CHAPTER I

INTRODUCTION

The genus *Salmonella* is bacterial organism, a gram- negative facultative anaerobic bacilli of the Family *Enterobacteriaceae*, has been recognized as a cause of foodborne disease (Ewing, 1986). *Salmonella* consists of two species, *S. enterica* and *S. bongori*. *S. enterica* is also divided into six subspecies, *S. enterica* subspecies *enterica*, *S. enterica* subspecies *salamae, S. enterica* subspecies *arizonae, S. enterica* subspecies *diarizona, S. enterica* subspecies *houtenae,* and *S. enterica* subspecies *indica* and these subspecies are further subdivided into serovars, characterized by O, H, and Vi antigens. There are over 2,500 known serovars which current classification (Minor and Popoff, 2001). Clinical syndromes include gastroenteritis (*Salmonella* food poisioning), enteric fever (Typhoid fever and typhoid-like fever), and septicemia. *Salmonella* distributes and can live in environment everywhere worldwide, and also found in intestinal tracts of human, animals, birds, reptiles including insects. It can be transmitted by the oral-fecal route. Most people get *Salmonella* infection by eating contaminated foods such as food animals, vegetables, and drinking water. *Salmonella* is also found to be one of the most important pathogen of foodborne diseases, which can survive in meats and food products delivered from animals that are not thoroughly or inadequately cooked. Food products delivered from animal especially poultry are the main vehicle of transmission for human (Schothorst *et al.*, 1974; Rigby *et al.*, 1982; Humphey *et al.*, 1988; Perales and Audicana, 1989; Oboegbulem *et al.*, 1990; Anonymous, 1992; Vugia *et al.*, 1993; and Boonmar *et al.*, 1998). World Health Organization (WHO) announced that salmonellosis is a major public health problem of all countries in the world (WHO, 1985). Such as incidence and epidemic report of

Salmonellosis in the United State which estimated 1.4 million cases each year or 560 cases per 100,000 inhabitants (Mead *et al.*, 2000). Malaysia, Indonesia, and Thailand have reported the highest incidences of typhoid fever of the world which more than 1,000 cases per 100,000 inhabitants each year (Thong *et al.*, 1995). The annual estimation of human salmonellosis in Thailand is 76-1,043 cases per 100,000 inhabitants (Saithanoo and Bangtrakulnonth, 1998). The problem of salmonellosis has become more significance with increasing of incidence of antibiotic-resistant strains (Boonmar *et al.*, 1998). WHO has implemented Global *Salmonella* Surveillance program (GSS) since the year 2000 which sofar 106 countries have participated in this program (Braam *et al.*, 2002). According to *Salmonella* has more than 2,500 serovars, therefore serotyping should be performed on each outbreak for identifying the source of infection which is essential in the surveillance and control of salmonellosis.

Salmonella typing methods fall into 2 broad categories which are phenotypic and genotypic methods (Tenover *et al.*, 1997). Phenotyping involves properties of gene expression which have a tendency to be varied. Since they are based on changes in growth conditions, growth phase, and spontaneous mutation. The factors that enable bacteria to cause infection are oftenly non-uniformity distributed within a species. Thus, the organisms most commonly associated with infections are oftenly a smaller subset of many strains that constitute a species (Musser, 1996). As a consequence, this subset may exhibit relatively little genetic diversity, and it can be difficult to differentiate among strains. The epidemiologic typing of bacterial pathogens can be applied to answer a number of different questions: in case of outbreak, what is the extent and mode of transmission of epidemic clone(s)? In case of long-term surveillance, what is the prevalence over time and the geographic spread of epidemic and endemic clones in the population? Molecular typing methods (genotypic methods) can be used to classify bacteria based on genomic diversity into groups of closely-related isolates (presumed to arise from a common

ancestor in the same chain of transmission) and divergent, epidemiologically-unrelated isolates (arising from independent sources of infection) (Struelens, 1998). Since genotypic methods are based on an analysis of the genetic structure of an organism and include polymorphisms in DNA restriction patterns based on cleavage of the chromosome by enzymes that cleave the DNA.

Salmonella serotyoing, antimicrobial susceptibility patterns and phage typing can be used for epidemiological study. However, these findings are not the final confirmation of epidemiological relation from food sources, animal or another sources. Since each serovars of *Salmonella* has different genetic patterns. Epidemiological conclusion need data of chromosomal DNA patterns for confirmation. Methods that able to reveal chromosomal DNA polymorphism are the best options for comparative typing of most bacterial pathogens (Tenover *et al.*, 1997). Macrorestriction analysis resolved by pulsed-field gel electrophoresis (PFGE) has been prooved to be a gold standard for genome fingerprinting of microbial pathogens (Maslow and Mulligan, 1996; and Tenover *et al.*, 1997) and is believed to possess a discriminating capacity greater than ribotyping and other probe-based restriction fragment length polymorphism methods (Swaminathan and Mater, 1993).

Pulsed-field gel electrophoresis (PFGE) is based on analysis of the whole genome by restriction endonuclease enzyme digestion which be useful for investigation of sources of infection salmonellosis (Powell *et al.*, 1994). Numbers and sizes of DNA fragments (DNA patterns) of each strain of organism after cutting with restriction endonuclease enzyme that have specific pattern and stable, is a highly discriminatory method for the differentiation of bacterial isolates based on differences in DNA content.

There are many studies from several countries that using method of pulsed-field gel electrophoresis for analysis of *Salmonella* strain, such as comparative studied of DNA patterns of *Salmonella* Bovismorbificans isolated from human patients in Finland,

Sweden, England/Wales, Austria, and Germany which found an international distribution of *Salmonella* strains (Liesegang *et al.*, 2002). The DNA patterns of *Salmonella* Enteritidis phage type (PT) 1, 4, 6, and 8 isolated from human patients animals and environment in Denmark, England, and Spain had differential DNA patterns (Laconcha *et al.*, 2000). The DNA patterns of *Salmonella* Typhi in Southeast Asia were found that the genetic diversities among *Salmonella* Typhi strains obtained from Malaysia, Indonesia, and Thailand were shared the same genetic patterns. (Thong *et al.*, 1995). The DNA patterns of *Salmonella* Bredeney isolated from human patients and animals in Ireland and Northern Ireland which found that most *Salmonella* Bredeney were clonally related (Cormican *et al.*, 2002). Studied of the DNA patterns of *Salmonella* Derby isolated from human patients and foods in Hong Kong and found that the sources of human infections were foods. Since most isolates of both were belonged to the same clones and isolates from other sources such as animals or the environment would help elucidate how foods were contaminated (Ling *et al.*, 2001). Analyzed of molecular epidemiology of Chilean *Salmonella* Enteritidis which found that genetic diversity, replacement, and expansion of specific *Salmonella* Enteritidis subtypes were associated with epidemic changes (Fernandez *et al.*, 2003). PFGE method is also applied to confirm in many causes of *Salmonella* infection of human patients in Denmark on the Danish Integrated Zoonosis Surveillance program which found that 20-25% of *Salmonella* were caused from egg contaminated, 10-14% of chicken meats imported from neighbourly countries, 2-4% of chicken meats produced in Denmark, 4-6% of porks imported from neighbourly countries, 6-8% of porks were produced in Denmark. 2-4% of meats imported from neighbourly countries, 0.3-0.5% of meats were produced within country, and 2-4% of turkey and duck meat (Daniso, 2001). Epidemiological studied of *Salmonella* 4, 12:b:- from broiler chickens in Denmark which found that DNA patterns were related to the isolates from food animal factory (Chadfield *et al.*, 2001).

The prevalence of *Salmonella* in Thailand reported by The WHO National *Salmonella* and *Shigella* Center, the National Institute of Health (NIH), Department of Medical Science, Ministry of Public Health, Thailand revealed that *Salmonella* Enteritidis was the most common isolated from human patients in 1993-1997 and also the most common serovar isolated from chicken meat in 1993-2001. Chicken meat is not only one of favourite foods for Thai consumers but also an importance food-exportation of Thailand. In the year 2002, *Salmonella* Enteritidis is the most common isolated from human patients (WHO National *Salmonella* and *Shigella* Center, 2002). To find the epidemiological relationship of *Salmonella* strains isolated from human patients and other food sources cannot depend on the serovar findings. Since each serovar of *Salmonella* have genetic diversity. Therefore, the epidemiological relationship between *Salmonella* Enteritidis isolated from human patients in Thailand and *Salmonella* Enteritidis isolated from chicken meat is still unconfirmed. This study was the first report on DNA patterns of *Salmonella* Enteritidis isolated from human patients and chicken meat in Thailand of the year 2002 by PFGE method to identify the incidence of human *Salmonella* Enteritidis infections relative to chicken meat and to be guideline for surveillance to outbreak cause from *Salmonella* Enteritidis which may be occured.