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# EPIDEMIOLOGIC ATTRIBUTES OF HUMAN NONTYPHOIDAL SALMONELLOSIS IN MICHIGAN

By

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ABSTRACT

EPIDEMIOLOGIC ATTRIBUTES OF HUMAN NONTYPHOIDAL SALMONELLOSIS IN MICHIGAN

By

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Nontyphoidal *Salmonellae*, particularly serotypes Typhimurium, Enteritidis, Heidelberg, and Newport, are common causes of foodborne gastroenteritis in the United States (US). It is estimated that about 1.4 million people suffer from salmonellosis each year with an estimated annual cost of $0.5 to $2.3 billion. Infections with these serotypes are often associated with the consumption of undercooked chicken, eggs, pork and beef, and contaminated vegetables.

Consumption of foods varies among the different ethnic groups. Additionally, several demographic factors may influence food intake behavior. The objectives of this study were to identify the high-risk population by the determination of the incidence of human infections with the most common *Salmonella* serotypes in Michigan in terms of time, place of residence (urban vs. rural counties) and demographic (age, gender, race and ethnicity), and whether the incidence is associated with low socioeconomic status (SES). This study was conducted by analyzing the 1995 to 2001 surveillance data on salmonellosis reported to Michigan Department of Community Health. Data on salmonellosis were analyzed by host related factors (race, age, place of residence) and linked to *Salmonella* serotypes to reveal the trends and important risk factors for the infections. This study revealed that from 1998 to 2001, the incidence of Typhimurium and Enteritidis significantly decreased where as the incidence of Newport significantly increased. Infants aged <1 year had the highest incidence followed by children aged 1 to

4 years. Among cases whose race or ethnicity was known, African-Americans had a significantly higher incidence of Typhimurium, Enteritidis, Heidelberg, and Newport compared with Whites; Hispanics had a significantly higher incidence of Enteritidis compared with Non-Hispanics. The high incidence among African-Americans and Hispanics is largely driven by the high incidence among their infants; children aged 1-4 years and elderly in urban counties. Among African-American infants, the incidence of Typhimurium and Enteritidis peaked at 2 to 4, and 9 and 10 months of age. African-American infants have a significantly higher incidence of invasive salmonellosis compared with White infants. Among African-Americans, the incidence of Typhimurium and Enteritidis increased during the winter months (October-December) where as among whites the incidence decreased. People living in poor area indicated by the census tracts had a significantly higher incidence of Enteritidis compared with people living in economically better areas. Because about 40% and 70% of the cases did not have information on race and ethnicity respectively, the findings regarding the racial and ethnicity differences in the infections should be interpreted with caution. Nevertheless, this information can be used by the state and local health departments of Michigan as a basis to guide salmonellosis prevention efforts in specific population subgroups and to conduct further studies to determine the risk factors for the high incidence among African-American and Hispanic infants, children and elderly; and among people living in poor census tracts.

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# INTRODUCTION

## RATIONALE

Nontyphoidal salmonellosis (NTS) is one of the major of foodborne diseases in the United States (US) and worldwide (Altekruse et al., 1997; CDC, 2006a; Mead et al., 1999; Motarjemi and Kaferstein, 1997; Swartz, 2002; Tauxe, 1997; Todd, 1997). On a global scale, an estimated 1.3 billion cases of acute nontyphoidal gastroenteritis occurred annually, resulting in 3 million deaths (Pang et al., 1995). In the US, NTS was associated with an estimated 1.4 million cases of foodborne illnesses annually, resulting in >100,000-physician office visits (Voetsch et al., 2004), 16,000 hospitalizations, and 600 deaths (Mead et al., 1999).

Although most nontyphoidal *Salmonella* causes mild to moderate self-limited gastroenteritis, serious extraintestinal infection such as bacteremia, endocarditis, meningitis, arthritis, and urinary tract infection may occur (Pegues et al., 2005). Population who are at higher risk for NTS include infants, the elderly, and immunocompromised persons (including patients with malignancy, human immunodeficiency virus, or diabetes, and those receiving corticosteroid therapy or treatment with other immunotherapy agents) (Buzby, 2001; Buzby, 2002; Fisk et al., 2005; Hohmann, 2001; Olsen et al., 2001a; Voetsch et al., 2004).

In many countries, the number of reported human NTS has increased markedly. In the US, the incidence of reported cases of NTS have doubled in the last two decades (Figure 1) (CDC, 2005b). The sharp peak in 1985 was due to outbreak of *S.* Typhimurium in Illinois associated with consumption of contaminated pasteurized milk

(Ryan et al., 1987). However, in recent years, the trends in the incidence of reported NTS has shown a sustained decrease (Figure 1) (CDC, 2005b).

In England and Wales, the incidence of NTS increased from 34.2/100,000 in 1981 to a peak of 78.4/100,000 in 1989. The incidence increased again in 1992 and 1997. From 1997 to 2001, the incidence has continued to decrease (Figure 2) (Health Protection Agency). Calculations for the year 1995 resulted in an estimate of 102,227 indigenous cases, with 3,412 hospital admissions and 268 deaths (Adak et al., 2002).

Figure 1. Incidence (per 100,000 population) of reported cases of nontyphoidal salmonellosis, United States, 1944-2003 (CDC, 2005b)

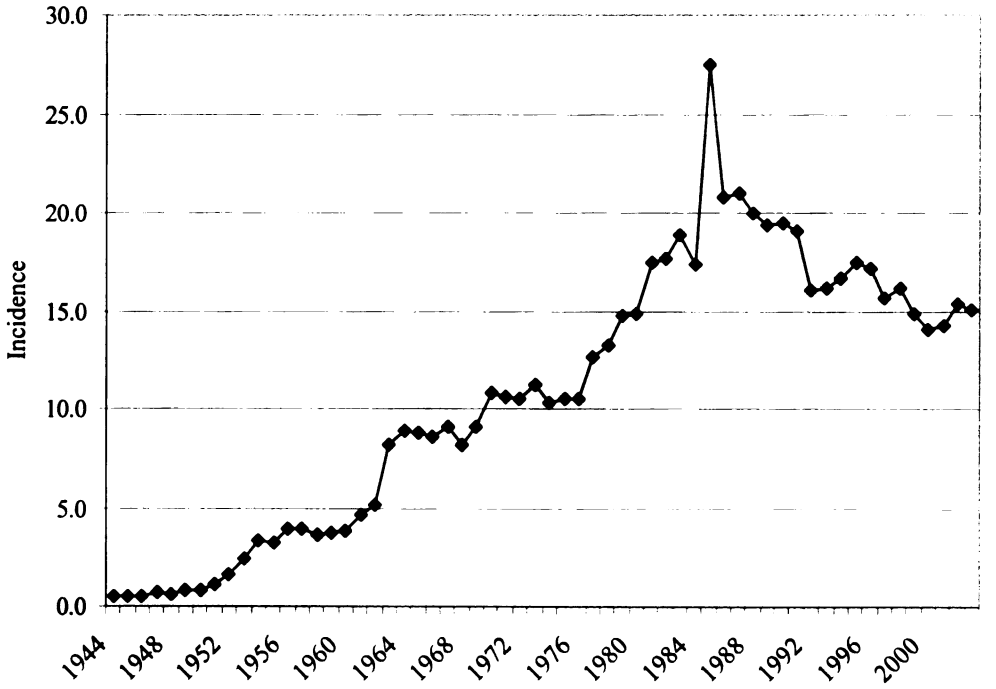
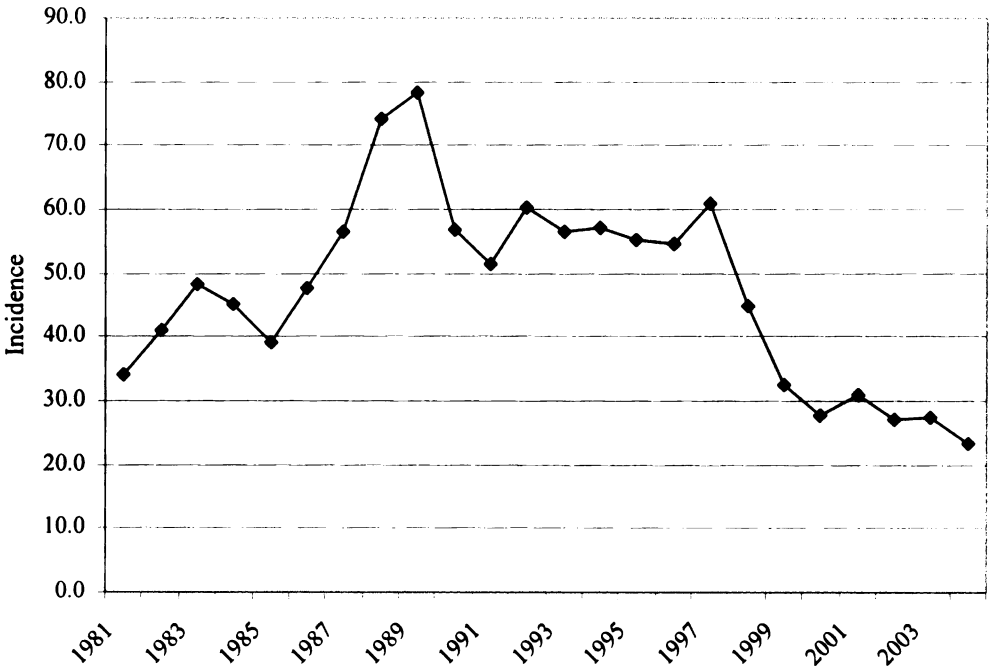


Figure 2. Incidence (per 100,000 population) of reported cases of nontyphoidal salmonellosis, England and Wales, 1981-2004 (Health Protection Agency)




Outbreaks and sporadic NTS have been associated with variety of food vehicles including foods of animal origin (raw or undercooked poultry, beef, pork, eggs and egg products, unpasteurized milk or dairy products), vegetables and fruits, chocolate, and peanuts (Kapperud et al., 1990; Kirk et al., 2004; Olsen et al., 2000; Sivapalasingam et al., 2004). The vehicles can also be non-food such as pet reptiles, farm animals or infected food handlers (CDC, 2001; CDC, 2003d; Friedman et al., 1998; Hundy and Cameron, 2002; Kimura et al., 2005; Maguire et al., 2000; Mermin et al., 2004; Wells et al., 2004). Other widespread outbreaks of NTS have been attributed to food produced in

centralized facilities such as commercially processed egg salad, unpasteurized milk, and ground beef. (CDC 2003c; CDC, 2004b; CDC 2006b).

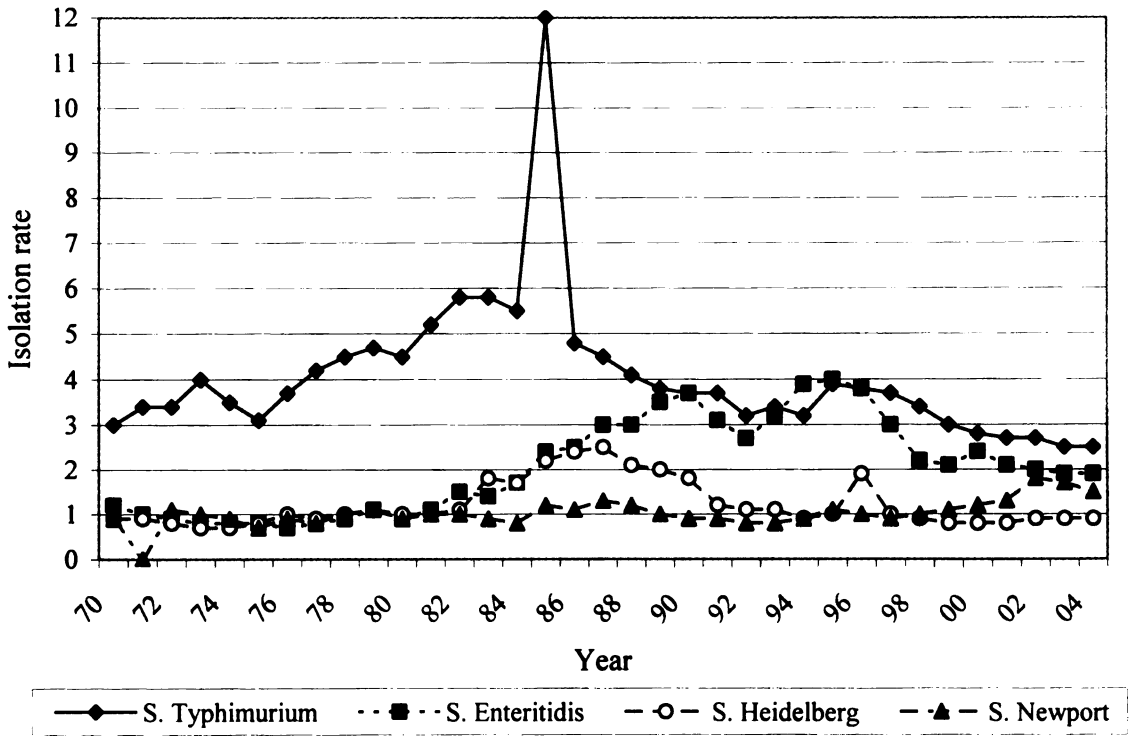
Although there are almost 2,400 *Salmonella* serotypes that have been identified (Brenner et al., 2000), only a few frequently cause the most cases of illnesses. The most common are *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport (CDC, 2005a).

The two major changes that occurred in the epidemiology of salmonellosis in the second half of the 20th century were: 1) the emergence of *S.* Enteritidis and 2) the emergence of multidrug-resistant *S.* Typhimurium DT104 (MR-DT104) (Velge et al., 2005).

*Salmonella* Enteritidis emerged as an important cause of human illness in the US during the 1980s. The incidence was 0.7/100,000 in 1976 and by 1985, the incidence increased to 2.4/100,000 (Figure 3). The number of outbreaks also increased during the 1980s, particularly in the northeastern US. Phage types (PT) 8 and 13a was the most common (Patrick et al., 2004).

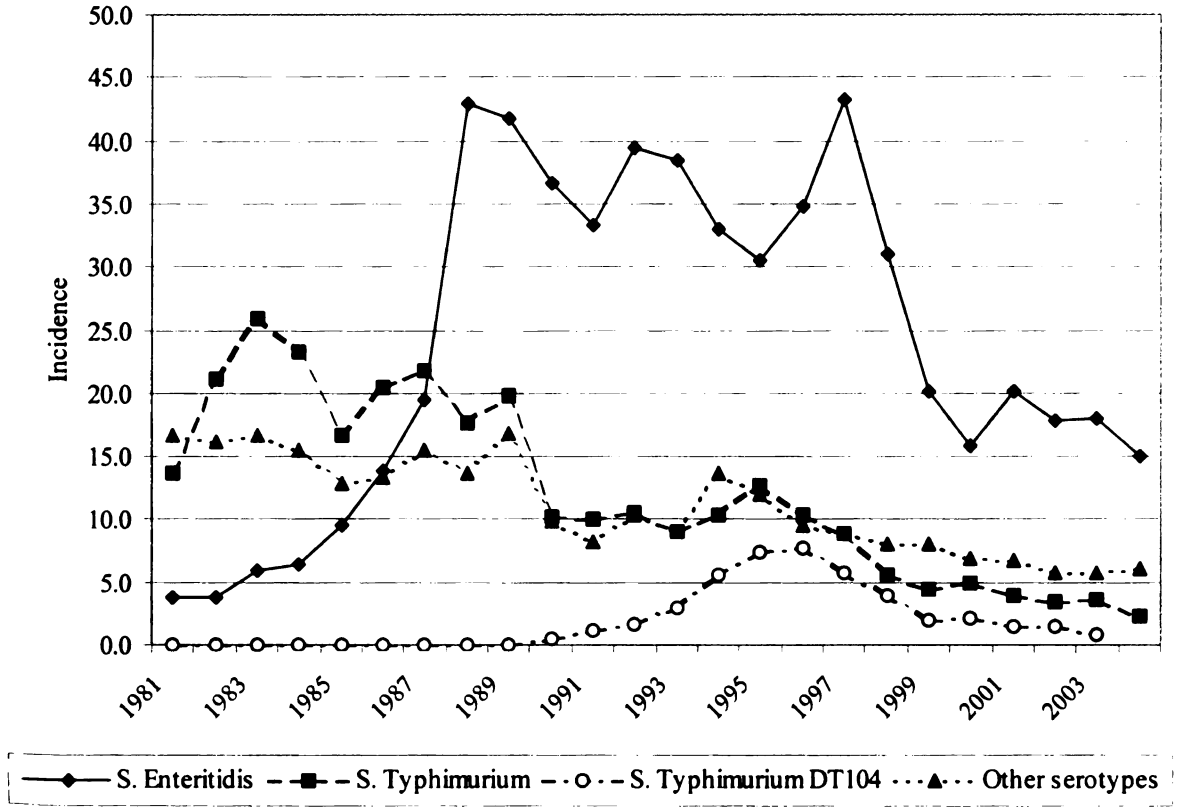
In England and Wales, the incidence of *S.* Enteritidis was 3.8/100,000 in 1981 peaking at 43.0 in 1988 and 43.2 in 1997 (Cogan and Humphrey, 2003; Ward et al., 2000) (Figure 4). Despite a subsequent decline in its incidence since 1997, *S.* Enteritidis continues to be the most frequently isolated *Salmonella* serotype in the United Kingdom (UK) with 16, 465 cases in 2001 (Cogan and Humphrey, 2003).

Figure 3. Isolation rates (per 100,000 population) of the four most common *Salmonella* serotypes, United States, 1970-2004 (CDC, 2005a)




In France, *S.* Enteritidis was the most commonly isolated serotype in 1993. The incidence of *S.* Enteritidis human isolates increased exponentially from 1987 to peak at 6,500 in 1994 and 1997 and subsequently declined to 4,500 cases in 1999 (Bouvet et al., 2001; Velge et al., 2005). Similar trends have also been reported from other countries in South America and Europe (Cogan and Humphrey, 2003; Rabsch et al., 2001).

Figure 4. Incidence (per 100,000 population) of *Salmonella* serotypes, England and Wales, 1981-2004 (Health Protection Agency & Threlfall et al., 1997)




Investigation of outbreaks and sporadic cases has repeatedly indicated that poultry and poultry derivatives, and undercooked and raw eggs are the most common sources of *S.* Enteritidis infection (Berghold et al., 2003; Hedberg et al., 1993; Irwin et al., 1993; Kimura et al., 2004; Mazurek et al., 2005; Morse et al., 1994; Tansel et al., 2003). Eggs are contaminated internally with *S.* Enteritidis as a result of infection in the hen's ovaries and oviducts (Gast and Beard, 1990a and 1990b; Thiagarajan et al., 1994; Timoney et al., 1989).

Multidrug-resistant *Salmonella* Typhimurium DT 104 (MR Typhimurium DT104) with chromosomally encoded antibiotic resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (R-type ACSSuT), emerged during the last decade as a global health problem in animals and humans (Threlfall, 2000). This strain was first detected in the UK in cattle and humans in the late 1980s, subsequently it became common in poultry, pigs and sheep (Threlfall et al., 1994). In the 1990s, the number of reported human isolates of MR Typhimurium DT 104 R-type ACSSuT increased from 259 in 1990, to 4,006 in 1996 (Figure 4) (Threlfall et al., 1997). During 1997 and 1998 the incidence began to decline.

Of additional concern, is the increasing proportion of human MR Typhimurium DT 104 R-type ACSSuT isolates which are resistant to antibiotics used for treatment of life-threatening invasive *Salmonella* infections. The proportion of MR Typhimurium DT104 isolates resistant to trimethoprim increased from <2% in 1993 to 24% in 1996, while the proportion resistant to ciprofloxacin increased from 1% in 1993 to 14% in 1996 (Threlfall et al., 1997).

In the US, MR Typhimurium DT 104 was first detected in 1985, and the prevalence increased to account for nearly 7% of *Salmonella* infections in 1998 (Rabatsky-Ehr et al., 2004; Ribot et al., 2002). Special studies conducted in selected counties in 1980, 1985, 1990, and 1995 revealed that the proportion of *S.* Typhimurium isolates which were R-type ACSSuT increased from 2% in 1980, to 4% in 1985, to 9% in 1990, and to 12% in 1995. The proportion of those R-type ACSSuT, which were DT 104, was 0% in 1980, 25% in 1985, 50% in 1990, and 85% in 1995 (Hougue et al., 1997a). In 1995, 28% (275/976) of the *S.* Typhimurium isolates were R-type ACSSuT, and

approximately 85% of those isolates were DT 104. In 1996, 33% (110/304) of *S.* Typhimurium isolates were R-type ACSSuT, and approximately 85% of those isolates were DT104 (Hougue et al., 1997a).

MR Typhimurium DT 104 is an animal pathogen and a foodborne pathogen of humans. The mortality rate for clinically affected cattle with this strain is reported to be 40-60% in some outbreaks in the UK (Hougue et al., 1997a). The case fatality rate in humans may be higher than with other *Salmonella* infections. In a study of 83 cases of MR Typhimurium DT 104 in the UK, 41% of patients were hospitalized and 3% died. In contrast case-fatality rate for other nontyphoidal *Salmonella* was approximately 0.1% (Hougue et al., 1997a).

Human infections with MR Typhimurium DT 104 have been associated with consumption of chicken, beef, pork, sausages and meat paste (Wall et al., 1994). The MR-DT104 epidemic is now worldwide with a considerable number of outbreaks since 1996 in the US and Canada (Besser et al., 2000; Glynn et al., 1998; Poppe et al., 1998).

Besides MR Typhimurium DT 104, multidrug-resistant strains of *S.* Newport (known as Newport-MDRAmpC) have emerged in the US (CDC, 2002c; CDC, 2004a; Gupta et al., 2001; Gupta et al., 2003). These strains are resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (Gupta et al., 2003). In addition, Newport-MDRAmpC is resistant to amoxicillin/clavulanic acid, cephalothin, cefoxitin, and ceftiofur, and exhibited decreased susceptibility to ceftriaxone (Gupta et al., 2003). Human infections with Newport-MDRAmpC have been associated with direct exposure to infected dairy cows and calves (Gupta et al., 2003), consumption of ground beef (CDC, 2002c) and handling contaminated pet treats (Pitout et al., 2003).

Although the major risk factors for NTS are consumption of undercooked high-risk foods including chicken, eggs, beef, pork, dairy products, and fresh produces (Olsen et al., 2000; Sanchez et al., 2002; Sivapalasingam et al., 2004), other modifiable risk factors such as low level of socioeconomic status (SES), including poverty and low level of education may also influence the incidence. For example, low level of income may lead to low level of education and thus may lead to less exposure to information on food safety and prevention of foodborne diseases (e.g. proper handling, preparing and cooking of high-risk foods (Shiferaw et al., 2000).

The social and economic impact of NTS is considerable. It imposes costs on the public sector, industry (in particular wholesale and retail food industry) and health costs (Socket and Roberts, 1991). In the US, the costs associated with NTS, including the costs of medical care and lost productivity, have been estimated to be $0.5-$2.3 billion annually (Frenzen et al., 1999). In England and Wales, the national cost of NTS in 1992 has been estimated between £350 and £502 million with an average cost per case between £789 and £861 (Roberts and Socket, 1994).

NTS has been a notifiable disease in the US since 1943 (Tauxe, 1991). In all states, the occurrence of this disease is required to be reported by physicians and laboratories to public health authorities. The reasons to notify the occurrence of NTS to public health authorities are: to identify outbreaks so that the local health authorities can investigate and control its spread, to enable preventive measures and/or education to be provided, to help target preventions, to evaluate the success of long term control efforts, to facilitate epidemiologic research to uncover a preventable cause, and to assist with national surveillance efforts (MDCH, Communicable Disease Reporting).

Even though salmonellosis surveillance in the states is often passive, analysis of salmonellosis surveillance data allows an estimate of overall incidence and trends, and identification of populations at higher risk.

## PROBLEM STATEMENT

From the above it is clear that nontyphoidal salmonellosis (NTS) remains an important public health problem in the US and worldwide causing substantial morbidity and economic impact. Although most infections cause mild to moderate self-limited gastroenteritis, serious extraintestinal infections resulting in death do occur.

## OBJECTIVES

In this dissertation, the principal objective was to determine the epidemiologic attributes of NTS in Michigan.

The specific aims of this study were:

1. To determine the incidence of infection with *Salmonella* serotypes in Michigan in terms of time (year), type of county of residence (urban vs. rural counties), and demographic characteristics (age, gender, race and ethnicity).
2. To determine whether incidence of salmonellosis in Michigan is associated with low socioeconomic status (such as poverty and low educational attainment).

In order to address these aims, there are several underlying key research questions that should be answered by the studies conducted. They are:

1. Has the incidence of infection with the most common *Salmonella* serotypes significantly changed from 1995 to 2001?

2. Did the incidence of infection with the most common *Salmonella* serotypes differ significantly between residence in urban and rural counties, between different racial and ethnic groups, between the two genders, and between different age groups?
3. Is the incidence of infection with the most common *Salmonella* serotypes among infants aged 6 months and older significantly higher than infants aged <6 months?
4. Did disparities exist in the incidence of infection with the most common *Salmonella* serotypes among racial and ethnic groups?
5. Is lower socioeconomic status (SES) significantly associated with higher incidence of infection with the most common *Salmonella* serotypes?

## HYPOTHESES TO BE TESTED

In order to address the above aims and answer the key research questions, a number of individual hypotheses were developed to be tested. They are:

1. There was no significant change in the incidence of infection with the most common *Salmonella* serotypes in Michigan from 1995-2001.
2. There is no significant difference in the incidence of infection with the most common *Salmonella* serotypes between residents in urban and rural counties, between different races and ethnic groups, between the two genders, and between different age groups.

3. There is no disparity in the incidence of infection with the most common *Salmonella* serotypes between racial and ethnic groups in Michigan by age, gender and type of county of residence.
4. There is no significant difference in the incidence of infection with the most common *Salmonella* serotypes between infants aged 6 months and older, and infants aged <6 months.
5. The incidence of infection with the most common *Salmonella* serotypes is not associated with low SES.

## OVERVIEW OF RESEARCH

Literature reviews on human and animal salmonellosis are presented in chapter one and two respectively. Chapter three addresses hypothesis 1 and 2 by analyzing salmonellosis surveillance data by year, type of county of residence, and demographic factors (age, gender, and race and ethnicity). Chapter four addresses hypothesis 3 by analyzing data for the role of age, gender, and type of county of residence in explaining racial and ethnic disparity. Chapter five addresses hypothesis 4 by analyzing data among infants aged <1 year by age in months. Chapter six is designed to addresses hypothesis 5 by analyzing cases of *Salmonella* Enteritidis in Wayne County, Michigan according to census tract.

# CHAPTER ONE

## LITERATURE REVIEW OF HUMAN SALMONELLOSIS

### General characteristics of *Salmonella*

*Salmonellae* are Gram-negative, rod-shaped bacteria belonging to the family Enterobacteriaceae (Pegues et al., 2005). They are facultative anaerobes (i.e. able to grow aerobically and anaerobically), and most are motile (the exceptions for non-motile serovars are *S.* Gallinarum and *S.* Pullorum). Currently, there are about 2,400 *Salmonella* serotypes that have been identified (Brenner et al., 2000). These are classified in two species, *S. enterica* and *S. bongori*. *S. enterica* is divided into six subspecies: I, *S. enterica* subsp. *enterica;* II, *S. enterica* subsp. *salamae;* IIIa, *S. enterica* subsp. *arizonae;* IIIb, *S. enterica* subsp. *diarizonae;* IV, *S. enterica* subsp. *houtenae;* and *S.* enterica subsp. *indica.*

Members of the seven *Salmonella* subspecies can be serotyped into one of over 2,400 serotypes according to somatic (O), surface (Vi), and flagella (H) antigens, and habitats (Table 1) (Pegues et al., 2005; Brenner et al., 2000). Most nontyphoidal *Salmonella* serotypes that cause diseases in animals and humans, such as *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport fall in the subspecies I, *S. enterica subsp. enterica*

*Salmonellae* are not fastidious organism. They can multiply under a variety of environmental conditions outside of living hosts. They can grow in many foods and water contaminated with feed or feces. Inhibition of their growth occurs at pH <3.8,

temperature <7°C, or water activity <0.94. *Salmonellae* can be killed by cooking and by pasteurization of milk (71.7°C, 15 seconds) and fruit juices (70-74°C, ≤ 20 seconds) (Gray and Fedorka-Cray, 2002).

Table 1: *Salmonella* species, subspecies, serotypes, and their usual habitats, Kauffmann-White scheme

| *Salmonella* species and subspecies | No.of serotypes within subspecies | Usual habitat |
|---|---|---|
| *S. enterica* subsp. *enterica* (I) | 1,454 | Warm-blooded animals |
| *S. enterica* subsp. *enterica* (II) | 489 | Cold-blooded animals and the environment |
| *S. enterica* subsp. *arizonae* (IIIa) | 94 | Cold-blooded animals and the environment |
| *S. enterica* subsp. *diarizonae* (IIIb) | 324 | Cold-blooded animals and the environment |
| *S. enterica* subsp. *houtenae* (IV) | 70 | Cold-blooded animals and the environment |
| *S. enterica* subsp. *indica* (VI) | 12 | Cold-blooded animals and the environment |
| S. *bongori* (V) | 20 | Cold-blooded animals and the environment |
| Total | 2,463 | |

(Adapted from Pegues et al., 2005; Brenner et al., 2000).

*Salmonellae* are zoonotic and can be pathogenic to humans and animals. *Salmonellae* are ubiquitous and have been recovered from insects and nearly all vertebrate species, especially humans, livestock, and companion animals. They can be found free living in nature and as part of the indigenous flora of humans and animals, and are also known to survive well in dry condition. Food animals are the primary reservoir for human nontyphoidal *Salmonella* (NTS) (Gray and Fedorka-Cray, 2002).

**Pathogenesis of *Salmonella* infection**

After ingestion of *Salmonella*-contaminated food or water, the bacteria colonize the lower intestine (ileum and cecum) (Pegues et al., 2005). Then they invade mucosal cells, which results in acute inflammation. This then leads to the activation of adenylate cyclase (AC), increased fluid production and release of fluid into the intestinal lumen, resulting in diarrhea. *Salmonella* gastroenteritis requires an 8-48 hour incubation period and may last from 2-5 days. Symptoms include nausea, vomiting and diarrhea (Darwin and Miller, 1999; Pegues et al., 2005).

After colonizing the small intestine, *Salmonella* can invade the intestinal epithelium through: 1) M-cells of the Peyer's patches. M cells are differentiated epithelial cells, which sample luminal antigens and pass these antigens to the underlying macrophages; 2) initial binding to specific receptors on the epithelial cells surface, induce the enterocyte membrane to undergo ruffling and thereby stimulate pinocytosis of the bacteria (Pegues et al., 2005). *Salmonella* then can multiply in macrophages and spread to mesenteric lymph nodes and throughout the body via the systemic circulation; and are

taken up by reticuloendothelial cells. Some organisms may infect the liver, spleen, gall bladder, bones, meninges, and other organs (Pegues et al., 2005).

**Virulence factors of *Salmonella***

Virulence factors are properties of *Salmonellae* that contribute to their ability to colonize, survive and cause disease (gastroenteritis and systemic infection) in the infected host (Baumler et al., 2000b). *Salmonellae* survive the acidic conditions in the stomach through an adaptive acid-tolerance response (Riesenberg-Wilmes et al., 1996). In the small intestine, the flagella help *Salmonella* swim chemotactically toward the mucosal surface, thus resisting removal by peristalsis (Baumler et al., 2000b). The long polar (LP) fimbriae help *Salmonellae* adhere to intestinal epithelium (Baumler et al., 2000b). LP fimbriae are encoded by five genes organized in the *lpf* (long polar fimbrial) operon located at 80 centisomes on the physical map of *S.* Typhimurium (Baumler, 2000b). Upon contact with intestinal epithelium, *Salmonellae* translocates bacterial effector proteins (SipB, SipC, SptP, SopE and AvrA) into the host cells cytosol via the type III secretion system (TTSS). TTSS is encoded by virulence genes (*inv*G, *prg*H, *prg*K) clustered in *Salmonella* pathogenicity island 1 (SPI1). Once the effector proteins are in the epithelial cell cytosol, they alter host cell signaling pathways that promote changes in the cytoskeleton, leading to cell membrane ruffling and subsequent bacterial internalization (Francis et al., 1993; Santos et al., 2003; Zhao, 2002). Invasion of epithelial cells results in an inflammation followed by neutrophil influx and secretion of fluids in the intestinal lumen (Santos et al., 2003).

Once *Salmonella* has breached the epithelial barrier they come into contact with cells of the reticuloendothelial system, particularly the macrophages (Zhao, 2002). The survival of *Salmonella* within macrophages is generally considered to be essential for the translocation of bacteria from the gut-associated lymphoid tissue to the mesenteric lymph nodes, liver and spleen. To encounter the hostile environment within macrophages such as acidic and low level of magnesium, *Salmonella* reside in membraneous vacuoles (Garcia-del Portillo and Finlay, 1994). The bacteria can block the maturation of phagosomes into phagolysosomes and the vacuoles have a reduced acidity (pH >5.0). The adjustment of the intravacuolar pH by the microorganisms is essential for the activation of distinct *Salmonella* virulence genes (Zhao, 2002).

*Salmonella* pathogenicity islands are required to modulate intracellular trafficking. The *Salmonella* pathogenicity island 2 (SPI-2) was identified as a gene cluster required for survival of *S.* Typhimurium inside host cells (Hensel et al., 1998; Zhao, 2002). The SPI-2 carries genes encode for a second type III secretion system (TTSS-2). *Salmonella* delivers proteins into *Salmonella*-containing-vacuoles or through the vacuolar membrane into the host cytosol. This process influences intracellular trafficking and contributes to the intracellular survival of *Salmonella* in macrophages (Zhao, 2002).

The SPI-3 and SPI-4 also play a role in the survival of *Salmonella* in macrophages (Marcus et al., 2000; Zhao, 2002). The *mgtC* and *mgtB* genes of SPI-3 encode for proteins that allow *S.* Typhimurium to acquire magnesium under low magnesium conditions. These genes were shown to be required for survival of the bacterium in macrophages and for virulence in mice (Blanc-Potard and Groisman, 1997). The SPI-4

contains 18 putative ORFs and some of these ORFs are homologous to the Type-I secretion system. Therefore it is expected that SPI-4 is involved in secretion of a cytotoxin (Wong et al., 1998). For at least one locus of SPI-4, it was demonstrated that it is required for intramacrophage survival (Baumler et al., 1994; Marcus et al. 2000; Zhao, 2002).

During the various stages of the infection, *Salmonellae* encounters a variety of environmental challenges, such as nutrient starvation, oxidative stress and digestive enzymes (Zhao, 2002). *Salmonellae* are equipped with a series of adaptive mechanisms that enable the bacteria to survive these challenges. One group of genes that play a role in metabolic adaptation is the starvation stress response genes (Kenyon et al., 2002; Spector, 1998). These genes encode for metabolic functions that are required for *Salmonella* to survive at a certain stage of infection. One example is the *aroA* gene of *S.* Typhimurium, which is involved in the synthesis of 5-enolpyruvylshikimate 3-phosphate that is required for synthesis of aromatic amino acids (Zhao, 2002). Mutation of the *aroA* gene results in attenuated virulence of *Salmonella.* Other metabolic genes that influence the adaptive abilities of *Salmonella* are the *narZ* gene that encodes a cryptic nitrate reductase important for the virulence of *S.* Typhimurium in the mouse model (Spector et al., 1999a), the *fadF* gene which is specifically induced in cultured epithelial cells and encodes an acyl-CoA dehydrogenase (Spector, et al. 1999b) and the *eutE* gene that probably allows the bacteria to utilize ethanolamine as a nitrogen and carbon source and is important for virulence in mice (Stojiljkovic et al., 1995; Zhao, 2002). Identification of genes that are required for *Salmonellae* survival may provide opportunities to develop attenuated strains, which can be used as a vaccine (Zhao, 2002).

**Clinical Manifestations**

Human infections with nontyphoidal *Salmonella* commonly result in self-limited acute gastroenteritis (Pegues et al., 2005). Within 6 to 48 hours after ingestion of contaminated food or water, nausea, vomiting, diarrhea and abdominal cramps occur. There may be fever from 38° to 39°C. Diarrhea is usually self-limited and usually subsides spontaneously in 3 to 7 days. In the US, NTS results in an estimated rate of hospitalization of 2.2/1,000,000 and 582 deaths annually (Mead et al., 1999). Death rate is highest among the elderly, especially in nursing homes, and among immunocompromised individuals, including those with lupus or AIDS (Celum et al., 1987; Levine et al., 1991a; Lim et al., 2001; Mishu et al., 1994).

After recover from gastroenteritis, the mean duration of carriage of nontyphoidal *Salmonella* in stool is 4-5 weeks. Antimicrobial therapy may increase the duration of carriage (Pegues et al., 2005).

Human infections with nontyphoidal *Salmonella* can also results in bacteremia. Bacteremia with sustained fever is more frequent with *S.* Choleraesuis and *S.* Dublin infections than other serotypes (Pegues et al., 2005). The risk of bacteremia is greater in infants, the elderly, and the immunocompromised individuals (Pegues et al., 2005). Among children, nontyphoidal *Salmonella* bacteremia is usually associated with gastroenteritis and prolonged fever, infrequently causes focal infections, and is fatal in less than 10% of cases (Pegues et al., 2005; Shimoni et al., 1999). Adults are more likely to have primary bacteremia and have a high incidence of secondary focal infections and death (Shimoni et al., 1999).

Nontyphoidal *Salmonella* tend to infect vascular sites, and high grade or persistent bacteremia suggests endovascular infections (Pegues et al., 2005; Parsons et al., 1983). The risk of endovascular infection that complicate *Salmonella* bacteremia is estimated to be 10% to 25% among persons aged > 50 years, which involves the aorta that resulted from seeding atherosclerotic plaques or aneurysms (Benenson et al., 2001; Cohen et al., 1987; Pegues et al., 2005). Mortality rates range from 14% to 60%.

Localized infection results when nontyphoidal *Salmonella* spreads from the intestine to other parts of the body such as intravascular lesions, skeletal system, and the meninges (Golberg and Rubin, 1988; Graman and Betts, 2003; Pegues et al., 2005). Extraintestinal complications of NTS such as osteomyelitis, septic arthritis, cholecystitis, muscle abscesses, endocarditis, pericarditis, vascular infection and meningitis have been reported (Banky et al., 2002; Benenson et al., 2001; Boyle et al., 2001; Can et al., 2004; Chen et al., 1998; Chen et al., 2005; Cohen et al., 1987; Collazos et al., 1999; Flannery et al., 2001; Gonen et al., 2004; Gunalingam et al., 2000; Juneja et al., 1996; Karim and Islam, 2002; Lee et al., 1999; Leonard et al., 2002; Lo et al., 2002; Minami et al., 2003; Moanna et al., 2006; Pegues et al., 2005; Santos and Sapico, 1998; Vaagland et al., 2004).

High grade bacteremia (more than 50% of three or more blood cultures) strongly suggests focal intravascular infection. *Salmonella* have a predilection for arterial atherosclerotic plaques and aneurysms. Osteomyelitis or suppurative arthritis can occur at any skeletal site. People with sickle cell anemia or skeletal prostheses are predisposed. Meningitis occurs principally in young children, especially newborn infants (Graman and Betts, 2003).

Chronic carrier state is defined as the persistence of *Salmonella* in stool or urine for periods more than 1 year (Pegues et al., 2005). Chronic carrier state occurs in 0.2% to 0.6% of patients infected with nontyphoidal *Salmonella* (Medina et al., 1989; Musher and Rubenstein, 1973). The carrier state may follow symptomatic disease or may be the only manifestation of infection. Women, persons with biliary abnormalities or concurrent bladder infection with *Schistosoma haematobium*, and infants have higher frequency of chronic carriage (Neves et al., 1971; Pegues et al., 2005).

Nontyphoidal *Salmonella* infections can result in chronic complications, reactive arthritis or Reiter syndrome, which is characterized by painful joints, irritated eyes, and painful urination (McDowell and McElvaine, 1997). Symptoms commonly begin approximately 7 to 30 days after gastroenteritis. In most individuals, the symptoms subside in less than 6 months (McDowell and McElvaine, 1997). The prevalence of reactive arthritis associated with nontyphoidal *Salmonella* outbreaks ranged from <1% (Urfer et al., 2000) to 29% (Dworkin et al., 2001).

**Nontyphoidal salmonellosis and HIV infection**

Human immunodeficiency virus (HIV)-infected persons have 20- to 100-fold increased risk of NTS compared with the general population (Celum et al., 1987; Pegues et al., 2005). Nontyphoidal *Salmonella* infection among individuals with acquired immunodeficiency syndromes (AIDS) is more likely to result in severe invasive diseases such as fulminate diarrhea, acute enterocolitis, rectal ulceration, recurrent bacteremia, meningitis, and death despite antimicrobial therapy (Fernandez et al., 1997; Pegues et al., 2005). Recurrent nontyphoidal *Salmonella* bacteremia is an AIDS-defining illness that

apparently results from incomplete clearance of the primary infection because of impaired cell-mediated immunity (Pegues et al., 2005).

**Vehicles of *Salmonella* infections**

Foods of animal origin that have been frequently implicated in outbreaks of human foodborne salmonellosis were beef, ham, pork, chicken, turkey, milk, cheese, and eggs (Bean et al., 1996; Gillespie et al., 2003; Kessel et al., 2001; Olsen et al., 2000; Smerdon et al., 2001). Other foods include ice cream, fruits and vegetables, salad, and baked foods (Bean et al., 1996; Olsen et al., 2000; Long et al., 2002). During the last three decades, the number of outbreaks caused by *Salmonella* associated with eating fresh produce has increased (Sivapalasingam et al., 2004). Fresh produce that has been implicated in the outbreaks was salad, seed sprout, melon, apple or orange juice, lettuce, tomato, precut celery, and mixed fruit (Sivapalasingam et al., 2004). The contributing factors that lead to outbreaks of foodborne NTS and other foodborne pathogens include improper holding temperature, inadequate cooking, contaminated equipment, food from unsafe sources and poor personal hygiene (Bean et al., 1996; Olsen et al., 2000).

Besides foods, infected food handlers, farm animals, pet animals, especially reptiles, young animals, animals with diarrhea, chicks and ducklings, kittens, hedgehogs, and rodents have also been implicated as sources of *Salmonella* infections in humans (Anonymous, 2000; Anonymous, 2005a; CDC, 1995; CDC, 2000; CDC. 2001; CDC. 2003d; CDC, 2005e; de Jong et al., 2005; Fey et al., 2000; Hendriksen et al., 2004; Hundy and Cameron, 2002; Maguire et al., 2000; Rice et al., 2003a; Schiellerup et al., 2001; Wells et al., 2004).

**Person-to-person transmission of *Salmonella***

Person-to-person spread of *Salmonella* infection can occur in closed communities such as hospitals, nursing homes, childcare center, residence hall, and home (Alam et al., 2005; Delarocque-Astagneau et al., 1998; Delarocque-Astagneau et al., 2000; Newcomb et al., 1997; Palmer et al., 1981; Palmer and Rowe, 1983; Rushdy et al., 1997; Standaert et al., 1994).

Person-to-person transmission of *Salmonella* typically occurs when a carrier's feces, unwashed from his or her hands, contaminates food during preparation or through direct contact with another person. Person-to-person transmission is particularly likely to happen between parent and infants (Loewenstein, 1975; Wilson et al., 1982). Health care workers who are carriers may also spread the infections to other health care workers or to patients. Especially if the health care worker continues to work despite feeling ill and shedding the bacteria (McCartney et al., 1993; Pegues et al., 2005). Fecal-oral transmission from person to person is important, especially when diarrhea is present; infants and stool incontinent adults pose a greater risk of transmission than asymptomatic carriers (Chin, 2000).

Chronic carriage poses a significant public health threat, especially when such carriage occurs in food handlers or hospital employees (Ziprin and Hume, 2001). Outbreak of *S.* Enteritidis phage type (PT) 4 in a hospital in 1995 might have been person-to-person transmission because seven asymptomatic excretors of *S.* Enteritidis were identified among the patient population and no common or continuing food source was located (Rushdy et al, 1997).

Person-to-person transmission of *Salmonellae* is particularly likely in the acute diarrhea phase, when the infected persons eliminate a great number of bacteria (Rushdy et al., 1997).

Nosocomial transmission of *S.* Hadar gastroenteritis among laundry staff who handled soiled linens from incontinent patients during an outbreak in a nursing home has been reported (Standaert et al., 1994). However, the actual route of transmission in the laundry workers could not be determined. Ingestion of the organism from contaminated hands is likely, either directly or after handling of food, with subsequent multiplication of the organism prior to consumption.

Using portable toilets may have played a role in person-to-person spread of infection during the entire outbreak of *S.* Hadar at a building site canteen in Italy (Faustini et al., 1998). Poor hygienic conditions and a high ratio of users:toilets support the results. The author did not succeed in explicitly demonstrating the transmission route. However, after the excreting workers had been suspended from work, no further case of salmonellosis was identified. The author also hypothesized that the index case, the cook, transmitted the infection to her fellow workers person-to-person and that more than one food handler at the canteen contaminated the foods.

Three of 11 *S.* Enteritidis hospital outbreaks in England and Wales (1992-1994) were mainly person-to-person transmission (Wall et al., 1996). In a 10-year review of *Salmonella* infections in hospitals in England and Wales (1978-1987), 70 of 248 (30%) outbreaks were reported as due to person-to-person spread (Joseph and Palmer, 1989). Thirty-one were traced to admission of a patient with diarrhea.

**People at higher risk of *Salmonella* infections**

Identifying subpopulations at risk of foodborne NTS and other foodborne illnesses is important for targeting and developing consumer education to reduce risks (Ralston, 1995). Several subpopulations have been identified of having higher risk for NTS because of conditions affecting immune function or exposure to nontyphoidal *Salmonella*. Individuals at higher risk because of lower immune function include young children, the elderly, cancer patients undergoing chemotherapy, organ transplant patients, AIDS patients (Table 2). Frequent consumers of foods associated with nontyphoidal *Salmonella* are also at higher risk because they are more likely to encounter a food portion with sufficient number of the bacteria to cause illness (Table2).

Table 2. Factors associated with the risk of *Salmonella* infections

| Factors | Reasons | References |
|---|---|---|
| **Host-related:** | | |
| 1.Age: | | |
| a) Infants aged < 1 year | Immature immune systems, produced little gastric acid/rapid gastric emptying | Buzby et al., 2001; Kim et al., 2004; |
| b) Children 1-4 years | Immature immune systems | Buzby et al., 2001 |
| c) Elderly ≥60 years | Immune system failing, weakened by chronic ailments; achlorhydria | Buzby et al., 2002; Smith, 1998; Kim et al., 2004 |
| 2.Race/Ethnicity: | | |
| a) African-American | Frequent consumption of high-risk foods (raw or undercooked animal products, fruits, vegetables) | Lee et al., 1994; Marcus et al., 2002; USDA, ARS |
| b) Hispanics | Frequent consumption of high-risk foods | Banerjee et al., 2002; Riley et al., 1984; USDA, ARS |
| 3. Concurrent medications: | | |
| a) Consumption of antibiotic before illness | Alteration of normal intestinal flora | Banatvala et al., 1999; Doorduyn et al. 2006; Dore et al., 2004; Glynn et al., 2004; Kass et al., 1992; Lee et al., 1994; Neal et al., 1994; Pavia et al., 1990 |
| b) Consumption of antacids | Reduce stomach acidity | Doorduyn et al., 2006; Kass et al., 1992; Neal et al., 1994 |

(Adapted from Ralston, 1995).

Table 2 (contd')

| Factors | Reasons | References |
|---|---|---|
| 4. Immunocompromised individuals: | Immune system inadequate to prevent infection | |
| a) Persons with AIDS | Impaired cell mediated immunity<br>Neutropenia | Fisk et al., 2005; Gordon et al., 2002; Leonard, et al., 2002; Levine et al., 1991b; Puthucheary et al., 2004 |
| b) Persons receiving chemotherapy | | Canney et al. 1985; Delaloye et al. 2004; Philippczik et al., 1999; |
| c) Persons receiving long- term therapy with glucocorticoids or other immunosuppressants | | Allard et al., 1992; Kanra et al., 2000; |
| d) Persons who have received organ transplant. | Taking immunocompromising drugs | Gruter et al., 2000; Srikantiah et al., 2005 |
| e) Malnutrition | | Sirinavin et al., 1988 |
| 5. Health problem: | | |
| a) Diabetes mellitus | Reduced gastric acid secretion and motility | Kass et al., 1992; Telzak et al., 1991. |
| b) Heart disease | | Kass et al., 1992 |
| c) Systemic lupus erythematous | Use of immunosuppresive agents (steroids) | Lim et al., 2001; Lovy et al., 1981; Shahram et al., 1993; Tsao et al., 2002 |
| d) Sickle cell anemia | Hemolysis, complement defects | Gomez et al., 1998; Rodgers et al., 2002; Ware, 1997 |
| **Diet-related:** | | |
| a) Frequent consumption of *Salmonella*-high risk foods (raw or undercooked animal products, fruits and vegetables). | Increases the probability of ingesting infectious doses of *Salmonella* | Olsen et al. 2000 |
| b) Poor food handling and preparation practices | | Kohl et al., 2002 |

**Treatment**

Each of clinical syndromes caused by *Salmonella* requires a different series of management described in the Table 3.

Table 3. Management of *Salmonella* infections (Adapted from Kim et al., 2004).

| Clinical syndrome | Treatment | Antibiotics of choice |
|---|---|---|
| Gastroenteritis<br>Normal host | No antibiotics | |
| Newborn infants<br>Persons older than 50 yr<br>Lymphoproliferative disease<br>Cardiovascular disease<br>Bone and joint disease<br>Sickle cell disease<br>Transplant recipient<br>HIV infection | Antibiotic prophylaxis until patient is afebrile for 24 hr | Ciprofloxacin, trimethoprim-sulfamethoxazole, amoxicillin, ceftriaxone, cefoperazone, ampicillin |
| Typhoid fever | Antibiotic therapy for 10-14 days | Ciprofloxacin, trimethoprim-sulfamethoxazole, amoxicillin, ceftriaxone, cefoperazone, ampicillin |
| Bacteremia<br>Without metastatic infection | Antibiotic therapy for 7-14 days | |
| With extraintestinal nonvascular metastatic infection | Antibiotic therapy for 2-4 weeks and drainage of focal infection | Chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, ceftriaxone, cefoperazone |
| Vascular metastatic infection | Antibiotic therapy for 4-6 weeks and excision of infected sites where possible | |
| Chronic carrier state<br>Normal biliary tract | Antibiotic therapy for 4-6 weeks | Ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, ciprofloxacin |
| Biliary tract disease | Parenteral antibiotics for 10-14 days and cholecystectomy | Chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, ceftriazone, cefoperazone |

**Recent trends in human nontyphoidal salmonellosis**

Despite improved sanitation and interventions such as hazard analysis critical control points (HACCP) system-regulation in meat and poultry slaughter and processing plants (Rose, 2002), increased attention to fresh produce safety through proper handling (FDA, 1998 and 2000), regulation requiring the refrigeration and safety labeling of shell eggs (FDA, 2001a), guidance for sprout safety (FDA, 1999), implementation of HACCP in juice industries (FDA 2001b), food safety education (FIGHT BAC), increased regulation of imported food, and industry efforts, including new intervention technologies, to reduce food contamination, NTS remains a major public health problem. This may be due to microbial adaptation and dissemination of the pathogen through increasing global distribution of food and international travel, increasing susceptible populations, as well as lifestyle changes leading to an increase in food service establishments and food outlets combined with inadequate knowledge of food handling (Gomez et al., 1997).

In many countries, there was a marked upsurge in the number of cases of human NTS between the mid-1980s and mid-1990s. Much of this can be attributed to *S.* Enteritidis (Humphrey, 2000). In the US, based on the National Notifiable Disease Surveillance System, the incidence of NTS steadily increased from 1955 to 1985 (CDC, 2005b). Since then, with the exception of 1985, when there was *S.* Typhimurium outbreak associated with contaminated pasteurized milk in Illinois, there was a decreasing trend until 1992 (Figure 1-1). The incidence increased again from 1992 to 1995. Much of this increase has been associated with increased in the number of cases of *S.* Enteritidis (CDC, 2005a). In 1994 and 1995 the incidence of *S.* Enteritdis surpassed *S.*

Typhimurium (Figure 1-2). Since 1995 there was a decreasing trend in the incidence of NTS cases.

Figure 1-1. Incidence (per 100,000 population) of reported nontyphoidal salmonellosis cases, United States, 1944-2003 (CDC, 2005b)

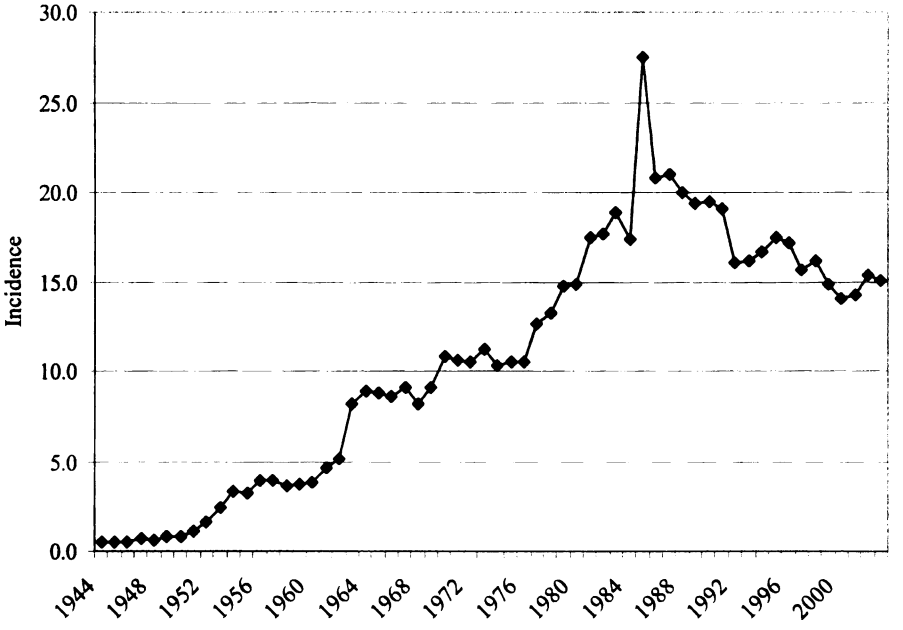
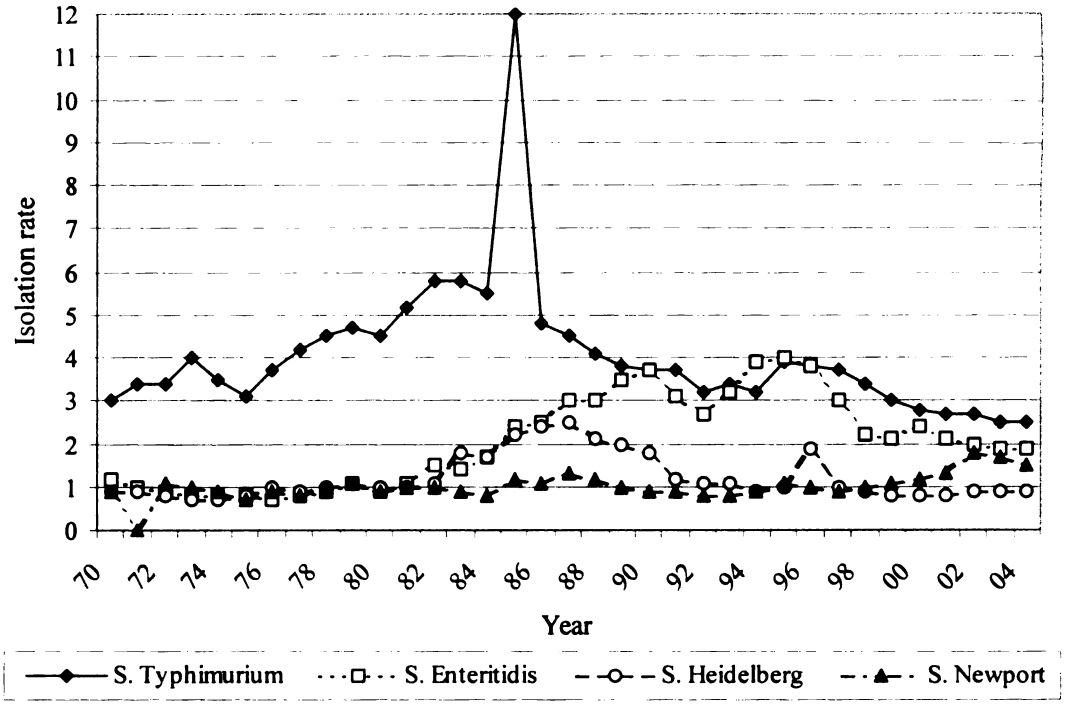


Figure 1-2. Isolation rates (per 100,000 population) of the four most common *Salmonella* serotypes, United States, 1970-2004 (CDC, 2005a)




Many cases of NTS and other foodborne illnesses are not reported because the ill person does not seek medical care, the health care provider does not obtain a specimen for diagnosis, the laboratory doses not perform the necessary diagnostic test, or the illness or laboratory findings are not communicated to public health officials (Mead et al., 1999). For NTS, the degree of underreporting has been estimated at ~38 fold (Chalker and Blaser, 1988).

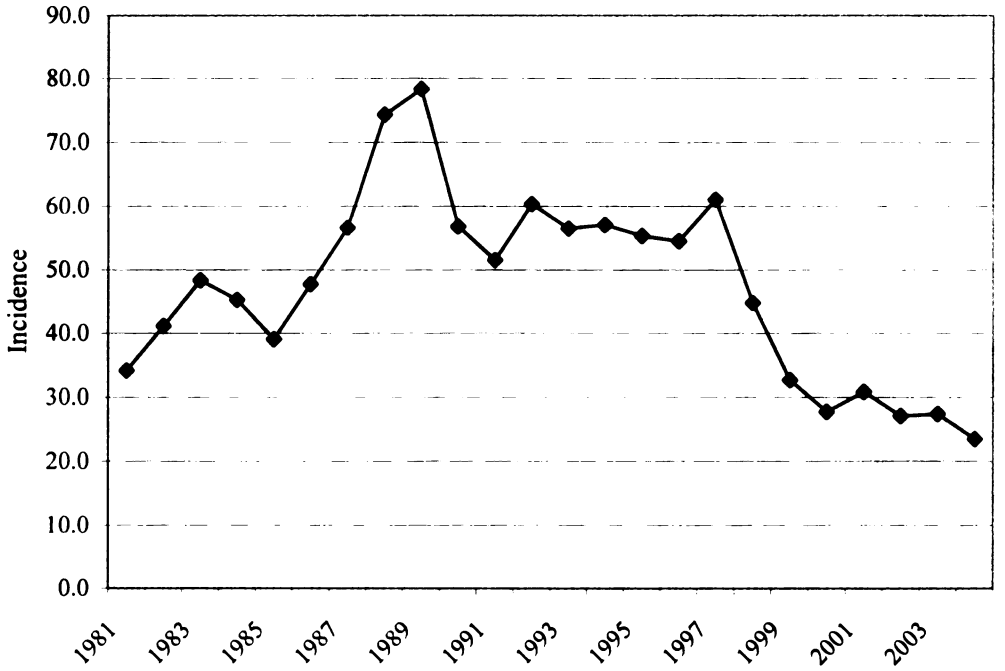
Based on FoodNet surveillance data, the estimated incidence of NTS decreased significantly from 1996 to 2003 (CDC, 2004c). During 1996 to 2003, the estimated incidence of *S.* Typhimurium decreased by 38% (95% CI = 47% to 27% decrease). The incidence of *S.* Enteritidis, *S.* Newport, and *S.* Heidelberg showed considerable variation

by year and did not change significantly (CDC, 2004c). The decline in human NTS during 1996 to 2003 accompanies a decline in the isolation of nontyphoidal *Salmonella* from meat and poultry by US Department of Agriculture's (USDA) Food Safety Inspection Service (FSIS) (Rose et al., 2002). The Food and Drug Administration (FDA) has introduced additional interventions to prevent foodborne diseases. These include implementing HACCP regulations for the juice industry beginning in 2002 (FDA, 2001b), publishing sprout safety guidance in 1999, publishing produce safety guidance in 1998 (FDA, 2000), and implementing regulations requiring the refrigeration and safety labeling of shell eggs in 2001 (FDA, 2001a).

By using FoodNet surveillance data from 1996 to 1999 and related survey, it was estimated that, ~1.4 million people are infected with nontyphoidal *Salmonella* each year in the US, resulting in ~15,000 hospitalizations and ~400 deaths (Voetsch et al., 2004).

In England and Wales, the incidence of NTS increased from 34.2/100,000 in 1981 to a peak of 78.4/100,000 in 1989. Since 1997, the incidence has continued to fall (Figure 1-3) (Health Protection Agency). Calculations for the year 1995 resulted in an estimate of 102,227 indigenous cases, with 3,412 hospital admissions and 268 deaths (Adak et al., 2002).

Figure 1-3. Incidence (per 100,000) of reported cases of nontyphoidal salmonellosis, England and Wales, 1981-2004 (Health Protection Agency)




The number of reported cases of human NTS in Canada decreased from 9,055 cases in 1991 to 4,950 in 2003 (CIDPC, 2003). In Argentina, the number of nontyphoidal *Salmonella* isolated from human decreased from 1,171 in 1998 to 453 in 2003 (WHO, 2000). In Denmark, the number of human NTS decreased from 3,259 in 1996 to 2,075 in 2002 (EU, 2003). In the Netherlands, the number of cases decreased from 2,889 in 1996 to 1,588 in 2002 (EU, 2003). In Australia, the number of cases increased from 5,145 in 1990 to 7, 008 in 2003 (CDA, 2006). In New Zealand, the number of cases increased from 1,244 in 1990 to 1,601 in 2003 (ESR, 2003). In Japan, the number of cases decreased from 16,576 in 1997 to 5,833 in 2003 (MHLW, 2003). In South Korea, the number of nontyphoidal *Salmonella* isolates from humans decreased from 1,451 in 1996

to 676 in 2003 (WHO, 2000). In Thailand, the number of human isolates increased from 3,664 in 1996 to 4,067 in 2003 (WHO, 2000).

## Economic impact of *Salmonella* infections

The social and economic impact of NTS is considerable, imposing costs upon the public sector, industry (in particular wholesale and retail food industry), and infected people. The infection may result in admission to hospitals and in a small proportion of cases, death (Sockett and Roberts, 1991). The economic cost of NTS in the US, England and Wales, Canada and Germany are shown in Table 4.

Table 4. Economic cost of human salmonellosis by country

| Country | Cost | Source |
|---|---|---|
| United States | $0.5 to $2.3 billion | Frenzen et al., 1999 |
| England and Wales | £996,339 | Sockett and Robert, 1991 |
| Canada | $486 | Todd, 1989 |
| Germany | DM 108 million | Krug and Rehm, 1983 |

## Common nontyphoidal *Salmonella* serotypes

The most common nontyphoidal *Salmonella* serotypes that cause NTS in human in the US and other countries were *S.* Typhimurium, *S.* Enteritidis, *S.* Newport and Heidelberg (CDC, 2005a; EU, 2003; Olsen et al., 2001a; WHO, 2000; WHO, 2003).

***Salmonella* Typhimurium.** *Salmonella* Typhimurium can be found in a broad range of species as well as the environment. In the US, from 1995 to 2001, 36%, 18%, 8% and 6% of clinical isolates of *S.* Typhimurium from non-human sources were from cattle, swine, chickens (including eggs), and turkeys respectively (CDC, 2002b). From

1995 to 2001, the food vehicles that were implicated in *S.* Typhimurium outbreaks in the US were pork, beef, chicken, unpasteurized milk, homemade cheese, fresh fruit, vegetables, clover sprouts (CDC, Foodborne Outbreak Response and Surveillance Unit).

Epidemiological investigations have shown that outbreaks of *S.* Typhimurium were associated with consumption of commercially processed egg salad, unpasteurized milk, raw eggs, cheese made of raw milk, lamb kebab, raw ground beef, chocolate, and salami sticks (CDC, 2003c; CDC, 2004b; Cowden, 1989; De Valk et al., 2000; Evans et al., 1999; Hall, 2002; Kapperud et al., 1990; Roels et al., 1997).

Sporadic *S.* Typhimurium infections have been associated with consumption of undercooked ground beef and undercooked eggs or egg-containing foods (Delarocque-Astagneau et al., 2000; Hedberg et al., 1993).

The recent public health concern of this serotype was the emergence of MR-DT104 that are resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT) (Threfall et al., 1994). This strain was first isolated from humans in 1984 in the UK (Threfall et al., 1994). The number of reported human isolates increased from 259 in 1990 to 4,006 in 1996 (Threlfall et al., 1997). The animal reservoirs of this strain in the UK include cattle, swine, sheep, poultry, and cats (Hogue et al., 1997). Also of concern is the increasing trend of additional resistance of DT104 to trimethoprim and ciprofloxacin (a fluoroquinolone) in England and Wales (Threlfall et al., 1997).

In the US, special studies in selected counties revealed that the proportion of R-type ACSSuT that were DT104 was 0% in 1980, 25% in 1985, 50% in 1990, and 85% in 1995. A study in 1995, where all states participated in a National Antimicrobial

Resistance Study, revealed that 85% of R-type ACSSuT were DT104 (Hogue et al., 1997). Results of the 1996 National Antimicrobial Susceptibility Monitoring System (NARMS) showed that 85% of R-type ACSSuT were DT104 (Hogue et al., 1997). Besides cattle, this strain has been isolated from other animals including swine, sheep, chickens, turkeys, horses, goats, emus, cats, dogs, elks, mice, coyotes, ground squirrels, raccoons, chipmunks, and several species of birds (Besser et al., 1997). Outbreaks of MR-DT104 in humans in the US have been reported (CDC, 2001; Cody et al., 1999; Villar et al., 1999).

***Salmonella* Enteritidis.** *Salmonella* Enteritidis is a leading cause of foodborne salmonellosis worldwide, particularly in developed countries (Rodrigue et al., 1990). Epidemiological investigation of sporadic infections and outbreaks showed that consumption of raw or undercooked eggs and foods containing raw or undercooked eggs were the major risk factors (Mishu et al., 1994; Passaro et al., 1996; St Louis et al., 1988) In the US, *S.* Enteritidis emerged as an important cause of human illness during the 1980s. In 1976, the isolation rate was 0.55/100,000. By 1985, the rate increased to 2.4/100,000 (CDC, 2002b). The rate peaked at 3.8/100,000 in 1995. The rate declined to 1.9 in 1999. However, the rates did not decline further through 2001, and outbreaks continue to occur (CDC, 2003e).

Recently, eating chicken prepared outside of the home was associated with sporadic *S.* Enteritidis infections in the US (Kimura et al, 2004) and raw almonds contaminated with *S.* Enteritidis caused outbreak in the US and Canada (Isaacs et al., 2005).

***Salmonella* Heidelberg.** In the US, *S.* Heidelberg was frequently isolated from chickens, swine and turkeys (CDC, 2002b), and was the fourth most common *Salmonella* serotype isolated from humans from 1995 to 2001 (CDC, 2002b). Epidemiolgical investigation showed that outbreaks of *S.* Heidelberg were associated with consumption of chicken, eggs, and cheddar cheese (Fontaine et al., 1980; Layton et al., 1997; Mahony et al., 1990; Schonei et al., 1995). Recently, it was reported that eating eggs prepared outside the home and eating frozen processed chicken nuggets and strips were associated with sporadic *S.* Heidelberg infections (Hennessy et al., 2004; MacDougall et al., 2004). A more recent report revealed that most common food vehicles implicated in *S.* Heidelberg outbreaks in the US were poultry, eggs, egg-containing food item, pork, and beef (Chittick et al., 2006). In Canada, frozen chicken nuggets and strips and eggs are the leading risk factors of *S.* Heidelberg infections (Currie et al., 2005).

***Salmonella* Newport.** In the US, *S.* Newport was frequently isolated from cattle (CDC, 2002b) and was the third most common *Salmonella* serotype isolated from humans from 1995 to 2001 (CDC, 2002b). Epidemiological investigations showed that outbreaks of *S.* Newport were associated with consumption of hamburger, peanuts, mango, cured ham, alfalfa sprouts, undercooked eggs, and ham or pork sandwiches (Aseffa, et al., 1994; Kirk et al., 2004; Lyytikainen et al., 2000; Narain and Lofgren, 1989; Sivapalasingam et al., 2003; Spika et al., 1987; Van Beneden et al., 1999).

Since 1996, there has been an increasing number of *S.* Newport isolates that are resistant to nine antimicrobial agents: amoxicillin/clavulanate, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (CDC, 2002c). This multidrug-resistant strain is known as Newport-MDRAmpC. This

strain also exhibits decreased susceptibility to ceftriaxone, an extended spectrum cephalosporin. The increase of Newport-MDRAmpc infections in humans was associated with exposure to dairy farms, ill cattle, cheese made from unpasteurized milk, and raw or undercooked ground beef (CDC, 2002c; Gupta et al., 2001; Gupta et al., 2003; McCarthy et al., 2002; Rankin et al., 2002a).

**Antimicrobial Resistance in *Salmonella***

In recent years, antibiotic-resistant *Salmonella*, which are associated with increased rates of hospitalization and greater morbidity and mortality, have emerged in many countries, including the US (Gupta et al., 2003; Rabsch et al., 2001). In the US in 1996, high percentages of nontyphoidal *Salmonella* isolates were resistant to sulfamethoxazole (20.3%), tetracycline (24.2%), streptomycin (20.6%), ampicillin (20.7%), chloramphenicol (10.6%) (CDC, 2006c). In 1999, low percentages of nontyphoidal *Salmonella* isolates were resistant to broad-spectrum β-lactam (ceftriazone, 0.4%) (CDC, 2006c). Ceftriazone is an important drug for the treatment of invasive salmonellosis in the pediatric population. In 2000, 0.4% of nontyphoidal *Salmonella* isolates were resistant to fluroquinolone (ciprofloxacin) (CDC, 2006c). The emergence of antibiotic-resistant *Salmonella* is favored by the overuse or misuse of antibiotics in human and veterinary medicine (Miriagou et al., 2006).

**Molecular mechanism of antibiotic resistance in *Salmonella***

1. Antibiotic inactivation

Resistance to ampicillin and cephalosporins

Ampicillin is a β-lactam antibiotic that acts by inhibiting penicillin-binding proteins (PBPs): transpeptidases, transglycosylases, and carboxypeptidases of bacteria that are essential for peptidoglycan synthesis (Yao and Moellering, Jr., 2003). Inhibition of PBSs interrupts cell wall synthesis, which inhibit cell growth (Rice et al., 2003b). However, the interaction of β-lactam molecules with PBPs triggers the activity of cell wall-degrading molecules, autolysins, which rupture the cells, leading to cell death (Rice et al., 2003b).

In *Salmonella*, antimicrobial resistance against β-lactam antibiotics (penicillins and cephalosporins) is mainly mediated by β-lactamase enzymes, which inactivate the antibiotics (Michael et al., 2006). Other mechanisms of β-lactam resistance described are changes in outer membrane proteins, leading to a lowered permeability for the enzyme (porin deficiencies) or the export of β-lactam via multi-drug transporters (Michael et al., 2006). Alterations in penicillin binding proteins, as described in some Gram-positive bacteria, have not been detected and are most probably not a relevant mechanism for resistance in *Salmonella*.

At least 10 different subgroups of β-lactamase genes (*bla*) coding for TEM-, SHV-, PSE-, OXA-, PER-, CTX-M-, CMY-, ACC-, DHA-, or KPC-type β-lactamases have been identified (Michael et al., 2006). Ampicillin resistance in *Salmonella* has been attributed to $bla_{TEM}$ β-lactamase gene (Livermore, 1998; Michael et al., 2006). This gene

is located in the resistance plasmid. It has been reported that the *bla* (TEM-135) gene for ampicillin resistance contained in transposon, Tn3, located in plasmid pFPTB1 (Pasquali et al., 2005). It has also been reported that an OXA-1 β-lactamase gene, located in an integron, was responsible for resistance to ampicillin in *S.* Typhimurium from fish (Ruiz et al., 1999). In *S.* Typhimurium, the ampicillin resistance *bla* (TEM) gene can be transferred by conjugation (Livermore, 1998).

Cephalosporins (e.g. ceftriaxone) are derivatives of the fermentation products of Cephalosporium acremonium. Cephalosporins act by binding to PBPs of susceptible bacteria and interfere with the synthesis of peptidoglycan of the bacterial cell wall (Yao and Moellering, Jr., 2003).

In recent years, reports of *Salmonella* isolates resistant to extended-spectrum cephalosporins (ESCs) have increased. The majority of such isolates produce extended spectrum β-lactamases (ESBLs) (Allen and Poppe, 2002; Winokur et al., 2000). These enzymes hydrolyze oxyimino cephalosporins. ESBLs are inhibited by clavulanic acid. The *bla*TEM genes that encode for ESBLs include *bla*TEM-3, *bla*TEM-4, *bla*TEM-20, *bla*TEM-27, *bla*TEM-52, *bla*TEM-63, and *bla*TEM-131 (Michael et al., 2006). The *bla* genes that encode for SHV-type β-lactamases, which are ESBLs, include *bla*SHV-2, *bla*SHV-2a, *bla*SHV-5, *bla*SHV-9, and *bla*SHV-12. Two types of PER β-lactamases, PER-1 and PER-2, which are ESBLs have been identified in *Salmonellae*. The *bla* genes that code for PER-1 and PER-2 β-lactamases, which are ESBLs include *bla*PER-1 and *bla*PER-2. These genes are commonly found on multiresistance plasmids. The *bla* genes that code for CTX-M β-lactamases, which are ESBLs include *bla*CTX-M-2, *bla*CTX-M-3, *bla*CTX-M-4, *bla*CTX-M-5, *bla*CTX-M-6, *bla*CTX-M-7, *bla*CTXM-9, *bla*CTX-M-14,

*bla*CTX-M-15, blaCTX-M-17 or *bla*CTX-M-18, *bla*CTX-M-27, *bla*CTXM-28, and *bla*CTX-M-32 (Michael et al., 2006).

Recently, a second mechanism of resistance to ESCs in *Salmonella* has emerged and strains producing *Amp*C-like cephalosporinases have been isolated. Several different groups of AmpC β-lactamases, such as CMY-, AAC- and DHA-β-lactamases, have been identified in *Salmonella*. Three different types of CMY β-lactamases have been detected with the gene *bla*CMY-2 being most widespread among the various *Salmonella* enterica. The remaining two *bla*CMY genes were *bla*CMY-4 and *bla*CMY-7. Only one representative gene of each of the remaining two groups is known to occur in salmonellae: *bla*ACC-1 and *bla*DHA-1 (Michael et al., 2006).

These β-lactamases genes in *Salmonellae* are usually encoded on transmissible plasmids, which could be acquired from other multidrugresistant Enterobacteriaceae, such as *Klebsiella pneumoniae* or *Escherichia coli* (Gupta et al., 2003). For example *S.* Newport-MDRAmpC strains in the US were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline. In addition, Newport-MDRAmpC isolates were resistant to amoxicillin/clavulanic acid, cephalothin, cefoxitin, and ceftiofur, and exhibited decreased susceptibility to ceftriaxone.

Resistance to streptomycin

Streptomycin is a bacteriacidal antibiotic of the aminoglycoside. It acts by inhibiting bacterial protein synthesis by binding irreversibly to the bacterial 30S ribosomal subunit (Yao and Moellering, Jr., 2003). The streptomycin-bound bacterial

ribosome then become unavailable for translation of mrNA during protein synthesis, thereby leading to cell death.

Resistance to streptomycin and other aminoglycosides can occur by one of four mechanisms: reduced uptake or decreased cell permeability, alteration in the target site (ribosomal binding sites), expulsion by efflux pumps, and enzymatic inactivation by aminoglycoside modifying enzymes (AMEs) (Rice et al., 2003b; Shaw et al., 1993).

In *Salmonella*, as in most other bacteria, resistance to aminoglycosides is most frequently mediated by AMEs, which attaches certain groups to the aminoglycoside molecule thereby destroying its antibacterial activity (Michael et al., 2006). The AMEs are phosphotransferases (APHs), adnyltransferases (ANTs), and acetyltransferases (AACs). Enzymatic modification results in high-level resistance. The genes encoding for AMEs are usually found on plasmids and transposons. The phosphotransferase *aph(6)-Ia* gene (also named *strA*) and the *aph(6)-Id* gene (also named *strB*) appear to be widely distributed in *Salmonella* and other gram-negative bacteria (Madsen et al., 2000; Pezzella et al., 2004). These genes have been described as being part of transposon Tn*5393* and are frequently located on plasmids.

Resistance to chloramphenicol

Chloramphenicol is a broad-spectrum antimicrobial agent. It is a bacteriostatic agent that inhibits protein synthesis by binding reversibly to the peptidyltransferase component of the 50S ribosomal subunit and preventing the transpeptidation process of peptide chain elongation (Yao and Moellering, Jr., 2003).

Resistance to chloramphenicol is mainly due to the production of inactivating enzymes, the chloramphenicol acetyl transferase (CAT) (Arcangioli et al., 2000; Rice et al., 2003b; Schwarz et al., 2004). The production of CAT is encoded by the acetyltransferase (*cat*) genes (Bolton et al., 1999; Gallardo et al., 1999; Ruiz et al., 1999).

In *Salmonella*, the dominant resistance mechanisms are enzymatic inactivation by type A or type B CAT and the export of chloramphenicol or chloramphenicol/florfenicol by specific efflux proteins (Michael et al., 2006). The *cat* genes are located on plasmid.

The chloramphenicol exporter gene *cml*A is also a cassetteborne gene that has been found in plasmid-located class 1 integrons in *S.* Typhimurium, serotype [4,5,12:i:-], and *S.* Muenchen. A new *cml*A4 variant has recently been identified in a plasmid-borne class 1 integron of *S.* Agona. (Michael et al., 2006).

A new chloramphenicol resistance gene from *S.* Typhimurium DT104, designated *flo*R, also conferring resistance to florfenicol. The *flo*R gene was surrounded by two class 1 integrons (Arcangioli et al., 1999). In contrast to the *cml*A-encoded efflux proteins, those encoded by *flo*R genes can also export florfenicol (Michael et al., 2006).

2. Alteration of the target site

Resistance to sulfonamides (e.g. sulfamethoxazole)

Sulfonamides represent the oldest group of antimicrobial agents and have been available since the 1930s (Guerra et al., 2004). Sulfonamides were the first effective systemic antimicrobial agents used in the United States. (Yao and Moellering, Jr., 2003). They act by inhibiting the bacterial enzyme dihydropteroic acid synthetase (DHPS) and thus interfere with bacterial folic acid synthesis.

In *Salmonella*, sulfonamide resistance arises from the acquisition of sulphonamide resistance genes-*sul1*, *sul2*, and *sul3*, encoding forms of dihydropteroate synthase that are not inhibited by the drug (Antunes et al., 2005; Guerra et al., 2004; Michael et al., 2006). The *sul1* gene is normally found linked to other resistance genes in class 1 integrons, *sul2* is usually located on small nonconjugative plasmids or large transmissible multiresistance plasmids, and *sul3* is found large plasmid (Antunes et al., 2005; Guera et al., 2004; Michael et al., 2006).

Resistance to quinolone (e.g. ciprofloxacin)

The primary bacterial target of quinolones is DNA gyrase and type IV topoisomerase (Yao and Moellering Jr., 2003). These enzymes are essential for DNA replication, recombination, and repair. These enzymes exist as tetramers composed of two different subunits (GyrA and GyrB of DNA gyrase; ParC and ParE of topoisomerase IV) (Rice et al., 2003b). The DNA gyrase A subunit is the main target in gram-negative bacteria, whereas topoisomerase IV is the primary target in gram-positive bacteria. Inhibition of these enzymes cause relaxation of the supercoil DNA, leading to termination of chromosomal replication and interference with cell division and gene expression. (Yao and Moellering Jr., 2003). The DNA gyrase A subunit is encoded by *gyr*A gene.

In *Salmonella*, raised minimal inhibitory concentrations (MICs) or resistance to quinolones has mainly been attributed to single point mutations in the quinolone resistance-determining region (QRDR) of the *gyrA* gene that result in the alterations of the target enzyme (DNA gyrase), which prevent binding of fluroquinolones to the target

enzyme (Cloeckaert and Chaslus-Dancla, 2001; Eaves et al., 2004; Kilmartin et al., 2005; Piddock, 2002; Seminati et al., 2005).

The most common amino acid substitutions in the GyrA subunit associated with quinolone resistance occur at codons Ser83 and Asp8. Double mutations at both residues 83 and 87 have been identified in clinical isolates of *S.* Typhimurium showing high-level resistance to fluoroquinolones (e.g., MIC of ciprofloxacin: 32 μg.mL–1) (Cloeckaert et al., 2001; Ling et al., 2003).

*Salmonella* isolates highly resistant to fluoroquinolones from a clinical case with three point mutations in the QRDR of the *gyrA* and *parC* has also been recently reported (Nakaya et al., 2003). In Hong Kong, transferable fluoroquinolone resistance in *S.* Enteritidis isolates due to a new *qnr* allele, which appeared to be linked to *bla* (CTX-M-14) has been reported recently (Cheung et al., 2005; Michael et al., 2006). In addition, a considerable number of *Salmonellae* isolates from 1990 to 2001 had a mutation in *parC* gene has been identified in Hong Kong (Ling et al., 2003).

3. Energy-dependent removal of antimicrobials via membrane-bound efflux pumps

Resistance to tetracycline

Tetracyclines are broad-spectrum bacteriostatic antibiotics that act against susceptible bacteria by inhibiting the attachment of aminoacyl-tRNA to the RNA-ribosome complex, preventing bacteria polypeptide synthesis (Chopra and Roberts, 2001; Yao and Moellering, Jr., 2003). Widespread use of tetracycline to treat clinical infections and to promote growth in livestock has been associated with the emergence and dissemination of a variety of resistance determinants (Rice et al., 2003b).

Most of tetracycline resistance determinants fall into one of two classes: efflux proteins or ribosomal protection proteins (Rice et al., 2003b). Tetracycline efflux proteins expel tetracycline from the cell by exchanging a proton for a tetracycline-cation complex. The efflux proteins in gram-negative bacteria consist of *tet* (A), *tet* (B), *tet* (C), *tet* (D), *tet* (E), *tet* (G), *tet* (H), *tet* (I). Ribosome protection protein act by binding to the ribosome, thereby changing its conformation and inhibiting the binding of tetracycline (Rice et al., 2003b). Ribosome protection proteins consist of *tet* (M), *tet* (O), *tet* (S), and *tet* (W).

In *Salmonella*, five tetracycline resistance genes - *tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(G) - have been reported. All of these *tet*- genes code for a membrane-associated efflux protein consisting of 12 transmembrane segments, which can export tetracycline, oxytetracycline, chlortetracycline and doxycycline (Frech and Schwarz, 1998; Michael et al., 2006; Pasquali et al., 2005; Pezzella et al., 2004). The *tet*(G) has exclusively been detected in *Salmonella* Genomic Island 1 (SGI1), located within the *S.* Typhimurium DT104 chromosome, but later on chromosome of other serotypes, such as *S.* Agona, *S.* Albany, *S.* Paratyphi B, and *S.* Newport. The two genes *tet*(A) and *tet*(B) are associated with small, non-conjugative transposons Tn1721 and Tn10, respectively, and are widespread among the different *Salmonella* serotypes. Complete or truncated copies of these transposons have been identified on large multiresistance plasmids, but also on plasmids conferring resistance to tetracyclines only, tetracyclines and penicillins, or tetracyclines and streptomycin. Plasmids conferring *tet*(C)- or *tet*(D)-mediated tetracycline resistance have been identified only in single cases (Pezella et al., 2004; Michael et al. 2006).

Multiresistant *Salmonella Typhimurium DT104*

The complete spectrum of antimicrobial resistance in *S.* Typhimurium DT 104, R-type ACSSuT (resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline), is chromosomally encoded (Threlfall et al., 1994). In 1998, two different integrons were identified (Cloeckaert and Schwarz, 2001; Ridley and Threlfall, 1998; Sandvang et al., 1998). The first integron carried the *aadA2* gene conferring resistance to streptomycin and spectinomycin, and a deletion in the *sulI* resistance gene. The second integron carried the β-lactamase gene $bla_{PSE-1}$ and a complete *sulI* gene conferring resistance to ampicillin and sulfonamides respectively. In 1999, the chloramphenicol and florfenicol resistance gene (*floR*) and tetracycline resistance genes (*tetR* and *tetA*) were identified (Arcangioli et al., 1999; Bolton et al., 1999; Briggs and Fratamico, 1999; Ng et al., 1999). These genes were located between the two integrons.

More recently this chromosomal antimicrobial resistance gene cluster has been shown to lies within 43-kb SGI1 (Boyd et al., 2000; Boyd et al., 2001; Cloeckaert and Schwarz, 2001).

Multidrug-resistant *S.* Typhimurium DT 104 (MR-DT104) has also acquired resistance to trimethoprim (R-type ACSSuTTm) and decreased susceptibility to ciprofloxacin (R-type ACSSuTCp) (Threlfall et al., 1997; Threlfall, 2000). Resistance to trimethoprim may be encoded by non-conjugative but mobilizable plasmid, which also encode resistance to sulphonomides (Cloeckaert and Schwarz, 2001; Threlfall et al., 1996; Threlfall, 2000). It has been suggested that resistance to trimethoprim may have resulted from the use of trimethoprim-containing compounds in cattle in attempts to

combat infection with *S.* typhimurium DT 104 of R-type ACSSuT (Threlfall et al., 1997; Threlfall et al., 1999; Threlfall, 2000).

Decreased susceptibility to ciprofloxacin in MR-DT104 is chromosomally encoded (Ridley and Threlfall, 1998). The emergence and spread of MR-DT104 with decreased susceptibility to ciprofloxacin in the UK followed the licensing for veterinary use of enrofloxacin in November 1993 (Threlfall et al., 1999; Threlfall, 2000). This antimicrobial was subsequently used for treatment and prophylaxis in both cattle and poultry (Piddock and Jong, 1999). A possible consequence of this has been the rapid development of resistance to nalidixic acid in strains of MR-DT104 in food-producing animals in the UK, particularly turkeys, chickens and cattle (Davies et al., 1999). This is because the mechanism of resistance against nalidixic acid (first generation fluoroquinolone) and enrofloxacin (more modern fluoroquionolone) is the same, single point mutation in the quinolone resistance-determining region of the topoisomerase gene *gyr*A (Butt et al, 2006; Davies et al., 1999; Hakanen et al., 1999).

The use of antimicrobial agents in agriculture might have contributed to the emergence of DT104 antibiotic resistance gene cluster (Angulo and Griffin, 2000). Because the genes included in the multiresistant gene cluster of DT104 strains confer resistance to tetracycline, β-lactams, aminoglycosides and sulfonamides, which are most frequently used in veterinary medicine, co-selection of the entire cluster may result from the use of any of these drugs (Cloeckaert and Schwarz, 2001). MR-DT104 may acquire resistance genes horizontally from nosocomial pseudomonads because the $bla_{PSE-1}$ encoded β-lactamase is a common feature of hospital *P.* aeruginosa isolates (Cloeckaert and Schwarz, 2001). The *tet*(G) also occurs in *Pseudomonas* sp. and *flo*R is closely

related to the *P.* aeruginosa chlaramphenicol-resistance gene *cml*A (Cloeckaert and Schwarz, 2001).

Whatever the origin of multiresistant DT104, we must consider the importance of the presence of the DT104 resistance gene cluster within SGI1 because the spread of multiresistant DT104 might occur without the selective pressure imposed by the used of antimicrobial agents (Cloeckaert and Schwarz, 2001). This is because the DT104 resistance gene is associated with the presence of two class-1 integrons (Ribot et al., 2002), a group of apparently mobile elements that can contain one or more antimicrobial resistance gene. Intergrons represent an important and efficient mechanism by which many bacteria, including *S.* Typhimurium DT104, can acquire resistance to antimicrobial agents (Ribot et al., 2002).

Multidrug-resistant *S.* Newport

Multidrug-resistant *S.* Newport is resistant to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (CDC, 2002c). In addition, this strain exhibited decreased susceptibility to ceftriaxone, an extended-spectrum cephalosporin (ESC) used in humans. This strain has plasmids that carry a $bla_{CMY-2}$ gene. These genes produce AmpC-type β-lactamse, which confer resistance to amoxicillin/clavulanic acid, cefoxitin, and extended-spectrum cephalosporins (ceftriaxone and ceftiofur) (CDC, 2002c; Dunne et al, 2000; Rankin et al., 2002b; Winokur et al., 2000; Zhao et al., 2003).

Integrons have been detected from multidrug-resistant *S.* Newport-AmpC (Pitout et al., 2003; Rankin et al., 2002; Zhao et al., 2003). These integrons contained *aad*A gene, which confers resistance to streptomycin, or *aad*A and *dhfr* gene, which confer

resistance to trimethoprim-sulfamethoxazole. Florfenicol resistance mediated by conjugative plasmid related to R55 from *Klebsiella* pneumonia has been detected in multidrut-resistant *S.* Newport isolated from Turkey (Meunier et al., 2003). It has been demonstrated that florfenicol resistance gene (*flo*R) exist on the $bla_{CMY-2}$-carrying plasmid of multidrug-resistant *S.* Newport (Doublet et al., 2004a). The presence of these resistance genes on the same plasmid may allow the bacterial host to be positively selected by either individual antimicrobial agent. Thus, the application of a chloramphenicol selective pressure in food animals could contribute to the dissemination of the $bla_{CMY-2}$-plasmids, and vice versa (Doublet et al., 2004a).

Recently, a new variant SGI1 antibiotic resistance gene cluster has been identified in two multidrug-resistant *S.* Newport (Doublet et al., 2004b). In these strains, the streptomycin/spectinomycin resistance gene cassette *aad*A2 inserted at the first *att*I1 site was replaced by two other aminoglycoside resistance gene cassettes. The first one contains a new resistance gene encoding an AAC(3)-I aminoglycoside 3-N-acetyltransferase that confers resistance to gentamicin and sisomicin. This gene has been named *aac*(3)-Id. The second one harbors the streptomycin/spectinomycin resistance gene *aad*A7. This gene cassette replacement in the SGI1 complex integron of *S.* Newport strains constitutes a new variant SGI1 antibiotic resistance gene cluster named SGI1-H. The occurrence of SGI1 in different *Salmonella* serotypes, such as *S.* Newport, strengthens the hypothesis of horizontal transfer of SGI1 (Doublet et al., 2004b).

More recently, a $bla_{SHV}$ extended-spectrum β-lactamase has been identified in Newport MDR-AmpC (Rankin et al., 2005). The presence of multiple β-lactamase genes

in Newport MDR-AmpC could effectively limit all β-lactam therapeutic options (Rankin et al., 2005).

**Acquisition of resistance gene by *Salmonella***

*Salmonellae* can acquire resistance gene by mutation or by acquisition of new DNA.

1. By Mutation

In the presence of antibiotics, *Salmonella* can mutate resulting in alteration of the antibiotic target site. For example, mutation of *gyr*A gene in quinolone resistance (Cloeckaert and Chaslus-Dancla, 2001). In the selective environment of the antibiotic, *Salmonella* that carry such a mutation is at a huge advantage as the susceptible *Salmonella* (wild type or non mutants) that are rapidly killed by the antibiotic, leaving a resistant subpopulation (resistant mutant) to grow and flourish (Helmuth, 2000; Tenover and McGowan, Jr., 1998).

2. By acquisition of new DNA

*Salmonella* can acquire resistance genes from another organism that has a gene for resistance to certain antibiotics. Or, more likely, some bacterium develops antibiotic resistance through the process of mutation and then donates these genes to *Salmonella* through conjugation or transduction (Helmuth, 2000). Conjugation involves cell-to-cell contact as DNA crosses a sex pilus from donor to recipient. The importance of conjugation in the spread of resistance gene via plasmid in *Salmonella* is well established (Helmuth, 2000).

Plamsids can carry and transfer multiple resistance genes, which may be located on a section of DNA capable of transfer from one plasmid to another or to the genome, a transposon (or "jumping gene"). Because the range of bacteria to which plasmids can spread is often limited, transposons are important in spreading resistance genes across such boundaries (Helmuth, 2000).

During transduction, bacteriophages (viruses that infect bacteria) can also transfer resistance genes between mating bacteria. When bacteria die they release DNA, which can be taken up by competent bacteria. It is important in *Salmonella* because many of the strains carry prophages or inhabit biotopes where transducing phages are found (Helmuth, 2000).

**Factors that favor the development of antibiotic-resistant *Salmonella***

In order for antibiotic resistance to develop in *Salmonella*, two conditions are needed: 1) *Salmonella* must come into contact with the antimicrobials; 2) Resistance against the antimicrobials must develop, along with a mechanism to transfer the resistance to daughter cells or directly to other *Salmonella* (Khachatourians, 1998).

Antimicrobials are extensively used in human medicine, the production of food animals, the treatment of companion animals, aquaculture and spraying of crops, particularly fruit trees, to eliminate surface bacteria (Benbrook, 2002; Khachatourians, 1998; Prescott et al., 2000; Snary et al., 2004; Wise et al., 1998).

In the veterinary context, antimicrobials can be administered to: 1) treat ill animals on the basis of animal health and welfare, and to prevent economic losses associated with death or decreased productivity (Prescott et al., 2000; Snary et al., 2004);

2) to prevent anticipated disease and, in the veterinary context, this is usually carried out by group administration; 3) to treat incipient disease in individuals and to prevent further outbreak in the group; and 4) to increase the rate of growth and to optimize the feed conversion rate for the rearing of food-producing animals.

In human medicine, the majority of antimicrobial use is for the treatment of clinical disease on an individual basis (Snary et al., 2004).

Because antimicrobials are so widely used in humans, food and companion animals, and aquaculture, bacteria including *Salmonella* are widely exposed to them. Eventually bacteria strains mutate or acquire new DNA to overcome or resist the antibiotic, rendering the antibiotics ineffective (Khachatourians, 1998; Rice et al., 2003b; Tenover and McGowan, Jr., 1998). For example, one or more mutations might be acquired that change the target of action, the uptake, efflux or extrusion of antibiotic, or the ability of *Salmonella* to inactivate or modify antibiotic (Khachatourians, 1998). Thus, producing antimicrobial-resistant *Salmonella*. *Salmonella* serotypes that have been frequently reported to develop antimicrobial resistance include *S.* Typhimurium, *S.* Typhimurium DT104, *S.* Newport, *S.* Agona, *S.* Hadar, and *S.*Virchow (Davis et al., 2002b; Fluit, 2005; Michael et al., 2006; Parry CM, 2003; Threlfall et al. 2000; Threlfall, 2002).

Furthermore widely used of antibiotics, imposed a selective pressure that select for resistant *Salmonella* and other bacteria. Selective pressure refers to the environment created through the use of antibiotics that enhances the ability of *Salmonella* and other bacteria that become resistance by mutation or through acquisition of new DNA to

survive and proliferate (Tenover and McGowan, Jr., 1998). The use of antimicrobials eradicates the susceptible strains, but the resistant strains will survive and proliferate.

Many of the currently known resistance genes in *Salmonella* are located on mobile genetic elements such as plasmids, transposons, gene cassettes and genomic islands. These resistance genes are easily exchanged between bacteria living in the same habitat, e.g. the Enterobacteriaceae in the gastrointestinal tract of humans and animals (Michael et al., 2006). Thus, *Salmonella* may play an important role as either acceptor or donor of resistance genes and thus are of relevance for the dissemination of resistance genes.

Coupled with the use of antimicrobials, the disposal of human sewage sludge and farm and abattoir waste may also be important in the dissemination of resistant bacteria. Vast quantities of such waste are spread over agricultural land, therefore potentially spreading resistant bacteria more widely in the environment, and in livestock and human populations (Snary et al., 2004). As a result of the widespread use of antimicrobials, be it for human or veterinary use, it is now clear that there exists a reservoir of resistant bacteria, including *Salmonella*, in humans, animals and the environment, and that this reservoir may impact on human health (Snary et al., 2004).

Resistant *Salmonella* from food animals can reach humans through: 1) eating meat contaminated with resistant *Salmonella*, 2) eating eggs or milk contaminated with resistant *Salmonella* that have not been properly pasteurized or cooked, 3) eating contaminated fruits or vegetables which have been grown in manure contaminated with resistant *Salmonella*, and 4) direct contact with animals having resistant *Salmonella* (Angulo et al., 2000b).

**Human health consequences of antimicrobial-resistant *Salmonella***

There are several human health consequences associated with antimicrobial-resistant *Salmonella*: 1) Early empirical treatment may fail, 2) The choice of drugs will be limited after the establishment of microbial diagnosis, 3) Increased transmission supported by the use of antimicrobials in humans, and 4) Increased virulence (Molbak, 2005). These factors may contribute to excess morbidity and mortality observed in outbreaks and in sporadic cases of infection with antimicrobial-resistant *Salmonella* (Helms et al., 2002; Molbak, 2005).

**Prevention of the development and dissemination of antimicrobial-resistant *Salmonella***

The emergence and increasing prevalence of antimicrobial-resistant *Salmonella* complicates the treatment of salmonellosis in humans and animals. Therefore, additional measures are needed to reduce the emergence and dissemination of resistant-*Salmonella*. This include prudent use of antimicrobials in human and veterinary medicine, adequate infection control in hospitals, improvements in food hygiene, supply of clean water and sanitation in affected areas, and considering for the use of vaccination (Angulo et al., 2000b; Parry, 2003). Potential components of prudent use of antibiotics include defining the need for the treatment and ensuring the appropriate therapeutic doses and regimes (Angulo et al., 2000b). In the farms, measures should include efforts to control the spread of pathogens such as improve hygiene and sanitation, feed safety and the use of probiotics (Angulo et al., 2000b).

**Prevention of nontyphoidal salmonellosis in humans**

Human NTS is an increasing public health problem. Prevention and control is a challenge to public health authorities. The prevention and control of NTS require both an understanding of the complex cycles of transmission and ongoing surveillance to characterize trends in *Salmonella* occurrence and identify outbreaks (Pegues et la., 2005). Barriers to the introduction and multiplication of *Salmonella* must be implemented along the route from farm to table.

Several approaches could be instituted. First, reduce infection in food animals via good animal husbandry practices and biosecurity. Second, reduce carcass contamination during slaughter and processing via good management practices and HACCP. Third, reduce contamination at retail via proper storage and refrigeration. Finally, by cooking the foods of animal origin thoroughly. In addition, *Salmonella* surveillance in humans and animals is important for determining trends and distribution of infections and to evaluate prevention programs.

Guideline for the prevention of NTS for the public that is outlined by the Center for Disease Control and Prevention (CDC), Atlanta include: 1) People should not eat raw or undercooked eggs, poultry, or meat. Poultry and meat, including hamburgers, should be well cooked, not pink in the middle; 2) Persons also should not consume raw or unpasteurized milk or other dairy products; 3) People should wash produce thoroughly before consuming; 4) People should avoid cross-contamination of foods. 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. Hand should be washed before handling any food, and

between handling different food items; 5) 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; 6) People should wash their hands after contact with animal feces; 7) Everyone should immediately wash their hands after handling reptiles; 8) People should not give pet reptiles (including turtle) to small children; and 9) Pet reptiles should not be in the same house as an infant (CDC, National Center for Infectious Diseases/Division of Bacterial and Mycotic Diseases).

# CHAPTER TWO

## LITERATURE REVIEW OF SALMONELLOSIS IN FOOD ANIMALS

### Introduction

Nontyphoidal Salmonella (NTS) in humans is often foodborne but can also be contracted through contact with infected animals. Foods containing products from farm animals, especially from poultry, pigs, and cattle, are an important source of human NTS (Gomez et al., 1997; Olsen et al., 2000; Sanchez et al., 2002). This is because food animals are often unapparent carriers, latently infected, or less frequently, clinically ill, excreting *Salmonellae* in their feces as a reservoir of contamination among animals, humans, and the environment (Wray and Wray, 2000). In addition, NTS are transmitted during slaughter and dressing of animal products and thence to consumers. The purpose of this review is to describe the source of NTS and routes of transmission in poultry, pigs, and cattle, the public health importance and prevention.

### Poultry

*Salmonella* infections in poultry can be grouped into two categories. First, infection with the two nonmotile serotypes, *S.* Pullorum and *S.* Gallinarum, which are host-specific for poultry (Gast, 2003). *Salmonella* Pullorum caused pullorum disease, which is an acute systemic disease of chicks and poults. *Salmonella* Gallinarum caused fowl typhoid, which is an acute or chronic septicemic disease of mature birds. These diseases have been responsible for serious economic losses in the past and have been

eradicated in many countries by implementing extensive testing and eradication program (Gast, 2003).

The second group includes numerous motile NTS, such as *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport. These serotypes are widespread in wild and domestic animals (Gast, 2003). These serotypes seldom cause acute systemic disease except in highly susceptible young birds that are subjected to stressful conditions. In very susceptible young chicks and poults, these serotypes can lead to high frequency of illness and death. Infection with these serotypes in chickens and turkeys lead to asymptomatic colonization of the intestinal tract, sometimes persisting until slaughter and subsequently contaminates the carcasses (Gast, 2003). *Salmonella* Enteritidis can be deposited in the contents of clean and intact eggs through transovarian infection (Gast and Beard, 1990a and 1990b; Thiagarajan et al., 1994). Other serotypes that can contaminate intact eggs are *S.* Typhimurium DT104 and *S.* Heidelberg (Gast et al., 2004; Gast et al., 2005; Leach et al., 1999; Williams et al., 1998).

*Sources of infection and routes of transmission*

Nontyphoidal *Salmonella* can be introduced into poultry flocks from many sources, including day-old chicks, feed, rodents, wild birds, insects, humans (Bailey et al., 2001 and 2002; Gast, 2003; Poppe, 2000). Horizontal transmission can be mediated by direct bird-to-bird contact, ingestion of contaminated feces or feed or litter, contaminated water, or personnel and equipment (Gast, 2003; Poppe, 2000).

Vertical transmission of nontyphoidal *Salmonella* to the progeny of infected breeder flocks can occur by internal or external contamination of eggs. Some *Salmonella*

serotypes such as *S.* Pullorum, *S.* Gallinarum, *S.* Enteritidis, *S.* Typhimurium DT104, and *S.* Heidelberg can infect the ovaries and oviducts and contaminate the eggs via transovarian transmission (Gast, 2003; Gast et al., 2004 and 2005; Gast and Beard, 1990a and 1990b; Leach et al., 1999; Thiagarajan et al., 1994; Williams et al., 1998). Contamination of egg contents may also occur by contamination of the eggshell with feces from hens excreting *Salmonella* (Timoney et l., 1989). *Salmonella* in the feces may penetrate the eggshell pores as the eggs cool and before the establishment on its surface of the proteinaceous cuticular barrier, which prevents bacterial invasion of the eggs. Alternatively, fecal matter adherent to the shell may contaminate the egg contents when the eggs have cracks.

*Public health importance.*

Poultry are an important reservoir of *Salmonella* for humans. Surveys of broiler flocks in the US have reported the isolation of *Salmonella* from 44 of 196 (22%) houses sampled (Byrd et al., 1999). More recently, Bailey et al. reported 973 (9.1%) of 10,740 samples collected from 32 integrated broiler operations from four states were *Salmonella* positive (Bailey et al., 2001). The most frequently encountered serotypes were *S.* Senftenberg, *S.* Thompson, and *S.* Montevideo. In another study, *Salmonella* were recovered from 60% of samples collected from grow-out broiler flocks (Bailey et al., 2002).

Surveys of poultry carcasses in the early 1990s showed that *Salmonella* was isolated from 43% of ready-to-cook broiler carcasses and 29% of frozen broiler carcasses obtained from retail stores (Bokanyi et al., 1990; Izat et al., 1991). More recently, the US

Department of Agriculture has reported that a national-pathogen reduction program reduced the incidence of *Salmonella* contamination on broiler carcasses from 20% to 10.2% between 1998 and 2000 (Rigney et al., 2004; Rose et al., 2002). A study by Bailey et al. showed that 49 of 798 (6.1%) carcass rinse samples were positive for *Salmonella* (Bailey et al., 2001). In another study, 7% of carcass rinse samples were positive for *Salmonella* (Bailey et al., 2002).

Survey of layer flocks in the US has reported the recovery of *Salmonella* from 72% of laying house environmental samples in one study (Jones et al., 1995) and from 68% of egg farms in another (Riemann et al., 1998). In studies involving pooled cecal samples from spent laying hens in several regions of the US, *Salmonella* was detected in 86% of tested flocks in 1991 and 98% in 1995 (Hogue et al., 1997).

Studies on unpasteurized liquid egg samples collected at 20 eggs breaking plants throughout the US demonstrated that 52% of samples collected in 1991 and 48% from 1995 were positive for *Salmonella* (Hogue et al., 1997c). Studies on environmentally positive commercial layer flocks showed that *S.* Enteritidis egg contamination frequency was less than 0.03% (Kinde et al., 1996; Schlosser et al., 1999). The US Department of Agriculture estimated that the national incidence of egg contamination with *S.* Enteritidis at approximately 0.005% (Ebel and Schlosser, 2000).

Even though the incidences of *Salmonella* in poultry and eggs are low, improper handling of these foods before consumption can permit the multiplication of *Salmonella* to the level that can cause severe gastrointestinal disease in humans. Thus, poultry products are consistently identified as important sources of *Salmonella* that cause human illness. More than one-third of foodborne salmonellosis outbreaks in humans in the US

were associated with poultry meat or eggs (Bean et al., 1996; Olsen et al, 2000; Tauxe, 1991). Between 1985 and 1996, 79% of *S.* Enteritidis outbreaks in the US that could be attributed to a specific food vehicle were associated with eggs (Angulo and Swerdlow, 1999).

Many of the human *S.* Enteritidis infections have been traced to contaminated eggs. Shell eggs, scrambled eggs, soft-boiled eggs, lightly cooked omelettes, food products containing raw or partly cooked eggs, including mayonnaise, tartar sauce, egg-nog, milk shakes, mousses, egg sandwiches, dishes containing raw egg white, ice-cream containing uncooked eggs and poultry meat have all been implicated in outbreaks of *S.* Enteritidis infection (Patrick et al., 2004). More recently, eating contaminated chicken has been associated with sporadic *S.* Enteritidis infection (Kimura et al., 2004).

*Prevention of salmonellosis in poultry (Gast, 2003)*

Because poultry are an important reservoir of nontyphoidal *Salmonella*, preventive measures in poultry farms could reduce the incidence of NTS in poultry and reduce the risk of foodborne NTS due to the consumption of contaminated poultry and poultry products. Preventive measures that should be employed include: 1) purchasing of eggs and chicks or poults from demonstrably *Salmonella*-free breeding stocks, 2) disinfecting hatching eggs properly and hatching them according to stringent sanitation standards, 3) cleaning poultry houses properly and disinfecting by recommended procedures, 4) incorporating rodent and insect control measures into house design and management, 5) implementing rigid biosecurity practices to restrict the movement of personnel and equipment into poultry housing premises and between houses, 6) using

only pelleted feed or feed containing no animal protein to minimize the likelihood of using contaminated rations, 7) providing water that come from a treated sources, 8) using competitive exclusion cultures, or vaccination to reduce the susceptibility of birds to *Salmonella* infection, and 9) testing *Salmonella* status of poultry and their environment frequently (Gast, 2003).

## Pigs

Pigs are an important reservoir of *Salmonella* spp, especially the host-adapted serotype, *S.* Choleraesuis, and other serotypes such as *S.* Typhimurium and *S.* Derby. *Salmonella* Choleraesuis is usually associated with septicemia in pigs and humans, but the prevalence has declined in many countries (Chiu et al., 2004; Fedorka-Cray, 2000). Infection with *S.* Typhimurium occasionally cause gastroenteritis, in general, infected pigs remain healthy carrier. Besides *S.* Typhimurium, *S.* Derby is also predominant in pigs.

### *Sources of infection*

Sources of infection in pigs include rodents, insects, humans and contaminated feed and feedstuffs (Fedorka-Cray, 2000).

### *Public health importance*

Many outbreaks of NTS in humans have been associated with contaminated animal products, including pork and pork products (Bean et al., 1996; Olsen et al, 2000). This is because pigs are an important reservoir of *Salmonella* (Wray and Wray, 2000). There were many reports on the isolation of *Salmonella* from pigs, porks and pork

products. For example, a national study of US pork producers in 1995 by USDA's National Animal Health Monitoring System showed that *Salmonella* in fecal samples was found on 58 (38.2%) of the operations and in 173 (17.5%) of the pens sampled. A total of 398 fecal samples (6.0%) were positive for *Salmonella*. Frequent serotypes isolated were *S.* Derby, *S.* Agona, *S.* Typhimurium copenhagen, *S.* Brandenberg, and *S.* Mbandaka (APHIS, USDA).

In the US, 12.4% of fresh pork sausages were positive for *Salmonella* (Jonhston et al., 1982). Recent survey indicated that the prevalence of *Salmonella* in pork was low (Zhao et al., 2001). Of 209 samples of pork tested in Greater Washington D.C. area, only 7 or 3.3% positive for *Salmonella*. More recently, Rigney et al. evaluated *Salmonella* contamination on pork carcasses and found that 7.0% of 8,483 cooled market hog carcasses sampled between January 1998 and December 2000 yielded *Salmonella* (Rigney et al., 2004). The five most commonly identified serotypes were *S.* Derby (24.1%), *S.* Typhimurium var Copenhagen (13.8%), *S.* Johannesburg (6.5%), *S.* Infantis (5.9%), and *S.* Heidelberg (5.7%). Data on foodborne disease outbreak suggest that 6-9 percent of foodborne *Salmonella* infections are associated with pork and pork products (Bean et al., 1996; Olsen et al., 2000).

*Prevention of salmonellosis in pigs* (Fedorka-Cray, 2000)

1. Biosecurity: Changes of clothing and boots for visitors, bird and rodent control, foot-baths containing active disinfectant outside houses, limiting access to the site by visitors and lorries, etc.

2. All-in, all-out system: Farms should operate all-in, all-out policy, with adequate cleaning and disinfection after the pen is empty.
3. Feeding system: Naturally fermented feed is now being recommended for reduction of *Salmonella* infection in pigs.
4. Vaccination: Live, attenuated, orally administered *Salmonella* vaccines provide the best protection against *Salmonella* infection.

## Cattle

*Salmonella* infections are an important cause of mortality and morbidity in cattle (Wray and Davies, 2000). *Salmonella* Typhimurium and *S.* Dublin appear to be the predominant serotypes isolated from cattle. Acute salmonellosis in adult cattle is characterized by sudden onset of fever, dullness, lost of appetite and depressed milk yield. In untreated cases, the fatality rate may reach 75%, although this may be reduced to 10% by treatment (Wray and Davies, 2000).

Sick, pregnant cows may abort. Cows may abort from *S.* Dublin infection without showing clinical signs. However, field observation and experimental studies have suggested that abortion is preceded by a period of pyrexia, when the organism is multiplying in the placenta (Hall and Jones, 1977).

*Salmonella* infections in calves is characterized by fever, dullness and loss of appetite, followed by a brown scour with fluid, offensive feces, which often contain blood or mucus (Wray and Davies, 2000). Affected calves quickly loose condition and become dehydrated, weak and emaciated. Infection with *S.* Dublin is predominated by bacteremia and respiratory signs. Some calves may suffer septicemia and collapse with

no diarrhea. Affected calves show profound depression, dullness, prostration, high fever (40.5-42°C) and death within 24-48 h (Wray and Davies, 2000). The infection also commonly causes pneumonia. Jaundice is a feature in some cases and nervous signs of encephalomeningitis may be seen in other cases (Wray and Davies, 2000). Polyarthritis and osteitis have also been described, and a sequel to some cases of enteric salmonellosis is the development of dry gangrene of the extremities, including ear tips, tail tip and the limb from the fetlock down. Calves from dams infected with *S.* Dublin may be stillborn, non-viable or sickly from birth (Wray and Davies, 2000).

*Sources of infection*

Most infection is introduced into *Salmonella*-free herds by the purchase of infected cattle, either as calves for intensive rearing or adult cattle for replacement. Purchased animals may have acquired infection on their home-farm premises, in transit or on dealers' premises (Wray et al., 1990 and 1991).

Some adult cattle, which recover from *Salmonella* infection, especially in the case of *S.* Dublin, may become active carriers and excrete the organism continuously or intermittently in their feces for years (Wray and Davies, 2000). Latent carriers result in persistence of the organism on farms. Latent *S.* Dublin carriers may become active carriers or even clinical cases during stress, especially during pregnancy, when abortion may occur and the organism may be excreted in the genital discharge, urine and milk. Cattle with chronic *Salmonella* infection of the udder may shed the organism in feces and milk. Such milk may be source of infection for calves.

Other sources of infection include the persistence of *Salmonella* in cattle house after depopulation, contaminated pasture, rodents, birds and feed (Wray and Davies 2000).

*Public Health Aspects*

Subclinical *Salmonella* infections frequently occurred in cattle and thus constitute an important reservoir for human infections. *Salmonella* serotypes have been isolated from the feces of healthy beef cattle. In the US, of the 4,977 fecal samples from 100 feedlot cattle operations, 273 (5.5%) yielded *Salmonella* (Fedorka-Cray et al., 1998). The most common serotypes recovered were *S.* Anatum (27.9%), *S.* Montevideo (12.9%), *S.* Muenster (11.8%), *S.* Kentucky (8.2%), and *S.* Newington (4.3%). Of the 5,049 fecal samples from 187 cow-calf operations, 70 (1.4%) yielded *Salmonella* (Dargatz et al., 2000). The 5 most common serotypes recovered were *S.* Oranienburg (21.8%), *S.* Cerro (21.8%), *S.* Anatum (10.3%), *S.* Bredeney (9.0%), and *S.* Mbandaka (5.1%). More recently in the United States, of the 960 fecal samples from dairy cattle in four states, 93 (9.96%) yielded *Salmonella* (Callaway et al., 2005). Serotypes *S.* Montevideo and *S.* Muenster were the most frequent and widespread (Callaway et al., 2005)

Carcasses may be contaminated with *Salmonella* from the intestinal tract during slaughter. In the US, of the 50,515 analyzed ground beef samples collected from January 1998 to December 2000, 1,873 (3.7%) yielded *Salmonella* (Rigney et al., 2004). The 5 most commonly isolated serotypes were *S.* Montevideo (16.4%), *S.* Anatum (8.7%), *S.* Typhimurium var Copenhagen (6.4%), *S.* Newport (6.2%), and *S.* Seftenberg (6.2%). Of the 3,695 bull and cow carcass samples from January 1998 to December 2000, 78 (2.1%) yielded *Salmonella* (Rigney et al., 2004). The 5 most common recovered serotypes were

*S.* Muenster (12.9%), *S.* Kentucky (10.0%), *S.* Montevideo (10.0%), *S.* Newport (10.0%), and *S.* Typhimurium (10.0%) (Rigney et al., 2004).

Even though the prevalence of *Salmonella* in live cattle and beef are low, *Salmonella* outbreaks in humans have been reported to be associated with the consumption of improperly cooked and stored beef (CDC, 2006b; Dechet et al., 2006; McLaughlin et al., 2006; Roels et al., 1997)

*Salmonella* serotypes have also been isolated from feces of healthy dairy cattle (Callaway et al., 2005; Edtrington et al., 2004; Fossler et al., 2005; Hume et al., 2004; Wells et al., 2001). *Salmonella* serotypes frequently detected were *S.* Montevideo, *S.* Muenster, *S.* Newport, and *S.* Anatum. Thus infected feces from either a clinical case or healthy carrier may contaminate milk during the milking process (Wray and Davies, 2000). Several studies have shown the presence of *Salmonella* serotypes in milk (Karns et al., 2005; Van Kessel et al., 2003). Many outbreaks of human salmonellosis have been associated with drinking of raw (unpasteurized) milk or products produced from raw milk, such as cheese (CDC, 2003c; Cody et al., 1999; Maguire et al., 1992; Olsen et al., 2004; Villar et al., 1999).

Pasteurization (heat treatment) of milk has contributed to dramatic declines in many milkborne infections. However, outbreaks associated with pasteurized milk continue to occur. For example, in 1985 a massive outbreak of *S.* Typhimurium (>16,000 cases) traced to pasteurized milk occurred in Illinois (Olsen et al., 2004; Ryan et al., 1987). The outbreak was caused by contamination of milk after pasteurization.

Humans may be infected by direct contact with infected cattle. Skin lesions caused by *S.* Dublin and other serovars have been described in veterinarians following

obstetrical manipulations (Visser, 1991). On farms, where *S.* Typhimurium DT104 infection was present, possible or confirmed, associated human illness occurred in farm workers or their families (Evans and Davies, 1996). A study of human infection with *S.* Typhimurium DT104 found that 30% (9 of 23 human cases) was associated with infected cattle (Fone and Barker, 1994). In a case control study, 10% of cases of *S.* Typhimurium DT104 in humans were associated with contact with ill farm animals (Wall et al., 1994 and 1995).

*Control Measures (Wray and Davies, 2000)*

*Herd disease security measures.* A closed-herd policy should be maintained. However, if cattle are purchased or return to the farms from markets, they should be kept in isolation for 4 weeks. The quarantine building should be located far away from the resident herd, and good hygiene and disinfection procedures must be practiced. Animals should be inspected daily for signs of diarrhea or other illness, and after 3 weeks, feces may be cultured for the presence of *Salmonella.* In the case of adults, a positive serological test for *S.* Dublin or *S.* Typhimurium will indicate that the animal should be kept in isolation and its feces cultured. Effective rodent and bird control should be carried out because these animals have been shown to contaminate stored feed.

## CHAPTER THREE

# EPIDEMIOLOGY OF HUMAN INFECTIONS WITH COMMON *SALMONELLA* SEROTYPES IN MICHIGAN, 1995- 2001


## ABSTRACT

Nontyphoidal *Salmonella* serotypes are among the most common bacterial causes of foodborne gastroenteritis in the United States (US); associated with ~1.4 million human illnesses annually. Studies on trends of the serotypes and host related factors are necessary for the development of effective prevention plan for the foodborne diseases caused by these pathogens. Cases of culture-confirmed salmonellosis at the Michigan Department of Community Health (MDCH) from 1995 to 2001 were analyzed in order to determine the epidemiologic trends of human infections with the most common *Salmonella* serotypes in Michigan. A total of 6,797 cases were reported, with an average annual incidence (AAI) of 9.9/100,000. Among cases for whom information on *Salmonella* serotype were available (6,292 cases or 93%), the most common serotypes were *S.* Typhimurium (1,596 cases or 26%), followed by *S.* Enteritidis (1,309 or 22%), *S.* Heidelberg (466 or 8%) and *S.* Newport (222 or 4%). After an increasing trend from 1995 to 1998, the incidence of infections with all *Salmonella* significantly decreased by 25% (95% CI = 31% to 18% decrease), *S.* Typhimurium decreased by 39% (95% CI = 49% to 26% decrease), and *S.* Enteritidis decreased by 32% (95% CI= 44% to 18% decrease) from 1998 to 2001. The incidence of *S.* Newport significantly increased by 101% (95% CI = 25% to 225% increase) from 1998 to 2001 and *S.* Heidelberg increased by 4% (95%

CI = 25% decrease to 46% increase). The infections were more frequent in the summer months. The AAI per 100,000 populations was highest among infants for all *Salmonella* (75.0), *S.* Typhimurium (21.9), *S.* Enteritidis (14.0), *S.* Heidelberg (5.4), and *S.* Newport (1.7). Among cases for whom race was reported, African-Americans had a significantly higher age-adjusted AAI per 100,000 compared with Whites for *S.* Typhimurium (2.5 vs. 1.3; RR, 2.3, 95% CI, 1.6-3.3), *S.* Enteritidis (1.4 vs. 1.1; RR, 1.4; 95% CI, 1.1-1.6), *S.* Heidelberg (0.8 vs. 0.3; RR, 3.6; 95% CI, 2.8-4.6), and *S.* Newport (0.3 vs. 0.1; RR, 2.8; 95% CI, 1.9-4.2). Among cases for whom ethnicity was reported, Hispanics had a significantly higher age-adjusted AAI of *S.* Enteritidis compared with Non-Hispanics (1.0 vs. 0.5/100,000; RR, 1.9; 95% CI 1.2-3.0), but not different for *S.* Typhimurium, *S.* Heidelberg, and *S.* Newport. The age-adjusted AAI per 100,000 of the most common serotypes was not different significantly between residents in urban and rural counties. In conclusion, this information can be used by the state and local health departments of Michigan to guide salmonellosis prevention efforts in specific age, racial or ethnic groups, by rationalizing the allocation of appropriate public health resources and personnel. Further studies should be conducted to identify the risk factors for the high incidence of infections with the most common nontyphoidal *Salmonella* among infants, African-Americans and Hispanics, and the emergence of *S.* Newport.

## INTRODUCTION

Nontyphoidal *Salmonellae* are among the most common bacterial causes of foodborne gastroenteritis in the United States and worldwide (Altekruse et al., 1997; CDC, 2006a; Mead et al., 1999; Motarjemi and Kaferstein, 1997; Swartz, 2002; Tauxe,

1997; Todd, 1997). On a global scale, an estimated 1.3 billion cases of acute nontyphoidal gastroenteritis occurred annually, resulting in 3 million deaths (Pang et al., 1995). In the US, nontyphoidal *Salmonella* caused an estimated 1.4 million cases of foodborne illnesses annually, resulting in >100,000-physician office visits (Voetsch et al., 2004), 16,000 hospitalizations, and ~ 600 deaths (Mead et al., 1999).

Human infections with nontyphoidal *Salmonella* usually result in an acute self-limiting diarrhea that does not warrant antimicrobial therapy (Pegues et al., 2005). However, these infections can also develop into life-threatening systemic diseases including meningitis and endocarditis that require effective chemotherapy (Pegues et al., 2005). The estimated costs associated with nontyphoidal salmonellosis (NTS) in humans in the US, including the costs of medical care and lost productivity, was $ 0.5 to $2.3 billion annually (Frenzen et al., 1999).

Human NTS often resulted from the consumption of contaminated foods of animal origin such as chicken, eggs, beef, pork, turkey, milk, and cheese (Gomez et al., 1997; Olsen et al., 2000; Sanchez et al., 2002). Other less commonly identified food vehicles include fish, shellfish, fresh fruits and juice, spices, chocolate and vegetables/produce (Gomez et al., 1997; Olsen et al., 2000; Sivapalasingam et al., 2004). Other sources of human NTS infection include person-to-person transmission and contact with animals and pets (Alam et al., 2005; CDC, 2000a; CDC, 2001; CDC, 2003d; Gomez et al., 1997; Hendriksen et al., 2004; Palmer et al., 1981; Wells et al., 2004).

From 1995 to 2001, *S.* Typhimurium, *S.* Enteritidis, *S.* Newport and *S.* Heidelberg were the most common *Salmonella* serotypes in humans in the US (CDC, 2002b). Studies

on trends of the serotypes and host related factors are necessary for the development of effective prevention plan for the food borne diseases caused by these pathogens.

From 1996 to 2001, the national incidence of infection with all *Salmonella* decreased by 11% (CDC, 2002a) although large outbreaks still occur, particularly of *S.* Enteritidis (Patrick et al., 2004). The incidence of *S.* Typhimurium infection decreased 24% from 1996 to 2001 (CDC, 2002a). The incidence of *S.* Enteritidis infection increased markedly from 1980 to 1995 (Olsen et al., 2000), but has decreased by 22% from 1996 to 2001 (CDC, 2002a).

The incidence of *S.* Newport has increased by 32% from 1996 to 2001 (CDC, 2002a) to become the third most frequent serotype in the US. The incidence of *S.* Heidelberg increased by 34% from 1996 to 2001 (CDC, 2002a).

Surveillance data allow estimation of overall incidence and trends, and identify population sub-groups at risk. The objectives of this study were to determine the trends in the incidence of the most common *Salmonella* serotypes infections in Michigan from 1995 to 2001 and to identify the population subgroups at risk for the infections.

## MATERIALS AND METHODS

### Data sources: *Salmonella* cases

Culture-confirmed salmonellosis case reports from 1995 to 2001 at the Michigan Department of Community Health (MDCH), Bureau of Epidemiology were used in this study. To determine the *Salmonella* serotype for each case, the cases were linked with the database at the MDCH Laboratory, which performs complete serotyping. Cases were linked by first and last name using EpiInfo 2004 (version 3.3; Centers for Disease

Control and Prevention, Atlanta, GA). Cases remaining unlinked due to spelling errors of the first or last name were linked manually on a case-by-case basis, by considering date of birth and place of residence. The names were removed from the final database used for analysis. The final database includes patient's address, age, gender, race, ethnicity, event date, and *Salmonella* serotype. Use of this data was approved by all relevant Community Research Institutional Review Boards (CRIRB) at the Michigan State University and MDCH.

**Data analysis**

Age-adjusted annual incidence rates (cases per 100,000 population) of infection due to all *Salmonella* and the most common serotypes were calculated based on the appropriate population estimates of Michigan from1995 to 2001 (NCHS). The United States year 2000 standard million population was used for age standardization (Anderson and Rosenberg, 1998).

Poisson regression analysis was used to estimate change in incidence rates from 1995 to 2001 and 1998 to 2001 (CDC, 2004c; Hardnett et al., 2004; Kleinbaum et al., 1998). By using this analysis, the year-specific relative rates were estimated, with the number of cases as the dependent variable, the population as the offset variable, and year and county as independent variable. The estimated change in incidence (relative rate) between 1995 and 2001, and 1998 to 2001 were calculated, along with a 95% confidence interval (CI). The analysis was conducted by using Proc Genmod in SAS (version 8.0; SAS Institute Inc., Cary, NC) (SAS Institute Inc.).

All cases were used to examine age, gender, and type of county of resident differences (N = 6,797 cases for all *Salmonella*; N = 1,598 for *S.* Typhimurium, N = 1,309 for *S.* Enteritidis; N = 466 for *S.* Heidelberg; and N = 222 for *S.* Newport). To examine racial differences, only cases who have information on race (White, African-American, Asian/Pacific Islander, or Native American) were used (n = 3,982 [59%] for all *Salmonella*; n = 925 [58%] for *S.* Typhimurium; n = 771 [59%] for *S.* Enteritidis; n = 254 [55%] for *S.* Heidelberg; and n = 114 [51%] for *S.* Newport. To examine ethnicity differences, only cases who have information on ethnicity (Hispanic or Non-Hispanic) were used (n = 1,850 [27%] for all *Salmonella*; n = 418 [26%] for *S.* Typhimurium; n = 364 [28%] for *S.* Enteritidis; and n = 146 [31%] for *S.* Heidelberg.

To determine if cases included in racial and ethnicity analysis are systematically different from those who were excluded, a comparison was made between cases with information on race and ethnicity, and cases without those information by age, gender and type of county.

To examine age differences, average annual incidences (AAI) were calculated for the following age categories: <1, 1-4, 5-9, 10-19, 20-29, 30-39, 40-49, 50-59, and ≥60. Age-standardized AAI were calculated to study gender, racial (White, African-American, Native-American, and Asian/Pacific Islander); ethnicity (Hispanics and Non-Hispanics); and county of residence (urban or rural counties) differences. The counties were grouped into urban or rural based on US Census Bureau's definitions i.e. urban county is any county containing a city of greater than 50,000 people or an area that has at least 100,000 people and has a substantial commuting interchange with a city of greater than 50,000 people (MDCH, Appendix A).

Adjusted relative rates (RR) with 95% confidence interval (CI) were determined by using Poisson regression analysis, with the number of cases as the dependent variable, the population as the offset variable, and age, gender, race, ethnicity and type of county as the independent variable.

## RESULTS

From 1995 to 2001, there were 6,797 culture-confirmed salmonellosis cases reported to MDCH, Bureau of Epidemiology; the average annual incidence was 9.9/100,000; and 93% had a known serotype. Among cases for whom information on *Salmonella* serotype were available (6,292 cases or 93%), the most common serotypes were *S.* Typhimurium (1,596 cases or 26%), followed by *S.* Enteritidis (1,309 or 22%), *S.* Heidelberg (466 or 8%) and *S.* Newport (222 or 4%) (Table1). These serotypes accounted for 57% of the total cases with known serotype.

*Temporal trends.* After an increasing trend from 1995 to 1998, the incidence of infection with all *Salmonella* significantly decreased by 25% (95% CI = 31% to 18% decrease), *S.* Typhimurium decreased by 39% (95% CI = 49% to 26% decrease), and *S.* Enteritidis decreased by 32% (95% CI = 44% to 18% decrease) from 1998 to 2001 (Figure 3-1). The incidence of *S.* Newport significantly increased by 101% (95% CI = 25% to 225% increase) from 1998 to 2001, where as *S.* Heidelberg increased by 4% (95% CI = 25% decrease to 46% increase).

*Seasonality.* Overall, most number of *Salmonella* cases occurred between May and September with the peak in July (Figure 3-2). This general pattern was consistent for the four most common serotypes.

*Place*. The age-adjusted AAI of infection with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport was not different significantly between residents in urban and rural counties (Table 3-4, 3-5, 3-6, 3-7).

*Age*. Information on patient age was available for 99% of reported cases. Average annual incidence of infection with all *Salmonella* serotypes was highest among infants aged <1 year (75.0/100,000) (Table 3-3). Incidence decreased abruptly after infancy, remained relatively constant through the adult years, and increased slightly among persons 60+ years old. Similar pattern of age-specific incidences was noted for infections with *S.*Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport (Table 3-4, 3-5, 3-6, 3-7).

*Gender.* Age-adjusted AAI of infection with all *Salmonella* serotypes was marginally significantly higher among females than among males (10.1 vs. 9.3/100,000; RR, 1.08; 95% CI, 1.03-1.13) (Table 3-3). Age-adjusted AAI for *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport was not different significantly between females and males (Table 3-4, 3-5, 3-6, 3-7).

*Race*. There was no significant difference between *Salmonella* cases with and without information on race for most age groups except for persons aged 20-29 and 30-39 years (Table 3-2). Information on patient race was available for 59% of reported cases. Among cases whose race was known, African-Americans had a significantly higher age-adjusted AAI per 100,000 populations than Whites for infection with all *Salmonella* (6.8 vs. 5.6; RR, 1.30; 95% CI, 1.20-1.41), *S.* Typhimurium (2.5 vs. 1.3; RR, 2.27; 95% CI, 1.98-2.61), *S.* Enteritidis (1.4 vs. 1.1; RR, 1.35; 95% CI, 1.13-1.62), *S.* Heidelberg (0.8 vs. 0.3; RR, 3.56; 95% CI, 2.77-4.58) and *S.* Newport (0.3 vs. 0.1; RR, 2.83; 95% CI,

1.92-4.18) (Table 3-3, 3-4, 3-5, 3-6, and 3-7). For Native-Americans, and Asian/ Pacific Islanders the age-adjusted AAI were significantly lower than Whites (Table 3-7).

*Ethnicity.* Information on ethnicity was available for only 27% of reported cases. Among cases whose ethnicity was known, Hispanics had a significantly higher age-adjusted AAI than Non-Hispanics for infection with all *Salmonella* serotypes (3.5 vs. 2.7/100,000; RR, 1.30; 95% CI, 1.03-1.65) and *S.* Enteritidis (1.0 vs. 0.5/100,000; RR, 1.9; 95% CI, 1.21-2.98) (Table 3-3, 3-5). For *S.* Typhimurium and *S.* Heidelberg, the age-adjusted AAI was not different significantly between Hispanics and Non-Hispanics (Table 3-4, 3-6).

## DISCUSSION

In this study, 6,797 cases of human infections with *Salmonella* serotypes were reported to the MDCH from 1995 to 2001 with an average of 971 cases annually and AAI of 9.8/100,000 populations. Compared to the national surveillance data, the overall incidence rate in Michigan during the study period was 36% lower (9.8 vs. 15.4/100,000) (CDC 2003a).

Similar to other reports (Olsen et al., 2001; EU, 2003; WHO, 2000; WHO, 2003), the most common *Salmonella* serotypes in this study were *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport. The involvement of these serotypes in human infections is to be expected because *S.* Typhimurium is widespread in food animals and environment, *S.* Enteritidis and *S.* Heidelberg have chicken as the primary reservoir, and the main reservoir of *S.* Newport is cattle (CDC, 2002b; Ferris et al., 2000; van Duijkeren et al., 2002; Wray and Wray, 2000). Thus, human infections with these serotypes are usually

foodborne and associated with undercooked beef, chicken, eggs, and unpasteurized milk (CDC, Foodborne Outbreak Response and Surveillance Unit; Bean et al., 1996; Olsen et al., 2000; Sanchez et al., 2002).

In this study, the incidence of infections with all *Salmonella*, *S.* Typhimurium and *S.* Enteritidis decreased significantly from 1998 to 2001. The decline in the incidence may suggest that an important progress towards the reduction of salmonellosis to achieve the national health objectives for salmonellosis, 6.8/100,000 populations (CDC 2002a; USDHHS, 2003) are being made. The decline in the incidence rates are unlikely due to changes in surveillance because no modifications for the diagnostic criteria for salmonellosis were made and only passive surveillance was conducted throughout the study period.

The decline in the incidence of all *Salmonella*, *S.* Typhimurium and *S.* Enteritidis in this study is consistent with the trend of salmonellosis in the US. For example, in the FoodNet sites, the incidence of all *Salmonella*, *S.* Typhimurium and *S.* Enteritidis decreased by 15%, 24%, and 22% respectively from 1996 to 2001 (CDC, 2002a).

The exact reasons for the decreased in the incidence rates in Michigan are not clear. However, several reports have suggested that the nationwide decrease in the incidence of many *Salmonella* serotypes, including *S.* Typhimurium and *S.* Enteritidis in the US may be partly attributed to several control measures including the implementation of the Pathogen Reduction/Hazard Analysis Critical Control Point (HACCP) systems regulations in meat and poultry slaughter and processing plants by US Department of Agriculture's (USDA) Food Safety Inspection Service (FSIS) on July 25, 1996 (CDC, 2002a; USDA: FSIS). Additional interventions that have been introduced that may have

resulted in decreased of the incidence rates include egg-quality assurance programs for *S.* Enteritidis, increased attention to fresh produce safety through better agricultural practices, regulation of fruit and vegetable juice, industry efforts including new intervention technologies to reduce food contamination, and public health food safety education such as FIGHT BAC targeting consumers and food handlers (CDC, 2002a; FIGHT BAC). The availability of produce and sprout safety guidance in 1998 and 1999 (FDA, 1998; FDA, 1999) may also have resulted in the decrease in the incidence rates.

In Michigan, the decline in the incidence rates may have occurred in the context of availability of food safety programs and guidelines at the Michigan Department of Agriculture (MDA) and MDCH such as protecting food as it grows, protecting food at the processing plants and the stores, protecting food at home, protecting food throughout the food chain, protecting food as it leaves the farm gate, and food safety training for food prepares and servers (MDA). The decline in the incidence in Michigan may have also occurred in the context of enhancement of pathogen management in the farms such as egg, poultry meat, pork, beef, and dairy quality assurance program and enhancement of good management practices in food service establishments.

Even though the incidence of all *Salmonella* decreased significantly from 1998 to 2001, the incidence of *S.* Newport increased significantly during this period. This increase is consistent with the trend of *S.* Newport infection in the US. The number of *S.* Newport isolates reported to Center for Disease Control and Prevention (CDC) increased from 2,566 in 1995 to 3,158 in 2001 (CDC, 2002b). In the FoodNet sites, the incidence of *S.* Newport increased by 32% from 1996 to 2001 (CDC, 2002a). The reasons for the significant increase in the incidence of *S.* Newport from 1998 to 2001 in Michigan are not

known. In the US, increased in the incidence of *S.* Newport was due to the emergence of multidrug-resistant *S.* Newport (CDC, 2002c, Gupta et al., 2003). The increase in the incidence of infection with *S.* Newport in Michigan should be further investigated for the possible risk factors and for the presence of multidrug- resistant strains. This is important because persons infection with multidrug-resistant strains of *Salmonella*, such as *S.* Typhimurium DT104, have worse outcomes than those infected with antimicrobial-susceptible strains (Helms et al., 2002; Martin et al., 2004; Varma et al., 2005).

Even though the incidence of *S.* Heidelberg remained stable, the public health importance of this serotype cannot be underestimated. This is because eating eggs and frozen processed chicken nuggets and strips were associated with sporadic *S.* Heidelberg infections (Hennessy et al., 2004; MacDoughall et al., 2004). A more recent report revealed that most common food vehicles implicated in *S.* Heidelberg outbreaks in the United States were poultry, eggs, egg-containing food item, eggs and poultry, pork and beef (Chittick et al., 2006). In Canada, frozen chicken nuggets and strips and eggs are the leading risk factors of *S.* Heidelberg infections (Currie et al., 2005).

Similar to other studies (Olsen et al., 2001; Schutze et al., 1995. Trevejo et al., 2003), infections with all *Salmonella* and the most common serotypes in this study occurred more frequently in the summer months. This may be due to increase in outside activities where more people are cooking foods of animal origin at picnics, barbecues, and on camping trips where safety control that kitchen usually provides such as thermostat-controlled cooking, refrigeration, and washing facilities are usually not available (Olsen et al., 2001). Furthermore, humid and hot conditions in summer favor the rapid growth of *Salmonella* in foods, which were not kept refrigerated. Therefore,

people should handle and cook foods of animal origin properly during picnic in the summer months. For example, beef, chicken, eggs and other perishable foods should be kept in coolers containing ice or cold packs. Beef, chicken, and hamburgers should be grilled until they are well done and the juices run clear when they are poked with a fork (MDA).

The high incidence of human salmonellosis during summer may also be due to increase in the number of flies in the environment. In the warmer weather, the flies begin to increase in numbers rapidly as their generation time within manure, foods, water and decaying materials becomes increasingly shorter (Lysyk, 1993). Flies are known to be important vector for rapid, widespread dissemination of *Salmonella* in the environment (Davies and Wray, 1996; Winfield and Groisman, 2003). *Salmonella* survives in flies for up to 4 weeks, which is the life span of the flies (Mian et al., 2002). Thus flies that come in contact with contaminated materials (i.e., manure, food, and water) are capable of transmitting *Salmonella* to human foods during outside activities during the summer (Mian et al., 2002).

This study shows that the age-standardized AAI of *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport did not differ significantly between residents in urban and rural counties. These findings suggest similar levels of exposure to potential sources of these serotypes such as eggs, poultry, beef, pork, and vegetable consumption.

The incidence of infections with all *Salmonella* and the most common serotypes in this study were highest among infants aged <1 year. Similar findings were reported in other studies (Olsen et al., 2001; Schutze et al., 1995; Trevejo et al., 2003; Voetsch et al., 2004). Besides the immature immune system, other reasons for the high incidence among

infants in Michigan are largely unknown. Olsen et al. suggested that the reasons for the high incidence among infants aged <1 year may include host susceptibility and exposure differences (Olsen et al., 2001).

Besides immature immune systems (Buzby et al., 2001; Gomez et al., 1998; Olsen et al., 2001), intrafamilial transmission and contaminated home environment have been emphasized as important risk factors for infant salmonellosis (Delarocque-Astagneau et al., 2000; Schutze et al., 1999; Wilson et al., 1982). However, other vehicles of *Salmonella* have also been implicated such as pet reptiles, contaminated infant formula, and solid foods that may contain undercooked meat or eggs (CDC, 2003d; Delarocque-Astagneau et al., 2000; Espie et al., 2005; Park et al., 2004). Infants' behavior of putting things in their mouth and low level of socioeconomic status of their parents such as poverty and low education level may also have contributed to the high incidence of infant salmonellosis. Low education level among the parents may lead to less exposure to information on prevention of foodborne diseases.

The high incidence may also be due to detection bias because of a greater likelihood that sick infants would more likely be brought to see the physicians and who order laboratory work for confirmation than cases among adults. The finding that infants have a 3–10-fold higher rate of salmonellosis than do other age groups in this study highlights the need for studies to determine the risk factors so that appropriate preventive measures can be formulated.

Only 59% of the cases in this study provided information on race. Among cases whose race was known, the incidence of all *Salmonella* was significantly higher among African-Americans than whites. The incidence of infection with *S.* Typhimurium, *S.*

Enteritidis, *S*, Heidelberg, and *S*. Newport, were also significantly higher among African-Americans compared with whites. An analysis of FoodNet data for the year 1998 to 2001 also demonstrated a higher incidence of *S*. Enteritidis among African-Americans (Marcus et al., 2002). Outbreaks of these serotypes were frequently associated with eating chicken, beef, eggs, and pork (CDC, Foodborne Outbreak Response and Surveillance Unit). This is because the primary reservoirs of these serotypes are chicken, cattle, and pigs (Ferris et al., 2000; CDC, 2002b). The USDA Continuing Survey of Food Intakes by Individuals (CSFII) in 1994 to 1996 showed that African-Americans eat chicken and ground beef more frequently than other ethnic groups (USDA: ARS). African-Americans also eat eggs more frequently than the US overall population.

We speculate that the higher incidence of infection with these serotypes among African-Americans in Michigan may be due to high-risk food consumption, handling and preparation practices such as eating undercooked meat and eggs, inadequate hygienic practices such as not washing hands with soap after handling raw meat and shell eggs, and not washing cutting board with bleach after using it for cutting raw meat. The high incidence may be also due to different in socioeconomic status such as poverty and level of education. Less education, for instance, may lead to less access or understanding to information on food safety such as proper handling and preparing high-risk foods for foodborne diseases.

The finding that the incidence was higher among African-Americans compared to whites in this study should be interpreted with caution because only 59 % of the cases have information on race. However, the results indicate the magnitude of salmonellosis

problem among African-Americans in Michigan and further studies should be conducted to determine the actual risk factors.

Only 27% of the cases in this study have information on ethnicity. Among cases whose ethnicity was known, the incidence of infection with all *Salmonella* among Hispanics was marginally significantly higher than Non-Hispanics. However, the incidence of *S.* Enteritidis among Hispanics was significantly higher than Non-Hispanics. It has been reported that Hispanics frequently eat high-risk foods for foodborne diseases, such as undercooked eggs, uncooked fruits and vegetables, and unpasteurized juices and dairy products (Banerjee et al., 2002). An analysis of FoodNet data for 2000-2001 demonstrated that Hispanics were more likely than Non-Hispanics to eat sprouts, parsley, cilantro, mangoes, yogurt, soft ice cream, unpasteurized milk, runny eggs, and oysters (Banerjee et al., 2002). The CSFII in 1994 and 1996 showed that Hispanics consumed more eggs than other ethnic groups (USDA: ARS). We speculate that the high incidence of *S.* Enteritidis among Hispanics in Michigan may be due to frequent consumption of undercooked eggs or foods containing undercooked eggs such as mayonnaise, hollandaise sauce, ice creams, and desserts. Since only 27% of the cases in this study have information on ethnicity, the significantly higher incidence of *S.* Enteritidis in Hispanics in this study should be interpreted with caution. However, the results indicate the magnitude of the *S.* Enteritidis problem among Hispanics in Michigan.

This study shows that the incidence of invasive salmonellosis in Michigan was highest among infants aged <1 year old. This finding is a public health concern because invasive salmonellosis can results in meningitis, osteomyelitis, endocarditis, arthritis, urinary-tract infection and pneumonia (Pegues et al., 2005). The reasons for the high

incidence among infants in Michigan are not known. However, other studies suggested that immunocompromised and predisposing clinical conditions (e.g., hematological malignancy or sickle-cell hemoglobinopathy) as the possible risk factors (Yang et al., 2002).

The main limitation of this study is that it was based on passive surveillance data. Although most culture-confirmed cases are reported to the MDCH, this surveillance system unavoidable underestimates the actual incidence (Voetsch et al., 2004). To be identified as a *Salmonella* case, a person must be symptomatic that is sever enough, consult a physician, and provide a clinical specimen in order to be categorized as laboratory-confirmed *Salmonella* case. To be counted as a case, the physicians or laboratory must report the case to the local health department. The degree of underreporting of salmonellosis has been estimated to be from 19- to 38-fold (Chalker and Blaser, 1988; Mead et al., 1999; Voetch et al., 2004). This is not unexpected because it has been estimated that 50 percent of persons with intestinal infectious diseases would neither consult a physician nor experience a full day of restricted activity (Garthright et al., 1988).

Missing information on race and ethnicity is another limitation in this study. Because race and ethnicity was reported for only 59% and 27 % respectively of reported *Salmonella* cases, these estimated race- and ethnicity-specific rates should be interpreted with caution. The surveillance data set contained both sporadic and outbreak cases. Therefore large outbreak may lead to certain demographic characteristics to be more represented.

In conclusion, this study shows that *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport were the most common *Salmonella* serotypes that caused human NTS in Michigan from 1995 to 2001. The incidence of *S.* Typhimurium and *S.* Enteritidis significantly decreased from 1998 to 2001, where as the incidence of *S.* Newport significantly increased. The incidence of infections with the most common serotypes were highest among infants aged <1 year. Among cases whose race was known, the incidence of the most common serotypes was significantly higher among African-Americans. Among cases whose ethnicity was known, the incidence of *S.* Enteritidis was significantly higher among Hispanics. The incidence of invasive salmonellosis was highest among infants.

Despites the limitations of the data, information from this study can be used by the state and local health departments of Michigan to focus salmonellosis prevention efforts among infants, African-Americans, and Hispanics by rationalizing the allocation of appropriate public health resources and personnel. Further studies should be conducted to determine the risk factors for the emergence of *S.* Newport and the high incidence of *Salmonella* serotypes among infants, African-Americans, and Hispanics in order to enhance the prevention efforts.

Table 3-1. The 15 most common *Salmonella* serotypes causing human salmonellosis in Michigan, 1995 – 2001

| Rank of total | *Salmonella* serotype | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | Total | % |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Typhimurium | 188 | 259 | 232 | 311 | 221 | 200 | 185 | 1596 | 25 |
| 2 | Enteritidis | 158 | 161 | 202 | 247 | 184 | 189 | 168 | 1309 | 21 |
| 3 | Heidelberg | 65 | 80 | 55 | 67 | 56 | 72 | 71 | 466 | 7 |
| 4 | Newport | 30 | 32 | 18 | 25 | 34 | 32 | 51 | 222 | 4 |
| 5 | Java | 24 | 31 | 13 | 27 | 21 | 33 | 29 | 178 | 3 |
| 6 | Thompson | 17 | 23 | 38 | 31 | 34 | 19 | 16 | 178 | 3 |
| 7 | Oranienburg | 30 | 23 | 17 | 45 | 23 | 18 | 18 | 174 | 3 |
| 8 | Agona | 29 | 16 | 37 | 40 | 14 | 10 | 11 | 157 | 3 |
| 9 | Muenchen | 19 | 13 | 17 | 13 | 50 | 5 | 13 | 130 | 2 |
| 10 | Braenderup | 12 | 14 | 11 | 8 | 10 | 58 | 6 | 119 | 2 |
| 11 | Saintpaul | 18 | 19 | 6 | 13 | 13 | 9 | 26 | 104 | 2 |
| 12 | Infantis | 14 | 9 | 11 | 12 | 29 | 18 | 8 | 101 | 2 |
| 13 | Montevideo | 11 | 14 | 8 | 18 | 21 | 18 | 9 | 99 | 2 |
| 14 | Stanley | 55 | 9 | 4 | 14 | 5 | 5 | 4 | 96 | 2 |
| 15 | Javiana | 18 | 7 | 12 | 13 | 15 | 7 | 9 | 81 | 1 |
| | Other serotypes | 161 | 206 | 145 | 178 | 139 | 122 | 123 | 1074 | 17 |
| | Unknown | 14 | 21 | 20 | 35 | 29 | 31 | 58 | 208 | 3 |
| | Total | 863 | 937 | 846 | 1097 | 898 | 846 | 805 | 6,292 | |

Table 3-2. Number of cases of all *Salmonella* (N = 6,797): comparison between cases with and without information on race by age, gender and type of county

| Demographic characteristics | Cases with race n = 3,982 (59%) | % | Cases without race n = 2,815 (41%) | % | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| Age (Years) | 3,957 | 58 | 2,632 | 39 | 191.75 | <0.001 |
| Gender | 3,982 | 59 | 2,815 | 41 | 0.06 | 0.81 |
| Type county | 3,982 | 59 | 2,815 | 41 | 0.06 | 0.81 |

Type of county: urban vs. rural

**Note:** To determine the $\chi^2$, one was added to cases without gender and without type of county for cases with and without information on race

Table 3-3. Average annual incidence of human infection with all *Salmonella* serotypes by age, gender, race, ethnicity and type of county of residence, Michigan 1995-2001

| Variables | No. cases | AAI (per 100,000) | Adjusted RR (95% CI) |
|---|---|---|---|
| Total | 6,797 | 9.9 | - |
| Age | | | |
| <1 | 690 | 75.0 | 10.57 (9.51-11.74)* |
| 1-4 | 842 | 22.1 | 3.14 (2.84-3.47)* |
| 5-9 | 534 | 10.3 | 1.49 (1.33-1.67)* |
| 10-19 | 688 | 6.8 | Referent |
| 20-29 | 880 | 9.5 | 1.32 (1.20-1.46)* |
| 30-39 | 853 | 7.9 | 1.14 (1.03-1.26)* |
| 40-49 | 743 | 7.1 | 1.02 (0.92-1.13) |
| 50-59 | 534 | 7.3 | 1.07 (0.95-1.20) |
| 60+ | 974 | 8.8 | 1.26 (1.14-1.39)* |
| Gender | | | |
| Male | 3,178 | 9.3 | Referent |
| Female | 3,567 | 10.1 | 1.08 (1.03-1.13)* |
| Race | | | |
| White | 3,207 | 5.6 | Referent |
| African-American | 737 | 6.8 | 1.30 (1.20-1.41)* |
| Native-American | 13 | 2.5 | 0.48 (0.28-0.82) |
| Asian/Pacific Islander | 25 | 1.6 | 0.37(0.25-0.54) |
| Other races | 54 | - | - |
| Not stated | 2,761 | - | - |
| Ethnicity | | | |
| Hispanic | 71 | 2.9 | 1.30 (1.03-1.65)* |
| Not Hispanic | 1,779 | 2.7 | Referent |
| Not stated | 4,947 | - | - |
| Type of county | | | |
| Urban | 5,436 | 9.8 | 1.05 (0.99-1.11) |
| Rural | 1,359 | 9.5 | Referent |

AAI: Average annual incidence
AAI for gender, race, ethnicity, and type of county were age-standardized
RR: Relative rate (Adjusted for all variables in Poisson regression analysis).
* Denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites and Hispanics compared with Non-Hispanics should be interpreted with caution because only 59% and 27% of the cases have information on race and ethnicity respectively.

Table 3-4. Average annual incidence of human infection with *Salmonella* Typhimurium by age, gender, race, ethnicity and type of county of residence, Michigan 1995-2001

| Variables | No. cases | AAI (per 100,000) | Adjusted RR (95% CI) |
|---|---|---|---|
| Total | 1,598 | 2.3 | - |
| Age | | | |
| <1 | 165 | 17.9 | 12.99 (10.37-16.29)* |
| 1-4 | 271 | 7.1 | 5.14 (4.22-6.27)* |
| 5-9 | 195 | 3.8 | 2.72 (2.20-3.37)* |
| 10-19 | 179 | 1.8 | 1.29 (1.04-1.60)* |
| 20-29 | 191 | 2.1 | 1.50 (1.21-1.85)* |
| 30-39 | 162 | 1.5 | 1.09 (0.87-1.36) |
| 40-49 | 154 | 1.5 | 1.06 (0.85-1.33) |
| 50-59 | 113 | 1.5 | 1.12 (0.88-1.43) |
| 60+ | 153 | 1.4 | Referent |
| Gender | | | |
| Male | 747 | 2.2 | Referent |
| Female | 833 | 2.3 | 1.07 (0.82-1.38) |
| Race | | | |
| White | 760 | 1.3 | Referent |
| African-American | 154 | 2.5 | 2.30 (1.60-3.31)* |
| Native-American | 4 | 0.7 | 1.16 (0.16-8.32) |
| Asian/Pacific Islander | 7 | 0.3 | 0.46 (0.06-3.33) |
| Other races | 8 | - | - |
| Not stated | 663 | - | - |
| Ethnicity | | | |
| Hispanic | 19 | 0.8 | 1.55 (0.98-2.46) |
| Not Hispanic | 399 | 0.6 | Referent |
| Not stated | 991 | - | - |
| Type of county | | | |
| Urban | 1,284 | 2.3 | 1.09 (0.79-1.52) |
| Rural | 312 | 2.1 | Referent |

AAI: Average annual incidence
AAI for gender, race, ethnicity, and type of county were age-standardized
RR: Relative rate (Adjusted for all variables in Poisson regression analysis).
* denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 59% of the cases have information on race.

Table 3-5. Average annual incidence of human infection with *Salmonella* Enteritidis by age, gender, race, ethnicity and type of county of residence, Michigan 1995-2001

| Variables | No. cases | AAI (per 100,000) | Adjusted RR (95% CI) |
|---|---|---|---|
| Total | 1,309 | 1.9 | - |
| Age | | | |
| <1 | 131 | 14.2 | 11.86 (9.26-15.18)* |
| 1-4 | 188 | 4.9 | 4.10 (3.26-5.15)* |
| 5-9 | 101 | 1.9 | 1.62 (1.25-2.11)* |
| 10-19 | 121 | 1.2 | Referent |
| 20-29 | 176 | 1.9 | 1.58 (1.26-2.00)* |
| 30-39 | 141 | 1.3 | 1.09 (0.85-1.39) |
| 40-49 | 143 | 1.4 | 1.13 (0.89-1.44) |
| 50-59 | 109 | 1.5 | 1.24 (0.96-1.61) |
| 60+ | 187 | 1.7 | 1.40 (1.12-1.77)* |
| Gender | | | |
| Male | 619 | 1.8 | Referent |
| Female | 684 | 1.9 | 1.07 (0.80-1.42) |
| Race | | | |
| White | 617 | 1.1 | Referent |
| African-American | 147 | 1.4 | 1.35 (1.13-1.62)* |
| Native-American | 2 | 0.2 | 0.38 (0.09-1.52) |
| Asian/Pacific Islander | 5 | 0.1 | 0.38 (0.16-0.92) |
| Other races | 10 | - | - |
| Not stated | 528 | - | - |
| Ethnicity | | | |
| Hispanic | 20 | 1.0 | 1.90 (1.21-2.98)* |
| Not Hispanic | 344 | 0.5 | Referent |
| Not stated | 945 | | |
| Type of county | | | |
| Urban | 1034 | 1.9 | 1.00 (0.70-1.42) |
| Rural* | 276 | 1.9 | Referent |

AAI: Average annual incidence
AAI for gender, race, ethnicity, and type of county were age-standardized
RR: Relative rate (Adjusted for all variables in Poisson regression analysis).
* denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites and Hispanics compared with Non-Hispanics should be interpreted with caution because only 60% and 28% of the cases have information on race and ethnicity respectively.

Table 3-6. Average annual incidence of human infection with *Salmonella* Heidelberg by age, gender, race, ethnicity and type of county of residence, Michigan 1995-2001

| Variables | No. cases | AAI (per 100,000) | Adjusted RR (95% CI) |
|---|---|---|---|
| Total | 466 | 0.7 | - |
| Age | | | |
| <1 | 51 | 5.5 | 12.70 (8.55-18.88)* |
| 1-4 | 62 | 1.6 | 3.72 (2.55-5.44)* |
| 5-9 | 39 | 0.8 | 1.72 (1.13-2.64)* |
| 10-19 | 60 | 0.6 | 1.36 (0.93-2.00) |
| 20-29 | 65 | 0.7 | 1.61 (1.11-2.34)* |
| 30-39 | 47 | 0.4 | Referent |
| 40-49 | 53 | 0.5 | 1.15 (0.78-1.71) |
| 50-59 | 36 | 0.5 | 1.13 (0.73-1.74) |
| 60+ | 49 | 0.4 | 1.01 (0.68-1.51) |
| Gender | | | |
| Male | 205 | 0.6 | Referent |
| Female | 258 | 0.7 | 1.22 (0.75-1.99) |
| Race | | | |
| White | 156 | 0.3 | Referent |
| African-American | 98 | 1.0 | 3.61 (1.85-7.05)* |
| Native-American | 0 | 0.0 | - |
| Asian/Pacific Islander | 0 | 0.0 | - |
| Other races | 5 | - | - |
| Not stated | 196 | - | - |
| Ethnicity | | | |
| Hispanic | 4 | 0.2 | 0.92 (0.34-2.48) |
| Not Hispanic | 142 | 0.2 | Referent |
| Not stated | 271 | - | - |
| Type of county | | | |
| Urban | 388 | 0.7 | 1.31 (0.69-2.51) |
| Rural | 77 | 0.5 | Referent |

AAI: Average annual incidence
AAI for gender, race, ethnicity, and type of county were age-standardized
RR: Relative rate (Adjusted for all variables in Poisson regression analysis).
*denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 58% of the cases have information on race.

Table 3-7. Average annual incidence of human infection with *Salmonella* Newport by age, gender, race, ethnicity and type of county of residence, Michigan 1995-2001

| Variables | No. cases | AAI (per 100,000) | Adjusted RR (95% CI) |
|---|---|---|---|
| Total | 222 | 0.3 | - |
| Age | | | |
| <1 | 16 | 1.7 | 8.51 (4.44-16.30)* |
| 1-4 | 15 | 0.4 | 1.94 (1.00-3.77) |
| 5-9 | 17 | 0.3 | 2.68 (1.41-5.08)* |
| 10-19 | 19 | 0.2 | 0.96 (0.52-1.78) |
| 20-29 | 29 | 0.3 | 1.52 (0.86-2.66)* |
| 30-39 | 45 | 0.4 | 2.08 (1.24-3.49)* |
| 40-49 | 21 | 0.2 | Referent |
| 50-59 | 21 | 0.3 | 1.46 (0.80-2.67) |
| 60+ | 41 | 0.4 | 1.84 (1.09-3.12)* |
| Gender | | | |
| Male | 97 | 0.29 | Referent |
| Female | 124 | 0.35 | 1.23 (0.61-2.48) |
| Race | | | |
| White | 76 | 0.1 | Referent |
| African-American | 38 | 0.4 | 2.83 (1.92-4.18) |
| Native-American | 0 | 0.0 | - |
| Asian/Pacific Islander | 0 | 0.0 | - |
| Other races | 2 | - | - |
| Not stated | 106 | - | - |
| Ethnicity | | | |
| Hispanic | 0 | 0.00 | - |
| Not Hispanic | 41 | 0.06 | Referent |
| Not stated | 143 | - | - |
| Type of county | | | |
| Urban | 36 | 0.7 | 0.71 (0.27-1.83) |
| Rural | 186 | 0.5 | Referent |

AAI: Average annual incidence
AAI for gender, race, ethnicity, and type of county were age-standardized
RR: Relative rate (Adjusted for all variables in Poisson regression analysis).
* denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 52% of the cases have information on race.

Figure 3-1. Relative rates compared with 1995 of infection with all *Salmonella* and the four most common serotypes, Michigan, 1995-2001

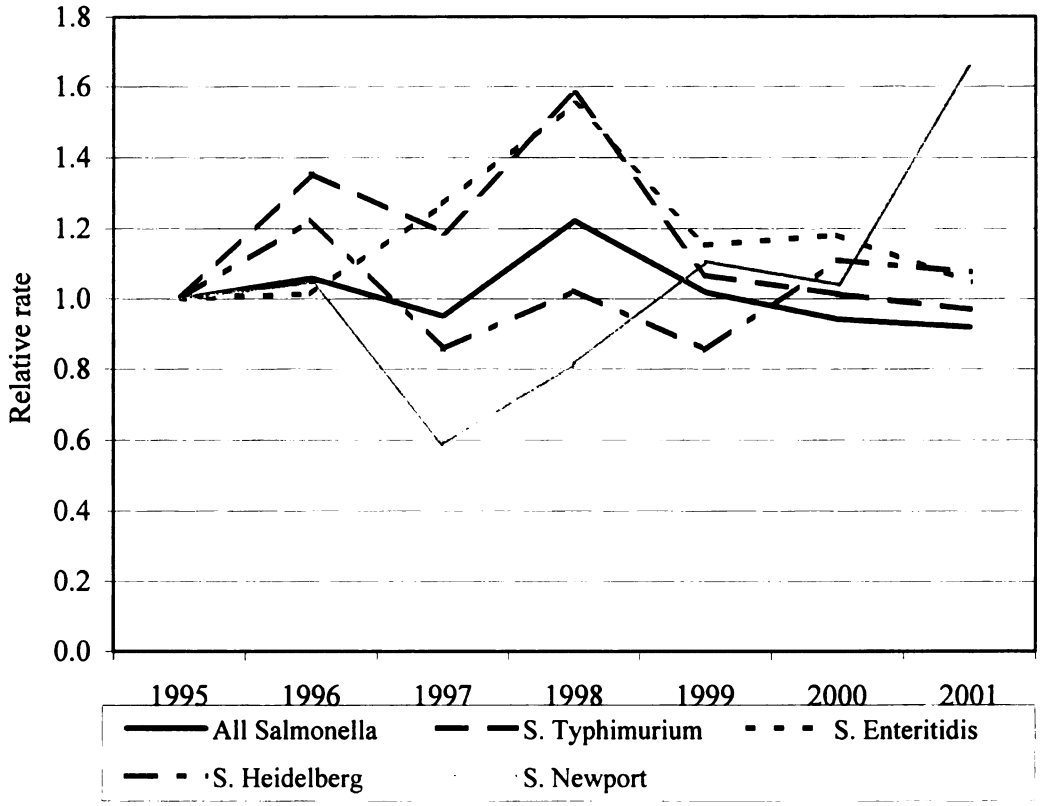
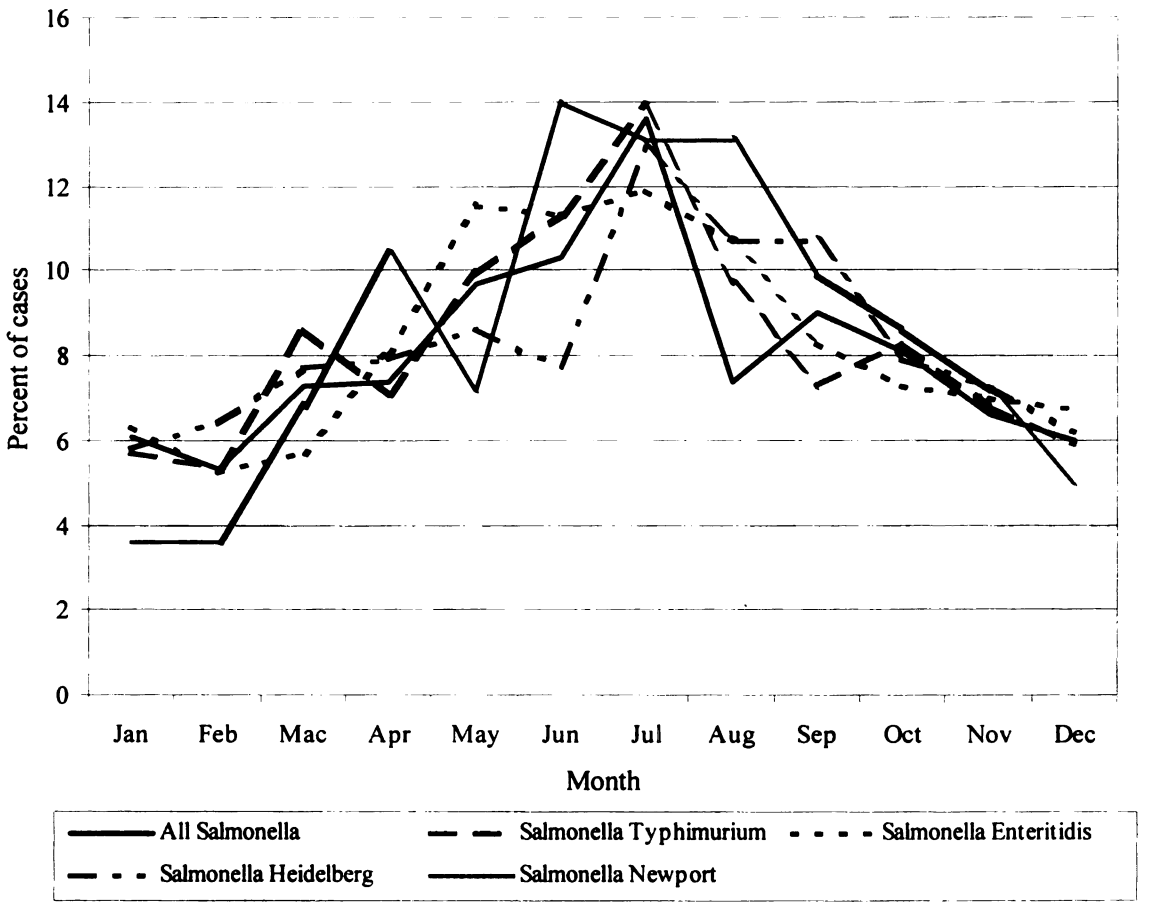


Figure 3-2. Percentage of cases of infections with all *Salmonella*, *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Heidelberg, and *Salmonella* Newport by month, Michigan, 1995-2001



## CHAPTER FOUR

# RACIAL AND ETHNIC DIFFERENCES IN INFECTIONS WITH COMMON *SALMONELLA* SEROTYPES IN MICHIGAN, 1995- 2001


## ABSTRACT

The study presented in Chapter Three showed that African-American-White differences in infection with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport, and Hispanic-Non-Hispanic differences in *S.* Enteritidis exist in Michigan. The objective of this study was to determine the interracial and interethnic incidence patterns of infections with these serotypes by age, gender, type of county of residence (urban or rural), and month of occurrence; that are necessary for understanding the causes of the variation. All cases of *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport that have information on race, and all cases of *S.* Enteritidis that have information on ethnicity were used to calculate age-, gender-, type of county- and month- specific average annual incidence (AAI) of these serotypes by race and ethnicity. The cases were from the 1995 to 2001 *Salmonella* surveillance database at the Michigan Department of Community Health. African-American infants aged <1 year, children aged 1-4 years, and elderly aged ≥60 years in urban counties have a significantly higher AAI of *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport compared to their White counterparts. Hispanic infants, children aged 1-4 years and elderly have a higher AAI of *S.* Enteritidis compared to their Non-Hispanic counterparts. Overall, African-Americans have a significantly higher AAI of *S.* Typhimurium and *S.* Enteritdis during the winter months (November,

December and January) compared with Whites. This study highlights the magnitude of the problem of infections with the most common *Salmonella* serotypes among African-Americans and Hispanics in Michigan. The results show that African-American-White differences in infections with the most common serotypes and Hispanic-Non-Hispanic differences in *S.* Enteritidis infection in Michigan is due to the high incidence among African-American and Hispanic infants, children aged 1-4 years and elderly in urban counties. Compared to Whites, the incidence of *S.* Typhimurium and *S.* Enteritidis among African-Americans was higher during the winter months. Salmonellosis prevention efforts should be focused on African-American and Hispanic infants, children aged 1-4 and elderly in the urban counties. Further studies should be conducted to determine the risk factors in order to enhance the prevention efforts.

## INTRODUCTION

In the United States (US), nontyphoidal *Salmonella* caused an estimated 1.4 million cases of foodborne illnesses annually, resulting in > 100,000-physician office visits (Voetsch et al., 2004), 16,000 hospitalizations, and ~ 600 deaths (Mead et al., 1999).

Although most infections with nontyphoidal *Salmonella* cause mild to moderate self-limited gastroenteritis, serious infections resulting in death such as meningitis and endocarditis do occur (Pegues et al., 2005). The estimated costs associated with nontyphoidal salmonellosis (NTS) in humans in the US, including the costs of medical care and lost productivity, was $ 0.5 to $2.3 billion annually (Frenzen et al., 1999).

Most outbreaks of NTS and sporadic illness have been caused by *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport. Infections with these serotypes were frequently associated with consumption of foods of animal origin such as beef, poultry, eggs, and dairy products (CDC, Foodborne Outbreak Response and Surveillance Unit). This is because specific nontyphoidal *Salmonella* are typically associated with particular food vehicles. For example, *S.* Typhimurium was frequently associated with beef, pork and dairy products (CDC, Foodborne Outbreak Response and Surveillance Unit), *S.* Enteritidis with eggs and chicken (Kimura et al., 2004; Patrick et al., 2004), *S.* Heidelberg with chicken and eggs (Chittick et al, 2006; Hennessy et al., 2004; Layton et al., 1997), and *S.* Newport with ground beef (CDC, 2002c). Furthermore, Hispanic origin and race are among many demographic factors that may influence food intake behavior (USDA, ARS).

In the US, the national surveillance data demonstrated that the incidence of reported salmonellosis was highest among African-Americans and Hispanics (CDC, 2005b). In the FoodNet areas, US, the incidence of NTS was significantly higher among African-Americans than among whites in 2000 (Lay et al., 2002) and from 1998 to 2000, the average annual incidence of *S.* Enteritidis infection was highest among African-Americans (2.0/100,000) followed by Hispanics (1.2/100,000) and whites (1.1/100,000) (Marcus et al., 2002).

The US Department of Health and Human Services (USDHHS, 2003), in its publication Healthy People 2010, states two overarching goals for the first decade of the 21st century: (a) increase quality and years of healthy life and (b) eliminate health disparities among different segments of the population. Health disparities between non-

Hispanic whites and members of racial and ethnic minority groups across all ages have been widely documented (CDC, 2005c; Richardus and Kunst, 2001).

Even though information on race and ethnicity in salmonellosis surveillance data is often not complete (CDC, 1999), analysis of race and ethnic variables is important for identifying high-risk groups. Once identified public health officials can address the differential health status of target populations (Chorba, 2001).

Similar to other states, human salmonellosis in Michigan is required to be reported by physicians and laboratories to public health authorities under the Communicable Disease Rules of the Michigan Public Health Code (MDCH; Wells et al., 2004). Local Health Departments routinely contact all patients, if possible, and collect epidemiologic information such as age, gender, race/ethnicity (Wells et al., 2004). Analysis of the surveillance data (Chapter Three) of this dissertation showed that among cases whose race was known, African-Americans have a significantly higher incidence of infection with *S.* Typhimurium, *S.* Enteritdis, *S.* Heidelberg and *S.* Newport compared with whites, and Hispanics had a significantly higher incidence of *S.* Enteritidis infection compared with Non-Hispanics.

This study was conducted to determine the interracial and interethnic incidence patterns of infections with the most common *Salmonella* serotypes by age, gender, type of county of residence (urban or rural), and month of occurrence that are prerequisite to understanding the causes of the interracial and interethnic variation.

## MATERIALS AND METHODS

All cases of *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport that have information on race were used to calculate age-, gender-, type of county- and month- specific average annual incidence (AAI) of those serotypes by race (African-American vs. white). All cases of *S.* Enteritidis that have information on ethnicity were used to calculate age-, gender-, and type of county-specific AAI by ethnicity (Hispanic vs. Non-Hispanic). Within gender and type of county variables, age-specific AAI were calculated by race and ethnicity. The cases were from the 1995 to 2001 *Salmonella* surveillance database at the Michigan Department of Community Health (MDCH).

The 1995 to 2001 race- and ethnicity-specific population estimates of Michigan by age, sex, and county were used as the denominator (NCHS). Sex- and type of county-specific AAI by race and ethnicity were age-standardized by using the United States year 2000 standard million population 2000 (Anderson and Rosenberg, 1998).

*Statistical analysis.* Poisson regression analysis (CDC, 2001; Hardnet et al., 2004; Kleinbaum et al 1998) was used to determine the relative rates with 95% confidence interval, with the number of cases as the dependent variable, the population as the offset variable and age-, sex-, race, ethnicity, type of county as the independent variable. The analysis was conducted by using Proc Genmod in SAS (SAS Institute Inc. 1997).

**Infections with *S.* Typhimurium**

From 1995 to 2001, there were 1,596 cases of *S.* Typhimurium infection reported to MDCH, Bureau of Epidemiology. The overall AAI was 2.2 cases /100,000. Of the 1,596 cases, 708 (46%) were Whites, 284 (18%) were African-Americans, 4 (<1%) were Native-Americans, 4 (<1%) were Asian/Pacific Islanders, 20 (1%) were other races and for 576 (36%) the race variable was not reported. A total of 518 cases (90%) without information on race were from urban counties.

Among cases for whom race was reported, African-Americans had a significantly higher age-adjusted AAI compared with whites (2.5 vs. 1.3/100,000; RR, 2.27; 95% CI, 1.98-2.61) (Table 4-1).

African-American infants aged <1 year, children aged 1-4 years, and the elderly aged 60 years and above have a significantly higher AAI per 100,000 than their White counterparts (32.4 vs. 6.8; RR, 4.77; 95% CI, 3.24-7.01: 10.9 vs. 3.5; RR, 3.10; 95% CI, 2.32-4.15: and 1.9 vs. 0.8; RR, 2.29; 95% CI, 1.43-3.66 respectively) (Table 4-1). African-American males and females have a significantly higher AAI than white males and females (2.4 vs. 1.2; RR, 2.23; 95% CI, 1.83-2.73 and 2.5 vs. 1.3; RR, 2.28; 95% CI, 1.88-2.76 respectively) (Table 4-1). African-American boys aged <5 years, and elderly men aged 60 years and above have a significantly higher AAI than their white counterparts. African-American girls aged 9 years and below, and elderly women 60 years and above have a significantly higher AAI than their white counterparts.

African-Americans in urban counties had a significantly higher age-adjusted AAI than their white counterparts (2.5 vs. 1.1/100,000; RR, 2.8; 95% CI, 2.4-3.3), especially

among infants aged <1 year, children aged 1-4 years, and elderly aged 60 years and above (Table 4-1). Age-adjusted AAI among African-Americans in rural counties was not different significantly from their white counterparts (0.9 vs. 1.8; RR, 2.3; 95% CI, 0.6-9.1).

**Infections with *S.* Enteritidis**

From 1995 to 2001, there were 1,309 cases of *S.* Enteritidis infection reported to MDCH, Bureau of Epidemiology. The overall AAI was 1.9 cases/100,000. Of the 1,309 cases, 617 (47%) were whites, 147 (11%) were African-Americans, 2 (<1%) were Native-Americans, 5 (<1%) were Asian/Pacific Islanders, and for 528 (40%) the race variable was not reported. A total of 480 cases (91%) without information on race were from urban counties.

Among cases for whom race was reported, African-Americans have a significantly higher age-adjusted AAI than whites (1.4 vs. 1.1 cases/100,000; RR 1.4, 95% CI 1.13-1.62) (Table 4-2).

African-American infants aged <1 year and elderly aged 60 years and above have a significantly higher AAI per 100,000 than their white counterparts (20.6 vs. 6.7; RR, 3.10; 95% CI, 2.00-4.79 and 1.7 vs. 1.0; RR, 1.73; 95% CI, 1.06-2.83 respectively) (Table 4-2).

African-American males have a significantly higher age-adjusted AAI than white males (1.5 vs. 1.0; RR, 1.57; 95% CI, 1.21-2.03) (Table 4-2). Among males, African-American infants aged <1 year and elderly aged 60 years and above have a higher AAI than their white counterparts. Overall age-adjusted AAI among African-American

females did not differ significantly from white females. However, African-American female infants aged <1 year had a significantly higher AAI than their white counterparts.

African-American in urban counties had a significantly higher age-adjusted AAI than their white counterparts (1.4 vs. 0.9/100,000; RR, 1.62; 95% CI, 1.34-1.95), particularly among infants aged <1 year, and elderly aged 60 years and above (Table 4-2). Age-adjusted AAI for African-Americans in rural counties was not different significantly from their white counter parts.

**Infections with *S.* Heidelberg**

From 1995 to 2001, there were 466 cases of *S.* Heidelberg infections reported to MDCH, Bureau of Epidemiology. The overall AAI was 0.7 cases/100,000. Of the 466 cases, 156 (34%) were Whites, 98 (22%) were African-American, 5 (1%) other races, and for 207 (44%) the variable race was not reported.

Among cases for whom race was reported, African-Americans had a significantly higher age-adjusted AAI than among whites (0.8 vs. 0.3 /100,000; RR 3.56, 95% CI, 2.77-4.58) (Table 4-3).

African-American infants aged <1 year and children aged 1-4 years have a significantly higher AAI than their white counterparts (17.1 vs. 1.5; RR, 11.20; 95% CI, 5.38-23-81 and 3.1 vs. 0.5; RR, 5.73; 95% CI, 3.03-10.48 respectively) (Table 4-3). African-American males and females had a significantly higher age-adjusted AAI than white males and females (0.9 vs. 0.3; RR, 4.23; 95% CI, 2.97-6.01 and 0.8 vs. 0.3; RR, 3.00; 95% CI, 2.08-4.31 respectively) (Table 4-3).

African-Americans in urban counties had a significantly higher age-adjusted AAI than their white counterparts (0.8 vs. 0.3/100,000; RR, 3.74; 95% CI, 2.85-4.90), especially among infants and children aged 1-4 years. (Table 4-3). Age-adjusted AAI among African-Americans in rural counties did not differ significantly from their white counterparts.

**Infections with *S.* Newport**

From 1995 to 2001, there were 222 cases of *S.* Newport infection reported to MDCH, Bureau of Epidemiology. The overall AAI was 0.3 cases/100,000. Of the 222 cases, 76 (34%) were Whites, 38 (17%) were African-American, 2 (1%) were other races, and for 106 (48%) the race variable was not reported.

Among cases for whom race was reported, African-Americans had a significantly higher age-adjusted AAI than among whites (0.3 vs. 0.1 /100,000; RR 2.83, 95%CI 1.92-4.18) (Table 4-4).

African-American infants, children aged 1-4, and elderly have a significantly higher AAI than their white counterparts (4.12 vs. 0.97; RR, 4.31; 95% CI, 1.51-12.30: 1.48 vs. 0.17; RR, 9.14; 95% CI, 3.18-26-32: 0.53 vs. 0.12; RR, 4.25; 95% CI, 1.59-11.31 respectively) (Table 4-4).

African-American males and females have a significantly higher age-adjusted AAI than white males and females (0.34 vs. 0.1; RR, 3.54; 95% CI, 2.06-6.08 and 0.30 vs. 0.14; RR, 2.26; 95% CI, 1.29-3.98 respectively) (Table 4-4).

African-Americans in urban counties had a significantly higher age-adjusted AAI than their white counterparts (0.33 vs. 0.12; RR, 3.03; 95% CI, 2.10-4.59), especially among infants, children aged 1-4 years, and elderly. (Table 4-4).

### *Salmonella* Enteritidis infections among Hispanics

From 1995 to 2001, there were 20 (2%) cases of *S.* Enteritdis among Hispanics and 344 (26%) among Non-Hispanics. For 945 (72%) cases the ethnicity variable was n0t reported.

Among cases for whom ethnicity was reported, Hispanics have a significantly higher age-adjusted AAI than Non-Hispanics (1.0 vs. 0.5/100,000; RR, 1.90; 95% CI, 1.21-2.98) (Table 4-5). Hispanic infants, children aged 1-4 years and elderly have a higher AAI than their Non-Hispanic counterparts (7.6 vs. 2.9; RR, 2.65; 95% CI, 0.92-7.62: 2.6 vs. 1.1; RR, 2.42; 95% CI, 0.96-6.15 and 1.6 vs. 0.6; RR, 2.91; 95% CI, 0.71-11.89 respectively) (Table 4-5). Hispanics in urban counties have a significantly higher age-adjusted AAI than their Non-Hispanic counterparts, especially among infants, children aged 1-4 years and elderly (Table 4-5).

### Seasonality

African-Americans had a significantly higher AAI of infections with *S.* Typhimurium and *S.* Enteritidis relative to whites during the winter months (November, December, and January) (Figure 1 and 2).

# DISCUSSION

This study shows that the significantly higher incidence of infections with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport among African-Americans compare with whites is contributed by the higher incidence among African-American infants, children aged 1-4 years and elderly aged 60 years and above in urban counties.

The reasons for the significantly higher incidence of infections with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport among African-American infants in urban counties compare to their White counterparts are not clear. This may be due to exposure-related factors. It may be that the infants contract the infections from infected older children or adults, contaminated surfaces in the house such as kitchen floor, contaminated infant formula, or contaminated solid food. These reasons is conceivable because the USDA Continuing Survey of Food Intakes by Individuals (CSFII) showed that more African-Americans frequently consumed pork, chicken, and eggs compared to whites (Table 4-6; USDA, ARS). These foods have been frequently implicated in human outbreaks associated with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport (CDC, Foodborne Outbreaks Response and Surveillance Unit).

Improper handling, preparing, and cooking of these foods may result in infection among older children and adults in the African-Americans households. But they may be asymptomatic or undiagnosed because of better resistance to the infections. However, they may transmit the bacteria to the infants if they are not washing their hands properly before preparing infant formula or food, or before handling the infant's pacifier.

Improper handling of these foods may result in contamination of kitchen surfaces and hands of food handlers. Since infants are very inquisitive, are orally fixated, and constantly exploring their environment (Schutze et al., 1998), they may contract the infection if they are allow to roam in the kitchen. The food handlers who may be also a caregiver may also transmit the bacteria to the infants if they did not was their hands before preparing infant formula or solid foods.

The importance of intrafamilial transmission and contaminated home environment in infant salmonellosis has been reported by Schutze et al. and Wilson et al. (Schutze et al., 1999; Wilson et al., 1982). More recently, it has been reported that high number of cases of salmonellosis among African-American infants and toddlers in Atlanta was associated with preparation of chitterlings (pork intestines) in African-Americans homes (Georgia Department of Human Resource, 2001). Chitterling consumption is customary among many African-American households. Cleaning raw chitterlings is a time-consuming process (median 5 hours in the above study), providing ample opportunity for cross-contamination of surfaces and hands. This example emphasized the importance of contaminated home environment due to preparation of foods of animal origin in infants and young children salmonellosis.

The high incidence among African-American infants relative to White infants may also be due to host-related risk factors. It may be that African-American infants in this study do not get breast milk. This reason is conceivable because the Michigan Pregnancy Risk Assessment Monitoring System (PRAMS) showed that lower percentage of African-American mothers breast-feed compared to non-African-Americans (44.7%

vs. 67.1%; Reznar et al., 2003). Bottle feeding is associated with increase risk for *Salmonella* infections among infants (Haddock et al., 1991; Rowe et al., 2004).

The high incidence may also be due to the presence of underlying disease such as sickle cell anemia among the African-American infants in this study. In has been reported that sickle cell anemia is more common among babies of African-American heritage (Ashley-Koch et al., 2006; Davies et al., 1997). It has been reported that a significant association exists between sickle cell anemia and *Salmonella* infections (Atkins et al., 1997; Katzman and Bozentka; Rodgers et al., 2002). Children with sickle cell disease are prone to development of septicemia and osteomyelitis when infected with nontyphoidal *Salmonella* (Cleary, 2003).

This may also due to low birth weight among the African-American infants. Low birth weight infants appear to be at higher risk of acquiring *Salmonella* than do full-term infants (Gomez and Cleary, 1998). In Michigan, the prevalence of low birth weight was highest among African-Americans compared to whites (11.9% vs. 5.9%; MDCH, 2002).

The high incidence may also due to socioeconomic status (SES) of the parent. It may be that parents of the African-American infants in this study have low educational level. In 2000, 23.5% and 27.9% of African-American women and men in Michigan respectively had less than 12 grade of education compared to 14.3% and 14.6% white women and men respectively (Anonymous, 2005b). Low educational level may lead to less exposure to information on food safety and prevention of foodborne illness (Shiferaw et al., 2002).

The high incidence of *S.*Typhimurium, *S.* Enteritidis, *S.* Hiedelberg, and *S.* Newport among African-American children aged 1-4 may due to consumption of

undercooked chicken, pork or eggs. This reason is conceivable because the USDA CSFII showed that higher percentages of African-American children aged ≤5 years consume beef, pork, chicken and eggs compared to their White counterparts (Table 4-6; USDA, ARS). The SES of their parents may also play a role in the high incidence of these infections among African-American children. Low educational level, for instance, may lead to less exposure to food safety educational materials and may result in improper cooking of high-risk foods. The children may also contract the infection from the infants.

The reasons for the high incidence of *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg and *S.* Newport among elderly African-Americans aged 60 years and above in urban counties relative to whites are not known. This may be due to exposure-related risk factors. It may be that elderly African-Americans more frequently consume high- risk food, such as chicken, eggs, and pork. They may also contract the infection from infected children in the households. This may also be due to low level of education, which may lead to less exposure to information on food safety and prevention of foodborne diseases.

The high incidence among elderly African-American may also due to the presence of underlying diseases such as gastric ulcer, which result in increase susceptibility to foodborne infections. In the US, gastric ulcer due to *H. pylori* is more prevalent among older adults, African Americans, Hispanics, and lower socioeconomic groups (CDC, Division of Bacterial and Mycotic Diseases; Fennerty et al., 1992). Individuals with gastric ulcer will take antacids or acid suppression drugs, which is risk factor for *Salmonella* infection.

This study shows that African-Americans have a significantly higher incidence of *S.* Typhimurium and *S.*Enteritidis during the winter months relative to whites. This may be due to improper handling, preparing, and cooking of foods such as chicken, turkey, and foods containing eggs during holidays and family gatherings (FDA, 2003).

Hispanics are a growing subgroup of the US population. People of Hispanic origin are expected to nearly double their share of the US population – from 13% to 24% between 2000 and 2050, and triple in number (US Census Bureau, Census Bureau projects tripling). On average, there were 292,782 Hispanics residing in Michigan from 1995-2001. Eighty-five percent % of the Michigan's Hispanics population lives in metropolitan areas (Wayne, Oakland, Kent, Ingham, Saginaw County). Common occupations among Michigan Hispanics are in service and labor sector (Coupe, et al, University of Michigan). In the United States in 2004, Hispanics have a higher incidence of salmonellosis than Non-Hispanics (10.6 vs. 7.5/100,000; CDC, 2005b).

This study shows that the significantly higher incidence of *S.* Enteritidis infections among Hispanics compare to Non-Hispanics is contributed by the higher incidence among Hispanic infants, children aged 1-4 years and the elderly. The reasons for the higher incidence of *S.* Enteritidis infection among Hispanic infants relative to Non-Hispanics in this study are not known. It may be that the infants contract the infections from infected older children and adults, contaminated surfaces in the house such as kitchen floor, contaminated infant formula, or contaminated solid food. These reasons are conceivable because the USDA Continuing Survey of Food Intakes by Individuals showed that more Hispanics frequently consumed chicken and eggs compared to Non-

Hispanics (Table 4-6; USDA, ARS). Furthermore, a study in FoodNet sites from 2000-2001 showed that Hispanics were more likely than Non-Hispanics to eat runny eggs (Banerjee et al., 2002). Chickens and eggs have been frequently implicated in human outbreaks associated with *S.* Enteritidis (CDC, Foodborne Outbreaks Response and Surveillance Unit).

Improper handling, preparing, and cooking of chickens and eggs may result in infection among older children and adults or contamination of home environment in the Hispanics households. These will be the sources of infection for the infants.

The higher incidence of *S.* Enteritidis infections among Hispanics children aged 1-4 years relative to Non-Hispanics children may be due to consumption of undercooked chicken meat or eggs. This reasons is conceivable because the USDA CSFII showed that more Hispanic children aged 5 years and under consume chicken and eggs compared to their Non-Hispanic white counterparts (Table 4-6; USDA, ARS).

The higher incidence of *S.* Enteritidis infections among elderly Hispanics compare to elderly Non-Hispanics may be due to consumption of undercooked chicken or eggs. It may also due to low educational level that may result in less exposure to or understanding of food safety educational materials and proper cooking of foods of animal origin.

The main limitation of this study is that it was based on passive reporting of *Salmonella* cases to MDCH, Bureau of Epidemiology and passive submission of *Salmonella* isolates to MDCH laboratory. This can result in an underestimate of the annual incidence rate. Incomplete information of patients' race and ethnicity is another limitation of our study and may contribute to lower race- and ethnicity-specific incidence

rate. Other limitations include the inability to control for socioeconomic status (SES), because data for salmonellosis surveillance did not have SES indicators for the individual cases.

Reporting accurate and complete race and ethnicity data in public health surveillance systems provides critical information to target and evaluate public health interventions, particularly for minority populations (CDC, 1999). In this study, of 6,797 *Salmonella* cases, 41% and 73% did not have information on race and ethnicity respectively. Race and ethnicity data may not be reported by health-care providers or clinical laboratories because of: 1) providers may not know what the federal standards are for data collection about the race and ethnicity of their patients for surveillance purposes; 2) if a health-care provider forgets or is reluctant to ask a patient's racial/ethnic background, this information may not be recorded; 3) patients may choose not to provide information about their race and ethnicity; and 4) clinical laboratory staff may not report race and ethnicity data because they do not have access to that information. Furthermore, resource constraints at the local and reporting area level may limit the ability of surveillance staff to follow up on reports with missing race and ethnicity data (CDC, 1999).

The percentage of *Salmonella* cases with information on race was not different significantly from percentage of cases without race in term of gender (59% vs. 41%; $\chi^2$, 0.06; *P*, 0.81) and type of county (59% vs. 41%; $\chi^2$, 0.06; *P*, 0.81) (Table 3-2). The percentage of cases with information on race was different significantly from percentage of cases without race in term of age (58% vs. 39%$\chi$2, 191.75; *P*, <0.001).

In summary, despite many cases that did not have information on race and ethnicity, this study shows that African-American-white differences in the incidence of infections with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport, and Hispanic-Non-Hispanic differences in *S.* Enteritidis infection is contributed by the higher incidence of the infections among African-American and Hispanic infants, children aged 1-4 years, and elderly living in urban counties. This information can be used by local and state health departments of Michigan to enhance targeted food safety educational programs in this population sub-groups in order to reduce the incidence of the infection. MDCH should recommend to physicians evaluating patients with enteric bacterial infections to ask about food habits that may be unique to the patient's race and to consider incorporating food safety education into routine health maintenance. The actual reasons for the high incidence of infection with *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport among African-American infants, children and elderly, and high incidence of *S.* Enteritidis among Hispanics in this study are not exactly known. Further study is necessary to determine the risk factors in order to enhance prevention efforts.

Table 4-1. Average annual incidence of *S.* Typhimurium infections among Whites and African-Americans by age, sex and type of county, Michigan, 1995-2001

| Variables | White* (n =708) | African-American (n =284) | Relative rate (95% CI) |
|---|---|---|---|
| Total | 1.3 | 2.5 | 2.27 (1.98-2.61)** |
| **Age** | | | |
| < 1 | 6.8 | 32.4 | 4.77 (3.24-7.01)** |
| 1-4 | 3.5 | 10.9 | 3.10 (2.32-4.15)** |
| 5-9 | 2.4 | 3.5 | 1.43 (0.98-2.10) |
| 10-19 | 1.2 | 1.1 | 0.88 (0.53-1.46) |
| 20-29 | 1.1 | 1.3 | 1.14 (0.70-1.85) |
| 30-39 | 0.8 | 1.3 | 1.65 (1.00-2.70) |
| 40-49 | 0.7 | 1.5 | 2.10 (1.28-3.44)** |
| 50-59 | 0.9 | 0.8 | 0.91 (0.42-2.00) |
| 60+ | 0.8 | 1.9 | 2.29 (1.43-3.66)** |
| **Sex** | | | |
| Male | 1.2 | 2.4 | 2.23 (1.83-2.73)** |
| < 1 | 7 | 37 | 5.28 (3.06-9.13)** |
| 1-4 | 3.9 | 9 | 2.32 (1.52-3.53)** |
| 5-9 | 3 | 3.4 | 1.15 (0.68-1.95) |
| 10-19 | 1.2 | 1.2 | 0.94 (0.48-1.84) |
| 20-29 | 0.8 | 1.0 | 1.15 (0.51-2.62) |
| 30-39 | 0.7 | 0.7 | 0.98 (0.38-2.51) |
| 40-49 | 0.7 | 1.9 | 2.91 (1.48-5.70)** |
| 50-59 | 1 | 0.8 | 0.77 (0.23-2.50) |
| 60+ | 0.6 | 1.9 | 3.02 (1.42-6.42)** |
| Female | 1.3 | 2.5 | 2.28 (1.88-2.76)** |
| < 1 | 6.6 | 27.5 | 4.20 (2.36-7.49)** |
| 1-4 | 3.1 | 12.3 | 3.94 (2.61-5.96)** |
| 5-9 | 1.8 | 3.5 | 1.90 (1.08-3.35)** |
| 10-19 | 1.2 | 1.0 | 0.82 (0.39-1.74) |
| 20-29 | 1.5 | 1.6 | 1.10 (0.60-2.01) |
| 30-39 | 0.9 | 1.8 | 2.10 (1.16-3.81)** |
| 40-49 | 0.8 | 1.2 | 1.51 (0.73-3.15) |
| 50-59 | 0.8 | 0.9 | 1.09 (0.38-3.13) |
| 60+ | 1 | 1.9 | 1.94 (1.06-3.55)** |

Table 4-1 (cont'd).

| Variables | White* (n =708) | African-American (n =284) | Relative rate (95% CI) |
|---|---|---|---|
| **Type of county** | | | |
| Urban | 1.1 | 2.5 | 2.80 (2.40-3.30)** |
| < 1 | 5.2 | 32.9 | 6.34 (4.04-9.94)** |
| 1-4 | 3.2 | 11.0 | 3.46 (2.51-4.75)** |
| 5-9 | 2.4 | 3.4 | 1.42 (0.95-2.13) |
| 10-19 | 1.1 | 1.1 | 0.99 (0.59-1.67) |
| 20-29 | 1 | 1.3 | 1.39 (0.83-2.31) |
| 30-39 | 0.7 | 1.4 | 1.90 (1.13-3.18)** |
| 40-49 | 0.6 | 1.5 | 2.75 (1.62-4.69)** |
| 50-59 | 0.8 | 0.8 | 1.02 (0.46-2.29) |
| 60+ | 0.6 | 2.0 | 3.13 (1.88-5.21)** |
| | | | |
| Rural | 1.8 | 0.9 | 2.3 (0.6-9.1) |
| < 1 | 12.3 | 0.0 | - |
| 1-4 | 4.7 | 7.8 | 1.67 (0.23-12.23) |
| 5-9 | 2.5 | 6.2 | 2.51 (0.34-18.55) |
| 10-19 | 1.5 | 0.0 | - |
| 20-29 | 1.8 | 0.0 | - |
| 30-39 | 1.1 | 0.0 | - |
| 40-49 | 1.2 | 0.0 | - |
| 50-59 | 1.1 | 0.0 | - |
| 60+ | 1.4 | 0.0 | - |

*denotes reference variable in Poisson regression analysis
**denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 59% of the cases have information on race.

Table 4-2. Average annual incidence of *S.* Enteritidis infections among Whites and African-Americans by age, sex and type of county, Michigan, 1995-2001

| Variables | White* (n = 617) | African-American (n = 147) | Relative rate (95% CI) |
|---|---|---|---|
| Total | 1.1 | 1.4 | 1.35 (1.13-1.62)** |
| **Age** | | | |
| < 1 | 6.7 | 20.6 | 3.10 (2.00-4.79)** |
| 1-4 | 2.7 | 4.0 | 1.49 (0.98-2.27) |
| 5-9 | 1.1 | 1.1 | 0.98 (0.50-0.89) |
| 10-19 | 0.8 | 0.7 | 0.78 (0.41-1.49) |
| 20-29 | 1.2 | 0.8 | 0.73 (0.41-1.31) |
| 30-39 | 0.7 | 0.5 | 0.63 (0.29-1.37) |
| 40-49 | 0.8 | 0.7 | 0.85 (0.44-1.65) |
| 50-59 | 0.9 | 1.2 | 1.28 (0.65-2.51) |
| 60+ | 1.0 | 1.7 | 1.73 (1.06-2.83)** |
| **Sex** | | | |
| Male | 1.0 | 1.5 | 1.57(1.21-2.03)** |
| < 1 | 5.7 | 25.5 | 4.49 (2.47-8.17)** |
| < 1 | 2.4 | 3.4 | 1.41 (0.75-2.66) |
| 1-4 | 1.2 | 1.5 | 1.25 (0.56-2.77) |
| 5-9 | 0.9 | 0.2 | 0.26 (0.06-1.06) |
| 10-19 | 0.9 | 0.5 | 0.64 (0.23-1.80) |
| 20-29 | 0.6 | 0.6 | 0.96 (0.33-2.73) |
| 30-39 | 0.8 | 0.8 | 1.00 (0.39-2.56) |
| 40-49 | 0.8 | 1.0 | 1.27 (0.44-3.64) |
| 50-59 | 0.8 | 2.2 | 2.66 (1.32-5.39)** |
| 60+ | | | |
| Female | 1.2 | 1.3 | 1.18(0.92-1.52) |
| < 1 | 7.7 | 15.6 | 2.02 (1.04-3.92)** |
| 1-4 | 3.0 | 4.6 | 1.56 (0.89-2.73) |
| 5-9 | 0.9 | 0.6 | 0.63 (0.19-2.15) |
| 10-19 | 0.7 | 1.1 | 1.52 (0.72-3.22) |
| 20-29 | 1.5 | 1.1 | 0.76 (0.37-1.54) |
| 30-39 | 0.9 | 0.4 | 0.42 (0.13-1.36) |
| 40-49 | 0.9 | 0.7 | 0.74 (0.29-1.87) |
| 50-59 | 1.0 | 1.3 | 1.28 (0.53-3.05) |
| 60+ | 1.1 | 1.3 | 1.23 (0.61-2.48) |

Table 4-2. (cont'd).

| Variables | Whites* (n = 617) | African-American (n = 147) | Relative rate (95% CI) |
|---|---|---|---|
| **Type of county** | | | |
| Urban | 0.9 | 1.4 | 1.62(1.34-1.95)** |
| < 1 | 6.6 | 20.9 | 3.16 (1.99-5.02)** |
| 1-4 | 2.2 | 4.0 | 1.80 (1.14-2.85)** |
| 5-9 | 1.0 | 1.1 | 1.05 (0.53-2.09) |
| 10-19 | 0.6 | 0.7 | 1.03 (0.53-2.00) |
| 20-29 | 1.1 | 0.9 | 0.83 (0.46-1.51) |
| 30-39 | 0.7 | 0.5 | 0.64 (0.29-1.40) |
| 40-49 | 0.6 | 0.7 | 1.25 (0.62-2.49) |
| 50-59 | 0.8 | 1.1 | 1.31 (0.64-2.72) |
| 60+ | 0.6 | 1.7 | 2.70 (1.58-4.62)** |
| | | | |
| Rural | 1.6 | 1.3 | 0.53 (0.13-2.12) |
| < 1 | 6.8 | 0.0 | - |
| 1-4 | 4.4 | 7.8 | 1.78 (0.24-13.07) |
| 5-9 | 1.2 | 0.0 | - |
| 10-19 | 1.3 | 0.0 | - |
| 20-29 | 1.5 | 0.0 | - |
| 30-39 | 0.7 | 0.0 | - |
| 40-49 | 1.6 | 0.0 | - |
| 50-59 | 1.2 | 7.7 | 6.40 (0.86-47.83) |
| 60+ | 1.9 | 0.0 | |

*Reference variable in Poisson regression analysis
**denotes statistically significant results
**Note:** Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 60% of the cases have information on race.

Table 4-3. Average annual incidence of *S.* Heidelberg infections among Whites and African-Americans by age, sex and type of county, Michigan, 1995-2001

| Variables | White* (n = 156) | African-American (n = 98) | Relative rate (95% CI) |
|---|---|---|---|
| Total | 0.3 | 0.8 | 3.56 (2.77-4.58)** |
| **Age** | | | |
| < 1 | 1.5 | 17.1 | 11.20 (5.38-23.81)** |
| 1-4 | 0.5 | 3.1 | 5.73 (3.03-10.84)** |
| 5-9 | 0.3 | 0.8 | 2.81 (1.13-6.98)** |
| 10-19 | 0.2 | 0.5 | 2.70 (1.19-6.11)** |
| 20-29 | 0.4 | 0.5 | 1.47 (0.66-3.24) |
| 30-39 | 0.2 | 0.3 | 1.48 (0.50-4.43) |
| 40-49 | 0.3 | 0.3 | 1.11 (0.39-3.22) |
| 50-59 | 0.1 | 0.9 | 6.49 (2.51-16.83)** |
| 60+ | 0.3 | 0.4 | 1.28 (0.45-3.66) |
| **Sex** | | | |
| Male | 0.3 | 0.9 | 4.23(2.97-6.01)** |
| < 1 | 1.3 | 18.5 | 13.73 (5.03-37.47)*8 |
| 1-4 | 0.5 | 3.4 | 7.47 (2.98-18.72)*8 |
| 5-9 | 0.3 | 0.9 | 2.79 (0.89-8.80) |
| 10-19 | 0.2 | 0.6 | 2.44 (0.83-7.13) |
| 20-29 | 0.3 | 0.4 | 1.27 (0.36-4.52) |
| 30-39 | 0.2 | 0.4 | 2.15 (0.58-7.95) |
| 40-49 | 0.2 | 0.5 | 2.11 (0.58-7.66) |
| 50-59 | 0.1 | 1.0 | 7.91 (1.98-31.62)** |
| 60+ | 0.2 | 0.2 | 0.91 (0.12-7.07) |
| Female | 0.3 | 0.8 | 3.00(2.08-4.31)*8 |
| < 1 | 1.7 | 15.6 | 9.00 (3.42-23.68)*8 |
| 1-4 | 0.6 | 2.7 | 4.38 (1.78-10.79)** |
| 5-9 | 0.2 | 0.6 | 2.85 (0.64-12.75) |
| 10-19 | 0.2 | 0.5 | 3.15 (0.89-11.16) |
| 20-29 | 0.4 | 0.6 | 1.60 (0.58-4.44) |
| 30-39 | 0.2 | 0.1 | 0.78 (0.10-6.35) |
| 40-49 | 0.3 | 0.1 | 0.45 (0.06-3.46) |
| 50-59 | 0.2 | 0.9 | 5.44 (1.46-20.26)** |
| 60+ | 0.3 | 0.4 | 1.47 (0.43-5.03) |

Table 4-3 (cont'd).

| Variables | Whites* (n = 156) | African-American (n = 98) | Relative rate (95% CI) |
|---|---|---|---|
| **Type of county** | | | |
| Urban | 0.3 | 0.8 | 3.74 (2.85-4.90)*8 |
| < 1 | 1.4 | 29.0 | 12.12 (5.54-26.50)** |
| 1-4 | 0.4 | 23.0 | 7.16 (3.41-15.04)** |
| 5-9 | 0.3 | 8.0 | 2.37 (0.94-6.01) |
| 10-19 | 0.2 | 9.0 | 2.18 (0.95-4.99) |
| 20-29 | 0.3 | 8.0 | 1.83 (0.79-4.24) |
| 30-39 | 0.2 | 4.0 | 1.72 (0.55-5.41) |
| 40-49 | 0.3 | 4.0 | 1.05 (0.36-3.08) |
| 50-59 | 0.1 | 7.0 | 5.55 (1.95-15.82)** |
| 60+ | 0.2 | 4.0 | 1.60 (0.54-4.79) |
| Rural | 0.3 | 0.9 | 1.40 (0.19-10.18) |
| < 1 | 1.8 | 0.0 | - |
| 1-4 | 0.9 | 0.0 | - |
| 5-9 | 0.1 | 0.0 | - |
| 10-19 | 0 | 0.0 | - |
| 20-29 | 0.6 | 0.0 | - |
| 30-39 | 0.2 | 0.0 | - |
| 40-49 | 0.2 | 0.0 | - |
| 50-59 | 0.1 | 7.7 | 60.83 (5.52-670.86) |
| 60+ | 0.4 | 0.0 | - |

*Reference variable in Poisson regression analysis

**denotes statistically significant results

**Note:** Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 58% of the cases have information on race.

Table 4-4. Average annual incidence of *S.* Newport infections among Whites and African-Americans by age, sex, and type of county, Michigan, 1995-2001

| Variables | White* (n = 76) | African-American (n = 38) | Relative rate (95% CI) |
|---|---|---|---|
| Total | 0.10 | 0.30 | 2.83 (1.92 - 4.18)** |
| **Age** | | | |
| < 1 | 0.97 | 4.12 | 4.31 (1.51-12.30)** |
| 1-4 | 0.17 | 1.48 | 9.14 (3.18-26-32)*8 |
| 5-9 | 0.17 | 0.19 | 1.10 (0.23-5.31) |
| 10-19 | 0.11 | 0.12 | 1.07 (0.23-4.94) |
| 20-29 | 0.03 | 0.32 | 11.73 (2.28-60.44)** |
| 30-39 | 0.13 | 0.13 | 0.99 (0.22-4.41) |
| 40-49 | 0.15 | 0.07 | 2.00 (0.26-15.32) |
| 50-59 | 0.11 | 0.00 | |
| 60+ | 0.12 | 0.53 | 4.25 (1.59-11.31)** |
| **Sex** | | | |
| Male | 0.13 | 0.34 | 3.54 (2.06-6.08)** |
| < 1 | 1.35 | 5.79 | 4.29 (1.24-14.82)** |
| 1-4 | 0.13 | 2.12 | 16.08 (3.42-75.74)** |
| 5-9 | 0.29 | 0.19 | 0.65 (0.08-5.41) |
| 10-19 | 0.12 | 0.12 | 0.98 90.11-8.35) |
| 20-29 | 0.03 | 0.41 | 15.30 (1.59-147.08)*8 |
| 30-39 | 0.09 | 0.00 | - |
| 40-49 | 0.07 | 0.16 | 2.34 (0.24-22.51) |
| 50-59 | 0.10 | 0.00 | - |
| 60+ | 0.12 | 0.22 | 1.81 (0.21-15.50) |
| Female | 0.13 | 0.3 | 2.26(1.29-3.98)** |
| < 1 | 0.57 | 2.39 | 4.20 (0.59-29.85) |
| 1-4 | 0.21 | 0.82 | 3.94 (0.80-19.54) |
| 5-9 | 0.05 | 0.20 | 3.81 (0.24-60.85) |
| 10-19 | 0.10 | 0.12 | 1.18 (0.13-10.56) |
| 20-29 | 0.03 | 0.25 | 0.91 (0.08-9.99) |
| 30-39 | 0.18 | 0.25 | 1.37 (0.29-6.43) |
| 40-49 | 0.22 | 0.00 | - |
| 50-59 | 0.13 | 0.00 | - |
| 60+ | 0.12 | 0.74 | 0.60 (0.19-1.88) |

Table 4-4 (cont'd).

| Variables | White* (n = 76) | African-American (n = 38) | Relative rate (95% CI) |
|---|---|---|---|
| **Type of county** | | | |
| Urban | 0.12 | 0.33 | 3.03 (2.01-4.59)** |
| < 1 | 0.89 | 4.19 | 4.68 (1.49-14.74)** |
| 1-4 | 0.22 | 1.51 | 6.85 (2.38-19.70)** |
| 5-9 | 0.20 | 0.20 | 0.99 (0.20-4.90) |
| 10-19 | 0.10 | 0.12 | 1.21 (0.24-6.01)** |
| 20-29 | 0.03 | 0.34 | 8.71 (1.69-44.88)** |
| 30-39 | 0.12 | 0.14 | 1.18 (0.25-5.58) |
| 40-49 | 0.16 | 0.07 | 0.45 (0.06-3.51) |
| 50-59 | 0.06 | 0.00 | - |
| 60+ | 0.10 | 0.54 | 5.49 (1.84-16.33) |
| Rural | 0.15 | 0.00 | - |
| < 1 | 1.23 | 0.00 | - |
| 1-4 | 0.00 | 0.00 | - |
| 5-9 | 0.10 | 0.00 | - |
| 10-19 | 0.14 | 0.00 | - |
| 20-29 | 0.00 | 0.00 | - |
| 30-39 | 0.20 | 0.00 | - |
| 40-49 | 0.09 | 0.00 | - |
| 50-59 | 0.25 | 0.00 | - |
| 60+ | 0.19 | 0.00 | - |

*Reference variable in Poisson regression analysis

**denotes statistically significant results

Note: Significantly higher AAI among African-Americans compared with Whites should be interpreted with caution because only 52% of the cases have information on race.

Table 4-5. Average annual incidence (per 100,000) of *S.* Enteritidis infections Among Hispanics and Non-Hispanics by age, sex, and type of county, Michigan, 1995-2001

| Variables | Non-Hispanic* (n=344) | Hispanic (n=20) | Relative rate (95% CI) |
|---|---|---|---|
| Total | 0.5 | 1.0 | 1.90 (1.21-2.98)*8 |
| **Age (Years)** | | | |
| <1 | 2.9 | 7.6 | 2.65 (0.92-7.62) |
| 1-4 | 1.1 | 2.6 | 2.42 (0.96-6.150 |
| 5-9 | 0.5 | 0.9 | 1.97 (0.47-8.37) |
| 10-19 | 0.4 | 0.5 | 1.22 (0.29-5.04) |
| 20-29 | 0.5 | 0.7 | 1.41 (0.44-4.54) |
| 30-39 | 0.3 | 0.6 | 2.02 (0.48-8.41) |
| 40-49 | 0.4 | 0.0 | - |
| 50-59 | 0.4 | 0.0 | - |
| 60+ | 0.6 | 1.6 | 2.91 (0.71-11.89) |
| **Sex** | | | |
| Male | 0.5 | 0.9 | 2.02 (1.06-3.83)*8 |
| <1 | 2.5 | 7.4 | 2.99 (0.66-13.47) |
| 1-4 | 1.1 | 1.0 | 0.94 (0.13-7.04) |
| 5-9 | 0.5 | 0.9 | 1.62 (0.21-12.35) |
| 10-19 | 0.4 | 1.0 | 2.34 (0.55-9.97) |
| 20-29 | 0.3 | 0.9 | 2.63 (0.60-11.50) |
| 30-39 | 0.3 | 0.6 | 2.31 (0.30-17.63) |
| 40-49 | 0.4 | 0.0 | - |
| 50-59 | 0.4 | 0.0 | - |
| 60+ | 0.5 | 1.7 | 3.86 (0.52-28.67) |
| Female | 0.6 | 1.0 | 1.81 (0.96-3.43) |
| <1 | 3.3 | 8.0 | 2.41 (0.55-10.62) |
| 1-4 | 1.1 | 4.3 | 3.97 (1.35-11.68) |
| 5-9 | 0.4 | 0.9 | 2.51 (0.32-19.83) |
| 10-19 | 0.4 | 0.0 | - |
| 20-29 | 0.7 | 0.5 | 0.78 (0.11-5.68) |
| 30-39 | 0.4 | 0.7 | 1.85 (0.25-13.84) |
| 40-49 | 0.5 | 0.0 | - |
| 50-59 | 0.4 | 0.0 | - |
| 60+ | 0.6 | 1.5 | 2.38 (0.33-17.32) |

Table 4-5 (cont'd).

| Variables | Non-Hispanic* (n=344) | Hispanic (n=20) | Relative rate (95% CI) |
|---|---|---|---|
| **Type of county** | | | |
| Urban | 0.3 | 1.0 | 3.25 (2.00-5.28)** |
| <1 | 2.3 | 6.7 | 2.96 (0.86-10.16) |
| 1-4 | 0.4 | 2.4 | 5.50 (1.79-16.86)** |
| 5-9 | 0.3 | 1.1 | 3.29 (0.74-14.58) |
| 10-19 | 0.2 | 0.6 | 2.59 (0.60-11.15) |
| 20-29 | 0.4 | 0.8 | 2.33 (0.71-7.71) |
| 30-39 | 0.3 | 0.7 | 2.71 (0.64-11.53) |
| 40-49 | 0.2 | 0.0 | - |
| 50-59 | 0.3 | 0.0 | - |
| 60+ | 0.3 | 1.8 | 6.11 (1.45-25.78) |
| Rural | 1.3 | 0.7 | 0.55 (0.14-2.23) |

*Reference variable in Poisson regression analysis

**denotes statistically significant results

**Note:** Significantly higher AAI among Hispanics compared with Non-Hispanics should be interpreted with caution because only 27% of the cases have information on ethnicity.

Table 4-6. Percentage of individuals consuming beef, pork, chicken, and eggs by race and ethnicity, United States, 1994-1996 (Adapted from USDA, ARS)

| Age (years) | Beef | | | | Pork | | | |
|---|---|---|---|---|---|---|---|---|
| | African-American | White | Mexican-American | Other Hispanic origin | African-American | White | Mexican-American | Other Hispanic origin |
| Males and females ≤5 | 19.9 | 13.3 | 17.9 | 12.3 | 14.4 | 9.9 | 7.8 | 11.9 |
| Males: | | | | | | | | |
| 6-11 | 13.8 | 24.4 | 19.7 | 19.9 | 18.1 | 12.1 | 8.2 | 8.5 |
| 12-19 | 30.5 | 23.5 | 23.8 | 26.7 | 30.5 | 13.3 | 9.7 | 18.6 |
| ≥20 | 23.1 | 24.4 | 33.9 | 30.6 | 27.3 | 16.9 | 17.6 | 19.6 |
| Females: | | | | | | | | |
| 6-11 | 16.8 | 19.8 | 21.5 | 21.8 | 19.6 | 8.7 | 9.5 | 8.9 |
| 12-19 | 25.5 | 20.2 | 27.8 | 25.8 | 15.1 | 10.6 | 19.5 | 7.1 |
| ≥20 | 17.1 | 17.6 | 24.7 | 26.4 | 24.4 | 15.3 | 19.6 | 16.8 |
| All individuals | 20 | 20.5 | 25.9 | 25.3 | 23.1 | 14.7 | 15.1 | 15.5 |

Table 4-6 (contd')

| Age (years) | Chicken | | | | Eggs | | | |
|---|---|---|---|---|---|---|---|---|
| | African-American | White | Mexican-American | Other Hispanic origin | African-American | White | Mexican-American | Other Hispanic origin |
| Males and females ≤5 | 31.7 | 18.7 | 20.7 | 26 | 23.7 | 14 | 38 | 26.6 |
| Males: | | | | | | | | |
| 6-11 | 32.2 | 17.1 | 18.2 | 18.8 | 17.3 | 13.5 | 26.2 | 19.9 |
| 12-19 | 24.2 | 16.1 | 18.2 | 24.1 | 25 | 13.3 | 29 | 27 |
| ≥20 | 27.8 | 17.3 | 15.3 | 21 | 29.8 | 19.6 | 30.3 | 30.9 |
| Females: | | | | | | | | |
| 6-11 | 23.6 | 16.4 | 27.4 | 22.9 | 17.3 | 11.1 | 16 | 25.9 |
| 12-19 | 29.5 | 17.1 | 24.1 | 16.1 | 20.9 | 11.7 | 23.9 | 22 |
| ≥20 | 32.8 | 16.5 | 19.3 | 22 | 24.2 | 17.1 | 30.5 | 17.3 |
| All individuals | 29.9 | 17 | 19 | 21.6 | 24.7 | 16.9 | 29.8 | 24.4 |

Adapted from USDA Continuing Survey of Food Intakes by Individuals (USDA, ARS.)

Figure 4-1. Average monthly incidence (per 100,000) of *Salmonella* Typhimurium infections by month and race, Michigan, 1995-2001

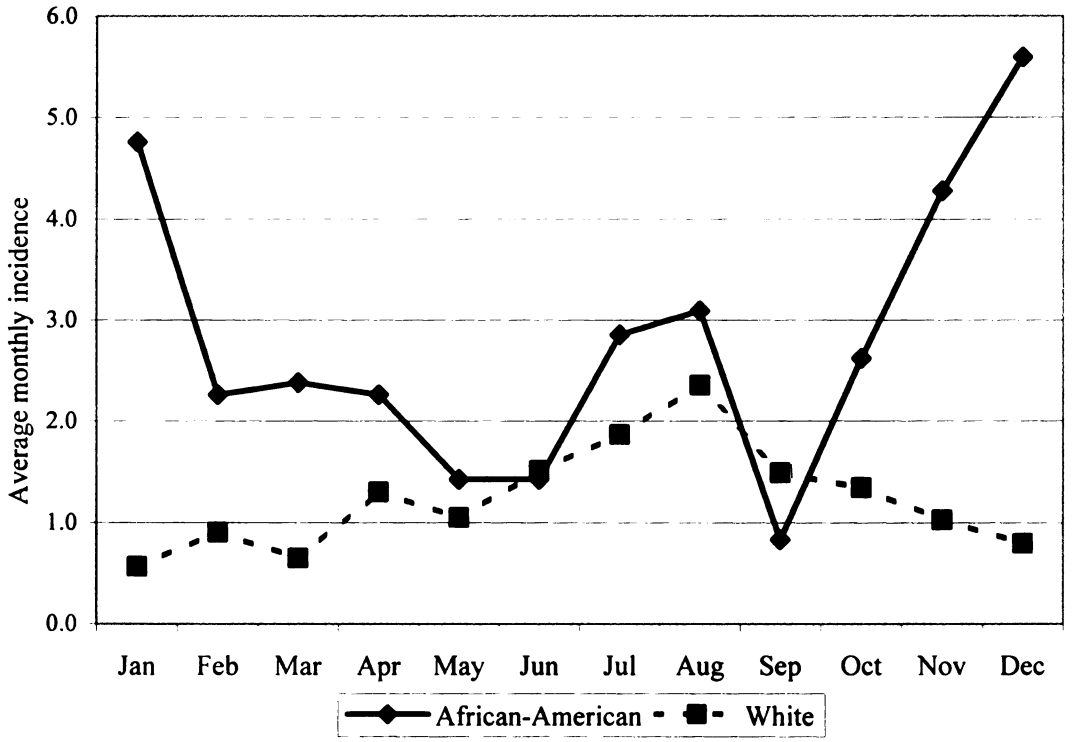
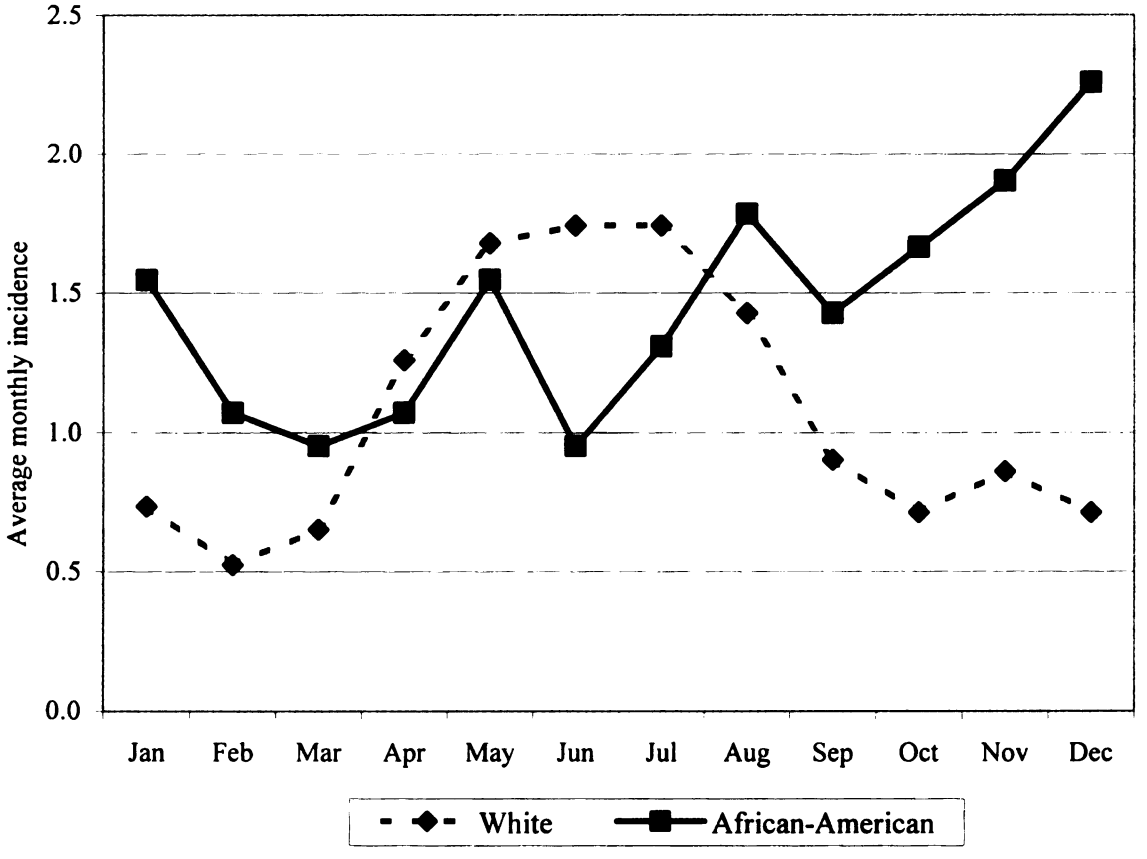


Figure 4-2. Average monthly incidence (per 100,000) of *Salmonella* Enteritidis infections by month and race, Michigan, 1995-2001



## CHAPTER FIVE

# EPIDEMIOLOGY OF INFECTIONS WITH COMMON *SALMONELLA* SEROTYPES AMONG INFANTS IN MICHIGAN, 1995-2001


## ABSTRACT

Nontyphoidal salmonellosis (NTS) is one of the major foodborne diseases in the United States. The incidence was highest among infants. The objective of this study was to determine the epidemiologic patterns of the most common *Salmonella* serotype infection among infants in Michigan from 1995 to 2001. Culture-confirmed salmonellosis cases from 1995 to 2001 at the Michigan Department of Community Health (MDCH) were analyzed. Of 6,797 cases 690 (10%) were infants aged <12 months. The average annual incidence (AAI) was 73.7/100,000. The most common serotypes were *S.* Typhimurium (25%) and *S.* Enteritidis (20%). The AAI per 100,000 of *S.* Typhimurium and *S.* Enteritidis infections were higher among African-American compared to white infants (32.4 vs. 6.8; RR, 4.77; 95% CI, 3.24-7.01 and 20.6 vs. 6.7; RR, 3.10; 95% CI, 2.00-4.79 respectively). For African-American infants, the AAI of *S.* Typhimurium was highest at 2 to3 months and 9 to 10 months old and for *S.* Enteritidis at 3 to 4 months old. For African-American and white infants, the AAI of *S.* Typhimurium and *S.* Enteritidis were higher during the summer months. For white infants, the AAI of *S.* Typhimurium and *S.* Enteritidis decreased during the winter months where as for African-American infants the incidence markedly increased. The high incidence of *S.* Typhimurium and *S.* Enteritidis among African-American infants during the winter months may be due to improper food handling and preparation practices in African-American households

during holidays/family gatherings. This suggests the need to enhance holiday food safety education among African-American households and for further studies to determine the actual risk factors.

## INTRODUCTION

Nontyphoidal salmonellosis (NTS) is one of the major foodborne diseases in the United States (US) (Alterkruse et al., 1997; CDC, 2006a; Mead et al., 1999). In the US, NTS was associated with an estimated 1.4 million cases of foodborne illnesses annually, resulting in over 100,000-physician office visits (Voetsch et al., 2004), 16,000 hospitalizations, and nearly 600 deaths (Mead et al., 1999). The estimated annual costs of medical care and lost productivity due to foodborne salmonellosis ranged from $0.5 billion to $2.3 billion (Frenzen et al., 1999).

Most nontyphoidal *Salmonella* caused mild-to-moderate gastroenteritis, but severe infections, including bacteremia and meningitis, also occur (Pegues et al., 2005). Groups at higher risk of severe illness and death from nontyphoidal salmonellosis are infants, elderly persons, and persons with impaired immune systems (Hohmann, 2001). In particular, invasive disease and death from nontyphoidal salmonellosis occur more frequently among infants than among older children (Stutman, 1994). It has been reported that serious complications of extraintestinal nontyphoidal salmonellosis among infants include bacteremia, meningitis, arthritis and osteomyelitis (Huang et al, 1991; Nelson and Granoff, 1982; Zaidi et al, 1999).

In 2001, the incidence of nontyphoidal salmonellosis in the US was highest among infants aged <1 year (CDC, 2003a). In 2003, data from the Foodborne Diseases Active Surveillance Network (FoodNet) revealed differences between age groups in the incidence rate of nontyphoidal salmonellosis. The overall annual incidence of NTS was 14.4 cases per 100,000 population; the highest age-specific incidence was among infants aged <1 year (131.9 cases per 100,000 infants) (CDC, 2004c).

The study presented in Chapter Three of this dissertation showed that the incidence of infection with all *Salmonella*, *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport in Michigan were highest among infants aged <1 year. Specific *Salmonella* serotypes are typically associated with particular food vehicles. For example, *S.* Typhimurium was frequently associated with beef, pork and dairy products (CDC, Foodborne Outbreak Response and Surveillance Unit), *S.* Enteritidis with eggs and chicken (Kimura et al., 2004; Patrick et al., 2004), *S.* Heidelberg with chicken and eggs (Chittick et al, 2006; Hennessy et al., 2004; Layton et al., 1997), and *S.* Newport with ground beef (CDC, 2002c). Hispanic origin and race are among many demographic factors that may influence food intake behavior (USDA, ARS).

Analysis of salmonellosis surveillance data by age in months, race, and common *Salmonella* serotypes may suggest the source or mode of transmission of *Salmonella* infections among infants. The objective of this study was to determine the incidence patterns of infections with the most common *Salmonella* serotypes among infants by age in months, race and the month of occurrence.

## MATERIALS AND METHODS

Data from the Michigan Department of Community Health Salmonellosis case report forms collected from 1995 to 2001 were used in this study. All cases of common Salmonella serotypes among infants aged <1 year were used to calculate the incidence by age in month, race, and month of occurrence.. The incidence of infections with all *Salmonella* and the most common serotypes was calculated by using the population of infants in Michigan from 1995 to 2001 as the denominator (NCHS). Average annual incidence (AAI) by race was also calculated. To calculate age-specific incidences by age (in month) and race, a denominator of one-twelfth the race-specific population of infants was used (Olsen et al., 2001). The AAI of the most common serotypes by month and race was also calculated.

Poisson regression was used to compute the relative rates (RRs) and their 95% CI for determining whether there was significant difference in the AAI of infection with the most common serotypes between races. The analysis was conducted by using Proc Genmod in SAS (SAS Institute Inc. 1997).

## RESULTS

Of 6,797 culture-confirmed salmonellosis cases reported to MDCH, Bureau of Epidemiology from 1995-2001, 690 (10%) were infants aged <1 year. The average annual incidence (AAI) was 73.7/100,000. The most common serotypes were *S.* Typhimurium (25%), *S.* Enteritidis (20%), and *S.* Heidelberg (8%) (Table 5-1).

From 1995 to 2001, the incidence per 100,000 of infection with all *Salmonella* decreased by 2% for white infants (32.6 to 31.9; $\chi^2$, 2.13; *P*, 0.14), but increased by 45%

for African-African infants (82.8 to 120.2; $\chi^2$, 0.86; *P*, 0.35) (Figure 5-1). The incidence per 100,000 of *S.*Typhimurium infection increased by 60% for white infants (4.8 to 7.7; $\chi^2$, 0.71; P, 0.40) and decreased by 46% for African-American infants (36.1 to 19.6; $\chi^2$, 1.23; P, 0.27) (Figure 5-2). The incidence per 100,000 of *S.* Enteritidis infection increased by 4 % for white infants (4.7 to 4.9; $\chi^2$, 0.00; P, 0.62) and decreased by 41% for African-American infants (20.1 to 11.8; $\chi^2$, 0.54; P, 0.50) (Figure 5-2).

From 1995 to 2001, there were 160 cases of *S.* Typhimurium among infants with and AAI of 16.9/100,000. Among cases whose race was known, African-American infants had a higher AAI per 100,000 compared to white infants (32.4 vs. 6.8; RR, 4.77; 95% CI, 3.24-7.01).

For African-American infants, the AAI peaked at 2 and 10 months of age producing a sharply bimodal curve (Figure 5-5). The AAI for white infants were almost lower at every age. The AAI for African-American infants aged <6 months was higher than that of white infants (21.5 vs. 10.6/100,000; RR, 2.03; 95% CI, 1.16-3.55). The AAI for African-American infants aged 6 months and above was higher than that of white infants (16.7 vs. 7.1/100,000; RR, 2.37; 95% CI, 1.24-4.54).

For both white and African-American infants, the incidence is higher during the summer months (Figure 5-3). The incidence among white infants decreased during the winter months where as for the African-American infants the incidence increased.

There were 131 cases of *S.* Enteritidis infection among infants, with an AAI of 14.2/100,000. Among cases whose race was known, African-American infants had a significantly higher AAI per 100,000 compared to white infants (20.6 vs. 6.7; RR, 3.10; 95% CI, 2.00-4.79).

For African-American infants the AAI of *S.* Enteritidis infections peaked at 3 and 4 months of age (Figure 5-6). For white infants, the AAI were lower at all ages. The AAI for African-American infants aged <6 months was significantly higher than that of white infant (28.7 vs. 9.0/100,000; RR, 3.20; 95% CI, 1.89-5.42). The AAI for African-American infants aged 6 months and above was higher than that of white infants (13.2 vs. 4.1/100,000; RR, 3.23; 95% CI, 1.48-7.03).

For white infants, the incidence is higher during the spring and summer months and lower during the winter months (Figure 5-4). For African-American infants, the AAI was higher during the winter months and lower during the summer months.

Of 604 infant cases whose site of isolation of *Salmonella* was known, 33 cases (5%) the isolate was from blood. *Salmonella* serotypes frequently isolated from blood were *S.* Heidelberg (21%), *S.* Typhimurium (15%) and *S.* Montevideo (12%). Of the 33 cases, 7 (21%) were from infants 2 months of age. Among infants whose race was known, seven were African-Americans (4.1/100,000) and 11 were whites (2.7/100,000) (RR, 2.7; 95% CI, 1.05-6.97).

## DISCUSSION

In this study, the AAI of infections with all *Salmonella* among infants in Michigan from 1995-2001 was 73.7/100,000. From 1997 to 2001, the AAI of *Salmonella* infections in the FoodNet sites was 122.4/100,000 (CDC, FoodNet Reports), in the United States was 130.31/100,000 (CDC, Summary of Notifiable Diseases), and in Michigan was 71.8/100,000. The most common *Salmonella* serotypes in this study were *S.* Typhimurium and *S.* Enteritidis.

*Salmonella* Typhimurium can be found in intestinal tract of many food animals such as cattle, pigs, chickens, and turkey (Wray and Wray, 2000). Thus, beef, pork, chicken, and turkey meat may be contaminated with this serotype during slaughter (Rigney et al., 2004). *Salmonella* Enteritidis is more strongly associated with chicken and eggs (Kimura et al., 2004; Mishu et al., 1994). Chicken meat may be contaminated with this serotype during slaughter and eggs are known to be contaminated with *S.* Enteritidis via transovarian transmission (Rigney et al., 2004; Thiagarajan et al., 1994).

This study shows that where as the incidence of infection with *S.* Typhimurium and *S.* Enteritidis among African-American infants decreased in 2001, the incidence of infection with all *Salmonella* increased. This was due to increased in the incidence of non-Typhimurium and non-Enteritidis such as *S.* Heidelberg, *S.* Newport and *S.* Java.

This study shows that even though the incidence of *S.* Typhimurium and *S.* Enteritidis among African-American infants is decreasing, the AAI of infection with these serotypes over the seven-year study period was significantly higher among African-Americans compared to white infants. The high incidence of *S.* Typhimurium and *S.* Enteritids infections among African-American infants is contributed by the high

incidence among the African-American infants aged 2-4 months and 9-10 months. For the African-American infants, the AAI of *S.* Typhimurium and *S.* Enteritidis were highest between 2-4 months of age, where as the AAI for white infants were almost lower at every age.

The reasons for the high incidence of *S.* Typhimurium and *S.* Enteritidis among African-American infants aged 2 to 4 months are not known. Since infants age to 2-4 months are still on a liquid diet, the high incidence may be due to transmission from infected older children or adults in the African-American households. This reason is conceivable because the USDA Continuous Survey of Food Intakes for Individuals (CSFII) showed that more African-Americans frequently consumed pork, chicken and eggs compared to whites (USDA, ARS). Improper handling, preparing and cooking of these foods may result in infection among older children and adults in African-American households. Thus, they may become the source of the infection for the infants if they do not practice proper personal hygiene. Intrafamilial transmission of *Salmonella* to infants has been previously reported (Delarocque-Astagneau et al., 1998 and 2000; Wilson et al., 1982; Rowe et al., 2004). The infants may also acquire the infection if they were given weaning foods earlier (e.g. cereal) and cross contamination occur during preparation (Rushdy et al., 1998).

The infants may also contract the infection from intrinsically contaminated formula (Anonymous 1997; CDC, 1993; Espie et al., 2005; Park et al., 2004; Usera et al., 1996) or the formula may be contaminated if a food handler who is also the caregiver does not wash her/his hands properly after handling meats or eggs, and subsequently prepares formula for the infants.

The infant may also contract the infection from the home environment such as the kitchen floor. Improper handling and preparing of meat or eggs may result in contamination of the kitchen floor. Infants may contract the infection if they are allow to crawl or play in the kitchen. The contribution of a contaminated home environment to infant salmonellosis has been previously reported (Haddock and Nocon, 1994; Schutze et al., 1999).

The AAI of *S.* Typhimurium is also higher among African-American infants aged 9-10 months compared to their white counterparts. This may be due to consumption of weaning foods that may contain undercooked meat contaminated with *S.* Typhimurium.

This study shows that the AAI of *S.* Typhimurium and *S.* Enteritidis among African-American infants increased during the winter months. This may be due to frequent preparation of foods of animal origin such as chitterlings (pork intestines), chicken, turkey and beef in the African-American households during holidays or family gatherings. Even though the infants may not consume the foods, transmission to infants may have occurred during preparation of the foods because of improper handling or from infected older children and adults who are asymptomatic or undiagnosed because of better resistance to infections. Recently, it has been reported that high number of salmonellosis cases among African-American infants in Atlanta during the winter months was associated with preparation of chitterling (pork intestine) (Georgia Department of Human Resource, 2001).

This study reveals that African-American infants had a significantly higher AAI of invasive salmonellosis compared to white infants. This may be due to lack of access to health care and distrust of health care profession among the African-American families.

Lack of access to health care among African-American families may have resulted *Salmonella* infections in the infants to progress to invasive disease. However, further studies to determine the actual risk factors should be conducted in order to enhance preventive measures because invasive salmonellosis in infants often leads to meningitis (Totan, 2001; Vaagland et al., 2004; Yang et al., 2002).

In conclusion, the high incidence of *S.* Typhimurium and *S.* Enteritidis among African-American infants aged <6 months may be due to intrafamilial transmission, contaminated infant formula or contaminated home environment. The high incidence of *S.* Typhimurium among African-American infants aged 6 months and above may be due to consumption of foods that may contain contaminated undercooked meat. The high incidence of *S.* Typhimurium and *S.* Enteritidis among African-American infants during the winter months may be due to improper handling, preparing and cooking of foods of animal origin in the African-American households during holidays or family gatherings. These results suggests the need to enhance food foodborne diseases prevention programs in African-American households and the need to conduct further studies to determine the actual risk factors for the high incidence among the African-American infants.

Table 5-1. The 15 most common *Salmonella* serotypes among infants, Michigan, 1995-2001

| Rank of total | *Salmonella* serotype | 1995 | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | Total | % |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Typhimurium | 18 | 24 | 28 | 25 | 23 | 24 | 18 | 160 | 25 |
| 2 | Enteritidis | 18 | 20 | 19 | 23 | 19 | 19 | 13 | 131 | 20 |
| 3 | Heidelberg | 5 | 7 | 10 | 8 | 7 | 7 | 7 | 51 | 8 |
| 4 | Java | 2 | 4 | 5 | 6 | 0 | 4 | 3 | 24 | 4 |
| 5 | Agona | 5 | 4 | 5 | 4 | 1 | 1 | 1 | 21 | 3 |
| 6 | Newport | 2 | 6 | 1 | 0 | 5 | 1 | 6 | 21 | 3 |
| 7 | Oranienburg | 2 | 1 | 5 | 3 | 1 | 1 | 2 | 15 | 2 |
| 8 | Muenchen | 2 | 1 | 3 | 3 | 5 | 0 | 0 | 14 | 2 |
| 9 | Infantis | 4 | 3 | 1 | 2 | 3 | 0 | 0 | 13 | 2 |
| 10 | Braenderup | 0 | 1 | 1 | 0 | 2 | 7 | 1 | 12 | 2 |
| 11 | Stanley | 8 | 2 | 1 | 0 | 0 | 1 | 0 | 12 | 2 |
| 12 | Montevideo | 1 | 0 | 1 | 2 | 1 | 4 | 2 | 11 | 2 |
| 13 | Saintpaul | 2 | 2 | 1 | 1 | 0 | 3 | 2 | 11 | 2 |
| 14 | Thompson | 2 | 0 | 3 | 2 | 1 | 1 | 0 | 9 | 1 |
| 15 | Hadar | 1 | 2 | 2 | 2 | 0 | 0 | 1 | 8 | 1 |
| | Other serotypes | 21 | 21 | 13 | 20 | 12 | 12 | 15 | 114 | 18 |
| | Unknown serotypes | 0 | 4 | 0 | 0 | 1 | 5 | 4 | 14 | 2 |
| | Total | 93 | 102 | 99 | 101 | 81 | 90 | 75 | 641 | 100 |

Figure 5-1. Incidence of infection with all *Salmonella* among infants by race, Michigan, 1995-2001

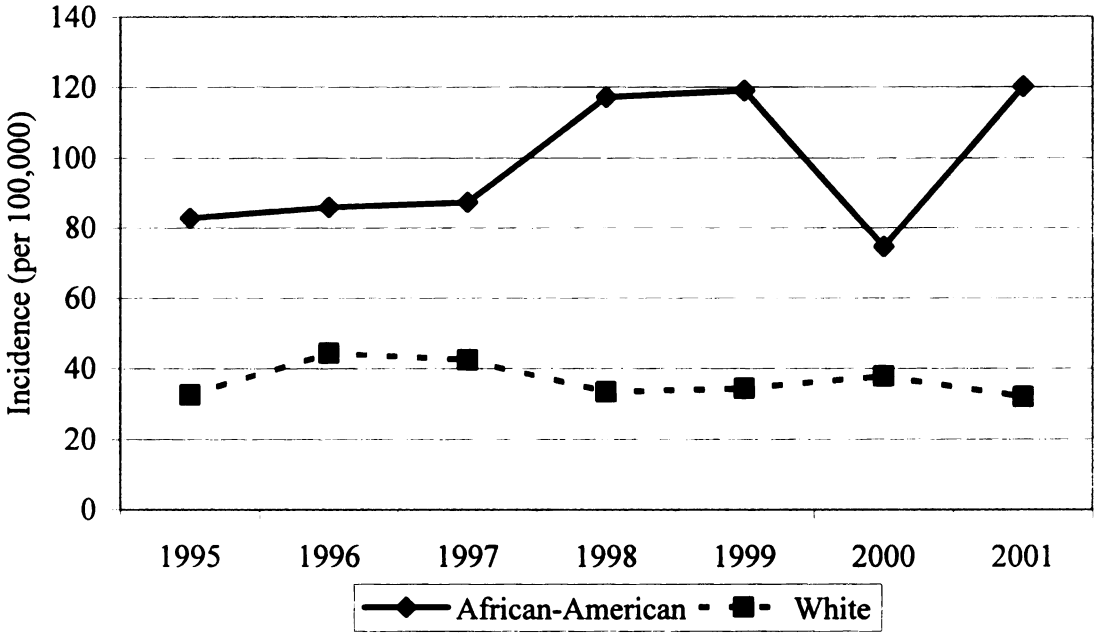


Figure 5-2. Incidence of *Salmonella* Typhimurium and *Salmonella* Enteritidis infections among infants by race, Michigan, 1995-2001

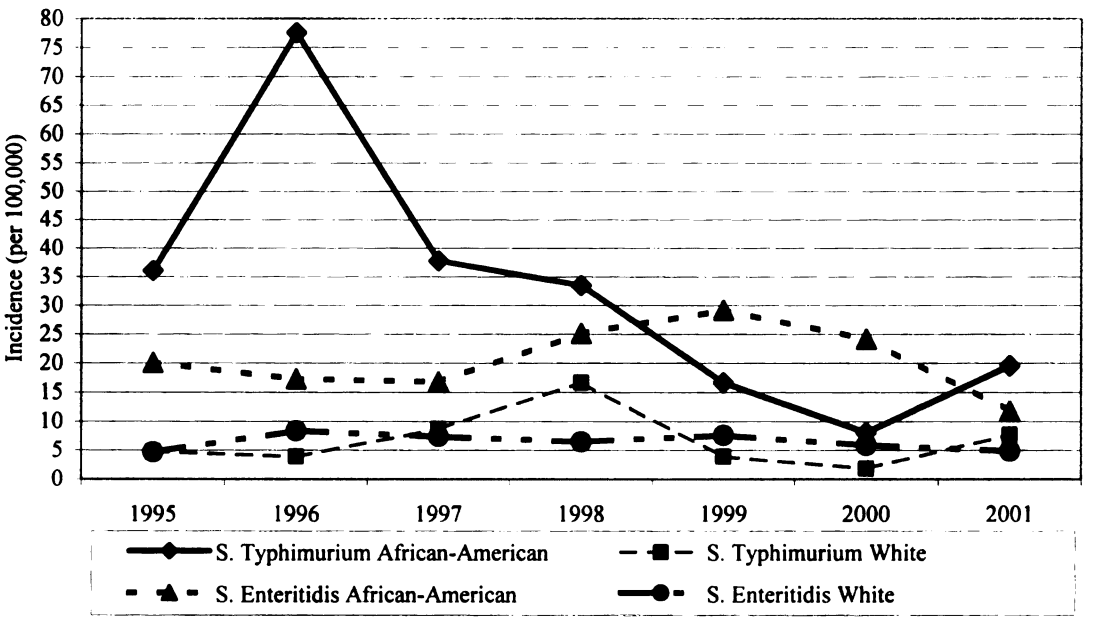


Figure 5-3. Average monthly incidence (per 100,000) of *Salmonella* Typhimurium infection among infants by month and race, Michigan, 1995-2001

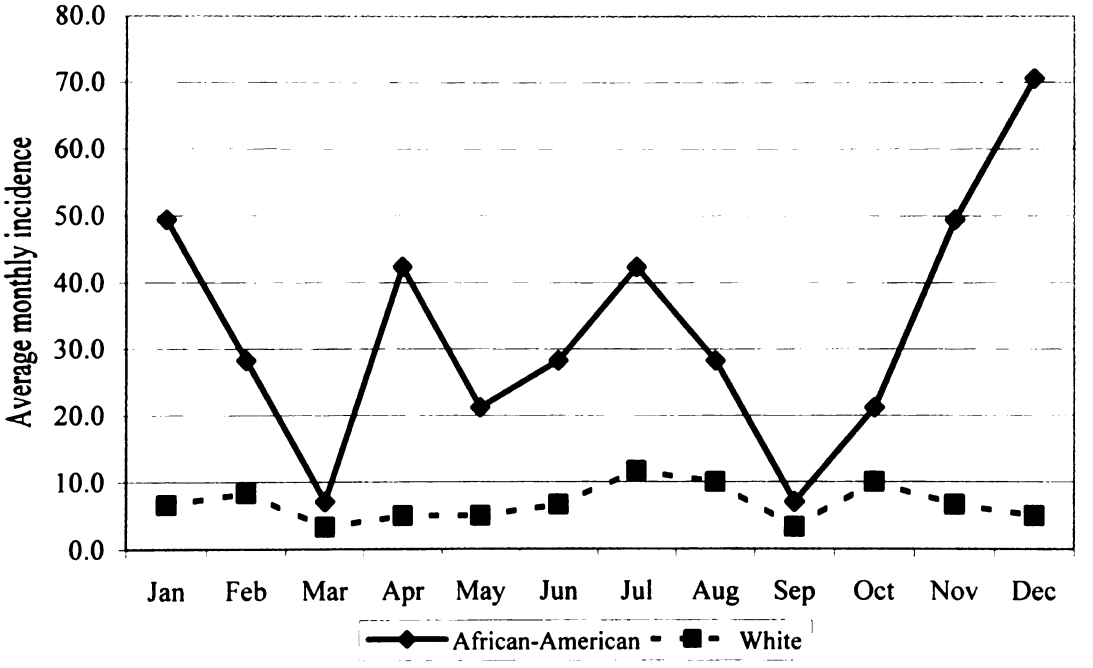


Figure 5-4. Average monthly incidence (per 100,000) of *Salmonella* Enteritidis infections among infants by month and race, Michigan, 1995-2001

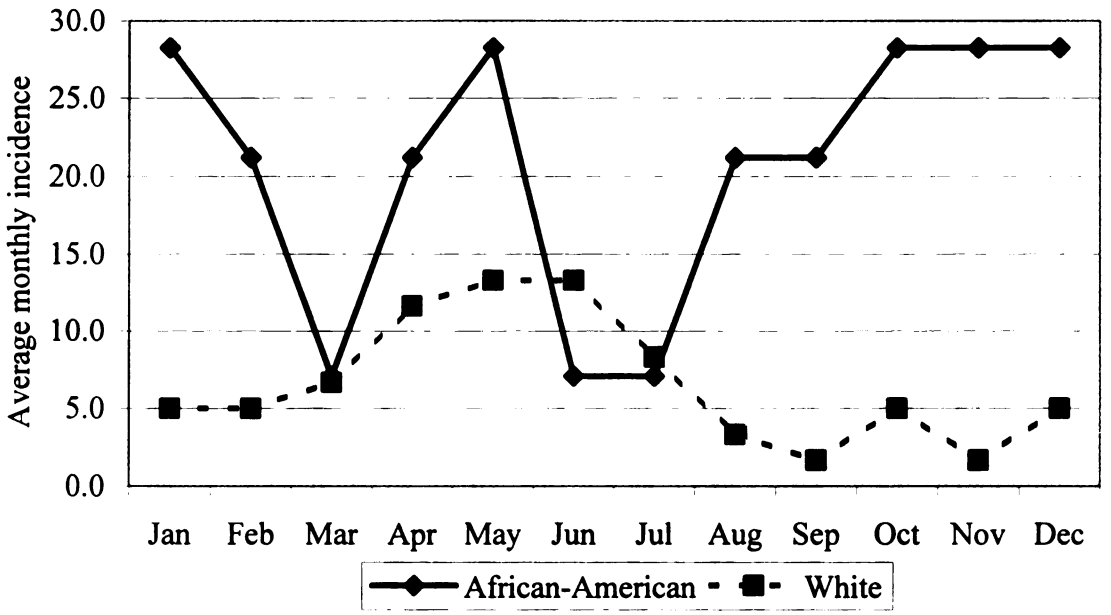
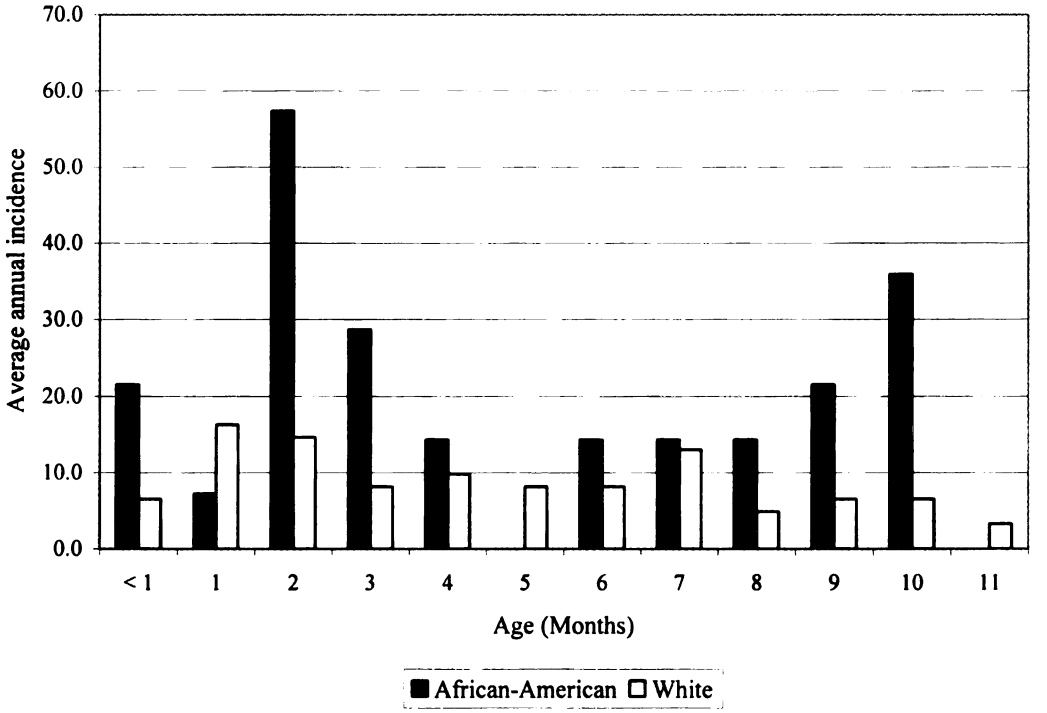


Figure 5-5. Average annual incidence (per 100,000) of *Salmonella* Typhimurium infection among African-American and White infants by month of age, Michigan, 1995-2001

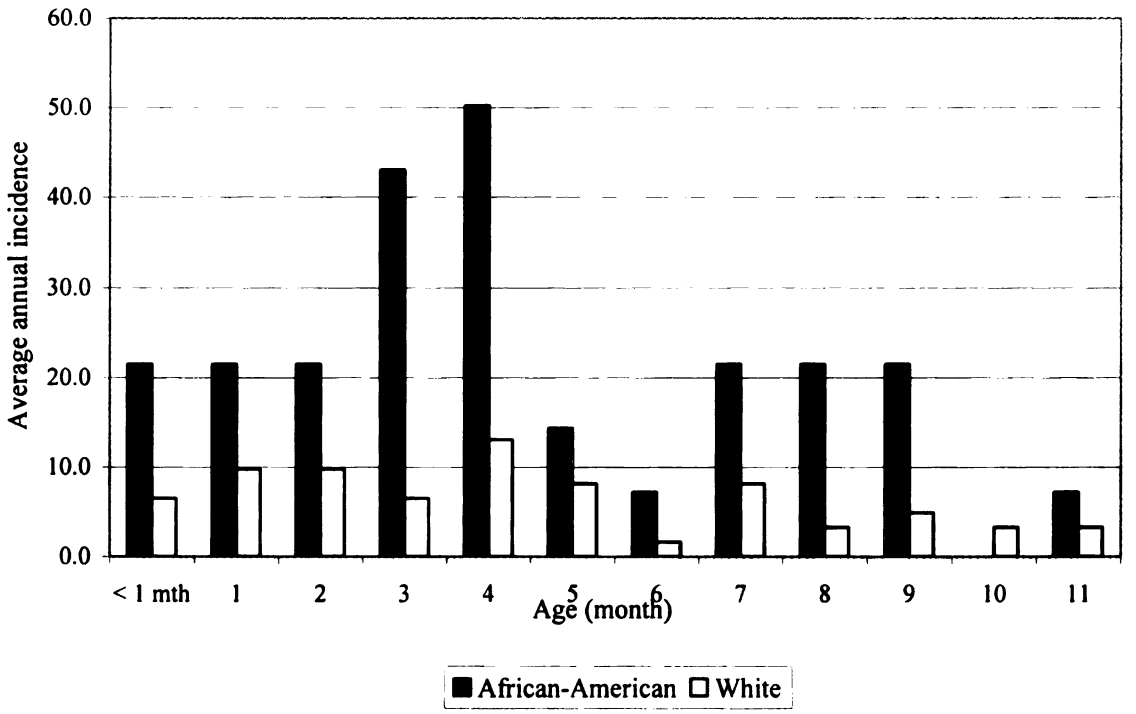



**Note:**

At 2 months of age, AAI for African-American was significantly higher than Whites (RR, 3.91; 95% CI, 1.51-10.14).

At 10 months of age, AAI for African-American was significantly higher than Whites (RR, 5.50; 95% CI, 1.48-20.49).

Figure 5-6. Average annual incidence (per 100,000) of *Salmonella* Enteritidis infection among African-American and White infants by month of age, Michigan, 1995-2001




**Note:**

At 3 months of age, AAI for African-American was significantly higher than whites (RR, 6.60; 95% CI, 1.86-23.40).

At 4 months of age, AAI for African-American was significantly higher than whites (RR, 3.85; 95% CI, 1.40-10.62).

# CHAPTER SIX

## RACE AND SOCIOECONOMIC STATUS IN *SALMONELLA* ENTERITIDIS INFECTIONS IN WAYNE COUNTY, MICHIGAN, 1995-2001


### ABSTRACT

*Salmonella* Enteritidis is one of the leading causes of foodborne salmonellosis in the United State (US). To determine whether socioeconomic disparities exist in the incidence of *S.* Enteritidis infection in Wayne County, Michigan, the address of *S.* Enteritidis cases from salmonellosis surveillance data base at the Michigan Department of Community Health (MDCH) were matched to their corresponding census tract (CT). For each CT, information is available regarding the number of persons below federal poverty line. The CTs were categorized according to percent of persons below poverty level: 0-4.9, 5-9.9, 10-19.9, and 20.0-100.0%. The average annual incidence (AAI) of *S.* Enteritidis infection among Whites and African-Americans, stratified by the CT categories was calculated. Incidence of *S.* Enteritidis in Wayne County was highest in people residing in the poorest CTs (20-100% persons below poverty line). Among Whites, the AAI in poorest CTs was higher than the least poor CTs (<5% persons below poverty level) but not different significantly. The same results were found among African-Americans. However, within the poorest CTs, the incidence among African-Americans was significantly higher than among Whites. The high incidence of *S.* Enteritidis infection in poor CTs suggests that further studies are needed to determine the underlying contributing factors.

## INTRODUCTION

In the past three decades, *S.* Enteritidis emerged from being a minor serovar of *Salmonella* to become one of the leading causes of foodborne illnesses in the US (Baumler et al., 2000; CDC 2003e; Guard-Petter, 2001; Herikstad et al., 2002; Mead et al., 1999; Mishu et al., 1994; Olsen et al., 2000; Patrick et al., 2004). Between 1993 and 1997, *S.* Enteritidis was responsible for higher number of foodborne gastroenteritis outbreaks and deaths than any other bacterium in the US (Olsen, et al., 2000). From 1990 to 2001, state and territorial health departments reported 677 outbreaks of *S.* Enteritidis, which accounted for 23,366 illnesses, 1,988 hospitalizations, and 33 deaths (CDC 2003e). The costs associated with *S.* Enteritidis infections in humans have been estimated to range from $150 million to $870 million annually (FSIS, 1998).

Most of the cases are associated with eating undercooked contaminated eggs (CDC, 2003e; Mishu et al., 1994; Olsen et al., 2000; Patrick et al., 2004). Recently, eating chicken has been implicated as risk factor for sporadic *S.* Enteritidis infection in the US (Kimura et al., 2004). Other host risk factors include consumption of antibiotics and antacids, extremes of age (infants, the elderly), and having a compromized immune system (Hohmann, 2001).

Another modifiable risk factor, which may influence the incidence of *S.* Enteritidis infection is socioeconomic status (SES). Socioeconomic status may provide a new focus for salmonellosis control efforts. The principal measures of SES are education, occupation, and income or combinations of these. Lower SES has been reported to be associated with increased risk for infectious diseases such as AIDs, Helicobacter pylori infection, infectious disease mortality, infectious illness in adulthood, H. Influenzae

infections, sexually transmitted diseases, and respiratory diseases (Cohen, 1999; Cohen et al., 2004; Ellen et al., 1995; Krieger et al., 2003a; Malaty and Graham, 1994; Olowokure et al., 2003; Stockwell et al., 1997).

Few studies addressing the association between low SES and incidence of foodborne diseases have been published. This is may be due to lack of socioeconomic data in most US public health surveillance systems. Recently, however, the methodology of geocoding residential addresses and using area-based socioeconomic measures (ABSM) has been shown to be useful in assessing the association between SES and health outcomes (Krieger, 2003b; http://www.hsph.harvard.edu/thegeocodingproject/). In this approach, both cases (numerators) and the catchments population (denominators) are classified by the socioeconomic characteristics of their residential area, thereby permitting calculation of rates stratified by the ABSMs (Krieger et al., 2003b). Therefore, the purpose of this study was to determine whether socioeconomic inequalities exist in the distribution of *S.* Enteritidis infection in Wayne County, Michigan from 1995 to 2001 by using ABSM.

## MATERIALS AND METHODS

### Data Sources: *Salmonella* Enteritidis cases

The study base comprised populations and areas in Wayne County, Michigan enumerated at or around the 2000 census (US Census Bureau). *Salmonella* Enteritidis data were from the salmonellosis surveillance database at the Michigan Department of Community Health (MDCH), Bureau of Epidemiology. Use of this data was approved by all relevant Community Research Institutional Review Boards (CRIRB) at Michigan

State University and MDCH. In the salmonellosis surveillance system, reporting is mandatory, and data on age, gender and race/ethnicity were obtained. We obtained the *S.* Enteritidis data for all cases recorded among residents of Wayne County from January 1, 1995 to December 21, 2001 (n = 315).

**Data sources: area-based socioeconomic measures**

We obtained 2000 census data for census tracts (CTs) from US Census Bureau, Census 2000 Summary File 3. According to the US Census, CTs on average contain 4,000 persons and are a small, relatively permanent statistical subdivision of a county. Census tracts are designed to be relatively homogenous with respect to population characteristics, economic status and living conditions.

Census tract-level measure of economic deprivation, percentage of persons below poverty, has been shown to be most sensitive to expected socioeconomic gradients in health, with the most consistent results, and maximal geocoding linkage evident for tract-level analysis (Krieger, 2003b). Percentage of persons below federally defined poverty line in census tracts in Wayne County were used as the area-based socioeconomic measures (ABSM) for this study. The federally defined poverty line is a threshold, which varies by size and age composition of the household. In Census 2000, the poverty line was based on 1999 income. In 1999, the federally defined poverty level (weighted average threshold) for a family of four was $17,029 (US Census Bureau, poverty). Poverty areas are federally defined as regions where 20% or more of the population is below the US poverty line (Krieger et al., 2003a). Percentage of persons below poverty line by census tracts in Wayne County was obtained from US Census Bureau, Census

2000 Summary File 3, Dataset P87 (poverty status in 1999 by age), P159A (poverty status in 1999 by age, White alone), and P159B (poverty status in 1999 by age, Black alone) (US Census Bureau). This data were downloaded from Census Bureau, USA, American Factfinder (www. http://factfinder.census.gov). All cases with valid addresses were matched to their corresponding census tract with ArcView 8.3 (ESRI, GIS and mapping software).

**Data Analysis**

The census tracts in Wayne County were categorized according to percentage of persons below federal poverty line (0.0 – 4.9, 5.0 – 9.9, 10.0 – 19.9, and 20.0 – 100.0) (Krieger et al., 2003a). The number of *S.* Enteritids cases in Wayne County from 1995 to 2001 were aggregated by race and stratified by category of census tract (CT). Populations of White and African-American by categories of CT were obtained from Dataset P159A and P159B of the census 2000. The populations were multiplied by seven years. Age-adjusted average annual incidence (AAI) of *S.* Enteritidis infection for White and African-American overall and stratified by age, gender, and categories of CT were calculated. For age standardization, the year 2000 standard million population was employed (Anderson and Rosenberg, 1998).

Relative rates for comparing incidence between African-American and White populations within each CT category and comparing people living in areas with the least and most resources were computed by using Poison regression analysis in SAS (version 8.0; SAS Institute Inc., Cary, NC) (SAS Institute Inc.).

## RESULTS

Based on Census 2000, Wayne County has 620 census tracts (CTs). The total population of Wayne County was 2,061,162: 50% Non-Hispanic White, 42% Non-Hispanic African-American, and 4% Hispanic (Figure 6-1). For six CTs the population is zero. Thus, only 614 CTs were categorized according to percentage of persons below poverty line (0-4.9%, 155 CTs; 5-9.9%, 101 CTs; 10-19.9%, 109 CTs; and 20-100%, 249 CTs). The population of CT with 0-4.9% poverty level were 559,276 (27.1%) and population of CT with 20-100% poverty level were 749,321 (36.3%). As shown in Figure 6-2, more than half of White population in Wayne County lived in areas where fewer than 5% of persons lived below poverty line. In contrast, almost 70% of the African-American population lived in areas where the poverty rate was 20% or more.

From 1995 to 2001, there were 315 cases of *S.* Enteritidis infection in Wayne County. The average annual incidence was 2.2/100,000. Of these, 103 (33%) were African-Americans, 42 (13%) Whites, 1 (0.3%) Asian/Pacific Islanders, 1 (0.3%) Native-Americans, 2 (0.6%) other races, and for 166 (53%) the race variable were not reported. The overall age-adjusted AAI of *S.* Enteritidis infection among African-Americans was significantly higher than Whites (1.5 vs. 0.5/100,000; RR, 3.10; 95% CI, 2.10-4.31) (Table 1). African-American infants aged <1 year, children aged 1-4 years and the elderly aged 60 years and older had a significantly higher AAI than their White counterparts (Table 6-1).

Among the total 315 *S.* Enteritidis cases for Wayne County, the proportion successfully geocoded to census tract was 93%. The number of *S.* Enteritidis cases and AAI among Whites and African-Americans by deprivation categories of census tract

(percent persons below poverty line) is shown in Table 6-1. Whites and African-Americans residing in poorest census tracts had a significantly higher age-adjusted AAI of *S.* Enteritidis infection than population residing in least poor census tract (<5% below poverty) and the socioeconomic gradient was steeper among African-Americans compared to Whites (Figure 6-3).

## DISCUSSION

This study shows that the incidence of *S.* Enteritidis infection in Wayne County was highest among populations residing in low SES census tracts. This pattern applied to both whites and African-Americans. However, in the low SES census tracts the incidence of *S.* Enteritidis was higher among African-Americans compared to whites. The gradient of socioeconomic inequalities in *S.* Enteritidis infection in Wayne County was steeper in African-Americans than in whites.

The association between increasing incidence of poor health outcomes and low SES is well established (Cohen, 1999; Malaty and Graham, 1994), but little work has been published specifically addressing foodborne disease and SES. Persons with low SES or living in areas of poverty may be at higher risk for foodborne disease such as *S.* Enteritidis infection because of intrinsic factors making them more susceptible to clinical illness and severe outcomes such as comorbidities and low plane of nutrition. Persons with a low SES may also be exposed to *S.* Enteritidis more often or at a higher dose than wealthier populations due to unhygienic living conditions, the frequent consumption of inexpensive fast foods and limited availability of fresh food options. Limited access to

primary health care may also allow the disease progress without treatment, resulting in emergency treatment and/or more severe clinical sequela.

In this limited study, it is difficult to determine the potential contribution of race vs. SES. The USDA Continuing Survey of Food Intakes by Individuals (CSFII), which is a national scale food consumption survey, showed that African-Americans more frequently eat chicken (29.9% vs. 17.0%) and eggs (24.7% vs. 16.9%) than their White counterparts (USDA, ARS). This is because chicken and eggs are the least expensive source of protein in the market. Therefore race may be contributing to the risk of infection independent of SES. However, because SES is a rough measure and the race variable was missing for over half (53%) of the cases, our current data does not allow any definite conclusions. These results do indicate a need for further investigation into the roles of poverty, race, education, ethnicity, access to health care, and cultural eating and cooking practices on the incidence of foodborne disease. These factors coupled with a clear understanding of disease surveillance and reporting patterns could better elucidate the complex relationships suggested in this paper.

Table 6-1. Average annual incidence (per 100,000) of *S.* Enteritidis infections among Whites and African-Americans by age, gender, and percent of persons below poverty, Wayne County, Michigan, 1995-2001

| Demographic characteristics | White | | African-American | | RR (95% CI) |
|---|---|---|---|---|---|
| | Cases | AAI | Cases | AAI | |
| **Total** | 42 | 0.5 | 102 | 1.6 | 3.10 (2.10 - 4.31)* |
| **Age** | | | | | |
| < 1 | 6 | 5.5 | 25 | 25.0 | 4.5 (1.85-10.97)* |
| 1-4 | 7 | 1.6 | 23 | 5.2 | 3.20 (1.37 - 7.45)* |
| 5-9 | 2 | 0.4 | 5 | 0.8 | 2.19 (0.43 - 11.29) |
| 10-19 | 3 | 0.6 | 10 | 1.0 | 3.42 (0.94 - 12.44) |
| 20-29 | 9 | 1.6 | 8 | 0.9 | 1.02 (0.39 - 2.63) |
| 30-39 | 5 | 0.8 | 4 | 0.4 | 1.13 (0.30 - 4.21) |
| 40-49 | 3 | 0.5 | 8 | 0.9 | 3.83 (0.80 - 12.74) |
| 50-59 | 3 | 0.6 | 6 | 1.1 | 3.19 (0.80 - 12.74) |
| 60+ | 4 | 1.6 | 13 | 1.7 | 6.11 (1.99-18.73)* |
| **Gender** | | | | | |
| Male | 13 | 0.3 | 50 | 1.7 | 5.32 (2.89 - 9.79)* |
| Female | 26 | 0.6 | 45 | 1.3 | 2.12 (1.31 - 3.43)* |
| **Poverty, %** | | | | | |
| 0.0 - 4.9 | 19 | 0.4 | 0 | 0.0 | - |
| 5.0 - 9.9 | 10 | 0.5 | 5 | 1.0 | 2.02 (0.69 - 5.92) |
| 10.0 - 19.9 | 4 | 0.4 | 19 | 1.2 | 2.79 (0.95 - 8.20) |
| 20.0 - 100.0 | 6 | 0.7 | 72 | 1.7 | 2.30 (1.00 - 5.30) |

AAI: Average annual incidence
For gender and level of poverty, AAI was standardized by using the year 2000 standard million population
RR: Relative rate comparing African-American with White
* denotes statistically significant results

Figure 6-1. Population of Wayne County, Michigan by race and ethnicity, Census 2000

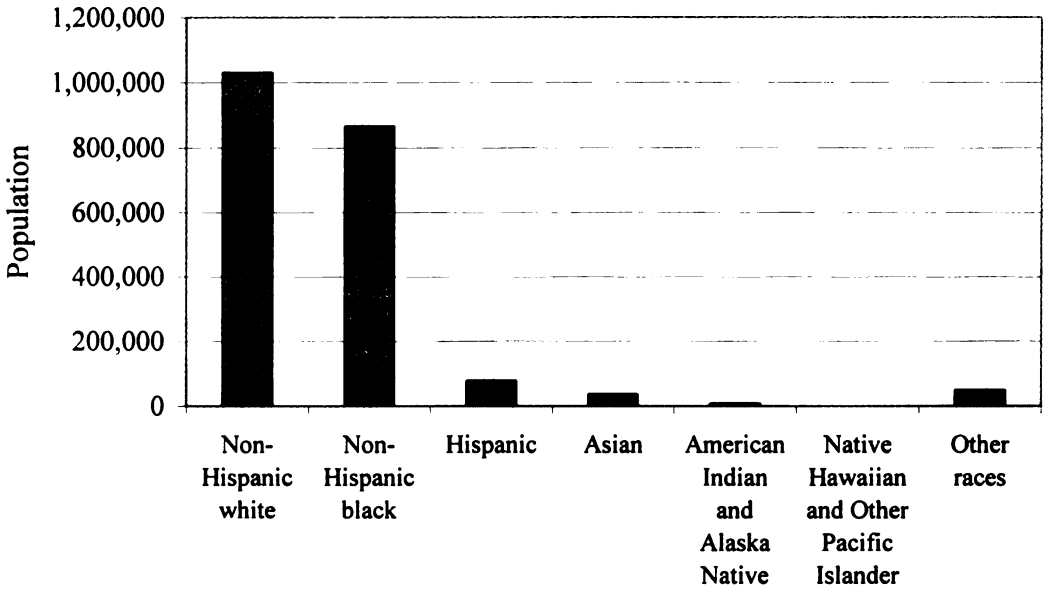


Figure 6-2. Population distribution of Wayne County by race and census tract category (percent persons below poverty)

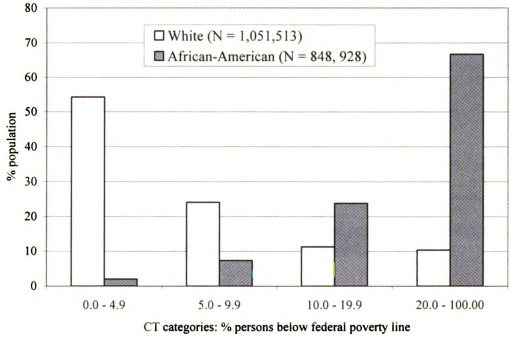
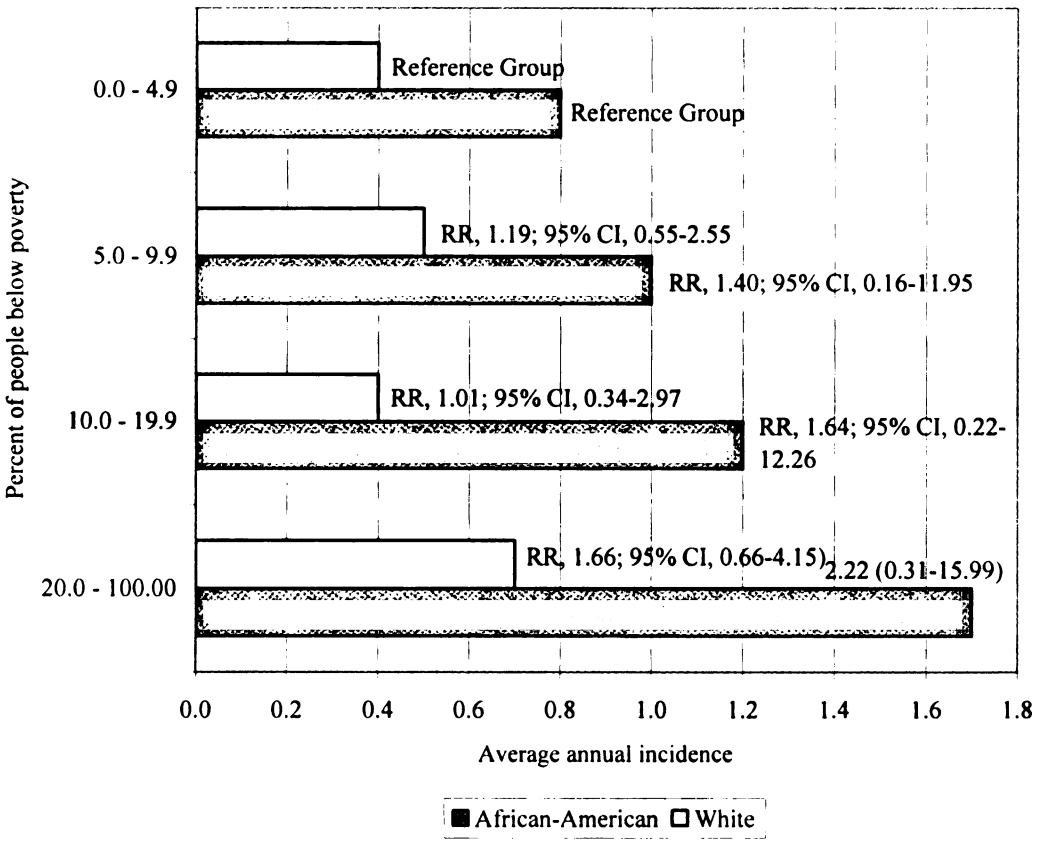


Figure 6-3. Socioeconomic gradients in age-standardized average annual incidence (per 100,000) of *S.* Enteritidis infections among Whites and African-Americans, Wayne County, Michigan, using census tract measures "percent of persons below poverty"




**Note:**

RR, relative rate, comparing incidence of *S.* Enteritidis infections between population (White and African-American) residing in poor census tract with population residing in the least poor census tract (<5% below poverty).

To determine the relative rate between African-American residing poor census tract and least poor census tract, one was added to cases of African-American residing in least poor census tract.

# CONCLUSION

Nontyphoidal *Salmonella* infections are important public health problem in the United States and worldwide (CDC, 2006a; Todd, 1997). Nontyphoidal *Salmonella* may cause gastroenteritis in people of all ages and severe invasive disease in infants, elderly persons, and immunocompromised persons (Hohmann, 2001). The most common *Salmonella* serotypes that were associated with nontyphoidal *Salmonella* gastroenteritis were *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport (CDC, 2005a). Human infections with these serotypes are often associated with consumption of chicken, eggs, pork, beef, and vegetables (CDC, Foodborne Outbreak Response and Surveillance Unit).

Hispanic origin and race are among many demographic factors that may influence food intake behavior (USDA, ARS). Thus, analysis of salmonellosis surveillance data by the most common *Salmonella* serotype that caused foodborne gastroenteritis (*S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport) and host related factors (race, age, place of residence) may reveal the trend of the infection, may identify the populations that are at higher risk for the infection, and may suggest the possible risk factors, sources and mode of transmission of the infections. Information generated from such a study can be used as a guide for the development of effective prevention plan for the foodborne diseases caused by these pathogens.

Therefore, this study was conducted to determine the epidemiologic attributes of human infection with the most common *Salmonella* serotypes in Michigan. The study

was based on the analyses of Michigan Department of Community Health Salmonellosis Surveillance data from January, 1995 to December 31, 2001.

The objectives of the study were to determine the incidence of infection with common *Salmonella* serotypes in Michigan in terms of time (year), type of county of residence (urban vs. rural counties), and demographic characteristics (age, gender, race and ethnicity) and to determine whether incidence of infection with *Salmonella* serotypes in Michigan is associated with low socioeconomic status (such as poverty and low educational attainment).

The main limitation of this study is that it was based on the analysis of passive surveillance data to estimate the incidence of salmonellosis in Michigan. The surveillance data used in the current study probably underestimates the true incidence of *Salmonella*-associated morbidity. This is because most of *Salmonella* infections are asymptomatic. When people become ill, they may not seek medical treatment because salmonellosis is usually a self-limited disease. If they seek medical treatment, physicians often do not take clinical samples for bacteriologic culture. If no sample were taken, probably the case would not be reported. The degree of underreporting of NTS has been estimated to be from 19- to 38-fold (Chalker and Blaser, 1988; Mead et al., 1999; Voetch et al., 2004). This is not unexpected given that an estimated 50 percent of persons with intestinal infectious disease would neither consult a physician nor experience a full day of restricted activity (Garthright et al., 1988).

Missing information on race and ethnicity is another major limitation in this study. This will under estimate the race- and ethnicity- specific incidence rate. Because race and ethnicity was reported for only 60% and 27% respectively of reported

*Salmonella* cases, these estimated race- and ethnicity-specific rates may be seriously biased and should be interpreted with caution. The surveillance dataset contained both sporadic and outbreak cases. Therefore large outbreak may lead to certain demographic characteristics to be more represented.

Despite the limitations, the results of this study demonstrated the magnitude of human nontyphoidal salmonellosis in Michigan. This study shows that the incidence of human infections with the most common *Salmonella* serotypes, *S.* Typhimurium and *S.* Enteritidis, significantly decreased from 1998 to 2001. However, the incidence of *S.* Newport significantly increased from 1998 to 2001. The incidence of *S.* Heidelberg remains stable. Infants aged <1 year had the highest incidence of infection with these serotypes, followed by children aged 1 to 4 years.

This study reveals that among cases whose race was known, African-Americans had a significantly higher incidence of infection with these serotypes compared with Whites. Among cases whose ethnicity was known, Hispanics had a significantly higher incidence of *S.* Enteritidis compared with Non-Hispanics. People living in poor census tracts had a significantly higher incidence of *S.* Enteritidis compared with people living in least poor census tracts.

This study also shows that the high incidence of infection with the most common *Salmonella* serotypes among African-Americans and Hispanics is driven by the high incidence among their infants, children aged 1-4 years and elderly in urban counties. Among the African-Americans, the incidence of *S.* Typhimurium and *S.* Enteritidis increased during the winter months (October-December).

Due to high percentages of the cases in this study do not have information on race, the finding regarding the African-American-white differences and Hispanics-Non-Hispanic differences in the incidence of infections with the most common *Salmonella* serotypes should be interpreted with caution.

Nevertheless, this information can be used by the state and local health departments of Michigan as a guide to enhance targeted salmonellosis prevention efforts in specific population subgroups and as an initial step for conducting further studies to determine the risk factors for the high incidence of the most common *Salmonella* serotypes among African-American and Hispanic infants, children and elderly; among people living in poor census tracts; and the emergence of *S.* Newport.

# APPENDIX A[1]

## RISK FACTORS FOR *SALMONELLA* ORANIENBURG OUTBREAK IN A NURSING HOME, MICHIGAN


### ABSTRACT

*Salmonella* Oranienburg is a relatively uncommon serotype. However, it has been associated with outbreaks in the United States and other countries. Besides gastroenteritis, it can cause vertebral osteomyelitis and paravertebral abscess, soft tissue and cartilage infection, and retroperitoneal abscess. The objectives of this study were to describe an outbreak of *Salmonella* Oranienburg in a nursing home in Michigan from June, 1994 to October, 1995 and analyze patient-level risk factors for the infection. This descriptive and case-control study was conducted in a 200-bed nursing home. For the case-control study, eighteen symptomatic residents with *S.* Oranienburg cultured from their stool were the cases and 22 asymptomatic residents with culture-negative stools were the controls. Data were abstracted from medical records for age, sex, symptoms, stool-culture result, co-morbidities, and medications. Cases in 1994 and 1995 were infrequently distributed over time, suggesting person-to-person rather than common source transmission. After adjusting for arthritis, diabetes mellitus, gastrointestinal disorders, hip fracture, and other neurological disorders, dementia remained significantly associated with infection (OR 41.9; 95% CI, 4.0-439.6). In conclusion, residents with dementia were at higher risk for *S.* Oranienburg infection in this Michigan outbreak. A


[1] A condense version of this chapter was published as Letter to the Editor in the Journal of the American Geriatrics Society (April 2006; vol. 54, No. 4:715-717).

concerted effort should be made to provide health care to institutionalized individuals diagnosed with dementia, as they may overlook basic preventive hygienic precautions. During this outbreak asymptomatic carriers may be the source of the infection that may lead to person-to-person spread. Even though a food vehicle could not be identified in this outbreak, due to ubiquity of nontyphoidal *Salmonella*, foods of animal origin, or fresh produce, should be offered with caution to residents of nursing homes. Surveillance for *Salmonella* carriers, hygienic practices in food preparation areas and personal hygiene among nursing staff and residents should be emphasized.

## INTRODUCTION

Each year an estimated 1.4 million nontyphoidal *Salmonella* infections occur in the United States (Mead et al., 1999). Most occur as a result of eating contaminated foods, particularly foods of animal origin (Bean et al., 1996; Olsen et al., 2000; Tauxe, 1991). Outbreaks of *Salmonella* infection have also been associated with eating fresh produce and person-to-person transmission (Palmer et al., 1981; Sivapalasingam et al., 1994). The most common *Salmonella* serotypes that cause infection in humans in the United States were *S.* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, and *S.* Newport (Olsen et al., 2001).

*Salmonella* Oranienburg is a relatively uncommon serotype ranking 11$^{th}$ in frequency in the United States from 1987 to 1997 (Olsen et al., 2001). This serotype has been associated with outbreaks in Germany, Denmark, Japan, Canada, Austria, United States, Norway, and Maldives Island (Allerberger et al., 2000; Deeks, 1998; Ethelberg,

2002; Gustavsen and Breen, 1984; Hedberg et al., 1992; Ray et al., 1983; Tsuji and Hamada, 1999; Werber et al., 2005).

Besides gastroenteritis, *S.* Oranienburg can cause bacteremia. Niizuma et al. found that 41% of cases due to *S.* Oranienburg infection had bacteremia compared to 5-10% of cases with infection by other nontyphoidal *Salmonella* serotypes (Niizuma et al, 2002). Infection with *S.* Oranienburg can progress to bacteremia or can cause cholecystitis, gonarthritis, osteomyelitis of the spine, femoral artery aneurysm, vertebral osteomyelitis and paravertebral abscess, soft tissue infection, and retroperitoneal abscess (Akiba et al., 2001; Hamada and Tsuji, 1999; Katsuno et al., 2003; Mjaaset et al., 1986; Okumara et al., 1999; Porcalla and Rodriguez, 2001). *Salmonella* Oranienburg can survive in dry conditions as evidenced by outbreaks in Japan, due to consumption of dry cuttlefish chips (Tsuji and Hamada, 1999). The infection can be transmitted person-to-person by the fecal-oral route (Niizuma et al., 2002).

*Salmonella* outbreaks in nursing homes have been associated with *S.* Enteritidis, *S.* Newport, *S.* Heidelberg, *S.* Hadar, and fluoroquinolone-resistant *S.* Schwarzengrund (Anand et al., 1980; Choi et al., 1990; Farley et al., 1988; Gradus and Scuh, 1988; Layton et al., 1997; Mishu et al., 1994; Olsen et al., 2001b; Standaert et al., 1994; Taylor et al., 1993). This study described an outbreak of *S.* Oranienburg in a nursing home in Michigan and analyzed patient-level risk factors for the infection.

# MATERIALS AND METHODS

## Outbreak

From June 16 to September 26, 1994, 13 laboratory-confirmed cases of *S.* Oranienburg infection were identified in a nursing home in Michigan (Figure 1). Cases 1 through 9 occurred between June 16 and July 13, and cases 10 through 13 occurred between September 3 and 26. Twelve (92%) of the cases were female. Cases ranged in age from 76 to 97 years old. Clinical signs of illness included diarrhea (69%), fever (53%), vomiting (30%), and nausea (15%). Three (23%) of the cases were asymptomatic. *Salmonella* Oranienburg was isolated from stool samples in 12 cases and from urine in one case. The duration of infection (from positive to negative culture) ranged from 6 to 52 days.

The nursing home is divided into four stations, and had approximately 200 beds with 200 total staff. All stations are in the same building. Station A and B are on the western, and C and D on the eastern part of the building. In between the eastern and western parts of the building are diversional activities, kitchen and linen area. Cases number 2, 6, 10, 12, and 13 were in station A. Case number 11 was in station B. Cases number 5, 7, and 9 were in station C. Cases number 1, 3, 4, and 8 were in station D. Cases number 1 and 4, 2 and 6, and 5 and 7 were roommates.

All cases were suffering from preexisting chronic illness; the most common diagnoses were cerebrovascular accident (CVA) and dementia. All cases were taking several different medications as part of their daily routine. All cases were mobile in wheel chairs. Most cases were on a soft diet with pureed foods. Two additional cases

were detected in November, resulting in a total of 15 cases in 1994. Case number 14 and 15 were in station D.

On July 8, 1994 the food service facilities at the nursing home were inspected by a certified food service sanitarian. The inspection included an interview with the food service manager and her assistant; taking food histories from the menu for the weeks of June 12 through June 25, 1994; taking food acceptance records of four residents who were positive for *S.* Oranienburg infection; physical inspection of the kitchen and food holding facilities, and taking food samples for bacterial culture. The inspection revealed that the kitchen was well run and *Salmonella* was not found in any food sources.

From January to October 1995, however, 75 more cases of *S.* Oranienburg infection were detected (Figure 1). Stool culturing conducted in February, March and May yielded 15, 34 and 3 cases respectively. Four culture-confirmed diagnoses occurred in nursing home staff: one in March and three in May.

**Case-Control Study**

Our case-control study tried to determine resident-level risk factors for the infection. Cases were defined as residents of the nursing home who had diarrhea and *S.* Oranienburg was isolated from their stool samples between June, 1994 and October, 1995. Controls were the nursing home residents who were asymptomatic and stool culture negative for *Salmonella.* Information about cases and controls was abstracted from their medical records using a standardized data collection form. Information collected included demographic data, the length of stay, use of antimicrobial agents,

gastrointestinal symptoms, results of laboratory cultures, underlying illnesses and routine medication.

**Statistical analysis**

Mann-Whitney U test was used to compare group means for nonparametric data. Differences in proportions between cases and controls were first compared using $\chi 2$ or Fisher's exact test, and an odds ratio with a 95% confidence interval (CI) was determined. Logistic regression was used to adjust simultaneously for potentially confounding factors. EpiInfo 2004 (version 3.3; Centers for Disease Control and Prevention, Atlanta, GA) was used for all calculations.

## RESULTS

**Demographic and Clinical characteristics**

Data collection forms of 28 residents whose stool cultures were positive for *S.* Oranienburg and 22 residents whose stool cultures were negative for *Salmonella* were received. Of the 28 residents, 18 met the case definition and were included as cases in our case-control study. The 18 cases did not differ significantly from the 22 controls with respect to sex, race and age (Table 1). All cases had diarrhea and abdominal cramps; 15 (83%) had fever.

**Epidemic curve**

The number of cases by month of infection in 1994 and 1995 are shown in Figure 1. Lack of clustering in time suggests person-to-person transmission rather than common source.

**Potential risk factors: univariate analysis**

Arthritis, atrial fibrillation, CVA, dementia, diabetes mellitus, gastrointestinal tract disorder, hip fracture, and other neurological disorder were more common among cases than controls (Table 1). However, only dementia had a strong and significant association with *S.* Oranienburg infection (odds ratio [OR], 12.50; 95% confidence interval [CI], 2.23 – 70.18) (Table 1). No routine medications were associated with the infection.

**Multivariate analysis using logistic regression**

Variables addressing co-morbidities and routine medications with an OR>1 from univariate analysis was evaluated for the effect of confounding by using an unconditional logistic regression model. Variables with OR >1 from logistic regression analysis were further evaluated in the final model. After adjusting for the analgesic used, arthritis, diabetes mellitus, gastrointestinal disorder, hip fracture, and other mental disorder, dementia remained significantly associated with the infection (OR 41.9; 95% CI, 4.0 – 439.6) (Table 2).

## DISCUSSION

Approximately 5% of persons 65 years of age and 20% of persons of 85 years or older are in nursing homes (Buzby, 2002). Nursing home residents are particularly vulnerable to *Salmonella* infection and other foodborne diseases because of age-related decreases in immune function, underlying illnesses, nutritionally acquired immunodeficiency syndrome, diminished physiological functions, immobility, decreased gastric acidity, and treatments involving antimicrobial agents (Smith, 1998). Reduced

gastric acid acidity can be due to disease, such as gastric achlorhydria, and medication, such as taking antacids and H2 antagonists (Roccaforte, 1994). Decreased intestinal motility can also predisposed the elderly to gastrointestinal infection (Roccaforte, 1994). Taking opiate analgesics and anticholinergics can decrease gastrointestinal motility.

Residents of nursing homes also face a higher risk of more severe outcomes from *Salmonella* infection. Case-fatality rates from specific foodborne pathogens were 10-100 times higher for nursing home residents than the general United States population (Buzby, 2002). Of 52 foodborne diseases outbreaks with a known cause in nursing homes from 1975 to 1987 in the United States, 27 (52%) were caused by *Salmonella*, causing illness in 1004 persons (36%) and death in 38 (81%) (Levin et al., 1987).

In this case-control study we found that residents of the nursing home with dementia were at higher risk of acquiring *S.* Oranienburg infections than controls without dementia. Generally, residents with dementia had a variety of presentations ranging from memory loss in the early stage, to complete dependence for their activities of daily living (ADLs), and may be less able to observe basic self-care and hygiene and were often bedridden with urinary and bowel incontinence (Dharmarajan and Ugalino, 2003).

Residents with CVA and other neurological disorders such as Alzheimer's, Parkinson's Disease, and depression may also be at higher risk for *Salmonella* infection due to their inability to provide basic self-care and hygiene. Residents with gastrointestinal disorders such as peptic ulcer disease and GERD also may be at higher risk due to taking medications such as antacids and H2 antagonists, which reduced gastric acidity (Roccaforte, 1994). Residents with arthritis and orthopedic disorders may be at

higher risk due to frequent taking of opiate analgesics, which can reduce intestinal motility (Roccaforte, 1994).

As in many *Salmonella* outbreaks in nursing homes (Taylor et al., 1993; Gradus and Scuh, 1988; Choi et al., 1990) the food vehicles or sources for *S.* Oranienburg infection in this outbreak could not be identified. In outbreaks where sources of infection could be identified, the most common vehicles were undercooked eggs or food containing eggs (Tribe et al., 2002). Other sources of infection included pureed food, food containing egg whites, and an asymptomatic infected cook (Holtby and Stenson, 1992; Layton et al., 1997; Standaert et al., 1994;).

Point source, person-to-person spread or endemic transmission not associated with a single source have been described in *Salmonella* outbreaks in nursing homes (Farley et al., 1988; Layton et al., 1997; Olsen et al., 2001b; Standaert et al., 1994). The epidemic curve in this outbreak suggests person-to-person transmission. Person-to-person spread by asymptomatic residents may be possible because three (23%) of the 13 cases detected between June 16 and September 26, 1994 were asymptomatic despite positive stool culture. However, the index case could not be ascertained. The infection may have been spread among nursing home residents because of close confinement with others who may be ill or carriers. Asymptomatic carriers and ill and convalescent patients have been suggested as a source of infection in salmonellosis outbreak in a nursing home (Gradus and Scuh, 1988; Olsen et al., 2001b).

The nursing home staff also may have played a role in the person-to-person transmission of *S.* Oranienburg to susceptible residents because four asymptomatic staff were also positive on stool culture during the mass stool screening in February, March

and May 1995. However, the staff may have contracted the infection from the ill residents.

Foods of animal origin, such as poultry, eggs, meat, milk and other dairy products, fresh produce, such as alfalfa sprouts, cilantro, tomatoes, cantaloupes, and unpasteurized orange juice have been implicated in *Salmonella* outbreaks in the United States (Campbell et al., 2001; CDC, 2002e; CDC, 2005d; Krause et al., 2001; Tauxe, 1991; Winthrop et al., 2003). Food vehicles that have been implicated in *S.* Oranienburg outbreaks in non-institutional settings included chocolate, cuttlefish chips, cantaloupe, cheese, black pepper and salad containing carrots and peas in a creamy base (Allerberger et al., 2000; Deeks, 1998; Ethelberg, 2002; Gustavsen and Breen, 1984; Hedberg et al., 1992; Ray et al., 1983; Tsuji and Hamada, 1999; Werber et al., 2005).

Even though the food vehicle could not be identified in this outbreak, it is conceivable that foods, that were pureed, may have been the potential source for the transmission of *S.* Oranienburg in the nursing home. Furthermore, improper food handling and inadequate preparation may have allowed multiplication of the bacteria. Cooked food might have been cross-contaminated with raw food items (e.g. in blender or meat grinder) (Matsui et al., 2004), or contamination from asymptomatic food handlers who have poor hygiene (Dryden et al., 1994; Hedberg et al., 1991).

A limitation of this study was the small number of cases that resulted in wide confidence intervals, particularly for dementia. However, in a case-control study of *Salmonella* infection in a nursing home, Olsen et al, 2001b used 5 cases and 13 controls and reported that cases were more likely than controls to have taken fluoroquinolones (OR, 22.0; 95% CI, 1.06 to 1177; $P$=0.02).

In conclusion, nursing home residents with dementia were at higher risk of contracting *Salmonella* infection in this Michigan outbreak. Therefore nursing home residents with dementia may need an elevated level of attention to prevent exposure to *Salmonella*. During this outbreak asymptomatic carriers may have been the source of the infection that lead to person-to-person spread. Even though food vehicles could not be identified during this outbreak, known sources for *Salmonella*, such as foods of animal origin and fresh produce, should be offered with caution to residents of nursing homes. All kitchen equipment used to process raw or potentially high-risk food items must be disassembled, cleaned, and sanitized properly after use. Cooked food should not be returned to utensils that still contain raw food items and hands and food preparation surfaces should be washed thoroughly. Furthermore, surveillance for *Salmonella* carriers among residents and employees should be considered, and personal hygiene among residents and hygienic practices by nursing staff should be emphasized.

Table 1. Univariate analysis of characteristics of cases with *Salmonella* Oranienburg infection and controls in a nursing home, Michigan, 1994-1995

| Characteristics | Patients (n=18) | Controls (n=22) | Odd ratio for *S.* Oranienburg infection (95% CI | *P*-value |
|---|---|---|---|---|
| Female sex - no. (%) | 14 (78) | 15 (68) | 1.63 (0.39-6.81) | 0.38 |
| Age yr | | | | |
| Mean | 85 | 80 | | 0.17 |
| Range | 71-97 | 60-93 | | |
| White race - no. (%) | 16 (89) | 20 (91) | 1.25 (0.16 - 9.88) | 0.62 |
| Underlying diseases – no. (%) | | | | |
| Anemia | 0 (0) | 4 (18) | undefined | 0.08 |
| Arthritis | 5 (28) | 5 (23) | 1.31 (0.25 - 6.85 | 0.73 |
| Atrial fibrillation | 4 (22) | 4 (18) | 1.29 (0.27 - 6.07) | 0.75 |
| Congestive heart failure | 3 (17) | 6 (27) | 0.53 (0.08 - 3.09) | 0.48 |
| Cerebrovascular accident | 4 (22) | 4 (18) | 1.29 (0.21 - 7.74) | 1.00 |
| Dementia | 10 (56) | 2 (9) | 12.50 (2.23 - 70.18) | 0.004 |
| Diabetes mellitus | 5 (28) | 5 (23) | 1.31 (0.25 - 6.85) | 0.73 |
| Gastrointestinal tract disorder | 4 (22) | 3 (14) | 1.81 (0.27 - 12.55) | 0.68 |
| Hip fracture | 2 (11) | 1 (5) | 2.63 (0.16 - 80.71) | 0.58 |
| Hypertension | 2 (11) | 11 (50) | 0.13 (0.02 - 0.80) | 0.02 |
| Hypothyroid | 1 (6) | 2 (9) | 0.59 (0.02 - 9.54) | 1.00 |
| Other cardiovascular diseases | 4 (22) | 14 (64) | 0.16 (0.14 - 0.88) | 0.02 |
| Other neurological disorder | 6 (33) | 4 (18) | 2.25 (0.43 - 12.41) | 0.30 |
| Other musculo skeletal disorder | 4 (22) | 7 (39) | 0.61 (0.12 - 3.11) | 0.72 |
| Respiratory problems | 2 (11) | 7 (32) | 0.27 (0.03 - 1.80) | 0.15 |

Table 1 (contd')

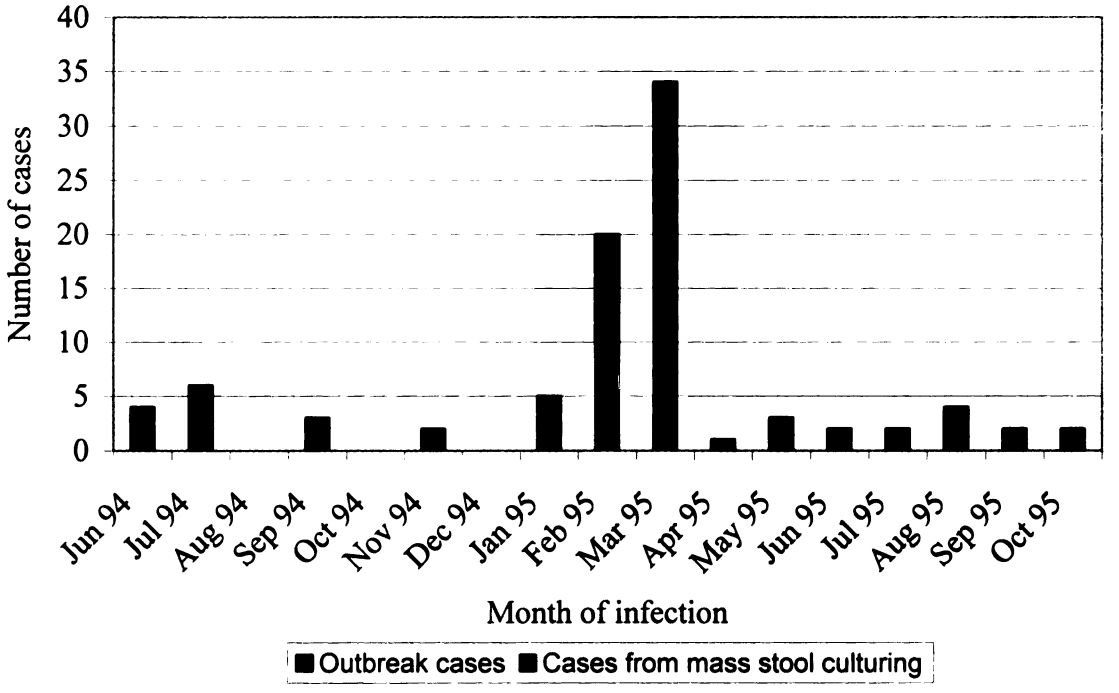
| Characteristics | Patients (n=18) | Controls (n=22) | Odd ratio for *S.* Oranienburg infection (95% CI) | *P*-value |
|---|---|---|---|---|
| Routine medication – no. (%) | | | | |
| Analgesic | 12 (67) | 8 (36) | 3.50 (0.79 - 16.26) | 0.11 |
| Antidepressive medications | 7 (39) | 6 (27) | 1.7 (0.37 - 7.94) | 0.66 |
| Antibiotics | 2 (11) | 5 (23) | 0.43 (0.05 - 3.08) | 0.43 |
| Cardiovascular drugs | 11 (61) | 16 (73) | 0.59 (0.13 - 2.71) | 0.66 |
| Antacids | 4 (22) | 7 (32) | 0.61 (0.12 - 3.11) | 0.72 |
| Nonsteroid anti-inflammatory drugs | 3 (17) | 7 (32) | 0.43 (0.07 - 2.40) | 0.46 |
| Prednisolone | 0 (0) | 2 (9) | 0 (0.00 - 5.18) | 0.49 |

Gastrointestinal tract disorder: colitis, chronic diarrhea, diverticulitis, esophagitis, GERD, hiatal hernia, pancreatic cancer, peptic ulcer disease, retroperitoneal abscess, and chronic liver failure.
Other neurological disorder: Alzheimer disease, anxiety, ataxia, brain tumor, depression, multiple sclerosis, peripheral neuropathy, Parkinson's Disease, and seizure.
Other cardiovascular diseases: angina, aorticstenosis, ASHD, ASVD, CAD, CM, thrombosis, myocardial infarct, PVD
Other musculoskeletal disorder: gout, OP, RA, Spinal OP, sacrolitis, amputated leg, septic knee, spinal stenosis.

Table 2. Logistic regression analysis of characteristics of cases with *Salmonella* Oranienburg infection and controls in a nursing home, Michigan, June 1994-October 1995

| Characteristic | OR (95% CI) | *P* value |
|---|---|---|
| Underlying diseases | | |
| Arthritis | 5.0 (0.5-50.6) | 0.18 |
| Dementia | 41.9 (4.0-439.6) | 0.0018 |
| Diabetes mellitus | 7.2 (0.5-99.9) | 0.14 |
| GIT disorder | 3.7 (0.2-58.6) | 0.36 |
| Hip fracture | 2.2 (0.04-112.4) | 0.67 |
| Other neurological disorder | 6.4 (0.7-61.8) | 0.11 |
| Routine medication | | |
| Analgesic used | 3.9 (0.6-24.4) | 0.14 |

Figure 1. Number of cases of *Salmonella* Oranienburg infection in a nursing home, Michigan, June 1994-October 1995



# APPENDIX B[1]

## ASSESSING EVOLUTION OF VIRULENCE AND GENETIC RELATEDNESS OF *SALMONELLA* ENTERITIDIS ISOLATES FROM HUMAN AND NON-HUMAN SOURCES


## ABSTRACT

*Salmonella* Enteritidis is among the leading causes of foodborne illness in the United States. To assess evolution in virulence and clonal relatedness among *S.* Enteritidis isolates from human and non-human sources, 353 selected *S.* Enteritidis isolates collected over a time that spans the observed emergence of this pathogen (1978-2004) were tested for attachment and invasion assay using HEp-2 cells. A select number of these isolates were tested by pulsed-field gel electrophoresis (PFGE) and multilocus enzyme electrophoresis (MLEE). Attachment-positive strains were common among mice, chicken, eggs and humans isolates indicating that attachment is a crucial property for *S.* Enteritidis. Invasion-positive strains were common among isolates from mice (100%), followed by humans (58%), and chicken (49%). By PFGE, using Xba I restriction enzyme, 34 isolates were grouped into 11 distinct PFGE types (PFT), designated PFT1 to PFT11. The most prominent was PFT1 (18 of 34 isolates, 53%). All mouse, 60%, 57%, and 33% egg, human, and chicken isolates respectively belong to PFT1. By MLEE, 150 isolates were grouped into 21 electrophoretic types (ET). The most prominent was ET-3 (110 of 150 isolates, 73%). All mouse, 97%, 72%, and 68% egg, chicken, and human


[1] Part of this chapter (or data here in) was published as Clonal Structure and Variation in Virulence of *Salmonella* Enteritidis Isolated from Mice, Chickens, and Humans. A. Mahdi Saeed, Seth T. Walk, Mokhtar Arshad, Thomas S. Whittam. J AOAC International. 2006; 89 (2):504-511.

isolates respectively belong to ET-3. Results of this study suggest that ET-3 is the ancestoral origin of most *S.* Enteritidis isolates. However, other ETs may have evolved, perhaps due to adaptation of the organisms in different hosts.

## INTRODUCTION

In the past three decades, *S.* Enteritidis emerged from being a minor serovar of *Salmonella* to become among the leading causes of foodborne illnesses in the United States (Baumler et al., 2000; CDC 2003e; Guard-Petter, 2001; Herikstad et al., 2002; Mead et al., 1999; Mishu et al., 1994). Between 1993 and 1997, *S.* Enteritidis was responsible for higher number of foodborne gastroenteritis outbreaks and deaths than any other bacterium in the United States (Olsen, et al., 2000). From 1990 to 2001, state and territorial health departments reported 677 outbreaks of *S.* Enteritidis, which accounted for 23,366 illnesses, 1,988 hospitalizations, and 33 deaths (CDC 2003e). The costs associated with *S.* Enteritidis infections in humans have been estimated to range from $150 million to $870 million annually (FSIS, 1998). Most of the cases are associated with eating contaminated eggs (CDC, 2003e; Olsen et al., 2000; Patrick et al., 2004). The United States table egg industry produced 67.3 billion eggs in 1998 (USDA, 1999). It is estimated that 1 in 20,000 eggs produced in the United States is contaminated with *S.* Enteritidis (Ebel and Schlosser, 2000). Thus, every year, more than 3 million eggs in the United States are potentially contaminated.

Unsolved questions regarding the epidemiology of *S.* Enteritidis include why it has emerged in the last 30 years as a major *Salmonella* serovar in industrialized countries and why it is associated with egg products (Baumler et al., 2000; Guard-Peter, 2001).

Other *Salmonella* serotypes, including *S.* Typhimurium, infect chickens and may cause human infection when contaminated cracked eggs and other poultry products are consumed. In contrast, *S.* Enteritidis contaminates the contents of intact eggs and is the major egg-associated human pathogen (Baumler et al., 2000; Keller et al., 1997; Mead et al., 1999; SE Pilot Project. 1995). This is because *S.* Enteritidis colonizes the ovarian tissue and oviducts of chickens and subsequently contaminates eggs as they form (Gast and Beard, 1990a and 1990b, Humphrey et al., 1991a and 1991b; Shivaprasad et al., 1990). Keller et al. showed previously that clinical strains of both *S.* Enteritidis and *S.* Typhimurium colonized the tissues of hen reproductive tracts and forming eggs. However, all of the *S.* Typhimurium and the majority of the *S.* Enteritidis were killed by the time eggs were laid (Kellet et al., 1995; Keller et al., 1997). This study suggested that the ability of *S.* Enteritidis to survive in eggs is crucial for the transmission of this bacterium and may have contributed to the emergence of *S.* Enteritidis as a major cause of human salmonellosis in industrialized countries.

*Salmonella* Enteritidis pandemics involve interactions of the bacteria with multiple environments: the hen house, the bird, the egg, and the human host (Guard-Peter, 2001). Risk factor for egg contamination is the presence of resident infected mouse population, which is a rich source of organ-invasive *S.* Enteritidis (Davies and Ray 1995; Guard-Peter et al., 1997; Henzler and Opitz, 1992 and 1999). Factors that may have resulted in the dramatic increase in the incidence of *S.* Enteritidis in the past 4 decades include: change in farm practices such as molting strategies, eradication of competing *Salmonella* strains (*S.* Pullorum and *S.* Gallinarum), and virulence evolution of the pathogens (Baumler et al., 2000; Rabsch et al., 2000; Saeed et al., 2006).

Because of its ability to contaminate the internal contents of eggs and increased frequency of human outbreaks, it is conceivable that *S.* Enteritidis has an enhanced virulence in animals and humans. The objectives of this study were to assess the evolution of virulence and the genetic/clonal relationship between *S.* Enteritidis isolates from human and non-human sources over the past 25 years in the Midwestern United States, including Michigan.

For these objectives, attempt was made to test the hypothesis that there is no difference in the proportion of attachment and invasion positive *S.* Enteritidis strains from human and non-human sources and there is no clonal relationship between *S.* Enteritidis isolates from human and non-human sources.

## MATERIALS AND METHODS

### *Salmonella* Enteritidis isolates

*Salmonella* Enteritidis isolates collected over a time that spans the observed emergence of this pathogen (1978-2004) (Truchanowicz *et al.*, 1970) were examined. Included are 353 isolates that were recently collected from animals and humans (Table 1). All isolates were confirmed biochemically and serologically at the National Veterinary Service Laboratories (Ames, IA) and were stored at –80°C in tryptic soy broth (TSB) containing 15% glycerol. Three *S.* Enteritidis strains from the *Salmonella* reference collection B (SARB) (Boyd *et al.*, 1993) were obtained from E. Fidelma Boyd (National University of Ireland - Cork) and served as genetic control strains.

**Refreshing the frozen isolates**

The frozen isolates were inoculated into TSB and incubated at 37°C for 24 to 48 hours. One loop full of the broth was inoculated onto XLT4 agar and incubated for 24 h at 37°C. One black colony was inoculated into triple sugar iron agar (TSIA) slant and incubated for 24 h at 37°C. *Salmonella* growth resulted in black butt and pink slant to the TSIA. Small amount of bacterial growth from the slant was subjected for slide agglutination test using D1 poly O *Salmonella* antigen. The isolates were tested for attachment and invasion assay by using HEp-2 tissue culture cell model, pulsed-field gel electrophoresis (PFGE) and multiloccus enzyme electrophoresis (MLEE).

**Assessing evolution of virulence of *S.* Enteritidis from human and non human sources**

The virulence phenotypes include tissue culture assays of attachment and invasion using HEp-2 tissue culture cell model.

**1) Attachment assay with HEp-2 cells**

Attachment assays were conducted according to standard operating procedure (SOP) of qualitative attachment assay in our laboratory (Appendix C).

**2) Invasion assay with HEp-2 cells**

Invasion assays were conducted according to SOP of qualitative invasion assay in our laboratory (Appendix D).

**Determining clonal (genetic) relatedness of *S.* Enteritidis isolates from human and non-human sources**

Clonal relatedness among select number of isolates was determined by PFGE using XbaI restriction enzymes and multilocus analysis of conserved genes with housekeeping functions.

**1) Pulse-field gel electrophoresis**

Pulse-field gel electrophoresis was conducted at the Michigan Department of Community Health (MDCH) bureau of laboratories as described by PulseNet laboratories (http://www.cdc.gov/pulsenet/protocols.htm) (Appendix E).

**2) Multilocus enzyme electrophoresis (MLEE)**

This procedure was performed by Seth Walk, graduate student, in the Microbial Evolution Laboratory of Dr. Thomas Whittam at the National Food Safety and Toxicology Center of Michigan State University.

To make quantitative estimates about the genetic relationships among isolates, the electromorphs with alleles at an enzyme locus and electrophoretic types (ETs) was equated with multilocus genotypes. The assumption is that isolates with the same ET owe their genotypic similarity to recent descent from a common ancestral cell; that is, they are members of a naturally occurring bacterial clone.

Enzyme extraction, gel electrophoresis, and specific enzyme staining was carried out as described in Selander *et al.* (Selander *et al.*, 1986). Briefly, lysates (whole cell

enzymes) were extracted from overnight nutrient broth cultures and frozen at –80° C. Lysate samples were individually loaded and electrophoresed under non-denaturing conditions in a buffered starch gel matrix at the appropriate concentration for the particular enzymes being stained. Gel slices were incubated in enzyme specific staining solutions and fixed for analysis.

The mobilities of 18 housekeeping enzymes were recoded for 150 isolates spanning the years and sources of the collection (Table 1). Mobilities were scored relative to previously characterized *Salmonella* strains from the SARB collection. The enzymes used in this study were: ADH (alcohol dehydrogenase), THD (threonine dehydrogenase), SKD (shikimate dehydrogenase), G6P (glucose-6-phosphate), MPI (mannose phosphate isomerase), GLUD (glutamate dehydrogenase), MDH (malate dehydrogenase), NSP (nucleoside phosphorylase), PEP (peptidase), GOT (glutamic oxalacetic transaminase), CAK (carbamylate kinase), AK (adenosine kinase), MPD (mannitol-1-phosphate dehydrogenase), PGD (6-phosphogluconate dehydrogenase), PGI (phosphoglucose isomerase), IDH (isocitrate dehydrogenase), ACO (aconitase), LDH (lactate dehydrogenase). Electrophoretic mobility variants (electromorphs) were assigned scores in side-by side comparisons relative to the SARB standards. Electromorphs were equated with alleles at the corresponding enzyme locus based on the assumption that each mobility difference reflects at least one amino acid replacement in the protein. Electrophoretic types (ETs) were assigned to isolates with indistinguishable allele profiles across all enzyme loci studied. Allele frequencies and ETs were used to estimate population genetic parameters as described in Selander *et al.* (Selander *et al.*, 1986).

**Statistical analysis**

To test the hypothesis that there is no significant difference in the proportion of positive attachment and invasion between *S.* Enteritidis isolates from human and non-human sources, a chi-square test was used (Fleis et al., 2001).

## RESULTS

**Attachment assay**

Of 156 human isolates, 140 (90%) attached to HEp-2 cells (Figure 1). Compared to human isolates, the percentage of attachment-positive was significantly lower for egg isolates (52% vs. 90%; $\chi^2 = 17.74$, $P < 0.001$), did not differ significantly for chicken isolates (98% vs. 90%; $\chi^2 = 3.31$, $P = 0.08$), mice isolates (100% vs. 90; $\chi^2 = 0.19$, $P = 0.60$), and isolates from other animal (100% vs. 90%; $\chi^2 = 0.80$, $P = 0.37$).

**Invasion Assay**

Of 159 human isolates, 93 (58%) invaded HEp-2 cells (Figure 2). Compared to human isolates, percentage of invasion-positive strains was significantly lower for environment and egg isolates respectively (11% vs. 58%; $\chi^2 = 7.76$, $P = 0.01$ and 17% vs. 58%; $\chi^2 = 18.01$, $P < 0.001$), did not differ significantly for chicken isolates (49% vs. 58%; $\chi^2 = 1.35$, $P = 0.24$), and was significantly higher for mice and other animal isolates respectively (100% vs. 58%; $\chi^2 = 5.18$, $P = 0.007$ and 88% vs. 58%; $\chi^2 = 5.15$, $P = 0.02$).

Of 28 isolates from 1978, 26 (79%) invaded HEp-2 cells (Figure 3). Compared to 1978 isolates, percentage of invasion-positive strains did not differ significantly for 1987 isolates and >year 2000 isolates respectively (50% vs. 79%; $\chi^2 = 0.85$, $P = 0.42$ and 81% vs. 79%; $\chi^2 = 0.00$, $P = 0.95$), was significantly lower for the 1990 to 1995 and 1996 to 2000 isolates respectively (50% vs. 79%; $\chi^2 = 6.89$, $P = 0.009$ and 18% vs. 79%; $\chi^2 = 18.31$, $P < 0.001$).

**Ability to Attach and Invade**

Isolates from mice and other animals have high percentage of Att+ Inv+ (Figure 5). Isolates from humans, eggs, and chicken have high percentage of Att+ only.

**Pulse-field Gel Electrophoresis**

A total of 34 *S.* Enteritidis isolates from human and non-human sources were tested by pulsed-field gel electrophoresis (PFGE) using Xba I restriction enzymes. Digestion with XbaI revealed 11 distinct PFGE types (PFT), designated PFT1 to PFT11. Eighteen isolates (53%) had distinct PFT1 and 3 (9%) had distinct PFT3 and FFT4 (Table 2). All mouse isolates and 60%, 57%, and 33% egg, human, and chicken isolates belong to PFT1.

**Multilocus enzyme electrophoresis**

A total of 150, *S.* Enteritidis isolates from human and non-human sources were tested by MLEE of 18 housekeeping enzymes. MLEE grouped the isolates into 21 electrophoretic types (ETs) (Table 3). The most common were ET-3 (110 or 73%)

followed by ET-22 (15 or 10%). All mouse, 97%, 72%, and 68% egg, chicken, and human isolates belong to ET-3.

## DISCUSSION

The results of the attachment assay show that high percentages of *S.* Enteritidis isolates from mice, chicken, eggs and humans and other animals attached to HEp-2 cells. The results indicate that attachment is a crucial property of the human pathogenic *S.* Enteritidis strains. The frequencies of attachment positives phenotypes are common among *S.* Enteritidis recovered from mice, chicken and humans. Significantly lower frequencies of positive attachment egg isolates compared to human isolates may be due to unsuitability of the HEp-2 cells. This is because it has been shown that high proportion of attachment positive *S.* Enteritidis from eggs when chicken ovarian granulosa cells were used (Thiagarajant et al., 1996).

The ability of *S.* Enteritidis to invade the intestinal epithelium is a necessary step for the initial phase of the infection. The hen house provides a number of niches (organic material, rodents and insects) in which bacteria survive and multiply (Guard-Peter, 2001). This study shows that environmental samples from chicken house harbor low frequency of invasive *S.* Enteritidis strains. As the cycles of *S.* Enteritidis take place between the environment and mice, more invasive *S.* Enteritidis will multiply in the mice. This theory is supported by this study where all mice isolates were invasive. This association has been suggested by others (Henzler and Opitz, 1992). The reason for high number of invasive *S.* Enteritidis from mice may be due to their effort to overcome barriers in the new host, the mice, especially the immune system such as gut-associated lymphoid

nodules, B-lymphocyte, B cells and antibody, and peripheral lymphoid filter (lymph nodes and macrophages) (Baumler et al., 1998). This may lead to expression of *S.* Enteritidis invasion gene, such as *inv*A, *inv*B, *inv*C gene in the mice population (Baumler et al., 1998; Porter and Curtis III, 1997). Further more it has been shown that mouse isolates from chicken farms were capable of producing unprecedented amount of high molecular weight (HMW) lipopolysaccharide (LPS), which enhanced its migration capability (Guard-Peter et al., 1997).

Our results shows that chicken also harbor considerably high number of invasive *S.* Enteritidis. The presence of resident infected mouse population, which is a rich source of organ-invasive *S.* Enteritidis, is a risk factor for egg contamination (Davies and Ray 1995; Guard-Peter et al., 1997; Henzler and Opitz, 1992). Mice had easy access to feed bins in chicken houses and the feed bins were contaminated with their droppings (Kinder et al., 1996). Laying hens exposed to mice droppings contaminated with *S.* Enteritidis may likely become infected with the organisms or such an exposure could exacerbate a previous infection. Exposure to *S.* Enteritidis as a chick or pullet may result in subsequent shedding of the bacterium by a laying hen and the production of infected eggs as layers (Henzler and Opitz, 1999).

The results show that eggs harbor low number of invasive *S.* Enteritidis. The reason is largely unknown. It is possible that *S.* Enteritidis change its gene expression in eggs in order to transverse the immune system in the avian host, and to survive in the hostile egg albumen environment. Recently, it has been reported that the presence of *yafD* gene in *S.* Enteritidis, enhanced resistance to egg albumen, while disruption of this

gene rendered the organism more susceptible to egg albumen (Lu et al., 2003). The yafD is homologous to members of an exonuclease-endonuclease-phosphatase family, including some enzymes involved in DNA repair. Thus YafD provides a survival advantage to *S.* Enteritidis in eggs by repairing DNA damage caused by egg albumen and that it may be one of the biologic determinants that contribute to the epidemiological association of *S.* Enteritidis with egg products (Lu et al., 2003).

The results show that human isolates consist of high percentages of invasive strains. Humans get the infection through the consumption of undercooked contaminated eggs or foods containing raw or undercooked eggs(Olsen et al., 2000; Patrick et al., 2004). Once in the human intestinal tract, *S.* Enteritidis have to adapt and survive in the hostile gastrointestinal environment. Once again it expresses the *inv* gene (Porter and Curtis III, 1997).

Analysis by time revealed that high percentage of *S.* Enteritidis isolated in 1978 (79%) invaded the cells. These isolates were from chick embryos. The results indicate that *S.* Enteritidis before the human epidemic (before 1980s) were already highly virulent and could invade the intestinal epithelial cells. During the years 1947 to 1958, *S.* Enteritidis was the fifth most common (3.6% of all *Salmonella* isolates) from animals in the United States, (Galton et al., 1964). This indicate that before *S.* Enteritidis epidemic in humans, which began in 1980s, *S.* Enteritidis was already circulating in the animal population, including poultry and was likely introduced into poultry houses from rodents because mice and rats captured in hen-houses frequently carried this organism (Guard-Petter et al., 1997; Henzler and Opitz, 1992). The results of our in vitro attachment and

invasion assay demonstrated that high percentages of *S.* Enteritidis isolate from poultry in 1978 and isolates from mice in 1991 (79% and 100% respectively) were invasive for HEp-2 cells. This finding indicates that *S.* Enteritidis circulating in poultry industry before the human epidemic already has the invasive capability. Why *S.* Enteritidis strains did not cause epidemics in humans before the 1980s may be due to the possibility that these strains were not widespread in the poultry industry before the 1980s. One hypothesis is that this niche was occupied by avian-adapted *S.* Pullorum and *S.* Gallinarum, which are endemic in poultry flocks in the early 1960s (Baumler et al., 2000). *Salmonella* Pullorum, *S.* Gallinarum and *S.* Enteritidis contain O12 antigen (a sugar backbone composed of O-polysaccharide repeating units) and the O9 antigen (a tyvelose sugar chain). Thus *S.* Pullorum and *S.* Gallinarum may have generated flock immunity against the O9-antigen at the beginning of the 20$^{th}$ century, thereby excluding *S.* Enteritidis from circulating in poultry flocks (**Baumler et al., 2000; Rabsch et al., 2000**).

Results of PFGE using XbaI enzyme reveal that most of the isolates from humans, eggs, chicken and mice belong to PFT1. The results indicate that regardless of location, time and phage type, *S.* Enteritidis isolates from humans, eggs, chicken, and mice were genetically related or clonal. The results of MLEE show that most isolates from human, egg, chicken, and mice belong to ET-3. These MLEE results are consistent with the previous findings of Boyd *et al.* (Boyd *et al.*, 1993), and indicate that most *S.* Enteritidis strains belong to a single widespread clone of which ET-3 appears to be a progenitor. The results of PFGE and MLEE are in agreement with the epidemiology of *S.* Enteritidis infections in human whereby the source of the infection are eggs laid by infected hens

and infection in hens was due to transmission from house mouse (De Buck et al., 2004; Guard-Peter, 2001; Henzler and Opitz, 1999;).

In conclusion, results of this study suggest that ET-3 is the ancestoral origin of most *S.* Enteritidis isolates. However, other ETs may have evolved, perhaps due to adaptation of the organisms in different hosts.

Table 1. Sources of *Salmonella* Enteritidis isolates by year of isolation

| Source | 1978 | 1987 | 1990 | 1991 | 1992 | 1994 | 1995 |
|---|---|---|---|---|---|---|---|
| Human | 0 | 0 | 26 | 23 | 20 | 13 | 41 |
| Chicken | 28 | 0 | 0 | 35 | 8 | 0 | 24 |
| Egg | 0 | 0 | 0 | 19 | 0 | 22 | 0 |
| Mice | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| Environment | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| Other animals | 0 | 2 | 0 | 1 | 0 | 0 | 3 |
| Total | 28 | 2 | 26 | 98 | 28 | 35 | 68 |

Table 8-1 (contd').

| Source | 1996 | 1997 | 1998 | 1999 | 2000 | 2004 | Total |
|---|---|---|---|---|---|---|---|
| Human | 0 | 31 | 2 | 0 | 0 | 24 | 180 |
| Chicken | 0 | 0 | 0 | 0 | 0 | 0 | 95 |
| Egg | 0 | 0 | 0 | 0 | 0 | 0 | 41 |
| Mice | 1 | 0 | 0 | 0 | 0 | 0 | 10 |
| Environment | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Other animals | 0 | 0 | 0 | 1 | 1 | 8 | 16 |
| Total | 1 | 31 | 2 | 1 | 1 | 32 | 353 |

Chicken isolates (1978 from chicken embryo; 1991 and 1995 from ceca of spent hens)

Other animals: Cattle (3), Dog (1), Mink (4), Mule deer (7), Sea lion (1).

Table 2. XbaI pulsed-field gel electrophoresis types of *Salmonella* Enteritidis isolates by source

| Source | XbaI PFGE pattern | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 00Sen Xb.001 | % | 2 00Sen Xb.004 | 3 04Sen Xb.002 | 4 02Sen Xb.007 | 5 05Sen Xb.007 | 6 00Sen Xb.004 |
| Human | 8 | 57 | 3 | 0 | 0 | 0 | 0 |
| Mouse | 4 | 100 | 0 | 0 | 0 | 0 | 0 |
| Egg | 3 | 60 | 0 | 0 | 0 | 2 | 0 |
| Chicken | 2 | 33 | 0 | 3 | 0 | 0 | 0 |
| Bovine | 1 | 100 | 0 | 0 | 0 | 0 | 0 |
| Mule Deer | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Environment | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| Mink | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 18 | 53 | 3 | 3 | 2 | 2 | 1 |

Table 2 (contd').

| Source | Xba I PFGE pattern | | | | | Total |
|---|---|---|---|---|---|---|
| | 7 00Sen Xb.016 | 8 02Sen Xb.001 | 9 05Sen Xb.008 | 10 05Sen Xb.009 | 11 05Sen XB.010 | |
| Human | 1 | 1 | 0 | 0 | 1 | 14 |
| Mouse | 0 | 0 | 0 | 0 | 0 | 4 |
| Egg | 0 | 0 | 0 | 0 | 0 | 5 |
| Chicken | 0 | 0 | 0 | 1 | 0 | 6 |
| Bovine | 0 | 0 | 0 | 0 | 0 | 1 |
| Mule Deer | 0 | 0 | 1 | 0 | 0 | 1 |
| Environment | 0 | 0 | 0 | 0 | 0 | 2 |
| Mink | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 1 | 1 | 1 | 1 | 1 | 34 |

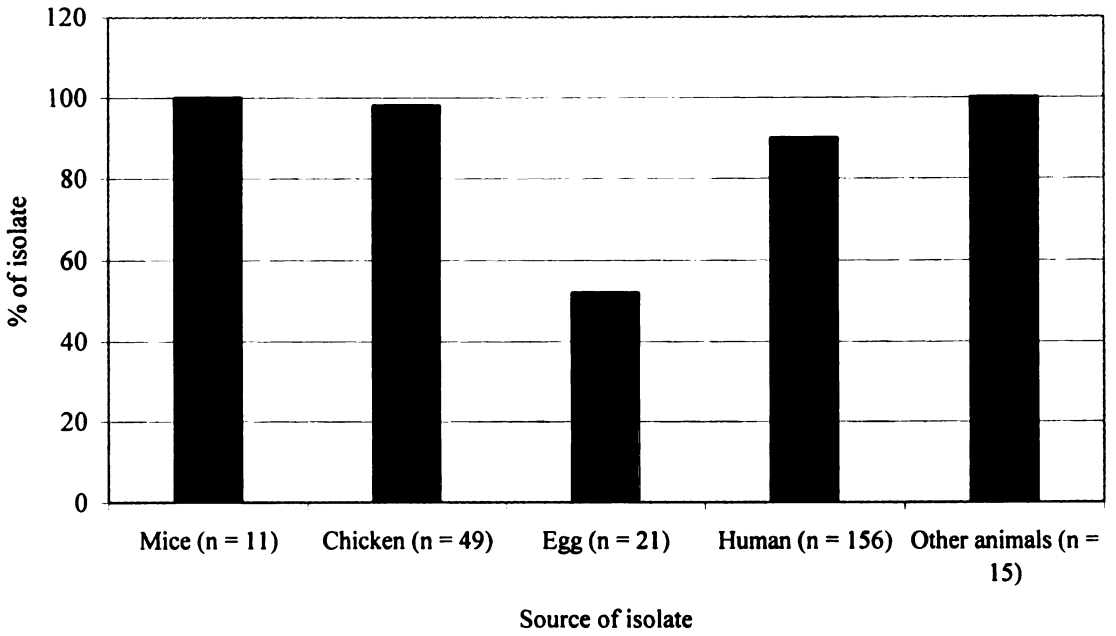
Table 3. Electrophoretic type (ET) of *Salmonella* Enteritidis isolates by source

| Source | Electrophoretic type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ET3 | % | ET4 | ET5 | ET6 | ET7 | E8 | ET9 | ET10 | ET11 | ET12 | ET13 |
| Cattle | 1 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Dog | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chicken | 33 | 72 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| Egg | 32 | 97 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Human | 34 | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| Mink | 4 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mice | 5 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mule deer | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sealion | 1 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 110 | 73 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 |

Table 3 (contd').

| Source | Electrophoretic type | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ET14 | ET15 | ET17 | ET18 | ET19 | ET20 | ET22 | ET24 | ET25 | ET26 | |
| Cattle | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 3 |
| Dog | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Chicken | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 46 |
| Egg | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 |
| Human | 0 | 0 | 3 | 0 | 0 | 0 | 5 | 1 | 2 | 2 | 50 |
| Mink | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| Mice | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Mule deer | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 7 |
| Sealion | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 1 | 1 | 3 | 2 | 1 | 1 | 15 | 1 | 2 | 2 | 150 |

Figure 1. Percentage of *Salmonella* Enteritidis isolates from human and non-human sources that attached to HEp-2 cells



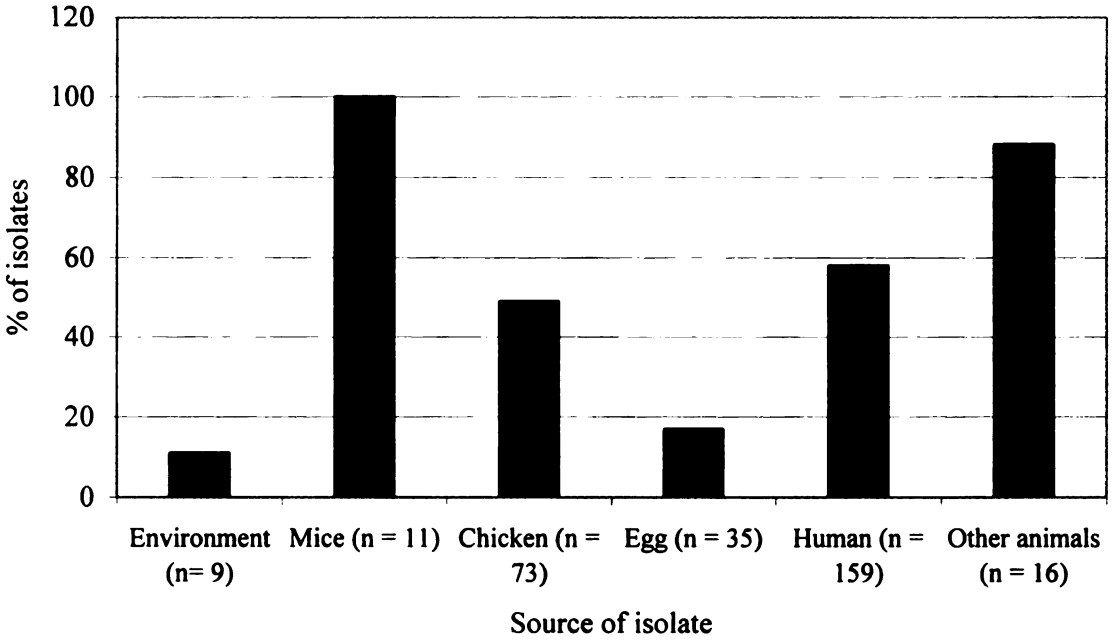

Other animals: Cattle (3), Dog (1), Mink (3), Mule deer (7), Sealion (1)

Compared to human isolates: Egg, $\chi^2 = 17.74$, $P < 0.001$;

Chicken, $\chi^2 = 3.31$, $P = 0.08$; Mice, $\chi 2 = 0.19$, $P = 0.60$;

Other animals, $\chi^2 = 0.80$, $P = 0.37$

Figure 2. Percentage of *Salmonella* Enteritidis isolates from human and non-human sources that invaded HEp-2 cells



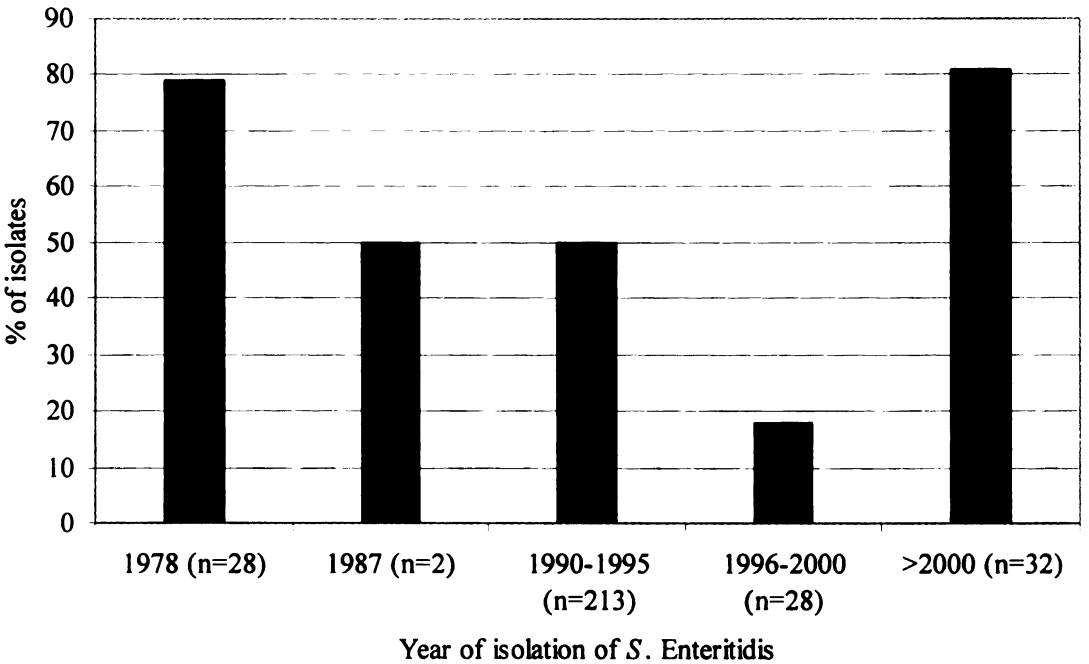

Other animals: Cattle (3), Dog (1), Mink (4), Mule deer (7), Sealion (1)

Compared to human isolates: Egg: $\chi^2 = 18.01$, $P < 0.001$;

Chicken: $\chi^2 = 1.35$, $P = 0.24$; Mice: $\chi^2 = 5.18$, $P = 0.007$;

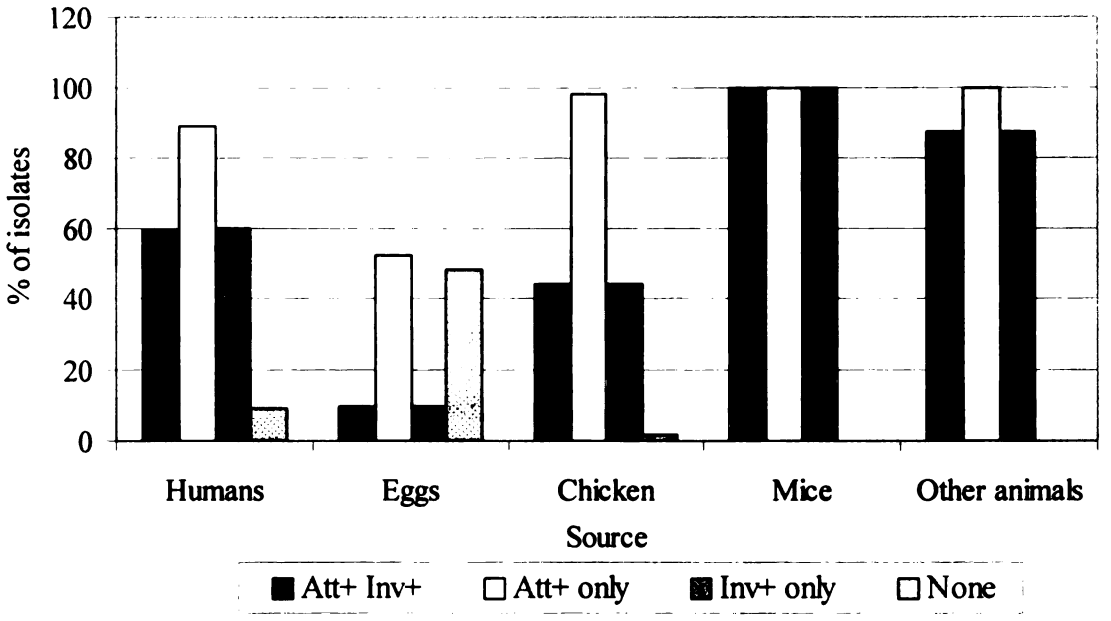
Environment ($\chi^2 = 7.76$, P = 0.001); Other animals, $\chi^2 = 5.15$, $P = 0.02$

Figure 3. Percentage of *Salmonella* Enteritidis isolates from different time of isolation that invaded HEp-2 cells




1978 isolates were from chicken embryo; 1987, dog (1) and mink (1); 1990 to 1995, human, chicken, egg, mice, environment, mink, and cattle, 1996 to 2000, human, mice and cattle; >2000, human, mule, sealion. Compared to 1978 isolates: 1987, $\chi^2 = 0.82$; $P = 0.42$; 1990-1995, $\chi^2 = 6.89$; $P = 0.009$; 1996-2000, $\chi^2 = 18.31$; $P < 0.001$; >2000, $\chi^2 = 0.00$, $P = 0.95$

Figure 4. Percentage of attachment-positive (Att+) and invasion-positive (Inv+) *Salmonella* Enteritidis strains by source of isolation



## APPENDIX C

**Standard Operating Procedures for Qualitative Attachment Assay using HEp-2 cells**

Bacterial cells attach to eukaryotic cells in different patterns. Certain receptors used for attachment are blocked by the presence of mannose. This procedure describes the incubation of different *Salmonella* Enteritidis isolates with HEp-2 cells to determine the attachment pattern of the bacterial isolate. Attachment sensitivity to mannose is determined by incubation with and without mannose.

**Process specifications:**

1. Grow Hep-2 cells and seed 24 well tissue culture (TC) trays with them as described in steps below:

1. Grow Hep-2 cells in a 75-$cm^2$ flask to 70-80% confluence.
    i. Prepare four 24 well TC treated trays and one control tray by using sterile tweezers to aseptically transfer one 22mm round glass cover slip to the bottom of each well (Cover slips are washed in acetone, rinsed twice with water, then autoclaved in a glass Petri dish). Set TC aside in Bio-safety cabinet. Add approximately 3mL of 0.05% Trypsin with EDTA (1:250) to the cell layer. Allow the Trypsin/EDTA solution to cover the cell layer for 1-2 minutes and then remove the solution. Incubate the flask at 37°C and 5% $CO_2$ for 10-20 min. Hep-2 cells should begin to slough off the flask bottom when the flask mouth is tilted to an upright position before proceeding to the next step. Add 10mL of MEM with 5% FBS (no

antibiotics). Make a uniform cell suspension using a pipette to mix cells. Add an additional 90mL of MEM with 5% FBS (no antibiotics) to the flask and mix. Transfer suspension to two 50mL plastic multi-channel pipette basins. Use a multi-channel pipette to transfer 1.0mL of cell suspension to each well of four 24 well TC Trays and 6 wells of the control tray. Five TC treated trays will accommodate 16 isolates and one control. Assay is done in triplicate. Cover trays and incubate at 37°C and 5% $CO_2$ overnight.

2. Prepare cultures of isolates by inoculating two tubes for each bacterial isolate and prepare two negative control tubes which will be treated the same as the inoculated tubes but will receive no bacteria. One in 10mL of brain heart infusion broth (BHI) and one in 10mL BHI with 1% mannose. BHI with 1% mannose is prepared by adding 1mL of a filter sterilized 10% mannose stock to 10mL of BHI. Incubate bacterial cultures at 37°C overnight.
3. The following day streak the media from one of the wells onto blood agar. Make sure the inoculating loop touches the bottom of the well before streaking with the loop. This will ensure that any non-motile bacteria that may be at the bottom of the well will be picked up by the loop. Incubate the blood agar plate overnight at 37°C in a $CO_2$ incubator, after 48 hours it should show no growth. If growth is observed the results of the entire assay are suspect.
4. Wash the HEp-2 cells in the 24 well trays with MEM three times to remove the fetal bovine serum. Remove MEM with a pasteur pipette attached to a gentle vacuum pump being careful not to touch the cover slips. Add 1mL of media to

each well. Half the trays receive plain MEM the other half receive MEM plus 1% mannose. Label the trays. [Note: A 10% stock solution of mannose in MEM is kept in the refrigerator and diluted to make the 1% mannose in MEM]. Write isolate identification name/number directly on tray covers. The assay is done in triplicate so three wells in every tray are devoted to one isolate. The isolates, grown in BHI with mannose, go in the TC trays filled with 1mL MEM plus mannose. The isolates grown in BHI without mannose go in the TC trays with only 1mL MEM.

5. Vortex each test tube of bacterial culture. Inoculate each well with 0.02mL of bacterial culture, and inoculate the control wells with 0.02mL of the control BHI. Incubate the trays for 3 hours at 37°C and 5% $CO_2$.
6. Streak the media from the control wells on to blood agar then remove media from the wells. Incubate the blood agar plate overnight at 37°C in a $CO_2$ incubator, after 48 hours it should show no growth. If growth is observed the results of the entire assay are suspect.
7. Wash cover slips in wells with 1mL of MEM three times to remove bacteria that have not attached to the Hep-2 cells. After final wash is removed, fix the cells on the cover slips by adding 1mL of absolute methanol to each well for 10-15 min. Remove methanol.
8. Stain with 1mL of Giemsa for 50 minutes. Giemsa stain is prepared by diluting 1:10 with PBS. Giemsa stock solution should be stored in a tightly sealed bottle that will protect it from light. PBS pH is reduced to 6.8 using 1 N HCl. Filter the PBS using a 60mL syringe and syringe driven filter. Add Giemsa to PBS and mix

thoroughly. Use a funnel and Whatman filter paper to filter solution a second time. The dilution should be prepared the day of the assay and any dilution that is not used that day should be discarded. Using a needle or a wood applicator stick tilt cover slip off the bottom of the well on to its side. [Note: be careful not to break cover slip – incompetence is not a virtue.] Grasp cover slip with metal hair clip (label clip with identification of bacterial isolate) taking care not to disrupt stained layer of cells. Slide the hair clip onto a wood applicator stick so the cover slip hangs down. Rinse cover slips by resting the edges of the applicator stick on the edge of a square container filled with water. Soak the cover slips for five minuets then place them in another container and let dry.

9. The next day, mount cover slips on microscope slides using paramount. Transfer identification, treatment type, and date stained to slides. Three cover slips fit on one slide.

## APPENDIX D

### Standard Operating Procedures for Qualitative Invasion Assay Using HEp-2 Cells

Some bacteria have the ability to invade eukaryotic cells. This procedure describes the incubation of different *Salmonella* Enteritidis isolates with HEp-2 cells to determine the invasiveness of bacterial isolates.

### PROCESS SPECIFICATIONS

1. Grow HEp-2 cells and seed 24 well TC trays with them as described below:
    i. Grow HEp-2 cells in a 75-$cm^2$ flask to 70-80% confluence.
    ii. Prepare four 24 well tissue culture (TC) treated trays and one control tray by using sterile tweezers to aseptically transfer one 22mm round glass cover slip to the bottom of each well (Cover slips are washed in acetone, rinsed twice with water, then autoclaved in a glass Petri dish). Set TC aside in Bio-safety cabinet. Add approximately 3mL of 0.05% Trypsin with EDTA (1:250) to the cell layer. Allow the Trypsin/EDTA solution to cover the cell layer for 1-2 minutes and then remove the solution. Incubate the flask at 37°C and 5% $CO_2$ for 10-20 min. HEp-2 cells should begin to slough off the flask bottom when the flask mouth is tilted to an upright position before proceeding to the next step. Add 10mL of MEM with 5% FBS (no antibiotics). Make a uniform cell suspension using a pipette to mix cells.

Add an additional 90mL of MEM with 5% FBS (no antibiotics) to the flask and mix. Transfer suspension to two 50mL plastic multi-channel pipette basins.

Use a multi-channel pipette to transfer 1.0mL of cell suspension to each well of four 24 well TC Trays and 3 wells of the control tray. Five TC treated trays will accommodate 32 isolates and one control. Assay is done in triplicate. Cover trays and incubate at 37°C and 5% $CO_2$ overnight.

2. For each bacterial isolate to be assayed inoculate one 10mL BHI test tube and incubate overnight at 37°C. Also include a negative control BHI tube which is treated the same as the bacterial cultures but does not actually receive bacteria.
3. The following day streak medium from one of the wells onto SBA making sure the inoculating loop touches the bottom of the well. Incubate the SBA plate in a $CO_2$ incubator at 37°C for 48 hours. Using gentle vacuum suction, remove the media from the cell layer and wash the wells twice with MEM. Remove the MEM and replace with 1mL of infection media. For 200mL infection media, combine 140mL MEM (70%), 40mL heat inactivated fetal bovine serum (20%), and 20mL BHI (10%).
4. Inoculate each well of the TC trays with 50μL of bacterial culture from vortexed 18 hour BHI broths. This assay is done in triplicate so a single isolate is inoculated in three TC wells. Incubate the TC trays for 2 hours at 37°C and 5% $CO_2$ to allow invasion to occur. Streak the media from the negative control wells onto blood agar. Remove infection media from wells. Incubate the blood agar plate overnight at 37°C, the following day it should show no growth. If growth is observed the results of the entire assay is suspect. Wash the cover slips two times with MEM (no additions) to remove excessive extra cellular bacteria.

5. Prepare the antibiotic media add 1mL (MEM + 10%FBS + 0.3 mg/mL lysozyme (from 100 X stock in PBS, filter sterilized) + 100 μg/ml gentamicin) to each well.

**Components of Invasion Media**

| | 50mL total | 100mL total |
|---|---|---|
| MEM | 44mL | 88mL |
| FBS | 5mL | 10mL |
| 100 X lysozyme | 0.5mL | 1mL |
| 10mg/ml gentamicin | 0.5mL | 1mL |

6. Incubate 2 hours 37°C and 5% $CO_2$ to allow the antibiotic time to kill extracellular *Salmonella*. Remove antibiotic media and wash cover slips twice with MEM. After final wash is removed, fix the cells on the cover slips by adding 1mL of absolute methanol to each well for 10-15 min. Remove methanol.
7. Stain with 1mL of Giemsa for 50 minutes. Giemsa stain is prepared by diluting 1:10 with PBS. Giemsa stock solution should be stored in a tightly sealed bottle that will protect it from light. PBS pH is reduced to 6.8 using 1 N HCl. Filter the PBS using a 60mL syringe and syringe driven filter. Add Giemsa to PBS and mix thoroughly. Use a funnel and Whatman filter paper to filter solution a second time. The dilution should be prepared the day of the assay and any dilution that is not used that day should be discarded.
8. Using a needle or a wood applicator stick tilt cover slip off the bottom of the well on to its side. Grasp cover slip with metal hair clip (label clip with identification

of bacterial isolate) taking care not to disrupt stained layer of cells. Slide the hair clip onto a wood applicator stick so the cover slip hangs down.

9. Rinse cover slips by resting the edges of the applicator stick on the edge of a square container filled with water. Soak the cover slips for five minuets then place them in another container and let dry. The next day, mount cover slips on microscope slides using paramount. Transfer identification, treatment type and date stained to slides. Three cover slips fit on one slide.

## APPENDIX E

# ONE-DAY STANDARDIZED LABORATORY PROTOCOL FOR MOLECULAR SUBTYPING OF NON-TYPHOIDAL *SALMONELLA* SEROTYPES BY PULSED FIELD GEL ELECTROPHORESIS (PFGE)

(Source: The National Molecular Subtyping Network (Pulsenet) for Foodborne Diseases, CDC, available at: http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf)

**Preparation Of PFGE Plugs From Agar Cultures**

Day 0

Streak an isolated colony from test cultures to Trypticase Soy Agar with 5% defibrinated sheep blood (TSA-SB) plates (or comparable media) for confluent growth; stab or streak small screw cap tubes of TSA, HIA, or similar medium, using the same inoculating needle/loop. This will ensure that the same colony can be retested if necessary. Incubate cultures at 37°C for 14-18 h.

Day 1

1. Turn on shaker water bath or incubator (54°C), stationary water baths (55- 60°C) and spectrophotometer (or equivalent instrument such as the Dade Microscan Turbidity meter or bioMérieux Vitek colorimeter).

2. Prepare TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0)1 as follows:

10 ml of 1 M Tris, pH 8.0

2 ml of 0.5 M EDTA, pH 8.0

Dilute to 1000 ml with sterile Ultrapure (Reagent Grade Type 1) water

Note: The TE Buffer is used to make the plug agarose and also to wash lysed PFGE plugs.

3. Prepare 1% SeaKem Gold:1% SDS agarose in TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0) for PFGE plugs as follows:

a. Weigh 0.50 g (or 0.25 g) SeaKem Gold (SKG) into 250 ml screw-cap flask.
b. Add 47.0 ml (or 23.5 ml) TE Buffer; swirl gently to disperse agarose.
c. Remove cap, cover loosely with clear film, and microwave for 30-sec; mix gently and repeat for 10-sec intervals until agarose is completely dissolved. Place flask in 55-60°C water bath for 5 minutes before adding SDS.
d. Add 2.5 ml (or 1.25 ml) of 20% SDS (pre-heated to 55°C) and mix well.
e. Recap flask and return to 55- 60°C water bath until ready to use.

SAFETY WARNING: Use heat-resistant gloves when handling hot flasks after microwaving.

Note: SeaKem Gold agarose works well for making PFGE plugs because it provides added strength to the plugs that are cast in reusable plug molds, minimizing breakage of plugs during the lysis and washing steps. The time and temperature needed to completely dissolve the agarose is dependent on the specifications of the microwave used, and will have to be determined empirically in each laboratory.

4. Label small tubes (12-mm x 75-mm Falcon tubes or equivalent) with culture numbers.

5. Prepare Cell Suspension Buffer (100 mM Tris:100 mM EDTA, pH 8.0) as follows:

10 ml of 1 M Tris, pH 8.0

20 ml of 0.5 M EDTA, pH 8.0

Dilute to 100 ml with sterile Ultrapure (Reagent Grade Type 1) water.

6. Transfer ≈2 ml of Cell Suspension Buffer (CSB) to small-labeled tubes. Use a sterile polyester-fiber or cotton swab that has been moistened with sterile CSB to remove some of the growth from agar plate; suspend cells in CSB by spinning swab gently so cells will be evenly dispersed and formation of aerosols is minimized.

Note: The minimum volume of the cell suspension needed will depend on size of the cuvettes or tubes used to measure the cell concentration and are dependent on the manufacturer's specifications for the spectrophotometer, turbidity meter, or colorimeter. Keep suspensions on ice if you have more than 6 cultures to process or refrigerate cell suspensions if you cannot adjust their concentration immediately.

7. Adjust concentration of cell suspensions to one of values given below by diluting with sterile CSB or by adding additional cells.

a. Spectrophotometer: 610 nm wavelength, absorbance (Optical Density) of 1.35 (range of 1.3-1.4)

b. Dade Microscan Turbidity Meter: 0.48 - 0.52 (measured in Falcon 2054 tubes)
0.68-0.72(measured in Falcon 2057 tubes)

c. bioMérieux Vitek colorimeter: ≈14-15% transmittance (measured in Falcon 2054 tubes)

Note: Cell suspensions need to be at room temperature when concentration is checked. The values in Steps 7a, 7b and 7c give satisfactory results at CDC; if different instruments or tubes are used, each laboratory may need to establish the concentration needed for satisfactory results.

## Casting Plugs

Label wells of PFGE plug molds with culture number. When reusable plug molds are used, put strip of tape on lower part of reusable plug mold before labeling wells.

Note 1: Unused plug agarose can be kept at room temperature and reused 1-2 times. Microwave on low-medium power for 10 -15 sec and mix; repeat for 5 -10 sec intervals until agarose is completely melted. This agarose melts rapidly!

Note 2: Proteinase K solutions (20 mg/ml) are available commercially, or a stock solution of Proteinase K can be prepared from the powder in sterile Ultrapure (Reagent Grade Type 1) water, aliquoted in 300-500 µl amounts, and kept frozen. Just before use, thaw appropriate number of vials needed for the samples; keep Proteinase K solutions on ice. Discard any thawed Proteinase K stock solution that was prepared from powder by the user at end of work day. Store commercially prepared Proteinase K solutions according to directions provided by the supplier.

1. Transfer 400 µl (0.4 ml) adjusted cell suspensions to labeled 1.5-ml microcentrifuge tubes. If cell suspensions are at room temperature, agarose can be added directly without pre-warming cell suspensions. If cell suspensions are cold, place tubes containing cell suspensions in plastic holders (floats); incubate in a 37°C water bath for a few minutes.

2. Add 20 µl of Proteinase K (20 mg/ml stock) to each tube and mix gently with pipet tip. (200 µl are needed for 10 cell suspensions.)

3. Add 400 µl (0.4 ml) melted 1% SeaKem Gold:1% SDS agarose to the 0.4-ml cell suspension; mix by gently pipetting mixture up and down a few times. Maintain temperature of melted agarose by keeping flask in beaker of warm water (55-60°C).

4. Immediately, dispense part of mixture into appropriate well(s) of reusable plug mold. Do not allow bubbles to form. Two plugs of each sample can be made from these amounts of cell suspension and agarose. Allow plugs to solidify at room temperature for 10-15 min. They can also be placed in the refrigerator (4°C) for 5 minutes.

Note: If disposable plug molds are used for making plugs with 1% SeaKem Gold:1% SDS agarose, use 200 µl cell suspension, 10 µl of Proteinase K (20 mg/ml stock) and 200 µl of agarose; up to 4 plugs can be made from these amounts of cell suspension and agarose.

Lysis Of Cells In Agarose Plugs

Note: Two plugs (reusable plug molds) or 3 - 4 plugs (disposable plug molds) of the same strain can be lysed in the same 50-ml tube.

1. Label 50-ml polypropylene screw-cap or 50-ml Oak Ridge tubes with culture numbers.

2. Prepare Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) as follows:

    25 ml of 1 M Tris, pH 8.0

    50 ml of 0.5 M EDTA, pH 8.0

    50 ml of 10 % Sarcosyl (N-Lauroylsarcosine, Sodium salt)2

    Dilute to 500 ml with sterile Ultrapure (Reagent Grade Type 1) water

3. Calculate the total volume of Cell Lysis/Proteinase K Buffer needed as follows:

    a. 5 ml Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) is needed per tube (e. g., 5 ml x 10 tubes = 50 ml).

b. 25 µl Proteinase K stock solution (20 mg/ml) is needed per tube of the cell lysis buffer (e. g., 25 µl x 10 tubes = 250 µl).

c. Measure correct volumes into appropriate size test tube or flask and mix well.

Note: The final concentration of Proteinase K in the lysis buffer is 0.1 mg/ml, and is different from the concentration that was added to the cell suspension (0.5 mg/ml).

4. Add 5 ml of Proteinase K/Cell Lysis Buffer to each labeled 50 ml tube.

5. Trim excess agarose from top of plugs with scalpel or razor blade (optional). Open reusable plug mold and transfer plugs from mold with a 6-mm wide spatula to appropriately labeled tube.

If disposable plug molds are used, remove white tape from bottom of mold and push out plug(s) into appropriately labeled tube. Be sure plugs are under buffer and not on side of tube.

Note: The excess agarose, plug mold, spatula, etc. are contaminated. Discard or disinfect appropriately.

6. Remove tape from reusable mold. Place both sections of plug mold, spatulas, and scalpel in 70% isopropanol (IPA) or other suitable disinfectant. Soak them for 15 minutes before washing them. Discard disposable plug molds or disinfect them in 10% bleach for 30-60 minutes if they will be washed and reused.

7. Place tubes in rack and incubate in a 54°C shaker water bath or incubator for 1.5 - 2 h with constant and vigorous agitation (150-175 rpm). If lysing in water bath, be sure water level is above level of lysis buffer in tubes.

8. Pre-heat enough sterile Ultrapure (Reagent Grade Type 1) water to 50°C so that plugs can be washed two times with 10-15 ml water (200-250 ml for 10 tubes).

**WASHING OF AGAROSE PLUGS AFTER CELL LYSIS**

Lower the temperature of the shaker water bath or incubator to 50°C.

1. Remove tubes from water bath or incubator, and carefully pour off lysis buffer into an appropriate discard container; plugs can be held in tubes with a screened cap or spatula.

**Note**: It is important to remove all of the liquid during this and subsequent wash steps by touching edge of tube or screened cap on an absorbent paper towel.

2. Add at 10-15 ml sterile Ultrapure (Reagent Grade Type 1) water that has been pre-heated to 50°C to each tube and shake the tubes in a 50°C water bath or incubator for 10-15 min.

3. Pour off water from the plugs and repeat wash step with pre-heated water (Step 2) one more time.

    a. Pre-heat enough sterile TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0) in a 50°C water bath so that plugs can be washed four times with 10-15 ml TE (300-350 ml for 10 tubes) after beginning last water wash.

4. Pour off water, add 10-15 ml pre-heated (50°C) sterile TE Buffer, and shake the tubes in 50°C water bath or incubator for 10-15 min.

5. Pour off TE and repeat wash step with pre-heated TE three more times.

6. Decant last wash and add 5-10 ml sterile TE. Continue with step 1 in "Restriction Digestion" section or store plugs in TE Buffer at 4°C until needed. Plugs can be transferred to smaller tubes for storage.

**Note**: If restriction digestion is to be done the same day, complete Steps 1-3 of next section (**RESTRICTION DIGESTION OF DNA IN AGAROSE PLUGS WITH *Xba*I**) during last TE wash step for optimal use of time.

## RESTRICTION DIGESTION OF DNA IN AGAROSE PLUGS WITH *Xba*I

**Note**: A small slice of the plug or the entire plug (made in disposable plug molds) can be digested with the restriction enzyme. Restriction digestion of a small slice of the plug is recommended because less enzyme is required and other slices of the plug can be subjected to restriction analysis with other enzymes, such as *Avr*II (*Bln*I), *Spe*I, etc. This is important when the PFGE patterns obtained with the primary enzyme from two or more isolates are indistinguishable, and confirmation is needed to determine that the PFGE patterns of these isolates are also indistinguishable with additional enzymes.

1. Label 1.5-ml microcentrifuge tubes with culture numbers; label 3 (10-well gel) or 4 (15-well gel) tubes for *Salmonella* ser. Braenderup H9812 standards.

   a. **Optional Pre-Restriction Incubation Step**: Dilute 10X H buffer (Roche Molecular Biochemicals or equivalent) 1:10 with sterile Ultrapure (Reagent Grade Type 1) water according to the following table.

| Reagent | µl/Plug Slice | µl/10 Plug Slices | µl/15 Plug Slices |
|---|---|---|---|
| **Sterile Reagent Grade Water** | 180 µl | 1800 µl | 2700 µl |
| **H Buffer** | 20 µl | 200 µl | 300 µl |
| **Total Volume** | 200 µl | 2000 µl | 3000 µl |

b. Add 200 µl diluted H buffer (1X) to labeled 1.5-ml microcentrifuge tubes.

c. Carefully remove plug from TE with spatula and place in a sterile disposable Petri dish or on large glass slide.

d. Cut a 2.0- to 2.5-mm-wide slice from test samples with a single edge razor blade (or scalpel, cover slip, etc.) and transfer to tube containing diluted H buffer. Be sure plug slice is under buffer. Replace rest of plug in original tube that contains 5 ml TE buffer. Store at 4°C.

**Note:** The shape and size of the plug slice that is cut will depend on the size of the comb teeth that are used for casting the gel. PulseNet recommends that the combs with larger teeth (10-mm-wide teeth) be used to cast the gels because computer analysis of the gel lanes is more accurate and less tedious than analysis of gel lanes cast with combs with the smaller teeth (5.5-mm). The number of slices that can be cut from the plugs will depend on the skill and experience of the operator, integrity of the plug, and whether the slices are cut vertically or horizontally (plugs made in disposable molds).

e. Cut three or four 2.0-mm-wide slices from plug of the *S.* ser. Braenderup H9812 standard and transfer to tubes with diluted H buffer. Be sure plug slices are under buffer. Replace rest of plug in original tube that contains 5 ml TE buffer. Store at 4°C.

f. Incubate sample and control plug slices in 37°C water bath for 5-10 min or at room temperature for 10-15 min.

g. After incubation, remove buffer from plug slice using a pipet fitted with 200-250 µl tip all the way to bottom of tube and aspirate buffer. Be careful not to cut plug slice with pipet tip and that plug slice is not discarded with pipet tip.

2. Dilute 10X H buffer 1:10 with sterile Ultrapure (Reagent Grade Type 1) water and add *Xba*I restriction enzyme3 (50 U/sample) according to the following table. Mix in the same tube that was used for the diluted H buffer

| Reagent4 | µl/Plug Slice | Ml/10 Plug Slices | µl/15 Plug Slices |
|---|---|---|---|
| **Sterile Reagent Grade Water** | 175 µl | 1750 µl | 2625 µl |
| **H Buffer** | 20 µl | 200 µl | 300 µl |
| **Enzyme (10 U/µl)** | 5 µl | 50 µl | 75 µl |
| **Total Volume** | 200 µl | 2000 µl | 3000 µl |

**Note**: Keep vial of restriction enzyme on ice or in insulated storage box (-20°C) at all times.

3. Add 200 µl restriction enzyme mixture to each tube. Close tube and mix by tapping gently; be sure plug slices are under enzyme mixture.

4. Incubate sample and control plug slices in 37°C water bath for 1.5-2 h.

5. If plug slices will be loaded into the wells (Option B, page 9), continue with Steps 1-4 of the next section (**CASTING AGAROSE GEL**) approximately 1 h before restriction digest reaction is finished so the gel can solidify for at least 30 minutes before loading the restricted PFGE plugs.

**CASTING AGAROSE GEL**

**A. Loading Restricted Plug Slices on the Comb:**

1. **Confirm that water bath is equilibrated to 55- 60°C.**

2. Make volume of 0.5X Tris-Borate EDTA Buffer (TBE) that is needed for both the gel and electrophoresis running buffer according to one of the following tables.

**5X TBE**:

| **Reagent** | **Volume in milliliters (ml)** | | | | | |
|---|---|---|---|---|---|---|
| **5X TBE** | 200 | 210 | 220 | 230 | 240 | 250 |
| **Reagent Grade Water** | 1800 | 1890 | 1980 | 2070 | 2160 | 2250 |
| **Total Volume of 0.5X TBE** | 2000 | 2100 | 2200 | 2300 | 2400 | 2500 |

**10X TBE**:

| **Reagent** | **Volume in milliliters (ml)** | | | | | |
|---|---|---|---|---|---|---|
| **5X TBE** | 100 | 105 | 110 | 115 | 120 | 125 |
| **Reagent Grade Water** | 1900 | 1995 | 2090 | 2185 | 2280 | 2375 |
| **Total Volume of 0.5X TBE** | 200 | 2100 | 2200 | 2300 | 2400 | 2500 |

3. Make 1% SeaKem Gold (SKG) Agarose in 0.5X TBE as follows:

a. Weigh appropriate amount of SKG into 500 ml screw-cap flask.

b. Add appropriate amount of 0.5X TBE; swirl gently to disperse agarose.

c. Remove cap, cover loosely with clear film, and microwave for 60-sec; mix gently and repeat for 15-sec intervals until agarose is completely dissolved.

d. Recap flask and place in 55-60°C water bath.

Mix 1.0 g agarose with 100 ml 0.5X TBE for 14-cm-wide gel form (10 or 15 wells)

Mix 1.5 g agarose with 150 ml 0.5X TBE for 21-cm-wide gel form (≥15 wells)

**SAFETY WARNING**: Use heat-resistant gloves when handling hot flasks after microwaving.

4. A small volume (2-5 ml) of melted and cooled (50-60°C) 1% SKG 1% SKG agarose may be wanted to seal wells after plugs are loaded. Prepare 50 ml by melting 0.5 g agarose with 50 ml 0.5X TBE in 250 ml screw-cap flask as described above. Unused SKG agarose can be kept at room temperature, melted, and reused several times. Microwave for 15-20 sec and mix; repeat for 10-sec intervals until agarose is completely melted. Place in 55-60°C water bath until ready to use. Alternatively, save approximately 5 ml of the melted agarose used to cast the gel in a pre-heated (55-60°C) 50 ml flask and place in 55-60°C water bath until used.

**Note:** Confirm that gel form is level on leveling table, that **front** of comb holder and teeth face the bottom of gel, and that the comb teeth touch the gel platform.

5. Remove restricted plug slices from 37°C water bath. Remove enzyme/buffer mixture and add 200 µl 0.5X TBE. Incubate at room temperature for 5 min.

6. Remove plug slices from tubes; put comb on bench top and load plug slices on the bottom of the comb teeth as follows:

a. Load *S.* ser. Braenderup H9812 standards on teeth (lanes) 1, 5, 10 (10-well gel) or on teeth 1, 5, 10, 15 (15-well gel).

b. Load samples on remaining teeth.

7. Remove excess buffer with tissue. Allow plug slices to air dry on the comb for ≈5 minutes or seal them to the comb with 1% SKG agarose (55-60°C).

8. Position comb in gel form and confirm that the plugs slices are correctly aligned on the bottom of the comb teeth, that the lower edge of the plug slice is flush against the black platform, and there are no bubbles (if allowed to air dry).

9. Carefully pour the agarose (cooled to 55-60°C) into the gel form.

10. Put black gel frame in electrophoresis chamber. Add 2 -2.2 L freshly prepared 0.5X TBE. Close cover of unit. (The amount of buffer needed depends on whether residual buffer was left in tubing or if unit was flushed with water after the last gel was run.)

11.Turn on cooling module (14°C), power supply, and pump (setting of ≈70 for a flow of 1 liter/minute).

12. Remove comb after gel solidifies for 30-45 minutes.

13. Fill in wells of gel with melted and cooled (55- 60°C) 1% SKG Agarose (optional). Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.

**B. Loading Restricted Plug Slices into the Wells:**

1. Follow steps 1-4 in Option A on pages 7 and 8 (**Loading Restricted Plug Slices on the Comb**).

**Note**: Confirm that gel form is level on gel-leveling table before pouring gel, that front of comb holder and teeth face bottom of gel, and the bottom of the comb is 2 -mm above the surface of the gel platform.

2. Cool melted SKG agarose in 55-60°C water bath for 15-20 min; carefully pour agarose into gel form (casting stand) fitted with comb. Be sure there are no bubbles.

3. Put black gel frame in electrophoresis chamber. Add 2-2.2 L freshly prepared 0.5X TBE. Close cover of unit. (The amount of buffer depends on whether residual buffer was left in tubing, or if unit was flushed with water after the last gel was run.)

4. Turn on cooling module (14°C), power supply, and pump (setting of ≈ 70 for a flow of 1 liter/minute) approximately 30 min before gel is to be run.

5. Remove restricted plug slices from 37°C water bath. Remove enzyme/buffer mixture and add 200 µl 0.5X TBE. Incubate at room temperature for 5 minutes.

6. Remove comb after gel solidifies for at least 30 minutes.

7. Remove restricted plug slices from tubes with tapered end of spatula and load into appropriate wells. Gently push plugs to bottom and front of wells with wide end of spatula. Manipulate position with spatula and be sure that are no bubbles.

   a. Load *S.* ser. Braenderup H9812 standards in wells (lanes) 1, 5, 10 (10-well gel) or in wells 1, 5, 10, 15 (15-well gel).

   b. Load samples in remaining wells.

**Note**: Loading the plug slices can be tedious; each person has to develop his/her own technique for consistently placing the plug slices in the wells so the lanes will be straight and the bands sharp.

8. Fill in wells of gel with melted 1% SKG Agarose (equilibrated to 55- 60°C). Allow to harden for 3-5 min. Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.

**ELECTROPHORESIS CONDITIONS**

1a. Select following conditions on **CHEF Mapper** for *Escherichia coli* O157:H7 and *Shigella* sonnei5 strains restricted with *Xba*I or *Avr*II (*Bln*I):

Auto Algorithm

30 kb - low MW

600 kb - high MW

Select default values except where noted by pressing "enter".

**Change run time to 18 - 19 h** (See note below)

(Default values: Initial switch time = 2.16 s; Final switch time = 54.17 s)

1b. Select following conditions on **CHEF-DR III**

Initial switch time: 2.2 s

Final switch time: 54.2 s

Voltage: 6 V

Included Angle: 120°

Run time: 18-19 h (See note below)

1c. Select following conditions on **CHEF-DR II**

Initial A time: 2.2 s

Final A time: 54.2 s

Start ratio: 1.0 (if applicable)

Voltage: 200 V

Run time: 19-20 h (See note below)

2a. Select following conditions on **CHEF Mapper** for non-typhoidal *Salmonella* strains restricted with *Xba*I or *Avr*II (*Bln*I):

Auto Algorithm

30 kb - low MW

700 kb - high MW

Select default values except where noted by pressing "Enter".

**Change run time to 18 - 19 h** (See note below)

(Default values: Initial switch time = 2.16 s; Final switch time = 63.8 s)

2b. Select following conditions on **CHEF DR-III**

Initial switch time: 2.2 s

Final switch time: 63.8 s

Voltage: 6 V

Included Angle: 120°

Run time: 18-19 h (See note below)

2c. Select following conditions on **CHEF DR-II**.

Initial A time: 2.2s

Final A time: 63.8 s

Start Ratio: 1.0 (if applicable)

Voltage: 200 V

Run time: 19-20 h (See note below)

**Note**: The electrophoresis running times recommended above are based on the equipment and reagents used at the CDC. Run times may be different in your laboratory and will have to be optimized for your gels so that the lowest band in the *S.* ser. Braenderup H9812 standard migrates 1.0 - 1.5 cm from the bottom of the gel.

**Day 2**

**STAINING AND DOCUMENTATION OF PFGE AGAROSE GEL**

1. When electrophoresis run is over, turn off equipment; remove and stain gel with ethidium bromide. Dilute 40 µl of ethidium bromide stock solution (10 mg/ml) with 400 ml of reagent grade water (this volume is for a staining box that is approximately 14-cm x 24-cm; a larger container may require a larger amount of staining solution). Stain gel for 20 - 30 min in covered container.

**Note**: Ethidium bromide is toxic and a mutagen; the solution can be kept in dark bottle and reused 3-5 times before discarding according to your institution's guidelines for hazardous waste or use the destaining bags recommended for disposal of ethidium bromide (**Section 10**).

2. Destain gel in approximately 500 ml reagent grade water for 60 - 90 min; change water every 20 minutes. Capture image on Gel Doc 1000, Gel Doc 2000, or equivalent

documentation system. If background interferes with resolution, destain for an additional 30-60 min.

**Note**: If both a digital image and conventional photograph are wanted, photograph gel first before capturing digital image.

3. Follow directions given with the imaging equipment to save gel image as an ***.img** or ***.1sc** file; convert this file to ***.tif** file for analysis with the BioNumerics software program (Additional information is in **Section 11** of the PFGE Manual).

5. Drain buffer from electrophoresis chamber and discard. Rinse chamber with 2 L reagent grade water or, if unit is not going to be used for several days, flush lines with water by letting pump run for 5-10 min before draining water from chamber and hoses.

**Please note the following if PFGE results do not have to be available within 24-28 hours:**

1.Plugs can be lysed for longer periods of time (3-16 hours).

2.The washing steps with TE to remove the lysis buffer from the PFGE plugs can be done for longer periods of time (30-45 min) and at lower temperatures (37°C or room temperature). They can be started on Day 1 and finished on Day 2 after overnight refrigeration of the plugs in TE.

3. The restriction digestion can be done for longer periods of time (3-16 hours).

4. If the lowest band in the H9812 standard does not migrate within 1 -1.5 cm of the bottom of the gel, the run time will need to be determined empirically for the conditions in each laboratory.

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**Formulas of Selected Reagents used in PulseNet Standardized Laboratory Protocol for PFGE**

**Tris:EDTA Buffer, pH 8.0 (TE, 10 mM Tris:1 mM EDTA, pH 8.0)**6

10 ml of 1 M Tris, pH 8.0

2 ml of 0.5 M EDTA, pH 8.0

Dilute to 1000 ml with sterile Ultrapure (Reagent Grade Type 1) Water

**Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosine + 0.1 mg/ml Proteinase K)**

25 ml (50 ml) of 1 M Tris, pH 8.0

50 ml (100 ml) of 0.5 M EDTA, pH 8.0

50 ml (100 ml) 10% N-Lauroylsarcosine, Sodium salt (Sarcosyl)

**or**

5 g (10 g) of N-Lauroylsarcosine, Sodium salt (Sarcosyl)7

Dilute to 500 ml (1000 ml) with Sterile Ultrapure (Reagent Grade Type 1) Water

Add 25 µl Proteinase K stock solution (20 mg/ml) per 5 ml of cell lysis buffer just before use for a final concentration in the lysis buffer of 0.1 mg/ml Proteinase K.

**Use the following calculations for *Avr*II (*Bln*I) or *Spe*I (30 Units/plug slice):**

| Reagent8 | µl/Plug Slice | µl/10 Plug Slices | µl/15 Plug slices |
|---|---|---|---|
| Sterile Ultrapure Water | 177 µl | 1770 µl | 2655 µl |
| H Buffer | 20 µl | 220 µl | 300 µl |
| Enzyme (10U/µl | 3 µl | 30 µl | 45 µl |
| Total Volume | 200 µl | 2000 µl | 3000 µl |

**Note**: Keep vial of restriction enzyme on ice or in insulated storage box (-20°C) at all times.

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