CHAPTER 2
LITERATURE REVIEW

2.1 The situation of food poisoning in Vietnam

Food poisoning and diarrheal illnesses are among the leading causes for world-wide morbidity and mortality (WHO, 1998). Food safety was identified as a high priority area in the years 2001 – 2005. Member countries of the OIE considered that the organization should be more active as far as public health and consumer protection is concerned and that this should include more involvement in the area of diseases or pathogens transmissible through food (Droppers, 2006). Meat and meat products are of particular importance regarding food-borne diseases. Food-borne pathogens can be transferred to food during the processing, distribution and storage from infected humans who handle the food or by cross-contamination from some other raw agricultural products (Hedberg *et al.*, 1994).

In recent years, the Vietnam government has shown considerable attention to reducing the risks to food safety. The most significant event was the creation of the Food Administration within the Ministry of Health in 1999 (Kim, 2002). According to the statistics of the Food Safety Administration in 2000-2006 there were 174 food poisoning cases at collective kitchens with 14,650 patients, 97 cases at industrial and export processing zones with 9,900 patients, 58 cases in schools with 3,790 victims (two died) and 161 street food poisoning cases with 7,688 victims (7 died). In Vietnam the Ministry of Health, which is responsible for food inspection of domestic and imported food, reported over 4 million cases of severe enteric disease, e.g., typhoid, cholera and shigellosis from 1997 to 2000. There were 1,391 outbreaks involving over 25,000 cases and 217 deaths (Kim and Phuong, 2001). There is a dramatic increase in those diseases from last year, which saw nearly 8,000 cases of food poisoning with 61 deaths. In the first quarter of 2009 Vietnam reported 229 cases of food poisoning, including two fatalities according to the Food Safety and Hygiene Department. An overview is given in Tables 1 and 2.

Table 1: The situation of food-borne infectious outbreaks in Vietnam, 1999 - April 2009

Year	Number of food borne infectious outbreaks	Number of infected humans	Number of deaths	Percentage of deaths
1999	327	7,576	71	0.9
2000	213	4,233	59	1.4
2001	245	3,901	63	1.6
2002	218	4,984	71	1.4
2003	238	6,428	37	0.6
2004	145	3,584	41	1.1
2005	144	4,304	53	1.2
2006	139	5,564	49	0.9
2007	248	7,329	55	0.75
2008	205	7,828	61	0.78
2009	11	229	2	-

Table 2: Food-borne infection and intoxication in Vietnam, 1999 -2008

Year	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
Biological agent (%)	48.3	32.8	38.4	42.2	49.2	55.8	51.4	35.4	38.6	7.8
Chemical agent (%)	11.0	17.4	16.7	25.2	19.3	132.	8.3	20.0	27.2	0.5
Food-borne infection (%)	6.4	24.9	31.8	25.2	21.4	22.8	27.1	21.5	29.7	25.4
Unknown (%)	34.3	24.9	13.1	7.4	10.1	8.2	13.2	23.1	4.5	66.3

Source: Annual summary reports, Food Safety and Hygiene Department, Ministry of Health, Vietnam.

2.2 The pork meat production and consumption in Vietnam

Pork is a popular meat and it is produced under a wide variety of production systems ranging from simple backyard pigs to large-scale integrated pig. Most of the pork is predominantly produced for domestic demands and consumption. It is estimated that 95% of the households consume pork in their daily diets (Dinh *et al.*, 2005). Therefore pigs are slaughtered on a daily basis and most of the pork is consumed as fresh meat within a day. According to a master plan of MARD the aim is to raise the animal production (including the development in the pig sector) to 30% of the total agricultural value by 2010 and to 35% by 2015 (GAIN, 2006). Pig production exhibits strong comparative advantages in both the north and the south of the country. Together with the income the pork consumption is increasing very fast (12.3 % from 2000 to 2005).

2.3 Current situation of the slaughterhouses in Vietnam

2.3.1 Supply of animals

Transportation is divided into two parts. The first is from the farmer to the slaughterhouse; the second from the slaughterhouse to the wet market or to the retail shops. The transport is mostly arranged by the traders. Some transportation from the slaughterhouse and to the wet market is done by the stall owners of the wet market. The living pigs are transported in small one ton lorries. These lorries do not provide much space to the animals transported. The load is well ventilated. But it is a very unstable mode of traveling for animals in traffic and on bad roads. The transport of meat to the market or to the retail shops is done by motorbikes or by one ton lorries. The lorries are often not chilled (Landell Mills Ltd. and VIN Consultants, 2007) .

The transport can be a very rough experience for the animals. The drivers show clearly that they have no idea of how to treat them. The better the treatment the better is the quality of meat. Transportation is driven too fast with a lot of stopping and starting. When the animals arrive at the slaughterhouse after a hard and may be a long journey. Since there is no unloading ramp the animals are forced to jump from the floor of the lorry onto the ground or they have to have a little “help” from the

driver. It is necessary for the animals to rest after this stressful transport. If the animals are gently treated this results in a positive effect on the quality of the meat. If an animal, in particular cattle, is exhausted before slaughtering the meat will often be dark, firm and dry. If the pig is stressed the meat will often be pale, soft and exudative (Landell Mills Ltd. and VIN Consultants, 2007).

2.3.2 Methods of sourcing the livestock

Since the majority of the pig farmers raised only some few pigs the market must assemble pigs from a large number of small-scale farms. Pig farmers use two marketing channels: 1. Pigs sold to assemblers and 2. Pigs sold to slaughterhouses. Assemblers are individuals specialized in the collection of fattened pigs from farmers and their onward sold to larger assemblers or slaughterhouses (Dinh *et al.*, 2005).

There were signed contract systems between and among supply chains for butcher shop owners in big markets. Only large scale producers had contracted with middlemen to sell their pork retail markets. Stallholders in the supply chain had signed contracts with them regarding constant supply (Dinh *et al.*, 2005).

2.3.3 General characteristics of pig slaughterhouses

Basically, there are two kinds of pig slaughterhouses. Modern slaughterhouses have a complete slaughter line starting with stunning, hoisting the unconscious animals onto overhead rail, sticking and bleeding are done by vacuum aspirators into blood collecting barrels, pre-washing the carcasses with potable water, scalding at 60 to 64^0C and dehairing by machine, singeing, polishing, evisceration, splitting the carcasses, chilling and further processing of the carcasses. The advantage of a modern slaughter line is minimization of the cross-contamination during the process. Modern slaughterhouses are common in developed countries; they exist in some meat exporting companies in developing countries.

So-called traditional slaughterhouses are popular in developing countries. Infrastructure is frequently old and obsolete, especially cold chain storage of the carcasses is rarely available. The process to slaughtered animals can be summarized as: stunning of animals by head hitting or no stunning, sticking with knives, bleeding, scalding by pouring boiling water on carcasses or dipping carcasses into a hot water

vat, dehairing with knives, pre-evisceration washing of carcasses by tap water, evisceration, then washing carcasses again, transporting the carcasses or primal directly to markets without chilling. All stages of pig slaughtering are done on the ground, products are dragged across the floor from one area to another for further processing. Parallel processes of slaughtering are done simultaneously by large number of butchers and meat handlers. Here, butchers can use the same knife for stickings, dehairings and eviscerations.

The term “slaughter point” implies that pig slaughtering is done in the house of meat traders or on pig farms. The slaughter area can be the house yard, nearby water well or somewhere inside the pig farms. The area for slaughtering is normally narrow. The method to slaughter animals is obviously floor dressing. Amounts of pork supplied to domestic market outlets from slaughter points even much higher than from traditional slaughterhouses in some developing countries where rural area occupies the most in the country and small scale of pig production is dominant (Heinz, 2008).

2.4 The source for microbiological contamination in fresh meat

2.4.1 Livestock

All animals carry a very large number of bacteria in their stomachs and intestines which are excreted in their feces. Bacteria are also present on the skin, the hide fleeces and feathers of the animals, including those from direct contact with feces or from indirect contact with the environment of the farm, transport vehicles or lairage (Lo Fo Wong *et al*., 2002; Mossel *et al*., 1998, Warrier *et al*., 2002; Botteldoorn *et al*., 2003). The bacteria in or on animals may include those which can cause food poisoning in humans and which are recognized as hazards from the meat. Most of these bacteria do not cause disease in meat producing animals, which means that these animals will appear “healthy” upon meat inspection (Paulsen and Smulders, 2004). Although ante-mortem inspection will enable clinically ill animals to be detected it is not possible to identify healthy carriers of pathogenic organisms. Therefore, it must be assumed that all animals entering the slaughterhouse have the potential to carry pathogenic organisms in or on them.

Gastrointestinal tract

Accidental puncture of the stomach and intestines is a source of contamination on occasions, as is spillage from the rectum and oesophagus. It has been estimated that the mixed bacterial flora of the gastrointestinal tract may reach 10^{10} cfu/g of contents

Sticking point

During the act of sticking, bacteria can enter the jugular vein or anterior vena cava and travel in the bloodstream to the muscles, lungs and bone marrow.

Operatives

All persons working in the slaughterhouse are an important, and extremely mobile, source of contamination for the meat. Movement of all personnel about the plant must be strictly controlled.

Equipment and utensils

The equipment used within the slaughter floor is a potential source of contamination. This includes knives, saws and hock cutters which come into direct contact with the meat (Gracey *et al.*, 1999).

2.4.2 Carcasses

Bacteria from the surface or digestive tract of an animal may be transferred onto the carcasses or onto other carcasses during slaughter and dressing. This transfer may be caused by direct contact or through cross-contamination by slaughterhouse staff, equipment, surfaces (Loncaric *et al.,* 2009), water or aerosols (Prendergast *et al.,* 2004).

2.4.3 Processed meat

The further processing of meat into minced meat, meat preparations and meat products provides an opportunity for any dangerous bacteria on the surface of the carcass meat to be spread throughout the product and also for new bacteria to be introduced from the environment, handling and processing.

In particular, bacteria will be spread into the centre of the food where they will be less easily destroyed by cooking. If the production process does not contain a pathogen reduction step such as cooking then any bacteria on the carcass meat will be present in the processed meat. If the product is intended to be eaten raw or not

thoroughly heated, then special care will be needed to ensure the absence of *Salmonella* and the safety of the food. For minced meat and meat preparations intended to be eaten cooked, the absence of *Salmonella*, although ultimately desirable, is not practical with the current prevalence of *Salmonella* in animals.

Processed meat products may contain a range of the microorganisms that can cause infection or intoxication of humans. The skill of the slaughterhouse workers is an important means for preventing the contamination of muscles with gut contents and fecal matter which may contain high numbers of infectious and toxigenic pathogens. The input level of microorganisms to products can be minimized by good layout, hygienic operational practices and rapid chilling. It may be increased by poor hygiene and badly controlled storage conditions. If carcasses are cooled too slowly, stand for long periods and if microbiological contamination of deep muscle or bone has occurred, there is a good chance for the growth of mesophilic bacteria, especially *Salmonella*, *Clostridia* and *bacilli*, leading to spoilage (Brown, 2000).

2.4.4 Environmental and ecological considerations

Salmonella spp. can survive and proliferate outside their hosts. The "*Salmonella* problem" is basically a continuous fecal oral cycle and thus also a hygiene problem. Many *Salmonella* spp. can survive for several months in soil and for several weeks on stems and leaves of feed crops. In and on insects and rodents and on the surfaces of building materials such as wood, concrete, iron, steel and brick many *Salmonella* can survive for at least several days and for as long as 9 months (Mitscherlich and Marth, 1984, Wray *et al*.,, 1987) Due to ecological and environmental circumstances it is sometimes possible to observe geographical differences in the prevalence of positive farms and *Salmonella* spp. isolated.

2.5 An overview of Aerobic Plate Count

2.5.1 Indicator organisms

Indicator organisms are larger groups of bacteria, including certain pathogenic bacteria, which are relatively easy to measure as a group and whose presence is likely to indicate the presence of pathogenic bacteria ("index bacteria" in the sence of

Mossel, 1982). Aerobic Plate Count (APC) is a general measure of the microbiological status of meat. But APC results and the number of present pathogens may not always be related. Testing for *Enterobacteriaceae* a group of indicator organisms that live in the intestines of animals and the environment will give a better indication of the likelihood of pathogenic organisms being present. Control measures that reduce the number of *Enterobacteriaceae* and the APC should thus reduce the risk of the presence of pathogenic bacteria on meat. Aerobic and *Enterobacteriaceae* counts are used as indicator organisms in meat and food products. A high APC on carcasses usually indicates the degree of care taken during slaughter and unsuitable time or temperature conditions during storage of the meat. It can also indicate heavy post-slaughter and post processing contamination.

APC will allow the growth of almost all organisms present that are capable of growth under the selected incubation conditions. APC is normally incubated aerobically at 20^{O}C and 35^{O}C to assess the levels of mesophilic gram positive and gram negative organisms present. The plated samples can also be incubated anerobically to assess anaerobic and facultative anaerobic populations. On completion of incubation the number of organisms per gram of sample may be back calculated depending on the original sample size and the levels of dilution employed. The colonies on these primary plates can be subcultured to purify bacterial colonies in order to help identify the organisms (Gracey *et al.,* 1999).

The APC is the best method for estimating the microbial population on raw foods and provides the most accurate index of the microbiological condition of food. Also, the determination of APC is a quite easy and cheap analytical method. Thus, the APC is one of the most commonly used indices of the microbiological condition of food (Avery, 1991).

The APC can be determined by pour plates, spread plates, spiral plating. Three types of bacteria, defined by their temperature of growth, are found on meat. Mesophiles grow at 37^{0}C. Psychrotrophs and psychrophiles have lower optimum growth temperatures than mesophiles. Grau (1986) defines psychrotrophs as having an optimum temperature of 20^{0}C or more with many unable to grow at 35 to 37^{0}C. Psychrophiles are capable of growth below 0^{0}C (Morita, 1975). The micro flora of meat and meat products changes with time and conditions of storage. Carcasses are

initially contaminated with mesophilic bacteria. Thus, determining the APC at 37°C is sometimes used for testing foods from animal sources. A high count of mesophilic bacteria on carcasses occurring immediately after the dressing is evidence of poor hygiene.

Aerobic plate counts serve as a useful indicator for the conditions of sanitation, holding temperatures and of time elapsed during food production and transportation. However, there are limitations to the value of any counts, particularly in fermented foods where a large population of bacteria is required for the fermentation or ripening process.

2.5.2 Meat spoilage and pathogenic microorganisms

There are many factors which affect the storage life of fresh meat and the keeping quality of meat. It can be predicted by monitoring for spoilage microorganisms (Gill and Bryant 1992). Temperature plays a crucial role in meat spoilage (Narashimha Rao and Ramesh, 1998). Microorganisms are classified as psychotrophs, mesophiles and thermophiles, see Table 3. However, the most relevant pathogenic bacteria are mesophiles (Table 4).

Table 3: Temperatures for microorganisms

Group	**Temperature (^{0}C)**		
	Minimum	**Optimum**	**Maximum**
Thermophiles	40 - 45	55 - 65	60 - 90
Mesophiles	5 - 10	30 -45	35 - 47
Psychrotrophs	-5 - +5	25 - 30	30 - 45
Psychrophiles	-5 - +5	12 - 15	15 - 20

Fresh meats may be initially contaminated from many different sources: soil, dust, faeces, water, equipment, the hands and clothing of personnel. And although it was originally thought that the flesh of healthy animals at slaughter was sterile, it can habour organisms mostly gram positive mesophiles. Depending on the types of

bacteria present, meat-borne disease or spoilage or both may result especially if substandard handling methods are adopted (Gracey *et al.*, 1999).

Meat is recognized as a source for several bacterial pathogens that cause food poisoning in human although the source of the infection is not determined in the majority of outbreaks of food-borne infectious diseases investigated (Hinton, 2000).

Table 4: Approximate minimum, maximum and optimum temperature values in ^{O}C permitting growth of selected pathogen relevant to food

Organism	Temperature (^{0}C)		
	Minimum	**Optimum**	**Maximum**
Bacillus cereus	5	28 to 40	55
Campylobacter spp.	32	42 to 45	45
Clostridium botulinum	10 to 12	30 to 40	50
Clostridium perfringens	12	43 to 47	50
Listeria monocytogenes	0	30 to 37	45
Enterotoxigenic *Escherichia coli*	7	35 to 40	46
Salmonella spp.	5	35 to 37	45 to 47

ICMSF 1996 ; Lund *et al*., 2000; Doyle *et al*., 2001.

2.6 An Overview of *E. coli*

2.6.1 Biochemical characteristics

Escherichia coli are Gram negative, non-spore forming rod shaped organisms. The organisms' ferments glucose with the production of acid and gas can motile through peritrichous flagella. The limits of temperature for the growth of *E. coli* are 7 – $46^{0}C$ and the optimum growth temperature is approximately $37^{0}C$. The bacteria grow at pH levels between 4.4 and 9.0 at a water activity (a_w) of at least 0.95. *E. coli* is a member of the family *Enterobacteriacaea*. *E. coli* is oxidase negative, catalase

positive fermentative (glucose, lactose, D-mannitol, D-Sorbitol, arabinose, maltose reduce nitrate and is ß galactosidase positive. Approximately 95% of the strains are indole and methyl red positive (Fratamico and Smith, 2006). All strains of *E. coli* are negative in the Voges-Proskauer test. Most strains do not hydrolyse urea or produce H_2S in triple sugar iron (TSI) medium, Simmon's citrate negative (Willshaw *et al.,* 2000). Biochemical tests of IMViC reaction (IMViC stands for the capital letters of Indole, Methyl red, Voges Proskauer and Citrate, respectively). A total of 96–98% of *E. coli* strains express the enzyme β-D-glucuronidase which reacts with the enzyme substrate BCIG (5-bromo-4-chloro-3-indollyl β-D-glucuronic acid) to produce a deep blue colour in the colonies. *E. coli* O157:H7 colonies remain colourless (Okrend *et al.,* 1990).

It is found in the gastrointestinal tract of humans and other warm blood animals. Consequently, the presence of *E. coli* in food has been regarded as an indication of fecal contamination (Todar, 2005; Willshaw *et al.,* 2000).

Most *E. coli* strains are harmless: Some, however, such as serotype 0157:H7 can cause serious food poisoning in humans and are occasionally responsible for costly product recalls. The harmless strains are part of the normal flora of the gut. They can benefit their hosts by producing vitamin K or by preventing the establishment of pathogenic bacteria within the intestine (Todar, 2005).

Overall, there are more than 170 different known serogroups, over 200 types of O antigens, 100 K antigens and 50 H antigens (Jay, 1997; Willshaw *et al.,* 2000). The O antigen is the polysaccharide that projects from the core polysaccharide capsular antigen. The H antigen is the flagella protein. There are considerably less H antigens because the carbohydrate side chains are less heterogeneous than that of the O antigen. The different O, H and K antigen formula give each strain its name (Jay, 1997).

E. coli has been used as an indicator to assess the hygiene and cleanliness of many food processes. Although the presence and numbers of *E. coli* in foods, especially raw meat, can not be directly related to the presence of pathogens, the detection of high levels of *E. coli* certainly indicates a high risk of "possible" pathogen contamination (ICMSF, 1978).

E. coli is commonly found in food of animal origin and is an indication of sewage pollution of water and unhygienic methods of preparation. The factors responsible for the onset of food poisoning, mainly lack of hygiene and careless storage of cooked and uncooked food at temperatures suitable for bacterial growth, are virtually the same as those that lead to the spoilage of food.

Further classification of the bacteria categorizes them into several groups: enterohemorrhagic *E. coli* (EHEC) or Shiga toxin producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC). EHEC or STEC organisms cause hemorrhaging and loss of blood due to the production of one or more toxins that are identical to that produced by Shigella dysenteriae. ETEC are often associated with outbreaks in childcare centers and with traveler's diarrhea. The toxin produced by ETEC is similar to the cholera toxin producing diarrhea without a fever. EPEC organisms do not contain the heat labile or heat stable toxin that the ETEC organism contain nor do they require the colonization factors. However, EPEC is similar to ETEC by its production of watery diarrhea. EaggEC are non invasive. They cause no inflammation and they are identified by the cells' tendency to clump together. EaggEC cause persistent non bloody, watery diarrhea in young children (Todar, 2005; Willshaw *et al.*, 2000).

2.6.2 Virulence properties

Enterotoxigenic *E. coli* (ETEC): is a causative agent of diarrhea without fever in humans, pigs, sheep, goats, cattle, dogs and horses. ETEC uses fimbrial adhesions to bind enterocyte cells in the small intestine. ETEC can produce two proteinaceous enterotoxins:

- The larger of the two proteins, thermolabile LT enterotoxin, is similar in structure and function to the cholera toxin
- The smaller protein heat stable ST enterotoxin causes camp accumulation in the target cells and a subsequent secretion of fluid and electrolytes into the intestinal lumen.

ETEC strains are non-invasive. They do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhea in children in the developing world, as well as the most common cause of traveler's diarrhea. Each year, ETEC causes more than

200 million cases of diarrhea and 380.000 deaths, mostly in children in developing countries (Steffen *et al.,* 2005).

Entero Pathogenic *E. coli* (EPEC) is a causative agent of diarrhea in humans, rabbits, dogs, cats and horses. Like ETEC, EPEC also causes diarrhea but the molecular mechanisms of colonization and etiology are different. EPEC lack fimbriae, heat stable toxin (ST) and heat-labile enterotoxin (LT). But they utilize an adhesion known as intimin to bind host intestinal cells. This virotype has an array of virulence factors that are similar to those found in *Shigella* and may possess a shiga toxin. Adherence to the intestinal mucosa causes a rearrangement of actin in the host cell causing significant deformation. EPEC cells are moderately-invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in the intestinal cell ultra-structure due to "attachment and effacement" are likely the prime cause of diarrhea in those afflicted with EPEC.

Enteroinvasive *E. coli* (EIEC) are found only in humans. EIEC infection causes a syndrome that is identical to Shigellosis with profuse diarrhea and high fever. EIEC are highly invasive. They utilize adhesin proteins to bind and to enter intestinal cells. They produce no toxins but severely damage the intestinal wall through mechanical cell destruction.

Enterohemorrhagic *E. coli* (EHEC) are found in humans, cattle, and goats. The sole member of this virotype is strain O157:H7 which causes bloody diarrhea and no fever. EHEC can cause the hemolytic-uremic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment, is moderately-invasive and possesses a phage-encoded Shiga toxin that can elicit an intense inflammatory response. It is important to note that not all *E. coli* possess ß-D-glucuronidase, in particular EHEC serotype 0157:H7 which forms white colonies.

Enteroaggregative *E. coli* (EAggEC) are found only in humans. They have fimbriae which aggregate tissue culture cells. EAggEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAggEC are non-invasive. They produce a hemolysin and an ST enterotoxin similar to that of ETEC (Todar, 2005; Willshaw *et al.,* 2000).

2.6.3 Epidemiology of gastrointestinal infection

The transmission of pathogenic *E. coli* often occurs via fecal oral transmission. Common routes of transmission include: unhygienic food preparation, raw sewage, feral pigs on cropland or direct consumption of sewage contaminated water. Dairy and beef cattle are primary reservoirs of *E. coli* 0157:H7. They can carry it, asymptomatically, and shed it in their feces. Food products associated with *E. coli* outbreaks include raw ground beef, raw milk and food contaminated by food worker via the fecal oral route (IMNA, 2002).

According to the U.S. Food and Drug Administration the fecal oral cycle of the transmission can be disrupted by cooking food, properly, preventing cross-contamination, implementing barriers such as gloves for food workers, health care policies and proper hand washing requirements. In order to reduce and/or eliminate from the food chain, education and training in food hygiene are important for commercial food handlers and consumers. Increased public awareness generated by government and retailer could reduce sporadic cases and small family outbreak that may be the result of poor food handling (Willshaw *et al.,* 2000).

2.7 Salmonella spp.

2.7.1 Morphology

Salmonella is a genus of rod-shaped, gram negative, non-spore forming and motile bacteria with diameters around 0.7 x 1.5µm, lengths 2 x 5µm and flagella which project in all directions. They are chemoorganotrophs obtaining their energy from oxidation and reduction reactions using organic sources and are facultative anaerobes. Most species produce hydrogen sulfide. *Salmonella* are mesophiles and prefer room temperature (35^0C) as the optimum growth temperature. Nevertheless, this group has a temperature of 10^0C minimum and 48^0C maximum but grow optimally at 37^0C (range 5.2 - 46^0C) (Yan *et al.,* 2003; Todar, 2005).

2.7.2 Nomenclature/ Taxonomy

The genus *Salmonella* belongs to the family *Enterobacteriaceae*. *Salmonella* are motile, non spore forming, facultative anaerobic, gram negative, oxidase negative,

rod shaped bacteria. According to the latest nomenclature, which reflects recent advances in *Salmonella* taxonomy, the genus *Salmonella* consists of two species: *Salmonella bongori* and *S. enterica* (Le Minor and Popoff, 1987). *S. bongori* contains less than 10 serovars that are extremely rare. Grimont and Weill (2007) reported that 2579 serovars belong to the *S. enterica* species and are divided into six subspecies: *Salmonella* subsp. enterica (1531 serovars); *Salmonella* subsp. *salamae* (505 serovars), *Salmonella* subsp. *arizonae* (99 serovars), *Salmonella* subsp. diarizonae (336 serovars), *Salmonella* subsp. *houtenae* (73 serovars), *Salmonella* subsp. indica (13 serovars), *S. bongori* (22 serovars). All are distinguishable by certain biochemical characteristics. Some correspond to previous subgenera. All *Salmonella* strains belong to a serovar based flagella H-antigens of protein nature (heat labile). Each antigenic variant is a serovar in the Kauffmann – White Scheme. The current taxonomy is shown is the Tables 5 and 6.

Table 5: *Salmonella* species, subspecies, serotypes and their usual habitats, Kauffmann-White Scheme

***Salmonella* species and subspecies**	**Usual habitat**	**Number of serotypes within subspecies**		
		2001	**2004**	**2007**
S. enterica subsp. *enterica* (I)	Warm-blooded animals	1,491	1,504	1,531
S. enterica subsp. *salamae* (II)	Cold-blooded animals and the environment	500	502	505
S. enterica subsp. *arizonae* (IIIa)	Cold-blooded animals and the environment	95	95	99
S. enterica subsp. *diarizonae* (IIIb)	Cold-blooded animals and the environment	331	333	336
S. enterica subsp. *houtenae* (IV)	Cold-blooded animals and the environment	71	72	73
S. enterica subsp. *indica* (VI)	Cold-blooded animals and the environment	13	13	13
S. bongori (V)	Cold-blooded animals and the environment	21	22	22
Total		2,523	2,541	2,579

Source: Popoff *et al.,* 2003, 2004; Grimont and Weill, 2007.

Table 6: *Salmonella* nomenclature currently seen in the literature

Complete name	**CDC designation**	**Other designations**
S. enterica[a] *subsp. enterica* ser. Typhi	*Salmonella* ser. Typhi	*Salmonella typhi*
S. enterica[a] *subsp. enterica* ser. Typhimurium	*S.* ser. Typhimurium	*Salmonella typhimurium*
S. enterica[a] *subsp. salamae* ser. Greenside	*S.* ser. Greenside	*S.* II 50:z:e,n,x, *S. green side*
S. enterica[a] *subsp. arizonae* ser. 18:z_4,z_{23}:−	*S.* IIIa 18:z_4,z_{23}:	"*Arizona hinshawii*" ser. 7a,7b:1,2,5:−
S. enterica[a] *subsp. diarizonae* ser. 60:k:z	*S.* IIIb 60:k:z	"*A. hinshawii*" ser. 24:29:31
S. enterica[a] *subsp. houtenae* ser. Marina	*S.* ser. Marina	*S.* IV 48:g,z_{51}:−, *S. marina*
S. bongori ser. Brookfield	*S.* ser. Brookfield	*S.* V 66:z_{41}:−, *S. brookfield*
S. enterica[a] *subsp. indica* ser. Srinagar	*S.* ser. Srinagar	*S.* VI 11:b:e,n,x, *S. srinagar*

[a] S. Choleraesuis and *S.*Enteritidis are also used *Salmonella* Nomenclature according to Brenner *et al.* (2000).

Salmonellae cause disease in both humans and animals. The serovar *S.* Typhi and most *S.* Paratyphi strains (A, B and C) which cause serious systemic infections in humans are specific human pathogens. They have no animal reservoir (Hafez and Jodas, 2000).

Group1: Highly host adapted and invasive serovars. This group includes species restricted and invasive *Salmonella* such as *S.* Pullorum, *S.* Gallinarum in poultry and *S.* Typhi.

Group 2: Non host adapted and invasive serovars. This group consists of poultry and may be capable of infecting humans. Currently, the most important serovars are *S.* Typhimurium, *S.* Hadar, *S.* Arizonae and *S.* Enteritidis.

Group 3: Non host adapted and non invasive serovars. Most serovars of the genus *Salmonella* belong to this group and are harmless for animals and humans.

2.7.3 Distribution of *Salmonella* serovars

2.7.3.1 Distribution of *Salmonella* serovars in the world

Among 1.100.000 *Salmonella* isolates from human and non-human (animal, food, feed and environment) sources, *Salmonella* Enteritidis and *Salmonella* Typhimurium are identified as the most common serotype, globally, during 2000 – 2005 (WHO, 2006), see Figure 1.

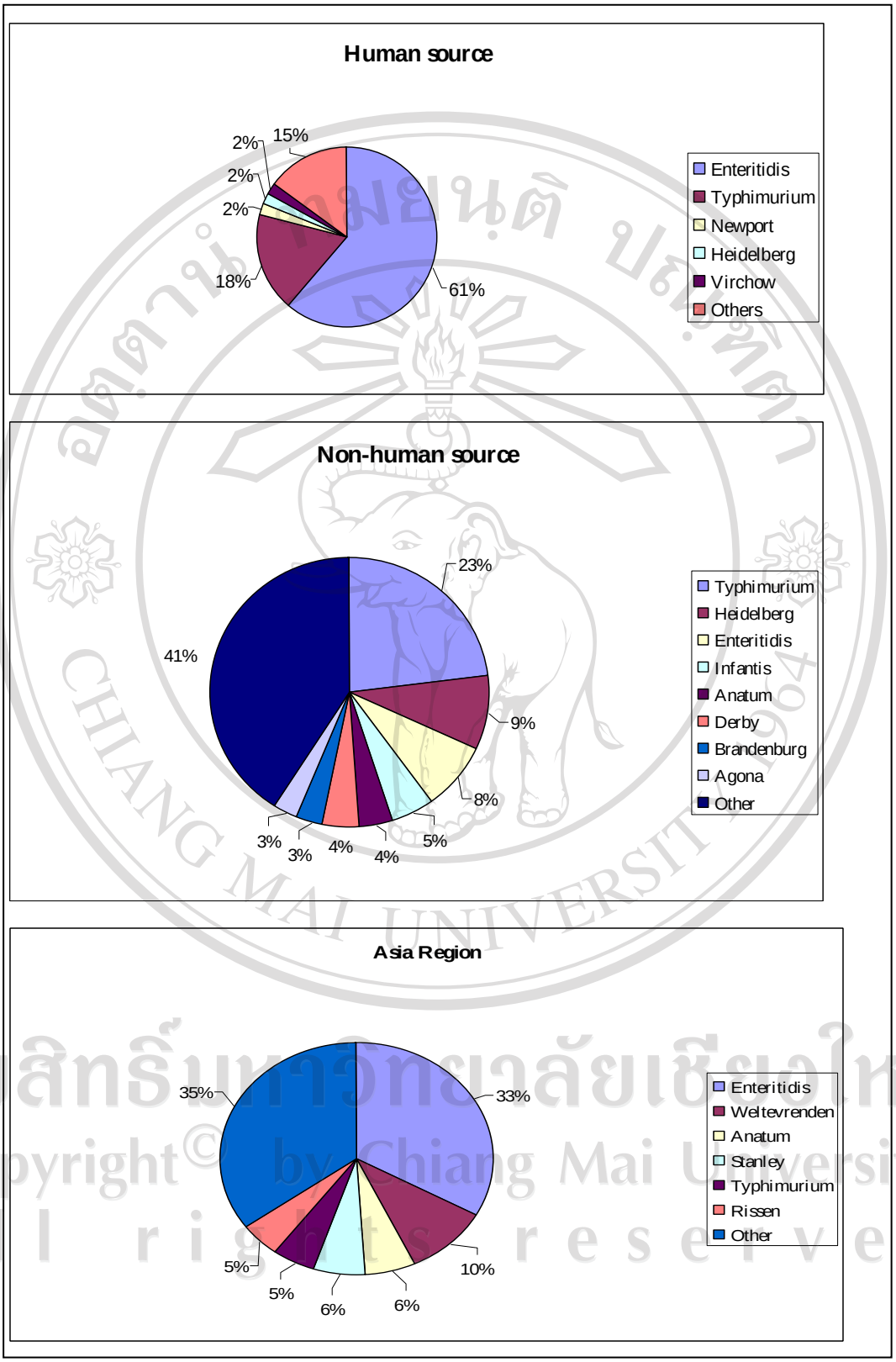

Figure 1: Distribution of global *Salmonella* serotypes reported to the Country Databank; Distribution of human *Salmonella* serotypes by the Asia region, 2000-2004

2.7.3.2 Distribution of *Salmonella* serovars in Vietnam

Vo *et al.* (2006) reported that the proportion of *Salmonella* Typhimurium, Anatum, Weltevreden is 15.8%, 15.5% and 11.4%, respectively, among 297 *Salmonella* isolates from human, cattle, pigs and poultry (Table 7).

Table 7: Distribution of the most common *Salmonella* serovars isolated in Vietnam from humans, cattle, pigs and poultry

***Salmonella* serovars**	**Humans**	**Cattle**	**Pigs**	**Poultry**	**Total (%)**
Typhimurium	21	3	23 (3[a])		47 (15.8)
Anatum	1	15 (7[a])	29 (4[a])	1	46 (15.5)
Weltevreden	4	11 (2[a])	17 (3[a])	2	34 (11.4)
Emek	2			26	28 (9.4)
Rissen		5	13	1	19 (6.4)
Derby		3 (1[a])	13 (1[a])	1	17 (5.7)
Blockley	1			14	15 (5.1)
S. (I), 4, 5, 12: b, -	2	3	4 (2[a])	1	10 (3.4)
Lexington		10 (1[a])			10 (3.4)
Hadar	2			6	8 (2.7)
Newport	1	1	6	0	8 (2.7)
London		1	2	4	7 (2.4)
Enteritidis	7				7 (2.4)
Albany	1			3	4 (1.3)
Panama	1		2		3 (1.0)
Rubislaw		3 (3[a])			3 (1.0)
Kedougou	2				2 (0.7)
Schwarzengrund				2	2 (0.7)
Tallahassee				2	2 (0.7)
Others	11	8 (2[a])	2 (1[a])	4	25 (8.3)
Total	56	63	111	67	297

[a] Isolates from animals with diarrhea. (Vo *et al*., 2006)

2.7.4 Salmonellosis

2.7.4.1 Epidemiology

The reservoir for salmonellae is the intestinal tract of warm and cold blooded animals. Salmonellae have mastered, virtually, the attributes necessary to ensure wide distribution, including abundant reservoir hosts, efficient fecal shedding from carrier animals, persistence within the environment, and the effective use of transmission vectors (feed, vehicles, etc.). Apparent long – term carriers that can shed salmonellae in feces continuously or intermittently, often in high numbers, are common in most host species. Shedding of the organism can be exacerbated by a long list of stressors including commingling of pigs, transportation, concurrent diseases and food deprivation.

The epidemiology of *Salmonella* infections in swine causes two relatively separate problems: *Salmonella* infection of pork carcasses and retail products and infections that cause salmonellosis in swine. Infection of swine by one or more serotype is common. Primary clinical disease, however, caused by serotype other than *S.* Choleraesuis or *S.* Typhimurium is uncommon. It is important to understand that swine can be infected with a variety of serotypes that do not cause disease in swine but do represent a source of infection for pork products (Schwartz, 1999).

Animals infected after exposure to infected animals, feed or environmental condition excrete *Salmonella* bacteria by fecal shedding. Fecal contamination of carcasses is the principal source of human food borne infections. The exception is when *Salmonella* is directly transmitted into the food product for example *S.* Enteritidis into eggs and sometimes other *Salmonella* serovars into milk. Humans excrete the microbe as animals do. *Salmonella* bacteria can survive for long periods in the environment, although in general no significant multiplication occurs. *Salmonella* are usually secondary to the infection of farm animals, even though infection cycles may continue, independently, of any continuous input of *Salmonella* bacteria from farm animals (Henzler and Opitz, 1992).

Pork and pork products are recognized as one of the major sources for human salmonellosis. In the Netherlands it was estimated that approximately 15% (5–25%) of human cases of Salmonellosis were associated with the consumption of

contaminated pork (Berends *et al*., 1998). The risk of the transmission of *Salmonella* from pork to humans can be quantified (Berends *et al*, 1997). Hald and Wegener (1999) used Danish estimation principles to quantify sources of human salmonellosis and assessed the role of pork in the transmission of *Salmonella* to humans in Denmark, Germany, Sweden and England. Although *Salmonella* types can occur in almost all food made from animal products there are often rather strong associations between certain types and a particular animal reservoir (Hald *et al.,* 2003).

2.7.4.2 *Salmonella* infections in humans

Salmonella infections in humans vary with the serovar, the strain, the infectious dose, the nature of the contaminated food and the host status. Certain serovars are highly pathogenic for humans. The virulence of more rare serovars is unknown. Strains of the same serovar are also known to differ in their pathogenicity. An oral dose of at least 10^5 *Salmonella* Typhi cells is needed to cause typhoid in 50% of human volunteers, whereas at least 10^9 *S.* Typhimurium cells (oral dose) are needed to cause symptoms of a toxic infection. Infants, immunosuppressed patients and those affected with blood disease are more susceptible to *Salmonella* infection than healthy adults (D'Aoust, 2000; Todar, 2005).

Non-typhoid salmonellosis in humans is usually manifested as a localized enterocolitis. The incubation period ranges from five hours to seven days. Clinical signs, however, usually begin 12 h to 36 h after ingestion of contaminated food. Shorter incubation periods are generally associated with either higher doses of the pathogen or highly susceptible people. Clinical signs include diarrhea, nausea, abdominal pain, mild fever and chills. The diarrhea varies from a few thin vegetable-soups like stools to massive evacuations with accompanying dehydration. Vomiting, prostration, anorexia, headache and malaise may also occur. The syndrome usually lasts for two to seven days. Systemic infections sometimes occur, and usually involve the very young, the elderly or the immuno-compromised. A fatal outcome is rare. The excreta of infected patients contain large numbers of *Salmonella* spp. at the onset of illness. Those numbers decrease with the passing of time. Some patients become carriers and some are still excreting *Salmonella* spp. after three months. Non-typhoid salmonellosis can later give rise to chronic diseases including localized infections in

specific tissues or organs and reactive arthritis, as well as neurological and neuromuscular illnesses. Subclinical infections and/or carriers also occur and investigations have found that 7% to 66% of the infected humans are subclinical carriers (Anon., 2005; Forshell and Wierup, 2006).

2.7.4.3 *Salmonella* infections in animals

Salmonella infected animals may or may not develop disease. Those serovars that were initially observed to cause disease were found to be adapted to specific animal species for example *S.* Choleraesuis (pigs).

These serovars cause disease in the species to which they are adapted and are considered less pathogenic to people. A group of more frequently isolated serovars such as *S.* Typhimurium, *S.* Enteritidis, *S.* Hadar and *S.* Infantis affect both humans and animals. In food animals these serovars manifest themselves, clinically, through per acute septicaemia, acute enteridis or chronic enteridis. In the subclinical form of the disease the animal may either have a latent infection or may become a temporary or persistent carrier. The remaining, less frequently isolated serovars can colonize animals, usually, without significant clinical signs. But they are all considered to be capable of causing gastrointestinal infection of varying severity in humans.

In most food animal species, Salmonellae, usually, establish a clinically unapparent infection of variable duration which is significant as a potential zoonosis. However, under various stress conditions, serovars that are usually non pathogenic may also cause disease in food animal species.

2.7.4.4 Serological aspects of *Salmonella*

The genus *Salmonella* has three kinds of major antigens with diagnostic or identifying applications: somatic, surface, and flagella.

Somatic (O): Somatic antigens are heat stable and alcohol resistant. Cross-absorption studies individualize a large number of antigenic factors, 67 are used for serological identification. O factors labeled with the same number are closely related although not always identical, antigenically.

Surface antigens: Surface antigens in *Salmonella* may mask O antigens. The bacteria will not be agglutinated with O antisera. One specific surface antigen is well

known: the Vi antigen. The Vi antigen occurs in only three *Salmonella* serovars (out of about 2,200): Typhi, Paratyphi C, and Dublin. Strains of these three serovars may or may not have the Vi antigen.

Flagellar (H) antigens: Flagellar antigens are heat-labile proteins. Mixing salmonella cells with flagella-specific antisera give a characteristic pattern of agglutination. Antiflagellar antibodies can immobilize bacteria with corresponding H antigens.

A few *Salmonella enterica* serovars (e.g. Enteritidis, Typhi) produce flagella which always have the same antigenic specificity. Such an H antigen is then called monophasic. Most *Salmonella* serovars, however, can alternatively produce flagella with two different H antigenic specificities. The H antigen is then called diphasic (Todar, 2005). The so called phase 1 and phase 2 antigens (D'Aoust *et al*., 2001).

2.8 The change of intrinsic parameters of fresh minced meat and the change of microorganism

Fresh meat is a rich nutrient matrix that provides a suitable environment for proliferation of meat spoilage microorganisms and common food-borne pathogens. Fresh meat is a highly perishable food product and unless appropriate action is taken during storage, distribution, sale and handling at retail it can be spoiled in a relatively short period of time. Factors affecting meat spoilage include intrinsic conditions (pH, water activity, composition, type and extent of initial contamination). Temperature is considered to be the most important factor. Although most countries have established regulations with maximum temperature limits for refrigeration storage they are often violated in practice (Giannakourou *et al*., 2001).

Implicit parameters are the result of the development of microorganisms which may have a synergistic or antagonistic effect on the microbial activity of other microorganisms present in the food products (Mossel *et al.,* 1995). Synergistic effects include the production or availability of essential nutrients due to the growth of a certain group of microorganisms, allowing development of other organisms which otherwise were unable to grow. Likewise, changes in the pH value, redox potential

and water activity may enable the development of microorganisms less tolerant to these inhibitory factors.

Moisture content

Microorganisms need water to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservations (Jay, 2000). The a_w of a food on this scale from 0.00 to 1.00 is related to the equilibrium relative humidity above the food on a scale of 0 to 100%. Thus, the percentage of the equilibrium of the relative humidity is (ERH) = a_w x 100. The a_w of food describes the degree to which water is "bound" in the food, its availability to participate in chemical/biochemical reactions and its availability to facilitate growth of microorganisms. Most of the fresh meat has a_w values that are close to the optimum growth level of most microorganisms (0.97 to 0.99) (Mossel *et al*., 1995).

pH and Acidity

Increasing the acidity of foods either through fermentation or the addition of weak acids has been used as a preservation method. In its natural state fresh meat is slightly acidic. The pH is a function of the hydrogen ion concentration in the food. In general, pathogens do not grow or grow very slowly at a pH level below 4.6. Many pathogens can survive in food at a pH level below their growth minimum. It should be noted that changes in pH can transform a food into one that pathogens can grow (ICMSF, 1980).

Table 8: Approximate pH value permitting the growth of selected pathogens in food

Microorganisms	**Minimum**	**Optimum**	**Maximum**
Clostridium perfringens	5.5 to 5.8	7.2	8.0 to 9.0
Vibrio vulnificus	5.0	7.8	10.2
Bacillus cereus	4.9	6.0 to 7.0	8.8
Campylobacter spp.	4.9	6.5 to 7.5	9.0
Shigella spp.	4.9	9.3	
Vibrio parahaemolyticus	4.8	7.8 to 8.6	11.0
Clostridium botulinum			

Microorganisms	Minimum	Optimum	Maximum
toxin	4.6	8.5	
growth	4.6	8.5	
Staphylococcus aureus			
growth	4.0	6.0 to 7.0	10.0
toxin	4.5	7.0 to 8.0	9.6
Enterohemorrhagic			
Escherichia coli	4.4	6.0 to 7.0	9.0
Listeria monocytogenes	4.39	7.0	9.4
Salmonella spp.	4.2	7.0 to 7.5	9.5
Yersinia enterocolitica	4.2	7.2	9.6

Source: ICMSF, 1980.

Nutrient Content

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depends on the microorganisms. These nutrients include water, a source of energy, nitrogen, vitamins and minerals (Mossel *et al*., 1995; Jay, 2000). Meat contains protein, lipids, minerals and vitamins. Most muscle food has low levels of carbohydrates.

Food borne microorganisms can derive energy from carbohydrates, alcohols and amino acids. Most microorganisms will metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates like glycogen found in muscle foods. Some microorganisms can use fats as an energy source. Amino acids serve as a source of nitrogen and energy. They are utilized by most microorganisms.

The Gram negative bacteria are generally able to derive their basic nutritional requirement from the existing carbohydrates protein, lipids, minerals and vitamins (Jay, 2000). The microorganisms that usually predominate in food are those that can most easily utilize the present nutrients. In general, the simple carbohydrates and amino acids are utilized first followed by the more complex forms of these nutrients.

Redox Potential

The oxidation reduction or redox potential of a substance is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing

(electron donating) power of the substance. The redox potential (Eh) is dependent on the pH of the substrate, normally the Eh is taken at pH 7.0 (Jay, 2000). The major groups of microorganisms based on their relationship to Eh for growth are aerobes, anaerobes, facultative aerobes.

These Eh values can be highly variable depending on changes in the pH of meat, microbial growth, packaging, the partial pressure of oxygen in the storage environment and composition. Another important factor is the poising capacity of the food. Poising capacity which is analogous to buffering capacity relates to the extent to which a food resists external affected changes in Eh. The poising capacity of the food will be affected by oxidizing and reducing constituents in the food as by the presence of active respiratory enzyme systems. Muscle food will continue to respire which results in low Eh value (Morris, 2000).

Pale, Soft, Exudative muscle

In healthy, rested pigs the amount of lactic acid produced shows a gradual increase after slaughter and the muscle pH falls from 7.0 to about 5.5 over a period of 4 - 8 hours. Watery pork is a direct result of a rapid fall in muscle pH after death. A muscle which is normal in its appearance at the time of death can become affected and this occurs when pH values of about 5.5 are attained within an hour after slaughter while the temperature of the muscle is still above 55^0C. Under these conditions changes occur in the properties of the muscle protein and the flesh becomes watery, changes to a pale, unattractive color and a lack of flavor. Many factors have been associated with this condition such as a high environmental temperature, rough ante mortem, handling, fighting, physiological differences between breeds and individual muscles in efficient slaughtering techniques and handling of carcasses. Pale, soft, exudative muscle is a serious problem if pork has to be sold fresh because of the amount of drip, which causes weight loss (up to 10%) and may necessitate repackaging and to a lesser extent its pale color. Such meat is more likely to be tougher when cooked and to have higher cooking losses (Gracey *et al*., 1999).

Besides pre-slaughter stress there are indications that the actual slaughter procedure may influence the onset of dark, firm, dry (DFD) and pale, soft, exudative (PSE). The following procedures will help to reduce the incidence of PSE and DFD meat.

- Animals should be handled gently and quietly at the farm and the meat plant.
- Loading ramps should be provided at the farm and efficient unloading facilities at the meat plant
- Stock should be kept in their original social groups as far as possible and there should be no mixing within the period of the last vital 24 -48 hours before the slaughter.
- Lairage pen design and race arrangements should allow for an easy and efficient movement of the stock
- Water should be provided at all times in the lairage, food if necessary
- The use of sticks and electric goads should be avoided
- Aggressive animals should be isolated in the lairage
- The use of overhead fine sprays of water in lairages reduces the incidence of PSE (Gracey *et al*., 1999).

Preservation of meat

The primary purpose of food preservation is to prevent food spoilage whether mild or extreme. The primary cause is the action of the microorganisms' bacteria, moulds or yeast aided by enzymes. As living organisms they can survive and develop only under particular environmental conditions. Under unfavorable conditions they die or fail to develop.

After the slaughter of a healthy animal, decomposition eventually develops in the parts exposed to the air. The time that the process takes depends in particular on the temperature and the humidity of the environment. The primary surface growth is initiated by aerobic bacteria like *Pseudomonas*, *Achromobacter* and some *Coliforms*. Those organisms extract oxygen from the meat surface and produce conditions suitable for the growth of anaerobic bacteria for example *Clostridium sporogenes* which can also grow within the deeper tissues where there is no oxygen. After the

surface putrefaction of meat has commenced the process spreads, gradually, via the nerve and connective tissue sheaths and along the surfaces of blood or lymph vessels. The rapidity of the extension of the putrefactive process throughout a carcass is greatly influenced by the condition of the animals before the slaughter (Gracey *et al.,* 1999).

2.9 Studies on microbiological contamination in fresh minced meat in Vietnam and overseas.

There have been some studies on the contamination of micro-organisms in fresh meat (Le, 2003; To, 1999; Chu, 2007; Nguyen, 2007) but there is only some few researches done on the contamination with *Salmonella* spp., *Escherichia coli* and the total aerobic count of fresh minced pork in Vietnam where no comprehensive national surveillance on *Salmonella* exists.

In Vietnam it is reported that the prevalence of *Salmonella* in pork at the market is 69.9% and 64%, respectively (Phan *et al.,* 2005; Van *et al*., 2007). Dr. Phan reported that 69.9% of the retail pork samples were contaminated with *Salmonella* spp. in the Mekong Delta, Vietnam.

Prevalence of *Salmonella* in the fresh pork samples in Vietnam were found to range from 14.1%; 41.7%; to 69.9% (Le, 2003; To, 1999; Phan *et al.,* 2005).

In Denmark, the Netherlands and Germany it was estimated that 10 to 15%, 14 to 19%, and 18 to 23%, respectively, of human infections could be attributed to pork and pork products; while pork was presumably no important factor in England and Wales and was negligible in Sweden (Hald *et al*., 2003).

The reported prevalence of *Salmonella* in retail meat varies widely in different countries. *Salmonella* is found less frequently in retail meats in developed countries. The rates of *Salmonella* contamination in pork appear to be much lower, ranging from 0.8 to 10.4% in the United States (Duffy *et al.*, 2001); 1.8% in Austria (Paulsen *et al.*, 2006); 2.6% in Ireland (Prendergast *et al*., 2009); 11.1% in Belgium (Ghafir *et al.,* 2005); 16 % in 3 retail supermarkets from Washington D.C. area (White *et al.,* 2001); 29% in Thailand (Padungtod and Kaneene, 2006).