CHAPTER 1

GENERAL INTRODUCTION

1.1 *Salmonella* classification and identification

Salmonella spp. is one of the most important bacterial-zoonotic pathogens that causes acute food-borne diseases in humans [1], and is recognized as a major public health problem [2]. Salmonellosis is the group of clinical conditions caused by *Salmonella* spp., with an estimated 80.3 million cases of foodborne salmonellosis occurring worldwide annually [3]. The Bureau of Epidemiology estimated 105,028 human cases of salmonellosis in Thailand in 2012 [4]. Clinically, salmonellosis in humans may start with an acute onset of fever, nausea, headache, vomiting and profuse diarrhea within 8 ~ 48 h of ingesting the pathogen. The severity of the disease depends on the ingested dose and the host's immune status [5].

Salmonella are Gram-negative, motile rods, facultative anaerobic bacteria belonging to the family Enterobacteriaceae [6]. Peritrichous flagella provide motility for most of them, except *Salmonella* pullorum-gallinarum [7]. *Salmonella* spp. characteristically ferment glucose and mannose, but fail to ferment lactose or sucrose. The bacteria tend to produce hydrogen sulfide with sometimes gas (Table 1.1). *Salmonella* can grow within a temperature range of 8-45^{o}C and a pH range of 4-8 [6, 7]. They can survive freezing in water for long periods and are resistant to many chemicals, including Brilliant Green, Sodium Tetrathionate and Sodium deoxycholate; these compounds inhibit coliform bacteria and, as a result, are useful for isolating *Salmonella* from feces [8].

The genus *Salmonella* includes two species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is divided into six subspecies, which are referred to by a Roman numeral and subspecies name (Table 1.2) [9]. Most of the serotypes belong to

enterica species causing human salmonellosis [10]. *Salmonella* are initially detected by their biochemical characteristics; groups and species must be identified by antigenic analysis [7]. *Salmonella* reveal several O antigens (more than 60) and different H antigens in one or both of two phases [6]. Some of them have capsular antigen (Vi antigen) that may interfere with agglutination by O antisera and may be associated with virulence [7].

The Kauffmann-White classification scheme is a practical summary of the antigenic structure of different *Salmonella* serotypes [11]. It is based on agglutination tests with absorbed antisera [7]. Examples of antigenic formulas are shown in Table 1.3.

For disease surveillance and outbreak investigation, serotyping is the most common technique for characterizing *Salmonella*. However, serotyping is based on immunological typing, and thus requires a large number of specific sera [12]. As a result, other techniques have been used for identifying *Salmonella*, including "Phage typing" that helps trace isolates by lysis by a set of specific bacteriophages [13-14] and "Repetitive Sequence-based Polymerase Chain Reaction", or rep-PCR, which provides a DNA fingerprint based on amplifying the non-coding region [15]. However, the discriminatory power of these methods is quite low [16]. "Pulse Field Gel Electrophoresis", or PFGE, is a DNA-fingerprinting comparison technique based on gel separation of large DNA fragment from the whole genome [17]. This method has long been accepted for molecular characterization of a wide range of bacterial species [18]. This technique clearly and precisely distinguishes bacterial genotypic diversity and is more appropriate for epidemiological investigations of foodborne pathogens, such as *Salmonella* spp. [19].

1.2 *Salmonella* in farm animal origin products

Although contaminated farm foods are the primary sources of salmonellosis in humans, a wide range of foods, especially those originating from farm animals, have been implicated. [8]. Contamination can occur during any process along the food production line such as farm, processing plants, warehouse or the restaurants [7, 20].

In most cases, *Salmonella* spreads horizontally between animals, via the fecal-oral route, through fecal contamination of the environment on the farm [3, 21-23]. The slaughtering process is a major point for spreading the organism from animal's intestinal tract to slaughtered carcasses directly be themselves or via contaminated slaughtering-equipment with the improper practices [1, 24]. As a result, infected farm animals in pre-harvest levels are considered as the first origin of the contaminated products that lead to human infections [25-27].

1.3 *Salmonella* in pig production chain

Salmonella isolates associated with pig production cause an estimated one-fifth of all cases of foodborne human salmonellosis [28]. In Thailand, three common *Salmonella* serotypes isolated from swine (Rissen, Weltevreden and Anatum) overlapped with the most common serotypes identified from human *Salmonella* infections [29].

Salmonella-infected pigs, which carrying the organisms in their intestinal tracts, can increase the risk of contamination of carcasses during slaughter [3, 30]. Farm pigs are the origin of the *Salmonella* reservoirs in the production chain [1]. Inadequate hygiene practices, broad-spectrum antibiotic use and contaminated feed play the primary role in *Salmonella* infection at the pre-harvest level. In addition, several other factors have been considered risks for salmonellosis in farm pigs [31]. Changes in feed structure can affect a variety of physiologic and metabolic processes in the GI tracts of pigs. *Salmonella* survival and colonization may be hostile from irregular changes in gut pH and the microbial ecosystem [32]. Other infections have been reported to favor *Salmonella* infections in pigs, including *Lawsonia intracellularis* [33], porcine reproductive and respiratory syndrome virus [34] and parasite infestation [35]. Moreover, farms that receive pigs from suppliers is also relates to *Salmonella* than farms that breed their own replacement stocks [36].

The high contamination pressure on farms is directly related to the number of contaminated carcasses [21, 24, 26]. About 70% of all carcasses contamination, results from the animal itself being the carrier, and 30% because other animals are

carriers [31]. The cross contamination occurs from inadequately routines practices and contaminated materials in the slaughtering line [24, 37]. Each step in the slaughtering line can affect the role to vary in *Salmonella* numbers in this level. Stress due to longer waiting periods before slaughtering could result in pigs shedding more pathogens in the intestinal lumen [37]. Scalding and singeing reduces the probability of contamination and the number of carcasses contaminated [27, 38]. Improper evisceration technique is the most important risk for carcass contamination from *Salmonella* via contaminated intestinal contents [31]. After slaughtering, chilling is a suitable method for arresting colonization by *Salmonella* spp. [39].

At the post-harvest stage (slaughtering plants, butcher shops or retail shops), the important parameters for reducing *Salmonella* contamination are proper handling, general hygiene, proper time-temperature parameters for product storage [30] and hygienic disinfection and manipulation [40]. These are essential to avoid cross contamination, as well as maintain any contamination at as low a level as possible [25].

1.4 Overview of the study and further plan

Based on the information mentioned above, *Salmonella* characteristic, distribution and risk were explored, mainly in the harvest or slaughtering level. All of information obtaining in this step was used to compare with the knowledge acquired from the previous level (pre-harvest or farm level). To create appropriate preventive measures and help to control Salmonellosis in the region.

About the further plan, the results of this study could form a basis for risk assessment of *Salmonella* in the whole pork production chain. It is a methodology used to establish and evaluate scientific information to estimate the probability and severity of an adverse event [41], such as the number of salmonellosis infected cases per year or mortality rates per meal in YOPI persons (young, old, pregnant or immunocompromised) [42]. The methodology can also help to identify those stages in the manufacturing, distribution, handling and consumption of foods that contribute to

an increased risk of several foodborne illnesses, and help focus resources and efforts to most effectively reduce the risk of foodborne pathogens, such as *Salmonella* spp. [41]. The model will be generated from completed information (Fig 1.1) in the whole production line [43]. Therefore, it would be enhancing a domestic consumer protection from *Salmonella*, promoting *Salmonella* free pork production and good practicing in consumption *Salmonella* free pork. In addition, the results can be used as baseline information to develop standard and guideline for prevention and control of *Salmonella* contamination in pork production chain both for domestic consumption and export.

1.5 Objectives

The objectives of dissertation research were

Chapter 2:

- To determine the prevalence and quantitative loads of *Salmonella* spp. among farm and slaughtering levels
- To compare the prevalence and quantitative loads from the representative samples from pig, environment and person among farm and slaughtering level

Chapter 3:

- To quantify contamination levels of *Salmonella* spp. on pig skins and carcasses in each step during the slaughtering process
- To evaluate the outcomes from different in pig supply sources and with different practices in three critical slaughtered-processing steps
- To define the risk of *Salmonella* spp. contamination in pork products after slaughtering level in three representative slaughterhouses

Chapter 4:

- To characterize *Salmonella* spp. isolated in pig production lines both at farms and slaughterhouses by focusing on the association of serotypes, antimicrobial resistance patterns and pulse field gel electrophoresis (PFGE) patterns

Table 1.1 Biochemical reactions of certain gram-negative enteric bacteria

Organisms	Motility	Glucose	Lactose	Sucrose	Mannitol	H_2S	Tripple sugar iron agar		Lysine Decarboxylase
							Slant	Butt	
E. coli	+	AG	AG	±	AG	-	A	AG	±
***S.* Typhi**	+	A	-	-	A	±	Alk	A	+
***S.* Paratyphi A**	+	AG	-	-	AG	-	Alk	AG	-
***S.* Typhimurium**	+	AG	-	-	AG	+	Alk	AG	+
V. cholerae	+	AG	-	A	A	-	A	A	+
P. aeruginosa	+	±	-	±	-	-	Alk	±A	-

(±) Variable (+) Positive (-) Negative (A) Acid

(AG) Acid with gas (Alk) Alkaline

Table 1.2 Present number of *Salmonella* serovars in each species and subspecies

***Salmonella* species and subspecies**	**No. of serotypes**
S. enterica **subsp.** *enterica* (I)	1,531
S. enterica **subsp.** *salamae* (II)	505
S. enterica **subsp.** *arizonae* (IIIa)	99
S. enterica **subsp.** *diarizonae* (IIIb)	336
S. enterica **subsp.** *houtenae* (IV)	73
S. enterica **subsp.** *indica* (VI)	13
S. bongori (V)	22
TOTAL	2,579

Table 1.3 Antigenic formula of *Salmonella* spp.

O Group	***Salmonella* serotypes**	**Antigenic Formula (O antigen : Phase 1 H antigen : Phase 2 H antigen)**
A	*S.* Parattphi A	1,2,12 : a : 1,5
B	*S.* Stanley	1,4,5,12 : d : 1,2
B	*S.* Typhimurium	1,4,5,12 : i : 1,2
C_1	*S.* Cholerasuis	6,7 : c : 1,5
C_1	*S.* Rissen	6,7 : f,g : -
D	*S.* Enteritidis	1,9,12 : g,m : -
D	*S.* Typhi	9,12,[Vi] : d : -
E_1	*S.* Weltevreden	3,10 : r : z6

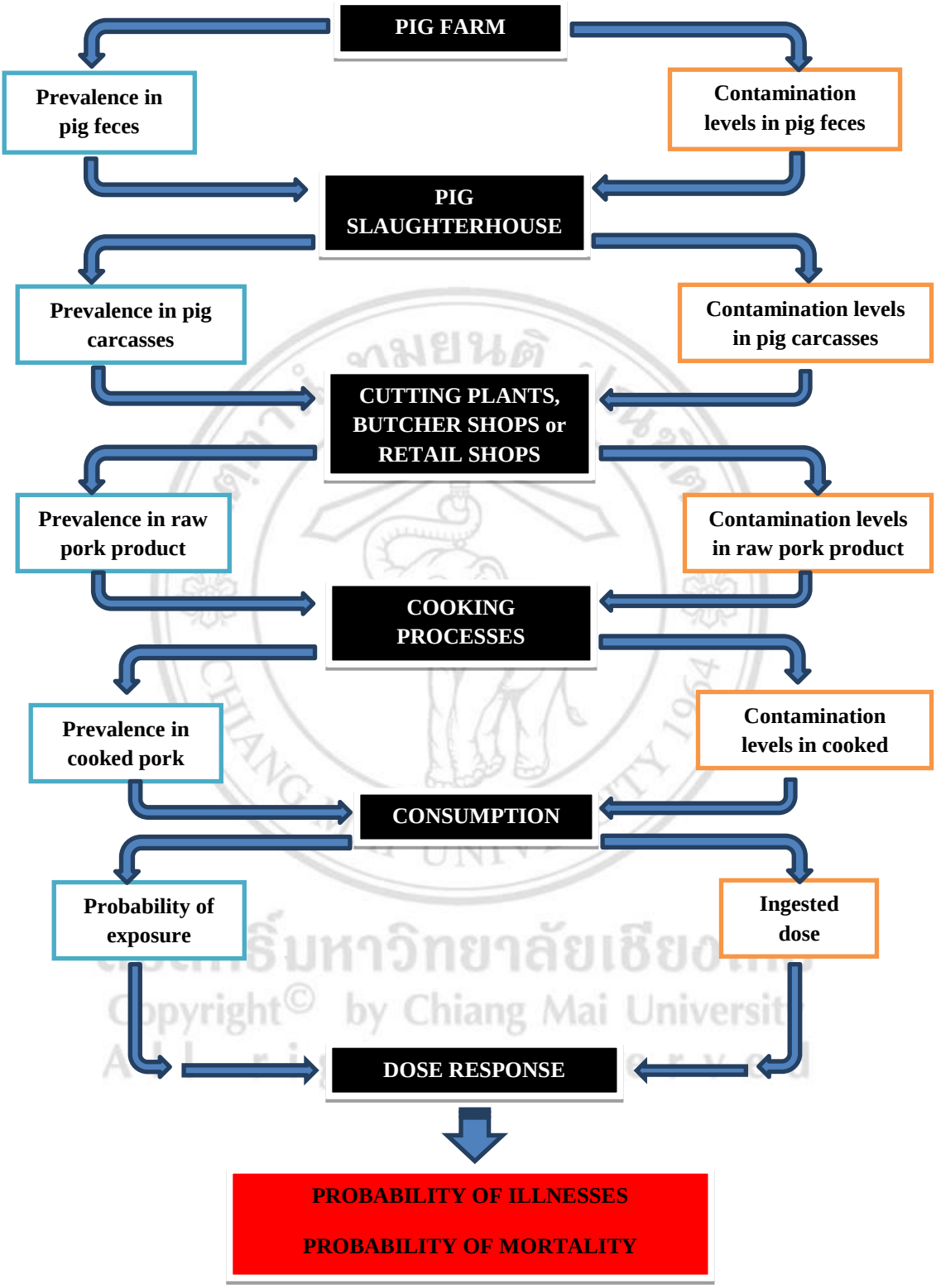

Figure 1.1 Flow diagram of the mathematical model of exposure assessment and dose–response for *Salmonella* spp. in pork products