MEAT & LIVESTOCK AUSTRALIA

On farm

A review of process interventions aimed at reducing contamination of cattle carcasses

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Final Report prepared for MLA by:

H. Haines[b]
J. Bobbitt[b]
J Simons[b]
D. Rowland[a]
K. Coates[b]

Agriculture Victoria – Rutherglen[a]
Victorian Institute of Animal Science - Attwood[b]

Meat and Livestock Australia Ltd
Locked Bag 991
North Sydney NSW 2059

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Feedlots

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Executive summary

Livestock may be colonised by potentially pathogenic microorganisms prior to slaughter, and if care is not taken during the procedures of handling, transporting, slaughtering and dressing livestock, the edible portions of the meat carcass surface can become contaminated with organisms capable of causing foodborne illness in humans. Of particular concern to consumers, and thus to producers and processors, are *Salmonella* spp. and enterohaemorrhagic *E.coli* (EHEC). Major outbreaks of foodborne illnesses in recent years have lead to the implementation of Hazard Analysis Critical Control Point (HACCP) based quality systems to control the microbial contamination of carcasses.

It is important to consider all aspects of carcass hygiene when developing contamination control strategies. This will include pre-slaughter treatment of livestock, which has been shown to influence the microbial contamination of meat surfaces. It is largely accepted as logical that livestock with high faecal contamination, mud, or dirt pose a significant threat of possible bacterial contamination on the resultant carcass. It is presumed that this threat can be minimised by presenting clean cattle to slaughter, and some work supports this contention. However, other studies indicate that the level of cleanliness of the presented animal bears little relationship to the microbial contamination of the subsequent carcass. This may be due to the effectiveness of processing interventions available to the processor. Difficulties arise when comparing the results of different studies, related to differing sampling techniques and methodologies, and different microbiological analysis of samples. Studies in which accepted standard testing and sampling practices have been used, in accordance with the Australian regulatory authorities and USDA MegaRegs, may be expected to provide more relevant information for Australian processors than other studies.

It is suggested that these discrepancies in studies which attempt to establish direct correlation between presented cattle and the microbial load on the final product are due to the many confounding contributions within the production line itself, including different line speeds and the general hygiene of the processing environment. Most studies completed within operating abattoirs may exclude excessively dirty cattle from the chain, or slow the chain to take extra care or extra trimming of the carcass in order to comply with current industry regulation.

Processing interventions that have been shown to reduce microbial contamination on carcasses include knife trimming, carcass washing using hot water, UV irradiation, steam vacuuming, steam pasteurisation, and use of chemical washes (including organic acids, chlorine, and trisodium phosphate). The effect of these treatments on the meat and hide quality of the product must be considered in any implementation strategy.

Early HACCP plans for beef processing would often identify multiple critical control points in the process. Codex Alimentarius has revised the definition of a critical control point (CCP) as a stage of processing in which control is necessary to prevent or eliminate a food safety hazard (to an acceptable level). The generally accepted CCP in the beef slaughter plant are bactericidal washes or other intervention strategies, and chilling. This does not mean that other stages of processing at which contamination may occur should not be controlled, but rather that this control is part of Good Manufacturing Process or Standard Operating Procedures.

This change in approach to CCP designation will impact on HACCP planning, as it is possible that presentation of clean livestock pre-slaughter will not be designated as a critical control point in current systems. The HACCP system is designed to provide an outcome of safe food, and the evidence suggests that this outcome can be attained despite the presentation state of cattle. However, the appeal of deleting this control point from HACCP plans may not marry well with industry obligations to meet regulatory requirements, nor maximise profitability to the producer by returns on hides.

Introduction

In the production of meat from farm animals there are a number of critical control points which influence the final microbial quality of the carcass. Quality systems, such as the Hazard Analysis Critical Control Point (HACCP) system, are increasingly used to minimise the type or extent of that contamination. The influence of processing interventions on the microbiological quality of meat has been well studied. However, clear correlation of these interventions to carcass microbiological contamination is not always possible. This report aims to provide background on the significant food poisoning organisms associated with red meats along with identification of the major sources of microbiological contamination, review the relationships established between intervention strategies on the processing line and final carcass contamination levels, and the impact of these strategies on HACCP planning.

Background

The microbiology of red meats depends upon the conditions under which animals are slaughtered and these have been well-studied (Sierra *et al* 1995). However, little attention had been given to the effects of handling practices of stock or carcasses on the resultant carcass hygiene (McGrath and Patterson 1969). The microorganisms that are of concern on carcasses are those that limit the shelf life of meat and those that cause disease in the consumers or handlers of that meat. Pathogens are of concern not only for the effects that food poisoning outbreaks have on consumer health, the meat industry, and subsequent consumer confidence, but also for the impact these organisms have on the ability of the product to meet regulatory requirements.

Outbreaks of enteric disease are generally caused by a lack of adequate controls throughout the human food chain, increasing the risks of foodborne disease to humans (USDA 1993). Livestock may be colonised by potentially pathogenic microorganisms prior to slaughter (Ayers 1955; Clegg *et al* 1986; Galton *et al* 1954; McGrath and Patterson 1969; USDA 1993). If care is not taken during the procedures of handling, transporting, slaughtering and dressing livestock, the edible portions of the meat carcass surface can become contaminated with organisms capable of causing foodborne illness in humans. Of particular importance are *Salmonella* spp. and enterohaemorrhagic *E.coli* (EHEC) (Ayers 1955; Sparling 1996; USDA 1993). Additionally, once microorganisms have been introduced into the environment of an abattoir establishment, the organisms may be easily spread (USDA 1996a).

Major outbreaks of foodborne illnesses in recent years, particularly caused by enterohaemorrhagic *Escherichia coli* (EHEC) serotypes and *Salmonella* spp. have increased public concern, leading to the implementation of new regulations governing the production of food (USDA 1996a; USDA 1996b). Many countries have responded by introducing HACCP based quality systems to control the microbial contamination of carcasses. Under these schemes, indicator organisms are used to monitor the level of faecal contamination of carcasses. The levels of these organisms are used as a guide to the likely contamination of the carcasses with pathogenic organisms that are also of faecal origin. The USDA (USDA 1996b) has not made HACCP planning mandatory at production stages prior to slaughter, but has recognised the value of (voluntary) commitment to HACCP-based food safety plans in reducing the risk of foodborne illness. Australian quality programs that impact on the provision of clean livestock include CATTLECARE™, Flockcare™, the National Saleyards Quality Assurance Program (NSQAP) and the National Feedlot Accreditation Scheme (NFAS), amongst others (Brett 1995).

Faecal contamination of carcasses occurs during the slaughter process. Carcasses can be contaminated with up to 10^6 colony forming units (cfu)/cm^2 even when faecal contamination is not a visible defect (Roberts 1980). It is therefore essential to design HACCP systems to reduce the contact of faecal pathogens with carcasses rather than rely on visual inspection systems (Hathaway 1997b). Steps can be taken to minimise faecal contamination of carcasses, but contamination cannot be entirely avoided. In establishing critical control points it is important to reduce the levels of potential foodborne pathogens in the faecal material as well as limiting the amount of faecal material on the carcass. In addition to pathogens of faecal origin, food-borne pathogens may also be introduced from the environment or process workers.

Meatborne Pathogens

The main meatborne pathogens of humans are *Salmonella* spp., EHECs, *Listeria monocytogenes*, *Campylobacter* spp., and *Staphylococcus aureus*. *Aeromonas* spp. are believed by some to be potentially significant causes of foodborne disease, and will also be described.

Salmonella spp.

Salmonella spp. are enteric pathogens (Baird-Parker 1990) which have been typically associated with faecal material. These organisms are also found in the environment, including sewage, farm effluent and other sites that have been contaminated with faeces. *Salmonella* spp. have also been isolated from the hair, wool, hides and skins, and hooves of livestock which have been either directly contaminated with faeces, or indirectly contaminated via the environment (Stolle 1981).

Salmonellosis is common in domestic animals, including cattle, sheep and pigs. The incidence of salmonella in feedlot cattle (USA) is reported as "over 8%", but the reviewer notes that "it is reasonable to expect vast differences in the carrier rate to exist between groups of feedlot cattle" (Griffin 1998). Animals at most risk of symptomatic infection are very young and pregnant animals. Animals are frequently infected with *Salmonella* spp. without exhibiting clinical symptoms (Jay *et al* 1997). The incidence of salmonellosis is increased when animals are stressed, as may occur when the animals are held in small areas such as lambing pens, on farm holding yards, at auction markets, or during transport (Grau and Smith 1974; Johnston 1990). Control of salmonellosis in feedlots requires segregation of diseased animals and good hygiene practices (Griffin 1998)

Research (Brownlie and Grau 1967; Grau and Smith 1974; Rings 1985; Wray 1989) has demonstrated that *Salmonella* spp. are capable of surviving lengthy periods of time in animal facilities at all production points from farm gate to the abattoir, although the survival time varies. Poor cleaning of yards between lots of livestock may result in dissemination of pathogens, even if the mobs are not mixed in the yards. Both Grau *et al* (1968), and Brownlie and Grau (1967) found high *Salmonella* numbers in the rumen of livestock over lengthy periods (up to 168 hours) of time post-farm gate. These authors also found that feeding after a period of starvation increased the percentage of cattle with *Salmonella* spp. in the rumen.

Escherichia coli

Similarly to *Salmonella* spp., *Escherichia coli* (*E.coli*) are enteric organisms that are also typically associated with faecal material and can be isolated from the hides and hooves of livestock (Stolle 1981). Generally, *E.coli* strains that colonise the intestine of mammals, including humans, contribute to the natural, non-pathogenic microflora of the intestinal tract (Johnston 1990; Doyle 1990). As a common intestinal organism, *E.coli* is frequently used as an indicator of faecal contamination of carcasses or meat.

However, there are many pathogenic strains of *E.coli* that have been associated with enteric disease in warm-blooded animals including food-borne disease in humans. Such strains are categorised into four main groups based on several distinct virulence properties. These are described as enteropathogenic, enteroinvasive, enterotoxigenic, and enterohaemorrhagic *E.coli* (Doyle 1990). Enterohaemorrhagic *E.coli* or EHECs have been increasingly associated with outbreaks of food-borne disease in humans.

Enterohaemorrhagic *Escherichia coli* (EHEC)

The main clinical symptoms of infection in humans by EHEC strains range from asymptomatic carriage through clinically non-specific diarrhoea, to the serious forms of the infection,

characterised by bloody diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome caused by one or more toxins (Armstrong *et al* 1996; Robins-Browne 1997). The nomenclature of these toxins is confusing: they have been known variously as "Shiga-like toxins", "verocytotoxins", verotoxins, and most recently as Shiga toxins (Stx) 1 and 2 (Robins-Browne 1997). The ability to produce the more serious disease symptoms is ascribed to some shiga-toxin producing *E.coli* (STEC), and the term EHEC is given to this subset of the STEC population. A number of EHEC have been recognised as causes of foodborne disease particularly the serotype O157:H7 in the USA (Doyle and Padhye 1989; Padhye and Doyle 1992) and serotypes O111 or O126 in Australia (Bettelheim 1996; Robins-Browne 1997). Outbreaks of O157 related disease have been linked to the consumption of undercooked ground beef and raw milk (reviewed by Armstrong *et al* 1996; Doyle 1990).

Studies have shown that verotoxin producing *E.coli* (VTEC) of many different serotypes can be isolated from animal species including sheep, pigs, goats and domestic pets (Beutin *et al* 1993). The incidence of VTEC in cattle and dairy herds has prompted the most intense study (Blanco *et al* 1996; Garber et a, 1995; Montenegro *et al* 1990; Wells *et al* 1991; Zhao *et al* 1995b) because of the links between beef products, unpasteurised milk and EHEC O157 human disease (Armstrong *et al* 1996; Borczyk *et al* 1987; Doyle and Schoeni 1987; Martin *et al* 1986; Padhye and Doyle 1992; Whipp *et al* 1994).

Studies in the USA found low incidence of EHEC O157 in beef, lamb, pork, and poultry (Doyle and Schoeni 1987; Kudva et a. 1995). Many carriers of this pathogen shed the organism into their faeces for long periods (Wang *et al* 1996) and careful handling of bovine faeces to prevent increasing transmission between animals or species is recommended. Clavero *et al* (1994) have demonstrated long-term shedding of O157 in chickens experimentally infected with small numbers of organisms.

E.coli O157 has been isolated from numerous herds of cattle in USA (Buntain 1996; Johnston 1990) and has been reported to occur at irregular intervals on most cattle farms in the USA with variable prevalence in those herds (Buntain 1996). This may be because natural colonisation in cattle is believed to be short-lived (Buntain 1996; Johnston 1990). Most researchers report higher incidence of verotoxin producing *E.coli*, including serotype O157, in young cattle post weaning (Garber *et al* 1995; Wells *et al* 1991; Wilson *et al* 1992; Zhao *et al* 1995b). Other authors have found similar proportions of VTEC in adult and young animals (Blanco *et al* 1996). The disparity between these reports may reflect the different detection methods for O157 used in these studies.

Livestock management practices that impact on incidence and shedding of O157 in cattle include:

- Weaning calves.

Garber *et al* (1995) demonstrated that post-weaning calves (greater than 8 weeks of age) are three times more likely to shed O157 than pre-weaning calves (less than 8 weeks of age). One reason put forward by these researchers for this increase is that the animals are stressed from crowding and competition at this stage, and this increases faecal shedding of pathogens.

- Contamination of yards, trucks and pasture.

  Wang *et al* (1996) demonstrated that O157 could survive in faeces for up to 70 days and still produce verotoxins. Thus, infected bovine faeces is a potential vehicle for transmitting O157 (and possibly other EHECs), allowing spread of this organism to other livestock and the surrounding environment.

- Feeding stock.

  Kudva *et al* (1995) demonstrated using a sheep model that animals negative for *E.coli* O157 may be induced to shed the organism in faeces when feed was withheld. Hancock *et al* (1997) also reported higher *E.coli* O157 prevalence in feed lot cattle where cattle had spent the shortest time on feed. This is suggested to have resulted from a triggering of the growth of the organism undetected in the gastrointestinal tract, or perhaps by increasing the animal's susceptibility to colonisation. Analysis of these findings is not simple, as Kudva *et al* (1995) indicated that while use of a low fibre-high nutrient diet may predispose animals to initial colonisation with the organism, this is followed by rapid elimination of the organism from the gastrointestinal tract. These workers feel that such feeds may thus significantly reduce the risk of animals carrying *E.coli* O157.

  The effects of different feed types on colonic pH and microflora in cattle has been studied (Diez-Gonzalez *et al* 1998), and these authors contend that feed changes may be used to affect the acid resistance (and therefore the ability to survive stomach acidity) of *E.coli*. Other researchers (Hancock *et al* 1999) have queried this finding. Work continues on the effects of feed on carriage and virulence of food borne organisms. A recent letter to the American Society for Microbiology (Russell 2000) suggests that this work supports the early findings of Diez-Gonzalez *et al* (1998), but that further research is warranted into the effect of dietary changes on the acid resistance of *E.coli* O157:H7 shedding from cattle.

It is important to note that serotypes of EHEC associated with foodborne illness in Australia are not readily identified by routine culture, although more specialised culture media have been developed (Bettleheim, 1995). At present complex molecular techniques are generally used to detect these organisms. These techniques are not suitable at this stage for rapid monitoring of critical control points (Buntain 1996).

Listeria monocytogenes

Listeria monocytogenes is wide spread in the environment (Siragusa *et al* 1992). The organism has been found to persist in soil, mud, fresh and salt water, decaying vegetation, animal feeds, fresh and processed foods, and faecal deposits from animals including humans (Gellin and Broome 1989; Gray and Killinger 1966; Lovett 1989; Woolford 1990).

More than 42 species of wild and domestic animals, as well as 17 avian species, have been found to harbour *Listeria* spp. The organism has also been detected in the faeces of both healthy and unhealthy animals (Gellin and Broome 1989; Low 1985). Although they do not produce endospores *Listeria* spp. are all relatively persistent in the environment (Woolford 1990). Environmental contamination, particularly in food processing areas, is considered the primary source for the dissemination of *Listeria* spp. onto meat (Jones 1990).

These bacteria do not cause typical enteric disease in humans (Johnston 1990), however they contribute to both consumer and producer concern. *Listeria monocytogenes* may cause significant disease in immunosuppressed individuals, and infection of pregnant women may lead to late abortion of the foetus or death of the neonate.

Campylobacter spp.

The human pathogens *Campylobacter jejuni* and *Campylobacter coli* are thermophilic pathogenic bacteria that are capable of surviving at cool (refrigeration) temperatures (Sierra *et al* 1995). They are the leading cause of bacterial gastroenteritis in developed countries (Menning 1988; Beuchat 1995).

Campylobacter spp. are enteric organisms (Van Donkersgoed *et al* 1990) that have been isolated from domestic and wild animals, birds and plants (Meanger and Marshall 1989; Luechtefeld *et al* 1981; Grau 1987; Beuchat 1995; Briesman 1985). Meanger and Marshall (1989) have confirmed cross-infection of at least two different species of *Campylobacter* between cattle and sheep. Manser and Dalziel (1985) found that enteropathogenic *Campylobacter* spp. commonly inhabit the gastrointestinal tract of livestock. This study found carriage rates of around 20% for cattle and sheep (Manser and Dalziel 1985). Although *Campylobacter* enteritis is associated with food from animal and plant origin, poultry is the most common source of infection (Beuchat 1995; Butzler and Skirrow 1979).

Staphylococcus aureus

These pathogenic staphylococci are primarily associated with food handlers, although they can also be isolated from hides or the udders of mastitic cows (Stolle 1981). *Staphylococcus aureus* is a coagulase positive staphylococcus (CPS) which produces heat stable enterotoxins that

cause food poisoning after ingestion. Foods commonly associated with *S. aureus* food poisoning include meats (beef, pork and poultry), processed meat products, dairy products and salads. Staphylococcal related food poisoning results from human, animal, or environmental sources, such as dust and water. The organism is a part of the normal flora of the upper respiratory tract, perineal region and skins of many healthy people, as well as being a cause of infections (boils, styes) and infected wounds. Animal sources of this organism, apart from the udders of mastitic cows, include the tonsils and skin of pigs, and the skins of poultry (Ash 1997). Carcass contamination of red meat with this organism may occur via dust prior to slaughter, contact with animals harbouring the organism, or via human contact during post-slaughter preparation (Lancette and Tatini, 1992). Desmarchelier *et al* (1999) found that contamination of beef carcasses with CPS increased after evisceration, with the most likely means of introducing the organism identified as the hands of the abattoir workers in the evisceration area. It is interesting that this study found CPS in foot baths in the evisceration area, and in air samples collected from around the slaughter line. They suggest that the abattoir environment may contribute to the contamination of beef carcasses with *S.aureus*.

Aeromonas spp.

Aeromonas spp. are psychrophilic organisms also found in the environment, especially in fresh and salt water, and sewage (Palumbo *et al* 1992). Initially, *Aeromonas* spp. were thought to be only pathogens of cold-blooded animals, and have been isolated from wild and farmed fish and shellfish (Fricker and Tompsett 1989).

Although the importance of aeromonads as a cause of gastroenteritis remains unclear (Hudson and DeLacy 1991), they appear to have the potential to cause infection in humans (Beuchat 1995). *Aeromonas sobria* and *Aeromonas hydrophila* have been reported as causative agents of human gastroenteritis, particularly in young children, and most often associated with contaminated water (Deodhar *et al* 1991; Joseph *et al* 1979; Daily *et al* 1980). Screening of red meat animals has demonstrated a low rate of *Aeromonas* spp. carriage (Stern *et al* 1987), but these organisms have been detected in red meat products (Hudson and DeLacy 1991; Okrend *et al* 1987; Palumbo *et al* 1985), and dairy products (Palumbo *et al* 1985). The implications of this detection are unclear.

Sources of Pathogens for Contamination of Carcasses

There are three major sources of food-borne pathogens on carcasses. These are:

- Faeces/ingesta from the animals that were slaughtered (eg. *Salmonella* spp., EHECs and others)
- slaughter environment (eg. *Listeria monocytogenes*)

- workers on the processing line (eg. *Staphylococcus aureus*)

Prior to slaughter, livestock may be colonised by potentially pathogenic microorganisms (Ayers 1955; Clegg *et al* 1986; Galton *et al* 1954; McGrath and Patterson 1969; USDA 1993). Many potential human pathogens colonise their animal hosts without causing clinical signs, making it difficult to detect carriers (Buchanan *et al* 1995). In apparently healthy livestock going to slaughter, pathogenic microorganisms are confined primarily to the gastrointestinal tract and exterior surfaces, such as the hooves, hide and skin, hair or fleece (Ayers 1955; Sparling 1996; USDA 1993), while internal organs and intact internal muscle are free of microorganisms (Gill, 1991; Johnson and Tompkin 1992). Faecal contamination is considered the primary avenue for dissemination of pathogens on the farm (Mawdsley *et al* 1995) and within the abattoir establishments (Grau 1987; USDA 1996a).

A number of husbandry practices can contribute to colonisation of livestock with potential human pathogens. These include:

- applying animal waste to pasture
- contamination of feed and water with faecal material
- use of poor quality feed (particularly silage)
- rodent or avian activity near feed stores, in pens and other livestock areas.

Once livestock are colonised with these bacteria, the faeces become a potential source of contamination for meat (Stern *et al* 1987).

Sources of Carcass Contamination

For the pre-slaughter production system under review the main areas of concern for reduction in contamination are transport, production and marketing systems.

Reducing Pathogens in Faeces

The presence and the shedding rates of pathogens in faeces are directly influenced by on-farm factors. Factors that contribute to the initial contamination of livestock are the environment (muddy, dusty), livestock types/breeds, transportation and lairage conditions at the abattoirs (Wescombe 1994). Handling methods for livestock can increase the rate of transfer, survival and growth of those pathogenic microorganisms. These may include selection of feed type, high animal stocking rates, or grouping unfamiliar animals together (as may be seen in feedlots or small paddocks). Poor handling practices may also contribute to microbial contamination, including use of uncleaned livestock pens/trucks, and mixing sick animals with healthy ones. Such factors can also affect the stress levels in livestock and increase pathogen shedding, therefore increasing the risk of exposure of animals to human pathogens (USDA 1993). By improving techniques of livestock handling systems, stress related immune suppression associated with animal processing procedures is reduced (Grandin 1984).

Management tools that can influence the incidence of colonisation of livestock with potential human pathogens include on farm animal identification and data management systems such as the (Australian) National Livestock Identification Scheme and the Beef Trading Information Systems (NLIS and BTIS). Other management practices may include diet modifications, changes in yarding practices and facilities, and in transportation and marketing systems (Buchanan *et al* 1995; Hancock *et al* 1993; USDA 1993). Salman *et al* (1993) state that such a program must be “quality assured and market driven”; that the government should act as facilitator, setting minimal standards; and that the feedback of processed information provided to the farmer is an essential tool to reinforce quality assurance.

Although the number of pathogens that can be controlled at the farm level is hard to estimate, control at the farm gate may prove a cost-effective means of reducing dangerous microorganisms (Hueston and Fedorka-Cray 1995), thereby reducing the risk of cross-contamination post farm gate to other livestock prior to slaughter. To reduce the prevalence of meatborne pathogens this approach must be followed by control further down the chain to ensure that hygiene gains made at farm level will not be lost (Zhao *et al* 1995b). These control points are identified during transport, on feedlots and in the livestock marketing systems.

Reducing Hide/Skin Contamination

The state of livestock leaving the farm or feedlot can be influenced by environmental conditions (USDA 1993). The type of weather conditions largely influence the amount of physical contamination on livestock 'on-farm' and prior to slaughter. Livestock are more susceptible to pollutants such as mud, faeces, and other foreign matter, when exposed to wet and moist conditions (Wescombe 1994; USDA 1993). Australian data (Alliance 1998) indicated that microbial counts were highest on cattle hides during winter and spring.

Dry climatic conditions reduce the risk of contamination by preventing livestock from falling, flicking mud or faeces on other animals, and splashing dirty water (Wescombe 1994). Holding yards can become dusty and this may also be a problem, as disturbed dust does eventually attach to the hide or fleece of livestock, and with it, potentially pathogenic bacteria (Wescombe 1994).

The exposure of infected livestock to other animals 'on-farm' or 'post farm-gate' increases the risk of livestock transferring microbial contaminants to others. It is usually considered that controlling this exposure and possible cross contamination depends, in part, on cleaning livestock immediately prior to (and after) transportation (USDA 1993).

Other effective approaches, as described by Zhao *et al* (1995a), include vaccination of animals and competitive exclusion. Competitive exclusion is based on the principle that the normal, non-pathogenic microflora of mature animals protects them from colonisation by enteropathogens such as EHEC. In chickens inoculation of chicks with mixed bacterial cultures derived from the gastrointestinal tracts of adult animals has been used to control *Salmonella* spp. in poultry (Fukata *et al* 1999). This technique may have application in the pre-harvest control of other enteric pathogens (Hancock *et al* 1993).

Legislation/Standards

The Federal and State regulatory authorities have a number of legislation and codes of practice that prescribe conditions of hygiene for storage and transport of animals for slaughter. In addition to effects on the hygiene of these processes, these requirements also impact on the stress of the animals, and therefore on the health and physiological condition of the animal prior to slaughter.

Australian Codes of Practice

The Standing Committee on Agriculture and the Animal Health Committee formulated Model Codes of Practice for the Welfare of Animals for animals at saleyards, transport of animals, and other issues related to the welfare of livestock (Sub-Committee on Animal Welfare 1991). Where State or Territory governments have formulated Codes of Practice for livestock handling, they are based on these national Codes, and in some states (New South Wales, Queensland and South

Australia) the national Codes are the state Codes. In so far as these codes provide guidelines for resting, feeding, watering stock, and cleaning transport and holding facilities they can affect the levels of stress and cleanliness of animals, and thus may have impact on the microbiological quality of the product.

Australian Standards

The Agricultural and Resource Management Council of Australia and New Zealand (ARMCANZ) have endorsed the following documents:

- Australian Standard for Hygienic Production of Meat for Human Consumption; AS4461:1997 (Australian Standard 1997a),
- Australian Standard for the Construction of Premises Processing Animals for Humans; AS4462:1997 (Australian Standard 1997b).

These documents set mandatory standards applicable to all processors of stock used for human consumption. AS4462:1997 (Australian Standards 1977b) provides a set of objectives for the construction of processing facilities. Section 5.6 of this standard states that:

> ***"facilities shall be provided to effectively wash or treat animals to remove contamination from the hide or skin where necessary."***

AS4461:1997 (Australian Standard 1997a) takes the processing of animals through to the chiller. The standard deals with the interaction of the supply of stock to the kill floor and their cleanliness (through ante-mortem inspection). It also deals with the interactions between the cleanliness of stock and processing rates. Statements from the standard to note are:

Section 6.1(c):

> ***"The specific aims of antemortem inspection are to prevent animals that are grossly contaminated with extraneous matter from entering the slaughter floor."***

Section 8.7 (a):

> ***"Slaughter shall proceed at a rate which allows adequate time for bodies to be dressed in a hygienic and orderly manner."***

Export Meat Orders

At a Federal level, the Australian Quarantine and Inspection Service (AQIS 1999) provides the following guidance to the interpretation of the Export Meat Orders (EMO's):

> ***"To produce and process microbiologically safe meat, it is important for a slaughtering establishment to receive clean and healthy livestock for slaughter. AQIS, through the provisions of the legislation (EMOs), restricts slaughter of cattle that are soiled or unclean, as well as daggy animals from feedlots as these animals pose a risk of contamination of meat. In addition to the requirements of the EMOs, the Australian red meat industry, under its CATTLECARE program, has undertaken the task of educating and increasing the awareness of livestock owners of the importance of clean livestock for slaughter in the delivery of safe products to meat consumers."***

Management Practices and Colonisation of Livestock by Pathogens

The colonisation of livestock by pathogens may be affected by a number of management practices.

Feed and Water

Animals are often deprived of feed and water prior to and during transport. Fluharty *et al* (1996) found that feed/water deprivation did not affect the total bacterial concentration in the rumen of calves 7-8 months old. However, as a result of denying calves feed and water prior to initial transportation Hutcheson and Cole (1986) demonstrated that the feed intake of calves after transportation was reduced which may result in increased shedding of microorganisms. As discussed previously, the impact of different feeding strategies is still being evaluated for pathogens such as *E.coli* O157: H7.

Animal feed and water sources are also critical factors as they may harbour pathogenic organisms (Johnston 1990). Water has also been identified as a source of pathogens, especially Campylobacter spp., for livestock (Jones and Watkin 1985) and *E.coli* (Whitehouse and Mele, personal communication).

Despite how carefully livestock feed is prepared, there may be subsequent contamination by wild animals such as birds during transport and/or storage of the feed (Fenlon 1985, 1986; Johnston 1990). Microorganisms have been transferred via contaminated feed to livestock on-farm and/or in feedlots all over America (Menning 1988), increasing the probability of further cross-contamination during lairage at the abattoirs (Menning 1988).

Woolford (1990) demonstrated that the prevalence of *Listeria monocytogenes* in the faeces of animals is correlated to foodstuffs given to livestock. For example in the UK, livestock fed a ration with a high proportion of silage have been linked to a high incidence of faecal excretion of *Listeria* spp. and of listeriosis (Lovett 1989; Woolford 1990). Gray (1960) found an epidemiological relationship in which the same *Listeria* serotype was isolated from the brain of infected sheep and from the oat silage on which the flock was feeding. *Listeria* spp. survive best within silage spoilt by mould growth, but in which an anaerobic environment is maintained (Woolford 1990). Where the silage has been preserved by lactic fermentation (Fenlon 1986), and the pH of the silage is less than 4, few *Listeria* are isolated. The presence of *Listeria* spp. in silage may be due to contaminated soil (Woolford 1990) and/or bird droppings acting as vectors for *Listeria* (Fenlon 1985).

Stress

Stress commonly occurs in livestock in the period between leaving the farm and slaughter. It has also been noted by many authors (Breazile 1988; Buntain 1996; Garcia *et al* 1985; USDA 1993) that various forms of stress can increase the risk of livestock shedding potential human pathogens. The responses of cattle subject to pre-slaughter stressors (including dehydration) reflect specific behavioral and physiological responses. Stress related problems are generally caused by an increase in body temperature, heart rate and circulating corticosteroid levels which affect the quality of meat by stimulating the pituitary-adrenal axis and plasma glucose levels (Warriss 1990). Pre-slaughter stressors generally include mixing of unfamiliar livestock in lairage (Wescombe 1994), prolonged transportation and depriving animals of food and water (Warriss 1990), changes in feed, or climatic changes (Buntain 1996).

As well as increasing the shedding rates of pathogens in faeces, stress responses have the potential of lowering disease resistance in livestock, making them more susceptible to contracting a clinical disease (Breazile 1988).

Pre-slaughter stress factors that not only increase shedding of bacteria in faeces but which also affect meat quality (based on high ultimate pH) include;

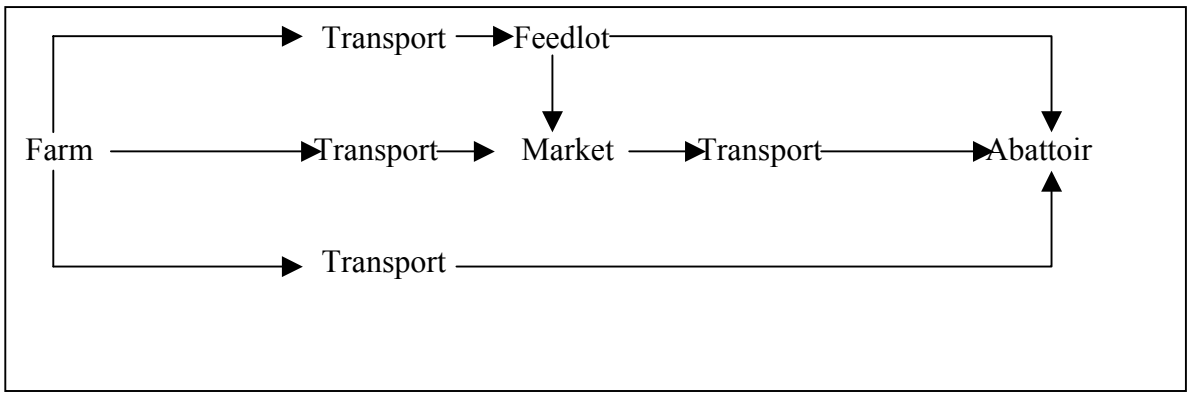
- age (Wescombe 1994)
- animal health (Wescombe 1994)
- exhaustion (Tarrant and Sherington 1980)
- insufficient rest (Tarrant and Sherington 1980)
- irregular metabolism (Thornton and Gracey 1974)
- trauma, ie. bruising as a result of rough transit, washing, etc. (Tarrant and Sherington 1980; Wescombe 1994)
- psychological stress (Van den Heever In: McLean 1984)

- poor quality feed prior to slaughter (Thornton and Gracey 1974)
- climatic conditions (Wescombe 1994)
- cattle cleaning process (Rowland *et al* 1999)

Transport

The production-cycle of farming livestock typically involves time spent in two or more locations prior to slaughter. As a result, transportation is often necessary (Figure 1) (Wescombe 1984; Hutcheson and Cole 1986). Transport can be very stressful for livestock (Johnston 1990), resulting in increased shedding of pathogens in faeces and, in some cases, a degree of immune suppression. Therefore, it is very important that correct transport practices are used. Vehicles must be designed in a way that they can substantially reduce stress and therefore shedding and contamination of animals destined for slaughter (Johnston 1990).

The most common cause of stress related illnesses is motion stress, which has been found to deplete muscle glycogen and blood glucose level during transportation (Van den Heever 1959; McLean 1984). Warriss *et al* (1990) demonstrated that physiological stress responses of livestock transported for over 3-6 hours had no significant effect on liveweight. This experiment also



indicated reduced muscle glycogen concentration (muscle pH was 5.72 - 5.70), which may result in reduced meat quality.

Figure 1: A flowchart showing the distribution channels for farmed livestock.

The duration of transport has a direct effect on increased shedding of *Salmonella* spp. in livestock. Marketing stress, including transport stress and feeding stress, was found to markedly increase the shedding of *Salmonella* spp. by feeder calves (Corrier *et al* 1990). The export of live sheep exposes the animals to many stressors, and increased stress has been associated with high shedding rates of *Salmonella* spp., and mortality from salmonellosis (Higgs *et al* 1993). Morgan *et al* (1988) found that pigs transported for less than 200km had the lowest incidence of *Salmonella* in faeces.

In addition to stress-related transport problems, the movement of livestock can contribute to the increased microbial contamination due to exposure to potential meatborne pathogens. This occurs when animals contact faeces and urine on the floor and wall rails of trucks, particularly when transported with infected livestock (Buntain 1996; Garcia *et al* 1985; Wescombe 1984). The Meat Research Corporation (MRC) (Alliance 1998) reported in a survey of ovine and bovine processing establishments that where animals were transported more than 200 kilometres to slaughter the microbial loads of carcasses were higher.

Garcia *et al* (1985), found that the growth and transmission (shedding) of *Campylobacter jejuni* in feedlot steers may have been encouraged by the stress of long distance transportation and overcrowding.

In summary, the duration of transport to the abattoir and waiting for slaughter should be kept to a minimum to reduce the dissemination of bacteria between animals and thus to meat (Shuppel *et al* 1996). Although transport stress will increase shedding of microorganisms from animals, cleaning of animals prior to transport will reduce the risk of potential contamination of animals. Cleaning of the environment, equipment and transport vehicles is also important in minimising the risk of contamination.

Marketing Systems

Livestock are constantly moved through the saleyards to the abattoirs. As a result, animals from multiple sources are frequently mixed at one or more points during the process. This common livestock marketing system can expose livestock to many types of stress (Hoerlin and Marsh 1957) and can often result in the transfer of infectious diseases between animals predominantly via increased faecal shedding (USDA 1993). Stressors that livestock are subjected to during auction sales at the saleyard include the mixing of unfamiliar livestock (Warriss 1990), overcrowding, excessive transportation and the deprivation of essential feed and water sources (Hoerlin and Marsh 1957).

The duration of the time spent in the saleyards should ensure that the livestock reach other production facilities and abattoirs in accordance with the relevant Codes of Practices.

Grau (1987) has demonstrated the increase in enteropathogenic bacteria in the faeces of animals in saleyards in a study involving 23 young calves, all of which tested positive for *C. jejuni* at slaughter. Prior to faecal sampling, the calves had passed through various sales centres, providing opportunities for cross-contamination from other livestock or exposure to other stressors leading to increased shedding rates.

Marketing alternatives available to the producer such as over the hooks, paddock sales, Computer Aided Livestock Marketing (CALM) and off farm transfers can minimise stressors to

animals associated with disrupted social settings, unusual noise and environment, and the risk of cross-contamination.

Feedlots

Grazing livestock usually have cleaner hides and fleece than animals produced in intensive feedlot systems (McGrath and Patterson 1969; Wescombe 1994). In addition, carcasses from feedlot cattle have been found to be more contaminated with enteropathogenic organisms than those from paddocked livestock (Grau 1987). This is due to high stocking densities and the potential for unpaved floors to become a quagmire after wet weather (Wescombe 1994).

Wet climatic conditions, heavy clay soils and poorly drained land, usually result in livestock arriving at the abattoirs wet with very dirty hides/fleeces which may harbour faecal contaminants (McGrath and Patterson 1969, Wescombe 1994). Livestock exposed to dry windy environments usually have a dusty exterior that may also include potential pathogenic bacteria (Wescombe 1994). Some feedlots located in dry regions of Australia use sprinkler systems to reduce airborne dust caused by the movement of cattle. The combination of dust, excess water, mud and faeces usually results in the formation of dags on the animals (Wescombe 1994). Thus, livestock in a feedlot production system present a greater problem for microbial contamination that may threaten the ability to meet domestic and international food safety regulations (McKinnon 1996).

A survey of pre-slaughter cleaning requirements conducted by Rowland *et al* (1999) indicated that for most regions of Australia, livestock cleanliness is a seasonal problem.

McGrath and Patterson reported in 1969 that the most common flooring for a production system of intensive feedlotting was solid flooring. In Australia most, if not all, feedlots have solid flooring, the limitation of which is that build-up of animal faeces can lead to contamination of livestock feed, hides, fleeces and hooves (McGrath and Patterson 1969).

The incidence of the different food-borne pathogens in feedlot cattle varies. Studies performed by Siragusa *et al* (1992) demonstrated that the level of *Listeria monocytogenes* isolated from a healthy population of feedlot beef cattle in America was low. This suggests that *Listeria monocytogenes* may not be of major concern in the feedlotting industry, provided that silage used is of good quality (Fenlon 1986; Low and Renton 1985).

However, researchers (Grau 1987) have demonstrated that feedlot finished cattle had a higher incidence of *Campylobacter jejuni* and *Campylobacter hyointestinalis* than free-ranging (paddocked) livestock. The reason for this higher incidence of *Campylobacter* spp. in feedlot cattle is unknown. It is thought that greater animal density in feedlots facilitates animal to animal spread, or that there is indirect spread via contaminated water, or that feeding practices favour the spread of these organisms (Garcia *et al* 1985; Grau 1987; Lichacz 1985).

To improve and maintain a quality assured product, feedlots must be encouraged to identify potential hazard points and approve the use of slatted flooring as a possible means for reducing the faecal contamination of housed or yarded livestock (McGrath and Patterson 1969; McKinnon 1996). Dirty livestock must also not be ignored (McKinnon 1996). A strategy suggested by McGrath and Patterson (1969) was that dirty livestock which had been cleaned should be housed post-cleaning on slatted floors, or on clean straw, for up to 24 hours prior to entering the abattoirs to reduce post-cleaning re-contamination.

Aside from reducing the incidence of pathogens in slaughter stock and limiting faecal contamination of the skins/hides of that animals pre-slaughter, the next most important control point is considered to be the cleaning of livestock as they leave the last point before the abattoirs. Literature in the field of washing livestock prior to slaughter is very scarce, however, cattle washing is common practice in Australia. The aim of cleaning is to reduce the amount of dirt/mud, and faecal contamination on livestock hides and hooves.

Pre-slaughter Washing of Cattle

The Cattle Council of Australia Guidelines for Preparation and Presentation of Cattle and Vealers for Slaughter say that cattle should be cleaned immediately prior to sending for slaughter and that these animals should be off-feed for 6-8 hours prior to transport. This is to ensure emptying of the gut, minimising defecation and cross-contamination of animals with faeces. Cattle washing can be done by either manual hosing and/or by soaking. Cattle subjected to a soaking period are sprayed with water over a longer period, enabling dags to become moistened and easier to remove (Wescombe 1994). Although manual hosing can be an effective method of removing excess dirt/mud and faeces prior to slaughter, it has limited success in cleaning secluded areas of the animal such as the underside, brisket and the inner-flanks. It has also been demonstrated that hosing for a short duration does not remove dags accumulated on the animal (Wescombe 1994).

Most abattoirs in Australia move cattle in small groups (5-20 beasts) via a wash pen from the holding areas to the knocking-pen. The wash pen has a combination of overhead, wall and floor mounted jet sprays which attempt to remove any dust, mud, and faeces from the hide of livestock prior to slaughter (Wescombe 1994).

Wescombe (1994) has also suggested that effective cleaning chemical solutions could be developed which would adhere to the hides of cattle for a desired period of time and would effectively reduce or prevent the formation of dags on cattle. Many chemical products have been tested for adherence to cattle hides over a period of several months. However, there was no microbiological evaluation of the hides during that period and, as chemical residues are a problem, more research and development in this field is required (Wescombe 1994). Commercial

chemical products that have been tested include Hoescht Nuvalb, Wool Grease, Dow Corning Silicon, ICI Polyethylene Glycol (PEG), Mobil Vital Bunca 'A' and 'B', Johnson and Johnson Baby Oil, Johnson and Johnson Cream, water based paint, Dupont MW133 and All Dull Colour (oil paint with and without kerosene).

In conclusion, pre-slaughter washing of cattle is capable of removing some dirt/mud and faeces from animals. However, in a majority of cases, dags attached to cattle (particularly the long and fine haired *Bos taurus* breeds) are not successfully removed (Wescombe 1994). More research is required to identify the best processes to remove or limit faecal contamination of animals pre-slaughter, with enzymic methods offering a possible alternative to traditional methods (Auer *et al* in press).

Hygiene status of Australian Beef

Over the last decade studies have been conducted into the hygiene status of Australian meat (CSIRO 1996; Sumner 1997; Vanderlinde 1999). In a report which analysed published data, "The Hygiene Status Of Victorian Meat (1993 – 1997)", it was concluded that

> ***"(i) in world terms, Australian meat has superior hygiene profile to that of its customers and competitors; and***
>
> ***(ii) Victorian meat has improved significantly in the past three years to equal that produced at other Australian abattoirs" (Sumner 1997).***

CSIRO (1996) reviewed microbiological levels of beef carcasses from both export and domestic work. The sampling method was not specified, other than that it was designed to "produce data compatible with recent international efforts particularly in the USA" (CSIRO 1996). Total viable counts at 25^{o}C (TVC 25) showed slightly higher levels on carcasses from domestic abattoirs when compared to export works. However, 88% of works that participated in the survey were rated as excellent, good or acceptable on basis of TVC 25. When results were compared with overseas studies, there was no significant difference between results from Australian, New Zealand, or USA product. Pathogen levels on carcasses were very low. In an international comparison reported by CSIRO (1996), USA product is reported as having generally higher levels of *Salmonella*, *Listeria* and *Campylobacter* spp. than Australian product, while higher levels of *S.aureus* and *E.coli* O157:H7 were noted on Australian carcasses than on carcasses in USA. It is important to consider all aspects of carcass hygiene in developing contamination control strategies. This will include pre-slaughter treatment of livestock, which is considered to be a major factor determining the amount of microbial contamination appearing on meat surfaces (McGrath and Patterson 1969). However the benefits of the intervention strategies applied during slaughter that are aimed at reducing visible and microbiological contamination should also

be examined. The literature on this aspect of carcass hygiene is reviewed in the following section of this paper.

Processing improvements and microbial carcass contamination

Presentation status of cattle

Strong ties exist between the visual cleanliness of cattle presented for slaughter, the production costs of processing and commercial value received for the hides. However there is still confusion in the literature regarding the correlation between the cleanliness (or rather dirtiness) of presented cattle and the bacterial load of the resultant carcass. It is largely accepted as logical that livestock with high faecal contamination, mud, or dirt (high dag/tag scores) pose a significant threat of possible bacterial contamination on the resultant carcass (Jordan *et al*, 1999; Dixon *et al* 1991; McGrath & Patterson, 1969; Newton *et al,* 1978). It is presumed that this threat can be minimised by presenting clean cattle to slaughter. Research undertaken by Grau *et al* (1968), and Grau and Smith (1974) support this contention.

Ridell and Korkeala (1993) in a Finnish study found that a solid layer of dung on the cattle hide led to significantly greater microbial contamination of the carcass. This happened despite slowing line speed to allow greater care in slaughtering procedures. In this study 21 "excessively dungy" cattle were assessed against 90 controls with animals sampled at "the end of the processing line" (presumably before chilling). Samples were excised from the shoulder and brisket for aerobic plate count at 25^{o}C. The authors argued that the exclusion of excessively dirty or dungy (sic) cattle is reasonable from the point of view of meat hygiene and that the higher microbial surface contamination of carcasses caused by excessive hide dunginess could not be compensated for by greater care in work procedures.

These findings differed from those of Van Donkersgoed *et al* (1997) who, in a Canadian study, found that carcass contamination could not be directly correlated to the tag score as a measure of the dirtiness of the animal. In this study 624 samples were taken in total from a high-line-speed (HLSP) abattoir (285 carcasses per hour) and an abattoir designated as "slow-line-speed" (SLSP) abattoir (135 carcasses per hour). The line speed of this slow abattoir runs at approximately twice the speed of most Australian abattoirs (Rowland, personal communication). The cattle were sampled by swabbing the rump and sacrum immediately after hide removal and from the brisket and top of shoulder after carcass splitting. These samples were pooled for each carcass and analysed for aerobic mesophilic organisms, coliforms and *E. coli* counts by hydrophobic grid membrane filtration (HGMF). No consistent association between tag and bacterial load could be established. It is important to note that in the HLSP abattoir the line speed was reduced for carcasses with a high (dirty) tag score, and tag was shaved off in response to that score. No line speed changes were made at the SLSP abattoir since the slower speed enabled the workers to take extra care when needed. Thus in contrast to the findings of

Ridell and Korkeala (1993) the higher microbial surface contamination of carcasses caused by excessive tag contamination could possibly be compensated by greater care in work procedures. Van Donkersgoed *et al* (1997) recognised that although the level of tag or dirtiness of cattle may not be directly linked to bacterial load after careful processing, there still remains issues of the impact that dung and dirt have on visual contamination and process costs. High levels of tag can affect the cost of production by decreasing line speeds and increasing labour cost for additional trimmers. High tag levels can also damage the leather of the hide, and adversely affect consumer perception of the beef industry. The observations of Van Donkersgoed *et al* (1997) are consistent with those of several others. Bell *et al* (1994) for example observed that the microbial contamination of carcasses was lower in abattoirs of lower line speed. Gill *et al* (1998) and Gill & McGinnis(1999) reported that microbial load of the carcass was linked to the hygiene of the processing line and to the establishment and maintenance of appropriate HACCP systems. Elder *et al* (2000) have recently reported that the prevalence of *Escherichia coli* O157:H7 or O157:nonmotile (EHEC O157) in faeces or hides correlated significantly with carcass contamination. In this study, the carcasses were sampled at pre-evisceration, post-evisceration before “antimicrobial intervention” and post-processing after the carcass had entered the cooler. The prevalence of EHEC O157 at each of the 3 post-processing sample times was 43%, 18% and 2% respectively. Reduction in carcass prevalence from pre-evisceration to the chiller suggests that procedures were effective within the processing plants in minimising microbial load.

Australian Studies

A recent report by Rowland *et al* (1999) in the “Preparation and Delivery of Clean Livestock” project conducted for MLA found no direct correlation between the dag loading of the live animal and the microbiological quality of the carcasses. In this study, 320 cattle (over winter and summer) were presented for slaughter in either an uncleaned state or after a cleaning treatment. The cleanliness of the presented cattle (before the cleaning treatments) was assessed using the UK Clean Livestock Grading Scheme that has a score ranging from 1 (cleanest animals) to 5 (excessively dirty animals). Uncleaned dirty cattle presented for this study had scores between 1 and 3. However no microbiological differences were observed between the cleaned and uncleaned treatment groups.

This could be attributed to a generally slower line speed (compared with EU and Canadian systems), that allows time for more careful removal of hides and adequate trimming of visible contamination. In addition, the samples were assessed after 12 hrs of chilling (USDA 1996b) and therefore a reduction in mesophilic bacterial load is expected as a result of 12 hrs of surface drying at low temperature (as also seen by Elder *et al* (2000)).

In contrast to the work of Rowland *et al* 1999, Alliance (1998) did observe a correlation between the microbial load of the hide and that of the carcass. In this study hides were removed from the cattle then assessed for cleanliness using ARMCANZ and AQIS carcass hygiene assessment systems before any further cleaning treatment. In assessing microbial load, swab samples were collected from brisket, flank and topside of the hides (prior to removal), after hide/skin removal and evisceration (but prior to trimming and washing), and after trimming and washing. Although microbiological loads on the carcass were low ($<10^4$cfu per cm^2) there was a statistically significant difference between the microbial loads of carcasses from either dirty or clean hides Although both Rowland *et al* (1999) and the Alliance studies were carried out in Australian abattoirs utilising HACCP plans, it is difficult to compare these studies due to the many otherwise different experimental conditions. Rowland *et al* directly compared the post chilling carcass microbial load to the cleanliness of the presented cattle before slaughter. Alliance compared the carcass microbial load to the visual cleanliness of the hide after its removal. The studies used different assessment schemes for determining cleanliness. Alliance (1998) did not indicate whether final bacterial assessment of the carcass was conducted pre or post chilling (and if post chilling, the time spent in the chiller). Chilling of the carcass for at least 12 hrs is a regulatory requirement since it has been shown to reduce the carcass bacterial load (Adams & Moss 1995, Vanderlinde 1999). It is worth noting that some studies indicate that the reduction in TVC 25 is not consistently observed (Roberts 1980), however, the same authors noted that Enterobacteriaceae numbers “generally fell” after chilling.

Other findings from the two Australian studies (Alliance 1998; Rowland *et al* 1999) were in agreement. Both studies found a link between hair length and hide contamination, and also that animals processed during winter were more likely to present with greater hide contamination than those processed during summer.

It is suggested that discrepancies seen across different studies, attempting to establish direct correlation between presented cattle and the microbial load on the final product, are due to the many confounding contributions within the production line itself. In order to be practically relevant, most studies are completed within an operating abattoir that still has to comply with current industry standards to ensure the safety of the plant. This may mean the exclusion of excessively dirty cattle from the chain, or slowing the chain to take extra care or extra trimming of the carcass. Line speeds can vary greatly between countries and even between individual abattoirs, and is recognised as a major contributor to transfer of microbial loads from hide to carcass (Van Donkersgoed *et al* 1997, Bell *et al* 1995). Hygiene of processing will also impact on the final product (Gill *et al* 1998; Gill & McGinnis, 1999). The contribution of these and other factors will impact on the level of microbial load on the carcass and whether the correlation to the presented animal can be established.

There is confusion within the industry regarding the application of microbiological studies where the carcass samples have been collected by different methods, processed differently, or sampled from carcasses at different stages of different processes (Sumner 1997; Rinehart and Foster personal communication). Recent Australian studies (Rowland *et al* 1999) have used current industry standards such as the sampling protocols specified in the USDA/FSIS "Final rule on pathogen reduction and Hazard Analysis and Critical Control Point (HACCP) systems" (Megaregs) (USDA 1996b).

Comparing Sampling techniques

A wide range of sampling techniques has been used for the collection of microbiological samples. These include:

- 3 site sponge sampling (Bacon *et al* 1999, Rowland *et al* 1999)
- Excision method (Dorsa *et al* 1997; Jericho *et al* 1997; Kain *et al*, 1999; Sofos *et al* 1999a)
- 2 site swab sampling (Van Donkersgoed 1997)
- Diverse carcass sites (i.e. other than the US/Aust. standard sites of rump, flank, brisket) (Gill and McGinnis 1999; Gill and Jones 1999, Lasta 1993).

Some work has been undertaken to compare different sample collection methodologies. Coates (1997) and Dorsa *et al* (1997) have compared the microbiological results obtained from excision samples with those obtained from three site sponge samples. Both studies indicated that sponge sampling recovers fewer microorganisms from carcasses than excisions, a finding supported by Kain *et al* (1999) and Untermann *et al* (1997) in a review of the literature. Of particular note is the finding that recovery rates of sponge and excision samples are related (in general sponge recovery is approximately 50% of that of excision) and operator dependent (Coates 1997). In contrast, however, Gill and Jones (2000) determined that there was no significant difference between samples collected from beef and pig carcasses collected by excision of sponge sampling at the end of the carcass-cooling period. Coates (1997), Dorsa *et al* (1997) and Gill and Jones (2000) used different microbiological methods and temperatures for their assessment (standard plate count, spiral plating method and Hydrophobic Grid Membrane Filtration, respectively) which may account for some of the difference in these findings. Gill and Jones (2000) also note that comparison of samples from freshly dressed and cooled carcasses with samples taken at later stages of processing "on which a spoilage flora has developed" is not valid. These issues are discussed in following sections of this review.

In order to compare information, Sumner (1997) correlated swab, sponge and excision results in a report on the hygiene status of Victorian (Australia) meat. In this report, Sumner used a ratio of 0.5:1.0 (sponge: excision) to equate excision samples to sponge results, while a conversion

factor of 1:5 (swab: sponge) was used to equate swab results to those obtained by sponge sampling.

Despite the recognition that sponge sampling recovers fewer bacterial counts, it has been adopted as the industry standard by the US MegaRegs and implemented in Australian export meat works.

Microbiological testing methods

Incubation temperature

Much of the US microbiological data is generated using incubation temperatures of 37°C (TVC 37) (Dorsa *et al* 1997; Sofos *et al* 1999a). TVC 37 will only account for the mesophilic component of the total bacterial population, as psychrophilic organisms will not grow at these temperatures. By using incubation temperatures of 25°C both mesophilic and psychrophilic organisms are more likely to grow. Bell and Hathaway (1996) estimate that incubating at 37°C will result in a reduction of log 0.65 against samples incubated at 25°C. This may in part account for the apparently lower TVC of US product when compared with Australian product. Comparison of Australian and US findings must recognise the different incubation temperatures used. Rowland *et al* (1999) and other Australian studies have used incubation of 25°C in accordance with current Australian standards and US MegaRegs.

Testing methodology

The comparisons between different testing methodologies may also contribute to the apparent difference in results. The Hydrophobic Grid Membrane Filtration (HGMF) technique utilises a square membrane filter (60 x 60 mm) with a pore size of 0.45μm, with a black hydrophobic grid that outlines 1600 small squares (Cox and Fleet, 1997). Samples are usually filtered onto the membrane, which is then placed onto culture media, whereupon interpretation and processing of data follows similar principles to most probable number (MPN) techniques. Alternatively, the organisms may be detected using fluorescent or other staining methods. HGMF methods have a number of advantages over more traditional methods of bacterial enumeration. They are filtration methods and so inhibitors or interfering substances can be removed from the culture environment. Some authors report that these methods offer increased precision and reproducibility than more traditional cultural methods (Peterkin *et al* 1989). In a study of the HGMF method (Jericho *et al* 1993) excised carcass surface samples that were then washed, the suspension inoculated onto the HGMF filter and the filter cultured at 37°C. Cultures were assessed using an automated counting method (described in more detail by Parrington *et al* 1993). The concentration of the vital stain routinely used in culture media for this method, triphenyltetrazolium chloride (TTC), studies been shown to be inhibitory to some organisms

(Parrington *et al* 1993). A number of studies have been conducted to compare HGMF with standard plate count methods. Greer and Dilts (1997) compared HGMF to conventional plate count methods as a means of enumerating meat borne spoilage bacteria and found no significant difference between the methods on either artificially or naturally contaminated meat. It is worth noting that TTC was not incorporated into the culture media in this study, but added to the filter post incubation and allowed to develop for 15 minutes prior to reading (Greer and Dilts 1997; Parrington 1993).

These results differ from those of Jericho *et al* (1996), in which samples excised and processed by HGMF were compared with both a plate count method (TVC 37) and a relatively new method, flow cytometry. In this latter study, it was found that HGMF with TTC in the culture media produced significantly lower aerobic counts than the plate count method. Application of TTC to the membrane after incubation gave higher counts, but these were still significantly lower than the TVC 37 method. Flow cytometry results did not correlate well with viable cell numbers. These authors (Jericho *et al* 1996) conclude that HGMF may be appropriate in situations where "absolute counts" are not essential (such as HACCP or process monitoring), but that consistent choice of method is necessary, as comparison of data between methods is difficult.

Dorsa *et al* (1997) utilised yet another culture method for their analysis of carcasses during processing. The Spiral plating system is essentially an automated plate count system, in which a liquid sample is inoculated in a spiral pattern onto a rotating agar plate, creating a "three-log dilution effect" (http://www.spiralbiotech.com/refurb.html). The elimination of the requirement to prepare serial dilutions of samples, and thus the labor and potential error associated with standard plate count methods, is seen as a major advantage of the spiral plating system (http://www.microbiology-intl.com/wasp.htm). After incubation plates can be counted manually, or using an automated system. The spiral plate system is an AOAC and APHA recognised method for the detection of microorganisms in food, milk and cosmetics (http://www-seafood.ucdavis.edu/haccp/compendium/chapt09.htm#Spiral Plate Method).

There is little information comparing the use of this system with standard methods for meat carcass testing, probably reflecting that this method is essentially an automated variation on the standard plate count method.

The difficulties in comparing different methodologies highlight the necessity of adopting and consistently using standard methods (as regulated) to make fair and accurate comparisons of abattoirs.

Time of sampling

The point of the process at which sampling is undertaken also serves to make comparisons difficult. In the literature reviewed a number of locations along the sampling chain were cited as the sampling points. These locations include:

- Post hide removal (Schnell *et al* 1995, Sofos *et al* 1999a, Van Donkersgoed *et al* 1997)
- Pre-wash (Dorsa *et al* 1997, Jericho *et al* 1995, Jericho *et al* 1997)
- Pre-trimming (Reagan *et al* 1996)
- Prior to chilling (at the end of the process chain) (Dorsa *et al* 1997; Gill and Jones 1999; Gill and Jones 2000; Riddell and Korkeala 1992)
- After chilling (12 or 24 hours) (Bacon *et al* 1999, Dorsa *et al* 1997, Kaine *et al* 1999, Rowland *et al* 1999, Sofos *et al* 1999a)

Determination of the risk factors associated with production is intrinsic to the development of HACCP plans. Dorsa *et al* (1997) and Sofos *et al* (1999a) have undertaken studies to evaluate the impact of processing on the microbiology of carcasses, each sampling at 3 sites along the chain. Dorsa *et al* (1997) assessed the carcasses at pre-wash, post –wash and after 24 hours in the chiller, using an automated spiral-plating system to prepare cultures, incubating at 37^{o}C . The results of this study indicated that total aerobic bacterial counts were significantly different at the three processing stages tested and highest results were obtained after 24 hours chilling. The authors noted that “this result was not expected” but the significance of this finding is difficult to assess. The authors suggest the increasing bacterial numbers post chilling may be attributed to moisture on the carcasses from sponge sampling prior to chilling. Alternative explanations for these findings include the growth of psychrotrophic spoilage organisms on carcasses in the chiller, influenced by other processing factors such as the crowding of carcasses in the chillers, or condensation problems for the chiller. Sofos’s group (1999a) conducted microbiological studies at post hide removal, post wash and after 24 hours in the chiller. In this study, the distribution of *Salmonella* spp. was a particular focus, the findings across 7 processing plants led the authors to suggest that plant contamination from dirty animals is important in the transmission of enteropathogenic organisms. These authors used a plate count method (Petrifilm™) to enumerate TVC 37, and found TVC 37 was significantly higher post hide removal compared with samples collected at the final carcass wash and the post 24 hours chilling. Sofos *et al* (1999a) found there was no significant difference between TVC 37 at the final carcass wash stage or after 24 hours chilling, and concluded that

> ***“individual plants will need to assess their operations and determine procedures that will help them consistently slaughter and dress carcasses of low microbiological contamination”*** **(Sofos *et al* 1999a)**

Intervention strategies

Trimming

Studies have shown that intervention practices along the processing line may reduce bacterial load caused by faecal contamination. Reagan *et al* (1996) showed that knife-trimming to remove visual contamination and washing of the carcass consistently resulted in low bacterial populations and visual scores for faecal contamination. In their study, cattle were deliberately contaminated with faecal material obtained from the external surface of the hide of each carcass, and were subjected to standard knife trimming in accordance "with USDA-FSIS zero tolerance standards for faeces and other visible material". The carcasses were sampled at several points of the processing line by excision at the "inside round" and analysed for pathogens and general hygiene indicators. Visual scoring of the carcasses using an arbitrary scale (applied by trained personnel) and microbiological analyses were conducted. A significantly lower visual score (indicating "cleaner") was seen in the carcasses that were trimmed and washed (i.e. processed to current US industry practices). Trimmed carcasses, those treated with hot water (74 – 88^{o}C), ozone and peroxide treatments had higher visual scores than the trimmed and washed carcasses. Both trimming and washing and use of hot water decontamination produced around 2-log reduction in aerobic bacteria. Ridell and Korkeala (1993) found, in a very small study, that increased microbial contamination of excessively dirty cattle was seen despite careful trimming of the carcasses from these animals.

Carcass washing

In the study by Reagan *et al* (1996) hot water (74 – 88^{o}C) decontamination appeared to produce a more uniform reduction in the bacterial load of carcasses. Gill *et al* (1999) found that treatment of beef carcass sides with water of 85°C for 10 seconds would substantially reduce the numbers of bacteria on the meat without unacceptable damage to the appearance of the product. Carcass sides were assessed after treatments and cooling by swabbing technique and analysis for *Escherichia coli* O157:H7, aerobic plate counts and *E. coli* counts.

Jericho *et al* (1995) studied the effect of washing carcasses and found that washing did not make a major impact on bacterial contamination of carcasses, however, in this study the water temperature was only 38^{o}C. In a laboratory based study on the efficacy of washing meat contaminated with a "faecal paste" Cabedo *et al* (1996) found that time of contact between the faecal material and the meat was significantly associated with the extent to which trial treatments removed bacteria. They concluded, however, that in general washing with 74^{o}C was "the most effective treatment for reduction of bacteria numbers" compared with water at 34^{o}C, 2% acetic acid, 5% hydrogen peroxide, or 12% trisodium phosphate solution.

Chemical de-hairing of carcasses

The objective of chemical de-hairing of carcasses is to remove all external contaminants such as mud and faeces prior to hide removal, and thus to reduce the potential bacterial contamination of the carcass (Sofos and Smith, 1998). Schnell *et al* (1995) found that the use of chemical de-hairing of cattle during the processing chain decreased the amount of visual contamination on beef carcasses, but that the de-haired carcasses had no lower bacterial load than the conventionally slaughtered animals. They further concluded that visual contamination did not correlate well with bacterial cleanliness. In this study cattle were sampled at several points of the processing line, by excision, at the brisket, flank and inside round and analysed for *Salmonella* spp., *Listeria* spp., *Escherichia coli* O157:H7, aerobic plate counts and *E. coli* counts. The study made no comment on the level of dirt on the cattle presented at the time of slaughter. Other work (reviewed by Sofos and Smith, 1998) has shown that hair-coat coliform contamination was reduced on chemically de-haired carcasses before removal of the hide, and also on the skinned carcass surface. An important consideration with the use of chemical de-hairing is the loss of profit from the resulting carcass hide.

Other carcass treatments

As these treatments have been recently reviewed (Dorsa 1997; European Commission 1996; Sofos and Smith 1998; Sofos *et al* 199b), they will be discussed only briefly here.

Ultra-violet (UV) irradiation

The use of UV irradiation as a carcass decontamination method has been investigated by a number of authors (reviewed by Thayer *et al* 1986). The use of UV in fresh meat processing has been limited by adverse organoleptic changes in the product, including colour and rancidity changes. A commercial operations (http://cvpsystems.com/) asserts that UV irradiation can extend the shelf life of modified atmosphere packaged (MAP) pork, there is little data regarding the use of this technology with beef carcasses. One Australian processor has incorporated an UV decontamination step, as part of the process chain (Haines, personal communication), but no further information is available at this time.

Steam vacuuming

This cleaning system was reviewed by Dorsa (1997). This equipment utilises the properties of both hot water and steam to remove visual and microbiological contamination via vacuum. A number of researchers have found this technique to be effective in reducing faecal bacterial numbers on the carcass, although variation in the reduction levels obtained was noted (Dorsa, 1997; Sofos and Smith 1998), suggesting that equipment and usage factors impact on the

efficacy of this technique. The process is approved by the USDA as an alternative to knife trimming for removal of visible contamination on the carcass, and is used in at least one Australian processor (Bobbitt, personal communication).

Steam pasteurisation

A number of studies have been conducted on the use of steam as a decontamination technology for red meat surfaces, with early studies indicating that unacceptable changes in the meat colour resulted (reviewed by Dorsa 1987). This technology has been increasingly adopted in US beef processing plants, with Frigoscandia claiming that steam pasteurisation is used to treat 60% of the US beef carcasses (Research and extension news from Kansas State University, http://www.oznet.ksu.edu/dp_news/sty/3meat3a.htm). In their review, Sofos and Smith (1998) found that steam pasteurisation reduced the mean numbers of organisms on beef carcasses where used, but that the impact of this expensive technology depends on other processing factors, particularly the potential re-contamination of meat during subsequent processing.

Organic acids

The European Commission (1996) has reviewed the use of lactic and other organic acids. They note that these compounds have the advantage of being generally regarded as safe (GRAS), and well accepted by consumers. They note that experimental studies do not indicate any superiority of action of these compounds, but that they appear to be more effective when used in conjunction. A number of factors affect the efficacy of these compounds, including the pH of action, the time and duration of treatment, and the nature of the meat surface involved. Antibacterial activity is more pronounced on fat surfaces of meat, and the presence of organic material such as blood or intestinal contents will reduce the efficacy of the compounds.

Chlorine

The use of chlorine as a decontaminating chemical for carcasses has also been part of other reviews (European Commission, 1996; Sofos and Smith 1998). The view expressed by European Commission (1996) regarding chlorine and chlorine dioxide is that the rapid inactivation of chlorine by organic material renders the technology ineffective in carcass decontamination. This is at odds with the material reviewed by Sofos and Smith (1998) citing studies in which 200 parts per million (ppm) chlorine reduced Salmonella on beef forequarters, and 800 ppm used in a spray washing system reduced *E.coli* O157:H7 by 1.3 log cfu/cm^2. Sofos and Smith (1998) also note the issues of high concentration chlorine use in carcass

contamination, including corrosion of metals in the processing environment, and the potential to form harmful reaction compounds with organic materials present on the carcass.

Trisodium phosphate (TSP)

Trisodium phosphate is a highly alkaline compound (European Commission 1996) used to decontaminate poultry (Coppen *et al*, 1998) and beef carcasses (Sofos and Smith 1998). Coppen *et al* (1998) found that use of AvGard™ (a trisodium phosphate immersion carcass wash) reduced Salmonellae, Enterobacteriaceae and total aerobic counts of broiler carcasses after 15-second immersion in tanks. Experimental studies reported by Sofos and Smith (1998) using artificially contaminated beef also indicated that the compound was efficient in reducing pathogen numbers. A study was conducted in lambs in which excised lamb breasts were inoculated with faecal paste, spray washed and then decontaminated with either lactic acid, 12% TSP or a combination of both techniques. TSP produced a reduction in *E.coli* and APC, but this reduction was less than that achieved with the combination treatment (Savell J.W., Texas A&M University reported in "Executive summaries of research project progress, fiscal year 1998-1999" http://www.utexas.edu/depts/bbr/natfiber/tffc/progress/progress98-99.html).

In a study of multiple processing interventions, Bacon *et al* (1999) studied the effects of steam vacuum, pre-evisceration carcass washing, organic acid application and thermal pasteurisation (71 – 76^{o} C) at 8 different beef slaughtering operations. Their results support the concept that multiple decontamination processes are effective in reducing carcass bacterial loads.
Kain *et al* submitted a report to the US National Cattlemen's Beef Association by in 1997, which found that

> **"factors such as extent of mud presence on animal hide, manure wetness, ambulatory score and body condition of live animals had no major influence on bacterial counts of resulting carcasses" (reviewed by Sofos *et al* 1999b).**

The literature reviewed here, and by others (Dorsa 1997; European Commission 1996; Sofos and Smith 1998; Sofos *et al* 1999b) supports the contention that interventions during processing can effectively reduce the microbial contamination of carcasses, in the context of hygienic processing operations.

Application of HACCP to reduce slaughter line contamination

The Hazard Analysis Critical Control Point (HACCP) system aims to identify problems and define measures for the control of microorganisms at all critical stages in production to ensure a safe product (Hueston and Fedorka-Cray 1995; Notermans *et al* 1995). The design and implementation of an effective HACCP program involves a systematic approach to identify, assess and control hazards at all steps of the food production chain, including pre-slaughter treatment of livestock.

Livestock production practices have been identified as playing a significant role in the control of microorganisms of public health concern (USDA 1993). Procedures are required to help livestock industries to prevent, eliminate or reduce the levels of zoonotic pathogens colonising animals throughout the livestock production system (USDA 1993). HACCP systems that have been implemented in the Australian livestock production system include the CATTLECARE™ and Flockcare™ programs.

An effective pre-slaughter HACCP plan is dependent upon the identification of those individual steps where contamination can best be controlled (Biss and Hathaway 1996; Hancock *et al* 1993). The contamination on the hide of livestock, which according to Hancock *et al* (1993) is the major source from which cross-contamination occurs. This should be considered as a critical control point (CCP) in the HACCP plan. Another potential CCP may be identified during carcass dressing at the abattoir, where potential cross contamination is again dependent (in part) on the condition of the skin or hide of the stock at slaughter. Therefore during the period between the farm-gate and the abattoir it is critical to reduce both the shedding of pathogens in faeces and the extent of faecal contamination of the skin/hide of the animal pre-slaughter.

The quality of the incoming material is pivotal to the successful application of most HACCP plans, and in many HACCP plans the quality of these ingredients, in this case live animals, is considered the first CCP for the process. The level of dag/tag contamination on live animals entering the slaughter plant is a quality issue that should be assessed by the quality assurance or regulatory personnel on-site as currently occurs in Australian abattoirs. Acceptance of particularly contaminated animals may require slowing the chain speed to allow for trimming that would not otherwise be required on a carcass originating from a less heavily contaminated animal (Van Donkersgoed 1997).

The potential food safety hazards associated with the live animal are biological (e.g. the form of pathogenic bacteria), chemical, such as residues, and physical (e.g. broken needles) (Troutt *et al* 1995, USDA 1999). Troutt *et al* (1995) highlighted the need for livestock production systems to incorporate "pre-harvest food safety programmes (sic) for residue avoidance and selected microbial reduction or elimination" into the pre-existing veterinary procedures of the farm/feedlot. Programs to control the spread of enteropathogens include

- ensuring good farming practices are followed
- veterinary monitoring
- identification of disease outbreaks
- certification of herd health (Mackey and Roberts 1993).

The authors also warned of the potential for spread of these pathogens when new animals are introduced into the feedlot, and risk of disease spread is also via contaminated feed (hence the need for elimination of *Salmonella* from feed) and poor waste disposal practices (Mackey and Roberts, 1993). Recognition of conditions that can be controlled on the farm/feedlot contributes to ensuring the ultimate quality of the carcass. These conditions include abscesses, excessive bruising, foreign objects, and "filth" on animals (Troutt *et al* 1995).

Although on-farm practices will differ when animals presented for slaughter are sourced from feedlots, extensive (pasture) farming or are mature animals previously used for milk or calf production (NACMCF 1993), the potential for contamination of the final product is similar regardless of the farming system. The likelihood of human pathogens entering the slaughter line can be controlled somewhat by implementation of the strategies detailed below.

Mixing of animals from different origins and social groups at markets, during transport and in lairage (Mackey and Roberts, 1993) contributes to the risk of contaminating animals with foodborne pathogens.

Transportation contributes to increased incidence of contamination by stress on the animals and increased risk of exposure of cattle to potential human pathogens (NACMCF, 1993). Limiting the likelihood of contamination involves:

- Minimising transport time
- ensuring that trucks are maintained in order that animals cannot be injured during transport

- ensuring the hygiene of the trucks are maintained so that they are free of faecal material prior to loading cattle and are cleaned and sanitised once animals have been loaded out (Mackey and Roberts 1993; NACMCF, 1993).

Faecal contamination of animals during transport can be limited by withholding feed 3-6 hours prior to transport. (Mackey and Roberts 1993). Multi-level trucks should be designed so that faeces does not fall from the upper levels to the lower levels.
The marketing system in use may have bearing on the health status and consequent risk of contamination of a particular animal destined for slaughter, therefore the highest standards must be maintained to ensure the quality of the cattle is not compromised when moving through these systems (NACMCF 1993).
Holding animals for long periods in lairage is undesirable as this increases the chance of cross contamination of animals from different origins (Mackey and Roberts 1993). One strategy for avoiding excessive contamination via the hide of the live animal is to exclude grossly contaminated animals from the abattoir (Mackey and Roberts 1993). Alternatively systems for scoring cattle according to the amount of faecal contamination on arrival at the abattoir may be introduced, and highly contaminated animals are then processed at the end of the slaughter line (Jordan *et al* 1999b).
Stress plays a role in increasing the risk of shedding potential human pathogens as stressed animals show a reduced resistance to disease, and are therefore more susceptible to pathogens (NACMCF 1993). Factors such as animal density, frequency of feedlot pen use, and co-mingling of sick animals can affect stress levels and so the risk of human pathogen exposure (NACMCF, 1993).
Feed and water contamination are potential sources of microbial contamination to cattle, and feed must be free of *Salmonella* and water must be from clean sources (NACMCF, 1993). It is now recognised that feeding food animals rendered by-products is an unacceptable practice (http://www.affa.gov.au/ocvo/pub/mfsi/chap3.html#3.2).
The ability to trace animals from farm to fork (traceback) is an increasingly important issue for the industry. Mechanisms such as the National Livestock Identification Scheme supported by MLA and DNRE are commendable and will contribute to the accountability of not only the slaughter plant but also the farmer for the safety of the consumers of their beef products.

Jordan *et al* (1999a) proposed a number of potential on-farm strategies to reduce the likelihood of contamination of beef carcasses with foodborne pathogens. These included

- efforts to reduce pathogen prevalence in infected herds ***"or reduce the prevalence of infected animals within infected herds (or both)"***.

- reducing ***"the opportunity for cross-contamination of animals and carcasses by acting on knowledge of the pathogen status of animals lots, trucks or herds".***
- control ***"the transfer of faeces onto carcasses by reducing the extent of faecal contamination present on the hide of animals prior to slaughter"*** by encouraging producers to market cattle with less faecal contamination of hides.
- reduce ***"the number of pathogens per gram of faeces"*** by modifying feed rations, using chemotherapeutic agents against the pathogen in question, or by the use of probiotic bacteria.

One strategy that is proving successful in the pig industries of various countries (Denmark, Australia, and the Netherlands) is the introduction of nation wide serological testing of pigs for *Salmonella* to indicate the prevalence of the pathogen within herds. This information can be utilised in various ways such as shunting test-positive animals to the end of the slaughter line or excluding test-positive animals from the slaughter consignment (Jordan *et al* 1999).
It has been recognised that contamination of carcasses results principally from the gastrointestinal tract or the hide of cattle (Hathaway 1997b; Mackey and Roberts, 1993; Troeger, 1994). The slaughtering process is designed in such a way as to minimise contamination. The implementation of HACCP systems, and their management components, at the slaughter process gives further assurance of the industry's commitment to the production of high quality meat. These systems identify the potential human health hazards and the associated risk of infection from consumption of the foodstuff in question, and identify steps in the process at which these hazards can be controlled (critical control points) (Hathaway 1997a). It is important to note that prior to 1997 Codex Alimentarius defined a critical control point (CCP) as a step at which control can be applied and is essential to **prevent, eliminate, reduce or minimise** a food safety hazard to an acceptable level. This definition was amended in 1997 (Codex 1997) to a step where control can be applied and is essential to **prevent or eliminate** a food safety hazard to an acceptable level, where an acceptable level is based on industry level/data or standards. In light of the 1997 amendment fewer of the operational processes on a slaughter line can be defined as a CCP, hence the move from plans with multiple CCPs to plans with only a few 'true' CCPs.
Areas of the process which have been documented as ones at which contamination is more likely to occur include

- receival/holding of the animals
- stunning
- bleeding
- head removal

- rodding
- hide/fleece removal
- evisceration and handling of viscera
- splitting of the carcass (NACMCF 1993, Mackey and Roberts 1993, Toeger 1994, Hathaway 1997a).

The receival and holding of the animals is a potential point for contamination and was often considered a CCP in early HACCP plans. At this stage animals are checked for clinical symptoms of disease. Co-mingling of animals occurs, providing opportunities for cross contamination, and can also induce stress in the animal (Brunner *et al* 1996). Stunning has been implicated in contamination if the delivery area and trap is not regularly cleaned (Troeger 1994). The entry wound following sticking may be problematic in terms of microbial contamination, as may be the head removal if inadequate cleaning and hosing is undertaken (Troeger 1994). Unskilled tying of the oesophagus may result in spillage of ingesta, contaminating the carcass (Mackey and Roberts 1993; Troeger 1994). Flaying is a site of carcass contamination, as if this is done ineffectually the hide can contact the freshly exposed carcass. Other problems that may occur at this stage include the knife cuts through the hide onto the carcass surface, or hands or equipment of the slaughterman touching the carcass and transferring bacteria (Mackey and Roberts 1993, Troeger 1994, Brunner *et al* 1996). Contamination can be minimised at this point with the use of skilled slaughterman who use a downward pulling action for hide removal, and the employment of hygiene and sanitisation standard operating procedures such as dipping knives as often as is practicable in hot (82°C) water (Mackey and Roberts 1993). Evisceration and viscera handling poses a microbiological problem as unskilled removal may result in spillage of ingesta or faecal material (Troeger 1994), or the gut may be punctured by a knife (Mackey and Roberts 1993). This potential contamination can be controlled by tying off the gullet and closing off the anal end of the gastrointestinal tract in a plastic bag (Mackey and Roberts 1993). Splitting the carcass may pose a minor point of contamination (NACMCF 1993) as bone dust and cross-contamination can easily fall onto the carcass.

Given the multitude of potential contamination points along the beef slaughter chain, in excess of 7 CCPs could be stipulated in early generic or proposed HACCP plans (NACMCF 1993, Troeger 1994, Brunner 1996). Now, in line with the revised Codex definition of a CCP, there has been a shift from multiple-CCP plans to more easily monitored plans with fewer CCPs (NACMCF 1993; USDA 1999). It is now recognised that many of the steps in the slaughter line, though they may be either minor or major points of contamination, are not in fact CCPs as they are not designed specifically to reduce or eliminate microbial contamination. This does not imply that these (non CCP) stages of processing should not be controlled. Control is necessary, although not critical,

to limit the amount of contamination as part of Good Manufacturing Practices and Standard Operating Procedures. These practices are essential complements to the HACCP system. Generally accepted CCPs in the beef slaughter plant are:

1. Bactericidal washes or other intervention strategies which have been approved by the relevant regulatory body at the end of the slaughter line (USDA 1999)
2. Chilling (NACMCF 1993, Mackey and Roberts 1993, Troeger 1994, Brunner *et al* 1996, Jericho *et al* 1998, USDA 1999) aimed at reducing the opportunity for enteropathogens to multiply despite the fact that this CCP is at the end of the process and in effect results in end product testing.

This change in approach to CCP designation will impact on HACCP planning, as it is possible that presentation of clean livestock pre-slaughter will not be designated as a critical control point in current systems. The HACCP system is designed to provide an outcome of safe food, and the evidence suggests that this outcome can be attained despite the presentation state of cattle. However, the appeal of deleting this control point from HACCP plans may not marry well with industry obligations to meet regulatory requirements, nor maximise profitability to the producer by returns on hides.

References

Adams, M.R., Moss M.O. (1995). *Food Microbiology*, The Royal Society of Chemistry, Cambridge U.K.

Alliance Consulting and Management (1998). *Contamination of carcases during slaughter and dressing.* Final report to Meat Research Corporation MSHE.006

Armstrong, G.L., Hollingsworth J., Morris Jr., J.G. (1996). *Emerging foodborne pathogens: Escherichia coli O157: H7 as a model of entry of a new pathogen into the food supply of the developed world*. Epidemiol Rev 18(1): 29-50

AQIS Clean animal Strategy, (1999). http://www.AQIS.gov.au/docs/mid/msep12.htm

Ash, M. (1997). "*Staphylococcus aureus* and staphylococcal enterotoxins" *In:* A. Hocking *et al Foodborne microorganisms of public health significance*. Fifth Edition, AIFST

Auer, N., Covington, A.D., Evans, C.S. (in press) *Enzymatic removal of dung from hides*. Proceedings of 25th IULTCS Congress

Australian Standard AS 4461:1997 – (1997a). *Hygienic production of meat for human consumption* (2nd edition), SCARM Report No. 54. CSIRO Publishing, Collingwood, Vic. Australia

Australian Standard AS 4462:1997 – (1997b). *Construction if premises processing animals for human consumption*, SCARM Report No. 55. CSIRO Publishing, Collingwood, Vic., Australia

Ayers, J.C. (1955). *Microbiological implications in the handling, slaughtering and dressing of meat animals.* Adv Food Res 6: 109-161.

Bacon, R.T., Sofos, J.N., Belk, K.E., Reagan, Reagan, J.O., Smith, G.C. (1999). *Commercial evaluation of multiple-sequential interventions for decontamination of beef carcasses.* http://www.colostate.edu/Depts/AnimSci/ran/meat/index.html

Baird-Parker, A.C. (1990). *Foodborne salmonellosis*. Lancet 336:1231-1235.

Bell, R.G., Harrison, J.C., Rogers, A.R., Roux, G.J.le (1995). *Bacterial contamination on carcasses.* Meat Industry Research Institute of New Zealand publication no. 963: 19

Bell, R.G., Hathaway, S.C. (1996). *The hygienic efficiency of conventional and inverted lamb dressing systems.* J Appl Bacteriol 81: 225-234

Bettelheim, K.A. (1995). *Identification of enterohaemorrhagic E.coli by means of their production of enterohaemolysin.* J App Bacteriol 79: 178-180.

Bettelheim, K.A. (1996). *Enterohaemorrhagic Escherichia coli: a new problem, an old group of organisms.* AVJ 73(1) 20-26.

Beuchat, L.R. (1995). *Pathogenic microorganisms associated with fresh produce.* J Food Prot 59: 204-216.

Beutin L., Geier, D., Steinruck, H., Zimmerman, S., Scheutz, F. (1993). *Prevalence and some properties of verotoxin (shiga-like toxin) – producing Escherichia coli in seven different species of healthy domestic animals*. J Clin Micro 31(9): 2483-2488.

Biss, M.E. and Hathaway, S.C. (1996). *Microbiological and visible contamination of lamb carcasses according to preslaughter presentation status: implications for HACCP.* J Food Prot 58(7): 776-783.

Blanco, M., Blanco J.E., Gonzalez, E.A., Mora, A., Prado C., Fernandez L., Rio, M., Ramos, J., Alonso, M.P. (1996). *Prevalence and characteristics of Escherichia coli serotype O157:H7 and other verotoxin producing E.coli in healthy cattle.* Epidemiol Infect 117: 251-257.

Borczyk, A.A., Karmali, M.A., Lior, H., Duncan, L.M.C. (1987). *Bovine reservoir for verotoxin-producing Escherichia coli O157:H7.* Lancet, Jan.10, 1987 p98 (letter)

Breazile, J.E. (1988). *The physiology of stress and its relationship to mechanisms of disease and therapeutics.* Veterinary Clinics of North America: Food Animal Practices. 4: (3) 441 - 445

Brett, D.J. (1995). Quality management programs to assure meat safety in Australian domestic and export abattoirs. *In: 41st Annual International Congress of Meat Science and Technology: Proceedings volume 2, San Antonio, Texas:* American Meat Science Association p. 323-325.

Briesman, M.A. (1985). *The epidemiology of Campylobacter infection in Christchurch 1981-1983.* N Z Med J 98: 391-393.
Brownlie, L.E. and Grau, F.H. (1967). *Affect of food intake on growth and survival of Salmonellas and Escherichia coli in the bovine rumen.* J Gen Microbiol 46:125-134.
Brunner, B., Marx, H., Stolle, A. (1996). *Process safety at slaughter.* Die Fleischeri 47(9): III-IV.
Buchanan, R.L., Acuff, G.R., and Halbrook, B. (1995). Data needed to develop microbial food safety systems for slaughter, processing, and distribution. *In: United States Department of Agriculture conference proceedings. Tracking foodborne pathogen data from farm to retail.* (3) 71-80.
Buntain, B. (1996). *Public Veterinary Medicine - Food Safety and Handling: Preharvest food safety issues.* J A V M A 208: 1396-1397.
Butzler, J.P. and Skirrow, M.B (1979). *Campylobacter enteritis.* Clin Gastroenterol. 8: 737-765.
Cabedo, L., Sofos, J.N., Smith, G.C. (1996). *Removal of bacteria from beef tissue by spray washing after different times of exposure to fecal material.* J Food Prot 59(12): 1284-1287.
Clavero, M.R., Monk, J.D., Beuchat, L.R., Doyle, M.P., Brackett R.E. (1994). *Inactivation of Escherichia coli O157: H7, Salmonellae and Campylobacter jejuni in raw ground beef by gamma irradiation.* App Environ Micro 2069-2075.
Clegg, F.G., Wray, C., Duncan, A.L., and Appleyard, W.T. (1986). *Salmonellosis in two dairy herds associated with a sewage farm and water reclamation plant.*
J Hygiene 97: 237-247.
Coates, K. (1997). Final report to Meat Research Corporation Microbial Food safety Key Program MSHS.003 *Sampling and test protocols.*
Codex Alimentarius Commission. (1997). *Hazard Analysis and Critical Control Point (HACCP) system and guidelines for its application. Annex to Appendix II – ALINORM 97/13* pp 66 – 76.
Corrier, D.E., Purdy, C.W., DeLoach J.R. (1990). *Effects of marketing stress on faecal excretion of Salmonella spp in feeder calves.* Am J Vet Res 51: 866-869.
Coppen, P., Fenner, S., Salvat, G. (1998). *Antimicrobial efficacy of AvGard™ carcase wash under industrial processing conditions.* Brit Poultry Sci 39: 229-234.
Cox, J.M., Fleet, G.H. (1997). New directions in the microbiological analysis of foods In *Foodborne microorganisms of public health significance*. pp 71-138 (Eds. A. Hocking, G. Arnold, I. Jenson, K.Newton, P. Sutherland) Australian Institute of Food Science and Technology Inc. NSW Branch, Food Microbiology Group, Australia
CSIRO Division of Food Science and Technology (1996). *Microbiological Quality of Australian Meat.* Final Report CS.196 to Meat Research Corporation
Daily, O.P., Joseph, S.W., Coolbaugh, J.C., Walker, R.I., Merrell, B.R., Rollins, D.M. Seidler, R.J., Colwell, R.R., and Lissner, C.R. (1980). *Association of Aeromonas sobria with human infection.* J Clin Microbiol 13: 769-777.
Deodhar, L.P., Saraswathi, K., Varudkar, A. (1991). Aeromonas spp. and their association with human diarrheal disease. J Clin Micro 29(5): 853-856.
Desmarchelier, P.M., Higgs, G.M., Mills, L., Sullivan, A.M., Vanderlinde, P.B. (1999). *Incidence of coagulase positive Staphylococcus on beef carcasses in three Australian abattoirs.* Int J Food Microbiol 47:221-229.
Diez-Gonzalez, F., Callaway, T.R., Kizoulis, M.G., Russell, J.B. (1998) *Grain feeding and the dissemination of acid resistant Escherichia coli from cattle.* Science 281: 1666-1667.
Dixon, Z.R., Acuff, G.R., Lucia, L.M., Venderzant, C., Morgan, J.B., May, S.G., Savell, J.W. (1991). *Effect of degree of sanitation from slaughter through fabrication on the microbiological and sensort characteristics of beef.* J Food Prot 54(3): 200-207.
Dorsa, W.J. (1997). *New and established carcass decontamination procedures commonly used in the beef-processing industry*. J Food Prot 60(9): 1146-1151.
Doyle, M.P. (1990). *Foodborne Illnesses: Pathogenic Escherichia coli, Yersinia enterocolitica, and Vibrio parahaemolyticus.* Lancet 336: 1111-1115.

Doyle, M.P., Padhye, V.V. (1989). *In:* Doyle M.P. *Foodborne Bacterial Pathogens.* New York. Marcel Decker. p 236 – 281.
Doyle, M.P., and Schoeni, J.L. (1987). *Isolation of Escherichia coli O157:H7 from retail fresh meats and poultry.* Appl Environ Microbiol 53: 2394-2396.
Elder R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Khoomaraie, M., Laegreid, W.W. (2000). *Correlation of enterohaemorrhagic Escherichia coli* O157 *prevalence in feces, hides, and carcasses of beef cattle during processing.* Proc Nat Acad Sci (PNAS) 97(7): 2999-3003.
European Commission Concerted Action CT94-1456. (1996). *Microbial control in the meat industry 3 – Decontamination of meat.* Series editors: M.H. Hinton, G.C. Mead, C. Rowlings. Volume editors J.E.L. Corry, G.C. Mead. Editorial board for Volume 3 R.A.A.W. Mulder, F.J.M. Smulders, J.M.A. Snijders. University of Bristol Press, UK.
Fenlon, D.R. (1985). *Wild birds and silage as reservoirs of Listeria in the agricultural environment.* J Appl Bacteriol 59: 537-543.
Fenlon, D.R. (1986). *Rapid quantitative assessment of the distribution of listeria in silage implicated in a suspected outbreak of listeriosis in calves.* Vet Rec 118: 240-242.
Fluharty, F.L., Loerch, S.C. and Dehority, B.A. (1996). *Effects of feed and water deprivation on ruminal characteristics and microbial population of newly weaned and feedlot-adapted calves.* J Animal Science 74(2):465-474.
Fricker, C.R., and Tompsett, S. (1989*). Aeromonas spp. in foods: A significant cause of food poisoning?.* Int J Food Microbiol 9: 17-23.
Fukata, T., Sasai, K., Miyamoto, T., Bapa, E. (1999). *Inhibitory effects of competitive exclusion and fructoologosaccharide, singly and in combination, on Salmonella colonization of chicks.* J Food Prot 62(3):229-233.
Galton, M.M., Smith, W.V., McElrath, H.B. and Hardy, A.B. (1954). Salmonella in swine, cattle and the environment of abattoirs. J Infect Dis 95: 236-245.
Garber, L.P., Wells, S.J., Hancock, D.D., Doyle, M.P., Tuttle, J., Shere, J.A. and Zhao, T. (1995) *Risk factors for faecal shedding of Escherichia coli O157:H7 in dairy calves.* JAVMA 207(1) 46-49.
Garcia M.M., Lior, H., Stewart, R.B., Ruckerbauer, G.M., Trudel, J.R.R. and Skljarevski, A. (1985). *Isolation, characterisation, and serotyping of Campylobacter jejuni and Campylobacter coli from slaughter cattle.* Appl Environ Microbiol 49(3) 667-672.
Gill, C.O. (1991). Microbial principles in meat processing. *In World Animal Science: Microbiology of animals and animal products.* pp. 249–270 (Ed. J.B. Woolcock) Elsevier, Amsterdam.
Gill, C.O., Deslandes, B., Rahn, K., Houde, A., Bryant, J. (1998). *Evaluation of the hygienic performances of the processes for beef carcass dressing at 10 packing plants.* J Appl Microbiol 84: 1050-1058.
Gill, C.O., Jones, T. (1999). *The microbiological effects of breaking operations on hanging beef carcass sides.* Food Res International 32: 453-459.
Gill, C.O., Jones, T. (2000). *Microbiological sampling of carcasses by excision or swabbing*. J Food Prot 63(2): 167-173.
Gill, C.O., McGinnis, J.C. (1999). *Improvement of the hygienic performance of the hindquarters skinning operations at a beef packing plant.* Int J Food Microbiol 51: 123-132
Gill, C.O., Bryant J., Bedard, D. (1999). *The effects of hot water pasteurizing treatments on the appearances and microbiological conditions of beef carcass sides*. Food Microbiol. 16: 281-289.
Gellin, B.G., and Broome, C.V. (1989). *Listerosis.* JAMA. 216: 1313-1320.
Grandin, T. (1984). *Reduce stress of handling to improve productivity of livestock.* Vet Med 79: 827 - 831.
Grau, F.H. (1987). *Campylobacter jejuni and Campylobacter hyointestinalis in the intestinal tract and on the carcasses of calves and cattle.* J Food Prot 51: (11) 857-861.
Grau, F.H., Brownlie, L.E. , Robwerts, E.A. (1968). *Effect of some pre-slaughter treatments on the Salmonella populations in the bovine rumen and faeces.* J Appl Bacteriol 31:157-163.

Grau, F.H. and Smith M.G. (1974). *Salmonella contamination of sheep and mutton carcasses related to preslaughter holding conditions.* J Appl Bacteriol 37, 111-116.
Gray, M.L. (1960). *Isolation of Listeria monocytogenes from oat silage.* Science. 132: 1767-1768.
Gray, M.L. and Killinger, A.H. (1966). *Listeria monocytogenes and listeria infections.* Bacteriol Rev. 30: 309-382.
Greer, G.G., Dilts, B.D. (1997). *Enumeration of spoilage bacteria with hydrophobic grid membrane filtration.* J Food Prot 60(11): 1388-1390.
Griffin, D. (1998). Feedlot Diseases *In*: *The Veterinary Clinics of North America- Food Animal Practice – Feedlot medicine and management* Volume 14, number 2.
Hancock, D.D, Rice, D.H., Thomas, L.A., Dargatz, D.A., Besser, T.E. (1995). Epidemiology of *E.coli* O157 H7 in feedlot cattle. J Food Prot 60(5): 462-465
Hancock, D.D., Besser, T.E., Gill, C., Bochach, C.H.; Russell, J.B., Diez-Gonzales, F. (1999). Science 284:49(g) (letter).
Hathaway, S.C. (1997a). *Risk analysis and international standards for meat hygiene.* Presented at the 43rd International Congress of Meat Science and Technology, Auckland New Zealand.
Hathaway, S.C. (1997b). *Intensive (pasture) beef cattle operations: the perspective of New Zealand.* Rev Sci Tech Off Int Epiz 16(2): 382-390.
Higgs, A.R.B., Norris R.T., Richards R.B. (1993). *Epidemiology of salmonellosis in the live sheep export industry.* Aust Vet J 70: 330-335
Hoerlin, A.B. and Marsh, C.L. (1957). *Studies on the epizootiology of shipping fever in calves.* JAVMA 131: 123.
Hudson, A.J. and De Lacy, K.M. (1991). *Incidence of motile aeromonads in New Zealand retail foods.* J Food Prot 54: (9) 696-699.
Hueston, W.D. and Fedorka-Cray, P.J. (1995). Pathogen identification on the farm and the impact of farm management strategies. *In: United States Department of Agriculture conference proceedings. Tracking foodborne pathogen data from farm to retail:* (3) pp 55-69.
Hutcheson, D.P., and Cole, A.N. (1986). *Management of transit-stress syndrome in cattle: nutritional and environmental effects.* J Animal Sci 62: 555-560.
Jay, S., Grau, F.H., Smith, K., Lightfoot, D., Murray C., Davey G.R. (1997). *Salmonella In* Eds .Hocking, A., Arnold, G., Jenson, I., Newton, K., Sutherland,P. *Foodborne microorganisms of public health significance.* 5th Ed AIFST (NSW Branch) Food Microbiology Group
Jericho, K.W.F., Bradley, J.A., Gannon, V.P.J., Kozub, G.C. (1993). *Visual demerit and microbiological evaluation of beef carcasses: methodology*. J Food Prot 56(2): 114-119.
Jericho, K.W., Bradley, J.A., Kozub, G.C. (1995). *Microbiological evaluation of carcasses before and after washing in a beef slaughter plant.* JAVMA 206(4): 452-455.
Jericho, K.W.F., Kozub, G.C., Loewen, K.G., Ho, J. (1996). *Comparison of methods to determine the microbiological contamination of surfaces of beef carcasses by hydrophobic grid membrane filters, standard pour plates of flow cytometry.* Food Microbiol. 13(4): 303-309.
Jericho, K.W.F., O'Laney, G., Kozub, G.C. (1997). *Verification of the hygienic adequacy of beef carcass cooling processes by microbiological culture and the temperature-function integration technique.* J Food Prot 61(10): 1347-1351.
Johnson, R.W., and Tompkin, R.B. (1992). Meat and poultry products. *In:* Eds Vanderzant, C., and Splittstoesser, D.F. *Compendium of methods for the microbiological examination of foods.* 3rd Ed. American Public Health Association. Washington DC.
Johnston, A.M (1990). *Foodborne Illness: Veterinary sources of foodborne illness.* Lancet. 336: 856-858.
Jones, D. (1990). *Foodborne Illness: Foodborne Listeriosis*. The Lancet. 336: 1171-1174.
Jones, F., and Watkin, C. (1985). *The water cycle as a source of pathogens*. J Appl Bacteriol (supplement): 275-365.
Jordan, D., McEwen, S.A., Lammerding A.M., McNab, W.B., Wilson, J.B. (1999a). *A simulation model for studying the role of pre-slaughter factors on the exposure of beef carcasses to human microbial hazards.* Prev Vet Med 41: 37-54.

Jordan, D., McEwen, S.A., Wilson, J.B., McNab, W.B., Lammerding, A.M. (1999b). *Reliability of an ordinal rating system for assessing the amount of mud and faeces (tag) on cattle hides at slaughter*. J Food Prot 62(5):520-525

Joseph, S.W., Daily, O.P., Hunt, R.J., Seidler, R.J., Allen, D.A., and Colwell, R.R. (1979). *Aeromonas primary wound infection of a river in polluted waters.* J Clin Microbiol 10: 46-49.

Kain, M.L., Sofos, J.N., Reagan, J.O., Smith, G.C. (1999). *Microbiological contamination baselines of beef carcasses, wholesale cuts and retail cuts.* http://www.colostate.edu/Depts/AnimSci/ran/meat/index.html

Kudva, I.T., Hatfield, P.G. and Hovde, C.J. (1995). *Effect of diet on the shedding of Escherichia coli O157: H7 in a sheep model.* Appl Environ Microbiol 61(4) 1363-1370.

Lancette, G.A, and Tatini, S.R. (1992). *Staphylococcus aureus*. *In:* Vanderzant, C. and Splittstoesser, D.F. *Compendium of methods for the microbiological examination of foods.* 3rd Ed. American Public Health Association.

Lasta, J.A., Gimeno, E.J. (1993). *Hygiene conditions of beef carcasses and cuts in Argentine meat packing plants.* Fleischwirsch 73(2): 169-171.

Lichacz, R. (1985). *Another unseen threat in drinking water – Campylobacter.* National Parks Journal 29(1): 14-15, 17.

Longdell G.R. (1996). *Recent developments in sheep and beef processing in Australasia.* Meat Sci 43 (supp) S165-S174.

Lovett, J. (1989) *Listeria monocytogenes*. *In:* Ed. Doyle, M.P. *Foodborne Bacterial Pathogens* pp 283-310.

Low, J.C. and Renton C.P. (1985). *Septicaemia, encephalitis and abortions in a housed flock of sheep caused by Listeria monocytogenes type 1/2.* Vet Rec 116: 147-150.

Luechtefeld, N.W., Cambre, R.C., Wang, W-L.L. (1981). *Isolation of Campylobacter fetus subsp. Jejuni from zoo animals.* JAVMA 179(11): 1119-1122.

Mackey, B.M., Roberts, T.A. (1993). *Improving slaughter hygiene using HACCP and monitoring.* Fleischwirtsch 73(1): 58-61.

Manser, P.A. and Dalziel, R.W. (1985). *A survey of Campylobacter spp. in animals.* J Hyg 95: 15-21.

Martin, M, L., Shipman, L.D., Wells, J.G., Potter, M.E., Hedberg K., Wachsmuth I.K., Tauxe R.V. (1986) *Isolation of Escherichia coli O157: H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome.* Lancet, November 1,1986, page 1043.

Mawdsley, J.L., Bardgett, R.D., Merry, R.J., Pain, B.F. and Theodorou, M.K. (1995). *Pathogens in livestock waste, their potential for movement through soil and environmental pollution.* Appl Soil Ecol 2:1-15.

McLean, K.J.E. (1984). *Ultimate pH of veal and beef: effect of distance travelled and rest prior to slaughter.* J Sth Afr Vet Assoc 55(1): 19-22.

McGrath, J.F. and Patterson, J.T. (1969). *Meat Hygiene: The pre-slaughter treatment of fatstock.* Vet Rec 85: 521-524.

McKinnon, J. (1996). *Canadian Beef - A focus on quality.* Department of Animal and Poultry Science, University of Saskatchewan. http://eru.usask.ca/saf_corp/livestok/Beef_pap/

Meanger, J.D. and Marshall, R.B. (1989) *Seasonal prevalence of thermophilic Campylobacter infections in dairy cattle and a study of infection in sheep*. N Z Vet J 37: 18-20.

Menning, E.M. (1988). *Danger lurks in your supermarket meat cases.* JAVMA 192: 494-497.

MHS Clean Livestock Policy (1997). *Meat Hygiene Services Operations Manual, Chapter 4, Annex 12, Amendment no. 40*, United Kingdom

Montenegro, M.A., Bülte, M., Trumpf T., Alexic , S., Reuter, G., Bulling, E., Helmuth, R. (1990). *Detection and characterization of fecal verotoxin – producing Escherichia coli from healthy cattle.* J Clin Micro 28(6): 1417-1421.

Morgan, I.R., Krautil, F.L., Craven, J.A. (1988). *Reduction of Salmonella contamination on pig carcasses. Research Report Series 57,* Department of Agriculture and Rural Affairs, Vic., Australia.

NACMCF (National Advisory Committee on Microbiological Criteria for Foods), USDA. (1993). *Generic HACCP for raw beef.* Food Microbiol. 10:449-488.
Newton, K.G., Harrison, J.C.L., Wauters, A.M. (1978). *Sources of psychrotrophic bacteria on meat at the abattoir.* J Appl Bacteriol 45:75-82.
Notermans, S., Gallhoff, G., Zwietering, M.H. and Mead, G.C. (1995). *Identification of critical control points in the HACCP system with a quantitative effect on the safety of food products.* Food Microbiology. Academic Press Limited.
Okrend, A.J.G., Rose, B.E. and Bennett, B. (1987). *Incidence and toxigenicity of Aeromonas species in retail poultry, beef and pork.* J Food Prot 50: 509-513.
Padhye N.V., Doyle, M.P. (1992). *Escherichia coli O157: H7: epidemiology, pathogenesis, and methods for detection in food.* J Food Prot 55(7): 555-565.
Palumbo, S. Abeyta, C. and Stelma, G. (1992). *Aeromonas hydrophila* group*. In:* Vanderzant, C. and Splittstoesser, D.F. *Compendium of methods for the microbiological examination of foods.* 3rd Ed. American Public Health Association.
Palumbo, S.A., Maxino, F., Williams, A.C., Buchanan, R.L., Thayer, D.W. (1985). *Starch-ampicillin agar for the quantitative detection of Aeromonas hydrophila.* Appl Environ Microbiol 50:1027-1030
Parrington, L.J., Sharpe, A.N., Peterkin, P.I. (1993). *Improved aerobic colony count technique for hydrophobic grid membrane filters.* Appl Environ Microbiol 59(9): 2784-2789.
Peterkin, P.I., Conley, D., Foster, R., Lachapelle, G., Milling, M., Purvis, U., Sharpe, A.N., Malcolm, S. (1989). *A comparative study of total coliform recovery from foods by most probable number and hydrophobic grid membrane filter methods*. Food Microbiol 6(2):69-84
Reagan, J.O., Acuff, G.R., Buege, D.R., Buyck, M.J., Dickson, J.S., Kastner, C.L., Marsden, J.L., Morgan, J.B., Nickelson II, R., Smith, G.C., Sofos, J.N. (1996). *Trimming and washing of beef carcasses as a method of improving the microbiological quality of meat.* J Food Prot. 59(7): 751-756.
Ridell, J., Korkeala, H. (1993). *Special treatment during slaughtering in Finland of cattle carrying an excessive load of dung: meat hygienic aspects.* Meat Science 35:223 - 228
Rings, D.M. (1985). *Salmonellosis in calves*. The Veterinary Clinics of North America: food animal practice. pp 529-539. Philadelphia, W.B.
Roberts, T.A. (1980). *Contamination of meat: the effects of slaughter practices on the bacteriology of the red meat carcass.* Royal Soc Health. 100: 3-9.
Robins-Browne, R.M. (1997). *STEC: food-borne pathogens of increasing public health significance.* Microbiol Australia 18(5): 8-9.
Rowland, D., Phillips M., Whitehouse J., Isgro D., Barlow, S., Bobbitt, J., Isaac, J., Kondekas N., Haines, H., Coates, K. (1999). *Preparation and Delivery of Clean Livestock.* Final Report submitted to Meat and Livestock Australia, MSQS.001, FLOT.302, TRBF.005
Russell, J.B. (2000). *Hay, Grain and E.coli revisited.* American Society for Microbiology (ASM) News (in letters). 66(1):1
Salman, M.D., Akey, B.L. (1993). Report of the committee on animal disease surveillance and animal health information systems. *In*: *Proceedings of the 97th annual meeting of the United States Animal Health Association*, Las Vegas, Nevada.
Schnell, T.D., Sofos, J.N., Littlefield, V.G., Morgan, J.B., Gorman, B.M., Clayton, R.P., Smith, G. (1995). *Effects of postexsanguination dehairing on the microbial load and visual cleanliness of beef carcasses.* J Food Prot 58(12):1297-1302
Shuppel, H., Salchert, F. and Schuppel, K.F. (1996). *Investigations into the influence of mastitis and other organ changes on microbial contamination of the meat of slaughter cows.* Fleischwirtschaft 76: 1:61-63.
Sierra, M., Gonzalez-Fandos, E., Garcia-Lopez, L., Fernandez, M.C.G. and Prieto, M. (1995). *Prevalence of Salmonella, Yersinia, Aeromonas, Campylobacter, and cold-growing Escherichia coli on freshly dressed lamb carcasses.* J Food Prot 58: 1183-1185.
Siragusa, G.R., Dickson, J.S. and Daniels, E.K. (1992). *Isolation of Listeria spp. from faeces of feedlot cattle.* J Food Prot 56: 102-105, 109.

Sofos J.N., Smith, G.C. (1998). *Nonacid meat decontamination technologies: model studies and commercial applications*. Int J Food Microbiol 44:171-188.
Sofos, J.N., Kochevar, S.L., Bellinger, G.R., Buege, D.R., Hancock, D.D., Ingham, S.C., Morgan, J.B., Reagan, J.O., Smith, G.C. (1999a). *Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants.* J Food Prot 62(2): 140-145.
Sofos, J.N., Belk, K.E., Smith, G.C. (1999b). *Processes to reduce contamination with pathogenic microorganisms in meat.*
http://www.colostate.edu/Depts/AnimSci/ran/meat/processes.htm
Sparling, P.H. (1996). *Public Veterinary Medicine - Food Safety and Handling: Postharvest food safety issues.* JAVMA 208: 1397-1398.
Stern, N.J., Drazek, E.S. and Joseph, S.W. (1987). *Low incidence of Aeromonas spp. in livestock faeces.* J Food Prot 50: (1) 66-69.
Stolle, A. (1981). *Spreading of Salmonella spp. during cattle slaughtering.* J Appl Bacteriol 50:239-245.
Sub-Committee on Animal Welfare (1991). *Model Code of Practice for the Welfare of Animals.* CSIRO Publications, Vic Australia.
Sumner, J. (1997). *The hygiene status of Victorian meat (1993-1997).*
Tarrant P.V. and Sherington, J. (1980). *An investigation of ultimate pH in muscles of commercial beef carcasses.* Meat Sci 4: 287-297.
Thayer, D.W., Lachica, R.V., Huhtanen, C.N., Wierbicki E. (1986). *Use of irradiation to ensure the microbiological safety of processed meats.* Food Technol April 1986
Thornton, H. and Gracey J.F. (1974). *Textbook Of Meat Hygiene* 6th ed. Balliere Tindall, London.
Troeger, K. (1994). *Evaluating hygiene risks during slaughtering.* Fleishchwirtsch 74(6): 624-626.
Troutt, H.F., Gillespie, J., Osburn, B.I. (1995) Implementation of HACCP programs on farms and ranches. In: *HACCP in meat, poultry and fish processing: Advances in meat research series Volume 10.* Ed: A.M. Pearson, T.R. Dutson. Blackie Academic and Professional, UK.
Untermann, F., Stephan, R., Dura, U., Hofer, M., Heimann, P. (1997). *Reliability and practicability of bacteriological monitoring of beef carcass contamination and their rating within a hygiene quality control programme of abbatoirs.* Int J Food Microbiol 34: 67-77.
USDA (1993) Generic HACCP for raw beef. *In: National Advisory Committee on Microbiological Criteria For Foods*, Academic Press Limited. p. 449-479.
USDA (1996a). *Pathogen Reduction: Hazard Analysis and Critical Control Point (HACCP) System.* National Technical Information Services, VA, USA.
USDA (1996b). *The final rule on pathogen reduction and Hazard Analysis and Critical Control Point (HACCP) systems*. Food Safety and Inspection Service, USDA.
http://www.fsis.usda.gov/OA/background/finalrul.htm
USDA/FSIS (1999). *Generic HACCP model for beef slaughter*.
http://www.fsis.usda.gov/index.htm
Van Den Heever, L.W. (1959). *The pH of meat.* J Sth Afr Vet Med Assoc 30(3): 271-288.
Vanderlinde, P.B., Shay, B., Murray, J. (1999). *Microbiological status of Australian sheep meat.* J Food Prot 62(4):380-385
Van Donkersgoed, J., Janzen, E.D., Chirino-Trejo, M., Berry, C., Clark, E.G. and Haines D.M. (1990). *Campylobacter jejuni abortions in two beef cattle herds in Saskatchewan.* Can Vet J 31. 373-377.
Van Donkersgoed, J., Jericho, K.W.F., Grogan, H., Thorlakson, B. (1997). *Preslaughter hide status of cattle and the microbiology of the carcasses.* J Food Prot 60(12):1502-1508
Wang, G., Zhao, T. and Doyle, M.P. (1996). *Fate of Enterohemorrhagic Escherichia coli O157:H7 in bovine feces.* Appl Environ Microbiol 62 (7):2567-2570.
Warriss, P.D. (1990). *The handling of cattle pre-slaughter and its effects on carcass and meat quality.* Appl Animal Behav Sci 28:171-186.

Wells, J.G., Shipman, L.D., Greene, K.D., Sowers, E.G., Green, J.H., Cameron, D.N., Downes F.P., Martin, M.L., Griffin, P.M., Ostroff, S.M., Potter, M.E., Tauxe R.V., Wachsmuth, I.K. (1991). *Isolation of Escherichia coli serotype O157:H7 and other shiga-like-toxin-producing E.coli from dairy cattle.* J Clin Micro 29(5): 985-989.
Wescombe, G.L.J. (1994). *Cattle Cleaning*, Sydney: Meat Research Corporation.
Whipp, S.C., Rasmussen, M.A., Cray Jr., W.C. (1994). *Animals as a source of Escherichia coli pathogenic for human beings.* JAVMA 204(8) 1168-1175.
Wilson, J.B., McEwen, S.A., Clarke R.C., Leslie, K.E., Wilson, R.A., Walter-Toews, D., Gyles, C.L. (1992). *Distribution and characteristics of verocytotoxigenic Escherichia coli isolated from Ontario dairy cattle.* Epidemiol Infect 108: 423-439.
Woolford, M.K. (1990). *The detrimental effects of air on silage*. J Appl Bacteriol 68 101-116.
Wray, C. (1989). *Salmonellosis in animals and its control*. Outlook on Agriculture 18(3):104-109.
Zhao, S., Meng, J., Zhao, A. and Doyle, M.P. (1995a). *Use of vaccine and biological control techniques to control pathogens in animals used for food.* J Food Safety 15:193-199.
Zhao, T., Doyle, M.P., Shere, J. and Garber, L. (1995b). *Prevalence of Enterohemorrhagic Escherichia coli O157:H7 in a survey of dairy herds.* Appl Environ Microbiol 61: 4:1290-1293.