CHAPTER VI

DISCUSSION

Salmonella enterica is one of the most common causes of human gastroenteritis worldwide. *Salmonella* Enteritidis is one of serovar which widely recognized as a major cause of foodborn gastroenteritis in human and has been isolated from cases of human disease in increasing numbers worldwide during the past 20 years (Rodrigue *et al.*, 1990). Animals and their products, particularly meat from chickens, are considered major sources of human infection (Perales and Audicana, 1988; and Rampling, 1993), which has pointed to an urgent need to effectively monitor the epidemic spread of this pathogen (Phillips, and George, 1994). The increased incidence of human infection with this pathogen may be related to the ingestion of raw or undercooked meat.

An epidemic or disease outbreak is the occurrence of disease at an unusual (unexpected) frequency. Because the word "epidemic" tends to create fear in a population, that term is usually reserved for a problem of wider than local implications, and the term "outbreak" is usually used for a localized epidemic. Nevertheless, the two terms are often used interchangeably (Raymond *et al.*, 1996). The emergence of a disease outbreak requires immediately action to determine the origin of the problem, and ultimately, to prevent other persons from becoming affected (James *et al.*, 1996).

After reviewing the report of prevalence of *Salmonella* Enteritidis in Thailand, increased from 1.3% in 1990 to around 14 % in 1993-1994 (Boriraj *et al.*, 1997). Thus, Thailand has also been a part of the global pandemic of *Salmonella* Enteritidis observed in the late 1980'ties (Rodrigue *et al.*, 1990). The 1995 global survey conducted by WHO showed that the global pandemic was continuing and expanding (Herikstad *et al.*, 2002).

The *Salmonella* Enteritidis pandemic appears to have ended in 1997 and has very much on its return since, which fits nicely with the observed decrease observed in Thailand where the relative importance of *Salmonella* Enteritidis has decreased in Thailand during the last decade. However, this serovar is still an important cause of human infections. Because of *Salmonella* Enteritidis had been found that the most common isolated in the year 2002. The frequent occurrence of this serovar among chickens found in this report suggests chicken meat as the main reservoir of this serovar. (Guard-Petter, 2001). The chicken meat source may be epidemiological related to human patients, source of chicken meat was selected to considered.

The study in the past of *Salmonella* Enteritidis was prevalence and compared with each source, and now is still used which was not enough to typing. Hence, the molecular typing by pulsed-field gel electrophoresis (PFGE) was done to complete this part of the study. The DNA patterns of *Salmonella* Enteritidis isolated from human patients and from chicken meat were compared to study in order to obtain support of epidemiological data.

For pulsed-field gel electrophoresis to analysis *Salmonella*, if enzymes which recognize only few restriction sites within a given genome ("rare cutter") are used for digestion of chromosomal DNA, the fragments produced are long and require special techniques for separation (pulsed-field gel electrophoresis, PFGE) involving alternating electric fields. Maslow *et al* (1993) provided rules on how to interpret PFGE results: (i) strains with identical patterns are considered clonal; (ii) strains with band shifts consistent with a single genetic event (point mutations affecting restriction sites, insertions, deletions, inversions) are also considered clonally related.

PFGE for *Salmonella* Enteritidis using three different restriction enzymes (*Xba*I, *Spe*I, *Avr*II) revealed two major patterns for each of the enzymes (Thong *et al.*, manuscript submitted). However, each pair of patterns was found in both PT4 and PT8 isolates. This indicates that phage types cannot be reliably separated by PFGE and that the two phage

types might be closely related. In this study, *Xba*I restriction endonuclease enzyme that was selected because *Xba*I restriction endonuclease enzyme was superior to *Spe*I and *Avr*II restriction endonuclease enzyme in discriminating (Thong *et al.*, 1995; and Murase *et al.*, 1995). And DNA patterns generated by *Xba*I restriction enzyme in this study revealed 42 DNA PFGE patterns that had difference of number DNA patterns to *Salmonella* Enteritidis isolated from human patients and chicken meat in Thailand between 1993 and 1997, nine different DNA patterns (Sumalee *et al.*, 1998), because the first was using different restriction enzyme, *Bln*I restriction enzyme, had differential recognition site and other conditions, such as model of contour-clamped homogenous electric field apparatus, initial switch time, or and final switch time, and eight different phage type had indistinguishable pattern with PFGE, belonged to one single PFGE-type. From these results it can be conclude that PFGE is neither a method that would allow separating strains of different phages types nor does it allow reliable discrimination within a given phage type.

Abiodun *et al* (2000) used *Xba*I restriction enzyme to digestion genome of *Salmonella* Enteritidis isolated from gastroenteritid cases in four Caribbean countries and analyzed with PFGE method. DNA digested by *Xba*I restriction enzyme revealed 13 distinctive PFGE patterns which had some patterns related to in this study (Abiodun *et al.*, 2000).

Most differences in the patterns found can be explained by single genetic events indicating a close relationship of the strains. Using dendrogram to cluster analysis should be correlated to criteria for interpreting PFGE patterns of Tenover *et al.* because dendrogram had discriminatory power higher, but can set position tolerance and optimization.

Dendrogram was indicated that had two major clusters (A and B) of human patients and chicken meat. they were 42.8 % similarity relation. Jorge *et al.* (2003) analyzed *Salmonella* Enteritidis isolated from Chilean, revealed two major clusters in the

same with this study. Cluster A in this study was the predominant strains and could be related to epidemic in Thailand because profile 1 was in cluster A and were the most found in both sources which distributed every part of Thailand (Department of Medical Science, 2002) and had close relation in both human patients and chicken meat, which sugested that were the same clonal identity.

However, from highly differential proportion of number of samples, suggested that if had the same number or proportion in both, could be found the same strain between human patients and chicken meat more.

Recently PFGE has been proved to be more accurate in discriminating between unrelated organisms than biotyping, serotyping, phage typing and plasmid analysis (Hoyen, *et al.*, 1999). PFGE technique must be performed on specialized, expensive equipment. Additionally the technique requires that the restriction enzyme used cuts the genomic DNA into 10-20 easily distinguishable fragments, to allow accurate identification of polymorphism. The appropriate restriction enzymes have been determined for most clinically relevant pathogens. The procedure is labbor-intensive and requires experience and technical expertise, but the analysis can be completed within approximately 10 days with standard protocols to yield a very accurate assessment of genetic relatedness.