CHAPTER II

LITERATURE REVIEW

2.1 Human foodborne disease situation

The World Health Organization (WHO) has described foodborne disease as one of the most widespread public health problems of the contemporary world (DVS, 2007). Foodborne diseases continue to be a common and serious threat to public health all over the world being a major cause of morbidity. On a global basis, incidence of food borne diseases appears to be increasing, with both industrialized and developing countries suffering large numbers of illnesses (Blackburn and McClure, 2002).

The continuing globalization of the food trade now allows food produced in one country to be sold and consumed halfway around the world implying that contaminated food product can cause disease outbreaks in many countries at the same time (CAC, 2005). Thus, there is growing concern that increased international trade in both raw materials and finished goods may contribute to the introduction of disease to areas currently free from a given hazard, or may increase the likelihood that some new or emerging microbiological hazard will spread (Stringer, 2005). Such globalization of the food supply complimented by advances in food production and processing technologies, changes in agricultural and animal husbandry practices, demographic changes and changes in lifestyle all contribute to the increased incidence of food borne diseases with widespread outbreaks, the emergence of new food borne pathogens and the development of antimicrobial resistance (FAO, 2004a).

Diseases which are considered foodborne are those that cause illness in humans wherein food is or contains the causative agent and which usually manifest in episodes of gastro-intestinal disease (DVS, 2007). It is estimated that each year two

million people die worldwide from diarrheal diseases, mostly attributed to contaminated food and drinking water. Furthermore, foodborne diseases, and more specifically diarrheal diseases, represent not only a significant health problem but also one with important economic consequences (WHO, 2005).

The worldwide incidence of food borne diseases is difficult to estimate although many people fall ill and die as a result of eating unsafe food. In developed countries, it is estimated that 30% of the people are affected by food borne diseases annually, and the problem is likely to be even more widespread in developing countries. WHO reported that in 2000, 2.1 million people died from diarrheal diseases, most of whom were children in the less developed countries (FAO, 2004b). As it is, most of the cases of foodborne disease are not reported, hence, the true dimension of the problem is unknown. The absence of reliable data on the burden of foodborne diseases impedes understanding about its public health importance and prevents the development of risk-based solutions to disease management (WHO, 2002).

Throughout the 1990s up to the present, three major food-borne bacterial targets, namely: *Salmonella*, *Campylobacter* spp. and *E. coli* have persisted, commanding the most intensive research and surveillance attention from government agencies and, to a large extent, the most awareness from the food industry (Newell et al., 2010). There has been dramatic increase in human infections with *Salmonella enteritidis* in Europe and North America in the past 20 years, as has the increase in *Campylobacter* infections in many countries throughout the world. In developed countries, much of this disease is considered to be preventable (Stringer, 2005).

Most diseases which are considered food-borne are caused by microorganisms that initially contaminate a living plant or animal or recontaminate the food during handling or processing (Sinell, 1995). Traditionally, meat has been viewed as the cause of a significant proportion of human food-borne disease. Despite a change in the spectrum of meat-borne diseases of public health importance with the changing production and processing systems, in recent years human surveillance studies of

specific meat-borne pathogens have shown that the problem persists (CAC, 2005). Studies have determined major pathogens of concern that need to be controlled specifically in fresh meat and poultry including *Salmonella*, *Campylobacter*, and enterohemorrhagic *E. coli* O157:H7 (FAO, 2004b, Sofos and Geornaras, 2010, Newell et al., 2010) and *Listeria monocytogenes* in ready to eat meat products (Sofos and Geornaras, 2010, Newell et al., 2010).

The most common food and water borne diseases in the Philippines are bacterial, the most reported of which are typhoid fever and cholera, according to the Food and Waterborne Diseases Prevention and Control Program of the country's Department of Health (DOH, 2013). In 2011, there were one hundred fifteen confirmed typhoid fever cases with two fatalities reported nationwide. Meanwhile, one hundred twenty confirmed cholera cases with three fatalities were reported nationwide in the same year (PIDSR, 2011).

A study by Azanza (2004) presented details about 60 reported Philippines foodborne outbreaks for the period of 1995–2004 using data from Field Epidemiology Training Program, Department of Health (DOH) of the Philippines and the health advisories released by the DOH. It was determined that meat-containing dishes such as spaghetti with meat sauce, stewed and roasted pork dishes, fried processed meats, water-buffalo meat dish and fried chicken were the leading causes of the outbreaks evaluated. *Salmonella* and *Vibrio* spp. were cited as the primary causes of infections with school food services and workplaces identified as the common risk settings for the outbreaks (Azanza, 2006).

2.2 Classification of slaughterhouses in the Philippines

Slaughterhouses in the Philippines undergo an accreditation process based on guidelines by the National Meat Inspection Service (NMIS) in compliance with the Standard Sanitary Operating Procedures (SSOP), Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP) Standards.

Upon satisfaction of the Audit Checklist set by NMIS, accredited abattoirs are classified according to distribution as follows:

Table 1 Classification of slaughterhouses by the National Meat Inspection Service (NMIS), Philippines (source: NMIS)

NMIS Classification	Area allowed for trade and distribution of products
A	allows the abattoir to cater only to the locality and trade within the city or municipality
AA	allows the transport and trade of the carcasses for domestic trade
AAA	complies with the NMIS' stringent evaluation and allowed to transport meat products for international trade

Non-accredited slaughterhouses are regulated by Local Government Units (LGU) and managed by the City/Municipal Treasurer's Office as these establishments are income generating enterprises. Operation of these slaughterhouses in terms of hygiene and meat inspection is governed by local ordinances. Slaughterhouse enterprises begin this way until such time that the local units would strive for accreditation to address the public's demand for safe food and to generate more income for the locality.

2.3 Pig slaughter lines in the Philippines

In most of the pig-slaughter facilities in member countries of the Animal Production and Health Commission for Asia and the Pacific (APHCA), which includes the Philippines, modern slaughter systems are not in place. However, semi-

line systems which enable hygienic slaughtering and carcass dressing without electro-mechanical equipment have been developed to good standards in the Philippines (Heinz, 2008).

In such systems, small and medium scale pig abattoirs feature a design that facilitates simple but efficient carcass movement off the floor. The design principle is a tiered, or terraced, slaughter floor where holding pens (lairage), the stunning area and scalding vats are located on one level; scraping tables are placed on another lower level. The difference in elevation enables the top of the scraping table reaching the level of the scalding vat, but with operators situated at a lower position.

In small scale facilities, a two-tier system may be sufficient. This characteristic multi-tiered floor design enables easy loading and discharge of the carcass from the scalding vat without mechanical elevating equipment. However, in such a set-up, evisceration as well as carcass splitting must be done with the carcass in a horizontal position after completing the manual dehairing. Carcasses are suspended only after such steps.

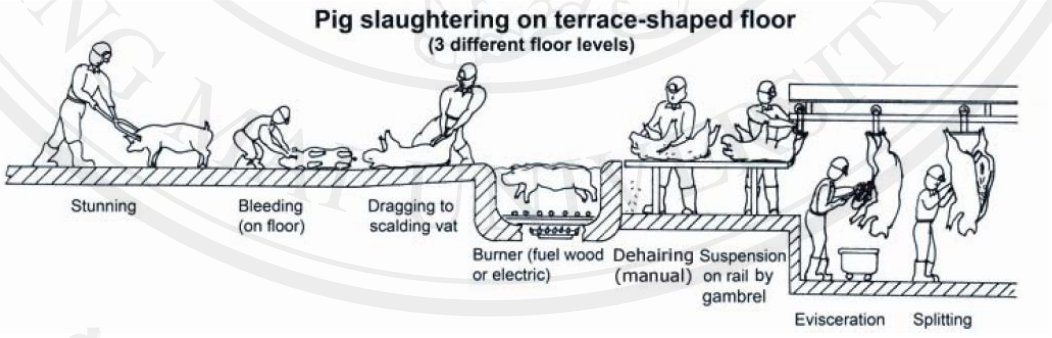

Figure 1 Medium-scale pig slaughtering on a tiered floor (source: Heinz, 2008)

In contrast, railing systems in medium scale facilities usually begin at another lower floor, with operators there standing at the lowest position of the floor. Pig carcasses can easily be manually maneuvered from the scraping tables to the start of the railing system, which is in a convenient height to hook the hind legs manually and without the use of elevating equipment from the scraping table position. Evisceration

and carcass splitting is conducted on the rail, with operators standing on the lowest floor level, providing them the most convenient position to perform the procedure without the need for platforms for these operations (Heinz, 2008).

The described multi-tiered system works very well for local slaughter as it prevents any floor contact of carcasses, beginning at the scalding stage. However, it would still be ideal for such pig abattoirs to be equipped with sufficient overhead rails that enable eviscerating and splitting on each carcass in a vertical position (Heinz, 2008).

2.4 Hygiene indicator microorganisms

The term "indicator organisms" is frequently associated with organisms of intestinal origin which are frequently employed to assess food safety and hygiene (Forsythe, 2010). Many organisms or groups of organisms have been suggested as indicator organisms such as fecal microorganisms which include enteric bacteria, viruses, and protozoa, with the latter two generally more difficult to enumerate. Common indicator organisms include coliforms, *E. coli*, *Enterobacteriaceae, enterococci* (formerly known as fecal streptococci) and bacteriophages (Forsythe, 2010). In addition, *Pseudomonads, Clostridia, Staphylococci*, and the Aerobic Plate Count (APC) have also been suggested as indicator microorganisms (Banwart, 1998).

The safety and quality of raw meat products can be estimated based on indicator microorganism counts. Furthermore, evaluation of the microbiological quality of raw meat and the hygienic-sanitary conditions of processing plants can be done based on the presence of indicator microorganisms in meat, equipment and processing facilities (Barros et al., 2007).

2.4.1 Total Viable Count (TVC)

One of the most frequently employed microbiological tests for foods is the Total Viable Count (TVC) which is also known as the standard plate count or aerobic

plate count. This involves plating of suitable dilutions of food on or in agar-based media containing complex nutrients which support growth of as wide a range of nutrients as possible (Forsythe and Hayes, 1998). Conventional methods for the enumeration of bacteria are colony count methods wherein the total number of bacteria in a product is determined by inoculating dilutions of suspensions of the sample onto the surface of a solid growth medium by the spread-plate method or by mixing the test portion with the liquefied agar medium in Petri dishes (Jasson et al., 2010). The purpose of obtaining the total count for aerobic bacteria is to determine the number of living micro-organisms per unit of the investigated substrate. It estimates only a portion of the total viable micro-organisms since only those that can grow under the conditions of the procedure will manifest themselves as colonies (DVS, 2007).

The total plate count is a good indicator for the overall bacterial load of meat and meat products. Critical hygienic dimensions are reached when the total number of bacteria on fresh meat is between 10,000 ($1.0x10^4$) and 100,000 ($1.0x10^5$) per gram However, the total number does not allow any conclusions on the nature of the microorganisms, for instance, if the bacteria are harmful or harmless (Heinz and Hautzinger, 2007).

2.4.2 *Enterobacteriaceae*

One of the most common tests in the food microbiology laboratory for examination of foods, ingredients and raw materials for such contamination is the total number of *Enterobacteriaceae* as well as detecting the presence of marker groups such as coliforms. This is partly because of the relative ease and speed with which the tests can be accomplished (Blood and Curtis, 1995).

Enterobacteriaceae is a family of bacteria consisting of Gram-negative rods up to 3µm in length which ferment glucose and a wide range of other sugars and are oxidase-negative and catalase-positive (Quinn et al., 2012). Bacteria in this family are facultatively anaerobic and non-sporeforming and constitute a large genetically and

biochemically related group showing substantial heterogeniety in ecology, host range and pathogenic potential (Blood and Curtis, 1995). *Enterobacteriaceae* are widely distributed and found in soil, water, vegetation, are also part of the normal gut flora found in the intestines of humans and many animals (Blood and Curtis, 1995, Quinn et al., 2012), are often isolated from fecal material (Allaby, 1998 as cited by (Long et al., 2009) and some of the pathogenic organisms can be disseminated by clinical and subclinical excretors and through survival in the environment (Quinn et al., 2012). This large family of bacteria includes pathogens such as *Escherichia coli* and *Salmonella*, as well as *Citrobacter* spp, *Enterobacter* spp, *Klebsiella* spp, *Morganella morganii*, *Proteus* spp, *Providencia* spp, *Serratia* spp, *Shigella* spp, and *Yersinia* spp (Engelkirk and Engelkirk, 2007).

Mosse1 in 1982 emphasized the indicator function of *Enterobacteriaceae* in stating that where it is claimed that a food has been processed for safety, the finding of such organisms demonstrated a failure of the process or indicates post-process contamination (Mossel, 1982).

2.4.3 Coliforms and *E. coli*

The coliform bacteria group or simply, coliforms, are a large group of gram-negative, non-sporeforming, rod-shaped bacteria that all belong to the family *Enterobacteriaceae*. Within the coliform group, are thermotolerant coliform bacteria, the main and most prominent of which is *E. coli* which is found exclusively in the faeces of humans, other mammals, and birds (Paruch and Mæhlum, 2012).

The occurrence of *E. coli* in the environment does not necessarily lead to occurrence of disease but their mere presence definitely reveals pollution with fecal matter (Haller et al., 2009). *E. coli* is regarded as the most sensitive indicator and the most appropriate measure of fecal contamination in the natural environment of water (Edberg et al., 2000). Among the coliform group, *E. coli* is the only member that satisfies most of the criteria for an ideal bacterial indicator of fecal pollution, namely: fecal origin, universally present in large numbers in feces of human and warm-

blooded animals, present in sewage but does not grow in natural waters, and readily detectable by simple methods (EnvironmentAgency, 2002).

As result of the successful use of coliforms as indicators in water, they have been employed as indicators of possible fecal contamination of foods (Banwart, 1998). Consequently, the presence of coliforms such as *E. coli* in food indicates post-processing contamination from some source or, less likely, process failure (DVS, 2007).

2.5 Foodborne pathogenic bacteria

2.5.1 Pathogenic *E. coli*

Pathogenic strains of *E. coli* are classified according to symptoms and the various mechanisms of pathogenesis into several groups which vary in their incubation periods and duration of illness (Forsythe, 2010). *E. coli* is considered a highly successful gut colonizer in various host species. Strains of *E. coli* isolated from intestinal diseases have been grouped into at least six different diarrheagenic *E. coli* (DEC) groups based on specific virulence factors and phenotypic traits (Forsythe, 2010, Newell et al., 2010); these include:

Enteropathogenic *E. coli* (EPEC) – causes watery diarrhea in infants and vomiting
Enterotoxigenic *E. coli* (ETEC) – also commonly known as traveler's diarrhea; causes watery diarrhea, rice water-like and a low-grade fever
Enteroinvasive *E. coli* (EIEC) – cell-to-cell spread and causes disease similar to dysentery
Enteroaggregative *E. coli* (EAggEC) – causes diarrhea and for some strains, HUS
Diffusely adherent *E. coli* (DAEC) – associated (but not consistently) with diarrhea
Enterohemorrhagic *E. coli* (EHEC) - includes Vero cytotoxin-producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC); causes bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombocytopenia purpura

Food associated outbreaks have been particularly associated with VTEC, and to a lesser extent EPEC, ETEC and EaggEC strains (Newell et al., 2010).

2.5.2 *Salmonella*

Bacteria that belong to the genus *Salmonella* are characterized as Gram-negative, facultatively anaerobic, non-spore forming, which are usually motile with peritrichous flagella (Blackburn and McClure, 2002).

Genus *Salmonella* consists of only two species, S. *enterica* and S. *bongori*. In turn, S. *enterica* is further subdivided into six subspecies, namely: S. *enterica* subsp. *enterica*, S. *enterica* subsp. *salamae*, S. *enterica* subsp. *arizonae*, S. *enterica* subsp. *diarizonae*, S. *enterica* subsp. *houtenae*, and S. *enterica* subsp. *indica* (Popoff et al., 2004).

Table 2 Present number of serovars in each *Salmonella* species and subspecies (source: Guibourdenche et al., 2010)

***Salmonella* species and subspecies**	**Number of serovars**
S. *enterica*	
subsp. *enterica*	1547
subsp. *salamae*	513
subsp. *arizonae*	100
subsp. *diarizonae*	341
subsp. *houtenae*	73
subsp. *indica*	13
S. *bongori*	23
Total	**2610**

The antigenic formulae of *Salmonella* serovars are listed in a document called the White-Kauffmann-Le Minor scheme, updating of which is the responsibility of the WHO Collaborating Center for Reference and Research on *Salmonella* (WHO-Salm), Institut Pasteur, Paris, France (Guibourdenche et al., 2010).

In 2002, WHO-Salm recognized and characterized 18 new serovars: 12 were assigned to S. *enterica* subspecies *enterica*, 2 to subspecies *salamae*, 2 to subspecies *diarizonae*, 1 to subspecies *houtenae* and 1 to S. *bongori*. As of 2010, there are 2610 known *Salmonella* serovars (Guibourdenche et al., 2010).

Salmonella spp. have been recognized for over a century now as the cause of diseases ranging from mild to severe food poisoning (gastroenteritis), and even more severe typhoid (enteric fever), paratyphoid, bacteremia, septicemia and a variety of associated longer-term conditions (sequelae). High rates of morbidity and mortality can be incurred in some of these severe conditions and such can occur in outbreaks involving large numbers of people, particularly in relation to typhoid outbreaks and septicemic conditions (Blackburn and McClure, 2002). Illness caused by *Salmonella* represents an important foodborne disease that continues to pose a major and unacceptable threat to human public health in both developed and developing countries (EFSA, 2010). *Salmonella* is one of the major food pathogens worldwide leading several countries to establish surveillance and control programs for the bacteria (Arguello et al., 2013).

*Salmonella*e are non-fastidious as they can multiply under various environmental conditions outside the living hosts (Pui et al., 2011). *Salmonella* spp. infect a wide range of hosts and all the major livestock species can become colonized, frequently asymptomatically, eventually producing contaminated meat and other food products. Thus, there is routine observation and reporting of food-borne outbreaks of salmonellosis (Newell et al., 2010). Poultry (including birds such as chicken, duck and turkey), beef, lamb and pork are all commonly contaminated with *Salmonella* and, not surprisingly, have all been associated in outbreaks of salmonellosis (Blackburn and McClure, 2002).

Salmonella in pigs, pork and pork products

Salmonella has the potential to colonize the guts of pigs and most often pigs are healthy carriers of *Salmonella* (Botteldoorn et al., 2003). Farmers are unaware when their pigs are infected with *Salmonella* as healthy pigs are often infected without showing any symptom of disease. In turn, pig carcasses, contaminated with *Salmonella*, will not be recognized during veterinary inspection after slaughter. Thus, to confirm that a carcass is contaminated with *Salmonella*, it is necessary to isolate *Salmonella* from that carcass (Swanenburg et al., 2001).

Several studies have identified carrier pigs as a predominant source of *Salmonella* contamination of pig carcasses during the slaughtering process (van Hoek et al., 2012, Baptista et al., 2010, Berends et al., 1997, Borch et al., 1996). The role of the slaughter process in carcass contamination has been indicated by such studies focused on the slaughterhouse environment. Pigs from infected farms and newly acquired or recrudescent infections in pigs at the subsequent stages of transport and lairage are important sources of *Salmonella* at the slaughtering plant. This continuous introduction of *Salmonella* into the slaughterhouse, inappropriate slaughter practices and the potential of *Salmonella* to become resident flora constitute a risk for carcass contamination (Arguello et al., 2013).

Pork products are among the main sources of *Salmonella* infection in humans (Arguello et al., 2013) and contribute significantly to the public health disease burden (van Hoek et al., 2012).

2.6 Contamination during slaughter

The external surface of animals is normally contaminated with a variety of microorganisms as are their gastro-intestinal tract. Each step from the beginning of the slaughter process subjects the carcass to opportunities for contamination with microorganisms: from the exterior surfaces, utensils and equipment and, most importantly, from the gastrointestinal tract. Furthermore, the cutting of carcasses also

involves the use of utensils and equipment which transfer microorganisms to the cut surfaces (DVS, 2007). Bacterial contamination can occur along the swine slaughter line, the intensity depending on the type of operation and performance of hygiene and sanitation procedures in the different stages of the process.

Contact with unclean surfaces is the main source of meat contamination, and floors are among the most contaminated surfaces. In many pig-slaughter facilities in member countries of the Animal Production and Health Commission for Asia and the Pacific (APHCA) which includes the Philippines, there are no proper lifting devices and carcasses are dragged along the floor from the stunning and bleeding stages to the scalding and onto the scraping tables. In many pig-butchering facilities in APHCA-Member Countries, entire pig-slaughter operations are still carried out on the ground which causes tremendous hygiene problems with heavy meat contamination (Heinz, 2008).

2.6.1 Contamination in lairage

NMIS requirements for accreditation state that slaughterhouses should provide the following facilities: holding pens for animals to be slaughtered, separate butchering facilities for swine and ruminants, hot water and rail systems, and covered bins for segregating slaughter by-products. Holding pens help in avoiding disease transmission and injury, minimize stress, and prevent animals from roaming around the facility (Maranan et al., 2008). Borch et al. in 1996 suggested that during lairage, pathogenic bacteria may spread from infected to non-infected pigs, thus herds should be handled separately and cleaning and disinfection should be performed between herds. The same study however acknowledged that this would be difficult to achieve in practice, and measures taken on the slaughter line are likely to be of higher significance to limiting the contamination of the carcasses with pathogenic bacteria.

Reduction of cross-contamination can be achieved by general control measures at the abattoir level. These include physical separation of areas considered “dirty” like lairage and the stages of the slaughter line as “clean” areas so as to

prevent mixing of staff, equipment and tools and air between them, as well as application of proper cleaning and disinfection regimes (Buncic and Sofos, 2012).

2.6.2 Contamination in the slaughter process

Inadequate hygienic practices during slaughtering or carcass handling result in high levels of microbial contamination in meat, will reduce shelf life and thus, meat value, adversely affecting the sensory properties of products for further processing (NMIS, 2010). Such shortcomings in general meat hygiene are to some extent due to lack of adequate facilities as well as carelessness and lack of training and skills on the side of personnel.

Stunning and bleeding

FAO in 2008 identified as one of main principles of slaughter hygiene avoiding contact between a carcass and the floor where it is important for the carcass to be off the ground as soon as possible during the first steps of the slaughtering process. This would be best achieved if carcasses are already off the ground at the point of bleeding. Considering that not all slaughterhouses may have the supporting technical devices to hoist up carcasses for bleeding, such step can be done on the ground because the carcass is still fully covered by the skin. However, once the protective cover of the skin is cut open or removed, it is necessary to prevent any contact with the floor.

Scalding and dehairing

Removal of hair from pig carcasses may be done by scalding the pigs by pouring hot water or submerging it in a scalding vat filled with hot water (60°–62°C). Advanced operations achieve this by steaming of the skin. These procedures loosen the hair, which is then removed by a scraping process. In small- to medium-sized operations, a knife is used for scraping, in larger operations machines are used (Heinz, 2008). The reduction of bacterial numbers on the carcass during scalding which

usually takes 6 – 8 minutes depends on the time-temperature conditions applied (Borch et al., 1996).

Evisceration and splitting

The main bacterial contamination risks particularly by *Salmonella* during the process of evisceration relate to possibilities of a spillage of the intestinal content onto the carcass surface due to leakage from natural openings such as the anus or by accident such as punctures (Buncic and Sofos, 2012). During the steps subsequent to scalding and dehairing, pathogenic bacteria may spread to the carcass from the intestines, stomach content, oral cavity and esophagus and maybe from the lungs in the case of vat scalding. Particular critical operations include circumcising of the rectum, removal of the intestinal tract and removal of the pluck set (Borch et al., 1996).

Steps in slaughter that may lead to an increase in *Enterobacteriaceae* counts are dehairing, polishing after singeing, and evisceration (Berends et al., 1997); while scalding and singeing are steps which result in a considerable decrease in numbers of microorganisms on carcass surfaces. It is mainly the process of evisceration that leads to contamination of carcasses with *Enterobacteriaceae* along with the bacteriological condition of the polishing machines and the way evisceration, further dressing and meat inspection are performed. All these factors determine the final load of the carcass with *Enterobacteriaceae* (Berends et al., 1997).

2.7 Slaughter of swine and cleaning and disinfection

Swine slaughter is an open process that includes steps where the bacterial number may be reduced, but does not contain any point where hazards are completely eliminated. It is a process with many opportunities for the contamination of the pork carcass with potentially pathogenic bacteria. Slaughtering and dressing are performed at an ambient temperature, while the carcass temperature is high. Therefore, during

this processing period, there is a great potential for extensive growth of bacteria (Borch et al., 1996).

The number of bacteria at the end of a production period can be drastically reduced provided that cleaning and disinfection routines are performed efficiently. However, in environments where disinfection is not conducted properly, additional growth occurs and endemic flora may develop (Borch et al., 1996). Cleaning and disinfection should minimize the usually high number of microorganisms, mainly bacteria on premises and equipment to an insignificantly low level, thus achieving reduction rates of approximately 5 to 8 log cycles (Reuter, 1998).

After disinfection, monitoring of hygienic conditions can be done by assessing the microbial status of equipment and premises. A reasonable method of doing this is the cultural determination of the remaining total mesophilic count. Furthermore, counting of *Enterobacteriaceae* may be advisable when heavier loads are expected (Reuter, 1998).