J. Hyg., Camb. (1980), **85**, 115
Printed in Great Britain

Growth of *Salmonella* on chilled meat

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(*Received* 12 *November* 1979)

SUMMARY

Growth rates of a mixture of *Salmonella* serotypes inoculated on beef from a commercial abattoir were measured at chill temperatures. The minimum recorded mean generation times were 8·1 h at 10 °C; 5·2 h at 12·5 °C and 2·9 h at 15 °C. Growth did not occur at 7–8 °C. From these data the maximum extent of growth of *Salmonella* during storage of meat for different times at chill temperatures was calculated. Criteria for deciding safe handling temperatures for meat are discussed. Maintaining an internal temperature below 10 °C during the boning operation would be sufficient to safeguard public health requirements.

INTRODUCTION

Salmonellas are the most common cause of food poisoning in England and Wales and in many other countries (W.H.O., 1974; Watson, 1976; Center for Disease Control, Atlanta, Georgia, 1977; Vernon, 1977). A high proportion of reported outbreaks have been attributed to the consumption of contaminated meat, although the statistics are sometimes prejudiced by the inclusion in annual summaries of cross infection in the very young, for example in hospitals. Between 1973 and 1975 approximately one quarter of those outbreaks in England and Wales, where the source could be identified, were traced to meat or meat products (Vernon, 1977).

Meat may become contaminated at the abattoir by accidental contact with hides, hooves or gut contents of infected animals and at retail or in the home by contact with other contaminated food. The importance of abattoirs as a source of contamination is difficult to assess because the incidence of *Salmonella* on carcasses varies widely between abattoirs and may vary from week to week at the same abattoir (Carpenter, Elliot & Reynolds, 1973; Edel *et al.* 1973; Meara, 1973). A further complication is that data from many abattoir surveys are based on examining gut contents, lymph nodes, spleens, etc., which are removed at slaughter, and may therefore overestimate the incidence of *Salmonella* on the carcass meat proper.

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0022-1724/80/0102-1979 $01.00 © 1980 Cambridge University Press

Efforts are made to exclude *Salmonella* from abattoirs by rejecting for slaughter those animals with clinical symptoms of salmonellosis. However some apparently healthy animals (symptomless excreters) are also a source of salmonellas and cannot be readily detected by ante-mortem inspection. Control therefore depends on hygienic slaughter practices and stringent temperature control to prevent growth of salmonellas during meat handling.

Meat exported to the EEC from 'Third Countries' and meat in intra-community trade must be produced in abattoirs conforming with the standards of hygiene laid down in Council Directives (1964, 1972, 1975) which specify temperatures to be maintained during meat handling. Carcass meat must be chilled immediately after the post-mortem inspection to an internal temperature of 7 °C, and the internal temperature of chilled meat must not rise above 7 °C during cutting and subsequent operations. 'Immediately' is open to interpretation as the rate of cooling is not specified. If a carcass is chilled to an internal temperature of 7 °C within 24 h of slaughter, as the Directive appears to recommend, the outer portions may undergo an irreversible form of toughening known as 'cold shortening' (Dransfield & Jones, 1978), while maintaining the internal temperature of meat at 7 °C during cutting necessitates a surface temperature of 1–3 °C which often results in meat that is difficult to cut. This has caused industrial problems in many parts of the world, hence the necessity of bringing the internal temperature of the meat to 7 °C prior to cutting is being questioned.

The choice of a safe storage temperature for foods is usually based on the minimum growth temperatures of bacteria associated with food-borne illness likely to be found on those foods (Michener & Elliott, 1964). Since the minimum growth temperature of a micro-organism is greatly influenced by pH, water activity and the presence of competing micro-organisms and may thus vary considerably between foods (Ingram & Mackey, 1976), a 'safe' storage temperature is not necessarily a reflexion of the lowest temperature recorded for growth of the microbe in question under laboratory conditions.

Most food poisoning bacteria asociated with meat are mesophiles, whose growth is prevented by refrigeration to below 5–10 °C. At temperatures a few degrees above their minimum, the growth rates of mesophiles are slow and often preceded by a long lag. Hence it may be possible to handle or store food for specified short periods at a temperature above the minimum for growth of bacteria of public health significance without their significant proliferation.

The minimum growth temperature and growth rates of *Salmonella* at chill temperatures have been determined with pure cultures inoculated on sterile muscle slices (Shaw & Nicol, 1969) but growth on chilled meat carrying the normal microflora has been tested at only a few temperatures (Alford & Palumbo, 1969; Goepfert & Kim, 1975) or for inadequately short incubation periods (Gomutputra & Fabian, 1953). The present work aimed at providing data on growth rates of *Salmonella* on beef from a commercial abattoir to enable the extent of growth during storage for different times at temperatures between 8 and 15 °C to be predicted.

MATERIALS AND METHODS

Media

Nutrient broth (Oxoid CM1), plate count agar (Oxoid CM325) and modified brilliant green agar (Oxoid CM329) containing 0·0125% (w/w) sulphadiazine (BGAS) were prepared according to the manufacturer's instructions. Buffered peptone diluent (BPD) contained (per l) KH_2PO_4, 0·0425 g; NaOH, 0·017 g; peptone 0·5 g.

Organisms

The following serotypes of *Salmonella* were used, all having been isolated from meat products and their identities confirmed by the Central Public Health Laboratory, Colindale: *S. agona*; *S. anatum*; *S. enteritidis*; *S. heidelberg*; *S. indiana*; *S. infantis*; *S. montevideo*; *S. panama*. *Salmonella senftenberg* NCTC 9959 was also used.

Preparation of inocula

Each serotype was grown in 18 ml of nutrient broth in static culture at 37 °C for 6 h (growing cells) or 48 h (stationary phase cells). 'Cocktails' of either growing or stationary phase cells of all the serotypes listed above were produced by mixing together 1 ml of broth culture of each serotype and diluting 1/100 in nutrient broth. The effect on growth at low temperatures of including *S. heidelberg* in the inoculum was tested by omitting this serotype from the cocktail and using it alone in pure culture.

Inoculation of meat

Method 1. Thin beef flanks from freshly slaughtered animals at a local abattoir were brought to the laboratory within 30 min of dressing, and either cooled to the storage temperature and inoculated with a bacterial suspension or stored in polythene bags at −10 °C until required. Frozen thin flanks were defrosted at 2–3 °C for 24 h and warmed to the storage temperature before inoculation.

Method 2. In an attempt to simulate the temperature changes which would occur when carcass meat was contaminated by *Salmonella* at slaughter, cooled overnight and cut up the following day, frozen thin flanks were defrosted, warmed to 33–36 °C, inoculated with *Salmonella*, cooled to 1 °C overnight and finally warmed to the required storage temperature before removing the first sample.

In both methods, organisms were applied to the meat surface thus: Whatman No. 1 filter paper cut to the size of the meat (*ca.* 32 × 26 cm) was immersed in bacterial suspension, drained and placed in an enamel tray. The piece of thin flank, previously stretched to remove any folds, was unrolled on the filter paper taking care to ensure even contact. The meat was transferred, inoculated side up, to a fresh sterile tray and the filter paper removed. The tray was then placed inside an oxygen-permeable polythene bag supported on an inverted wire basket, with a beaker of water inside the bag to maintain high relative humidity.

Incubation and temperature measurement

The temperature of meat before inoculation was monitored with a Dependatherm electronic thermometer type MRC/SP (Kane-May Ltd, Burrowfield, Welwyn Garden City, Hertfordshire) with a chisel-type probe inserted immediately below the meat surface. After inoculation, the temperatures of meat and incubator were monitored separately with Grant miniature recorders (Grant Instrument (Developments) Ltd, Toft, Cambridge) fitted with type HM probes. Incubation was in water-cooled incubators (Astell Ltd, Catford, London) fitted with standard thermometers. In experiments at 7·5, 10, 12·5 and 15 °C, meat temperatures were maintained within ± 0·5 °C.

Sampling and enumeration of Salmonella

Quadruplicate meat disks (10 cm^2) were excised from the surface with sterile instruments at zero time and thereafter at 6, 24, 30, 48, 54 and 72 h. Each disk was homogenized separately in 100 ml BPD in a Colworth Stomacher 400 (A. J. Seward, Blackfriars Road, London) and duplicate 0·5 ml volumes of decimally diluted homogenate were spread on plates of BGAS. Colonies were counted after overnight incubation at 43 °C.

To check recognition of *Salmonella* colonies, 100 colonies presumed to be *Salmonella* and 50 considered not to be *Salmonella* were checked using the API 20E System (API Laboratory Products Ltd, Farnborough, Hants) and serology. The presumed identities proved to be correct in all cases.

Measurement of pH

pH was measured after homogenizing 5 g samples of meat in 5 mM sodium iodoacetate plus 150 mM-KCl (Bendall, 1975).

Relative humidity

Relative humidity, measured inside the polythene bag with a dial hygrometer, remained at *ca.* 98 % throughout incubation.

Statistical treatment of data

The time at which a statistically significant increase in number had occurred was tested by comparing the counts ($\log_{10}/cm^2$) in successive samples with the zero time count, by a two-way analysis of variance between times and experiments, using duplicate counts from each of four meat disks (i.e. eight counts) at each time interval.

Mean generation times were calculated from growth curves, drawn using geometric means of each eight replicate bacterial counts. The steepest part of the curve was selected by eye and the best line fitted to it by least squares regression analysis.

Table 1. *Growth of* Salmonella *on beef at chill temperatures*

Temperatures (°C)	Proportion of trials where growth was observed
7·5	0/5*
10	15/19
12·5	7/7
15	5/6

* In two trials incubation was for 30 h only.

Table 2. *Minimum temperatures for growth of* Salmonella *in laboratory media*

Organism	Minimum growth temperature (°C)		Reference
	Agar	Broth	
S. heidelberg	5·2	5·9	Matches & Liston (1972*b*)
	5·5	5·9	Matches & Liston (1968*b*)
S. typhimurium	6·1	5·9	Matches & Liston (1968*b*)
	—	6·4	Matches & Liston (1972*b*)
S. derby	6·1	7·5	Matches & Liston (1968*b*)
	—	9·0	Matches & Liston (1972*b*)
S. aertryke	6·1	—	Matches & Liston (1968*b*)
S. montevideo	5·5	—	Matches & Liston (1968*b*)
S. newport	6·5	—	
S. thompson	6·4	—	
Several strains	5–10	—	Stokes & Bayne (1957)

RESULTS AND DISCUSSION

The frequencies with which growth took place on meat at the different chill temperatures are listed in Table 1. Growth was not observed at 7–8 °C but occurred in most instances (15 of 19) at 10 °C.

Shaw & Nicol (1969) reported growth of *S. oranienburg* at 8 °C but not 7 °C on sterile beef slices, whereas Gomutputra & Fabian (1953) reported no growth of *Salmonella* on beef or pork at 10 °C after 24 h. This incubation time seems too short to expect detectable growth to occur. Growth on minced beef has been reported at 12·5 °C but not 7 °C (Goepfert & Kim, 1975) and on minced pork at 10 °C but not 4 °C (Alford & Palumbo, 1969).

For comparison, published minimum growth temperatures in laboratory media and various other foods are given in Tables 2 and 3. The lowest recorded temperature for growth is 5·2 °C on laboratory medium (Matches & Liston, 1972*b*) and 6·7 °C in a food product (Angelotti, Foter & Lewis, 1961).

The mean generation times for growth on meat at chill temperatures calculated from our data are shown in Table 4. Factors which influence growth, e.g. pH or the presence of competing micro-organisms, have more pronounced effects at temperatures near the minimum for growth (Michener & Elliott, 1964) and might explain the wide variation in growth rates at 10 °C and the occasional failure of inocula to grow at this temperature.

Table 3. *Minimum temperatures for growth of* Salmonella *in foods**

Food	Growth (°C)	No growth (°C)	References
Beef and pork	—	4·4–10	Gomutputra & Fabian (1953)
Chicken à la King	6·7	5·6	Angelotti, Foter & Lewis (1961)
Ham salad	—	10	Angelotti, Foter & Lewis (1961)
Cream custard	—	10	Angelotti, Foter & Lewis (1961)
Crab meat	—	5	Berry (1942)
Crab meat (sterile)	8	5	Matches & Liston (1968*a*)
Sole	8	6	Matches & Liston (1968*a*)
Egg	10	2	Ayres & Taylor (1956)
Egg	10	5	Stokes, Osborne & Bayne (1956)
Liquid egg	11·1	7·2	Gibbons, Moore & Fulton (1944)
Ground pork	10	4	Alford & Palumbo (1969)
Ground pork	—	7	Sulzbacher (1952)
Ground beef	12·5	7	Goepfert & Kim (1975)
Ground beef	—	5	Tiwari & Maxcy (1972)
Luncheon meat	—	5	Goepfert & Chung (1970)
Bacon	16	5	Farrell & Upton (1978)
Ground beef (vacuum packed)	12	5	Davidson & Witty (1977)
Sterile beef	8	7	Shaw & Nicol (1969)

* This table is based on that given by Michener & Elliott (1964) but includes more recent data.

Table 4. *Mean generation times of salmonellas measured during the period of most rapid growth on meat at chill temperatures*

Mean generation times (h) at a temperature of		
10°C	12·5°C	15°C
8·1 (c)*	5·2 (c)	2·9 (b)
9·3 (a)	5·4 (a)	3·2 (c)
10·6 (a)	5·5 (c)	3·4 (c)
10·7 (a)	6·4 (a)	3·5 (c)
10·9 (a)	7·2 (b)	
11·1 (a)	7·6 (a)	
13·6 (a)	10·2 (a)	
14·5 (a)		
18·2 (a)		
20·1 (a)		
25·5 (a)		

* Each tabulated figure is from a separate experiment. Letters in parentheses denote which serotypes were included in the inoculum: (a) a mixture of all serotypes listed in the Methods section; (b) the mixture with *S. heidelberg* omitted; (c) *S. heidelberg* alone.

The pH of meat, measured before inoculation, varied between pH 5·6 and 6·5. There was no effect of pH on growth rate at 12·5 or 15 °C, but at 10 °C the most rapid rates usually occurred on low pH meat (i.e. pH 5·8 or less). High pH meat contains less soluble carbohydrate (Newton & Gill, 1978; R. H. Dainty, personal communication) and might, therefore, be more restrictive to microbial growth under the exacting conditions of low-temperature incubation.

Table 5. *Growth rates of* Salmonella *at chill temperatures*

Temperature (°C)	Organism	Substrate	Generation time (h)	References
8	*S. heidelberg*	Broth + 1 % NaCl	19*	Matches & Liston (1972*a*)
	S. heidelberg	English sole	28*	Matches & Liston (1968*a*)
	S. heidelberg	Sterile crab	31*	Matches & Liston (1968*a*)
	S. oranienburg	Sterile beef	35	Shaw & Nicol (1969)
10	*S. oranienburg*		9·4	Shaw & Nicol (1969)
	3 strains	Ground pork	12*	Alford & Palumbo (1969)
	S. derby	Ground pork	19*	Alford & Palumbo (1969)
	S. enteritidis	Ground pork	22*	Alford & Palumbo (1969)
	S. thompson	Ground pork	17*	Alford & Palumbo (1969)
	Mixture of strains	Beef surface	8–26	This paper
12	*S. heidelberg*	Broth	12*	Matches & Liston (1972*a*)
	S. typhimurium	Broth	12*	
	S. derby	Broth	12*	
12·5	Mixture of strains	Beef surface	5·2–10·2	This paper
15	Mixture of strains	Beef surface	2·9–3·5	This paper
	S. oranienburg		4·6	Shaw & Nicol (1969)

* Mean generation times were calculated from published growth curves and are, therefore, approximate.

It was inferred from previous work (Matches & Liston, 1968*b*) that *S. heidelberg* might grow better at low temperatures than some other serotypes. However, its inclusion in or omission from the inoculum did not affect the frequency with which growth was initiated or its subsequent rate.

Since there are few published data on growth rates of *Salmonella* at chill temperatures we have calculated generation times from the growth curves illustrated in some papers (Table 5). Growth rates on beef were similar to rates on other substrates at the same temperatures.

Lag times were not determined precisely, but there was evidently wide variation in times taken to achieve a statistically significant increase in number (Table 6). In the two instances where growth was detectable after 6 h at 10 °C, the meat had been inoculated by Method 2, i.e. it had been inoculated whilst warm, cooled to 1 °C overnight and warmed to 10 °C before taking the first sample. The metabolic events which occur during lag presumably took place during warming, hence allowing immediate growth at 10 °C. In most cases growth at 10 °C started only after a much longer lag and was sometimes preceded by a decline in viable count. Similar long lags occurred before growth of *Salmonella* in cooked foods stored at chill temperatures (Angelotti *et al.* 1961).

Meat must be stored and handled at temperatures which prevent growth of salmonellas, but what constitutes 'growth' when meat is handled for short periods is open to question. For example, if meat kept below 7 °C was transferred to an environment at 10 °C and if growth proceeded at the maximum rate observed at 10 °C, numbers would double in 8 h. In practice this increase would normally be undetectable because routine microbiological counting techniques are seldom

Table 6. *Times taken to achieve a statistically significant increase in number*

Time (h)	Number of experiments where growth was detectable at the time shown		
	10 °C	12·5 °C	15 °C
6	2/11	—	—
24	1/11	4/4	2/2
30	6/11	—	—
48	1/11	—	—
54	1/11	—	—

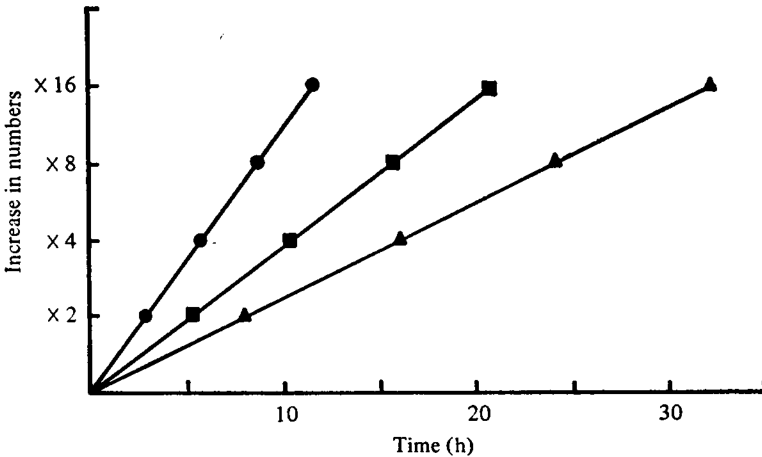

Fig. 1. Predicted maximum increase in numbers of *Salmonella* on beef held at 15 °C, ●—●; 12·5 °C, ■—■; or 10 °C, ▲—▲.

precise enough to detect so small a difference; and a twofold difference in count due to growth is insignificant compared with differences due to the uneven distribution of bacteria on the surface of meat. Hence, under some circumstances meat might be handled at temperatures above those normally regarded as the minimum for growth of *Salmonella*, without detectable growth occurring.

The statutory requirement to keep meat below 7 °C, therefore, seems unnecessarily stringent for short-term handling operations such as boning and cutting, and an alternative approach is to define 'safe' temperatures in relation to the duration of handling and storage. Fig. 1, based on our data, shows how this might be done; for example, if an increase in numbers of not more than twofold were deemed 'insignificant growth' then temperatures up to 12·5 °C could be tolerated for 5 h or 10 °C for 8 h. Similarly, if a four fold increase in numbers is considered insignificant, temperatures up to 15 °C for 5 h, 12·5 °C for 10 h or 10 °C for 16 h would be safe. The calculations include an additional margin of safety because they assume no lag before the start of growth; in fact a lag was evident in most of our experiments.

There would have to be effective precautions to ensure that the specified times and temperatures were not exceeded, but cutting plants approved for trading

within the Community or similar establishments from 'Third Countries' are under the constant supervision of the veterinary authorities appointed by the state concerned, and it is one of their duties to see that meat brought into the cutting plant should be processed as quickly as possible and that no unnecessary accumulation of meat occurs. Under practical conditions cutting and packaging should take only 2–3 h, thus a temperature of 10 °C would be entirely adequate to ensure that there was no significant multiplication of *Salmonella*, since we have shown that even a two fold increase in numbers at 10 °C takes at least 8 h.

If the temperature required in intra-Community and 'Third Country' legislation were amended in such a way that meat must be cooled to an internal temperature of 10 °C and maintained at or below that temperature during cutting, difficulties in cutting and the possibility of cold shortening would be virtually eliminated without significantly increasing the risk of *Salmonella* growing.

We thank Dr H. J. H. MacFie for statistical analyses and Mr P. Manley for his skilled technical assistance, and the Union International Company Ltd for providing the facilities and funds for the work to be carried out.

Note added in proof

We have recently learned of work (Catsaras, M., *Bulletin de l'Académie Vétérinaire de France*, **51**, 155–65, 1978) reporting that *Salmonella typhimurium* inoculated into 'la viande hachée' (minced beef) grew at 10 °C with a mean generation time of approximately 2 h, ultimately reaching *c.* 8×10^9/g. This claim should be viewed with caution since the counts on which it is based were made on deoxycholate citrate lactose agar, apparently without serological confirmation of *Salmonella* colonies. Some other lactose-negative enterobacteria can produce *Salmonella*-like colonies on this medium (Fagerberg, D. J. & Avens, J. S. *Journal of Milk and Food Technology*, **39**, 628–46, 1976).

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