Studies on the growth and survival of *Staphylococcus aureus* in corned beef

By J. M. MANSFIELD, G. FARKAS*

Union International Research Centre, Old London Road, St Albans, Herts AL1 1PX

ANTONNETTE A. WIENEKE AND R. J. GILBERT

Food Hygiene Laboratory, Central Public Health Laboratory, London NW9 5HT

(Received 23 June 1983; accepted 11 July 1983)

SUMMARY

The growth of an enterotoxin A producing strain of Staphylococcus aureus in corned beef was investigated. In the inoculated 6 lb. canned product the bacteria spread throughout the meat and attained high numbers. The rate of spread of the organisms was related to the temperature and length of storage of the cans and the numbers of bacteria inoculated. Cans which had been stored for more than four months showed high counts of the bacteria throughout the meat. It was noted that with the long term contaminated product counts of S. aureus on some selective media may give falsely low results.

Numbers of S. aureus on meat inoculated by handling after removal from the can were initially extremely variable. More uniform distribution and higher counts were attained only if the meat was exposed for some hours at ambient temperature or above. The significance of the results to the investigation of outbreaks of food poisoning suspected of being associated with canned corned beef is discussed.

INTRODUCTION

Freshly opened cans of corned beef originating from various countries have been incriminated in several outbreaks of staphylococcal food poisoning in this country (Stersky, Todd & Pivnick, 1980; Gilbert, Kelvin & Roberts, 1982). Post-processing leakage of micro-organisms into the cans was a possible cause of these outbreaks. Between February and June 1979 nine separate incidents affecting > 85 persons occurred after the consumption of corned beef. The meat was bought ready sliced from shops or was eaten at canteens, and said to come from recently opened 6 lb. cans. At least five different brands of corned beef were implicated but all came from the same production plant in Brazil. In each incident *Staphylococcus aureus* was isolated from the suspected meat, and strains lysed by phage 29 only and, which produced enterotoxin A, were isolated from four of them (de Saxe, Coe & Wieneke, 1982). One unusual aspect of these nine incidents was that the number of S. aureus

* Present address: 11, Clifton Hill, St John's Wood, London NW8.

isolated (< 100 to $1.0 \times 10^5/g$ of meat) were generally well below the levels normally reported (1.0×10^6 to $1 \times 10^{10}/g$) in outbreaks of staphylococcal food poisoning (Gilbert, 1983).

Little information is available on the growth or survival of S. aureus in either sealed cans of corned beef or the freshly-opened product, or on the spread of the organism throughout the meat. When outbreaks of food poisoning or foodborne illness associated with any freshly-opened canned food occur they can warrant both national and international concern and there is a costly economic impact on the food industry. The purpose of the present studies, therefore, was to assess (a) the fate of S. aureus inoculated into 6 lb. cans of corned beef which were then stored under varying conditions for up to 1 year, and (b) the numbers and spread of S. aureus on corned beef contaminated by handling on removal from the can and subject to varying conditions of abuse.

MATERIALS AND METHODS

Culture media

Non-selective media: Brain Heart Infusion (BHI) Broth Oxoid CM 225, Plate Count Agar (PCA) Oxoid CM 325 and Horse Blood Agar (BA), Oxoid Blood Agar Base no. 2 (CM 271) plus 7 % defibrinated horse blood. Selective media for *S. aureus*: Baird–Parker Agar (BPA) Oxoid CM 275 and Kranep Agar (KR) Oxoid CM 441. All media were prepared according to the manufacturers' instructions.

Peptone buffered diluent (PBD) contained (per l) – KH_2PO_4 , 0.0425 g; NaOH, 0.017 g; peptone (Oxoid L37), 0.5 g. Toluidine Blue DNA Agar (TDA) was prepared as described by Lachica *et al.* (1971).

Victoria Blue Corned Beef Fat Agar (VBCBA) was prepared as described by Jones & Richards (1952), except that corned beef fat was used as the lipid base.

Rabbit plasma for coagulase tests (MR28) was supplied by Wellcome Reagents, 303 Hither Green Lane, London SE13 6TL.

Corned beef

The 6 lb. cans of corned beef used were from consignments commercially produced in Brazil.

Culture

The enterotoxin A producing strain of S. aureus used in all the experiments was isolated in 1979 from a food poisoning outbreak associated with contaminated Brazilian corned beef.

Methods

(a) Investigations into the fate of S. aureus within canned corned beef. The stock S. aureus culture maintained on nutrient agar slopes was checked for purity, and suspensions and dilutions prepared in sterile 0.85% sodium chloride solution to give ca. 50, 250 or 500 organisms per 0.2 ml. The actual levels of viable organisms in the suspensions were checked by preparing counts on BA incubated at 37 °C for 48 h.

The surfaces of the cans to be inoculated were cleaned with detergent solution, followed by 0.5 % hypochlorite solution and dried with paper towels. The cans were

then laid side seam uppermost and the areas to be inoculated were disinfected by swabbing with industrial methylated spirit. Using a sterile small nail, sterile hammer and forceps, a pin hole was punched next to the side seam near to the filling end of each can. Checks were carried out to assess for gross contamination within the cans by inserting a sterile needle into the meat via the pin hole, removing and incubating the needle in BHI broth overlaid with mineral oil at 30 °C for 1 week. The meat within the cans was inoculated with 0.2 ml of the appropriate suspension of *S. aureus* using a syringe, the needle of which was inserted through the pin holes onto the meat surface.

	Storage conditions					
	Set 1 RT 15–22 °C	Set 2 30 °C	Set 3 30 °C 3 weeks then RT	Set 4 37 °C	Set 5 37 °C 3 weeks then RT	
Total number of cans inoculated and incubated	12 (9a, 3b)	6 (5a, 1b)	8 (c)	7 (6a, 1b)	8 (c)	
Number of cans opened and examined after:						
1 week	2 (1 a, 1 b)	1, b	0	2 (1 a, 1 b)) 0	
1 month	2 (1a, 1b)	1, a	0	1, a	0	
3 months	2 (1a, 1b)	1, a	2, c	1, a	2, c	
6 months	2, a	1, a	2, c	1, a	2, c	
9 months	2, a	1, a	2, c	1, a	2, c	
12 months	2, a	1, a	2, c	1, a	2, c	

Table 1. Storage c	conditions and	timetable of	' sampling o	f stored cans
--------------------	----------------	--------------	--------------	---------------

a, inoculum 520 S. aureus A29 per can; b, inoculum 52 S. aureus A29 per can; c, inoculum 250 S. aureus A29 per can.

The pin holes were subsequently sealed with Araldite adhesive which was previously shown to be non-inhibitory to S. aureus when set and free of viable micro-organisms. Inoculated cans were left for 1 h to allow setting of the adhesive and absorption of the inoculum so that artificial spread did not occur when the cans were stood upright. All cans were stored with the side seam vertical and either the filling end or can makers' end uppermost on a random basis as might occur in manufacture. The cans were stored, opened and examined as shown in Table 1. Before opening all cans were chilled overnight at 4 °C. Cans were opened aseptically at the opposite end to the point of inoculation and the corned beef drawn out of the can so as not to spread material from the inoculated end of the can onto the uninoculated end. The appearance, odour and texture of the meat was noted and between six and 24 samples were taken from the meat from each can using sterile knives and sampling equipment. The higher number of samples were taken from cans stored for shorter periods (up to 3 months). The areas sampled and order in which samples were taken is shown in Fig. 1.

To determine the number of S. aureus present at least one 10 g portion of meat per sample was taken. Samples were homogenized with 90 ml PBD in a Colworth Stomacher for $1\frac{1}{2}$ min and further diluted with PBD. Aliquots (0.5 ml) of dilutions were spread on surface dried plates (1 h in laminar flow cabinet) of BPA, BA, KR and PCA which were incubated for 2 days at 37 °C. Where BA or PCA counts varied 470

significantly from selective agar counts, colonies on the non-selective media were checked for identity as *S. aureus* by coagulase tests.

In samples from cans stored for up to 3 months, tests were also carried out for the presence of S. *aureus* per gram of meat, by inoculating BHI broth, incubating at 37 °C for 2 days and checking the identity of any organisms isolated. Selected samples of meat from cans stored at 37 °C for 1 week and 3 months were examined for the presence of thermostable DNAase (Lachica *et al.* 1971) and staphylococcal enterotoxin (Gilbert & Wieneke, 1973).

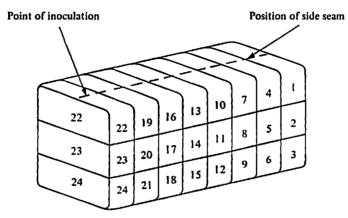


Fig. 1. Sampling of meat from cans. The numbers refer to the areas of the block of meat samples. When the whole block was sampled, samples were taken in numerical order. When eight samples were taken, the areas sampled were 3, 5, 7, 11, 14, 22, 23 and 24. When six samples were taken, the areas sampled were 2, 3, 11, 14, 22 and 23.

(b) Investigations into the fate of S. aureus inoculated onto corned beef by handling on removal from the can. Cans of corned beef were chilled overnight, opened aseptically and the meat partially removed from the cans. Suspensions of the S. aureus were prepared and diluted, in sterile 0.85% sodium chloride, to contain around 10^4 organisms/ml and 1 ml inoculated onto volunteers' hands using cotton wool swabs. The corned beef was then removed from the cans and placed on plastic trays by the volunteers, during which process it became contaminated.

In tests to assess the effect of repeated slicing with an unsterilized knife and storage at room temperature, a block of meat was exposed at 18-22 °C and at 0, 2, 4 and 6 h at least three slices of meat were cut and samples from the slices examined for *S. aureus* using BA, KR and BPA as previously described. The slicing knife was not sterilized between cutting of the slices.

To assess the effect of storage of the meat under abuse conditions, one third of a block of meat was immediately sliced with an initially sterile knife and slices of the meat were placed at room temperature (18–22 °C), 30 and 37 °C covered loosely with polythene to prevent dehydration. Duplicate samples were taken from the slices at 0 h and after 2, 4 and 6 h exposure to the above conditions and examined for *S. aureus* as previously described. The remaining meat was stored at room temperature for 6 h and the temperature monitored just below the surface, every 2 h, using a Dependatherm electronic thermometer and probe (Kane May Ltd, Welwyn Garden City, Herts). After 6 h the meat was refrigerated overnight

471

at 4-6 °C. The next day the second third of the block of meat was sliced and the day one procedure repeated. The remaining meat was again exposed at room temperature for 6 h, the temperature monitored and the meat chilled overnight. On the third day, the final portion of the meat was sliced and exposed at room temperature, 30 and 37 °C, and sampled every 2 h for 6 h for *S. aureus* as previously described.

RESULTS

(A) Growth and spread of S. aureus within the canned corned beef

Meat appearance

In all the cans opened the meat appeared normal. There were no off odours or marked discolorations even in portions containing in excess of 10^7 S. aureus per gram.

Growth and spread within the meat

Levels of S. aureus inoculated into the cans were ca. 52 organisms per can (low inoculum trials) and 250 or 520 organisms per can (high inoculum trials).

A summary of the S. *aureus* levels in the meat within the cans, after storage under various conditions for 1 week to 12 months, is shown in Table 2. Under all the conditions tested, the organisms had spread throughout the meat in the can by 3 months. The rate of spread was slower at the lower storage temperature and where lower numbers of organisms were inoculated. Counts increased to a maximum of *ca*. 10^8 per gram and then slowly declined. Maximum numbers and onset of decline was reached first in samples stored at the higher temperature. At 37 °C the growth phase was completed between 1 week and 1 month, whilst at RT a similar stage was not reached until between 1 and 3 months. At 30 °C the growth phase was completed by about 1 month.

Maximum counts were not obtained simultaneously throughout the meat but occurred sooner in areas nearest the point of inoculation and later in areas furthest away. This was more noticeable in cans stored at the lower temperatures, where maximum counts were shown in one area of the meat (from the 1 month RT samples), before spread throughout the can had occurred. Pre-incubation of cans for 3 weeks at 30 and 37 °C before storage at RT advanced the early stages of the growth cycle.

Onset of slow decline in numbers occurred first in cans stored at higher temperatures – after around 1 month in samples stored at 37 °C and between 1 and 3 months in samples stored at RT. Decline did not occur uniformly throughout the meat but occurred first in the areas nearest the point of inoculation which reached maximum numbers first. This was again more noticeable in cans stored at lower temperatures where ranges of counts within individual cans were wide compared to those found in cans stored at higher temperatures for the same length of time.

The rate of decline in counts was slow under all the storage conditions, and after 1 year the levels of *S. aureus* were still greater than 10^4 organisms per gram and the majority were greater than 10^5 per gram. Towards the end of the storage period (6–12 months) counts were higher in product stored at RT than in product stored at 30 or 37 °C.

1	F	(~	1		100	<u>100</u>	2	100	
	nen R	(8)	1		Ξ	Ξ	Ξ	¥	
	37 °C 3 weeks then RT	E	ł	. 1	6.4-7.2	5.6 - 6.6	5.2-5.8	4.2-5.7	ganisms.
	37 °C 3	(E)	I	ł	6.98^{b}	6.25^{b}	5.54^{b}	5.16 ^b	520 orį
		(®	50-100	100	100	100	100	100	her with
	37 °C	£	< 1-8-5	$6 \cdot 3 - 6 \cdot 4$	$5 \cdot 5 - 6 \cdot 1$	5.5-5.8	5.1-5.5	4.2-5.2	ms, the ot organisms
ires)		(Î	7.56ª	6.40^{d}	5.82^{d}	5.62^{d}	5.40^{d}	4-83 ^d	organisı 50–520 (sms. isms.
nperatu	en RT	(®	ł	}	100	100	100	100	vith 52 with 22 8 organi 20 organ
Storage conditions (temperatures) 30 °C 3 weeks then RT	£	I	ł	6.8-7.7	2.5-8.4	$5 \cdot 5 - 6 \cdot 0$	5.2-5.9	 (m) Log mean S. aureus counts as determined on blood agar. (r) Range log S. aureus counts as determined on blood agar. (s) Spread, % meat in cans sampled shown to be infected with S. aureus. ^a Results based on counts from 32 samples from 2 cans - one inoculated with 52 organisms, the other with 520 organisms. ^b Results based on counts from 12 samples from 2 cans - both inoculated with 52 organisms. ^c Results based on counts from 8 samples from 1 can - inoculated with 52 organisms. ^d Results based on counts from 6 samples from 1 can - inoculated with 52 organisms. 	
age con	ge cond 30 °C 3	Ē	١	١	7.28^{b}	7.42^{b}	2.79^{b}	5.42^{b}	agar. agar. ed with - one ir - both inoculation
Stor		(®	50	100	100	100	100	100	n blood blood a e infecta 2 cans 2 cans 1 can - 1 can -
	30 °C	(r)	< 1-5.6	7-2-7-5	$6 \cdot 3 - 7 \cdot 2$	$6 \cdot 2 - 6 \cdot 7$	5.9 - 6.3	5.6 - 6.0	 (m) Log mean S. aureus counts as determined on blood agar. (r) Range log S. aureus counts as determined on blood agar. (s) Spread, % meat in cans sampled shown to be infected with S. aureus. (a) Spread, % meat in cans samples from 2 cans - one inoculated the Results based on counts from 12 samples from 2 cans - both inoculated c Results based on counts from 8 samples from 1 can - inoculated with 5 and 4 Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 1 can - inoculated with 5 d Results based on counts from 6 samples from 6 d Results based on counts from 6 d Results ba
		Ē	4·75°	7-40 ^d	6.90^{d}	6.46^{d}	6-11 ^d	5.95^{d}	a as dete as dete npled s n 32 san 1 12 sar 1 8 sam 1 6 sam
	C	(8)	27-75			<u>100</u>		100	us counts s counts cans sar ints from ints from ints from
RT, 15-22 °C	T, 15-22	Έ	< 1-6.6	< 1-8-2	5.0-8.4	6-1-7-9	5 - 4 - 6 - 5	$5 \cdot 3 - 6 \cdot 6$	n S. aureu g S. aureu 6 meat in sed on cou sed on cou sed on cou
	Ē	5.62^{a}	7-278	#70-T	7.42 ^b	$6.28^{\rm b}$	6.24^{b}) Log mea Range log Spread, % cesults baa cesults baa cesults baa cesults baa	
	CL and CL	time	1 week	1 month	3 months	6 months	9 months	1 year	

473

Of the 41 cans examined, two (high inoculum 30 °C 3 weeks and RT 1 month, RT for 6 months) showed atypical results compared to the other cans in terms of spread and counts of organisms. Both cans showed fat accumulations at the end around the point of inoculum, suggesting that if inoculation occurs into larger areas of fat rather than meat, growth and spread of the organisms may be delayed despite the fact that the inoculum was shown to possess lipolytic activity against corned beef fat as tested on VBCBA.

Toxin and thermostable DNAase detection

Table 3 shows the results of tests for detection of enterotoxin and thermostable DNAase in meat from cans stored at 37 °C for 1 week and for 3 months. In samples from cans stored for 1 week at 37 °C toxin and thermostable DNAase were detected where *S. aureus* counts were greater than 5.7×10^6 per gram. In samples from cans stored for 3 months at 37 °C where *S. aureus* counts were all below 1.3×10^6 per gram, enterotoxin could not be detected.

		Detection of				
Samples from cans (high inoculum) stored at 37 °C	Log S. <i>aureus</i> counts per gram	Enterotoxin A	Thermostable DNAase			
1 week	6.00	_	NT			
	6.31		NT			
	6.44	_	NT			
	6-60	_	_			
	6.76	+	+			
	6-90	+	+			
	6.95	+	NT			
	7.20	+	NT			
	8.20	+	NT			
3 months	5.48	_	+			
	5.73	_	+			
	6.06		+			

Table 3. Detection of enterotoxin and thermostable DNAase in corned beef inoculated with S. aureus and stored at 37 °C for 1 week and 3 months

Recovery of S. aureus in selective media

It was noted that the recovery of *S. aureus* on BPA varied substantially from the numbers recovered on BA. Recovery of the organisms on BPA was less the longer the cans were stored, as shown in Table 4. Recovery on KR was not reduced, although there was some increase in variation compared to the levels on BA as the period of storage lengthened.

The low recovery on BPA could not be reversed by resuscitation of the organisms in BHI broth for 1 h or by allowing growth of the organisms in BHI broth for 5-18 h at 20-22 °C. However, the stock culture which had not been exposed in the corned beef during the experiments gave good recovery on BPA.

Samples from inoculated	Range of counts on selective media, expressed as % of equivalent counts on blood agar					
corned beef stored:	' Baird–Parker agar	Kranep agar				
1 week	86-123	68-99				
1 month	13.9-61	54-102				
3 months	2.7 - 29.5	NT				
6 months	< 0.0007-0.03	69-105				
9 months	NT	48-120				
12 months	NT	36-166				
	NT, Not tested.					

Table 4. Recovery of S. aureus from corned beef using selective media

Table 5. Log S. aurcus counts per gram of corned beef contaminated after removal from the can, stored at room temperature (18-22 °C) and sliced with an unsterilized knife

Time of slicing and	Log co	Temperature of meat				
sampling (h)	(г)	(a)	(m)	(v)	(c)	block (°C)
0	< 1-3.99	3.21	< 1·55	1.45	< 3.22	5
2	< 1-3.68	2·91	< 1.70	1.16	< 2.84	12
4	< 1-3.66	2.95	< 1.95	1.13	< 3.25	15
6	2.48 - 4.20	3.60	3.28	0.32	3.65	18
	 (r) Range of I (a) Log of ave (m) Mean log (v) Variance. (c) Calculated 	erage count count.		on Kilsby &	Pugh (1981).	

(B) Growth and spread of S. aureus on corned beef contaminated by handling on removal from the can

Levels of S. aureus inoculated onto the blocks of corned beef by handling during removal of the meat from the can, ranged from < 10 to 9700 per gram. The levels of S. aureus on slices of corned beef taken, without sterilizing the knife, from blocks of meat stored at RT over a 6 h period, are shown in Table 5. Samples taken at 0, 2 and 4 h showed wide variation of counts due to uneven contamination.

Statistical analysis, using the methods of Kilsby & Pugh (1981) was applied to the results to assess any increases in counts taking account of variance. After 6 h there was evidence that both significant spread of the organisms and increases in counts had occurred.

The numbers of S. aureus on slices of corned beef taken from a block of meat on three consecutive days and stored at RT, 30 and 37 °C for up to 6 h, are shown in Table 6. Initial samples taken on the first day showed a wide variation in counts, as might be expected due to uneven distribution of contamination arising from handling of the meat. The variation in counts decreased with time, being less on initial samples taken on days two and three, suggesting gradual growth and spread of the organisms on the stored block of meat. More definite evidence of growth was shown on slices kept at RT, 30 and 37 °C, particularly on those cut on day three.

As might be expected, the greatest increases in counts were shown on slices stored at 37 °C where samples, taken 6 h after slicing on day three, showed numbers of S. aureus around 3.6×10^6 per gram.

The results shown in Table 6 are confirmed S. aureus counts on BA. Counts were also determined on KR and BPA. The percentage recovery of organisms on selective media was similar to that obtained with samples from inoculated canned corned beef stored for 1 week.

	Le	T				
Time of		nitial	(m) ^t	Temperature of remaining block of		
sampling (h)	(m) ^a	(r)	RT	30 °C	37 ℃	meat (°C)
Day 1: first third of meat sliced	3•4	< 1-3.81	NT	NT	NT	10
2	NT	NT	2.59	3.80	2.94	17.5
4	NT	NT	2.87	3.53	3.46	21
6	NT	NT	2.76	4-11	4.85	22
Day 2: second third of						Remaining block chilled overnight
meat sliced	2.18	1.6-2.48	NT	NT	NT	11
2	NT	NT	4.42	3.32	3.21	17
4	NT	NT	2.69	4.02	4.02	21
6	NT	NT	3.26	3.99	4.70	22
Day 3: final third of						Remaining block chilled overnight
meat sliced	4.01	3 3.3-4.32	NT	NT	NT	10
2	NT	NT	5.23	5.62	4.53	NT
4	NT	NT	3.92	5.71	5.87	NT
6	NT	NT	5.15	4.69	6.26	NT
		(r) Range	nean counts log counts x samplings	per gram.		

Table 6. Counts of S. aureus on sliced corned beef exposed at room temperature, 30 or 37 °C, following contamination of the meat by handling on removal from the can

a ...

. .

~

DISCUSSION

^b From two samplings. NT, Not tested.

(A) Growth of S. aurous within canned corned beef

The results show that S. aureus grows within sealed cans of corned beef, eventually spreading throughout the meat, and persists in considerable numbers even 1 year after inoculation. Such contamination could not be recognized by changes in appearance or odour and on this basis consumers would accept the contaminated meat. The multiplication of S. aureus within the corned beef showed discernible log growth, semi-stationary and slow decline phases. As expected, the growth cycle was more rapidly completed at higher storage temperatures but even after storage for 1 year at 37 °C, there was no evidence of a rapid decline in numbers of viable cells. The rate of spread of the organisms through the meat within the can was affected by:

(i) The temperature of storage of the cans. Spread was faster at higher temperatures, presumably due to more rapid growth but probably also due to softening of the fat.

(ii) The numbers of organisms inoculated. Spread was more rapid in cans inoculated with a higher number of organisms. The difference in the number and the spread of organisms was still present in cans stored at RT for 1 month but not in cans stored at RT for 3 months.

(iii) The texture of the product. Whilst the texture of the product is fairly uniform, fat may accumulate at one end of the can depending on can position during cooling. Atypical results obtained from two cans suggests that inoculation into large areas of fat may restrict or delay growth and spread of organisms. It is also possible that variation in product moisture could have an effect although this was not determined.

Whilst only limited tests to assess for distribution of staphylococcal enterotoxin were carried out, the results obtained were rather unusual. The detection of toxin and thermostable nuclease in meat from a can stored 1 week at 37 °C where counts exceeded 5.7 million per gram, and the non detection of toxin in meat from a can stored at 37 °C for 3 months, even though the presence of thermostable nuclease suggests that counts had been sufficiently high, cannot be fully explained, but could be due to insensitivity of the method of detection. It is possible that changes in the culture which resulted in the loss of recovery of the organisms on BP agar might also have been associated with loss of ability to produce enterotoxin. However, colonies giving very weak or atypical reactions on BPA were shown to produce enterotoxin A when tested in culture medium.

The progressive decrease in the recovery of *S. aureus* on BPA during the course of the experiments could not be attributed to difference in batches of media but suggests that prolonged exposure in corned beef may render some strains of *S. aureus* sensitive to some selective media. Low recovery of thermally stressed, frozen or dried *S. aureus* cells on certain selective media has been reported (Busta & Jezeski, 1963; Erwin & Haight, 1973; Gray *et al.* 1974; Hurst *et al.* 1974; Jackson, 1974; Smolka *et al.* 1974). Most note increased sensitivity to salt and pH following stress from sub lethal heating, drying or storage. Baird-Parker & Davenport (1965) noted that recovery of stressed cells was best on media which supply catalase or otherwise neutralize peroxides. However, in our experiments, catalase deficiency alone would not appear to be the cause of low recovery as both blood agar and BPA contain agents to neutralize effects of peroxides. The effect could be due to increased sensitivity to potassium tellurite since KR, which does not contain this material, showed no marked evidence of decreased recovery of organisms.

A possible reason for the development of sensitivity could be prolonged contact with agents such as low levels of nitrite or its reaction compounds which may be present in corned beef, but this was not investigated further. However, it would appear that if only selective media, such as BPA, are used in testing corned beef

which has suffered long term contamination, counts of S. *aureus* may appear to be misleadingly low.

The significance of these experiments to a commercial operation is that if post-processing contamination of large cans of corned beef occurs with S. *aureus* it can lead to growth and spread of the organisms throughout the meat in the can, and to development of high counts – above those regularly able to cause food poisoning – without any readily discernible changes in odour and appearance of the product or cans. In practice, however, it is unlikely that can infection levels would be as high as the lowest inoculum levels used (for practical reasons) in these tests. Contamination could occur with only one or two cells per can.

As the rate of spread of the organisms is related to the inoculum levels, temperature of storage of the product and meat texture, it is calculated that with low levels of inoculation (one or two cells per can) and product storage at 18-22 °C, spread throughout the meat could take up to 16 weeks and possibly longer if infection happened to occur in an area of fat. A product suffering post-process leaker contamination with *S. aureus* could show variable counts from 0 to 1×10^8 per gram over a period up to about 4 months. Once spread has occurred, counts increase rapidly even at 18-22 °C; a product which is 5 months to 1 year old could be expected to show generally high counts throughout the meat, ranging from around 2×10^5 per gram to 1×10^8 per gram.

When examining older cans for the presence of S. *aureus* assessment should not be based solely on counts recovered on selective media. The distribution of enterotoxin which might occur in infected product is less clear. Areas of meat in cans less than 4 months old, where contamination has not spread, probably would not contain toxin. Whether at later stages, with more uniform distribution of organisms throughout the can, toxin would occur throughout the meat remains uncertain due to the limited number of tests carried out.

(B) Growth of S. aureus on corned beef contaminated by handling after opening of the can

Initially, levels of contamination are variable, reflecting the sites of meat infected and not necessarily restricted to one end of the block of meat as might occur in the early stages of leaker infection. Counts would also be generally low. Maltreatment of meat contaminated by handling would be necessary to produce the levels of organisms encountered in outbreaks of food poisoning. Such treatment would appear to require a considerable time lapse between contamination and sale or consumption of the meat and of storage of the product at room temperature or above.

Repeated slicing of a block of meat contaminated by handling, without sterilizing the knife, was shown to spread the contamination and render counts less variable.

When investigating outbreaks of food poisoning or foodborne illness associated with canned foods it is important to ascertain whether contamination occurred from post process leakage or from handling of the meat after removal from the can.

Considering that S. American corned beef is usually 3 or more months old by the time it is on sale in the United Kingdom, such product, contaminated by

J. M. MANSFIELD AND OTHERS

post-process leaker infection, could be expected to show generally higher and more uniform counts than would be likely if contamination occurred post opening of the can.

The findings reported here, together with information on the age of the canned product, distribution and magnitude of counts found, any handling of the opened can, may be of help in clarifying the source of S. *aureus* contamination of corned beef and, as such, it may be possible to use greater selectivity in withdrawing suspect cans based on batch code numbers.

We wish to express our thanks to the Directors of the Union International Company for allowing the work to be published, to Mr R. F. Looney for his advice and help, Mr P. Boyes for his technical assistance and Mrs M. Ward for preparing the manuscripts.

REFERENCES

- BAIRD-PARKER, A. C. & DAVENPORT, E. (1965). Effect of recovery medium on the isolation of S. aureus after heat treatment and after the storage of frozen or dried cells. Journal of Applied Bacteriology 28, 390-402.
- BUSTA, F. F. & JEZESKI, J. J. (1963). Effect of sodium chloride concentration in an agar medium on growth of heat-shocked *Staphylococcus aureus*. Applied Microbiology 11, 404-407.
- DE SAXE, M., COE, A. W. & WIENEKE, A. A. (1982). The use of phage typing in the investigation of food poisoning caused by *Staphylococcus aureus* enterotoxins. In *Isolation and Identification Methods for Food Poisoning Organisms* (ed. J. E. L. Corry, D. Roberts and F. L. Skinner), pp. 173-197. Society for Applied Bacteriology Technical Series no. 17. London: Academic Press.
- ERWIN, D. G. & HAIGHT, R. D. (1973). Lethal and inhibitory effects of sodium chloride on thermally stressed Staphylococcus aureus. Journal of Bacteriology 116, 337-340.
- GILBERT, R. J. (1983). Foodborne infections and intoxications recent trends and prospects for the future. In Advances and Prospects in Food Microbiology. Society for Applied Bacteriology Symposium Series no. 11. London: Academic Press. (In the Press.)
- GILBERT, R. J., KOLVIN, J. L. & ROBERTS, D. (1982). Canned foods the problems of food poisoning and spoilage. *Health and Hygiene* 4, 41-47.
- GILBERT, R. J. & WIENEKE, A. A. (1973). Staphylococcal food poisoning with special reference to the detection of enterotoxin in food. In *The Microbiological Safety of Food* (ed. B. C. Hobbs and J. H. B. Christian), pp. 273–285. London: Academic Press.
- GRAY, R. J. H., GASKE, M. A. & ORDAL, Z. J. (1974). Enumeration of thermally stressed Staphylococcus aureus MF-31. Journal of Food Science 39, 884-886.
- HURST, A., HUGHES, A. & COLLINS-THOMPSON, D. L. (1974). The effects of sub lethal heating on *Staphylococcus aureus* at different physiological ages. *Canadian Journal of Microbiology* 20, 765-768.
- JACKSON, H. (1974). Loss of viability and metabolic injury of Staphylococcus aureus resulting from storage at 5 °C. Journal of Applied Bacteriology 37, 59-64.
- JONES, A. & RICHARDS, T. (1952). Night blue and Victoria blue as indicators in lipolysis media. Proceedings of the Society of Applied Bacteriology 15, 82.
- KILSBY, D. C. & PUGH, M. E. (1981). The relevance of the distribution of micro-organisms within batches of food to the control of microbiological hazards from foods. *Journal of Applied Bacteriology* 51, 345-354.
- LACHICA, R. V. F., GENIGEORGIS, C. & HOEFRICH, P. D. (1971). Metachromatic agar diffusion methods for detecting Staphylococcal nuclease activity. *Applied Microbiology* 21, 485–487.
- SMOLKA, L. R., NELSON, D. E. & KELLEY, L. M. (1974). Interaction of pH and NaCl on enumeration of heat-stressed Staphylococcus aureus. Applied Microbiology 27, 443-447.
- STERSKY, A., TODD, E. & PIVNICK, H. (1980). Food poisoning associated with post-process leakage (PPL) in canned foods. Journal of Food Protection 43, 465-476.