EVALUATION OF HYGIENIC QUALITY OF MARKET MEAT

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "EVALUATION OF HYGIENIC QUALITY OF MARKET MEAT" is a bonafide record of research work done by me during the course of research and that the thesis had not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "EVALUATION OF HYGIENIC QUALITY OF MARKET MEAT" is a record of research work done independently by P.Kuttinarayanan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

(M. SOMAN)

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Dedicated to my beloved parents

P. N. PANICKER and P. P. AMMA

INTRODUCTION

INTRODUCTION

The tropical and subtropical environments are highly favourable for the growth and multiplication of a wide variety of micro-organisms on account of high temperature and humidity. Meat is an excellent source of nutrients necessary for the proliferation of the microbes. They enter the meat during pre-slaughter, peri-slaughter and post-slaughter operations. The factors responsible for contamination of meat are dirty air, soil, water and equipments, besides unclean animals and operators.

The organisms gaining entry into meat or meat products are either spoilage organisms or potential pathogens. Some of the pathogenic organism elaborate toxins causing food poisoning while others multiply and cause infection to the consumer. The spoilage organisms influence the keeping quality and shelf life. Therefore the bacterial load in meat is important both from commercial as well as Public Health point of view.

In India, meat is obtained from different species of animals. In many states in India as in the case of Kerala, bulk of the meat is being marketed without proper

storage or preservation. The slaughter houses are premitive, mode of slaughter operations are unsatisfactory and the means of transport of meat to the market is inadequate. No considerable improvement occurred in recent past in these fields and therefore the bacterial quality of meat produced in such environment is to be viewed with suspicion.

The hygienic quality of meat is dependent on the load and type of bacteria present. Total viable count will indicate the load of living bacteria in meat. Coliforms, Faecal Streptococci, and Clostridium perfringens are indicators of faecal contamination.

The bacterial quality of water used for slaughter operations greatly influence finished meat. Analysis of water supplied in the slaughter house will help in assessing one of the source of contamination in meat.

In India, the work done on the bacteriological quality of market meat are not many. Therefore, the present work was taken up with the object of gathering information on the hygienic quality of market meat in the prevailing conditions. The efficiency and desirability of washing carcasses using sanitizers for the

reduction of bacterial load in meat was also thought worth studying. The relation between the bacterial load in carcase and the water used in the meat stalls was also thought worth studying.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Kircher as early as 1659 suggested the role of micro organisms in spoilage of foods. Kerner (1820) described sausage poisoning and its high fatality rate. It was Pasteur who in 1837 showed that souring of milk was caused by micro organisms and he appreciated and understood the role of micro organisms in food spoilage. Gaertner in 1888 first isolated *Salmonella enteritidis* from meat which caused food poisoning in Germany in 57 persons.

Total Viable Count

Total viable count is an indication of hygienic quality of fresh meat. Kirsch *et al.* (1952) used this method in frozen lean beef.

Stringer *et al.* (1969) used total viable count in judging the bacterial quality of beef. Kotula *et al.* (1972) examined beef and got variability in microbial counts in different locations of the beef carcases. They recorded more count of mesophilic and psychrophilic organisms from neck region (1.2×10^5) than abdominal cavity and other locations (2.5×10^3). Surkiewicz *et al.* (1975) analysed raw beef patties, 76 per cent

of which had APC with in 10^7, 84 per cent of the samples had less than 100 coliforms and 92 per cent had less than 100 E.coli per gram. Sixty one samples of raw ground beef examined by Al-Delaimy and Stiles (1975) observed a mean APC 3.8×10^6 per gram of meat, while 41 per cent of the samples had count above 10 million per gram. One sample had coliform count more than 100 per gram of meat and 54 per cent of the samples were positive for faecal E.coli. Shelef (1975) examined refrigerated liver and got mean 6.8 log 10 count per gram.

Geopfert (1976) observed APC ranging from 100,000 to more than 50×10^6 organisms per gram of raw ground beef in 955 samples tested. Ingram and Roberts (1976) is of opinion that total count of a sample give an idea of the contamination and tell upon the hygienic quality of meat as well as the abattoir conditions. Westhoff and Feldstein (1976) recorded 2.0×10^6 organism per gram of beef. Misra and Gupta (1976) examined precooked accelerated freeze dried meat and found 90 to 100 organism per gram in 71.3 per cent of the samples. Geopfert (1977) examined ground beef patties and had count less than 10^6 per gram in two third of the samples and 30.5 per cent of the samples had count above 10^6 organism per gram.

Wojton and Kossakowska (1977) used aerobic plate count and count of indicator bacteria to assess the hygienic quality of meat. Kramer and Gilbert (1978) used five different methods to assess the APC of meat and found the variability is less than 0.5 log 10. By using pour plate method these workers recorded 7.32 log 10 count per gram of meat.

Oblinger and Kennedy (1978) examined frozen beef and got 10^3 to 10^4 organisms per gram of meat. Paradis and Stiles (1978) did not observe any correlation between APC and indicator bacteria. Raccach and Henrickson (1978) observed a count 10^3 per gram of meat obtained from electrically stimulated hot-boned carcases. Roy et al. (1978) observed an initial load of 2 to 3 log10 in beef muscle, coliforms and enterococci also detected. Summer (1978) stated various unhygienic practices which cause an increased bacterial load in meat.

According to Jay (1978) total plate count would probably give much information as any other microbial index for determining sanitory quality.

Examination of meat was done by using plate count agar, recommended by Ammerican Public Health Association

(1953). Harrigan and Mc Cance (1978) suggested incubation of plates at 35°C for 48 hours and to count bacterial colonies when plates were to have 30 to 300 colonies.

Indicator Bacteria

Certain bacteria which are natural inhabitants of gastro-intestinal tract of man and animals, denoted as indicator bacteria, when found in any other materials is a clean indication of its contamination with faecal matter. According to Buttiaux and Mossel (1961) organisms selected as indicators should possess specificity ie. the bacteria should occur in the intestinal tract, should occur in high numbers in the faeces, possess resistance to extra-enternal environment and permit easy and reliable detection.

Coliforms, Faecal Streptococci and *Clostridium perfringens* are considered as the common indicator bacteria and are considered as pathogens - Harrigan and Mc Cance (1976).

Coliform and *Escherichia coli*.

The presence of coliforms with *Escherichia coli* in food is an indication of possible faecal contamination. *E.coli* as an indicator of water-borne pathogen was first suggested by Schardinger (1892).

According to Burton (1949) contamination prior to freezing of foods is efficiently detected by the presence of coliforms while enterococci were superior indicators in frozen vegitables.

Wilson and Mc Closkey (1951) suggested E.coli count will tell upon the sanitary quality of oysters. Chartley et al. (1969) advocated testing for the presence of coliforms and other indicators for evaluating the hygienic quality of raw milk. Bachhil and Ahluwalia (1973) used E.coli and E.freundii as a tool to examine the hygienic quality of raw meat and stated that the presence of E.coli type I indicate that the meat had been directly or indirectly contaminated with human or animal faeces. Al-Delaimy and Stiles (1975) reported that faecal contamination leads to increase in coliform and E.coli count and decrease in shelf-life of raw ground beef.

Cutherts et al. (1976) and (1977) considers count of coliform and E.coli as well as aerobic plate count as a tool for judging the hygienic quality of turkey meat.

Linton (1977) tried presumptive coliform count to detect faecal contamination of pig carcases and stated that E.coli in pig and man are similar in type and antibiotic sensitivity. Wojton and Kossakowska (1977) adopted E.coli count to assess the hygienic quality of carcases.

American Public Health Association (APHA 1953) recommended Brilliant green bile broth 2 per cent (BGB broth) and Eosin Methylene blue (EMB) agar for the detection of coliforms and E.coli.

Fishbein et al.(1953) used rapid technique for presumptive count and confirmatory test using Lauryl Sulphate tryptose medium and EMB Agar from cream and pie. Krishnaswamy and Lahiry (1964) examined market meat and obtained a coliform count of 2.6 to 4.5 log10 per gram of meat. Bhatta (1966) used BGB broth for quantitation of coliforms and EMB Agar for E.coli from water. E.coli was identified by IMViC test. Hall et al.(1967) followed most probable number (MPN) method for detection of coliform using BGB broth. They studied growth character on EMB Agar and IMViC test for detection of E.coli.

Mossel and Ratto (1970) found BGB broth was better than violet red bile agar (VRB) for isolation of coliform from dried food and drugs. Moussa et al.(1973) used MPN technique for quantitation of coliforms in freeze dried food stuff using BGB broth. Shelef (1975) using VRB Agar, in liver got a 2log count in the initial day.

Fowler and Clark (1975) used BGB broth for estimating coliforms in delicatessen salads and IMViC reactions for testing the isolated E.coli. Chambers et al.(1976) using VRB Agar and recorded a mean log count from 2.64 to 2.97. Edmund and Latt (1977) used IMViC test for identifying E.coli which were isolated from foods. Newton et al.(1977) conducted IMViC tests for identifying E.coli which were isolated from hides and meat. They got 160 samples positive for coliforms and E.coli was isolated from 100 of them. Wojton and Koscakowska (1977) using BGB broth tested E.coli group in meat to test the hygienic quality.

Rajagopalan (1978) used BGB broth for detection of coliforms and EMB Agar for enumeration of E.coli from frozen fish, meat, chicken and ice-cream.

Coliform counting by MPN procedure was recommended by APHA (1953) Cruickshank et al.(1975) Harrigan and Mc Cance (1976) using BGB broth. Cruickshank et al.(1975) Oblinger and Coburger (1975) preferred five tube method to three tube method for determination of MPN.

Cruickshank et al.(1975) found that Eijkman test with BGB broth at 44°C is a useful method in identifying typical coliform (E.coli) and stated that BGL broth

supresses the growth of anaerobes especially Cl. Welchii. The same method was followed by Harrigan and Mc Cance (1976).

Faecal Streptococci

Faecal Streptococci differ from most other gram positive cocci in that it is catalase negative (Wilson and Miles, 1975) but Streptococcus faecalis which is one of the members of faecal streptococci is catalase positive (Cowan, 1974).

The group D streptococci or Faecal Streptococci are also known as enterococci and consist of distinct species such as:-

Streptococcus faecalis, Streptococcus faecium, Streptococcus bovis and Streptococcus equinus.

On the basis of the present knowledge it appears that coliform count as an index of sanitary quality is applicable to some foods whereas the use of this index for food, brings objection from some investigators who feel that enterococci rather than coliforms better reflect the sanitary quality (Jay, 1978).

Buttiaux (1959) compared the presence of coliforms and Faecal Streptococci in water and concluded that group D Streptococci are excellent indicators of faecal contamination. Bartly et al(1960) isolated Faecal Streptococci

from water, sewage and faeces and considered that they were the best indicators of pollution even in the absence of coliforms.

Different workers used different techniques for estimating the number of faecal streptococci in food stuffs. Barnes (1959) tested media containing 0.10 per cent thallus acetate and triphenyl tetrazolium chloride as an indicator and tried slanetz agar, azide broth and packers crystal violet broth for testing food stuffs. Lachica and Hart-man (1968) used selective media like Tween carbonate media, Thallus acetate citrate Agar, M-enterococcus Agar (Slanetz) Thallus acetate Agar, K.F. Agar, and citrate Agar and found the first two are the best for isolating and enumerating Faecal Streptococci in frozen foods.

Wojton and Kossakowska (1977) used sodium azide broth to enumerate Faecal Streptococci in carcases. Pierson et al.(1978) tried azide dextrose broth, crystal violet azide broth and K.F.Agar, to isolate group D streptococci from ground beef and got variable results. Rey et al.(1978) used K.F. Agar for isolating enterococci from excised muscle and obtained 1.2 log10 count on the initial day.

Stiles et al. (1978) analysed raw and processed meat to detect group D streptococci using K.F. Agar. They observed no meaningful relationship with other indicator organism and concluded that contamination with group D streptococci were principally from packing plants rather than at retail level.

Clostridium perfringens

Organisms belonging to the species Clostridium perfringens (Cl.perfringens) are gram positive rods, when exposed to adverse condition they form spores.

Cl. perfringens are normally present in the intestinal tract of men and animals. It is also found in soil, dust and sewage. Presence of this organism indicate past contamination in the absence of coliform or E.coli.

Cl.perfringens was demonstrated as ethiological agent in food poisoning first by Mc Clung (1945). The food poisoning strains of Cl. perfringens primarily belongs to the type A (Jay, 1978).

According to Marshall et al.(1968) Tryptone sodium sulphite Neomycin (TSN) Agar gave the maximum count at lesser time when compared to sodium sulphite polymixin sulfadiazine Agar (SPS). Addition of thioglycollate

buffer enhances the anaerobiosis and it should be added when TSN Agar is used, under aerobic conditions.

Gibbs and Freame (1965) used sulfadiazine and polymixin as selective anti-bacterial agents for the isolation of Cl. perfringens. Hall et al. (1969) tried SPS Agar to detect the total number of Cl. perfringens in foods. Harmon et al. (1971) stated that TSN Agar was the best selective medium for isolation of Cl. perfringens, when compared to SFP agar and SPS Agar, and it gave least false positive results.

Jayne and William (1973) tried D. cycloserin and Neomycin to supress the group D streptococci during the isolation of Cl. perfringens.

Labee and Duncan (1975) used, starch, glucose, or maltose in the culture media and obtained a 100 fold increase in the recovery rate of Clostridium perfringens.

Harmon and Kattur (1976) and (1977), Martin et al. (1976) used the enzyme catalase to increase the number of Cl. perfringens isolated in order to assess the quantity more accurately.

Clostridium perfringens may get access into meat by contamination of the carcase with polluted water, dung and air-borne dust. Many authors attempted to isolate Cl. perfringens from meat and meat preparations.

Lepovetsky et al. (1953) tried Heart infussion broth to isolate clostridia from muscle, bone marrow, and lymphnodes. Mossel (1959) used sulphite polymixin agar to detect the presence of Cl.perfringensin foods.

Strong et al.(1963) tried thio-glycollate medium for raw meat, poultry and fish and observed 16.4 per cent of them contained Cl.perfringens. Hall and Angelloti (1965) used thio glycollate medium to detect Cl.perfringens in meat and meat products and noted 70 per cent of the beef and 82 per cent of the veal were positive. Emsweiler et al.(1976 b) tested meat using neutral-red cooked meat medium and Fruin (1978) testing 339 isolates from ground beef and pork and found 320 (94%) were type A. Paradis and Stiles (1978) observed less than 10 Cl.perfringens per gram of bologna tested.

Various other products were examined by different authors to detect the presence of Cl.perfringens.

Strong et al. (1963) isolated Cl.perfringens from poultry and fish, Christiansen and King (1971) from selds and Guthertz et al. (1976); (1977) from turkey meat.

Water Analysis

The quality of water used for slaughter operations is very much important to produce good quality meat. The water supplied to the slaughter house should be of potable type - Blair (1975).

Ritter (1956) used BGB broth for testing the presence of coliform in well water and azide dextrose broth for enterococci.

Gilcreas (1975) is of opinion that Faecal Streptococci can be used as indicators of pollution in addition to E.coli.

Water being a source of contamination to meat, assessing the hygienic quality of water supplied to slaughter house, will help to trace out the source and extent of contamination of meat prepared in such areas.

Decontamination

Fresh meat is an ideal medium for bacterial growth thereby leading to its spoilage and food-borne infection. The cheapest method to reduce the surface bacterial load of carcase is by washing them with some harmless sanitisers.

Chlorine which is a by-product in many chemical industry can be effectively utilised for this purpose. In the form of bleaching powder it can be easily stored.

According to Mercer and Somoner (1957), Wilson and Miles (1975), the hypochlorus acid formed by chlorine in solution is a good germicide.

Zeigler and Stadelman (1955) found that cooling of chicken in 10 or 20 ppm chlorine water was effective in increasing shelf-life. Heys et al. (1963) found acidified chlorine water increases the bactericidal effect.

Benardo et al. (1967) found that E. coli in suspension were destroyed by exposure to different strength of chlorine water at different temperature. The bactericidal effect was found increased with increased dose of chlorine and higher temperature of water.

Patterson (1968) used water at a strength of 200 and 400 ppm residual chlorine for immersing poultry carcase. The shelf-life was expected to enhance by 20 per cent in the case of treated carcase.

Murray (1969) indicated the use of 10 ppm chlorine in inplant water supply of slaughter house was a considerable aid to the improvement in the bacterial quality of meat. Dainty (1971) advocated spraying with hot water (75 to 90°C) to reduce 80 per cent of bacteria.

Jagger (1972) reported water with 22 ppm residual chlorine was used for spraying and got reduction in the bacterial load, without any tainting effect in the carcases.

According to Kotula *et al.* (1974) reduction in total count were evident in 45 minutes and 24 hours after spray washing with water containing 200 ppm chlorine. The pressure of the spray were 4.2 and 24.6 kg per cm^2. The reduction attained were 1.5191 and 2.3097 for samples at 45 minute after wash and 2.3901 and 3.0716 after 24 hour washing respectively for the above treatments.

Thronton and Gracey (1974) recommended hypochlorite solution containing 250 ppm chlorine can be used effectively to disinfect equipments in the meat stalls. Mead *et al.* (1975) used 20 ppm chlorine in the water supply of the processing plants and noted approximately 10 fold reduction in faecal and spoilage bacteria.

According to American Meat Science Association (1975) washing of carcases with 150 ppm and 200 ppm chlorine water reduces the bacterial load by 81.9 and 81.8 per cent respectively.

Patterson (1975) recommended dipping poultry carcases in hypochlorite solution of 220 ppm for 1 to5 minutes is as effective in reducing bacterial load, as by any other method. Kelly (1975) stated chlorination of washing water would reduce the carcase contamination and bacterial load without any effect on keeping quality.

Emsweiler et al. (1976 a) observed a significant reduction in total aerobic count of beef forequarters within 24 hours after spray wash with 100, 200 and 400 ppm chlorine water.

A two log reduction was noticed by Smith et al. (1976) in lamb carcases with 0.02 per cent chlorine water. Kelly (1976) suggested washing with chlorinated warm water reduced total count significantly. Morton (1976) advocated aqous solution of nascent chlorine spray to reduce the bacterial load to a nil state.

Lazarus (1977) used hypochlorus acid in acetate-acetic acid buffer 25 to 200 ppm to reduce the surface microbial load significantly. Pothiraj (1978) reported a significant reduction in microbial load in sheep carcases by potable water spray, at 3 kg per cm^2 for one minute with brushing. It was reported that spray washing of carcase using heated water at 90°C gave a reduction in bacterial load by 99 per cent (Anon., 1978). Smith and Graham (1978) used water at 80°C for 10 seconds without any objectionable colour on beef and mutton carcases. Above 99 per cent of inoculated Salmonellae and E.coli were destroyed.

MATERIALS AND METHODS

MATERIALS AND METHODS

Eighty four samples of meat for this study were collected from different meat stalls in and around Municipal Town, Trichur such as Municipal meat stalls at East Fort (20 samples), West Fort (21 samples), Panchayat meat stall, Mannuthy (33 samples) and Kerala Agricultural University Slaughter house, Mannuthy (USH) (10 samples).

Approximately fifty grams of meat was collected each time in separate polythene bags weighed and sterilised by ultra violet rays. Samples were placed in thermosflask over ice cubes. The laboratory test was started within one hour from the time of collection of samples. All glass wares and instruments were sterilised suitably before the work. Processing of meat for bacteriological examination was done under sterile conditions using sterile instruments and agents. The composition and preparation of reagents and media is appended separately.

Total Plate Count (APC)

Preparation of inoculam.

Meat samples collected in polythene bags were brought to the laboratory and weighed accurately. It was then triturated with measured volume of phosphate buffer in order to get a uniform suspension using mortar and

pestle. From the minced meat suspension serial dilutions were made in phosphate buffer to get one in 10 million dilution (10^{7}). This was used for all tests to follow.

One milli litre each of the diluted samples was carefully transferred to duplicate petridishes (110 x 17 mm) and approximately 12-15 ml of melted standard plate count agar (APHA 1953) having a temperature of 45°C was poured in to the petridish, to get a uniform suspension. The contents were mixed properly by clockwise, anticlockwise forward and backward movements in that order of the petridish. It was allowed to solidify at room temperature, and kept inverted in the incubator at 35°C for 48 hours (APHA 1953).

After incubation, the plates were examined for the growth of the organism by the presence of colonies, which were counted by the help of colony counter. The plates having 30 to 300 colonies were selected for counting. The count was taken as the average of the two plates after applying dilution factor and expressed as number of viable organisms per gram of meat.

Coliform Count

For detecting coliform, most probable number (MPN). Brilliant green bile broth (BGB broth) as prescribed by

APHA (1953) with 5 ml of one per cent neutral red as indicator per litre of the media was used. Estimates of MPN of the diluted meat suspension was made following five tube method.

The prepared media were kept at 35°C for two hours. before meat suspension was added to it. To 20 ml one and half strength BGB broth 10 ml of the suspension was added. Similarly to 10 ml single strength media one ml of meat suspension was added, and to another set of 10 ml media tubes 0.1 ml meat suspension was added.

The contents were incubated at 35°C for 48 hours.

After incubation the tubes were examined for acid and gas production. The result was compared with the table for MPN prescribed by Cruickshank et al. (1975) to estimate MPN in the 100 g of meat sample.

Escherichia coli (E.coli) count

Eosin Methylene blue agar (EMB agar) (APHA 1953) was used for isolation of E.coli. One ml each of the meat suspension to which approximately 12 to 15 ml of melted and cooled (~45°C) EMB agar was added and mixed properly as in the case of standard plate count. The contents were allowed to solidify and then incubated at 35°C for 18-24 hours.

After incubation, the plates were examined for the presence of colonies with metalic lusture, which were considered as *E.coli* and their count was recorded. The average of the two plates was taken and applying the dilution factor the *E.coli* count per gramme of meat was worked out.

Random colonies were selected and grown in nutrient agar slants. IMViC tests were conducted to ascertain the identity of *E.coli*.

Eijkman Test

This test was done to assess the percentage of typical coliform in relation to total *E.coli* in the sample of meat as recommended by Bhatta (1966) and Cuickshank *et al.* (1975).

Five ml each of BGB broth 2 per cent with inverted durham's tube was taken in five test tubes and one each of the randomly selected five colonies in the plate, was transferred to the medium kept at 35°C. The contents were incubated at 44°C for 48 hours. After incubation, the tubeswere observed for gas production. The percentage of gas producing colonies are considered as typical coliforms.

Faecal Streptococci count

Slanetz medium was used for the isolation of Faecal Streptococci.

One millilitre each of the meat suspension was pipetted into duplicate petridishes. Approximately 12-15 ml medium was added to each of the petridishes and mixed thoroughly by shaking the plate as mentioned earlier. The contents were allowed to solidify. It was then kept for incubation at 37°C for 48 hours.

After incubation the plates were examined for the presence of colonies with varying appearence on account of the varieties of Faecal Streptococci. Average of the total count of the two plates were taken. Applying the dilution factor, number of Faecal Streptococci per gram of meat was recorded.

Isolated colonies were randomly checked by gram staining, nitrate reduction, mannitol fermentation and catalase production (Cowan, 1974) for confirmation.

<u>Clostridium perfringens</u> Count

TSN Agar with thioglycollate buffer was used for isolation of <u>Cl.perfringens</u>.

One millilitre each of the meat suspension was pipetted into duplicate 25 x 200 mm tubes. Approximately 25 to 30 ml of melted medium (47°C) was added into the tube. Contents were mixed properly. A layer of liquid paraffin (Sterilized) was made over the contents to maintain anaerobiosis.

The tubes were incubated at 37°C for 48 hours. After incubation, the tubes were examined for the presence of black colonies indicating the growth Cl. perfringens. The colonies were counted, average count was recorded. By applying the dilution factor number of Cl.perfringens per gram of meat was recorded.

Random colonies were tested by gram staining, motility, nitrate reduction, and lactose fermentation for confirmation.

Water Analysis

Twenty water samples collected from the tanks of all the meat stalls were tested for aerobic plate count, coliform count, count of E.coli, Faecal Streptococci and Clostridium perfringens using respective media as in the case of meat samples.

Decontamination

Calcium hypochlorite (Bleaching powder) was used to prepare chlorine water. The strength of chlorine in

the solution was determined by iodometric titration (APHA 1953) and further dilutions were made in order to get 10, 20, 50 ppm of chlorine in water respectively.

The solutions were used for spraying on the carcase 30 to 45 minutes after the preparation of the solution.

Initially meat samples from the carcase were collected from near the area where spraying was to be done. Subsequently chlorine water were applied in the area adjacent to that from where the meat samples were collected using a hand sprayer (Poly sprayer - Bayer) at a constant pressure from a distance of one foot from carcase surface. About 100 to 110 ml of the solution was sprayed over an area of 30 cm square within 30 seconds. Fifteen minutes was allowed for proper dripping and drying. There after samples were collected from the treated areas in similar manner as above.

The meat samples collected before and after chlorine treatment were processed for determination of total aerobic plate count as in the case of earlier samples. For chlorine treated samples 0.05 per cent of sodium thiosulphate was added to the peptone water to neutralise the residual chlorine if any in the meat (Patterson, 1968).

RESULTS

RESULTS

The result of total plate count, presumptive coliform count, count of *Escherichia coli*, Faecal Streptococci and *Clostridium perfringens* in respect of 84 samples of meat tested are shown in table 1.

Aerobic Plate Count

Result of 10 samples from USH has shown that the average aerobic count is $36.40 \times 10^6 \pm 3.03$. The maximum count obtained was 55×10^6 organisms per gram of meat and minimum was 26×10^6. Ninety per cent of the samples from USH had count between 30×10^6 and 50×10^6 and in only one sample (10%) the count was 55×10^6. (Table II a).

Out of 33 samples from Mannuthy, the average count was $65.03 \times 10^6 \pm 3.91$. The aerobic count ranged between 30×10^6 and 103×10^6 organisms per gram of meat. Out of the 33 samples, 11 (33.33%) had a count between 30×10^6 and 50×10^6. For seven (21.21%) samples the count was between 51×10^6 and 70×10^6 and in 10 (30.3%) samples, it was 71×10^6 to 90×10^6. In the remaining 5 (15.15%) samples, the count was 91×10^6 to 110×10^6.

The result of 21 samples collected from West fort, the mean APC was $94.48 \times 10^6 \pm 5.46$. The count ranged

from 37 x 10^6 to 150 x 10^6 organisms per gram of meat. One (4.76%) of the samples came in the range of 30 x 10^6 to 50 x 10^6 and for another (4.76%) the count was within the range of 51 x 10^6 to 70 x 10^6. But eight (38.08%) samples had count between 71 x 10^6 and 90 x 10^6. Whereas in the case of nine (42.88%) samples count ranged between 91 x 10^6 and 110 x 10^6 and only in two (9.52%) samples the count was more than 111 x 10^6 organisms per gram of meat.

In twenty samples collected and examined from East fort the APC ranged between 63 x 10^6 and 127 x 10^6 organisms per gram of meat, with an average of 89.2 x 10^6 $\pm$ 3.77. Three (15%) of the samples had count between 51 x 10^6 and 70 x 10^6, for 10 (50%) it was 71 x 10^6 to 90 x 10^6 and for five (25%) the count obtained was 91 x 10^6 to 110 x 10^6. In two (10%) samples the APC was more than 111 x 10^6 organism per gram of meat.

Coliform Count

The average presumptive coliform count (MPN) in the meat samples from USH was 2.91 x 10^5 $\pm$ 0.372 per 100 gram meat. The maximum MPN detected in the samples from the same source was 4.5 x 10^5 and the minimum 1.2 x 10^5 coliforms per 100 gram of meat. All the samples had MPN less than 5 x 10^5 (Table II b).

The average MPN of coliforms among Mannuthy (33) samples was $13.52 \times 10^5 \pm 2.24$ coliform per 100 gram of meat. The count ranged between 2.0×10^5 and 42.5×10^5. Of the 33 samples 12 (36.36%) had count less than 5×10^5. In the case of 15 (45.45%) samples the count was between 5.1×10^5 and 25×10^5. The remaining six (18.19%) had 25.1×10^5 and 50×10^5 coliform per 100 gram of meat. No sample had count more than this.

The mean coliform count in 21 samples collected from West fort was $77.60 \times 10^5 \pm 16.40$ coliforms per 100 gram of meat. The maximum and minimum were 180×10^5 and 4.5×10^5 respectively. Only one (4.67) had the count below 5×10^5. Nine (42.88%) of the samples had MPN between 5.1×10^5 and 25×10^5 and 3(14.18%) had 25.1×10^5 to 50×10^5. But eight (38.08%) of the samples had MPN more than 100×10^5 coliform per 100 grams of meat.

In the case of 20 samples from East fort the average MPN was $45.75 \times 10^5 \pm 13.94$ coliform per 100 grams of meat. The maximum value observed was 160×10^5 and minimum was 2.0×10^5. In the case of five (25%) samples MPN was less than 5×10^5 whereas eight (40%) had a value ranging between 5.1×10^5 and 25×10^5.

One sample (5%) each had MPN ranging between 25.1×10^5 to 50×10^5, 50.1×10^5 to 75×10^5, and 75.1×10^5 to 100×10^5. All the remaining four (20%) had MPN value more than 100×10^5.

Escherichia coli count

Out of the 84 samples tested none had a count less than 800 *E.coli* per gram of meat.

The average *E.coli* count of 10 samples collected from USH was $20.5 \times 10^2 \pm 3.001$. The maximum count of E.coli was $33. \times 10^2$ and the minimum was 8×10^2 per gram of meat. Four (40%) of the samples the count ranged between 800 and 1500. An equal number had count 1501 to 3000. Remaining two (20%) samples contained *E.coli* between 3001 and 5000 per gram and all were within 5000 *E.coli* per gram (Table II c).

The average *E.coli* count of 33 samples collected from Mannuthy was $35.27 \times 10^2 \pm 1.90$. The minimum count was 1400 and the maximum 7000 *E.coli* per gram meat. In all the samples the count was 800 or more. One (3.03%) had count between 800 and 1500, whereas in 10 (30.30%) it ranged between 1501 and 3000. Twenty one (63.64%) samples were in a range of 3001 to 5000, and the remaining one had *E.coli* between 5000 and 7500.

The mean *E.coli* count of 21 samples from West fort was $53.24 \times 10^2 \pm 2.46$ *E.coli* per gram of meat. The minimum and maximum count were 3000 and 8800 respectively. No sample had a count less than 1500 per gram. In one of the sample (4.76%), the count fell in the range of 1501 and 3000. The count in 12 (57.16%) samples was 3000 to 5000, whereas in two (9.52%) it was 5001 to 7500. Six (28.56%) had counts above 7500.

Altogether 20 samples were tested from East fort. They gave an average count of $40.35 \times 10^2 \pm 3.64$ *E.coli* per gram of meat. The maximum count was 7500 and the minimum was 1200. In all the samples the count was above 800. Out of these in three (15%) of the samples the count was between 800 and 1500. In 14 (70%) of the samples the *E.coli* count was 3001 to 5000 and three (15%) had 5001 to 7500 *E.coli* per gram of meat.

Colonies of *E.coli* isolates were tested at random by Indole, methyl red, Voges-proskauer and citrate utilization test. It was found that all the isolates tested from USH Mannuthy, Eastfort and West fort were Indole and MR positive and V.P. and citrate negative.

Eijkman Test

The *E.coli* isolated were tested for production of gas at 44.0°C (Eijkman test). Ninety percent of the colonies, so tested were found to be positive to the

test indicating they are typical coliforms. The details of the result of Eijkman test is shown in (Table III).

Faecal Streptococci

The average count of Faecal Streptococci in 10 meat samples collected from USH was $28.11 \times 10^3 \pm 6.67$ organism per gram of meat (Table I). The minimum and maximum count was zero and 66000 respectively. One (10%) of the sample was negative. In two (20%) of the samples the count ranged between 1001 and 10000. Three (30%) of the sample had a count between 20,001 and 30,000. In another 3 samples, it was between 30,001 and 50,000 and in the remaining one it was between 50001 and one lakh. None of the samples had a count above one lakh (Table IId).

The mean Faecal Streptococci count of 33 samples collected from Mannuthy was $40.05 \times 10^3 \pm 3.75$ per gram of meat. The minimum count was 30,001 and maximum was 101,000. None of the samples had a count below 1,000. Five (15.15%) of the samples had a count between 1001 and 10,000 whereas in four (12.12%) of the samples the count ranged between 20001 and 30,000. Seventeen(51.52%) samples were in the range of 30,001 and 50,000 and in six (18.18%) samples the count ranged between 50,001 and one lakh. In one (3.03%) of the sample the count was above one lakh.

In 21 samples tested from West fort gave an average count of $57.71 \times 10^3 \pm 4.22$ organism per gram of meat. The minimum count observed was 33000 and maximum count was 1,10,000. None of the samples had count below 30,000. In nine (42.84%) of the samples, the count was between 30,001 and 50,000 whereas in 11 (52.40%) it was between 50,001 and one lakh and in one (4.76%) of the sample the count exceeded one lakh.

Twenty samples collected from East fort revealed an average Faecal Streptococci count $52.6 \times 10^3 \pm 4.15$ organism per gram of meat. The maximum count observed was 107,000 and minimum was 33,000. All the twenty samples had count above 30,001. Twelve (60%) of the samples had count between 30,001 and 50,000. In seven (35%) samples the count ranged between 50,001 and one lakh and in the remaining one sample it was above one lakh.

The isolated colonies were randomly tested by gram staining, nitrate reduction, mannitol fermentation and catalase production. All the tested colonies were gram positive, short chain cocci. The result of nitrate reduction, mannitol fermentation and catalase production are shown in (Table IV). All of the tested organism reduced nitrate. About 32 per cent fermented mannitol. Approximately 21 per cent of the tested colonies were catalase positive rest 70 per cent were negative.

Clostridium perfringens Count

The average count of *Cl.perfringens* from UCH was $0.82 \times 10^2 \pm 0.79$ organism per gram of meat. The maximum recovery from one sample was 800 per gram. Seven (70%) of the sample did not have *Cl.perfringens* and in two (20%) the count ranged from 10 to 100 (Table II e).

Thirty three samples tested from Mannuthy had shown an average of $3.82 \times 10^2 \pm 0.75$ *Cl.perfringens* per gram of meat. The maximum recorded count was 1800. In seven (21.21%) samples, *Cl.perfringens* was not detected and six (18.18%) samples had count 10 to 100, whereas 12 (36.37%) samples had count between 101 and 500. In six (18.18%) samples the count was 501 to 1000 and in one (3.03%) sample it was in the range of 1001 and 1500. In another sample the count was between 1501 and 2000.

The average *Clostridium perfringens* count for 21 samples collected from West fort was $6.94 \times 10^2 \pm 1.33$. The maximum count was 2,000 and minimum was 20 *Cl.perfringens* per gram of meat. Three (14.25%) samples had count between 10 and 100. In 8 (38.12%) samples the count ranged between 501 and 1000 and in another 4 samples 1000 to 1,500. The remaining two (9.52%) sample it was between 1501 and 2000.

Average count of Clostridium perfringensin 20 samples collected from East fort was $5.96 \times 10^{2} \pm 1.0$. The maximum count observed was 1900 organism per gram. One (5%) of the samples did not reveal the presence of Cl.perfringens. Two (10%) had count between 10 and 100. In seven (35%) samples the count ranged between 101 and 500, whereas in eight (40%) samples it was between 501 and 1000. Out of the remaining, in one sample the count ranged between 1001 and 1500 and for another it was between 1501 and 2000 Cl.perfringens per gram of meat.

The isolated colonies randomly tested for confirmation by gram staining, motility, nitrate reduction and by lactose fermentation. It was observed that all the tested colonies were gram positive, non motile, nitrate reducing and lactose fermenting.

The mean value of the aerobic plate count, coliform count, count of E.coli, Faecal Streptococci and Clostridium perfringens were compared with respect of the source of collection by analysing the data statistically using the 't-test'.

The samples obtained from USH was found significantly lower from others at 1% level in respect of APC, coliform, E.coli, Faecal Streptococci and Cl.perfringens (Table V).

Samples from Mannuthy were highly significantly lower from that of East fort and West fort with respect to counts of all organisms enlisted.

The samples of West fort and East fort were significantly lower in respect of APC , coliform E.coli and Faecal Streptococci at both levels. In respect of Cl. perfringens the significance of difference was only at 5 per cent level for samples from the above sources. Average counts of APC and other bacteria in samples from different sources are presented in table I.

Water Analysis

Results of bacteriological examination of water is given in (Table VI).

The mean and standard error of aerobic plate count of 5 samples from USH was $40.2 \times 10^3 \pm 6.88$, for Mannuthy samples $79.4 \times 10^3 \pm 7.34$, for West fort samples, $106 \times 10^3 \pm 6.55$ and for East fort samples $93.6 \times 10^3 \pm 5.96$ per millilitre of water.

Coliform count per 100 ml of water from USH was 330 ± 210.10, Mannuthy 1172 ± 210.2, West fort 1398±430.28 and for East fort 1772 ± 729.79. All the twenty samples contained coliform in 100 ml of water.

Escherichia coli count per ml of water tested show a mean and standard error as, for USH 1.0 ± 1.0, Mannuthy 10.2 ± 1.93, for West fort 108± 3.02 and for East fort 7.0 ± 1.10. Only one of the five samples from USH had

E.coli, whereas all the five samples each from the remaining three sources were positive for E.coli. None of the E.coli isolates were positive in Eijkman test.

Faecal Streptococci count of water analysed is shown in Table VI. The average count from USM samples was 7.6 ± 1.9, Mannuthy 24.2 ± 9.1, for West fort 35.0±14.74 and for East fort 19.4± 2.5 per ml of water. All the samples were positive for Faecal Streptococci.

Clostridium perfringens was not detected in all the five water samples from USM. Out of five samples from Mannuthy two gave on Cl.perfringens per ml of water, with a mean of 0.40±0.25. In the case of five West fort samples three of them had two Clostridium perfringens per ml and one sample contained one Cl.perfringens and others were negative. This gave an average of 1.4+0.4 Cl.perfringens per millilitre. In the case of water from East fort out of five, two of the samples had two Clostridia per ml and one sample had only one Cl.perfringens per ml of water examined with a mean of 1.0±0.45.

On statistical analysis of the data it was found aerobic plate count of USM water sample were significantly lower than that of Mannuthy, East fort and West fort. There was no significant difference between the samples from Mannuthy, West fort and East fort with regard to APC (Table VII).

In case of coliform count, there was significant difference between USH and Mannuthy samples at 5 per cent level and no significant difference between the samples between other sources.

Escherichia coli count of samples from USH significantly lower than that of Mannuthy, East fort and West fort and there was no significant difference between Mannuthy, West fort and East fort samples.

Faecal Streptococci count of USH was significantly lower than that of East fort. There was no significant difference between samples from other sources.

In the case of *Cl.perfringens*, the count in samples from USH was significantly lower than that from West fort. There was no significant difference noticed between other samples.

Decontamination

The effect of spraying the carcase with water containing 10, 20 and 50 ppm residual chlorine was studied by testing the initial bacterial load and after treatment load of the meat (Table VIII). The effect of decontamination with 3 levels of chlorine was compared with pre-treatment counts by statistically analysing the data using 't-test'. It was found that the difference was highly significant.

On calculating the percentage of reduction of count, 10 ppm chlorine water reduced 24.8 per cent of the bacterial load, 20 ppm reduced 59.9 per cent and 50 ppm reduced 77.9 per cent.

Covariance analysis of the data (Table IX) has shown that 10, 20 and 50 ppm are significantly different in their effect. Paired 't-test' showed that 50 ppm water was better than 20 ppm and 10 ppm in that order. But water containing 10 ppm residual chlorine significantly reduced the bacterial load, though not as much as the other two.

DISCUSSION

DISCUSSION

In order to evaluate the hygienic quality of meat and meat products various tools have been accepted. Aerobic plate count of the material under investigation is one of the foremost of such tools. It is an indirect indication of the bacterial load, though the anaerobic bacteria and psychrophilic bacteria may not come to light.

The load of coliforms in the sample is another indication of the total hygienic quality of meat. The contamination of meat of animals often occurs from faecal matter either from within the animal or from the external sources, directly or indirectly.

The three types of bacteria viz. *Escherichia coli*, Faecal Streptococci and *Clostridium perfringens* are often incriminated as indication of faecal contamination and hence known as indicator bacteria.

In the present study the market meat samples were tested for hygienic quality by aerobic plate count, presumptive coliform count and count of other indicator bacteria. This methodology was accepted in general and followed by many workers in the past.

Out of the total 84 samples brought for the study from four different places, ten samples were from the

University Slaughter house (USH) which was manned and managed by professionally qualified persons and not like the other three places such as meat stalls at Mannuthy, East fort and West fort. The last three having more or less similar environment and practices.

Aerobic Plate Count

The aerobic plate count(APC) of the samples ranged between 30×10^6 and 150×10^6 per gram of meat but in 90 per cent of the samples from USH the APC was between 30×10^6 and 50×10^6 whereas only 12 of the remaining 74 samples could fall in this range. Thirty three per cent of the samples from Mannuthy and 4.76 per cent of the samples from West fort belong to this group (Table IIa). Fifteen per cent of the samples from Mannuthy, 25 per cent from East fort and 43 per cent from West fort had a count in the range of 91×10^6 to 110×10^6 indicating a much higher bacterial load.

According to International Commission for Microbiological Specification of Food (ICMSF, 1974) general viable count in fresh meat should not exceed 10^7 per gram of meat. None of the samples under study could claim this quality but under tropical conditions and general practice this is a norm not easy to fulfil. But the quality of the samples from USH in general seems

satisfactory even if they were not in par with international standard. This is lower than 6.8 log10 reported by Sholef (1975) after examining fresh liver. Al-Delaimy and Stiles (1975) observed count above 10 million per gram of meat in 41 per cent of the samples. Goepfert (1976) observed APC ranging from 10^5 to more than 50×10^6 organisms per gram of raw ground beef.

Coliforms

Presence of coliforms and their count is an indication of contamination from environment. No sample was free from coliform. The MPN ranged between 1.2×10^5 and 180×10^5 per 100 gram of meat. But 33.3 per cent of the sample had a count less than 5×10^5, in which cent per cent of the samples from USM were covered. Forty to forty five per cent of the samples from other three places, the MPN ranged between 5×10^5 and 25×10^5 (Table II b). In the case of samples from West fort and East fort the MPN was more than 100×10^5 in considerable numbers. The result pointed to a significant difference in the coliform count between the products of USM and other places. The coliform count in USM sample is lower than that reported by Krishnaswamy and Lahiry (1964).

Escherichia coli

Among the indicator bacteria *E.coli* was detected in all the samples, which is a clear indication of their faecal contamination. All of them had a count more than 800 per gram of meat. As seen from (Table II c) the load of *E.coli* from the USH samples were comparatively low, as none of them was having a count higher than 5,000. The *E.coli* count between 3001 and 5000 was observed in 20 per cent of USH samples where as the corresponding figures from others were 63.64 per cent for Mannuthy, 57.16 per cent for West fort and 70 per cent for East fort. Here again a significant difference was noticed for USH samples from the others. Al-Deleimy and Stiles (1975) also found presence of *E.coli* in 54 per cent of meat samples tested.

Though the presence of *E.coli* was detected in the samples, the results of 420 colonies randomly tested from all the samples indicate that 377 (90%) were typical coliform as evidenced from the result of Eijkman test (Table III). But there is no significant difference of the isolates from the samples from the four different sources. A high percentage of the strains of typical coliforms indicate the potential danger to the consumers' health.

Usually Faecal Streptococci is found existing in materials during contamination with faecal matter. All except one contained Faecal Streptococci. The count varied from 1000 to more than one lakh per gram of meat (Table II d). More than 91.7 per cent of the total samples had count above 20,000. Thirty per cent of USH, 51.2 per cent of Mannuthy, 42.84per cent of West fort and 60 per cent of East fort were in the range of 30000 to 50000. About 52.4 per cent of the west fort samples, 35 per cent from East fort and 18.18 per cent from Mannuthy had a count between 50,000 and one lakh, whereas one (10%) of the USH sample had this count. In general there was considerably high count of Faecal Streptococci in the meat sample obtained from places other than USH. This is more or less in agreement with Chou and Marth (1969) who observed Faecal Streptococci in 93 per cent of frozen meat in the range of 11000 to 1000000 per gram of meat.

From the biochemical reaction (Table IV) of the Faecal Streptococci in their ability to ferment mannitol and catalase production, the species variation could be determined. Most of the catalase producing strains could be *Streptococcus faecalis* (Cowan, 1974). The mannitol fermenters include *Streptococcus faecalis* as well as *Streptococcus faecium*. The other being generally

Streptococcus bovis or Streptococcus durans (Wilson and Miles, 1975). In the present study an average 33 per cent of the isolates were mannitol fermenters and about 22.5 per cent were catalase positive, indicating that the majority of the isolates were Streptococcus bovis or Streptococcus durans found in the gastro-intestinal tract of bovines. Their presence in the meat could be due to peri slaughter or post slaughter faecal contamination.

Clostridium perfringens

Clostridium perfringens is another set of bacteria normally present in soil and faecal matter. Sixty nine (82.1%) of the samples contained Cl.perfringes. Seventy per cent of the USH samples, 21.21 per cent of the Mannuthy samples, five per cent of the East fort samples were free from this bacteria. The load of Clostridium was with in 2000 per gram of meat and the bulk of the samples from places other than USH had count between 100 and 1000 (Table II c). Hall and Angelloti (1965) found 82 per cent of the veal and 70 per cent of beef were positive for Clostridium perfringens. In the present study also the samples positive for Clostridium was 82 per cent.

The isolates, besides having grown in the selective medium satisfied the characteristics of Cl.perfringens from the biochemical reaction.

Stringer *et al.* (1969) noticed a significant rise in APC in meat during transport to the retail market. Moulder and Krol (1976) observed the transportation of dressed carcases may lead to increase in bacterial load due to poor hygiene in transit. Shelef (1975) observed that the APC increases on spoilage of meat. Chambers *et al.* (1976) was of opinion that bacterial count in market meat was due to poor sanitation practices. Westhoff and Feldstein (1976) attributed the higher bacterial load in meat to improper handling and poor sanitation in the abattoir. In the opinion of Summor (1978) the source of contamination in meat were mainly due to the absence of proper working surface, knife sterilisers, hand washing facilities, un-restricted acess of outside personnel and dirty area. Absence of protective clothing for the working personnels, the practice of undesirable acts during working such as smoking and unprotected vehicles and mode of transport were some of the contributory factors to a high bacterial load in the market meat. Similar causes were attributed by Thronton and Gracey (1974).

In the case of meat stalls under our study the conditions were unsatisfactory in respect to the type of construction, management facilities and practices. Only

exception was that the University Slaughter house (USH) which had an improved structure than the rest. Animals slaughtered in this place were from those maintained in the university farms disposed for slaughter for reasons other than diseases. The supervision of the operations was done by professionally qualified persons paying more attention to cleaning of surfaces and keeping the meat in polythene bags during transport. The distance involved in transport was also limited and the factors mentioned above might have contributed to the comparatively low APC in the meat obtained from there.

In the case of Mannuthy the only difference from West fort and East fort was that the slaughter was conducted in an area a few yards distant from the meat stall. It could not claim any improvement in the facilities or environment when compared to others. But the place where meat was kept for sale was not exposed to the roads where there is frequent traffic movement, whereas both at West fort and East fort the slaughter area was about 3 KM away from the stall. The carcases being transported exposing to the dust and vehicles in the road and further displaying them nearer to the roads so that air-borne dust could get access to the carcases. This could be a reason for the higher APC in meat samples of East fort and West fort, when compared to that of Mannuthy. It was not possible

to assess the health status of the animals slaughtered in all the three places and their role if any on the bacterial load.

The result of analysis of bacterial quality of water indicated that there was significant difference in the load of bacteria between that of USH and other 3 places, being minimum in the former. The difference in the bacterial load in meat could be partially attributed to this factor.

The presumptive coliform count of thememeat samples, more or less correspond to the APC. It is well known that coliforms are widely distributed in the environment. There is no wonder if the carcases which had been soliberally exposed to unrestricted environment had a high coliform count. The presence of large number of coliforms indicates an unsatisfactory hygienic quality.

Escherichia coli are abundently found in the normal gastro-intestinal tract of man and animals. The meat samples under observation invariably had E.coli. The only difference was generally in the number, where it was minimum in USH and increasing in the samples from Mannuthy, East fort and West fort in that order, probably due to the reasons explained for high bacterial load. The result of Eijkman test conducted randomly on colonies of E.coli show more than 90 per cent positive indicating their potential

pathogenicity. All of them were either of human or animal origin which gain access to the meat during slaughter operations.

Faecal Streptococci another indicator bacteria was also detected from all but one sample. But only a difference in the load was detected, being less in USH samples and an increase in samples from Mannuthy, East fort and West fort respectively.

Presence of Clostridium perfringens show a different picture of the other two indicator bacteria. The absence in 15 of the samples is conspicuous. About 70 per cent of the samples from USH and 21.21 per cent from that of Mannuthy, had shown freedom from Cl.perfringens. But generally samples except that from USH the load was considerably significant. The reason that could be attributed so, is that Clostridium is generally found in the soil and excreta. The operations of slaughter when conducted on the dirty and pervious floor will increase the possibility of more contamination on the carcass. A satisfactory impervious clean floor in the USH might have broken the channel of contamination from the floor and whatever Clostridium found in small number in few carcases could have been originated from the faecal contamination during slaughter operations. If Clostridium count was

more in carcasses from other sources it may be due to contamination both from the floor and from the animal.

The general picture of the APC, coliform count, and count of other indicator bacteria in the meat samples collected from four different sources justifies the reasons under-lying in the maintenance, management and practices in the slaughter houses and its environment, the mode of transport and display of the carcase in the meat stall.

Water Analysis

As water is abundantly used in slaughter houses and meat stalls its quality directly influence the load and type of bacteria present in the meat. Since there was significant difference in the bacterial load, in the water tested from different sources and corresponding difference in the bacterial load of meat samples collected, the relation between the bacterial quality of water and the meat seems strengthened.

Decontamination

Though there is controversy regarding the desirability of washing carcase after flaying to reduce bacterial load, it was suggested by workers like Patterson (1968), Dainty (1971), Morton (1976) to wash the carcase to reduce the bacterial load and to enhance the keeping quality.

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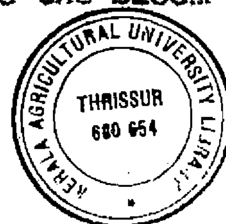
Chlorine water was the detergent of choice for most of the workers. Dainty (1971) used hot water at 75 to 90°C and claimed a 80 per cent reduction bacterial load. Patterson (1968) used water containing 200 and 400 ppm chlorine and expected 20 per cent increase in shelf life.

American Meat Science Association (1975) obtained a reduction of 81.9 per cent by using water containing 150 and 200 ppm of chlorine.

In the present study water for spraying carcase was used with varying strength of chlorine such as 10, 20 and 50 ppm. It was necessary to assess the efficiency of these treatments on bacterial load of the carcases. A contact period of fifteen munutes was allowed after spraying. The reduction in bacterial load achieved was 24.8, 59.9 and 71.9 per cent respectively.

Emsweiler *et al.* (1976 a) after using water containing 50 ppm chlorine generated from calcium hypochlorite, observed significant reduction in bacterial load in the carcase within one hour, and also for 100, 200 and 400 ppm chlorine.

Though Dainty (1971) used water having a temperature of 75 to 90°C to reduce the bacterial load by 80 per cent, such a high temperature can change the bloom of the carcase



and give a bleached appearence as observed by Anon...(1978). Therefore, it was not felt desirable to use hot water for this operations.

The present result by using 50 ppm chlorine water achieving a reduction of 77.9 per cent in the bacterial load could well be compared with more or less similar effect using 150 and 200 ppm chlorine by American Meat Science Association (1975).

Morton (1976) achieved a bacterial load to zero on the carcase by using equous solution of nascent chlorine. But it is more expensive and combursome.

The criteria for selecting the agent and mode of operation should be the availability, cost, operation facility, efficacy and after effects. By using the detergent, there should not be any considerable change in the physical quality and acceptability. A high concentration of chlorine in the water used for washing is likely to leave a taint in the carcase. Therefore it is felt desirable if more or less the same effect is achieved with a lesser concentration of chlorine in water, the latter is preferable. In the present operation this 20 ppm and 50 ppm chlorine water has achieved the same effect as that of 150 or 200 ppm water used by other workers. Therefore, this is a satisfactory method to reduce the bacterial load and may improve the keeping quality though

it was not attempted to compare the keeping quality of carcases in the present work. So it is suggested that washing carcases with water containing 50 ppm chlorine is a method which can be adopted to improve the hygienic quality of meat obtained even in carcases prepared under tropical conditions.

SUMMARY

SUMMARY

Beef is one of the most important food of animal origin marketed in Kerala. Infection is possible to the consumer if the meat consumed is contaminated with pathogenic bacteria. Higher bacterial flora in meat will adversely affect its keeping quality leading to economic loss to the nation. In order to assess the extent of bacterial contamination and to select suitable method for reduction of bacterial load in meat, the present study was undertaken.

Eighty four samples of beef obtained from meat stalls in and around Trichur Town was processed and tested using standard methods for the detection of aerobic plate count, coliform count, count of *Escherichia coli*, Faecal Streptococci and *Clostridium perfringens*.

All the eighty four samples had aerobic plate count more than 30×10^6 per gram of meat. Coliform count and *Escherichia coli* were present in all the samples. About 90 per cent of the isolated *Escherichia coli* were Eijkman test positive. Only one meat sample was free from Faecal Streptococci and fifteen samples were free from *Clostridium perfringens*. There was significant difference between the counts obtained from University slaughter house (USH), Mannuthy, East fort and West fort samples.

The minimum count noticed was in the samples collected from USH and maximum in the case of West fort samples.

The result of water samples tested from the above meat stalls did not show variation in bacterial count, corresponding to that noticed in the case of meat samples. There was significant difference in aerobic plate count in the water collected from USH and other places. The minimum count noticed was in the water samples taken from University slaughter house.

Seventeen carcases were subjected to spraying with chlorine water to study its effect on bacterial load. A reduction of 24.8 per cent, 59.9 per cent and 77.9 per cent in bacterial load was achieved by using water containing 10, 20 and 50 ppm available chlorine respectively. The chlorine water at the above strengths reduced the bacterial load significantly. Water containing 50 ppm available chlorine was found to be the best for its efficiency in reducing bacterial load without affecting the physical quality and consumers acceptability. Therefore, washing carcases with water containing 50 ppm residual chlorine, is suggested as an effective and safe measure when meat is prepared under low hygienic environment and to render the meat safe for the consumer and to enhance its keeping quality.

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TABLES

Table I

Mean of Bacterial count of meat collected from various sources

Place	Sample		APC x 10^6 per gram	Coliform (MPN) x 10^5 per 100 gram	*E.coli* x 100 per gram	Faecal Streptococci x 1000/gram	Cl.P perfringens x 100/gram
	No.	Percentage					
USH	10	11.95	36.4±3.03	2.91±0.37	20.5±3.00	28.11±6.67	0.82±0.79
Mannuthy	33	39.25	65.03±3.91	13.52±2.24	35.27±1.90	40.05±3.75	3.82±0.75
West fort	21	25.00	94.48±5.46	77.60±16.40	53.24±4.26	57.71±4.22	6.94±1.33
East fort	20	23.80	89.20±3.77	45.75±13.94	40.35±3.64	52.60±4.15	5.96±1.06

Table II a

Distribution of aerobic plate count (APC) in Meat from various sources

Source	USH		Mannuthy		West fort		East fort	
Range (count/g)	No. of sample	Per cent	No. of sample	Per cent	No. of sample	Per cent	No. of sample	Percent
30×10^6 to 50×10^6	9	90	11	33.33	1	4.76	-	-
50.1×10^6 to 70×10^6	1	10	7	21.22	1	4.76	3	15.00
70.1×10^6 to 90×10^6	-	-	10	30.30	8	38.08	10	50.00
90.1×10^6 to 100×10^6	-	-	5	15.15	9	42.88	5	25.00
Above 100.1×10^6	-	-	-	-	2	9.52	2	10.00
Total samples	10		33		21		20	

Table II b

Distribution of Coliform (MPN) in Meat from various sources.

Source	USH		Mannuthy		West fort		East fort	
Range (count/100 g)	No.of sample	Per cent	No.of sample	Per cent	No.of sample	Per cent	No.of sample	Per cent
$<5 \times 10^5$	10	100	12	36.36	1	4.76	5	25.00
5 to 25×10^5	-	-	15	45.45	9	42.93	8	40.00
25.1×10^5 to 50×10^5	-	-	6	18.19	3	14.18	1	5.00
50.1×10^5 to 75×10^5	-	-	-	-	-	-	1	5.00
75.1×10^5 to 100×10^5	-	-	-	-	-	-	1	5.00
Above 100×10^5	-	-	-	-	8	38.13	4	20.00
Total samples	10		33		21		20	

Table II c

Distribution of Escherichia Coli in Meat from various sources.

Source	USH		Mannuthy		West fort		East fort	
Range (count/g)	No.of sample	Per cent	No.of sample	Per cent	No.of sample	Per cent	No.of sample	Per cent
Below 800	-	-	-	-	-	-	-	-
800 to 1500	4	40	1	3.03	-	-	3	15.00
1501 to 3000	4	40	10	30.30	1	4.76	-	-
3001 to 5000	2	20	21	63.64	12	57.16	14	70.00
5001 to 7500	-	-	1	3.03	2	9.52	3	15.00
Above 7500	-	-	-	-	6	28.56	-	-
Total samples	10		33		21		20	

Table II d

Distribution of Faecal Streptococci in Meat from various sources

Source	USH		Mannuthy		West fort		East fort	
Range (count/g)	No.of sample	Per cent	No.of sample	Per cent	No.of sample	Per cent	No.of sample	Per cent
Zero	1	10	-	-	-	-	-	-
1 to 1000	-	-	-	-	-	-	-	-
1001 to 10000	2	20	5	15.15	-	-	-	-
10001 to 20000	-	-	-	-	-	-	-	-
20001 to 30000	3	30	4	12.12	-	-	-	-
30001 to 50000	3	30	17	51.52	9	42.83	12	60.00
50001 to 1 lakh	1	10	6	18.18	11	52.41	7	35.00
Above 1 lakh	-	-	1	3.03	1	4.76	1	5.00
Total samples	10	33	33		21		20	

Table II e

Distribution of *Clostridium perfringens* in Meat from various sources

Source	USH		Mannuthy		West fort		East fort	
Range(count/g)	No.of sample	Per cent	No.of sample	Per cent	No. of sample	Per cent	No. of sample	Per cent
Zero	7	70.00	7	21.21	-	-	1	5.00
10 to 100	2	20.00	6	18.18	3	14.28	2	10.00
101 to 500	-	-	12	36.37	8	38.12	7	35.00
501 to 1000	1	10.00	6	18.18	4	19.04	8	40.00
1001 to 1500	-	-	1	3.03	4	19.04	1	5.00
1501 to 2000	-	-	1	3.03	2	9.52	1	5.00
Total samples	10		33		21		20	

Table III

Reaction of <u>E.coli</u> to Eijkman test

Source	No. of colonies tested	No.of colonies positive	Per cent
USH	50	43	86.00
Mannuthy	165	150	90.90
West fort	105	94	89.50
East fort	100	90	90.00
Total	420	377	90.00

Table IV

Result of certain biochemical tests for differentiation of Streptococci isolates

Source	No. of colonies tested	Gram staining	Nitrate reduction		Mannitol fermentation		Catalase production	
			Number positive	Number negative	Number positive	Number negative	Number positive	Number negative
USH	50	Positive	50 (100)	-	15 (30)	35 (70)	8 (16)	42 (84)
Mannuthy	165	-do-	165 (100)	-	60 (36.36)	105 (63.64)	45 (27.27)	120 (72.73)
West fort	105	-do-	105 (100)	-	35 (33.33)	70 (66.67)	23 (21.90)	82 (78.10)
East fort	100	-do-	100 (100)	-	30 (30.00)	70 (70.00)	19 (19.00)	81 (81.00)

Note: The values in the bracket indicate the percentage.

Table V

Comparison of bacterial counts in meat from different sources

(t-values)

Source	APC	Coliform	*E. coli*	Faecal Streptococci	*Cl. perfringens*
USH V/s Mannuthy	21.24**	14.77**	18.67**	7.26**	10.87**
USH V/s West fort	31.53**	14.40**	21.97**	15.21**	13.48**
USH V/s East fort	39.76**	9.97**	15.39**	12.84**	14.01**
Mannuthy V/s West fort	19.99**	19.24**	18.37**	13.91**	12.33**
Mannuthy V/s East fort	19.62**	11.61**	5.93**	10.06**	7.62**
West fort V/s East fort	3.28**	6.11**	9.35**	3.57**	2.38*

** Significant at 1% level   * Significant at 5% level

Table VI

Result of bacteriological examination of water collected from Meat Stalls
(Mean values)

Source	No. of samples	APC x 1000 per ml	Coliform per 100 ml	*E. coli* per ml	Faecal streptococci/ml	*Cl. perfringens* per ml
USH	5	40.2±6.88	330±210.10	1.0±1.0	7.6±1.94	0±0
Mannuthy	5	79.4±7.34	1172.0±210.20	10.2±1.93	24.2±9.1	0.40±0.25
West fort	5	106.8±6.55	1398±430.24	10.8±3.02	35±14.74	1.4±0.40
East fort	5	93.6±5.96	1772±729.79	7.0±1.10	19.4±2.58	1.0±0.45

Table VII

Comparison of bacterial count in water from different sources
(t-values)

Source	APC	Coliform	*E.coli*	Faecal Streptococci	*Cl.perfringens*
USH V/s Mannuthy	3.9**	2.83*	4.22**	1.78	1.67
USH V/s West fort	7.01**	2.23	2.89*	1.85	3.5**
USH V/s East fort	5.87**	1.9	4.05**	3.65**	2.22
Mannuthy V/s West fort	1.50	0.47	0.17	0.62	2.13
Mannuthy V/s East fort	2.78*	0.79	1.29	0.51	1.18
West fort V/s East fort	1.49	0.49	1.18	0.96	0.67

** Significant at 1% level   * Significant at 5% level

Table VIII

Effect of Decontamination of carcases with different strength of chlorine

(APC x 10^3 before and after spraying)

Replication	10 ppm chlorine		20 ppm chlorine		50 ppm chlorine	
	Before	After	Before	After	Before	After
1	75	8.5	55	1.2	95	0.3
2	193	181.0	200	12.5	198	66.0
3	275	218.0	258.	130.0	215	54.0
4	2400	1800.0	2180	750.0	2600	400.0
5	2890	2500.0	2400	800.0	2810	270.0
6.	2930	2180.0	2160	1100.0	2900	850.0
7	1700	1100.0	1710.	820.0	2880	670.0
8	1100	850.0	1640	750.0	2100	580.0
9	150	72.0	162	41.0	175	44.0
10	141	80.0	165	37.0	103	30.0
11	165	99.0	185	48.0	195	57.0
12	210	190.0	228	144.0	240	101.0
13.	230	210.0	218	101.0	219	107.0
14	73	43.0	164	31.0	140	45.0
15	142	107.0	267	44.0	62	30.0
16	158	68.0	150	39.0	196	40.0
17	192	79.0	141	66.0	209	37.0
Total	13024	9785.5	12283	4914.7	15337	3381.3
Percentage reduction	24.8		59.9		77.9	

Table IX

Result of covarience analysis of decontamination of carcases.

Source	df	S.S. pre-treatment	Sum of products	S.S. post treatment	Residual S.S.	df	M.S.S.	F
Chlorine	2	29858.35	-311503.05	1315485.99				
Replication	16	50098925.49	22763326.28	10505173.25				
Error	32	1765446.98	2144600.86	3640868.95	1035693.61	31	33409.47 (E_1)	
Total	50	52162920.82	22830977.12	15461528.20				
Replication + Error	48	51864437.47	24907927.15	14146042.21	12949714.46	47		
Replication adjusted					11914020.86	16	744626.30 (T)	T/E_1 = 22.29
Chlorine + Error	34	2063995.33	1833097.81	4956354.95	1628029.16	33		
Chlorine Adjusted					592335.55	2	296167.78 (B)	B/E_1 = 8.86**

** Significant at 1% level

APPENDIX

APPENDIX

1. Biochemical tests - Sugar fermentation

a) Preparation of basal media. (Cowan, 1974).

Peptone - 10 g

Sodium chloride - 5 g

Dist. water - 1000 ml

Dissolved the ingredients in warm water, adjusted the pH 8.0 to 8.4 and boiled to 10 minutes. Filtered, adjusted the pH 7.2 to 7.4 and sterilized at 115°C for 20 minutes.

b) Sterilization of sugars.

Dissolved the sugar in distilled water and sterilized by filteration using Seitz filter.

c) Preparation of sugar media.

To 900 ml of peptone water already prepared added 10 ml of 0.2 per cent phenol red indicator and sterilized at 115°C for 20 minutes. Added aseptically 90 ml of the appropriate sugar solution which was previously prepared, sterilized and mixed. About 5 ml of the mixture was distributed in to sterile test tubes with inverted Durham's tubes and steamed for 30 minutes.

2. Brilliant Green bile broth 2% (APHA, 1953).

Peptone - 10gh

Oxbile - 200 ml

Lactose - 10 g

Brilliant green
1% aq.sol. - 13 ml

Dist. water - upto 1000 ml

Dissolved peptone in 500 ml water, added oxbile and lactose adjust pH to 7.4. Add brillient green solution and 5 ml 1% neutral red solution. Made up the volume to 1000 ml.

One and half strength media was prepared and distributed in 20 ml quantities with inverted Durham's tubes. Single strength media prepared and distributed in 5 ml quantities with inverted Durham's tube and autoclaved at 115°C for 15 minutes.

3. Catalase test (Cowan, 1974).

To an overnight incubated nutrient broth culture of the organism, was added one ml 3% H_2O_2 and examined immediately and after five minutes for evolution of gas, which indicated catalase activity.

4. Citrate utilization test

<u>Preparation of Simon's citrate</u> (Cowan, 1974)

Koser's citrate - 1000 ml

Agar - 20 g

Bromthymole blue 0.2% solution - 40 ml

Dissolved the ingredients, dispensed, autoclaved at 121°C for 15 minutes and allowed to set as slopes.

5. EMB agar (Levine) (APHA, 1953)

Peptone	-	10 g
Lactose	-	5 g
KH_2PO_4	-	2 g
Agar	-	13.5 g
EosinY	-	0.4 g
Methylene blue	-	0.065 g
Dist. water	-	1000 ml

Dissolved peptone, KH_2PO_4 by heating in 500 ml water, added Lactose and made upto 1000 ml. Adjusted pH 7.0±0.1. Then added EosinY, Methylene blue and agar, heated to dissolve and distributed in 200 ml quantities and autoclaved at 115°C for 15 minutes.

6. Gram's method of Staining (Cruickshank et al., 1975).

a) Solutions required

i. Ammonium oxalate crystal violet solution

Crystal violet - 20 g

Methylated spirit - 200 ml

Ammonium oxlate 1% in water - 800 ml

ii. Iodine solution

Iodine - 10 g

Potassium iodide - 20 g

Dist. water - 1000 ml

iii. Liquour iodi fortis

Iodine - 10 g

Potassium iodide - 6 g

Methylated spirit - 90 ml

Dist. water - 10 ml

iv. Iodine - acetone solution

Liquour iodi fortis - 35 ml

Acetone - 965 ml

v. Ziehl - Neelsen's carbol fuschin

Basic fuschin - 10 g

Absolute ethanol - 100 ml

5% phenol in water - 1000 ml

Dissolved the dye in the alcohol and added to the phenol solution.

vi. Dilute carbol fuschin

Ziehl-Neelson's carbol fuschin - 50 ml

Dist. water - 950 ml

b) Staining prodedure

1. Covered the slide with ammonium oxalate crystal violet and allowed to act for 30 seconds.

2. Poured off crystal violet stain and washed with iodine solution, covered with iodine solution and allowed to act for about 30 seconds.

3. Poured off iodine solution and washed with iodine acetone, covered with iodine acetone and kept for about 30 seconds.

4. Washed throughly with water.
5. Counter stained with dilute carbol fuschin for about 30 seconds.
6. Washed with water, blotted, dried and examined.

It was essential that the whole slide was flooded with each reagent in turn and that previous reagent was thoroughly removed at each step.

7. Indole test (Cruickshank et al., 1975).

i) Medium

Peptone - 20 g
Sodium chloride - 5 g
Dist.water - 1000 ml

Adjusted the pH to 7.4. Dispensed and sterilized by autoclaving at 121°C for 15 munutes.

ii) Kovac's reagent.

Isoamyl alcohol - 150 ml
p-Dimethyl-aminobenzal dehyde - 10 g
Con.Hydrochloric acid - 50 ml

Dissolved the aldehyde in the alcohol and slowly added the acid. Stored in the refrigerator. Shaken gently before used.

8. Motility test (Cruickshank et al. 1975).

The selected colonies were tested for motility by hanging drop method.

9. Methyl-Red test (Cruickshank *et al.* 1875).

i. Medium (glucose phosphate peptone water)

Peptone - 5 g

K_2HPO_4 - 5 g

Water - 1000 ml

Glucose, 10% solution
(Sterilized separately) - 50 ml

Dissolved the peptone and phosphate, adjusted the pH to7.6, filtered, dispensed in 5 ml amounts into the test tubes and sterilised at 121°C for 15 minutes. Added 0.25 ml glucose solution to each tube (final concentration 0.5%).

ii. Methyl Red indicator solution

Methyl red - 0.1 g

Ethanol - 300 ml

Dist.water - 200 ml

10. Nitrate broth (Cowan, 1974).

Potassium nitrate (KNO_3) - 1 g

Nutrient broth - 1000 ml

Dissolved KNO_3 in nutrient broth and distributed into tubes and sterilised at 115°C for 20 minutes.

11. Nutrient broth (Cowan, 1974).

Beef extract - 10 g

Peptone - 10 g

NaCl - 5 g

Dist. water - 1000 ml

Dissolved the ingredients by heating in the water. Adjusted to pH 8.0 to 8.4 with N-NaoH and boiled for 10 minutes. Filtered and adjusted to pH 7.2 to 7.4 and sterilized at 115°C for 20 minutes.

12. Nutrient agar (Cowan, 1974)

Nutrient agar was prepared by adding 20 g of agar for every 1000 ml of nutrient broth.

13. Peptone water

Peptone - 10 g

Dist. water - 1000 ml

Dissolved the peptone by heating in water, adjusted the pH 7.0. Autoclaved at 121°C for 20 minutes.

14. Phosphate Buffer (APHA, 1953).

i) Stock solution

Dissolved 34 g of KH_2PO_4 in 500 ml distilled water. Adjusted pH 7.2 with 1N NaOH and made up the volume to one litre.

ii) Working solution

Pipetted 1.25 ml stock solution and made up the volume to 1000 ml. Dispensed in 200 ml quantities and sterilize in autoclave for 15 minutes.

15. Plate count agar (APHA, 1953).

P Tryptone - 5 g

Yeast extract - 2.5 g

Dextrose - 1 g

Agar - 15 g

Aqa.Dist. - 1000 ml

Dissolved tryptone, yeast extract and dextrose, adjusted the pH 7.0±0.1 with 0.1N NaOH added, agar and autoclaved at 121°C for 15 minutes.

16. Slanetz medium

Peptone - 20 g

Yeast extract - 5 g

Glucose - 2 g

$Na_2HPO_4.2H_2O$ - 4 g

Sodium azide - 0.4 g

Agar - 10 g

Dist. water - 1000 ml

Dissolved all ingredients except agar. Cooled and adjust pH 7.2, added agar and autoclaved at 121°C for 10 minutes in 200 cc quantities. This media should prepare freshly.

Triphenyl tetra zolium chloride solution 1% (TTC)

Weighed accurately 1 g of TTC, dissolved in distilled water, made up the volume to 100 ml and sterilized by

filteration. This solution 1 cc was added to melted and cooled slantez agar before use

17. TSN agar (Mossel, 1956 and Marshall et al., 1965)

Peptone - 15 g
Yeast extract - 10 g
Sodium sulphite - 10 g
Iron citrate - 0.5 g
Polymixin B.sulphate - 0.02 g
Neomycin sulphate - 0.05 g
Agar - 13.5 g

Dissolved all ingredients except agar, adjusted the pH 7.2±0.02 and added agar. Sterilized in the autoclave for 10 minutes at 121°C.

To the liquid media at 47°C added 25 ml of thio glycollate buffer, mixed.

Thioglycollate Buffer

Weighed 5.7 g of dipotassium hydrogen phosphate (AR) and 2.8 g sodium hydrogen carbonate and added distilled water 100 ml. Prepared 13.3% sodium thio glycollate solution. Mixed 35 ml of the first solution and 15 ml of second solution to prepare thio glycollate buffer.

18. Voges-Proskauer test (Cruickshank et al. 1975).

i. Medium - glucose phosphate peptone water

ii. O' Meara reagent

Potassium hydroxide	- 40 g
Creatine	- 0.3 g
Dist. water	- 100 ml

Dissolved the ingredients in 100 ml water by shaking.

EVALUATION OF HYGIENIC QUALITY OF MARKET MEAT

By

P. KUTTINARAYANAN

ABSTRACT OF A THESIS

submitted in partial fulfilment
of the requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and Animal Sciences
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ABSTRACT

With a view to investigate the hygienic quality of market meat in and around Trichur town the present study was undertaken. Eighty four samples of meat were tested for detecting total aerobic plate count, count of coliforms, Escherichia coli, Faecal Streptococci and Clostridium perfringens.

Eighty four samples of beef obtained from different meat stalls had aerobic plate count ranging from 30×10^6 150×10^6 organism per gram of meat. Kerala Agricultural University Slaughter House (USH) gave the minimum count in comparison with other three places such as Panchayat meat stall, Mannuthy, Municipal Meat stall at West fort and East fort. The maximum count recorded from West fort sample had a count 150×10^6 organism per gram of meat.

Coliform count (MPN) ranged between 1.2×10^5 and 160×10^5 per 100 grams of meat. The maximum count obtained was from samples of East fort and minimum was that from USH. E.coli count ranged from 800 to 8800 organisms per gram of meat. The proportion of E.coli in the samples was corresponding to the APC in respect of the source of collection. All the randomly tested E.coli colonies were

indole producing, MR positive and VP and citrate negative. Ninety per cent of them were Eijkman positive indicating they were typical coliforms.

Faecal Streptococci were present in all except one sample. The count ranged between 0 and 110000 organisms per gram of meat. On testing the colonies by certain biochemical test, the result that the isolates were mostly of animals origin. About 15 samples were free from <u>Clostridium perfringens</u> and count ranged between zero and 2000 organism per gram of meat.

The counts had shown that among the samples collected, those from USH had significantly low count in respect of all organisms studied. Similar result was seen in case of the bacterial quality of water samples collected from all the four places.

Seventeen carcases were subjected to spray washing with different levels of chlorine such as 10, 20 and 50 ppm. The meat samples were collected from the surface, before and 15 minutes after spraying, and they were processed for estimating the bacterial load. A reduction in bacterial load was observed by spray washing with water containing 10, 20 and 50 ppm chlorine to the extent of 24.0, 59.9 and 77.9 per cent respectively and were significant at one per cent level.

Water with 50 ppm chlorine was found to be the best for washing carcase reduction in bacterial load without affecting its physical appearence and acceptability.