HYGIENIC INVESTIGATION OF ANIMAL FOOD PRODUCTS IN KENYA WITH EMPHASIS ON THE DETECTION OF CLOSTRIDIUM BOTULINUM BOTH IN FOODS AND THE ENVIRONMENT.

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A thesis submitted in part fulfilment for the Degree of Master of Science in the University of Nairobi.

1977

DECLARATION:

This thesis is my original work and has not been presented for a degree in any other University.

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This Thesis has been submitted for examination with my approval as University Supervisor.

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ABSTRACT

With the establishment of a Bureau of Standards in Kenya, there is an accelerated interest in setting up numerical bacterial standards for the various categories of foods. At the moment the standards for milk and milk products have already been finalised. Of necessity, the standards should be based on factual studies in the concerned country (WHO, 1974). Such studies do not appear to have been carried out in Kenya previously. The aim of this research was to assess the existing microbial load in both meat, meat products, milk and milk products. After the study, microbiological limits were to be recommended based on the results of the research. For this purpose a survey of the microbiological quality of meat and meat products was carried out on 104 samples taken from varying communities in and around Nairobi. The butcheries which were sampled were distributed such that they included varying socio-economic classes. From them, grab samples were taken, but the plan was not designed to fit a statistical pattern.

Forty samples of market milk and milk products were examined for microbial contents. The samples included raw, pasteurized, and fermented milk, cream, yoghurt, assorted ice creams, and cheese. From a local dairy farmers co-operative society milk collecting centre (Sigona), 114 samples of raw milk were examined. There were 39 farmers in

the Society and each farmer's milk was sampled separately.

The methods used were of two types - (a) those aimed at assessing the level of general sanitation of the food. These tests were: the total viable acrobic plate count (TPC) after 48 hours incubation at 37C, the coliforms count after 24 hours incubation at 37C; and random detection of Escherichia coli. (b) Those tests aimed at assessing the safety of the food. These included an attempt to isolate Salmonella, Clostridium perfringens, Clostridium botulinum, and E. coli. Effort was also made to detect botulinal toxin in 104 meat samples and 137 samples of mud, sand and fishes.

The results show that no sample of meat exhibited less than 10,000 colonies on the total plate count (TPC). Forty-three per cent (43%) of the samples had TPC between 10-100 million , of which 83% were whole meat chops, 10% were ground meat and 7% were raw sausages. The greatest number of meats (52%) showed a coliform count between one thousand and 50 thousand coliforms per gram of the meat samples. Neither Salmonella nor Cl. botulinum was isolated. Random testing for Cl. perfringens and E. coli revealed their presence in the meat. Clostridium perfringens was tentatively diagnosed in mud and sand as well. Concerning the microbial counts

from the milk samples picked at the milk collecting centre, there were great fluctuations with the coliforms varying from a low 50 to a high 358,000 per millilitre at 37C after 24 hours incubation. However, of 39 farmers, 15 (38%) showed a mean coliform count less than 1,000 per ml, 3 (8%) farmers had a mean over 100,000 and 20 (51%) had mean counts over one million with 7 (18%) of them showing counts above five million organisms per ml. Thirty nine (39) samples tested for Salmonella were all negative. Escherichia coli was frequently detected. All the dairy centre milk samples were screened for antibodies against brucellosis using the milk ring test. All were negative.

In conclusion, there was a relatively high number of bacteria present in the meat although the meat was still marketable. According to the Kenya Bureau of Standards' milk grading scales, 79% of the Sigona raw milk was of good or very good bacteriological quality based on TPC.

No Salmonella or Cl. botulinum were isolated. Escherichia coli and Cl. perfringens were isolated from meat. Escherichia coli were isolated from milk too. Concerning the screening for Cl. botulinum or its toxin in mud and sand, one mud sample out of 137 samples yielded what was possibly Cl. botulinum toxin.

ACKNOWLEDGEMENTS

I would like to express my gratitude to all those who contributed directly or indirectly in the course of this work. Noteworthy was the effort of Professor Tjaberg, Chairman, Department of Public Health, Pharmacology and Toxicology (PHPT) who as my sole University Supervisor relentlessly encouraged and guided me through the research project and finally read and constructively critisized the manuscript. His other indirect contributions are far too many to be enlisted here.

My sincere thanks are also extended to Dr. Nderu of the same Department for his participation in the initial stages of the research by helping me plan the project and also for accompaning me on several occasions to collect the meat samples.

Respectful thanks are also paid to Dr. Gathuma and Mbadi of the same Department for reading the manuscript and suggesting hints regarding scientific writing. The skilful technical assistance, typewriting and other services rendered by the departmental staff are gratefully acknowledged.

Outside the department, I would like to register my gratitude to the Norwegian Agency for International Development (NORAD) for providing all the research funds utilised. Also the same Agency financed my

three-month course in Diagnostic Veterinary Bacteriology and Immunology at the Norwegian College of Veterinary Medicine. This gratitude is extended to Professor Fossum, Chairman of the Institute of Microbiology and Immunology under whose supervision the study in Norway was undertaken.

The services provided by the Librarians especially at Kabete and Norwegian College of Veterinary Medicine are highly appreciated.

I am grateful too to the University Administration (including the Dean, and the Chairman of the Department) for granting me three-month leave for the study in Norway.

Finally, I would like to thank Miss T.M. Njuguna for typing the thesis.

INTRODUCTION

In Kenya, the average consumer has a meagre appreciation of the potential microbial hazards that may result from badly handled foods. And even for the few who comprehend the danger, the physical appearance of the food cannot always foretell the ensuing catastrophy. The consumer places trust on regulatory bodies to check on the hygiene of the food offered in markets.

It was for this purpose that the Kenya Bureau of Standards was established in order to regulate the producers and thereby safeguard the consumers. In executing its duties, the bureau has to consider the fact that food processing operations vary to a certain extent from country to country according to variations in the climate, habits, and the level of development. Only a few years ago, in Kenya, food was consumed directly on preparation, as each family was the "producer" as well as the consumer. However as more and more people moved to the towns, more customers now frequent centralised kitchens or consume food that has been prepared in centralised kitchens and distributed from there.

As such it has become increasingly important to assess the hygienic quality of such products in view of the fact that many people will be affected when products of low quality are sold. To be effective, the control will need to consider the sources of the undesired causes of illness from food. Commonly these are contaminated food ingredients, or a failure

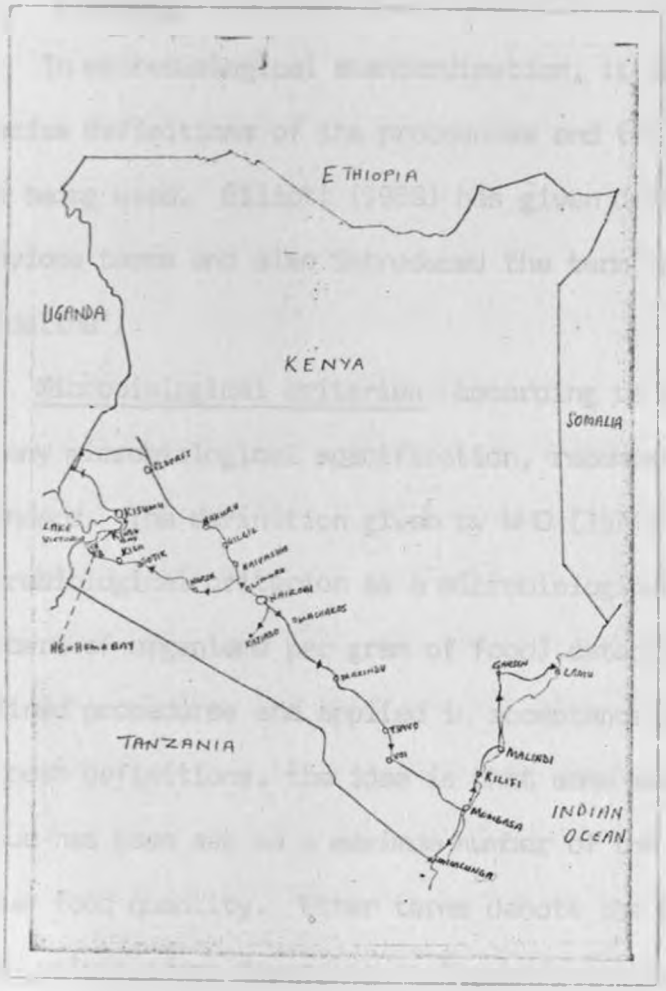
or poor manufacturing practices, unsanitary conditions in the factory or cross-contamination involving the product, faults with the handling during distribution or retailing.

It was in that background that this investigation was carried out. Meat and milk are the most perishable foods and this investigation concentrated on these two animal products. The objectives of the meat study was to find out the level of bacterial contents with a view to suggesting possible microbial limits for meat and some meat products. The study was concerned with limits for total counts and coliforms. As for pathogens, Salmonella, and Clostridium botulinum, were screened for. The Bureau has already proposed microbial limits for milk and milk products. The objective in this study was to try and see how well the suggested levels could be used. This was carried out on a few market milk and milk product samples as well as raw milk from a milk collecting station for a dairy co-operative society. In this case, no limits were suggested since those proposed by the bureau appeared practicable.

Of the food pathogens, Cl. botulinum was investigated the most. It is usual that the occurrences of human botulism has preceded and provided the impetus for surveys and studies of Cl. botulinum in the foods and the surrounding in the area that such outbreaks occurred. However only one case of suspect human botulism has been recorded in Kenya. This apparent abscence or very low

incidence of botulism prompted the survey. Whereas in the past local foods were not prepared in a manner encouraging botulism, a change to home-preservation of vegetables and, or, meats could lead to outbreaks if the spores happened to be already present in the soil from where they could contaminate the food. Hence the survey was carried out in meat, soils and sand with a few samples of fish. This survey was involving a large part of the country extending from the Coast to the lake basin, and Western Kenya as shown on the map.

Regarding the methods used, no new methods were tried and the investigation utilised well established procedures. Any scientific names have been used as referred to in the authors communication without putting effort at trying to convert them to the names in current usage.



Map of Kenya. The mud and sand samples were collected along the route indicated by the arrows.

REVIEW OF LITERATURE

1. Microbiological criteria for food quality

1.1 Glossary:

In microbiological standardisation, it is required to use precise definitions of the procedures and technical terms that are being used. Elliott (1969) has given definitions of some previous terms and also introduced the term "microbiological guideline".

Microbiological criterion according to Elliott (1969) is any microbiological specification, recommended limit, or standard. The definition given by WHO (1974) considers a microbiological criterion as a microbiological value (e.g. numbers of organisms per gram of food) established by use of defined procedures and applied in acceptance sampling of food. In both definitions, the idea is that some microbiological value has been set as a maximum number of the organisms per known food quantity. Other terms denote the authority that such values carry depending on the body setting them. Thus microbiological specification (Elliott, 1969) or Microbiological purchasing specification (WHO, 1974) is the maximum acceptable number of microorganisms or of specific type of microorganisms which have been laid down as conditions of acceptance of a specific food or food ingredient which have been determined by a purchasing firm or its agents. This differs from a Recommended Standard (Limit) which represents suggested values by an authoritative body as determined by specific methods. Of legal implications is the term Microbiol-

ogical Standard which is a part of a law/regulation which governs foods produced, processed or stored, in, or imported into, the area of jurisdiction of the regulatory agency.

Elliott (1969) suggested a new term: Microbiological Guideline defined as that level of bacteria in a final product, or in a shipped product, that requires identity and correction of causative factors in current and future production, or in handling after production.

1.2 Total microbial and coliform count

Microbiological criteria for foods are intended to assess the safety and quality of the food on a numerical basis. Whenever samples are analysed, criteria are essential for objective interpretation of results, and also for enforcing plant sanitation (Thatcher, 1958). The field of microbiological standards has been investigated by many authors (as reviewed by Elliott & Michener, 1961) and various suggestions put forward, but only those of pasteurized milk have been widely used (WHO, 1974). Weinzirl and Newton (1914a) after analysing bacteria in hamburger meat samples proposed a bacteriological standard for meat whose maximum limit was 10 million bacteria per gram. Meat containing over 112 million bacteria per gram was detectable organoleptically as being "very bad". Weinzirl and Newton (1914a), also noted that at putrefaction, the average bacterial count for summer samples was higher (200 million) as compared with that of the winter samples (25 million). However there was no close agreement between the actual number of bacteria and the extent of spoilage. Their maximum was higher than that of one million set previously by Marxer (1903).

Brewer (1925) concluded that prepared meats usually contained more bacteria than fresh meats, and that the appearance of fresh meat could not be taken as an index of its bacterial content. Elford (1936) examined 41 samples of ground meat using 10 million bacteria per gram of the meat incubated at 37C for 48 hours as a standard. For samples whose counts were above this level he notified the retailer. He observed a general improvement and this value was subsequently made a legal limit.

Kirsch et al. (1952) found aerobic plate counts from 20 samples of market hamburgers to range from 1.4 million to 95 million cells per gram. Noticeable spoilage of stored hamburger (0-2C) occurred when the counts had reached 500 million or more per gram.

Ayres (1955) after considering the significance of the bacteria which exist on meat when it leaves the packing plant preferred standards for bacterial numbers. For whole-sale cuts he suggested an aerobic population ranging from 10,000 to 100,000 bacteria per sq. cm., anaerobic population ranging from 5,000 to 50,000 bacteria per gram. For the most probable number after heating for 20 minutes at 80C he suggested an aerobic count ranging from 10 to 100 spores per gram and less than one spore per gram after anaerobic incubation.

Rogers and McCleskey (1957) examined ground beef which they found to contain an average of 32,800,000 bacteria per gram after 48 hours incubation at 37C, and 192,500,000 when incubated for 7 days at 7C. Coliforms varied widely from 20 to 1,100,000 cells per gram and no marked correlation was noted between the coliform and plate counts.

Elliott and Michener (1961) tabulated various suggested microbial limits in chilled and frozen food. The values showed great diversity in all types of foods, e.g. for frozen precooked foods the range was 2,000 to 500,000 viable cells per gram, precooked meats 100,000 viable cells per gram, raw meats 1,000,000 to 100,000,000 viable cells per gram, showing that the limits for particular foods would differ according to the nature of the foods, their formulation, method of manufacture, and storage.

Rao (1970) observed standard plate counts that ranged from 11,000 to 36,533,000 per gram and coliform counts were 233 to 71,333 per gram on 15 ground beef samples from a retail market. Law et al. (1971) found that the bacterial load on 32 ground beef samples from 16 retail stores varied from 1,900 to 460,000,000 per gram. Duitschaever et al. (1975) reported that at the time of manufacture 76% of 74 sets of raw beef patties had aerobic counts less than one million per gram, and isolated salmonella in 3 (0.4%) of 735 beef patties. Emswiler et al. (1976) evauated the bacteriological quality of unfrozen raw ground beef from 0 to 18 days of storage at -0.7C ± 0.6C. At the time of manufacture, all of the ground beef samples had 10 or fewer total aerobic and psychrotrophic bacteria per gram, 81% had 100 or fewer coliforms per gram. Total aerobic and psychrotrophic bacteria increased by 1 log. between 3 and 18 days of storage.

Overall the field of bacterial standards has raised controversies. Those authors in favour of standards correlate low bacterial counts with safety from food pathogens (Heller, 1951).

Although low bacterial counts do not guarantee food safety, foods that are consistently within established microbiological standards are more likely to be safe (WHO, 1974). Thatcher (1958) stressed the need to set standards on foods imported from countries that have high disease rates and poor sanitation, while Straka and Stokes (1956) regarded food with high numbers of spoilage organisms not to be wholesome regardless of the presence or absence of disease organisms. Other arguments put forward are that reduction in bacteria loads can be achieved at no extra effort.

Elliott and Michener (1961) have reviewed the arguments for those in favour of the adoption of microbiological standards. These views can be summarised as :

1) Bacteriological standards are a convenience and a necessity.
2) Bacteriological standards enhance plant sanitation
3) Low bacterial counts are attainable
4) Low bacterial counts are associated with safe foods
5) Bacterial counts reflect sanitation level
6) Bacterial counts reflect degree of decomposition
7) Low bacterial counts will enhance shelf-life.

Ingram (1961) and Rowlands (1952) preferrred the use of appropriate procedures to get rid of bacteria from food rather than emphasizing specific bacteriological standards. Other authors are of the opinion that complete absence of pathogens is not necessary, as for example the presence of Clostridium botulinum spores in food will not cause botulism so long as conditions preferable for toxin release are not provided.

Staphylococci must multiply to many millions per gram before intoxication is possible (Dack et al., 1960) and so is the case with salmonella infection. Such standards lose their prophylactic value where small numbers of pathogens cause outbreaks (Thatcher, 1958).

A low bacterial count will not guarantee freedom from pathogens as total number of microorganisms are often unrelated to the presence or absence of pathogens. Hobbs and Wilson (1959) reported finding salmonella on meats which showed low counts and no fecal organisms. Salmonelloses outbreaks reported by Thatcher (1958) occurred from dried baby feed with low total bacterial counts while dried milk containing enterotoxin caused food poisoning in spite of lack of viable staphylococci. The organisms that formed this toxin may have perished during storage, or may have been destroyed by a processing treatment (Anderson and Stone, 1955; Armijo et al., 1957). This further shows that standards specifying absence of pathogens would be of little value in such an instance.

Spoilage of meat at refrigeration temperatures is consequent to massive proliferation of bacteria, in which case a total count standard would be unrelated to public health since no food poisoning bacteria can grow at such temperatures (Burr and Elliott, 1960). Sampling and analytical errors add to the disadvantages of set numerical standards. Michener et al. (1960) reported variations of 20 fold or more in plate count of packages of peas obtained from a commercial line at nearly the same time.

According to Elliott and Michener (1961) the authors who are against the adoption of microbiological standards present the following arguments:

1) There is no need for bacteriological standards, present control is adequate.
2) Bacteriological standards will not free foods of danger from pathogens.
3) A fecal indicator standard has limitations.
4) Total count is unrelated to danger or to spoilage.
5) Methods of sampling and analysis are inadequate.
6) Existing laboratory facilities and personnel are inadequate
7) Processing and storage influence viable counts.
8) Excessive sanitation will introduce a food poisoining hazard.
9) Foods will be overcooked or preservatives will be introduced to meet a standard.
10) More background information is needed.
11) Bacterial standards will be hard to defend in court.

1.3 Indicator organisms

Laboratory methods employed to determine pathogens are less sensitive when such a pathogen is found in low numbers in a food. Even with sensitive methods, the cost and time restrict their application. Consequently, groups or species of bacteria which are easily detected and which, beyond a certain limit, are considered to be associated with pathogens are used. Such groups or species so used are called indicator organisms and

are valuable in assessing both the microbiological safety and quality of foods (Thatcher & Clark, 1968). Other than the numerical criteria, this second type of standard is based on indicator organisms.

1.3.1 Coliforms: Testing water for coliform bacteria as indicators of faecal contamination and the possible presence of intestinal pathogens is a well established procedure. (APHA, 1971). To an extent the same indicator bacteria have been applied in milk. However the same rationale does not per se hold true in connection with other foods. McCoy (1961) exemplifies this notion by referring to the flesh of an animal slaughtered in the septicemic stage of a salmonella infection or the meat of an egg laid by a salmonella carrier hen. While in both cases fecal contamination indicators might be lacking there may be large numbers of salmonellae. Before the application of this indicator to foods is justified, several questions need to be answered.

Although the intestinal tract of man and animals serves as the main source of E. coli other coliform bacteria are widely distributed in nature (Jay, 1970) such that their presence in unprocessed food has no special sanitary significance (Foster, 1969), nor does the presence of E. coli in raw food indicate a recent pollution with human excreta (McCoy , 1961).

However, some standards have been set indicating maximum coliform values.

One has to note the difference between water and food especially regarding the fact that under suitable conditions of temperature, foods form excellent media for bacteria growth

(McCoy, 1961), and hence the number of organisms present at the time of examination may therefore afford no information about either the source or extent of contamination.

1.3.2 Enterococci

Enterococci are members of the genus Streptococcus which belong to the Lancefield serologic group D. The species are S. faecalis var liquefaciens and var zymogenes, and S. faecium var durans (Niven, 1963). The use of enterococcus index for food safety has been examined. According to Hartman (1960) enterococcus counts in frozen chicken pies were more related to total counts than to coliform counts. Other authors support this idea (Kereluk and Gunderson, 1959: Raj et al., 1961; Niven, 1963; Jay, 1970; and Zabarowski et al., 1956). A major advantage of enterococci is their persistence in food as compared with coliforms, e.g. Riemann (1957) demonstrated the survival of Group D stretococci in ham curing brines.

Niven (1963) has predicted the possible future use of enterococci instead of coliforms in assessing the safety of foods with respect to hazards from persistent food viruses e.g. the infectious hepatitis virus. Also due to their greater resistance, enterococci are sometimes preferred indicator of poor sanitation in plants with frozen foods (Niven, 1963).

1.3.3. Other indicators mentioned in literature include Streptococcus salivarius which has been used as an indicator of oral contamination in food factories (Thatcher and Clark, 1968). The same authors name Staphylococcus aureus as an indicator of skin, mouth and nasal contamination. Standards for Staphylococcus are also seen in Elliott and Michener (1961). Clostridia

in canned foods might indicate that heating was insufficient to destroy, for example, spores of Cl. botulinum had it been present (Thatcher and Clark, 1968).

Appendix VI shows a condensation of Elliot and Michener's microbial limits in chilled and frozen foods per gram except as indicated. The use of standards cannot be discarded. The experience in some countries shows that the establishment of standards has stimulated improvements in plant sanitation and quality control (Elford, 1936; Thatcher, 1958; WHO, 1974). Standards or other criteria are of great value in guiding as well as controlling food manufacturers. The standards will be helpful in changing food hygiene and sanitation.

2. Clostridium botulinum

2.1. Historical landmarks:

In 1895,in Belgium,three persons died out of a group of thirty-four who had eaten salted ham. From the remnants of the ham,van Ermengem isolated an anerobic rod which he named Bacillus botulinus (Dolman and Murakani, 1961). Since then there have been numerous reviews of botulism outbreaks (Meyer, 1956, Dolman, 1957 a,b,c; Nakamura, 1963; Eisenberg and Bender, 1976), to mention a few. Occurrence of the spores and the disease has been reviewed by Meyer (1956), Dolman (1960), Seelinger (1960), Nakamura (1963), Reed (1964), Gangarosa et al., (1971), Lynt et al. (1975), and Eisenberg and Bender (1976) while the properties of the lethal toxin have been characterised by Duff et al. (1956), Vermilyea et al. (1968), Eklund and Poysky (1972), Schantz and Sugiyama (1974),Tjaberg (1974), and

Hatheway (1976). Skulberg (1964) described the colonial features of Cl. botulinum cultures.

Leuchs (1910) demonstrated immunologic disparity in the toxins liberated by the Belgian and a German strain inspite of their almost identical cultural and morphological appearance. These are now regarded as type B and type A respectively (Dolman & Murakami, 1961). Bengtson (1922) isolated Cl. botulinum from larvae of the green fly (Lucilia caesar) and found that its toxin was not neutralised by antitoxins to types A or B, hence named it type C. A similar culture was isolated by Seddon (1922) from a bone. Pfenninger '1924) observed that antitoxin against Bengtson's strain neutralised the toxin from Seddon's strain but not "vice versa". To eliminate the discrepancy Gunnison and Meyer (1929) used the connotations C_{α} and C_{β} to distinguish the Bengtson and Seddon strains.

Theiler obtained impure toxic cultures of an organism of the botulinus type from a case of Lamziekte. On toxin-antitoxin basis it was found not to be related to either A,B or C (Robinson, 1930). Meyer and Gunnison (1929) designated it Cl. botulinum type D.

The significance of serotype E was not generally recognised until the mid 1930s. It is possible that this type had caused "fish poisoning" in Russia long before it was recognised (Dolman and Iida, 1963). Its reports first appeared when a meal of uncooked smoked salmon caused death (MacKenzie, 1934; Hazen, 1938) and two years later, German packaged sprats ("Kielersproten") were incriminated in a second outbreak.

At about the same time an intensive survey in Russia for botulinum spores was carried out in sturgeons and ten botulinus-like strains were recovered (Dolman and Iida, 1963). Gunnison et al. (1936) verified the unique characteristics of these strains and suggested that they be designated type E which was also applied to the strains from the German sprats and the smoked salmon.

On a Danish Island, (Langeland), home-made liver paste was incriminated in an outbreak of botulism in which one person died (Jensen and Hahnemann, 1959). From the sample, Moller and Scheibel (1960) isolated proteolytic Cl. botulinum and subsequent studies of the culture by Dolman and Murakami (1961) lead to its designation as type F. Later nonproteolytic type F has been found on the West Coast of U.S.A.

Gimenez and Cicarreli (1970) carried out surveys on the prevalence of Cl. botulinum in soils of Argentina. During the surveys one strain isolated from soil collected in a cornfield in Mendoza Province produced an antigenically specific, trypsin activated, heat labile botulinum-like toxin. As it was unneutralised by any of the known botulinum antisera, Gimenez and Ciccarelli (1970) considered the strain a prototype of Cl. botulinum type G.

2.2. World distribution of botulism or Cl. botulinum

Currently, the consensus is that botulinum spores are distributed all over the world. This generalization is mostly based on investigations centred in the northern hemisphere and extrapolated to those areas which have not been investigated as yet. A closer look at the literature reveals the absence

of extensive surveys for Clostridium botulinum spores or toxin in African soils, seas and foods. This might be expected in view of the lack of authenticated reports of outbreaks of botulism in this zone. As to whether this lack of outbreak reports is genuine or mere absence of health reports is hard to say, for while botulism in animals (caused by type D after licking "green carrions") has been reported in S. Africa for a long time, (Robinson, 1930) as well as type B spores in soil (Knock, 1952), reports of human botulism are lacking even from S. Africa. An outbreak of human botulism has been reported in Tchad where six people were at risk after eating raw salted ham which had been kept in a defective refrigerator (Demarchi et al., 1958). Type D was diagnosed at the Pasteur Institute in Brazaville and Paris to be the causative type. It was not until 1951 (Nakamura et al. 1952) that the first reports of botulism in Japan appeared. Now the country stands unrivalled anywhere else in the world in the number of type E outbreaks. It might be possible that the country used to experience the same magnitude (if not more) of fatal cases but passed unnoticed for it is hard to believe that these cases started in 1951. It might very well be that the same applies to the African continent.

Clostridium botulinum exists in seven antigenically distinct serotypes which are designated A-G with type C being further subdivided into C_{α} and C_{β}. The steps leading to the establishment of these types have been analysed earlier. The following is a presentation of the geographical distribution of the various types.

2.2.1 Types A and B

These are the types that were investigated in the initial stages. Thus, such reports as Leuchs (1910), Burke (1919), Meyer and Dubovsky (1922a,b,c,), were referring to type A and type B. Although these were emanating from the United States of America, subsequent classical soil surveys in California, (Meyer and Dubovsky, 1922 a); Alaska and Canada (Dubovsky and Meyer, 1922a); Belgium, Denmark, England, the Netherlands, and Switzerland (Meyer and Dubovsky 1922b) pointed to a generalised distribution of these spores. Since then there have been reports in soil from as far apart as South and South-West Africa, the U.S.A. where type A predominates in the West of the Rocky Mountains while type B dominates the area to the east of the Great Lakes (Dolman and Murakami, 1961; Gangarosa et al., 1971) as well as Latin America, Europe, and Asia (as shown in Table 1)

The geographical distribution of outbreaks shows an irregular pattern. As Dolman (1957a) has stated, this disease is not a major problem in any place for even in some countries it is unidentified. In the U.S.A. after a peak in the period 1930 to 1939 with 154 outbreaks due to commercially and home processed foods, it gradually declined to 78 outbreaks in the period 1960 to 1968, and 30 in the period 1970 to 1973. Lynt et al. (1975) have tabulated U.S.A. outbreaks between 1899 and 1973 showing a predominance of type A botulism with 154 outbreaks followed by type B with 42 outbreaks out of a total of 219 of which two were due to types A and B together. But in Canada during the period 1917 to 1957, only 8 out of 17 recorded outbreaks were due to type A. In France type B has

Table i. Main features of the different types of Cl. botulinum (Dolman & Murakami 1961 except type G)

TYPE	DIFFERENTIATED BY	YEAR	SPP MAINLY AFFECTED	COMMONEST VEHICLES	HIGHEST GEOGRAPHIC INCIDENCE
A	Leuchs	1910	Man, Chickens	Home canned vegetables	Western USA
	Burke	1919	(Limberneck)	and fruits, meat & fish	Soviet Ukraine
B	Leuchs	1910	Man, horses,	Prepared meats esp	France, Scandinavia
	Burke	1919	cattle	pork products	Eastern USA.
Cα	Bengtson	1922	Aquatic wild birds (Western duck sickness)	Fly larvae (Lucilia caeser), rotting vegetation of alkaline ponds.	Western USA. & Canada S. America, S.Africa, Australia
Cβ	Seddon	1922	Cattle (Midland cattle disease), horses(forage poisoning),mink	Toxicforage; Carrion; pork liver	Australia, S.Africa, Europe, N. America
D	Theiler/Robinson	1927	Cattle (Lamziekte)	Carrion	S. Africa
	Meyer/Gunnison	1928			

TYPE	DIFFERENTIATED BY	YEAR	SPP MAINLY AFFECTED
E	Gunnison, Cummings & Meyer	1936	Man, mink, birds
F	Dolman and Murakami	1961	man
G	Ginenez and Ciccarelli	1970	no case

COMMONEST VEHICLES	HIGHEST GEOGRAPHIC INCIDENCE
uncooked products of fish and marine mammals	N.Japan, British Columbia, Labrador, Alaska
preserved meat	Denmark, USA.
	Argentina

the highest recorded outbreaks, yet in the neighbouring British Isles, the Loch Maree tragedy was due to type A (Leighton, 1923),

Recently, three cases of type A botulism were reported in Alaska after consuming fermented salmon eggs. The three patients recovered after they were treated with trivalent botulinal antitoxin. This was the second outbreak of Cl. botulinum type A intoxication in Alaska (MMWR 1976a). In the same year (MMWR 1976b) four Californian infants all three or less months of age were found to be suffering from botulism. Three of the infants liberated botulinal toxin, and organisms of types A and B in the stool. A fourth infant liberated toxin and organisms of Cl. botulinum type A (MMWR 1976b). These recoveries signalise a new recognition of botulism caused by in vivo toxin production. This supports the earlier predictions by Hall (1945) who demonstrated Bacillus botulinus type B in a wound infection though there were no symptoms of botulism. He also recovered type A in debrided tissue in two additional cases of wounds with severe lacerations. Davis et al. (1951) reported a fatal wound infection in which Cl. botulinum type A was isolated. Hampson (1951) too reported a fatal case due to Cl.botulinum type A wound infection. Merson and Dowell (1973) investigated epidemiologic, clinical and laboratory aspects of nine cases of wound botulism. All the cases were due to botulinal toxin type A. Four were fatal. Russian researchers have emphasised the importance of wound botulism (Tjaberg, 1977).

In view of the reports of isolations in soils,wounds, infant stool, and outbreaks in various countries it appears logical to state that both types A and B have a distribution which approximates the whole world.

2.2.2 Type C

The first reports of this serotype were made in the United States of America by Bengtson (1922) and in Australia by Seddon (1922). Since then reports of C_{α} strain have come from Canada, South America, Western United States, South Africa and Australia (Dolman and Murakami, 1961). While C_{β} has been reported from Australia, south Africa, North America and Europe (Dolman and Murakami, 1961). More recently type C toxin has been detected in Norway in an outbreak in a herd of 20 cows, sixteen heifers and calves of which one cow, two heifers and a calf died (Ektvedt and Hansen, 1974). Although the skin of an incriminated hedgehog (Erinaceus europaeus) contained type C toxin, the examination of serum from the cow that died and six silage specimens failed to reveal any botulinal toxin.

Type C toxin has been detected in mud and fish samples in Latin America (Ward et al., 1967a), from the U.S. Gulf Coast (Ward et al., 1967b), the Atlantic Coast of the U.S.A. (Ward et al. 1967c), from the whole shrimps (Panaeus aztecus) collected from the Gulf of Venezuela and the Gulf of Darien (Carrol et al., 1966), and from mud samples collected from many of the lakes and waterways of London (Smith and Moryson, 1975). In the London survey, type C was reported in 16 out of 69 samples. The abscence of reports from Asia of type C Cl. botulinum cannot be taken to mean that this organism does not exist there in view of its wide distribution in other parts of the world.

2.2.3. Type D:

Unlike type C, type D has a limited distribution so far having been reported from South African cattle lamziekte victims (Robinson, 1930), Australia (Gray, 1948), Tchad (Demarchi et al., 1958) and from England (Smith & Moryson, 1975). In these reports, outbreaks have only been reported from Africa (Theiler and Robinson, 1927; Demarchi et al., 1958) in cattle and humans, respectively. Only toxin detections in mud, sand and oysters have been reported from the East Coast of U.S.A. and London, while hay, soil and rabbit carrion yielded the toxin in Australia.

2.2.4. Type E:

Since its verification in the mid-thirties, there has been a stream of reports pertaining to this serotype. McClung (1967) has tne view that this current dominance of type E has raised questions regarding the types recognised earlier.

It is interesting to note how frequently type E outbreaks are associated with marine sources as reported by Dolman (1957b), Dolman and Kerr (1947), Dolman and Chang (1952), all from Canada while Pedersen (1955), and Cann et al. (1965) have reported outbreaks and isolation in Scandinavia. And from Japan, it has been reported by Nakamura et al. (1952), Nakamura et al. (1956), and Iida et al. (1958). Outbreaks in the Unites States have been reported by Kautter (1964), Ager and Dolman (1964), Eadie et al. (1964), Craig and Pilcher (1965), and Bott et al. (1966) and others. Reports from the Soviet zone are hard to come by. Dolman and Iida (1963) have cited two cases from the Soviet

Republics. The above are the major areas with type E outbreaks connected with foods from marine sources as well as sea-sand and bottom sediments.

The early reports of type E were almost misleading concerning the geographical distribution of type E botulism for although the disease is now regarded as almost endemic in Japan, no outbreaks had been registered until 1951 (Nakamura et al., 1952). Other areas with high incidences are the British Columbia, Alaska, and Labrador (Dolman, and Murakami, 1961). This disease has a high predominance in the Northern Hemisphere but it is no longer true to say that it is limited to latitude 40°N and above (Dolman and Murakami, 1961) since Ward and Carroll (1965) and Ward et al. (1967b, c) have recovered it in mud from the American Atlantic Coast between Staten Island and the gulf of Mexico.

The ecological origin of type E spores appears to be an area of controversy. Early isolations were from foods of marine sources. However there is abundant evidence also in favour of a view that the spores are of terrestrial origin and are consequently washed to the sea where fish and marine mammals participate in the spread and perpetuation of these spores. The Baltic sea is heavily seeded with these spores. It is to be observed (Johannsen, 1963) that the sea is fed by waterways draining a land area three and a half times more than the surface of the sea itself and thereby providing a considerable concentration of the spores. Johannsen (1963) obtained toxic cultures of type E in garden soils (15%), soil, gravel or sand in towns (32%), potato peels (68%), and shore and sea bottom (44%).

Isolations from beach shorelines have been reported from Lake Michigan (Bott et al., 1966), Hokkaido, Japan (Nakamura et al., 1956), Elephant point, Kitzebue and Point Hope (Miller et al. 1972). Fatalities in Minneapolis due to type E spores contamination in smoked cisco further support terrestrial origin of the spores as this station is far from any ocean, and so was the isolate from intestines of three mullets caught far inland in Manitoba (Dolman and Iida, 1963), and more recently, it has been isolated from lakes, disused reservoir and canals in London (Smith and Moryson, 1975).

2.2.5. Type F:

Since the Langeland outbreak of 1958 mentioned earlier, only one more outbreak has been documented so far. In California in 1966 (Midura et al., 1972), 20 people ate venison jerkey which had been prepared at home. Of the 20 people 15 were asymptomatic, three developed botulism while the other two developed mild gastro-enteritis. Another report refers to toxin identification in mud samples from a river in North Dakota (Wertz et al., 1967) and from a fish caught in Louisiana (Ward et al., 1967b). Isolations have been reported from marine sediments collected from the coast of Oregon and Washington (Eklund, et al., 1967), from the gills and viscera of a salmon that was caught 32 km. upstream of Columbia River (Craig and Pilcher, 1966), crabs caught on the eastern coast of the U.S.A. (William-Walls, 1968) and from soil taken in Buenos Aires Province of Argentina (Gimenez and Ciccarelli, 1968). However,

other than the outbreak due to liver paste (Jensen and Hahnemann, 1959) and Venison jerky (Midura et al., 1972) the other isolations and toxin detections have not been associated with outbreaks of botulism.

2.2.6. Type G

This strain has never been incriminated in food-borne outbreaks. So far, this serotype has only been reported in Argentinan soils. It was documented only six years ago (Gimenez and Ciccarelli, 1970) and has not been found in other countries.

2.3. A review of the enrichment and toxin detection methods

The growth of Cl. botulinum is influenced by growth of other microorganisms. The influence could be stimulatory as for example Salmonella typhimurium and Cl. perfringens (Munsey et al., 1973) and some lactic acid bacteria (Benjamin et al., 1956) lower the redox potential as well as supplying growth factors. The inhibitory effect is exerted by lactic acid bacteria in fermented foods (Saleh and Ordal 1955), free fatty acids produced by Brevibacterium linens (Gracz et al., 1959a, b) nisin produced by Streptococcus lactis and bioticin E produced by type E-like Cl. botulinum (Ellison et al., 1971). Kautter et al. (1966) reported that a bacteriocin-like substance from a non-toxic organism identical to Cl. botulinum type E inhibited the type E. Kwan and Lee (1974) investigated a bacteriocin produced by a species of Moraxella and found that it inhibited the outgrowth of Cl. botulinum type E. Smith (1975) also reported

inhibition of Cl. botulinum by Cl. perfringens. The existence of such inhibitory factors in a specimen renders the detection of the toxins difficult.

Dubovsky and Meyer (1922b), and Burke (1919) broadly established the basic methods for successful isolation of the botulinum organism from soil and heavily contaminated samples which formed a foundation for a generalised survey of the botulinum spores in many regions. Dobovsky and Meyer (1922b) studied the composition and reaction of culture media and found that beef heart digest medium was satisfactory, but found no need for meticulous anaerobiosis since Cl. botulinum will grow in hay or loose soil. They recommended the use of freshly boiled beef heart digest without wax cover, and incubation at 35 C for 10 days, while using a second buffered beef peptic digest veal infusion broth at 37 C and 10 days incubation for enrichment of weakly toxic cultures. The conspicous shortfall of the early procedures was the heat treatment of the samples which injured spores of low thermal resistance especially in view of the current knowledge regarding heat resistance of spore of Cl. botulinum type E (Johnston et al., 1964).

It was Eales and Turner (1952) who, copying Van Goidsenhover and Bertraud, incorporated 0.1% of sodium luminal on to blood agar to reduce swarming. Though not an enrichment method it facilitated development of separate colonies. To eliminate possible contaminants in the toxic enrichment cultures before streaking on egg yolk medium of McClung and Toabe (1947), Johnston et al., (1964) mixed the toxic enrichment cultures with

an equal amount of absolute alcohol. Isolation of type E cultures using the technique of Johnston et al. (1964) was accomplished by Chapman and Naylor (1966).

2.3.1. Indicators for toxin production

The use of laboratory animals for the detection of botulinal toxin has been exercised for a long time. Dubovsky and Meyer (1922b) in their studies of methods of botulinal toxin detection from soils utilized three guinea pigs per sample. They also tested a limited number of cultures by intraperitoneally injecting three mice with 0.1 ml of the centrifuged and filtered supernatant. The guinea pigs were injected subcutaneously with 1.2 ml of the preparation. After thorough investigations they concluded that toxic enrichment cultures should be considered positive for Cl. botulinum only when a decisive toxin-antitoxin reaction is obtained; observation of intoxication signs after feeding; or isolation of pure cultures. For many years the use of mice has been the standard reference method both for diagnostic purposes and in research laboratories. However, the method has shortfalls. Steward et al. (1968) doubted the accuracy of inoculating material intraperitoneally, and showed that of 150 mice injected intraperitoneally, 21 (14%) showed all or part of the inoculum in a site other than the peritoneal cavity. They considered this error inherent in the technique and not easily correctable. Miner et al. (1969) repeating Steward and colleagues' work tried variations in technical procedures such as the size of the needle, site of penetration, angle of needle to the abdominal wall and speed

of injection. They found no appreciable reduction in the error. Baillie et al (1973) enumerate disadvantages of using mice such as unspecific deaths due to protein shock and other factors (also observed by Haines, 1942) and secondly a long observation period usually exceeding three days.

Botulinal toxins are simple proteins. They can be demonstrated in vitro by immunological methods. Johnson et al. (1966) demonstrated a hamagglutination technique sensitive to one LD_{50} mouse unit of botulinal toxin. Vermilyea et al. (1968) were able to detect toxin titres 370 to 557 mouse LD_{50} per ml. in food samples using gel diffusion. Though a useful method it is difficult to prepare and store slides for testing large numbers of samples. Consequently Mestrandrea (1974) developed a microcapillary agar gel diffusion method and tried it at the New York District office of the Food and Drug Administration. It easily picked out the causative type B toxin. Where the toxin titres are low it can be concentrated using "Millipore ultra filtration chamber" (Sonneschein and Bisping, 1976) or a sephadex G25 column (Vermilyea et al. 1968). Concentration is necessary since the immunodiffusion test is 100,000 less sensitive than the mouse toxicity test (Schantz and Sugiyama, 1974). Serological methods while appearing to hold future promise are currently restricted by non-specificity. Kalitina (1960) stained a film of pure cultures of Cl. botulinum rabbit antiserum and fluorescein isocyanate. He obtained an intense green luminescence which was specific for Cl. botulinum types A to E cells and not other cells in the suspension. Aalvik et al. (1973) applied a direct

fluorescent staining technique using equine antiserum prepared against purified toxin to detect type E botulinal toxin in cultures. Only type E strains of Cl. botulinum were stained whereas cells of Cl. botulinum strain B17 which is genetically related as well as nontoxigenic E-like cells could not be stained. Bulatova and Kabanova (1960) found that types A and B would react but types C, D and E were negative with the same sera. They also reported that Cl. sporogenes (3 out of 17 strains) reacted although they did not share agglutinating antigens with the botulinum strains. Cross reaction has also been reported by Boothyroyd and Georgala (1964). Similar cross-reactions in proteases of Cl. botulinum have been reported by Tjaberg (1974). The advantage of these immunological methods is the rapidity with which results can be obtained. Vermilyea et al. (1968) in spite of confirming in mice, could complete the procedure in 24 hours. In conclusion, full acceptance of the fluorescent antibody technique must await a more complete study involving a large collection of botulinal strains as well as those of Cl. sporogenes (cClung, 1967).

2.3.2. Isolation of Cl. botulinum

In the earlier studies on soil for Cl. botulinum. colony isolation was only attempted from cultures which were found to be toxic to animals (Dubovsky and Meyer, 1922b; Knock, 1952). This practice was critized by McClung (1967) because contaminating organisms are known to destroy the toxin. Dubovsky and Meyer (1922b) could isolate Cl. botulinum using the enriched

cultures which were used for toxin production. They either heated this culture at 80C for one hour or boiled it for 10 minutes. Samples from the heated specimens were plated on sheep blood agar, or inoculated into deep liver agar tubes. Such specimens could also be enriched for 24 hours in beef heart peptic digest broth, then cultivated in deep agar. Alternatively they used soil from which toxic Cl. botulinum had been detected.

Knock (1952) made a 1:10 dilution of raw soil in a sterile tube then placed the tube in boiling water. Growth was observed in the tube heated longest. A major problem with isolation of discrete colonies on solid media is their swarming on the media. Eales and Turner (1952) utilised "sodium luminal" and successfully isolated discrete well separated colonies of Cl. botulinum type D. Robinson (1930) recommended the use of deep glucose agar or glucose liver agar shake for the isolation of types C and D.

These early methods emphasised the use of heat, but this cannot be applicable to spores of Cl. botulinum type E (Johnston et al., 1964). This has lead to the use of absolute alcohol in their isolation (Johnston et al., 1964). This method does not seem to have gained popularity. Instead, researchers such as Gimenez & Ciccarelli (1967) and Smith and Moryson (1975) separated the samples into two, heated one and left the other unheated. After incubation of both samples, Gimenez and Ciccarelli (1967) streaked inocula on dried agar plates and examined them after anaerobic incubation. Suspicious colonies were subcultured into cooked meat medium and incubated for 6 days after which they were tested for toxicity in mice. In this way

they recovered an unknown type of Cl. botulinum . These authors, (1968, 1970) also used single cells from well isolated colonies to prepare pure colonies.

Eklund et al. (1967) after investigating some media for the isolation of type F, recommended peptone, trypticase,beef infusion - Egg yolk agar plus 1% yeast extract and 0.1% sodium thioglycollate.

MATERIALS AND METHODS:

1. Materials

1.1. Meat

1.1.1. Description of the sampled area

Nairobi is a cosmopolitan city bordered to the north and west by high population density peasant farming areas. To the south and east are drier terrains roamed in by wildlife, having minimal agricultural productivity. The city has attracted rural people looking for job opportunities, consequently resulting in the springing up of many unhygienic urban and suburban residential centres. There are two major export slaughter houses a few kilometers away from the town. Within and around the town there are about five slaughter slabs providing meat mainly for local consumption. During sampling the city butcheries were divided into four groups depending upon the type of customers they were serving, this being related to financial status of the customers. And from the neighbouring rural areas to the west, two groups were allotted but more on geographical location than on residential status.

The zones were:

i) Low income class urban & suburban centres:
Kangemi ,Kibera, Riruta, Kawangware, Ndumbuini, Dagoretti-Corner, and Kabete.

ii) High income class urban centres :
Hurlingham, Karen, Westlands, Woodley and City Market.

iii) Middle class butheries:
Kariokor, Westlands (Stalls) Eastleigh and River road.

(iv) Mathare Valley: This is a shanty town occupying the Nairobi river valley between the Psychiatry hospital and the Kenya Airforce base. This shanty town has very poor health facilities and was deemed unique to constitute a sampling zone.

(v) The Lower Kabete zone: Lower Kabete, Kamutiiini and Wangige Villages in Kiambu District.

(vi) The Kikuyu zone: Kinoo, Gitaru and Kikuyu townships in Kiambu District.

1.1.2. Meat Sampling:

Equipment needed: Sterile beakers, frozen "ice paks", Coleman icebox, 70% alcohol, cotton wool and a dial thermometer with a sharp end for piercing the meat sample.

Due to mutilation of the carcass, the butchers were not very flexible with regard to the choice of the sample to be taken. To an extent this created a bias in the sampling. From the carcass a piece of meat over 300 grams (gm) was collected and placed inside a sterile beaker whose mouth was immediately covered with aluminium foil. When a convenient size of meat was already available it was taken in as a whole without having to handle it any more by further cutting.

As for the raw ground meat, the butchers usually had some already in stock which was kept in a cool place or in the open as the case was in some butcheries.

In most cases the minced meat was lying on an aluminium tray or on concrete tables. Rarely was it necessary to have to wait to get freshly fabricated minced meat. Three hundred to five hundred gram samples were weighed and emptied into a sterile

beaker. The shovel used usually was left lying carelessly, in some cases the butcher used his hand to add on more minced meat to make up the weight. Some pork sausages had been factory packaged, in which case a full package was purchased so as to avoid having to handle the sausages any more. For all sample, a piercing metal thermometer was used to record the internal temperature of the sample before the sample was placed into the cooling box. On withdrawing the thermometer from the meat, it was wiped with cotton wool moistened with 70% alcohol, allowed to evaporate then replaced into the thermometer case. When removing a segment of the sample for bacteriological investigation, the pierced site was avoided. At the same time the butcher was questioned as to where and when the animal was slaughtered. Occasionally this information was not forthcoming or the wrong information was given.

The sample and the containing beaker were placed inside the icebox containing frozen "icepaks" and were not removed again until the time of processing. The samples on reaching the laboratory (usually less than two hours after collection) were immediately processed as the media and diluent had already been prepared. A total of 104 meat samples were collected and processed.

1.2. Milk

Equipment needed: Coleman icebox, sterile universal bottles.

The samples included raw and factory processed milk products. When a processed product was purchased, the whole piece was placed inside the icebox. That was the case for pasteurised fresh and fermented milk prepared at the Kenya Co-operative Creameries, pure jersey cream in polythene bags prepared in a farm dairy and icecream in paper cups. Ice cream cone was placed inside a sterile beaker, then covered with sterile aluminium foil. For wrapped chocolates, the wrapper was removed without touching the bar itself. The bar was dropped into a sterile beaker and treated like the other samples. A piece of cheese about 200g was cut off from the sale bar. This piece was placed in a sterile beaker also.

Raw milk from hotels and a dairy co-operative society (Sigona) was directly poured into a sterile universal bottle, and the cap replaced.

All the samples were transported to the laboratory in an icebox and were processed within four hours after sampling.

1.3 Mud/sand/fishes:

The survey for Cl. botulinum spores in the environment was intended to be representative of many ecological zones extending from the coastal beach sand through the savannah river mud and the highlands to the lake basin as depicted on the map. Ideally fish samples should have been refrigerated or frozen immediately. But lack of a field refrigerator necessitated the use of a cool box cooled with frozen icepaks. Unfortunately these were not effective and the fish samples putrified fast before they reached the laboratory.

1.3.1 Sampling:

Equipment needed: icebox and frozen icepaks, tough polythene bags, sterile disposable gloves, thermometer, 70%alcohol.

The samples were taken from the banks of flowing rivers whose sizes varied greatly, man-made and natural water pools some of which were disused and others were used for consumption by wild and domestic animals as well as human beings. In some pools and rivers, people were bathing at one end while cattle were drinking at the other end. Sand was collected from coastal beaches and the shores of Lake Victoria at Homa-bay and Kisumu town. Some coastal beaches were utilised for holiday makers but others were disused. The few samples of fish were collected from the coast, Lamu and Kisumu town fish markets.

The sample consistency varied from place to place in some areas being dark or brown with offensive smell, to white sand. At some sites, the pool was considered to be having many, though different, small ecological zones. In such areas more than one sample was taken at different points no less than ten meters apart. The samples were given the same number but differentiated by alphabetical letters. A hand was aseptically passed into a sterile plastic glove and the fingers folded to form a "bowl". The material being sampled was scooped by the gloved hand. By holding the free end of the glove with the other hand, the glove was pulled out over the palm inside out such that it was removed inside out and the material that was

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outside the glove on the palm was now within the glove. The open end of the glove was tightly sealed with cotton threads, and checked for leakages before it was put in a bigger polythene bag which was holding over ten of such samples. Once full, the bag was also sealed and placed in the icebox. Great care was taken during sampling and at all other stages to avoid cross contamination between different samples.

On reaching the laboratory, the material was stored in a deep freezer at - 20C (Smith and Moryson, 1975) until one day before laboratory examination commenced. At that time, the bag holding ten or so samples was transferred from the deep freezer to the refrigerator or left at room temperature so as to thaw out gradually.

2. Methods

2.1 Sterilisation of Equipment:

2.1.1 Preparation of Glassware for sterilisation:

2.1.1.1 Decontamination: Before washing, all the glassware containing infective material or cultures were either placed in 3% lysol solution or autoclaved at 121C, (15 pounds per square inch) for 15 to 20 minutes. Except where otherwise stated, all autoclaving was carried out under these conditions.

2.1.1.2 Cleaning, drying and wrapping: Any gross particles on the glassware were removed and the equipment thoroughly rinsed with cold or hot water before it was brushed in detergent solution. Tap water was used to clean off the detergent from the article which was subsequently rinsed for three more times using fresh distilled water for every rinse. The glass was then allowed to drain and dry in the hot air oven with the mouth placed downwards. After drying, the tops, screw-caps and lids in case of petridishes were replaced. Flasks, bottles, and test tubes without tops were plugged with cotton wool and wrapped with a piece of aluminium foil before sterilization. Wide mouthed glass ware such as beakers and porcelain mortars were wrapped in aluminium foil. Pipettes contaminated with infective material were placed in 3% lysol but other pipettes were place in distilled or tap water in a tray and submerged in cleaning fluid in a "kartell" pipette washer where they were left overnight. Finally they were washed in tap water and rinsed preferably in hot distilled water before they were dried, cotton plugged and placed into cylindrical aluminium containers or wrapped in aluminium foil ready for sterilization.

2.1.2 Preparation of steel ware for sterilization :

These were washed following the same protocol as for the glassware. The blades of the homogenizer shaft were covered with cotton wool to prevent them from piercing the outer layer of aluminium foil. Scapel holders, pairs of forceps, and porcelain pestles were wrapped in aluminium foil. Factory sterilized surgical scapel blades were used and each sample was fragmented with only one blade. In between the cuttings, the pair of forceps and the scapel blade were dipped in 70% alcohol and flamed twice.

2.1.3 Sterilization of the equipment :

Glass and steel equipment were sterilized by hot air in an oven (Memmert) which is capable of raising the air temperature in the oven to 220C. Most of the time, sterilization was carried out at 160C for one hour except for a few occasions when the equipments were urgently required and a temperature 180C for 20 minutes was used. The temperature was maintained for the entire period of time after which the oven was allowed to cool down slowly without opening the doors.

2.2 Bacteriological methods.meat

2.2.1 Dilution methods

An MSE homogeniser was used to macerate the samples. The shaft bearing the blades, which had been sterilized, was fixed to the motor stem and the wrapping aluminium foil and cotton wool aseptically removed. Pieces of the samples were aseptically cut using pairs of sterile forceps and scapel blades, and loaded into an already weighed 100 millilitres (ml.) vortex glass beaker. In total, 10 grams of the sample were thus removed. Into the beaker holding the sample fragments, 90g of 0.1%

peptone water were added. This diluent has advantages in that it retards death of many cell species and has a low surface tension so that any aggregated cells will disperse easily (Jayne-Williams, 1963). The beaker and its contents were fitted into the bakelite container and the cap fitted on. The bakelite container with the beaker was connected to the homogenizer with the blades inside the flask and into the diluent. The shaft was spun at 14,000 revolutions per minute (R.P.M.) and for not more than three minutes (Rao, 1970) to avoid over-heating of the sample (Sharf, 1966).

The beaker was disconnected and covered with sterile aluminium foil, after which it was allowed to stand for about 15 minutes to permit resuscitation of the microorganisms

Thus a 1:10 dilution of the samples was obtained. The beaker was shaken thoroughly before pipetting of the suspension for dilution. Using aseptic technics, 10 ml. of the suspension were transferred into a bottle containing 90 ml. of 0.1% peptone water. The bottle was thoroughly shaken by rotating it in a clockwise-anticlockwise direction for 5-8 times. Immediately, 1 ml. was removed and transferred into a test tube containing 9 ml. of the diluent. This tube was labelled 1:1000 (10^{-3}). The pipette used for this transfer was not allowed to touch the diluent in the next tube while releasing the suspension into the latter tube from the pipette. A fresh sterile pipette was used to mix the diluent and the suspension as well as the transferring from this tube to the next. A second fresh

pipette was used for mixing. To secure plates which meet the required standard range of colonies (usually 30-300) per plate, this serial dilution was carried on to 10^{-6}. On attaining a dilution whose total bacterial and coliform quantity was to be tested the suspension in this dilution was thoroughly shaken and 1 ml was carefully pipetted into each of appropriately marked duplicated culture petridishes. This was done before proceeding further with the serial dilution.

2.2.2. The pouring methods:

After autoclaving, the Plate Count Agar (PCA) (Oxoid Manual, 1973) and Violet Red Bile Agar (VRB) (Oxoid Manual, 1973) were cooled to 45 degrees centrigrade in a water bath. When pouring, the aluminium foil and cotton wool mouth plugs were removed and the mouth of the flask flamed. The inverval between inoculating the material into the petridish and pouring of the agar was short so as to avoid dehydration of the sample. On pouring the molten agar, the petridish was swirled in a fashion tracing a figure of eight for five times, avoiding over-spilling. During swirling, the dish lid was held partly covering the bottom component. Once mixed, the dish lids were replaced such that only a tiny slit was left between the bottom and top lid to allow for evaporation while the agar was solidifying. Once solidified, the lid was properly fitted and the plates incubated in inverted positions at 37C for 24 and 48 hours for coliforms and total counts respectively. For coliform counts 1 ml of both 10^{-1} and 10^{-3} were inoculated into petridishes to which about 15 ml of liquid VRB agar was poured. Total viable

aerobic count was done at 10^{-4} and 10^{-6} using 1 ml aliquots and 0.1 ml aliquot from 10^{-6} to give a 10^{-7} dilution. About 15 ml of PCA were poured into the petridish, followed by the general procedure explained earlier. As a sterility check , two plates were poured with the diluent and the appropirate agar medium alone. No growth was expected on these two control plates.

2.2.3 Counting procedure

After the stated incubation period was over, counts were taken. As far as possible only those plates with 30-300 colonies were considered (Nickerson and Sinskey, 1972). On occasions when the colonies were well laid out, it was possible to count over 300 colonies by subdividing the plate into 4 or 8 segments using a felt marker on the back of the plate and enumerating the colonies on each segment at a time. After multiplying the colony count with dilution factor the results were considered to represent the number of viable bacteria (or coliforms as the case may be) per gram of the meat.

The plates were placed on a dark background and all the colonies in all the 4 VRB or the 6 PCA plates counted manually by placing an ink mark on the base of the bottom half of the petridish. On the PCA such colonies were white and varied widely in their diameters. Generally the more the colonies on a plate the smaller they appeared. On the VRB plates, positive results were indicated by dark-red colonies with a surrounding zone of precipitated bile of at least 0.5 mm in diameter (Sharf, 1966)

2.2.4 Testing for salmonella

A piece of the sample weighing over 10g was aseptically cut from the sample in the collecting beaker and inoculated into two liquid selective enrichment media. The two media recommended by Huhtanen and Naghski (1972), Tetrathionate broth (Oxoid) and Selenite broth (Difco) were used. The media were held in bottles or conical flasks each containing over 45 ml of either of the two media. After vigorous shaking of the bottles or flasks they were incubated at 37C for both 24 and 48 hour periods. After 24 hours incubation the sample-containing vessels were again shaken by hand. About 5 minutes after mixing (Huhtanen and Naghski, 1972), a loopful of the supernatant fluid was picked on a bacteriological wire loop both from the tetrathionate and selenite broths and streaked carefully on bromo-thymol blue lactose agar (BBLA) plates.

The BBLA plates as well as the enrichment broths were incubated at 37C for 24 hours ± 1 hour. At the end of the reincubation, enrichment in the two media had already been going on for a total of 48 hours enrichment. In this way isolation was attempted after 24 and 48 hours of enriching. Two of those colonies showing a blue pH reaction in the BBLA plates were picked carefully and sown into Triple Sugar Iron (TSI) (Oxoid) and Urea slants (Oxoid) making a stab with a flamed inoculating straight wire, and incubated for 24 hours ± 1 hour at 37C. Tryptone water (Oxoid) was inoculated too. This procedure, that far, was able to eliminate all the suspects

so that it was not necessary to try serological methods.

2.2.5. Tentative test for the presence of E. coli

When an E. coli detection was to be attempted from a sample, the Violet Red Bile Agar plate used for the enumeration of coliforms was again used. Ten representative coliform colonies were randomly picked with a bacteriological loop from a plate showing at least 30 of such colonies. The inoculum was streaked on the dry surface of an Endo Agar plate (Oxoid) and subsequently incubated for 18-24 hours at 37 C. Raised colonies two to three millimetres in diameter and showing a flat or concave dark centre and a metallic sheen were tentatively taken to be E. coli. Such colonies were further subjected to an IMViC test following the methods described in the standard methods for the examination of water and waste water (APHA, 1971).

2.2.6. Tentative test for the presence of Clostridium perfringens.

The presence of Cl. perfringens was randomly tested but no quantitation of the organism was attempted. For detecting its presence, a sterile bacteriological loop was used to pick some material from the cooked meat medium tubes, the same tubes that had been inoculated and incubated for the detection of Cl. botulinum. The loopfuls were streaked on Neomycin Blood Agar and Egg yolk Agar as suggested by Sutton et al. (1971). The plates were incubated for 24-48 hours anaerobically at 37 C. A glass slide smear was gram stained too.

2.2.7 Detection of botulinal toxin

A piece of meat weighing about 10g was aseptically obtained from the original sample in the beaker, and transferred into tubes of Robertsons Cooked Meat medium which had been prepared, autoclaved and cooled rapidly just before use. For each sample, four tubes were inoculated. Two of them were heated at 80C for 20 minutes after which they were rapidly cooled. All the four tubes per sample were placed in a "BBL Gas Pak Anaerobic system" jar. A "Gas Pak" envelope was cut at one corner and placed in an upright position in the jar. Ten ml of tap or distilled water were pipetted down through the open corner of the envelope. The jar lid was quickly replaced and sealed tight. The jar was incubated at 37C for 6 days

Thereafter, using sterile 1 ml pipettes, 1 ml of the broth culture was taken from each of the four tubes and transferred into one centrifuge tube, and the combined aliquots centrifuged at 1730xg for 20 minutes. In the earlier stages, trypsin was not used to activate any precursors (Duff et al., 1956). When used in later extracts, a 10 mg/ml solution of bovine pancreatic enzyme (BDH lot no 1804720) was prepared; 0.1 ml of the trypsin was added to 1 ml of the centrifuged extract, and incubated at 37C for 1 hour. After trypsinization, the extract was injected intraperitoneally into a 20 g white mouse (from the breeding house of the Veterinary Research Laboratories, Kabete) using a sterile needle (Gauge 23 to 25)

and 1 ml graduated syringes. Many mice were dying within one hour since inoculation. The mice showed convulsive spasms with protracted stretching of the hind limbs, raised tails, excitement, respiratory arrest and death. Similar drawbacks were reported by Dubovsky and Meyer (1922b) and Haines (1942). Haines (1942) observed that diluting the toxic cultures over a wide range merely delayed onset of the characteristics symptoms. Smith and Moryson (1975) diluted their supernatants 1:4 and this was adopted in this survey to reduce the frequency of the side deaths. The activated, diluted culture-centrifugate was inoculated into mice intraperitoneally on either left or right side since Miner et al (1969) found no difference in the inoculation sites. The mice were observed for 4 to 5 days after which survivors were euthenised and incinerated. If a mouse died, a second one was inoculated. If it too died, the extract was suspected and boiled for 10 minutes (Haines, 1942) then inoculated into a mouse. If it died, there was no more verification on that extract. On a few occasions, an extract was inactivated by heat and neutralisation with polyvalent or specific types A, B, C, D and E antisera carried out as described later.

2.2.8. Isolation of pure Cl. botulinum cultures

When a sample supernatant was toxic to mice and suspected to be containing botulinal toxin, isolation of the agent was attempted. This involved picking a loopful of the supernatant, aseptically, and streaking it on 3% blood agar. An attempt at isolation was carried out on each of the four enriched tubes.

Each tube's supernatant was streaked onto one blood agar plate. The plates were incubated anerobically for 48 hours. After incubation, the plates were scrutinized for the presence of typical colonies of any of the types of Cl. botulinum. Suspect colonies were picked and transferred to newly heated tubes of cooked meat medium and incubated anerobically for four days at 37 C after which the tubes were tested for toxin production.

2.3. Bacteriological methods - milk and milk products

Liquid milk was thoroughly shaken after which serial dilutions using physiological saline (0.85% sodium chloride) were made. The 10^{-1} and 10^{-3} dilutions were used for coliform counts at 37 C after 24 hours incubation. Total aerobic counts were made from dilutions 10^{-3} and 10^{-5} after 48 hours incubation at 37 C.

Frozen ice creams and chocolates were allowed to thaw out at room temperature and then thoroughly shaken. Serial dilutions were then made as for liquid milk. A 10 gm piece of cheese was homogenised in 90 gm of 0.1% peptone water. Further processing was similar to that described for meats.

The tests carried out for Salmonella from the first 39 samples collected at Sigona were the same as explained in the section on meats. Likewise the tests for E. coli in milk were similar to the tests carried out on meats.

Milk Ring Test was performed as follows:

0.025 ml of a stained brucella antigen was added to 1 ml of the sample milk in a thin tube, the tube was inverted a

number of times to mix the antigen together with the milk. The tube was placed in a water bath at 37C for 1½ hours before noting the presence (positive) of a blue ring at the top with the rest of the milk being white. A homogeneously bluish milk was taken to be negative.

2.4 Bacteriological methods - mud/sand/fish

2.4.1 Enrichment:

The method used follwed very closely that utilised by Smith and Moryson (1975) in their survey for Cl. botulinum in lakes and waterways of London.

2.4.1.1 Mud and sand :

A quantity of the sample weighing about 50 gm was placed into a tared porcelain mortar and 50 gm of 0.1 molar phosphate buffer at pH 7.0 was added. When such mud was of low water content, more phosphate buffer was added to arbitrary amounts so as to provide at least 30 ml of supernatant. The sample was mixed thoroughly with a pestle and allowed to stand for some time. The supernatant was divided into two equal parts of at least 15 ml each and pipetted into a universal bottle for centrifugation in an MSE GF eight swing-out head centrifuge which was spun for 30 minutes at a speed giving 1730xg centrifugal force. The resultant supernatant was discarded and the two pellets transferred each one to a separate tube containing cooked meat medium which had just been autoclaved and cooled. One tube only was heated at 60C for one hour. Both tubes were then placed in an anaerobic aluminium jar which was evacuated and

filled with nitrogen gas. The jar was incubated at 30C for 6-8 days for enrichment and toxin production. Examination for botulinal toxin and Cl. perfringens was then attempted.

2.4.1.2 Fish:

An enterior 1/3 of the whole fish (7 - 10 gram) was chopped off and placed in a sterile plastic bag which was incubated anaerobically for 6-8 days. This was followed by grinding of the enriched sample in 50 ml (roughly) of sterile physiological saline (0.85% saline) to extract the toxin. The supernatent was centrifuged at 1730xg for 15 minutes. The resultant fluid was trypsinised as before, diluted 1:4 and injected intraperitoneally in mice.

2.4.2 Toxin detection:

Initially, two ml of the culture broth from each of the two tubes was removed and centrifuged at room temperature for 20 minutes at 1730xg. One ml of each of the centrifugates was trypsinised as below and all the four extracts from each sample, i.e (1) Heated, (2) Heated trypsinised, (3) Non-heated, (4) non-heated trypsinised, were diluted 1:4 (Smith and Moryson, 1975) and injected intraperitoneally to one mouse each so that four mice were utilised per sample.

However, this procedure was modified (to save on mice) so that two ml of the fluid culture from each tube were pipetted and pooled together. The four ml pooled broth was spun at 1730xg for 30 minutes. Two ml of the centrifugate were mixed with 0.2 ml of a 10 mg/ml solution of bovine pancreatic trypsin (BDH)

then incubated for one hour. A 1:4 dilution (Smith and Moryson, 1975) was tried using sterile physiological saline and found to reduce the non-specific deaths previously observed by Haines (1942). The mice were observed for at least 5 days for the development of clinical signs of depressed abdomen, distress, paralysis and subsequent death. When death occurred, the inoculation was repeated this time using two mice. If these died too, the toxic fluid which had been stored in a refrigerator was diluted 1:100, and heated at 80 C for 10 minutes. Neutralisation tests were done too as follows: The specific sera were diluted such that each ml contained 1,000 mouse protecting units. Half an ml of this solution was injected into one mouse for each sero-type and a control. Half an hour later the suspect extract was injected into all the five protected mice except the control. The mice were observed for signs of botulism and death.

2.4.3. Tentative diagnosis for Cl. perfringens in mud/sand/fish

Initially a loopful of the enriched cooked meat medium broth was streaked on blood agar and egg yolk medium in duplicate. One of the duplicate plates was incubated aerobically and the other one anaerobically at 37 C for 24 hours. The usefulness of the results was doubted and this was abandoned. From the enriched tube of cooked meat medium and the fish, material was picked using a bacteriological loop. This was streaked on to Sulphite-polymixin - sulphadiazine (SPS) egg yolk (EY) agar plate which had been dried overnight in an incubator at 37 C.

Plain SPS was overlain on the streaked SPS - EY plate and the plate incubated anaerobically overnight at 30C. The next day, black colonies with a zone of white precipitate were inoculated into lactose motility agar tubes and litmus milk tubes. The media were incubated at 37C for 24 hours. Motility and lactose fermentation were checked in lactose motility medium (Shahidi and Ferguson, 1971). Stormy fermentation was tested for in Litmus milk (sterilised through tyndallisation)

Arbitrary grading of the butcheries and meat samples:

The cleanliness of a butchery was assessed and an arbitrary value given on a scale with the highest value five and the lowest value one. Meat appearance was also graded between three (best) and zero (worst putrifying).

RESULTS

1. Meats:

During this survey of the microbiological quality and quantity of meat and meat products, 104 meat samples were collected from varying communities in and around Nairobi. The butcheries providing the meats were distributed such that they included varying socio-economic classes (Appendix I and II). The samples included 75 beef chops, six pork chops, four goat and one mutton chop, 11 ground beef samples, six packets of raw pork sausages, and one raw beef sausage.

Grab samples were taken but the experiment was not fitted to a statistical design due to the large distances between randomly picked butcheries and consequently this would have delayed the transportation of the samples back to the laboratory. The complete data of each sample including the results will be found in Appendix III.

Tables ii and iii show total plate count and the coliforms found in the various meat samples collected for this survey. The results for all the 104 samples shows that there was no sample contaminated with less than 10 thousand mesophilic Total Plate Count (T P C) microorganisms per gram of the sampled meat (Table ii). There were 40% of the samples contaminated with total microorganisms in the range 10 - 100 million per gram. Of those samples in this 10 - 100 million range, 83% were whole meat cuts as opposed to 10% for ground beef and 7% for sausages.

It is observed that of all the whole meat chops, 35 (41%) had T P C between 10 and 50 million, with a mean of 32 million.

Table ii shows the frequency of meat samples whose total aerobic plate counts were falling within the indicated ranges of bacteria.

TOTAL PLATE COUNT	WHOLE MEAT CUTS	RAW SAUSAGES	GROUND BEEF	TOTAL
Range x 10^3				
<10	0	0	0	0
10-500	5(6%)*	1(14%)*	0	6(6%)*
500-1000	8(9%)	1(14%)	1(9%)*	10(10%)
1000-1500	4(5%)	1(14%)	0	5(5%)
1,500-2,000	6(7%)	0	2(18%)	8(8%)
2,000-2,500	5(6%)	0	0	5(5%)
2,500-3,000	0	0	0	0
3,000-10,000	6(7%)	0	2(18%)	8(8%)
10,000-100,000	35(41%)	3(43%)	4(36%)	42(40%)
>100,000	17(20%)	1(14%)	2(18%)	20(19%)

* NB. The percentages(in Brackets) in the column do not add up to exactly 100 due to rounding off.

Table iii shows the frequency of meat samples whose coliform counts were falling within the indicated ranges of coliforms.

COLIFORMS RANGE $\times 10^3$	RAW SAUSAGE	GROUND BEEF	WHOLE CUTS	TOTAL
< 1	0	0	10(12%)*	10(10%)
1-50	5(71%)*	7(64%)	41(49%)	53(52%)
50-100	1(14%)	1(9%)	7(8%)	9(9%)
100-150	0	2(18%)	3(4%)	5(5%)
150-200	0	0	1(1%)	1(1%)
250-300	0	0	1(1%)	1(1%)
> 300	1(14%)	1(9%)	20(24%)	22(22%)

* NB. The percentages in the column do not add up to exactly 100 due to rounding off.

Table iii shows the frequency of meat samples whose coliform counts were falling within the indicated ranges of coliforms.

COLIFORMS RANGE $\times 10^3$	RAW SAUSAGE	GROUND BEEF	WHOLE CUTS	TOTAL
< 1	0	0	10(12%)*	10(10%)
1-50	5(71%)*	7(64%)	41(49%)	53(52%)
50-100	1(14%)	1(9%)	7(8%)	9(9%)
100-150	0	2(18%)	3(4%)	5(5%)
150-200	0	0	1(1%)	1(1%)
250-300	0	0	1(1%)	1(1%)
> 300	1(14%)	1(9%)	20(24%)	22(22%)

* NB. The percentages in the column do not add up to exactly 100 due to rounding off.

Twenty per cent (20%) of the whole meat cut samples had over 100 million microorganisms per plate. When the beef, goat, and mutton whole meat cuts were grouped together they showed a TPC mean of 33.5 million (Table iv) but pork chops averaged only 19 million. For the ground unfrozen cooled beef, no sample contained less than half a million micro-organisms per gram aerobically. Thirty six per cent (36%) of all the 11 ground meat samples had a count within the range 10 million to 100 million and two samples (18%) had over 100 million organisms per gram. The general TPC mean for all the ground beef was 35 million (Table iv).

As for the sausages 43% of them had counts within the range 10 million to 100 million organisms per gram as compared to the T P C general mean for the sausages which was 37 million organisms per gram (Table iii).

The greatest number of the meats (52%) showed a coliform count between 1 thousand and 50 thousand, of which 77% of the samples in this range were whole meat cuts (Table iii). Twenty-two per cent (22%) of all the meat samples had over 300 thousand coliforms per gram, while 71% of all the samples showed less than 100 thousand coliforms per gram. Sixty nine per cent (69%) of the whole meat cuts were having less than 100 thousand coliforms per gram compared with 85% for raw sausages and 73% for ground beef (Table iii). Table iv shows the total plate counts and number of coliforms in relation to the type of meat sampled. From this table it is seen that the coliform means for all the beef, goat and mutton chops was 11 thousand, for ground beef the coliform mean was 72 thousand , 104 thousand for pork chops, and 54 thousand for raw sausages.

Table iv. The mean total plate and coliform counts for each of the Meat Sample types sampled during the survey.

TYPE OF SAMPLE	NO OF SAMPLES	MEAN TOTAL Count x 10^3	MEAN COLIFORMS
Beef, Goat and mutton chops	80	33,500	11,000
Ground Beef	11	35,000	72,000
Sausages	7	37,000	54,000
Pork Chops	6	19,500	104,000

The influence of the abattoir hygiene on the meat contamination is shown by table v. The values are the means for all the samples that came from a certain abattoir which were collected from a certain butchery. During sampling the sample temperatures were noted. Table vi is showing the number of samples whose temperature was within the indicated ranges. The mean counts for all the samples whose temperatures were within a range are also shown in the last two columns of the table. A subjective assessment of the cleanliness of the butchery and the appearance and smell of the meat is shown on an arbitrary scale in table vii. The high class butcheries had high scores both in meat and the general cleanliness. Other centres show a close relationship between both scores except for Kawangware where though the butcheries were fair, the meat was bad. Salmonella was not isolated from any of all the 104 meat samples surveyed. Although E. coli and Cl. perfringens were not screened in all the samples,a few random samples yielded organisms which were tentatively diagnosed as E. coli and Cl. perfringens. While testing for botulinal toxin from the meats, no sample was confirmed as containing botulinal toxin. On a few occasions some mice were suspected but this was not reproduceable and therefore not considered positive.

2 Milk:

Table viii summarises the mean TPC and coliforms count obtained from the various types of milk samples taken from the market. Usually the raw milk in eating houses, was boiled

Table v . The influence of the abattoir on meat contamination as judged by total and coliform counts.

ABATT-OIRS / BUTCH-ERIES		KMC	DAGORETTI
MATHARE	Coliforms	5000*	34279
	Total Count	$39,500 \times 10^3$	51756×10^3
KARIOKOR	Coliforms	2880	81330
	Total Count	4237×10^3	41199×10^3

NB. (1) Uncountable and over 300 counts per plate were left out.

(2) * Only one sample analysed

(3) Mathare and Kariokor contributed 33% of the total meat samples. 50% of all the sampled meat originated from both the KMC and Dagoretti abattoirs.

Table vi. Summary of the recorded meat sample temperature ranges at the time of sampling and the means of the total plate and coliforms counts.

RANGE OF DEGREES CENTIGRADE	NO. OF SAMPLES	MEAN PLATE COUNT X 10^6	MEAN COLIFORMS X 10^3
12 - 13 C	1	1	1.5
14 - 15	0	0	0
16 - 17	7	33	59
18 - 19	16	109	82
20 - 21	20	83	93
22 - 23	25	49	176
24 - 25	23	41	58
26 - 27	9	38	13
28 - 29	2	61	300
30 and above	1	145	-

N.B. Decimals of a centigrade degree were rounded to the nearest whole number.

Table vii. Arbitrary quality score for the meats and the butcheries from which the meat samples were collected.

BUTCHERY	MEAN SCORE	
	MEAT	BUTCHERY
High class	2.80 out of 3.00	4.00 out of 5.00
Kabete	1.70 " " "	2.00 "
Kibera	1.70 "	2.00 "
Ndumbuini	1.50 "	2.00 "
Kangemi	1.25 "	2.00 "
Dagoretti Corner	1.25 "	2.25 "
Mathare	1.05 "	1.20 "
Kawangware	1.00 "	2.35 "
L.Kabete/Kikuyu	1.30 "	1.80 "

Table viii. Summary of the mean total plate and Coliform Counts of the sampled market milk. The values are the means for each type of whole milk or milk product.

SAMPLE TYPE	NO. OF SAMPLES	MEAN TPC	MEAN COLIFORMS
Raw milk	8	1,600.000	32,000
Whole pasteurised milk	5	500	200
Unprocessed cream	3	1,100,000	38,000
Fermented milk	3	7	2
Yogurt	4	3	2
Cheese	2	102,500	1,100
Icecream	16	290,000	2,100

before it was sold. The table shows that the mean for the raw milk and unprocessed cream are close and high while that of fermented milk and yogurt are very low.

However the number of samples taken for most of the market milk types was too small (Appendix IV) to represent the overall picture. Fermented milk and yogurt showed negligible TPC and coliform numbers. Raw milk, unprocessed cream and cheese had TPC over one million. High coliform counts were encountered in raw milk and unprocessed cream.

Concerning the milk from the Sigona collecting station, 73% of the samples had a TPC which was falling within the range 500 thousand to one million (Table ix). Only 11% showed a TPC over 5 million. The major part of the samples (62%) showed a coliform count less than one thousand per ml. Ninety-three per cent (93%) of all the samples had a coliform count less than 50 thousand and only one sample showed a count over one million. To investigate the possibility that certain farms produced constantly bad milk, the colony counts from samples of milk which came from the same farm were averaged, the averages so obtained were grouped into ranges (Table x).

The TPC was evenly distributed between the farms. There were six (6) farms (15%) with less than 100 thousand TPC/ml. There were seven (7) farms with milk containing over 5 million

Table ix. The frequency of the raw milk samples collected at Sigona Dairy. The frequencies are grouped according to the total plate count and coliform counts which fall within the shown ranges of counts.

COLIFORMS			TOTAL PLATE COUNT (TPC)		
RANGE X 10^3	FREQUENCY	PERCENTAGE OF TOTAL	RANGE X 10^3	FREQUENCY	PERCENTAGE OF TOTAL
<1	68	62	<500	67	64
1-50	34	31	500-1000	10	9
50-500	7	6	1000-1500	5	5
500-1000	0	0	1500-2000	1	1
>1000	1	1	2000-2500	1	1
			2500-3000	5	5
			3000-5000	4	4
			$>$ 5000	12	11

microorganisms per ml.

Of the individual Sigona dairy raw milk samples 40 of them had a T P C less than 100 thousand and 12 milk samples had a T P C over 5 million per ml, while 86 samples had less than 2 million TPC per ml. Fifteen farms (38%) (table x) had mean coliform counts less than one thousand and 68 (62%) of the individual samples were in this range. Eighty-four (74%) of the individual Sigona raw milk samples contained less than 4 thousand coliforms per ml. There were 23 farms whose mean coliform count per ml was falling within this range of 4 thousand coliforms per ml. Table xi shows the possible outcome of the Sigona raw milk when graded according to the proposed raw milk standards for Kenya. Based on both the coliform and T P C, a lot of the milk was of good or very good quality (79% and 93% respectively).

In the milk from the Sigona dairy no antibodies against brucellosis were detected using the milk ring test. Salmonella were tested for in the first 39 samples only but none was isolated. E. coli were frequently encountered from the milk during random testing.

3. Mud/Sand/Fish

Of the 137 samples tested, only one yielded botulinal toxin. This mud sample had been picked at the Kilifi Creek on the bank proximal from Mombasa. Typing was attempted using antisera from the Pasteur Institute (kindly provided by the Norwegian Food Research Institute, Norway). During the initial trial at typing, all the protected mice died. On repeat they again all died. After this a titration was carried out so as to assess

Table x. The frequency of farms as well as milk samples which exhibited mean total plate and Coliform Counts within the indicated ranges.

MEAN COLIFORMS RANGE X 10^3	FREQUENCY		MEAN TPC RANGE X 10^3	FREQUENCY	
	NO. OF FARMS	NO. OF INDIVIDUAL SAMPLES		NO. OF FARMS	NO. OF INDIVIDUAL SAMPLES
< 1	15	68	< 100	6	40
1 - 2	5	6	100 - 200	4	13
2 - 4	3	10	200 - 400	6	12
4 - 6	1	1	400 - 600	0	6
6 - 6	2	1	600 - 800	2	5
8 - 10	0	4	800 - 1,000	1	2
10 - 12	1	0	1,000 - 2,000	7	8
12 - 14	2	1	2,000 - 3,000	2	6
14 - 16	1	1	3,000 - 4,000	3	3
> 16	6	11	4,000 - 5,000	1	1
> 100	3	7	>5,000	7	12

Table xi. The percentages of the raw milk samples collected at Sigona Dairy that fall within ranges of total plate and coliform counts which have been proposed as standards for milk by the Kenya Bureau of Standards.

BUREAU'S TPC. RANGE X 10^6	OBSERVED TPC IN PERCENTAGE	COMMENT BY BUREAU
0-1	73%	Very good quality
1-2	6%	Good quality
2-5	10%	Bad quality
> 5	11%	Very Bad quality
BUREAU'S COLIFORMS. RANGE X 10^3	OBSERVED COLIFORMS IN PERCENTAGE.	COMMENT BY BUREAU
0-1	62%	Very good quality
1-50	31%	Good quality
50-500	6%	Bad quality
> 500	1%	Very bad quality

the preferable toxin/antitoxin protective range. By now, the previously toxic culture was no longer toxic and it was impossible to type or titrate the toxin. A bacteriological loop with a droplet of the enriched toxic broth had been streaked on a horse blood agar (3%) plate and incubated anaerobically. A suspect colony was inoculated into a cooked meat medium tube and incubated over-night, anaerobically. This culture broth was found not to be toxic to mice. Cl. perfringens was frequently tentatively diagnosed in muds and sand.

DISCUSSION

In Kenya the subsistence diet of a society was determined by the climatic and physical geographical barriers. With regard to meat as food a clan used to slaughter an animal and consume it within a few hours after slaughter while it was still fresh. This minimised contamination and reduced the period that contaminants stayed multiplying in the carcass. However, the advent of the village groups introduced a system whereby a central slaughter slab was established and meat from this source was sold to a larger population. Due to better communication, meat from these small slaughter slabs finds its way to urban centres, and in the process contamination and bacterial proliferation occurs.

A major part of the locally consumed meat is handled without any cooling at all. Under ideal conditions bacteria can multiply by binary fission every 20 minutes (Hobbs, 1968). At this rate, a single bacterial cell could increase to 2,097,152 within seven hours. A chemical analysis of meat (Lawrie, 1966) shows that water (% wet weight) makes 75%, proteins 18.0%, soluble non protein substances including nitrogen, carbohydrate, inorganic substance and traces of glycolytic intermediates, trace metals, and vitamins make up 3.5% and fat 3.0%. With all these nutrients for bacteria, yeasts and molds to grow, meats together with milk are considered the most perishable of all important foods.

The spoilage or destruction of meat which has been held at room temperature is predominantly caused by flora (especially

aerobic spore formers) which are different from those causing spoilage at low temperatures (Jay, 1970). Most meat at slaughter is considered to be sterile. However it soon gets contaminated after slaughter. The main sources of contamination are the hides and skins, soil, contents of the alimentary tract, airborne and aqueous sources. This initial bacterial contamination will influence the bacterial load on the finished stored carcass. An on-the-spot observation of the care exercised in handling meat in the small butcheries revealed that the meat was poorly handled and consequently exposed to contaminants. The already contaminated meat was held at room temperatures which varied from 12 to 30C with most samples showing 18-27C which encourages bacterial multiplication. These facts explain the high bacterial loads on the sampled meats.

The safety and keeping quality of foods are related to their microbial content. This realisation has lead to establishment of microbiological food criteria as a valuable means of improving the hygienic quality and safety of foods. Elliott and Michener (1961) have tabulated various suggested microbial limits in chilled and frozen foods. For raw meats, the value given by 13 authors or groups varies from 10,000 to 10 million (Jay, 1970). Thatcher (1974) gave the maximum for chilled raw meat at 10 million per gram. These values are for chilled and frozen meat and cannot compare with those for Nairobi considering the differences in handling of the meats. Standards for raw non-cooled meat were no where

available. Hobbs (1959) investigated unfrozen meat cuts and obtained a TPC 2 million at 37C.

It is observed from table ii that only 42% of all the samples were within the maximum of 10 million TPC per gram of meat, the maximum for Thatcher as mentioned above. But since the 10 million/gm is for chilled meat, a higher value would be expected for the non chilled meat samples that were processed, in which case those with 10 million maximum count could be regarded as fair. TPC over 10 million but less than 100 million was obtained from 42 (40%) samples so that a maximum allowance at 100 million would include 84 samples. In setting the standards, an economic factor has to be considered. Taking 2.5 million TPC as the maximum bacterial load allowed per gram, one observes that 42% of the sausages, 27% of the ground beef and 33% of the whole cuts were within the limit but the major part of the samples would have been condemned. If the limit was set at 100 million, only 14%, 18% and 20% of the sausages, ground beef and whole cuts respectively would be condemned. At first this rate of condemnation would appear exaggerated, but tables vi and vii reveal the poor handling that the meat is subjected to. To recommend a microbiological limit of 100 million might appear as a submission to defeat and acceptance of poor sanitation in the meat industry. To lower the limit to for example 10 million/gram then more meat (52%) will be condemned than consumed (48%). Considering Hobbs (1968) demonstration of bacterial multiplication in ideal conditions, and remembering the way meat is handled in the zone of this research, 100 million microbes

per gram would be a realistic recommendation. At a constant rate of binary fission, it would take less than nine hours for one bacterial cell to reach 100 million. Where cooling of meat was non-existent (and this is common), a total count is not very meaningful.

Table vi shows the distribution of the temperatures of the samples during sampling, It is of interest to note that only one sample had a thermometer reading less than 16C. This is because most meat is handled at room temperature. About 2/3 of the samples showed a temperature reading between 20 and 25C. There was a general though unproportional increase in the TPC and coliforms as the temperature recorded increased. Such inconsistency could be explained by the fact that the temperature recorded might not have been prevailing for a long time and the fact that the age of the samples since slaughter varied. This means that though a carcass might be having a specific temperature, if it is old, any bacteria on it will have had enough time to multiply to high values whereas a recently slaughtered carcass though showing high temperatures will need some time for such a bacterial load to be attained. It is therefore not possible to compare different samples from different carcasses unless one knows the interval between slaughter and sampling. Although such information was recorded, it was rather unreliable since most butchers reported that the carcass was purchased "today" or "yesterday".

Silliker (1963) has stated that total counts most effectively evaluate the sanitary quality of foods which do not support microbial growth. In such cases high counts could be used to

indicate the type of sanitary control exercised in their production, transportation and storage. However not all the total count bacteria will indicate sanitation since some are pathogens unless the food is stored in an environment (e.g. low temperature) which will not allow the growth of the pathogens. While setting microbiological standards for foods, the basic interest is the product safety and shelf - life. Thus, it is common to see total counts maxima indicated in control programs standards (Elliott and Michener, 1961). Silliker (1963) in conclusion states that total counts are of limited value in the evaluation of food quality.

As category of meats, comminuted meats unvariably have higher numbers of microorganisms than non-comminuted meats (Law et al., 1971; Brewer, 1925; Duitschaever et al., 1973). Elliott and Michener (1961), Weinzirl and Newton (1914 a,b) have suggested standards for raw hamburger meat ranging from 2.25 to 10 million total viable aerobes per gram. If 10 million was the standard, 54% of the samples in this research would be rejected. This value is very close to the 64% rejectable figure of Duitschaever et al. (1973). The higher counts observed in mince meat can be explained on the grounds that generally commercial comminuted meat is made up of trimmings from various cuts and thus represents pieces that have had excessive handling. In the process of grinding, surface bacteria are transferred into the admixtures whose surface area has been

increased by reducing the particle size, and thereby favours the growth of aerobic bacteria. Also poor hygiene in some plants such that grinders, knives and storage facilities are rarely cleaned (it was common to see a butcher cleaning the grinders free hand), in case one piece is heavily contaminated, it will contaminate others while in the grinder. Other than the above reasons for high counts in comminuted meats, sausages do get contaminated from microorganisms in the seasoning and formulating ingredients that are usually added (Richmond and Fields, 1966; Jay, 1970). In any case microbiological standardisation of sausages poses a problem due to the diversity in manufacturing procedures and compositions, e.g. heat treatment, water activity and final pH of the product. For these reasons Thatcher (1974) did not propose any microbiological criteria for sausages.

Another possible explanation for this unexpected similarity in the TPC of different meat types might be based on the different levels of cleanliness of the butcheries providing the meats. Although meat kiosks in Mathare Valley scored poorly on the subjective scores (table vii),this zone provided 18 (17%) of the samples which were all whole meat cuts. The ground beef samples were collected from butcheries which were of high cleanliness. Consequently, the whole meat cuts had been poorly handled and hence had a big mean TPC count while the ground meat was more fairly handled.

Finally another explanation is that this high TPC in whole cuts and relatively lower in the other types is due to the small number of samples used in calculating the means for sausages and the ground beef as compared with the larger sample total for whole cuts.

The coliform picture is different in that most of the samples had relatively a lower count. Thus if a figure of 150×10^3 per gram was to be taken as a maximum coliform count, 77% of the samples would be passed. And even if the maximum was to be set at 50,000 coliforms per gram of meat, 63% of the total samples would have been passed. Although this value is still very high when compared with suggested coliform ranges in temperate lands, (Appendix VI) the same reasoning used in connection with TPC as related to tropical climate can be repeated here. It would be possible to compare these values to those of temperate lands only when the meat is constantly handled in the cold until it is sampled or it reaches the kitchen. Thatcher (1974) has not suggested standards based on coliform counts in standardising of raw meats.

The detections of E.coli in the meats cannot be taken with great seriousness since they occurred so frequently in the meats. This can only be considered in the total plate and coliform count to elucidate the fact that so long as the meat in general is roughly handled, it is going to be difficult to know what maximum limits for total, coliform or specific pathogens counts will be more practicable.

Although the subjective score table cannot be heavily emphasised, there is an obvious contrast in meat scores between the major group of butcheries scoring between 1.00 and 1.70 and the high class butcheries scoring 2.80 out of 3.00. The same is observed in connection with the scores for the butcheries' cleanliness. This demonstrates an obvious experience that the

bulk of the population is provided with meat of low bacteriological quality. These scores combined with the high sampling and storage temperatures help to explain the resultant high microbial counts in the meats. These low hygiene scores very closely reflect the butchers' attitude which to a great extent is due to ignorance and slackness. They need to be supervised and guided more closely.

Elliott and Michener (1961) presented the salient precautionary arguments from different individuals relevant to the adoption of microbiological standards.

Different standards should be set for different groups of foods since the methods of preparation of the foods tend to differ. Priority in standardisation should be directed to the hazardous types of foods after sufficient data has established the expected bacterial levels while considering variations in composition, processing procedures, and time of frozen storage. It is important that when standards are chosen, there should be a definite relation between the standards and the hazard against which it is meant to protect the public. Methods of sampling and analysis should be carefully studied for reliability and reproducibility among different laboratories are requested to aid in processing samples, the different results whould be comparable. Tolerance should be included in the standard to account for inacura-

cies of sampling and analysis. At first, the standard should be applied on a tentative basis to allow for voluntary compliance before becoming a strictly enforced regulation. This will give people a chance to prepare to change. Microbiological standards will be difficult to enforce, and if they are unwisely chosen, they will not stand in courts of law.

These recommendations should be heeded if the Kenya Bureau of Standards considers setting any microbiological standards in meats.

Empey and Scott (1939) suggested ways in which meat contamination could be reduced. Such methods included cold water spraying in a race to clean soil from the hides. This is best if done just before slaughter. Whereas this is feasible at the export slaughter houses, no such facilities are currently available in the local slaughter slabs. May be the cost of a race is prohibitive to the latter group of slaughter slabs. Empey and Scott (1939) found that by using 100-120 litres of water per cow, the effect was equivalent to reducing the skin flora by half the initial number. Water-borne contamination can be eliminated by heating the possibly contaminated water to 60C for one minute. This heat treatment will clear off the low temperature microbes. The butchers' and their equipments' hygiene is important. From the killing floor, the carcasses

should be handled in the cold till it reaches the kitchen. As stated earlier, these conditions can only be adhered to in the export slaughter houses,but not in the small slabs which are much more difficult to controll. These small slabs will remain a potential hazard to the public. A better move would be to close them down and replace them with one or two larger slaughter houses which will be easier to controll.As Thatcher (1963) has noted, the controll of food poisoning and infection throughout the world leaves much to be desired.

Most meat is usually cooked enough to destroy salmonellae. This does not remove the possibility that the organisms, when in raw meat, may spread to cooked products by the hands of food handlers, or by cutting utensils and other equipments and surfaces.Insufficient cooking of raw fresh material, or failure to increase the cooking time of frozen or partially thawed meat, may permit the survival of salmonellae. It would be desirable to have all raw meats, poultry, and edible organs free from salmonellae, as the presence of salmonellae in these raw foods is a disease risk. Out of 104 meat and meat product samples, no salmonellae were isolated using standard enrichment, plating and biochemical methods. There has been great variations in the reported recoveries of salmonellae in meat and meat products. Weissman and Carpenter (1969) reported Salmonellae

in 56% of pork carcasses, and 74% of beef carcasses; Felsenfield et al. (1950) reported attempts to find the distribution of Salmonella in different parts of a carcass: from 7% of the loin area, 7% from the rib area, 5.6% from the shoulder and 8% from tenderloin. Beef carcasses are less contaminated than pork carcasses. Felsenfield et al. (1950) reported finding only 0.2% positive incidence in 512 beef samples. Angelotti et al., (1961) could not isolate Salmonella in any cured meat products.

Duitschaever et al., (1973) could not isolate salmonellae out of 213 samples of raw refrigerated ground beef whose TPC was in the range 10-95 million. This failure to isolate Salmonella in the sampled carcasses does not indicate that this bacterium is not found in the carcasses. As the abattoirs varied very much and each abattoir's carcasses were sampled only on a few occasions, it is possible that the few samples taken missed the contaminated carcasses. Meat is among the major vehicles for salmonella infection and as such poses a threat when not well handled. More risk is with meat which has been contaminated after cooking since such food will not necessary be boiled again so that salmonellae may die. Salmonellae being poor competitors will flourish better on boiled meat than on the rather heavily contaminated raw meat. If such bacterial cells were of low numbers and considering the high bacterial load recorded, it is possible that such few cells were present but never detected.

The Kenya Bureau of Standards has suggested 100 thousand bacterial TPC per gm as the maximum allowed level of contamination

for processed liquid milk. Appendix IV shows that this maximum was adhered to, except for one packet which had not been kept in a cooled space. This packet contained 140,000 colonies per ml. Similarly, the coliform count maximum is 10 per ml with no E. coli recovered. However four out of five pasteurised whole milk samples showed counts above the suggested 10 coliforms. The other sample had no viable coliforms. The raw milk in eating houses had a mean TPC 1.6 million per ml which is good quality milk according to the Bureau's suggested figures.

Based on the suggested TPC grades of the Kenya Bureau of Standards, 73% of the samples of the Sigona milk would be graded as very good quality and only 11% was very bad quality. If the maximum had been set at 100,000 TPC/ml, 40 samples (37%) would have been in the very good quality bracket. This would have meant that 43% would be in the 100 thousand to 2 million range. Raising bad quality range from a maximum of 5 million to 7 million would have reduced the percentage of very bad milk from 11 to 5%.

From a personal assessment of the collecting station's hygiene and a visit to a few of the farms during milking time, it would appear that not 73% of the farms should produce "very good" milk. It must be a smaller fraction. This can be explained by accepting that my recoveries were far too low or that the Bureau is very submissive and hence its values need a reappraisal.

Comparing with the American milk standards (Jay, 1970) the Kenya Bureau's limit is far too high. This greater allowance, might have considered the climatic effect on bacterial

multiplication as discussed in the section on meats.

Salmonellosis has been traced to chocolate (D'Aoust et al., 1975)even though this is not a common vehicle. There was no salmonellae isolated from 47 samples of raw milk and 32 samples of processed milk and milk products.

The use of a milk ring test (MRT) to screen for brucellosis in cattle in Kenya has previously been reported (Oomen and Wegener,1974; Waghela, 1977). The present screening was based on a small scale farming co-operative society. Though no brucellosis antibodies were detected, the limitation in the size of the sampled area and number of herds (39) with an unknown number of cattle minimises the importance of the results in terms of the epidemiology of the disease. The extent of positive (MRT) reactors is seen in Philpott (1971) where he observed 50% positive creamery milk samples at Mariakani and 20% at Nairobi creamery. Although these figures are high, the high rate of false positives with MRT offsets the significance of such values. Another factor to consider is the fact that a history of past vaccination is important, and if such a history is not available or reliable, it becomes difficult to interpret the results.

For the first time in Kenya, detection of possibly Cl. botulinum in soil is reported. The detection of botulinal toxin from a mud sample collected at the Kilifi creek at the Kenya coast is very significant. Lack of reports on the disease might be taken to imply that the disease is non-existent here. For the past over 32 years, there has not been recorded a fatal human case of botulism in Kenya. However, 12 years ago a patient at Machakos (about 64 Km to the east of Nairobi)

was suspected of being suffering from botulism. This patient recovered fully without treatment (Dr. Mann, 1974).

Many reasons might explain this absence of the condition. The association of botulism with food and eating habits of a community has been recognised for many years. Meyer (1956) emphasised that without exception the disease is as a result of carelessness in the preparation and preservation of vegetable and animal foods. This is together with the local customs encouraging eating of such food uncooked in the form of salads, watery conserves, poorly cured or inadequately smoked pork and salted fish products. Generally it would appear like the type of food in which the causative toxin has been liberated affects the magnitude of a botulism attack more than the effect of the toxin type that was involved (Meyer, 1956). As shown in the review of literature home processed food has been incriminated in most of the cases, a large part of the cases involving contaminated vegetables. An association was also observed between the food and the botulinal toxin type that was incriminated. Gangarosa et al. (1971) showed that botulinum toxin type A involved vegetables in 93 (65%) of type A outbreaks, yet 16 (94%) of type E outbreaks involved fish and fish products. These reports support the view that the type of food is important in the occurrence of outbreaks of this food poisoning. In Kenya, rural communities by virtue of local customs, geographical and climatic factors, are restricted to certain types of crops or food staples. In the past, foods and eating habit of these communities were not conducive to botulinal intoxication.

The foods and eating customs of two tribes will be described briefly. The Agikuyu (comprising a major section of the population) traditionally were hunters gradually changing to livestock rearing and crop farming. Their food was therefore meat which was either roasted or boiled and later on vegetables, cereals and tubers. Any left over food was stored in a barn ("Ikumbi") and usually consumed within three days. The Masai are a nomadic tribe and used their cattle as source of food, namely milk, blood and meat, raw or heat treated. In both tribes, except for milk, no other fermentation or home canning takes place. Home processing contributed 72% of all outbreaks in the USA betwen 1899 and 1969. And in Japan mostly "Izushi" a pickled relish made of raw fish, rice and diced vegetables was incriminated in 45 out of 49 episodes (Nakamura, 1963).

Clearly the role exercised by food in botulism outbreaks is unquestionable. The fact that the Kenyan tribes' way of preparing their food differs so much from the preparation of the commonly incriminated foods is possibly one of the major reasons for the low occurrence or abscence of the disease. If that be the case, and since Meyer (1956) related the incidence of human botulism to the percentage of soils producing toxic cultures, then occurrence of the spores in the soil and other places could pose a threat since these spores could easily contaminate the foods. This supposition becomes more real when it is observed that more and more people will gradually rely on canned foods. Even though commercial canning has had a good record concerning outbreaks of botulism (Lynt et al., 1975), and although the

essential parameters for processing canned foods have been established, there still occurs some reports of botulism from commercially canned foods where this is commonly done(e.g Lynt et al., 1975). Should home canning spread among people who were not used to it, botulism is likely to be a recognised problem (Johannsen, 1965).

Of interest is the possibility of the existence of non-toxic variants in the environment. However since a major characteristic of Cl. botulinum is the formation of toxin, it is difficult to ascertain that a nontoxic culture is actually Cl. botulinum (Skulberg and Hausken, 1965). Such variants were initially described by Dolman (1957c)and Hobbs et al. (1965). A chance exists that the toxin level is so low that it cannot be easily detected. This difficulty was recorded by Dubovsky and Meyer (1922b) and Gimenez and Ciccarelli (1970).

It is to be observed that the presence of certain substances and organisms do inhibit the growth of Cl. botulinum. The absence of Cl. botulinum in soil or sediment samples cannot therefore be taken as proved unless the absence of inhibiting organisms has been demonstrated. In the limited time of this research it was not possible to accomplish such a prove that these inhibitors were absent. Cl. perfringens were detected in mud but no controlled experiments with known Cl. botulinum were carried out to prove that the Cl. perfringens isolated was or was not inhibitory to Cl. botulinum.

An outbreak of botulism in Tchad due to type D poses interesting points in that type D is usually taken to be non-

toxic to humans, this outbreak being the first in record (Demarchi *et al.*,1958). To the present time this is also the first record of confirmed outbreak of human botulism in Africa. It would have been helpful to know from where the ham was prepared. If it had been prepared in Tchad then contamination might have occurred in Tchad. This is not strange since type D spores have been reported in S. Africa before. If the ham had been prepared in France it might have been contaminated in France or Tchad. If contamination had occurred in Tchad showing that the spores are present then it could mean that other people catch the disease and are never reported, it is generally accepted that diagnosis of anerobic organisms tends to be more difficult than for aerobic ones (McClung 1967). This means that it might not be confirmed in most hospital laboratories. There is a possibility that Africans might be resistant to botulism. Quantitative *per os* tests in primates have shown low toxicity in case of types C and D (Meyer, 1956). Although some carrions examined in South Africa were found to be very toxic, Africans eating such carrions did not suffer from botulism. It may be that the indigenous people are resistant to the prevalent type D toxin.

The only suspected case of botulism in Kenya in the last 32 years occurred at Machakos 12 years ago. The patient recovered fully and it was never confirmed. To my knowledge,no spores have been isolated or toxin deteced previously in East Africa. This is the first report of detection of *Cl. botulinum*

toxin in East Africa. However the prevalence of the spores in our soils/muds is low. In this case, unless the population changes its dietary customs drastically, the fear of botulism is still not immediate but considering the tremendous increase in international food trade, it will be necessary to appreciate the hazard of botulism. May be more attention will be given to wound botulism, Hall, (1945), Davis et al. (1951) and Merson and Dowell (1973).

CONCLUSIONS:

This investigation utilised meat cuts which were sampled from meat shops most of which had no refrigeration facilities. As a result, a relatively high total bacteria and coliform counts were obtained from the samples. In spite of containing such high bacterial loads, most of the samples were still marketable.

An attempt to grade raw milk from a local milk collecting station using the scales that have been proposed by the Kenya Bureau of Standards revealed that 79% of the milk was good or very good based on total plate count.

Of the pathogens tested for in the meat, milk and their products, no *Salmonella* or *Cl. botulinum* were recovered. *Escherichia coli* and *Cl. perfringens* were isolated from meat and *E. coli* was isolated from milk too. The recovered pathogens are not a major danger to the consumers as most of them eat their meat after it has been boiled or roasted. Milk too is usually boiled before it is drunk.

Although the number of mud and sand samples that were screened in the survey was relatively small to draw a generalised conclusion, it would appear like the prevalence of *Cl. botulinum* is low since only one mud sample out of 137 samples yielded what was possibly *Cl. botulinum* toxin.

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KEY TO THE ABBREVIATIONS AND INITIALS USED IN THE APPENDIXES:

Bana ------ Banana Hill, Limuru

Dag. --------Dagoretti

D.Corner ----Dagoretti Corner

Gikamb. ---- Gikambura

Gita. ------ Gitaru

Hurg. ----- Hurlingham

Kab. ------ Kabete

Kang. ------ Kangemi

Kar. ---- Kariokor

Kawang. --- Kawangware

KMC -------- Kenya Meat Commission

Lint. ----- Lintano

L.Kab. ----- Lower Kabete

Mat. ------ Mathare Valley zone

Mkt. ------ Market

Nyonj. ----- Nyonjoro

O/R. ------ Ongata Rongai

Thig. ----- Thigio

Unk . ------ Unknown

Upl. ------ Uplands Bacon Factory

Wang. ------ Wangige Market

Wai. ------- Waithaka

West. Sta. -- Westlands Stalls

Wsb. ------ Westlands Super butcheries.

p.s.	--------	pork sausage
b.c.	--------	beef cut
b.s.	--------	beef sausage
g.b.	--------	ground beef
-	--------	no count taken
- ve	--------	negative, no isolate
+ ve	--------	positive colonies isolated
X	--------	unknown abattoir
D	--------	test mouse died
L	--------	test mouse was sacrificed after four days pastinoculation

APPENDIXES

Appendix I. Zoning of butcheries.

A) Urban Centres: Dagoretti Corner, Kangemi, Kawangware Kabete, Kibera, Ndumbuini, and Riruta.

B) Zones with High Class butcheries: City Market, Hurlingham, Karen Westland, Woodley.

C) Open City Markets: Kariokor, Westlands, (Eastleigh and River Road)

D) Mathare Valley: Mathare River Valley near the Mental Hospital in Nairobi

E) Lower Kabete: Lower Kabete, Kamutiini, and Wangige

F) Kikuyu-Kinoo: Kikuyu, Gitaru and Kinoo centres.

Appendix II

Showing the origin (abattoirs) of the sample(s) and where the sample(s) was taken from

	ABATTOIR															
	DAG	KMC	WAI	OR	UPL	NYONJ	GITA	LINT	BANA	L.KAB	THIG	NGONG	GIKAMB.	WANG	UNKN	TOTAL
Math.	15	1	1	0	0	0	0	0	0	0	0	0	0	0	1	18
Kar.	6	5	1	2	0	0	0	0	0	0	0	0	0	0	0	14
Wsb.	0	6	0	0	3	0	0	0	1	1	0	0	0	0	2	13
Dag.	0	0	3	3	0	1	0	0	0	0	0	1	0	0	1	9
West.(Sta.)	1	2	2	0	0	0	0	0	0	0	1	0	0	0	0	6
L. Kab.	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Kang.	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	4
City Mkt.	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Kabete	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	3
Karen	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	3
Hurg.	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	3
Kawang.	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	3

	DAG	KMC	WAI	OR	UPL	NYONJ	GITA	LINT	BANA	L.KAB	THIG	NGONG	GIKAMB.	WANG	UNKN	TOTAL
Kibera	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	3
Kenyatta	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	3
Woodley	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	4
Ndumbuini	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Kikuyu	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	2
Gitaru	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
Eastleigh	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Kamutiini	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Kinoo	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Riruta	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
River Road	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Wangige	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

Appendix III — The comprehensive data on each of the 104 meat samples

SAMPLE NO.	SAMPLE TYPE	SUBJECTIVE SCORE OUT OF 3 MEAT	SUBJECTIVE SCORE OUT OF 5 BUTCHERY	SAMPLING AREA	ABATTOIR	SAMPLE TEMP.C	TPC X 10^3	COLIFORMS	BOTULINAL TOXIN
1	b.c	2	3	West. Stall	KMC	24	220	15	D
2.	b.c	2	3	"	DAG.	26	285	2630	D
3.	b.c	2	3	"	WAI	24.5	67,500	19,500	D
4.	pork	3	5	Wsb	BANA	21	520	430	D
5.	g.b	3	5	"	KMC	17	73,500	1,675	D
6.	g.b.	2	4	City Mkt.	"	19.5	37,500	77,000	D
7.	skin(cow)	1	1	Kar.Mkt.	O/R	27	24,000	300,000	L
8.	b.c	2	3	"	DAG	27	875	34,000	D
9.	b.c	1	2	Kang.	DAG	27	70,000	68,000	L
10.	b.c	1	2	"	WAI	27	820	3000	L
11.	b.c	1	3	Kawang.	O/R	20	1,960	70,500	D
12.	b.c	1	2	Riruta	WAI	24.5	3,075	2005	D

NO.	TYPE	3 MEAT	5 BUTCH-ERY	SAMPLING AREA
13	coat	2	3	D.Corner
14.	b.c	3	5	Woodley
15.	g.b	2	5	"
16.	p.s	2	5	"
17.	b.c	1	1	Mathare
18.	b.c	1	1	"
19.	b.c	1	2	"
20.	b.c	1	1	"
21.	b.c	1	1	Math
22.	b.c	1	1	"
23.	b.c	1	2	Kar.
24.	b.c	2	1	"
25.	g.b	2	4	Wsb
26.	p.s	2	4	Wsb

ABATTOIR	SAMPLE TEMP.C	TPC X 10^3	COLI-FORMS	BOTULINAL TOXIN
O/R	25	420	3,550	D
X	17	36,000	42,000	D
X	18.5	1,700	104,000	D
UPL	16	25,000	1,885	L
KMC	25	39,500	5,000	L
DAG.	23	79,000	2,170	L
"	22	1,120	1,110	L
"	24	uncountable	105,000	L
"	23	125,500	2235	L
"	23		300,000	L
"	21	1570	1500	L
"	23	122,000	300,000	D
X	24	120,000	1285	D
UPL	19	69,500	55000	

NO.	TYPE	SUBJECTIVE SCORE OUT OF 3 MEAT	OUT OF 5 BUTCH-ERY	SAMPLING AREA	ABATTOIR	SAMPLE TEMP.C	TPC X 10^3	COLI-	BOTULINAL TOXIN
27.	b.c	1	1	Mat.	WAI	27.5	55,500	300,000	D
28	b.c	1	2	Mat.	DAG.	26.5	42,000	99,000	L
29.	b.c	1	1	Mat.	DAG.	22.5	23,000	22,500	D
30.	b.c	1	1	Mat.	"	25	39,500	1,375	L
31.	g.b	2	4	Wsb.	X	26.5	88,000	367,500	D
32.	b.c	1	2	L.Kab.	KMC	20.5	27,000	16,500	
33.	b.c	0	1	L.Kab.	DAG.	21	82,500	300,000	
34.	b.c	1	2	Ndumbuini	'	24	335	None	
35.	b.c	2	2	"	"	22	795	355	
36.	b.c	1	2	Kab.	O/R	23	2,275	1,000	
37.	g.b	2	4	Wsb	KMC	22.5	3,730	44,000	D
38.	Pork	3	4	Wsb	L.Kab.	20.5	39,000	300,000	D
39.	b.c	3	5	Wsb.	KMC	23	8,500	61,500	D
40.	g.b	2	5	Wsb	KMC	22	1,665	18,000	D
41.	b.c	1	3	West.Sta.	WAI	29.5	48,500	300,000	
42.	b.c	1	3	"	KMC	29	74,000	300,000	D

NO.	TYPE	OUT OF 3 MEAT	OUT OF 5 BUTCH-ERY	SAMPLING AREA
43.	b.c	1	1	Kang.
44.	b.c	2	3	"
45.	g.b	2	4	City Mkt
46.	b.c	2	4	"
47.	b.c	1	2	Kar.
48.	b.c	1	2	Kar.
49.	b.c	1	2	Kar.
50.	Skin(sheep)	0	1	Kar.
51.	p.s	2	4	Wsb.
52.	b.c	1	4	"
53.	pork	3	5	Karen
54.	p.s	2	5	"
55.	b.s	2	5	"
56.	b.c	1	2	D.Corner

ABBATTOIR	SAMPLE TEMP.C	TPC X 10^3	COLI-FORMS	BOTULINAL TOXIN
Nyonj	22	72,000	9,500	L
x	24	34,000	33,560	L
KMC	20	3,235	136,500	D
KMC	18.5	262,000	156,000	L
DAG	25.5	uncountable	-	D
KMC	30*	145,000	300,000	D
KMC	26	20,500	2,880	D
O/R	25	19,500	10,000	D
UPL	25.5	430	1,115	D
KMC	25.5	19,500	300,000	L
Lint	17	2,120	17,500	D
"	16	640	13,500	D
"	16.5	112,000	300,000	D
WAI	18		300,000	D

SAMPLE NO.	SAMPLE TYPE	OUT OF 3 MEAT	OUT OF 5 BUTCH-ERY	SAMPLING AREA	ABATTOIR
57	b.c	1	2	D.Corner	NYONJ
58.	b.c	1	2	"	WAI
59.	Goat	1	2	"	O/R
60.	Goat	0	2	"	X
61.	b.c	0	2	"	O/R
62.	b.c	1	2	Kab.	X
63.	b.c	1	1	Mat.	DAG
64.	B.C	L	L	"	"
65.	b.c	2	2	"	"
66.	b.c	1	1	"	"
67.	b.c	1	1	"	"
68.	b.c	1	1	"	X
69.	b.c	2	3	Kar.	DAG
70.	b.c	2	3	"	WAI
71.	g.b	3	4	Wsb	KMC
72.	p.s	2	4	"	UPL

SAMPLE TEMP.C	TPC X 10^3	COLI-FORMS	BOTULINAL TOXIN
20	31,000	7,500	D
21	67,500	295,000	D
20.5		300,000	D
20.5		300,000	D
19	18,500	300,000	
20.5	1,140	1,345	D
23	1,390	1,515	L
21	31,000	58,000	L
21	865	7,500	L
22	124,000	148,000	L
24	40,500	107,000	L
23.5	30,500		D
22.5		300,000	L
21	1,870	26,500	L
22	925	1,170	L
22	50,000	6,500	L

SAMPLE NO.	SAMPLE TYPE	OUT OF 3 MEAT	OUT OF 5 BUTCH-ERY	SAMPLING AREA	ABATTOIR
73.	b.c	2	2	Kibera	O/R
74.	b.c	2	2	"	DAG
75.	b.c	1	2	"	KMC
76.	g.b	3	5	Hurg.	"
77.	pork	3	5	"	UPL
78.	b.c	2	2	Kenyatta	NYONJ
79.	b.c	1	2	"	"
80.	b.c	2	3	West Sta.	THIGIO
81.	b.c	2	3	Eastleigh	DAG
82.	b.c	1	2	Kar.	"
83.	b.c	1	2	"	KMC
84.	b.c	1	1	"	"
85	b.c	1	2	"	"
86.	b.c	1	3	Eastleigh	X
87.	b.c	1	1	Mat:	DAG
88.	b.c	1	1	"	"

SAMPLE TEMP.C	TPC X_{10}^3	COLI-FORMS	BOTULINAL TOXIN
19	645	3,645	D
21	2,165	3,295	L
22	65,000	276,500	L
16	21,500	38,000	D
18	68,500	300,000	L
22	29,000	91,500	D
22	785	12,500	
25	228,000	300,000	
18.5	38,650	None	D
22.5	40,350	208,500	L
23	1,600	None	L
20.5		"	D
18	2,400	"	D
21.5	3,450	10,000	D
24	7,950	12,500	D
25	160,000	11,000	D

OUT OF 3 OUT OF 5

SAMPLE NO.	SAMPLE TYPE	MEAT	BUTCHERY	SAMPLING AREA	ABATTOIR
89.	b.c	1	2	Kawang.	O/R
90.	b.c	1	2	"	WAI
91.	mutton	3	2	D.Corner	NGONG
92.	b.c	2	3	"	WAI
93.	pork	3	5	Woodley	UPL
94.	g.b	3	5	Hurg.	KMC
95.	b.c	1	2	Kenyatta	NYONJ
96.	p.s	3	2	Kab.	UPL
97.	b.c	1	2	Kinoo	DAG
98.	b.c	1	2	Kikuyu	GITA
99.	b.c	1	2	"	GIKAMB
100.	b.c	2	2	Gita	GITA
101.	b.c	2	2	"	"
102.	pork	2	2	Wang.	WANG
103.	b.c	1	2	kamutiini	DAG
104.	b.c	1	2	L.Kab	DAG

SAMPLE TEMP.C	TPC X 10^3	COLI-FORMS	BOTULINAL TOXIN
25	18,100	93,000	L
24	225,000	300,000	
25	1,350	None	L
23.5		300,000	L
24	4,550	2,500	L
18.5	uncountable	48500	L
23.5	201,000	300,000	L
12	1,000	1,500	L
18	36,400	79,000	L
19.5	5,850	1,360	L
20	650	1,210	L
24.5	2,250	34,500	L
18.5	39,400	3,000	L
19	1,500	3,000	L
20.5	2,000	34,000	L
19	190,000	33,000	L

Appendix IV. Showing microbial counts and E.coli. isolations from marketed liquid milk

SAMPLE NO.	SAMPLE TYPE	MEAN COLIFORMS	MEAN AEROBIC PLATE COUNT	E. COLI
1.	Raw	38	1.530	- ve
2.	Fermented	5	25	- ve
3.	Chocolate	80	700	-ve
4.	Icecream	159	266	-ve
5.	Pasteurised fermented milk	0	38	-ve
6.	Icecream	45	184	2
7.	Yogurt	0	0	-ve
8.	Pasteurised whole milk	340	63,870	-ve
9.	Raw	312	5,1200	-ve
10.	Raw	50	6,850	-ve
11.	Icecream	300	4,800	-ve
12.	Icecream	642	16,300	-ve
13.	Icecream	2,200	30,000	+ve
14.	Cheese	1480	7,260,000	-ve
15.	Yogurt	8	0	-ve
16.	Pasteurised whole	240	140,000	-ve
17.	Raw	6000	6,400,000	5
18.	Yogurt	8	10	-ve
19.	Pasteurised whole milk	57	24,900	-ve
20.	Icecream	61	351,000	-ve
21	Icecream	55	332,000	+ve

SAMPLE NO.	SAMPLE TYPE	MEAN COLIFORMS	MEAN AEROBIC PLATE COUNT	E.COLI
22	Raw Fermented milk	1		
23	Cheese	730	94,500	-ve
24	Raw milk	5,200	100,000	-ve
25	Pasteurised whole milk	55	30,000	-ve
26.	Icecream	8,300	31,000	-ve
27.	Icecream	16,150	57,500	-ve
28.	cream	21,150	338,000	-ve
29.	Icecream	580	8600	-ve
30.	Yogurt	0	0	-ve
31.	Icecream	600	300,000	+ve
32.	Icecream	-	29,500	-ve
33.	Raw milk	15,300	710,000	+ve
34.	Raw milk	55,000	2,460,000	+ve
35.	Raw milk	123,000	2,960,000	+ve
36.	Icecream	400	3,000,000	-ve
37.	Icecream	600	110,500	
38.	Icecream	1500	364,500	
39.	Pasteurised whole milk	300	68,500	+ve
40.	Cream	54,500	1,865,000	

APPENDIX V. The total plate count and coliform count of Sigona Dairy Milk samples

SAMPLE NO.	COLIFORMS	TPC	SAMPLE NO.	COLIFORMS	TPC
1	36,150	$460x10^3$	25	60	25,550
2	600	$705x10^3$	26	400	$170x10^3$
3	2,100	135,000	27	560	$1.850x10^6$
4	1,750	$2.9.10^6$	28	2,220	$225x10^3$
5		$155x10^3$	29	395	$70x10^3$
6	20,100	$6.0x10^6$	30	475	$50x10^3$
7	900	$280x10^3$	31	1,365	21,900
8	750	$155x10^3$	32	$44x10^3$	585,000
9	0	$370x10^3$	33	250	8,900
10	27,850	$750,10^3$	34	390	17,800
11	100	$2.55x10^6$	35	60	3,250
12	50	$1.35x10^6$	36	0	1,130
13	8,300	$1.35x10^6$	37	775	17,000
14	2,050	$750x10^3$	38	20	8,100
15	50	$30x10^3$	39	65	5,600
16	150	$700x10^2$	40	795	76,000
17	0	$150x10^3$	41	50	85,000
18	250	$600x10^3$	42	125	115,000
19	150	$3.5x10^6$	43	1,070	915,000
20	5,100	$4.85x10^6$	44	-	-
21	150	$360x10^3$	45	8,150	$1.71x10^6$
22	24,350	$11.95x10^6$	46	-	-
23	9,400	$350x10^3$	47	-	-
24	300	$430x10^3$	48	35	$255x10^3$

SAMPLE NO.	COLIFORMS	TPC	SAMPLE NO.	COLIFORMS	TPC
49	805	357,000	74	490	112×10^3
50	2,005	1.55×10^6	75	785	29.5×10^3
51	45,500	215,500	76	90	22×10^3
52	355	119,000	77	1,430	1.2×10^6
53	435	605,000	78	400	350×10^3
54	30,000	3×10^6	79	355	22.10^3
55	42,500	510×10^3	80	885	61×10^3
56	2,700	-	81	60	1.05×10^6
57	8,000	38×10^6	82	45	18×10^3
58	75,500	450×10^3	83	2,065	29×10^3
59	1,025,000	$2,945 \times 10^6$	84	530	24.5×10^3
60	210	5.450×10^6	85	290	2.7×10^6
61	161,500	30×10^6	86	35	900×10^3
62	2,305	$5.150.10^6$	87	105	340.5×10^3
63	250×10^1	15.2×10^6	88	935	57.5×10^3
64	475×10^3	-	89	405	93×10^3
65	390×10^3	-	90	80	108×10^3
66	15×10^3	1.9×10^6	91	0	21×10^3
67	89.5×10^3	13×10^6	92	145	25×10^3
68	8.5×10^3	17.8×10^6	93	45	38×10^3
69	200×10^3	7.45×10^6	94	110	1.5×10^6
70	1,240	71,500	95	380	167×10^3
71	275	27×10^3	96	240	$39.x0^3$
72	40	15.5×10^3	97	25	37×10^3
73	165	18.5×10^3			

NO.	COLIFORMS	TPC
98	50	67×10^3
99	450	67.5×10^3
100	135,000	266×10^3
101	60	74.5×10^3
102	24,000	144.5×10^3
103	135	22×10^3
104	180	42×10^3
105	590	29×10^3
106	10	31.5×10^3
107	120	47.5×10^3
108	2,620	3.5×10^6
109	3,195	3.2×10^6
110	945	165.5×10^3
111	1,160	2.3×10^6
112	50	330×10^3
113	13,500	5.8×10^6
114	2,395	5.25×10^6

Appendix VI. Summary of suggested microbial limits in chilled and frozen foods/g, except as indicated (from Jay, 1970)

FOODS	NO. OF AUTHORS OR GROUPS	RANGE OF TOTAL VIABLE AEROBES	RANGE OF COLIFORMS	RANGE OF STAPH.	RANGE OF SALM. AND/OR SHIGEL	RANGE OF ENTEROCCOCCI
Frozen precooked foods	12	2,000-500,000	0-100	0-1,000	0 to absent in 50 g	1,000
Precooked meats	3	10-10,000	absent in 0.1 g	-	None	absent in 1 g
Raw meats	13	100,000-10,000,000	absent in 0.01-0.1 g	absent in 0.01-0.1 g	absent in 50g	-
Frozen whole eggs	4	200,000-10,000,000	-	-	-	-
Fish, shellfish & waters	10	50,000-1,000,000	0,7-1,600	100	-	1,000
Vegetables	10	50,000-500,000	absent in 0.1 g	-	-	-
Icecream & frozen desserts	25	100-4,000,000	0-200	-	-	0 to absent in 0.001 ml.

Staph. -staphylococci; Salm. - Salmonella; Shigel.- Shigella