World Journal of Agronomy, Food Science & Technology
Vol. 1, No. 1, January 2014, PP: 1- 13
Available online at www.wjafst.com

Research article

Characterization of Salmonella Isolates from Beef Cattle, Broiler Chickens and Human Sources

Desalegn Amenu

Wollega University, College of Natural and Computational Science, P.Box, 395, Nekemte, Ethiopia

E-mail: wadadesalegn@gmail.com

Abstract

Non typhoid Salmonella serovars rernain a potential threat to human and animal health. Infection with Salmonella may not lead to fatal disease but rather it may remain localized in the gastro-intestinal tract resulting in gastroenteritis or may take a septicemia form that can affect several organ systems. Infected food animals that do not develop salmonellosis and those that recover from the disease may become carriers of Salmonella and serve as sources of infection to humans and animals. **Apart** from being a source of foodborne illness for humans, Salmonella-contaminated food animal carcasses are also of concern because they are a source of antibiotic-resistant Salmonella. In addition, drug resistance patterns and virulence characteristics of beef, chicken and human isolates during 1996-1 997 were compared and the molecular aspects of virulence genes were investigated. These findings were confirmed by plasmid DNA isolation using different protocols and by sequence analysis of the spvC positive isolates. It may be speculated that this **could** have happened as a result of chromosomal integration of **spvC** sequence. To the best of our knowledge this is the **first** report demonstrating the occurrence of the spvC gene sequence on the chromosome.

Keywords: Cattle, Broiler Chickens, Human Sources

Introduction

Food borne bacterial diseases are a continuing challenge to human health world-wide. *Escherichia coli* 01 57:H7 has emerged in the past few years as a pathogen of public health importance. Although most E. *coli* are normal Rom, several strains are capable of causing disease in humans and other warm-blooded animal. Salmonellosis is an important zoonotic disease of world-wide significance.

Food borne bacterial pathogens

Over the past **two** decades, **the** epidemiology of food borne diseases **has** changed rapidly as a consequence of changes in the social environment and the ability of pathogens to adapt to new niches (Altekruse *et.al*., 1998). Several well recognized pathogens such as *Salmonella, Escherichia coli 0* 1 57: H7, *Campylobacter,* and *Yeninia*

enterocolitica, have emerged as public health problems and have increased in prevalence or become associated with new food vehicles (Altekruse et.al., 1998). Of these pathogens, *Salmonella* is the most commonly reported cause of foodbome disease in the **UK** and the USA (Eley., 1996). In Canada, *Salmonella* is the second leading cause of human disease after *Campylobacter* (Health Protection Branch, Health Canada, 1997).

Disease associated with ***E.*** *coli* 0157:H7 was first reported in the USA in 1982 and subsequently outbreaks and sporadic cases have been reported in Canada, the UK and Japan *(*Eley. 1996*). C. jejuni is* considered to be a very frequent cause of foodborne illness in the **US** and other industrialized nations. *Yersinia* is an emerging pathogen which is the major cause of diarrhea in many countries, with high incidence in Scandinavia and japan **(**Miller, 1998**).** Infection can occur most often via ingestion of contaminated foodstuffs such as red and white meat products, eggs, untreated mil k, **dain/** products and any other foods or water that are contaminated with faecal material. In addition, foodborne infections with Salmonella and E. coli 0157 **can also** be transmitted through fruits and vegetables contaminated with faecal material from diseased or carrier animals. Contamination of these foods can occur du ring production, processing, distribution, retail marketing and handling (Gomez. et.al., 1997). The primary sources of contamination are usually food animals and possibly human carriers.

Salmonellosis in humans

Salmonellosis is an important zoonotic disease of world-wide significance. In particular, food animals with subclinical infection constitute a vast reservoir for disease in humans (Khakhrla et.al.,1997). Disease is caused by various *Salmonella* serovars and it occurs in a wide variety of forms presenting a broad clinical spectrum. In humans salmonellosis may occur as an acute, self-1imiting gastroenteritis or as systemic infection characterized by septicaernia and ultimate localization in extra-intestinal sites. The gastrointestinal form is often referred to as the "food poisoning syndrome." This is a misnomer as the disease is an infection rather than intoxication. The systemic disease is referred to as enteric fever, with typhoid being the classic form. All *Salmonella* serovars are presumably pathogenic to humans **(**Tletjen and Fung, 1995**).** *Salmonella* infections can cause important morbidity, mortality and econornic burden and are particularly severe in infants, the elderly and imrnunocomprornised individuals.Although the outbreaks of human salmonellosis are frequently lirnited to single cases or confined to one family, the global data of *Salmonella* infection are impressive (D'aoust, 1989***).*** In Canada about 8,000 cases of human salmonellosis are reported yearly by the National Laboratory for Enteric Pathogens. In the United States **sa1**monellosis accounts for more than 40,000 reported **cases,** 500 deaths and financial **costs** are greater than $ 50 million each. The magnitude of salrnonellosis has sparked a great interest in the identification and classification of these microorganisms, with emphasis on how they associate and establish themselves to cause disease in humans and animals, particularly food animals (D'aoust, 1989***).***

Isolation and characterization of *Salmonella* species

Isolation and identification of strains involved is **an** important **step** in controlling *Salmonella* outbreaks or sporadic clinical cases. Numerous **typing** schemes have been used to identify Salmonella species, including biochemical and

serological identification; the latter differentiate *Salmonella* into serovars. Further identification by phenotypic characteristics has also been used both independently and in combination for subdividing serovars. These include phage typing, antibiotic resistance patterns, and colicin typing and plasmid characterization. These methods are usually supplemented by genotypic characterization such as plasmid finger print and chromosomal analysis. Phenotypic and genotypic characterization can provide information on the strain implicated; demonstrate an epidemiological link between cases and associate cases with a potential source. Furthermore, these typing schemes can also be used as diagnostic tools and for the assessment of the pathogenic properties of *Salmonella* (Gonzalez and Mendoza, 1995).

Isolation of *Salmonella*

Salmonellae are widely distributed in natural environment however, the main reservoir of *Salmonella* is the intestinal tract of infected or carrier animals particularly meat animals (Gomez. et.al., 1997). Carriers of *Salmonella* are often difficult to detect by routine culture protocols (Nolan et.al., 1995). Failure to detect *Salmonella* complicates the estimation of this organism's impact on the productivity of food animals and makes the control of salmonellosis difficult Since Salmonellae often colonize the intestinal tract, faecal or cecal samples seem to be better than extra-intestinal samples for detection of carriers (Nolan et.al., 1995). The detection of *Salmonella* in faecal sample can be complicated because the organism may be greatly outnumbered by other competing microflora **(**Nolan et.al., 1995). **As** a consequence, isolation and detection of *Salmonella* either by conventional or rapid methods involves a selective enrichment step *(*Nolan et.al., 1995*).* A selective enrichment medium contains inhibitory reagents that allow *Salmonella* to grow while restricting the growth of other microflora. This selectivity is based on the synergism between the inhibitory reagent and the incubation temperature which varies between **3** 7°C and 43 OC (7, 10). Many inhibitors are in use, the most common being bile, tetrathionate, sodium selenite, and either brilliant green or malachite green dyes. Two or more of these inhibitors may be **used** in combination. The selectivity of tetrathionate broth depends on the ability to suppress the growth of coliform bacteria, while Salmonella species possess the enzyme tetrathionate reductase and consequently are able to grow in the medium **(**Miller, 1998***).***

In the standard protocol for isolation, incubation in selective broth is usually followed by recovery of viable Salmonella **on** selective solid media. Several selective solid media have recently been described for the recovery of non-typhoid Salmonella from stool specimens. These include Rambach agar **(Ra),** novobiocin-brilliant green glycerol lactose agar (NBGL), xylose- lysine Tergitol 4 agar **(XLT4),** Hektoen agar (HE) and modified semisolid Rappaport-Vassiliadis medium (MSRV) (Nolan et.al.,1995). The sensitivity of these media before and after enrichment was studied and compared by Aitwegg et. al (Nolan et.al., 1995), who found that MSRV was the most sensitive medium tested for the isolation of *Salmonella* from stool specimens. The selectivity of this medium is based on the presence of malachite green and novobiocin that inhibit most coliforms. The efficiency of the MSRV medium lies in the ability of *Salmonella* to migrate through the semisolid agar ahead of competing flora at the high incubation temperature of **41** - 43 **OC.** Other rapid methods that have been developed for detection of Salmonella particularly in food samples include immunological conductance impedance D NADNA hybridization and DNA

amplification **(**Stone et.al., 1994**).** However, the sensitivities of these methods also rely on the initial number of Salmonella.

Biochemical identification

The classification of the genus *Salmonella* has been controversial for many yean. In the Kauffmann scheme it has been subdivided into four subgenera (1-IV) **(**Stone, et al., 1994). This subdivision is based on their biochemical characteristics. Le Minor (Stone, et al.,1994) proposed that the genus *Salmonella* consists of only a single species, S. *enterica* which is divided into subspecies based on biochemical reactions of strains with dulcitol, lactose, O-nitrophenol-PD galactosidase (ONPG), salicin, d-tartrate, mucate, maltose, gelatinase, sorbitol and potassium cyanide (KCN). Seven subspecies have been identified, S. *enterica* subsp. *enterica* (l), S. *enterica.* subsp. *salamae* (Stone, et al.,1994), *S. enterica* subsp. *arizona* **(**lia), *5-enterica* subsp. *diarizona* **(**Il), *S. enterica* subsp. *houtenae* (IV), S. *enterica* subsp. *bongori* (VI and *S.enterica* subsp *indica.(VI).* More recently Popoff et al. (Stone, et al., 1994) divided the genus *Salmonella* into two species *S. enterica* and *S. bongori.* Thus, the former species S. *enterica* is subdivided into six subsp. *enterica* **I** subsp. *salamae* subsp. *arizona* (Il la), subsp. *diarizona* (1II), subsp. *houtenae* (IV) and subsp *indica* **(V).** Each subspecies is divided into serovars based on their antigenic determinants, somatic and flagellar antigens *(*Stone, et al., 1994*).*

Serological identification

Salmonella strains have been classified into serovars on the basis of extensive diversity of the somatic *(0)* antigens, capsular antigens **(Vi)** and the flagellar (H) antigens. The O antigens are heat stable polysaccharides located in the cell wall and are shared with other members of the *Enterobacteriaceae.* In contrast, the H antigens are heat labile proteins contained in the flagella and the surface polysaccharide antigens, and often occur in **two** forms phase **I** and phase II. The flagellar antigens are highly specific to *Salmonella.* Thus, a positive reaction, with polyvalent H antiserum is sufficient to presume that the organism is *Salmonella.* The Vi antigen, the surface antigen of the typhoid organism, is the most important example of heat sensitive surface polysaccharide antigens.

The serological scheme of classification **was** established by White in 1926 and extended elaborated by Kauffmann du ring **1** 972-1 978. Over 2,200 antigenical**ly** distinct serovars have now been recognized. Approximately 1289 serovars of subspecies I have been identified and about **99 */O** of *Salmonella* strains isolated from humans belong to this subspecies. The species names given to the serovan were formerly derived from the disease condition either in human [e-g., *Salmonella* typhi or *Salmonella para* typhi] or in animals [e.g., *Salmonella choleraesuis* 1. Since the host specificity suggested by these names was not always true, serovars names were later based on the geographic origin of the first strains to be found [e-g., *Salmonella dublin]* (Stone, et al.,1994**).** However, serovan belonging to subspecies 11, III, IV, V and VI are frequently isolated from reptiles but are rarely from warning blooded airmails. The O antigens have been used to separate *Salmonellae* into groups. Fifty serologically distinct groups have been assigned (A, B, *C,*, ***C,,*** **D,** E, **E,,** E, F, *G,* H, **1** and others) al1 of which have O antigen and some of which have common antigens.

For example, group A contains somatic antigen 2, group B contains somatic antigen 4, group Cl contains somatic antigen 7, group C2 contains somatic antigen 8. In addition, al1 strains in group B contain somatic antigen 12 as do strains in group D, and group Cl and C2 al1 contain somatic antigen 6, as well as others mentioned above. The O antigens are designated by Arabic numerals and H antigens by lower case **letter** in phase one and Arabic numerals in phase two. For instance, *Salmonella typhimurium* has been grouped as **(**Guthrie,2000**).** The identification of *Salmonella* to the serovar level enables the observation of overall changes in the prevalence of particular serovars in a certain geographical area. For example, serological typing has demonstrated that *Salmonella typhimurium* displaced *Salmonella enteritidis* as the most common serovar causing disease in human and animais in Canada (**Poppe,1994**).

Phage typing

Phage typing has proven to be a valuable adjunct to serological identification. Early studies in the 1920s demonstrated that phages could not only be used for serovars identification, but by varying the host strains on which the phage was propagated, adapted phages could be isolated which could form the basis of a typing scheme (32). The phage typing scheme based on principal of phage adaptation was first applied in 1938 to differentiate strains of *Salmonella typhi* (Gomez et al.,1995**).** This scheme uses progressive adaptation of Vi-phage II which is specific for the capsular antigen of S. ***typhi.*** These adaptations are used as the routine test dilution (RTD), the highest dilution that produces confluent lysis on homologous bacterial phage type. Eleven phage types were initially identified. In 1947 the method of S. *typhi* Vi phage typing was characterized and by 1986, with further adaption of Vi-phage II, a further **95** types were defined and internationally recognized, bringing the number **to** 106 phage types of *Salmonella typhi* **(**Gomez et al.,1995***).*** In contrast, phage typing schemes for other ***Salmonella*** serovars are **based** on the patterns of the lysis produced by serologically distinct phages isolated from a variety of sources. Over *200* phage types of **L** *typhimurium* have been specified (**Poppe, 1994**).

Phage typing has been the method of choice in the reference laboratory for differentiation within serovan. It is a highly discriminatory method for the subdivision of serovars into distinct phage types (PT) which facilitates the identification of the strains involved in human outbreaks. For example, phage typing has demonstrated that **an** upsurge in the incidence of S. *enteritidis* infection in humans in many European countries was caused exclusively by strains belonging to PT4 and this phage type was found in poultry. In the United States and Canada, PT 8 and PT **13** are predominant (D'aoust. et al., 1994*).* Recently, phage typing demonstrated that S. *typhimurium* PT **1 04** also known DT 104 is the commonest cause of sahonellosis in England and Wales and this phage type has emerged in Canada and the United States as well (Threlfall et al., 1996*).*

Antibiotic resistance typing

Antibiotic resistance in ***Salmonella*** strains is commonly encoded by resistance plasmids (R-plasmids) that have been acquired as a result of antibiotic selective pressure in human or veterinary medicine. In turn, the majority of R-plasmids have acquired their resistance genes by a mechanism called transposition in which mobile elements of DNA are inserted into nonhomologous regions of chromosome or plasmid DNA. These elements or transposons can either be acquired from other plasmids in the same strains, from **the** chromosome or from plasmids carried by other bacteria in the host organism. Resistance genes can also be acquired from other bacteria in the host organism either by conjugation in which the resistance gene is transferred from donor to recipient cell via specialized structure of the donor called pilus or by transduction via a carrier such as bacteriophages. Another mechanism by which resistance genes can arise is via spontaneous mutation of the chromosomal genes, again as a result of selective pressure (Espinasse, 1993).

Although there is a growing need to update information on drug resistance trends in Salmonella, antibiotic resistance typing as such is not a satisfactory method for discrimination within the serovars as resistance plasmids and transposons are unstable traits. Nonetheless, antibiotic resistance typing can be performed in conjunction with serotyping, phage typing and plasmid profile analysis for epidemiological purposes. This method has been used in identifying clones of chloramphenicol resistant S. ***typhi*** belonging to different phage types which caused major outbreaks in Mexico, India and South-east Asia since the **1970s (**Espinasse, 1993). **1.3.7 Plasmid characteriration** Plasmids are covalently-closed, circular, doublestranded extrachrornosomal elements of **DNA** that replicate independently of the bacterial chromosome.

Plasmid profile involves screening of the strain for presence of plasmid(s) and characterizing the plasmids in terms of number per strain and their molecular weight. Plasmid DNA extracts are analysed by electrophoresis on agarose gels where they are separated into clearly defined bands according to their molecular weights. Plasmid profile typing has been used for strain differentiation within serovan and within a phage type **(**Threlfall and Frost, 1990). In Britain the method was first applied to investigate the epidemiology of multi drug resistant Salmonella ***typhimurium*** phage type 204c which was resistant to apramycin and gentamicin. Seven plasmid profiles have subsequently been identified in the strains which have been isolated from cattle in Britain **(**Threlfall and Frost, 1990). Similarly plasmid profile typing has been successfully used for dividing S. ***typhimurium*** DT ***104*** into plasmid profiles.

Plasmid DNA fingerprinting

Plasmid profile can only provide information on the size and the number of the plasmids carried on the strains under investigation. For example, the presence of a single 60 MDa plasmid in each of two strains under test does not necessarily establish epidemiological relatedness because these plasmids could have completely different DNA sequences (**Mayer, 1998**). A further degree of identification **can** be attained by using restriction enzyme analysis. The restriction enzyme analysis is used to differentiate between strains that have the same plasmid profile. The enzyme recognizes **a** specific base sequence of DNA and cleaves DNA whenever it finds its recognition sequence,

thus generating DNA fragments. The number and the size of the fragments refled the DNA sequence of the plasmid under question. The specificity of this analysis is increased if two or more restriction enzymes are studied.

Virulence determinants of *Salmonella*

The ability of *Salmonella* to colonize relevant parts of the alimentary tract is the first stage of infection. Close association and penetration of the intestinal mucosa is a prerequisite for the pathogenesis (Lester, 1995). Recent research on adhesion and invasion has focused on tissue culture systems as models for invasion and the identification by mutational analysis of the **gens** involved in this process. The facultative intracellular growth and survival of ***Salmonella*** demand a large number of genes distributed around the chromosome, and **at** least 60 virulence genes including those responsible for nutrient biosynthesis have been identified. Galan and Curtiss identified a genetic locus (***inv)*** which affects the entry of S. ***typhimurium*** into epithelial ce1 1s. Furthermore, ***inv*** mutants were found to produce elevated LD, values in orally challenged mice and were found to colonize Peyer's patches less efficiently than the wild type strains.

Virulence plasmids

Salmonella species with the notable exception of S. ***typhi*** often have a large plasmid (30-60MDa) which is serovar specific. This plasmid has been shown to be essential for virulence in many *Salmonella* species. However, the large plasmid enables the organism to penist within the reticuloendothelial cells, while cured strains or strains that have no plasmid are quickly eliminated by the host immune system (). Cald-well and Gulig identified a 8-kbp region of a 9Bkbp (60-MDa) S. ***typhimurium*** virulence plasmid capable of conferring the organism's complete virulence plasmid phenotype. This 8kbp virulence region encompasses five genes collectively designated *Salmonella* plasmid virulence gens spv **RABCD.** Only ***spvC*** gene has been conclusively shown to be a virulence gene. The **exact** function of ***spvC,*** formerly known as ***virA*** is not known, although it seems to increase the survival and the growth of *Salmonella* within the reticuloendothelial cells (Swamy et al., 1996).

Antibiotic resistance patterns

The use of antibiotics in human and veterinary medicine has resulted in a spectacular decrease in the mortal**ity** rate of infectious diseases. However, this contribution to therapeutics has not been without disadvantages, one of the most outstanding is the evolution of antibiotic resistance. This has resulted in the dissemination of resistance genes to sensitive species and the emergence of new resistance determinants. One of the reasons for emergence of drug resistant pathogens in humans may be the intensive use of antibiotics in food animais both for therapeutic purposes and to improve growth rates (Poppe et al., 1996). The widespread use of antibiotics as feed additive in poultry, cattle, and pigs facilitates intestinal colonization by resistant *Salmonella.* Such practices often lead to emergence and persistence of resistant strains of *Salmonella* and increase the potential for cross-contamination of animals through prolonged faecal shedding. Antibiotic resistance also poses a concern to both human healths in the possible transmission of drug resistant *Salmonella* to humans via food derived from animal sources. Three possible routes by

which the use of antibiotics in animals could pose a risk to human health include: (i) pathogenic antibiotic-resistant bacteria transmitted to humans via contaminated food of animal origin can compromise the therapeutic value of antibiotic (ii) non pathogenic resistant bacteria can be contracted from animal sources, and these could serve as pools of resistance genes for bacteria in the human gut, and (iii) antibiotics could remain as residue in animal products which allow the selection of antibiotic resistant bacteria in humans. Though the use of antibiotics is extensively regulated in Canada, the question of whether feed antibiotic use in animals contributes to emergence of anti biotic resistant human pathogens **has** become an issue (Canadian Animal Health **i**nstitute **1** 99 7**).**

Antibiotic resistance in Salmonella, *Escherichia coli, Shigella* and other genera of *Enterobacteriaceae* is often encoded by resistance plasmids, some of which are self-transmissible whereas others may be CO-transferred by conjugative plasmids **(**Poppe, 1996**).** However, it **has** been reported that serovar-specific plasmids such as the 60-**MDa** of S. *typhimurium* and the 36-MDa of S. *enteritidis* do not usually encode drug resistance. Antibiotic resistance in *Salmonella* has also been found to be encoded by chromosomal gens. There has been great concern regarding the emergence of multiple drug resistance and resistance to new drugs in Salmonella species. It is also of interest to know whether the resistance is plasmid or chromosomally mediated. Previous studies have shown that multiple resistance of S. ***typhimurium*** DT **104** to ampici**ll**in, chloramphenicol, streptomycin, sulfonamides, and tetracycline are encoded by chrornosomal DNA (36). Development of resistance to new antibiotic is not uncommon. F1uoroquinolone-resistant salmonellae have already emerged in the UK and have been isolated from animals, foodstuffs and humans. Although ciprofloxacin has never been used in food animals, the use of the related drug enrofloxacin can cause cross-resistance (48). Threlfai**l** and colleagues (Threlfall et al., 1996**)** have identified a strain of S. ***typhimurium*** DT 104(R-type ACSSuTCp) with resistance to ciprofloxacin at MIC: 0.25 mg/L, and this resistance **has** been shown to be mediated by the chromosomal genes. Although antibiotic resistance is not considered a virulence factor, it may be related to the bacteriai existence and persistence in a host. It has also been proven that the dissemination of virulence gens could occur under selective pressure of antibiotic use (Balls et al., 1996). The antibiotic resistance patterns may **vary** from one geographical area to another, therefore, data need to be gathered on locally isolated strains.

Communicability and transmissibility of salmonellosis in humans

Although salmonellae are ubiquitous, the primary reservoir of *Salmonella* is the intestinal tract of infected or colonized domestic and wild animals and humans **(**Eley, 1996**).** The primary source of *Salmonella* infection in humans worldwide is contaminated food and water. Food of animal origin such as poultry, egg, milk, beef and pork are the main sources. *Salmonella* is in the faeces of both infected animals with clinical signs and healthy carriers. When livestock are slaughtered and dressed, faecal contamination of the carcasses may occur. The importance of red meat and poultry meat as vehicles of salrnonellosis lies in their physical contamination with the organism and in failure of the consumer to handle the **raw** food so **as** to prevent infection. Other significant sources of human salmonellosis are shellfish harvested from water contaminated with faecal materials and also contaminated fresh fruits and vegetables. Water contaminated with sewage is always a hazard as it was responsible for a massive

outbreak of S. *typhimurium* in 1971 in the US. Person to person spread of salmonellosis is possible particularly in hospitals (Eley, 1996).

Epidemiological aspects of human salmonellosis

Of the 2200 serovan of Salmonella identified, most human and animal disease is caused by a handful of serovars that belong to subspecies **1.** The distribution of the serovars involved change over time for usually quite unknown reasons (Lax et al., 1996). Salmonellosis is more common and severe in young children, elderly and individuals with underlying chronic diseases. The data from many countries have provided evidence of a steady and significant increase in salmonellosis during the past **two** to three decades. In Canada, during the period 1983 and 1992 a total of 89,760 Salmonella strains from humans were reported to the National Laboratory for Enteric Pathogens. Furthermore, there were 2,180 reported outbreaks associated with 10,065 cases during the IO-year period. Likewise, in the United States, approximately 25,000 cases of Salmonella infection were reported annually in the 1970s to the Centre for Disease Control **(CDC)** with continuai increase to more than 50,000 reported infections in 1985. In 1995, 41,222 infections were reported to the CDC. The major recent change in the epidemiology of *Salmonella* **has** been the emergence and increase of *Salmonella enteritidis* in industrialized nations and *Salmonella typhimurium* definitive phage type DT 1 **O4** in the United Kingdom, the United States and **Canada.** An important feature of this increase has been the spread of a strain of *Salmonella typhimurium* DT *104* with multi-resistance patterns characterized by resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (R-type ACSSuT). Recent studies have shown that infection with R-type ACSSuT was associated with higher hospitalization and fatality rates than other *Salmonella* serovars. Studies have identified farm animals as the source of human infection and consumption of improperly handled red meat **as** a risk factor for salmonellosis **(**Threlfall et al., 1996).

Prevalence of *Salmonella* serovars

Salmonella typhimurium

Salmonella typhimurium has been recognized worldwide as the most common human pathogen and etiological agent associated with the environment and the food chain**.** The largest outbreak of waterborne salmonellosis reported in the United States **was** associated with this serovars. However, between **1985** and 1991, S. *typhimurium* **was** the most prevalent pathogen implicated in food borne salmonellosis in the United States, accounting for more than 21 **O/.** of the total ***Salmonella*** isolates. However, as discussed, the importance of this serovar is in the emergence of multi drug resistant S. *typhimurium* R-type **ACSSuT.** The rate of isolation has dramatically increased from ***4Of0*** in 1989 to 43% in 1994. Furthermore, **S.** *typhimurium* isolated from cattle before 1986 did not have this R-type compared to 13% of the isolates obtained between 1986 and 1 991, and 64% of the isolates obtained in 1992 and 1995 (4). In addition, a high number of isolates from either human or cattle sources with the R-type **ACSSuT** that were phage typed were found to be phage type 1 **04.** In Canada, S. *typhimurium* has been the most common serovar isolated between 1983 and 1992. S. *typhimurium* **was** associated with 1,622 human cases reported from 222 outbreaks. This serovar accounted for the highest number of *Salmonella* isolates implicated in recent food borne diseases from **1** 992

and 1 993, representing 20.396 of the total *Salmonella.* Thirty-seven percent of *S. typhimurium* isolates from humans were found to be S. *typhimurium* DT 104 (Ontario Animais Health Surveillance Network 1997).

In Europe, the increase in prevalence of S. *typhimurium* in food borne disease continued to be significant (56). In Spain the frequency of the isolation of S. *typhimurium* has increased over the last few years from 13 **O/O** in 1988 to 25.1 O/O in 1994 **(57).** There have been continual increases in the isolation of S. typhimurium DT 104 in the United Kingdom from *259* **in** 1990 to 2,873 **in** 1994 and 3,837 in 1995. Moreover, ***S.*** typhimurium DT 104 is the second most prevalent Salmonella in humans after **S** .enteritidis, although a few years ago S. typhimurium Phage type 204 **was** predominant in the United Kingdom (Gomez et al., 1997).

S. *heidelberg,* S. *hadar* and S. *infanfis*

In Canada during 1 983-1 992, S. heidelberg and S. hadar ranked the second and third most commonly isolated serovars from humans after S. typhimurium. The reported isolation rate of S. heidelberg and 5. hadar in humans were 12.0 % and 10.9% respectively. There were 35 reported outbreaks of S. heidelberg infection that affected 205 people. Forty-six outbreaks of S. hadar infection occurred that affected 421 people. The frequency of S. hadar isolations from human and non-human sources reached a peak during the years 1987-1990 and declined thereafter. Seventeen outbreaks of S. infantis were reported during 1983-1 993 which affected **124** persons (Miller, 1998).

Salmonella en*teritidis*

Human infection with Salmonella enteritidis has significantly increased du ring the last few years in many parts of the world (**Nastasi, 1996**). The increases in foodborne illness during the last two decades have largely been attributed **to** S. enteritidis in a number of countries such as Argentina, Austria, Bulgaria, Finland, France, Hungary, Italy, Spain, Switzerland, and the United Kingdom **(**Guthrie, 2000). In Europe the prevalence of S. *enteritidis* infection surpassed *S. typhimurium* and became the most commonly isolated serovar. In **England** and Wales *Salmonella* isolation reported from human infection doubled from 10,251 in 1981 to 22,627 in 1991. This increase was because of the increase in S. *enteritidis,* particularly phage type **4.** It accounted for **53%** of ail *Salmonella* isolated in 1989 (1 5). In the United States, the proportion of total *Salmonella* isolates that were S. *enteritidis* increased from **5%** in 1976 to ***26%*** in **1994** and decreased to 25% in 1995.

In Canada the frequency of S. *enteritidis* isolations varied between 4.2 to 9.2% of ail the serovars isolated between **1** 979-1 989. However, this percentage increased in 1 991 to 1 2.5% and S. *enteritidis* was the second most common serovar after S. *typhimurium.* While large outbreaks associated with this serovar have rarely occurred in Canada, a notable exception occurred among patients and staff of a regional hospital in Ontario, where the outbreak was associated with S. *enteritidis* phage type (PT). In a period of 1983-1 991, there were 73 outbreaks of S. *enteritidis* infection associated with 568 cases. The infections caused by PT **4** were less common in Canada and were almost exclusively found in people who had travelled abroad.

In many countries outbreaks of S .*enteritidis* infection in humans have often been associated with consumption of eggs, food prepared from eggs, poultry **meats** and other poultry products contaminated with S .*enteritidis* .In Canada, **most** of S. ***enteritidis*** isolates from humans **belong** to PT 8 (56A0h), followed by PT **4** (18.3961, PT 13 (8.3% and PT 13a (5.1 %) and these phage types have been frequently isolated from poultry and their environment. On the other hand, most recent European isolates of S. *enteritids,* from both humans and animals have been phage **type 4.** Like the phage types that are common in the United States and Canada, PT **4** has been associated with significant egg-transmitted disease in humans. PT **4** infection of broiler chickens has also been linked to the contamination of finished carcases **(Nastasi, 1996).**

Salmonellosis in food animals

Members of the genus Salmonella remain a potential threat to human and animal health. Human outbreaks have been associated with consumption of contaminated animal products. Animal-adapted Salmonella serovars can advisedly affect the health and produdivity of a herd or a flock and contrat sharply with healthy carrier animals infected with non-host adapted serovar that may cause salmonellosis in human population. Salmonellosis in food animals including cattle, poultry, swine, and sheep arises from intensive rearing pradice, the use of contaminated and medicated feeds and infected or carrier animals. At the abattoir, the initial source of contamination is the carrier animal. Transmission at the abattoir occurs by direct contact between carrier and non-carrier animals and also by exposure to contaminated environment. It has been suggested that stress associated with transportation, overcrowding and feed withdrawal experienced by animals before slaughter increases shedding of Salmonella. This effect was demonstrated in a study in which 30% of Salmonella-free pigs became Salmonella carriers after a mock transportation to an abattoir. Nonetheless, there appear to be no published data that associate feed withdrawal with an increase in Salmonella shedding in cattle. During slaughtering operations these carrier animals are able to contaminate the area, the equipment and personnel, and eventually the final products. Other environmental factors such as insects, rodents and wild birds have also been implicated in the infection of herds and contamination of processing **areas(Vela, 1996)**

Bovine salmonellosis

Salmonellosis is a serious infectious disease of cattle causing enteritis, septicaemia, pneumonia and abortion (**Vela, 1996**). The disease has primarily been associated with S. *dubiin* and *S. typhimurium* with the former being more common in adult cattle and the latter more common in calves. As an indication of the importance of bovine salmonellosis, it **has** been cited by Vela and Cuschieri (**Vela, 1996**) that between 10,000 and 20,000 incidents of salmonellosis are recorded annually in cattle in the UK. In Denmark, during the period of 1980-1 *992*, more than 70% of *Salmonella* outbreaks in cattle were caused by S. *dublin. Salmonella* infection in cattle originates from various sources, such **as** importation of infected animals or introducing infected or carrier animals to a healthy herd, contamination of feed and water, and cross-infection from other domestic or wild animals (**Vela, 1996**).

In conclusion, the results of this yielded hitherto unpublished information on the characteristics and drug resistance patterns of Salmonella serovars in beef cattle, broiler chickens and **humans** in PEI, which will serve as a baseline for future surveillance. It also highlights the adverse effects of fasting prior to slaughter **on** Salmonella shedding by apparently healthy carriers beef **cattle.** Finally, the experiments at the molecular level revealed the occurrence of ***spvC*** virulence plasmid gene sequence in the chromosome of Salmonella strains, a finding not previously documented in the literature.

References

[1] Altekruse .S.F., Swerdlow.D.l. Wells S.J. ,1998. Factors in the emergence of food borne diseases. Vet Clin North Am Food Anim Pract. 1998; 14:l-15.

[2] Eley R.A., 1996. Infective bacterial food poisoning. In: Eley R.A. ed. Microbial food poisoning. Chamman and Hal l,2-6 Boundary Row, London SEI 8HN, UK, 1 996: 1 5-33.

[3] Miller, M.A., Page .J,C.,1998. Other food borne infections. Vet Clin North Am Food Anim Pract. 1998; l4:71-89.

[4] Gomez .T.M., Motarjemi.Y., Miyagawa .S., Kaferstein .F.k., Stohr .K. ,1997. Foodborne salmonel losis. World Health Stat Q. 1997; 5O:8 1-89.

[5] Khakhrla .R., Woodward .D., Johnson .W.M., Poppe .C.,1997. Salmonella isolated frorn humans, animals and other sources in Canada, 1983-92. Epidemiol Infect. 1997; 1 l9:15-23.

[6] Tletjen .M, and Fung .D.Y. 1995.Salmonellae and food safety. Crit Rev Microbiol. 1995; 21 :53-83.

[7] D'aoust J,.Y. ,1989. Salmonella. In: Michael P.Doyle, ed. Food borne Bacterial Pathogens. New York and Basel: Marcel Dekker, INC, 1989:327-445.

[8] Gonzalez Hevla MA and Mendoza MC. Differentiation of strains from a food-borne outbreak of *Salmonella enterica* by phenotypic and genetic typing methods: Eur j Epidemiol. 1995; 1 1 :479-482.

[9] Nolan .L.k., Giddings .*C.w.*, Boland .E.w., Steffen .D.j., Brown .J.,. Misek. A.,1995. Detection and characterization of *Salmonella typhimoriurn* from a dairy herd in North Dakota. Vet Res Commun. 1995; 19:3-8.

[10] Stone .*C.c.*, Oberst .R.d., Hays .M.p., Mcvey .S., Chengappa .M.m.,1994. Detedion of *Salmonella* serovars from clinical samples by enrichment broth cultivation-PCR procedure. J Clin Microbiol. 1994; 32:1742-1749.

[11] Guthrie Rk. *Salmonella.* Florida: CRC, Press, Inc., 2000 corporate Blvd., Boca Raton, Florida, 3343 1 . 1 992: 1-220.

[12] Poppe C. *Salmonella enteritidis* in Canada. lnt J Food Microbiol. 1 994; 21 : 1-5.

[13] Threlfall €1, Hampton Md, Schofield Sl, Ward Lr, Frost Ja, Rowe B. Epidemiological application of differentiating multiresistant *Salmonella* typhimurium DT104 by plasmid profile. Commun Dis Rep CDR Rev. 1996; 6:R155-9.

[14] Espinasse J. Responsible use of antirnicrobials in veterinary medicine: perspectives in France. Vet Microbiol. 1993; 35:289-301.

[15] Threlfall Ej, Frost Ja. The identification, typing and fingerprinting of Salmonella: laboratory aspects and epidemiological applications. J Appl Bacteriol. 1990; 68:5-16.

[16] Mayer Lw. Use of plasmid profiles in epidemiologic surveillance of disease outbreaks and in tracing the transmission of antibiotic resistance. Clin Microbiol Rev.1988;1-228-224

[17] Lester A, Bruun Bc, Husum P, Kolmos Hj, Nielsen Bb, Schelbel Jh, Skovgaard N, Th U Ne-Stephensen F. *[Salmonella dublin]*. Ugeskr Laeger. *1* 995; 157:2û-24.

[18] Swamy Sc, Barnhart Hm, Lee Md, Dreesen Dw. Virulence determinants invA and spvC in *Salmonellae* isolated from poultry products, wastewater, and human sources. Appl Environ Microbiol. 1 996; 62:3768-3 771.

[19] Poppe C, Mcfadden Ka, Demczu K Wh. Drug resistance, plasmids, biotypes and susceptibility to bacteriophages of *Salmonella* isolated from poultry in Canada. Intj Food Microbiol. 1996; 30:325-344.

[20] Threlfall €1, Hampton Md, Schofield Sl, Ward Lr, Frost Ja, Rowe B. Epidemiological application of differentiating multiresistant *Salmonella* typhimurium DT104 by plasmid profile. Commun Dis Rep CDR Rev. 1996; 6:R155-9.

[21] Balls E, Vatopoulos Ac, Kanelopoulou M, Mainas E, Hatzoudis *G*, Kontogianni V, Malamou-Lada H, Kitsou-Kiriakopoulou S, Kalapothaki V. Indications of in vivo transfer of an epidemic R plasmid from *Salmonella enteritidis* to *Escherichia col;* of the normal human gut flora. J Clin Microbiol. 1996; 34:977-979.

[22] Eley R.A. Infective bacterial food poisoning. In: Eley R.A. ed. Microbial food poisoning. Chamman and Hal l,2-6 Boundary Row, London SEI 8HN, UK, 1 996: 1 5-33.

[23] Lax Aj, Barrow Pa, Jones Pw, Wallis Ts. Current perspectives in salmonellosis Br Vet 1. 1995; 151 :351-377.

[24] Threlfall Ej, Frost Ja, Ward Lr, Rowe B. lncreasing spedrum of resistance in mu [tiresistant *Salmonella typhimurium* [letter]. Lancet. 1 996; 347: 1 053-1 054.

[25] Nastasi A, Mammina C. Epidemiology of *Salmonella enterica* serotype Enteritidis infections in southern ltaly during the years 1980-1 994. Res Microbiol. 1996; 147:393-403.

[26] Guthrie Rk. *Salmonella.* Florida: CRC, Press, Inc., 2000 corporate Blvd., Boca Raton, Florida, 3343 1 . 1 992: 1-220.

[27] Vela L, Cuschlerf *P. Salmonella* excretion in adult cattle on the Maitese island of Gozo. Rev Sci Tech. 1 995; 14:777-787.