BACTERIOLOGICAL SAFETY ISSUES IN RED MEAT AND READY-TO-EAT MEAT PRODUCTS, AS WELL AS CONTROL MEASURES.

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Abstract

The importance of *Eschericha coli* O157, *Listeria monocytogenes* and *Salmonella typhimurium* DT104 as meat-borne pathogens is well established. Pathogenic bacteria such as *Aeromonas* spp., *Arcobacter spp.,* psychrotrophic *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum* and non-invasive *Listeria monocytogenes* can be regarded as rookies, but not yet firmly associated with today's production of red meat and meat products. The development of PCR and other DNA-based techniques will shed new light on so called emerging pathogens. Important safety issues in meat production, such as insufficient cleaning and disinfection (including the stable/lairage, processing environment), carcass decontamination and chilling, and cross contamination are discussed. Furthermore, probability modelling of survival and growth is identified as an important way to achieve a better understanding of how to deal with the complexity of further processing, including heat treatment and storage.

Keywords: meat, meat products, safety, safety issues, pathogenic bacteria, minimal processing, sous vide cooking, probability modelling

Foodborne disease - Oldies and rookies relevant to meat and meat products

There is no doubt that foodborne pathogenic bacteria are the cause of illness and death for many people each year, at great economic cost and human suffering. Most of the pathogens we are struggling with today are not newcomers. *Salmonella, Staphylococcus aureus, Bacillus cereus* and *Clostridium botulinum* have been associated with foodborne illness for decades. For example, in 1953 a large Swedish outbreak involving 8,845 cases and 90 deaths was caused by *Salmonella typhimurium* PT 8 in raw meat (D´Aoust, 1989). The typical symptoms of staphylococcal food poisoning had already been described in 1936 (Bergdoll, 1989). One of the earliest food poisoning outbreaks caused by *Bacillus cereus* was reported in 1906 (Kramer & Gilbert, 1989). As early as during the 19th century, botulism was closely associated with the consumption of sausages and home-cured hams, and in the 1890s the bacteria responsible for a large outbreak, probably a nonproteolytic *C. botulinum* type B, was isolated (Hauschild, 1989). *Yersinia enterocolitica* emerged in the 1970s, while *Campylobacter* spp., *Listeria monocytogenes* and *Escherichia coli* O157 all emerged during the 1980s.

Two major sources of bacteria causing foodborne disease in meat and meat products may be identified. The living animal carries pathogenic bacteria while the processing environment harbours them. In addition, the human being is also an important source of pathogenic bacteria, most frequently indirectly by cross contamination. Bacteria originating from the animal may, during slaughter, contaminate the carcass, and subsequently be distributed via cut meat or meat raw material intended for further processing into meat products. Limiting the contamination and subsequent inactivation of occurring pathogenic bacteria will be decisive to the safety of meat and meat products.

Living animals carry bacteria in the intestinal tract (Table 1). A variety of properties among the large repertoire of pathogenic bacteria entering the slaughterhouse via the animal determines when they may become a safety risk, and moreover how to address the risk. Properties of particular importance are an ability to become established within the processing environment, good survival during subsequent processing, e.g. heat treatment, and growth at refrigeration temperatures. The entire processing chain, including further processing and storage, must be taken into account.

Campylobacter spp. are the most frequent cause of registered human cases of foodborne bacterial disease in Sweden (94 cases/100,000 inhabitants) followed by *Salmonella* (54 cases/ 100,000 inhabitants), as in e.g. Denmark and UK (Anon. 2000a; Anon. 2000b; Anon. 2001). *Campylobacter* spp. are normally found in the intestinal tract of pigs and cattle. Up to 96% of the samples from pig faeces have been found to be positive (Borch, Nesbakken & Christensen, 1996a). *C. jejuni* was isolated from 38 % of individual dairy cattle samples (Wesley et al., 2000). In a Swedish study, *Campylobacter* spp. were found in 16%-83% of cattle faecal samples; the lowest occurrence was found in older cattle (≥ 2 years) while the highest was in young cattle (< 1 year; Borch & Blixt, unpublished results). In spite of this high occurrence in the intestinal content of pigs and cattle, *Campylobacter* spp. have not as yet been firmly associated with the consumption of red meat. However, eating pork and barbecuing were identified as risk factors for indigenous campylobacter infection in a Swedish case-control study (Studahl & Andersson, 2000). Facts about occurrence in animal faeces, together with lessons learned from other pathogenic bacteria in terms of slaughter hygiene and the likelihood of carcass contamination, indicate the need for more research in order to elucidate the controlling factors.

Escherichia coli O157, as well as other serotypes of *E. coli,* may be attributed to the group verotoxigenic *E. coli* (VTEC). Within this group the genes *vt*1 and *vt*2 are important virulence factors. Using PCR and other nucleic acid based detection techniques the occurrence of these genes is now recognised in several serotypes of *E. coli*. Ruminants are considered to act as a reservoir of this bacterium. The long list of outbreaks occurring since the mid 1980s reveals the transmission routes, e.g. person-to-person, intake of food, contact with farm environment, or some other form of environmental transmission, e.g. swimming in contaminated water (Chapman, 1999). Several VTEC-serotypes are present in cattle faeces (Table 2), e.g. O8, O91 and O113, which have been isolated from human cases. In 2000, a total of 137 cases of enterohaemorrhagic *E. coli* were reported in Sweden, of which 71% were caused by serotype O157 (Anon. 2000a).

Salmonella typhimurium phagetype DT104 is a multiple antibiotic-resistant pathogen which emerged in the mid 1990s and is now widespread in Europe, North America and the Middle East (Humphrey, 2001). Foodborne outbreaks via cooked meat, undercooked liver, pork and improperly pasteurised milk are reported. *S. typhimurium* is associated with pigs, cattle, sheep, chickens and turkeys. It survives well in both wet and dry environments, and is difficult to eradicate once a farm has become infected. In Denmark, *Salmonella* DT104 was isolated from 95 farms (Anon., 2000d) during 1996-2000.

There have been several reports implicating *Arcobacter* spp. in human illness, and in animal disease such as abortion, mastitis and diarrhoea (Philips, 2001). The symptoms of *Arcobacter* infection in humans are similar to campylobacteriosis. *Arcobacter* belongs to the *Campylobacteraceae* family. Aerotolerance and the ability to grow at 15°C are the main characteristics that distinguish *Arcobacter* and *Campylobacter*. A high occurrence of 97 % is reported for pork (Collins, Wesley & Murano, 1996). The inter-plant variation was high, indicating the possibility of varying hygiene practices in slaughterhouses, or a high variation in pigs. *Arcobacter* spp. were found in 14 % of faecal samples from dairy cattle, *Arcobacter butzleri* was found in 51 % of the positive samples (Wesley et al., 2000).

A few cases of foodborne outbreaks associated with *Aeromonas* spp. have been reported, implicating fish, oysters and seafood (Kirov, 1993). Toxin formation has been demonstrated in meat extract at low temperature (Majeed & MacRae, 1991). Furthermore, *Aeromonas* spp. have been isolated from pig faeces, and in pig carcass processing equipment (Gray & Stickler, 1989; Gill & Jones, 1995). They are recognised as opportunistic bacteria and may be present in clinical samples as commensals rather than as primary pathogens. A study of 350 isolates of *Aeromonas* spp. revealed that 65 % harboured virulence genes for cytotoxic enterotoxin and aerolysin using PCR detection (Bin Kingombe et al., 1999). Among the meat (beef, pork, minced meat) isolates, 80 % were virulent. In comparison, 50 % of the clinical isolates were virulent, and 50 % of the environmental water isolates. Virulent *Aeromonas bestiarum* and *Aeromonas veronii,* but not *Aeromonas hydrophila,* isolated from meat, belonged to the same subgroups as clinical isolates. However, the number of isolates was very low, and further studies are needed in order to draw any wider conclusions. PCR and other DNA-based techniques enable microbiologists to detect important virulence genes, and to specifically characterise bacterial isolates. The development of these tools will shed new light on so called emerging pathogens.

Cooked meat products and pâté have been responsible for outbreaks of listeriosis caused by *Listeria monocytogenes*. The implicated products have typically been contaminated post heat treatment. Two variants of the disease are recognised today. The invasive form mainly affects susceptible population groups; the clinical symptoms are infections of the central nervous system, sepsis, abortion and stillbirth. The more recently described non-invasive listeriosis causes febrile gastroenteritis. Cold salad of corn and tuna fish, and cold-smoked rainbow trout are examples of foods identified in non-invasive listeriosis (Miettinen, Siitonen, Heiskanen, Haajanen, Björkroth & Korkeala, 1999; Franciosa, Tartaro, Wedell-Neegaard, & Aureli, 2001). The occurrence of non-invasive listeriosis is, presumably, underestimated since the bacterium is not routinely screened for in patients with gastrointestinal illness. It is documented that *Listeria monocytogenes* may become established within the processing environment (Boerlin & Piffaretti, 1991; Suihko et al., 2002), surviving cleaning and disinfection routines. Strains isolated from the processing environment needs a further characterisation, in terms of invasive and non-invasive capability.

C. botulinum is widespread in nature and has been retrieved from soil, aquatic environments and animals. The occurrence of non-proteolytic *C. botulinum* type B was reported to be 62 % in pig faeces (Dahlenborg, Borch & Rådström, 2001) and 73 % in cattle faeces (Dahlenborg et al., unpublished results). Low numbers of spores were demonstrated; most samples contained less than 4 spores/g. A combined selection and enrichment PCR procedure was used to detect the type B neurotoxin gene. The contamination of carcasses with *C. botulinum* spores thus seems likely to occur during slaughter. Many types of food have been associated with botulism. Home-preserved food has traditionally been an important risk factor. During the past 20 years, the epidemiology of botulism now includes commercially produced food, including restaurants. Foods implicated are hazelnut yoghurt, eggplant in oil, potato dips and vegetable soup, dry-cured ham and meat beef pot pie (Anon, 1983; Bell & Kyriakides, 2000; Pavic, Lastre, Bukovski, Hadziosmanovic, Miokovik & Kozacinski, 2001). Consumer and marketing demands on convenient food, calling for home-style production, have led to drastic changes in terms of less processing and less use of preservatives, placing a new focus upon *C. botulinum*. Further processing, including mild heat treatment, anaerobic packaging atmosphere and prolonged storage at chill temperatures, is a factor identified and also experimentally verified as beneficial for growth and toxin formation in psychrotrophic, non proteolytic *C. botulinum* (Peck, 1997).

B. cereus has been found in many different foods, including meat and meat products (Konuma et al., 1988; van Netten, van de Moosdijk, van Hoensel, Mossel & Perales, 1990 ; Te Giffel, Beumer, Leijendekkers & Rombouts, 1996). It is the psychrotrophic strains that are of concern for food stored at refrigeration temperatures. Some strains of *B. cereus* have been shown to grow at temperatures below 10°C. These strains have mainly been isolated from dairy products (van Netten., van de Moosdijk, van Hoensel, Mossel & Perales, 1990; Andersen Borge, Skeie, Sørhaug, Laingsrud & Granum, 2001). Some strains grow at low temperatures when they are in broth medium, as opposed to food (Choma *et al.* 2000). Toxin production is reported to be higher at 12-15°C than at 30°C (Finlay, Logan & Sutherland, 2000). The combination of heat resistance and the ability to grow at low temperatures makes *B. cereus* a potential hazard for chilled heat-treated food, and needs further evaluation.

Safety issues in the production of meat

Herd level

In Sweden, there is a long-standing tradition of controlling salmonella in farm animals, consisting of the identification and slaughter of salmonella-carriers, combined with the cleaning and disinfecting of stables. A similar strategy was applied in a specialised beef herd, which had been found to be positive for *E. coli* O157, with 22 % of the calves (n=40) harbouring the bacterium (Borch, Nesbakken & Christensen, 2001). The results demonstrated that *E. coli* O157 was established in the barn or stable environment. The cattle herd was purged of *E. coli* O157 by slaughtering positive animals, together with the cleaning and disinfection of the stable environment. However, through the exchanging of calves with dairy herds, new contamination was introduced. The risk of transmitting *E. coli* O157 by trading calves should be controlled, as the continuous inflow of calves originating from dairy herds plays an important role in the introduction of this organism into beef herds. Based on knowledge of the prevalence of the bacterium in individual calves and the sensitivity of the analytical method used to detect the bacterium, an estimate may be made of the status of a group of calves before being traded. Serological testing of the living animal could be one way of sorting and destining animals according to infection level (Nesbakken, 2001). This could be applied before slaughter, or before being transferred to another herd/farm. *Salmonella* spp. and *Yersinia enterocolitica* are examples of bacteria that may be detected using the serological testing of pigs. In conclusion, on the herd level, the cleaning/disinfection of stables should be improved and the possibility of sorting animals on the basis of infection level before transportation should be evaluated in order to reduce the spreading via the buying and selling of animals between farms.

Slaughterhouse

The usual cleaning and disinfection applied in the lairage of a pig slaughterhouse reduced the occurrence of *Salmonella* from 90 % positive environmental samples to 25% positives (Swanenburg, Urlings, Keuzenkamp & Snijders, 2001). The intranasal inoculation of pigs may lead to a rapid transfer of *Salmonella typhimurium* to tonsil, cecum and colon (Fedorka-Cray, Kelley, Stabel, Gray & Laufer, 1995). The waiting period in the lairage is thus a risk for the infection of the pigs. Improved cleaning/disinfection routines would limit this infection.

Slaughter is a difficult process from a hygienic point of view. HACCP and GMP must be focused on controlling the sources of contamination during slaughter, including meat inspection (Borch, Nesbakken & Christensen, 1996a; Nesbakken 2001). For most operations, it is only possible to reduce the extent of contamination during processing, i.e. improving the hygiene level.

Cross contamination routes involve faeces to carcass, carcass to carcass and environment to carcass (sources such as machinery, operator, wall, air). At a beef slaughterhouse, VTEC was retrieved from 32% of the unchilled carcasses, whereas 57% of the faecal samples contained VTEC (Table 3). By matching the results from faeces with the ones of the carcasses, an indication of the direct contamination from faeces to carcass, and the indirect contamination from faeces via tools equipment and operators could be estimated. The presence of VTEC in faeces resulted in a presence on the fresh carcass in 26% of cases, whereas a negative faeces-sample gave a positive fresh carcass in 39% of cases (unpublished results Borch & Hofvendahl). For post exsanguinated pigs, 80 % of carcasses were *Salmonella*-positive, when the faeces were *Salmonella*-positive. The corresponding figure for faeces-negative carcasses was 46 % (Tamplin, Feder, Palumbo, Oser, Yoder & Luchansky, 2001). Cross contamination during slaughter thus has a significant effect on the bacterial status of carcasses.

The decontamination of carcasses using various techniques enables a 1-2 log reduction approximately in total viable counts and *E. coli* (Sofos & Smith, 1998). The effectiveness in reducing the number of *E.* coli decreases in the following order: knife-trimming = hot water (>74-87.8°C) > water spray-washing (28-42°C) = ozonated water spray-washing= hydrogen peroxide spray-washing. Furthermore, during chilling, the concentration of bacteria and the number of positive carcasses are reported to be reduced (Jensen & Christensen, 2000). The blast chilling of pig carcasses is reported to reduce the count of *E. coli* by 1 log unit. The proportion of *Salmonella*-positive pig carcasses is reported to be reduced from 33% before chilling to 17% after blast chilling (Jensen & Christensen, 2000). Chilling reduced the occurrence of VTEC from 32% to 7%, and presumptive *E. coli* from 42% to 22% on beef carcasses (Table 3). The optimal conditions required for no bacterial growth/bacterial inactivation on carcasses, in terms of airspeed, airflow, relative humidity and temperature profiles for individual carcasses, are not known (Bolton & Sheridan, 2001). By combining hot water decontamination and blast chilling, the bacterial reduction is higher than if the effect of decontamination alone is estimated. The reduction in *E. coli* on pig carcasses as a result of blast chilling, hot water (80°C) decontamination and hot water decontamination plus blast chilling was less than 1, more than 2 and more than 2.7 log units, respectively (Jensen & Christensen, 2000). One measure among several that has been taken in Denmark to battle *Salmonella* DT104, is the mandatory use of hot water decontamination on pig carcasses originating from herds with DT 104 (Anon., 2000d). According to Jensen & Christiansen (2000), a combination of decontamination and blast chilling will reduce *Salmonella* to undetectable levels in 90% of the cases.

It is well documented that *Listeria* spp., *Staphylococcus aureus, Yersinia enterocolitica, Salmonella* and *Aeromonas* spp. may be detected in the slaughterhouse environment, for example on floor and walls, cold room floor, hand basins, splitting saws, chopping blocks (Gill & Jones, 1995; Fenlon, Wilson & Donachie, 1996; Samarco, Ripabelli, Ruberto, Iannitti & Grasso, 1997; Autio, Sateri, Fredriksson-Ahomaa, Rahkio; Lundén & Korkeala, 2000). The possibilities of cross contamination are substantial.

Mild processing methods evoke safety concerns for meat products

Processing methods

During processing, most cured meat products are first heat treated and then packaged. The heat treatments reduce the bacterial count and select the type of bacteria which survives and which will possibly grow at cold storage temperature. Post heat treatment contamination, e.g. with *L. monocytogenes*, is a major concern for, in particular, products being peeled or sliced. In the production of ready-to-eat food, different techniques are used, e.g. cook-and-chill and sous vide cooking. During cook-and-chill processing, the product is heat-treated and subsequently hot or cold filled into the package. Cold filling will increase the risk of contamination. During sous vide cooking, raw materials are vacuum packaged and subsequently cooked under controlled conditions as regards temperature and time. The temperature is usually below 100°C, and the cooking time is longer than during traditional cooking. The main advantages of sous vide cooking is an improved juiciness and flavour, and the possibility of using muscles that are less tender and thus not suitable for conventional cooking.

Hazards

Psychrotrophic *C. botulinum* and *B. cereus* are of particular concern in minimally processed foods, e.g. sous vide cooked products, due to their ability to survive the mild heat treatment and their subsequent growth during cold storage. The growth of these spore-forming bacteria depends on many factors. Experimental data also shows a high strain-to-strain variation, in many situations. Extrinsic parameters such as heat treatment, storage temperature and gaseous atmosphere, and intrinsic parameters such as pH, salt and other additives are of importance. Furthermore, the combination of factors needs to be accounted for.

Heat treatment

The heat resistance varies from strain to strain, and with the composition of the heating substrate. The heat resistance, determined as D_{95}-values, varies between 1.2 and 36.2 minutes for *B. cereus* (Kramer & Gilbert, 1989). The $D_{82.2}$-values for non-proteolytic *C. botulinum* type E spores are reported to vary between 0.25 and 0.84 minutes in buffer, and in food products between 0.2 and 6.6 minutes (Betts & Gaze, 1995). A similar variation between buffer and meat was observed for *B. cereus*, the D_{90}-value was 11-17 minutes in buffer and 2-17 minutes in meat (Faille, Gavini & Maigonnat, 1997). At high pH- values, *B. cereus* spores are more heat resistant than at low levels. In a study by Couvert, Leguerinel & Marfart (1999), the D_{95}-values of *B. cereus* spores were 0.6 minutes at pH 5.0, and 2.8 minutes at pH 7.0. These variations in heat resistance result in difficulties when assessing the reduction of bacteria during processing.

Cooling

The cooling rate is important to consider when ensuring the safety of minimally processed foods. When heated food is cooled down, it passes temperatures that are suitable for spore germination and the growth of vegetative cells. Juneja, Snyder & Marmer (1997) studied the effect of cooling from 54°C to 7°C, at rates varying from 6 to 21 hours, on bacteria in ground beef. The results did not show any growth within this time-temperature range for *B. cereus* spores, *S. aureus* or *Salmonella*. However, spore germination and growth were shown for *C. botulinum* after 8 hours, and when a temperature of 22°C had been reached. In another study, Juneja, Snyder & Cygnarowicz-Provost (1994) found that pasteurised cooked beef had to be cooled from 60°C to 7°C within 15 hours to prevent the germination and growth of *Clostridium perfringens*.

Storage

It is difficult to produce safe food while relying only on heat treatment and still obtain good quality, and it is unrealistic to rely only on a very low temperature during storage. Consequently, it is necessary to use a combination of heat treatment and storage conditions to ensure safety. Heating non-proteolytic spores of *C. botulinum* strains for 1 minute at 90°C resulted in a 10^6 reduction and no subsequent growth during 23 weeks of storage at 5°C (Stringer; Fairbairn & Peck, 1997). Storage at 10°C required heating for 60 minutes at 90°C to ensure no

subsequent growth. Growth of non-proteolytic *C. botulinum* spores was detected after heat treatment for 10 minutes at 90°C and subsequent storage at 5°C, 8°C and 12°C after 90 days, 54-75 days and 38-42 days, respectively (Fernandez & Peck, 1999).

The effect of heat treatment (mild: $P_{85}\leq$ 2 min, increased: P_{85}= 67-515 min; z= 7.0 °C) in combination with storage temperature was evaluated for ground beef and pork cubes inoculated with non-proteolytic *C. botulinum* spores and subsequently sous vide processed (Lindström et al., 2001). *C. botulinum* was detected in 81 % of samples after the mild heat treatment, and storage at 4°C or 8°C up to 28 days, and toxin formation was observed in 17 % of the samples. After the increased heat treatment, toxigenesis was not observed, in comparison *C. botulinum* was detected in one sample (n=12). The temperature conditions used during commercial sous vide processing may thus not always eliminate non-proteolytic *C. botulinum* type B spores. During product and process development, a careful evaluation is necessary in order to obtain product safety.

Factors within the food may affect the apparent survival during heat treatment and the subsequent growth ability. In the presence of lysozyme, the measured heat resistance of non-proteolytic *C. botulinum* is increased (Peck, 1997; Graham, Mason & Peck, 1996). The germination system in non-proteolytic *C. botulinum* is heat sensitive and is relatively easily inactivated by heating. Lysozyme induces the germination of the heat-damaged spores surviving heat treatment (Haushild, 1989). Other factors/components present in food have a similar effect to lysozyme. Thus, the natural presence in foods of lysozyme, enzymes (chitinase, papain), egg yolk emulsion, extracts of fruit and vegetable and horse blood (Peck, 1997) may significantly increase the heat treatment required for sufficient spore inactivation.

The composition of the gaseous atmosphere in sous vide packaging is initially aerobic (albeit the gaseous volume is small), unless replaced by another gaseous mixture during packaging. During storage, the gaseous composition may change towards a higher CO_2 concentration, as in ordinary vacuum packages. In modified atmosphere packaging (MAP), the air in the headspace of the package is mainly replaced by CO_2 and N_2. MAP extends the shelf-life of refrigerated foods by selecting the microbial flora towards a composition that is less detrimental to product quality than the aerobic microflora (Borch, Kant-Muermans & Blixt, 1996b). However, reduced microbial competition and an anaerobic atmosphere can increase the growth potential of *C. botulinum.* Furthermore, the presence of CO_2 may act as a *C. botulinum* spore germinant (Foegeding & Busta, 1983). For cooked, uncured turkey inoculated with non-proteolyitic *C. botulinum* type B spores, toxin was detected after 14 days of storage at 4°C when packaged under 100% N_2, and after 28 days when packaged under 30% CO_2 and 70 % N_2 (Lawlor, Pierson, Hackney, Claus & Marcy, 2000). The study indicates a growth inhibitory effect of CO_2 on *C. botulinum*. Overall, it was concluded that MAP and refrigerated storage were insufficient to prevent the growth of *C. botulinum*.

Probability of growth

The high variability in bacterial behaviour, due to naturally occurring variations, suggests that the probability of growth, toxin formation and so on should be considered. For example, if an inoculation study of products performed on a low number of replicates shows no growth of a certain bacterium after specific treatment/ storage, the statistical uncertainty of this information is high, and it cannot be concluded that the studied conditions impair growth completely. Figure 1 shows the probability of the visible growth of proteolytic *C. botulinum* type A after heat treatment equivalent to F_{121}= 0.8-1.6 min and subsequent storage at 30°C. A high number of replicates (*n*= 40-98) were studied. For the lowest heat treatment, 95 % of the samples showed visible growth after 7 days of storage. For the severest heat treatment, 18 % of the samples showed visible growth (Arinder & Borch, unpublished results). It may be concluded that none of the applied F-values gave a completely safe product. In the experiment, a sterilized meat batter was inoculated with 10^{10} /g spores of proteolytic *C. botulinum* type A under anaerobic conditions. Heat treatment was performed at 100°C in an oil-filled water bath for times corresponding to F_{121}=0.8-1.6 minutes (z= 10 °C). In each treatment, 40-98 replicates were used. After heat treatment, the tubes were chilled under anaerobic atmosphere and agar was put on top of the meat batter. Subsequently the tubes were put in incubators at 30°C and studied each day for visible growth shown as visible gas production within the meat batter.

Figure 2 shows the probability of growth of psychrotrophic *B. cereus* under various conditions with respect to pH, NaCl-concentration and incubation temperature (Arinder & Borch, unpublished results). The first signs of growth occurred after 1-5 days of incubation. There is a delay in time of 4-15 days between the first observation of growth, that growth being observed in all replicates (*n*= 50). The information can be extended into an estimate of recommended storage times. Provided the objective that at least 95 % of products are to show no growth of *B. cereus*, the storage time for the combination pH 6.5 and 3.5 % NaCl (w/w), a shelf-life of 4 days can be set at 8°C. The experiment was performed by inoculating 4 log cfu/g spores of three strains of *B. cereus* in Brain Heart Infusion broth with adjusted levels of NaCl (0.5 and 3.5 w/w %) and pH (5.5, 6.5). Incubation was performed in microtitre plates at 8°C and 10°C. For each combination of pH, NaCl and storage temperature, 50 replicates were analysed by recording the absorbance during incubation. The time at which a change in the absorbance was observed was used as a measure of time to growth. This time included resuscitation, lag phase, and growth to a detectable level. In conclusion, probability distribution functions are needed which take into account the variability among strains and treatments in order to more precisely evaluate safety in terms of microbial inactivation, survival and growth.

Conclusion

There is a palette of measures when dealing with current safety issues in meat and meat products. Measures must be applied along the whole production chain, from the stable, via processing, to the table and applied as early as possible in the production chain. The epidemiology is not fully understood for many of the existing and potential foodborne pathogenic bacteria. Two examples are *Campylobacter* and *C. botulinum*; in spite of a high occurrence in pigs and cattle, the connection between foodborne disease and outbreaks caused by meat and meat products is tenuous. The points where safety may be assured need to be identified. New DNA-based tools for the specific detection/enumeration and characterization of bacteria will clarify which species and subgroups are of real concern. A probabilistic approach when evaluating the safety of processing methods will ensure more reliable results and, consequently, safer processes.

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Table 1. The occurrence of various bacteria in faeces from pigs and cattle.

	Sample	Occurrence (%)	Reference
Arcobacter spp.	Cattle	14	Wesley et al., 2000
Bacillus cereus	Cattle	33	1)
Campylobacter spp	Cattle	16-83	2)
Campylobacter spp.	Pig	96	Borch, Nesbakken & Christensen, 1996a
Clostridium botulinum type B	Pig	62	Dahlenborg, Borch & Rådström et al. submitted
Clostridium botulinum type B	Cattle	73	Dahlenborg, Borch & Rådström, 2001
Enterobacteriaceae	Cattle	81	1)
Presumptive *E. coli*	Cattle	88	1)
Enterococcus spp.	Cattle	52	1)
Clostridium perfringens	Cattle	23	1)

1) Borch & Nerbrink, Swedish Meats R&D, unpublished
2) Borch & Blixt, Swedish Meats R&D, unpublished

Table 2. Serotypes of *E. coli* harbouring *vt*1 and/or *vt*2 genes. The strains were isolated from cattle faecal samples (n=198).

Serotype of *E. coli*	Number (%) of isolates	Isolates positive for		
		*vt*1	*vt*2	*vt*1 + *vt*2
O8	8 (12)		8	
O22	1 (1)		1	
O73	1 (1)		1	
O91	11 (16)		9	2
O105ac	3 (4)			3
O113	16 (24)		12	4
O116	1 (1)		1	
O136	1 (1)			1
O162	3 (4)		1	2
O163	2 (3)		2	
O172	1 (1)			1
E8686/77	2 (3)	1	1	
E43478/86	5 (7)		5	
O?	14 (21)	1	3	10
Total	68	2	44	22

Nerbrink, Borch & Löfdahl; unpublished results. Serotyping was performed by T. Cheasty, PHLS, London, UK.

Table 3. The occurrence of VTEC and presumptive *E.coli* in a beef slaughterhouse. Samples comprising swab and sedimentation samples of the processing environment, faeces, carcasses (pelvic duct and thick flank incision about 650 cm^2 plus inside of fore shank about 350 cm^2), 25 g meat cuts were taken during 15 visits over a period of 19 weeks. (Borch & Hofvendahl, unpublished results)

Sample	Position	Occurrence (% positive samples)		No. of samples
		VTEC[a]	Presumptive *E. coli*	
Environment[b]	Slaughter	11	40	55
Environment[b]	Chilling	0	0	42
Environment[b]	Cutting	3	5	37
Faeces	Slaughter	57	100	60
Unchilled carcass	Slaughter	32	42	60
Chilled carcass	After chilling	7	22	60
Meat cuts	Cutting	3	43	60

[a] VTEC: detection of either of the genes *vt1* or *vt2* using PCR.
[b] Sampled during processing

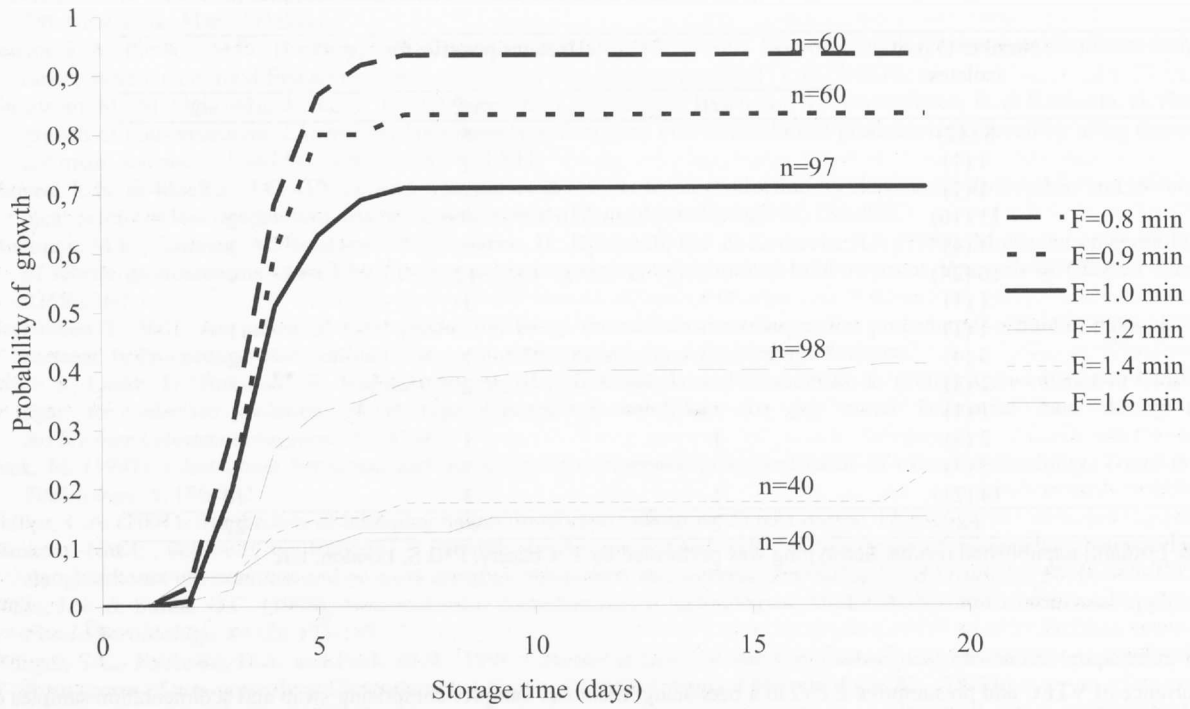

Figure 1. The probability of growth of non-proteolytic *C. botulinum* type A ($N_0=10^{10}$ spores/g) in meat sausage batter (pH 6.1; 38 ppm nitrite post heat treatment; 2.5 w/w % salt; 66 % water) after a heat treatment equivalent to $F_{121}^{10} = 0.8$-1.6 minutes, and subsequent storage at 30°C. Growth was indicated by visible gas production in the test tubes. The number of replicates is given in the Figure. (Arinder & Borch, unpublished results)

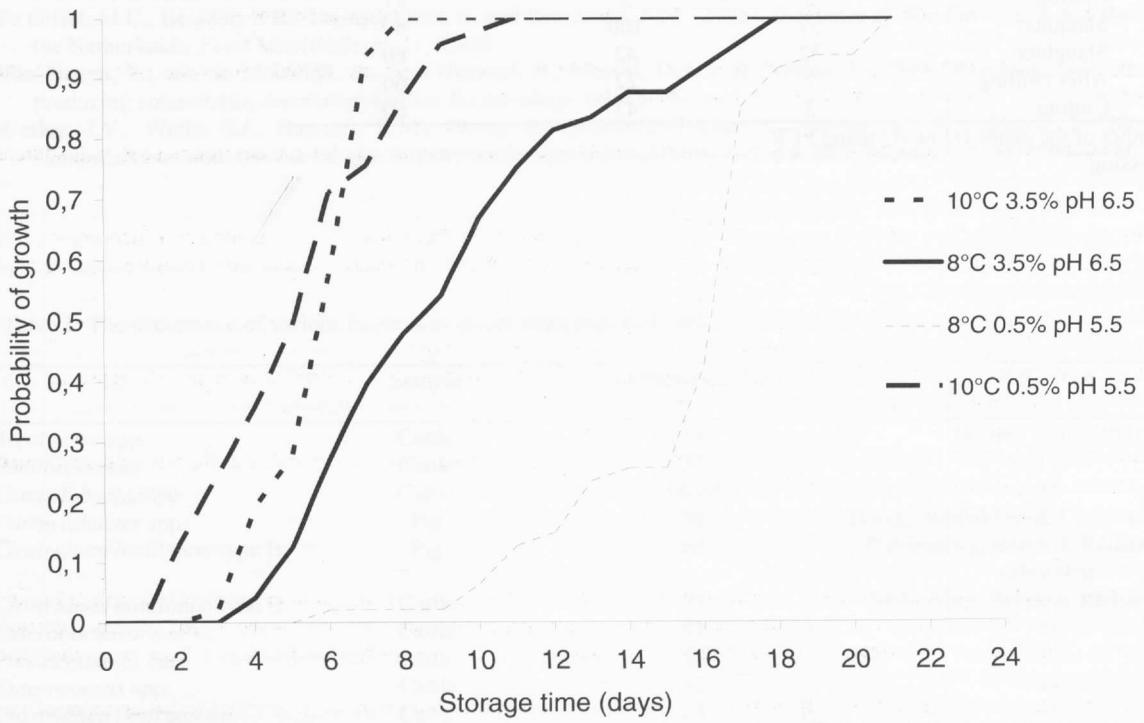

Figure 2. The probability of growth (n=50) of psychrotrophic *B. cereus* ($N_0=10^4$ spores/ml) in BHI-broth with varying pH and salt levels at 8°C at 10°C. Growth was recorded as change in absorbance. (Arinder & Borch, unpublished results)

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