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INVESTIGATION OF HYGIENE ASPECTS OF PIG PROCESSING USING THE HACCP CONCEPT

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ABSTRACT

Contamination of fresh meat by pathogenic and spoilage microorganisms can occur at any stage of the slaughter process. Pathogens which are frequently found in fresh meat and which pose a public health problem include *Salmonella* spp. *Campylobacter* spp. and *Yersinia* spp. Contamination with spoilage bacteria affects the storage stability and shelf life of meats.

Factors that contribute to meat spoilage include physical damage, biochemical changes in the meat tissues and the activity of microorganisms, of which bacteria are undoubtedly the most important. Fresh meats present a rich medium for the support of microbial growth and will ultimately be rendered unacceptable to consumers as a consequence of spoilage due to such growth. The source of spoilage bacteria can be the slaughter animals themselves, the environment, water and personnel working in the processing plants.

This study was conducted to determine the effect of some processing operations on the level of contamination of the pig carcass with aerobic bacteria and to establish microbial quality control points based on the Hazards Analysis Critical Control Point (HACCP) principles. As a component of the HACCP system and a step in the setting up of an HACCP plan for microbial quality control of fresh carcass meat, this study aims at identifying hazards at various stages of processing, evaluating preventive measures and establishing critical control points. Where appropriate, corrective measures to ensure that bacterial contamination is within an acceptable level are recommended.

The study was carried out at a processing plant in the North Island of New Zealand during the period April to July 1998. Based on observations at the plant, a flow chart of pig processing was drawn up. A number of processing stages were selected as points where potential risks of bacterial contamination were

most likely to occur. These points initially included dehairing, polishing and scraping, evisceration, and inspection. Eight visits to the abattoir were made and a total of 32 paired swab samples from carcasses at each process stage were collected. With four process stages selected for sampling, the total number of samples was 128. In addition, 12 scalding tank water samples were collected for analysis. All samples were processed in the Microbiology Laboratory at Massey University. The aerobic plate count (APC) technique employing incubation at 30°C for 3 days was used for enumeration of aerobic bacteria. A matrix table was designed for entering APC data after each count. The mean of colony forming units per square cm (CFU/cm^2) for pig carcass surfaces and CFU/ml for scalding water were calculated and $\log_{10}$ transformation was performed.

The highest mean APC was found after the carcasses had passed the dehairing machine (5.1 $\log_{10}$/cm^2, ST.D. = 0.57) and the lowest number before the dehairing step (4.31 $\log_{10}$/cm^2, ST.D. = 0.61). A rapid increase in APC at the dehairing stage indicated a heavy recontamination of the pig carcass with bacteria from the equipment and from detritus accumulated during the operation. After the operation, the count gradually decreased to 4.4 $\log_{10}$/cm^2, ST.D. = 0.38 at the post-evisceration point but then slightly rose again to 4.5 $\log_{10}$/cm^2, ST.D. = 0.4 at the post-inspection step. The increase in the APC at the dehairing stage by 0.8 $\log_{10}$/cm^2 ($p = 0.0002$, $n = 16$) is significant. There was little change in the APC at the polishing and scraping and evisceration stages. There was an insignificant difference of 0.2 $\log_{10}$/cm^2 in the APC between samples taken at the start and at the end of the shift.

The scalding water temperature fluctuated between 60°C and 67.5 °C (mean = 63.2, n = 12). Bacterial contamination of the scalding water remained almost unchanged with time (2.55 $\log_{10}$/ml at the beginning and 2.62 $\log_{10}$/ml at the end of the shift). An expected inverse correlation between scalding water counts and water temperature could not be verified.

Although this study is confined to the microbiological assessment of only a few operational stages that can contribute to the storage quality of fresh pork, the results showed that recontamination of the pig carcass at the dehairing stage is serious and may pose potential safety and quality hazards. Control of bacterial contamination at this step is likely to have a beneficial effect on the microbial quality and safety of the final products. A quality Critical Control Point should be established at the dehairing step which can be considered as a safety CCP as well. However, some technological modification at this step such as installation of hot water showers to make the operation "specifically designed", may be needed to meet the criteria for establishing a CCP. At the polishing and scraping step the results of the study indicated a slight decline in bacterial numbers, provided that brushing and washing of the carcasses was done properly. Any deviation from the normal procedure e.g. inadequate water supply to the brush and scraping table, reduced frequency of hand and knife washing, or increased frequency of touching the carcass by the worker's hands, is likely to result in an increased level of bacterial contamination. Monitoring measures and corrective actions at this stage could be crucial for maintaining an effective CCP. At the evisceration step, preventive measures such as plugging or tying the anus should be considered. This step could be an important CCP for both quality and safety. Further investigations are required to assess the effect of meat inspection procedures on the spread of bacteria from multiple incisions of lymph nodes, internal organs and tonsils. If this step were to be considered a CCP, it would mainly have safety implications.

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CHAPTER I

GENERAL INTRODUCTION

In many countries throughout the world pork contributes significantly to human nutrition. Pig husbandry also brings considerable financial benefit to farmers through domestic and international trade in live animals and fresh meat. Although pig production systems, slaughter technology and processed products may vary in different countries and between geographical locations, pig processing is in broad terms performed in a similar way and is generally being standardised to meet the hygienic requirements of the world market. Developments in pig processing technology are aimed at both productivity of the industry and safety of the consumer.

Pig processing is an operation with many opportunities for contamination of the carcass by a range of microorganisms, including spoilage bacteria, which may affect the shelf life of the product, and pathogenic bacteria, which pose a potential public health risk.

Meat spoilage is a complex event, in which a combination of microbial and biochemical activities may interact. The microbiology of meat spoilage has over the years received considerable attention, and the characteristics of the typical microflora which develop on meat during storage are well documented (Mossel *et al.* 1995). The contamination of pork with large numbers of spoilage organisms is undoubtedly a major factor influencing the storage stability of that meat in other type of packaging such as vacuum packaging (Shay and Egan 1986). In order to improve the storage life of fresh pork, the ecology of the spoilage flora on pig carcasses should be studied and better understood. Although there has been much progress in the characterisation of the total microflora and biochemical metabolites developing during spoilage, little is

known about the identities of specific microorganisms in relation to meat composition (Huis in't Veld 1996). Some of the most prevalent species of spoilage bacteria such as *Pseudomonas* spp., *Shewanella* spp., and *Enterobacteriaceae* need to be studied in more detail. Also, the factors that influence the development of spoilage such as storage temperature, humidity, pH, preservative agents etc. should be considered in connection with methods to prolong the shelf-life of meat.

During pig processing, contamination of the carcass with potentially pathogenic bacteria can occur at a number of operational stages. The source of contamination may be the animals themselves, the slaughterhouse environment, or personnel involved in handling meat. Like the general contamination, the pathogenic bacteria are mainly animal-related (Borch *et al.* 1996b), and they show differences in their mechanisms of distribution and survival. The potentially pathogenic bacteria that may be associated with pork include *Campylobacter coli* and *C. jejuni, Salmonella* spp., *Yersinia enterocolitica, Aeromonas hydrophila, Listeria monocytogenes, Staphylococcus aureus, Clostridium botulinum, Clostridium perfringens, Escherichia coli* etc. (Stringer *et al.* 1969; Rasch *et al.* 1978; Oosterom *et al.* 1985; De Boer and Nouws 1991; Kotula 1992; Borch *et al.* 1996b). Some operational procedures during pig processing can pose a risk of cross-contamination and spread of the above pathogens. These procedures include dehairing, scraping, evisceration, incision of lymph nodes and palpation of organs during meat inspection. The effect of traditional meat inspection procedures on the spread of pathogenic bacteria over the carcass has been studied previously (Chua and Chew 1991; Gill 1995; Borch *et al.* 1996b; Edwards *et al.* 1997)

Good Manufacturing Practice (GMP) has been successfully implemented for a long time in the food industry, especially in production of raw food materials, with the purpose of eliminating or minimising the contamination of food with both pathogenic and spoilage microorganisms. However, since many bacteria

including pathogenic ones are endemic in the processing environment (Nerbrink and Borch 1989; Borch *et al.* 1996b), a certain level of contamination of carcass meat during processing is unavoidable. Therefore, identification of specific bacteria, their sources, and the process steps where their occurrence is most likely to happen (risk analysis) is crucial for establishing effective preventative measures. To this goal, the HACCP Principles have been introduced to the food processing industry and in recent years much effort has been made to bring them into practice. Originally, the HACCP System was developed as a technique for the identification and control of food safety hazards (Mortimore and Wallace 1995). Later, its principles became applicable in a wider context, including non-food areas.

Using microbial spoilage of fresh meat as an example, the hazard analysis can be used to establish which spoilage organisms are likely to be present in the meat that might survive a certain process e.g. chilling or freezing, and which might cross-contaminate from the process environment. The quality control points can be established using the same procedure employed for identification of a safety CCP.

CHAPTER II

LITERATURE REVIEW

PROCESSING OF PIGS

In most countries where pork is consumed and pig production constitutes a significant part of the livestock industry, processing of pigs is carried out in basically similar ways but varies somewhat from technologically advanced operations in the developed countries to simple backyard methods in many countries of the third world. Breeds, live weight at slaughter, and carcass quality are also different, depending on livestock production systems and on traditional customs and habits. In most countries, pigs are processed by procedures which include stunning, bleeding, scalding, dehairing, washing and polishing, and evisceration. After evisceration, depending on the production purpose, there may or may not be additional steps such as deboning, chilling, packaging, freezing, and storage. The standard pig processing procedures which are applied in most modern pig abattoirs are described below (Dockerty *et al.* 1970; Wilson 1985; Grau 1986).

Pre-slaughter lairage

The main purpose of a lairage is to act as a holding area where a reservoir of animals can be held in preparation for slaughter. This enables the slaughter line to operate at a constant speed irrespective of variations in the delivery of stock from producers or markets (Warris 1987). Pigs are transported to slaughter plants by different types of transport and from different distances. Normally a certain period of time is required for animals to rest before they enter the slaughter line. Tradition has it that this will lead to the production of better meat.

The length of resting time varies from 15-20 minutes, as seen in New Zealand, to 1-2 days in other countries. Minimum 12-24 hours pre-slaughter rest is stipulated by law in some developing countries (Warriss 1987). Warris (1987) found that pre-slaughter rest could alleviate the effects of transport and stress, and that a period of at least 2h was suggested for groups of mixed animals. Duration of the pre-slaughter resting time needed also depends on animal breed. In a large survey of the influence of lairage times of up to 24 hours on Polish pigs, which appear fairly stress-resistant, McMeekin (1982) found no significant effect on the initial pH, but the ultimate pH increased significantly after 24 hours and there was a general improvement in water holding capacity with longer lairage.

It is believed that during the pre-slaughter holding time the content of the gastro-intestinal tract is reduced considerably and that this will consequently reduce the risk of contamination of carcasses by faecal bacteria during mechanical dehairing and evisceration (McMeekin 1982). Sufficient resting time, as shown by many studies, positively influences the ultimate pH value of the meat and, therefore, prolongs the keeping time and quality of meat. Resting time also improves the bleeding rate and thus improves the shelf-life of chilled or frozen meat, especially from stress-susceptible pigs. Shorter time of rest was not sufficient for recovery from the effects of transport.

Very long pre-slaughter resting time also has disadvantages. First of all, long duration lairage and high density of animals kept in the pre-slaughter holding areas create conditions for accumulation of manure and other discharge that in many cases contain high numbers of pathogenic bacteria, including *Salmonella* spp., *Campylobacter* spp., and *Enterobacteriaceae*., and therefore, pose a risk of cross contamination and spread of pathogens between animals (Smulders 1987). Prolonged resting time also causes additional stress to animals, increases weight loss and financial expenses. After prolonged rail transport (54 hours), rest for 24 hours pre-slaughter increased the incidence of watery meat

as well as reduced carcass yield. Patterson (1968) concluded that if pigs were not stressed on arrival at the factory, then resting in lairage would not improve meat quality. A short pre-slaughter holding period as practised in New Zealand does not mean that the potential risk of contamination of carcasses with intestinal contents is ignored. The compliance of farmers with the requirement not to feed animals immediately prior to transport to processing plants is well observed and monitored. Prior to animals entering the slaughter chain, washing or bathing is carried out in order to reduce the risk of spread of bacteria from the dust on the hair coat of the animal's body.

During the pre-slaughter holding time the *ante-mortem* inspection is carried out. Animals with infectious conditions or defects which make them unfit for slaughter for human consumption are withdrawn from the food chain.

Stunning of Pigs

The two main methods of stunning pigs are electrical and carbon dioxide stunning, of which the former is the most widely used (Wilson 1985). Electrical stunning can be broadly classified into two types: head-only and head-to-body. An electrical current of 75-80 volts is passed through the brain for at least 7 seconds, in so-called low-voltage head-only electrical stunning. In the high-voltage head-to-body method 300 volts are applied for 2-3 seconds (Wilson 1985). In both cases, electrical stunning results in a period of desynchronised neuronal activity resulting in an epileptiform seizure with a period of insensibility lasting approximately 45 seconds duration (Petersen *et al.* 1991). In New Zealand, head-to-body electrical stunning is widely used in pig abattoirs. In this method of stunning, a current is simultaneously passed through the head and the body by means of a third electrode usually applied to the back. This body current has the effect of causing cardiac dysfunction by ventricular fibrillation. Thus, as well as causing immediate insensibility by depolarisation of neurones,

permanent insensibility is ensured by cardiac dysfunction and subsequent inhibition of a cerebral circulation.

In many developing countries, as well as in some small meatworks in developed countries, the head-only method of electrical stunning is still practised. With respect to humane slaughter, head-only stunning is theoretically contra-indicated as animals may take up to 85 seconds to bleed to insensibility, while the duration of a temporary head-only stun is in the region of only 45 seconds (Petersen *et al.* 1991). It has been suggested that head-only stunning, followed by low voltage immobilisation immediately after sticking, prolongs the period of electrical induced insensibility. At present, there are still some scientific differences in opinion as to whether or not this hypothesis is valid (Petersen *et al.* 1991).

Since electrical stunning was introduced in the 1930s, it has received considerable attention from research workers but there is still a lack of knowledge on its efficiency in producing insensibility (Gracey 1986).

Research work on the meat quality in pigs subjected to captive bolt, carbon dioxide anaesthesia and electrical stunning has shown in general that the electrical stunning method is preferable, especially when it is of a high-voltage type (300V). It has also been demonstrated that the use of tongs operating at 300V in a restrainer-conveyor resulted in superior pig meat quality compared with automatic stunning with 680V, the incidence of the inferior meat quality known as soft pale exudative (SPE) meat being much less in the former method.

In carbon dioxide stunning pigs are passed through an atmosphere which is a mixture of CO_2 and air. The pigs are thus rendered unconscious. The advantage of this method is that blood splashing, which is the most common stunning defect in pigs, is eliminated (Wilson 1985). This method also cuts out the human element which is often at fault with electrical stunning.

Bleeding (Exsanguination, Sticking)

Approximately 8% of an animal's liveweight is blood and only half the total blood volume is lost by the common methods of bleeding (Petersen *et al.* 1991).There are two methods of bleeding: the gash cut which is a transverse incision of the neck that severs the carotid arteries, jugular veins and vagus nerves, and the thoracic stick which is the common method used for bleeding pigs. In the thoracic stick, a knife is inserted into the jugular furrow and thrust down the neck between the first two ribs. This should sever both the bicarotid trunk and anterior vena cava. Care should be taken not to insert the knife too far as it may penetrate into the shoulder, allowing blood and water from the scalding tank to run back into the shoulder 'pocket' beneath the scapula. Too large a sticking wound also facilitates the entry of microorganisms from contaminated scalding water into the carcass tissues (Troeger 1993).

The stunning of an animal by any means produces a rise in the blood pressure of the arterial, capillary and venous systems (Gracey 1986). This is accompanied by a transitory increase in the heart rate. Both of these factors facilitate bleeding. The importance of immediate bleeding is obvious when it is realised that the rate of flow from the cut vessel is five to ten times more rapid than in the intact vessel, and not until 20% of the blood has been lost does the pressure begin to fall. Delay in bleeding after stunning may cause imperfect bleeding and blood splashes in the carcass tissues (Gracey 1986).

Scalding

The purpose of scalding of pig carcasses with hot water after bleeding is to make the hair coat, as well as the scurf of the skin, easier to remove. The most

common methods of scalding are vat scalding and scalding with a hot water spray.

Vat scalding can be of two types: vat scalding with simultaneous dehairing or vat scalding followed by dehairing. Considerable attention is given to the temperature of scalding water, to the length of time each carcass is exposed to the hot water, and to the thoroughness with which the scalding water reaches all parts of the surface of the carcass. If the scalding water is too hot, the surface of the skin will appear cooked. If the scalding water is not hot enough, the scurf and the hair will be insufficiently loosened requiring vigorous scraping which can lead to the production of carcasses with an unclean and rough skin. Ideal scalding temperatures range from 58 to 62°C with exposure times ranging from 5 to 7 minutes. Temperatures of 63°C and above for 4-5 minutes damage the skin (ICMSF 1998). To maintain the supply of the large volume of hot water required in the scalding vat, recirculation of the scalding water is permitted, provided that water that is added from time to time is clean and potable. Use of the vat scalding system leads to the accumulation of soil, faeces, and blood in the tank, so pre- and post-operation removal of such contaminants, cleaning of the vat, and supply of clean hot water at the beginning of each production shift is very important. In most modern pig slaughterhouses, the scalding water is heated by electrical or gas heaters and the temperature is automatically controlled. But elsewhere manual control of scalding water temperature is still in use and both temperature and exposure times are often abused. In backyard slaughter of pigs, boiling water is poured directly onto the skin of carcasses from a pot or a tank and the temperature is controlled by the experience of butchers, so the subsequent dehairing is usually not adequate and there is often a need to shave carcasses with a sharp knife.

In the hot water spray method of scalding, instead of dipping the carcass into the scalding tank, it is sprayed with hot water in a horizontal position or moved on the rail, in a vertical position, through a tunnel with hot water showers.

Temperature of scalding water and exposure time are the same as in the vat scalding method. This method of scalding has an advantage that it eliminates the accumulation of mechanical contaminants such as hairs, scurf, dirt and soil in the scalding water. Results of a study showed that the risk of bacterial contamination of pig carcasses is lowest for vertical scalding by spraying (Troeger 1993). Scalding by spraying on the rail also reduces a risk of the penetration of microorganisms from scalding water into blood vessels, the lungs and livers (Troeger 1993).

Dehairing

The hair and scurf loosened by scalding is removed mechanically by a dehairing machine. The principle of mechanical dehairing is that after the pig carcass is removed from the scalding vat or from the scalding spray tunnel it is put into the dehairing machine, which has a central drum with a number of blades continuously rotating. Hair and scurf are removed by friction produced by the rotating blades beating the skin. While tumbling and rolling inside the machine, the carcass is sprayed with hot water, which removes the hair and scurf as they are rubbed free from the skin surface and improves the effect of cleaning and polishing. The process normally takes a similar time as required for scalding, which is about 5 to 7 minutes.

The scalding/dehairing process has long been known to be a hygienically weak point in the processing of pork (Troeger 1993). Most microorganisms are destroyed in the scalding water, but dehairing can be a major source of carcass contamination (Dockerty *et al.* 1970; Gill and Jones 1992; Gill and Bryant 1992; ICMSF 1998). The problem of bacterial contamination of carcasses due to the scalding and dehairing operations will be discussed in more detail later.

Singeing, Scraping and Polishing

The order of performing these operations varies between meat works. On some pig slaughter chains in New Zealand, scraping and polishing is carried out immediately after dehairing, and is followed by singeing, while in other slaughterhouses singeing is performed after dehairing, followed by scraping and polishing. The carcass is scraped manually or mechanically using knives and brushes to remove hair and scurf that remain after scalding. Some meatworks have special brushes, attached to a plastic tube connected to the water supply, so that washing of carcasses takes place during scraping. Care should be taken to avoid making scratches on the skin surface which reduce the visual appeal of the carcasses and create sites for attachment and penetration of bacteria into deeper tissue layers (Gracey 1986).

Singeing is performed to burn residual hair by a device that produces flames without affecting the skin. Some skill is needed for performing this process properly, otherwise hair will not be burned or skin can be damaged by the flame. Under some circumstances, a special kind of singeing, which includes scorching of the outer layer of the skin, is carried out (Gracey 1986).

In the United States, some meat plants supplement the work of the dehairing machine with an application of a mixture of resin and oil to the carcass. This mixture which consists of approximately 15% of paraffin or cottonseed oil and 85% of resin (Brandly *et al.* 1966) is held in a steam-heated storage tank. In some plants this mixture is applied only to parts of the carcass with large brushes. Other plants are equipped to immerse the entire carcass momentarily in the hot mixture with measures taken to prevent the mixture from entering the mouth and the nostrils of the carcass (Brandly *et al.* 1966). After the mixture is applied, the carcass is sprayed with cold water, which sets the coating. This coating, possessing some elasticity due to the oil content of the mixture, is then

peeled off by hand taking with it any hair or scurf which was missed in the dehairing machine.

Evisceration

First, the anal sphincter and rectum are freed from attachment to the carcass. At this point in some plants, ligation or closure of the rectum is made with a rubber stopper or a plastic bag to avoid contamination which would result from the escape of intestinal contents during the subsequent handling of the viscera. The carcass is then opened by a longitudinal incision in the median line extending from the pelvic region to the neck. Care must be exercised to avoid cutting into the viscera when the abdominal wall is opened. Also, the incision must not cut into the prepuce. This incision splits the pelvis at the symphysis and opens the thorax along the median line. Next, the urinary bladder, the uterus, and the penis are removed; again, care should be exercised to avoid cutting into the prepuce. The content of the prepuce can be a serious source of contamination (Brandly *et al.* 1966) should it be allowed to come in contact with edible portions of the carcass. The organs removed (penis, uterus, bladder), are handled as inedible. Even if they are used as edible products in some countries, they should be separated and kept in isolated areas to avoid spreading of bacteria. Organs in the thorax and abdomen are removed, and edible offal such as liver, kidneys and hearts are separated from the inedible viscera and inspected. The viscera must be traceable to the carcass they came from until after inspection.

After evisceration the dressing process as such is completed. Depending on the design of the slaughter chain and the purpose of the production of the final commodities there may be subsequent processing steps such as carcass splitting, deboning, chilling, packaging, and freezing.

Processing pork also includes inspection procedures, which consists of *ante-mortem* and *post-mortem* inspection. *Ante-mortem* inspection takes place before the pigs are brought to the stunning box and is carried out by a meat inspector in the pre-slaughter holding areas. In some countries, *post-mortem* inspection consists of inspection of the head, the viscera, and the carcass, which take place separately along the slaughter line and is carried out by different inspectors. In other countries, *post-mortem* inspection is performed only at the final step of the slaughter process after evisceration and all inspection procedures are carried out by one inspector. Procedures for *post-mortem* inspection also vary from country to country and depends on a number of factors such as animal disease status, local regulations, market requirements etc.

BACTERIAL CONTAMINATION OF PIG CARCASSES

Sources of Bacterial Contamination

The level of bacterial contamination of the live animal presented for slaughter depends on a number of factors. First of all, the hygienic conditions of different housing systems greatly affect the hygienic status of animals. Farms which are equipped with good sanitary and hygienic facilities or have an advanced hygienic management often produce healthy and clean animals with a low level of bacterial contamination (Gracey 1986). On these farms the animal health status is likely to be better and therefore, the risk of contamination and spread of pathogenic bacteria through slaughter of sick or diseased carrier animals is reduced.

One of the main sources of bacterial contamination of the carcass in slaughter plants is the live animal itself (Dockerty *et al.* 1970; Gill 1986; Gracey 1986; Borch *et al.* 1996b), particularly in the winter when animals on the farm are kept

inside under crowded conditions, which allows accumulation of faeces and, therefore, creates a favourable environment for microbial multiplication. In a study of microbiological characteristics of pig carcasses in Italian slaughterhouses Barbuti *et al.* (1992) found that the initial contamination of pork comes mainly from the animal's skin but also from faecal material and from the surrounding environment.

The risk of live animals being contaminated or recontaminated with bacteria also occurs when they are kept too long in the pre-slaughter areas. During lairage there is a great risk of accumulation and spread of *Salmonella* spp. among animals which creates a risk of further contamination of meat during processing (Lazaro *et al.* 1997). Live animals are also the main source of contamination of the carcass with psychrotrophic bacteria that are able to grow at refrigeration temperature and play an important role in chilled meat spoilage (Newton *et al.* 1978; Scholefield *et al.* 1981; Kraft 1986). Some psychrotrophic organisms originate from faeces, but most are inhabitants of the environment, including soil and water. Dirty, live animals pose a greater risk for contamination of carcasses if they are transported over long distances and kept for a long time in the pre-slaughter holding area.

Total bacterial counts of the surface of the carcass range from $5,6x10^2$ CFU/ cm^2 (colony forming units per cm^2) to $1.7x10^6$ CFU/ cm^2. Nortje *et al.* (1990a) in a study of the aerobic psychrotrophic population on meat and meat contact surfaces in a meat production system, as well as on meat stored at chill temperature, found that *Pseudomonas* spp. dominated the aerobic, Gram-negative bacterial population associated with carcasses. The skin of pigs sampled at the bleeding step of the slaughter process carries about 10^6-10^7 CFU/cm^2 of aerobic mesophiles, 10^3-10^4 CFU/cm^2 *Enterobacteriaceae*, 10^2- 10^3 CFU / cm^2 spores of *Bacillus* spp., 10^2 CFU or less per cm^2, *Clostridium* spp., and 10^4 per cm^2 of psychrotrophs (Dockerty *et al.* 1970; Scholefield *et al.* 1981). The skin may also be contaminated with *Salmonella* acquired on the farm, in

transport, or in lairage. During the act of sticking, bacteria can enter the jugular vein or anterior vena cava and travel in the blood to muscles, lung and bone marrow (Robert 1980; Borch *et al.* 1996b).

During the scalding/dehairing operation, most microorganisms are destroyed in the scalding water (Snijders and Gerats 1976; Scholefield *et al.* 1981). However, some bacteria, predominantly spores of bacilli and small numbers of clostridia can survive. The number of viable bacteria in the scalding water may vary from 10 to 10^4 per ml and is reduced as the temperature rises (Snijders and Gerats 1976). The surviving microorganisms in the scalding water can enter the sticking wound or the mouth and invade the vascular system and the lungs (Roberts 1980; Troeger 1993). Dehairing is a hygienically weak point in pig processing. Pig carcasses are recontaminated with faecal and other material from dehairing machines. Both dehairing machines and hand scrapers used for dehairing are frequently contaminated with salmonellae and, because they are often inadequately cleaned, can disseminate the bacterium (Borch *et al.* 1996b).

During singeing, the number of bacteria on the surface of the skin is considerably reduced due to the thermal effect of the flame. Dockerty *et al.* (1970) found that the mesophiles were reduced about two-fold during singeing. Other studies have shown that the reduction in both mesophiles and *Enterobacteriaceae* due to the effects of singeing was much greater and in the order of 10^3 CFU/cm^2 or more (Snijders and Gerats 1976; Gerats *et al.*1981). The number of mesophilic bacteria increases again after scraping, but this increase varies between plants (Rahkio and Korkeala 1996) and is mainly due to inadequate cleaning and sanitising of the scraping and polishing tools, thus scraping and polishing facilities (manual or mechanical) can be considered the sites where bacterial attachment and growth take place and, therefore, are the main sources of carcass contamination.

Under normal conditions, the heaviest and potentially the most dangerous load of bacteria is in the animal's digestive tract. During opening of the carcass and removal of the abdominal contents, any puncture of the intestinal tract or escape of material from the rectum will result in contamination of the carcass and cross-contamination of other carcasses as result of direct contact or via hands and knives (Grau 1986). The critical operation is circumcision of the anus and freeing of the rectum, but this is not practised in all the plants. Nesbakken *et al.* (1994) found that the use of a plastic bag for sealing off the anus at the evisceration stage reduced the incidence on the carcass of *Yersinia enterocolitica* O:3/biotype 4. Without the use of a plastic bag 10% of carcasses were contaminated with this bacterium, whereas only 0.8% were contaminated when plastic bags were used. During removal of the intestine, there is a potential risk of puncturing it so that faecal matter is spread over the carcass. However, with well-trained operators this should occur infrequently. Normally the stomach is removed with the intestinal tract and if the oesophagus is cut too close to the stomach there is a high risk of leaking of its contents, which will contaminate the carcass and internal organs. If visible contamination occurs this may be trimmed. This may improve the appearance of the carcass but will not eliminate the microbial contamination.

The tongue and the tonsils are removed together with the pluck, consisting of the kidneys, the diaphragm, the heart, the lungs, the oesophagus and the trachea. The spread of bacteria from the tonsils and the pharynx to the carcass and the pluck is unavoidable. Many studies have shown that tongue and pharynx with attached tonsils harbour pathogenic bacteria including *Yersinia* spp. and *Salmonella* spp. (Borch *et al.* 1996b).

Some equipment used during carcass splitting and head cutting such as electric saws and hammers, can also be the source of contamination if they are not properly cleaned and sanitised. Equipment has been found to be involved in

cross-contamination with pathogenic bacteria between carcasses (De Boer and Nouws 1991; Hayes 1992; Edwards *et al.* 1997).

Meat inspection procedures, usually carried out after evisceration, also represent a cross-contamination risk. The traditional *post-mortem* meat inspection comprises palpation, observation and incision of internal organs such as heart, liver, mesenteric and other lymph nodes, and lungs, to detect the presence of defects and disease. The inspection procedures are being revised by many countries due to their lack of sensitivity. Although the main criticism of traditional meat inspection is that it is of doubtful sensitivity, the very nature of the procedures, e.g., palpation and incision of lymph nodes, can pose a risk by spreading pathogenic bacteria (De Boer and Nouws 1991; Hayes 1992; Berends *et al.* 1993; Edwards *et al.* 1997). The problem is highlighted by *Salmonella* localised within the mesenteric lymph nodes, being potential source of contamination following detailed examination by multiple incisions (Edwards *et al.* 1997). Contamination from lymph nodes and possibly lungs of vat scalded pigs may also occur during meat inspection (Borch *et al.* 1996b). Due to cutting of lymph nodes and palpation of organs and other parts of the carcass performed by meat inspectors, pathogenic bacteria may be transported from an infected region to other parts of the carcass by knives and hands of the meat inspection personnel (Nesbakken 1988).

Pathogenic Bacteria

Pig processing is an operation with many opportunities for contamination of product with potentially pathogenic bacteria. Both the pathogenic and non-pathogenic contaminants are mainly animal-related (Borch *et al.* 1996b), and show differences in their distribution and survival abilities. The potentially pathogenic bacteria associated with pork include *Campylobacter jejuni* and *C. coli, Salmonella* spp., *Yersinia enterocolitica, Aeromonas hydrophila, Aeromonas sobria*, *Listeria monocytogenes, Staphylococcus aureus, Clostridium*

botulinum, Clostridium perfringens and *Escherichia coli* (Kotula *et al.* 1980; Kotula 1992).

Campylobacter spp. are common inhabitants of the intestinal tract of pigs and other domestic animals. Oosterom *et al.* (1985) found an average of 4.10^3 CFU/g of *Campylobacter* in intestinal content of pigs. *Campylobacter* spp. were isolated from the faeces of 75% to 85% of the pigs in a Dutch investigation (Oosterom *et al.* 1983). Another study showed that the prevalence of *Campylobacter* spp. was 9% and 0% of the carcasses before and after chilling respectively (Oosterom *et al.* 1985). In a study on the incidence of *Salmonella*, *Campylobacter*, and *Yersinia enterocolitica* in pig carcasses and the slaughterhouse environment in a Quebec abattoir, *Campylobacter* spp. were isolated from 247 (61.7%) of 400 specimens, and *C. coli, C. jejuni*, and *C. laridis* accounted for 97%, 2%, and 1% of isolates respectively (Mafu *et al.* 1989).

Campylobacter spp. have been isolated from all stages of the pig slaughter process. Gill and Bryant (1993) found that the detritus from dehairing machines of two pig slaughterhouses contained *Campylobacter* spp. in numbers ranging from $3x10^3$ to $1x10^6$ CFU/g, and that pig carcasses after leaving the dehairing machine contained 3-70 CFU/cm^2 *Campylobacter* spp..

Campylobacter spp. are microaerophilic, do not grow at temperatures below 30°C, have a low heat resistance and are sensitive to drying, freezing and an aerobic atmosphere (Stern and Kazmi 1989). Laboratory experiments have shown that the over-night reduction in numbers of *Campylobacter* on pig skin to undetectable levels during cold storage was mainly due to drying of the skin. If the humidity is high they could survive as long as a moistened surface could be observed. It is important to note that the survival time of *Campylobacter* spp. under similar storage conditions vary and is longer for poultry carcasses and pig liver than for pig carcass surfaces (Oosterom *et al.* 1983).

Pigs can be healthy carriers of *Salmonella* spp. which are frequently found in pig carcasses and on pork products. Based on reports from several countries, D'Aoust (1989) concluded that the incidence of *Salmonella* spp. on pig carcasses varies from 0.4% to 76.3%, with an average of 16.2%. A study in Denmark showed that 22,2% of faecal samples from pigs and 1.3% of samples of pork cuts from abattoirs contained Salmonellae (Anon 1995). The most frequently isolated serovar in both faecal and pork cut samples was *S. typhimurium*. The incidence of *Salmonella* spp. in tonsils, carcass fore-end and liver/diaphragm was in another Danish study 15%, 8%, and 17%, respectively (Christensen and Luthje 1994). In a Brazilian study of slaughtered pigs, Lazaro *et al.* (1997) found tonsil contamination in 77.5% of the *Salmonella*-positive pigs. *Salmonella* spp. can be found in the entire digestive tract and in the associated lymphatic tissues of pigs, but the numbers vary from section to section. The presence of *Salmonella* spp. reported in mesenteric lymph nodes varies from 2.4 to 32% (Watson 1975; D'Aoust 1989; D'Aoust 1994; Lazaro *et al.* 1997). *Salmonella* spp. can also be found in palatine tonsils and mandibular lymph nodes, and have been isolated from various slaughter and processing equipment including knives and gloves of meat inspectors (Nesbakken 1988; Edwards *et al.* 1997, Lazaro *et al.* 1997). As for *Campylobacter* spp., incision of mesenteric and portal lymph nodes and palpation of livers during *post-mortem* inspection can result in contamination of meat and cross-contamination of pig carcasses. Some pig processing procedures including scalding/dehairing, scraping, evisceration, splitting the carcass and cutting of head, can contribute to the potential spread of *Salmonella* spp.

Pigs are considered important reservoirs for pathogenic *Yersinia* spp.. Results of many surveys have demonstrated the common occurrence of *Yersinia enterocolitica* and related species in the tonsils and intestinal tract of healthy pigs at slaughter-age. There is however still debate about the source of infection and when pigs become infected (Bungay 1997).

For many years animals have been regarded as the main source of human *Y. enterocolitica* infections and pigs appear to be the principal carriers of the serovars that are pathogenic to humans (Schiemann 1989). *Yersinia. enterocolitica* O:3 is often found in the intestinal contents and faeces of pigs. Shiozawa *et al.* (1991) found that 4.3% of 140 pigs were carriers of this organisms in the caecum with counts varying from less than 300 to 110000 CFU/g of intestinal contents.

During lairage, pathogenic bacteria including *Yersinia* spp. may spread from infected to "clean" pigs. During subsequent processing, the bacteria may spread to the carcass from intestinal and stomach contents, oral cavity, and oesophagus (Nesbakken 1988; Nesbakken *et al.* 1994). The incidence of *Yersinia* spp. on tonsils, carcass fore-end and liver/diaphragm in a Danish study was found to be 56%, 40%, and 25%, respectively (Christensen and Luthje 1994). On pig carcasses, the incidence of pathogenic serotypes of *Yersinia enterocolitica* can range from undetectable level (Mafu *et al.* 1989; De Boer and Nouws 1991) to as high as 40% (Christensen and Luthje 1994).

Yersinia enterocolitica has been isolated from carcass surfaces with varying frequencies depending on the evisceration techniques. Manual evisceration was found to result in high frequencies of contamination: of 26.3% on the medial hind limb and 12.9% on the split sternum. The use of a mechanized bung cutter reduced the rate of contamination, especially when it was used in connection with enclosing the anus and rectum in a plastic bag to minimize faecal contamination. When carcasses were eviscerated in this way, it was possible to reduce carcass contamination with *Yersinia enterocolitica* to 1.9% on the medial hind limb, 1.0% in the pelvic duct, and 2.2% on the split sternum (Andersen 1988).

Although a proof of a link between the consumption of pork and human illness caused by *Y. enterocolitica* is not yet established, it has been suggested that pigs play an important role in human infection (Mafu *et al.* 1989). Epidemiological studies have associated human yersiniosis with the consumption of minced pork (Nesbakken 1988).

Pork was found to be the source of 38.7% of meat-borne staphylococcal infections in the USA (Schraft 1992). It is likely that this is the most common form of food poisoning but is underreported because of its relatively mild nature and short duration. The most important source of *Staphylococcus aureus* is probably the human body, the principal reservoir being the nose. Bryan (1980) pointed out that many lesions such as boils, carbuncles, septic cuts and abrasions abound with *Staphylococcus aureus* and that the carriage rate in hair may be as high as that of the nose.

Staphylococcus aureus on pig carcasses are a mixture of survivors of the original flora on the skin of the pig and contaminants acquired during slaughter (ICMSF 1998). In a study on contamination of pig hindquarters with *Staphylococcus aureus,* Schraft (1992) found 33.6% of carcasses of chilled pork were contaminated with this organisms and the source of contamination was traced back to the processing plant. Rasch *et al.* (1978) observed that the slaughter technique could affect the *Staphylococcus aureus* counts on the skin of pig carcasses and Narucka (1979) showed that pig heads, of which up to 35% were contaminated with *Staphylococcus aureus* immediately after slaughter, had rates of contamination of 75% after cooling and deboning.

Listeria monocytogenes is the most important human pathogen within the genus *Listeria* (Borch *et al.* 1996b). Certain groups of people including infants, the elderly, persons immunocompromised by drugs or diseases and pregnant

women are most susceptible to the organism (Borch *et al.* 1996b). *Listeria* spp. are widely distributed in the environment. Skovgaard and Nørrung (1989) suggested that faecal contamination may play and important role in the dissemination of *L. monocytogenes* in raw meat.

It has been found that the prevalence of *L. monocytogenes* in the environment of different meat processing plants varies widely between 0% and 93% (mean value 13%) (Borch *et al.* 1996b). This variation has been attributed to variations in hygienic conditions. *L. monocytogenes* may proliferate in the processing environment, and has been isolated from food contact surfaces, floors and drains (Schiemann 1989).

The transmission of *L. monocytogenes* does not seem to occur via the animal, but seem to be a problem localized to the slaughterhouse and other food processing plants and, therefore, *Listeria* spp. and *L. monocytogenes* may be useful as indicators of the general hygienic status of slaughterhouses and other food plants (Borch *et al.* 1996b).

Clostridium botulinum: most clostridia that occur in raw meat are harmless putrefactive mesophiles. Most cases of meat-born botulism have been from improperly preserved, home-processed meats that have been eaten without prior cooking. *Cl. botulinum* is widely distributed in soil and the types causing human botulism are more prevalent in the northern hemisphere than in the southern (Hayes 1992).

The epidemiology of botulism in humans is different from continent to continent. While in Europe and Asia, human botulism is more likely to be linked to consumption of pickled and canned animal food, most of the cases in the United

States were traced to the consumption of bottled and canned food and beverage of vegetable and fruit origin (Hayes 1992).

Clostridium perfringens type A is readily isolated from soil, natural water, intestinal contents of animals and humans, from a wide range of foods and most frequently from raw meats and poultry (Patterson 1967; Grau 1986). Food poisoning caused by *Cl. perfringens* normally occurs when there is temperature abuse during the preparation of food, which allows multiplication to a sufficiently high number for survival and release of toxin in the digestive tract. This type of food poisoning is generally associated with catering malpractice and often involves large numbers of persons. Outbreaks of *Cl. perfringens* food poisoning frequently occur in institutions, hospitals, canteens and similar establishments. *Cl. perfringens* spores can survive refrigeration and usual cooking temperatures.

Because of the ubiquitous nature of the bacterium, it is impossible to eliminate *Cl. perfringens* from foods. The control measures should be aimed at, first of all, minimising contamination of raw meat and, subsequently, restricting both spore germination and the proliferation of vegetative cells during cooling and storage (Smulder 1987; Hayes 1992).

Aeromonas hydrophila and *Aeromonas sorbia* are considered potential foodborne pathogens causing mild, self-limiting diarrhoea and occasional vomiting, mainly associated with young children, the elderly or with immunocompromised people (Kirov 1993). Both raw and cooked foods are potential sources of human infection. *A. hydrophila* is widely distributed and is very common in water. However, many foods carry *A. hydrophila* and to lesser extent *A. sorbia*. Newton *et al.* (1978) isolated *Aeromonas* spp. frequently from carcasses of food animals after dressing. Fricker and Tompsett (1989) found that the carriage rates of *A. hydrophila* and *A. sorbia* were 69% in poultry, 21% in pork and raw salads, 17% in beef, and 15% in fish.

Being heat-sensitive, *Aeromonas* spp. are unlikely to survive the scalding in the pig slaughter process, but they are found in high numbers on dehairing machines, in dehairing water, trimming belts and mesh gloves (Gill 1995). *Aeromonas* spp. could be used as an indicator of processing hygiene with respect to the hygienic standards of the slaughtering and processing equipment's, such as dehairing machines, mesh gloves and trimming belts.

Escherichia coli include non-pathogenic and pathogenic strains. Non-pathogenic strains can be isolated from the intestines of warm-blooded animals (Hayes 1992). Pathogenic strains of *E. coli* are divided into four groups (Olsvik *et al.* 1991). The first group, enteropathogenic *E. coli* (EPEC) cause severe diarrhoea in infants. The second group, enterotoxigenic *E. coli* (ETEC), cause diarrhoea in human, both infants and adults. ETEC strains produce heat-labile and heat-stable enterotoxins. These organisms have been found in various animal meats and poultry and caused many large outbreaks of infection. The third group of pathogenic *E. coli*, enteroinvasive *E. coli* (EIEC) produce a cytotoxin and often induce rather more severe illnesses like colitis and a form of dysentery. Many outbreaks have been attributed to EIEC strains, with cheeses, milk and meats most frequently incriminated. The final group, enterohaemorrhagic *E. coli* (EHEC) also produce cytotoxins which cause more severe symptoms. EHEC have been isolated from ground beef and minced pork. Hamburgers are the food most often involved in outbreaks of infection caused by EHEC strains (Hayes 1992).

Attachment of Bacteria to Pig Carcass Skin

The attachment of microorganisms to meat surfaces has become of great interest to the food industry and to researchers during the last two decades (Benedict 1988). Many studies have been done on the attachment ability of both

pathogenic and spoilage bacteria to the meat surfaces (Butler *et al.* 1979; Stone and Zottola 1985; Benedict 1988).

Adhesion of bacteria to surfaces depends on characteristics of the microorganisms, the substrate surface to be colonized, and the surrounding fluids (Benedict 1988). There are relatively few studies of the mechanisms of microbial attachment to meat surfaces as compared with those on attachment and adhesion of bacteria to live mucosal membrane. However, results of studies of the relationship between the extent of attachment of microbes to the meat and meat products and the initial population of bacteria in the environment (water, air, contact surfaces) showed that the higher the initial population in the environment, the greater was the attachment (Butler *et al.* 1980, Piette and Barriga 1994). Farber and Idziak (1984) reported on the attachment of psychrotrophic meat spoilage organisms to *Longissimus dorsi* muscle. It was noted that fascia was the best surface for bacterial attachment. The mechanism for attachment of the psychrotrophic spoilage organisms to the meat surface was not examined in these studies, but the presence of mucoid material produced by some organisms on the agar plates was noted as possibly important in attachment.

In a study on attachment of microorganisms to pork skin and surfaces of beef and lamb using embedding techniques to examine exposed pork skin and surfaces of beef and lamb after rinsing in the bacterial suspensions, Butler *et al.* (1979) reported a direct relationship between bacterial counts of the skin and concentration of bacterial cells in the attachment medium. Temperature and pH of the attachment medium had little effect on bacterial attachment firmness, but Gram-negative motile organisms showed a greater attachment to the surfaces than Gram-positive non-motile species (Benedict 1988). This conclusion is consistent with the result of a study by Notermans and Kampelmacher (1974)

who reported that non-flagellated bacteria did not attach or only in low numbers, whereas flagellated bacteria showed a very marked attachment.

Butler *et al.* (1980) concluded that contamination of pork carcasses with bacteria at points in slaughter-dressing preceding dehairing are of little consequence in determining the ultimate bacterial load. Neither singeing nor washing, after the carcass has been dehaired, decreased bacterial count by more than about 1 log.

Factors Affecting Bacterial Contamination of Carcass Meat

Microbial growth

Bacteria multiply by the process of binary fission. The rate of growth depends on a number of factors that influence the interaction between bacteria and the medium in which they grow. Under normal conditions, bacterial growth can be divided into four phases (Figure 1): lag, exponential, stationary, and death (Montville 1997). The lag phase is a time period during which there is little growth of bacteria. Bacteria just increase in size and produce new material needed for further development and multiplication. The length of the lag phase is extremely variable (Hayes 1992) depending on a number of factors such as temperature, the inoculum size (larger inocula usually have shorter lag phases), and the physiological history of the organisms (Montville 1997).

The next phase of growth, termed the logarithmic or exponential phase, is characterized by a constant specific growth rate. During this phase, each cell in the population is duplicating itself and the cells formed are all viable and of constant size. In food microbiology, the term "doubling times" or "generation times" (t_{gen}) is often used to describe the rate of logarithmic growth.

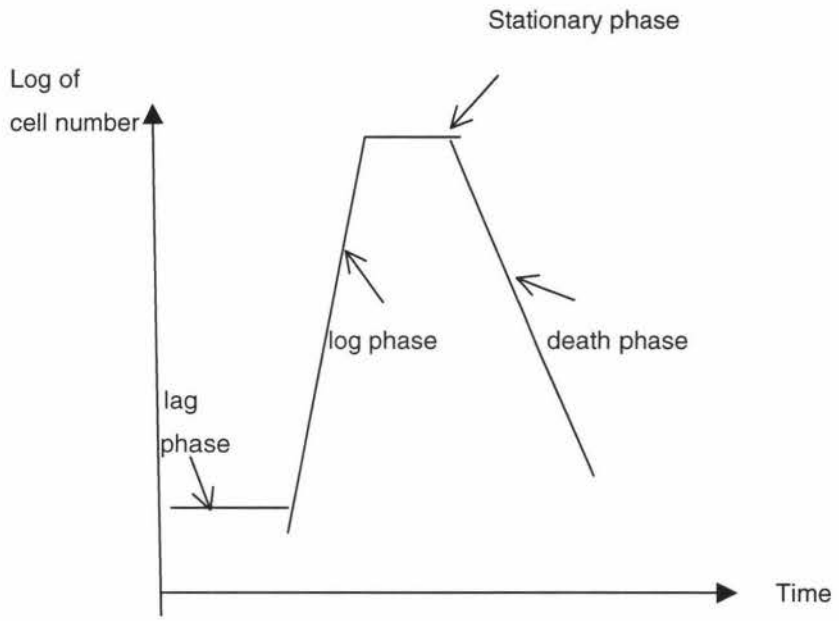

Figure 1: Growth curve of bacteria showing the four phases of growth.

With time and as growth of bacteria increases, the environment will change due to the depletion in nutrients and accumulation of waste products. These changes result in a decrease in the growth rate until the population enters the stationary phase. During this period the number of viable bacteria remain constant; this may be due to the complete cessation of growth or to the fact that any growth may be offset by the death of an equal number of bacteria. The final phase of the bacterial growth curve is known as the decline or death phase and represents a period when the death rate exceeds the rate of multiplication. Death of bacterial cells is often accompanied by lysis so that if old cultures are viewed under the microscope a large amount of cell debris may be seen with few intact cells in evidence (Montville 1997).

Nutrition

Bacteria depend on nutrients for both energy and growth. They differ greatly in their nutritional requirements but certain elements are essential such as carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus; smaller quantities of iron,

magnesium, potassium, and calcium are also required. Bacteria may require one or more organic compounds for cellular material that they are unable to synthesise from simpler compounds.

Temperature

Temperature affects the growth and metabolic activity of all living cells. At high temperature all bacteria are destroyed. At freezing temperatures many bacteria also die but some just "slow down" their activities. Microorganisms of concern in meat production are classified into three main groups depending on their temperature requirements for growth (Table 1).

Table 1: Classification of bacteria according to temperature requirement for growth. From Hayes (1992); *all data in parentheses from Mountney and Gould (1988).

Bacterial Groups	Temperature required for growth (°C)		
	Minimum	Optimum	Maximum
Thermophilic	25-45	50-55	70-90
	(35-45)*	(45-70)	(60-80)
Mesophilic	10-25	30-40	35-50
	(5-20)	(30-45)	(40-50)
Psychrotrophs	0-5	15-20	30
	(0-5)	(20-35)	(25-40)

The definition of the temperature ranges of these three groups is still the subject of much controversy and it should be emphasized that some overlapping of the groups do occur. Thus, for example, a variety of mesophilic bacteria with optimum growth temperature of 30-35°C are able to grow, albeit slowly, at 5°C or less; such organisms sometimes are called psychrotrophic mesophiles. A fourth group of specialized organisms, the psychrophiles, is also recognized. They are characterized by extremely low optimum growth temperature (5-20 °C) and the ability to grow well at 0°C. They play some role in the shelf life of

refrigerated and frozen meat and are believed to contribute to the spoilage of chilled meat, but in nature and in other kinds of foods they are of little importance (Hayes 1992).

Moisture

Water accounts for some 80-90% of the total weight of living cells and all bacteria require it for growth, although requirements vary widely for different groups of bacteria. It is the amount of available water and not total tissue water that determines the rate of bacterial growth in meats. The available moisture, expressed as water activity (a_w), is the vapour pressure of the solution divided by the vapour pressure of pure water. The a_w of pure water is 1.0, that of fresh meat is 0.99 and that of many bacteriological media is in excess 0.995. Most bacteria grow well with water activities of 0.990-0.998 (Hayes 1992).

Oxygen

Bacteria can be characterized on their oxygen requirements. Those depending on the presence of free oxygen in the air are called obligate or strict aerobes. Few bacteria are strictly aerobic but many, such as pseudomonads growing on the surface of meats are generally considered aerobic. Bacteria that can only grow in the absence of free oxygen are obligate anaerobes. The majority of bacteria fall between these two groups and can grow in either the absence or presence of free oxygen, and although they often display a preference for aerobic conditions they are called facultative anaerobes.

Hydrogen Ion Concentration

Microorganisms vary in their pH requirements for growth. Most bacteria grow at a pH near neutral (Rhodehamel 1992). The pH, a measure of the hydrogen ion concentration, is one of the key factors in determining the survival of microorganisms and bacterial spores. Fresh meat has a neutral pH and is an ideal medium for bacterial grow, but the pH changes due to biological and chemical activities that take place during the immediate post slaughter period.

Changes of pH in meats occur mainly due to breakdown of ATP and accumulation of lactic acid during *rigor mortis*, thus pH of meats falls from about 7, which is the normal value for live tissues, to an ultimate value of about 5.5 of meat (Petersen *et al.* 1991).

Microbial death

Death of bacteria in meats is mainly the result of measures applied during processing and storage with the aim to minimize or eliminate both pathogenic and spoilage microorganisms. Treatments such as thermal processing, cooking, warming, chilling, freezing, drying, pickling, irradiation, exposure to sanitizing agents or various preservatives all have the purpose of killing or reducing the number of bacteria initially present in meat or at least inhibiting microbial growth.

Bacteria may be injured rather than killed by the above treatments, so recognition of sublethally injured (or damaged) microorganisms is important for accurate interpretation of microbiological data on meat and meat products. Sublethal injury induced by exposure to processing treatments often is observed as a loss of one or more of the abilities of the microorganisms to function normally under conditions that are satisfactory for cells that have not been exposed to treatments. Thus injury is characterized by decreased resistance to selective agents or by increased nutritional requirements (ICMSF1980).

Heat injury has been reported in many species of bacteria including *Streptococcus faecalis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli, Aerobacter aerogenes, Pseudomonas fluorescens, , Bacillus subtilis,* and *Clostridium botulinum* (Busta 1978). Reduced temperature above 0°C can injure *S. aureus, Streptococcus lactis,* and *Cl. perfringens*. Storage temperature below 0°C induces injury in numerous bacteria such as *Shigella sonnei, Staphylococcus faecalis, Pseudomonas fluorescens* (Busta and Smith 1976).

Injury is important to food quality and safety for several reasons. Some spoilage bacteria that were injured during heat treatment such as scalding can become non-culturable but viable. Results of the APC may, therefore, underestimate the level of bacterial contamination of the skin. Some pathogens may be injured during processing and not detected at post-processing microbiological examination at suboptimal temperatures. Later such cells may be repaired, grow, and produce toxin if storage temperature alters. They will subsequently pose a public health risk.

Effect of Processing Procedures in Pork Meat

Scalding and singeing result in a reduction in the bacterial levels on the pork carcass. The extent of this reduction for a specific bacterial species depend on its thermal sensitivity and on time/temperature combinations used in these operations (Sorqvist 1986 and 1988; Stern and Kazmi 1989). During normal scalding procedures (5-6 min, 60-65°C) a several log reduction of *Aeromonas* spp., *Campylobacter* spp., *L. monocytogenes, S. aureus*, and *Y. enterocolitica* is achieved (Dockerty *et al.* 1970; Sorqvist 1986; Nerbrink and Borch 1989). The heat resistance of *Salmonella* spp. is somewhat higher than of the other common pathogens (D'Aoust 1989). The thermal sensitivity and growth range for some pathogens in pork is presented in Table 2.

The slaughtering process of pigs is carried out at ambient temperature, while carcass temperature is high. Thus, there is a great potential for an intensive growth of bacteria during the processing period.

Table 2: Heat resistance and growth range for selected pathogenic bacteria from pigs and the environment. * D is decimal reduction time. All values are approximate since combination of environmental factors have a big influence on the growth and survival. Adapted from Borch *et al.* (1996b)

Bacteria	Heat resistance	Growth range	
		PH	t°C
Aeromonas spp.	2 min 55°C, 8 log reduction	4-10	4-12
Campylobacter spp.	D*60 = 12-24 s	4.9->8.0	30-47
L. monocytogenes	D60 = 40-190 s	4.5-9.6	1-45
Salmonella spp.	D60 = 33s-9.5 min	4.5-9.0	5-47
S. aureus	D55 = 66 s	4.0-9.8	6-44
Y.. enterocolitica	D60 = 27 s	4.2-9.0	-2-42

In addition, bacterial growth can occur during storage of pork products. *A. hydrophila, L. monocytogenes*, and *Y. enterocolitica* are reported to grow on meats stored at low temperatures, but the growth rate is dependent on environmental factors such as temperature, pH value, available moisture and oxygen. Growth may be limited by appropriate storage conditions that kill bacteria or inhibit their growth.

Spoilage of Meat

Definition

Fresh meat is an ideal medium for the support of microbial growth and will ultimately be rendered unacceptable to consumers as a consequence of such growth unless the meat is preserved (Gill 1986).

Jay (1992) stated that spoilage mechanisms cannot be properly understood without a sound definition of the term. Spoilage of meat and of foods in general,

may be defined as any change which renders the products unacceptable for human consumption and may result from a variety of causes (Hayes 1992). With regard to meat, the term "spoilage" is used to signify a condition which has rendered the products objectionable to consumers because of colour, odour, and flavour changes (Petersen, *et al.* 1991).

Spoilage is not a single, particular, objective state but, in fact, it describes the subjective evaluation of a multiplicity of conditions and, therefore, it is not always possible to usefully equate "spoilage" and "nonacceptability" as the question arises: "not acceptable" - to whom. Gill (1986), in his work on control of microbial spoilage in fresh meats, gave the following definition: "The term spoilage is used to signify any single symptom or group of symptoms of overt microbial activity, manifest by changes in meat odour, flavour, or appearance, without considering whether any particular consumers would find these changes objectionable, although some almost certainly would".

Types of spoilage

Meat spoilage may be caused by the following factors:

- physical damage including insect bites and injury due to bruising, pressure, heating, freezing, drying and radiation;
- The activities of indigenous enzymes in live tissues. If these enzymes are not destroyed they continue to function during processing and storage;
- Chemical changes not induced by microbial or naturally occurring enzymes. These changes usually involve oxygen and, except from microbiological spoilage, are the most common cause of spoilage;
- The activity of microorganisms, particularly bacteria, yeasts and moulds.

Spoilage caused by microorganisms is undoubtedly the most important of the above.

Meat spoilage, and food spoilage in general, is a complex event, in which a combination of microbial and biochemical activities may interact. The

microbiology of meat spoilage has over the years received considerable attention, and the characteristics of the typical microflora which develop on different types of meat products during the storage has been well documented (Mossel *et al.* 1995).

The most common manifestation of meat spoilage is the formation of odour as a result of microbial breakdown of low molecular weight components of meat. Hydrogen sulfide (H_2S) and other organic sulfides have strong and offensive odours and are organoleptically detectable in very low concentrations. Organisms capable of producing substantial quantities of these substances are therefore regarded as having a high spoilage potential. Detection of the presence of H_2S by a tissue tape moistened with zinc acetate, which reacts with hydrogen sulfide and changes colour, is used as a laboratory qualitative method for evaluation of meat spoilage. Production of H_2S in aerobically stored meats can be used as an indicator of high numbers of *Enterobacteriaceae*, and therefore potential hygiene problems. Apart from the sulfides, a number of other volatile compounds have been ascribed to the production of off-odours associated with spoilage. Many of these are produced either by deamination or by decarboxylation of amino acids and the odours are described as being fruity, cheesy, fishy, etc. Large numbers of volatile compounds can be produced as a result of the growth of pseudomonads, which are responsible for proteolysis, especially when their numbers are in excess of 10^9 per cm^2 (Dainty 1996). Compounds detected most consistently with stored meats include methanol, acetone, methyl ethyl ketone, dimethyl sulfide and dimethyl disulfide, ammonia and certain amines (Dainty *et al.* 1983). Two particular volatiles, acetoin and diacetyl, have been suggested as useful indicators of the microbial spoilage of pork stored in aerobic and anaerobic conditions (de Pablo *et al.* 1989; Ordonez *et al.* 1991).

Spoilage may also be manifested by a change in colour. Under certain conditions where hydrogen sulfide has been produced, it may combine with

myoglobin to form the pigment sulphmyoglobin which is seen as green discolouration of the meat. Change in colour of meats as a symptom of spoilage can also be produced by some bacterial species such as *Photobacterium* and *Pseudomonas* that cause phosphorescence. Certain microorganisms can produce pigments, which are visible on the meat surface. Examples of this are the red spot condition caused by *Serratia marcescens* and the blue colour caused by *Pseudomonas syncynea*. Other pigment producing microorganisms are *Micrococcus* (pink) and *Chromobacterium* (yellow) (Mountney and Gould 1988).

Microorganisms engaged in meat spoilage have the ability to form slime as a result of coalescence of large numbers of colonies. Surface slime is characterized by a shiny, viscous, moist covering on the surface of the meat. The numbers of bacterial cells required to form visible slime depend on the type of organisms involved. However, it is generally considered that a count of 10^7 to 10^8 CFU per cm^2 is required (Petersen *et al.* 1991). *Pseudomonas, Achromobacter, Streptococcus, Leuconostoc*, and *Micrococcus* are generally considered as slime producers, some lactobacilli can also produce slime (Mountney and Gould 1988). Off-odour and slime are first evident when the total counts have reached 10^6 CFU/cm^2, and 10^8 CFU/gr., respectively (Hayes 1992). Growth of the aerobic bacteria ceases with bacterial numbers in excess of $10^9/cm^2$, possible because the rate of diffusion of oxygen and nutrients into the bacterial slime is not sufficient to support further growth (Gill and Newton 1977).

Factors Affecting Microbial Spoilage

The meat substrate

Meat comprises various proportions of muscle, fat, and connective tissues. Fat and connective tissues are very different from muscle, and, under most circumstances, spoilage of muscle tissues is the critical factor determining the

storage life of meat (Gill 1986). The composition of muscle tissues changes after death with the development of *rigor mortis*. The quantities of the bulk materials, protein and fat, do not alter during rigor development. Neither are they substrates for microbial attack before the development of spoilage (Gill and Newton 1980; Dainty *et al.* 1983). Instead, bacteria grow on meat initially by metabolizing low molecular weight soluble components, mainly carbohydrates. The concentrations of several of these microbial nutrients alter considerably during the development of rigor, with the major changes resulting from the degradation of glycogen via glycolysis. Most of the glycogen is converted into lactic acid, the accumulation of which causes the muscle pH to fall. Glycolysis finally ceases when deamination of adenosine monophosphate has removed this substance, which is an essential cofactor for the activity of some glycolytic enzymes. There is usually some residual glycogen left in the post-rigor muscle, which normally has an ultimate pH between 5.5 and 5.5 (Gill and Newton 1977; Borch *et al.* 1996a). High pH meat spoils more rapidly than normal pH meat since amino acids are rapidly attacked (Borch *et al.* 1996a).

Bacterial Contamination

As the rate of bacterial growth on meats at a given temperature follows a prescribed pattern, the lower the initial number of bacteria present on the meat the longer it will take for the bacterial flora to reach spoilage level (Hayes 1992). Thus, with beef stored at 5°C, if the initial count exceeds 10^5CFU/cm^2 spoilage can be expected within 6 days whereas with a count of 10^3CFU/cm^2 spoilage would not occur until the 10th or 11th day. Meat processed under hygienic conditions is normally contaminated by an initial flora of 10^3 to 10^4 CFU/cm^2 of which less than 10%, and often only 1%, are capable of growth at chiller temperatures (Newton *et al.* 1978). Since the hide is probably the source of most microorganisms on dressed carcasses, animals brought to the abattoir should be freed of adhering dirt by washing before slaughter. The reduction in bacterial numbers on carcasses achieved by spray or other form of washing is typically between 50 and 90% (Crouse *et al.* 1988). Further reduction in the microbial

load can be achieved by spraying the dressed carcasses with hot water alone or by inclusion of decontaminants such as chlorine, lactic acid or other approved chemicals in the water (Smulders *et al.* 1986; Dickson and Anderson 1992) but discolouration and unpleasant odours of the treated meat have limited the practical application of these procedures. Implementation of Good Manufacturing Practice (GMP), Hazard Analysis Critical Control Point (HACCP), and strict sanitation standards in the abattoir will help to prevent contamination of product.

Bacterial growth on the surface or near the surface of the meat is most rapid. The flora is dominated by mesophilic, aerobic or facultatively anaerobic, Gram negative rods (Hayes 1992). Most of these organisms are enteric in origin and include genera such as *Escherichia, Aeromonas, Acinetobater, Proteus* and *Enterobacter*, together with other genera such as *Staphylococcus, Micrococcus*, and *Bacillus* (Hayes 1992; ICMSF 1998). *Acinetobacter* are strict aerobes and commonly form a significant portion of any aerobic spoilage flora on meat. Pure culture of some strains of *Acinetobacter* have been described as imparting fishy odour to meat, but any direct effects of these bacteria are usually minor and subordinate to the spoilage activities of pseudomonads (Gill 1986). However, when organisms of this group are the major components of a flora approaching its maximum density, it is possible that they enhance the spoilage activities of the pseudomonads and *Shewanella putrefaciens* by restricting the oxygen available to these latter organisms (Gill 1986). The group of *Enterobacteriaceae* includes a wide range of facultatively anaerobic organisms, including potential pathogens. *Enterobacteriaceae* preferentially utilize glucose and metabolism of this substance produces strong catabolite repression of the enzymes of amino acid degradation. Only when these carbohydrates are exhausted are amino acids attacked (Gill and Newton 1977). Organic sulfides can be produced from amino acids and many strains can release H_2S, although not in the quantities produced by *Shewanella putrefaciens* (McMeekin 1982). Organisms of this

group have a high spoilage potential and can be important in spoilage if conditions favour their growth.

Microorganisms of significance in the spoilage of fresh chilled meat are mainly psychrotrophs and, of these, pseudomonads are the leading spoilage bacteria (Gill and Newton 1977; Dainty and Mackey 1992; Dainty 1996; Neumeyer *et al.* 1997) Pseudomonads normally account for more than 50% of the spoilage flora with *P. fragi, P. lundensis* and *P. fluorescent* being the most important species (ICMSF 1998).

Like most of the other Gram-negative rod shaped bacteria, *Pseudomonas* spp. usually comprise only a small proportion of the initial microflora of fresh meat. They are, however, widely distributed in the environment and may contaminate meat from many sources. Food spoilage due to pseudomonads may occur in a number of ways. *Pseudomonas* spp. are able to utilize a wide range of substrates for growth. In the case of foods of animal origin such as fresh meat and meat products, the non-protein nitrogen fraction will be first metabolized. Subsequently, the production of lipases or proteases will liberate fatty and amino acids which after metabolism can result in formation of malodorous sulfides, esters, acids, etc. (McMeekin 1982). The unpleasant odour of such products is usually the first symptom of spoilage. At later stages, when bacterial numbers approach 10^9CFU/cm^2, the production of extracellular slime and the development of often pigmented growth becomes visible (Dainty and Mackey 1992; Dainty 1996; Huis in't Veld 1996). Growth of the aerobic flora ceases with bacterial numbers in excess of 10^9 CFU/cm^2, apparently because the rate of diffusion of oxygen into the bacterial slime is not sufficient to support further growth (Gill and Newton 1977).

A number of other Gram-negative rods may also grow rapidly at chill temperatures and spoil meat, such as *Aeromonas, Photobacterium,* and

Shewanella. Like *Pseudomonas*, these bacteria cause spoilage due to non-protein nitrogen fraction metabolism.

Some Gram-positive, spore-forming bacteria such as *Bacillus* and *Clostridium* can survive heat processing and are able to grow at chill temperatures. These organisms play a significant role in the spoilage of processed foods. *Bacillus* spp. are largely aerobic in nature. Perhaps best recognized is *B. cereus* which can grow at low temperatures (5°C or less), other *Bacillus* spp. can grow at temperatures of 0-2°C (Gill 1986). A bacterium of interest which can grow to high numbers on the surface of meat stored at chill temperature is *Brochotrix thermosphacta.* It is unusual in being Gram-positive in a largely Gram-negative flora and being able to grow well on fatty tissues as well as lean meat down to 5°C or below. Some of the off-odours which develop in the early stage of spoilage are often attributed to this organism (Skovgaard 1985).

Another Gram-positive bacterial group involved in meat spoilage are lactic acid bacteria. These anaerobic, but aerotolerant organisms usually dominate the flora of meat stored anaerobically and utilize glucose and arginine in meat for growth. They tend to grow slowly at refrigeration temperature and under aerobic conditions are generally out-competed by pseudomonads. Normally they are present in the initial microflora in low numbers and are rarely responsible for the spoilage of fresh proteinaceous food such as fresh meat (Huis in't Veld 1996). Lactic acid bacteria, however, have been identified as the major spoilage microorganisms of vacuum-packed meat and poultry (Dainty *et al.* 1983; Borch *et al.* 1996b). Typical lactic acid bacteria are identified as *Lactobacillus, Leuconostoc* and *Pediococcus* spp. Spoilage caused by lactic acid bacteria becomes apparent only after maximum numbers of bacteria have been attained, so adjusting conditions to favour development of a *Lactobacillus*-dominated flora of low spoilage potential greatly extends the shelf life of chilled meat (Gill and Newton 1978).

Some types of microorganisms have a greater impact on the organoleptic characteristic of a food than others due to the presence of different enzymes acting upon the food constituents. Examples of these types of bacteria may be *B. thermospacta* and *Carnobacterium* spp. which cause off-odour and *Lactobacillus* spp., *Leuconostoc* spp., and *C. divergens* which cause dicolouration in fresh meat (Borch and Molin 1989). In addition to the effects of certain levels and types of spoilage organisms, changes in quality characteristics will also vary depending on the products and conditions of storage such as temperature and gaseous atmosphere.

Interactions between Microorganisms

The contribution that any group of organisms makes to spoilage will depend, to some extent, on their relative numbers in the spoilage flora. In aerobic floras there appears to be no significant interaction between species until maximum numbers are approached (Gill 1986). At that time some species sequester the limited volume of available oxygen at the expense of competitors. In an anaerobic flora, competition for glucose can produce similar effects when maximum numbers are approached (Gill and Newton 1978). In addition, lactobacilli produce antimicrobial agents that inhibit competitors (Raccach and Baker 1978), but even in this case bacterial numbers must be relatively high before such effects become apparent. For most of the time they are growing, organisms are indifferent to the presence of competitors. Under these circumstances, dominance is primarily determined by the relative rates of growth of competing organisms, the fastest-growing species ultimately predominating. The relative rates of growth of various groups will be affected by the storage conditions. To prolong the shelf life of meat it is, therefore, desirable not only to retard the total growth of all bacteria, but to adjust conditions to produce a greater selective retardation of otherwise fast-growing organisms of high spoilage potential (Gill 1986).

Indicator Organisms

The presence of microorganisms in foods is not necessarily an indicator of hazards to the consumer or of inferior quality (ICMSF 1978). Meat and other perishable foods become potentially hazardous to the consumer only when the principles of sanitation and hygiene are violated. If food has been subjected to conditions that could allow entry and growth of infectious agents, it may become the vehicle for transmission of diseases such as salmonellosis, campylobacteriosis, and yersiniosis.

The routine examination of foods for a wide range of pathogenic bacteria is, in most laboratories, impossible either because they are inadequately equipped or because the sample size would be impractical to handle (Hayes 1992). Thus it has become normal practice to examine food products for bacteria whose presence indicates the presence of hazardous bacteria. These bacteria, termed "indicator organisms", are often regarded as being of great significance when assessing the microbiological safety and quality of foods. However, routine tests for selected pathogens and toxins, such as salmonellae and staphylococcal enterotoxins, are necessary whenever epidemiological or other evidence suggests the occurrence of a specific agent in a particular type of food. For some food-borne diseases, such as infectious hepatitis, reliable methods for detecting the causative agent are not available to the microbiologist. For other food-borne infections, such as shigellosis, the methods may be unreliable, especially when the pathogens are sparsely or unevenly distributed in foods heavily contaminated with other organisms (ICMSF 1978). Even when sensitive methods are available, some laboratories may not have the technical capabilities required, or the tests are too expensive and unpractical to perform. Such difficulties have led to the widespread use of groups (or species) which are more readily enumerated, and whose presence in foods indicates exposure to conditions that might introduce hazardous organisms and allow growth and

multiplication of pathogenic species. These indicators are of value in assessing both the microbiological quality and the safety of foods.

Indicators of Microbiological Quality

The shelf life of a perishable product, and of meat in particular, is often determined by the number and types of microorganisms initially present. As a general rule, a food containing a large population of spoilage organisms will have a shorter shelf life than the same food containing fewer numbers of the same spoilage organisms.

The number of spoilage organisms reflects the microbiological quality, or wholesomeness, of a product as well as the effectiveness of measures used to control or eliminate such organisms (Smoot and Pierson 1997). Most indicator organisms are primary spoilage microorganisms. Counts of viable bacteria are commonly based on the number of colonies that develop in nutrient agar plates which have been inoculated with known amounts of diluted food and then incubated under prescribed conditions. Such counts are sometimes called the Total Plate Count, when in fact only those bacteria which will grow under the particular conditions selected are enumerated. By changing the composition of the culture medium, the gaseous atmosphere, and the time and temperature of incubation, a wide variety of conditions can be obtained. For example, incubation at temperatures between 0° and 7°C favours the growth of psychrotrophic bacteria. Many of these organisms cannot grow at 30-37°C, which are the temperatures most suitable for incubation of mesophilic organisms, including both the pathogenic and saprophytic species. Temperatures between 20 and 30°C are suitable for growth of most of the psychrotrophic and mesophilic bacteria and therefore are commonly used for evaluation of sanitary conditions in fresh meat processing plants (Hayes 1992; Smoot and Pierson 1997). Incubation at still higher temperatures (50-60°C) allows the development of thermophilic organisms but inhibits the mesophilic and psychrotrophic species. Other groups can be selected for counting by

adding selective inhibitors, such as sodium chloride, surface-active agents, or dyes to the agar medium, or by modifying the composition of the atmosphere in the incubator, such as excluding oxygen. Each type of viable count is potentially useful for special purposes, but the Aerobic Plate Count (APC) is most commonly employed to indicate the sanitary quality of food.

When evaluating results of APCs for a particular food, it is important to remember that:

- APC's only measure live cells, and therefore it would be of value, for example, to determine the quality of raw materials used for a heat-processed food;
- APC's are of little value in assessing organoleptic quality, since high microbial counts are generally required prior to organoleptic quality loss;
- Since different bacteria vary in their biochemical activities, quality loss may also occur at low total counts depending on the predominant organisms present (Smoot and Pierson 1997).

The use of APCs as an index of sanitary quality in meat will be discussed in more detail in the next paragraph.

In addition to APC's, anaerobes can be used as indicators of the microbiological quality of foods. The use of this method is however limited because it is much more complicated to incubate bacteria under anaerobic than under aerobic conditions (ICMSF 1978). Some systems have been developed to facilitate and to improve anaerobic counting by the use of anaerobic work-chambers. These chambers are made of clear plastic, pre-reduced agar plates, and deep agar in impermeable plastic bags. Anaerobic procedures usually measure not only obligate anaerobes, but also facultatively anaerobic organisms among the *Enterobacteriaceae*, faecal streptococci, and staphylococci, unless selective media are used (ICMSF 1978).

Indicators of Food-borne Pathogens and Toxins

Microbiological criteria for food safety may be based on tests for indicator organisms which suggest the possibility of the presence of a microbial hazards. Presence of *E. coli* in foods, for example, indicates possible faecal contamination of water or meat during operations and, therefore, the potential presence of enteric pathogens. Direct tests for pathogenic microorganisms and their toxins, excepting *Salmonella* spp. and *S. aureus*, are not routinely applied to foods for quality purposes (Smoot and Pierson 1997).

Some of the more common indicator microorganisms used for assuring food safety are described below.

Enteric Indicator Bacteria

Escherichia coli and faecal coliforms. The term coliform includes *E. coli* and several species from other genera of the *Enterobacteriaceae*. Practically speaking, coliform are the microorganisms that are detected by coliform tests (ICMSF 1978). The faecal coliforms comprise a group of organisms selected by incubating inocula derived from a coliform-enrichment broth at temperature higher than normal (44 - 45.5°C). Such enrichment culture usually contain a high proportion of *E. coli* type I and II which are useful for indicating a probable faecal source of contamination (Mossel *et al.* 1963).

Faecal coliforms, including *E. coli*, are easily destroyed by heat and may die during freezing and frozen storage of foods (Smoot and Pierson 1997). Microbiological criteria involving *E.coli* are useful in those cases where it is desirable to determine if faecal contamination may have occurred. The native habitat for *E. coli* is the enteric tract of humans and animals, thus its presence in foods generally indicates direct or indirect pollution of faecal origin (ICMSF 1978). Because *E. coli* readily proliferate in meat the numbers can be influenced

by many factors such as natural contamination, actual growth, poorly cleaned equipment, and contamination from personnel. Thus, all that can be concluded is that faecal contamination, direct or indirect, occurred at some stage and that the safety of the food is in question (Hayes 1992). The failure to detect *E. coli* in a food does not however guarantee the absence of enteric pathogens (Silliker and Gabis 1976). In many raw foods of animal origin, such as fresh meat and dairy products, small numbers of *E. coli* can be expected because of the close association of these bacteria with the animal environment and the likelihood of contamination of carcasses from faecal material, hides, and skin during slaughter and dressing.

Other *Enterobacteriaceae* comprise many genera including those characterised by lactose fermentation (e.g. *Escherichia* and *Enterobacter*) and those not fermenting lactose (e.g. the non-enteropathogenic *Proteus* and *Serratia* as well as *Salmonella* and *Shigella*). Mossel *et al.* (1963) claimed that a close correlation exists between counts obtained for total numbers of the family *Enterobacteriaceae* and the extent of faecal pollution, particularly in relation to contamination by *Salmonella*. Because many discrepancies were found when the more conventional tests for coliforms were employed, it was suggested that a test for total *Enterobacteriaceae* would be more reliable.

The enterococci comprise two *Streptococcus* species found in human and animal intestines, namely *S. faecalis* and *S. faecium.* Sources of enterococci include faecal material from both warm-blooded and cold-blooded animals and some plants. Enterococci differ from coliforms in that they are salt tolerant (grow in presence of 6.5% NaCl) and are relatively resistant to environmental changes (Jay 1992). In food, it has often been argued that enterococci are better indicators of the sanitary quality than *E. coli* (ICMSF 1978). They are generally more resilient than coliforms, particularly in frozen and dried foods (Hayes 1992). Enterococci can establish and persist in the food processing environment for long periods of time.

Other Indicators

Staphylococci. The presence of *staphylococcus aureus* in food is usually taken to indicate contamination from the skin, mouth or nose of workers handling food, but inadequately cleaned machinery, tools, and equipment as well as animal carcasses may also be sources of contamination. The presence of large numbers of staphylococci is, in general, a good indication that sanitation and temperature control of meat products have been inadequate (ICMSF 1978). Because only 50% of *S. aureus* strains are enterotoxin producer (Hayes 1992) it is important that tests for the presence of the enterotoxin in food be carried out. The result of tests for toxin is more conclusive when investigating food-poisoning outbreaks.

Mesophilic spore-forming bacteria. The presence of mesophilic bacilli in canned foods indicates that either the container was not hermetically sealed or that heat-processing was insufficient to destroy the spores. When spore-forming bacteria are present in chilled meat in unusually high numbers, or comprise an unusually large proportion of the bacterial population, there is a risk that they may include *C. perfringens, C. botulinum,* or *B. cereus.* These organisms could present a hazard in both fresh and preserved food.

The Aerobic Plate Count as an Index of Food Quality

One of the most common and maybe the best microbiological tests used for estimating the microbial population on raw meat and carcasses is the aerobic plate count (APC) which is also known as the standard plate count (SPC), total viable count (TVC), total plate count (TPC), or aerobic viable count (AVC). In this work the term "aerobic plate count" (APC) will be used.

In the APC method, suitable dilutions of meat samples, taken by appropriate sampling methods, are placed on or in agar-based media containing complex

nutrients which support the growth of as wide a range of microorganisms as possible. The pH of the medium is usually adjusted to 7.0-7.4 so that bacteria, rather than yeasts or moulds, are recovered (Hayes 1992). The medium used for the APC should not be altered, however, the temperature of incubation should suit the product under examination and the conditions under which it is stored (Avery 1991). The individual bacterial cells transferred to the plate in the diluent divide during incubation. Thus an estimate of the total number of viable cells in the dilution can be calculated by counting the total number of bacterial colonies following incubation.

Depending on optimum growth temperatures, bacteria are divided into thermophiles, mesophiles, psychrotrophs, and psychrophiles. Commonly used incubation temperatures are 55°C for thermophiles, 35-37 °C for mesophiles, 20-30 °C for many spoilage bacteria, which largely consist of psychrotrophs and some mesophiles. Lower temperatures (5°C) are used to incubate psychrophiles. The definition of psychrotrophic in relation to growth temperatures is still disputed. Eddy (1960) defines psychrotrophs as organisms that can grow at low temperatures, such as 0°C, although their optimum growth temperatures may be 10 - 30°C or higher. In contrast, Grau (1986) defines psychrotrophs as having an optimum temperature of 20 °C or more with many unable to grow at 35 °C and higher. A draft proposal for the determining of APC's at 30 °C was drawn up by the International Standardisation Organisation (ISO) in 1964. This draft became a standard and has since been adapted to suit more modern equipment and views on plating techniques (Barraud *et al.* 1967; ISO2293 1988). Presently, the spread-plate technique, using 0.1ml of a suitable decimal dilution on pre-dried agar plates, incubated at 30 °C for 3 days, is regarded as the standard technique for determining APC's on meat and meat products (Nottingham *et al.* 1975; Ingram and Roberts 1976; ISO2293 1988).

The microflora of meat and meat products changes with time and conditions of storage. Since carcasses are initially contaminated with mesophiles at the time

of slaughter, the mesophilic count is sometimes used when examining foods from animal sources. A high count of mesophiles on carcasses immediately after slaughter is evidence of poor hygiene. When meat is stored at chill temperatures, the flora changes from a predominant mesophilic to a flora composed mainly of psychrotrophs and psychrophiles. At this stage, bacterial counts at 20-30 °C, at which mesophiles and psychrotrophs both grow, will be the highest (Avery 1991). The international reference method for the APC from meat and meat products recommends an incubation temperature of 30 °C (ISO2293 1988). The ISO recommendation should be used only where applicable since the temperature of incubation should suit the products under examination and the conditions under which they are stored. Nortje *et al.* (1990b), in their work on the influence of incubation temperatures on bacterial counts in a meat production system, showed that counts obtained at 30 °C incubation yielded higher numbers of bacteria than those acquired at 25 °C. Other workers found that the total counts at 20 °C are usually similar to those at 30 °C or other temperatures between 20 °C and 30 °C (Ingram and Roberts 1976). To predict the size of the spoilage population, a count at 25 °C for 2-3 days can be a time saving and fairly accurate tool. The conditions regarding habitat, environmental temperatures, and vectors should be considered in the interpretation of results (Nortje *et al.* 1990b).

The APC can be a useful indicator of conditions of sanitation, holding temperatures, and of time elapsed during food production and transportation. However, there are limitations to the value of any counts. For example, there are many foods, such as fermented sausages and cheeses, wherein the growth of bacteria is necessary for the development of characteristic odours, flavours, and textures. In these products, a high total count of the desired microorganisms is advantageous; conversely, the absence of large numbers of microorganisms is associated with the absence of characteristic organoleptic attributes (Silliker 1963). For perishable foods, including fresh meat, spoilage is developed as a natural consequence of not being consumed and is always attended by

extensive microbial growth. Thus, the total count on these products represents composite reflections of raw materials, processing procedures, and handling conditions. The point at which the sample is taken determines whether its total count can reasonably be used as an index of sanitary quality (Silliker 1963).

Aerobic plate counts are, at best, an estimate of the number of microorganisms present in a food. Only the organisms capable of growth within the selected parameters (temperatures, time, pH etc.) will proliferate and be counted. It is difficult to select parameters that will support the growth of all types of microorganisms present in a given product. For each meat product tested, the most ideal parameters must be identified which will give the best estimate of the total microbial load. Consistently accurate and meaningful results will be obtained only if the same pre-set standard methods are used each time that particular meat product is examined (Avery 1991).

Many plating techniques are employed for enumerating total numbers of viable bacteria and the results must be expressed as colony forming units (CFU) per gram for meat and meat products or per cm^2 for contact surfaces. Enumeration of colonies is traditionally performed manually using an illuminated colony counter with the operator counting each individual colony. This can be a tedious operation and, unless a suitable number of colonies has developed in the growth medium (ideally below 300, although for statistical reasons a minimum of 30 colonies is required), it can also be inaccurate (Hayes 1992). More details of counting techniques and adjustment for computing and recording of APC's can be found in the ICMSF recommendations (ICMSF 1978).

The following is a brief description of the most common methods employed for APC's:

The Spread Plate Method

In this method, the plate agar is pre-poured and allowed to solidify in the Petri dishes; 0.1 ml aliquots of the dilutions are spread evenly over the whole surface of the medium using a sterile glass spreader. The plates are incubated at the required temperature for a period of time depending on the incubation conditions as shown below:

- thermophiles: 50-55°C for 24 hours
- mesophiles: 37-40 °C for 24 hours
- psychrotrophs: 15-30 °C for 24-96 hours
- psychrophiles: <15 °C for 48-168 hours
- sanitation: 20-37 °C for 24-48 hours

Incubation parameters as recommended by ISO for the APC for hygienic assessment are 30 °C for 72 hours.

After incubation, plates containing 30-300 colonies should be counted and the number of viable cells per gram or per cm^2 of food calculated. Counting technique, formulas for expressing CFU's per gram or per cm^2, and principles of rounding numbers are described in the ICMSF manual mentioned above. This method is the most common for APC's.

The Pour Plate Method

In this method, duplicate sets of Petri dishes are inoculated with 0.1 ml volumes of appropriate dilutions of the food being examined. Melted plate agar is then poured over the plate. The dishes are left until the agar becomes solid before incubation at the temperature and time as described in the previous method. Counts are calculated in the same way.

The Drop Plate Method

Solidified medium is again used in this method. Specially calibrated pipettes delivering 0.02 ml per drop are used and five separate drops are delivered onto

the surface of the agar, the drops being dried before incubation. Dilutions giving less than 20 colonies per drop should be counted.

The Agar Droplet Method

This is a miniaturised method for determination of the aerobic count. Compared with the above methods, the agar droplet method is more rapid and uses far smaller quantities of materials. In this technique the dilutions are prepared in molten agar and colonies develop in the solidified 0.1 ml droplets during incubation. A viewer screen which magnifies the droplets approximately ten-fold is used for counting of colonies of the most suitable dilution. It is suitable for use with hygiene and meat swabs and meat samples. However, since this method involves mixing the inoculum with molten agar, which may damage the bacteria through heat shock, it is not recommended for counts from frozen and chilled meat (Avery 1992).

The Spiral Plate Method

The spiral plate method is a semi-automatic technique for enumeration of aerobic counts. This is an internationally recognized method, in which a machine continuously plates a known volume of sample on the surface of a rotating agar plate. The amount of sample deposited decreases as the dispensing stylus is moved from the center to the perimeter of the rotating plate. Counting can be performed manually using a counting grid but laser-based automatic colony counters have been developed specifically for use with this technique.

All the methods described above are classical procedures for counting microorganisms from meat samples. They normally involve: homogenisation of samples, dilution, plating onto appropriate media, incubation at selected temperatures for a specific period of time (24 hours or more), and counting of CFU's. This traditional approach to microbiological analysis in the meat industry is the basis of the retrospective use of the results in order to improve the microbiological quality of future lots of meat products. However, the modern

approach of food quality assurance (HACCP and similar systems) demand procedures by which the microbiological status of raw materials or end products may be rapidly assessed so as to allow the employment of active measures before marketing of meat products.

Several technological advances enable the automation of classic procedures. The gravimetric diluter (automatic dispenser) considerably reduces errors arising from manual weighing and dispensing. Several ready-to-use systems such as Redigel and Petrifilm™ can be used for reducing materials: (Beuchat *et al.* 1998). Petrifilm plates can be used for counting mesophiles, psychrotrophs, coliforms, *E. coli*, and yeasts and moulds (Otero *et al.* 1998). In the Hydrophobic Grid Membrane Filter (HGMF) procedure, a special filter, a most probable number-like system of enumeration of microorganisms and specific media are combined. HGMF-based techniques are available for counting viable organisms, coliforms, faecal coliforms, *E. coli*, enterococci, yeasts and moulds, lactic acid bacteria, *Salmonella*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Cl. perfringens*, as well as several spoilage bacteria (Otero *et al.* 1998). Rapid methods based on the inoculation of liquid media and the quantification of microbial numbers after modifications in optical or electrical properties of the media and very rapid methods such as Direct Epifluorescent Filter Technique (DEFT), Flow Cytometry (FCM), and bioluminescence methods have been developed and are available for employment (Otero *et al.* 1998).

HACCP Principles in the Meat Industry

HACCP is an abbreviation of Hazard Analysis Critical Control Point - a system that identifies specific hazards and establishes preventative measures for their control.

The term "hazard" in the HACCP concept includes any factor present in a food that can cause harm to the consumer, either through injury or illness. Hazards may be biological, chemical or physical. The risk of a hazard is the probability of occurrence of that specific hazard.

The HACCP concept argues that safety and quality of a product can be assured only when a systematic approach to the identification, assessment and control of hazards is undertaken prior to and during production, distribution and sale. It is an approach which shifts the emphasis from identifying failed product units through end-product testing to avoidance of product deficiencies through risk assessment, monitoring, corrective action and validation at all stages up to the sale of food products.

The HACCP concept has been applied in the food industry for some time but has recently undergone some major changes (Mortimore and Wallace 1995). These developments mean that the HACCP techniques have progressed considerably since their introduction, and have meant that some companies have found their systems to be rapidly out of date.

HACCP was originally developed as a microbiological safety system in the early days of the US manned space program, as it is of vital importance to ensure the safety of food for the astronauts. The original system was pioneered in the 1960s by the Pillsbury Company, working alongside the National Aeronautics and Space Administration (NASA) and the US Army Research and Development Laboratories at Natick (WHO; 1980; Adams 1990; Sperber 1991). It was based on the engineering system Failure, Mode, and Effect Analysis (FMEA), which looks at what could potentially go wrong at each stage in an operation along with possible causes and the likely effects, before developing effective control measures (WHO 1980). Like FMEA, HACCP looks for hazards, or what could go wrong, but in sense of the safety of products. Control and management systems are then implemented to ensure adherence with established control measures.

HACCP Principles

The HACCP System is based on seven principles which outline how to establish, implement and maintain a HACCP Plan for the operation under study (WHO 1980; Adams 1990; Sperber 1991; Mortimore and Wallace 1995). These are:

- *Principle 1*: Hazard analysis and assessment of the magnitude of the hazards and the risks associated with each operational step of production of raw materials, including processing, manufacturing, distribution, marketing, preparation, and consumption of foods; and identification of the preventative measure that can be taken at each step to eliminate or reduce the risk to acceptable levels.
- *Principle 2*: Determination of critical control points (CCP's) at which identified hazards can be controlled.
- *Principle 3*: Establishment of critical limits or criteria that indicate whether an operation is under control at a particular critical control point.
- *Principle 4*: Implementation of procedures to monitor each CCP to check that it is under control.
- *Principle 5*: Implementation of appropriate and immediate corrective actions when the monitoring results indicate that a particular CCP is not under control.
- *Principle 6*: Establishment of effective record-keeping procedures that document the HACCP System.
- *Principle 7*: Establishment of procedures for verification that HACCP is working properly.

Hazards

Identification and classification of hazards are the basis of every HACCP System. Chemical hazards in the form of residues of pollutants and agricultural

remedies are often looked at by the consumer as the most important. In reality, these pose a relatively low health risk at the levels likely to be found in food. Biological hazards, on the other hand, usually represent the greatest immediate danger to the consumer, through the potential to cause food poisoning (Mortimore and Wallace 1995). Most food processing operations will be at risk from one or more biological hazards, either from raw materials or during the process, and the HACCP plan will be designed to control these. Biological hazards can be either macrobiological or microbiological.

1. **Macrobiological hazards**, such as flies or insects, while unpleasant if found, rarely pose a risk to product safety. However, they may be an indirect risk through harbouring pathogenic microorganisms and introducing these to the product. For example, an insect harbouring *Salmonella* would pose a major risk to consumers if it gained access to a fresh, ready-to-eat product (Mortimore and Wallace 1995).

2. **Microbiological hazards** are comprised of microorganisms such as pathogenic bacteria, yeasts and moulds. Pathogenic or food-poisoning microorganisms exert their effect either directly or indirectly on humans. The direct effects result from an infection or invasion of body tissues and are caused by the organism itself, e.g. bacteria, viruses and protozoa. Indirect effects are caused by the formation of toxins (or poisons) that are usually preformed in the food, e.g. bacteria and fungi. Summary information of most pathogenic bacteria or bacterial groups that pose significant public health hazards and consumption of foods of animal origin are involved, is presented in the paragraph "Pathogenic Bacteria" of this Chapter (pp 17-24). In addition, some viruses and parasites which also play significant roles in foodborne illness should be taken into consideration. The greatest number of outbreaks of food-borne viral infection are due to Hepatitis A and Small Round Structured Viruses (SRSV) such as Norwalk virus. Shellfish are the most common source of these viruses because they concentrate the virus from contaminated water. The larvae of some pathogenic

roundworms, tapeworms and flukes can infect humans via the consumption of infected meat and fish. Examples include *Taenia saginata, Trichinella spiralis*, and *Chlonorchis sinensis* (trematode or fluke from Asian fish). Protozoa such as *Toxoplasma gondii* produce encysted forms which can infect humans from ingestion of undercooked meat.

3. **Chemical contamination** of foodstuffs can happen at any stage of their production, from growing of the raw materials through to immediately prior to consumption of the finished product. The effect of chemical contamination on the consumer can be long-term through accumulation of chemicals which can build up in the body over many years, or it can be short-term such as the effect of allergenic substances in foods. Chemicals that are of major public health concern include cleaning chemicals, allergens, toxic metals, pesticides, veterinary remedies, chemical food additives, and some chemicals of industrial and agricultural application.

4. **Physical hazards** may enter a food product the some way as biological and chemical ones and include glass, metal, stones, sand, or any other material foreign to the food product.

Preventative Measures

When all potential hazards have been identified and analyzed, a list of the associated preventative measures must be made. The measures are the control mechanisms for each hazard and are normally defined as those factors which are required to eliminate or reduce the occurrence of hazards to an acceptable level (Mortimore and Wallace 1995; NACMCF 1998). For example, intact packaging, pest control, secure buildings, and logical process flow are preventative measures of cross-contamination by vegetative pathogens, e.g. *Salmonella spp. L. monocytogenes , V. parahaemolyticus,* and *Yersina enterocolitica*. More than one preventative measure may be required to control a

particular hazard which occurs at different stages of the process. On the other hand, more than one hazard might be effectively controlled by one preventative measure.

Critical Control Point (CCP)

A CCP is a step or a procedure at which a preventative or control measure can be exercised and a food safety hazard prevented or reduced to an acceptable level (Roy 1993). Critical Control Points are sometimes referred to as CCP1 or CCP2:

- CCP1 that will assure control of a hazard (elimination of a hazard)
- CCP2 that will minimize but cannot assure the control of a hazard

In some references one may also see CCPe, CCPp and CCPr (Roy 1993):

- CCPe that will eliminate hazards and no further problem exists up to this point of processing
- CCPp is an operation at which hazards are prevented but not necessarily eliminated. For example, freezing prevents bacterial growth but does not eliminate bacterial cells.
- CCPr is an operation at which a hazard is significantly reduced, minimized or delayed but not eliminated or even prevented. Handling of foods with sanitized utensils rather than with hands is an example of this sort of CCP.

Identifying CCP's is a very important step in a HACCP plan, however, it is also the least understood aspect of the HACCP approach. The location of CCP's using judgement alone may lead to more points being identified as CCP's than are really necessary. On the other hand, too few CCP's would be even more disastrous and could cause the sale of unsafe food. It is important that control points are located where they are essential for food safety and so care should be employed to ensure that the CCP's are correctly identified. To assist in finding where the correct CCP's should be, a tool known as the CCP tree is available. Some versions of the CCP decision tree have been published by

NACMCF (1992) and Codex Alimentarius (1993). A simplified version of the CCP tree is shown in Figure 2.

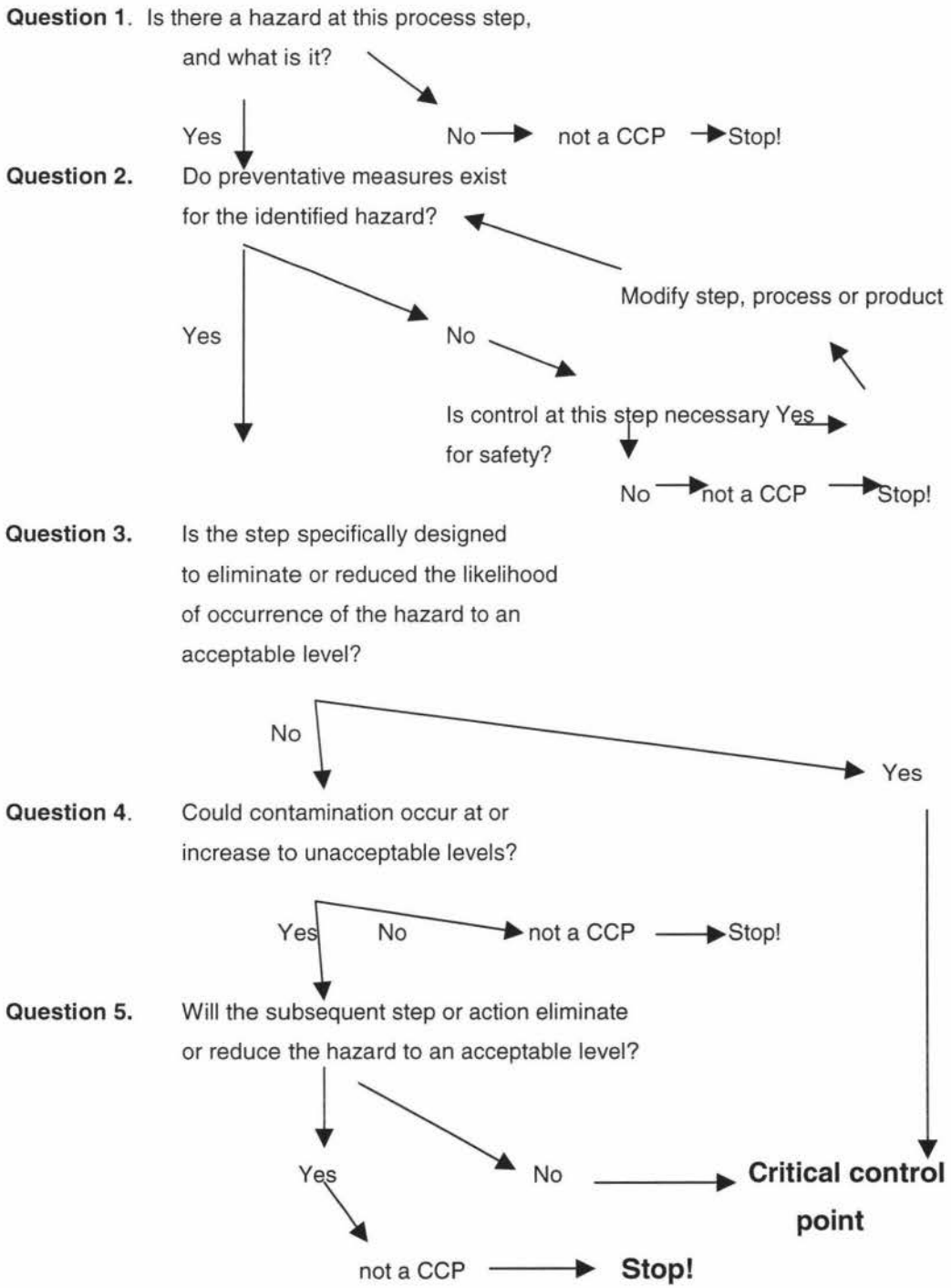

Figure 2: The CCP Decision Tree

Determination of a CCP is a complex issue and the result depends on a number of factors. There may be different answers to the same question depending on the actual situation. For example, to question 1: does a hazard exist? and what is it?, the answer may be "yes" if a responder requires a nil tolerance while another may say "no" having in mind that there could be some acceptable level of a hazard. Another example relates to question 3 "is this step specifically designed to eliminate or reduce a hazard to an acceptable level?". The conclusion may depend on one's view of "specifically designed". Many steps in the processing of meat can be controlled to minimize contamination (therefore, the answer can be "yes") even though they may not be specifically designed to do so (Tomkin 1994). Since these steps are not specifically designed to eliminate or reduce contamination with enteric pathogens, the answer to the question is "no". Thus, the steps in slaughtering which are not specifically designed to eliminate or reduce contamination are not CCP's.

Perhaps this was the intended result of those who designed the decision tree. On the other hand, since these steps are not CCP's they may receive less attention and, thus, less effort to control the level of contamination (Tomkin 1994). We can see a similar problem in application of hot water washing during dehairing of pig carcasses.

Critical Limits

Setting up critical limits is another difficult issue in the preparation of an HACCP plan. Depending on the particular control criteria, the CCP may have just one Critical Limit, or there may be an upper and lower Critical Limit (NACMCF 1992 and 1998; Mortimore and Wallace 1995). Since the Critical Limit defines the boundaries between safe and unsafe product, it is vital that they are set at the correct level for each criterion. Those who are responsible for design and implementation of HACCP systems must have an in-depth knowledge of the hazards and control mechanisms of the process, however, information and

advice on the matters can be obtained from different sources such as published data, expert advice, experimental data, and mathematical modeling.

Monitoring

Monitoring is a very important part of the HACCP System, ensuring that a product is manufactured safely at all times. There are two basic types of monitoring procedures (Mortimore and Wallace 1995):

- On-line systems, where the critical factors are measured during the process. These may be continuous systems where critical data are continuously recorded, or discontinuous systems where observations are made at specified time intervals during the process.
- Off-line systems, where sample are taken for measurement elsewhere of the critical factors. Off-line monitoring is normally discontinuous and has the disadvantage that the sample taken may not be fully representative of the whole batch.

Ideally, monitoring procedures should provide information in time for corrective measures to be taken to regain control of the process before there is a need to reject the product (Roy 1993). The data derived from monitoring must be evaluated by a responsible individual who has the authority to take corrective actions. If the monitoring is not continuous then the frequency of the monitoring must be sufficient to guarantee that the CCP is under control.

Specific corrective actions for an HACCP plan should be worked out in advance in order to minimize any confusion or disagreement which might otherwise occur if and when action needs to be taken. A corrective action may be one which adjusts the process to maintain and prevent a deviation at the CCP or it may be one which should be taken after some deviation has occurred to bring the process back under control.

Record Keeping

Efficient and accurate record keeping is essential to the successful application of an HACCP System. Records must be kept to demonstrate that the HACCP system is operating and that appropriate corrective actions have been taken in connection with any deviation from the critical limits. This will demonstrate safe product manufacture. Examples of HACCP records are associated with: ingredients, product safety and quality, processing, packaging, storage and distribution, deviation file, modification to the HACCP system etc.

It is important to check that the HACCP plan, which consists of a HACCP control chart and process flow diagram with all CCP's being highlighted, is correct and valid. This should be carried out soon after the plan is completed so that implementation can follow without delay. It may be appropriate to use experts to cross-check the design of the plan to ensure that no issue has been missed (NACMCF 1992 and 1998; Mortimore and Wallace 1995).

Application of HACCP Principles in Determination of Microbial Quality Critical Control Points in Pig Processing

Processing of pigs includes a number of operational steps at which bacterial contamination can occur. However, it does not contain any point at which hazards (both pathogenic and spoilage microorganisms) can be completely eliminated (Borch *et al.* 1996b). The major points of contamination during manufacturing of pork are related to the animals and to the environment.

The effective design and implementation of HACCP systems for fresh meat depend on identification of process steps that potentially contribute most of the bacterial contamination on the carcass, rather than designating as CCP's all those process steps that contribute only minor contamination (Biss and Hathaway 1995). The identification of hazards in pig processing typically

involves a listing of pathogenic and spoilage bacteria associated with pork carcasses from the moment animals enter the slaughter chain to the chilling of carcasses in the chilling rooms. During the manufacturing process, the microflora on the carcasses is affected by the various processing stages with respect to bacterial contamination and composition (Gerats *et al.* 1981). In order to achieve a good hygienic quality of pork with the HACCP approach, it is important to evaluate how the spoilage flora (hazards) is affected by the processing stages.

Before evisceration, pig carcasses are usually subjected to the operations of scalding, dehairing, singeing and polishing. Bacteria on the skin of carcasses are largely destroyed during scalding, but the carcasses are recontaminated with both pathogenic and spoilage bacteria during the subsequent dehairing operation (Gill and Bryant 1993; Gill and Jones 1997). A substantial proportion of the bacteria that survive the singeing operation will be redistributed over the carcass during polishing. Thus, the dehairing operation could be considered a CCP for both safety and quality aspects of pork meat. However, although recontamination at the dehairing stage is unavoidable, there is a need to modify this technique in order to minimise such contamination to an acceptable level. Sources and factors associated with subsequent recontamination of pork carcasses with pathogenic and environmental spoilage bacteria have been presented in the paragraph "Bacterial contamination of pig carcasses".

CHAPTER III

DETERMINATION OF CRITICAL CONTROL POINTS ON A PIG SLAUGHTER LINE

Introduction

Lakeview Abattoir is situated in a suburb of Levin town, 50 km south-west of Palmerston North. The plant was built in 1985 and was originally owned by a family group. Two years ago, in 1996, it was bought out by one family member. At present, the plant employs 200 people including administrative staff.

The plant is operated in two shifts: morning and afternoon with a maximum capacity of processing 160 cattle, 500 pigs and 1000 sheep per day. There are currently two continuous semi-automatic slaughter chains in use. One chain is designed specifically for beef, the other chain is used for processing both sheep and pigs. Equipment in the plant is modern and has not been changed significantly since the abattoir was established.

The number of animals slaughtered per shift is determined on a daily basis. The animals are brought to the works by trucks on the day of slaughter. Chilled and frozen meat produced at the plant is distributed locally to supermarkets and to retail butchers shops. In addition to meat, which is the main final product, the plant also produces some by-products such as casings and tripe for local consumption and export.

The plant is designed for a continuos product flow, and for edible and inedible product being handled and processed in separate areas. This facilitates the prevention of contamination of edible products by highly contaminated materials

such as contents of the gastro-intestinal and uro-genital tracts. The company also provides its working staff with sanitary facilities, including wash rooms, changing areas and showers. All sanitation facilities are designed and operated according to special regulatory requirements.

At present, edible departments of the plant are required to be cleaned and sanitised at the end of every working day by hosing and scrubbing with cold water and sanitisers, followed by a final hot hosing with 82°C water. A company supervisor is responsible for checking cleaning and sanitation at the end of each working day. He also has a duty to recheck all areas in the edible departments again the following day before work starts, to make sure that any dirty or inadequately cleaned areas are re-cleaned. The company has an ongoing hygiene improvement policy.

Ministry of Agriculture and Forestry (MAF) carries out periodical on-site checks. Any hygiene or food safety problems identified at the time of an audit are given a timeframe for correction. MAF also carries out a TB and residue surveillance programme at the plant by having samples taken and sent to laboratories for testing.

Materials and Methods

Study Design

The study was carried out at the Lakeview pig abattoir during the period April to July 1998. After the first introductory visit to the study site, subsequent visits were made for observation of the plant in general, and the pig processing chain in particular. During the observation time, interviews with the plant manager and meat inspection staff were carried out in order to study the production capacity, slaughter technology, carcass inspection procedures, sanitation and hygienic

monitoring plans currently employed at the plant. As a result of these observations, a flowchart of the pig processing was drawn up and verified by the supervisor and the supervising meat inspector. By reference to the literature and analysis of the manufacturing practices in the plant, a number of processing stages were identified as points where potential bacterial contamination or recontamination were most likely to occur. These points initially included dehairing, polishing and scraping, evisceration, and inspection (see Figure 3). Later, the hot scalding water was also included in the sampling plan in order to assess its level of bacterial contamination and to find out whether there was a significant change in bacterial contamination of the water with respect to its duration of use. The selected operations have the potential to cause microbial contamination of the surface of pork carcasses. Technical description of pig processing, potential microbial contamination and spread of both pathogenic and spoilage bacteria at each operation have been discussed in Chapter II.

The terms "critical control point" and "hazard" will be referred to as CCP and hazards for the microbial quality of pork carcasses, rather than safety, although it should be kept in mind that sometimes operations that are declared critical control points in the HACCP system may also be quality control points. Therefore control measures may simultaneously address both the safety and quality of the pork meat. However, it is possible that some operations have important effects on only safety or storage stability, or that controlling actions affect only one or other aspect of product hygiene (Gill and Jones 1997). For example, using the flowchart shown in Figure 3 and the HACCP concept, to determine CCP's for controlling the risk of some pathogenic microorganisms such as *Yersinia enterocolitica* or *Salmonella* spp., we would have the following CCP's: evisceration, meat inspection, and removal of head (Tomkin 1994). It is desirable that the systems for control of product safety and storage stability separate even when there is in practice a high degree of coincidence, to avoid regulatory confusion of the safety and commercial aspects of process hygiene.

Process steps	Hazards	Risks
Lairage and *ante-mortem* inspection	Diseased animals, pathogenic and spoilage bacteria	accumulation and spread of bacteria within animals
Stunning		
Sticking	Contaminated tools	contamination of sticking wounds
Scalding		
• **Dehairing**	Bacteria from the machine	spread of bacteria to carcasses
• **Polishing**	Bacteria on tools	spread of bacteria to and over carcasses
Singeing/flaming		
• **Evisceration, removing the head**	Bacteria from intestines, pharynx, tonsils, tools	spread of bacteria to carcasses
Splitting carcasses	contaminated saws	spread and cross-contamination
• ***Post-mortem* inspection**	bacteria from incised tissues and inspector's hands and knives	spread and cross-contamination
Chilling/freezing	surviving bacteria, temperature abuse	growth and multiplication of pathogens

• Proposed points for considering as CCP's

Figure 3: Flowchart of pig processing at the Lakeview plant with operational stages where major bacterial contamination can occur and CCP may be established.

As a component of the HACCP system and a step in the setting up of a HACCP plan for microbial quality control of fresh pork carcass meat, this study aimed to identify microbial hazards at various process stages, analyse preventative measures, establish critical control points and, if possible, recommend monitoring and corrective measures to ensure that the bacterial contamination remain within acceptable levels. For this purpose, the microbial levels on the surface of pork carcasses were assessed before they enter and after they exit each operation using a swab sampling technique and the APC in the laboratory. Data from the APC were collected for analysis using SPSS and Excel statistical software. Prior to statistical analysis, a logarithmic transformation (log_{10}) of the count data was performed to equalise the variance of different variables, while not affecting their means. The significance of the mean differences was tested using the T-test for paired samples for means.

Sampling Plan

As the study is designed to assess the effect of procedures of various operational steps on the microbial quality of carcasses, the number of pigs slaughtered per shift and the initial microbial status of the surface of a particular pig should not affect the results. The number of samples to be taken to obtain the desired statistical significance of any difference in APC found was based on the subsequent sampling technique. According to this method, samples are collected continuously, microbial contamination levels before and after each operation are assessed until a difference is found and the statistical significance is achieved. This sampling method does not require a prior calculation of the sample size, but it has the disadvantage that one is not sure when to stop if there is no significant differences between the paired variables (APC's before and after an operation) being studied.

During each visit to the abattoir, two pig carcasses were selected for sampling, one at the start and another at the end of a shift (10 minutes, approximately, after the first pig entered the chain and 10 minutes before the shift ends).

Each carcass was followed from the beginning to the end of the processing line. At the dehairing step, samples were taken after the carcass had been lifted out of the scalding tank and entered the dehairing machine, and again after it exited the dehairing machine. These two samples constituted a pair of "before and after" samples for the dehairing operation.

As scraping takes place on the table immediately after a carcass leaves the dehairing machine, samples for assessing this operation were taken after scraping was finished, the post-dehairing sample doubling-up as a pre-scraping sample (Figure 4).

Before reaching the evisceration point, the carcass goes through flaming and washing, therefore evisceration point samples were taken both before and after evisceration. For the inspection step, sampling was carried out similarly to that at the polishing and scraping step (post-evisceration sample doubling-up as pre-inspection sample). Thus after completion of sampling of one carcass, four paired samples for dehairing, scraping and polishing, evisceration, and inspection points were obtained, as shown in Figure 4.

Each sample consisted of pooled swabs taken from three sites on the carcass at the hind leg, brisket, and flap locations according to published protocol (ICMSF 1988). A sterile metal template with a $5cm^2$ hole was used to delineate the sampling site. After the metal template was placed on the skin surface at the sample site, one swab moistened with sterile dilution fluid (0.1% peptone and 0.85% NaCl) was used to rub the skin approximately 10 times up, down and across the entire exposed area. Then, a second dry swab was used to rub the

same sample area in a similar manner. The swabs were broken into a McCartney bottle containing 10 mls of sterile dilution fluid.

Process steps	Sampling method	Times of sampling	Number of samples each time	Sub-total
Scalding	Water sampling	2	1	2
• **Dehairing**	template swab	2	2 (before and after)	4
• **Polishing**	template swab	2	1 (after)	2
Singeing/flaming				
• **Evisceration**	template swab	2	2 (before and after)	4
• ***Post-mortem* inspection**	template swab	2	1 (after)	2
Chilling/freezing				
			Total	14

Figure 4: Sampling plan for APC assessment

Samples from three sites on the carcass were pooled together in one bottle. Thus the APC represents a count "average" for the whole carcass at one particular process point. Bottles containing the swabs were labelled and placed in a cool box with ice for transport to the laboratory. The time lapse between the completion of sampling at the abattoir and inoculation of APC plates in the laboratory at Massey University varied from one to two hours.

After the first two complete sets of samples had been collected, it was decided that the scalding water would also be sampled to assess its bacterial content. Water samples were taken by dipping an uncapped 10 ml McCartney bottle, with a string tied to the neck, into the scalding water. When full, the bottle was pulled out of the water, closed with a cap and stored in the cool box.

Eight visits to the abattoir were made and each time sets of samples from two pig carcasses were taken. In total, 32 samples from each process stage were collected during the experiment. With 4 process stages selected for sampling, the total number of samples were 128. However, as mentioned above, the "post-dehairing" and the "post-evisceration" samples were used as "pre-scraping" and "pre-inspection", respectively. So, in effect, only 96 carcass swabs were collected. In addition, 12 scalding water samples were collected for analysis.

Aerobic Plate Count

After collection, swab samples were stored in a cool box with ice and transported to the Microbiology Laboratory, Massey University for analysis. All samples were processed and incubation commenced on the day of collection.

Homogenates from the swabs were prepared using a mixer, each bottle being mixed for approximately 15-20 seconds. Ten-fold dilutions of the homogenates were then made by transferring 1ml of the initial dilution into 9ml saline to make subsequent dilutions of 10^{-1},10^{-2}, 10^{-3} etc. From each dilution, 0.1ml volumes were inoculated onto duplicate, dried plate count agar (see Appendix). Each inoculum was evenly spread over the agar surface using a sterile glass spreader.

After inoculation, all plates incubated at a temperature of 30°C. For the first samples, all dilutions were plated and CFU were calculated after 24, 48, and 72

hours. Following the results of the first APCs it was decided that counts should be done only from the 10^{-1} and 10^{-2} dilutions. This was because growth from the initial dilution was uncountable and a dilution of 10^{-3} produced very few CFU making the count statistically invalid. It was also decided that plates should be counted after 72 hours of incubation as this incubation time gave the highest number of CFU. The count was calculated using the following formula:

$$CFU/cm^2 = \frac{(C_1 + C_2) \cdot i}{2 \cdot T \cdot D \cdot V}$$

C_1 and C_2	counts of duplicate plates
I	initial volume of swab homogenate
T	total sampling area
D	rate of dilution
V	volume inoculated.

A table was designed for entering APC data after each count and variables were encoded. The average CFU/cm^2 from each count was calculated and the $\log_{10}$ transformation was performed after all samples had been examined (see Appendix).

Results and Discussion

The results of total aerobic counts of the surface of pig carcasses are shown in Table 3.

Table 3: Logarithmic means of APC of pig carcasses at different processing steps and of scalding water.

	N	Minimum	Maximum	Mean	Std. Deviation
W	12	1.70	3.33	2.5856	.5207
D1	16	3.12	5.19	4.2765	.6067
D2	16	3.78	5.87	5.0907	.5706
P3	16	4.09	5.90	4.9181	.4681
E4	16	3.75	5.90	4.5686	.5423
E5	16	3.67	4.94	4.4497	.3835
I6	16	3.80	5.13	4.4616	.4007

N Number of times sampled
W Scalding water
D1 Before dehairing
D2 After dehairing
P3 After scraping
E4 Before evisceration
E5 After evisceration
I6 After inspection.

Table 4 shows that the highest mean APC was found after the carcasses had exited the dehairing machine (5.1 $\log_{10}/cm^2$, S.D. 0.57) and the lowest number of bacteria on the skin surface was found before the dehairing step (4.3 $\log_{10}/cm^2$,

S.D. 0.61). The low APC's before dehairing were clearly due to the heat effect of the scalding water. The number of bacteria then increased rapidly after the carcass passed through the dehairing machine. This result is consistent with other studies of the effects of the dehairing machine on the bacterial population of pork carcasses (Dockerty *et al.* 1970; Gill and Bryant 1992). After the dehairing step, the count gradually decreased until the carcass reached the post-evisceration point (4.4 log_{10}/cm^2, S.D. 0.38). A slight rise to 4.5 log_{10}/cm^2 (S.D. 0.4) was found at the post-inspection step. The overall changes of APC's of the pork carcasses through the slaughter and dressing process are illustrated in Figure 5.

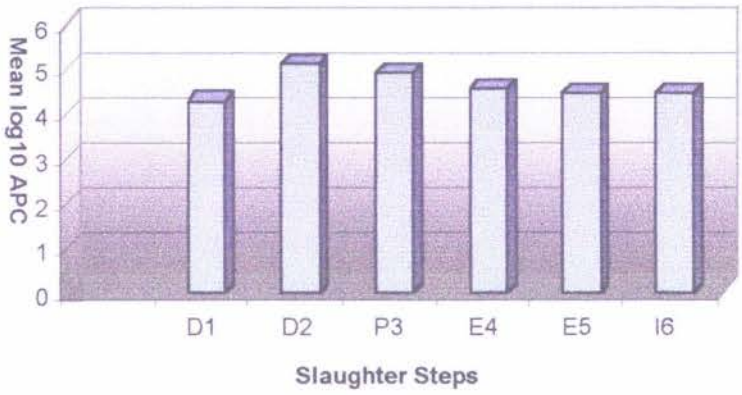

Figure 5: APC's of pig carcass skin at six processing steps.

Scalding Water

The scalding water temperature at the abattoir is maintained by gas heating and is controlled manually by slaughtermen. The actual temperature readings on the thermometer installed at the scalding vat were always lower than those checked by an electronic thermometer by at least 2°C. Scalding water temperature fluctuated greatly from 60 to 67.5°C. The average temperature of the scalding water at the beginning of the process was 63.27°C and at the end 66.27°C (n = 6). There was a significant increase in visible contamination of the water by soil,

hair, and other detritus. However, bacterial contamination remained almost unchanged with time (2.55 log_{10} at the beginning and 2.62 log_{10} per ml at the end). Due to the fluctuation of the scalding water temperature, the difference between counts of the water at the start and at the end of slaughter shifts were not comparable (Figure 6).

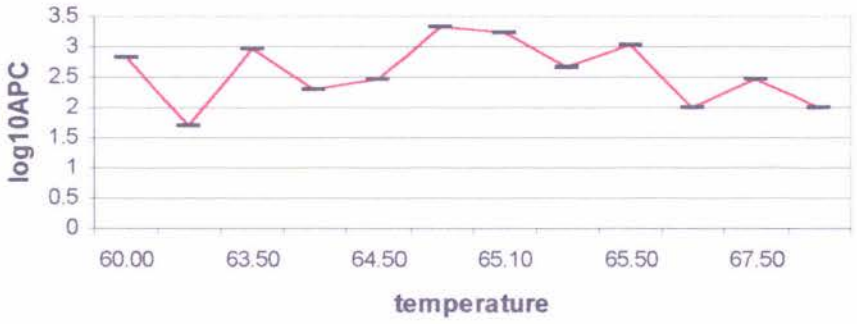

Figure 6: APC and temperature of scalding water

Dehairing

The difference in APCs at the dehairing step was assessed by comparing the count before the carcass entered with that after it came out from the dehairing machine. The APC increased significantly from 4.3 log_{10}/cm^2 to 5.1 log_{10}/cm^2 ($p = 0.0002$, $n = 16$) indicating a heavy recontamination of the carcasses with bacteria from the dehairing machine.

As discussed in Chapter II, the major sources of bacterial contamination which occur at this step, come mainly from contaminated equipment and from the accumulation of detritus during processing. These findings are consistent with the results of other studies (Dockerty *et al* 1970; Gill and Bryant 1992). Figure 7 demonstrates the changes in APC's of pork skin surfaces at the dehairing step.

Gill and Bryant (1992) stated that the domination of spoilage organisms on the dehairing equipment would seemingly account for the qualitative and quantitative similarity of the flora initially present on pork cuts prepared at different commercial slaughter plants.

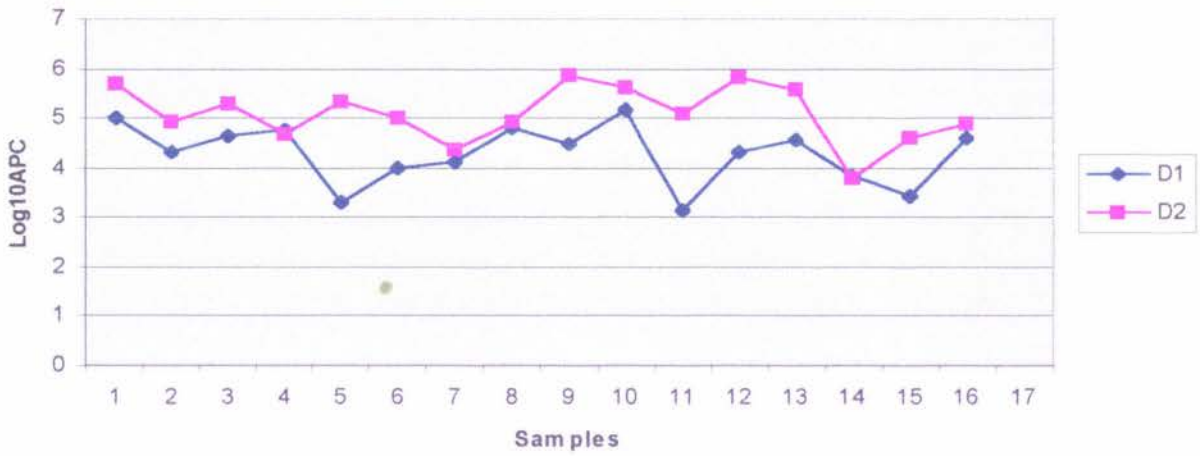

Figure 7: APC's before (D1) and after (D2) dehairing

Although singeing and polishing operations may modestly alter absolute numbers of spoilage organisms, those operations will not significantly affect the proportion of different spoilage organisms in a spoilage flora. Therefore, a substantial improvement in pork storage quality would seem to be attainable only when a means is identified to maintain the microbiological condition of freshly scalded carcasses during post-scalding processing.

Changes of APC'c with Process Time

Other studies have shown that bacterial levels at a particular slaughter step normally increase with process time, and higher numbers of bacteria are found at the end of the operational time than at the beginning (Troeger *et al.* 1987; Troeger 1994). The results of this study, however, did not show significant differences in APCs between the beginning and the end of a slaughter shift (Figure 8).

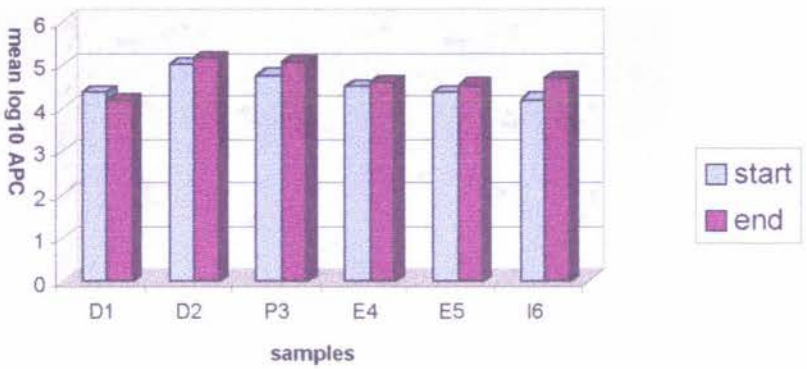

Figure 8: Differences in APC between start and end of the shift at six different process steps.

There were genenally increases in APC's of samples taken at the end (4.7 log_{10}/cm^2) in comparison with those taken at the start (4.5 log_{10}/cm^2) of processing. However the difference was small (0.2 log_{10}/cm^2) and not statistically significant ($p > 0.05$). This might be due to the small sample size.

Scraping and Polishing

In contrast to results of other studies (Dockerty *et al.* 1970; Grau 1986; Nerbrink and Borch 1989; Rahkio and Korkeala 1996), the scraping and polishing operations in this investigation had little effect on bacterial numbers. There were slight decrease in APC's of post-scraping and polishing samples (4.92 log_{10}/cm^2) in comparison with those of pre-scraping and polishing (5.1 log_{10}/cm^2). Only five samples (31%) had post-scraping APC's higher than pre-scraping, the remaining 11 samples (69%) produced lower post-scraping counts (Figure 9).

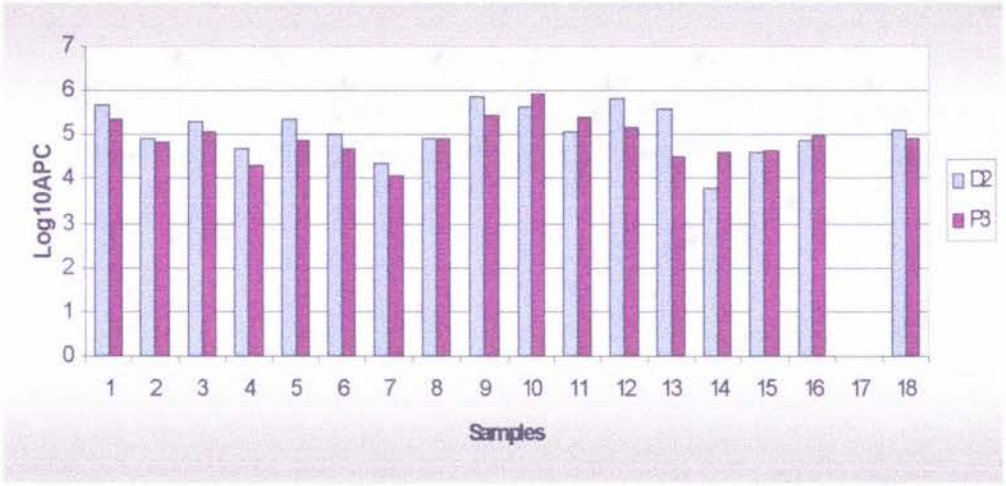

Figure 9: Changes in APC's between pre- (D2) and post-scaping and polishing (P3) operations.

Column 18 shows means of counts.

In principle scraping and polishing should contribute significant contamination and should facilitate the spread of bacteria over the carcass. Depending on facility type and hygienic practices the level of contamination and spread may vary between different plants. With manual "dry" scraping and polishing, followed by washing, widespread and heavy recontamination of the pig carcass can be expected. A study on the effects of operational procedures on bacterial contamination of hot pork carcasses in North Vietnam showed an increase in

APC's from 5.4 $x10^5$ (5.7 $\log_{10}$) to 3.7 x 10^6 (6.5 $\log_{10}$) CFU/cm^2 of the carcass skin surface after one minute of scraping (Vu 1992). At the study abattoir, scraping is performed after dehairing on an alloy table with continuous running water, which should have a double hygienic effect. Running water continuously washes away scurf, hair and other detritus from the table, thus it has a cleaning effect on the equipment. At the same time the running water also has a washing effect on the carcass while this is on the table and turned around during the operation.

One of the factors that certainly prevents an increase in bacterial contamination of pork carcasses at this step is that workers at the site are well trained in hygienic practices including frequent washing of knives and brushes and avoiding touching the carcass by hands. From the HACCP approach, the above mentioned factors may be included into Principle 1 (identification of preventative measures such as washing the table, knives, worker's hands etc.), Principle 3 (establishing critical limits such as frequency of knife and brush washing, frequency and location of the carcass touched by hands), and Principle 4 (monitoring of such operational procedures). A decrease in APCs at scraping and polishing as shown in this study does not necessarily mean that bacterial contamination does not exist, neither does it show that the operations reduce the contamination level.

Evisceration

There was no significant difference ($p > 0.05$) in APC's before and after evisceration. However, if counts of samples are considered separately, higher pre-evisceration counts were obtained from seven samples (43.7%) compared to post-evisceration. In nine samples (56.3%), an increase in total bacterial counts was observed (Figure 10).

Figure 10: Differences in APC's between pre- (E4) and post-evisceration (E5) operations.

As shown in Figure 10, there was an unusual rapid reduction in bacterial counts between pre- and post-evisceration in sample No.2 and sample No.15, which greatly affected the overall means. These abnormal drops may be due to sampling errors. Another sampling problem which should be considered is that, at this step, after the pre-evisceration samples had been collected, the carcasses went through the shower before they were subjected to the evisceration. Thus, the reduction in APC at this step, may be due to the effect of washing. If the sampling technique was improved, the pre-evisceration samples could be taken after the carcass had passed through the shower so that only the effect of the evisceration remained to be assessed, and if the sample size could be increased, the result might have been different.

In addition, bacterial contamination of the carcass surface is not uniform and depends on the location of the sample site. The evisceration procedure has a

greater effect on microbial contamination at lower parts of the carcass such as the head, shoulder, and brisket (Dockerty *et al.* 1970; Gill and Bryant 1992). If the evisceration step is chosen as a CCP in the HACCP plan for pig slaughter, more attention should be paid to the establishment of preventative measures such as training and application of measures to prevent the escape of the intestinal contents during the operation by tying up or plugging the anus. In this study, samples from three sites of the carcass were pooled so the distribution of aerobic bacteria on different parts of the pig skin surface could not be assessed.

Inspection

At the inspection step, there was an increase in APC's on the surface of the carcass from 4.4 $\log_{10}/cm^2$ (S.D. 0.38) to 4.5 $\log_{10}/cm^2$ (S.D. 0.40). Although this increase is considerable in absolute terms, the T-test showed that the variance was not statistically significant ($p > 0.05$).

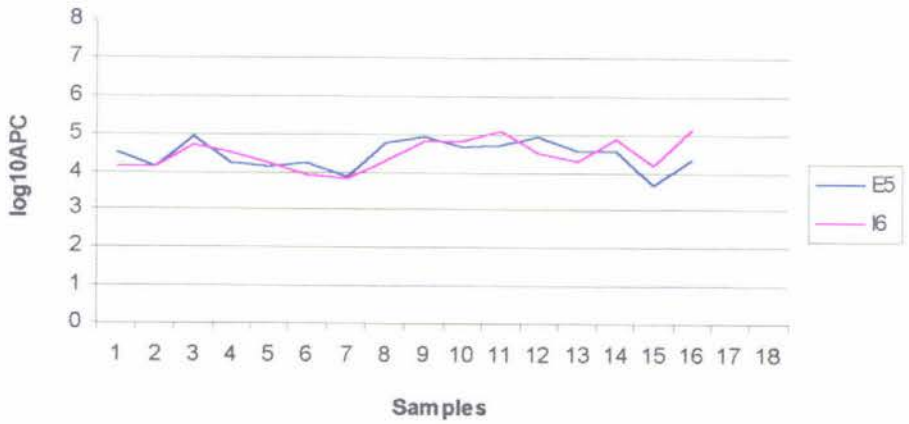

Figure 11: Changes in APC's before (E5) and after (I6) inspection

The APC's at the inspection point may not change significantly but the composition of the microflora may differ from that at other steps during processing. For example, incision of lymph nodes can have serious effect on the safety and quality of meat by spreading pathogenic bacteria directly from the incised node to the carcass and indirectly to other carcasses via contaminated knives. This has been shown with *Salmonella*, initially localized within the mesenteric lymph nodes, but spread to the carcass following detailed examination by multiple incisions (Edwards *et al.* 1997). During meat inspection, contamination from lymph nodes and possibly lungs of vat scalded pigs may also occur (Borch *et al.* 1996b). As a result of palpation of organs and the carcass by meat inspectors, pathogenic bacteria may be transported from an infected region to "clean" parts of the carcass by hands and knives of the inspection personnel (Nesbakken 1988). At the study abattoir, *post-mortem* inspection procedures for pigs are based on visual inspection with limited incisions. Only the submandibular lymph nodes are incised in every carcass. Others are incised only when necessary. A more detailed inspection, with multiple incision of lymph nodes, is applied to adult breeding animals or animals suspected of having tuberculosis. This practice is in line with the results of overseas studies on limitation of traditional meat inspection procedures and is based on the favourable animal health status of pigs in New Zealand.

As discussed in Chapter II, a HACCP plan and the number of CCP's may vary between plants depending on the technology, manufactured products and specific hazards. Meat inspection can be a CCP from a safety aspect but at the same time it may not be a quality CCP.

Conclusions and Recommendations

Surprisingly, there was no significant change in the APC's of scalding water with time, although visible contamination increased considerably. Because the temperature of the scalding water could not be controlled effectively, an association of bacterial contamination with time could not be made.

There was clear evidence of a quality hazard and a risk of recontamination of pig carcasses during dehairing. The increase in APC's of 0.8 $\log_{10}$ at this step indicates heavy bacterial recontamination of the carcass and implies that the source of contamination is the dehairing equipment. Since a hazard exists and the risk of carcass contamination is high, establishing a quality Critical Control Point at this step essential. On a conventional pig slaughter chain, the dehairing step is not specifically designed to reduce or eliminate hazards. From a HACCP approach, this process step should therefore be modified. As the level of bacterial contamination of pig carcasses at the dehairing step affects the bacterial count at the end of the slaughter process (Dockerty *et al.* 1970; Gill and Bryant 1992), control of the microbial load at this point could play an important role in the control of spoilage of fresh pork. Gill (1980) suggested that the installation of a dehairing apparatus designed to prevent carcass contamination, or post-dehairing decontamination treatment using heat or organic acids (Prasai 1992), could reduce contamination of pig carcasses. Treatment with hot water that reliably raises the temperature of all areas of the carcass surface above 80°C for at least 10 seconds is likely to be satisfactory (Davey 1989). Such a treatment could be by immersion of the carcass, as in scalding, but that may be commercially unattractive due to additional costs involved in modifying the existing technology and labour. On-rail decontamination can be achieved by subjecting the carcass to showers of recirculating water at 85°C. An appropriate apparatus has been designed, and has been shown to be reliable under commercial circumstances (Gill 1995).

As with the dehairing step, the polishing and scraping operations are not designed to eliminate or reduce microbiological hazards. The used of modified equipment and tools, for example the installation of running water at the polishing table or the use of a "shower" brush connected to the water supply, could prevent the accumulation of detritus and reduce the risk of recontamination of the carcass with bacteria from the equipment. Polishing and

scraping of the carcass can be selected as a microbial quality CCP, however, attention needs to be paid to setting up preventative measures (Principle 1) and establishing monitoring procedures (Principle 4) for operational activities at this step.

At the evisceration step, both safety and quality aspects of carcass meat are important . The main source of bacterial contamination at this step is the escape of contents from the intestinal tract of the animal. Effective preventative measures do exist at this step to minimise the hazards, although there is no way of eliminating them completely. Plugging and tying up the anus during evisceration have been specifically designed as preventative measures (Biermuller *et al.* 1973; Nesbakken 1988; Borch *et al.* 1996b), so if the CCP decision tree is followed, this processing step should be chosen as a CCP.

In addition, it is important to note that dehairing, scraping and polishing, and evisceration can only be CCP_2, or CCPr, because at all of the above steps the microbiological hazards can only be minimised or reduced to an acceptable level, but at none of them can the hazards be eliminated.

Although meat inspection procedures have been simplified in New Zealand, they may still pose a potential risk by spreading of pathogens of public health significance to the carcass. Inspection should not contribute to the microbial quality risk via incisions of lymph nodes and palpation of organs and the whole carcass by the meat inspector. Further studies may be needed to determine whether meat inspection should be chosen as a safety CCP in a HACCP system, which would be applicable to fresh pork processing. The objective of any study should focus on the identification of microbiological hazards which may be a threat to consumer's health, such as the spread of pathogenic *E. coli, Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. The implications of the existing traditional meat inspection procedures should be reviewed and tested

before any new technical and regulatory procedure for pig slaughter can be developed.

Finally, no HACCP system can be applied without prerequisite components of GMP (Tomkin 1994; Gill 1995; Mortimore and Wallace 1995). Therefore, if there is any intention of bringing a HACCP plan to the abattoir, some aspects of hygiene should be reviewed. These may include:

- hygienic presentation of pigs for slaughter ;
- *ante-mortem* inspection of animals;
- automatic control of scalding water temperature;
- cleaning the carcass by showering with hot water during the dehairing operation;
- cleaning by running water during polishing and scraping;
- plugging the anus or tying up the rectum during evisceration;
- maintenance of existing pre- and post-operational disinfection and sanitation procedures.

In any HACCP plan in food production, the production business (in this case the meat company) should be involved and play an active role in implementation of the plan. Regulatory authorities only provide the business guidance in setting up a HACCP team, providing training, and external experts if necessary. Once the system is functioning, the company itself is responsible for successful implementation and for proper quality assurance of its products.

APPENDIX

1. **Plate Count Agar formula**

Tryptone	5.0 g/l
Yeast extract	2.5 g/l
D-glucose	1.0 g/l
Agar	15.0 g/l
Distilled water	1000 ml

PH 7.0 ± 0.2.

2. **Table for APC data entry**

APC						
Date of sampling and incubation / /						
Date of count / /			**Incubation time** hrs			
Sample Points	Dilutions					
	10^{-2}			10^{-3}		
	Plate1	**Plate2**	**average**	**Plate1**	**Plate2**	**average**
shift start						
D1						
D2						
P3						
E4						
E5						
I6						
shift end						
D1						
D2						
P3						
E4						
E5						
I6						

D1 - before dehairing; D2 - after dehairing; P3 - after polishing
E4 - before evisceration; E5 - after evisceration; I6 - after inspection.
Time interval between shift start and shift end is 2 hours approximately.

3. Aerobic Plate Counts

APC's at shift start

Date	t°a	Wa	D1a	D2a	P3a	E4a	E5a	I6a
14/5/98			100000.00	500000.00	233333.30	20000.00	33333.30	13333.30
21/5/98			20000.00	83333.30	70000.00	803333.30	20000.00	13333.30
28/5/98	64.3	200	43333.30	190000.00	110000.00	16666.70	83333.30	30000.00
4/6/98	60.0	700	10000.00	46666.70	20000.00	66666.70	18333.30	30000.00
19/6/98	65.5	1050	201620.00	213333.30	73333.30	23333.30	14000.00	16666.70
25/6/98	61.8	50	10000.00	103333.30	50000.00	13333.30	23333.30	6666.70
3/7/98	63.5	950	13333.30	23333.30	12333.30	5666.70	7000.00	6333.30
9/7/98	64.5	300	64666.70	83333.30	81000.00	48333.30	61000.00	20333.30

APC's at shift end

Date	t°b	Wb	D1b	D2b	P3b	E4b	E5b	I6b
14/5/98			33333.30	766666.70	466666.70	366666.70	33333.30	66666.70
21/5/98			153333.30	413333.30	793333.30	156666.70	46666.70	63333.30
28/5/98	67.50	100	20000.00	120000.00	260000.00	13333.30	50000.00	116666.70
4/6/98	64.50	2150	20000.00	670000.00	150000.00	16666.70	86666.70	30000.00
19/6/98	67.50	300	36666.70	3886667.0	30000.00	16666.70	23333.30	20000.00
25/6/98	67.30	100	6666.70	6000.00	36666.70	10000.00	36666.70	76666.70
3/7/98	65.50	450	2666.70	40666.70	42333.30	80333.30	4666.70	16000.00
9/7/98	65.10	1750	39666.70	78000.00	95666.70	69000.00	21000.0	136000.0

4. Log Transformation of Aerobic Plate Counts

At shift start

Date	t°a	Wa	D1a	D2a	P3a	E4a	E5a	I6a
14/5/98			5	5.69897	5.367977	4.30103	4.522879	4.124939
21/5/98			4.30103	4.920819	4.845098	5.904896	4.14613	4.124939
28/5/98	64.3	2.30103	4.636822	5.278754	5.041393	4.221849	4.920819	4.696067
4/6/98	60.0	2.845098	4.74819	4.669007	4.30103	4.823909	4.263241	4.477121
19/6/98	65.5	3.021189	3.301032	5.329059	4.865301	4.367977	4.14613	4.221849
25/6/98	61.8	1.69897	4.000002	5.01424	4.69897	4.14613	4.230451	3.937854
3/7/98	63.5	2.977724	4.124939	4.367977	4.09108	3.753328	3.845098	3.801632
9/7/98	64.5	2.477121	4.81068	4.920819	4.908485	4.684247	4.78533	4.308209

At shift end

Date	t°b	Wb	D1b	D2b	P3b	E4b	E5b	I6b
14/5/98			4.477143	5.867293	5.431385	5.12496	4.937874	4.823909
21/5/98			5.185637	5.6163	5.899456	5.194977	4.669007	4.801632
28/5/98	67.5	2	3.124939	5.079181	5.414973	4.315273	4.69897	5.066947
4/6/98	64.5	3.332438	4.30103	5.826075	5.176091	4.293733	4.937852	4.477121
19/6/98	67.5	2.477121	4.564271	5.583577	4.477121	4.221849	4.535718	4.30103
25/6/98	67.3	2	3.823909	3.778153	4.564271	4	4.564271	4.884607
3/7/98	65.5	2.653213	3.425969	4.609239	4.626682	4.904896	3.669007	4.20412
9/7/98	65.1	3.243038	4.598426	4.892095	4.980761	4.838849	4.322219	5.133539

REFERENCE LIST

Adams, C. E. (1990). Use of HACCP in Meat and Poultry Inspection. Food Technology, 44, 169-170.

Andersen, J. K. (1988). Contamination of Freshly Slaughtered Pig Carcasses with Human Pathogenic *Yersinia enterocolitica*. International Journal of Food Microbiology, 7, 193-202.

Anon (1995). Annual Report on Zoonoses in Denmark, 1994. Copenhagen, Denmark: Danish Zoonosis Centre.

Avery, S.M. (1991). The Aerobic Plate Count. In: Cook, R.L. Microbiological Methods for the Meat Industry. (2nd ed.). Hamilton, New Zealand: Meat Industry Research Institute of NZ.

Barbuti, S., Manganelli, E., Ghisi, M., and Campanini, M. (1992). Microbiological Characteristics of Pig Carcasses in Italian Slaughterhouses. Proceedings of the 38th International Congress on Meat Science and Technology, 4, 617-620.

Barraud, C., Kitchell, A. G., Labots, H., Reuter, G., and Simonsen, B. (1967). Standardisation of the Total Aerobic Counts of Bacteria in Meat and Meat Products. Fleischwirtschaft, 12, 1317-1318.

Benedict, R. C. (1988). Microbial Attachment to Meat Surfaces. The 41th Annual Reriprocal Meat Conference of the American Meat Science Association, 41, 1-6.

Berends, B. R., Snijders, J. M. A., and van Logtestijn, J. G. (1993). Efficacy of Current EC Meat Inspection Procedures and Some Proposed Revisions with Respect to Microbiological Safety: a Critical Review. Veterinary Record, 133, 411-415.

Beuchat, L. R., Copeland, F., Curiale, M.S., Danisavich, T., Gangar, V., King, B.W., Lawlis, T.L., Likin, R.O., Okwusoa, J., Smith, C.F. and Townsend, D.E. (1998). Comparison of the SimPlate Total Plate Count Method with Petriflm™, Redigel™, and Conventional Pour-Plate Methods for Enumerating Aerobic Microorganisms in Food. Journal of Food Protection, 61, 14-18.

Biermuller, G. W., Carpenter, J. A., and Reynolds, A. E. (1973). Reduction of Bacteria on Pig Carcasses. Journal of Food Science, 38, 261-263.

Biss, M. E., and Hathaway, S. C. (1995). Microbiological and Visible Contamination of Lamb Carcasses According to Preslaughter Presentation Status: Implication for HACCP. Journal of Food Protection, 58, 776-783.

Borch, E., Kant-Muermans, M. L., and Blixt, Y. (1996a). Bacterial Spoilage of Meat and Cured Meat Products. International Journal of Food Microbiology, 33, 103-120.

Borch, E., and Molin, G. (1989). The Aeobic Growth and Product Formation of Lactobacillus, Leuconostoc, Bronchothrix and Carnobacterium in Batch Culture. Applied Microbiology and Biotechnology, 30, 81-88.

Borch, E., Nesbakken, T., and Christensen, H. (1996b). Hazard Identification in Swine Slaughter with Respect to Foodborne Bacteria. International Journal of Food Microbiology, 30, 9-25.

Brandly, P. J., Migaki, G., and Taylor, K. E. (1966). Meat Hygiene. New York, USA: Lee and Febiger.

Bryan, F.L. (1980). Foodborne Disease in the United States Associated with Meat and Poultry. Journal of Food Protection, 43, 140-150.

Bungay, A.A.C. (1997). Faecal and Tonsilar Carriage of *Yersinia* spp. by Pig. MVS Dissertation. Massey University, New Zealand.

Busta, F.F. (1978). Introduction to Injury and Repair of Microbial Cells. Advances in Applied Microbiology, 23, 195-201

Busta, F. F., and Smith, L. B. (1976). Bacterial Injury and Recovery. The 29th Annual Reriprocal Meat Conference of the American Meat Science Association, 301-315.

Butler, J. L., Stewart, J.C., Vanderzant, C., Carpenter, Z.L., and Smith, G.C. (1979). Attachment of Microorganisms to Pork Skin and Surfaces of Beef and Lamb Carcasses. Journal of Food Protection, 42, 401-406.

Butler, J. L., Vanderzant, C., Carpenter, Z.L., Smith, G.C., Lewis, R.E., and Dutson, D.R. . (1980). Influence of Certain Processing Steps on Attachment of Microorganisms to Pig Skin. Journal of Food Protection, 43, 699-705.

Christensen, H., and Luthje, H. (1994). Reduced Spread of Pathogens as a Results of Changed Pluck Removal Technique. Proceedings of the 40th International Congress on Meat Science and Technology, S-III. 06.

Chua, S. B., and Chew, S. T. (1991). Recent Trends in Meat Safty: The Singapore Perspective. Singapore Veterinary Journal, 15, 41-53.

Codex Alimentarius (1993). Guideline for the Application of the Hazard Analysis Critical Control Point (HACCP) System. Codex Committe on Food Hygiene, WHO/FNU/FOS/93.3 II.

Crouse, J. D., Anderson, M. E., and Naumann, H. D. (1988). Microbial Decontamination and Weight of Carcass Beef as Affected by Automated Washing Pressure and Length of Time in Spray. Journal of Food Protection, 51, 471-474.

D'Aoust, J.-Y. (1989). *Salmonella*. In: M.P. Doyle Foodborne Bacterial Pathogens (pp. 327-446). New York, USA: Marcel Dekker.

D'Aoust, J.-Y. (1994). *Salmonella* and the International Food Trade. International Journal of Food Microbiology, 24, 11-31.

Dainty, R. H. (1996). Chemical/Biochemical Detection of Spoilage. International Journal of Food Microbiology, 33, 19-33.

Dainty, R. H., and Mackey, B. M. (1992). The Relationship between the Phenotypic Properties of Bacteria from Chill-Stored Meat and Spoilage Process. Journal of Applied Bacteriology, 73, 703S-714S.

Dainty, R. H., Shaw, B. G., and Roberts, T. A. (1983). Microbial and Chemical Changes in Chill-Stored Red Meats. In: Roberts T.A. and Skinner F.A. Food Microbiology: Advances and Prospects. London, England: Academic Press.

Davey, K. R. (1989). Theoretical Analysis of Two Hot Water Cambinet Systems for Decontamination of Beef Sides. International Journal of Food Science, 24, 291-304.

De Boer, E., and Nouws, J. F. M. (1991). Slaughtered Pigs and Pork as a Source of Human Pathogenic *Yersinia Enterocolitica*. International Journal of Food Mocrobiology, 12, 375-378.

De Pablo, B., Asensio, M. A., Sanz, B., and Ordonez, J. A. (1989). The D(-) Lactic Acid and Acetoin/Diacetyl as Potential Indicators of the Microbial Quality fof Vaccum-Packed Pork and Meat Products. Journal of Applied Bacteriology, 66, 185-190.

Dickson, J. S., and Anderson, M. E. (1992). Microbiological Decontamination of Food Animal Carcasses by Washing and Sanitizing Systems: A Review. Journal of Food Protection, 55, 133-138.

Dockerty, T. R., Ockerman, H.W., Cahill, V.R., Kunkle, L.E., and Weiser, H.H. (1970). Microbial Level of Pig Skin as Affected by the Dressing Process. Journal of Animal Science, 30, 884-890.

Eddy, B.P. (1960). The Use and Meaning of the Term "Psychrophilic". Journal of Applied Bacteriology, 23, 189-192.

Edwards, D. S., Johnston, A. M., and Mead, G. C. (1997). Meat Inspection: An Overview of Present Practices and Future Trends. The Veterinary Journal, 154, 135-147.

Farber, J. M., and Idzak, E. S. Attachment of Psychrotrophic Meat Spoilage Bacteria to Muscles Surfaces. Journal of Food Protection, 47, 92.

Fricker, C. R., and Tompsett, S. (1989*). Aeromonas* spp. in Foods: A Significant Cause of Food Poisoning. International Journal of Food Microbiology, 9, 17-23.

Gerats, G. E., Snijders, J. M. A., and van Logtestijn, J. G. (1981). Slaughter Techniques and Bacterial Contamination of Pig Carcasses. Proceedings of 27th European Meeting of Meat Research Workers, Vienna, 2, 198-200.

Gill, C. O. (1980). Total and Intramuclular Bacterial Population of Carcasses and Cut. The 33th Annual Reriprocal Meat Conference of the American Meat Science Association, 33, 47-53.

Gill, C. O. (1986). The Control of Microbial Spoilage in Fresh Meat. In: Advances in Meat Research, 2, 49-88.

Gill, C. O. (1995). Current and Emerging Approach to Assuring the Hygienic Condition of Red Meat. Canadian Journal of Animal Science, 75, 1-13.

Gill, C. O., and Bryant J. (1992). The Contamination of Pork with Spoilage Bacteria during Commercial Dressing, Chilling and Cutting of Pig Carccases. International Journal of Food Microbiology, 16, 51-52.

Gill, C. O., and Bryant, J. (1993). The Presence of *Escherichia coli*, *Salmonella* and *Campylobacter* in Pig Carcasses Dehairing Equipment. Food Mcrobiology, 10, 337-344.

Gill, C. O., and Jones, T. (1992). Assessment of the Hygienic Efficiencies of Two Comercial Processes for Cooling Pig Carcasses. Food Mcrobiology, 9, 335-343.

Gill, C. O., and Jones, T. (1997). Assessment of Hygienic Characteristics of a Process for Dressing Pasteurized Pig Carcasses. Food Microbiology, 14, 81-91.

Gill, C. O., and Newton, K. G. (1977). The Development of Aerobic Spoilage Flora on Meat Stored at Chill Temperature. Journal of Applied Microbiology, 43, 189-195.

Gill, C. O., and Newton, K. G. (1978). The Ecology of Bacterial Spoilage of Fresh Meat at Chill Temperature. Meat Science, 2, 207-217.

Gill, C. O., and Newton, K. G. (1980). Development of Bacterial Spoilage at Adipose Tissue Surfaces of Fresh Meat. Applied and Environmental Microbiology, 39, 1076-1077.

Gracey, G. F. (1986). Meat Hygience, 8th Edition. London, England: Bailliere Tindall.

Grau, F. H. (1986). Microbial Ecology of Meat and Poultry. Advances in Meat Research 2, 17-24. AVI Publishing Co.

Hayes, P. R. (1992). Food Microbiology and Hygiene. England: Elsevier Science Publishers LTD.

Huis in't Veld, J. H. (1996). Microbial and Biochemical Spoilage of Foods: An Overview. International Journal of Food Microbiology, 33, 1-18.

Hunter, E. J. (1994). Pig Welfare and Carcass Quality: A Comparison of the Influence of Slaughter Handling Systems at Two Abattoirs. The Veterinary Record, 423-425.

ICMSF. International Committee of Microbiological Specifications of Food (1978). Microorganisms in Foods: Their Significance and Methods of Enumeration. Canada: University of Toronto Press.

ICMSF. International Committee of Microbiological Specifications of Food (1980). Injury and Its Effects on Recovery. In: Microbial Ecology of Foods, Factors Affectting Life and Death of Microorganisms, 1, 205-214. New York, USA: Academic Press Inc.

ICMSF. International Committee of Microbiological Specifications of Food (1988). Microoganisms in Food 2 - Sampling for Microbiological Alalysis: Principles and Specific Applications. Toronto, Canada: University of Toronto Press .

ICMSF. International Committee of Microbiological Specifications of Food (1998). Microorganisms in Food 6. Microbial Ecology of Food Commodities. London, UK: Blackie Academic and Professional.

Ingram, M., and Roberts, T. A. (1976). The Microbiology of the Red Meat Carcass and the Slaughterhouse. Royal Society Health Journal, 96, 270-276.

ISO 2293. (1988). Meat and Meat Products - Enumeration of Micro-organisms - Colony Count Technique at 30°C. International Organization for Standardization, (2nd ed.).

Jay, J. M. (1992). Mordern Food Microbiology. New York, USA: Chapman and Hall.

Kirov, S.M. (1993). The Public Health Significance of *Aeromonas* spp. in Food. . International Journal of Food Mocrobiology, 20, 179-198.

Kotula, A. V. (1992). Control of Extrinsic and Intrinsic Contamination of Pork. In: Elimination of microorganisms from meat and poultry, 181-201. Amsterdam, Netherlands: Elsevier Science Publisher.

Kotula, A. W., Ayres, J. C., Hutahnen, C. N., Stern, N. J., Stringer, W. C., and Tompkin, R. B. (1980). Guideline for Microbiological Evaluation of Meat. The 33th Annual Reriprocal Meat Conference of the American Meat Science Association, 33.

Kraft, A. A. (1986). Psychtrophic Organisms. Advances in Meat Research, 2, 191-208). AVI Publishing Co.

Lazaro, N. S., Tibana, A., and Hofer, E. (1997). *Salmonella* spp. in Healthy Swine and in Abattoir Environment in Brasil. Journal of Food Protection, 60, 1029-1033.

Mafu, A. A., Higgins, R., Nadeau, M., and Cousinneau, G. (1989). The Incidence of *Salmonella*, *Campylobacter*, and *Yersinia enterocolitica* in Swine Carcasses and Slaughterhouse Environment. Journal of Food Protection, 52, 642-645.

McMeekin. (1982). Microbial Spoilage of Meat. In: R. Davies, Development in food microbiology-1, 1, 1-40. Barking, Essex, UK: Aplied Science Publisher.

Montville, T. J. (1997). Principles Which Influence Microbial Growth, Survival, and Deathin Food. In: M. P. Doyle, L. R. Beuchat, and T. J. Montville, Food Microbiology - Fundamentals and Frontiers, 13-29. Washington DC, USA: American Society for Microbiology Press.

Mortimore, S., and Wallace, C. (1995). HACCP - A Practical Approach (1st ed.). London, UK: Chapman and Hall.

Mossel, D. A. A., Visser, M., and Cornelissen, A. M. R. (1963). The Examination of Foods for Enterobacteriaceae Using a Test of the Type Generally Adopted for the Detection of Salmonellae. Journal of Applied Bacteriology, 26, 444-452.

Mossel, D.A.A., Corry, J.E.L., Struijk, C.B., and Baid, R.M. (1995). Essentials of the Microbiology of Foods: A Text Book for Advanced Studies. Wiley, England.

Mountney, G. J., and Gould, W. A. (1988). Practical Food Microbiology and Technology. New York, USA: An AVI Book.

NACMCF. National Advisory Committee on Microbiological Criteria for Foods (1992). Hazard Analysis Critical Control Point System. International Journal of Food Mocrobiology, 16, 1-23.

NACMCF. National Advisory Committee on Microbiological Criteria for Foods (1998). Hazard Analysis and Critical Control Point Principles and Application Guidelines. Journal of Food Protection, 61, 762-775.

Narucka, U. J. (1979). Occurence and Health Significance of *Staphylococcus aureus* in a Pig Slaughter Line. Rotterdam, The Netherlands: A.A. Balkema.

Nerbrink, E., and Borch, E. (1989). Bacterial Contamination During the Pig Slaughtering Process. Proceedings of the 35th International Congress on Meat Science and Technology, Copenhagen, Denmark.

Nesbakken, T. (1988). Enumeration of *Yersinia enterocolitica* O:3 from the Porcine Oral Cavity, and its Occurrence on Cut Surfaces of Pig Carcasses and the Environment in a Slaughterhouse. International Journal of Food Microbiology, 6, 287-293.

Nesbakken, T., Nerbrink, E., Rotterud, O.J., and Borch, E. (1994). Reduction of *Yersinia enterocolitica* and *Listeria* spp. on Pig Carcasses by Enclosure of the Rectum during Slaughter. International Journal of Food Microbiology, 23, 197-208.

Neumeyer, K., Ross, T., and McMeekin, T. A. (1997). Development of a Predictive Model to Describe the Effects of Temperature and Water Activity on the Growth of Spoilage Pseudomonads. International Journal of

Food Microbiology, 38, 45-54.

Newton, K. G., Harrison, J. C. L., and Wauters, A. M. (1978). Sources of Psychotrophic Bacteria on Meat at the Abattoir. Journal of Applied Bacteriology, 45, 75-82.

Nortje, G. L., Nel, L., Jordaan, E., Badenhorst, K., Goedhart, E., and Holzapfel, W. H. (1990a). The Aerobic Psychrotrophic Population on Meat and Meat Contact Surfaces in a Meat Production System and on Meat Stored at Chill Temperatures. Journal of Applied Bacteriology, 68, 335-344.

Nortje, G. L., Nel, L., Jordaan, E., Badenhorst, K., Goedhart, G., Holzapfel, W. H., and Grimbeek, R.J. (1990b). The Influence of Incubation Temperature on Bacterial Counts in a Meat Production System. Journal of Food Protection, 53(5), 418-422.

Notermans, S., and Kampelmacher, E. H. (1974). Attachment of Some Bacterial Strains to the Skin of Broiler Chickens. British Poultry Science, 15, 573-585.

Nottingham, P. M., Rushbrook, A. J., and Jury, K. E. (1975). The Effect of Plating Technique and Incubation Temperature on Bacterial Counts. Journal of Food Technonogy, 10, 273-279.

Olsvik, O., Wasteson, Y., Lund, A., and Hornes, E. (1991). Pathogenic *Escherichia coli* Found in Food. International Journal of Food Microbiology, 2, 145-149.

Oosterom, J., Dekker, R., de Wilde, G. J. R., van Kempen-de Troye, F., and Engels, G. B. (1985). Prevalence of *Campylobacter jejuni* and *Salmonella* during Pig Slaughtering. Veterinary Quarterly, 7, 31-34.

Oosterom, J., Wilde, G. J. A., de Boer, E., de Blaauw, L. H., and de Karman, H. (1983). Survival of *Campylobacter jejuni* during Poultry Processing and Pig Slaughtering. Journal of Food Protection, 46, 702-706.

Ordonez, J. A., de Pablo, B., Perez de Castro, B., Asensio, M. A., and Sanz, B. (1991). Selected Chemical and Microbiological Changes in Refrigerated Pork Stored in Carbon Dioxyde and Oxygen Enriched Atmospheres. Journal of Agricultural Food Chemistry, 39, 668-672.

Otero, A., Garcia-Lopez, M.-L., and Moreno, B. (1998). Rapid Microbiological Methods in Meat and Meat Products. Meat Science, 49, S179-S189.

Patterson, J. J. (1967). Hygiene in Meat Processing Plants. Importance of Bacteria in Meat Processing Plants,16, 1-18. Northern Ireland: Rec. Agric. Res., Ministry of Agriculture.

Patterson, J. J. (1968). Hygiene in Meat Processing Plants III. Methods of Reducing Carcass Contamination, 17, 7-12. Northern Ireland: Rec. Agric. Res., Ministry of Agriculture.

Petersen, G. V., Madie, P., and Blackmore, D. K. (1991). Veterinary Aspects of Meat Quality. Palmerston North, New Zealand: Veterinary Continuing Education, Massey University.

Piette, J. P. G., and Barriga, M. I. (1994). Attachment of Bacteria to meat Surfaces: In Search of Mechanisms. Meat Focus International, October, 411-415.

Prasai, R. K., Acuff, G.R., Lucia, L.M., Morgan, J.B., May, S.G., and Savell, J.W. (1992). Microbiological Effect of Acid Decontamination of Pork Carcasses at Various Location in Processing. Meat Scince, 32, 413-423.

Raccach, M., and Baker, R. C. (1978). Lactic Acid Bacteria as an Antispolage and Safety Factor in Cooked, Mechanically Deboned Poulty Meat. Journal of Food Protection, 41, 703.

Rahkio, M., and Korkeala, H. (1996). Microbiological Contamination of Carccases Related to Hygienic Pratice and Facilities on Slaughtering Line. Acta Vet Scandinavica, 37, 219-228.

Rasch, B., Lie, O., and Yndestad, M. (1978). The Bacterial Flora in Pork Skin and Influence of Various Singeing Methods on this Flora. Nord. Veterinary Medicine, 30, 274-281.

Rhodehamel, E. J. (1992). Overview of Biological, Chemical and Physical Hazards. In:M.D. Pierson and A. Corlett Jr. (editors), HACCP Principles and Applications, 8-28. New York, USA: Chapman and Hall.

Roberts, T. A. (1980). Contamination of Meat. The Effects of Slaughter Practices on the Bacteriology of the Red Meat. Royal Society Health Journal, 100, 3-9.

Roy, G. (1993). Hazard Analysis Critical Control Point. Hanoi, Vietnam: TCP/VIE/2253.

Schiemann, D. A. (1989). *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. in: M.P. Doyle, Foodborne Bacterial Pathogens, 601-672. New York, USA: Marcel Dekker.

Scholefield, J., Menon, T. G., and Lam, C. W. (1981). Psychrotrophic Contamination of Pig Carcasses. Proceedings of the 27th European Congress of Meat Research Workers, Vienna, 2, 621-624.

Schraft, H., and Untermann, F. (1992). Contamination of Pig Hindquaters with *Staphylococcus aureus*. International Journal of Food Microbiology, 15, 191-194.

Shay, B. J., and Egan, E. A. F. (1986). Study of Possible Storage Techniques for Extending the Storage Life of Chilled Pork. Foodd Technology Australia, 38, 144-146.

Shiozawa, K., Nishina, T., Miwa, Y., Mori, T., Akahane, S., and Ito, K. (1991). Colonization in the Tonsils of Swine by *Yersinia enterocolitica*. Contrib. Microbiological Immunology, 12, 63-67.

Silliker, J. H. (1963). Total Count as Indexes of Food Quality. In: Microbial Quality of Foods . New York, USA: Academic Press.

Silliker, J. H., and Gabis, D. A. (1976). ICMSF Method Studies. VII. Indicator Tests a Substitutes for Direct Testing of Dried Foods and Feds for *Salmonella*. Canadian Journal of Microbioloy, 22, 971-974.

Skovgaard, N. (1985). *Bronchothrix thermosphacta*: Comments on Its Taxonomy, Ecology and Isolation. International Journal of Food Microbiology, 2, 71-79.

Skovgaard, N., and Norrung, B. (1989). The Incidence of *Listeria* spp. in Faeces of Danish Pigs and in Minced Pork Meat. International Journal of Food Microbiology, 8, 59-63.

Smoot, L. M., and Pierson, M. D. (1997). Indicator Microorganisms and Microbiological Criteria. In: M. P. Doyle, L. R. Beuchat, and T. J. Montville Food Microbiology - Fundamentals and Frontiers, 66-80. Washington DC, USA: American Society for Microbiology Press.

Smulders, F. J. M. (1987). Elimination of Pathogenic Organisms from Meat and Poultry. Elseviers Science Publishers B.V. The Netherlands.

Snijders, J. M. A., and Gerats, G. E. (1976). Pig Slaughter Hygiene. Fleischwirtschaft, 56, 717-721.

Sorqvist, S. (1986). Bacterial Contamination of Scalding Water during Vat Scalding of Pig. Fleischwirtschaft, 66, 1745-1748.

Sorqvist, S. (1988). Survival of *Campylobacter, Salmonella,* and *Yersinia* spp. in Scalding Water Used at Pig Slaughter. Fleischwirtschaft, 68, 381-383.

Sperber, W. H. (1991). The Modern HACCP System. Food Technology, 45, 116-120.

Stern, N. J., and Kazmi, S. U. (1989). *Campylobacter jejuni.* in: M.P. Doyle (editor) Foodborne Bacterial Pathogens, 77-110. New York, USA: Marcel Dekker.

Stone, L.S., and Zottola, E.A. (1985). Effect of Cleaning and Sanitizing on the Attachment of *Pseudomonas fragi* to Stainless Steel. Journal of Food Science, 50, 951-957.

Stringer, W. C., Bilskie, M.E., and Naumann. (1969). Microbial Profiles of Fresh Meat. Food Technology, 23, 97-102.

Tomkin, R. B. (1994). HACCP in the Meat and Poultry Industry . Food Control, 5, 153-161.

Touvinen, V. K. (1994). Control of Swine Diseases by Slaughterhouse Activities: Epidemological Studies. Helsinki, Finland: College of Veterinary Medicine, Department of Basic Veterinary Medicine Section of Animal Health.

Troeger, K. (1993). Scalding and Dehairing Technology. Influence on the Bacterial Count of Pig Carcasses. Fleischwirtschaft, 7, 1157-1160.

Troeger, K. (1994). Development of Bacteria Count in Scalding Water during Slaughter. Effect on Surface Bacterial Count in Pig Carcasses. Fleischwirtschaft, 10, 337-344.

Troeger, K., Deiter, R., and Montoya, C. (1987). Influence of Scalding and Dehairing during Pig slaughter on Meat Quality. Fleischwirtschaft, 67, 66-70.

Vu, N. Q. Unpublished Data.

Warriss, P. D. (1987). The Effect of Time and Conditions of Transport and Lairage on Pig Meat Quality. In: Tarrant, P.V: Evaluation and Control of Meat Quality in Pigs . The Netherland: Martinus Nijhoff Publisher.

Watson, W. A. (1975). Salmonelosis and Meat Hygiene: Red Meat. The Veterinary Records, 96, 374-376.

WHO. World Health Organisation (1980). Report of the WHO/ICMSF Meeting on Hazard Analysis Critical Control Point System in Food Hygiene. Geneva, Switzerland: WHO.

Wilson, A. (1985). Practical Meat Inspection. London, UK: Blackwell Scientific Publications.

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