2. LITERATURE REVIEW

2.1 Evolution of *Salmonella*

It is speculated that the genera of *Escherichia coli* and *Salmonella* diverged from a common ancestor about the time of the emergence of mammals, and emerge as mammalian and avian pathogens through the acquisition of pathogenicity islands and of a virulence plasmid, through variation in lipopolysaccharide antigens, through development of mechanism for flagellar antigen phase shifting, and in other ways (Wray *et al.*, 2001). Some writers estimate *Salmonella* diverged from the genus *Escherichia* 120–160 million years ago (Lawrence, 1999, Cotter *et al.*, 2000). Baumler *et al.*, found that the close DNA relatedness among *Salmonella* serotypes is evidence for their clonal origin, and based on the degree of sequence divergence, it can be estimated that a common ancestor of the genus existed about 25 to 40 million years ago (Baumler *et al.*, 1998). In 1892 Loeffler described the causative agent of murine typhoid, (then known as *Bacillus Typhi*) that caused an epidemic typhoid fever-like disease in mice (Santos *et al.*, 2001). Recently, *Salmonella* Typhi was identified in ancient skeletal material, thereby incriminating typhoid fever for the plague that devastated Athens in 430-426 B.C. It is hypothesized that accumulation of single mutations, insertions or deletions with the genome of modern-time *Salmonella* Typhi appears to have generated many pseudogenes, suggesting its recent evolutionary origin (Papagrigorakis *et al.*, 2007).

2.2 Genus *Salmonella*

*Salmonella* organisms are facultative anaerobic gram-negative rods within the family of Enterobacteriaceae (Yan *et al.*, 2003). Clasically, the members of this genus are motile by peritrichous flagella except *Salmonella* Pullorum and *Salmonella* Gallinarum, which lack flagella, however, the long standing fact that *Salmonella* Pullorum is non motile has been disproved and it has been shown that the motility can be induced under special medium conditions (Holt *et al.*, 1997). *Salmonella* grow
optimally at 35°C to 37°C, catabolize a variety of carbohydrates into acid and gas, use citrate as the sole carbon source, produce H₂S and decarboxylate lysine and ornithine to cadaverine and putrescine respectively (Barbara et al., 2000). Historically Salmonella catabolized glucose and lysine, but failed to metabolize lactose, sucrose and urea, however due to the widespread exchange of genetic elements between compatible bacterial strains in the environment, atypical Salmonella biotypes that cannot decarboxylate lysine (Muramatsu et al., 1992, Morita et al., 2006) or that readily use lactose (Falcao et al., 1975, Kohbata et al., 1983, Glosnicka et al., 1987) sucrose (Johnson et al., 1976, Reid et al., 1993) and urea, have been isolated. They are chemo-organotrophic organisms, having both a respiratory and a fermentative type of metabolism (John et al., 1994). Many serotypes in the group are closely related to each other by somatic and flagellar antigens and most strains show diphasic variation of the flagellar antigens.

The genus Salmonella comprises two species: (1) Salmonella enterica, which is divided into six subspecies: Salmonella enterica subspecies enterica (I), Salmonella enterica subspecies salamae (II), Salmonella enterica subspecies arizonae (IIIa), Salmonella enterica subspecies diarizonae (IIIb), Salmonella enterica subspecies houtenae (IV) and Salmonella enterica subspecies indica (VI); and (2) Salmonella bongori (formerly called Salmonella enterica subspecies bongori V). Species and subspecies can be distinguished on the basis of differential characters, and through antigenic formulae, into 2501 serovars (Solari et al., 2003). However, a recent report from the Centre for Infectious Disease Research and Policy classifies members of the Salmonella species into more than 2541 serotypes (serovars) according to their somatic (O) and flagellar (H) antigens (CIDRAP, 2006). The antigenic formulae of Salmonella serotypes are defined and maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on Salmonella at the Pasteur Institute, Paris, France (WHO collaborating centre), and new serotypes are listed in the annual updates of the Kauffmann-White scheme (Brenner et al., 2000).
2.3 Current nomenclature

Several schemes based on biochemical characteristics, DNA homology, and enzyme electrophoretic patterns have been used for the taxonomic classification of *Salmonella* (Barbara *et al.*, 2000). *Salmonella* nomenclature is complex, and scientists use different systems to refer to and communicate about this genus. The current usage often combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars) which causes confusion (Brenner *et al.*, 2000). The genus *Salmonella* has two systems of nomenclature in circulation. One system proposed by Le Minor and Popoff in the 1980s, which has received a wide acceptance although it does not conform to the rules of bacteriological code, and the other which conforms to the bacteriological code, but used by the minority. The present problem is that two systems of nomenclature are in the use for the members of the genus *Salmonella* (Tindall *et al.*, 2005).

The nomenclature of the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kauffmann on the basis of the serologic identification of O (somatic) and H (flagellar) antigens. A capsular polysaccharide, the Vi antigen is present on *Salmonella* Typhi and few other serovars of *Salmonella*, including *Salmonella* Dublin (Heyndrickx *et al.*, 2005). The defining development in *Salmonella* taxonomy occurred in 1973 when Corsa et al. demonstrated by DNA-DNA hybridization that all serotypes and subgenera I, II and IV of *Salmonella* and all serotypes of “Arizona” were related at the species level, thus belonging to a single species. The single exception subsequently described is *Salmonella bongori* previously known as subspecies V which by DNA-DNA hybridization is a distinct species. In 1986 the subcommittee of Enterobacteriaceae of the International Committee on Systematic Bacteriology at the XIV International Congress of Microbiology unanimously recommended the change of type species of *Salmonella* to *Salmonella enterica*, a name coined by Kauffmann and Edwards in 1952, however the Judicial committee denied with the fact that the *Salmonella* Typhi might be overlooked as it is one of the most important human pathogens (Brenner *et al.*, 2000).
The current nomenclature used by CDC is based on the recommendations from the WHO collaborating centre and it adequately addresses the concern and requirements of clinical and public health microbiologists (Deb and Kapoor, 2005). The nomenclature is summarized in the table 1. According to the CDC system, the genus *Salmonella* contains two species, each of which contains multiple serotypes (Brenner *et al.*, 2000) as shown in table 2.

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

<table>
<thead>
<tr>
<th><em>Salmonella</em> species and subspecies</th>
<th>No. of serovars within subspecies</th>
<th>Usual habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. enterica</em> subsp. <em>Enterica</em> (I)</td>
<td>1454</td>
<td>Warm blooded animals</td>
</tr>
<tr>
<td><em>S. enterica</em> subsp. <em>Salamae</em> (II)</td>
<td>489</td>
<td>Cold blooded animals and the environment</td>
</tr>
<tr>
<td><em>S. enterica</em> subsp. <em>Arizonae</em> (IIIa)</td>
<td>94</td>
<td>Cold blooded animals and the environment</td>
</tr>
<tr>
<td><em>S. enterica</em> subsp. <em>Diarizoneae</em> (IIIb)</td>
<td>324</td>
<td>Cold blooded animals and the environment</td>
</tr>
<tr>
<td><em>S. enterica</em> subsp. <em>Hauteneae</em> (IV)</td>
<td>70</td>
<td>Cold blooded animals and the environment</td>
</tr>
<tr>
<td><em>S. enterica</em> subsp. <em>Indica</em> (VI)</td>
<td>12</td>
<td>Cold blooded animals and the environment</td>
</tr>
<tr>
<td><em>S. bongori</em> (V)</td>
<td>20</td>
<td>Cold blooded animals and the environment</td>
</tr>
</tbody>
</table>
Table 2: *Salmonella* nomenclature in use at CDC, 2000.

<table>
<thead>
<tr>
<th>Taxonomic position</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus (italics)</td>
<td><em>Salmonella</em></td>
</tr>
</tbody>
</table>
| Species (italics)  | • enterica, which includes subspecies I, II, IIIa, IIIb, IV and VI  
|                    | • bongori (formerly subspecies V) |
| Serotype (capitalized, not italicized) | • The first time a serotype is mentioned in the text; the name should be preceded by the word “serotype” or “ser”.  
|                    | • Serotypes are named in subspecies I and designated by antigenic formulae in subspecies II to IV, and VI and *S. bongori*  
|                    | • Members of subspecies II, IV and VI and *S. bongori* retain their names if named before 1966 |

2.4 Morphology

Salmonellae are Gram-negative, straight rods not exceeding 1.5 micrometers in width. They are facultative anaerobes usually motile by peritrichous flagella (European Commission, 2000a). Most salmonellae form common fimbriae and most of them possess type-1 fimbriae associated with mannose-sensitive adhesive properties. These fimbriae are composed of fimbrillin subunits containing a high proportion of hydrophobic amino-acids (Old *et al.*, 1998). *Salmonella* are routinely classified by serotype on the basis of expression of three surface antigens, the somatic O antigen, the flagella H1 and H2 antigens and the capsular Vi antigen, according to the Kauffmann-White scheme (Scott *et al.*, 2002). The absence of flagella may consequently affect complete identification of the serotype; *Salmonella enterica* serovar Typhimurium exhibits morphological differences dependent on the peptone constituents of the culture medium however, in media containing soy-based peptone as the primary nutrient, *Salmonella* displays a normal flagellated morphology (Victoria *et al.*, 2006).
2.5 Serotyping

*Salmonella* express flagellar, polysaccharide and capsular antigens which determine strain pathogenicity and therefore variation of these antigens has formed the basis for *Salmonella* serotyping. The Kauffmann-White scheme, first published in 1929, divides *Salmonella* into more than 2500 serotypes according to their antigenic formulae (Mortimer *et al.*, 2004). Today, 57 O antigens and 117 H antigens have been identified and more than 2500 serotypes have been described. Some of the H antigens share common antigen factors. These antigens are clustered in five complexes, the E, G, L, Z4 and 1 complex. *Salmonella* H antigens are expressed in different phases. Most serotypes are diphasic, i.e. they express two flagella antigens, and a minor part are monophasic, i.e. express one flagella antigen. *Salmonella* Gallinarum is the only serotype in the Kauffmann-White scheme that does not express any flagella antigen and is therefore non-motile (Sonne-Hansen *et al.*, 2005). *Salmonella* serotyping methods recognize 63 distinct phase 1 flagellar antigenic factors and 37 phase 2 flagellar antigenic factors, although the latter are not always present. Some antigenic factors, denoted by square brackets in formulae, may be present or absent without affecting serotype designation. Serotyping methods are stable, reproducible and have high typability, yet there are several drawbacks, particularly the dependence on availability of antisera, considering the ethics, cost and quality control measures necessary to maintain such a supply (Mortimer *et al.*, 2004).

*Salmonella* isolates can be differentiated from one another in a wide variety of ways, and the number of *Salmonella* continues to increase. Epidemiologically, it is important to be able to distinguish *Salmonella* isolates, because definitive typing of *Salmonella* isolates may assist in tracing the source of an outbreak and monitoring trends in antimicrobial resistance associated with a particular type (Yan *et al.*, 2003). In addition to the conventional antigen-based serotyping, there are advanced techniques for serotyping currently being used to enhance the tracing of the individual isolates. The following techniques are currently available for serotyping.
2.5.1 Conventional serotyping

Conventional serotyping of *Salmonella* is the most commonly used method to differentiate strains, which are epidemiologically the smallest bacterial unit from which isolates share the same phenotypic and genotypic traits (Yan *et al.*, 2003). In most clinical studies, initial serotyping is done using polyvalent O antisera to allow *Salmonella* isolates to be grouped into different O groups designated in capitalized letters. Many *Salmonella* show diphasic production of flagellar antigens and each strain can spontaneously and reversibly vary between these two phases with different sets of H antigens. In phase 1 or the specific phase, the different antigens are designated by small letters, and in phase 2 or the group phase, the antigens first discovered are numbered. In a single cell, usually only one antigen is expressed at a time (Yan *et al.*, 2003). Conventional serotyping using the autoagglutination method has some limitations, such as limitations in detection of Vi antigens (Wain *et al.*, 2005), strains which are not typeable (Rasschaert *et al.*, 2005); it only allows detection of a single antigen-antibody reaction at a time, requires well-experienced technologists to perform, consumes relatively high amount of reagents, and takes a longer time (Cai *et al.*, 2005).

2.6 *Salmonella*: disease and pathogenesis

*Salmonella* are well-known pathogens, highly adaptive and potentially pathogenic for humans and/or animals. *Salmonella* infections are capable of producing serious infections that are often foodborne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in
bacteremia and serious extra intestinal disease (Fluit, 2005). The main reservoirs for non-typhoidal *Salmonella* are animals such as poultry, livestock, pets and reptiles. *Salmonella Typhi* and *Salmonella Paratyphi* colonize only in humans, so they can be acquired only from close contact with a person who has typhoid fever, from a chronic carrier, or from water or food contaminated by human feces (CIDRAP, 2006).

While certain serovars of *Salmonella enterica* cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting, to typhoid fever, a life threatening infection (Hensel, 2004). The outcome of *Salmonella* infections is determined by the host and the status of the bacterium. Whereas, age, genetic and environmental factors mainly determine the status of the host, the status of the bacterium is determined by so-called virulence factors (Alphons et al., 2005).

Serotypes adapted to man, such as *Salmonella Typhi* and *Salmonella Paratyphi*, usually cause severe diseases in humans as a septicaemic typhoidic syndrome (enteric fever). These serotypes are not usually pathogenic to animals. Serotypes that are highly adapted to animal hosts, such as *Salmonella Gallinarum* (poultry) or *Salmonella Abortus-ovis* (sheep), usually produce very mild symptoms in man. However, *Salmonella Choleraesuis* which has the pig as a primary host also causes severe systemic illness. In the same way, *Salmonella Dublin*, which has a preference for bovines, is primarily responsible for the systemic form of Salmonellosis. In young calves this disease causes high mortality, and in adult cattle it results in fever, reduced milk yield, diarrhea, abortion, and occasionally death. Ubiquitous serotypes, such as *Salmonella Enteritidis* or *Salmonella Typhimurium*, which affect both man and animals, generally cause gastrointestinal infections usually less severe than enteric fever. However, they also have the capacity to produce typhoid-like infections in mice and in humans, or asymptomatic intestinal colonization in chickens (Velge et al., 2005).
Salmonella avoid host defense in the stomach and reach the intestines, (CIDRAP, 2006) and the bacteria interact with the non-phagocytic cells such as the epithelial cells of the intestinal mucosa (Hensel, 2004). They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection (Alphons et al., 2005).

The mechanism of pathogenesis has been described in the following steps:

a) **Bacterial mediated endocytosis:** A highly coordinated series of interactions between proteins released by salmonellae and proteins of the host cell causes host cellular surface membrane ruffling and engulfment of bacteria in cellular vacuoles.

b) **Neutrophil recruitment and migration:** Salmonellae associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen.

c) **Epithelial cell cytokine secretion:** In tissue culture models of Salmonella Enteritidis, translocation of SPI-1 proteins into intestinal epithelial cells leads to synthesis and polarized secretion of inflammatory mediators and neutrophil chemoattractants.

d) **Fluid and electrolyte secretion:** Several translocated SPI-1 proteins contribute to intestinal inflammation and fluid secretion. Intestinal inflammation probably contributes to fluid secretion and diarrhea by disrupting the epithelial barrier and increasing water flux by an exudative mechanism. Innate immune system activation also contributes to intestinal inflammation.

e) **Systemic infection:** Salmonella Typhi invades macrophages and the migration of infected macrophages to reticuloendothelial organs via the lymphatic system and blood produces systemic illness with less diarrhea.
2.6.1 Salmonellosis in humans

With respect to human disease, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes, typhoid fever, bacteremia and enteritis (Santos et al., 2001). The non-typhoid *Salmonella* serotypes can cause protean manifestations in humans, including acute gastroenteritis, bacteremia, and extraintestinal localized infections involving many organs (Chiu et al., 2004). Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies *entericae*), the most common O-antigen serogroups are A, B, C1, C2, D and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment but rarely from humans (Velge et al., 2005).

Following ingestion of contaminated food or water, the pathogenesis of both typhoid and *Salmonella* enteritis begins with the intestinal phase, while only typhoid progresses to a systemic phase (Brown et al., 2005). Transmission of this disease within the human population is generally a result of poor sanitation of water and food supplies in developing nations. The broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the human food supply, and bacterial transmission generally results from consumption of raw or undercooked food products (Jones, 2005).

The vast majority of *Salmonella* infections are transmitted from animals to humans through food and occasionally from person to person through the fecal-oral route. In general, *Salmonella* cause one or more of four broad clinical syndromes such as gastro-enteritis, enteric fever, septicemia with associated focal lesions, and asymptomatic long-term carriage.

2.6.2 Salmonellosis in animals

*Salmonella* serotypes have a broad host range (Santos et al., 2003), prevalent in the warm-blooded animal population (Jones, 2005), including rodents (Porwollik et
snakes (Solari et al., 2003), and free living terrestrial and aquatic turtles (Vila et al., 2006), and the pathogenicity of Salmonella serovars is known to be specific for animal species (Ishibashi et al., 1996). Some serotypes are highly adapted to animal hosts, such as Salmonella Gallinarum in poultry and Salmonella Abortus-ovis in sheep. Many nontyphoidal Salmonella strains such as Salmonella Typhimurium and Salmonella Enteritidis infect a wide range of animal host including poultry, cattle and pigs (Ohl and Miller, 2001). These serotypes generally cause self-limiting gastrointestinal infections usually less severe than enteric fever in humans. However, they also have the capacity to produce typhoid-like infections in mice and in humans or asymptomatic intestinal colonization in chickens (Velge et al., 2005).

2.7 Salmonella: A public health perspective

Salmonellosis is an important global public health problem causing substantial morbidity, and thus also has a significant economic impact. Although most infections cause mild to moderate self-limited disease, serious infections leading to deaths do occur (Jong and Ekdahl, 2006). In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, foodborne diseases still dominate as the most important public health problem in most countries (Dominguez et al., 2002). Many foods, particularly those of animal origin, have been identified as vehicles for transmission of these pathogens to human beings and spreading them to the processing and kitchen environment (Uyttendaele et al., 1998). In developed countries food is recognized as the most frequently implicated vehicle of transmission and causes heavy financial burden on health care systems (Jordan et al., 2006). In the United States alone, an estimated 1.4 million non-typhoidal Salmonella infections, resulting in 168 000 visits to physicians, 15 000 hospitalizations and 580 deaths occur annually and the total cost associated with Salmonella is estimated at US$ 3 billion annually (WHO, 2005). Apart from the foodborne infections, the other major epidemiological development in Salmonellosis is the emergence of multiple-antibiotic resistant Salmonella, particularly in the developing countries (Okeke et al., 2005).
2.7.1 Global overview

In many countries incidence of human *Salmonella* infection has increased drastically over the years. The two most commonly isolated serotypes of concern and mostly implicated in disease outbreaks are *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype Enteritidis (Sadeyen *et al.*, 2004, Chiu *et al.*, 2004, Buck *et al.*, 2004). Besides the importance of this micro-organism for public health, another aspect is the cost generated by human Salmonellosis. During 1999, the cost linked to foodborne Salmonellosis ranged between 560 million and 2.8 billion € in Europe, where *Salmonella* was estimated to be responsible for nearly 166 000 cases (Korsak *et al.*, 2006). It is reported that the rate of Salmonellosis in the United States is between 15 to 20 cases per 100 000 people and approximately 10% of the Salmonellosis cases are caused by consumption of poultry meat (Oscar, 2004). The *Salmonella* species is one of the eight micro-organisms in the European Union Zoonoses Monitoring Directive, which shows it is a disease considered to have a high impact on human health in the Union (Jong and Ekdahl, 2006). The Enter-net surveillance program reported *Salmonella enterica* serotypes Enteritidis and Typhimurium, the most predominant organisms identified by the participating countries making up over 80% of all isolates during the period of 1998-2003. It also reported that for all *Salmonella* the general trend is declining with a reduction of 35.3% in 2003 over 1998 (Eurosurveillance, 2004).

2.7.2 Status in developing countries

Ensuring consumer health concerns by greater involvement of the health sector, development of Codex standards, guidelines and incorporation of the work of the Commission into the national legislation to promote food safety and fair trading practices are reflected in the priorities of the Codex Alimentarius Commission in the developing countries (Moy and Schlundt, 2005). The Food and Agriculture Organization of the United Nations and the World Health Organization jointly state that “illness due to contaminated food was perhaps the most widespread health problem in the contemporary world,” and “an important cause of reduced economic
productivity” (Kaferstein, 2003). With the increasing population in the developing world, there is an increasing demand for meat and meat products which will force the present resource driven system of livestock production to a demand driven system (Zessin, 2006) which will increase the disease transmission risks. There is a multifactorial risk of foodborne hazards in the developing countries due to poor sanitation and inadequate access to potable water (Henson, 2003b).

Poultry products have always topped the incidence of Salmonellosis in many developing countries including India, Egypt, Brazil and Zimbabwe (Henson, 2003b) and is the most seriously perceived food risks in chicken meat, even in the developed countries (Yeung, 2001).

The reported prevalence of *Salmonella* in chicken carcasses in South Asian countries varies from country to country. Studies in northern Thailand revealed 57% prevalence in chicken meat at the market during 2002-2003 (Padungtod et al., 2006), 14.5% prevalence in Kathmandu, Nepal (Maharjan et al., 2006), and 42.63% prevalence in Ho chi Minh city, Vietnam (Bao, 2005). Sero-prevalence of poultry *Salmonella* in Bangladesh has been reported to be 23.46% (Sikder et al., 2005). Not much literature has been available on the prevalence of *Salmonella* in chicken carcasses from India, few researches reports negligible (Vaidya et al., 2005) to as low as 5% (Rahman et al., 2004a), to a prevalence of 69% (Bajaj et al., 2003). However, the overall annual incidence of foodborne Salmonellosis in India is nearly 6 per 1000 inhabitants (Henson, 2003b).

2.7.3 *Salmonella* in food animals in India

In economically weaker developing Asian nations including India, in view of limited adherence to very high standards of hygiene, the probability of bacterial contamination of food commodities at various stages of processing and handling is very high (Goel et al., 2002). Indian food processing industries are not as developed as in the Western hemisphere, lack of proper cold chains, inadequate power supply, and low consumer perception of the risks of foodborne illness are great deterrents in
achieving food safety (Marthi, 1999). *Salmonella*, a major public health problem in India is noted to be in increasing trend (Bhattacharya et al., 2007, Shahane et al., 2007). In studies conducted in northern India, the prevalence of *Salmonella* in live and slaughtered goats was 17.6% and 46% by indirect ELISA (Chandra et al., 2005, 2007), and 3% in sheep meat in central India (Yadav et al., 2006). There are reports of *Salmonella* outbreaks in Japanese quails (Mathew et al., 1990), poultry (Praksah et al., 2005) and other species of animals including does and ewes (Verma et al., 1998).

2.7.4 *Salmonella* in Bhutan

Although there are no publications available, there are records of *Salmonella* isolation from different sources including poultry and food items in Bhutan (unpublished lab records). Outbreaks in poultry and pheasant farms have been recorded earlier. There are frequent outbreaks of Salmonellosis reported in humans, either through the water sources or from the food items (Kuensel, May 2007). There is no surveillance of *Salmonella* presently carried out in the country. Implementation and monitoring of *Salmonella* surveillance in food and food products will be an immense responsibility of the concerned agencies in the near future in Bhutan.

2.7.5 *Salmonella* in poultry products

Poultry meat and its derivatives are among the food products that cause the most concern to public health authorities, owing to the associated risks of bacterial food poisoning (Luiz et al., 2004). The modernization of chicken farms and globalization of the bird breeding trade also have played a role in infection (Velge et al., 2005). *Salmonella enterica* serovar Enteritidis is transmitted to the human food supply through eggs of hens that appear healthy (Porwollik et al., 2005). Contamination with *Salmonella* in poultry products can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing, handling and preparation (Cui et al., 2005a). *Salmonella* accounts for 19% in the fresh and frozen poultry products in South Africa (Nierop et al., 2005), 3.1% and 2.8% in chicken and turkey meat respectively in Ireland (Jordan et al., 2006), 6.5% in Albania
(Beli et al., 2001), 60% in Portugal (Antunes et al., 2003) and 49% in Spain (Capita et al., 2003).

2.7.6 Health and economic impact

While it is recognized that the prevalence of foodborne illnesses in developing countries is considerable, in most countries there is limited data through which the incidence of particular diseases and trends can be assessed over time (Henson 2003b). The growing movement of people, live animals, and food products across borders; rapid urbanization in developing countries; increasing numbers of immune-compromised people; changes in food handling and consumption; and the emergence of new or antibiotic-resistant pathogens all contribute to increasing food safety risks (Unnevehr, 2003). Food safety requirements in export markets have a profound impact on the way that supply chains for agricultural and food products in developing countries operate. Food safety regulations and standards are increasingly influencing the ability of developing countries to access markets for agricultural and food products, particularly in industrialized countries (Henson, 2003a).

Food safety is of particular concern in developing countries not only because of the high prevalence of the foodborne illness and other hazards associated with food, but also because of the considerable economic and social cost that, in turn, reflects prevailing levels of economic development. The majority of trade-limiting factors in developing countries relate to economics, poor infrastructure and lagging skills; food safety is still mainly the responsibility of the consumers. Improving food safety along western standards, however, may carry considerable costs and price food out of reach of the poor (Veen, 2005).

The Center for Disease Control and Prevention has estimated that *Salmonella* infections were responsible for 1.4 million annual illnesses, resulting in nearly 600 deaths in 2003 in United States (CDC, 2003). The proportion of illnesses attributed to *Salmonella*-contaminated meat and poultry is unknown. More severe cases of Salmonellosis tend to occur in the very old, the very young, and the immuno-
compromised. The estimated annual costs in dollars in 1998 and 2003 of medical care and lost productivity due to food-borne *Salmonella* infections in the United States were $2.3 and $2.9 billion (Frenzen *et al.*, 1999, ERS-USDA, 2003).

All together 150,165 cases of human Salmonellosis have been reported in 14 member states of the European Union in the year 2000 (European Commission, 2000b), and 192,703 cases in 2004 (Forshell *et al.*, 2006). In the year 2000, 16,983 laboratory confirmed cases of Salmonellosis were reported in the United Kingdom, most commonly associated with the consumption of chicken and undercooked egg dishes (UK zoonoses report, 2000). In the year 2001, China recorded that 17.9% of the total food poisoning was caused by *Salmonella* spp. (FAO/WHO, 2004). The trend in Salmonellosis in Australia has been increasing over the time, both in the number of cases recorded as well as in rate per population (Sumner *et al.*, 2004).

Apart from the impact on the health of the individuals, economic losses on international and national trade due to *Salmonella* in the poultry products and product recalls have direct economic and public perception effects on the processing industries. The recalls also have a negative effect on the demand of the product and effect a move towards non-meat products. In the United States, although the incidence has decreased, *Salmonella* accounts for 5% of USDA recalls (Kramer *et al.*, 2005).

### 2.8 Antimicrobial resistance

Drug resistance in foodborne bacterial enteric pathogens is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals, and specifically in the developing countries by use of medicines in humans (Threlfall *et al.*, 2000, Bogaard and Stobberingh, 2000). A major concern is that the high levels of antibiotic resistance are a result of the use of antibiotics in food animals. A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans (White *et al.*, 2001). In recent years the emergence and global dissemination of multi-drug resistant typhoidal strains
has posed major public health problems in the developing countries, and over the past
decade it has assumed epidemic proportions in South Asia (Okeke et al., 2005). 
Antimicrobial resistance among non typhoid *Salmonella* serotypes has been a serious
problem worldwide. The identification of antimicrobial-resistant *Salmonella* in food
has raised concerns on treatment of foodborne Salmonellosis especially the
development of ceftriaxone and ciprofloxacin-resistant *Salmonella*, as these are
important in treating *Salmonella* infections in children and adults, respectively. The
extent of global food trade and the intercontinental transmission of resistant
*Salmonella* via foods underscores the potential impact that local geographical
agricultural antimicrobial use may have on consumer health worldwide (Butaye et al.,
2006). Conventional antimicrobial agents, such as ampicillin, chloramphenicol, and
trimethoprim-sulfamethoxazole had been the drug of choice in the treatment of
Salmonellosis before the 1980s. However, multi-drug resistance, with rates of
resistance to these antimicrobial agents of more than 50% has been reported in many
areas of the world. Extended-spectrum cephalosporins and fluoroquinolones are
increasingly reported after 1991 (Chiu et al., 2004). The possible emergence and
spread of *Salmonella* strains resistant to antibiotics commonly used as treatment are
concerns, because these infections can be invasive and difficult to treat by the drugs
of choice for invasive *Salmonella* disease (Paterson, 2006). In developing countries,
household subsistence farming is common, which means that a large proportion of the
population has close contact with food animals; therefore, if resistant organisms are
common in animals, the chance that they will be transmitted to human beings is more
likely (Okeke et al., 2005). Some research studies indicate that the costs associated
with antimicrobial resistance are higher by several times (Howard and Scott, 2005).

The emergence of *Salmonella* strains that are resistant to commonly used
antimicrobials should be particularly noted by clinicians, microbiologists and those
responsible for control of communicable diseases, as well as food producers including
the food industry. Control of drug resistant *Salmonella* is most efficiently achieved
through the reduction of antimicrobial use. Prudent usage in food animals should be
combined with good husbandry, good abattoir practice and good hygiene at all stages
in the food production chain, from processing plants to kitchens and food service
establishments. Although some countries have succeeded in reducing the frequency of Salmonella in poultry dramatically, it is unlikely that the eradication of Salmonella in domestic animals is possible in the foreseeable future. The increased occurrence of drug-resistant pathogens in food of animal origin emphasizes the general need for cooking such foods thoroughly prior to consumption. Education of food handlers in the principle of safe food handling is an essential step towards reducing the incidence of foodborne diseases resulting from cross-contamination during processing and preparation of foods (WHO, 2005).

2.8.1 Global trends in resistance pattern

Antimicrobial resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions, poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil conflicts and bad governance (Byarugaba, 2004), misdiagnosis, counterfeit drugs and lack of education in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases (Walia, 2006). Most non-typhoidal Salmonella infections manifest as potentially self-limiting diarrhea. Antimicrobial resistance is clinically relevant because 3-10% of these infections can progress to life-threatening bacteraemia, particularly in young and immuno-compromised patients. In Indonesia, Salmonella Paratyphi isolates recovered between 1995 and 2001 were universally susceptible to commonly used antimicrobials, Salmonella Enteritidis isolates were resistant to most of the antimicrobials tested, with the exceptions of fluoroquinolones. A similar study in Zimbabwe reported much lower rates of resistance among Salmonella Enteritidis, and more than 50% of non-typhoidal Salmonella isolates from children in Kenya were multi-drug resistant (Okeke et al., 2005). One of the studies in Spain reported high percentages of resistance of Salmonella isolates to sulfadiazine, neomycin, tetracycline and streptomycin, which might be the result of use of antibiotics as a prophylaxis, growth promoter or treatment (Carraminana et al., 2004). A similar study in Alberta, Canada indicated high resistance of Salmonella isolates from food and food animals to ampicillin, streptomycin, sulfamethoxazole and
tetracycline (Johnson et al., 2005). In Ethiopia, resistance pattern of Salmonella isolates from chickens indicated large proportions of strains resistant to a variety of drugs (Molla et al., 2003). Over the past decade in Nepal, increasing antibiotic resistance in Salmonella enterica has lead to a shift in the antibiotics used against this organism from chloramphenicol and ampicillin to trimethoprim-sulfamethoxazole, fluoroquinolones and ceftriaxone, where only a 16-40% positive response to treatment has been achieved (Pokharel et al., 2006). In a study in the United States Salmonella isolated from pre-harvest turkey production sources were resistant to multiple antibiotics (Nayak et al., 2004). In another study, of 380 Salmonella isolates from animal origin in the US, 82% of the isolates were resistant to at least one antimicrobial, and 70% to three or more antimicrobials. Resistance was most often observed to tetracycline, followed by streptomycin, sulfamethoxazole, ampicillin, chloramphenicol, kanamycin, amoxicillin/clavulanic acid, and ceftiofur (Zhao et al., 2007). From 1999 to 2003, 34 411 Salmonella were isolated from animals in the USA, of which 10.9% were found to be resistant to ceftiofur, a third generation cephalosporin used in animals, whilst only 0.3% were resistant to ceftriaxone, a third generation cephalosporin used in human medicine. There was an increase in ceftiofur resistance (Frye and Fedorka-Cray, 2007). Increased antibiotic resistance among Salmonella is not only in the percentage isolates resistant to a particular antibiotic, but also the development of resistance against newer antibiotics (Fluit, 2005).

In a study in Nepal, 35 multi-drug-resistant strains out of 132 strains of Salmonella Typhi were observed showing simultaneous resistance to ampicillin, chloramphenicol, and co-trimoxazole. Although there were no isolates resistant to ciprofloxacin, 69.23% of 52 isolates tested for minimum inhibitory concentration of ciprofloxacin showed reduced susceptibility and 76% of 112 strains tested for nalidixic acid were resistant (Khanal B et al., 2007). There are reports of Salmonella resistant strains isolated from The Netherlands (Duijkeren et al., 2003, 2006), France (Weill et al., 2006), Portugal (Antunes et al., 2003) and many other countries.

Between the year 1999 and 2004, the number of publications reporting Salmonella resistant to β-lactams antibiotics has increased drastically. In 2004,
Salmonella resistant to extended spectrum cephalosporins were identified in 43 countries (Arlet et al., 2006). In a retrospective study in Korea, the resistance rate against chloramphenicol showed mild increase, but the ampicillin, trimethoprim/sulfamethoxazole, kanamycin or nalidixic acid remained at a similar level over 9 years (Yoo et al., 2004). Because the majority of human cases of non-typhoidal Salmonellosis are acquired through the consumption of contaminated food and water, data on the proportions of serotypes and their resistance patterns in different countries are important for global public health management, as food consumption practices vary in different countries and increasing global travel and food trade increase the likelihood of acquiring infections from non-domestic sources (Lauderdale et al., 2006).

2.8.2 Resistance pattern in India

In developing countries like India, easy availability of a wide range of drugs coupled with inadequate health services result in increased proportions of drugs used as self-medication compared to prescribed drugs resulting in impending health problems and antimicrobials resistance. Approximately, 78% of Salmonella Typhi isolates collected from infected patients between 1990 and 1991 demonstrated resistance to chloramphenicol, ampicillin and trimethoprim/sulfamethoxazole. Approximately 81% of the Salmonella enterica serotype Typhi isolates from northern India were resistant to chloramphenicol (Sharma et al., 2005b). A study in Calcutta in India revealed all Salmonella enterica serogroups were uniformly resistant to commonly used drugs with the exception to norfloxacin and ciprofloxacin (Saha et al., 2001). In a few of the studies, a changing pattern of the multi-drug resistant Salmonella isolates was noted (Madhulika et al., 2004, Das and Bhattacharya, 2006). In a recent study in England, Scotland and Wales, it was found that 70% of typhoid cases in returning travelers originated from India or Pakistan, with the highest level of antimicrobial resistance from the Indian subcontinent (Cooke et al., 2007). A study in south India revealed that Salmonella strains from egg and egg-storing trays were resistant to ampicillin, neomycin, polymyxin-B and tetracycline, with 8.9% resistance level to ciprofloxacin (Suresh et al., 2006). Although resistance patterns in Salmonella
have been increasingly observed, re-emergence of chloramphenicol sensitivity has been noted in few of the studies (Sood et al., 1999, Tankhiwale et al., 2003a, Achla et al., 2005, Mohanty et al., 2006).

2.8.3 Status in Bhutan

Although the Department of Health does antimicrobial sensitivity testing of the Salmonella isolates, the procedure is rather treatment based and the published data are not available.

2.9 Prevention and control of Salmonella: overview

Prevention and control measures should be designed based on the two distinct categories, Salmonella infections that have a direct negative impact on the poultry population, and Salmonella infections of importance to public health (Breytenbach, 2004). Prevention of Salmonellosis by the implementation of hygiene measures is difficult and use of antibiotics may give rise to the emergence of resistance problems (Mastroeni and Menager, 2003). Reducing Salmonella prevalence requires a multi-hurdle approach at all stages of breeding, hatching, grow-out, transportation and processing. No silver bullets can be added at a single point in production or processing that will completely eliminate Salmonella on chickens. Therefore, some researchers describe “competitive exclusion” as the most effective and harmless method to control Salmonella in poultry (Ragione and Woodward, 2003, Schneitz, 2005). Attenuated DNA recombinant live Salmonella vaccines (Kang et al., 2002), combined with comprehensive control strategy in animals, feed and animal food stuffs with restrictions on the infected flocks until they have been cleaned up from infections and mandatory testing before slaughter like that being implemented in Sweden (Boqvist and Vagsholm, 2005), will help reduce Salmonellosis. The prevention of paratyphoid Salmonella infection which has greater public health consequences requires a comprehensive control strategy including regular monitoring, strict biosecurity, sourcing feed containing no animal protein, and vaccination (Breytenbach, 2004).
2.9.1 Prevention and control of *Salmonella* in end products

Ensuring safe food production requires knowledge on the nature and origin of the animals, animal feed, the health status animals at the farm, the use of veterinary medicinal products, the results of any analysis of the samples taken at the farm and slaughter data regarding ante-mortem and post-mortem findings and the risks associated with post-harvest production stages (Snijders and Knapen, 2002), or no part of the food chain can be regarded alone but has to be seen as part of the whole. A holistic approach to fresh meat storage and retailing must therefore start with the living animal and cannot end with the sale of the meat. It must also include the consumers (Nowak *et al.*, 2006). Additional measures to control secondary contamination could be prevention of contamination by cleaning and disinfection, hygiene of personnel and proper processing (Sinell, 1995). Growth of microorganisms in meat and poultry products can be controlled by maintaining a cold chain at 10°C, especially for *Salmonella* during transport and storage (Coleman *et al.*, 2003).

Inactivation of potential microbiological pathogens in the end-product may appear attractive, as it reduces the spoilage micro-organisms too (Farkas, 1998), however, other options such as antimicrobial packaging (Quintavalla and Vicini, 2002), vacuum packaging, intelligent packaging and smart active labeling with freshness and spoilage indicators (Nowak *et al.*, 2006) might prove better choices for consumer health protection. Provided the initial production stages have low bacteria and/or are pathogen free, the approach to preventing spoilage of fresh meat is to keep the products at low temperatures, where in most countries the storage temperature is prescribed by law. Enabling rapid identification of microbial contamination to allow rapid response (Doyle and Erickson, 2006), knowledge and attitude of the consumers (Woteki *et al.*, 2001), personal hygiene of food handlers (Nowak *et al.*, 2006), consumer perception of food safety (Redmond and Griffith, 2004), and continuous further education are equally important to achieve food safety practices (Fischer *et al.*, 2006).