Observations on the microbiological flora of canned Parma ham

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SUMMARY

A significant proportion of a consignment of branded Parma Ham was found to have 'blown' tins; the associated bacterial flora was therefore investigated. No *Salmonella* or *Shigella* were found. *Clostridium welchii* type A and *Clostridium bifermentans* were isolated in moderate numbers only from enrichment cultures of the ham. *Staphylococcus* spp. and coryneform bacteria were obtained from all tins and Group D streptococci were present in a few. No food-poisoning cases were associated with this ham.

INTRODUCTION

Ham of the Italian type is a popular food in Britain and with entry into the European Economic Community there is every likelihood that its consumption will increase.

In the Parma province alone production reaches 35,000 metric tons per year and so far there have been few bacteriological studies of the food. Giolitti, Cantoni, Bianchi & Penon (1971) found that in lean hams the microflora counts remained at low figures during the curing process; slightly higher figures were found in ham fat. They concluded that in the ripening process the microflora exerted only a minor influence and that in normal and sour hams bacteriological spoilage appeared not to play a significant role.

The product which we examined was a sliced, salt-cured ham from the Parma region which had not been subjected to any cooking process before canning. A W.H.O. expert committee (1968) point out that with raw ham the major risk is the development of *Clostridium botulinum* infection.

Matveev, Nefedjeva, Bulatova & Sokolov (1967) in the U.S.S.R. indicated that during recent years a serious danger in connexion with botulism appeared to be various home-processed pork products, and in fact pork products were the third most common cause of botulism in Russia; *Cl. botulinum* type B was most commonly isolated from pork. There are no records of botulism in Canada from sliced, commercially packed meats (Thatcher, Erdman & Pontefract, 1967) although Reed, Butas & Gall (1965) described a small outbreak caused by canned liver paste.

Because of the possibility of pathogenic bacteria, particularly anaerobes, being responsible for the 'blowing' of the tins in the present study, an attempt was made

using routine laboratory methods to isolate the responsible organisms. In no way could this be considered a research investigation; it was an endeavour to solve an urgent public health problem with limited facilities and resources.

MATERIALS AND METHODS

The number of 6 oz. cans examined at the warehouse by the Public Health Inspector totalled 1,008; 218 of these tins were found to be 'blown'. Similarly, out of 144 12 oz. tins, 105 were 'blown'. The consignment was stored at between 0 and 2° C. in the warehouse but had been kept at 'room temperature' for some days before transference to the cold room. Twenty-three of the 6 oz. cans were collected by the Public Health Inspector for laboratory examination.

The cans were made from coated, pressed, mild steel with a crimped and soldered double seamed joint and were of the 'round flat' type, the exhaust hole being plugged with solder after processing. These were checked for defects but all were sound, none showing any evidence of 'blowing'. Storage instructions on the lid read 'Keep in a cool place'.

Three of the tins were examined for bacteriological culture direct from the warehouse. The remaining 20 were kept at three different temperatures for 2 days before bacteriological examination. Five were kept at 4°, 10 at 22° and 5 at 30° C. The tins were washed with soap and water and treated with 70 % alcohol before opening (Cruickshank, 1965). Duplicate 1 g. samples were taken from each tin using a sterile cork-borer. The samples were homogenized in 50 ml. (sample 1) and in 5 ml. (sample 2) of quarter-strength Ringer's solution.

Tenfold dilutions were made from sample 1 and pour plates made from this using Dextrose Tryptone Agar (Oxoid) (D.T.A.) and Plate Count Agar (Oxoid) (P.C.A.). The D.T.A. plates were incubated at 37° and 55° C. The P.C.A. plates were incubated at 22° and 37° C. Using the same dilution and a modified Miles & Misra (1938) technique (Sutton, Ghosh & Hobbs, 1971) plate counts were made on duplicate blood agar (B.A.) plates and incubated at 37° and 22° C. Special media for the selective isolation of the aerobic flora were not used. Sample 2 was examined by spreading 0·5 ml. on the surface of duplicate blood-agar plates which were incubated at 37° C. under anaerobic conditions for 48 hr. A total anaerobic count was made by adding together the colony counts from each plate. Two ml. of sample 2 was inoculated into a tube of cooked-meat broth and heated to 100° C. for 30 min., incubated at 37° C. overnight and subcultured on Neomycin (Upjohn) blood agar plates for the presence of heat-resistant spores. Further ham from each tin was inoculated into Robertson's cooked-meat broth, McConkey broth and Selenite F enrichment medium and incubated for 48 hr. at 37° C. and the subculture examined after a further 48 hr. at the same temperature.

On completion of the bacteriological studies four of the tins were examined for water content and NaCl concentration; the pH was estimated on 11 tins; sodium nitrite and nitrate concentrations were also determined on the meat.

Table 1. *Total viable bacterial plate count per gram of ham*

Media and incubation temperature	Storage temperature for 48 hr. before opening											
	4° C.			22° C.						37° C.		
D.T.A., 37° C.	1	1·6		6	93		11	1·2		16	72	
	2	4·6		7	86		12	13		17	22	
	3	150	×10³	8	19	×10³	13	1	×10³	18	6·4	×10³
	4	48		9	26		14	26		19	1·5	
	5	19		10	25		15	19		20	120	
D.T.A., 55° C.		Nil			Nil						Nil	
B.A./P.C.A., 37° C.	1	930		6	1250		11	1040		16	400	
	2	990		7	540		12	750		17	4500	
	3	680	×10³	8	1030	×10³	13	1520	×10³	18	480	×10³
	4	1280		9	1980		14	2050		19	440	
	5	450		10	1150		15	1560		20	600	
B.A./P.C.A., 22° C.	1	144		6	900		11	670		16	120	
	2	230		7	430		12	670		17	510	
	3	290	×10³	8	740	×10³	13	670	×10³	18	110	×10³
	4	350		9	730		14	980		19	20	
	5	100		10	800		15	700		20	280	

RESULTS

The degree of firmness of the tins was assessed after 48 hr. storage at different temperatures. All the tins stored at 30° C. felt soft to finger and thumb pressure. Several of those kept at 22° C. were likewise soft, and all those kept at 4° C. felt firm. Of the 12 soft tins, 11 produced cultures of anaerobes; from 2 of the 8 firm tins similar organisms were isolated. In each case *Cl. welchii* was isolated from a mixed anaerobic culture.

The average wet-weight NaCl content of the ham was 3·93 % and the moisture content 46·94 %, these figures giving a concentration of 8·4 g. salt/100 g water. The average pH of the tins examined was 5·95; the sodium nitrate concentration was less than 1 p.p.m. whilst the sodium nitrite concentration was 2·8 p.p.m.

Variation in storage temperatures (range 4–30° C.) for 48 hr. before sampling the tins appeared not to affect the concentration and the distribution of the bacterial content (Table 1).

Comparison was made with the total viable plate counts obtained from the blood agar plates and the pour plate method using Plate Count agar. The average of the counts was taken and is given as B.A./P.C.A. in Table 1.

Staphylococci accounted for 70 % of the total aerobic plate count, assessed from the blood agar plate. Packet-forming Gram-positive cocci with a typical colonial appearance and a diameter of not less than 0·5 mm. were selected for further study and classified as staphylococci according to Cowan & Steel (1966). The organisms were coagulase-negative and fermented glucose and mannitol. Enterotoxins A, B and C were not produced by the three cultures so examined.

Colonies having the morphological characteristics of *Streptococcus faecalis* were identified as Lancefield Group D streptococci and were isolated from seven of the

tins; this organism grew well on 10 % bile salt medium, fermented sorbitol and was grouped using the formamide extraction method of Fuller (1938). An organism with similar morphological appearances was identified as a Group C streptococcus using the same technique. This organism was isolated from only one of the tins and only in small numbers.

Small Gram-positive rods growing well aerobically were identified as coryneform bacteria. These organisms were present in all the tins and were the second largest group isolated. The presence of lactobacilli could not be demonstrated under aerobic or microaerophilic conditions on routine culture media.

A heat-resistant *Cl. welchii* type A was isolated from two of the tins. A proteolytic Gram-positive rod with oval subterminal spores was obtained from 13 of the tins. The results from a number of biochemical tests indicated that this organism was a *Cl. bifermentans*. This was confirmed by the Food Hygiene Laboratory at Colindale, where culture filtrates from this organism did not kill mice. In each case the anaerobes were isolated on subculture from cooked-meat medium. No other anaerobes were isolated.

Because of the local lack of suitable animals, adequate studies of possible *Cl. botulinum* toxin and spores were not feasible and the isolation of vegetative forms only was attempted.

DISCUSSION

Food-poisoning outbreaks due to staphylococcus enterotoxin and to *Cl. welchii* remain common (Thatcher, 1963) and deaths from botulism were recorded by both Dauer and Dolman in 1961.

A significant proportion of Parma ham tins were found to be 'blown' but the meat showed no evidence of serious spoilage or putrefaction; storage at 4° C. appeared to reduce the number of 'blown' tins; *Cl. botulinum* was never isolated.

The average pH of a small number of cans was 5·95; *Cl. botulinum* growth can occur if the pH is above 5·0 and the relative humidity above 94 % (W.H.O. Committee, 1968); on the other hand, it cannot germinate and grow in a strongly acidic environment, reduction of the pH could thus possibly be an extra safety factor. The W.H.O. Committee (1968) state that the limiting salt concentration for types A and B *Cl. botulinum* is 8–9 %; this percentage being based on the concentration of salt in the aqueous phase of the food. Moreover Spencer (1967) found that in cured meats 14–15 p.p.m. nitrite allied with 4·9 g salt/100 g water at a pH of 6·4 had an inhibitory effect on type A *Cl. botulinum* both in growth and toxin production. In comparison with these results, the Parma ham figures suggest that the sodium nitrite (2·8 p.p.m.) and sodium nitrate (less that 1 p.p.m.) are low but that the sodium chloride concentration (8·4 g/100 g water) is probably acceptable in a food where safety is partially dependent on the concentration of curing salts to prevent outgrowth of the bacteria.

Spencer (1967), however, found that the concentration of salts necessary to prevent growth of *Cl. botulinum* depends on the number of vegetative cells present, and as these are usually sparse in food the concentration of curing ingredients, although low, might well provide acceptable safety levels.

Cl. bifermentans was found in 13 of 20 tins, particularly those stored at 22° C. and over; this is not an organism likely to cause food-poisoning although it can produce an exotoxin; its role is more that of an indicator organism than a pathogen. *Cl. welchii* was isolated in moderate numbers from 2 of 20 tins in mixed culture with other anaerobes. Sutton *et al.* (1971), however, point out that large quantities of *Cl. welchii* (millions/g) are necessary to cause clinical food poisoning; Hobbs & Gilbert (1970) confirm this. Giolitti *et al.* (1971) isolated no anaerobes in their investigation of raw ham although the meat was 'untinned' salt cured ham, unlike the tinned slices in the present study.

The curing of the hams is done by external dry rubbing of salt followed by ripening for 6–12 months or longer; the exact preparatory process is a trade secret. *Staphylococcus*, the only other potential pathogen isolated, grows well at a pH above 4·5 and in a salty environment, both these conditions being fulfilled in this ham. No large numbers of staphylococci were isolated and no clinical symptoms would occur after eating this meat.

The cause of the 'blown' containers is difficult to assess; bacterial gas production is likely although putrefaction was not present. Both *Cl. welchii* and *Cl. bifermentans* have saccharolytic and proteolytic properties. Enzymic hydrolysis, suggested by Giolitti *et al.* (1971), may be a factor and expansion of trapped air within the cans may be another possible cause. Whether keeping this consignment at 'room temperature' for some days before cold room storage caused or increased the number of 'blown' tins is not known but could be of some significance in view of the experimental results. Neither the vacuum nor the head space gases in the cans were determined.

The bacteria present in the raw ham could have been introduced into the meat during the slicing or canning procedures, although lactobacilli have been found in pig carcasess and at all stages in the production of bacon (Riddle & Spencer, 1966; Kitchell & Ingram, 1967; Riddle, 1968). Mol *et al.* (1971), in a careful study of vacuum-packed cooked meats, state that unclassified streptobacteria eventually spoil the food because they grow well at low temperatures under anaerobic conditions and that variations in salt, pH and nitrite do not apparently curb their growth; similar organisms have also been isolated from raw cured meats (Cavett, 1962, 1963) in addition to lactic acid bacteria and micrococci (Kitchell & Ingram, 1963). Coryneform bacteria and *Staphylococcus* spp. were found in all the tins examined in this study and may play a part in eventual spoilage of meat, as may the streptococci Group D; they do not, however, produce food-poisoning in humans.

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