment and business behaviour of the farm are recorded. In Section 3, the veterinary laboratory is required to record what will be done or what situation they have found. For the form relating to avian samples, more details about where the animals are kept are required. After confirmation of identity by the veterinary laboratory, and when the results are known of tests and antibiotic resistance patterns for all *Salmonella*, the forms are distributed to the Scottish Agriculture College (SAC) and SSRL and then to Defra. Defra may arrange a formal visit if necessary.

In contrast to the human data, these statutory animal notifications are based on "incidents"; a term is defined by Defra as "An incident comprises the first isolation and all subsequent isolations of the same serovar or serovar and phage/definitive type combination of a particular *Salmonella* from an animal, group of animals or their environment on a single premises, within a defined time period (usually 30 days)" (Defra 2003). The purpose of

using "incident" is to assess more accurately data in the number of live-stock units. Finally, Defra arranges a visit in order to progress an order if necessary.

2.4 THE STRUCTURE OF DATASETS RECEIVED IN THIS STUDY

The surveillance of *Salmonella* infection in Scotland is based on cooperation between SSRL and HPS. Veterinary laboratories are involved in collecting the data from veterinary sources.

2.4.1 The structure of datasets derived from human sources

Two datasets were kindly supplied by HPS and SSRL: 1) Weekly recordings of all *Salmonella* cases between 1988 and 2002, 2) A different presentation of the data, recording the received dates of ST DT104 only, from 1988 to 2004.

Cases received from different National Health Service (NHS) boards were coded as "report board". In Scotland, there are fifteen Health Boards: Ayrshire & Arran (AA); Argyll & Clyde (AC); Borders (BR); Dumfries & Galloway (DG); Fife (FF); Forth Valley (FV); Greater Glasgow (GG); Grampian (GR); Highland (HG); Lanarkshire (LN); Lothian (LO); Orkney (OR); Shetland (SH); Tayside (TY); and Western Isles (WI) (Figure 1.1). AC has recently been integrated into GG.

The datasets supplied included both Home Cases and Imported Cases, that is, cases in which the patient declared that salmonellosis had been contracted overseas. The subsequent study deals only with Home Cases. The datasets were supplied in excel spreadsheet format, with columns detailing sample identification and further information,

such as phage type, gender of patient and other potentially useful epidemiological details as outlined in Table 2-2.

Table 2.2 The description of variables used in the human databases.

Variables	Dataset1	Dataset2	Description
Pathogen	Yes	Yes	Serotype of an isolate
Type	Yes	Yes	Phage type of an isolate
Year	Yes	Yes	The year of collection
Gender	Yes	Yes	Gender of a patient
Age	Yes	Yes	Age of a patient
Report board	Yes	Yes	Where the cases are from
Imported	Yes	Yes	Developed originally in the UK or not
Isolated site	Yes	Yes	Sampling sites of an isolate
Resistance	Yes	Yes	15 different antibiotic resistances
Received date	No	Yes	The date the case was received
Month	Yes	No	The month the case was received
Week	Yes	No	The week the case was received

'Yes' means the dataset has the variable and 'No' means that the dataset does not have the variable.

2.4.2 The structure of dataset derived from veterinary sources

In the veterinary dataset, samples originated from a wide variety sources - including from animals, foodstuffs and environment. According to the description from a DEFRA publication, since 1993, Salmonella reports from livestock have been separated into "isolations" and "incidents" (Defra 2003). By definition, "Isolations comprise individual reports of Salmonella made from samples and reported to Officers of the Minister" (Defra 2003). Repeat and duplicate samples were removed (Browning et al. 2001a; Defra 2003) Then, incidents and isolations from veterinary sources between July 1990 and December 2004 were added to the national dataset in HPS.

Poultry samples offer a further source of veterinary samples. A poultry monitoring system is operated in the poultry industry and therefore samples from poultry may contain many non-clinical diagnostic samples.

All records were kindly provided by HPS. The datasets were supplied in excel spreadsheet format, with column headings detailed in Table 2.3.

Table 2.3 Details of case information contained within the datasets provided by veterinary laboratories where *Salmonella* infection had been determined.

Variables	Description
Pathogen	Serotype of *Salmonella*
Type	Phage types of *Salmonella* Enteriditis and Typhimurium
Year	The year of collection
NO	The collection number - contains the reported week
Source	Which animal the case is from
True source	The categories of animal
Lab source	The veterinary laboratory of isolation
Resistance pattern	The 15 antibiotic resistance patterns
Week	The week the case was reported to laboratory
Pathogen2	*S.* Typhimurium, *S.* Enteritidis, and Others

2.5 CLEANING THE DATASETS

All data were checked by EXCEL 2000. The two datasets were compared and then the errors were corrected. A unique number was given to each record to trace the original data when necessary. In the human database, the postal district for each record was added using the electronic map enquiry system on the website: http://streetmap.co.uk. The records, which did not have address information, were marked as 'missing'.

In the animal database, the original dataset did not contain the date received. Instead of that, a reference number including the information on the year and week was used. A new variable, week, was generated from the reference numbers. The week ranged from 1 to 53 and the week number was used as the minimum time interval. Thus, a year was divided into 13 four-weekly months. The 53rd week was assigned to the 13^{th} four-weekly month.

Text categories were encoded as nominal variables and "Yes" or "No" answers were changed to binary format. Similarly, antibiotic resistant patterns were re-coded to binary format - 1 referred to resistant and 0 referred to sensitive. "Intermediate" was recoded as "0" – only three cases had intermediate resistance to tetracycline, trimethoprim, kanamycin, respectively. Cases for which there was missing information were considered individually, depending on the analysis being undertaken. Missing data were usually coded as '9'. No information in a variable was coded as '99'. Date format, such as 01/01/2005, was reclassified into two separate fields - "month" and "year".

2.6 EPIDEMIOLOGICAL ANALYSIS METHODS

Descriptive, cluster, spatial and time series modelling were used to conduct different analysis, some details of which will be presented in later chapters. Where appropriate, statistical significance was set at a p-value <0.05; all tests were two-tailed.

2.6.1 Descriptive statistics

Descriptive statistics help to summarise data and identify trends or patterns which can then be further explored using more sophisticated statistical techniques. Functions within the Microsoft Office software package, Excel version 2000, namely pivot table and pivot chart, were used to generate summary tables and charts. To further enhance the readability of figures, PowerPoint version 2000 was employed. The population statistics in the 2001 Census were used to calculate the cumulative incidence in an area or used as the base for other statistical analysis (General Register Office for Scotland 2002) (Table 2.4).

Table 2.4 Mid-year population estimates; Scotland and its Health Boards by single age and sex: 2001

Healthboard	persons	males	females	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59
Argyll & Clyde	425756	204532	221224	23134	26134	27977	26420	23061	24030	31302	34880	32574	28777	30508	25391
Ayrshire & Arran	368149	175486	192663	19186	22761	24614	22492	18961	20656	26096	28423	26942	24745	27194	22813
Borders	106764	51361	55403	5509	6601	6731	5627	4472	5311	7367	8359	8176	7324	8108	6817
Dumfries & Galloway	147765	71303	76462	7467	9001	9523	8247	6192	7344	9670	11254	10563	9973	11199	9893
Fife	349429	167628	181801	19214	21494	23247	22238	20834	19964	25616	26995	25692	23142	25381	20907
Forth Valley	279480	134444	145036	15864	17489	18047	17614	16954	16688	20988	22239	20559	18572	20396	16510
Grampian	525936	259735	266201	28194	31756	33264	33006	34500	34450	40741	42005	40112	36937	37918	28786
Greater Glasgow	855953	404134	451819	47126	51054	53654	56811	61878	59021	66282	69066	64049	54594	51588	43402
Highland	208914	102297	106617	11363	13070	13780	12122	9995	11264	14007	16213	15805	15221	16074	13498
Lanarkshire	558751	268109	290642	32762	35517	37051	37031	32438	35157	44030	45667	42648	37895	37515	31846
Lothian	778367	373045	405322	43221	45572	46007	47330	57846	57709	65390	63396	57968	49838	52196	40544
Orkney	19245	9497	9748	984	1218	1370	1107	796	1007	1356	1483	1404	1383	1428	1330
Shetland	21988	11071	10917	1328	1534	1642	1305	1191	1351	1570	1726	1574	1629	1625	1375
Tayside	389012	186770	202242	20228	22388	24177	24455	24113	21949	25976	29397	27964	25604	27956	23208
Western Isles	26502	13082	13420	1294	1549	1786	1468	1156	1402	1703	1851	1880	1835	2021	1679

Table 2.4 (continued) mid-year population estimates; Scotland and its health board areas by single age and sex: 2001

Healthboard	60-64	65-69	70-74	75-79	80-84	85-89	90-94	95-99	100+
Argyll & Clyde	23268	20777	17730	13605	8851	4967	1926	397	47
Ayrshire & Arran	20646	18922	15773	12822	8307	4584	1732	437	43
Borders	6213	5615	5074	4220	2796	1656	613	155	20
Dumfries & Galloway	9108	8302	7528	5832	3642	2020	792	193	22
Fife	17965	16305	14353	12084	7603	4394	1564	382	55
Forth Valley	14846	12898	10879	8799	5587	3107	1160	260	24
Grampian	25360	23390	20078	16136	10323	5851	2482	568	79
Greater Glasgow	42043	40311	35167	27823	17562	9758	3798	859	107
Highland	11827	10459	8972	7046	4337	2577	1001	246	37
Lanarkshire	29034	25645	21411	16022	9723	5030	1829	442	58
Lothian	36338	33181	29153	23824	15571	8841	3536	792	114
Orkney	1159	903	824	648	440	279	96	27	3
Shetland	1053	868	741	667	416	266	97	26	4
Tayside	21240	20077	18002	14858	9129	5466	2218	528	79
Western Isles	1633	1463	1232	1137	702	445	203	54	9

2.6.2 Cluster analysis

The purpose of cluster analysis is to understand the relationships amongst cases and variables, in this situation, the relationship between *Salmonellae* and antibiotics (SPSS Inc 2006). The clustered groups can be further assessed using more advanced statistical methods, such as temporal, spatial or spatio-temporal analyses. There are now a large number of database, spreadsheet and statistical analysis software packages available, and in this study, SPSS, Excel 2000, SatScan and MapInfo 5.5 were used where appropriate for analysis and presentation purposes. Excel 2000 spreadsheets were used for cleaning the datasets and to generate SPSS compatible files. In SPSS, hierarchical cluster analyses, K-means cluster analyses, McNemar and chi-square tests were undertaken. The results were exported to Excel 2000 and modified to fit the Mapinfo 5.5 format to present clusters or other information in the electronic map files, which were all kindly supplied by Dr. Dominic Mellor.

2.6.2.1 The test of independence

The McNemar sign test was used to determine the independence of antibiotic resistances. It is usually used for testing prior and post dichotomous results in the same sample. However, it can be used to test the response for a related pair of variables, e.g. antibiotic resistance patterns. If the null hypothesis is true, it is expected that the frequency table for 'yes, no' and 'no, yes' will be equal. If the p-value is less than 0.05, it means the pair show significantly different responses (SPSS Inc. 2006; Dohoo et al. 2003).

2.6.2.2 Hierarchical cluster analysis

In hierarchical cluster analysis, clusters are not formed in one simple step. It is a long procedure involving many calculations. The results are derived and presented according to the linkage and measure methods chosen by the researcher. Square Euclidean Distance (SED) is the most common means of measuring the distance between a pair of observations. Euclidean distance (ED) and simple matching are both used for calculating the distance between data in binary format (SPSS Inc. 2006). The difference among the measure methods lies in the description of the positions of outliers. Outliers are those results very dissimilar to the major patterns. In the clustering study, SED was chosen as the standard measure method. Simple matching and ED were also used in order to check the positions of outliers.

There are 7 different linkage methods supported by SPSS (SPSS Inc. 2006). However, for binary data, e.g., antibiotic resistant patterns, "between-group-linkage" is a more suitable method. The methods differ in the selection of the first observation (target) in the algorithm. Normally, if the dataset is big enough, different distance measurement or linkage methods will usually generate the same or very similar results. The "between-group-linkage" calculates the distance according to the average of distances between all pairs of objects (isolates). In the "between-group-linkage", $D(r,s)$ is computed as

$$D(r,s) = T_{rs} / (N_r * N_s)$$

Where T_{rs} is the sum of all pairwise distances between cluster r and cluster s. N_r and N_s are the sizes of the clusters r and s respectively

At each stage of hierarchical clustering, the clusters r and s, for which $D(r,s)$ is the minimum, are merged.

The first step in the algorithm is to separate all cases into different clusters containing one case only. Then, the two closest observations are joined. In the next step, a third observation can either join the cluster to form one group or joins another observation to create two different clusters. Each step results in one less cluster than in the previous step. This calculation continues until all cases are combined in one cluster. In this algorithm, missing values are not allowed, and so missing data were removed for the purposes of this particular analysis.

The final results of clustering are usually shown as a dendrogram. The decision maker needs to determine what degree (level) can be thought of as a group. This is known as 'cutting the dendrogram'. This answer can be got from checking the dendrogram or the score in the calculation. The pattern of how these scores change with the clustering steps can help the user to find the final grouping.

The main disadvantage of this type of analysis is that there are no exact rules about which distance measures and linkage methods to use. A sensible approach is to try different linkage methods and use different distance measures to assess the robustness of output; "K-means" analysis can be undertaken to validate conclusions.

2.6.2.3 K-means cluster analysis

Data can be classified by a method with no pre-assumptions, such as hierarchical clustering analysis, or classified by a pre-determined clustering method, such as K-

means clustering. K-means clustering analysis is another classifying package available in SPSS (SPSS Inc. 2006). This method can assist decision-makers in choosing the most appropriate cutting line in hierarchical clustering analysis.

In simple terms, K-means clustering analysis is an algorithm which may be used to classify or to group observations based on a given K number of groups, where K is a positive integer number. The result is given by minimizing the sum of squares of distances between data and the corresponding cluster centroid. The advantages of this method are that it helps users identify homogeneous groups of cases; it can handle large numbers of cases; the centres can be assigned *a priori*, if they are known; and users can select a method for classifying cases. In this method, analysis of variance F statistics will be generated. The weakness of this method is it is not robust with respect to outliers. The centroid may be influenced away from the true centre when some cases are very far from the centroid.

2.6.3 Spatial analysis methods

Spatial analysis is a method used for understanding relationships in a certain time and/or certain zone. It is a powerful, meaningful and useful tool in understanding and predicting the relationships between cases and space. There are many software packages with capabilities in this field, such as MapInfo and SaTScan. In this thesis, MapInfo was used for presenting the clusters on the electronic map. In the thesis, the cumulative incidence in NHS board level and post district level were shown in the thematic map format.

The main analyses were implemented in SaTScan. SaTScan was developed by Kulldorff and colleagues (Kulldorff, 1997). It is able to detect clusters of cases in time, space, and time-space using a random circling scanning method. In the Poisson-based model, the criterion is that the data must follow a Poisson distribution. The model can help users to detect clusters of any size anywhere in the study region. SaTScan uses the random circling method to identify the highest possible cluster(s) in time, space or time-space. The alternative hypothesis is that there is an increased risk of disease in the focused area; the null hypothesis is that the risk is not increased. For each random circle, a likelihood ratio is calculated until the system finds the most likely cluster, which has the largest likelihood ratio. The other possible clusters are listed and the p-value shown under each possible cluster. All random circle finding is obtained by Monte Carlo simulation.

In the Poisson model, census population data over time are necessary. In the thesis, the 2001 Scottish census population data were used. The census data in 1981, 1991, and 2001, did not show a dramatic changes and it was assumed that the population did not change dramatically over the study years. In the animal dataset, this underlying assumption could not be upheld and so the Poisson model was not used.

The second model used in the thesis was a Bernoulli model. It is suitable for binary data and is used as a case-control study model. The null hypothesis is the risk in the case group is the same as that in the control group. This model was more suitable for the animal data. The positions of laboratories were used as specific coordinative position and the assumption was that the proportion of cases at each laboratory was the same under a Poisson distribution.

SaTScan also supports three additional models but they were not used: 1) a space-time permutation model; 2) an ordinal model; or 3) an exponential model.

2.6.4 Time series method

Time series study is a technique used to identify the nature of the phenomenon in a series of observations in a fixed time interval. It was first developed by Yule and Slutsky in the 1920s and used to develop a comprehensive model called autoregressive integrated moving average model (ARIMA) by Box and Jenkins in the 1970s (Box and Jenkins 1976). It has four main purposes: modelling, forecasting, characterisation and classification (Borovkova 1998). The aim of modelling is to build a model which can describe historic data. Then, the generated model can be used for forecasting, providing basic estimates about the future. The fundamental characters of a model can be identified and models can be classified into groups according to the level of similarities (Borovkova 1998). It can focus on the series itself as a uni-variate time series study or take into account other factors as a multivariate study. There are linear and non-linear time series approaches. For example, the ARIMA model is a linear model and the self-threshold auto-regressive model is a nonlinear model (Watier 1995).

The most basic time series model is the simple additive regression form:

$$Z_t = b + S_t + T_t + E_t$$

Where b is the constant. E_t is the white noise, which explains the background information, S_t is the seasonal component and T_t is the trend (level) component.

There is another multiplicative form ($Z_t=b+E_t*S_t*T_t$) which, for disease researchers, is attractive given epidemic increases from one time point to the next. The trend package in SPSS 14 supports two models: the exponential smoothing model and the ARIMA model. The ARIMA model is a group of different forms. It contains: auto-regressive (AR), moving average (MA), auto-regressive and moving average (ARMA) and auto-regressive integrated moving average (ARIMA) models.

The AR model needs a time series in stationary form. In contrast, the MA model can deal with infinite time series. The ARIMA model combines the advantages of both processes and minimises the disadvantages. The general programmatic form is ARIMA(p,d,q), where p is the AR parameters, d is the differencing passes and q is the MA parameters. This thesis focuses on the ARIMA model because it also addresses some of the issues of exponential smoothing models. The ARIMA model is also advantageous because it has a low cost of development and maintenance and finally, it can generate lower root-mean-square-error (RMSE) compared with other models. This means that it has a better descriptive ability and in turn provides a good predictive ability (Abeku et al. 2002; Smelser and Baltes 2001). These issues are discussed further in later chapters.

CHAPTER III

SALMONELLOSIS, *S.* TYPHIMURIUM AND *S.* TYPHIMURIUM DT 104 IN HUMANS IN SCOTLAND, 1988-2004

CHAPTER III

SALMONELLOSIS, *S.* TYPHIMURIUM AND *S.* TYPHIMURIUM DT 104 IN HUMANS IN SCOTLAND, 1988-2004

3.1. INTRODUCTION

As outlined in the opening review, salmonellosis is well recognised as a zoonosis associated with symptoms such as diarrhoea and fever in infected animals and humans. Usually, the pathogen spreads by the faecal-oral route via contaminated water, food or direct contact with infected animals. Each year in the USA, 400 to 600 people die as a result of infection and between one and three million people are infected by one of the *Salmonella* spp. (Humphrey et al. 2000). In Scotland, most *Salmonella* cases are associated with two main serotypes: *Salmonella* Enteritidis is responsible for about 70% of reported cases and *Salmonella* Typhimurium (ST) is responsible for about 30% of cases (Browning et al. 2003; Browning et al. 1999; Browning et al. 2001a; Browning et al. 2001b). *S.* Typhimurium definitive type 104 (ST DT104) was first isolated in the 1960s. It was not a major concern at that time due to the small number of isolates but concerns grew in 1988 after a cassette of resistant genes was found in its genome. The aims of this study outlined in this chapter were to describe the trends of *Salmonella*, ST and ST DT104 infections in Scotland, based on human isolates between 1988

and 2004.

3.2 Material and methods

The surveillance of *Salmonella* infections in Scotland is based on cooperation between SSRL and HPS as outlined in Chapter 2. Based on these data and using the Scottish 2001 Census data, proportions, period prevalences and cumulative incidences were calculated for each of the categories described, stratifying by year, location and age of patient.

3.3 Results

3.3.1. The trends of *Salmonella* spp., *S.* Typhimurium, and *S.* Typhimurium DT104

Figure 3.1 shows the numbers of total *Salmonella*, *S.* Enteritidis, *S.* Typhimurium, ST DT104 isolates including Home Cases and Imported Cases. Figure 3.2 shows the proportion in each year. A total of 32,582 isolates were collected from humans between 1988 and 2004. The majority of isolates were *S.* Enteritidis, with the next most common serotype being *S.* Typhimurium. Third position was occupied by four different serotypes over the sixteen years of the study, or five if imported cases are excluded: *S.* Virchow (1988-1996, 2000-2002); *S.* Hadar (1997-1999); *S.* Bareilly (2003 – following a major outbreak); and *S.* Newport (2004). When

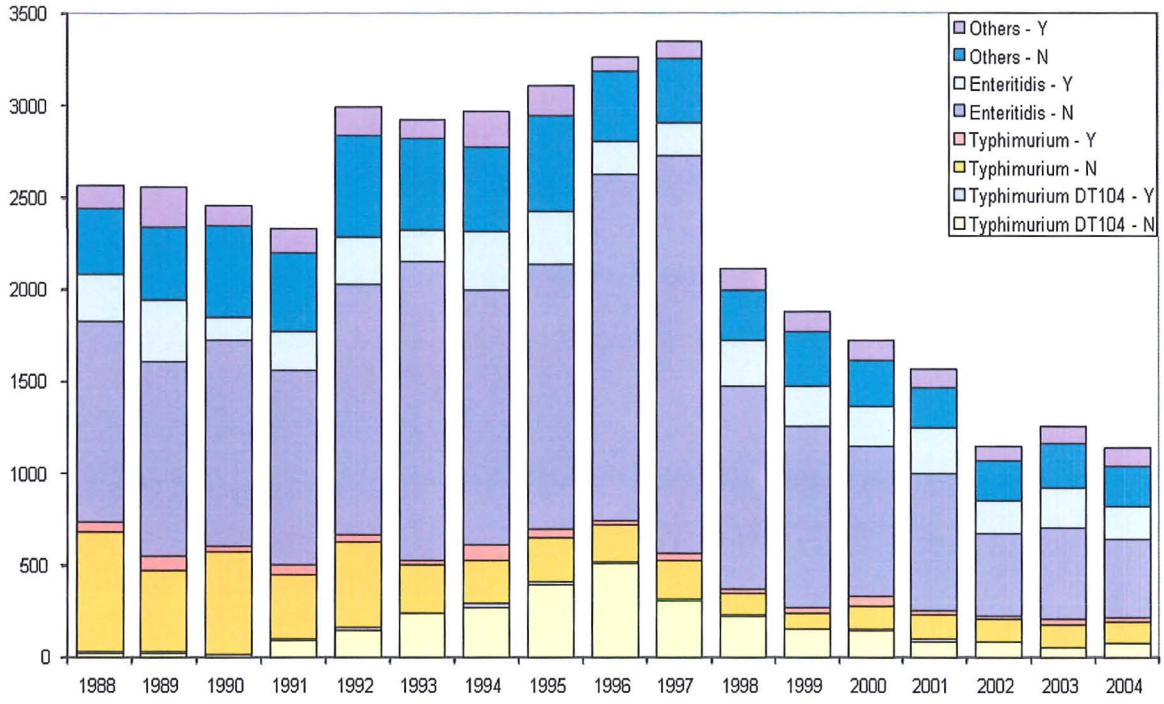


Figure 3.1 The numbers of the *Salmonella* Enteritidis and Typhimurium and its phage type ST DT104 from the Scottish human isolates, 1988-2004. Y: Home Cases; N: Imported Cases.

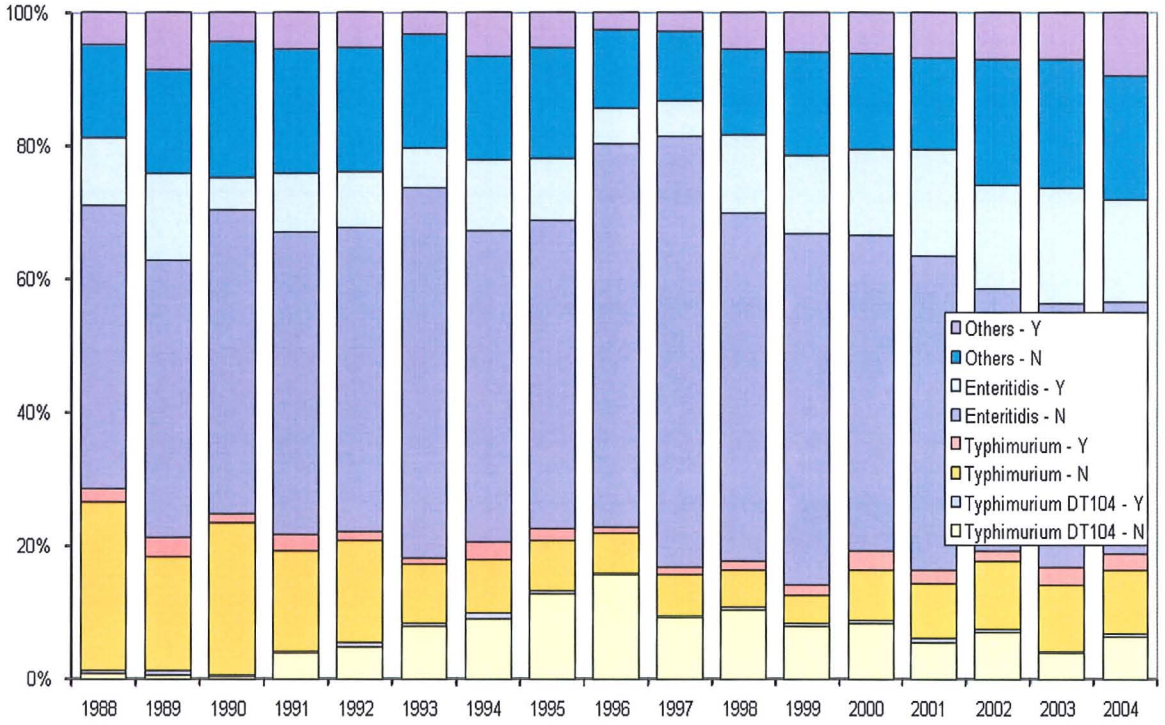


Figure 3.2 The proportion of different *Salmonella* serotypes from Scottish human isolates, 1988 – 2004. Y: Home Cases; N: Imported Cases.

imported cases are excluded, *S.* Wangata took third place in 1992. Based on these data the salmonellosis figures could be roughly divided into three periods: 1988-91, 1992-97, and 1998-2004. Case numbers increased gradually to 3,349 by 1997, and then dropped quickly to 1,143 by 2004.

S. Typhimurium isolates totalled 8,067 over the whole period of the study – 3,941 classified as female, 4,175 as male, and the remainder unclassified by gender. Home Cases made up 7,212 of the total (3,414 female, 3,751 male), with some imported cases being recorded every year. The *S.* Typhimurium trend line rose gradually from about 500 isolates to a peak of 744 in 1996. Recorded isolations then fell steeply, almost mirroring the trend line for salmonellosis. The proportion of *S.* Typhimurium of all cases of salmonellosis remained stable at about 20%.

S. Typhimurium DT104 isolates totalled 2,955, including 1,442 female and 1,494 male. Home Cases made up 2,798 of these (1,358 female and 1,422 male). *S.* Typhimurium DT104 case numbers increased gradually to 511 in 1996 before falling, but even in the last year of the study ST DT104 accounted for about 50% of all ST isolates. The isolates of ST DT104 could also roughly be divided into three periods: before 1991, when there were very few isolates; 1991-1998, the main infection period; and after 1998, when the figures began to decline, with just 73 cases recorded in the final year of the study in 2004. After 1991, ST DT104 began to

account for an increasing and significant proportion of total ST cases. While total ST cases were declining, the ST DT104 total increased sharply in both number and proportion of the ST total, reaching its highest number (520) and highest proportion (69.9%) in 1996. After 1996, even though both ST and ST DT104 declined somewhat, ST DT104 still accounted for a significant percentage of ST.

Figure 3.1 also shows that the isolate numbers of *Salmonella* and *S.* Typhimurium were quite stable and did not change dramatically before 1997. However, the trend line for isolates of ST DT104 shows a very traditional epidemic curve, rising from 20 to 511 in nine years. In 1997 the numbers began to drop, and remained below 100 from 2001 onwards. Imported cases of ST DT 104 were more stable than Home Cases, usually totalling less than ten per year (Figure 3.2).

3.3.2 Cumulative incidence of Home Cases in NHS Health Boards

The cumulative incidence of salmonellosis was 6.44 per thousand persons from 1988 to 2004 in Scotland. Table 3.2 shows the number of submitted isolates in the GG, GR, and LO Health Boards were the three highest. Two islands had the lowest isolates. The AA, WI, and SH Health Boards had the lowest cumulative incidence but the DG, BR, LN, LO, and GR Health Boards had higher cumulative incidences (Table 3.1, Figure 3.3). Every board had a similar trend;

Table 3.1 The submission of *Salmonella* from individual human health boards in Scotland, 1988-2004 (Home Cases only)

BOARD	AA	AC	BR	DG	FF	FV	GG	GR	HG	LN	LO	OR	SH	TY	WI	Total	C. incidence**
1988	71	166	61	77	148	105	281	269	74	249	479	2	0	129	5	2116	4.18
1989	74	158	64	68	91	70	239	351	76	207	365	9	0	141	1	1914	3.78
1990	83	200	57	95	101	82	289	439	80	272	309	3	0	176	8	2194	4.33
1991	53	209	67	78	102	90	327	272	40	237	310	5	0	136	7	1933	3.81
1992	107	239	57	96	161	106	396	373	82	290	434	5	17	159	3	2525	4.99
1993	161	192	53	106	172	111	277	387	104	361	438	11	6	217	14	2610	5.15
1994	102	162	108	104	139	158	344	338	87	227	354	10	18	193	6	2350	4.62
1995	143	172	94	125	196	129	335	365	96	305	415	20	13	181	7	2596	0.51
1996	125	255	65	103	216	165	428	386	112	319	524	14	8	235	16	2971	5.87
1997	127	217	154	99	186	154	377	401	133	352	476	25	12	305	12	3030	5.99
1998	72	161	39	51	77	110	278	259	49	205	276	11	8	100	9	1705	3.37
1999	64	132	27	53	68	92	296	175	53	199	263	1	4	70	17	1514	2.99
2000	59	112	32	30	66	66	213	156	50	260	237	2	11	41	2	1337	2.64
2001	47	92	19	37	54	77	187	145	72	158	191	1	7	84	5	1176	2.32
2002	28	54	20	23	34	66	160	110	35	122	143	2	4	67	1	869	1.72
2003	29	54	14	28	38	70	192	101	29	119	179	4	4	48	4	913	1.80
2004	36	66	10	17	51	51	127	102	25	124	144	6	1	66	3	829	1.64
Total	1381	2641	941	1190	1900	1702	4746	4629	1197	4006	5537	131	113	2348	120	32582	
C. incidence*	3.24	7.17	8.81	8.05	5.44	6.09	5.55	8.8	5.73	7.17	7.11	6.81	5.14	6.04	4.53	6.44	

* Cumulative incidence per 1000 persons; ** Cumulative incidence: per 10,000 persons

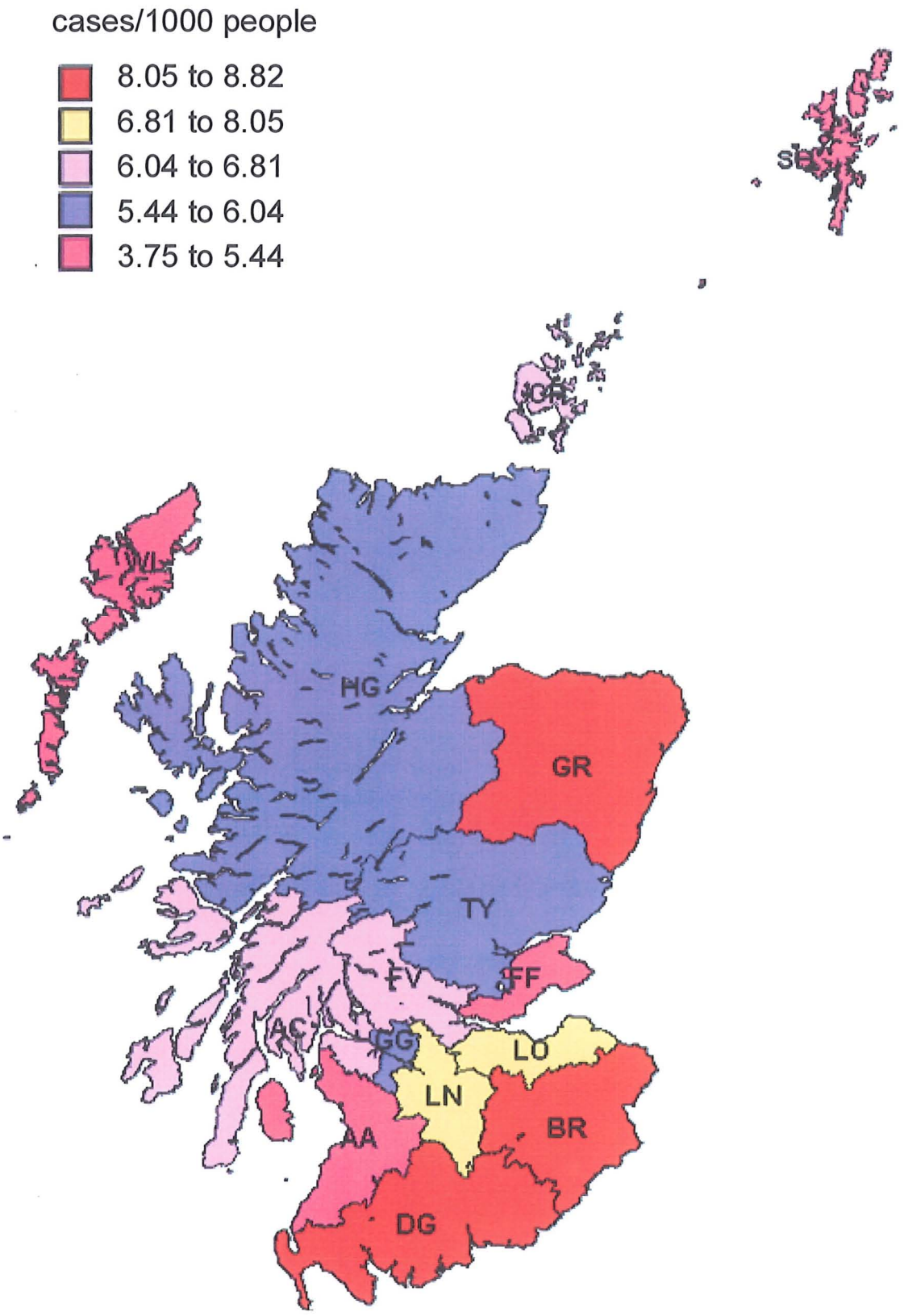


Figure 3.3 The cumulative incidences *of Salmonella* spp. infection in each Scottish Health Board, 1988 – 2004

initially rising and then falling roughly speaking at the same time. Generally, the southern and eastern parts of Scotland had higher rates and the middle and northern parts of Scotland lower rates.

The cumulative incidence of *S.* Typhimurium was 1.42 per 10 thousand persons. The DG, SH, and GG Health Boards had higher cumulative incidences than other Health Boards. In contrast, the TY, WI and AC Health Boards were the lower rate regions. The results are slightly different from salmonellosis (Table 3.2).

Table 3.3 shows the cumulative incidence of ST DT104 was 6.05 per 10 thousand persons. Although the GG Health Board had the highest submissions, the DG and OR Health Boards had very high rates. The cumulative incidence of the BR Health Board was as high as 7.21 per 10 thousand persons. The SH, AA and HG Health Boards had lower cumulative incidences. In addition, the SH Health Board only had 4 isolates in 14 years. In summary, the results showed the DG Health Board had higher cumulative incidence in salmonellosis, *S.* Typhimurium and ST DT104 infections.

Table 3.2 The submission of *S.* Typhimurium from individual human Health Boards in Scotland, 1988-2004 (Home Cases only)

YEAR	AA	AC	BR	DG	FF	FV	GG	GR	HG	LN	LO	OR	SH	TY	WI	Total	C. incidence^
1988	27	69	12	32	90	35	79	113	26	57	82			46	1	669	132
1989	36	29	23	24	23	15	68	66	22	45	78	2		25		456	90
1990	23	17	18	33	15	20	55	173	21	116	49			33	1	574	113
1991	24	36	18	42	26	27	65	50	9	59	57	2		26	2	443	88
1992	23	46	18	22	37	26	138	72	19	85	80	3	3	34		606	119
1993	28	26	7	34	33	31	67	65	10	102	64	1	4	21		493	97
1994	30	33	18	48	40	25	71	58	18	46	52	4		58	1	502	99
1995	32	31	34	64	44	27	96	80	17	76	93	5	1	36	1	637	125
1996	24	56	14	33	46	46	98	89	29	74	128	8	1	61		707	140
1997	15	38	9	20	28	40	64	104	14	78	60	10	2	30	1	513	101
1998	10	17	3	12	10	24	59	50	12	57	58	5		15	2	334	66
1999	15	17	3	7	7	13	37	25	4	32	40		2	16	14	232	56
2000	9	20	10	3	24	10	36	56	7	44	38	2	2	12		273	54
2001	10	21	3	7	11	13	33	28	9	35	27	1	1	16	1	216	43
2002	5	11	4	7	7	12	38	35	8	25	25	1	1	20	1	200	40
2003		10	3	10	11	13	36	19	5	20	32		1	13	1	174	34
2004	8	11	2	4	13	9	25	17	8	32	31	3		17	2	182	36
Total	319	488	199	402	465	386	1065	1100	238	983	994	47	18	479	28	7,211	142
C. incidence*	1.33	1.15	1.86	2.72	1.33	1.38	2.09	1.24	1.14	1.76	1.28	2.44	.82	1.23	1.06		

* Cumulative incidence per thousand people; ^ Cumulative incidence per million people

Table 3.3 The submission of ST DT104 from individual human health boards in Scotland, 1988-2004 (Home Cases only)

YEAR	AA	AC	BR	DG	FF	FV	GG	GR	HG	LN	LO	OR	SH	TY	WI	Total
1988		1	5	2		1	1	3		6			1			20
1989	1	1			4		2	3	3	2			1			17
1990		1	1							8						10
1991	12	10	1	14	1	6	10	4	4	14	8			6		90
1992	13	5	3	10	22	3	26	20	2	22	14			4		144
1993	14	10		15	12	12	36	24	4	72	23			12		234
1994	18	19	7	30	23	16	44	20	12	26	25	3		22		265
1995	23	21	26	57	30	17	56	47	7	37	51	5		20		397
1996	23	51	8	31	37	36	75	56	21	41	74	7		51		511
1997	11	19	8	14	21	28	38	73	8	34	29	4	1	19	1	308
1998	7	11	3	10	7	20	44	26	4	43	35	1		8		219
1999	12	11	2	5	4	10	22	17	3	17	24		2	7	14	150
2000	6	10	9	1	20	7	18	27	3	20	20			4		145
2001	2	4	1	2	3	4	20	14		15	12			7	1	85
2002	1	6	1	3	2	6	17	13	1	11	10		1	8	1	81
2003		2	1	4	3	1	11	3		12	8			4		49
2004	2	3	1	2	9	2	12	6	1	15	14			6		73
Total	145	185	77	200	198	169	432	356	70	382	363	20	4	180	17	2798
C. incidence*	3.41	5.03	7.21	13.54	5.67	6.05	6.67	5.05	3.35	6.84	4.66	10.39	1.82	4.63	6.41	

*:per 10 thousand persons

3.3.3. The distribution of ST phage types

The most common phage type was phage type 49 from 1988 to 1990 (Table 3.4). From 1991, the most common phage type was then DT104. The proportion of ST DT104 increased up to 70% in 1996. From 1996, the submissions of ST DT104 declined but still were more than 30% of all ST isolates. The second common type was usually RDNC (nonrecognised phage lysis pattern). The phage types 66, 204c, 170 and 104 were the second most common phage types in 1988, 1990, 1992 and 1996, respectively. ST DT104 was mainly of Scottish original except in 1992, when 17 ST DT104 isolates were recorded as being imported from other countries (Table 3.5, Figures 3.4 and 3.5)

Table 3.4 *S.* Typhimurium phage types isolated from humans in Scotland.

Year	Most common phage type	No. of isolates	No. of Home Cases	Second most common phage type	No. of isolates	No. of Home Cases
1988	49	173	173	66	91	86
1989	49	110	99	RDNC*	77	57
1990	49	148	147	204c	148	147
1991	104	95	95	RDNC	78	59
1992	104 170*	161	150	170 104*	152	144
1993	104	242	234	RDNC	62	47
1994	104	292	265	RDNC	96	63
1995	104	409	397	RDNC 104b*	78	63
1996	104	520	511	104b	53	48
1997	104	317	308	RDNC	67	58
1998	104	230	219	RDNC	47	37
1999	104	155	150	RDNC	39	27
2000	104	152	145	RDNC	39	27
2001	104	96	85	RDNC	35	26
2002	104	85	91	U302	23	22
2003	104	51	49	RDNC	36	29
2004	104	79	73	RDNC	33	28
Total	104	2955	2798	RDNC	987	768

The column 'All' is the total number of the named type; the number in 'Home' only includes the 'Home Cases'.
* means when only 'Home cases' were counted.
*RDNC: nonrecognised phage lysis pattern

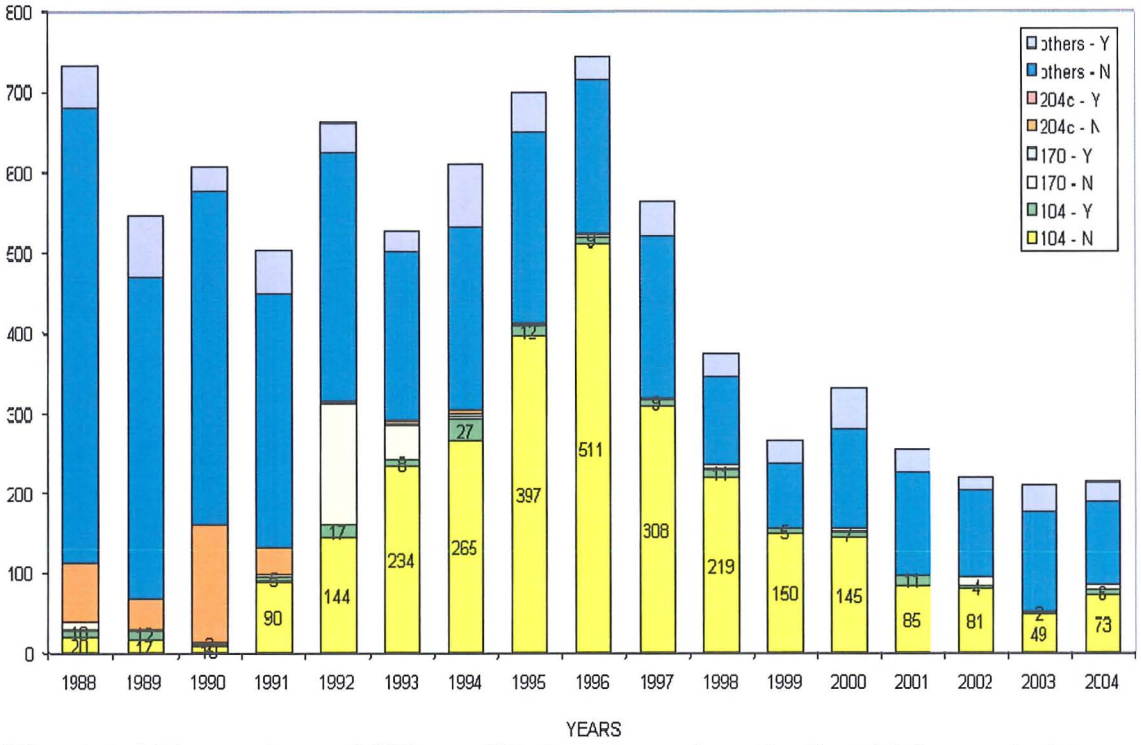


Figure 3.4 The numbers of different ST phage types from the Scottish human isolates, 1988-2004. Y: Home Cases; N: Imported Cases.

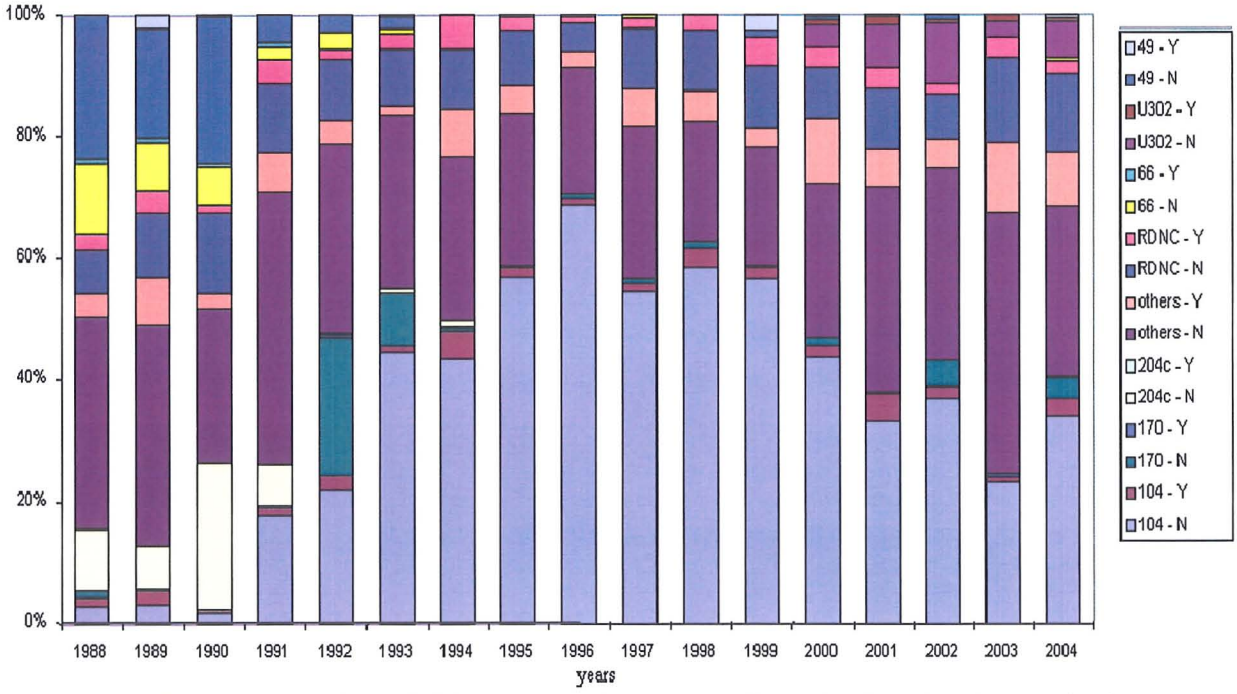


Figure 3.5 The proportion of different ST phage types from the Scottish human isolates, 1988-2004. Y: Home Cases; N: Imported Cases.

3.3.4 Seasonal effects

Figures 3.6 and 3.7 show the seasonal effects, classifying the number of isolates into 13 four-weekly months. Most human isolates were reported in the summer months (August to October) and the numbers of *Salmonella*, ST, and ST DT104 isolates all went up to their peak in the 9th period and then declined. There were also secondary peaks in the first and the 13th periods. The cases numbers of ST in all periods were between 20 and 150, except associated with an outbreak of phage type 49 in October 1990; When the data for ST DT104 were updated to 2004, the pattern was maintained (Figure 3.8). The results showed a strong seasonal change in the ST time series. These aspects will be explored further in later chapters.

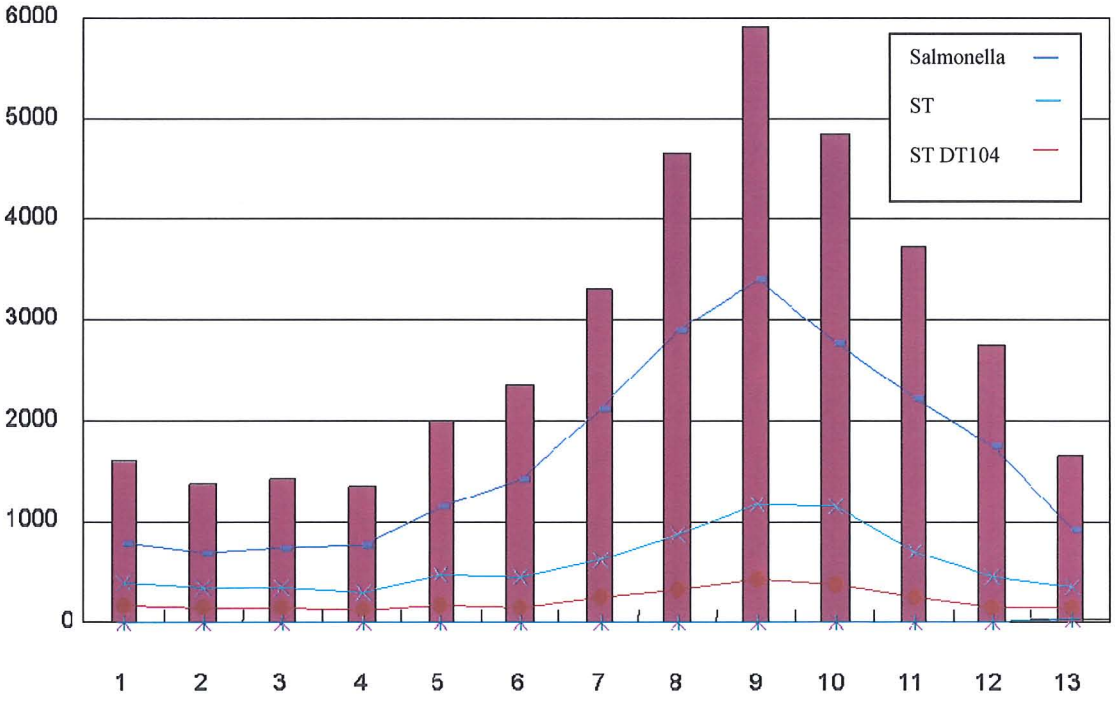


Figure 3.6 The numbers of the *Salmonella* spp. isolates in each four-weekly month, Scotland, 1988-2002

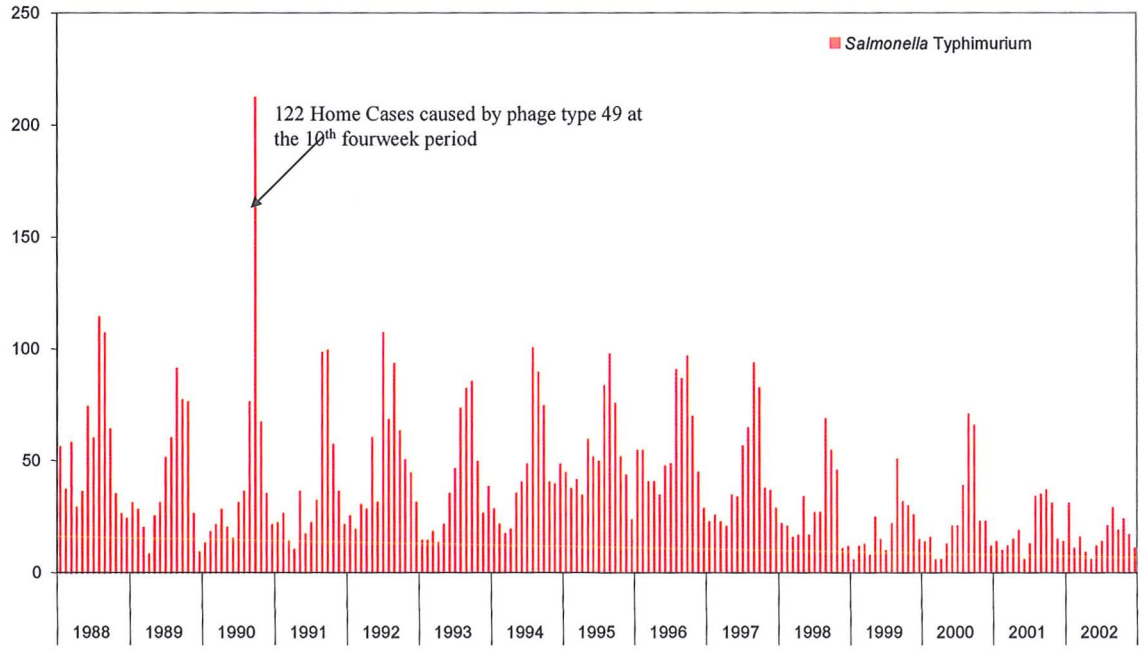


Figure 3.7 The time series of ST isolates in the four-week interval, Scotland, 1988-2002

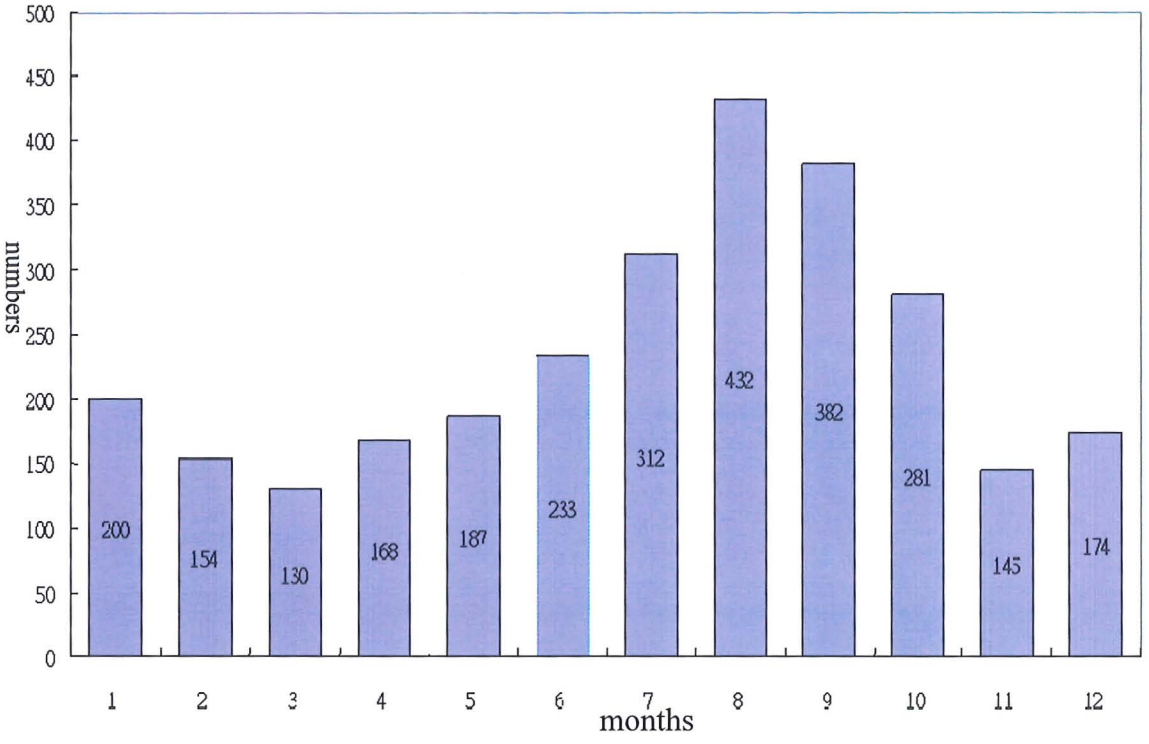


Figure 3.8 The numbers of the ST DT104 submissions in each calendar month, Scotland, 1988-2004

3.3.5. Age

Figure 3.9 shows the distribution of ST DT104 isolates from the different age groups of patients. Children younger than 10 years had significantly more ST DT104 reports than other age groups. Although the age group 65+ had 194 isolates, the cumulative incidence was only 2.4 per thousand persons due to the large population denominator in this group.

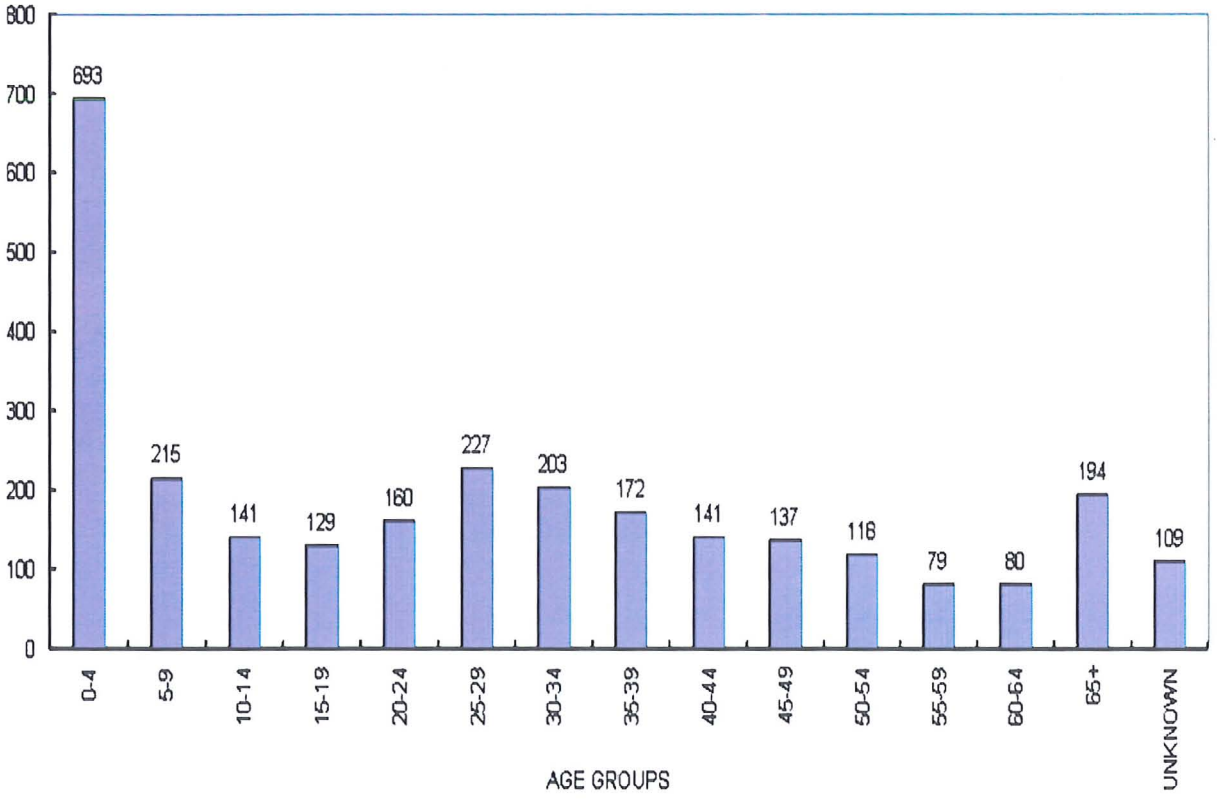


Figure 3.9 The numbers of reported isolations of ST DT104 from different age groups, Scotland, 1988-2004

3.4 Discussion

The purpose of this chapter was to describe the trends in *Salmonella*, ST, and ST DT104 infections in Scotland. Although there are some minor difference between Scotland and other European countries, the trends are broadly similar (Helms et al. 2005). In

and other European countries, the trends are broadly similar (Helms et al. 2005). In England and Wales, The trend of *Salmonella* spp. infection rose until 1998 and then declined in 1999 (Threlfall et al. 2000). In the rest of Europe, the proportion of ST of all *Salmonella* spp. cases declined in line with observations in this study but the proportions of ST DT104 and MR ST DT104 infections all increased particularly in 1996 (Helms et al. 2005).

There would appear to be good grounds for concluding that there has been something of an epidemic but whether the reported isolations are a true reflection of infections is a matter for debate. Diagnostic kits and methodology development is directly related to the ability to detect and report and in the early rise, the increased case numbers may reflect a detection bias (Humphrey et al. 2000). Add to this awareness (Humphrey et al. 2000) and planned surveillance and the rate of increase is likely to be exaggerated.

In Scotland, several factors might be associated with the decline of *Salmonella* isolate numbers from 1998. These would include improved biosecurity following BSE and the banning of bone meal in livestock feed in 1996; vaccination against *S.* Enteritidis PT4 in 1994; vaccination against *S.* Enteritidis in egg-laying flocks in 1997 (Defra 2003; Threlfall 2000; Helms et al. 2005). All of them might cause the total *Salmonella* cases to decline (Helms et al. 2005; Threlfall 2000). ST DT104 reflected the same general trends and the reasons for this are less clear although biosecurity and changes in farming practice are likely factors. For example The Over Thirty Month Scheme (OTMS), which was introduced in May 1996, forces cattle older than 30 months to go directly from farms to slaughter and incineration (Ternent 2002).

The seasonal trends are well-established and are related to increased temperature and summer related behaviours (Browning et al. 2003). Indeed these trends are in agreement with the situation for *E. coli* O157, which also has the highest incidence in June- August (Ternent 2002), as well as other food borne organisms. The age profile of cases is again well established and relates to immune status and exposure (Browning et al. 2003).

As regards spatial effects, the difference in the cumulative incidences in the different health boards is worthy of further investigation. Whether these relate to exposure or detection biases is difficult to establish. Certainly, it will interesting to relate these figures to livestock distribution and infection rates in livestock as suggest by Calvert et all (Calvert et al. 1998). Before exploring the situation in animals, the next chapter will address the resistant ST DT104 in more detail.

CHAPTER IV

DESCRIPTIVE EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN *S.* TYPHIMURIUM TYPE DT104 IN SCOTLAND (1988 -2004)

CHAPTER IV

DESCRIPTIVE EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN *S.* TYPHIMURIUM TYPE DT104 IN SCOTLAND (1988 -2004)

4.1 INTRODUCTION

A multi-resistant ST DT104 clone resistant to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline, viz., ACSSuT R-type, became a major cause of ST DT104 infection in humans in the UK from the late 1980s. It appeared in several European countries, USA, Canada, Israel, Turkey and Japan during the 1990s (Threlfall 2002). Chapter 3-1 showed that in Scotland, ST DT104 isolates accounted for 38.8% of ST isolates and caused 8.6% of Salmonella infections from 1988 to 2004. Nalidixic acid and trimethoprim resistances were identified from ST DT104 strains in England and Wales (Threlfall et al. 1996) and there has been a worrying in increase in ST DT104 isolates with trimethoprim and low-level ciprofloxacin resistance in many industrialised countries (Parry 2003).

One aim of Enter-Net, funded by the EU, is to investigate and evaluate *Salmonella* infections (Fisher 1999). The purposes of the surveillance system are to collect isolates and share information among participants to control salmonellosis. Many different resistance types (R-types) have been identified. This chapter describes the changes in ST DT104 resistances in Scotland from 1988 to 2004. It will be used as the initial information for the following hierarchical cluster and geographical cluster analyses.

4.2 MATERIAL AND METHODS

Isolates were routinely tested by the Kirby-Bauer disk diffusion method for sensitivity to a panel of 14 antibiotics (15 treatments) in the SSRL (Browning et al. 2001a). The 15 treatments used in the SSRL were described in Chapter 2. A total of 2,798 isolates of ST DT104 defined as Home Cases were examined.

Scores were assigned based on the numbers of antibiotics to which an isolate was resistant. Low level ciprofloxacin and cefotaxime resistances were excluded because they were added into the panel from 1999. Thus, the highest R-type scores were 13.

When the HPS publications were reviewed, 'multi-resistant type' before 2001 were defined as an isolate resistant to 3 or more antibiotics. After that year, the definition was changed. 'Multi-resistant type' was then defined as an isolate resistant to 4 or more antibiotics in the panel (Browning et al. 2001b; Browning et al. 1999; Browning et al. 2004; Browning et al. 2001a; Browning et al. 2005; Browning et al. 2003; Helms et al. 2002). In this Chapter, the latter definition was adopted i.e., resistant to 4 or more antibiotics.

Basic descriptive epidemiological analyses were performed as in Chapter 3.

4.3 RESULTS

Table 4.1 shows the changes of the R-type scores in each year. The proportions of different R-type scores are shown in Figure 4.1. The fully sensitive type (FS) remained present in high proportions from1988 to 1990 but numbers dropped sharply thereafter. In contrast, the percentage and number of resistant types increased rapidly, especially those R-types isolates with score 6. The ratio of the sensitive to the resistant type dropped from 80% to 14% after

1991. Sensitive isolates (R-type scores less than 4) almost disappeared after 1992. During the study period, the overall ratio of the R-score more than 4 against the R-score less than 4 was 89%.

Table 4.1 Numbers of isolates by their R-type scores using 13 antibiotics (Scottish human isolates, 1988-2004)

	R scores										
YEAR	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
1988	16			1	2	1					**20**
1989	6		3		1		7				**17**
1990	8					1	1				**10**
1991	9	1		3	2	1	69	5			**90**
1992	24		1		1		115	3			**144**
1993	6	1	6	1	1		217	2			**234**
1994	12		7	6			221	18	1		**265**
1995	8	2	11	9		2	286	78	1		**397**
1996	4	1	4	31		2	329	137	3		**511**
1997	7		1	4	2		213	61	20		**308**
1998	3		1	7	1		158	38	10	1	**219**
1999	9		1	2	3		110	25			**150**
2000	2		3	5	3		107	25			**145**
2001	2		4	2	1	1	30	45			**85**
2002	1	1	10	3		1	31	31	3		**81**
2003		1	2			1	29	16			**49**
2004			3	25			25	20			**73**
Total	**117**	**7**	**57**	**99**	**17**	**10**	**1948**	**504**	**38**	**1**	**2798**

Figure 4.1 shows that the proportion of MR ST DT104 was always higher than that of R-type score less than 4 from 1991. Although the case numbers declined from 1997, the percentage of MR ST DT104 remained high. From 2001, the proportion of MR ST DT104 went down. In 2004, the percentages of MR ST DT104 fell further and only 25 out of 73 isolates had R-type scores of 3.

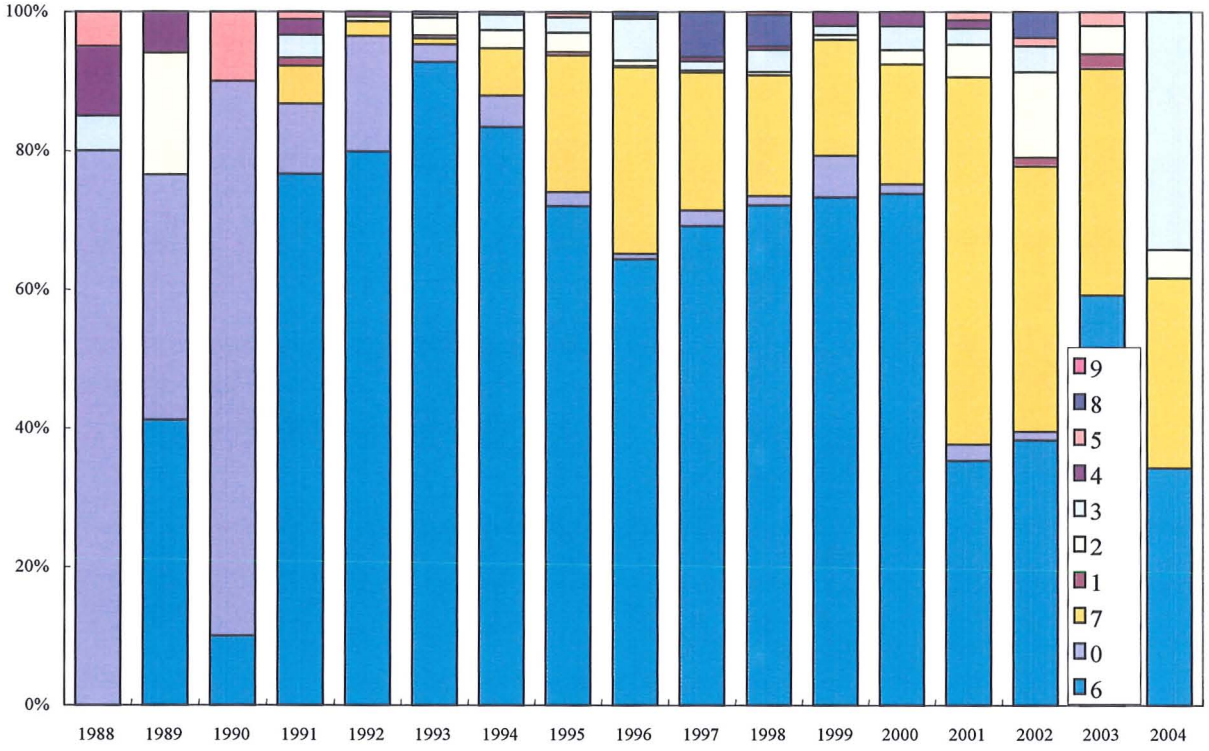


Figure 4.1 Changes in the R-type score proportions of human ST DT104 isolates, Scotland, 1988-2004. The numbers in the legend are the R-type scores.

Table 4.2 shows the R-types identified from 1988 to 2004. A total of 52 R-types were identified throughout the study period. The percentage of the fully sensitive type was 4%. The most common R-type score was 6 (69.6%). The ApClSpStSuTe R-type contained 1,941 isolates. It constituted 99.6% of all isolates with a R-type scores of 6. There were 17 and 10 isolates in the R-type score 4 and 5 group, respectively. 504 isolates were present in the R-type score 7 group. The first R-type score 7 isolate was identified in 1991. In this group, the most common R-type was the ApClSpStSuTeTm R-type (325 isolates). The second most common R-type was the ApClNaSpStSuTe R-type (169 isolates). A total of 38 isolates belonged to R-type score 8, in 6 different R-types. The first R-type score 8 isolate (ApClKaNaSpStSuTe R-type) was identified in 1994. The most common R-type in the R-type score 8 group was the ApClNaSpStSuTeTm R-type (10 isolates). Only 1 isolate was R-type

score 9.

Table 4.2 The ST DT104 resistance types from human isolates, Scotland, 1988-2004.

	R-types			
1	Na	Te	Ap	
2	ApSu	SpSt	StSu	SuTm
3	SpStSu	ApClSu	ApKaSu	ApNaSu
	ApStSu	ApSuTe	ApSuTm	StSuTe
	SuTeTm			
4	ApClSpSt	ApClSpSu	ApClSuTe	ApSpStSu
	ApStSuTe	NaSpStSu	SpStSuTe	SpStSuTm
5	ApClSpSuTe	ApClStSuTe	ApClSuTeTm	ApSpStSuTe
	ClFzSuTeTm	SpStSuTeTm		
6	ApClKaStSuTe	ApClNaStSuTe	ApClSpStSuTe	ApClSpStSuTm
	ApClStSuTeTm	ApCpSpStSuTe	KaNaSpStSuTm	
7	ApClFzSpStSuTe	ApClGmSpStSuTe	ApClKaSpStSuTe	ApClNaSpStSuTe
	ApClSpStSuTeTm	ApCpNaSpStSuTe	ApCpNaSpStSuTm	
8	ApClCpNaSpStSuTe	ApClFzNaSpStSuTe	ApClGmNeSpStSuTe	
	ApClNaSpStSuTeTm	ApClKaSpStSuTeTm(Lc)	ApClKaNaSpStSuTe(Lc)	
9	ApClKaNaSpStSuTeTmLc			

R-types are sorted according to their R-type scores, which are listed in the left hand column.

Table 4.3 shows the numbers of each R-type from 1988 to 2004. Each year, the numbers of R-types which appeared were slightly different. In 1988, there were 4 R-types and the fully

sensitive type. It was reported that R-types ACSSuT (ApClStSuTe) were most common in England and Wales (Threlfall et al. 1994). However, in the Scottish data, only three isolates belonging to the ApClStSuTe R-type were reported in three different years. In 1990, only 3 different R-types were reported. After 1990, the phenotype diversities increased. The highest diversity was recorded in 1996; 17 R-types were reported. There was no correlation between type numbers and case numbers every year (Spearman's rank test, p=0.003).

Table 4.4 shows the numbers of Imported cases from 1988 to 2004. 14 R-types and 157 isolates were recorded. The most common imported R-type was the ApClSpStSuTe R-type (112 isolates). The trend for the fully sensitive type was the same as for 'Home cases' and were only present in the earlier years if the study. The ApClKaNaSpStSuTeTmLc R-type (from Spain) and theFzTe R-type (from Majorca) are the two R-types which have not been found in Home Cases. The three most common places of origin of Imported Cases were Malta, Majorca, and Spain.

Table 4.3 The ST DT104 R-types identified by year, Scotland, 1988-2004

	Year																	
R-types	**1988**	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**Total**
Ap															1	1		2
ApClCpNaSpStSuTe										1								1
ApClFzNaSpStSuTe								1										1
ApClFzSpStSuTe				1	1		1								1			3
ApClGmNeSpStSuTe										1								1
ApClGmSpStSuTe							1											1
ApClKaNaSpStSuTe							1		2	2								5
ApClKaNaSpStSuTeLc										4								4
ApClKaNaSpStSuTeTmLc											1							1
ApClKaSpStSuTe							1				1				1			3
ApClKaSpStSuTeTm										3	1				3			7
ApClKaStSuTe															1			1
ApClNaSpStSuTe							4	22	62	13	12	1	4					118
ApClNaSpStSuTeLc										8	6	7	5	8	4	1	12	51
ApClNaSpStSuTeTm									1	8	1							10
ApClNaSpStSuTeTmLc										1	8							9
ApClNaStSuTe							1											1
ApClSpSt												1						1
ApClSpStSuTe		7	1	72	115	218	221	292	322	213	155	107	107	30	26	29	25	1940

Table 4.3 (continued) the ST DT104 R-types identified by year, Scotland, 1988-2004.

R-types	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total
ApClSpStSuTeLc										1								1
ApClSpStSuTeTm				4	2	2	12	66	73	32	18	15	16	37	29	11	8	325
ApClSpStSuTm														1				1
ApClSpSu				1														1
ApClSpSuTe				1				1										2
ApClStSuTe	1		1												1			3
ApClStSuTeTm															2			2
ApClSu	1																	1
ApClSuTe	1																	1
ApClSuTeTm									1									1
ApCpNaSpStSuTeLc												1						1
ApCpNaSpStSuTmLc												1						1
ApCpSpStSuTe												1						1
ApKaSu									1									1
ApNaSu									1									1
ApSpStSu						1												1
ApSpStSuTe								1								1		2
ApStSu						1	1								1			3
ApStSuTe	1	1		1	1													4
ApSu					1	6	6	10	3	1	1	1	3	4	8	1	3	48

Table 4.3 (continued) the ST DT104 R-types identified by year, Scotland, 1988-2004 (Continued)

R-types	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total
ApSuTe														1				1
ApSuTm									1						1			2
ClFzSuTeTm									1									1
KaNaSpStSuTmLc												1						1
Na				1					1									2
NaSpStSu												1						1
NaSpStSuLc												1	1					2
SpSt									1					2				3
SpStSu				2			5	9	27	4	7	2	5	1			25	87
SpStSuTe									1									1
SpStSuTeTm														1				1
SpStSuTm										1	1		2	1				5
StSu		3														1		4
StSuTe									1					1				2
SuTeTm				1														1
SuTm							2											2
Te						1		2										3
FS	16	6	8	9	24	6	12	8	4	7	3	9	2	2	1			117
Grand Total	20	17	10	93	144	235	268	412	503	300	215	149	145	89	80	45	73	2798
Types	5	4	3	10	6	7	13	9	17	12	12	13	8	12	14	7	5	

Table 4.4 The imported ST DT104 R-types by year, Scotland, 1988-2004

	Year																	
R-Type	**1988**	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**	**2004**	**Total**
ApClSuTe	1																	1
ApClFzKaNaSpStSuTeTmLc											1							1
ApClNaSpStSuTe							1			1		1	2					5
ApClNaSpStSuTe													1					1
ApClNaSpStSuTeLc										2					1	2	1	6
ApClNaSpStSuTeTm											1							1
ApClSpStSuTe		6	2	5	14	8	23	11	6	6	7	4	2	11	2		5	112
ApClSpStSuTeTm		1						1	1									3
ApClStSuTe	1																	1
FzTe									1									1
NaSpStSuLc													1					1
SpStSu					1		1				1							3
SpStSuTm													1					1
Fully Sensitive	8	5			2		2		1		1				1			20
Total	10	12	2	5	17	8	27	12	9	9	11	5	7	11	4	2	6	157
Types	3	3	1	1	3	1	4	2	4	3	5	2	5	1	3	1	2	14

Table 4.5 shows the resistance to each single antibiotic each year. According to the resistance percentage (R%) of each antibiotic, the 15 antibiotics can be roughly divided into three groups. The first group (Group 1) coutained Ap, Cl, Sp, St, Su, and Te. The second group (Group 2) contained Tm, Lc and Na. The other drugs, Ka, Fz, Cp, Gm, Ne and Cf were allocated into the third group (Group 3). The temporal distribution of resistance to each in each group is very similar. Group 1 were antibiotics to which resistance was common. Case numbers all went up sharply from 1991 and reached a peak in 1996. In contrast, 13.2%, 7.6% and 6.7% of isolates were resistant to Tm, Na and Lc, respectively. Na resistance was first discovered in 1991. The percentages resistant in Group 3 antibiotics were lower than 1%.

Figure 4.2 shows the cumulative percentage for each antibiotic resistance throughout the study period. The resistances of Ap, Cl, Sp, St, Su and Te had very similar trends. The cumulative numbers all rose sharply from 1991. From 1990 to 1999, the cumulative numbers for the isolates resistant to Group 1 antibiotics increased four fold. From 1992 to 1993, there was a two-fold increase. The cumulative trend of the increase in Tm and Na resistances increased after 1994, reducing again after 1998.

Table 4.5 The numbers of ST DT104 isolates resistant to each antibiotic in each year, Scotland, 1988-2004

Year	Ap	Cl	Sp	St	Su	Te	Tm	Na	Lc	Ka	Fz	Cp	Gm	Ne	Cf	Total
1988	4	3	N	2	4	3	0	N	N	0	N	N	0	N	N	20
1989	8	7	7	11	11	8	0	0	N	0	N	N	0	0	N	17
1990	2	2	1	2	2	2	0	0	N	0	0	0	0	0	N	10
1991	80	79	81	80	83	80	5	1	N	0	1	0	0	0	N	93
1992	120	118	118	119	120	119	2	0	N	0	1	0	0	0	N	144
1993	228	220	221	222	228	221	2	0	N	0	0	0	0	0	N	235
1994	249	242	246	248	256	242	14	6	N	2	1	0	1	0	N	268
1995	393	382	392	391	402	385	66	23	N	0	1	0	0	0	N	412
1996	467	462	489	490	497	464	77	67	N	3	1	0	0	0	N	503
1997	288	287	292	292	293	287	45	37	14a	9	0	1	1	1	N	300
1998	204	203	211	211	212	203	30	28	15	3	0	0	0	0	0b	215
1999	135	131	139	139	139	132	17	13	11	1	0	3	0	0	0	149
2000	135	132	140	140	143	132	18	10	6	0	0	0	0	0	0	145
2001	81	76	81	82	85	78	40	8	8	0	0	0	0	0	0	89
2002	79	68	64	69	78	68	35	4	4	5	1	0	0	0	0	80
2003	44	41	42	43	44	42	11	1	1	0	0	0	0	0	0	45
2004	48	45	70	70	73	45	8	12	12	0	0	0	0	0	0	73
TotalR	2565	2498	2594	2611	2670	2511	370	210	71	23	6	4	2	1	0	2798
TotalS	233	300	184	287	128	287	2428	2568	995	2775	2755	2757	2796	2777	687	
R%	91.7	89.3	93.4	90.1	95.4	89.7	13.2	7.6	6.66	0.82	0.22	0.14	0.07	0.04	0	

N: not done.

a: 30 isolates were not tested.

b: 109 isolates were not tested.

Total R: The numbers of all resistant isolates

Total S: The numbers of all sensitive isolates

R%: resistance percentage

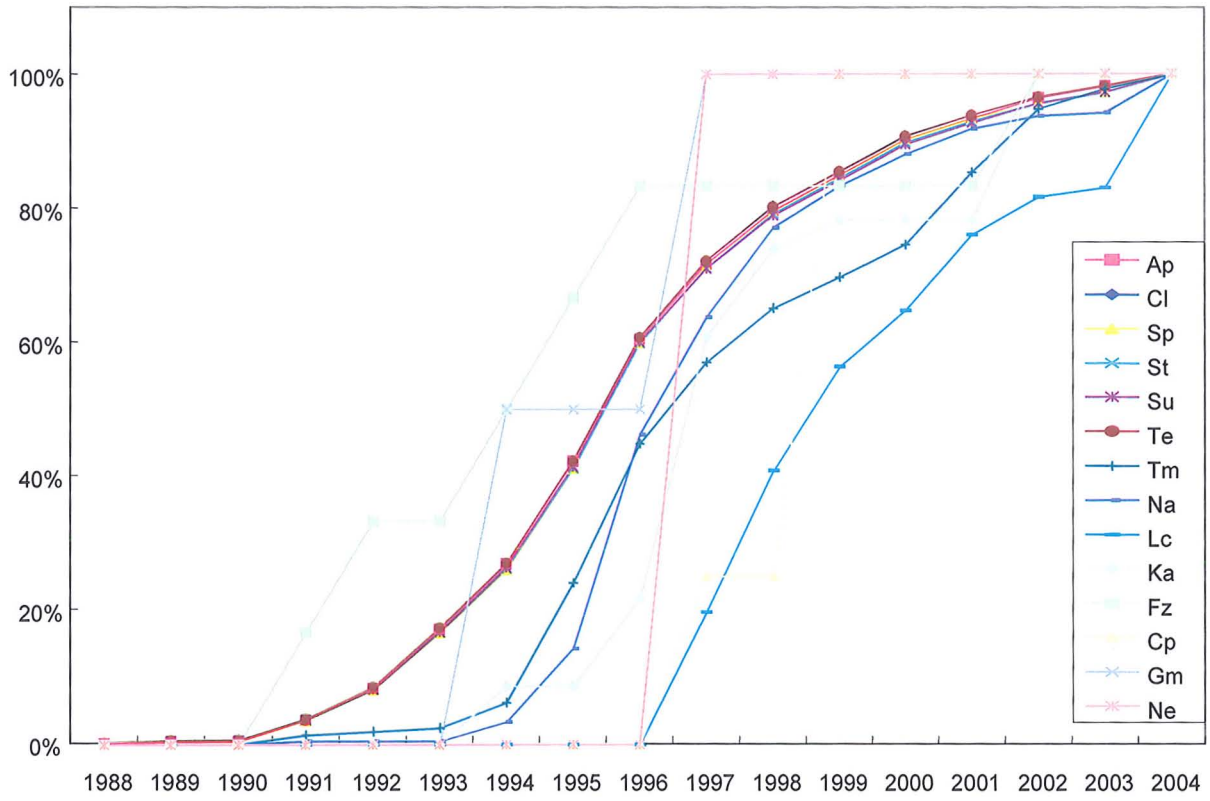


Figure 4.2 The cumulative percentages of ST DT104 isolates resistant to each antibiotic every year, Scotland, 1988-2004

4.4 DISCUSSION

The aim of this chapter was to set the emergence of resistance in the context of the epidemiological trends described in the last chapter, as well as highlighting that when considered as a whole, the resistant population itself is heterogenous. The factors related to the expression of antibiotic resistance patterns are complex. The phenotype diversities in a *Salmonella* serotype under the same antibiotic exposure experience have been documented (Davison et al. 2005). Nosocomial *Salmonella* outbreaks related to antibiotic treatment have also been reported (Olsen et al. 2001; Stephen et al. 2003). The fitness of the selected

population of micro-organisms in competition with others enhances the survival ability of multi-resistance strains (Dick 2003). External factors are therefore well recognised as playing an important role in the evolution of antibiotic resistance patterns in different regions. It is apparent that two neighbouring countries may present different distribution of MR ST DT104 (Helms et al. 2005). In England and Wales, 95% of ST DT104 were resistant to ApClSpStSuTe in 1996 (Helms et al. 2005), but in Scotland, it was 72% only. In other European countries the percentage of MR ST DT104 increased from 1994 to 2001 (Helms et al. 2005), a trend not reflected in Scotland. Nevertheless, for the fully sensitive R-type, the numbers reported have fallen everywhere (Helms et al. 2005).

In England and Wales, the most common R-type of ST DT104 historically has been the penta-form ApClStSuTe R-type, which was first reported in 1988 (Threlfall 2000). It is worthy of note that spectinomycin was not present in the test panel at that time. The present study has shown that the hexa-form, ApClSpStSuTe R-type, was the most common R-type in Scotland. In the following chapters the label ApClStSuTe, will be used. It is commonly abbreviated to ACSSuT because there was another ApClSpStSuTe R-type in the Scottish dataset. The first imported ApClSpStSuTe R-type was isolated in 1991 from a traveller who went to Spain but it is believed that the hexa-form R-type might be Scottish in origin and that this clone has then spread to other countries (Threlfall 2000).

Isolates were tested for sensitivity to a panel of 15 treatments. The combination of R-types may therefore be up to 2^{15} in number. Only 52 R-types were identified. This may the case for two reasons. Firstly, the database may not have captured all R-types. Only one-third of salmonellosis in humans is reported officially (Wheeler et al. 1999). Secondly, the appearance of selected antibiotic resistances is highly related, such as the ACSSuT R-type (Threlfall 2000). The results suggested that the 15 antibitotics could be classified into 3 groups. This assumption will be analysed by hierarchical cluster analysis in Chapter 5.

There were two core resistance patterns - the SpStSu or ApClSpStSuTe – with one or other identified in almost all isolates. However there is a significant diversity and for all the predominance of the clone resistant to Ap, Cl, Sp, St, Su, and Te (Threlfall 2000), the current study suggests that the contention of Lawson et al., (2004) that is that earlier MR DT104 strains were more heterogeneous than those of today based on genotype (Lawson et al. 2004). This study was, however, based on phenotypic observations.

There are issues of concern: In 2002, of 1,168 isolates of MR ST DT104, 11% had trimethoprim resistance, 10% low-level ciprofloxacin resistance and 1% both, in the England and Wales data (Parry 2003). Similar patterns are seen among animal isolates in England and

Wales (Jones et al. 2002). In contrast, in the Scottish data, 35 out of 80 isolates in 2002 (44%) had trimethoprim resistance and, 4 (5%) isolates were resistant to low level ciprofloxacin.

In summary, there has been an increase in MR DT104 throughout the study period. The nature of the resistance is varied and it is clear that besides the emergence of resistance to individual antibiotics, there are permutations and combinations of these resistance types. Of significant note is the decline of the fully sensitive isolate. The following chapters will examine these phenotypes in more detail, will explore the broad "three group" categorisation of this chapter and attempt to relate the patterns here with animal isolate data, as well examining the distribution of key R-types in time and space. In particular, the timing of emergence of individual resistance will be commented on in Chapter 10.

CHAPTER V

HIERARCHICAL CLUSTER ANALYSIS OF RELATIONSHIPS AMONG ANTIBIOTIC RESISTANT *S.* TYPHIMURIUM DT104 IN HUMANS IN SCOTLAND (1990 –2004)

CHAPTER V

HIERARCHICAL CLUSTER ANALYSIS OF RELATIONSHIPS AMONG ANTIBIOTIC RESISTANT *S.* TYPHIMURIUM DT104 IN HUMANS IN SCOTLAND (1990 –2004)

5.1 INTRODUCTION

Salmonella Typhimurium definitive type 104 (ST DT104) infection was first identified in 1952 but it became of major concern after the chromosomal encoded antibiotic resistant strain was identified by Threlfall (Threlfall 2000). After the discovery, there were significant increases in numbers of ST DT104 isolates as a proportion of *Salmonella* spp. It was believed that the phenomenon was directly related to the spread of this strain (Threlfall 2000; Threlfall et al. 1994; Malorny et al. 2001; Prager et al. 1999); however, many different resistant phenotypes were identified by the Scottish Salmonella Reference Laboratory (SSRL). Recently, more and more different genotypes with similar resistance phenotypes have been identified (Yang et al. 2002; Threlfall et al. 2005). To date, 15 antibiotics have been used to identify the antibiotic resistant properties of each isolate in the SSRL (Browning et al. 2003). As mentioned in Chapter 4, 52 R-types were found in 2,798 isolates from the human submissions.

The expression of antibiotic resistance relates to many factors. The presence/absence of genes, the qualitative and quantitative level of expression (Palzkill 2001), and the level of gene mutations all influence the responses in the antibiotic resistance tests. When a cassette of genes are operated by one initial gene (*INT*), the genes in the cassette will be translated and transcribed together. For example, the SGI1 cassette identified in the genome of ST DT104 contains 5 antibiotic resistance genes which translated together. In these circumstances,

although one antibiotic was used for treatment, the bacteria will translate all the potential relative functional genes in this cassette.

The expression of antibiotic resistance relates to the previous antibiotic exposure experience of a clone of bacteria (Sanchez et al. 1997; Levy 1994). This experience relates to treatments and the usage of growth promoters. There are many different kinds of multi-drug efflux pumps, which can actively and non-specifically move antibiotics out from bacteria (Gillespie and Bamford 2003). If a bacterium has a previous antibiotic exposure experience, it may respond faster when a drug is used. again

In Chapter 4, similar R-types were grouped according to the patterns identified. From the previous results, responses to 13 antibiotics were allocated to three groups: high, medium and low resistant sub-groups. The low resistance drugs have not been reported as being integrated into the ST DT104 genome. The results in Chapter 4 showed that R-types may share basic resistance cores, e.g., the SpStSu or the ApClSpStSuTe resistance pattern.

Hierarchical cluster analysis is a useful tool for classifying cases of interest into groups according to the user's enquiry. It is a non-parameter method free from *a priori* assumptions that links samples into suitable groups according to the link method and the measured distance. K-means cluster analysis is effective in dealing with a very large datasets and can be used to validate hierarchical cluster analysis.

The aims of the study in this chapter were to use cluster analyses to identify the relationship among antibiotic resistant patterns in the human isolate R-types from 1990 to 2004.

5.2 MATERIAL AND METHODS

There are 15 treatments used in SSRL to identify ST DT104. However, the treatments were introduced in different years (Chapter 2). Hierarchical cluster analysis does not allow for any missing information in a case. Thus, to obtain a better profile of the relationships, it was necessary to maximise the case and variable numbers. Of the 15 treatments used, only 796 isolates collected from 1998 to 2004 were included. In contrast, there were 2,761 isolates collected from 1990 to 2004 available for analyses when 13 drugs were used (Table 4.3). Only 37 isolates recorded in 1988 and 1989 were excluded with R-types ApClSuTe and ApClStSuTe (Table 4.2). The 9 and 15 treatment hierarchical cluster analyses were also performed but were used for reference only. The cluster analysis, linkage methods, distance measurements, data presentation and K-means cluster analysis were all carried out as described in Chapter 2. The McNemar test was used to analyse whether the antibiotic resistance patterns are matched (SPSS Inc. 2006; Dohoo et al. 2003).

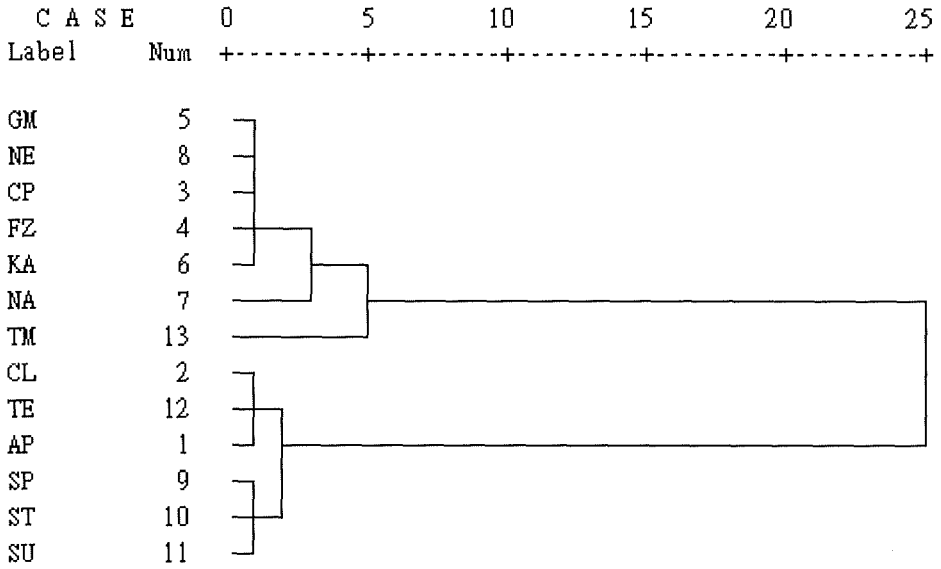
5.3 RESULTS

5.3.1 The relationships among the antibiotic resistance patterns

The 13 drugs were allocated into 2 distinct groups (Figure 5.1). The upper group was called Group H-A and the bottom group was called Group H-B. Group H-A was highly homogenous but had some minor diversities. Na and Tm are less similar to other Group H-A drugs. When the second line was set at cutting line 3/25, Group H-A was divided into group H-A1, H-A2, and group H-A2. Group H-A1 contained Gm, Ne, Cp, Fz and Ka. Group H-A2 contained Na and Group H-A3 contained Tm. In Group H-B, it was divided into 2 sub-groups: 1) Group H-B1 contained Ap, Cl and Te; 2) Group H-B2 contained Su, Sp and St. When 15 antibiotics

were put in the model, Cf was located in Group H-A1. Lc were located in Group H-A2 with Na. Tm was located in Group H-A3 but could be considered as part of group H-B.

Rescaled Distance Cluster Combination



Abbreviation: GM: Gentamicin, NE: Netilmicin, CP: Ciprofloxacin, FZ: Furazolidine, Ka: Kanamycin, Na: Nalidixic acid, TM: Trimethoprim, CL: Chloramphenicol, TE: Tetracycline, AP: Ampicillin, SP: Spectinomycin, ST: Streptomycin, SU: Sulphonamide.

Figure 5.1 Dendrogram constructed from the 13 antibiotic hierarchical cluster analysis using the 'between group' linkage method and the binary square Euclidean distance measurement.

In the K-means cluster analysis, with K set at 2, group 1 had Ap, Cl, Sp, St, Su and Te and the group 2 contained Cf, Cp, Fz, Gm, Ne, Ka, Na and Tm (with Lc). The result suggested that the cutting line used in the hierarchical analysis was suitable.

The dependence of variables was checked by the McNemar test (Dohoo 2003). The result showed that only six pairs of antibiotic resistances had similar responses. All of the drugs belonged to Group H-A and the drugs were not found to be highly resistant. There were 70, 1, 35, and 960 for Na + and Lc +, Na – and Lc +, Na + and Lc -, and Na – and Lc – isolates, respectively and as a consequence, the two were considered to be independent.

5.3.2 The relationships among R-types

Figure 5.2 shows 50 R-types aggregated into many groups. Three cutting sites were chosen based on the dendrogram. The first cutting site was chosen at the distance of 20/25. Thus, three groups were formed. They were named as Group A, B and C. The numbers and the resistance patterns are shown in Table 5.1. The name of each cluster was usually given according to the reference number of the first isolate in this R-type. Group A, B and C contained 29, 21 and 1 R-types, respectively. With only one isolate in Group C it could be assumed for practical purposes that there were 2 groups. This was confirmed by the K-means cluster analysis. The second cutting site was chosen at 15/25. Six clusters formed as follow: Group a, b, c, d, e, and f. Group a and b were listed in Group A and named as Group Aa and Ab. The third cutting site was chosen at 10/15 and then 10 groups were formed; 3 were in Group Aa, 2 were identified in Group Ab and 3 were found in Group B, respectively. Only Group B was divided to 2 clusters. The sub-groups were named from 1 to 8. Group C only had one isolate and did not contain any sub-group.

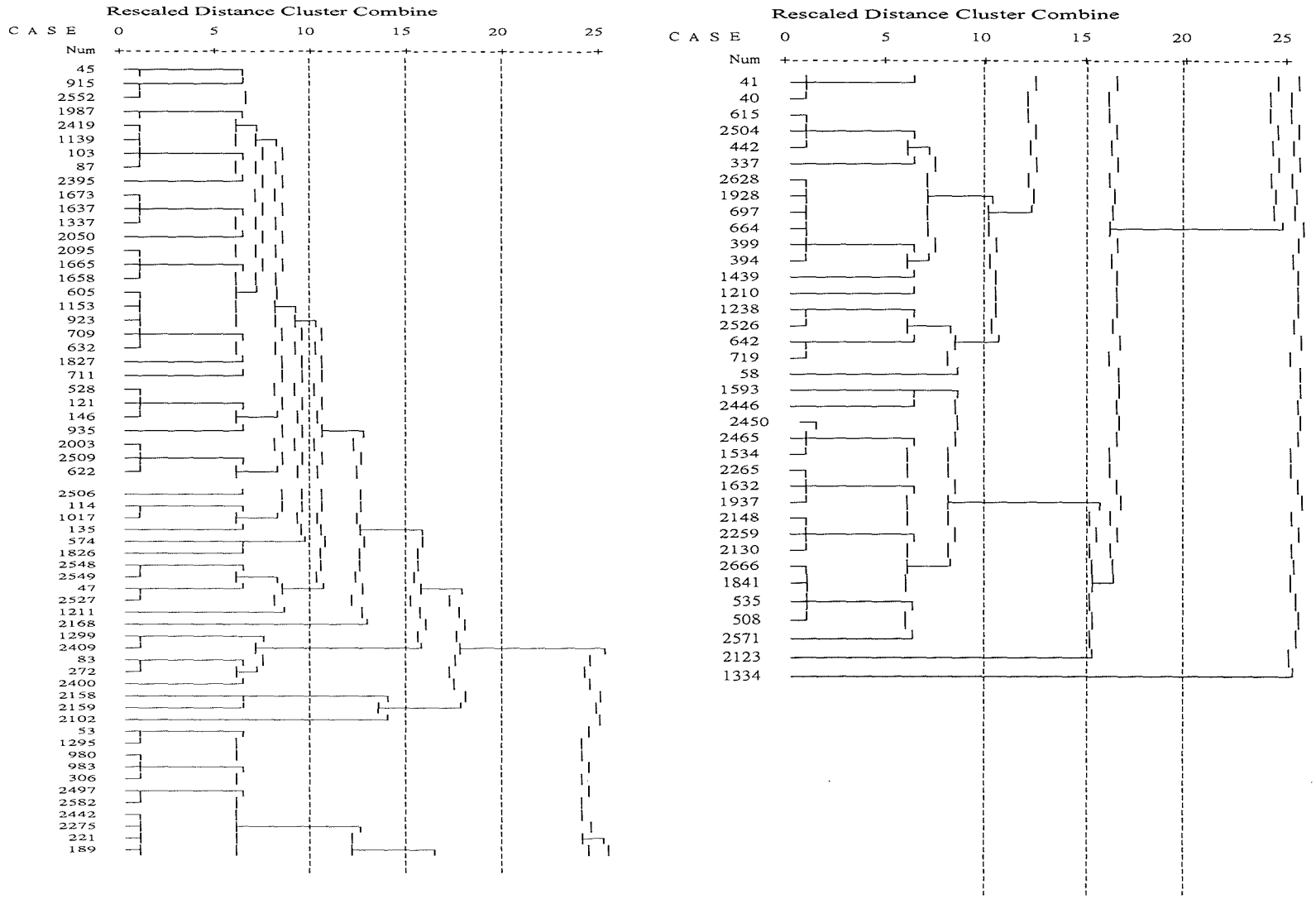


Figure 5.2 Dendrogram of the 13 antibiotic hierarchical cluster analysis from the human isolates between 1990 and 2004, which were constructed by the 'between groups' linkage method and the square Euclidean distance measurement.

Table 5.1 The hierarchical groups constructed by the human isolates between 1990 and 2004.

Id			No	R-type	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total
A	a	1	45	ApClSpStSuTe	0	7	1	72	115	218	221	292	322	214	155	107	107	30	26	29	25	**1941**
			915	ApSpStSuTe								1								1		**2**
			1987	ApClKaSpStSuTeTm										3	1				3			**7**
			52	ApClSpStSuTeTm	0	0	0	4	2	2	12	66	73	32	18	15	16	37	29	11	8	**325**
			2395	ApClSpStSuTm														1				**1**
			531	ApClKaNaSpStSuTe(Lc)							1		2	6								**9**
			2050	ApClKaNaSpStSuTeTmLC											1							**1**
			1708	ApClNaSpStSuTeTm(Lc)									1	9	9							**19**
			605	ApClNaSpStSuTe(Lc)	0	0	0	0	0	0	4	22	62	21	18	8	9	8	4	1	12	**169**
			1827	ApClCpNaSpStSuTe										1								**1**
			711	ApClNaStSuTe							1											**1**
			146	ApClFzSpStSuTe				1	1		1								1			**4**
			935	ApClFzNaSpStSuTe								1										**1**
			622	ApClKaSpStSuTe							1				1				1			**3**
			2506	ApClKaStSuTe															1			**1**
			114	ApClSpSuTe				1				1										**2**
			135	ApClSpSu				1														**1**
			574	ApClGmSpStSuTe							1											**1**
			1826	ApClGmNeSpStSuTe										1								**1**
			2548	ApClStSuTeTm															2			**2**
			18	ApClStSuTe	1		1												1			**3**
			1211	ApClSuTeTm									1									**1**

Table 5.1 (continued) the hierarchical groups constructed by the human isolates between 1990 and 2004.

Id		No	R-types	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total
			Total Aa1	1	7	2	79	118	220	242	383	461	287	203	130	132	76	68	42	45	2496
	2	2168	ApClSpSt												1						1
			TotalAa2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
	3	1299	StSuTe									1					1				2
		11	ApStSuTe	1	1		1	1													4
		2400	ApSuTe														1				1
			TotalAa3	1	1	0	1	1	0	0	0	1	0	0	0	0	2	0	0	0	7
			TotalAa	2	8	2	80	119	220	242	383	462	287	203	131	132	78	68	42	45	2504
	b	4	2158 ApCpNaSpStSuTeLc												1						1
			2519 ApCpSpStSuTe												1						1
			2102 ApCpNaSpStSuTmLc												1						1
			TotalAb	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	3
			TotalA	2	8	2	80	119	220	242	383	462	287	203	134	132	78	68	42	45	2507
B	c	5	53 Na				1					1									2
			306 Te						1		2										3
			2497 Ap															1	1		2
			38 Full sensitive	16	6	8	9	24	6	12	8	4	7	3	9	2	2	1	0	0	117
			TotalBc	16	6	8	10	24	7	12	10	5	7	3	9	2	2	2	1	0	124
	d	6	442 ApStSu						1	1								1			3
			337 ApSpStSu						1												1
			268 ApSu	0	0	0	0	1	6	6	10	3	1	1	1	3	4	8	1	3	48

Table 5.1 (continued) the hierarchical groups constructed by the human isolates between 1990 and 2004.

Id			No	R-type	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total
			1439	ApKaSu									1									1
			1210	ApNaSu									1									1
			1238	ApSuTm									1						1			2
			642	SuTm							2											2
			58	SuTeTm				1														1
				TotalBd	0	0	0	1	1	8	9	10	6	1	1	1	3	4	10	1	3	59
	e	7	1593	SpStSuTe									1									1
			2446	SpStSuTeTm														1				1
			1534	SpSt									1					2				3
			1632	SpStSuTm										1	1		2	1				5
			2148	NaSpStSu												2	1					3
			85	SpStSu	0	0	0	2	0	0	5	9	27	4	7	2	5	1	0	0	25	87
			22	StSu		3														1		4
				TotalBe7	0	3	0	2	0	0	5	9	29	5	8	4	8	5	0	1	25	104
		8	2123	KaNaSpStSuTmLc												1						1
				TotalBe8	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
				TotalBe	0	3	0	2	0	0	5	9	29	5	8	5	8	5	0	1	25	105
				TotalB	16	9	8	13	25	15	26	29	40	13	12	15	13	11	12	3	28	288
C	f	9	1334	ClFzSuTeTm									1									1
				TotalC	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
				TotalAll	18	17	10	93	144	235	268	412	503	300	215	149	145	89	80	45	73	2796

Group A contained 29 R-types and 2,506 isolates. Group B contained 20 R-types and 288 isolates. There were 3 sub-groups containing most of submissions and the 3 main groups were all located in Group A. They were Group No 45, No 52 and No 605. Basically, they share the same ApClSpStSuTe R-type structure. Group No 52 had an additional Tm resistance and Group 605 had an additional nalidixic acid resistance. There were 3 main sub-groups found in Group B as well. The cases in Group No 38 were sensitive to all treatments. Group No 268 was the ApSu R-type. Group No 85 was the SpStSu R-type. Basically, the isolates in Group Bd were all ampicillin sensitive. All isolates in Group 2 were resistant to Sp and St. Group Bc was sensitive to non-chromosomal related antibiotics. There were some differences between Group A and B. The main difference between Group A and B was the chloramphenicol resistance. Also, in Group B, the R-types were less resistant to Sp/St and Cp/Fz/Gm/Ka/Na. Many of the R-types in this group were sensitive to Ap. The dendrograms constructed from the 9 and 15 treatment hierarchical cluster analyses were similar.

Figure 5.3 shows the general trend was dominated by Group Aa, Ba, Bb, and Bc and their subordinate 6 R-types. Group No 45, the ApClSpStSuTe R-type, had the highest percentage from 1992. This R-type showed a very similar pattern corresponding to the pattern of all isolates throughout the study period. In contrast, the percentage of Group 38, the FS R-type, declined gradually. Group No 52, the ApClSpStTeTm R-type became a concern after 1991 and contributed about 40 per cent in 2001. Group 605, the ApClSpStSuTeNa R-type, became a concern after 1994 and had a surge in 2004. The major R-type in Group B was Group No 38, the FS R-type. Group No 268, the

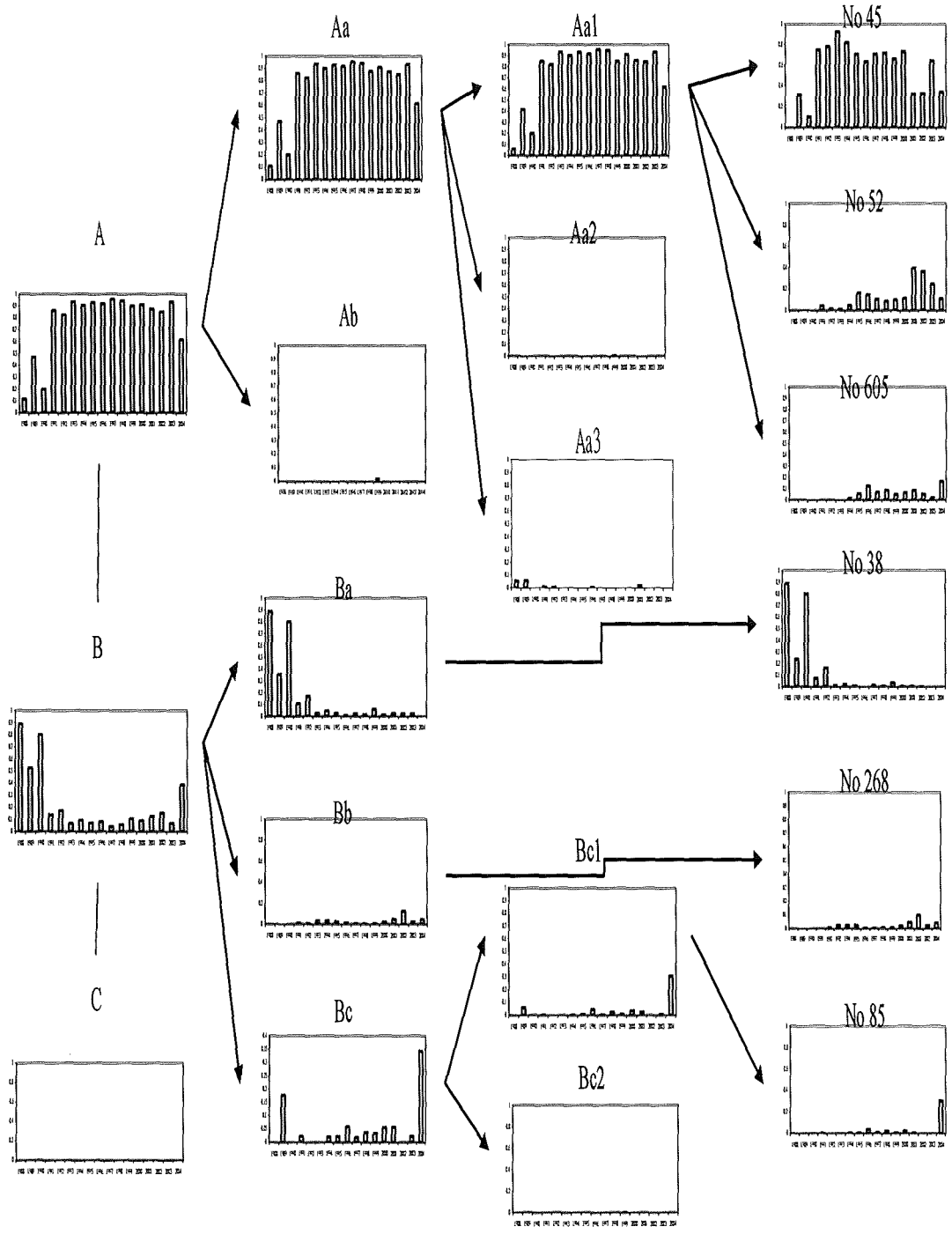


Figure 5.3 The percentages of groups, sub-groups, and R-types from 1988 to 2004; The Y-axis is the percentage ;the X-axis is year.

ApSu R-type and Group No 85, the ApStSu R-type had significant increases in 2003 and 2004, respectively.

5.4 DISCUSSION

This chapter has investigated the way in which the phenotypic expression of resistance occurs in ST DT104. In common with previous chapters the diversity and dynamic nature of resistance is evident, albeit that there are predominant R-types.

There were 50 R-types identified in the current study. The diversity of the R-types among ST DT104 isolates from the human dataset is high. However, they were also similarities and they can be grouped into two major distinct groups. Many R-types appeared and then disappeared. Threlfall reported they could be considered as variant forms caused by the mutation of the genome (Threlfall et al. 2003). Gene fragments lost or repeated may be caused by transposons during evolution, especially of those genes located on plasmids. Gene mapping of those few phenotypes would help to understand the relationship among phenotypes in each group.

The appearance of the additional Tm resistant isolates has been associated with the treatment of the ApClSpStSuTe R-type isolate infection in cattle by Tm (Threlfall et al. 1996) although it is not clear how much trimethoprim was prescribed for human treatments over the study period.

The emergence of quinolone resistance is an issue of concern. It is believed that the emergence of quinolone resistance is associated with use of enrofloxacin, a kind of

fluoroquinolone, for veterinary use from November 1993 in the UK (Threlfall et al. 1997). From this dataset, the first nalidixic acid resistant isolate identified in SSRL was in 1991, which was a Na R-type. Na resistance isolates were identified every year from 1994. There were 4 isolates identified as Cp resistant. All of them had the ApClSpStSu R-type. There were also 71 isolates identified as Lc resistant from 1997, when the test was introduced. The quinolone resistant mechanism is associated with the mutation(s) in *gyr*A and with the mutation in *gyr*B to get the full resistance to fluroquinolone (Aarestrup et al. 2003). In order to investigate further the ciprofloxacin resistance, there have been suggestions that the breakpoint of ciprofloxacin resistance should be reduced from 4 mg/L to 0.125 mg/L (Aarestrup et al. 2003; Threlfall et al. 1997). The low concentration ciprofloxacin (0.125mg/L) is used to compare with the MIC level of nalidixic acid (Aarestrup et al. 2003). There were 4 out of 2,761 Cp resistant isolates but 71 out of 1,066 isolates were resistant to low level ciprofloxacin. From the current data, the dependent relationship between the two drugs could not be established.

In summary the cluster analysis suggests that there are a number of phenotypes that are worthy of consideration when investigating the emergence of resistance. How these phenotypes relate to each other at the molecular level is a matter for speculation and how they relate to isolates from veterinary sources is an important next step in the investigation.

CHAPTER VI

TEMPORAL, SPATIAL AND SPATIO-TEMPORAL ANALYSES OF ST DT 104 HUMAN ISOLATES, 1988 TO 2004

CHAPTER VI

TEMPORAL, SPATIAL AND SPATIO-TEMPORAL ANALYSES OF ST DT 104 HUMAN ISOLATES, 1988 TO 2004

6.1 INTRODUCTION

The ApClSpStSuTe ST DT104 R-type is the main antibiotic resistance phenotype identified world-wide since it was first described (Threlfall 2000). However, the distributions of other variants have not been similarly addressed. The different ST DT104 R-types were investigated in Chapter 5 and the results suggested that the R-types could be allocated into two main groups. Group A contained many multi-resistant R-types. Group B contained those R-types which were more sensitive to the 13 antibiotics used at the SSRL. However, the distributions of these different groups in time, space and time and space have not been determined.

SaTScan is a program developed by Kulldorff in 1996 (Kulldorff 1997). It detects the most likely clusters in time, space and time-space by a method that compares the likelihood of circles chosen randomly and then lists the highest possible circle by order (Kulldorff 2006; Kulldorff 1997). It is a powerful tool that can be directed at identifying the most likely distributions of two groups and their subgroups. The program uses two models to approach the issue. Firstly, adopting assumptions associated with the Poisson distribution, the relative risk of a target group in time, space, or time-space can be compared with the general population in the same zone. Secondly, adopting a Bernoulli approach, a case-control analysis can be performed. The model compares the distributions of 'case' and the 'control' groups and then the results are presented as the location of the most likely high/low cluster of the

'case' group. The aim of this study was to discover the most-likely temporal, spatial, and spatio-temporal clusters for: 1) *Salmonella* submissions 2) *S.* Typhimurium submissions, 3) ST DT104 submissions, 2) Group A submissions, 3) Group B submissions, 4) Group Hex submissions and 5) Group FS submissions. The Group A, Group B, Group Hex and Group FS were as defined in Chapter 5.

6.2 MATERIAL AND METHODS

In this study, retrospective analyses based on Poisson and Bernoulli distributions were used to investigate the temporal, spatial, and spatio-temporal clusters in the population. The study was implemented at two different geographical levels: the NHS board and the postal district levels. The assumption was that the expected clusters would have the same or similar positions if the variance within each Health Board was low, i.e., were occurring randomly in each board. According to the requirements of SaTScan, three different datasets, in text format, were used. They were: 1) the population dataset, which supplied the population in space over time; 2) the co-ordinate dataset, which supplied the information about the grid position of the locations; 3) the case and control dataset, which included when, where and how many cases occurred. A duration of 1 year was used as the basic unit of analysis. In the Poisson model, only case, co-ordinate, and population datasets were required. In the Bernoulli model, a control rather than the population dataset was required.

The population data were originally from the 2001 Scottish census data. Comparison of the 1991 and 2001 censuses showed that the Scottish population was relatively stable (General Register Office (Scotland) 2002; General Register Office (Scotland) 1991), and for the purposes of this study, it was assumed that the population in each study zone did not change

between 1988 and 2004. The population dataset was presented in a x, y, z format, in which x was location, y was the corresponding population and z was the time when the cases occurred. The original dataset provided the population in 15 NHS boards (Chapter 2). In contrast, the population in each postal district needed further calculation. The basic unit in a census is called a 'census tract'. Generally, a unit is the same as a postal district; however, because of the limitation of natural borders, some postal districts are divided into two census tracts. Twenty-six such census tracts were merged into 13 postal districts using MapInfo.

SaTScan does not use the area of each zone in calculating clusters; instead, it uses the centroid of each zone. The latitude and longitude coordinate system was used in this study. The format of the coordinate dataset is also a (x, y, z) system, in which x is the name of the place, y is latitude, and z is longitude. MapInfo was used to calculate the centroid of each NHS board and then the locations were exported to a text format file. All names and locations of the NHS boards were described in Chapter 2.

The study subject was the Home Cases, which was defined as cases reported as occurring in the UK without a history of travelling abroad in the previous three months (Chapter 2). The cases were sorted, matched, and stacked according to their corresponding time and locations in Excel 2000. Each submission contained the Health Board of the case reported, i.e., the place of the case. In the case and the control datasets, the case numbers in each postal district were counted. Four isolates were recorded as being in England and 127 of 2,798 had no location recorded and were defined as "missing".

All *Salmonella*, ST, ST DT104, the ST DT104 ApClSpSStuTe R-type (named as Group Hex), ST DT104 FS type (named as Group FS), the ST DT104 Group A (named as Group A), and ST DT104 Group B (named as Group B) isolates were analysed separately.

Clusters identified in the Bernoulli model at the postal district level were listed according to the rank given from the model.

6.3 RESULTS

6.3.1 ST DT104 infections in Scotland from 1988 to 2004

Figure 6.1 shows all the cases with their corresponding postal districts in Scotland. The central belt of the Scotland is shown enlarged in Figure 6.2.

Figure 6.3 shows the cumulative incidences in each postal district. Generally, the cumulative incidences were higher in the southern and eastern parts of Scotland. The cumulative incidences in the outer islands and the central belts were also high.

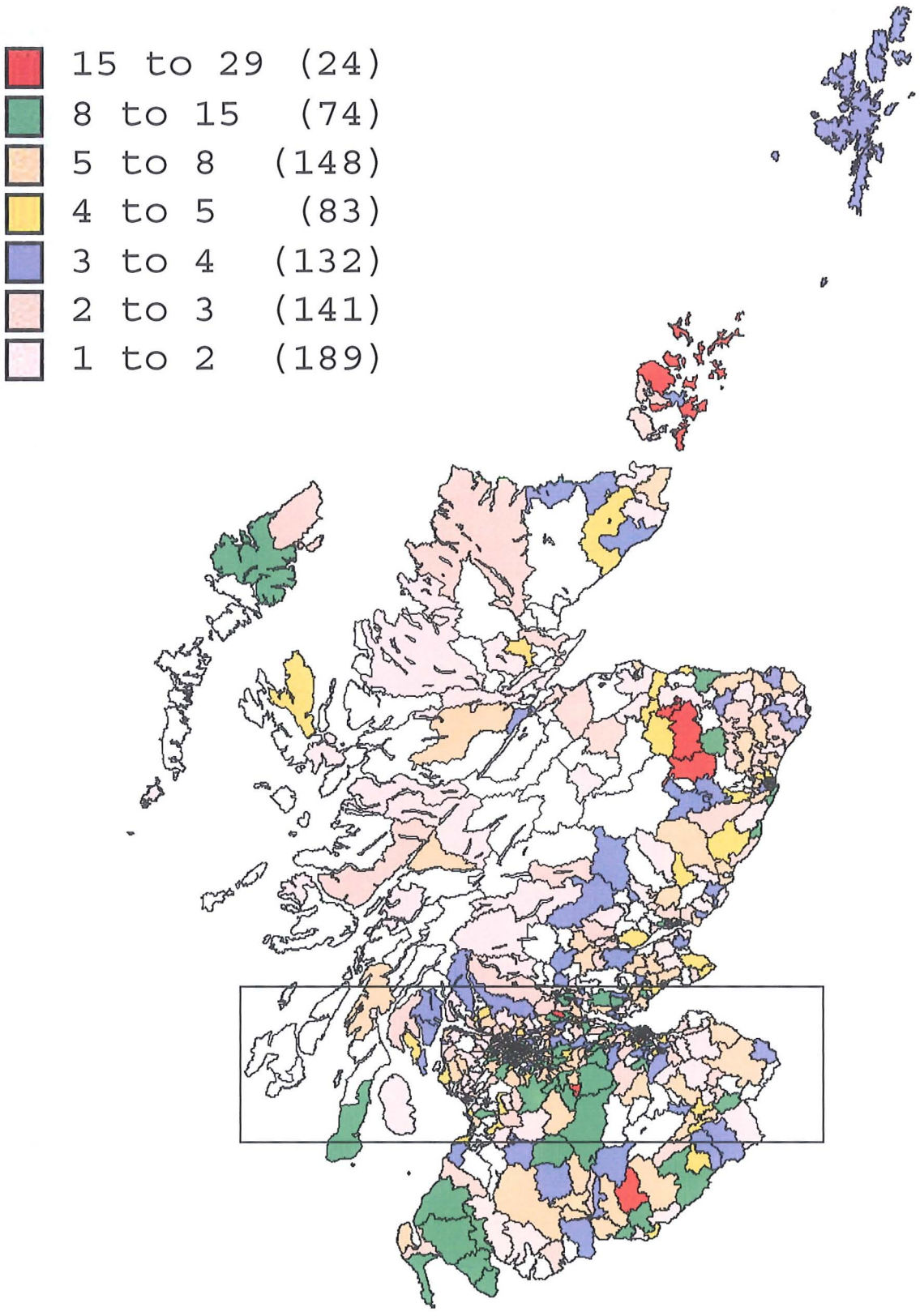


Figure 6.1 The case numbers of ST DT104 in each postal district in Scotland (1988 to 2004); blanks spaces are areas with no cases. The numbers of postal districts belonging to the group were shown in the brackets. The central belt of Scotland in the box is enlarged in Figure 6.2

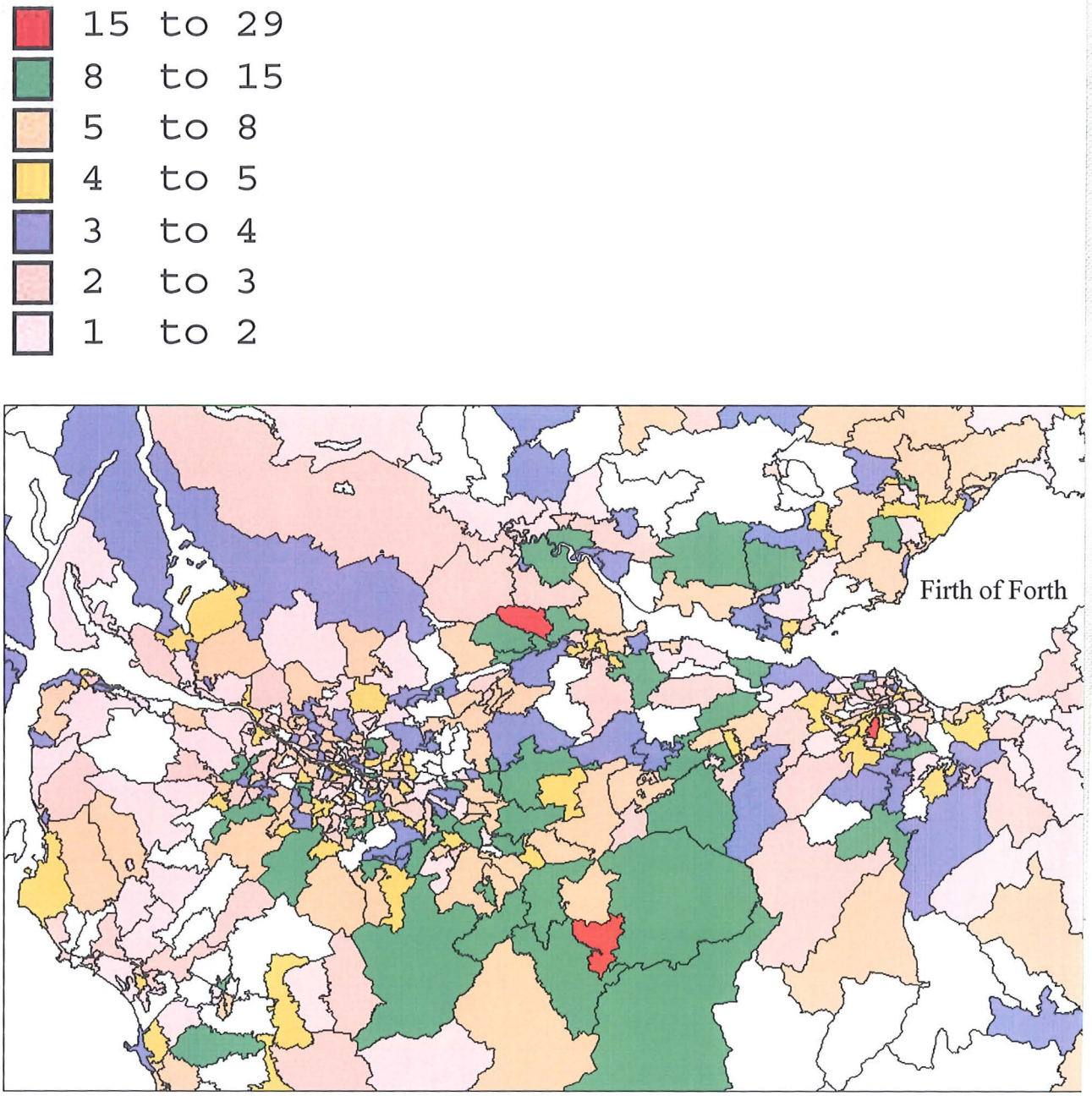


Figure 6.2 The case numbers of ST DT104 in each postal district in the central belt of Scotland (1988 to 2004) shown in detail. The blank areas do not have any case.

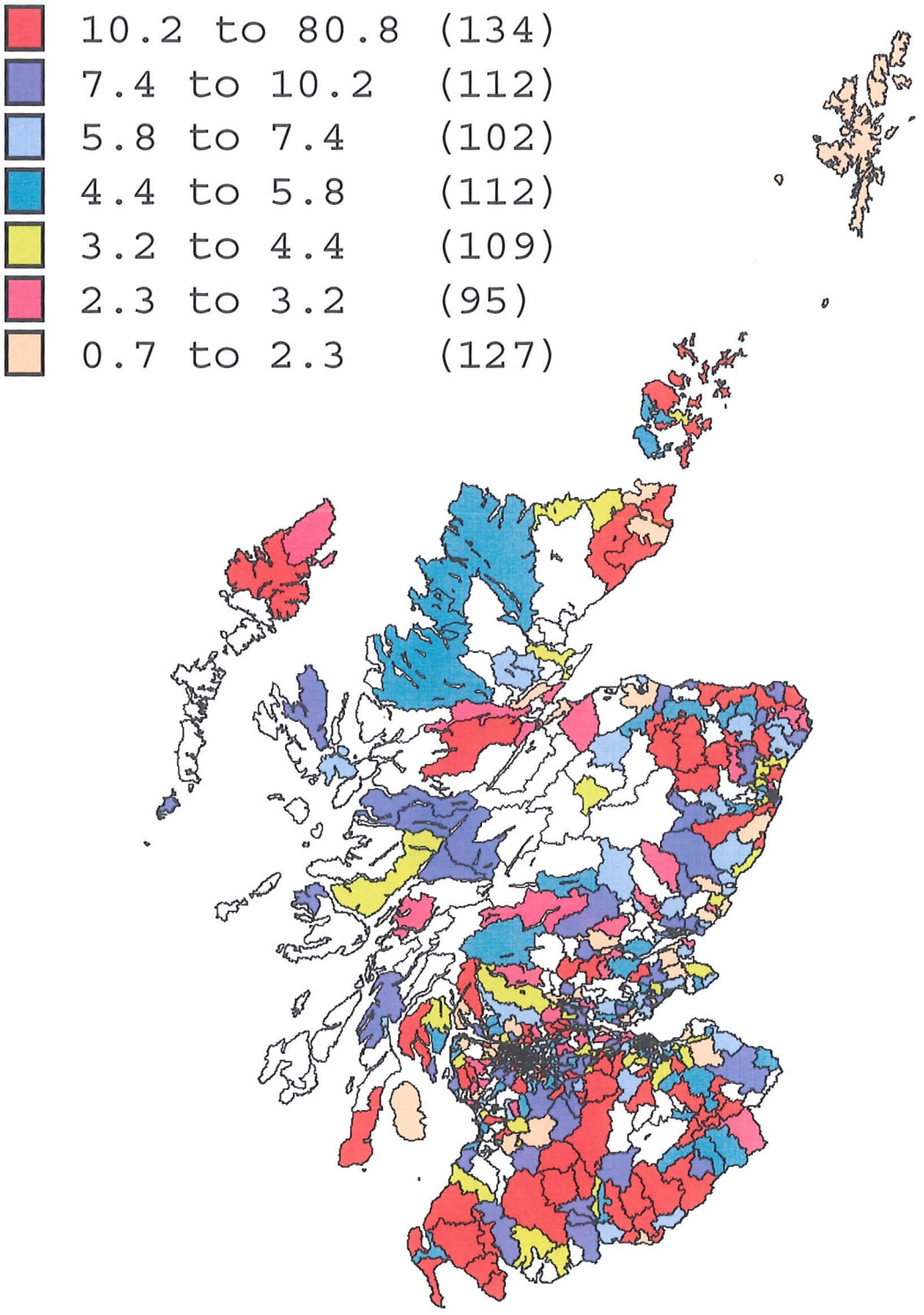


Figure 6.3 **The cumulative incidence in each postal district in Scotland from 1988 to 2004;** the blanks are the areas no any case. The numbers of postal districts belonging to the group were shown in the brackets.

6.3.2 Results of temporal analysis based on the Poisson model

The purely temporal cluster analysis was performed to detect the temporal clusters from the study groups. Table 2.2 shows the results from each group in the purely temporal study. In the period from 1998 to 2004, Group *Salmonella* and Group ST had a temporal lower risk cluster (TLRC). Group ST DT104 and Group A had a temporal higher risk cluster (THRC) in the period from 1993 to 1998. Group B had a THRC in the period 1994 to 1996. Group Hex had a THRC during the period from 1992 to 1999. The TLRC from Group FS was during 2000 to 2004.

Table 6.1 The most likely temporal clusters from Group *Salmonella*, Group ST, and its subgroups from 1988 to 2004.

Groups	Relative Risk	p-value	Period
Salmonella	0.411	0.001	1998-2004
S. Typhimurium	0.431	0.001	1998-2004
ST DT104	4.106	0.001	1993-98
Group A	4.639	0.001	1993-98
Group B	2.261	0.001	1994-96
Group Hex	6.275	0.001	1992-1999
Group FS	0.107	0.001	2000-04

6.3.3 Results of the spatial cluster analysis based on the Poisson model at the NHS board level

The purely spatial cluster analysis was performed to detect the spatial clusters from each study group. Table 6.2 shows the most-likely clusters in each study group.

Three spatial higher risk clusters (SHRCs) for Group Salmonella were identified in DG, GR, and LN. Two SHRCs were located in the southern part of Scotland and other SHRC was located in the East. Only two SHRCs were identified in Group ST. These clusters covered the

DG and GR Health Boards, respectively. The STRC from Group *Salmonella* and Group ST were the same. Two spatial lower risk clusters (SLRCs) were identified in LO and FF and AC, GG, FV and AA. The two circles were located in the eastern and western sides of the central belt.

Three SHRCs were identified for Group ST DT104. They were located in the DG, the GR, and the LN Health Boards. The locations were the same as the SHRCs identified from Group Salmonella. Three SLRCs from Group ST DT104 were found in the FF, LO and TY, the AC, GG, FV and AA, and the HG Health Boards. The first two circles were the same as those identified in Group Salmonella and Group ST.

The isolates in Group A, Group B, Group Hex and Group FS were allocated as described in Chapter 5. Three SHRCs were identified for Group A. The clusters were located in the DG; the SH, OR, and GR; and the LN Health Boards. The first circle was in the southern part of Scotland and the second circle, which included the outside islands, was located in the western part of Scotland. The third circle was located in the middle of the southern part of Scotland. Group A also had 3 SLRCs located around the AC, GG, FV, and AA Health Boards; around the FF, LO and TY Health Boards; and in the HG Health Board. The three SLRCs covered the northern part of Scotland. No SHRC for Group B were found but an SLRC was located in the circle of the WI, HG, OR, GR, TY, and AC Health Boards. The circle covered the northern part of Scotland. The most-likely clusters for Group Hex showed four SHRCs located in the DG; the LN; the OR; and the GR Health Boards. In addition, two SLRCs were located in the AC, GG, FV, and AA; and the LO Health Boards. There was also a higher rank SLRC identified in the HG Health Board with a p-value of 0.059. However, no significant SHRC or SLRC was identified for Group FS.

Table 6.2 The most likely spatial clusters from the Group *Salmonella* Group ST, and its subgroups, Scotland, 1988 - 2004.

Groups	Relative Risk	P-value
Salmonella		
DG	1.963	0.001
GR	1.552	0.001
LN	1.272	0.001
LO, and FF	0.885	0.002
AC, GG, FV, and AA	0.740	0.001
Salmonella Typhimurium		
DG	1.850	0.001
GR	1.601	0.001
AC, GG, FV, and AA	0.728	0.001
LO and FF	0.924	0.1
ST DT104		
DG	2.560	0.001
LN	1.274	0.001
GR	1.257	0.003
FF, LO and TY	0.842	0.003
AC, GG, FV, and AA	0.810	0.001
HG	0.596	0.001
GroupA		
DG	2.725	0.001
SH, OR, and GR	1.323	0.001
LN	1.298	0.002
FF, LO, and TY	0.814	0.001
AC, GG, FV, and AA	0.785	0.001
HG	0.618	0.004
GroupB		
WI, HG, OR, GR, TY, and AC	0.621	0.017
Group Hex		
DG	3.256	0.001
OR	2.592	0.023
LN	1.331	0.003
GR	1.264	0.038
LO	0.740	0.002
AC, GG, FV, and AA	0.736	0.001
HG	0.665	0.059
Group FS		
Purely Spatial	NONE	

6.3.4 Spatio-temporal cluster analysis based on the Poisson model at the NHS board level

Spatio-temporal cluster analysis based on a Poisson model was performed to detect possible clusters from the study groups relating to the corresponding areas over time. Table 6.3 shows the spatio-temporal clusters (STCs) from the study groups. The identified STCs were the same in both Group *Salmonella* and Group ST. The spatio-temporal higher risk cluster (STHRC) was found in the circle including the SH, OR and GR Health Boards in the period from 1990 to 1997. The spatio-temporal lower risk cluster (STLRC) was found in the circle of the AC, GG, FV, AA and LN Health Boards between 1999 and 2004. No STHRC was identified from Group ST, but a STLRC was located in the circle of the AC, GG, FV, AA, and LN Health Boards in the period 1991 to 2004. When the study focused on mainland Scotland only, Group Salmonella and Group ST generated different clusters. The two STLRCs for Group Salmonella were located in the HG, GR, TY, FV, FF and AC Health Boards between 1999 and 2004; and the DG, AA, LN, BR, and GG Health Boards between 2001 and 04.

There was one STHRC for Group ST DT104 located in the circle of the WI, HG, OR, GR, TY, AC, and FV Health Boards in the period from 1995 to 97. The circle covered the northern part of Scotland. The other STHRC from Group ST DT104 was located in the circle of the BR, LO, LN, FF, and DG Health Boards in the period from 1993 to 98. The circle covered the middle and southern parts of Scotland.

The spatio-temporal cluster analysis for Group A showed the same clusters as those identified in Group ST DT104. There were two STHRCs for Group Hex. The first STHRC covered the DG, AA, LN, BR, and GG Health Boards between 1993 and 98. The cluster covered the

Table 6.3 The most likely spatio-temporal clusters from Group *Salmonella*, Group ST, and its subgroups, Scotland, 1988 - 2004.

Groups	Relative Risk	P-value	Period
Salmonella			
SH, OR, and GR	2.039	0.001	1990-97
AC, GG, FV, AA, and LN	0.432	0.001	1991-2004
Excluding Islands-temporal-spatial			
HG, GR, TY, FV, FF, and AC	0.497	0.001	1999-2004
DG, AA, LN, BR, and GG	0.484	0.001	2001-04
Salmonella Typhimurium			
SH, OR, and GR	2.069	0.001	1990-97
AC, GG, FV, AA and LN	0.444	0.001	1999-2004
Excluding Islands-temporal-spatial			
AC, GG, FV, AA, LN	0.431	0.001	1991-2004
ST DT104			
WI, HG, OR, GR, TY, AC, FV	3.043	0.001	1995-97
BR, LO, LN, FF and DG	2.691	0.001	1993-98
GroupA			
BR, LO, LN, FF, and DG	2.841	0.001	1993-98
WI, HG, OR, GR, TY, AC, FV	3.210	0.001	1995-97
GroupB			
AA, LN, DG, GG, and AC	3.969	0.001	1996
GR, TY, FF, HG, and FV	0.258	0.001	1998-2003
Group Hex			
DG, AA, LN, BR, and GG	3.202	0.001	1993-98
HG, GR, WI, TY, FV, FF, OR, AC	2.797	0.001	1994-97
Group FS			
LO, FF, and BR	4.870	0.001	1988-92
GG, FV, LN, and AA	2.695	0.086	1991-94
WI, HG, OR, GR, TY, AC	0.069	0.016	1999-2004

middle and the southern part of Scotland. The second STHRC was located in the northern part of Scotland and covered the HG, GR, WI, TY, FV, FF, OR, and AC Health Boards between 1994 and 1997.

There was one identified STHRC for Group B which included AA, LN, DG, GG, and AC Health Boards in 1996. The circle covered the centre-western and the southern parts of Scotland. In contrast, a STLRC including the GR, TY, FF, HG, and FV Health Boards in the period from 1998 to 2003 was identified. The area covered the eastern and the northern parts

of Scotland. There was one STHRC and one STLRC identified for Group FS. The STHRC covered the LO, FF, and BR Health Boards between 1988 and 1992 and the STLRC WI, HG, OR, GR, TY, and AC Health Boards in the period 1999 to 2004.

6.3.5 Clusters from spatial cluster analysis based on the Poisson model at the postal district level

Table 6.4 shows the clusters from the different study groups. Clusters were named according to their group name and ranked order from the Bernoulli model. The SHRCs and the SLRCs in both Group ST DT104 and Group A had the same locations and radii. Three SHRCs and three SLRCs were identified from Group ST DT104 and Group A (Figure 6.4 and 6.5). The first SHRC, DT104all-1/Group A-1, had a centre located in the north-western side of Annan, DG, within a radius of approximately 93 km. The second SHRC, DT104all-2/GroupA-2/Hex-2, had a centre in Aberdeenshire, GR, with a radius of approximately 20 km. The third SHRC, DT104all-3/groupA-4, had a centre located in Lothian, with a radius of approximately 4.6 km. The first SLRC, DT104all-4/GroupA-3, had a centre located in the Western Isles (Figure 6.4) with a very large radius of approximately 245 km. The second SLRC, DT104all-5/GroupA-5, had a centre located in Fife, with a radius of approximately 48 km. The third SLRC, DT104all-6/GroupA-6, had a centre located in Greater Glasgow, with a radius of approximately 3 km.

There were three identified SHRCs and one SLRC for Group Hex. The first HRC identified from Group Hex was named Hex-1, whose centre was located in Dumfries and Galloway, and had a radius of 95 km. The centre and the radius were almost the same as those of DT104-1. The coverage area was in the southern part of Scotland. The second HRC from Group Hex, Hex-2,

Table 6.4 The most likely spatial clusters from Group ST DT104, and its subgroups, Scotland, 1988 – 2004; ordered in rank. The study was implemented at the postal district level.

Name	P-value	Location	Radius (km)	RR
Clusters from Group ST DT104				
DT104 all-1	0.001	55.004581 N, 3.319748 W	93.13	1.933
DT104 all-2	0.001	57.396759 N, 2.861479 W	20.28	4.917
DT104 all-3	0.001	56.002213 N, 3.895982 W	4.58	3.219
DT104 all-4	0.001	57.247871 N, 7.320584 W	244.82	0.759
DT104 all-5	0.001	56.217957 N, 2.913362 W	47.79	0.747
DT104 all-6	0.005	55.866852 N, 4.092675 W	2.82	0.061
Clusters from Group A				
Group A-1	0.001	55.004581 N, 3.319748 W	93.13	2.035
Group A-2	0.001	57.396759 N, 2.861479 W	20.28	5.338
Group A-5	0.001	56.002213 N, 3.895982 W	4.58	3.189
Group A-3	0.001	57.247871 N, 7.320584 W	244.82	0.682
Group A-4	0.001	56.217957 N, 2.913362 W	47.79	0.700
Group A-6	0.037	55.866852 N, 4.092675 W	2.82	0.068
Clusters from Group B				
Group B-1	0.001	57.267067 N, 3.414077 W	77.3	0.176
Group B-2	0.015	56.073265 N, 4.030589 W	15.76	2.860
Group B-3	0.027	55.880638 N, 3.159124 W	2.35	8.388
Group B-4	0.109	55.859253 N, 4.475159 W	0	11.518
Clusters from Group Hex				
Hex-1	0.001	55.001743 N, 3.195488 W	95.23	2.488
Hex-2	0.001	57.396759 N, 2.861479 W	20.28	6.15
Hex-4	0.039	59.069847 N, 3.177505 W	0	4.782
Hex-3	0.001	56.791744 N, 4.955959 W	125.57	0.656
Clusters from Group FS				
FS-1	0.003	55.832664 N, 4.348066 W	1.65	14.945
FS-2	0.003	55.900124 N, 3.144440 W	0	27.319
FS-3	0.069	55.265659 N, 2.816563 W	16.46	17.516

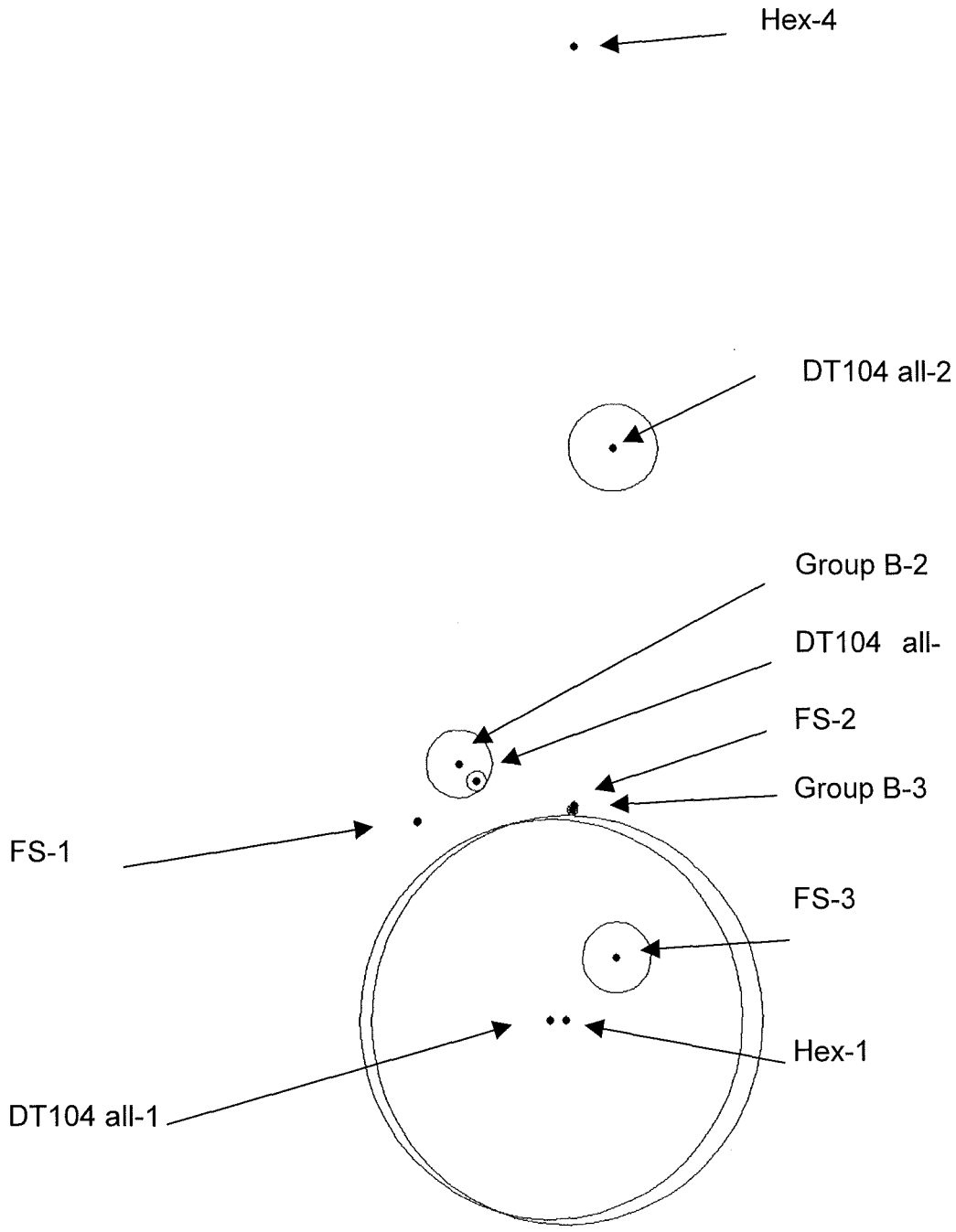


Figure 6.4 Spatial distribution of identified significant SHRCs of study groups using the maximum cluster size $\leq$ 50% of the total population in Scotland, 1988–2004.

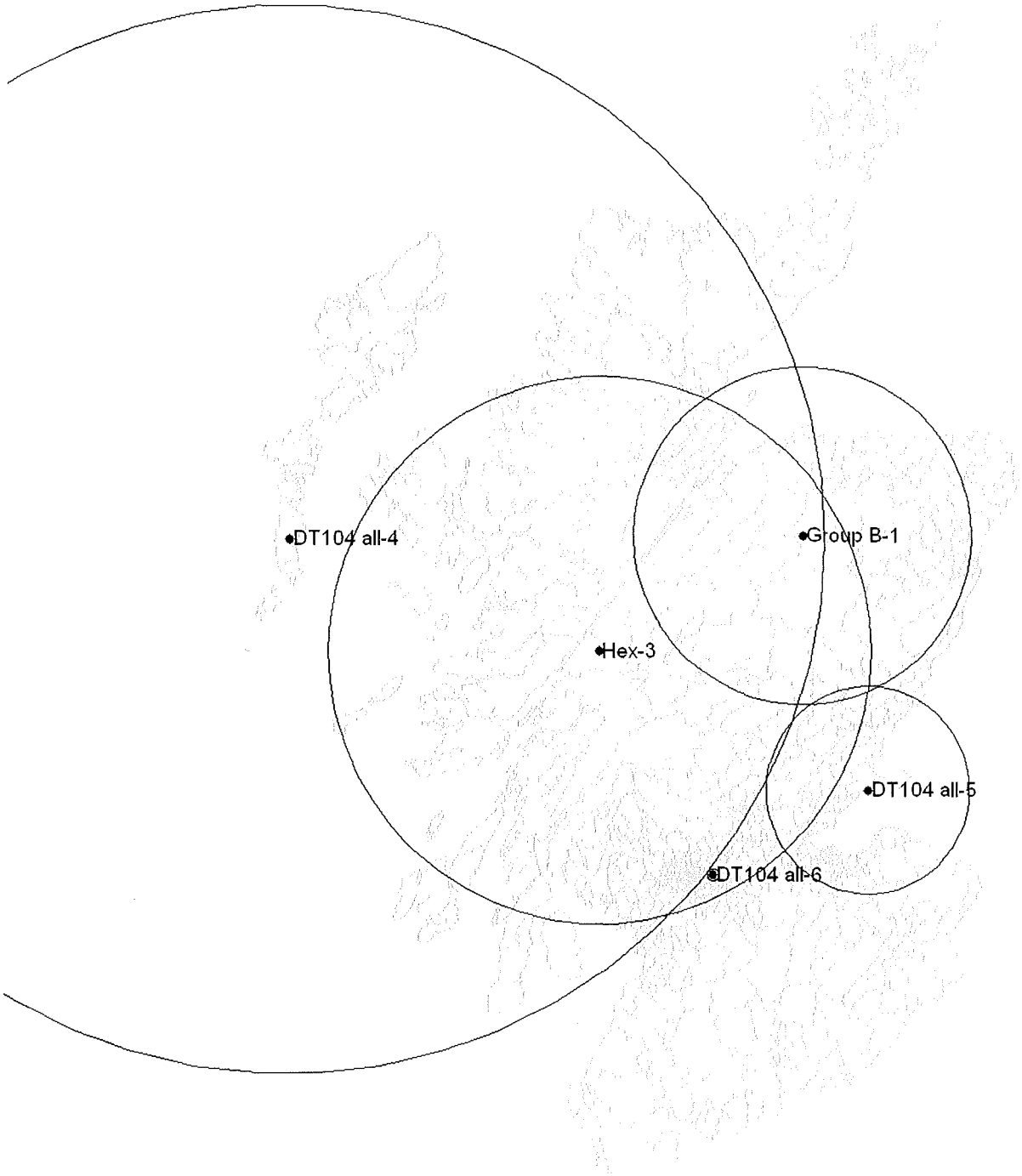


Figure 6.5 Spatial distribution of identified significant SLRCs of study groups using the maximum cluster size $\leqq$ 50% of the total population in Scotland, 1988 – 2004.

had the same position and radius as those of STDT104-2. The third HRC from Group Hex was called Hex-4 . Its centre was located in the Orkneys.

There were two SHRCs and one SLRC for Group B (Figure 6.4). The first SHRC from Group B was called Group B-2. The centre was close to Shielbrea, FV within a radius of approximately 16 km. The second SHRC from Group B was called Group B-3. The centre was in Lothian. The two SHRCs had small radii, about 2 km, which means that the clusters were highly aggregated. The SLRC from Group B was called GroupB-1. The centre was located in Grampian, within a radius of approximately 77 km.

There were two SHRCs identified from Group FS. The first one was FS-1 and had the same centre as FST-1 and Group BT-2. The point was located in the G53 postal district, south-east of Paisley, GG, within a radius of approximately 1.7 km. The centre of the second SHRC, FS-2/FST-2/Group BT-4, was located in the EH17 postal district, LO. There was one case submitted in 1989 and five cases were isolated in 1990.

6.3.6 Clusters from spatio-temporal cluster analysis based on the Poisson model at the postal district level

Table 6.5 shows the identified spatio-temporal clusters (STCs) from all study groups. There was only one STHRC identified from Group ST DT104 (DT104all-1). The location of the STHRC was centred around Hawick, Borders, from 1993 – 98 (Figure 6.6). The radius of this cluster is approximately 96 km. The centre of the identified STHRC from Group A (Group AT-1) was located between Edingham and Southwick, DG from 1993 to 98. Its subgroup, Group Hex, had two STHRCs identified. The first one was called HexT-1, with a

Table 6.5 The most likely temporal-spatial clusters from Group ST104 and its sub-groups from 1988 to 2004 at the postal district level; clusters in rank. The study was based on a Poisson model.

Name	P-value	Location	Radius	RR	Period
DT104 allT-1	0.001	55.510201 N, 2.839513 W	95.95	2.732	1993-98
Group AT-1	0.001	54.923302 N, 3.769124 W	116.87	2.897	1993-98
Group BT-1	0.001	55.859253 N, 4.475159 W	0.00	195.772	1996
Group BT-2	0.001	55.832664 N, 4.348066 W	1.65	87.115	1999
Group BT-3	0.001	56.144978 N, 3.849847 W	43.04	2.778	1992-96
Group BT-4	0.001	55.900124 N, 3.144440 W	0.00	152.965	1990
HexT-1	0.001	55.061913 N, 3.617562 W	99.59	3.290	1992-98
HexT-2	0.001	57.684399 N, 4.723242 W	197.99	2.815	1993-97
HexT100-1	0.001	55.503174N, 4.123856W	99.71	3.270	1993-98
HexT100-2	0.001	57.089546N, 2.410524W	75.14	4.020	1995-97
HexT100-3	0.001	58.602936N, 3.350581W	52.83	9.450	1994-97
HexT100-4	0.001	58.212681N, 6.378440W	23.51	27.706	1999
FST-1	0.001	55.832664 N, 4.348066 W	1.65	221.450	1999
FST-2	0.001	55.900124 N, 3.144440 W	0.00	384.518	1990
FST-3	0.005	55.265659 N, 2.816563 W	16.46	59.647	1988-92
FST-4	0.020	56.257370 N, 3.911094 W	47.97	7.775	1992

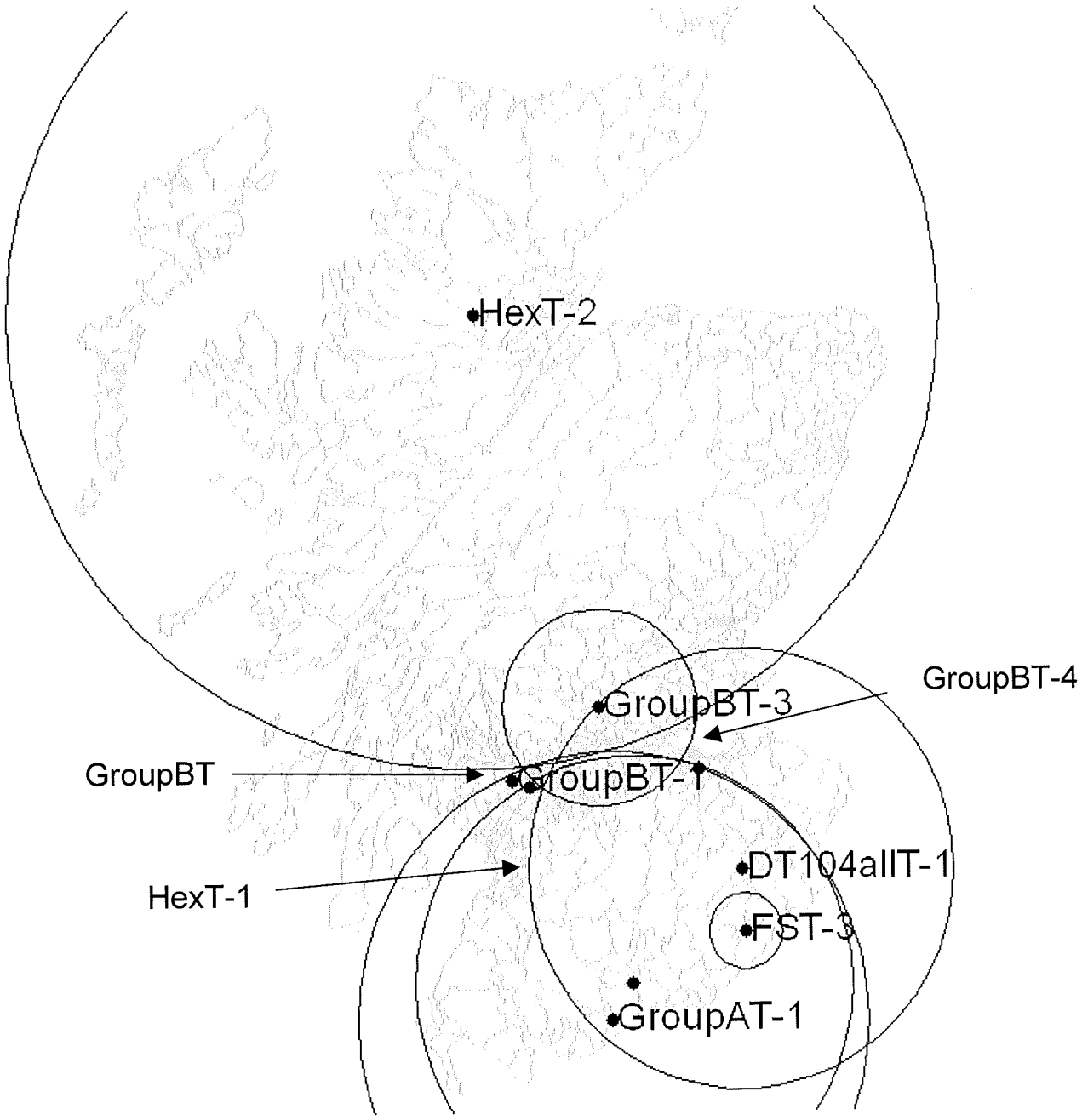


Figure 6.6 Spatio-temporal distribution of significant STHRCs and STLRCs of study groups using the maximum cluster size $\leqq$ 50% of the total population in Scotland, 1988 – 2004.

centre was located in Maxwelltown, DG in the period 1992-98. The radius of HexT-1 was approximately 99.6 km. The centre of this STHRC was very close to the centre of Group A-1 but the cluster was smaller. The second circle was called HexT-2. Its centre was located in the IV23 postal district, Lubfearn, HG, from 1993-97. The radius of HexT-2 was as large as 197.99 km. As a consequence of the radius being larger than 100 km, it was required to limit the maximum radius to 100 km. Thereafter, four identified STHRCs were identified instead of the original two STHRCs (Figure 6.7). The four clusters were called HexT100-1, HexT100-2, HexT100-3, and HexT100-4. HexT100-2, HexT100-3 and HexT100-4 were located in the margin of HexT-2. HexT100-1 was moved to the northern site of HexT-1 to fill the space where HexT-2 was original occupied. The point of HexT100-1 was centred on Smallburn, LN. The area also covered the southern part of Scotland. The other two circles were located in the northern part of Scotland. The point of HexT100-2 was located in Myrebird, GR within a radius of approximately 75 km. HexT100-3 were centred on Castletown, HG, within a radius of approximately 53 km. The centre of Hex100T-4 was located in Stornoway, Isle of Lewis.

There were four identified STHRCs from Group B. They were located in Group BT-1, Group BT-2 Group BT-3 and Group BT-4/FST-2. The centre of Group BT-1 was located in the PA3 postal district, the north-western area of Paisley in 1996. The data showed that although all isolates in this area were from 1995- 97, 14 out of the 21 isolates were reported in 1996 and 10 out of those 14 were isolated in June and July. The centre Group BT-2 (FST-1) was located at G53, the south-eastern area of Paisley in 1999. Seven out of 18 cases were submitted in 1999 and all the cases occurred in October, with four cases were marked as family contact (FC). Two cases were reported from the same address but were not marked as FC. It would be an outbreak centre. The centre of Group BT-3 was located near Menstrie, Stirling, FY, and the period was from 1992 to 1996. The circle covered the northern part of

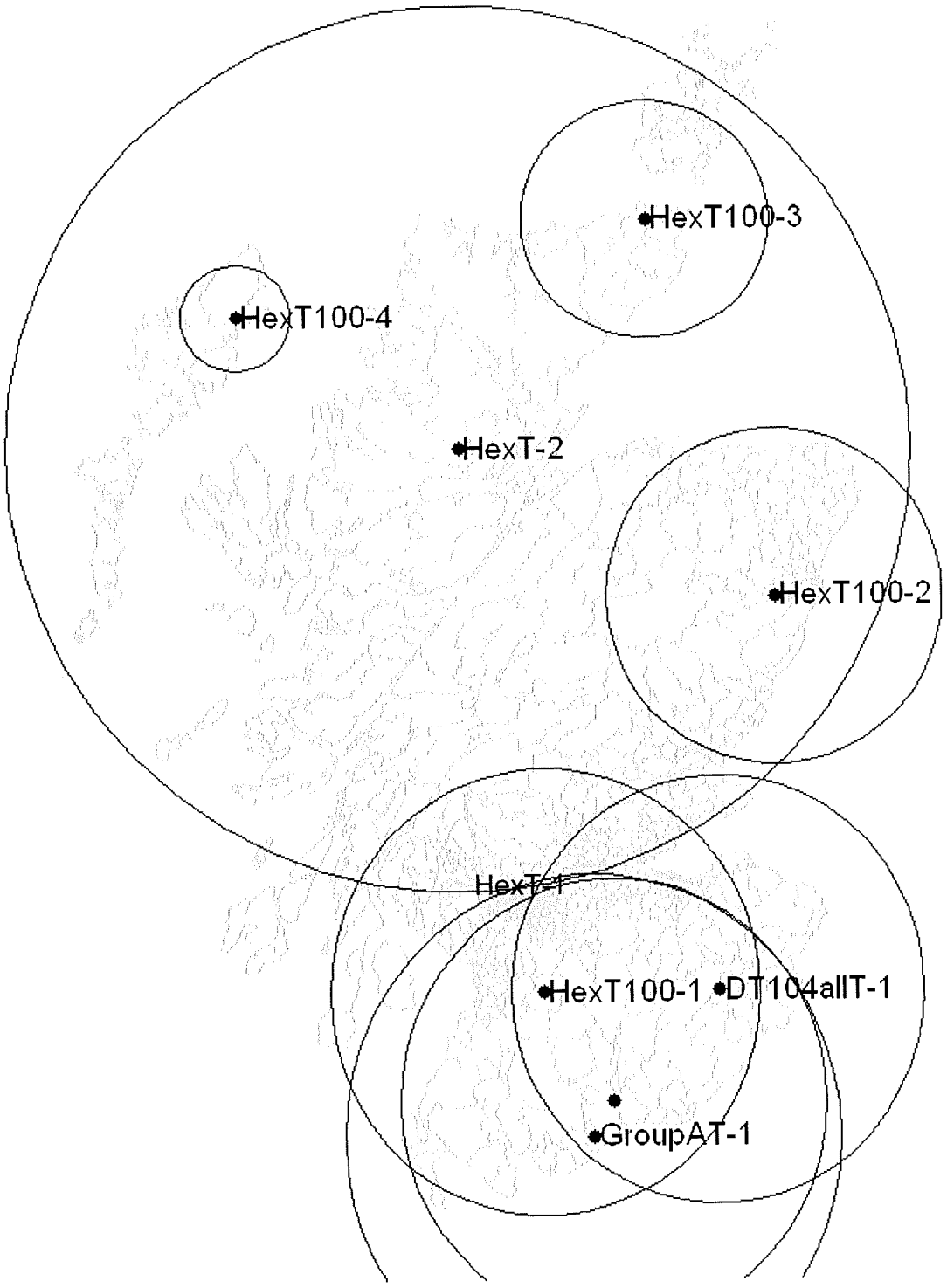


Figure 6.7 Spatio-temporal distribution of identified significant STHRCs and STLRCs of study groups using the limitation of 100 km and the maximum cluster size $\leqq$ 50% of the total population in Scotland, 1988 – 2004.

Edinburgh, Perth, and the north of Glasgow. The centre of Group BT-4/FST-2 was at EH17, LO and the circle contained 5 isolates from June to September in 1990. The 5 cases were isolated from children and were identified as FS type.

Two STHRCs from Group FS were the same as those from Group B. FST-1 had the same location and radius as in Group BT-2 and FS-1. FST-2 was the same as Group BT-4 and FS-2. The third cluster called FST-3 was located in the TD9 postal district, near Hermitage, Hawick, DG, from 1988 to 1992. Five out of 15 isolates occurred from 1988 to 1992 in this area. The fourth STHRC from Group FS was called FST-4. The centre of FST-4 was located in the FV15 postal district, near Greenloaning, FV. The radius of FST-4 was approximately 48 km. The circle covered the east-northern part of Glasgow, Stirling, Perth, and Falkirk, that is, located around the middle part of the central belt.

6.3.7 Temporal cluster analysis based on the Bernoulli model at the NHS board level

The most-likely clusters from the comparisons between Group ST, Group A, Group Hex, Group FS and their corresponding control groups: non Group ST, non Group A, non Group Hex, and non Group FS were identified. A temporal higher risk cluster (THRC) for Group ST was identified in the period 1997 to 2001. The HRTC for ST DT104 was identified between 1988 and 1992 (Table 6.6). The temporal lower risk cluster (TLRC) for Group A was identified between 1988 and 1990. There was a THRC for Group B in the same period. Group Hex had a TLRC from 2001 to 2004. Group FS had a THRC in the period from 1988 to 1992, when compared with other ST DT104 isolates (Table 6.6).

Table 6.6 The most likely temporal clusters from study groups based on Bernoulli distribution at the NHS board level.

Compared groups	Relative rate	P-value	Duration
Group ST and non Group ST	0.775	0.001	1997-01
Group ST104 and Non-Group ST104	0.181	0.001	1988-92
Group A and Non-Group A	0.328	0.001	1988-90
Group Hex and Non-Group Hex	0.529	0.001	2001-04
Group FS and Non-Group FS	10.450	0.001	1988-92

6.3.8 The most likely clusters from spatial cluster analysis based on the Bernoulli model at the NHS board level

The results from the comparison between Group ST and Non-Group ST showed that there were three SHRCs and two SLRCs. The centres of the SHRCs were located in the DG, LN and OR Health Board while the centres of the two SLRCs were located in the LO and AC Health Board (Table 6.7). There were two SHRCs from Group ST DT104 located in the eastern and the southern parts of Scotland. The SLRC from Group ST DT104 covered the HG and GR Health Boards (Table 6.7).

Table 6.7 The purely spatial clusters from study groups based on Bernoulli distribution at the NHS board level.

NHS Health Boards	Relative rate	P-value	Radius (km)
Group ST and Non-Group ST			
DG	1.557	0.001	0
LO	0.781	0.001	0
AC	0.823	0.001	0
LN	1.126	0.005	0
OR	1.625	0.013	0
Group ST104 and Non-Group ST104			
HG, GR	0.788	0.001	118.3
DG, AA	1.266	0.001	54.18

6.3.9 The most likely clusters from spatio-temporal cluster analysis based on the Bernoulli model at the NHS Health Board level

Table 6.8 shows the most-likely spatio-temporal clusters from the comparisons between pairs. In the period from 1988 to 95, there were two identified STHRCs from the Group ST and Non-Group ST comparison. The first STHRC covered the DG, AA, LN, BR, and GG Health Boards within a radius of approximately 97 km. The second STHRC covered large areas, including the northern part of Scotland and the Outer Islands, within a radius of approximately 183 km in 1988. After limiting the maximum radius to 100 km, four new STHRCs and one new STLRC were generated from the second STHRC. The HG Health Boards were identified as STLRCs from 1997 to 2001. The Health Boards of TY and FF (1988), GR (1988 – 1990), WI (1999), and OR (1996 – 2002) were identified as STHRCs.

Following the comparison of the rate of Group ST DT104 with that of Non-Group ST DT104, two STLRCs were identified. In the period from 1988 to 90, the first STLRC covered the northern part of Scotland within a radius of approximately183 km. The second STLRC was identified in the circle of the LN, GG, AA and LO Health Boards within a radius of approximately 52 km, in the southern part of Scotland between 1988 and 90. After breakdown, the first STLRC generated two STHRCs and one STLRC. The STLRC was located in the HG Health Board, 1988 - 1990. A STHRC covered the southern part of Scotland in the period 1993 to 2000. The two STHRCs were located in the Outer Islands - one STHRC was located in the WI Health Board (1999 – 2002) and the other high rank STHRC was located in the OR Health Board from 1994 to 96 (p=0.059).

Table 6.8 The most-likely cluster from spatio-temporal cluster analysis based on Bernoulli distribution at the NHS board level.

Health Board	Relative rate	P	Radius (km)	Duration
Group ST and Non-Group ST				
DG, AA, LN, BR, GG	1.367	0.001	97.61	1988-95
HG, GR, WI, TY, FV, FF, OR, AC	1.963	0.001	183.84	1988
Radius < less than 100km				
DG, AA, LN, BR, GG	1.367	0.001	97.61	1988-95
TY, FF	2.242	0.001	43.98	1988
GR	1.528	0.001	0.00	1988-90
WI	3.726	0.002	0.00	1999
HG	0.580	0.020	0.00	1997-01
OR	2.183	0.043	0.00	1996-02
Group ST104 and Non-Group ST104				
HG, GR, WI, TY, FV, FF, OR, AC	0.044	0.001	183.84	1988-90
LN, GG, AA, LO	0.075	0.001	52.45	1988-90
Radius < less than 100km				
GG, FV, LN, AA, AC, LO, FF, TY, DG	2.600	0.001	97.61	1993-00
HG	0.000	0.001	0.00	1988-90
WI	2.586	0.001	0.00	1999-02
OR	2.281	0.059	0.00	1994-96

6.3.10 The most likely clusters from spatial cluster analysis based on Bernoulli distribution at the postal district level

Table 6.9 shows the spatial clusters from the different comparisons. Figure 6.8 shows the SHRCs and Figure 6.9 shows the SLRCs from the comparison between different pairs. After Group A and Non-Group A were compared, the centre of the first SHRC, A-B-S-01, was located near Tomintoul, GR with a radius of approximately 77 km. There was an SLRC, A-B-S-02, located in Lothian with a radius of approximately 2 km. The third SHRC, A-B-S-03, had a centre located south of Hamilton, GG with a radius of approximately 20 km. The circle covered Wishaw, ML2 and Lanark, ML11.

Table 6.9 The most-likely spatial clusters from the comparison between different pairs; order in rank. The study was based on Bernoulli model at the NHS Health Board level.

Names	P-value	Location	Radius (km)	Relative risk
Group A and Non-Group A				
A-B-S-01	0.001	57.267067 N, 3.414077 W	77.3	1.106
A-B-S-02	0.013	55.880638 N, 3.159124 W	2.35	0.398
A-B-S-03	0.056	55.638416 N, 3.812780 W	20.66	1.103
Group Hex and Non-Group Hex				
Hex-N-01	0.001	54.939037 N, 4.189730 W	89.84	1.279
Hex-N-02	0.001	56.367844 N, 4.079036 W	70.11	0.841
Group FS and Non-Group FS				
FS-N-01	0.001	55.900124 N, 3.144440 W	0.00	18.493
FS-N-02	0.001	55.815506 N, 4.365403 W	2.19	11.124

There were two THRCs and one TLRC identified in the comparison of Group Hex and Non-Group Hex. The THRC Hex-N-01 was located in the DG7 postal district, the southern part of Galloway Park, DG, covering Stranraer and Dumfries. Hex-N-03 was located in the AB34 postal district, with a radius of 46 km and covering the area from the western side of Aberdeen to Glenmore Forest Park. The centre of the single TLRC, Hex-N-02, was located in the PH6 2 postal district, near Comrie with a radius of 70 km. The circle covered the central belt of Scotland.

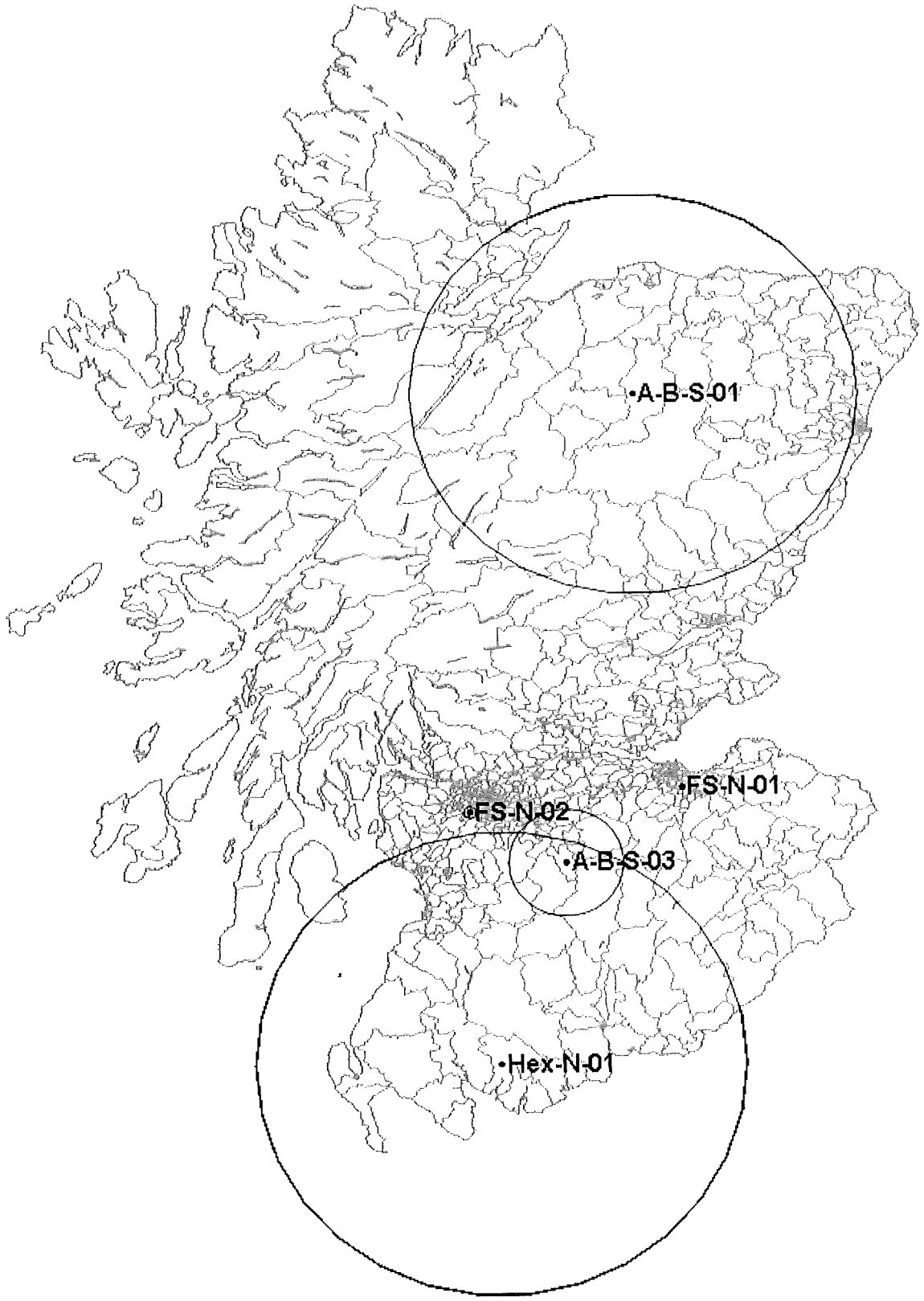


Figure 6.8 Spatial distribution of identified significant SHRCs of study groups based on Bernoulli model in Scotland, 1988 – 2004.

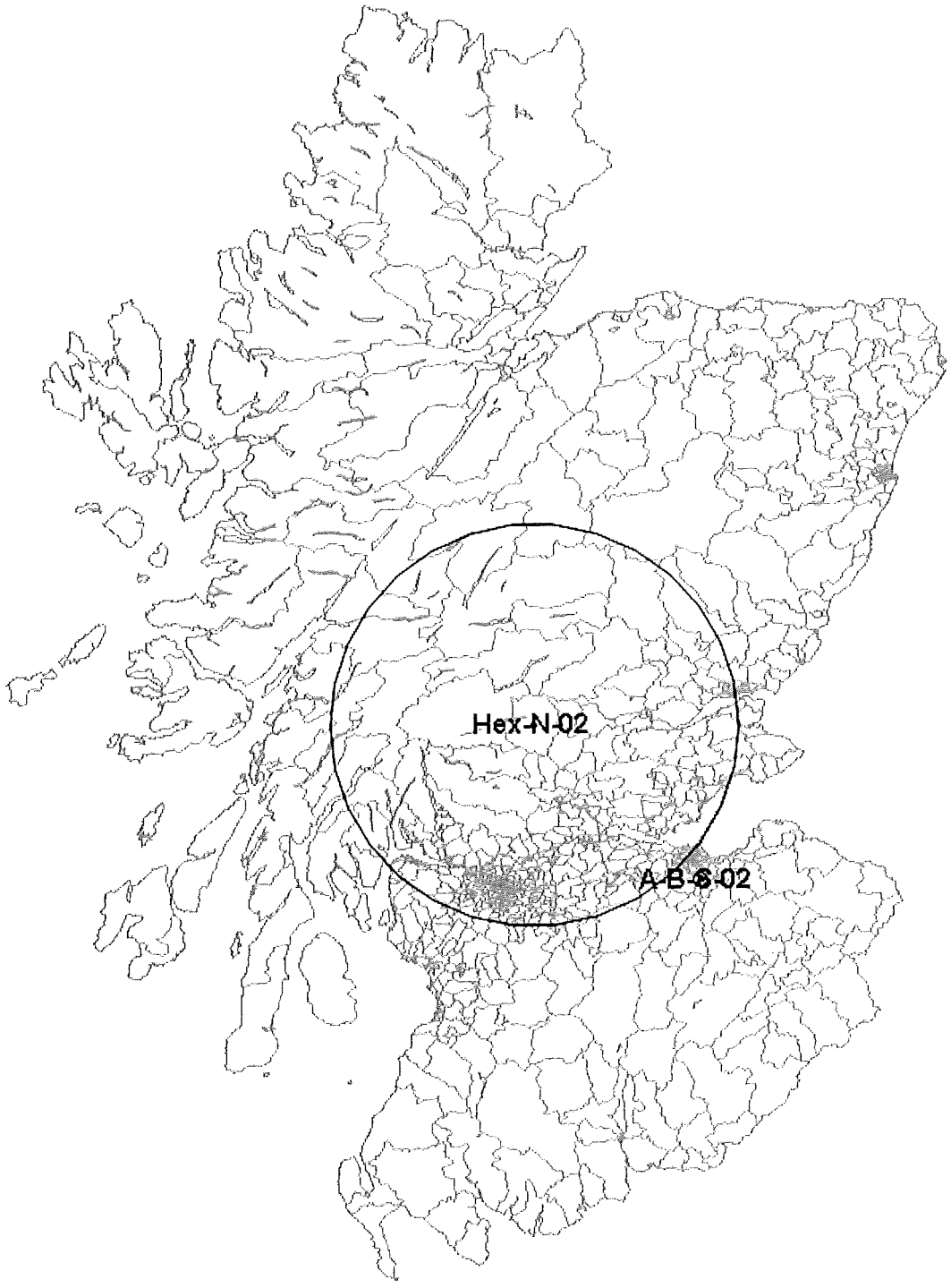


Figure 6.9 Spatial distribution of identified significant SLRCs of study groups based on Bernoulli model in Scotland, 1988 – 2004

Comparing Group FS and non Group FS, there were two identified THRCs. The first, FS-N-01, was located in the EH178 postal district, Edinburgh, LO, with six observed isolates, greater than the expected 0.34. The second THRC was centred near Nitshill within a radius of 2 km, an area which covered the G53 7, PA27, G53 5, and G78 1 postal districts.

6.3.11 The most likely clusters from spatio-temporal cluster analysis based on the Bernoulli model at the postal district level

Table 6.10 and Figure 6.10 show the spatio-temporal clusters from the different comparisons. The first, A-B-01, had a centre located in the DD8 postal district, near Glamis, Dundeen, TY with a radius of approximately 102 km in the period from 1988 to 1990. The circle covered Stirling, Perth, Dundee, the northern site of Edinburgh, and the southern side of Aberdeen. The second, A-B-02, was located in the G41 postal district, near Pollokshaws, Glasgow, GG, with a radius of approximately 4 km between 1999 and 2000. The north/south range of this area was from Kingston, G1 to Giffnock, G46. The east/west range of this area was from G73 to G52. In more general terms, the area was located in the marginal position of the Greater Glasgow Health Board. (Figure 6.10).

There was one STLRC, Hex-N-01, identified in the comparison of Group Hex and others. The centre of the circle was located in the PH6 postal district, near Dunira, FV, within a radius of approximately 70 km from 2001 to 2004. The area covered the central belt of Scotland, which was the same as the comparison between Group A and others.

A STHRC, FS-N-01 was identified in the comparison of Group FS and others. The centre of the STHRC was located in north-eastern Scotland (near Aberdeen) within a radius of 206 km in the period 1988 to 1992. After the maximum radius was limited to 100 km, one STHRC

was identified (FS-N-100-01). The centre of the circle was located near Edinburgh within a radius of approximately 99 km (Figure 6.10). The area covered the middle and the southern parts of Scotland.

Table 6.10 The spatio-temporal clusters from study groups based on Bernoulli model at the postal district level. Clusters were listed according to the ranks from the model.

Name	P-value	Location	Radius	Relative rate	Period
Group A and Non-Group A					
A-B-01	0.001	56.600147 N, 3.000730 W	101.92	0.221	1988-1990
A-B-02	0.001	55.828152 N, 4.282462 W	4.13	0.101	1999-2000
Group Hex and Non-Group Hex					
Hex-N-01	0.001	56.367844 N, 4.079036 W	69.81	0.378	2001-2004
Group FS and Non-Group FS					
FS-N-01	0.001	57.675259 N, 2.960798 W	205.9	11.735	1988-1992
Radius <100km					
FS-N-100-01	0.001	56.105236 N, 3.161780 W	99.10	10.307	1988-1992
FS-N-100-02	*0.063*	*57.166752 N, 2.153729 W*	*10.05*	*24.245*	*1988-1991*

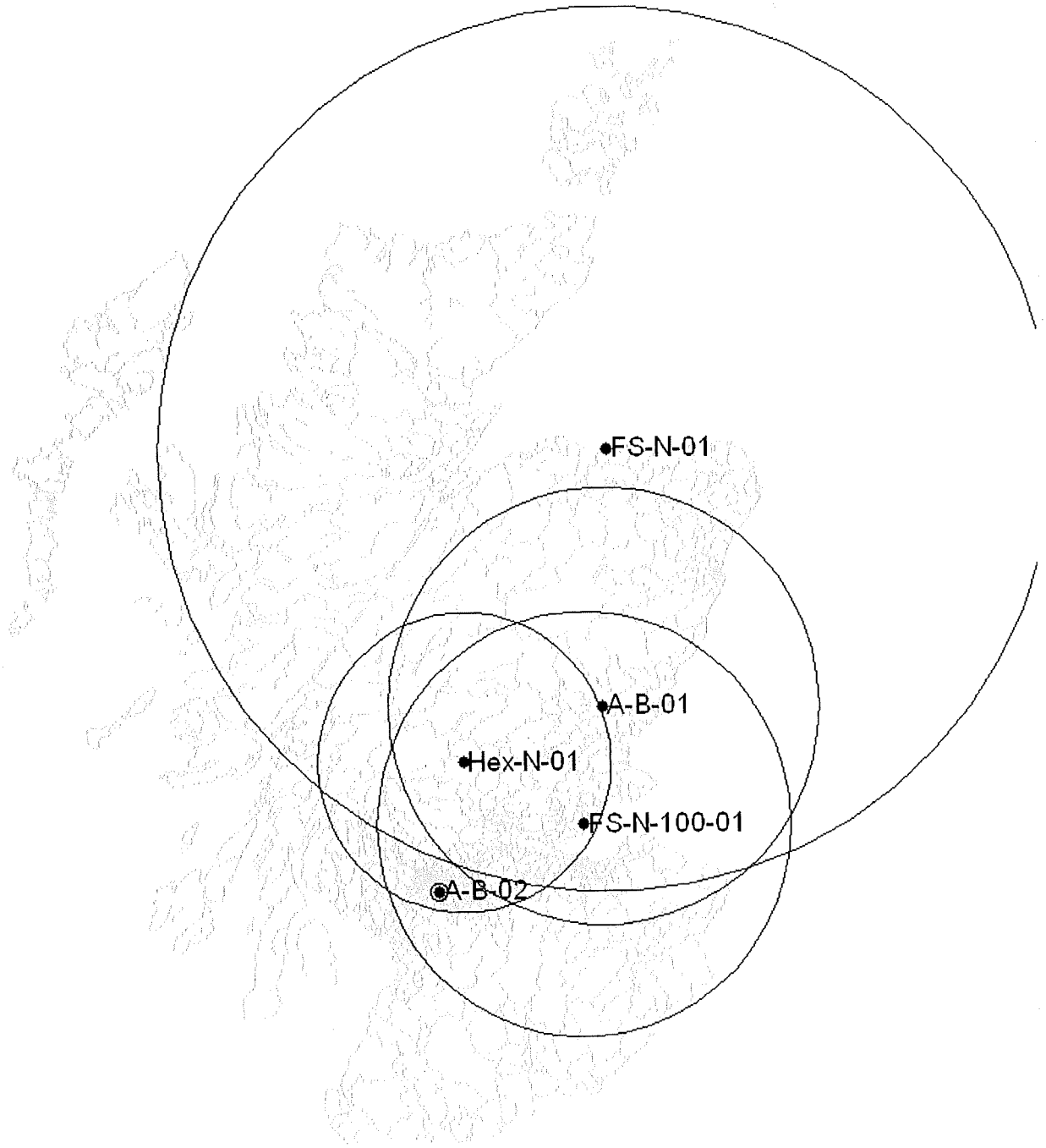


Figure 6.10 Spatio-temporal distribution of identified significant STHRCs and STLRCs from study groups using the maximum cluster size $\leqq$ 50% of the total population in Scotland, 1988 – 2004.

6.4 DISCUSSION

E. coli O157 has been investigated with regard to the case distribution in space at both animal health district and postal district levels (Toft et al. 2005; Ternent 2002). These studies suggested that to use a spatial scale with more regions is more complex but can reveal aggregations which may be invisible at a higher level structure (Toft et al. 2005). On the other hand, a study at a higher level may avoid problems caused by small sample sizes and missing data (Toft et al. 2005); may be time-saving and may still provide the necessary epidemiological inference for intervention. The current study has supported these observations. It has shown that the results at both geographical levels were similar in the higher case area, where the cases aggregate together, but were heterogeneous and most likely misleading where there were smaller higher/lower risk clusters analysed at a higher level of resolution.

Ideally, one would hope that the results at the two different geographical levels would be the similar. However, contrasting the NHS Health Board and the postal district levels, the results appear to be affected by variances within and between areas. There are several reasons; SaTScan tries to achieve as large a cluster as possible and maximises the circle that can fit the criteria given, such as 50% population. Thus, a most likely circle can be represented as a centre without any cases at the centre but with many smaller clusters at the margin of the circle (Kulldorff and Nagarwalla 1995). The problem arose particularly in the results from Highlands and the Island areas. The HG Health Board usually had fewer incidents, but the Outside Islands had more incidents. The results may then show an incorrect HRC/LRC including the HG, GR, OR, and SH Health Boards. with the centre located in the HG Health Board. The problem may be overcome by changing the criteria by limiting the radius or

reducing the percentage of the population covered, but this too presents problems as choosing these parameters is somewhat subjective. One benefit of using analysis at the NHS Health Board level is that it is less likely to compromise patient confidentiality; another is that missing addresses play less of a role as the case is assigned at NHS board level only.

Implementing the study at the postal district level also has benefits - it limits clusters to a smaller zone, so, where a higher rate cluster is found, a small scale investigation could be carried out in a short time. Further advantages include easier identification of an outbreak and the avoidance of mis-understandings caused by geographical issues. For example, the Grampian Mountains are located in the middle of Scotland and not many people live in this area and so the incidence would be very small. The higher rate clusters at postal district level usually did not include the mountains. In contrast, the centres of the lower rate clusters were located around mountains.

Mis-understandings usually occur where there is no prior knowledge of the study areas. However, in this study it was known *a priori* where topographical features were likely to affect population density and these influence the distribution of clusters. The results based on the Poisson model showed the LRCs were usually located in the Highlands or surrounding area and the HRCs were located in the southern and eastern parts of Scotland; this is of some comfort.

From the cattle census at postal district level, a large number of cattle are found in Dumfries (DG8, 7, 2, 11 and ML17), Grampian (AB54, 53, 41 and 51), and the Orkney Islands. The fact that the results in this study show higher rate clusters following a similar distribution to the cattle distribution rather than population density *per se* provides evidence similar to that

found in the case of *E coli* O157 (Ternent 2002). Calvert concluded that 37.8 per cent of submissions from DG may have regular contact with cattle (Calvert et al. 1998). However, caution is required; cattle density is an index of rurality and it may be that it is this feature alone that is being identified.

A virulence gene was identified from plasmids in multi-resistant types (Threlfall et al. 2005). The ApClSpStSuTe R-type was the main isolate from Scottish humans from 1991 to 2004. However, some scientists believe the general ApClSpStSuTe R-type is not a hyper-virulent strain, rather, it has a better ability to survive. The *rpo*S (Loewen and Hengge-Aronis 1994), *qac*E△1 (Cloeckaert et al. 2000), and *mar*A (George and Levy 1983) all enhance its survival ability. High virulence also means that a strain can spread more widely and survive longer. From the comparison results in the Bernoulli study, Group A and its subgroups had a wider circle and the multi-antibiotic resistance phenotypes appear to have a greater possibility of occurring in livestock areas. The results also suggest that the survival ability in Group A may be better. The study overall also supports the suggestion that Group B was more associated with urban areas, transferred more slowly and tended to be self-limiting. Accordingly the radii were usually small and the relative risks were usually near 1. On the other hand, Group FS had higher relative risks in temporal and spatio-temporal studies.

A disease can be epidemic or endemic. In an endemic disease, there are no higher/lower rate clusters in a purely temporal study. The results in this study showed that lower risk clusters were identified in the temporal cluster study of the *Salmonella* and ST groups. Control measures against salmonellosis, especially *S.* Enteritidis, were introduced from 1994. In 1994, *S.* Enteritidis PT 4 vaccine was introduced into broiler breeders. In 1997, live attenuated *S.* Enteritidis vaccine was allowed in egg-laying flocks (Defra 2003). Although vaccines were

used against *S.* Enteritidis, the purely temporal study for ST DT104 supports the argument that other factors could be responsible for the decline from 1998. For Groups Hex and FS, the purely temporal results support the contribution of other factors - improvement in catering practice, a cool summer, and lighter contamination of uncooked foods have all been suggested (Scottish Centre for Infection and Environmental Health 1999; Handysides 1999).

The argument about the advantages and disadvantages of the Bernoulli and Poisson models is the same as for the cohort and the case-control study. Generally, the results of the two models should be very similar (Kulldorff and Nagarwalla 1995). The Poisson model is more suitable for dealing with a rare disease because, by using the whole population as the denominator, it has greater statistical power (Kulldorff 1997). The results showed that the two studies had similar results. However, the Poisson model was able to identify more clusters, especially those clusters in Group B and its subgroups.

In summary and considering the results as whole, the dual approach of spatial resolution and adopting the two different modelling approaches, has permitted the identification of high and low risk clusters in space, time and space-time. The key issue is that there is some heterogeneity when one considers the groups of *Salmonella* as outlined in this and other chapters. It is vitally important not to over-interpret the individual results as issues of data quality, choice of time unit, data sparsity, statistical power and spatial resolution will prevent individual analyses from being particularly robust. Suffice to say, in this chapter, that there are differences, that these differences are consistent and will be interesting to revisit in the light of analyses based on the animal isolates.

CHAPTER VII

DESCRIPTION OF

SALMONELLA TYPHIMURIUM AND ITS PHAGE TYPE DT104

ISOLATES FROM VETERINARY SOURCES IN SCOTLAND

CHAPTER VII

DESCRIPTION OF *SALMONELLA* TYPHIMURIUM AND ITS PHAGE TYPE DT104 ISOLATES FROM VETERINARY SOURCES IN SCOTLAND

7.1 INTRODUCTION

Salmonella is an important pathogen associated with significant morbidity and mortality in livestock. In 2003, *Salmonella* Typhimurium (ST) was the second most common *Salmonella* serotype in England and Wales (Defra 2003). Economic loss caused by ST is considerable, consisting not only of animal mortality costs but also of disinfection costs, which, in a 400 sow farm with ST, may amount to £9,608 in a 3 week period (Anon 2007d). Clearly, ST infection in livestock needs to be controlled by the most efficient means possible.

Ever since antimicrobial drugs were first used to control bacterial infections in livestock, the development of antibiotic resistance has been inevitable (Henney 2007). Antibiotic resistance genes can be single gene segments or many genes located together, called gene cassettes, in a bacterial genome or in R-plasmids. The gene cassette can move among species via conjunction or simple direct intake from the environment (Tortora et al. 2001). A gene cassette called the *Salmonella* genomic island 1 containing 5 antibiotic resistance genes against ampicillin (Ap), chloramphenicol (Cl), spectinomycin (Sp), streptomycin (St), tetracycline (Te) and sulphonamide (Su) has been identified in a clone of *S.*

Typhimurium DT 104 (ST DT104), referred to as R-type ACSSuT (Threlfall 2000). When ST DT104 was identified in 1985, it was not a major concern because it did not contain any resistance genes. However, since the penta-resistant form of ST DT104 was discovered, the prevalence of multi-drug resistant ST DT104 in livestock has been increasing gradually (Browning et al. 2001b; Browning et al. 2004; Browning et al. 2005; Browning et al. 2003; Browning et al. 1999). Antibiotic use has been shown to influence the increase of resistant strains of ST DT104 in humans (Threlfall et al. 1994); thus, it is essential to understand the ecological and epidemiological patterns of ST DT104 infection in animals.

The aims of the study described in this chapter are to describe the epidemiology of *Salmonella* isolates from the animal population and veterinary sources in Scotland from 1990 to 2004 and to describe the antibiotic resistance patterns of all *Salmonella* isolates collected from veterinary sources in Scotland from 1990 to 2004.

7.2 MATERIAL AND METHODS

Salmonella isolates come from widely differing veterinary sources, ranging from animals and foodstuffs to environmental samples, collected via a passive surveillance system. Since 1993, Defra publications have separated *Salmonella* reports from livestock into isolations and incidents, as detailed in Chapter 2 (Defra 2003).

All records were kindly supplied by HPS. Data were re-checked using EXCEL version 2000. Table 7.1 lists the relevant variables used in this study. The original dataset did not contain the date the sample was received. The reference numbers included the year and week of submission. A new variable, week, was generated from the reference numbers.

The week ranged from 1 to 53 and the week number was used as the minimum time interval. Thus, a year was divided into 13 four-weekly months. The 53rd week was assigned to the 13th four-weekly month.

The antibiotic resistance patterns were re-coded as 1/0 where 1 referred to resistant and 0 referred to sensitive. R-type scores were used as described in Chapter 4. The calculation method for the resistance score was the same as described in Chapter 4. The variable "pathogen" was re-assigned into 3 categories: *S.* Typhimurium, *S.* Enteritidis, and others.

Table 7.1 The description of variables used in the study

Variables	Description
Pathogen	Serotype of *Salmonella*
Type	Phage types of *Salmonella* Enteriditis and Typhimurium
Year	The year of collection
NO	The collection number - contains the reported week
Source	Which animal the case is from
Trues ource	The categories of animal
Lab source	The veterinary laboratory of isolation
Resistant pattern	The 15 antibiotic resistance patterns
Week	The week the case was reported to laboratory
Pathogen2	*S.* Typhimurium, *S.* Enteritidis, and Others

In this study data quality and issues of confidentiality precluded using data at a fine level of spatial resolution. As a consequence, the Animal Health Divisions defined by the Scottish Agriculture College (SAC) were used as the unit of spatial aggregation (Ternent 2002). Scotland is divided into six animal health divisions: Highland, North East, Central, South West, South East and Islands (Figure 7.1). Cases reported by laboratories were assumed to have occurred in the corresponding Animal Health Divisions and were

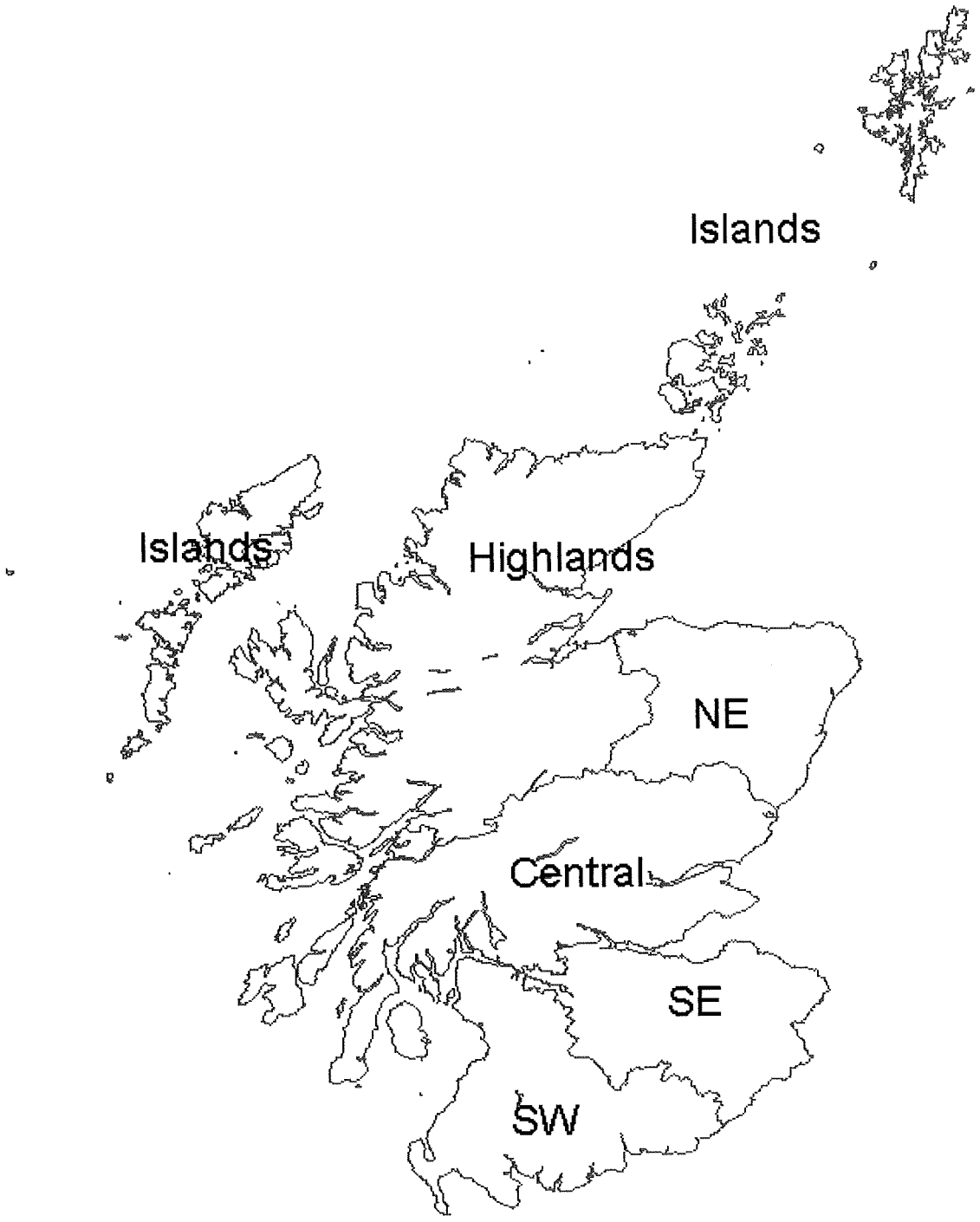


Figure 7.1 The regions of the Animal Health Divisions.

allocated accordingly. However, there is no laboratory in Islands and samples sent from the Islands were allocated into Highlands.

All data manipulation and basic statistical analysis were carried out in Microsoft Excel version 2000.

7.3 RESULTS

7.3.1 The trend of Salmonella, Salmonella Typhimurium, *and Salmonella* Typhimurium *DT 104 in Scotland between 1990 and 2004*

7.3.1.1 Sources of *Salmonella* cases

There were 12,384 *Salmonella* spp isolations reported from 1990 to 2004. All isolates were sorted into 19 categories ascribed by HPS (Table 7.2). The avian category contained 38 species of birds; the majority were caged birds and wild birds. In this category, 197 samples were from pigeons, 337 from pheasants and 272 from wild birds. The miscellaneous animal category contained 21 species, but the samples were mainly from porpoises (109 samples). The zoo animal category had 10 species. The reptile category had 19 species and cases were mainly from snakes (44). The feed category contained meal samples from three groups: poultry, fish and other animals, including samples from chicken products and mussels. The 'other water' category referred to water samples collected from reservoirs. The environment category contained 7 isolates from environments surrounding animals.

Table 7.2 The *Salmonella* spp. submissions to SSRL from each animal category during the study period.

SOURCE	Typhimurium	Enteritidis	Others	Total
BOVINE	2985	41	1932	4958
POULTRY	194	878	3072	4144
PORCINE	338	2	153	493
OVINE	160	1	727	888
CAPRINE	2			2
EQUINE	83	5	10	98
AVIAN	759	50	332	1141
CANINE	94	12	95	201
FELINE	84	16	13	113
PHEASANT			1	1
CERVINE			1	1
ZOO ANIMAL	10	1	5	16
REPTILE	1	3	115	119
OTHER WATER			1	1
MISC.ANIMAL	25	17	145	187
ANIMAL ENVIRONMENT			7	7
FOOD			4	4
FEED			8	8
NOT STATED	1		1	2
Total	4736	1026	6622	12384

There were many *Salmonella* serotypes identified throughout the study period. More than one third of all *Salmonella* samples were from cattle samples. A further third of samples were from poultry (Table 7.2). Almost all *S.* Enteritidis isolates were obtained from poultry samples (878/1026). In contrast, most cases of ST were from cattle (Table 7.2). Only 194 samples of ST were of poultry origin.

Table 7.2 shows that 94% of all *Salmonellae* cases (11,624 isolates) were from five main animal categories, bovine, poultry, avian, ovine and porcine; 4,736 samples were reported as ST, 2,985 of which were bovine related. The table also shows poultry isolates were mainly reported as non-ST.

The *Salmonella* spp. samples reported per year from the 5 animal categories are listed in Figure 7.2. It shows that there was an overall decline from 1993 to 1999, when only 284 cases were recorded by HPS. Isolates related to bovine sources were low in 1993 and 1998, respectively. However, isolates from poultry sources increased between 1995 and 1998. After 2000, the total submissions declined. There were fewer isolates sent from poultry samples; only 31 and 39 samples from poultry sources were sent in 2000 and 2001, respectively.

In 2002, there were 676 reports of *Salmonella* from different animal sources. This represents an increase of 30% compared with the previous years (Figure 7.2). The increase was due to isolates from cattle, which increased by almost 200% in 2002 (458) compared to 2001 (165) (Figure 7.2). There were only 3 poultry isolates sent to SSRL from 2003 to 2004.

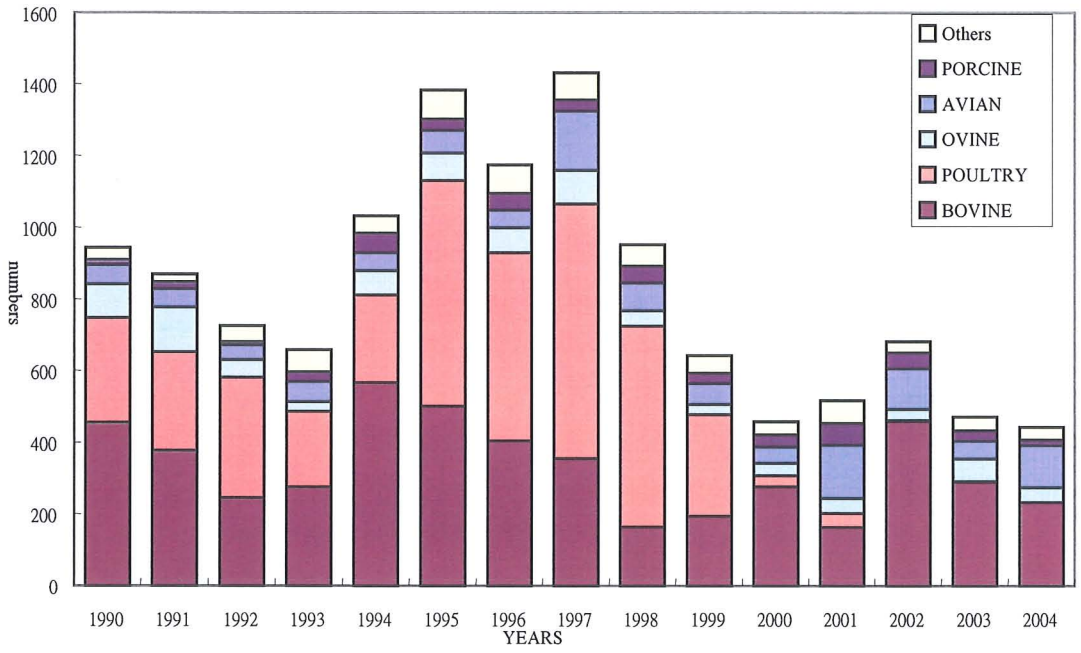


Figure 7.2 Chart showing the animal sources of *Salmonella* spp. submissions to SSRL from 1990 to 2004.

Figure 7.3 shows the number of submissions for *Salmonella*, ST and ST DT104, respectively. The *Salmonella* submissions declined in 1993, mainly caused by the decline in *S.* Enteritidis submissions. In 1997, the *Salmonella* submissions were the highest with 1431 isolates recorded. After 1997, the submissions of *Salmonella* spp. declined again. The number of isolations dropped in years 1993 and 2000, increasing again the following year.

The trend for ST DT104 was not similar to others. It had only 8 isolates in 1990, with numbers rising from 1991, with 509 the highest recorded number of isolates in 1994. After 1994, the submissions began decline with only 21 isolates in 2004.

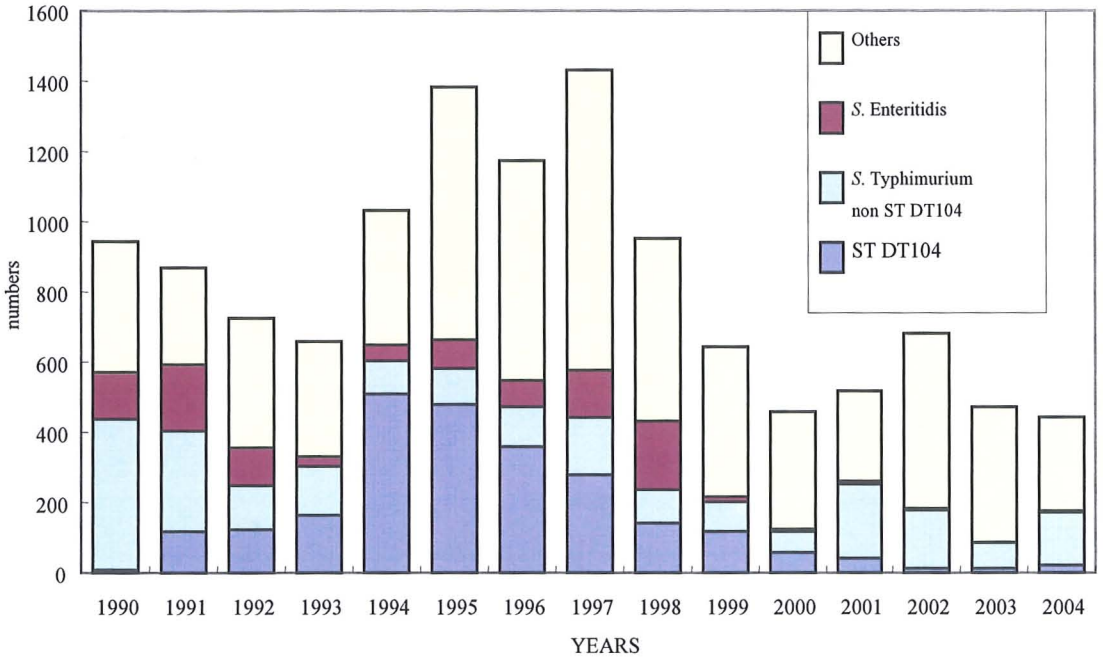


Figure 7.3 The submissions of *Salmonella*, ST and *S.* Enteritidis to SSRL in Scotland, 1990-2004

Figure 7.4 shows that trend of the proportion of ST DT104 in the isolates rose in 1991 and 1994. In 1994, 509 isolations in ST (about 80%) were of ST DT104. The isolation numbers and proportion of ST DT104 declined gradually after 1994.

There were 47 different phage types of *S.* Typhimurium isolated from Scotland throughout the study period and the proportions varied over time. However, there were 5 main phage types. In 1990, ST phage type 204c was the most common phage type. ST DT104 became predominant in 1992. The proportion of ST DT104 declined in 1994 and then ST Phage type 40 replaced it as the major type from 2000 (Figure 7.4).

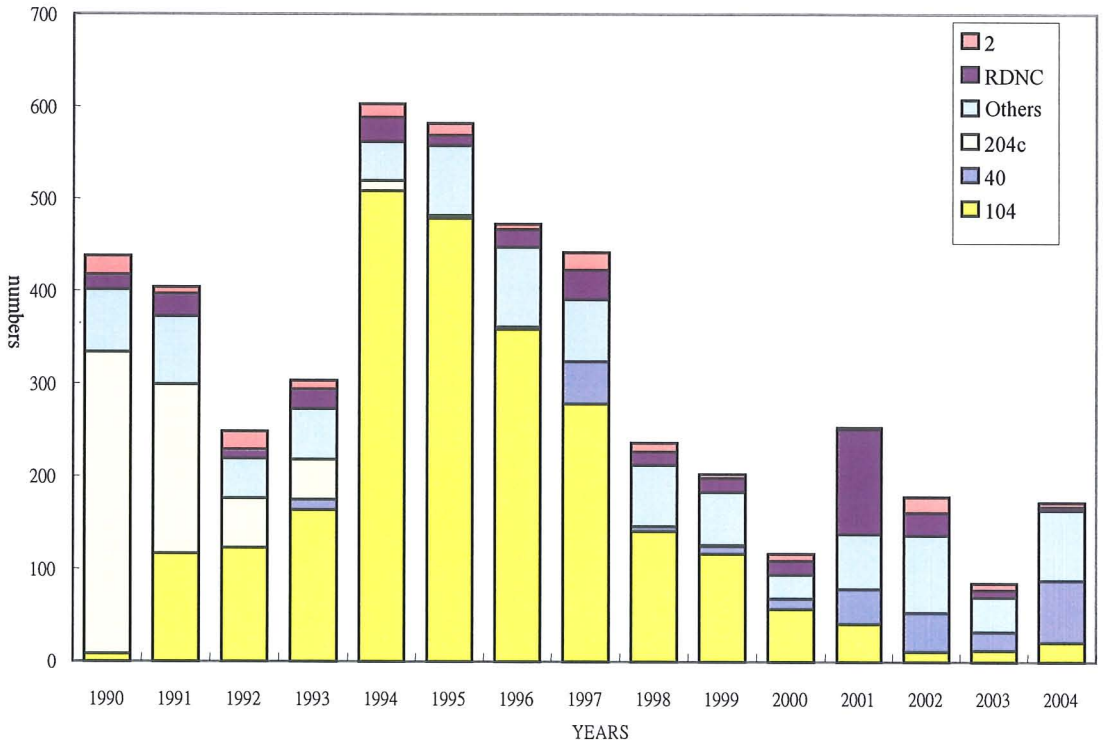


Figure 7.4 The phage types of ST submissions to SSRL in Scotland, 1990-2004.

Figure 7.5 shows the submissions in every four-week month from 1990 to 2004. Generally, the figure shows that there were 2 peaks in each year, the first peak was recorded in the 5th or the 6th four-week month. The second peak occurred in the 11th or 12th four-week month.

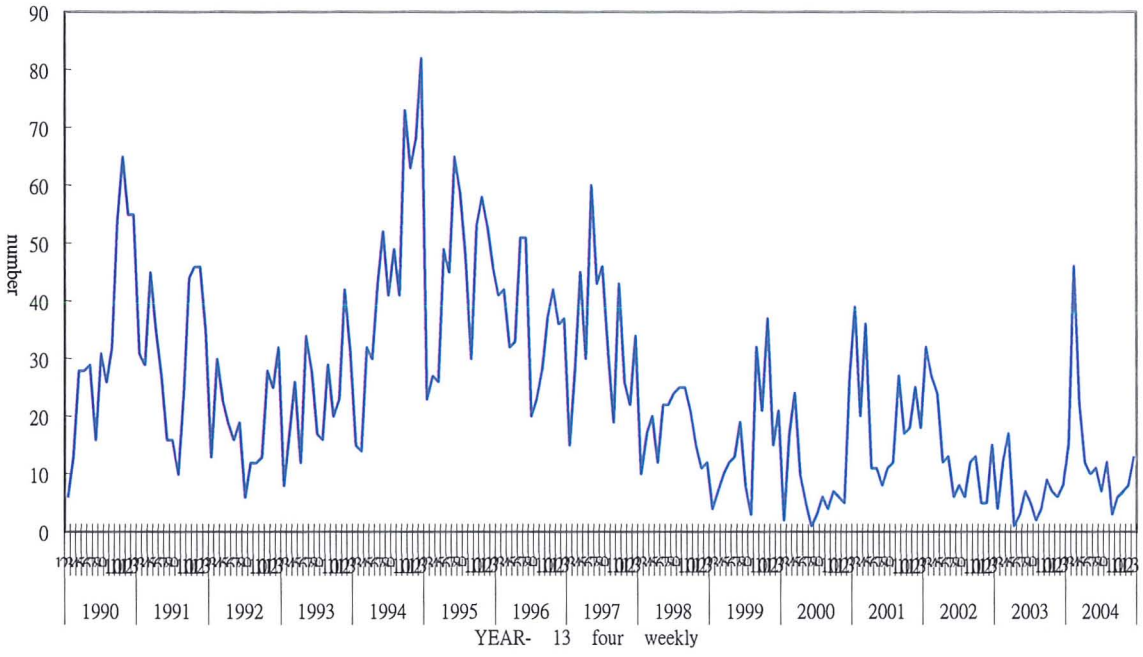


Figure 7.5 The submissions from *S.* Typhimurium in each four-weekly month period, Scotland, 1990-2004

7.3.1.2 *Salmonella* Typhimurium DT104

Tables 7.3 and 7.4 show that ST DT104 was isolated from 11 different categories and 38 animal species. There were a total of 1,930 bovine submissions, with half of the isolations (1094) from the South West Division. The region includes Dumfries, Ayr, and Paisley, and is a traditional livestock area. The preponderance of submissions from cattle was reflected in the correlation of the total number of ST DT104 with the number from bovine sources, whether analysing by Animal Health Division ($p=0.001$) or year.

Table 7.3 The ST DT104 submissions from cattle and total cattle numbers in each Animal Health Division, Scotland, 1990-2004.

Animal Health Divisions	Cattle	Total	Cattle population (2003)	Cumulative %
Highland	177	217	151,019	0.117
North East	244	370	368,998	0.066
Central	164	242	511,044	0.031
South East	339	514	198.996	0.170
South West	1006	1094	569,148	0.177
Total	1930	2437		

*Islands has 106,115 cattle, the population were added into the Highland animal district

Table 7.4 shows the ST DT104 submissions from different sources in each year. ST DT104 isolations were mainly reported from bovine sources (80%); the second most common was from pigs.

Table 7.4 Reported numbers of isolates of ST DT104 by species in the SSRL animal dataset, Scotland, 1990-2004

YEAR	Avian	Bovine	Canine	Caprine	Equine	Feline	Misc. Animal	Ovine	Porcine	Poultry	Zoo Animals	Total
1990		7							1			8
1991	3	108	1			2		3				117
1992	1	105	4		6	3	1	2	1			123
1993		147	2		2			3	10			164
1994	5	452	4		4	3	4	11	23	2	1	509
1995	10	388	11	1	3	13	1	30	10	12		479
1996	2	284	11		11	9	2	22	9	8	1	359
1997	1	209	12		5	13	3	13	12	4	6	278
1998	1	87	4		4	8		6	22	8	1	141
1999		49	1			1	2	9	6	49		117
2000		36	2		2	1		2	9	5		57
2001	1	25							8	7		41
2002		6			1		1		3			11
2003		7					1	1	3			12
2004		20	1									21
Total	24	1930	53	1	38	53	15	102	117	95	9	2437

Table 7.5 describes the ST DT104 submissions from veterinary sources in each of the 13 four-week periods. The seasonal trend was not significant between 1994 and 1997 but there were usually smaller peaks in the 6th and the 10th period. The first peak usually occurred from the 2nd to the 7th period. The second peak usually occurred from the 10th to the 13th period.

Table 7.5 The ST DT104 submissions reported in each four-weekly month from animal sources, Scotland, 1990-2004

Four-weekly period	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	Total
1		2	4	2	12	19	35	11	6	4	1	11	1	1	1	110
2		7	8	10	10	22	30	16	8	1	11			1	4	128
3		11	8	10	25	25	26	17	12		12	1	1		1	149
4		2	8	5	26	41	29	18	6	5	2		1	1		144
5		4	9	18	33	38	42	35	12	13	3	1			4	212
6		4	13	10	41	56	43	22	16	18		2	2		1	228
7		5	4	13	36	50	17	26	19	3	2	3	2	3	1	184
8	1	3	3	4	45	40	15	25	6	2	6	4	2		3	159
9		5	8	20	32	26	9	13	12	7	4	9	1	1		147
10	1	15	9	15	69	38	27	29	18	6	2			2		231
11	4	27	12	10	54	47	30	18	11	34	3	3		2	2	257
12	2	16	17	24	55	40	30	16	8	10		4		1	1	224
13		16	20	23	71	37	26	32	7	14	11	3	1		3	264
Total	8	117	123	164	509	479	359	278	141	117	57	41	11	12	21	2437

7.3.2 Antibiotic resistance results

Table 7.6 shows the R-type score of all ST DT104 submissions in each year. The hex-antibiotic resistant form was the most common and there were three R-types belonging to this form (Table 7.7), the majority (99.8%) being ApClSpStSuTe The R-type score 7 contained 7 R-types and 148 samples; many isolates (88.5%) belonged to the

ApClNaSpStSuTe(Lc) and ApClSpStSuTeTm R-types i.e., basically, ApClSpStSuTe with extra Na or Tm resistances. R-type score 8 was mainly of ApClGmNeSpStSuTe R-type (8 samples). Throughout the study period, the number of the fully sensitive type, FS, was very small. Moreover, only 50 isolates had R-type scores of 0 – 3 and all other isolates were multi-resistant types.

Table 7.6 The R-type score of the ST DT 104 submissions from animal sources, 13 antibiotics were used but the resistance pattern of low level ciprofloxacin was excluded, Scotland, 1990-2004

	R –type score											
YEAR	0	1	2	3	4	5	6	7	8	9	10	Total
1990	1				1		6					8
1991	2	1			1	1	108	4				117
1992	4				1		116	1	1			123
1993	5					1	153		1	4		164
1994	4	1	1	6	4	5	469	18			1	509
1995			1	3		1	450	22	2			479
1996			3	5			326	20	4		1	359
1997	2			2			242	30	1	1		278
1998	1		2		1		118	14	6	1		143
1999	2			1			102	10	1	1		117
2000				1			53	3				57
2001							24	16	1			41
2002							6	5				11
2003	1		1				7	3				12
2004						1	18	2				21
Total	22	2	8	18	8	9	2198	148	17	7	2	2439

Table 7.7 presents the resistance patterns identified throughout the study period. Isolates resistant to Lc were listed separately, although, they were grouped into one R-type. There were 25 R-types identified, despite the difference in Lc pattern. Most of the cases belong to the ApClSpStSuTe R-type. The second most common R-type was ApClSpStSuTeTm. It

Table 7.7 R-types of ST DT104 identified from animal sources, Scotland, 1990-2004

Antimicrobial	90	91	92	93	94	95	96	97	98	99	00	01	02	03	04	Total
FS	1	2	4	5	4			2	1	2				1		22
Te		1			1											2
SpSu														1		1
SuTm						1										1
ApSu					1		3		2							6
SpStSu					4	3	5	2		1	1					16
SuTeTm					2											2
ApClSpSt					1											1
ApSpStSu									1							1
ApStSuTe	1	1	1		3											6
ApClSpStSu					1											1
ApSpStSuTe						1									1	2
ClSpStSuTe					2											2
ApClSpStTe					1											1
ApClSpSuTe		1														1
ApKaStSuTe				1												1
KaSpStSuTe					1											1
ApClSpStSuTe	6	108	116	153	469	450	323	241	118	102	53	24	6	7	18	2194
ApClFzSpStSu								1								1
ApNaSpStSuTe							3									3
ApClGmSpStSuTe								5								5
ApClKaSpStSuTe					1	3	1	1		1						7
ApClNaSpStSuTe							9	6	4							19
ApClNaSpStSuTeLc								2	2	4		12			2	22
ApClNeSpStSuTe						1										1
ApClSpStSuTeTm		1	1		16	18	10	16	8	5	3	4	5	3		90
ApClFzSpStSuTe		3			1											4
ApClNaSpStSuTeTm									4							4
ApClNaSpStSuTeTmLc									2							2
ApClKaSpStSuTeTm										1						1
ApClGmNaSpStSuTe							1									1
ApClGmNeSpStSuTe			1	1		2	3	1								8
ApClCpNaSpStSuTeLc												1				1
ApClGmKaNeSpStSuTe				4					1							5
ApClKaNaSpStSuTeTmLc										1						1
ApClGmKaNeSpStSuTeTm					1		1									2
ApClGmNaNeSpStSuTeLc								1								1
Total	8	117	123	164	509	479	359	278	143	117	57	41	11	12	21	2439
Types	3	7	5	5	16	8	10	10	8	8	3	4	2	2	4	

was first isolated in 1991 but high numbers (90) were recorded in 1994. The SpStSu R-type was isolated 16 times after 1994 and was in 4th position behind the fully sensitive type. Since 1996, nalidixic acid resistance has been found in three R-types, ApClNaSpStSuTe, ApClGmNaSpStSuTe, and ApClNaSpStSuTe (Table 7.7). Nine R-types had Na resistance. Despite the resistances against Tm, Na, Ap, Cl, Sp, St, Su, and Te, other drugs were still effective against the ST DT104 isolates collected from the animal dataset. The number of R types found every year was different. From 1994 to 1999, more than 8 R-types were reported every year. In 1994, there were 16 R-types. Overall, two R-types, ApClSpStSuTe and ApClSpStSuTeTm, were usually identified every year.

Figure 7.6 shows the numbers of isolates resistant to each antibiotic from the animal ST DT104 submissions. According to the results shown in Figure 7.6 and Table 7.8, antibiotics can be separated into two sub-groups; high and low antibiotic resistance groups. The first group included Ap, Cl, Sp, St, Su and Te. The number of isolates resistant to those antibiotics sharply increased from 1991 and, from 1993 to 1994, the numbers more than doubled. The highest number was recorded in 1994. Thereafter, the numbers decreased, falling to less than 50 in 2004. The other antibiotics including Cp, Fz, Gm, Ka, Na, Ne, and Lc formed another group. The number of isolates resistant to single antibiotics in this group were usually few in each year. Generally, the two groups had very similar trends and patterns.

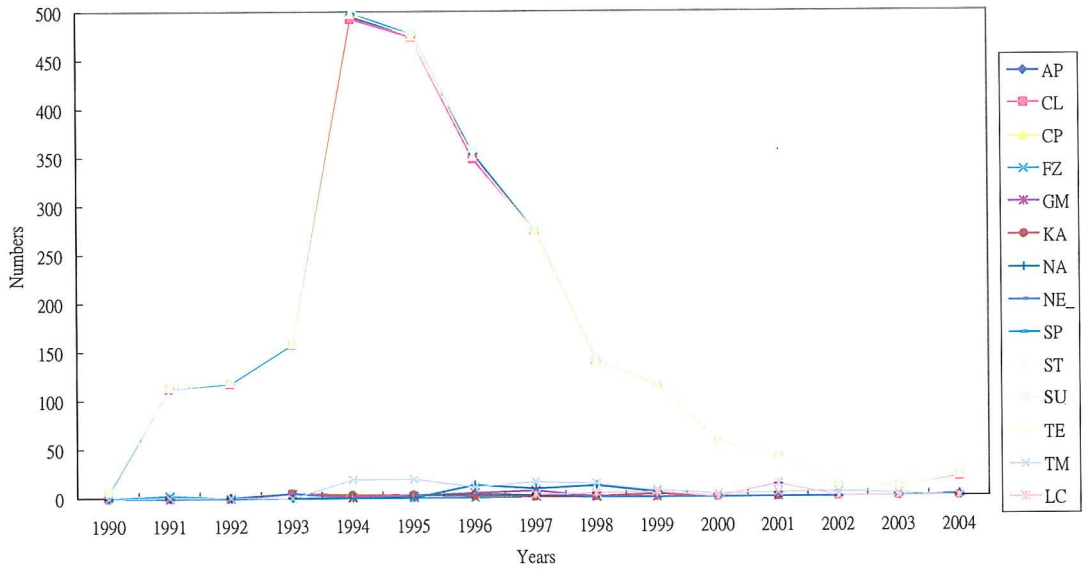


Figure 7.6 Trends of samples resistant to 13 antimicrobial agents, Scotland, 1990-2004

Table 7.8 The cumulative ST DT104 submissions from animal sources for each single antibiotic treatment, Scotland, 1990-2004

YEAR	Ap	Cl	Sp	St	Su	Te	Tm	Gm	Ka	Na	Ne	Fz	Cp	Lc	Total
1990	7	6	6	7	7	7	0	0	0	0	0	0	0	-	8
1991	121	119	119	120	121	122	1	0	0	0	0	3	0	-	125
1992	240	237	237	239	240	241	2	1	0	0	1	3	0	-	248
1993	399	395	395	398	399	400	2	6	5	0	6	3	0	-	412
1994	894	888	893	899	901	898	21	7	8	0	7	4	0	-	921
1995	1369	1362	1371	1377	1380	1373	40	9	11	0	10	4	0	-	1400
1996	1723	1710	1727	1733	1739	1724	51	14	13	13	14	4	0	-	1759
1997	1997	1984	2003	2009	2015	1997	67	21	14	22	16	5	0	3	2037
1998	2137	2121	2141	2147	2155	2134	81	22	15	34	17	5	0	7	2178
1999	2251	2235	2256	2262	2270	2248	88	22	18	39	17	5	0	12	2295
2000	2307	2291	2313	2319	2327	2304	91	22	18	39	17	5	0	12	2352
2001	2348	2332	2354	2360	2368	2345	95	22	18	52	17	5	1	25	2393
2002	2359	2343	2365	2371	2379	2356	100	22	18	52	17	5	1	25	2404
2003	2369	2353	2376	2381	2390	2366	103	22	18	52	17	5	1	25	2416
2004	2390	2373	2397	2402	2411	2387	103	22	18	54	17	5	1	27	2437
%	98.0	97.3	98.3	99.0	99.0	98.0	4.2	0.9	0.7	2.2	0.7	0.2	0.04	4.2	

7.4 Discussion

The trend in *Salmonella* isolations set out in this chapter could be influenced by many factors: the number of *Salmonella* isolates from veterinary sources sent to laboratories; changes in serotypes of *Salmonella* in a year; changes in the recording system; changes in the animal population and changes in the Food Hygiene legislation and its implementation.

Samples of *Salmonella* infection were sent from a wide range of veterinary sources, including foodstuffs, animals, and environment. Clearly, if more samples from one group were sent, the major serotype in that group would occupy a larger proportion of the total population of isolates. For example, poultry constituted the main contributors to *S.* Enteritidis but *S.* Typhimurium was mainly from bovine sources. Thus, when

isolates from poultry in a year were higher than the isolates sent from cattle, the isolates from *S.* Enteritidis were predominant. Submissions could also be affected by non cyclical or unexpected seasonal factors. For example, the submissions from sheep fell because of the dry summer between 2003 and 2004 (Browning et al. 2005).

As the ST submissions were mainly from bovine sources, the decline or increase in the number of samples from cattle also modified the trend. Cattle isolates fell considerably between 1998 and 2003, a drop which may have been related to movement restrictions put in place due to an outbreak of Food and Mouth Disease (FMD).

In addition, many poultry submissions were from the poultry surveillance system and, as such, the submissions were not clinical cases. Any change in this system would affect the number of isolates sent to laboratories. After 1993, laws were modified. Before 1993, under the Zoonoses Order 1989, Poultry Laying Flocks Order 1989 and Poultry Breeding Flocks and Hatcheries Order 1989, owners of poultry farms were forced to test for *Salmonella.* At the end of 1993, a decline in reported *S.* Enteritidis from the poultry industry resulted in the removal of this requirement (Defra 1999; O'Brien and de Valk 2003). In 1994, a *S.* Enteritidis PT 4 vaccine was introduced into broiler breeders. In 1997, live attenuated *S.* Enteritidis vaccine was allowed for egg-laying flocks and then in 2000, the Lion Quality Stamp was introduced as well (Defra 2003). All of these interventions will have reduced submissions from poultry livestock (Browning et al. 2001a). From 1993 to 1999, poultry isolates from the non-statutory surveillance system were high. However, since 2000, the proportion of

incidents resulting from non-statutory surveillance has decreased (Defra 2003), thus modifying the trend from 1993 to 2000.

The decline of submissions from animals may be a consequence of a reduction of disease in animals or a lack of case submissions because of economic pressures; for example, one impact may have been BSE (Browning et al. 1999). A slaughter order for cattle in 1997 was implemented against BSE. The order is likely to have directly reduced the number of samples sent to laboratories. The results in this chapter show a decrease in submissions from bovine sources in 1998 and 1999. Another example is the reduction of isolates from cattle in 2001, possibly due to the Foot and Mouth Disease outbreak (Browning et al. 2003).

The introduction of a ban on feeding mammalian meat and bone meal to livestock in March 1996 may be another factor in reducing Salmonella infections. The rate of contamination of domestic processed animal proteins with samonella has decreased from 25% in 1993 to 2.1% in 2000 (Defra 2003). This would appear to parallel the decline of Salmonella infections reported here.

Changes in the record structure used in this study are documented (Defra 2003) and the effect of this is difficult to assess.

The trend of ST

Factors which may influence the trend in *Salmonella* submission rate may also affect the trend in ST. ST was mainly isolated from cattle so the decrease in bovine submissions would result in a decline in the number of ST. The bovine submissions

were affected by BSE and FMD during the study period. The cattle population in Scotland remained stable from 1990 to 2004 according to the SERAD census records, except for a small drop in 2002; it is therefore more likely that submission rates were affected by economic pressures or possibly, in the case of FMD, movement restrictions rather than either numbers of cattle or fundamental changes in the prevalence of infection. However, again, this will remain a moot point.

Phage type change may be related to the antibiotic usage

ST DT104 replaced phage type 204c as the major ST phage type from 1992 to 2001; subsequently phage type 40 emerged. Although it is difficult to ascertain why the major phage type changed, the historic data suggested that antibiotic usage may be a possible reason. The most common phage types have changed many times since 1969 (Anon 1998). In 1969, ST DT 29 was the most common multi-resistant type in humans and cattle. It was related to the use of apramycin, which is a similar compound to gentamicin (Anon 1998). Then, the third wave was caused by ST DT104 and is presented in the current study. Its replacement by DT 40 would be another interesting topic for future research.

Seasonal effects were not very significant in the submissions from animal sources

Throughout the study period, two seasonal peaks were identified. However, a significant pattern was not observed in every year. From 1994 to 1998, the time series showed that the ST DT104 isolations were similar in every four-week period. The observation is similar to a publication in 1999, which mentioned that there was no significant seasonal effect for the samples from veterinary sources (Browning et al.

2001b). There were a lot of ST DT 104 isolations from 1994 to 1998; a seasonal effect may have been masked by a larger "epidemic" of submissions.

The south of Scotland had higher prevalence

Although it is impossible to trace the original location of submissions, the data showed that there was higher prevalence in the south east and the south west animal health districts. The results in Chapter 3 also showed that the same areas had a higher prevalence of human submissions. From 1993 to 1996, 37.8 % of human ST DT104 cases reported in the areas were from the people who had regular contact with cattle (Calvert et al. 1998). Cattle are the major ST DT104 reservoir and direct transmission of ST DT104 from cattle to human has been demonstrated (Calvert et al. 1998; Wall et al. 1995). The transmission in rural areas can occur not only by foodborne spread but also by direct contact (Calvert et al. 1998). The cluster statistical methods employed in the following chapters will test the hypothesis.

R-score 6 and 7 were the most common R-types

In total, 2,346 out of 2,439 isolates belonged to R-score 6 and 7 and 2,325 out of 2,346 isolates were ApClSpStSuTe (2,194), ApClSpStSuTeTm (109) and ApClSpStSuTeNa (22) R-types. The main clone ApClSpStSuTe R-type is disseminated across the EU (Threlfall 2000) and the main reason the R-type score 6 is the most common form in this study. The emergence of Tm resistance is believed to be associated with the treatment against the ApClSpStSuTe R-type (Threlfall 2000), which was the second most common R-type throughout the study period. The third most common R-type was the ApClNaSpStSuTe with or without the additional Lc resistance. Fluoroquinolones were licensed for therapeutic use in the 1990s and

resistant strains found in 1996; enrofloxacin was licensed for veterinary used in the UK in 1993 (Threlfall et al. 1996). An increase in fluoroquinolone resistance in ST has been described, which parallels the time for which fluoroquinolone has been used in veterinary medicine (Threlfall et al. 1996). However, the fact that human usage parallels this period is often ignored.

Aminoglycoside resistance is another important issue. The R-type score 7 had 2 R-types which had the additional Gm and Ka resistances with the ApClSpStSuTe resistance. The active efflux pumps may play a role in moving aminoglycosides out, non-specifically. The ApClGmNeSpStSuTe R-type was found in bovine and ovine isolations reported from laboratories located in Thurso, Perth, and Ayr.

Regardless of the antibiotic in question, the epidemiology of antibiotic resistance varies according to three main factors: the size of the population of micro-organisms; the pre-exposure prevalence of resistance genes; and the fitness of the selected population of micro-organisms in competition with other micro-organisms present in the environment which have not been exposed to antibiotics (Georgala et al. 1999). These reasons explain why the main R-types would have a better chance of survival. The higher the R-type score a strain has, the higher the chance of survival. All the above assumptions were based on antibiotics being used constantly in a limited population in a limited area and this has almost certainly not been the case in the study period or study region. The high number of confounding factors described in this chapter make definitive explanations elusive as to why numbers have risen and fallen; one would expect the proportion of resistant organisms to increase over time in the face of selection pressures.

Conclusion

Although the trends of *Salmonella* and ST infections from veterinary sources were masked by many factors, they showed declines after 1998. The trend of ST DT104 was highly related to the isolations of ST DT104 from bovine sources and may have been influenced by other diseases such as BSE and FMD. The surveillance system and vaccine programme instigated in poultry livestock have been successful in controlling Salmonella infections, at least at the reporting level. The changes of resistance patterns remains more elusive and there are many assumptions that must be made, even at the most basic level. The relationships among R-types will be the focus of study in the following chapters.

CHAPTER VIII

HIERARCHICAL CLUSTER ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERNS IN *SALMONELLA* TYPHIMURIUM DT104

CHAPTER VIII

HIERARCHICAL CLUSTER ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERNS IN *SALMONELLA* TYPHIMURIUM DT104

8.1 INTRODUCTION

The specific strain *S.* Typhimurium definitive type DT 04 (ST DT104) has been isolated widely from humans, cattle, goats, sheep, fish, pets and wild animals (Browning et al. 2005; Threlfall 2000). The phage type is well known for its genome containing a cassette containing multi-antibiotic resistant genes (Threlfall 2000). After the penta-antibiotic resistant strain was first isolated, it was to be expected that resistance against ampicillin, chloramphenicol, streptomycin/spectinomycin, sulphonamide, and tetracycline would occur together (Threlfall 2000). The relationships among different antibiotic resistant patterns from the human ST DT104 submissions have been identified in Chapters 5 and 6. It was hypothesised that similar relationships would be identified in the animal submissions.

Hierarchical cluster analysis is a powerful tool for exploring and sorting data into a number of groups, as has been mentioned in Chapter 2 and Chapter 3. The aims of this Chapter were: 1) to understand the resistance patterns of antibiotics in isolates from animals, 2) to understand the relationship of phenotypes isolated from animal sources, and 3) to discuss the similarity and dissimilarity between the human and the animal results. The following Chapter 9 uses these results to identify the relationships in time, space and space-time.

8.2 MATERIAL AND METHODS

The dataset is the same as that used in Chapter 7. The study method was the same as described in Chapter 5 and relationships among thirteen antibiotic resistance patterns were investigated. Low level ciprofloxacin and cefotaxime were introduced to test for resistance in the Scottish Salmonella Reference Laboratory from 1997 and 1998, respectively. However, only 311 isolates could be included for analysis when all 15 antibiotic treatments were used and so the two antibiotic resistance patterns were excluded in the major study. When 13 antibiotics were used, there were 2,439 isolates included and so the dendrogram constructed from 13 antibiotic resistance patterns was the focus of the analysis. The dendrogram constructed from 15 antibiotic resistance patterns was used as the reference to understand the whole set of relationships. A K-means cluster analysis was executed to check the results. The procedure of executing the K-means cluster analysis and the hierarchical cluster analysis were introduced in Chapter 5. The different distance calculating methods, the square Euclidean distance (SED), the Euclidean distance (ED), and the simple matching (SM), which were introduced in Chapter 5, were also used to explore the distances amongst outliers.

8.3 RESULTS

8.3.1 Relationships among antibiotic resistance pattern

The three distance measurement methods generated similar results but a minor difference was observed. The dendrograms were the same when SM and SED were used (Figure 8.1). When 13 antibiotics were used, Cp, Fz, Ka, Gm, Ne, Na, and Tm were in the upper group, which was referred to Group A-A. Tm is located in the position nearest the lower group (Group A-B), in which Sp, St, Su, Ap, Te, and Cl lie (Figure 8.1). Figure 8.2 shows the dendrogram to be a little different from Figure 8.1. When ED was used to calculate the distance, the basic

shape was still the same but the distances between pairs were greater (Figure 8.2). In addition, Sp, St, and Su were closer to each other and Ap, Cl, and Te were closer to each other.

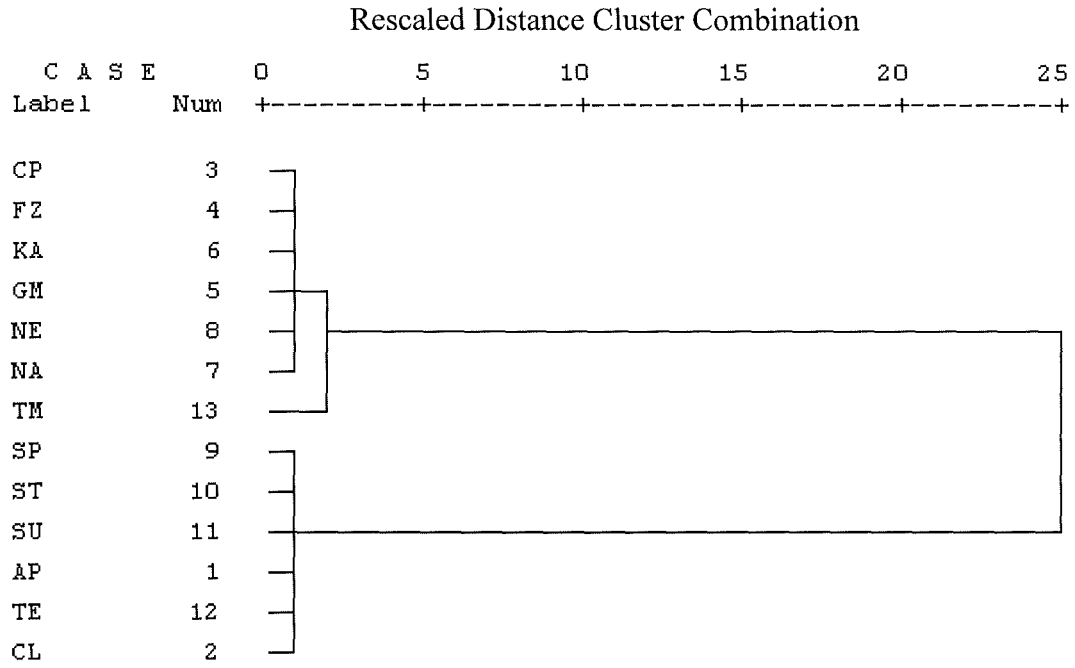


Figure 8.1 The dendrogram constructed from the 13 antibiotic hierarchical cluster analysis using the square Euclidean distance or the simple matching measurements, and the between group linkage method.

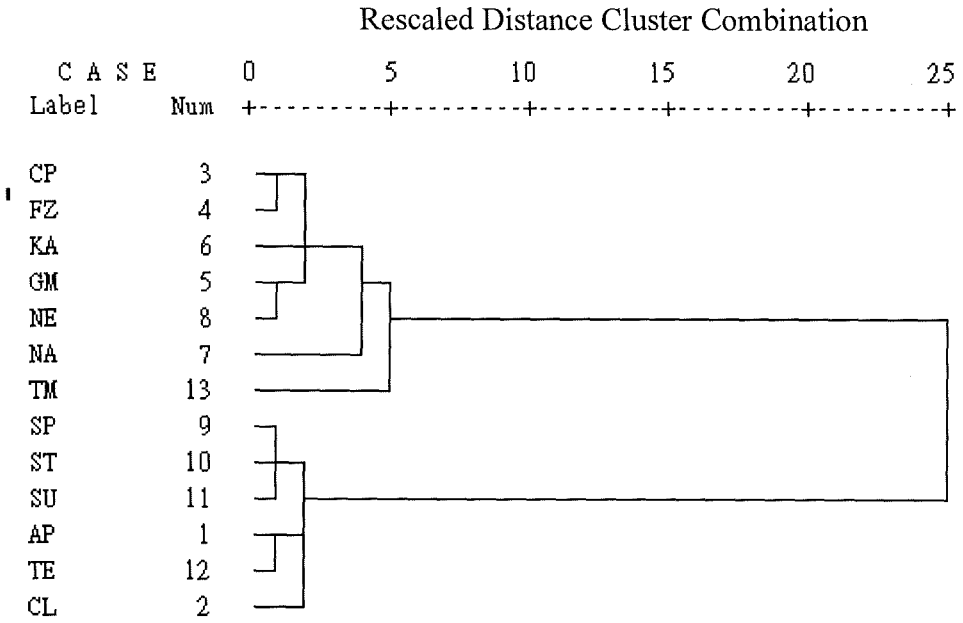


Figure 8.2 The dendrogram constructed from the 13 antibiotic hierarchical cluster analysis using Euclidean distance measurement, the between group link method.

8.3.2 The R-type relationships

Figure 8.3 shows the dendrogram of all clustered R-types. The figure shows there are two distinct groups, Group A and B. The distance between Group A and Group B is considerable. Thus, the first line to separate the two groups could be chosen from 15/25 to 20/25. The first line was set at 15/25 in the figure. The second line was set at the distance of 12/15. Thus, the line separated Group A into 4 subgroups, Group Aa, Ab, Ac, and Ad and Group B into two subgroups, Group Ba and Bb. The third line to separate the tree into 9 subgroups was set at 10/25. All the R-types in each different group are listed in Table 8.1. The results were checked by K-means cluster analysis.

When K=2, the results suggested that some subgroups in Group A were better classified in Group B. The second group contained 58 cases, which belonged to Group Ad without the subordinate Group No. 639, Group No. 217, Group No. 1 (from group Ab2), and Group 462.

Figure 8.3 The dendrogram of the R-types constructed from the 13 antibiotic hierarchical cluster analysis using SED measurement and the between group linkage method.

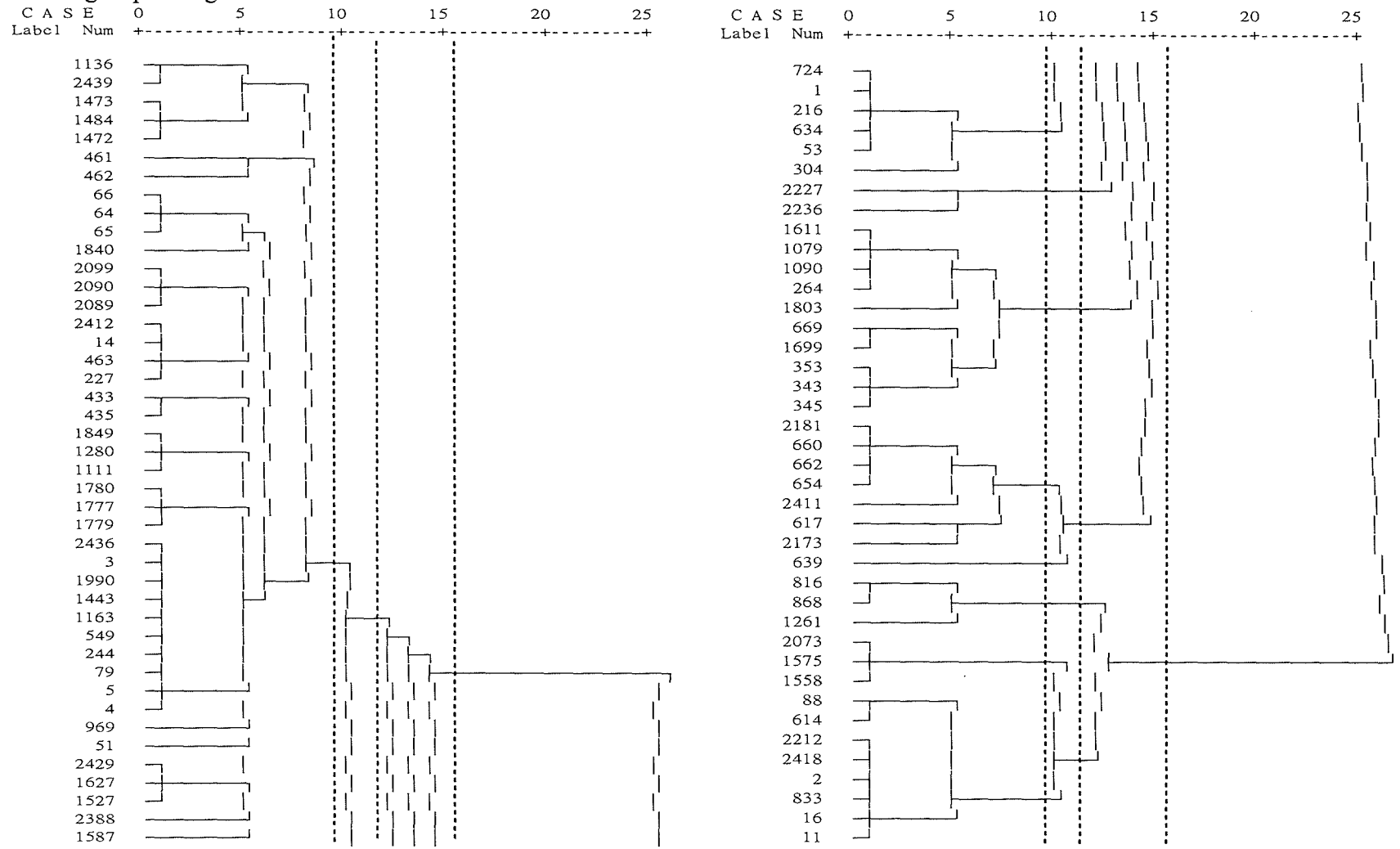


Table 8.1 The numbers of groups, sub-groups and R-types in each year

			No	R-types	Total	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
15	12	10	1136	ApSpStSuTe	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
A	a	1	1473	ApNaSpStSuTe	3	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
			461	ApClSpStTe	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			462	ApClSpSt	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			64	ApClFzSpStSuTe	4	0	3	0	0	1	0	0	0	0	0	0	0	0	0	0
			1840	ApClFzSpStSu	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
			2099	ApClNaSpStSuTeTm(Lc)	6	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0
			14	ApClSpStSuTeTm	90	0	1	1	0	16	18	10	16	8	5	3	4	5	3	0
			433	ClSpStSuTe	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
			637	ApClKaSpStSuTe	7	0	0	0	0	1	3	1	1	0	1	0	0	0	0	0
			1780	ApClGmSpStSuTe	5	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
			3	ApClSpStSuTe	2194	6	108	116	153	469	450	323	241	118	102	53	24	6	7	18
			969	ApClNeSpStSuTe	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
			51	ApClSpSuTe	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
			1452	ApClNaSpStSuTe(Lc)	41	0	0	0	0	0	0	9	8	6	4	0	12	0	0	2
			2388	ApClCpNaSpStSuTeLc	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
			1587	ApClGmNaSpStSuTe	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
				Total Aa1	2361	6	113	117	153	491	473	347	272	138	112	56	41	11	10	21
		2	1	ApStSuTe	6	1	1	1	0	3	0	0	0	0	0	0	0	0	0	0
			304	ApKaStSuTe	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
				Total Aa2	7	1	1	1	1	3	0	0	0	0	0	0	0	0	0	0
	b	1	2227	ApClKaNaSpStSuTeTmLc	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
			2236	ApClKaSpStSuTeTm	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
				Total Ab1	2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0

Table 8.1 (continued) the numbers of groups, sub-groups and R-types in each year.

		No	R-types	Total	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
	c	1	217 ApClGmNeSpStSuTe	8	0	0	1	1	0	2	3	1	0	0	0	0	0	0	0
			1803 ApClGmNaNeSpStSuTeLc	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
			669 ApClGmKaNeSpStSuTeTm	2	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
			353 ApClGmKaNeSpStSuTe	5	0	0	0	4	0	0	0	0	1	0	0	0	0	0	0
			Total Ac1	16	0	0	1	5	1	2	4	2	1	0	0	0	0	0	0
	d	1	653 SpStSu	16	0	0	0	0	4	3	5	2	0	1	1	0	0	0	0
			2411 SpSu	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
			617 ApClSpStSu	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			2173 ApSpStSu	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
			Total Ad1	19	0	0	0	0	5	3	5	2	1	1	1	0	0	1	0
		2	639 KaSpStSuTe	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			Total Ad2	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
			Total Ad	20	0	0	0	0	6	3	5	2	1	1	1	0	0	1	0
			Total A	2406	7	114	119	159	501	478	356	276	140	115	57	41	11	11	21
B	a	1	816 SuTeTm	2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
			1261 SuTm	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
			Total Ba1	3	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0
		2	835 ApSu	6	0	0	0	0	1	0	3	0	2	0	0	0	0	0	0
			Total Ba2	6	0	0	0	0	1	0	3	0	2	0	0	0	0	0	0
		3	88 Te	2	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
			2 FS	22	1	2	4	5	4	0	0	2	1	2	0	0	0	1	0
			Total Ba3	33	1	3	4	5	8	1	3	2	3	2	0	0	0	1	0
			Total	2439	8	117	123	164	509	479	359	278	143	117	57	41	11	12	21

Basically, the result was still similar to that in the hierarchical cluster analysis. When K=3, Group Ac and Ad were reallocated from Group A. Group Ac then formed the second group, and Group Ad together with Group B formed the third group. The results in the K-mean cluster analysis led to the conclusion that the first cutting site was suitable.

It is of note that Group A is much bigger than Group B and contains many different multi-resistant types. In total, 35 R-types were found but many R-types did not occur in large numbers during the study period. Only 5 R-types were present in numbers higher than 10, Group No. 14 (ApClSpStSuTe R-type), Group No. 3 (ApClSpStSuTeTm R-type), Group No. 1452 (ApClNaSpStSuTe(Lc) R-type), Group No. 653 (SpStSu R-type) and Group No. 2 (Fully sensitive type). Three main R-types, Group No. 14, Group No. 3, and Group No. 1452 were located in Group Aa. They are highly related and the distances between them are less than 5/25. Group No. 653 was located in Group Ad1 and Group No. 2 was located in Group Ba3 (Table 8.1). The other groups contained very few cases and represented less than 5% of the total per year. Figure 8.4 shows the patterns of each group by years. Group A, its subordinate group Group Aa1, and its grouped R-type Group No. 3 dominated the general pattern throughout the study period. From 2001, the proportions of another two R-types, Group No 14 (ApClSpStSuTeTm R-type) and Group No 1452 (ApClSpStSuTeNa R-type) in Group Aa1 became more significant. The Group No. 14 formed 45% and 25% of cases in 2002 and 2003, respectively. In 2001, there were 21% (14/57) of cases from Group No. 1452. All other groups, their sub-groups and related R-types formed a low proportion of cases and did not play significant roles throughout the study period.

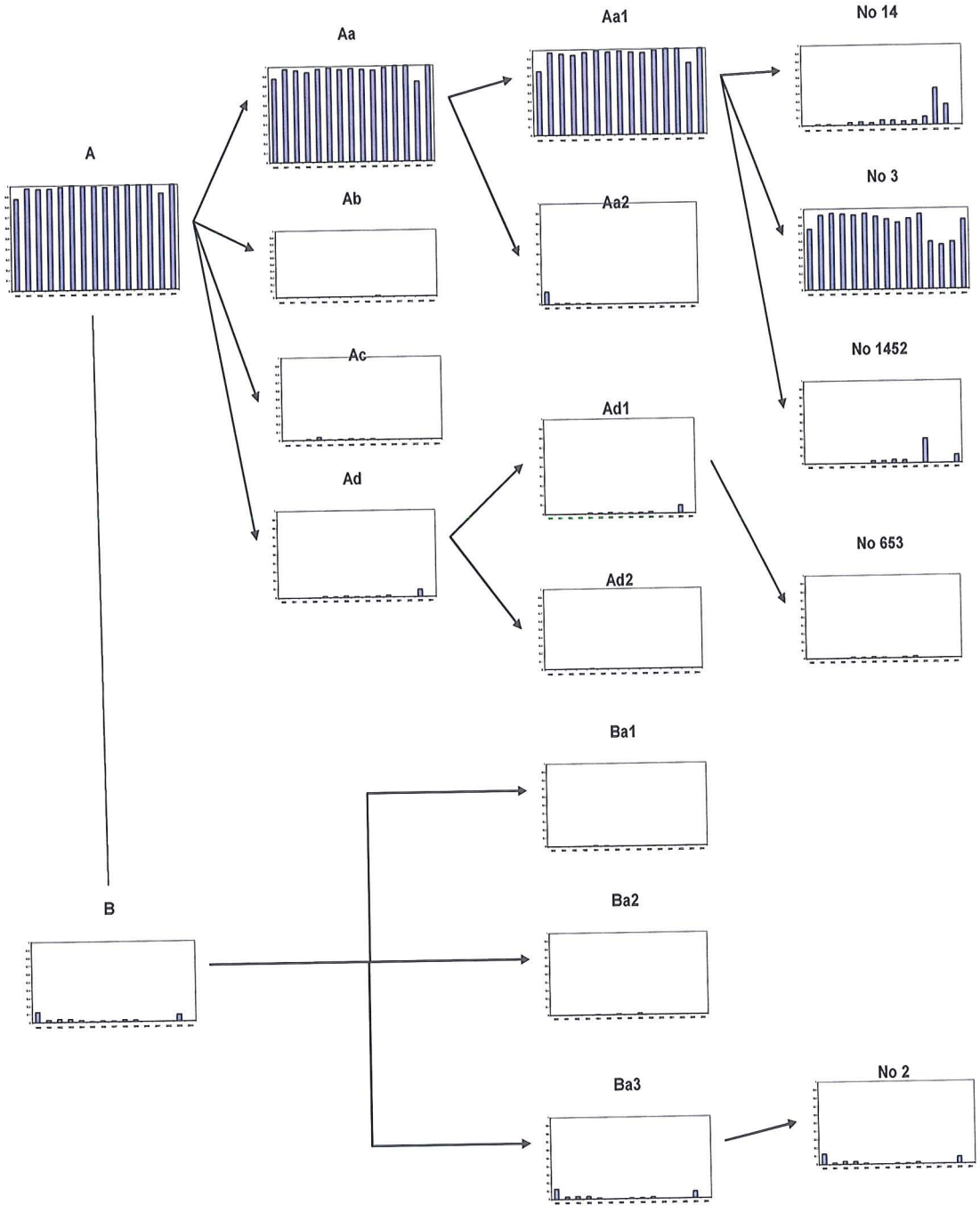


Figure 8.4 The proportions per year for each group, subgroups and R-types; Y-axis is the percentage; X-axis was the year from 1990 to 2004.

8.3.3. The comparison of the clustering results between human and animal datasets

The antibiotic and the R-type relationships from the human dataset have been identified in Chapter 5. The antibiotic relationships in the human submissions were the same as those in the animals (Figures 5.1, 5.2, 8.1, 8.2, and 8.3). Generally speaking, the pattern was the same in both the human and the animal datasets. Ap, Cl, Sp, St, Su, and Te were in the same group and the Cp, Cf, Fz, Ka, Gm, Ne, Na, Lc and Tm were located in the other group.

There were some differences in the R-type relationship although the main structure remained the same. There were 22 R-types found in both human and animal datasets despite the diversity in the Lc resistance pattern (Table 8.2). The SpStSu R-type was located in the animal Group A but it was located in the human Group B. It is the only R-type, which was located in a different group. All major patterns, the ApClSpStSuTe, ApClSpStSuTeTm, SpStSu, and FS R-types were identified in both human and animal databases. Although the orders of some R-types were a little different, the R-types still remained in the two distinct first order groups.

However, there were unmatched R-types; 29 in the human dataset and 13 in the animal dataset. Although the numbers of those unmatched R-types were few, the human R-types show higher diversity and the animal R-types show higher multi-resistance patterns (Table 8.3).

Table 8.2 The R-types found in both animal and human datasets. The R-types are sorted according to the sequence in the results of the human hierarchical cluster study.

Human NO	Animal No	Human NO	Animal No
Group A		Group B	
45 ApClSpStSuTe(LC)	3	306 Te	88
915 ApSpStSuTe	1136	38 FS	2
1987 ApClKaSpStSuTeTm	2236	268 ApSu	835
52 ApClSpStSuTeTm	14	642 SuTm	1261
2050 ApClKaNaSpStSuTeTmLc	2227	58 SuTeTm	816
1708 ApClNaSpStSuTeTm(Lc*)	2099	85 SpStSu	653
605 ApClNaSpStSuTe(Lc)	1452		
1827 ApClCpNaSpStSuTe(Lc*)	2388		
146 ApClFzSpStSuTe	64		
622 ApClKaSpStSuTe	637		
114 ApClSpSuTe	51		
574 ApClGmSpStSuTe	1780		
1826 ApClGmNeSpStSuTe	217		
2168 ApClSpSt	462		
11 ApStSuTe	1		
337 ApSpStSu	2173		

*: The animal type has Lc form but the human type does not

Table 8.3 Un-matched R-types in the animal and human datasets.

Human No.	N	Human	Animal	N	Animal No.
2395	1	ApClSpStSuTm	ApNaSpStSuTe	3	1473
531	9	ApClKaNaSpStSuTe(Lc)	ApClSpStTe	1	461
711	1	ApClNaStSuTe	ApClFzSpStSu	1	1840
146	4	ApClFzNaSpStSuTe	ClSpStSuTe	2	433
935	1	ApClFzNaSpStSuTe	ApClNeSpStSuTe	1	969
2506	1	ApClKaStSuTe	ApClGmNaSpStSuTe	1	1587
135	1	ApClSpSu	ApKaStSuTe	1	304
2548	2	ApClStSuTeTm	ApClGmNaNeSpStSuTeLc	1	1803
1211	1	ApClSuTeTm	ApClGmKaNeSpStSuTeTm	2	669
1299	2	StSuTe	ApClGmKaNeSpStSuTe	5	353
2400	1	ApSuTe	SpSu	1	2411
2158	1	ApCpNaSpStSuTeLc	ApClSpStSu	1	617
2519	1	ApCpSpStSuTe	KaSpStSuTe	1	639
2102	1	ApCpNaSpStSuTmLc			
53	2	Na			
2497	2	Ap			
442	3	ApStSu			
337	1	ApSpStSu			
1439	1	ApKaSu			
1210	1	ApNaSu			
1238	2	ApSuTm			
1593	1	SpStSuTe			
2446	1	SpStSuTeTm			
1534	3	SpSt			
1632	5	SpStSuTm			
2148	3	NaSpStSu(Lc)			
22	4	StSu			
2123	1	KaNaSpStSuTmLc			
1334	1	ClFzSuTeTm			
Total		29	13		

N: the numbers in the group

8.3.4 The independent test between the resistance patterns of nalidixic acid and low level ciprofloxacin

There were 27 isolates resistant to Na and Lc, 9 isolates resistant to Na but sensitive to Lc, no isolate resistant to Na and Lc, and 604 isolates sensitive to Na and Lc. The

McNemar test for independence showed that the responses were significantly different, ($p = 0.007$)

8.4 Discussion

The relationships among the different antibiotic resistant patterns from human isolates have been discussed in Chapter 5. They supported the assumption that the high levels of resistance of the Group B antibiotics are highly related to the emergence of chromosomally encoded antibiotic resistant ST DT104. The results in Chapter 5 also supported the assumption that the high levels of sensitivity of the Group A antibiotics was probably attributable to the genes in R-plasmids. From the results in Chapter 5 and the current study, the relationships among antibiotics in humans and animal isolates would appear to be similar. It suggested that some factors influencing the development of antibiotic resistant strains in humans could be the same as those in animals.

Whilst it is not possible from this study to confirm or refute a definitive relationship between the two populations of bacteria, what is clear is that there are many similarities when one clusters the different R-types. Speculation on why this might be the case requires more careful consideration of the relationships in time and space, as in particular, the temporal relationship between emergence in the human and emergence in the animal population may give some indication as to the direction of the spread of resistance types.

As outlined previously, It is suggested that the high usage of growth promoters in livestock, mutation and treatment are related to the development of antibiotic resistant strains (Threlfall et al. 2003). It would be a fair assumption that in the case of animal treatment, one might see emergence first in the animal population and then in the human. For example, Threlfall suggests that the emergence of the trimethoprim resistant ST DT104 strain was related to the treatment of ApClSpSuTe R-type (Threlfall et al. 1996). Although the Tm resistance was first identified in other phage types of *S.* Typhimurium, the trimethoprim resistance in ST DT104 was first recorded in 1991 in Scotland in the year of the increase of the ApClSpStSuTe R-type. The Tm resistance was recorded in the Scottish dataset one year earlier than that in England and Wales (Threlfall et al. 2005). There is as yet no any evidence to prove that the Tm resistance is associated with any chromosomally-encoded antibiotic resistance gene. Instead, evidence supports the association of the resistance with plasmids and Tm resistance associated with plasmids was reported as early as 1981 in California (Ikeda and Hirsh 1985). This study has shown a decline in the percentage of the ApClSpStSuTe R-type from 2001 and an increase in the percentage of the ApClSpStSuTeTm R-type. This may be due to competition and displacement. However, the decline of the percentages of ApClSpStSuTeTm R-type after 2002 may relate to the reduction in trimethoprim/sulphonamide use for therapeutic purposes in animals as the sales of trimethoprim/sulphonamide for therapy fell from 103 tonnes in 2001 to 77 tonnes active ingredient in 2004 (Anon 2007).

There is considerable evidence that the ST DT104 infections in humans relate to direct contact with animals and animal products (Threlfall et al. 1994; Wall et al. 1994; Threlfall et al. 2000). Thus, it is to be expected that 22 out of 35 R-types in the animal

dataset were found in the human dataset. In contrast, only 22 out of 50 human R-types were found in the animal dataset. What one cannot conclude, however, is where the resistance emerged first. Evidence of transfer of the organism does not necessarily imply origin of the resistance. For this temporal studies will be useful.

Hierarchical cluster analysis was a helpful tool for exploring a dataset and providing information about the structure of the dataset. The results identified the possible relationships among antibiotics and the R-types. The next chapters extend this using temporal, spatial, and spatio-temporal analyses.

CHAPTER IX

TEMPORAL, SPATIAL AND SPATIO-TEMPORAL CLUSTER ANALYSES OF ST DT 104 SUBMISSIONS FROM VETERINARY SOURCES, FROM 1990 TO 2004

CHAPTER IX

TEMPORAL, SPATIAL AND SPATIO-TEMPORAL CLUSTER ANALYSES OF ST DT 104 SUBMISSIONS FROM VETERINARY SOURCES, FROM 1990 TO 2004

9.1 INTRODUCTION

As outlined in earlier chapters in Scotland, samples from suspected cases of *S.* Typhimurium infection are analysed and then the confirmed results are stored at SSRL and HPS. According to the findings from the human dataset, the ST DT104 epidemic occurred from 1993 to 98. In addition, higher risk areas were found in the southern and eastern parts of Scotland. Higher risk areas were also identified in the outer islands. In contrast, Highlands and the central belt of Scotland were relatively lower risk areas. The previous results showed that the higher risk clusters in humans might be associated with the location of the cattle population. It is believed that 37.8 per cent ST DT104 cases which occurred in Dumfries and Galloway (DG) from 1993 to 96 may have regular contact with cattle (Calvert et al. 1998). In addition, for ST DT104, DG had the highest prevalence in both cattle and human (Calvert et al. 1998) and the assertion, though unsupported, was that the clustering of cases was related in the two species. The aim of this study was to use SaTScan to identify the most-likely clusters from the animal isolations in time, space, and time-space.

9.2 MATERIAL AND METHODS

Chapter 2 described the structure of the animal dataset. Chapter 6 described the general SaTScan methods that were used in both the animal and the human datasets. The main difference between the human and the animal datasets was the recording system. Each animal record contained the year and the week number when the isolation was sent. The basic time interval in this study was the year. In this study, it was assumed that clients usually sent samples to the laboratories near their work place. In addition, the study assumed that the locations of the laboratories, which identified the cases, were the locations where the cases occurred. The implications of these assumptions are discussed later

There are 17 veterinary laboratories involved in identifying animal *Salmonlella* spp. infections. The laboratories are scattered in 5 animal health districts (Table 9.1 and Figure 9.1). Because the true population of all animals was unknown, only a Bernoulli model was implemented to find the most likely clusters in time, space, and time and space. The study groups were previously generated as described in Chapter 8: Group ST, Group A ST DT104 isolations, Group Hex ST DT104 isolations, and Group FS ST DT 104 isolations were used as the 'case groups'. The corresponding 'control groups' were Non-Group ST, Group B ST DT104 isolations, Non-Group Hex ST DT104 isolations, and Non-Group FS ST DT104 isolations..

Table 9.1 The ST DT104 submissions from animal sources in 17 different laboratories, Scotland, 1990 – 2004.

Laboratories	Total
ABD:PHL	15
ABD:ROY	27
ABD:VET	328
AYR:ARL	1
AYR:VET	514
DMF:VET	580
DUN:ARL	1
EDI:AGR	235
EDI:VET	35
FIF:ARL	1
GLA:VET	36
INV:VET	65
MID:VET	73
OBA:LAB	1
PER:VET	203
ROX:VET	171
THU:VET	251
TOTAL	2,439

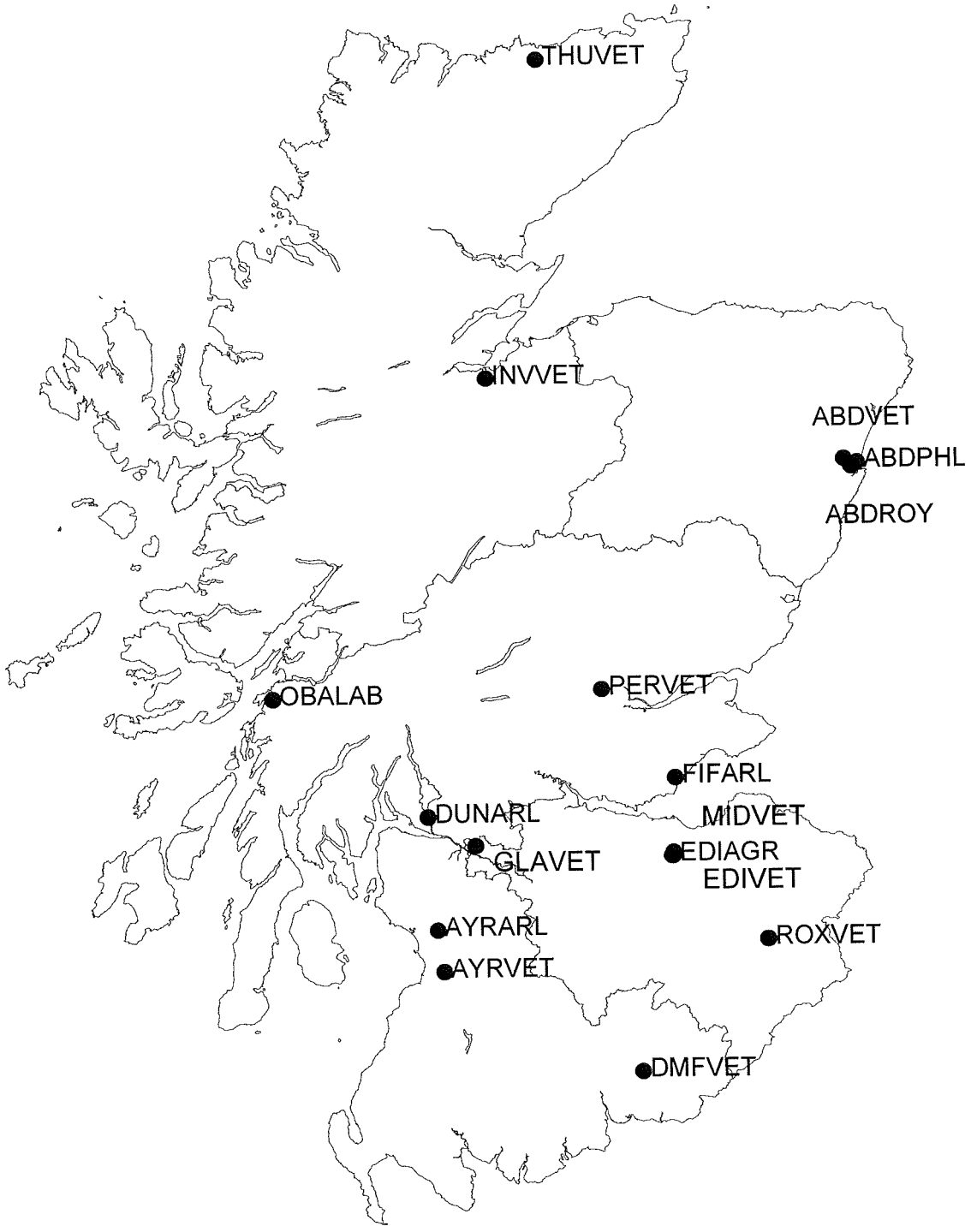


Figure 9.1 The positions of the 17 veterinary laboratories and the borders of the Animal Health Divisions

9.3 RESULTS

9.3.1 The comparison between Group *Salmonella* Typhimurium and Non-Group ST

The purely temporal study showed that Group ST had a lower relative risk cluster (LLRC) between 1997 and 2003 (p=0.001, RR=0.658): ST infections declined from 1997.

The results showed the centres of the circles from the comparison between Group ST and others were the same in the purely spatial and the spatio-temporal analyses. There was one SLRRC/STLLRC located at MIDVET. However, because there were very few ST isolates recorded in MIDVET this circle could be misleading. The SHRRC and the STHRC were identified as the same in both studies. They were located in the west-south part of Scotland (1990-96) and the east part of Scotland (1991-97) (Table 9.2).

Table 9.2 The radii of the identified circles from the comparison between Group ST and Non-Group ST based on Bernoulli model.

	Radius (km)	RR	P-value	Duration
Purely spatial				
MIDVET	0	*0.107*	*0.001*	
AYRVET, AYRARL, GLAVET, DUNARL, DMFVET	81.30	*1.859*	*0.001*	
ABDPHL, ABDROY, ABDVET, PERVET, INVVET, FIFARL, EDIVET, EDIAGR	159.76	*1.712*	*0.001*	
Spatio-temporal				
MIDVET	0	*0.052*	*0.001*	*1992-98*
AYRVET, AYRARL, GLAVET, DUNARL, DMFVET	81.3	*2.526*	*0.001*	*1990-96*
ABDPHL, ABDROY, ABDVET, PERVET, INVVET, FIFARL, EDIVET, EDIAGR	159.76	*1.834*	*0.001*	*1991-97*

9.3.2 The comparison between Group ST DT104 and Non-Group ST DT104

The purely temporal study showed a higher relative risk cluster from Group ST DT104 in the period 1992 to 1999 (p=0.001, RR=4.33). Thus, the study period was separated into 1990-91, 1992-99, and 2000-04. Table 9.3 shows the spatial and the spatio-temporal clusters from the comparison. There were 2 SLRRCs and 3 SHRRCs. The first most-likely SLRRC was in the southwest part of Scotland. The other SLRRC was in INVVET. The two SHRRCs were located in: 1) the central and the south west parts of Scotland and 2) in the south-east part of Scotland. The third SHRRC was located in the east part of Scotland (Aberdeen). The spatio-temporal analysis showed that only one STHRC was identified. The area covered the central belt and the south-east part of Scotland between 1994 and 98.

Table 9.3 The radii of the identified circles from the comparison between Group ST DT104 and Non-Group ST DT 104.

	Radius (km)	RR	P-value	Duration
Purely spatial				
AYRARL, AYRVET, GLAVET, DUNARL, MIDVET	88.91	*0.598*	*0.001*	
DMFVET, ROXVET	67.07	*1.436*	*0.001*	
THUVET	0	*1.618*	*0.001*	
INVVET	0	*0.656*	*0.002*	
ABDVET, ABDROY	3.79	*1.162*	*0.010*	
Spatio-temporal				
EDIAGR, MIDVET, EDIVET, FIFARL, ROXVET, PERVET, GLAVET, DMFVET, DUNARL	89.04	*1.888*	*0.001*	*1994-98*

9.3.3 The comparison between Group A and Group B

The purely temporal study showed that Group A had a THRRC in the period from 1995 to 1997 (p=0.032, RR=1.015). Table 9.4 shows the most likely spatial and spatio-temporal clusters identified in the comparison. The SHRRC was located in the southwest part of

Scotland. The SLRRC was located at INVVET. There were 3 STLRCs identified in the spatio-temporal analysis. The first STLRC was located in the southeast part of Scotland between 1990 and 93. The second STLRC was located at INVVET from 1995 to 2001. The third STLRC was identified at THUVET in the period from 1990 to 92. In contrast, the STHRC was located in the west-south part of Scotland between 1995 and 2001.

Table 9.4 The radii of the identified circles from the comparison between Group A and Group B.

	Radius (km)	RR	P-value	Duration
Purely spatial				
AYRVET, AYRARL, GLAVET, DUNARL, DMFVET	81.30	1.019	0.001	
INVVET	0.00	0.918	0.007	
Spatio-temporal				
ROXVET, EDIAGR, MIDVET, EDIVET	46.88	*0.841*	*0.001*	*1990-93*
INVVET	0	*0.562*	*0.001*	*1994*
AYRVET, AYRARL, GLAVET, DUNARL, DMFVET	81.3	*1.018*	*0.003*	*1995-2001*
THUVET	0	*0*	*0*	*1990-92*

9.3.4 The comparison between Group Hex and Non-Group Hex

In the period 2001 to 03, there was an identified TLRRC in the purely temporal cluster analysis (RR=0.637, p=0.001). Table 9.5 shows the identified spatial and spatio-temporal clusters from the comparisons. There was one identified SLRRC in the central belt of Scotland. The identified SHRRC was located in the southeast part of Scotland. The STLRC was identified in the circle composed by Glasgow, Dundee, Lothian, Oban, Perth, and Ayr within a radius of approximately 89 km from 1996 to 2002 in the spatio-temporal cluster analysis. The STHRC was found at DMFVET between 1994 and 96. It means a Group Hex higher risk cluster was located in the south part of Scotland.

Table 9.5 The radii of the identified circles from the comparison between Group Hex and Non-Group Hex.

	Radius (km)	RR	P-value	Duration
Purely spatial				
PERVET, FIFARL, EDIVET, MIDVET,EDIAGR, GLAVET, DUNARL	78.11	*0.895*	*0.001*	
DMFVET	0	*1.078*	*0.001*	
Spatio-temporal				
DUNARL, GLAVET, AYRARL, AYRVET, OBALAB, PERVET, MIDVET, EDIVET, EDIAGR	89.04	*0.854*	*0.001*	*1996-2002*
DMFVET	0	*1.107*	*0.001*	*1994-96*

9.3.5 The comparison between Group FS and Non-Group FS

The purely temporal study showed that Group FS had a TLRRC between 1995 and 96. Thus, the study period could be separated into three periods: 1990 to 94, 1995 to 96, and 1997 to 2004. Table 9.6 shows the identified spatial and spatio-temporal clusters from the comparison. There were 2 SHRRCs identified: 1) at INVVET, and 2) in the southeast part of Scotland in the purely spatial cluster analysis. In addition, a SLRRC was located in the southwest part of Scotland. The spatio-temporal analysis showed that there were 3 identified STHRCs and 1 identified STLRC. The three STHRCs were located in 1) in the southeast part of Scotland (1990 - 93), 2) at INVVET in the period (1994 - 99), and 3) at THUVET (1990 - 92). The STLRC was located in the southwest part of Scotland in the period from 1991 to 97.

Table 9.6 The radii of the identified circles from the comparison between Group FS and Non-Group FS based on Bernoulli model.

	Radius (km)	RR	P-value	Duration
Purely spatial				
AYRVET, AYRARL, GLAVET, DUNARL, DMFVET	81.30	*0.115*	*0.004*	
INVVET	0	*10.742*	*0.008*	
ROXVET, EDIAGR, MIDVET, EDIVET	46.88	*3.745*	*0.068*	
Spatio-temporal				
ROXVET, EDIAGR, MIDVET, EDIVET	46.88	*27.45*	*0.001*	*1990-93*
INVVET	0	*21.444*	*0.003*	*1994-99*
THUVET	0	*121.850*	*0.012*	*1990-92*
AYRVET, AYRARL, GLAVET, DUNARL, DMFVET	81.30	*0.069*	*0.036*	*1991-97*

9.4 Discussion

Although the original intention at the outset of the study was to investigate the animal cases at the same resolution as the human cases, this was not possible because of the form in which the data were collected and maintained. The approach adopted in this chapter has many short comings but was an attempt to address the problems associated with the structure of the animal data.

One disadvantage of the Bernoulli model is that it only presents relative risk clusters not the true epidemic curve. Generally speaking, the results of the Bernoulli and Poisson model should be very similar (Kulldorff and Nagarwalla 1995). However, the Bernoulli model study is like a case control study and has all of the disadvantages of a case-control study. The results are affected by control groups and show the relative risk clusters. Thus, it should not be considered the same as showing a epidemic curve as might be identified using other techniques. However, the study can present possible relative higher/lower risk clusters over time, in space or in time-space.

The quality and quantity of outcomes highly depend on the original data. The main errors can be identified into two categories. Firstly, from the spatial perspective, precision of case location affects the results. In this study, the locations of laboratories were used instead of the true locations of cases. Secondly, in this study, the cases were assumed to have occurred in the district around the laboratories. This was for two reasons: the location of cases was inconsistently recorded and the underlying population denominator was not available. In effect what was being examined was the features of the populations of isolates at each laboratory. It was an attempt to understand the distribution of higher/lower risk clusters with the laboratory as the unit of interest. Ideally, a study should be based on actual location of infected farms in Scotland and on bovine farms only. If that is not possible, using the location of farms and the average ST DT104 infection rate or individual infection rate for each farm (similar to how the laboratories have been treated here) would be a sensible approach.

The statistical power is different in each comparison because the denominator is different (Kulldorff, 1997), although the statistical power for each result is always satisfied in each test. Thus, fewer cases in the control group will dramatically affect the results and it will bring some bias to the results. For example, control data containing 1 case in each area was used (Group fake 1). When Group Hex was compared with Group fake 1, it showed a lower risk cluster from 2001 to 2004 ($p=0.001$, $RR=0.760$). When 100 cases were used in each area in the control group, the result showed that the higher risk cluster was from 1993 to 1997. When 200 cases were put in each area in the control group, the result showed that no HRC or LRC was found. The Bernoulli model is not suitable for the comparison between groups, when one group does not have enough cases (the statistical power is not sufficient). In SaTScan, the case and the control group can be reversible (Kulldorff 1997). Group FS did not contain any

cases in 1995 and 1996. Thus, the comparison between Group Hex and Group FS was not able to show any meaningful results and the results were not shown. .

Fewer cases in some laboratories may result in misleading clusters. Two LRRCs were identified at INVVET and MIDVET, with radii of 0 km. The two identified LRC laboratories have a common feature, which is that they are farther from other laboratories. The size of an area/location and the numbers in an area/location are factors which influence the results (Kulldorff and Nagarwalla 1995). This means the distance between locations and the numbers compared with other locations will have an effect. Thus, the MIDVET, identified as a LRC because it had very few cases, could be misleading. The cluster at INVVET could also be misleading. When the dataset was checked, 6 out of 65 cases belong to Non-Group Hex and 5 out of 6 cases belonged to Group FS. Thus, it appears that the LRC located at INVVET might relate to the large distance from others.

ST DT104 has replaced phage type 204c as the major type in the UK (Anon 1998). Both the human and the animal datasets showed that the proportion of ST DT104 surged from 1991 (Chapters 3 and 7). However, the epidemic duration appears to be different. The identified epidemic duration from the animal dataset (Bernoulli model) was from 1992 to 99 but that from the human dataset (Poisson model) was between 1993 and 98. From the human dataset based on the Bernoulli model, the temporal lower risk cluster was range from 1988 to 1992. Whether one year constitutes sufficient difference to be biologically meaningful (animal predating human) is doubtful but the co-occurrence at least suggests that there were similar factors influencing the spread of ST DT104 in both humans and animals.

The decline from 1997 in ST submission might relate to the restriction of movement in cattle. A decline from 1998 in humans was also identified (Chapter 6). A report suggests that the reasons for the fall in human cases in 1998 are still unclear but may be explained by improved catering practice, a cooler summer, and lighter contamination of uncooked foods (Scottish Centre for Infection and Environmental Health 1999; Handysides 1999).

Higher risk clusters from Group A and its subgroup Group Hex were identified in the southern part of Scotland; in contrast, the higher risk clusters from Group B and its subgroup Group FS were usually identified in the middle and eastern part of Scotland and Highland, which is the northern part of Scotland. A report published by Calvert et al. (1998) on ST DT104 submissions from 1993 to 96 concluded that the Highland and the Dumfries and Galloway (DG) had a higher prevalence of infection in animals although the human prevalence was generally uniform across mainland Scotland excluding DG (Calvert et al. 1998). The current study and the study described in Chapter 6 suggest that the southern area of Scotland, which covers the DG Health Board contained the higher risk clusters from mult-resistant ST DT104 (Group A and Group Hex). The two results in Chapters 6 and 9 also identified higher risk clusters from low resistant ST DT104 (Group B and its subgroup Group FS) in the northern part of Scotland.

In conclusion, not withstanding the short comings of the data available for study in this chapter, the analysis presents the first description of regional variarion in resistant ST DT104 and sub-groups. The results suggest that the animals in the southern parts of Scotland had more chance to become infected with multi-resistant STDT104 strains, especially in the epidemic period from 1992 to 99. It is worth remembering that very few FS strains would be submitted to veterinary laboratories because of low morbidity or mortality caused by the

strain due to successful therapeutic intervention. Further studies locating isolates to farm are clearly necessary.

CHAPTER X

TIME SERIES ANALYSIS OF HUMAN AND ANIMAL DATA RELATING TO *S.* TYPHIMURIUM PHAGE TYPE DT 104

CHAPTER X

TIME SERIES ANALYSIS OF HUMAN AND ANIMAL DATA RELATING TO *S.* TYPHIMURIUM PHAGE TYPE DT 104

10.1 INTRODUCTION

In Chapters 3 and 7, significant seasonal patterns in occurrence were observed in humans but were not observed in animals. Although the three stage time series lines were identified in Chapter 5, there currently is no tool to describe the general patterns of ST DT104 infection in Scotland. This Chapter describes the development of time series models, which can identify the seasonal effects and describe the general patterns. Thus, the generated model can be used to detect unusual increases in reported cases as rapidly as possible.

Time series analysis is a method used to: 1) identifying the characteristics represented by the series of observations, and 2) forecasting future values of a data stream (Pankratz 1983). The auto-regressive integrated moving average model (ARIMA) was set up by Box and Jenkins in the 1970s (Box and Jenkins 1976). The ARIMA model combines the benefits of auto-regressive and moving-average models and minimises the disadvantages of both. It describes the historic data well and can use the results generated to get a reasonable prediction ability (Pankratz 1983; Box and Jenkins 1976). In addition, it has two advantages: 1) low development and maintenance costs, and 2) it provides a baseline for prediction (Smelser and Baltes 2001).

Time series study has been used widely, especially in economic activities, such as setting policies and financial management (Smelser and Baltes 2001); however, it is still rarely used in understanding salmonellosis. Waltier and his colleagues attempted to develop time series models for non-typhoid *Salmonella* infections in France, *S.* Bovismorbificans infection, and *S.* Typhimurium infection, respectively (Watier et al. 1991; Watier et al. 1993; Watier 1995). The models are more complex but are based on an understanding of the ARIMA models. No time series model has yet been built for the Scottish *Salmonella* submissions. The ARIMA model is a good choice for building up the essential knowledge.

The aims of this study were to use the univariate Box-Jenkins time series model to find the best models for both human and animal data streams, to evaluate the good and bad points of the models and to assess them in an epidemiological context. Ideally, the aim was to compare the series and establish whether there was a direct relationship between the animal and human trends. The results were also expected to be a base for further time series studies.

10.2 MATERIAL AND METHODS

The SPSS 14.0 trend package was used in this study. It has two model builders: 1) the exponential smoothing model and 2) the ARIMA model, which were used to find the best models. Only the ARIMA models were used in this study.

Seasonal patterns can be detected using the auto-correlation function (ACF) and the partial auto-correlation function (PACF). ACF was used to investigate how the correlation between any two data points of the series changes as their separation changes. It shows two outcomes: whether the time series is stationary and whether a seasonal pattern is present. The ACF value

at lag 1 means the value of the correlation between an observation at the time *t* with the observation at the time *t-1*. The PACF removes the requirement for stationary in the ACF and shows clearer seasonal patterns in a fixed interval time period (Box and Jenkins 1976; SPSS Inc. 2006). When seasonal patterns are present, the graphs will show periodic peaks in fixed interval lags (months). The strength of peaks will decrease gradually to zero because the data points are not infinite. In this study, 36 lags (36 months) were used to understand the periodic cycle.

Data were cleaned and coded in Excel 2000 according to the requirements of time series study, which required cases at a fixed time interval. The month was the minimum time interval in this study and cases submitted in the same month were grouped together. The submissions from humans (1988 to 2004) and animals (1990/7 to 2004) were used, respectively. For model comparison, a second human model was generated using data from 1990/7 to 2004/12.

The human and animal record systems were different. Each record in the human dataset contained the date of submission, and so this submission date was used directly. In contrast, each animal submission only contained the week number (1 to 53) when it was submitted. Every animal case was assumed to be submitted on Monday. The date corresponding with the Monday was used to as the date of submission. Hence, cases were re-grouped into different months according to the newly recreated dates. A new time series containing the total cases in each month was formed and it was used in this study.

The expert modeller pack in the SPSS 14.0 trend package was used to select the best models automatically. The selected models were assessed by customer models again according to the

good model criteria described by Box and Jenkins: the lowest root-mean-squared error (RMSE), the lowest mean absolute percent error (MAPE), and the lowest normalized Bayesian index criteria (BIC) (Pankratz 1983; Box and Jenkins 1976; SPSS Inc. 2006). The independence of residuals of each model was measured according to the ACF and PACF of residuals. The residuals were graphed in Q-Q plots to ascertain if they were random. If many points in the tails in the Q-Q plots are far from the central line, it means the data stream may not be from a normal distribution (SPSS Inc. 2006).

The stationary R-square was used to assess the goodness-of-fit. When a model contains a trend or a seasonal pattern, the value is a suitable estimate of the proportion of the total variation in the series that is explained by the model (SPSS Inc. 2006). However, if there is no trend of seasonal pattern in the model, the R-square can be used directly. The higher the stationary R-square or R-square, the better the model.

A good time series model should have an acceptable quality in forecasting. In order to be able to assess a model's ability to forecast accurately, a function called 'holdouts' was used. In this study, new predictive values for 2004 were generated using data points from 1988 to 2003 in the human dataset. For the animal dataset, the same method was adopted for data from 1990/7 to 2003. The observations in 2004 were compared to understand the predictive ability for each group.

It became apparent that the quality of the animal related data was not as robust as the human isolate data. In order to carry out a comparison based on the resistance to different antimicrobials, simple cumulative plots of isolates over time were generated and the dates of emergence compared.

10.3 RESULTS

10.3.1 The model for the human dataset from 1988 to 2004

Figure 10.1 shows that there is a strong seasonal pattern in human data from 1988 to 2004. The ACF graph shows peaks occurred in lag 1, 12, 24 and 36. Each data point is highly related to the previous data point, the 12, 24, and 36 month earlier data point. The relationship between a data point with its previous fixed interval period data point decreased to zero as time passed. The PACF graph shows one big peak in lag 1 and the peaks still occurred in a 12 month interval. The pattern suggests that it might be a ARIMA$(0,1,1)(0,D,Q)_{12}$. Also, when checked the seasonal peaks, it still shows a gradual decrease. Thus, the seasonal component could be $(0,0,1)_{12}$ or $(0,1,1)_{12}$. After careful selection by the criteria given above, the ARIMA$(0,1,1)(0,1,1)_{12}$ is the best-fit (Table 10.1). The Q-Q plot shows that the residuals are from a normal distribution, although few data points are higher or lower than expected (Figure 10.2) and the residual ACF and PACF graphs show that there is no significant peak and periodic peak. It means all residuals are independent (Figure 10.3).

Figure 10.4 shows that the model could catch the peaks very well in the epidemic period, mainly from 1993 to 2000. From 1988 to 1991/6, it was still good at identifying the trend but not very good at tracing the peaks. After 2001, the model can detect peaks but over-weights the seasonal component quite often. The prediction values fall faster than observations in the non-season period.

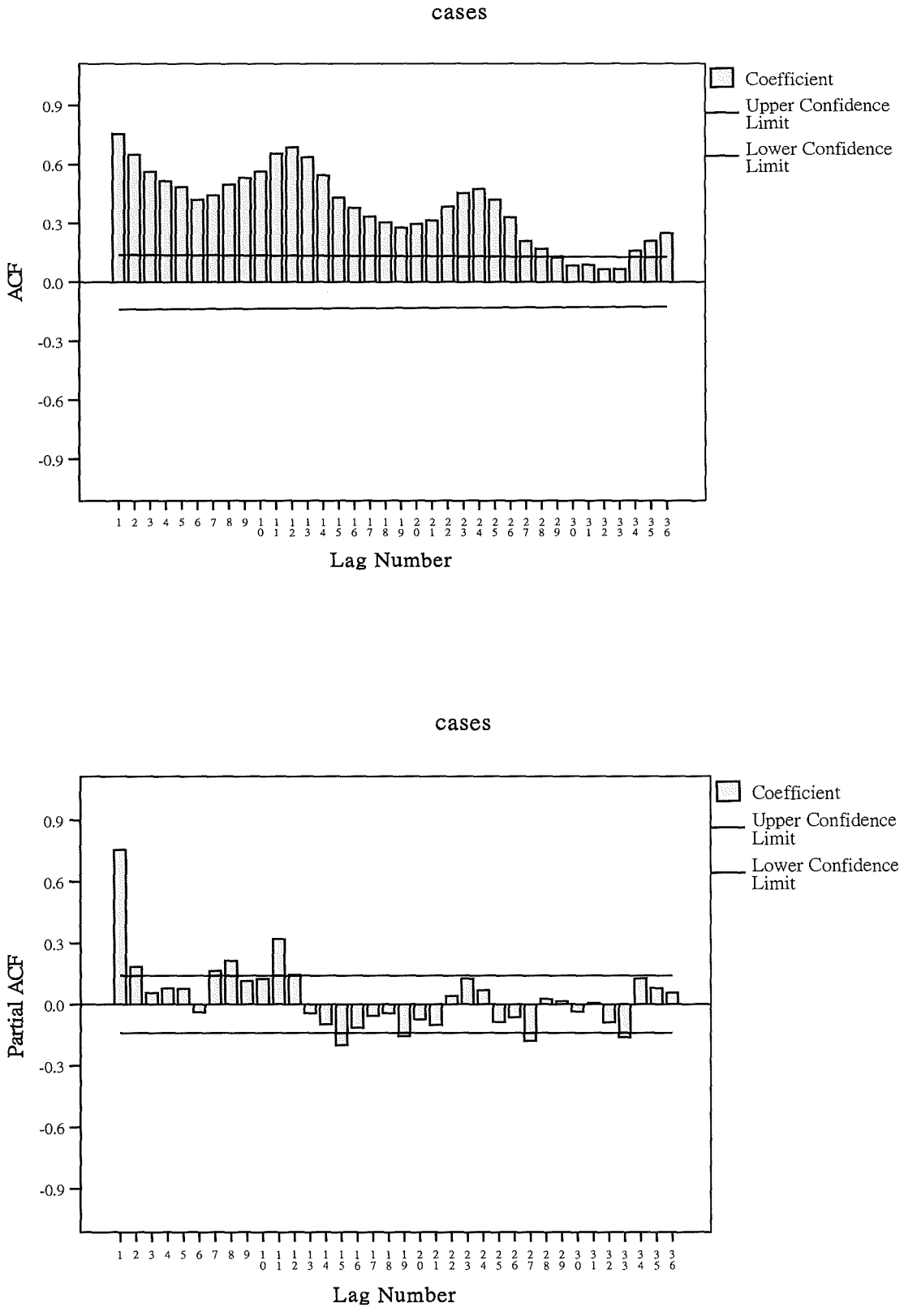


Figure 10.1 The ACF (above) and the PACF (below) graphs of human dataset from 1988 to 2004. The X-axis is the month.

Table 10.1 The information of human model parameters and criteria index

The ARIMA $(0,1,1)(0,1,1)_{12}$ model

From 1988/1 to 2004/12 (0,1,1)(0,1,1)			Estimate	SE	t	Sig.
Human model	Difference		1			
	θ 1	Lag 1	.744	.049	15.191	.000
	Seasonal Difference		1			
	θ 12	Lag 1	.741	.060	12.457	.000
Stationary R-square	0.591	MAPE	65.12	RMSE	6.99	

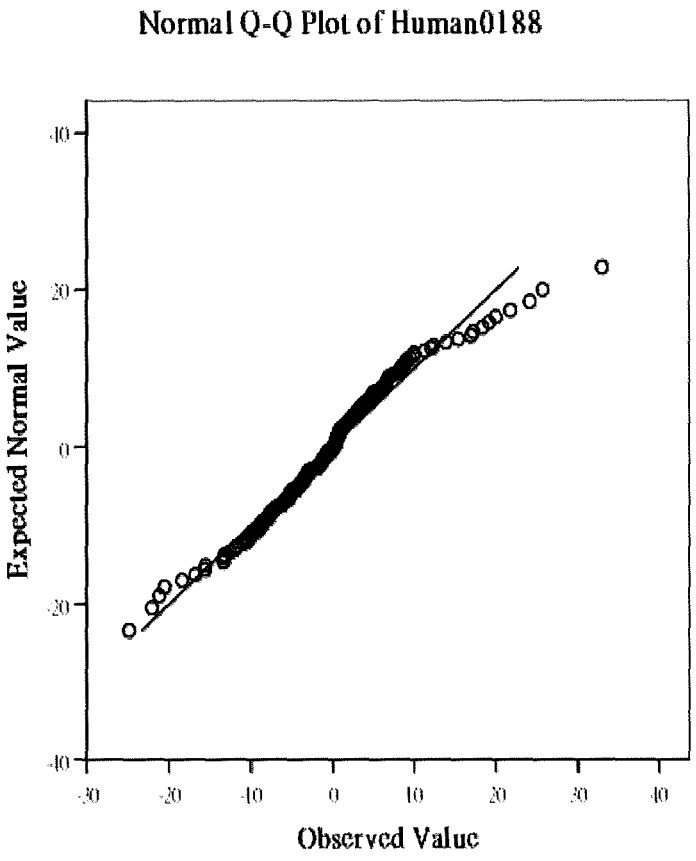


Figure10.2 The Q-Q plot of the residuals from the human model (1988 –2004)

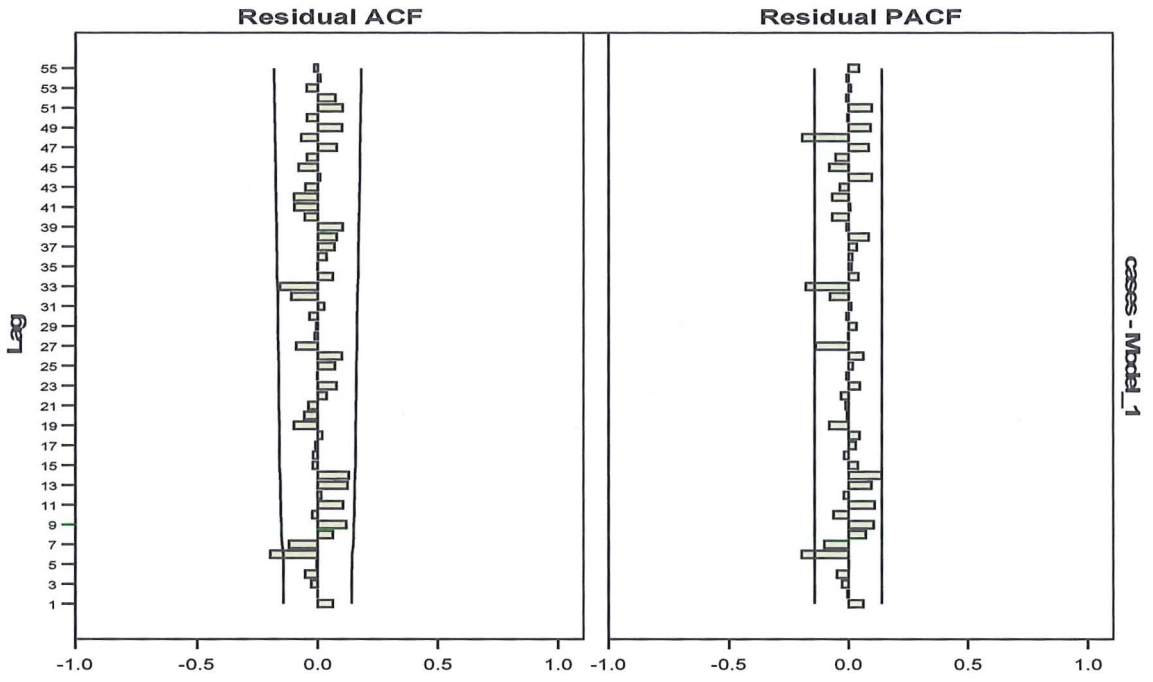


Figure 10.3 The residual ACF and PACF graphs from the human model (1988-2004)

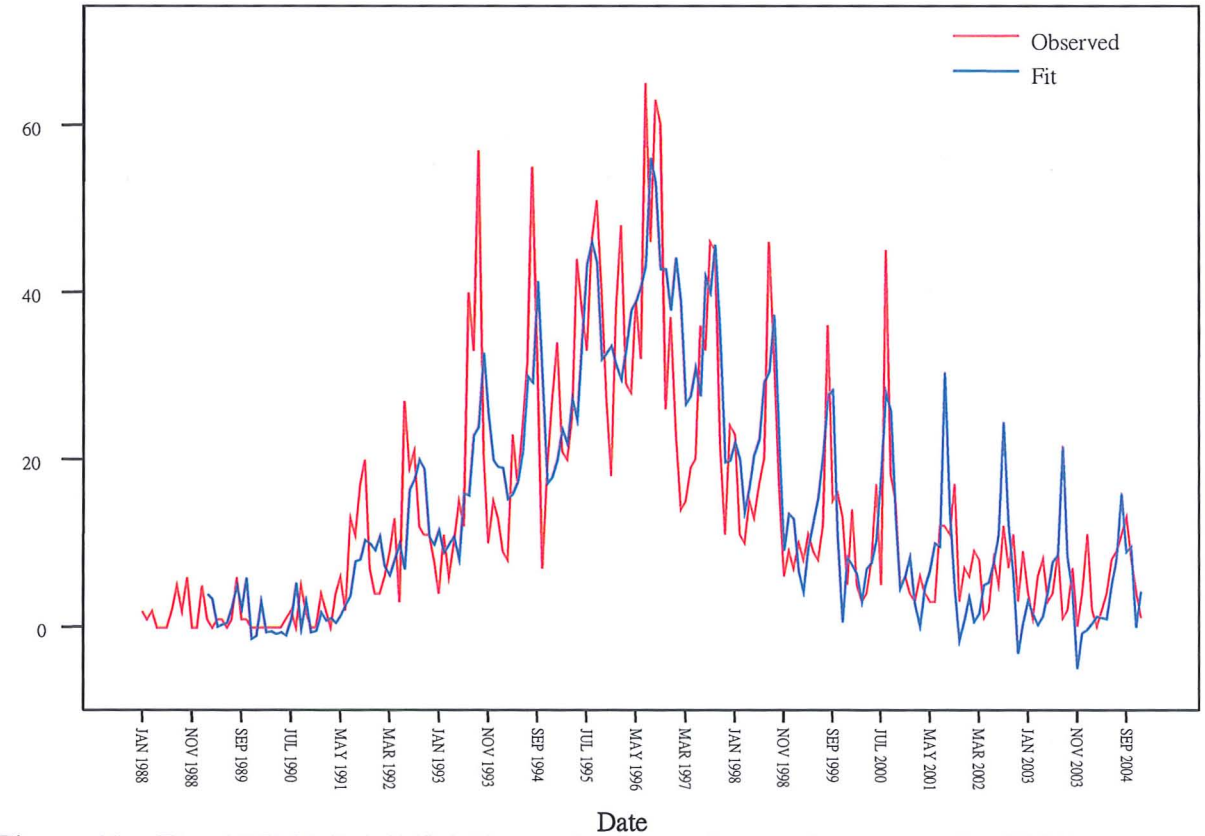


Figure 10.4 The ARIMA(0,1,1)(0,1,1)$_{12}$ model for the human dataset (1988 – 2004)

10.3.2. The model for the human dataset from 1990/7 to 2004

This model using human data from 1990/7 to 2004 was similar to the model using the whole data stream (Table 10.2 and Figures 10.5 to 10.7). Figure 10.6 shows the distribution of the residuals is almost from a normal distribution, although few data points are still higher than expected. Figure 10.8 shows the predictive values in this model are almost the same as the predictive values in the model shown in Figure 10.4. They also show almost the same pattern. However, the higher MAPE and RMSE and the lower stationary R-square show that this model is a little worse than the model using the whole time series (Table 10.2).

Table 10.2 The information of human model parameters and criteria index (1990/7 to 2004)

The ARIMA $(0,1,1)(0,1,1)_{12}$ model (1990/7 to 2004)

From 1990/7 to 2004/12 (0,1,1)(0,1,1)			Estimate	SE	t	Sig.
Human model	Difference		1			
	θ1	Lag 1	.737	.055	13.401	0.000
	Seasonal Difference		1			
	θ12	Lag 1	.803	.073	11.058	0.000
Stationary R-square	0.588	MAPE	79.21	RMSE	9.25	

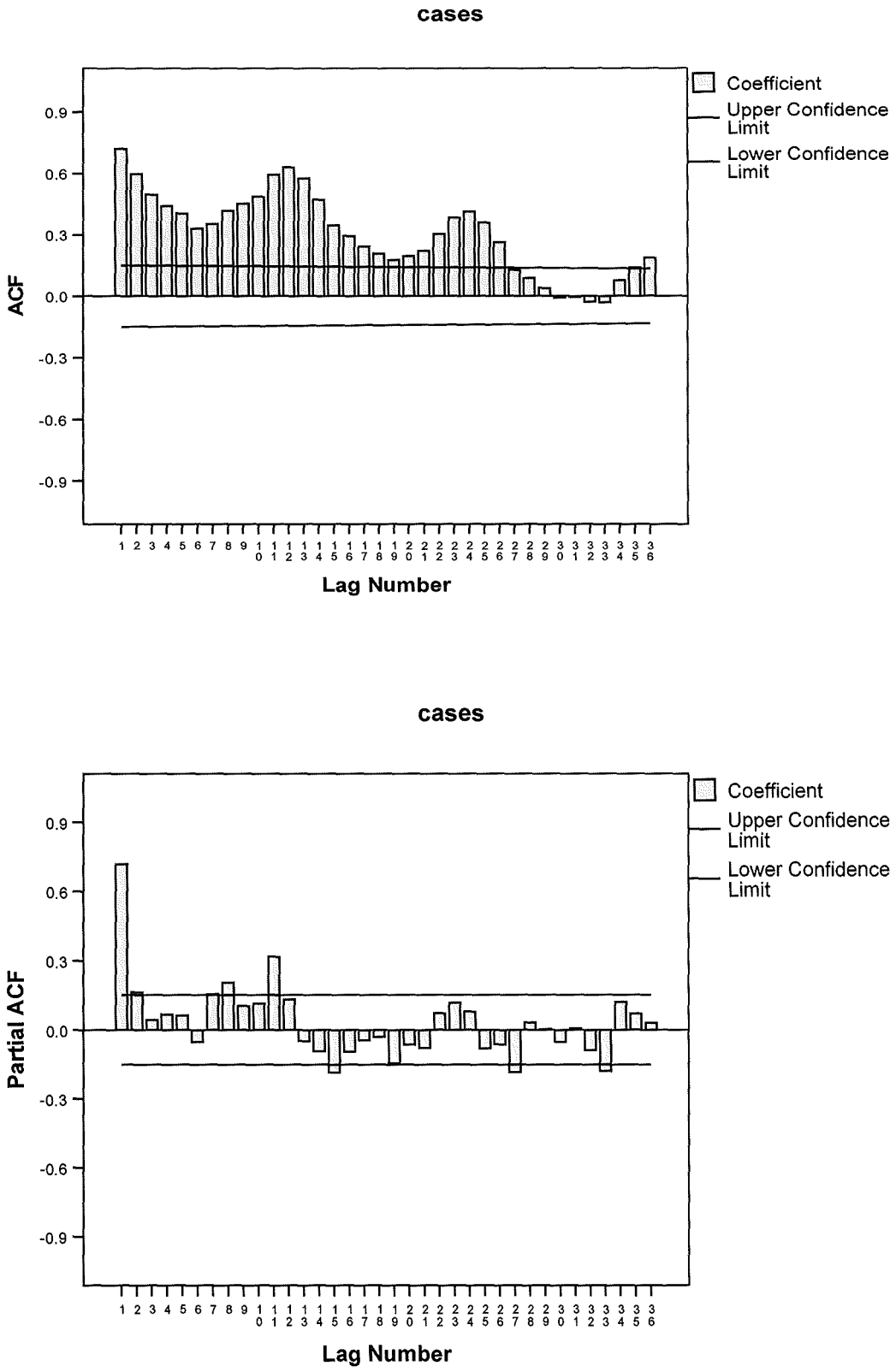


Figure 10.5 The ACF (above) and the PACF (below) graphs of the human dataset from 1990/7 to 2004. The X-axis is the month.

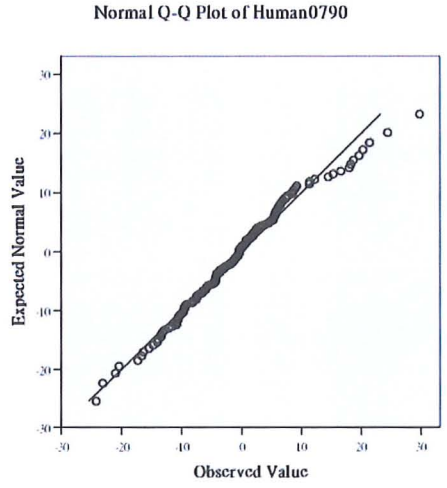


Figure10.6 The Q-Q plot for the residuals from the human model (1990/7 – 2004)

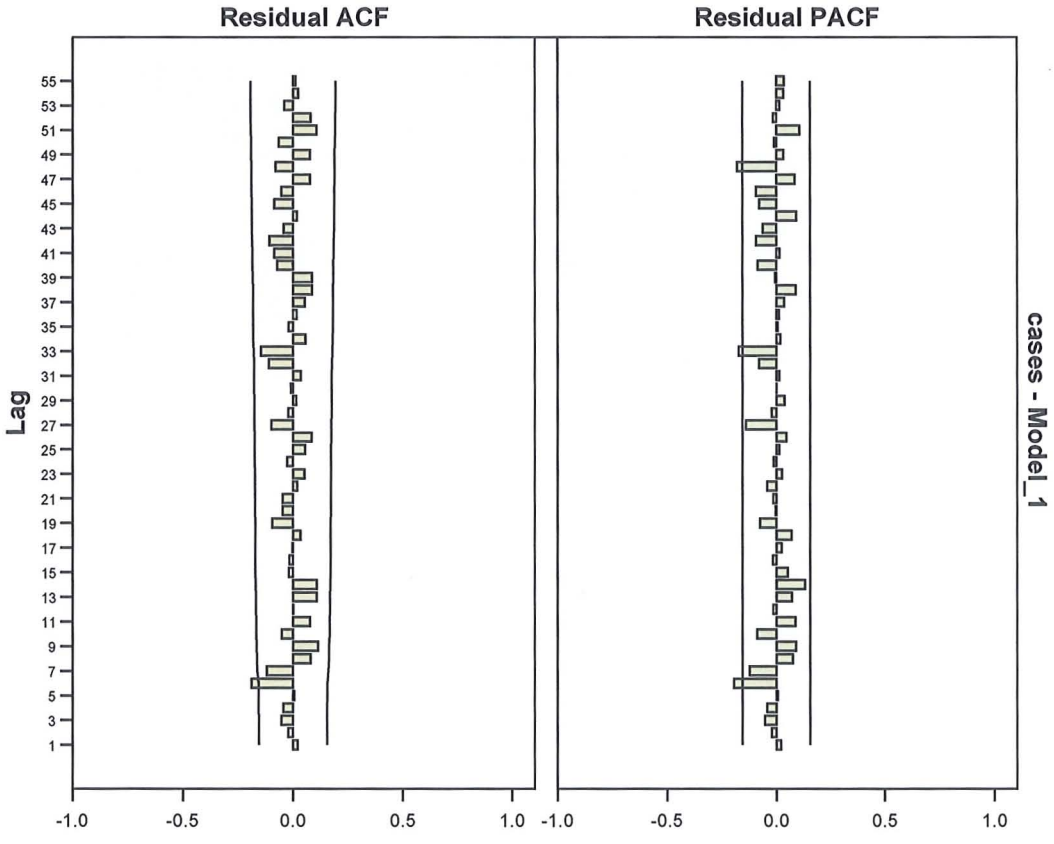


Figure 10.7 The residual ACF and PACF graphs from the human model (1990/7-2004)

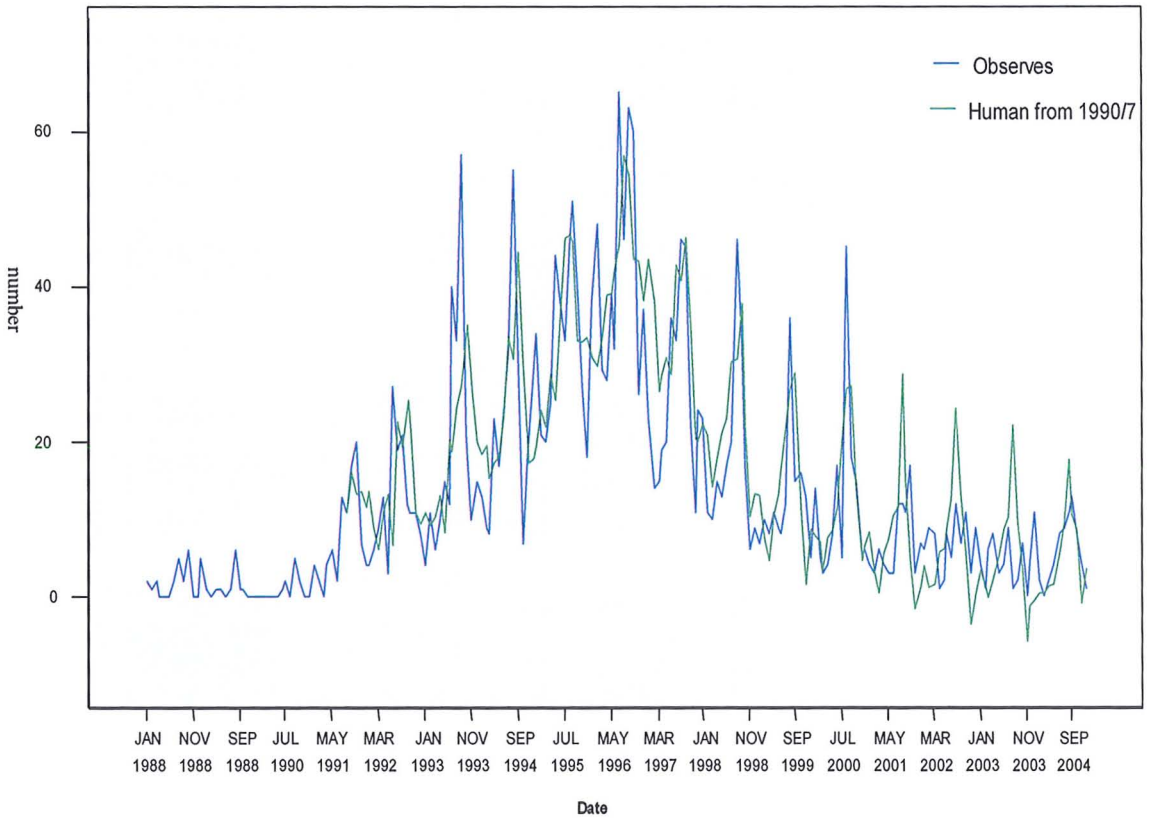


Figure10.8 The ARIMA $(0,1,1)(0,1,1)_{12}$ model for the human dataset (1990/7 – 2004)

10.3.3 The model for the animal dataset

A different model type was selected for the animal dataset. The ACF graph shows a series of decreasing values but there are still many small peaks occurring in the lag 1, 12, 24, and 36 (Figure 10.9). The PACF graph in the Figure 10.9 shows that there is a big peak in lag 1. It also shows that the values decrease slowly and very small peaks still appear in the 12-lag interval. All the information suggests that the time series is non-stationary and the model might be different from the human model. The model could be the ARIMA$(0,1,1)(P,D,Q)_{12}$. The patterns suggest that the parameters P, D and Q should be 0 or 1. After due consideration it would appear that the ARIMA$(0,1,1)(1,0,1)_{12}$ is the best-fit (Table 10.3). The dependence and randomness of residuals are identified in Figure 10.10 and 10.11, respectively. From the residual ACF and PACF graphs, the distribution of residuals are still from a normal distribution; however, many data points are higher and lower than expected. The stationary R-square shows the fit ability is low.

Figure 10.12 shows the model can trace the trends but does not fit the peaks before 2000 very well. After 2003, the model can still catch the trend but traces the peaks poorly.

Table 10.3 The information of animal model parameters and criteria index.

The ARIMA $(0,1,1)(1,0,1)_{12}$ Model

			Estimate	SE	t	Sig.
Animal model	Difference		1			
	θ1	Lag 1	.612	.061	10.011	0.000
	φ12	Lag 1	.740	.155	4.771	0.000
	θ12	Lag 1	.545	.197	2.761	0.006
Stationary R-square	0.299	MAPE	65.565	RMSE	8.442	

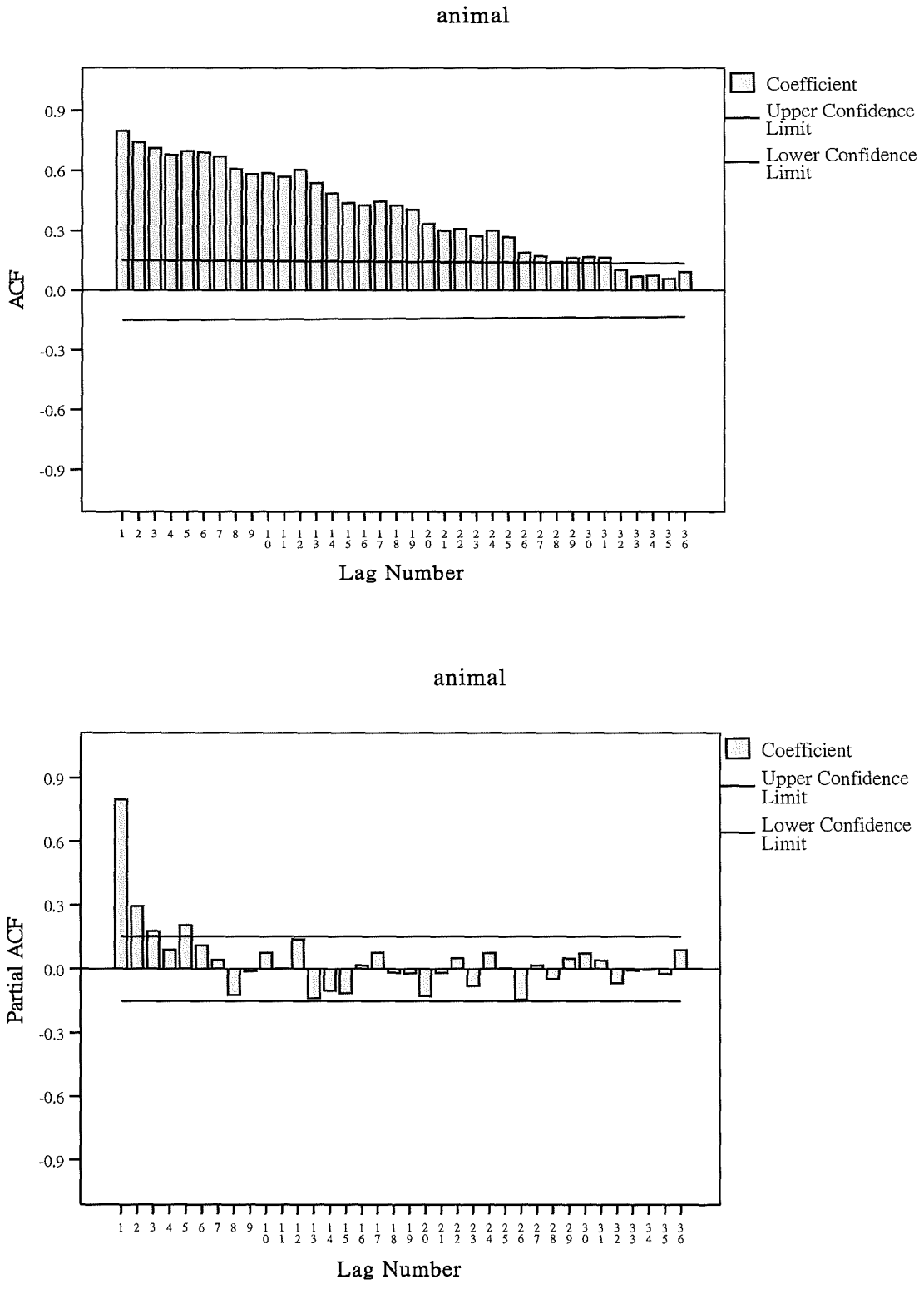


Figure 10.9 The ACF and PACF graphs of animal dataset from 1990/7 to 2004. The X-axis is the month

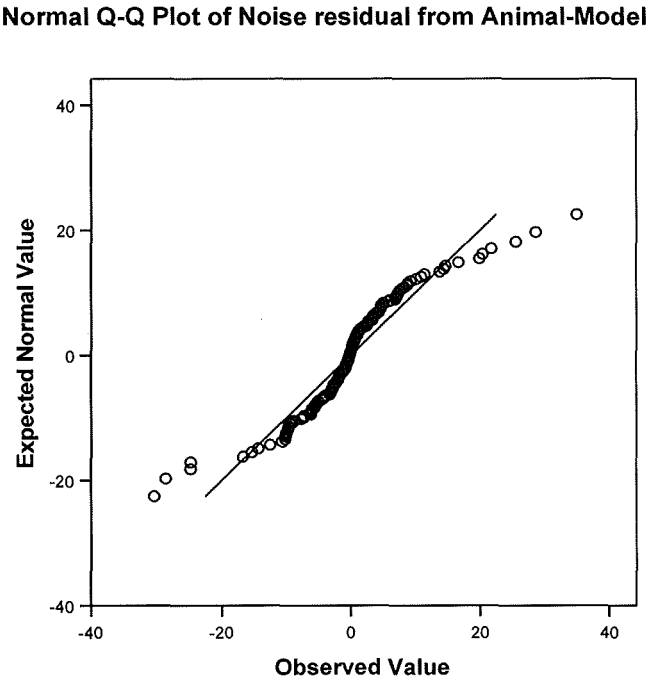


Figure 10.10 The Q-Q plot of animal residuals from the animal model

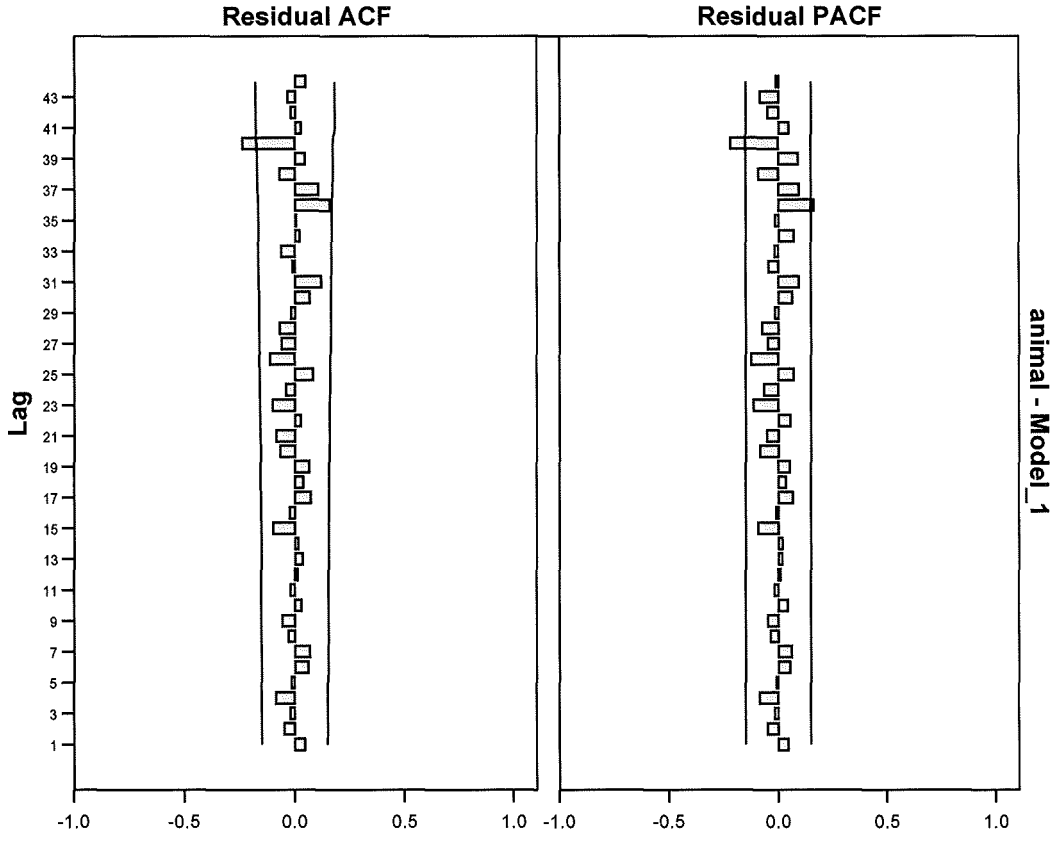


Figure 10.11 The residual ACF and PACF graphs from the animal model

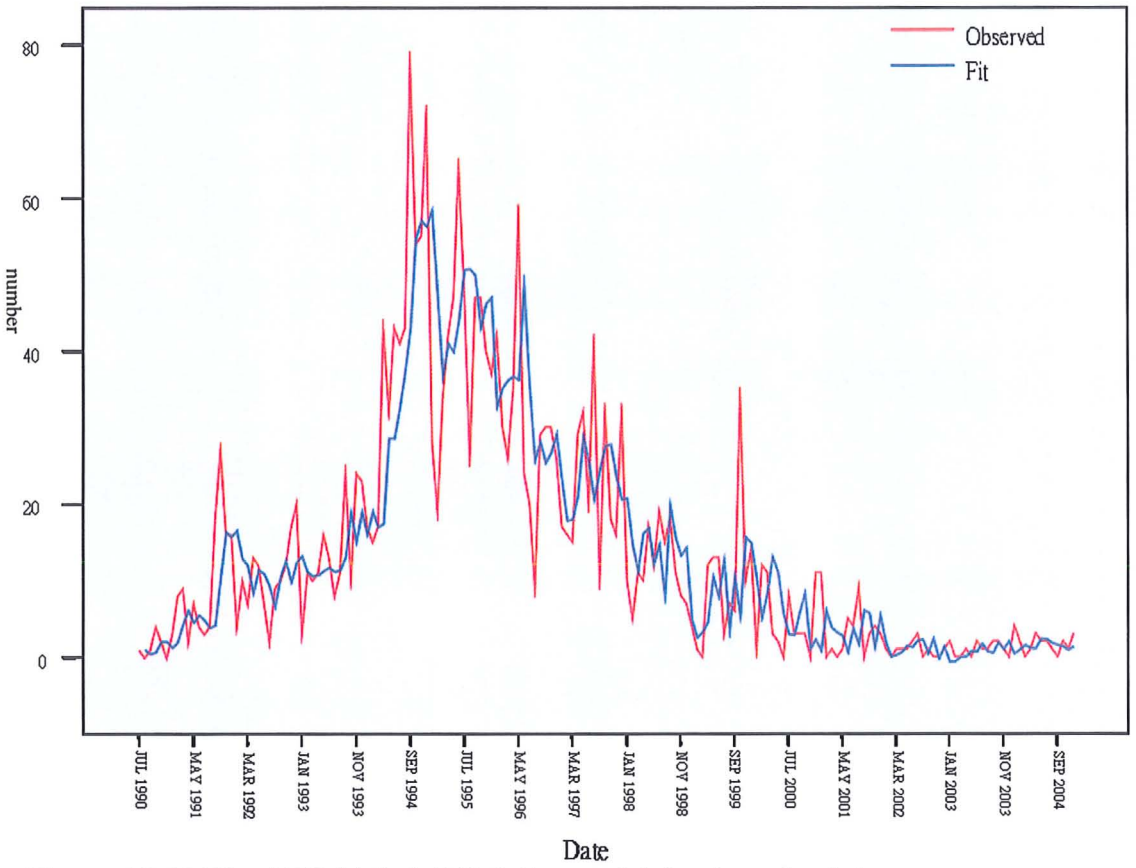


Figure 10.12 The ARIMA(0,1,1)(1,0,1)$_{12}$ model for the animal dataset

10.3.4 The qualities of the predictive abilities of both human and animal models

The holdout function was used to check the qualities of the models. Figure 10.13 and 10.14 show that the two human models have a similar predictive ability. They show similar trace abilities and can predict the peaks in summer. However, the model in Figure 10.14 shows higher predictive values in summer and lower values in February. The prediction abilities in both models are satisfactory.

In the animal model, the predictive values were almost zero. It has two very small peaks in July and October, respectively. At the same time, the observations were between 0 and 4. Generally speaking the quality of the predictive ability of this model is poor (Figure 10.15).

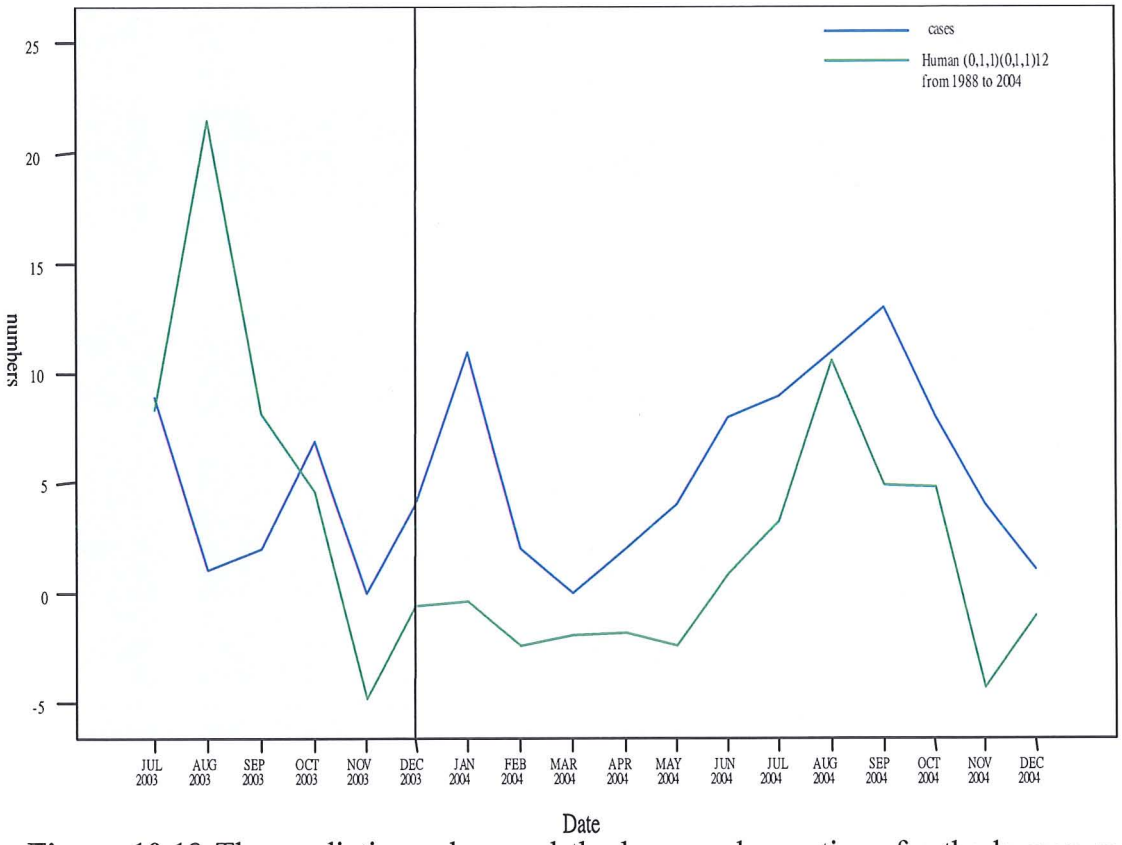


Figure 10.13 The predictive values and the human observations for the human model with data from 1988 to 2003

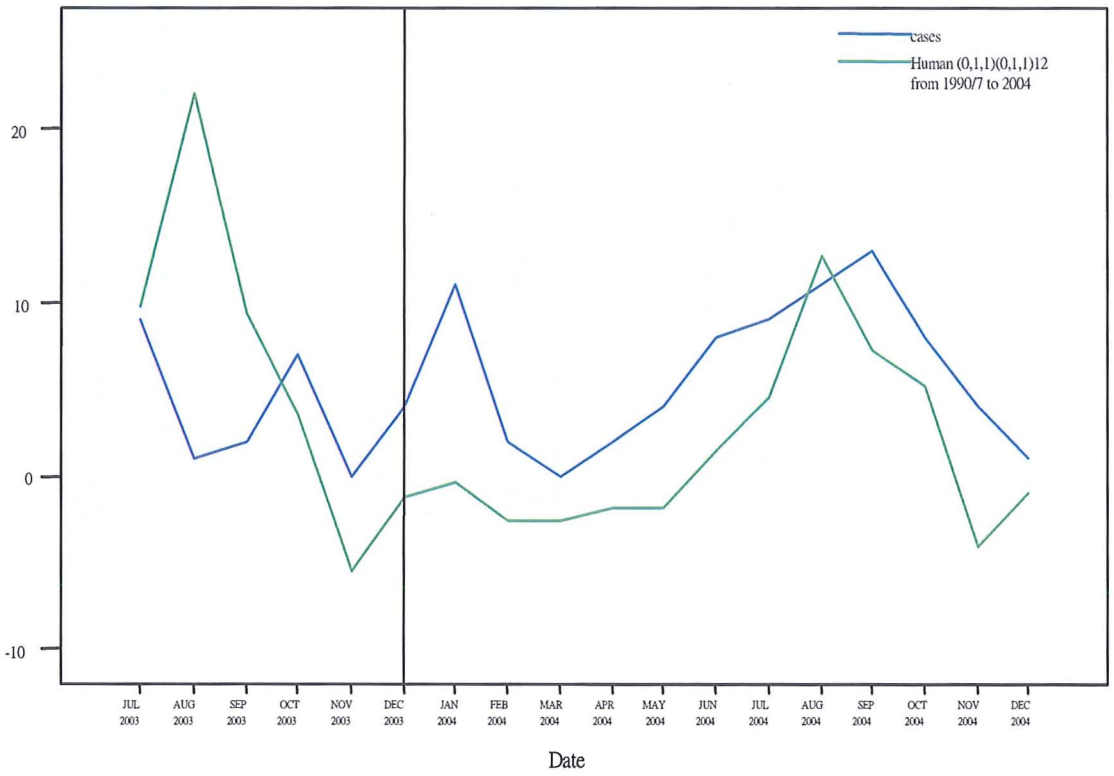


Figure 10.14 The predictive values and the human observations from the human model with data from 1990/7 to 2003/12

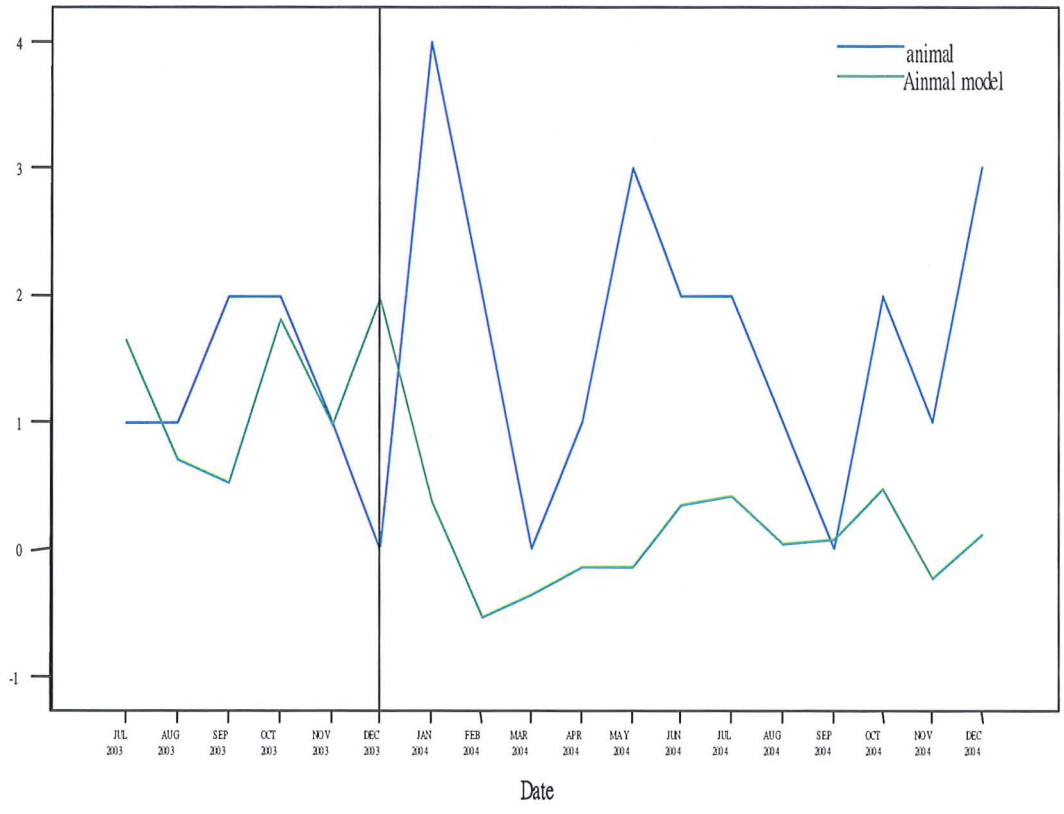


Figure 10.15 The predictive values and the animal observations from the animal model

10.3.5 Comparing the emergence of resistance in animal and human isolates

Figures 10.16 to 10.23 show the simple plots comparing the cumulative totals of isolates considered resistant to the antimicrobials in this study. As can be seen, there is no consistent evidence of animal isolates predating human isolates in terms of resistance. Table 10.4 summarises the timing of emergence in relation to each other.

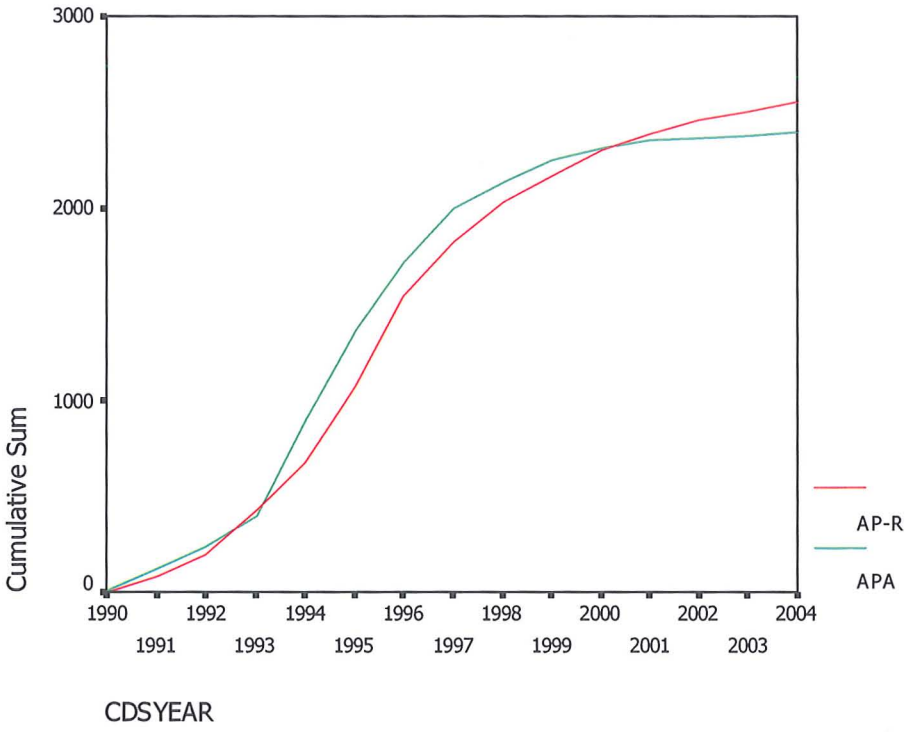


Figure 10.16 Cumulative sum of the isolates resistant to ampicillin in humans (red) and animals (green) in Scotland from 1990 to 2004. The shape and number of the cumulative sums for chloramphenicol, spectinomycin, streptomycin, sulphonamide and tetracycline were nearly identical.

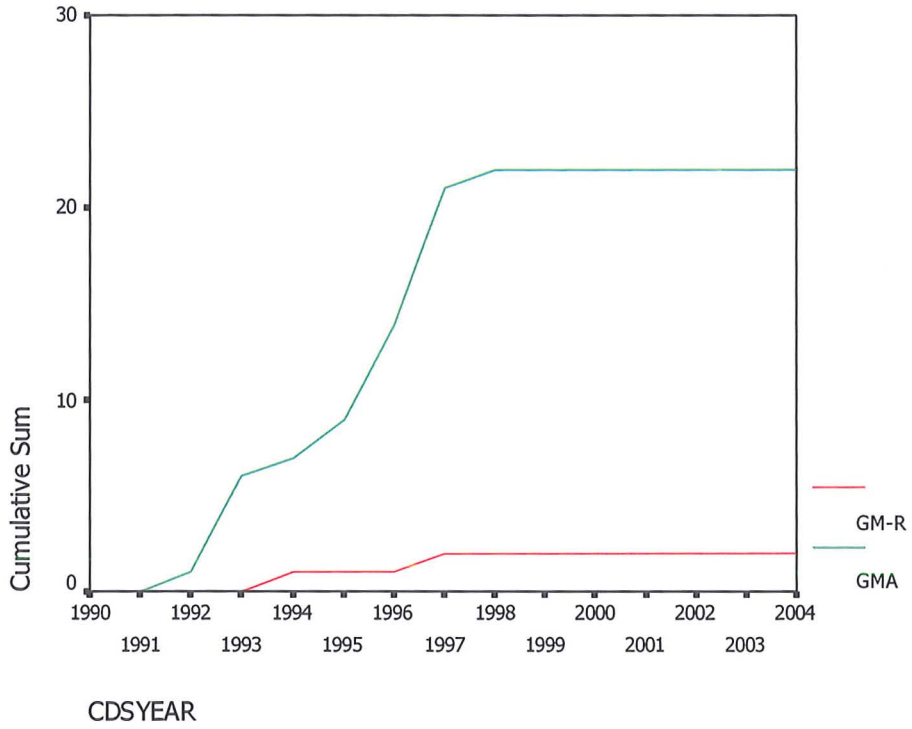


Figure 10.17 Cumulative sum of the isolates resistant to gentamicin in humans (red) and animals (green) in Scotland from 1990 to 2004.

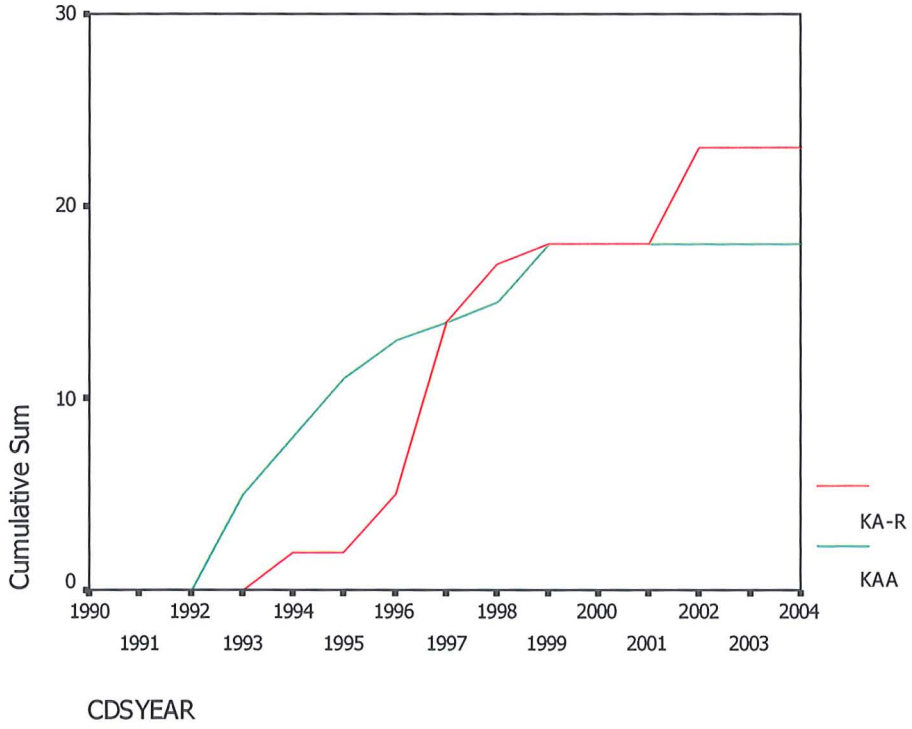


Figure 10.18 Cumulative sum of the isolates resistant to kanamycin in humans (red) and animals (green) in Scotland from 1990 to 2004.

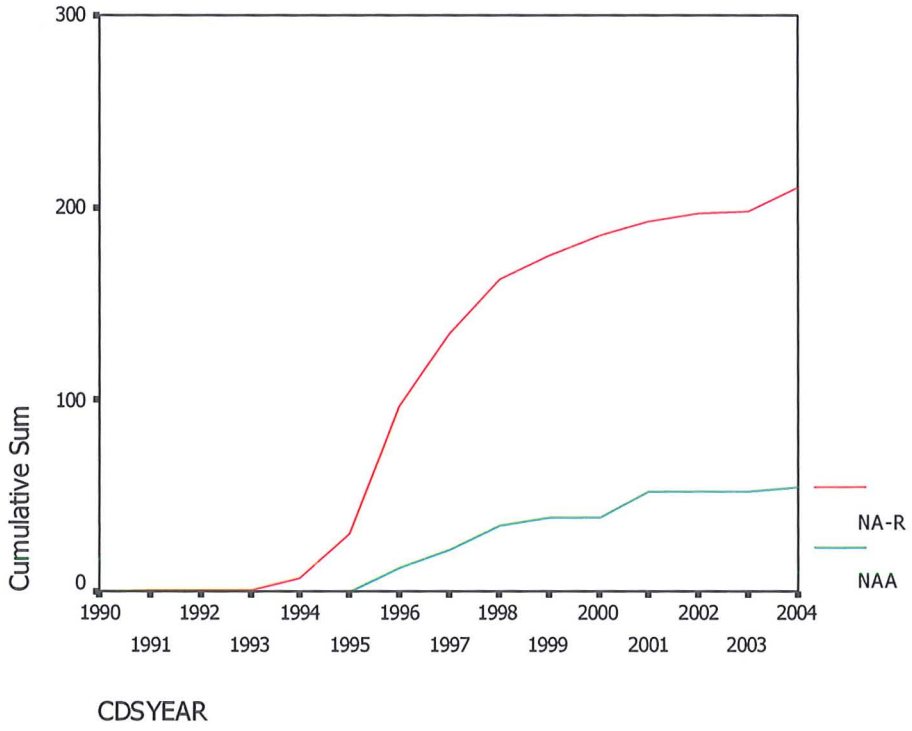


Figure 10.19 Cumulative sum of the isolates resistant to nalidixic acid in humans (red) and animals (green) in Scotland from 1990 to 2004.

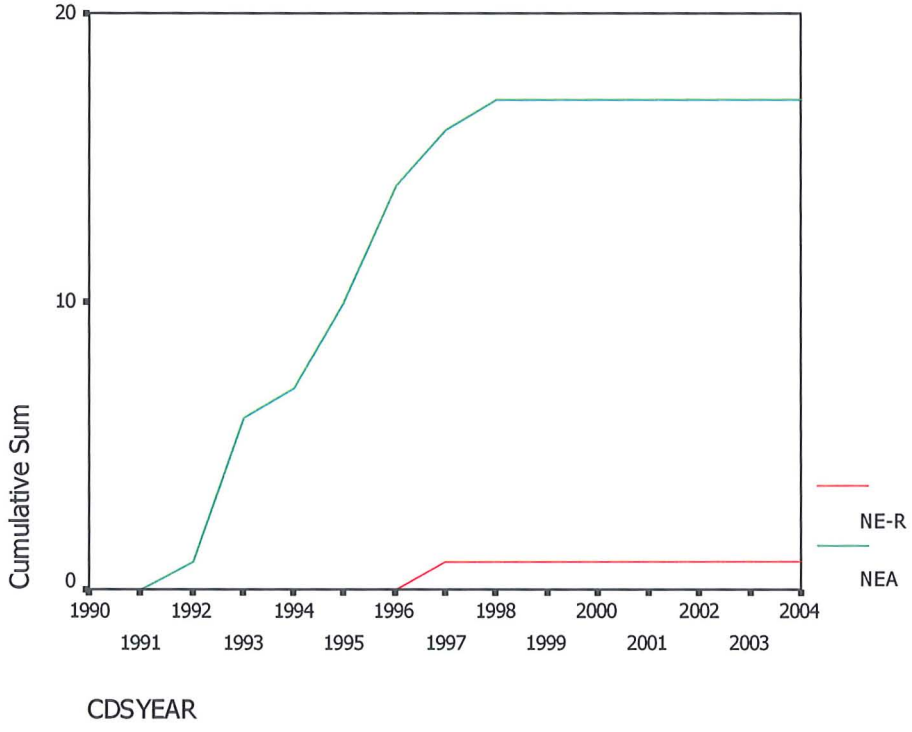


Figure 10.20 Cumulative sum of the isolates resistant to netilmicin acid in humans (red) and animals (green) in Scotland from 1990 to 2004.

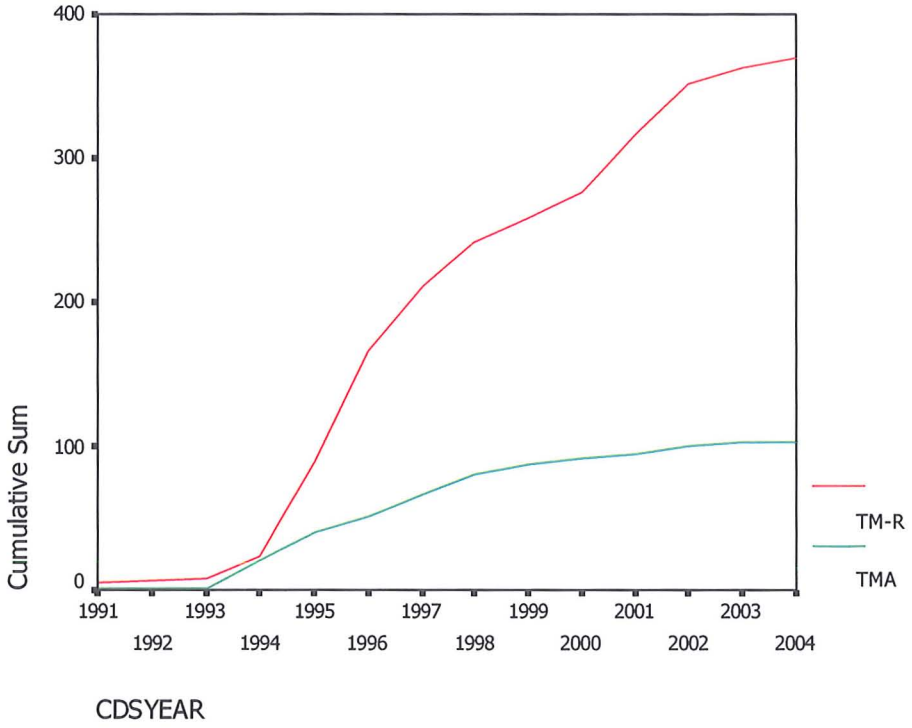


Figure 10.21 Cumulative sum of the isolates resistant to trimethoprim in humans (red) and animals (green) in Scotland from 1990 to 2004.

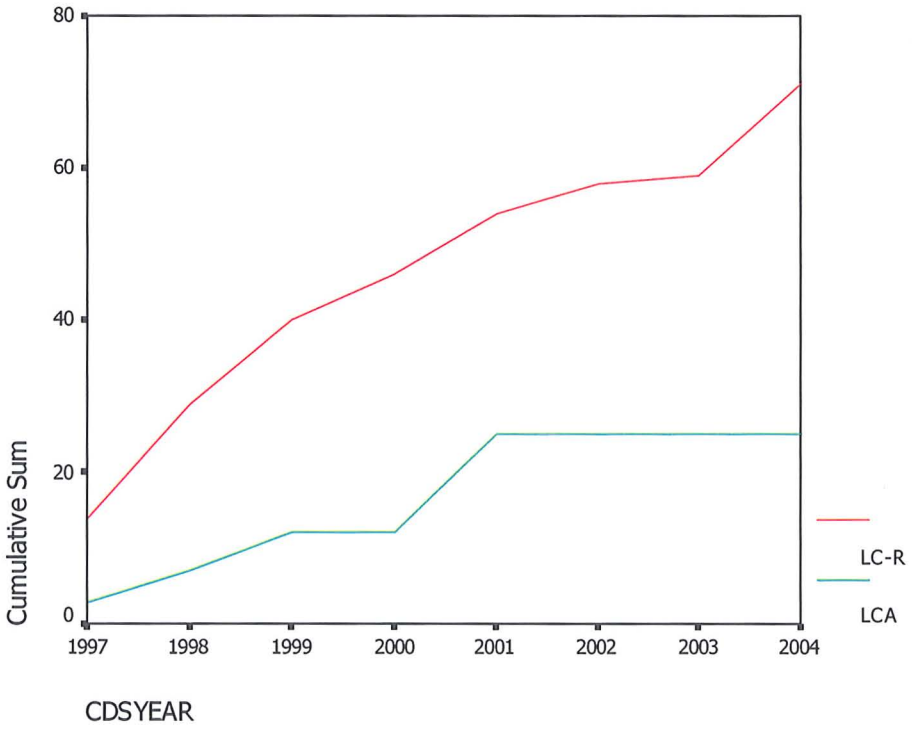


Figure 10.22 Cumulative sum of the isolates resistant to low level ciprofloxacin in humans (red) and animals (green) in Scotland from 1990 to 2004.

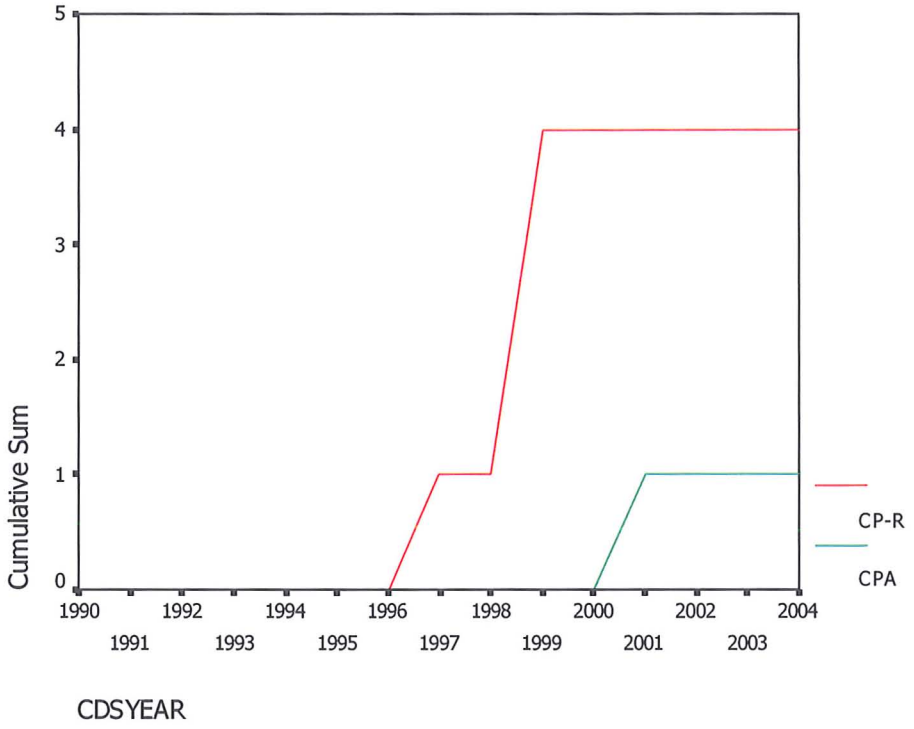


Figure 10.23 Cumulative sum of the isolates resistant to ciprofloxacin in humans (red) and animals (green) in Scotland from 1990 to 2004.

Table 10.4 The relationship between the emergence of resistance in human and animal isolates. Blank cells identify co-emergence; cells with figures identify the number of years between emergence in the two populations. The figure appears in the population of isolates where emergence occurring later.

	Antimicrobial												
	Ap	Cl	Sp	St	Su	Te	Tm	Gm	Ka	Na	Ne	Cp	Lc
Human								2	1		5		
Animal										2		4	-

10.4 DISCUSSION

This chapter set out to establish time series models for human and animal data of *Salmonella* data. To some extent this has been achieved, particularly in the case of the human data, but the ability to compare the time series meaningfully has been hindered by failure to establish a good animal data model.

The different submission systems cause the models to present different ideas. In the human dataset, each case was from every patient whose samples were sent to SSRL and was identified as ST DT104 infection. The human model presented the numbers of isolates per month. In contrast, the animal model presented the numbers of incidents per month. Thus, cases in the animal system could be under-estimated and the two sets are not directly comparable The periodic patterns shown in ACF and PACF were more significant in the human system, but less obvious in animals. There are a number of possible explanations for this. First, the submissions were from a number of different species and different species may peak at different times. The major source of cases was cattle, but the percentage of cattle cases did not remain the same throughout the study period, with the percentage of cattle cases dropping between 1998 and 2003. In addition, the total numbers ranged from hundreds before 1997 to tens after 1998. Second, it is likely that farmers or veterinarians may treat a series of cases as one incident and therefore submit only one case.

The structure of the data could directly impact the descriptive and predictive ability of the models (Smelser and Baltes 2001). As mentioned in previous studies, an increasing trend and a declining trend were identified in the animal and the human time series, respectively. It is believed that the sudden increase may be directly related to the surge of multi-resistant strains of ST DT104 (Threlfall et al. 1994) or phage type change (Anon 1988). Moreover, the decline

could be attributed to the introduction of the many *Salmonella* control orders, which have been mentioned in Chapter 3. The dramatic change in the submissions resulted in a lower descriptive ability.

The different models lead to different inferences, Briefly, the human models weighted the earlier data and seasonal effect more heavily than did the animal models: the ARIMA$(0,1,1)(0,1,1)_{12}$ model puts the same weight on the trend and the seasonal component. It means that the animal model relies more on the most recent observation. In addition, the animal model showed unmatched plots after 2000 because there were very few cases. There are some methods which use more factors to solve the problems and make better models. The self threshold auto-regressive model (SETAR) built by Watier and his colleagues for *Salmonella* Typhimurium infection used different equations when different conditions were observed (Watier et al. 1991).

The ARIMA$(0,1,1)(0,1,1)_{12}$ model was also adapted to the animal dataset to understand the difference. The model could identify the peaks during the epidemic period (1993 to 1999) very well but failed in describing data outside the epidemic period because of a stronger seasonal component. Thus, ARIMA$(0,1,1)(1,0,1)_{12}$ is a better model to avoid over-weighting of the seasonal component.

The prediction ability in the human models was good, but in the animal model comparatively poor. Again, the fewer case numbers and dramatic pattern changes affect the ability to predict. In addition, the few cases in the animal dataset, less than 5 and near to 0 from 2001/9, also may affect forecasting. The prediction ability may be improved in two ways: extending the

time series to get a better mean; and choosing a more suitable range of time series. However, it is difficult to see how this might be achieved with the data collection systems in place.

There are many ambitious models trying to get very good forecasting or description. However, the study attempted to build a reasonable model, which can present enough information for building a more complex model in the future. The univariate ARIMA model is easy to maintain, generates lower RMSE compared with other models (Smelser and Baltes 2001; Abeku et al. 2002). Also, it can supply basic information for multivariate ARIMA studies. After the basic model structure has been built and more and more data points have been added, the model may present better results.

There are often too many uncertainties to make a perfectly accurate prediction model possible (Smelser and Baltes 2001). However, the models based on the human data presented here satisfy the general basic principles of model building. They are parsimonious, the estimated alpha and theta are high quality, the RMSE and MAPE are acceptable, and the forecast ability is acceptable. Although a small part of this overall study, one might hope that these models might provide a starting point for other studies.

In considering the failure of the data to allow direct comparison of the models, the simple plots used to identify the date of emergence in the human and animal populations provided some of the most telling evidence. Firstly, there was no consistent pattern as to where population resistance first appeared. Secondly, there some evidence that the chromosomally mediated resistances were more likely to co-emerge than the plasmid mediated. Thirdly, there were some differences in the rate at which the isolates then accrued, as evidenced by the slope of the cumulative plots. The cumulative plots were used to give some feel for total

environmental history but should be viewed with caution particularly given the caveats regarding the animal data. One might argue that these simple plots provide the strongest evidence in this thesis that the role of isolates in the veterinary sector, in terms of being the root cause of the problem, is not as clear as some would suggest.

CHAPTER XI

GENERAL DISCUSSION

CHAPTER XI

GENERAL DISCUSSION

The emergence of antibiotic-resistant ST DT104 infections in humans and animals has been a topic of research that has received a lot of attention. In terms of resistance, one of the first significant studies of *Salmonella* Typhimurium infections in the UK was carried out by Anderson, who recorded the antimicrobial resistance situation in England and Wales (Jones et al. 2002). Following this, the increasing concerns regarding the emergence of antimicrobial resistance led the UK government to survey the issue. The Scottish *Salmonella* antimicrobial resistance surveillance is managed by the HPS and the SSRL. The data from 1996 to 2003 were published in the HPS Weekly Report (SCIEH Weekly Report) (Browning et al. 2004; Browning et al. 2001a; Browning et al. 2001b; Browning et al. 2005; Browning et al. 1999;Browning et al. 2003). To date no comprehensive systematic descriptive study focused on ST DT104 infections in Scotland has been undertaken.

In outline, Chapters 3 and 4 described the epidemiological trends of the *Salmonella*, and ST DT104, highlighting that there were clear epidemic curves and although there was heterogeneity in the microbial population, certain clones predominated. Chapter 7 confirmed that, broadly speaking, there were similar trends in isolates from animal sources. Chapters 5 and 8 described cluster analyses of human and animal isolates and, again, predominant types were similar and the clustering patterns suggestive of common ecological exposures and mechanisms. Although there were many similarities, there were

differences: fully resistant R-types were rarer in animals and multiple resistance more common. Chapters 6 and 9 then applied spatial and temporal analyses to the human and animal datasets, identifying in both populations, potential high and low risk clusters in time and space. With much less confidence in the inferences from the animal data, it would appear that MR isolates are more common in rural areas in both human and animals. Finally, in Chapter 10, in an attempt to apply time series approaches, robust models were achieved using the human data whilst, again, the animal data were of lesser quality. A simple plot of emerging resistance suggested that there was no clear uniform case to be made in support of resistance appearing first in animals and then in humans.

The study has described changes in isolation rates for ST DT104 between 1988 and 2004. The emergence of the multi-resistant ST strains in England and Wales is highly related to the emergence of ST DT104 infections from 1992 (Jones et al. 2002). Since 1991, the Scottish ST DT104 infections have emerged in humans (rather than phage type 49), and in animals (rather than the phage type 204c). The reason for this is unclear but one possible explanation is due to the fitness selection related to the usage of antimicrobial agents and its ability to infect a wide range of hosts (Davison et al. 2005). It is suggested that the chromosomally-encoded resistance is stable but that plasmid-mediated resistance is not, even if selective pressure is withdrawn (Threlfall et al. 1994). Chapters 4, 5 and 8 showed that the proportion of human and animal isolates against chromosomally-encoded antibiotic resistances were both high and stable. In contrast, the proportion of isolates with plasmid-mediated resistances is low. Some of the plasmid-mediated resistances appeared only sporadically in the isolates.

This thesis used hierarchical cluster analysis to explore the diversities at the phenotype level. Chapters 5 and 8 showed that isolates from humans or animals can be allocated to two very similar groups. A total of 22 R-types, especially those multi-resistance types, are common in the human and animal isolates. Although there were 52 R-types in the human isolates and 35 R-types in the animal isolates, the unmatched R-types were a very low proportion of the whole, suggesting that there is commonality in the microbiological environment in which humans and animals exist.

In addition, the clustering studies provided evidence of the disappearance of the R-types that are more susceptible. Although there may be a case to argue that the susceptible types are more likely to be successfully treated and less likely to be submitted, the trend is certainly downwards.

The spatial and temporal studies were helpful in establishing areas of risk and identifying for the most part that the more resistant types were from rural areas. However, the ecosystem is dynamic and the site of a case occurring may not necessarily be the place of infection. There is also a dynamic situation in relation to the transfer of resistance determinants. It has been suggested that ST DT104 isolates acquired the chromosomally-encoded resistance genes from fish (Doublet et al. 2003) or other species (Cloeckaert and Schwarz 2001). On the farm, an infected cow can shed 10^2 to 10^5 *Salmonella* Typhimrium organisms in a gram of faeces (Wray and Sojka 1978). The pathogen can transfer to other farms by trucks or even by dust (Woodhead et al. 2004) and can be found on most farms: Davison states that ST DT104 organisms could be identified on 63% of the dairy farms in England and Wales and that prevalence of ST DT104 infections are from 0.8 to 1.8.

(Davison et al. 2005). All this evidence suggests that there are other factors at play that lead to disease in animals, as well as exposure and disease in humans.

The results presented in this thesis do not refute the assertion that human multi-resistance R-types might be of animal origin (Calvert et al. 1998), especially when the results of the purely temporal study are considered. This pathogen can transfer to humans via direct contact with infected animals (Calvert et al. 1998) or contaminated products. The temporal study pointed out the emergence of ST DT104 infections in humans and in animals are closely related in time: The most-likely epidemic period of ST DT104 infections in humans was from 1993 to 1998 and in animals it was from 1992 to 1999. Similarly, the spatial and spatio-temporal studies confirmed that rural areas are more likely to have resistant isolates in animals and humans supporting the observations of Calvert et al. (1998). However, what is not clear is the direction of this relationship.

Working against the theory that there is a single explanation for the relationship between human and animal isolates are the differences in the time series models. Notwithstanding the deficiencies of the data, there were clear differences between the time series. When considering a simple time plot of when resistance to the individual antimicrobials emerged, there was no consistent temporal association between the emergence in humans and animals. This suggests that ecological and epidemiological drivers within the relationships are complex and not necessarily uni-directional.

The aim of this work was to investigate the relationships between the human and the animal ST DT104 isolates resistant to a number of antimicrobials. To some extent the aim has been achieved. However, more importantly, while attempting to meet this objective

several methodologies have been explored and many important questions relevant to the relationship between the cases from the two sources have been highlighted. In order to improve the understanding of the relevance of resistance in ST DT104 from humans and animals, there are several areas of work considered in this thesis that need to be expanded.

Firstly, there is a strong link between the quality of data, the ability to use this data and the quality of outcomes. Because the animal data did not contain sufficient information, the quality of the outcomes in the SaTScan analysis from the animal dataset is marginal, as was the animal based time series. A few methods could be used to improve the quality. Locating the farms on electronic maps might improve the quality of the study. More precise animal numbers in each postal district would be helpful too. Both of these issues are being addressed in the wake of Foot and Mouth Disease. However, the limiting factor in this study was the quality of the information accompanying the samples. Data were inconsistent, often missing and there is a danger that incidents, outbreaks and individual cases are confused. Whilst these deficiencies detracted from the analysis of the animal isolates, it severely hampered any meaningful comparison of human and animal results at all levels of the thesis. The study has effectively been constrained to a number of quantitative studies in the two species leading to a qualitative comparison.

Secondly, whilst useful at the level of phenotype, the cluster analysis could be expanded into a study at the level of genotype. A further study based on the heterogeneity of genotypes from the four main R-types: ApClSpStSuTe, ApClSpStSuTeTm, ApClSpStSuTeNa, and SpStSu, would provide important additional information. Threlfall reported the RFLP profile of the R-type ApClSpStSuTe (Threlfall et al. 2005) and this would lead to an ability to subdivide some of the more prevalent R-types.

In terms of geographical comparisons, it is important to recognise that Scotland is contiguous with the rest of the UK. Including England and Wales, UK based studies would be sensible but this would not be without problems. Surveillance techniques, data recording and laboratory methods would all need to be addressed in a standard fashion if the study was to address the spatial distribution of ST DT104 infections in the mainland UK properly. In terms of temporal issues, the ARIMA model has provided an insight into the ability to forecast and this might also be extended should the data quality be sufficient. It is worth remphasising that sometimes simple epidemiological analyses and plots over time can provide more relevant insight than more complex approaches.

The infections caused by ST DT104 have been a concern for over 14 years and changes in the phage types are well documented (Anon 1998). Although the submissions from ST DT104 have declined, recently, the emergence of type DT 40 has replaced DT104 as a matter of concern. It may be that this study has been conducted as DT104 begins to decline as a threat. What is certain is that its ability to spread resistance determinants in the ecosystem will remain and, regardless of the organism, it is perhaps on these that future studies should be directed.

REFERENCES

Aarestrup FM, Wiuff C, Molbak K, Threlfall EJ. (2003) Is it time to change fluoroquinolone breakpoints for *Salmonella* spp.? Antimicrob Agents Chemother 47:827-829.

Abeku TA, de Vlas SJ, Borsboom G, Teklehaimanot A, Kebede A, Olana D, van Oortmarssen GJ, Habbema JD. (2002) Forecasting malaria incidence from historical morbidity patterns in epidemic-prone areas of Ethiopia: a simple seasonal adjustment method performs best. Trop Med Int Health 7:851-857.

Almuth L, Prager R, Streckel W, Rabsch W, Gericke B, Seltmann G, Helmuth R, Tschape H. (1997) Wird der *Salmonella-enterica*-Stamm DT104 des Serovar Typhimurium der neue fuhrede Epidemietyp in Deutschland. Robert Koch Institue Info 1:10.

Alvseike O, Leegaard T, Aavitsland P, Lassen J. (2002) Trend of multiple drug resistant *Salmonella* Typhimurium in Norway. Euro Surveill 7:5-7.

Amyes SG, Towner KJ, Carter GI, Thomson CJ, Young HK. (1989) The type VII dihydrofolate reductase: a novel plasmid-encoded trimethoprim-resistant enzyme from gram-negative bacteria isolated in Britain. J Antimicrob Chemother 24:111-119.

Angulo FJ, Griffin PM. (2000) Changes in antimicrobial resistance in *Salmonella enterica* serovar Typhimurium. Emerg Infec Dis 6:436-438

Angulo FJ, Johnson KR, Tauxe RV, Cohen ML. (2000) Origins and consequences of antimicrobial-resistant non-typhoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. Microb Drug Resist 6:77-83.

Anon. (1986) Turtle-associated salmonellosis--Ohio. MMWR Morb Mortal Wkly Rep 35:733-4, 739.

Anon. (1992) Report on Vacuum Packaging and Associated Processes - Advisory Committee on the Microbiological Safety of Food. HMSO London

Anon. (1994) Salmonellosis, summary of isolations during 1993. Scottish Centre for Infection and Environmental Health.

Anon. (1997) Multidrug-resistant *Salmonella* serotype Typhimurium--United States, (1996) MMWR Morb Mortal Wkly Rep 46:308-310.

Anon. (1998) Science and technology - seventh report. http://www.parliament.the-stationery-office.co.uk/pa/ld199798/ldselect/ldsctech/081vii/st0701.htm. The House of Lords, London, UK. (25/05/2007)

Anon. (2006) The Merck Veterinary Manual. Whitehouse Station, NJ, USA: Merck & Co., Inc.

Anon. (2007a) Current health topics/Hand hygiene. http://www.nhsgg.org.uk/content/default.asp?page=s111_13. (25/05/2007)

Anon. (2007b) Disease Control. http://www.defra.gov.uk/animalh/diseases/control/index.htm . (25/05/2007)

Anon. (2007c) Summary profile for Salmonellosis. http://www.defra.gov.uk/animalh/diseases/vetsurveillance/profiles/salmonellosis.pdf . Defra. (25/05/2007).

Anon. (2007d) The economic benefit an Antec disinfection programme in the control of a *Salmonella* outbreak. http://www.antecint.co.uk/Main/pigsalm.htm . (25/05/2007).

Arcangioli MA, Leroy-Setrin S, Martel JL, Chaslus-Dancla E. (1999) A new chloramphenicol and florfenicol resistance gene flanked by two integron structures in *Salmonella* Typhimurium DT104. FEMS Microbiol Lett 174:327-332.

Baggesen DL, Aarestrup FM. (1998) Characterisation of recently emerged multiple antibiotic-resistant *Salmonella enterica* serovar Typhimurium DT104 and other multiresistant phage types from Danish pig herds. Vet Rec 143:95-97.

Baggesen DL, Sandvang D, Aarestrup FM. (2000) Characterization of *Salmonella enterica* serovar Typhimurium DT104 isolated from Denmark and comparison with isolates from Europe and the United States. J Clin Microbiol 38:1581-1586.

Balotescu C, Israil A, Radu R, Alexandru I, Dobre G. (2003) Aspects of constitutive and acquired antibioresistance in *Aeromonas hydrophila* strains isolated from water sources. Roum Arch Microbiol Immunol 62:179-189.

Bass L, Liebert CA, Lee MD, Summers AO, White DG, Thayer SG, Maurer JJ. (1999) Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. Antimicrob Agents Chemother 43:2925-2929.

Baumler AJ, Gilde AJ, Tsolis RM, van der Velden AW, Ahmer BM, Heffron F. (1997) Contribution of horizontal gene transfer and deletion events to development of distinctive patterns of fimbrial operons during evolution of *Salmonella* serotypes. J Bacteriol 179:317-322.

Bell C., Kyriakides A. (2002) *Salmonella*: a practical approach to the organism and its control in foods. Blackwell Science Ltd. Oxford, UK

Besser TE, Gay CC, Gay JM, Hancock DD, Rice D, Pritchett LC, Erickson ED. (1997) Salmonellosis associated with *S.* Typhimurium DT104 in the USA. Vet Rec 140:75.

Borovkova, SA. (1998) Estimation and prediction for nonlinear time series. University of Groningen.
Ref Type: Thesis/Dissertation

Box GEP, Jenkins GM. (1976) Time series analysis forecasting and control. Revised ed. Holden-Day Inc. San Francisco, USA

Boyd D, Peters GA, Cloeckaert A, Boumedine KS, Chaslus-Dancla E, Imberechts H, Mulvey MR. (2001) Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J Bacteriol 183:5725-5732.

Boyd DA, Peters GA, Ng L, Mulvey MR. (2000) Partial characterization of a genomic island associated with the multidrug resistance region of *Salmonella enterica* Typhymurium DT104. FEMS Microbiol Lett 189:285-291.

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B. (2000) *Salmonella* nomenclature. J Clin Microbiol 38:2465-2467.

Brown DF, Upson R, Brenwald NP, Rampling A. (1991) Mechanism of nitrofuran resistance in *Salmonella* Enteritidis phage type 4 and interpretation of nitrofuran susceptibility tests. J Antimicrob Chemother 27:23-28.

Browning LM, Brown DJ, Coia JE, Mather H, Reilly WJ, Cowden JM. (2005) Salmonella in Scotland, 2003. SCIEH Weekly Report 38:306-310.

Browning LM, Cola JE, Cowden JM, Mather H, Reilly WJ (2004) Salmonella in Scotland, 2002. SCIEH Weekly Report 38:22-24.

Browning LM, Cowden JM, Edwards G.FS, Reilly WJ, Mather H. (2001a) Salmonella in Scotland, 2000. SCIEH Weekly Report 35:306-309.

Browning LM, Cowden JM, Edwards G.FS, Reilly WJ, Mather H. (2001b) Salmonellosis in Scotland, 1999. SCIEH Weekly Report 35:186-188.

Browning LM, Edwards G.FS, Girdwood RWA, Mather H, Munro DS, Reilly WJ. (1999) Salmonellosis in Scotland 1996-1998. SCIEH Weekly Report 33:118-125.

Browning LM, Coia JE, Cowden JM, Mather H, Reilly WJ. (2003) *Salmonella* in Scotland, 2001. SCIEH Weekly Report 37:42-44.

Calvert N, Stewart WC, Reilly WJ. (1998) *Salmonella* Typhimurium DT104 infection in people and animals in Scotland: a collaborative epidemiological study 1993-96. Vet Rec 143:351-354

Carlson SA, Ferris KE. (2000) Augmentation of antibiotic resistance in *Salmonella* Typhimurium DT104 following exposure to penicillin derivatives. Vet Microbiol 73:25-35.

Carnevale R, Molbak K, Bager F, Aarestrup FM. (2000) Fluoroquinolone resistance in *Salmonella*: a web discussion. Clin Infect Dis 31:128-130.

Centre for Veterinary Medicine. (2003) Updated information on FDA's proposed withdrawal of approval of poultry fluoroquinolones.
http://www.fda.gov/cvm/NOOHB.htm. (25/05/2007)

Chaslus-Dancla E, Martel JL, Carlier C, Lafont JP, Courvalin P. (1986) Emergence of aminoglycoside 3-N-acetyltransferase IV in *Escherichia coli* and *Salmonella* Typhimurium isolated from animals in France. Antimicrob Agents Chemother 29:239-243.

Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, Chien MS, Lin TY. (2002) The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype Choleraesuis. N Engl J Med 346:413-419.

Chu C, Su LH, Chu CH, Baucheron S, Cloeckaert A, Chiu CH. (2005) Resistance to fluoroquinolones linked to *gyr*A and *par* C mutations and overexpression of *acr* AB efflux pump in *Salmonella enterica* serotype Choleraesuis. Microb Drug Resist 11:248-253.

Cloeckaert A, Schwarz S. (2001) Molecular characterization, spread and evolution of multidrug resistance in *Salmonella enterica* Typhimurium DT104. Vet Res 32:301-310.

Cloeckaert A, Sidi BK, Flaujac G, Imberechts H, D'Hooghe I, Chaslus-Dancla E. (2000) Occurrence of a *Salmonella enterica* serovar Typhimurium DT104-like antibiotic resistance gene cluster including the *flo*R gene in *S. enterica* serovar Agona. Antimicrob Agents Chemother 44:1359-1361.

Daly M, Fanning S. (2004) Integron analysis and genetic mapping of antimicrobial resistance genes in *Salmonella enterica* serotype Typhimurium. Methods Mol Biol 268:15-32.

Davies R. (2000) *Salmonella* monitoring and control in pigs. Zoonotic Infection in Livestock and the Risk to Public Health. Department of Food and Rural Affairs. London, UK

Davies J, O'Connor S. (1978) Enzymatic modification of aminoglycoside antibiotics: 3-N-acetyltransferase with broad specificity that determines resistance to the novel aminoglycoside apramycin. Antimicrob Agents Chemother 14:69-72.

Davison HC, Smith RP, Pascore SJ, Sayers AR, Davies RH, Weaver JP, Kidd SA, Dalziel RW, Evans SJ. (2005) Prevalence, incidence and geographical distribution of serovars of *Salmonella* on dairy farms in England and Wales. Vet Rec 157:703-711

De Angelis, I, Rossi L, Pedersen JZ, Vignoli AL, Vincentini O, Hoogenboom LA, Polman TH, Stammati A, Zucco F. (1999) Metabolism of furazolidone: alternative pathways and modes of toxicity in different cell lines. Xenobiotica 29:1157-1169.

Defra. (1999) Zoonoses Report United Kingdom. Department for the Environment Food and Rural Affairs, London, UK.

Defra. (2003) Salmonella in livestock production in GB 2003. London: Veterinary Laboratories Agency.

Dick SM. (2003) The epidemiology of antimicrobial resistance monitoring in pigs in relation to the growth promoter avilamycin. University of Glasgow.
Ref Type: Thesis/Dissertation

Dohoo I, Martin W, Stryhn H. (2003) Veterinary epidemiologic research. AVC Inc., Prince Edward Island, Canada.

Doublet B, Lailler R, Meunier D, Brisabois A, Boyd D, Mulvey MR, Chaslus-Dancla E, Cloeckaert A. (2003) Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster in *Salmonella enterica* serovar Albany. Emerg Infect Dis 9:585-591.

Enne VI, Livermore DM, Stephens P, Hall LM. (2001) Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. Lancet 357:1325-1328.

Fisher IS. (1999) The Enter-net international surveillance network - how it works. Euro Surveill 4:52-55.

Fluit AC, Schmitz FJ. (1999) Class 1 integrons, gene cassettes, mobility, and epidemiology. Eur J Clin Microbiol Infect Dis 18:761-770.

Food Standards agency (2007) Guidance on HACCP implementation. http://www.food.gov.uk/multimedia/webpage/haccparchive/haccpdoc03. (25/05/2007)

Furushita M, Shiba T, Maeda T, Yahata M, Kaneoka A, Takahashi Y, Torii K, Hasegawa T, Ohta M. (2003) Similarity of tetracycline resistance genes isolated from fish farm bacteria to those from clinical isolates. Appl Environ Microbiol 69:5336-5342.

General Register Office for Scotland. (2002) Scotland's Census - 2001 Population Report: Scotland: laid before the Scottish Parliament pursuant to section 4(1) of the Census Act 1920/ Registrar General for Scotland. Edinburgh, UK.

General Register Office for Scotland. (1991) 1991 Census User's Guide. 1, Preliminary reports for England and Wales and for Scotland: prospectus. Edinburgh, UK.

Georgala DL, Committe, Working Group. (1999) Report on microbial antibiotic resistance in relation to food safety. Stationery Office. London, UK.

George AM, Levy SB. (1983) Amplifiable resistance to tetracycline, chloramphenicol, and other antibiotics in *Escherichia coli*: involvement of a non-plasmid-determined efflux of tetracycline. J Bacteriol 155:531-540.

Gillespie SH, Bamford BK. (2003) Antibiotics in clinical use. Medical Microbiology & Infection at a Glance. Blackwell Publishing.p 18-19. Oxford, UK.

Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ. (2001) Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother 45:723-726.

Grinsted J, de la CF, Schmitt R. (1990) The Tn21 subgroup of bacterial transposable elements. Plasmid 24:163-189.

Gross U, Tschape H, Bednarek I, Frosch M. (1998) Antibiotic resistance in *Salmonella enterica* serotype Typhimurium. Eur J Clin Microbiol Infect Dis 17:385-387.

Hakanen A, Kotilainen P, Huovinen P, Helenius H, Siitonen A. (2001) Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. Emerg Infect Dis 7:996-1003.

Hakanen A, Kotilainen P, Jalava J, Siitonen A, Huovinen P. (1999) Detection of decreased fluoroquinolone susceptibility in Salmonellas and validation of nalidixic acid screening test. J Clin Microbiol 37:3572-3577.

Handysides S. (1999) From the editor: where have all the gastrointestinal infections gone? Eurosurveillance Weekly 3:990119.

Hall RM, Collis CM. (1995) Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. Mol Microbiol 15:593-600.

Helms M, Ethelberg S, Molbak K. (2005) International *Salmonella* Typhimurium DT104 infections, 1992-2001. Emerg Infect Dis 11:859-867.

Helms M, Vastrup P, Gerner-Smidt P, Molbak K. (2002) Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. Emerg Infect Dis 8:490-495.

Henny JE. (2007) Statement of Jane E. Henney M.D. Commissioner Food and Drug Administration department of Health and Human Services before the subcommittee on Labour, Health, and Human Services, and Education Committee on appropriation United States senate September 20,2000.
http://www.fda.gov/ola/2000/antimicro.html. (03/05/2007)

Herikstad H, Hayes P, Mokhtar M, Fracaro ML, Threlfall EJ, Angulo FJ. (1997) Emerging quinolone-resistant *Salmonella* in the United States. Emerg Infect Dis 3:371-372.

Herrero A, Rodicio MR, Gonzalez-Hevia MA, Mendoza MC. (2006) Molecular epidemiology of emergent multidrug-resistant *Salmonella enterica* serotype Typhimurium strains carrying the virulence resistance plasmid pUO-StVR2. J Antimicrob Chemother 57:39-45.

Hochhut B, Lotfi Y, Mazel D, Faruque SM, Woodgate R, Waldor MK. (2001) Molecular analysis of antibiotic resistance gene clusters in *Vibrio cholerae* O139 and O1 SXT constins. Antimicrob Agents Chemother 45:2991-3000.

Hollinger K, Wray C, Evans S, Pascoe S, Chappell S, Jones Y. (1998) *Salmonella* Typhimurium DT104 in cattle in Great Britain. J Am Vet Med Assoc 213:1732-1733.

Hudson CR, Quist C, Lee MD, Keyes K, Dodson SV, Morales C, Sanchez S, White DG, Maurer JJ. (2000) Genetic relatedness of *Salmonella* isolates from nondomestic birds in Southeastern United States. J Clin Microbiol 38:1860-1865.

Humphrey TJ, Mattick KL, Jorgensen F. (2000) Fit to eat? Food scares and safe food production. Microbiology Today 27:10-12.

Huovinen P, Sundstrom L, Swedberg G, Skold O. (1995) Trimethoprim and sulfonamide resistance. Antimicrob Agents Chemother 39:279-289.

Imberechts H, De Filette M, Wray C, Jones Y, Godard C, Pohl P. (1998) *Salmonella* Typhimurium phage type DT104 in Belgian livestock. Vet Rec 143:424-425.

Jones YE, Chappell S, McLaren IM, Davies RH, Wray C. (2002) Antimicrobial resistance in *Salmonella* isolated from animals and their environment in England and Wales from 1988 to 1999. Vet Rec 150:649-654.

Jorgensen F, Leach S, Wilde SJ, Davies A, Stewart GS, Humphrey T. (2000) Invasiveness in chickens, stress resistance and *Rpo*S status of wild-type *Salmonella enterica* subsp. enterica serovar Typhimurium definitive type 104 and serovar enteritidis phage type 4 strains. Microbiology 146 Pt 12:3227-3235.

Judicial Commission of the International Committee on Systematics of Prokaryotes. (2005) The type species of the genus *Salmonella* Lignieres 1900 is *Salmonella enterica* (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987, with the type strain LT2T, and conservation of the epithet enterica in *Salmonella enterica* over all earlier epithets that may be applied to this species. Opinion 80. Int J Syst Evol Microbiol 55:519-520.

Juneja VK, Eblen BS. (2000) Heat inactivation of *Salmonella* Typhimurium DT104 in beef as affected by fat content. Lett Appl Microbiol 30:461-467.

Karpiskova R, Benes D, Dedicova D. (1999) Emergence of multidrug resistant *Salmonella* Thyphimurium DT 104 in the Czech Republic. Euro Surveill 4:56-58.

Killalea D, Ward LR, Roberts D, de Louvois J, Sufi F, Stuart JM, Wall PG, Susman M, Schwieger M, Sanderson PJ, Fisher IS, Mead PS, Gill ON, Bartlett CL, Rowe B. (1996) International epidemiological and microbiological study of outbreak of *Salmonella agona* infection from a ready to eat savoury snack--I: England and Wales and the United States. BMJ 313:1105-1107.

Kulldorff M, Nagarwalla N. (1995) Spatial disease clusters: detection and inference. Stat Med 14:799-810.

Kulldorff M. (1997) A spatial scan statistic. Communication in Statistics: Theory and Methods 26:1481-1496.

Kulldorff M. (2006) SaTScan user guide. http://www.satscan.org/techdoc.html. (25/05/2007)

Kumar A.N., Jayaraman R. (1991) Molecular cloning, characterization and expression of a nitrofuran reductase gene of *Escherichia coli*. J Biosci 16:145-159.

Lacroix FJ, Cloeckaert A, Grepinet O, Pinault C, Popoff MY, Waxin H, Pardon P. (1996) *Salmonella* Typhimurium *acr*B-like gene: identification and role in resistance to biliary salts and detergents and in murine infection. FEMS Microbiol Lett 135:161-167.

Lawson AJ, Desai M, O'Brien SJ, Davies RH, Ward LR, Threlfall EJ. (2004) Molecular characterisation of an outbreak strain of multiresistant *Salmonella enterica* serovar Typhimurium DT104 in the UK. Clin Microbiol Infect 10:143-147.

Leegaard TM, Caugnat DA, Froholm LO, Hoiby EA, Lassen J. (2000) Emerging antibiotic resistance in *Salmonella* Typhimurium in Norway. Epidemiol Infect 125:473-480.

Levin BR, Perrot V, Walker N. (2000) Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. Genetics 154:985-997.

Levy SB. (1994) Balancing the drug-resistance equation. Trends Microbiol 2:341-342.

Liebana E, Garcia-Migura L, Clouting C, Clifton-Hadley FA, Lindsay E, Threlfall EJ, McDowell SW, Davies RH. (2002) Multiple genetic typing of *Salmonella enterica* serotype Typhimurium isolates of different phage types (DT104, U302, DT204b, and DT49) from animals and humans in England, Wales, and Northern Ireland. J Clin Microbiol 40:4450-4456.

Lindstedt BA, Heir E, Gjernes E, Kapperud G. (2003) DNA fingerprinting of *Salmonella enterica* subsp. enterica serovar Typhimurium with emphasis on phage type DT104 based on variable number of tandem repeat loci. J Clin Microbiol 41:1469-1479.

Loewe L, Textor V, Scherer S. (2003) High deleterious genomic mutation rate in stationary phase of *Escherichia coli.* Science 302:1558-1560.

Loewen PC, Hengge-Aronis R. (1994) The role of the sigma factor sigma S (*Kat*F) in bacterial global regulation. Annu Rev Microbiol 48:53-80.

Low JC, Angus M, Hopkins G, Munro D, Rankin SC. (1997) Antimicrobial resistance of *Salmonella enterica* Typhimurium DT104 isolates and investigation of strains with transferable apramycin resistance. Epidemiol Infect 118:97-103.

Malorny B, Schroeter A, Bunge C, Hoog B, Steinbeck A, Helmuth R. (2001) Evaluation of molecular typing methods for *Salmonella enterica* serovar Typhimurium DT104 isolated in Germany from healthy pigs. Vet Res 32:119-129.

Mammina C, Cannova L, Massa S, Goffredo E, Nastasi A. (2002) Drug resistances in *Salmonella* isolates from animal foods, italy 1998-2000. Epidemiol Infect 129:155-161.

Martinez-Martinez L, Pascual A, Jacoby GA. (1998) Quinolone resistance from a transferable plasmid. Lancet 351:797-799.

Metzer E, Agmon V, Andoren N, Cohen D. (1998) Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium phage-type DT104 among salmonellae causing enteritis in Israel. Epidemiol Infect 121:555-559.

Murray A, Coia JE, Mather H, Brown DJ. (2005) Ciprofloxacin resistance in non-typhoidal *Salmonella* serotypes in Scotland, 1993-2003. J Antimicrob Chemother 56:110-114.

Naas T, Mikami Y, Imai T, Poirel L, Nordmann P. (2001) Characterization of In53, a class 1 plasmid- and composite transposon-located integron of *Escherichia coli* which carries an unusual array of gene cassettes. J Bacteriol 183:235-249.

Nesvera J, Hochmannova J, Patek M. (1998) An integron of class 1 is present on the plasmid pCG4 from gram-positive bacterium *Corynebacterium glutamicum*. FEMS Microbiol Lett 169:391-395.

Nield BS, Holmes AJ, Gillings MR, Recchia GD, Mabbutt BC, Nevalainen KM, Stokes HW. (2001) Recovery of new integron classes from environmental DNA. FEMS Microbiol Lett 195:59-65.

Nielsen B, Alban L, Stege H, Sorensen LL, Mogelmose V, Bagger J, Dahl J, Baggesen DL. (2001) A new *Salmonella* surveillance and control programme in Danish pig herds and slaughterhouses. Berl Munch Tierarztl Wochenschr 114:323-326.

O'Brien SJ, de Valk H. (2003) *Salmonella* - "old" organism, continued challenges! Eurosurveillance 8:29-31.

Olsen SJ, DeBess EE, McGivern TE, Marano N, Eby T, Mauvais S, Balan VK, Zirnstein G, Cieslak PR, Angulo FJ. (2001) A nosocomial outbreak of fluoroquinolone-resistant *Salmonella* infection. N Engl J Med 344:1572-1579.

Pankratz A. (1983) Forecasting with univariate Box-Jenkins models: concepts and cases. Wiley. New York, USA.

Palzkill T. (2001) Impending doom: antibiotic exposure and bacterial gene expression. Genome Res 11:1-2.

Parry CM. (2003) Antimicrobial drug resistance in *Salmonella enterica*. Curr Opin Infect Dis 16:467-472.

Paulsen IT, Skurray RA, Tam R, Saier MH, Jr., Turner RJ, Weiner JH, Goldberg EB, Grinius LL. (1996) The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol Microbiol 19:1167-1175.

Pessoa-Silva CL, Miyasaki CH, de Almeida MF, Kopelman BI, Raggio RL, Wey SB. (2001) Neonatal late-onset bloodstream infection: attributable mortality, excess of length of stay and risk factors. Eur J Epidemiol 17:715-720.

Pfaller MA, Jones RN, Doern GV, Kugler K. (1998) Bacterial pathogens isolated from patients with bloodstream infection: frequencies of occurrence and antimicrobial susceptibility patterns from the SENTRY antimicrobial surveillance program (United States and Canada, 1997). Antimicrob Agents Chemother 42:1762-1770.

Pittet D, Wenzel RP. (1995) Nosocomial bloodstream infections. Secular trends in rates, mortality, and contribution to total hospital deaths. Arch Intern Med 155:1177-1184.

Poppe C, McFadden KA, Demczuk WH. (1996) Drug resistance, plasmids, biotypes and susceptibility to bacteriophages of *Salmonella* isolated from poultry in Canada. Int J Food Microbiol 30:325-344.

Prager R, Liesegang A, Rabsch W, Gericke B, Thiel W, Voigt W, Helmuth R, Ward L, Tschape H. (1999) Clonal relationship of *Salmonella enterica* serovar Typhimurium phage type DT104 in Germany and Austria. Zentralbl Bakteriol 289:399-414.

Ramos JM, Ales JM, Cuenca-Estrella M, Fernandez-Roblas R, Soriano F. (1996) Changes in susceptibility of *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Virchow to six antimicrobial agents in a Spanish hospital, 1980-1994. Eur J Clin Microbiol Infect Dis 15:85-88.

Rang HP, Ritter JM. (1971) The effect of disulfide bond reduction on the properties of cholinergic receptors in chick muscle. Mol Pharmacol 7:620-631.

Ridley A, Threlfall EJ. (1998) Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonell*a Typhimurium DT 104. Microb Drug Resist 4:113-118.

Rodrigue DC, Tauxe RV, Rowe B. (1990) International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol Infect 105:21-27.

Rossi A, Lopardo H, Woloj M, Picandet AM, Marino M, Galds M, Radice M, Gutkind G. (1995) Non-typhoid *Salmonella* spp. resistant to cefotaxime. J Antimicrob Chemother 36:697-702.

Rowe B, Threlfall EJ, Ward LR, Ashley AS. (1979) International spread of multiresistent strains of *Salmonella* Typhimurium phage types 204 and 193 from Britain to Europe. Vet Rec 105:468-469.

Rubino S, Muresu E, Solinas M, Santona M, Paglietti B, Azara A, Schiaffino A, Santona A, Maida A, Cappuccinelli P. (1998) IS200 fingerprint of *Salmonella enterica* serotype Typhimurium human strains isolated in Sardinia. Epidemiol Infect 120:215-222.

Ryan CA, Nickels MK, Hargrett-Bean NT, Potter ME, Endo T, Mayer L, Langkop CW, Gibson C, McDonald RC, Kenney RT. (1987) Massive outbreak of antimicrobial-resistant salmonellosis traced to pasteurized milk. JAMA 258:3269-3274

Rychlik I, Barrow PA. (2005) *Salmonella* stress management and its relevance to behaviour during intestinal colonisation and infection. FEMS Microbiol Rev 29:1021-1040.

Sanchez L, Pan W, Vinas M, Nikaido H. (1997) the *acr*AB homolog of *Haemophilus influenzae* codes for a functional multidrug efflux pump. J Bacteriol 179:6855-6857.

Sands LC, Shaw WV. (1973) Mechanism of chloramphenicol resistance in staphylococci: characterization and hybridization of variants of chloramphenicol acetyltransferase. Antimicrob Agents Chemother 3:299-305.

Sandvang D, Aarestrup FM, Jensen LB. (1998) Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol Lett 160:37-41.

Scottish Centre for Infection and Environmental Health. (1999) Surveillance report: gastrointestinal infections. SCIEH Weekly Report. 33:10-11.

Smelser NJ, Baltes PB. (2001) International Encyclopedia of the Scocial Behavioral Sciences. Elsevier Science Ltd. Oxford, UK.

Smith, K, Rachid, MA, Willingham, E, Sturtz, R, de Almeida, QC, Ferreira, F. (2007) Etiology (*Salmonella* Typhimurium DT104). http://www.vet.uga.edu/vpp/NSEP/Brazil2002/salmonella/eng/etiology.htm . (21/3/2007).

Sojka WJ, Wray C, Pritchard GC, Hedges RW. (1982) Gentamicin resistant *Salmonella*. Vet Rec 111:512.

SPSS Inc. (2006) SPSS trends 14.0 First ed. Prentice Hall, Chicago, USA.

Steinbach G, Hartung M. (1999) [Attempt to estimate the share of human *Salmonella* infections, which are attributable to *Salmonella* originating from swine]. Berl Munch Tierarztl Wochenschr 112:296-300.

Stephen JM, Toleman MA, Walsh TR, Jones RN. (2003) *Salmonella* bloodstream infections: report from the SENTRY Antimicrobial Surveillance Program (1997-2001). Int J Antimicrob Agents 22:395-405.

Stokes HW, Hall RM. (1989) A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol Microbiol 3:1669-1683.

Takkinen J, Nakari UM, Johansson T, Niskanen T, Siitonen A, Kuusi M. (2005) A nationwide outbreak of multiresistant *Salmonella* Typhimurium in Finland due to contaminated lettuce from Spain, May 2005. Euro Surveill 10:E050630.

Ternent, HE. An epidemiological study of *Escherichia coli* O157 Strains in Scottish fattening cattle. (2002) University of Glasgow.
Ref Type: Thesis/Dissertation

Threlfall EJ. (2000) Epidemic *Salmonella* Typhimurium DT 104--a truly international multiresistant clone. J Antimicrob Chemother 46:7-10.

Threlfall EJ. (2002) Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. FEMS Microbiol Rev 26:141-148.

Threlfall EJ, Fisher IS, Berghold C, Gerner-Smidt P, Tschape H, Cormican M, Luzz I, Schnieder F, Wannet W, Machado J, Edwards G. (2003) Antimicrobial drug resistance in isolates of *Salmonella enterica* from cases of salmonellosis in humans in Europe 2000: results of international multi-centre surveillance. Euro Surveill 8:41-45.

Threlfall EJ, Frost JA, Ward LR, Rowe B. (1994) Epidemic in cattle and humans of *Salmonella* Typhimurium DT 104 with chromosomally integrated multiple drug resistance. Vet Rec 134:577.

Threlfall EJ, Frost JA, Ward LR, Rowe B. (1996) Increasing spectrum of resistance in multiresistant *Salmonella* Typhimurium. Lancet 347:1053-1054.

Threlfall EJ, Hopkins KL, Ward LR. (2005) Diversification in *Salmonella* Typhimurium DT104. Emerg Infect Dis 11:980-981.

Threlfall EJ, Rowe B, Ferguson JL, Ward LR. (1983) Gentamicin resistant *Salmonella* Typhimurium. Vet Rec 113:627.

Threlfall EJ, Rowe B, Ferguson JL, Ward LR. (1985) Increasing incidence of resistance to gentamicin and related aminoglycosides in *Salmonella* Typhimurium phage type 204c in England, Wales and Scotland. Vet Rec 117:355-357.

Threlfall EJ, Rowe B, Ferguson JL, Ward LR. (1986) Characterization of plasmids conferring resistance to gentamicin and apramycin in strains of *Salmonella* Typhimurium phage type 204c isolated in Britain. J Hyg (Lond) 97:419-426.

Threlfall EJ, Ward LR, Rowe B. (1978) Spread of multiresistant strains of *Salmonella* Typhimurium phage types 204 and 193 in Britain. Br Med J 2:997.

Threlfall EJ, Ward LR, Rowe B. (1997) Increasing incidence of resistance to trimethoprim and ciprofloxacin in epidemic *Salmonella* Typhimurium DT104 in England and Wales. Euro Surveill 2:81-84.

Threlfall EJ, Ward LR, Rowe B. (1999) Resistance to ciprofloxacin in non-typhoidal salmonellas from humans in England and Wales-the current situation. Clin Microbiol Infect 5:130-134.

Threlfall EJ, Ward LR, Skinner JA, Graham A. (2000) Antimicrobial drug resistance in non-typhoidal salmonellas from humans in England and Wales in 1999: decrease in multiple resistance in *Salmonella enterica* serotypes Typhimurium, Virchow, and Hadar. Microb Drug Resist 6:319-325.

Ikeda JS, Hirsh DC. (1985) Common plasmid encoding resistance to ampicillin, chloramphenicol, gentamicin, and trimethoprim-sulfadiazine in two serotypes of *Salmonella* isolated during an outbreak of equine salmonellosis. Am J Vet Res 46:769-773.

Toft N, Innocent GT, McKendrick IJ, Ternent HE, Mellor DJ, Gunn GJ, Syne B, Reid SWJ. (2005) Spatial distribution of *Escherichia coli* O157-positive farms in Scotland. Prev Vet Med 71:45-56.

Tortora GJ, Funke BR, Case CL. (2001) The prokaryotes: domains Bacteria and Archaea. Microbiology: an introduction. London: Benjamin Cummings.p 303-330.

van Duijkeren E, Wannet WJ, Houwers DJ, van Pelt W. (2003) Antimicrobial susceptibilities of *Salmonella* strains isolated from humans, cattle, pigs, and chickens in the Netherlands from 1984 to 2001. J Clin Microbiol 41:3574-3578.

Van Pelt W. (1999) Surveillance of *Salmonella*: achievements and future directions. Euro Surveill 4:51.

Wall PG, Morgan D, Lamden K, Ryan M, Griffin M, Threlfall EJ, Ward LR, Rowe B. (1994) A case control study of infection with an epidemic strain of multiresistant *Salmonella* Typhimurium DT104 in England and Wales. Commun Dis Rep CDR Rev 4:R130-R135.

Wall PG, Morgan D, Lamden K, Griffin M, Threlfall EJ, Ward LR, Rowe B. (1995) Transmission of multi-resistant strains of *Salmonella* Typhimurium from cattle to man. Vet Rec 136:591-592.

Ward LR, de Sa JD, Rowe B. (1987) A phage-typing scheme for *Salmonella* Enteritidis. Epidemiol Infect 99:291-294.

Waltier L, Richardson S, Hubert B. (1991) A time series construction of an alert threshold with application to *S. bovismorbificans* in France. Stat Med 10:1493-1509.

Waltier L, Richardson S, Hubert B. (1993) *Salmonella* Enteritidis infections in France and the United States: characterization by a deterministic model. Am J Public Health 83:1694-1700

Watier L. (1995) [Time series analysis of non-typhoid salmonella infections]. Rev Epidemiol Sante Publique 43:173-185.

Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, Hudson MJ, Roderick PJ. (1999) Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ 318:1046-1050.

Woodhead M, Fleming D, Wise R. (2004) Antibiotics, resistance, and clinical outcomes. BMJ 328:1270-1271.

Wray C, Sojka WJ. (1978) Experimental *Salmonella* Typhimurium infection in calves. Res Vet Sci. 25:139-143.

Wray C, Davies RH. (1996) A veterinary view of *Salmonella* in farm animals. PHLS Microbiol Dig 13:44-48.

Yang SJ, Park KY, Kim SH, No KM, Besser TE, Yoo HS, Kim SH, Lee BK, Park YH. (2002) Antimicrobial resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from animals in Korea: comparison of phenotypic and genotypic resistance characterization. Vet Microbiol 86:295-301.

Zhao J, Aoki T. (1992) Nucleotide sequence analysis of the class G tetracycline resistance determinant from *Vibrio anguillarum*. Microbiol Immunol 36:1051-1060.