Opinion of the SCVPH on cooling of carcasses during transport - 15 - 16 March 1999

1. TERMS OF REFERENCE

Council Directive 64/433/EEC as amended by Directive 91/497/EC Chapter XV, Transport: Para 69. 3rd Indent (a) states - " by way of derogation from the 1st paragraph, carcasses, half carcasses, half carcasses cut into no more than three wholesale cuts, and quarters may be transported at temperatures higher than those laid down in Chapter XIV under conditions to be set after consultation of the Scientific Committee in accordance with the procedures laid down in Article 16 of this Directive".

The Committee was asked to evaluate whether, from a consumer health point of view, [hygienic equivalence] it is possible with the current available transport cooling equipment to cool during transport, carcasses, half carcasses, cuts into no more than three wholesale cuts, and quarters, as referred to in Directive 64/433/EEC, and if so under which conditions. The Committee is asked to assess the additional risk for consumer health introduced by cooling of carcasses during transport instead of the usual practiced stationary cooling.

2. BACKGROUND

The Commission requested the Scientific Veterinary Committee (SVC) to examine this question and make recommendations. In turn the SVC formed a working party to examine and report back to it. The working party's first meeting was 12 December 1991.

However, in 1985 and before this 1991 request the Commission Services had in the framework of the Cold Chain, begun to examine the possibility of allowing transport of fresh meat before this temperature (7ºC) had been achieved. This with the intention of laying down alternative rules while offering to the Consumers the same health guarantees. This matter was referred to the SVC which in turn reported (2951/V1/85 EN file 6.21.11). When their recommendation was passed to a Commission Working Group meeting (6-7 March 1985: Document 2011/V1/85 Rev 1) the proposal was not accepted. The grounds for its non-acceptance being that it was restricted only to pig meat; some Member States considered this a restriction and sought arrangements for meat of all species. The matter then remained in abeyance until the present Scientific Committee working party was established.

3. INTRODUCTION

Council Directive 64/433/EEC as amended by Directive 91/497/EEC Chapter XIV, Storage, Para 66 states: Fresh Meat must be chilled immediately after the post mortem inspection and kept at a constant internal temperature of not more than 7ºC for carcasses and 3ºC for offal. Derogations from this requirement may, for technical reasons relating to the maturation of meat, be granted by the competent authority on a case by case basis for the transportation of meat to cutting rooms or butcher shops in the immediate vicinity of the slaughter house, provided that such transportation takes not more than one hour. The Directive also provides for meat to be cut warm.

It was considered that the present provisions for cooling of carcasses, half carcasses, cuts into no more than three wholesale cuts, and quarters as referred to in Directive 64/433/EEC, require immediate chilling after the post-mortem inspection, without further specification of the conditions under which this needs to be carried out. This lack of specifications implies that also for cooling in stationary plants, no cooling kinetics have been provided, which may lead to a lack of uniformity in the actual process being used. However, the Committee compared the currently available possibilities for cooling during transport with those generally applied in stationary plants, with special emphasis on maintaining a high level of hygienic protection for the consumer.

4. REFRIGERATION EFFECTS (technical aspects)

The major risk to health from eating meat is the possibility of ingesting pathogenic organisms in numbers sufficient to cause disease. As it is possible for any healthy animal to carry potentially pathogenic organisms, it must be assumed that all meat may be contaminated. Regulatory requirements for handling of meat during and after carcass dressing aims to restrict the possibility of pathogenic organisms growing to numbers that might constitute a health hazard. The regulation of the cooling rate is a means of controlling the potential growth of pathogenic and spoilage organisms.

Too rapid chilling in the case of ruminant-carcasses may produce cold shortening, a biochemical event producing tough meat, and a product unacceptable to the consumer. However, currently there are usually alternative methods in practice to overcome cold shortening e.g. electrical stimulation.

Within 64/433/EEC no time is specified for the interval between a carcass departing a slaughter hall and entering refrigeration and its subsequent exit from these chillers now being at a temperature of no more than 7ºC. The single requirement is for fresh meat to be chilled "immediately " after the post mortem inspection. For some microbial species growth will continue at low rates down to 0 ^{o} C. The temperature of 7 ^{o} C could have been chosen to minimise the growth of Salmonella. Limited growth at lower temperatures has been documented (Mossel et al., 1981 and Catsaras and Grebot, 1984).

The surface temperature and dryness will be of most significance to microbiological growth. However the measurement of meat temperature is commonly made from within the depth of the hind leg muscle mass with respect to anaerobe and facultative anaerobe microbes and chilling reserve. It should be recognised that the temperature within the deep muscle is an important factor in the chilling process.

The microbiological load present on any carcass whatever the species, will be influenced by the hygienic practices during the slaughtering process and the initial cleanliness and health status of the livestock. Good manufacturing practices (GMP) must be applied throughout.

The existing regulatory framework for transport vehicles (e.g. DIN (Deutsche Industrie Norm)norm 8959 - testing of heat insulated transport means for foodstuffs) does not contain any specific requirements for the long distance transport of foods undergoing further cooling. It is based on the assumption that at loading the goods to be carried have attained the required temperature, and it can then be maintained in the transport vehicle. However, the requirements for transporters as described in the Agreement on the international carriage of perishable foodstuffs and on the special equipment to be used for such carriage often referred to as ATP provides for the requirements for transporters carrying either chilled or frozen products is clearly prescribed (Anon., 1991).

The refrigeration capacity of cooling units in stationary plants (abattoirs) required to extract heat from hot carcasses (40°C) received directly from the slaughter line, is much greater than that of cooling units fitted to lorries carrying refrigerated meat. This is to be expected, and explained by the different tasks required of each. One (abattoir) to remove the majority of heat, the other (transporter) to maintain and continue this carcass heat loss during transport for an already partially cooled carcass. Initially the surface temperature of the carcass is high so the temperature difference between it and the air is large. A considerable amount of heat transfer flows from the carcass into the air at various rates during the 24 hours after slaughter. In beef carcasses over four times the average rate of heat flow is found in the first hour. After approximately 8 hours the rate of release is approximately average for the total 24 hours of cooling. After 16 hours it is 0.4 of the average (Cox and Bailey 1978). Since the transport cooling is primarily concerned with the maintenance of a low temperature which has been obtained through stationary cooling, the cooling capacity of transport cooling units is generally significantly lower than the capacity of stationary units. This leads to lower cooling rates in the transport units (see Chapter 7).

Table 1 provides storage densities typically found during refrigeration for pigs, beef and sheep in the abattoir, and during transport. The storage density in the transporter exceed those of the abattoir where tighter packing is necessary to counter the motion of the lorry when driven. However, only transporters fitted with equipment to maintain separation of the carcasses should be used.

Table 1 Number of carcasses/sides per meter of Hanging Rail

Species Abattoir Transport (Stationary)
Pig 6-8 8-10 75kg carcass receiving fast chill 50 minutes before entering equilibration chiller.
Pig 3-4 7-8 No case hardening before enters abattoir chiller.
Sheep 4 9 5-6.
Beef 2 3 (hind 1/4).

Within transporters hanging rails are now fitted at intervals with stop/locks to prevent compaction. In addition, to ensure air circulation throughout vehicles are fitted with conduits extending from the cooling units to allow its distribution throughout.

Because they are mounted at the front wall of the loading space the refrigeration units in the vehicles are far less efficient than the ceiling evaporators used in stationary cold-storage plants.

There must be enough space between the walls and the transported goods to allow an appropriate flow of air. A further disadvantage is that there is a continuing trend to increase the effective loading capacity of chilling vehicles by saving space by installing smaller, compact refrigeration units. As a result, often units with a smaller chilling capacity are installed.

5. BACTERIOLOGICAL PARAMETERS TO TAKE INTO ACCOUNT

Refrigeration is systematically applied to meat. Reducing the bacterial growth on the meat exerts a beneficial influence on keeping quality, reducing the growth rate of spoilage bacteria and on consumer health. Therefore in order to assess the efficiency of different chilling temperatures it is necessary to consider the effect on the different microbial associations in the different parts of the carcass:

a. Inhibition of deep spoilage

Bacterial growth in the deep musculature, leading sometimes to phenomena like bone taint, can be avoided by early and rapid chilling of the carcasses.

b. Inhibition of the growth of superficial microflora

Most of the microbial population of fresh meat is on the surfaces of carcasses or cuts. After chilling storage the microbial population consists also of bacteria, able to develop at temperatures down to just above the freezing point, but their optimum growth temperature is usually 20 to 30ºC (range). They are often involved in meat spoilage.

Nevertheless, there are two reasons why a lower temperature reduces the growth of microflora on and in the carcasses:

- their lag phase is extended;
- their rate of growth is reduced

Therefore lowering the temperature and keeping it near to 0ºC improves the keeping quality of fresh meat.

c. Other factors influencing bacterial growth on fresh meat.

Temperature although of paramount importance, is one parameter influencing bacterial growth on fresh meat. Other

important factors, intrinsic and extrinsic to fresh meat, to take into account are:

- initial bacterial count
- time
- water activity (a_W)
- relative humidity (r.h.)
- pH
- pO_2 and pCO_2

All these factors either alone or in combination, are directly influencing the composition of the microbial flora on and/or in the meat. This includes strict anaerobes, aerobes and facultative anaerobic bacteria. Attention should be paid to facultative anaerobic bacteria because of their ability to colonise both deep and surface muscle tissue.

6. REFRIGERATION IN PRACTICE

Refrigeration facilities albeit stationary or during transport of fresh meat, can be an additional source of contamination. A failure to handle meat hygienically and contact with equipment not adequately cleaned can also be an additional source of contamination.

Furthermore, the following conditions could lead to an increase in bacterial population already present on and in the carcasses:

a. breakdown or insufficient capacity of cold chain;

b. accumulation of moisture on the meat, leading to increased surface water activity and to an increase in microbial association;

c. cross-contamination of carcasses.

Both capacity of the refrigeration equipment and sufficient air circulation to remove residual carcass heat are basic elements for ensuring appropriate and efficient refrigeration conditions.

Both these factors are critical issues for refrigeration during transport.

7. BACTERIOLOGICAL CHANGES ON CARCASSES DURING COOLING

7.1. Time-temperature relations during cooling in stationary and transport phase

The rate of cooling varies between stationary cooling and cooling during transport. A number of studies have been carried out, which are reflected in Tables 2, 3 and 4.

Table 2 The temperature on pig skin and in deep tissue after different methods of cooling (Wenzel et al.,1983):

Cooling techniques (temperature and duration)	Temperature in both sampling sites beneath 15 and 10 °C after	APC (log) per cm^2 Correspondingly
2 h at -8 °C and 17 h at +2 °C	< 15 °C after ca. 3 h < 10 °C after ca. 8 h	3.7 to 4.4 3.7 to 4.4
Generally at +4 °C	< 15 °C after ca. 8 h	4.2

	< 10 °C after 13 h	4.3
2 h at -8 °C and followed by +4 °C	< 15 °C after ca. 5 h < 10 °C after 10 h	4.0 4.0
2 h at -8 °C and followed by +10 °C	< 15 °C after ca. 10 h < 10 °C: not possible	about 4.2
2 h at -8 °C and followed by +16 °C	+15 °C not possible	./.
Generally at +10 °C	<15 °C after ca. 12 h	about 4.8
Sampling sites for temperatures: 0.5 cm beneath the surface 5.0 cm beneath the surface APC = Aerobic Plate Count		

Woltering (1990) monitored the temperature of pig carcasses 0.5 cm beneath the skin. Cooling conditions of -25 °C for ca. 40 min. (followed by a 2nd phase at +5 °C) resulted after 40 min. in -5 °C, followed by a successive increase to a temperature not passing +5 °C.

Table 3 The cooling time in different practical trials using stationary and transport cooling (Holzer and Ring, 1998)

	Test	Start temp. °C	Finish temp. °C	Temp. fall °C	Time taken h
1.	Test: pigs, hindquarters				
1.1.	Stationary:	14	12	2	1.5
1.2.	Transport:	14	12.2	1.8	6
2.	Test: beef				
2.1.	Stationary:	16.9	7	9.9	12
2.2.	Transport:	16.9	5.9	11	28
3.	Test: beef				
3.1.	Stationary:	15.4	7	8.4	11
3.2.	Transport:	15.4	6.5	8.9	29

Table 4 The cooling time in different experimental trials using stationary or transport cooling

Interval from approx. 40 °C to 15 - 17 °C (Wenzel et al. ,1983) Stationary: Pigs: 3-10 h Beef: Not available
Interval from approx. 15 °C to 7 °C (Holzer and Ring 1998) Stationary: Pigs: Not available Beef: Forequarters: 6 h

Hindquarters: 14 h Transport: Pigs: Not available Beef: Forequarters: 16 h Hindquarters: 29 h

7.2. Bacteriological associations on carcasses (qualitative and quantitative data)

7.2.1. Deep tissue of carcasses before or immediately after slaughter

It is recognised that the muscle tissues of a healthy animal are basically sterile whereas the surface is invariably subjected to a certain degree of contamination during even the most hygienic of dressing operations. It is obvious now that marketing stress may induce massive faecal excretion of salmonellae even in groups where a few pig were shedding low levels before slaughtering (Gray et al., 1996; Corrier et al., 1990). Internal organs which are most frequently infected in long-term carriers of Salmonella seem to be infected at a low level, but fairly stable (Wood and Rose, 1992): tonsils, mandibular nodes, intestinal walls, ileo-colic lymph nodes. These organs may even be reached by alternate routes of invasion as was shown experimentally in esophagotomised pigs (Fedorka-Cray et al., 1995).

A literature review on this subject was conducted by Schüppel et al., 1994. Questions remain to be solved as to whether these alternate routes of infections are the reason for infected internal organs or that translocation from the intestinal contents into epithelial cells and consequent infections of gut walls and other organs such as liver and spleen is a more important route.

Moreover, certain slaughter procedures such as penetrating captive bolt, stunning, sticking, pithing, scinning, scalding and dehairing of pigs may influence the bacteriological status of the deep tissues and organs negatively (Mackey and Derrick, 1979; De Zuniga et al., 1991).

7.2.2. Bacterial numbers on carcass surfaces

The overall hygiene of each process, slaughter, refrigeration etc. must be taken into account in any assessment for each process stage, and will have an impact on the bacteriological association (status) of the next and subsequent stages. The bacteriological status of a carcass depends on several factors including the performance of any technical devices and personnel involved.

The results of bacteriological examinations obtained with different sampling techniques, and sampling sites together with different laboratory techniques may provide different results. Thus caution is necessary when interpreting microbiological results.

a. Aerobic Plate Count (APC):

Generally the initial count should be about log 3-5/cm² (pork as well as beef), although higher data in beef and lamb (log 4-5/cm²) may be possible. In Table 5 only data from the last decade have been quoted, mirroring more recent facilities in the abattoirs.

Table 5: Bacteriological data immediately prior to chilling (per cm²) [1] :

	APC	Enterobacteriaceae	Source
Pork	5.3 x 10^3 log 3 - 3.5 < log 4	3 %	Gill and Bryant (1992) Rahkio et al. (1992) CMA (1998) required as a guideline

Beef	log < 3-4 < 5 x 10^3 10^2 - 6 x 10^3	1-10	Whelehan et al. (1986) Gustavsson and Borch (1993) CMA (1998); required as a guideline Sumner (1997)
Lamb	log 5.0	log 4.5 (psychrotroph.)	Prieto et al. (1991)

b. Taxonomic differentiation:

The bacteriological association on or in a sample is far more difficult to assess in relation to the different species or genera present. Whereas bacterial associations in general (total counts) can be explored by means of non-selective techniques, selective techniques are necessary to evaluate the level of pathogens or other specific groups of bacteria.

On beef carcasses from 10 packing plants, Gill et al. (1998) found an APC of 1.52 - 3.26/ cm ^{2}, correspondingly coliforms ranging from "Negative" to 1.44/ cm ^{2} resp. *E. coli* from "Negative" to 1.08/ cm ^{2} (= after cooling). Stolle (1985) reviewed the literature for beef during chilling and reported as main taxa: Pseudomonas, Acinetobacter, Moraxella, Enterobacteriaceae, Micrococcus and Brochothrix. Gill and Bryant (1997) found a predominating Gram-negative microflora on pork carcasses (Table 6). In both plants the APC increased with chilling, but the relation of Gram-positives and Gram-negatives remained quite similar.

Table 6: Microflora in % of total APC (swab techniques) on pork skin before and after chilling (Gill and Bryant 1992)

	APC/cm ^{2}	Micrococci	Acinetobact./ Moraxella	Pseudom.	Flavobact./ Alteromonas	Bacilli
Pre chiller wash	3.11	34%	20%	28%	14%	10%
* plant A	3.36	22%	47%	14%		
* plant B						
Exit from chiller	4.26	35%	31%	21%	4%	
* plant A	3.53	38%	28%	19%	13%	
* plant B						

Beef: Bläschke and Reuter (1984) assessed the bacterial associations on beef carcasses during cooling. The percentage of Gram-negatives was 884 Gram-negatives out of a total of 4221 isolates (i.e.: 20.9 %). Among Gram-negatives the authors found increasing numbers of isolates belonging to Pseudomonas genus over time (Table 7). However, the greatest number of isolates were Gram-positive (i.e.: 79.1 %).

Table. 7: Bacterial associations (in % of isolates) during the cooling procedure (Bläschke and Reuter, 1984):

	after 2 d (n = 295) (n = number of isolates)	after 7 d (n = 589) (n = number of isolates)
Flavobacterium	4	-
Enterobacteriaceae	11	3
Acinetobacter	23	2
Alcaligenes	3	1

Moraxella	22	2
Pseudomonas	36	91
Aeromonas	2	-

Pork: On pigskin Woltering (1990) found at the beginning of chilling predominantly Gram-positive, irregular rods and Micrococci. The pattern of the microflora changed related to relative humidity and time: higher humidity imposed higher amounts of Gram-negatives.

The main organisms on surfaces were Pseudomonas, Moraxella, Acinetobacter, Enterobacteriaceae. Among Gram-positives Micrococci and Gram-positive irregular rods were predominant. Quantitatively, in general Gram-positives were about 1/3 of the flora (Gill and Bryant 1992). However, Gram-positives constituted more than half the quantity in the beginning of chilling (Woltering, 1990). Results of Bläschke and Reuter (1984) yielded about 80 % of isolates being Gram-positive. Woltering (1990) found no Enterobacteriaceae on surfaces during chilling.

With a relative humidity of 80 as well as 90 % the percentage of Gram-positives declined sharply, beginning with two days of chilling (Feldhusen et al. 1992). The impact of relative humidity on the APC has also been demonstrated: bacterial growth was more intensive with a relative humidity of 90 % compared to 75-80 % r.h. in experiments reported by Buttiaux and Catsaras (1966).

Gill and Bryant (1997) strongly support the continuous monitoring of the temperature at all stages of the cold chain in order to avoid insufficient cooling and to prevent growth of *E. coli.*

From a health risk assessment point of view the most relevant bacteria to consider on carcasses are:

Salmonella spp.

Y. enterocolitica (pork)

E. coli (EHEC)

L. monocytogenes

Campylobacter, especially in poultry (will not grow on surfaces because of its sensitivity to a_W and O_2 as well)

Since the 7°C is likely to have been defined in relation to minimising Salmonella growth, the growth conditions for this genus is considered in greater detail in the following. Salmonella generation times are presented in Table 8.

Table 8: Salmonella Generation Times

Temperature °C	Environment	Generations/ h min/ Generation	Source
22	PH 7.3	50 min.	SCHEIBNER1994 (impedance)
20		72 min.(1.2 h) 1.4 h 5. h (lean beef) 1.1 h (fatty beef)	BROUGHALL et al. 1983 GRAU 1987 DICKSON et al. 1992 " " "
18	pH 7.3	100 min 112 min.(1.7 h)	SCHEIBNER 1994 (impedance) BROUGHALL . et al. 1983

16	100 % r.h.	132 min (2.2h) 156 min (2.6 h)	BROUGHALL et al. 1983 GIBSON et al. 1988
15	100 % r.h. pH 7.3	0.2 5 h 100 min 2.9 h 2.1 h (lean beef) 1.5 h (fatty beef)	GILL 1979 SCHEIBNER 1994 (impedance) GRAU 1987 DICKSON et al. 1992 " " "
14	100 % r.h.	186 min (3.1h) 246 min (4.1 h)	BROUGHALL et al. 1983 GIBSON et al. 1988
12	100 % r.h.	300 min. (5 h) 402 min (6.7 h) 5.4 h	BROUGHALL et al. 1983 GIBSON et al. 1988 GRAU 1987
10	100 % r.h. 100 % r.h.	0.1 10 h 10 h 11.5 h 9.9	GILL 1979 BROUGHALL et al. 1983 GIBSON et al. 1988 GRAU 1987
8	0.98 a_w 100 % r.h.	24 h 21.8 h 35 h	BROUGHALL et al. 1983 GIBSON et al. 1988 GRAU 1987

7.3. Concluding remarks for chapter 7

To summarise the results from technology and microbiology of carcass surface and to decide a starting point for further calculations, the following need to be considered:

- Time-temperature curves in and on carcasses:

The temperature patterns in carcasses during cooling have yet to be thoroughly assessed. The data available do not elucidate the situation completely. This may be born in mind when drawing conclusions from these results. It seems reasonably well documented, however, that transport cooling in general is significantly slower than stationary cooling.

- Aerobic Plate Count (APC):

APC concentrations are generally between log 1 and log 4/cm^2.

- Proportion of Gram-negatives vs. Gram-positives:

Although Gram-positive genera are dominating shortly after slaughter, as cooling commences the relative fraction of Gram-negatives will increase. Enterobacteriaceae may constitute 1-10% of the Gram-negative number. Also, as a_W increases so will the percentage of Gram-negatives.

Any bacterial strains present as a minority in a mixed flora may be suppressed by the presence of either faster growing or more numerous bacteria. These events can seriously complicate any predictions of bacterial patterns when results are interpreted.

8. CALCULATION OF BACTERIAL MULTIPLICATION RATES

8.1. Basic assumptions

Generally the terms of growth / multiplication should be separated from the term of "contamination" (Gustavsson 1997), meaning the mere presence of bacteria on or in a sample.

Multiplication of bacteria depends strongly on circumstances as described above. Although the particular environment cannot be defined and predicted entirely because of its dependence on history and further fate, the bacteriological association is of utmost importance for the growth pattern of a (pathogenic) microorganism on a carcass surface.

The refrigeration period is for the purpose of this calculation divided into a stationary phase (Section 8.2) and the following transporting phase (Section 8.3). The main body of the calculations relate to Salmonella, but there will be a number of examples of growth patterns for other relevant bacteria also.

Considering a potential inhibiting impact of competing bacteria on the growth of Salmonella, an initial concentration of Salmonella on the surface of the carcass of 10/cm² could be too high, but it will in these calculations serve as a conservative estimate.

8.2. Bacterial growth patterns during initial stationary cooling

During initial stationary cooling the carcass will normally pass a temperature interval of about 25-30 °C. Considering the deep chill temperature on the surface during the air blast, and the profound changes in environment (temperature and water-activity), bacterial growth is probably not realistic during this phase. On the contrary, a sub-lethal injury could be expected during this first and fast chilling period.

Consequently the number of Salmonella as well as other bacteria may remain constant or the bacteria may even suffer sub-lethal injury during this initial treatment, when assuming a cooling technology according to GMP principles.

From these assumptions it seems realistic to estimate a situation where the bacteriological risk from meat-borne pathogens does not increase during a normal stationary cooling phase.

8.3. Bacterial growth patterns during cooled transport

For this calculation an additional "worst case" assumption is introduced: it is assumed that from the beginning of transport no further decline of the temperature reached during stationary cooling will occur. Under these conditions a generation time slow enough, and therefore a temperature low enough, has to be ensured to keep the meat safe (with regard to pathogens) and stable (with regard to spoilage).

8.3.1. Generation times and resulting Salmonella concentrations at different temperatures

The generation times for Salmonella shown in Table 9 may serve as calculation aids:

Table 9 Resulting concentration of Salmonella on carcasses after a transport of 8 hours at different temperatures, assuming an initial Salmonella concentration of 10/cm²

Temperature	Permitting a generation time of	Generation cycles	Resulting conc. of

	(from Table 8)		Salmonella/ cm²
22° C	50 min.	9.6	10,000
20°C	70 min.	6.9	1,000
18°C	100 min.	4.8	300
16°C	130 - 160 min.	3.7 - 3	100
15°C	174 min.	2.8	70
14°C	190 - 250 min.	2.5 - 2	60 - 40
12°C	300- 400 min.	1.6 - 1.2	30 - 25
10°C 7°C	600 - 700 min No growth	8. - 0.7 0	20 - 15 10

Gill et al. (1991) calculated the following model for growth of *E. coli*, where y expresses the growth rate (as generations/ h) and x the temperature in °C. In this context this is relevant because the pattern of *E. coli* is in many cases used as a rough estimate of the fate of Salmonella.

a) $y = (0.0513x - 0.17)^2$ when x is between 7 and 30 °C

b) $y = (0.027x + 0.55)^2$ when x is between 30 and 40 °C

Using equation a) the results presented in Table 10 can be derived.

Table 10 Resulting concentration of *E. coli* on carcasses after a transport of 8 hours at different temperatures, assuming an initial *E. coli* concentration of 10/cm²

Temperature °C	Generations per hour	Generation time (min)	Resulting conc. of *E.coli* / cm ^{2}
22	0.92	65	1800
18	0.56	107	240
15	0.36	166	80
14	0.30	200	60
12	0.20	300	30
10	0.12	500	20
7	0.04	1500	10-15

Combining the theoretical data from Tables 9 and 10, taking into consideration that these data are derived from experimental and theoretical (modelling) data respectively, and accepting the extrapolation from E.coli to Salmonella growth data, it can be concluded: a starting temperature of 22 °C would enable a number of generating cycles between 7 and 10, when supposing a transport time of 8 hours, resulting in about 2000 - 10000 Salmonella cells per cm ^{2}.

From Table 4 it can be seen that the time necessary for cooling down from approx. 15 to 7°C is 16 h for beef forequarters, whereas it is 29 h for beef hindquarters. Hindquarters will need a longer time to cool down. As an example Table 11 presents the resulting concentration of Salmonella after a cooling transport at different temperatures and assuming a total transport time (with a constant temperature) of either 10 or 18 hours.

Table 11 Resulting concentration of Salmonella on carcasses after a transport of 10 and 18 hours at different temperatures, assuming an initial Salmonella concentration of 10/cm²

Temperature	Generation time	Assumed transport cooling	Generation	Resulting Conc. of

	(from Tables 9 and 10)	time	cycles	Salmonella/cm ^{2}
10°C	10h	600 min (10h)	1	20
10°C	10h	1080 min. (18h)	1.8	35
14°C	200 min.	600 min.	3	80
14°C	200 min.	1080 min.	5.4	460
15°C	160 min.	600 min.	3.8	140
15°C	160 min.	1080 min.	6.8	1000

8.3.2. Rates of bacterial concentration increase at different temperatures

Table 12 presents an example of an estimate of the duration in hours to obtain a 10-fold increase in the bacterial concentration on meat surface at temperatures from 5 to 20 ° C for four meat relevant pathogens. This time period is independent of the initial concentration, i.e. the results are true for an increase from 1/cm ^{2} to 10/cm ^{2} as well as from 100/cm ^{2} to 1000/cm ^{2}.

Table 12 Estimated time in hours* to obtain a 10-fold increase in the bacterial concentration on meat surface, i.e. from 1/cm2 to 10/cm2 or from 10/cm2 to 100/cm2. Lag phase not included. Data generated using USDA Pathogen Modelling Program, Version 5.1, entering a pH of 6,8 and a Sodium chloride % of 0,9.

Temperature (° C)	*Salmonella spp.*	*Escherichia coli* O157	*Yersinia enterocolitica*	*Listeria monocytogenes*
5	N.A.	N.A.	28	32
7	N.A.	N.A.	19	22
10	27	20	12	13
12	16	13	8.9	9.6
14	10	9.3	6.8	7.2
16	6.4	6.7	5.3	5.4
18	4.5	5.1	4.3	4.3
20	3.2	3.8	3.5	3.3
* uncertainty figures not included, since experimental generation time data vary considerably N.A.: not applicable, only limited data available				

A lag phase is not included in these estimations reflecting the worst case scenario, that the bacterial populations on the meat have already passed through this phase during stationary cooling. A pH value of 6,8 and a sodium chloride % of 0.9 corresponding to an a $_w$ of 0.995 were chosen again to reflect worst case situations, although the pH in meat is generally considerably lower and although the air-drying of carcasses is also likely to lower the a $_w$ at the meat surface.

The 10-fold increase was chosen to reflect a significant increase, but the corresponding human health risk increase has not been calculated. The data in Table 12 should therefore only be considered examples to compare the growth of relevant bacterial pathogens at different temperatures.

The USDA Pathogen Modelling Program has been validated by independent experts for E.coli O157 in raw ground

meat (Walls and Scott, 1996). The experimental data differed from the model primarily in relation to the lag phase. However, the estimation of the time for a 1000 fold increase was very close to the experimental data. The authors warn that similar experiments should be performed for carcass microbiology.

9. CONCLUSIONS

In calculating the number of bacteria on surface during cooling, various factors such as temperature, relative humidity, the starting number of bacteria as well as the duration of the particular environmental conditions should be taken into account.

Unfortunately only little data reflecting the time-temperature curves for carcass cooling are available. However, there is a clear indication that cooling during transport is less efficient than stationary cooling.

Likewise very little specific data considering the growth of pathogens during cooling of carcasses is available. However, from a number of general studies it is known that growth of some relevant pathogens such as *Yersinia enterocolitica* and *Listeria monocytogenes* will continue down to (and below) 5°C, whereas other relevant faecal bacteria such as *Salmonella spp*. and *E. coli* only show very limited growth below 7°C. At 10°C many faecal pathogens do grow slowly, and the growth rates increases significantly at higher temperatures. The chosen temperature of 7°C is therefore not a temperature preventing the growth of all relevant pathogens, if this was necessary the temperature should probably be below 0°C.

The growth of pathogens during rapid stationary cooling from 40°C is likely to be insignificant. The introduction of a less efficient cooling regime during transport is likely to increase the time period before 7°C is reached (the cooling time), thereby increasing the risk of pathogen growth. The importance of this cooling time increase will increase with the meat temperature at the transport initiation. In principle there will be no cooling time increase and therefore no risk increase if the meat is 7°C at transport initiation and this temperature is maintained during transport. A low level of risk increase will result at 10°C and a short total transport time, a higher level if the temperature or the total transport time is increased.

From the above considerations it is clear that every deviation from stationary cooling, using the transport cooling capacities, enhances the risk of bacterial multiplication. If a low level of risk increase is considered acceptable, preferably after an overall cost-benefit analysis of the stationary cooling capacity versus transport cooling, it is imperative that this level is managed strictly, considering the following:

To minimise additional bacterial growth the deep tissue temperature should be below 10°C before transport initiation. The transport equipment and conditions should ensure continuous cooling i.e. the air temperature should always be below 7°C. The surface meat temperature and the deep tissue temperature should always be below 10°C during transport, and the total transport time should never exceed 8 hours if meat temperature is above 7°C.

10. SUMMARY AND RECOMMENDATIONS

1. Cooling of carcasses, half carcasses, or carcasses cut into no more than three wholesale cuts and quarters, during transport with the current available transport cooling equipment, will introduce an *additional risk* for consumer health. This follows from observations which show that with the currently available equipment, it takes two to four times longer to reach the required temperature of 7 ° C, when starting with carcasses or parts thereof, precooled to an internal temperature of 16 ° C.

2. Should it however be considered to authorise cooling of carcasses or parts thereof during transport with the currently available equipment, the following recommendations concerning the procedure should be taken into account:

- The carcasses or parts thereof must be chilled immediately after post mortem inspection before transport is initiated, to reach an internal temperature of not more than 10 ° C
- The surface meat temperature should always be below 10 ° C during transport
- Total transport time should never exceed 8 hours if meat temperature is above 7 ° C

- The air temperature at any point within the mobile refrigeration unit must remain below 7 ° C throughout the duration of the transport, ensuring continuous cooling of the meat throughout the transport.
- This implicates that the mobile refrigeration unit before loading commences, must have precooled the internal environment below 7 ° C.
- The integrity of the cold chain must be maintained at all stages, including during activities of loading and unloading. This also implies that, at unloading, the mobile refrigeration unit must be linked immediately after the doors open with the docking bay in order to avoid entry of ambient air.
- The mobile refrigeration unit must be fitted with a regularly calibrated temperature registration system, which ensures appropriate air measurement throughout the cooling process.

3. The Committee stresses that the required most rapid chilling and an optimal airflow and implementation of GMP throughout the entire slaughter and cooling process is of utmost importance to maintain the necessary hygienic conditions as well as microbiological quality.

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[1] Data are presented as in the original publication