STUDY ON HYGIENIC QUALITY OF BUFFALO MEAT MARKETED IN DHARAN

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Food Technology Instruction Committee

Institute of Science Technology

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Study on hygienic quality of buffalo meat marketed in Dharan

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ABSTRACT

The present work was undertaken to assess the hygiene quality of Buffalo meat marketed in Dharan. Buffalo meat, swabs of Knives, swabs of Chopping cart and swabs of Hands of butchers were examined for microbiological parameters (TPC, total Coliforms, *E. coli, S. aureus*, *Salmonella* and *Shigella)*. A survey with the help of questionnaire was done to assess the sanitary condition and personal hygiene of meat shops and butchers.

Average value for TPC of meat sample was found to $3.59x10^7$cfu/g. The average coliform, *E. coli* and *Staphylococcus aureus* counts were $2.06x10^4$, $1.69x10^3$ and $9.67x10^3$cfu/g respectively. Except two samples, all samples were found to be infected with *Salmonella* where as all the samples were found to be *Shigella* positive.The average value for total plate count of Chopping cart, knives and palms of butchers were found to be $3.15x10^4$, $3.47x10^3$ and $2.01x10^4$cfu/cm^2 respectively. The average Coliform, *E. coli* and S*taphylococcus aureus* counts of chopping cart were found to be $1.11x10^3$, $9.8x\ 10^1$ and $6.2x10^2$cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of knives were found to be $1.31x10^3$, $1.66x\ 10^2$ and $2.83x10^2$cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of the palms of butchers were found to be $1.95x10^3$, $1.66x\ 10^2$ and $1.77x10^2$cfu/cm^2. Two swabs of chopping cart, three swabs of Knives and three swabs of hands were *Salmonella* free. Out of ten swab samples five samples of chopping cart, three samples of Knives and two samples of hands were detected for *Shigella.*

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CHAPTER I

INTRODUCTION

The microbiology of carcass meat is highly dependent on the conditions under which animals are reared, slaughtered and processed. The extent to which contamination occurs and the composition of the flora that results reflects the standard of hygiene in the slaughterhouse. The hide or skin of the particular animal and others being dressed in its close proximity is probably the major source of saprophytic species. In addition to skin, the gastro-intestinal and respiratory tracts, urine and milk are other important animal sources of infection. Generally *Escherichia coli* comprise a greater proportion of the total aerobic flora of the intestine than of the hide or fleece (Brown, 1982)

After slaughter and evisceration animal meat retains the general microbial characteristics that it had prior to slaughter. The surface of the animal is contaminated with soil, air and water borne organisms. Extremely high numbers of micro organisms are found in the animal's intestinal content, and it is expected that some of these will find their way to the surface of the carcass during the dressing operations. In addition, some apparently healthy animals may harbor certain microorganisms in the liver, kidneys, lymph nodes and spleen, and these micro organisms can get to the skeletal muscles via the circulatory system where they can be present in the muscle.

Contamination may also occur during the sticking operation during slaughter, and these microorganisms may be distributed via the circulatory system to the muscles. The meat carcasses are subsequently handled and moved into the commercial food distribution channels where they are cut into smaller units, and increasingly more numbers of micro organisms are added to the surfaces of the cut meat (Price *et al,* 1971).

Live bacteria may be present in lymph nodes, some of which remain attached to the carcass after evisceration. The gut of course contains enormous numbers of bacteria, many of which play a useful part in digestion. Some of these find their way to the carcass during slaughterhouse operations. Other organisms reach the carcass via butcher's hands, tools, clothing, etc. (Wilson *et al*, 1881)

The nature and level of microbial contamination in meat have important consequence in relation to public health, storage life and the type of spoilage of meat (Gracey and Collins, 1994).

The most important food poisoning bacteria belong to the genera *Staphylococcus, Salmonella, Clostridium* and *Campylobacter*. The Staphylococci are associated with the nasal cavities of man and animals as well as with other parts of the body. *Salmonella* are indigenous to the intestinal tract of man and animals but may enter foods from other sources contaminated from fecal matter. The Clostridia are from soil while *Campylobacter* spp. are animal associated. In addition to the above, *Bacillus cereus, Vibrio parahaemolyticus, Yersinia enterocolitca* and *Vibrio cholera* all cause gastroenteritis (Purohit, 1997)

Raw meat quality is often judged by the size of its microbial population able to grow at 30-37^{o}C. However, this count is most appropriately used to monitor hygiene, not quality (Brown and Baird-Parker, 1982).

Dharan Municipality (2112 hectare area), located in the eastern Terai of Nepal stretches from the edge of the northern Mahabharat hill range up to the Charkoshejhadi in the south, which separates it from the southern Terai. Currently (2005), Dharan has a population of 1.5 lakhs. They consume 1750 kg chicken, 1500 kg buffalo meat, 1400 kg pig meat, 1350 kg goat meat, and 540 kg fish and dried fish daily. Chickens are slaughtered at 179 places, pigs at 64, buffaloes at 10, and goats at 11 places. Buffalo meat shop in Dharan is spread all over city particularly saying 31 buffalo meat shops are found. These data imply that people of Dharan consume a significant amount of meat (Personal Communication).

1.1. Problem Statement

Evaluation of quality before consuming is a must for any food, and this is more so for commodities like meat, meat and egg: they are complete foods in themselves and support not only our life but also that of pathogenic microorganisms. Microbiological quality is important from public health point of view. Both consumers and sellers of Dharan are not very sensitive towards meat quality. Apparently the hygienic condition of meat sold in Dharan market is very poor. Although food poisoning incidences due to consumption of poor quality meat has not been recorded systematically to date, this does not necessarily imply that meat sold in Dharan is safe. In fact, no such systematic studies have been made on meat market of Dharan so far, let alone the recorded incidences of zoonotic diseases.

1.2. Objective of the study:

1.2.1. General Objective

The general objective of the present work is to study the general condition of meat market and hygienic quality of meat marketed in Dharan.

1.2.2. Specific Objective

The specific objectives are listed as follows

a. To enumerate the microbial load of buffalo meat marketed in Dharan
b. To enumerate the pathogenic flora of buffalo meat, e.g., *Salmonella*, *Shigella*, fecal coliforms, etc
c. To determine possible contaminating sources.
d. To enumerate the microbial load on possible contaminating sources.
e. To study the public awareness about meat hygiene

1.3. Limitation of the work:

- The study is limited to the investigation of some common pathogenic bacteria like *Salmonella* and hygiene indicators like fecal coliform.
- Investigation of parasites such as *nematodes, cestodes, trematodes,* etc., viruses and microbial toxins such as botulin toxin is not included in the present work.

CHAPTER II

LITERATURE REVIEW

2.1 The term Meat Quality

Quality is most easily defined as 'fitness for purpose' or 'conformation to specification'. In case of meat, the meat must be fresh, clean and wholesome to be at least acceptable (Anonymous, 2003)

There is not a single definition which can fully describe the "quality of meat". Health and ethical aspects may be as important as technological and sensory characteristics of the meat. Together they form what we call ‘meat quality’. To buy a piece of meat, factors like tenderness, juiciness, color and taste referred generally as eating quality will become more important. When it comes to processing factors like pH and water holding capacity will be of paramount importance. (Hambrecht, 2005)

It can be said that the quality of meat is the sum of chemical composition, physical properties, biochemical condition, morphological structure, sensory properties, nutritional value, processing and technological properties, hygienic condition and culinary properties and the result of their inter relationships or dependencies.(Ingr, 1990)

However, pathological condition and wholesomeness are essential parts of meat quality.

2.1.1 Factors affecting meat quality

2.1.1.1 Age

Change in muscle morphology and composition during growth greatly influences meat quality. Better quality buffalo meat can be obtained if the animals are slaughtered between 2 to 3 years of age. A slaughter weight of 250-300 kg or 26 months of age for male buffalo calves is considered to be optimum for meat production (Anjaneyulu *et al*, 1986).

Age affects the body composition of animals particularly amount of fat and also the proportion of muscle to bone. Meat from aged animals tends to be tougher than meat from young animals. This toughness is due to changes in the structure of connective tissue in older animals and not to the actual amount of fibrous type of tissue. In fact in very young animals the proportion of connective tissue is very high compared to that in mature animals.

Meat tends to be darker in older animals due to the deposition of brown pigments in muscle and also to the greater amounts of myoglobin. In very old animals senile atrophy occur which may be recognized by a reduction in a size of muscles, loss of fat and occasionally by a distinct dark brown color of muscle. (Wilson *et al,* 1981)

2.1.1.2 Sex

Carcasses from male animals have a lower proportion of fat and a higher proportion of lean meat than those of female animals of similar slaughter weight. (Wilson *et al,* 1981)

Castrated male buffaloes showed faster growth rate and higher dressing yields with better quality. Partial castration at 6 months of age was reported to be better in tenderness of meat (Anjaneyulu *et al*, 1986)

2.1.1.3 Plane of Nutrition

Nutrition may affect meat quality via feeding level and feed composition. A higher feeding level is said to have beneficial effects on tenderness and juiciness of the meat. Well-known are effects of dietary fat composition on the fatty acid profile in both the intramuscular fat and other fat depots. Fatty acid composition of the phospholipid fraction of the intramuscular fat may affect membrane stability, oxidation processes, flavor development and possibly water holding capacity.

A high degree of unsaturation causes the fat to become soft and susceptible to oxidation and makes it unsuited for production of e.g. salami or other sausages. Other feed ingredients do not directly influence meat or fat quality in the animal but affect its stress susceptibility. Although nutritional effects are most prominent with respect to fat quality, nutrition may also play a role in meat quality. (Hambrecht, 2005)

2.1.1.4 Breed/Genetics

There are significant breed effects for many meat quality traits such as water-holding capacity, pH or intramuscular fat. Meat from Piétrain pigs, for example, often exhibits the PSE condition due to the presence of the Halothane gene which causes high stress susceptibility. When a stress response is triggered, there is a striking increase in metabolism, intense production of heat and lactate and contraction of skeletal muscles. Animals show a higher muscle temperature both ante- and post-mortem and a more rapid

pH-decline post-mortem due to the increased turnover of glycogen to lactate. For economical reasons (high lean meat percentage), the gene is still present in some sire lines. Although it is recessive, carriers of the gene (only one copy of the gene present) still tend to have a worse meat quality than non-carriers

It leads to a decreased technological quality due to lower protein content in the meat and a low ultimate pH caused by an abnormal high glycogen content in the muscle cells which is converted to lactate. Duroc pigs, on the other hand, show a sometimes two-fold higher intramuscular fat content when compared to Landrace pigs and Large White which may have a positive effect on eating quality.

During the last years, a number of specific breed effects have been found to be caused by single major genes which have led to the search for specific major genes that influence meat quality. Selection by using modern DNA technology promises not only to improve meat quality but also to increase uniformity. (Hambrecht, 2005)

2.1.1.5 Transportation and handling of animal

In developing countries meat animals are transported from the farm to the slaughterhouse on foot, by road, or by rail. Frequently livestock must travel on foot for several days to reach the abattoir. Since the distances involved often are quite substantial and the management of the animals during this process is poor, transportation has deleterious effects that result in significant food losses. Livestock who have traveled long distances on foot or in transport frequently are insufficiently rested before slaughter, negatively affecting the quality of the meat. (http://files.hsus.org/web-files/PDF/soa_ii_chap12.pdf)

Often holding pens are overcrowded, causing unnecessary stress to the animals. The quality and condition of the carcass and its storage depend greatly on the care taken prior to slaughter. Nervous, tired, and excited animals may have a raised body temperature, causing imperfect bleeding. Muscular fatigue reduces glycogen content in the blood, which after slaughter changes into lactic acid, thus causing favorable conditions for spoilage and the growth of food-borne bacteria. Fatigue and excitement also cause penetration of bacteria from the intestinal tract to the meat. (Chambers and Grandin, 2001)

2.1.1.6 Slaughtering method

Slaughter methods vary widely and include, among others, simple decapitation, severing the medulla, and severing of the major blood vessels with or without previous stunning.

Animals going to slaughter should be rendered unconscious in order to make death as stress-free and painless as possible. Nevertheless, in the Jewish (kosher) and the Muslim (halal) slaughter of livestock, stunning generally is not allowed, and the animal is bled directly, using a sharp knife to cut the throat and sever the main blood vessels. This results in sudden and massive loss of blood, with loss of consciousness and death. These types of slaughtering can be very unsatisfactory since the animal may not be rendered unconscious and may suffer considerable discomfort and pain in the slaughter process. Many Muslim authorities permit some form of pre-slaughter stunning such as electric stunning of cattle, sheep, and poultry (Chambers and Grandin 2001).

The use of humane methods in the handling of livestock prevents needless suffering, results in safer working conditions, reduces meat losses, and improves meat quality. However, cruelty to animals exists in developing countries because of unsatisfactory slaughtering procedures and infrastructures. Animals may be pulled, beaten, or dragged on their way to slaughter and are allowed to see other animals being slaughtered. Animals frequently are slaughtered without being stunned. (Mann 1984).

2.2 Microbiology of Meat

Meat is an ideal culture medium for many organisms because it is high in moisture, rich in nitrogenous foods of various degrees of complexity, plentifully supplied with minerals and accessory growth factors, usually has some fermentable carbohydrate (glycogen), and is it a favorable pH (Frazier, 1997).

Meat demands strict hygiene during slaughter and further processing. It is an ideal culture medium for many microorganisms because it is high in moisture, rich in nitrogenous foods of various degree of complexity and plentifully supplied with minerals and accessory growth factors. Also it usually has some fermentable carbohydrate and is at a favorable pH for most microorganisms (Bacus and Brown, 1981).

Organisms will physically and chemically alter the substrate on which they grow, producing unwanted odors, tastes and colors. If mold contaminants are present, then

visible mold colonies may develop. Among some of these contaminants, frequently will be bacteria capable of causing disease or producing toxins dangerous to the human consumer. Ever since food poisoning statistics have been produced, meat and poultry dishes have been prominent as vehicles of illness (Wilson *et al.*, 1981).

The characteristic microbial populations developing in meat products are the result of the effects of the prevailing environmental conditions on growth of the types of microbes initially present in the raw materials or introduced by cross contamination or processing (Ford and Park, 1980). Factors affecting microbial growth in a food include both intrinsic and extrinsic factors (Mossel and Ingram, 1955). Intrinsic factors are predominantly chemical, including the concentration and availability of nutrients, pH, redox potential, buffering capacity, availability of water and structure of meat and meat products (Ayres, 1995). The extrinsic factors are concerned mainly with storage and processing conditions.

2.2.1. Sources of contamination

Meat may be contaminated by two ways viz. intrinsic contamination and extrinsic contamination. The word intrinsic was used to describe microbial flora occurring in deep tissues in contrast to extrinsic surface contamination received during dressing and handling (Ingram, 1972).

Bacterial contamination of carcasses may occur at virtually every stage of slaughtering and processing. Processing hygiene, however, aims at holding the initial bacterial numbers on a level as low as possible, since this affects shelf-life as the occurrence of pathogenic bacteria (Upmann *et al.*, 2000)

Microorganisms have been found in the lymph nodes, bone marrow and even flesh of healthy animals. Staphylococci, Streptococci, Clostridium and *Salmonella* have been isolated from the lymph nodes of red meat animals, *E coli* from intestine and hide, Clostridia spp from livers and pancreas of apparently healthy animals (Frazier, 1997; Nottingham, 1982). Ante-mortem infection may be increased by starvation, fatigue and shock.

The exterior of the animal harbors large numbers of many kinds of microorganism from soil, water and manure, as well as its natural surface flora. Molds, mainly *Cladosporium*, *Sporotrichum*, *Mucor*, etc; yeasts, mostly *Asporogenous* and bacteria,

mostly micrococcus, *Bacillus*, *Clostridium*, *Escherichia*, *Salmonella*, etc may reach the surface of meats and grow there.

The microbial spoilage of meat is influenced by the original bacterial content present in it and by secondary contamination during processing. Secondary contamination is mainly due to using contaminated equipments (cutting tools, chopping blocks, containers etc), the surrounding air and water and carrying agency. Man, as a carrier of different organisms, is the most important factor in the area of secondary contamination (Heinzal and KGaA, 1987).

Knives, cloths, air and hands and clothing of workers serve as intermediate sources of contamination. During handling of meat thereafter, contamination can come from carts, boxes, or other containers, form contaminated meat, from air, and from personnel (Frazier, 1997).

The essential problem in many developing countries is the failure to provide for hoists or hooks, hardware which permits the dressing of carcasses to take place off the floor. The contamination resulting from floor dressing of carcasses is considerable, especially where the removal of hides and the cleaning of stomachs are carried out in the same location as the dressing of the carcass itself (Mann, 1984).

Personal hygiene and particularly keeping the hands clean are important in relation to the spread of *Salmonella* of pathogenic varieties (Heinzal and KGaA, 1987).

Holding animals in vehicles or lairages without adequate litter and/or drainage frequently results in fecal soiling of the skin. Animal for slaughter are often very dirty, their legs covered with manure. In these cases, the knife will have to cut through manure and fecal residues, resulting in a great possibility for meat contamination (Chambers and Grandin, 2001).

Coliform bacteria, Gram negative mesophiles and psychrophiles and enterococci are often used as indicators of good plant hygiene (Brown and Baird-Parker, 1982).

2.2.2 Microorganisms of Public Health Concern

2.2.2.1 Aerobic mesophillic bacteria

The total plate count (TPC) expressed as organism/g on fresh meat or a meat product sets a limit to its shelf life. Meat will spoil with TPC at 10^6/g because of off odours. Slime and discoloration appear at 10^8/g. (Anonymous, 2003).

The most commonly used hygiene indicator to investigate the persistence of specific spoilage or pathogenic organisms is the total aerobic mesophillic count(30^{o}c) (Brown and Baird-Parker,1982).

Almost all food poisoning bacteria and most spoilage causing bacteria are mesophiles. A high TPC resulting from severe contamination during slaughter or processing will shorten the shelf life even in ideal conditions. It also indicates poor hygiene so that contamination with food poisoning bacteria is likely (FAO, 1991).

According to Inspected German Quality meat, maximum value for the TPC for fresh meat on cutting and packaging unit is $5x10^{6}$/sq. cm or g and the value is same for EU microbiological standards of cut meat for retail sale and further processing also. Danish Quality Assurance Warranty specifies freshly slaughtered meat must contain TPC on an amount less or equal to 10^{4}/sq. cm or g (Anonymous, 2003).

2.2.2.2 Coliforms

Members of total coliforms and fecal coliforms groups are referred to as indicator organisms since a quantization of their presence are used to indicate the potential presence of pathogens in foods. It is believed by some investigators that the higher the numbers of coliforms, the greater the possibility of pathogenic organisms being present. This indicator/pathogenic relationship however is scientifically debatable and by no means accepted unanimously by the scientific community.

Coliforms do not necessarily indicate contamination from a fecal source, in the sense of implying immediate contact with the feces. The presence of large numbers in a processed food indicates that the opportunity of proliferation might have occurred, which could also have allowed multiplication of *Salmonella,* Staphylococci*,* etc (Refai, 1979)

Coliforms (certain strains) can also produce illness in man, although meat has not been demonstrated as vector (Brown and Baird-Parker,1982).

Maximum limit for the Coliforms according to the EU Microbiological standards of cut meat and retail sale and further processing is $5x10^{3}$/g (Anonymous, 2003).

2.2.2.3 Spore formers

Spore formers are of two types viz. aerobic spore formers e.g. *Bacillus* spp such as *Bacillus cereus, B. subtilis,* etc and anaerobic spore formers e.g. *Clostridia* such as *Clostridium botulinum type* A and B, *Cl. perfringens* ,etc (Leistner, 1985).

Small number of *B. cereus* may be found in meat and poultry. Between 1960 and 1968, meat or meat products were implicated in majority of food poisoning outbreaks in Hungary. (Roberts, 1982)

Cl. perfringens type A is commonly found in meat, poultry and their products. The spores have various degrees of heat resistance ranging from a few minutes to several hours at 100^{o}c; both the heat resistant and heat sensitive strains have been implicated in food poisoning (Sutton and Hobbs, 1968).

2.2.2.4 *Salmonella*

Salmonella in red and white meat is a world wide problem. Food borne *salmonella* infection results from the ingestion of large numbers of the organism, which then multiply within the small intestine (Roberts, 1982).

Almost all members of the *Salmonella* genus are potentially pathogenic. *Salmonella* spp are common inhabitants of the intestinal tracts of many animals, especially cattle and during slaughter and dressing processes, they can easily contaminate food via fecal contamination.

Less than 1 to large numbers in foods have been implicated in outbreaks. Hence the presence of *Salmonella* at any level in meats is objectionable (Bachhil and Jaiswal, 1988).

The risk of *Salmonella* contamination to other foods and subsequent multiplication remains, even when the particular food in question is unable to support the growth. It is therefore undesirable in meat. Although one or few typhoid organisms are found to be sufficient to cause illness in human, it is believed that much higher number are required to cause food poisoning incidences (Corry, 1976).

When referred to EU Microbiological standards for cut meat and retail sale *Salmonella* should not be detected in 1 gram (Anonymous, 2003). The majority of the meat borne *Salmonella* incidences has been due to the live animal providing meat, and some cases due to under cooking of contaminated meat leading to survival of pathogens (Wilson *et al.,* 1981).

Salmonella can reach food from animal excreta at time of slaughter, from human excreta or from water polluted by animal or human sewage. They are brought into kitchen in raw meat and may be transferred to cooked foods via hands, surfaces, utensils and other equipment (Roberts, 1982).

2.2.2.5 *Staphylococcus aureus*

Meat is contaminated with *Staphylococcus aureus* by handling and by sneezing or coughing. Minute amounts of toxin will cause illness, which starts within 1-8 h of eating poisoned food. It does not produce off-odors or spoilage so it cannot be easily checked (FAO, 1991).

Counts of 10^5/g or less wouldn't be expected to result in enterotoxin production. Minor and Marth (1971) have shown that counts must be 10^7-10^8/g for detectable enterotoxin production. The greatest amount is produced at the optimum temperature for growth i.e. 35-37^{o}C (Roberts, 1982). $5x10^3$/g is the maximum limit for *S. aureus* on EU Microbiological standards of cut meat and retail sale (Anonymous, 2003).

2.2.2.6 *Clostridium botulinum*

As *clostridia* are part of the normal intestinal flora of animals there is a possibility that *Cl. botulinum* may be present. Human botulism is almost invariably caused by food which has been inadequately preserved, stored for some time and then consumed cold or without sufficient heating (Roberts, 1982).

Botulism, the most serious form of food poisoning, results from consuming food containing toxin of *Clostridium botulinum* Types A, B, E and F are the main causes in man. The spores, apart from type E are heat resistant and can withstand cooking procedures apart from steam under pressure. The toxins however, can be easily destroyed by heating (Hersom and Hulland, 1980)

2.2.2.7 *Listeria monocytogenesis*

Listeriosis occurs mainly in pregnant women, neonates, immunosuppressed patients and the elderly. The causative agent *Listeria monocytogenes* has been isolated from meat processing facilities including soil, sewerage, silage and raw meats. It is excreted on animal faeces. The presence of this pathogen on raw foods is likely to be unavoidable. The organism can grow at pH 4.6-9.6. It can grow in aerobic, micro aerophilic and anaerobic conditions and in the presence of CO_2 (Bobbitt, 2002, Gracey and Collins, 1994).

2.2.2.8 Yeasts and molds

They often manifest themselves in foods of low pH, low moisture, high salt or sugar content and can utilize organic acids, proteins and lipids. They spoil by causing off-color and flavor in meat products (Refai, 1979).

If insufficient oxygen is present, they use acid in the food and so increase the pH (Shapton and Shapton, 1991). Current evidence suggests that mycotoxins do not present a major health hazard (Brown and Baird-Parker, 1982).

2.2.2.9 Viruses and Parasites

Among the pathogenic viruses are those causing hepatitis A and gastroenteritis. In the UK, there are approximately 400 cases per year of Hepatitis A caused by viral infection, gastroenteritis cases total approximately 11,000 per year, having increased from approximately 4500 cases per year in the early 1980's. Hepatitis A is of importance in cold meats (Shapton and Shapton, 1991). However, the fate of viruses present in meat has received little attention.

The most important parasites in meat inspection are those transmissible to man by consumption of the flesh of affected animals, while other parasites, though not transmissible to man may render the flesh or organs repugnant and therefore unmarketable e.g. extensive muscular sarcosporidiosis.

The parasites of importance are *Nematodes* (round worm), *Cestodes* (tapeworm), *Trematodes* (flukes), Protozoa and Arthopoda or joint footed animals, including flies and linguatula (Gracey and Collins, 1994). Control of such infections can be achieved by avoiding unsanitary disposal of human faeces near cattle or swine feeding areas and by proper cooking.

Frequent consumption of raw or under cooked meat where there is little inspection can lead to the development of trichinosis in the consumer (Roberts,1982).

2.3. Meat production and consumption in Nepal

Nepal has considerable livestock resources, but meat is largely a product of non-commercial enterprises. Despite this, meat is an important part of the Nepalese diet; and lean meat is prized as a basic source of high quality protein and vitamins. However, the actual consumption of meat and meat products in the country is influenced by religious,

cultural and economic factors. For example, the Newar, Gurung, Limbu, Rai, Tamang, and Magar, in particular, are avid consumers of meat. Goat, Sheep, and duck meat is acceptable to almost all Nepali while buffalo meat is preferred by Newar and pig meat by Magar, Rai, Limbu and Tharu. Though earlier chicken meat was consumed by all castes other than Brahmin and Chettri, however, with the change of time, the ethnic barrier for meat consumption is loosened. Even so 2 % of the total population is estimated to be vegetarian (Singh, 1994; Anonymous, 2003).

Meat production in Nepal from various species of animals in fiscal year 2004/2005 is given in Table 2.3.

Table 2.3 Meat production in Nepal in 2004/2005

Meat Source	Production in M ton
Buffalo	138953
Sheep	2744
Goat	41698
Pig	15724
Chicken	15461
Duck	237
Total	214817

(Source: ABPSD, 2005)

Meat is one of the most expensive food items in Nepal. Meat consumption patterns depend on the level of income of individuals. Meat consumption is higher in cities and towns than in rural areas. Consumption of meat is higher during festivals. Most of the meat is consumed fresh (FAO, 1991).

As the data on meat production shows, meat production from buffalo is highest. Buffalo meat contributes 64.68 % of the total meat supply in the country. The main reason for its popularity may be because of its low cost in comparison to other meats and also its versatility.

The major products made from buffalo meat in Nepal are *sukuti, momo, keema* curry, *choyela, kachila,* meat balls and sausages (Majupuria, 1997). In Newar community, a popular meat item known as *Kachila* is eaten without cooking. If the meat is contaminated with pathogens, there is a potential danger of food poisoning.

Buffalo meat has been developed as an animal resource to produce meat in many countries e.g. Italy, Egypt, Bulgaria, Australia, Laos, Indonesia, etc. Emphasis on future research has been implied for exploitation of male buffalo calves as a potential source of meat production in India (Sharma and Mendiratta, 1999).

Buffaloes are kept for three purposes; work, milk and meat. Buffalo meat contributes 65% of the total meat supply in the country. Several buffalo breeds have been developed in India, but information about buffalo breeds in Nepal has not been found yet. However, mainly two breeds viz. Local and *Murrah*-Local crossbred is found in Nepal. *Mehsana* breeds are also domesticated in some parts of Nepal.

The meat animals are slaughtered on demand and have no regular and organized meat marketing. In the urban areas, the collection agents go to various villages, buy animals and supply to the butcher. In the hills and mountains, male buffalo calves are sold for slaughter at day zero or so to fetch more milk from the mother (Singh, 1994)

2.4. Meat hygiene in Nepal

In Nepal, lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses of meat as well as invaluable by-products. (Subba, 1996).

In developing countries, a high percentage of animal slaughter takes place in rural areas under very primitive conditions that do not meet even minimal technical and hygienic requirements. Animals are slaughtered in all kinds of places, such as converted buildings or rooms, under the shade of trees, and on open, bare ground. Animals that have been slaughtered on the ground are then hoisted via the gantry so that the carcass can be dressed. When rural slaughtering takes place on relatively small premises, very simple equipment, such as hooks or ropes for hanging animals and chopping blocks for breaking down carcasses, may be available. However, it remains a common practice to dress carcasses on the building floor. Under these conditions, the utilization of animal by-products generally is low or non existent, since the byproducts are considered a nuisance. (Hambrecht, 2005).

When meat is sold on one or two market days, meat stalls often are crowded, and customers lean on the stall; the meat becomes contaminated through contact with their hands, bank notes, baskets, clothes, and other objects. The behavior of butchers is not always the most appropriate from a hygienic point of view and may contribute to the

problem. In urban areas the traditional marketing of meat begins with early morning slaughter and delivery of the unchilled meat to the marketplace a few hours later. The FAO recommends that in the long term this be improved to a complete "cold chain" system, with the meat being cooled down at the slaughterhouse and then transported in refrigerated trucks to controlled butcher outlets. The development of the meat sector, in particular in the rapidly expanding population centers, will have to move in this direction for both public health and environmental reasons (Garcia de Siles *et al*. 1997).

Once the meat leaves the abattoir, its hygienic quality also is influenced by careless and poor handling. Carcasses, quarters, unwashed offal, and other items are placed together on the floor of the market or on dirty concrete or wooden tables in meat shops, increasing the microbiological contamination of the meat (Hambrecht, 2005).

Slaughtering places are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents and dirty effluents and are not protected against dogs, rodents and insects. Meat products produced under such conditions are generally spoiled due to bacterial contamination and may cause food poisoning.

Due to lack of meat inspection, meat from diseased or parasite- infected animals has been the source of infections and transferable diseases to humans as well as animals. Besides meat quality is adversely affected by careless handling conditions in slaughtering places as well as in the meat market or shops (Joshi, 1991). It is estimated that 1 gram of fresh bovine feces contains $5x10^8$ bacteria and fattening buffalo is known to pass approximate 40 to 42 kg of dung daily.

The butchers of Kathmandu valley utilize sides of roads, banks of rivers, often ground of their house or any available open places for slaughtering animals. In addition, meat is hung all day in unhygienic surroundings. Meat and gut are kept together for sale by vendors. Microorganism e.g. *E. coli, Staphylococcus aureus,* etc can be readily transferred to meat under such conditions, and there is a potential danger of food poisoning or intoxication (Karki, 1995).

65.7% of butchers of Kathmandu valley kept offal disposal container and 68.6% of butchers have dog proof provision on selling counter. Where as 64.9% of butchers do not know about meat borne diseases and 14.29% have refrigeration facility. Primal cuts of meat are transported to shops by rickshaw or in baskets (Joshi and Olesen, 1999).

Several reports have been published on microbiology of meat from different parts of the Nepal with different organism pattern. Karki 1995 studied the bacteriological quality

of poultry and buffalo meat of Kathmandu valley. He reported that *E. coli* and *Staphylococcus aureus* was found in all the samples. Other isolates were *Klebsilla oxytoca, Proteus vulgaris, Enterobacter aerogens, Salmonella arizone, Citrobacter diversus, Tautomella ptyseos, Providencia rettgeri, Plesiomonas shigelloides, Pseudomonas aeruginosa, Streptococcus fecalis* and *Bacillus spp.*

According to the annual report of NARC in the fiscal year 2044/45, *Enterobacter* and *Pasterulla spp* were found to be the most predominant organism from cow and Buffalo samples.

According to the annual report of NARC different bacterial samples were examined in the fiscal year 2047/48 and reported that *E. coli* was found the most predominant organism followed by *Salmonella spp*, *Staphylococcus spp*, *Citrobacter spp* and *Pseudomonas spp*.

Munankami 1998 has reported presence of *Enterobacteriaceae*, total Coliform, *Staphylococci* and *Salmonella* in a significant amount in the buffalo meat purchased from the local market of Dharan.

Prasai (2000) studied the microbiological pattern of raw meat of Kathmandu valley. He has reported *E. coli* as the most predominant organism. Other isolates were *Staphylococcus aureus*, *Salmonella spp*, *Bacillus spp*, *Pseudomonas aeruginosa*, followed by *Proteus vulgaris*, *Enterobacter aerogenes*, *Citrobacter diversus*, *Klebsiella oxytoca*, *Streptococcus faecalis*, *Citrobacter freundii*, *Providencia rettgeri*, *Proteus mirabilis* and *Enterobacter cloacae*.

2.5. Buffalo meat marketing system in Dharan

Buffalo are usually purchased in livestock markets from brokers and traders who obtain the animals from villages of Nepal as well as from neighbor country India. Butchers themselves purchase either from brokers, traders or agents at nearest collection points Railway, Ghopa (far western part of the city) and Inarua municipality (Western parts).

The meat shops are dispersed all over the city centering main market area. It is usual for butchers to slaughter clandestinely in early in the morning on open field. Slaughtering sites are usually unhygienic, often unpaved and poorly drained. Carcasses are held in the same areas as those used for slaughtering, often amongst the debris (blood, legs and heads, gut contents) of earlier slaughters. Animals are mistreated using cruel methods such as restraining their legs with tightly bound ropes. The water used for cleaning is often heavily

polluted with dung and the same water is forwarded to clean weapons and carcasses before they are transferred to the shop.

The condition of shops where meat is sold does not comply with minimum expected requirements for hygiene and quality. Animals do not under go any health inspection and the water used for cleaning is dirty. The transportation of buffalo meat from slaughtering site to meat shop is done with public vehicles which are unsuited to carrying food products. Auto-tempos, Rickshaws, Cars and Hand-carts all are used and meat is carried unwrapped exposing to flies and dust. Shops do not have refrigeration facility to keep meat in chilled condition. Even many shops do not have toileting facility (Personal Communication).

2.6 Chemical composition of Buffalo meat

As in other species, the degree of fatness and age at slaughter affect the proximate composition. Meat from fattened buffaloes, compared to the meat of leaner animals, has a lower percentage value for moisture, slightly lower values for protein and total ash and higher value for fat.

Table 2.6: Nutritional value of buffalo meat

Parameters	Amount
Moisture (%)	76.3
Protein (%)	21.9
Fat (%)	0.9
Ash (%)	0.9
Energy (cal/100g)	102
Cholesterol (mg/100g)	46.3
pH	6.4

(Source: Ranjhan, 1999)

Buffalo meat is leaner and has less fat as compared with beef. It has less cholesterol also. Although the data for buffalo meat are not so comprehensive, one may assume that the nutritive value is at least equal to that of beef and veal. Serdjerk and Bistritreki (1956) confirm that buffalo meat contains more proteins, phosphorus and iron (FAO, 1990).

2.7. Importance of HACCP in quality of Meat

An important priority in meat production is to minimize contamination with enteropathogenic organisms during slaughter, dressing and subsequent handling of meat (FAO, 1992). Although microbiological testing of foods is an important tool to ensure safety, such testing has the disadvantages that it normally requires time and it often detects problems only after they occur (Potter, 1996). A careful analysis of microbiological hazards can be made and an in-house, effective monitoring system for quality assurance applied.

HACCP plays an important role in retaining good microbiological quality and stability of meat. HACCP is basically a statement of a preventive system of controls based on hazard analysis and critical control points. This involves the identification and control over those processing parameters whose loss of control would result in on unacceptable risk to consumers (Frazier, 1997).

With regard to meat production, the HACCP concept systematically identifies potential hazards in the entire chain from animal production to consumption and ranks them according to severity and likely frequency. This covers facilities, equipment and operation and is intended to augment and refine the various codes of manufacturing practice undertaken industry. The procedure is intended to enable management to take preventive rather than depend on intensive testing of the end-products (FAO, 1992).

CHAPTER III

MATERIALS AND METHODS

3.1 Research design

The area of research reflecting the quality of buffalo meat marketed in Dharan consisted of three parts:

a. Laboratory analysis of meat samples,
b. Laboratory analysis of possible contaminating source, and
c. Sanitary Inspection and hygiene assessment by survey

3.2 Meat sample collection plan

The Dharan municipality is divided into 19 wards. Microbiological Quality of marketed meat was analyzed on the basis of domestic consumption and simple uses. There are 10 slaughtering sites and altogether 31 meat shops. About 1500 kg of buffalo meat/day is consumed in the city.

During sampling, it was assumed that the meat qualities do not change in the short time (2-3 hrs), and if any, are fairly regular. Buffalo meat samples were collected from randomly chosen 10 places (out of 31 shops).

A sample size of 250g from each place was collected in sterile polythene bags and analyzed within 2 hrs of collection. The samples were kept inside the sterile polythene plastic bags without touching by the collector.

Generally the samples were collected in the morning time of at 6-7 AM. Sample was processed immediately as soon as possible. An ice box was used during the collection to discourage the growth of microorganisms.

3.3 Chain of custody procedures

Properly designed and the executed chain of custody forms ensure sample integrity, from sample collection to data reporting. This includes the ability to trace possession and handling of sample from the time of collection through analysis and final disposition. This process is referred to as “Chain of Custody” and is necessary to demonstrate sample control when data are to be used for routine control of samples.

The following procedures were performed in the present study;

a. Manual sampling was done.
b. Sample containers were made of polythene plastic packages.
c. 250-300 gram samples were taken to comply with the sampling, handling, analysis, storage and preservation requirements.
d. Duplicate destructive samples were taken from each sampling sites.
e. Information on sample was collected from the seller himself and other relevant information on the site was noted by the collector.
f. Total sampling time took half an hour and the sample was transported in a protected condition (in an icebox) to the laboratory within an hour of completion of sampling.
g. During analysis, parameters were processed with the prime priority and analyzed immediately.

3.4. Preparation of Meat Sample

3.4.1. Homogenization

25 gram of meat sample was aseptically transferred into meat mincer(National meat grinder, Model-MK-G10N, Matsusiuta Electric Ind. Company Ltd.) and 225 ml sterile distilled water was also added in the same machine and homogeneous mixture of sample was obtained. Before starting the mincer, it was thoroughly washed with clean water, distilled water and finally with 70% alcohol (Lattuada and Dey, 1998 and Brown,1982)

3.4.2 Serial Dilution of Homogenate

1 ml of that sample homogenate was pipetted and mixed with a tube containing 9 ml distilled water. This was then shaken well and labeled as 10^{-2}.

From first dilution, 1 ml sample was transferred to the second tube containing 9 ml distilled water and shaken well and that tube was labeled 10^{-3}.

Similarly for 3rd, 4th, 5th and 6th tubes, the same process was repeated and the tubes were labeled 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively (KC and Rai, 2000).

3.5. Sample collection from possible contaminating source

Sample from hands of butcher, chopping block and knife was collected from 10 shops out of 31 meat shops. Cotton wool swabs of 4 cm length and 1.5 cm thickness were used. Distilled water was used as diluent (Harrigan and McCane, 1979).

3.6. Analysis

3.6.1. Total Plate Count (TPC)

Total plate count was determined by pour plate method according Harrigan and McCane (1979) using plate count agar and distilled water as diluent.

3.6.2. Total Coliform

Coliform count was determined by pour plate method according to Varadraj (1993).

3.6.3. Fecal Coliform

Enumeration of Fecal coliform was done according to Varadraj (1993).

3.6.4. *Staphylococcus aureus*

Staphylococcus aureus enumeration was carried out according to Brown (1982) and Varadraj (1993). Coagulase test was done for confirmation.

3.6.5 *Salmonella* detection

Salmonella was detected according to the Varadraj (1993**)** Maaβen and stolle (2005) with some modifications as following

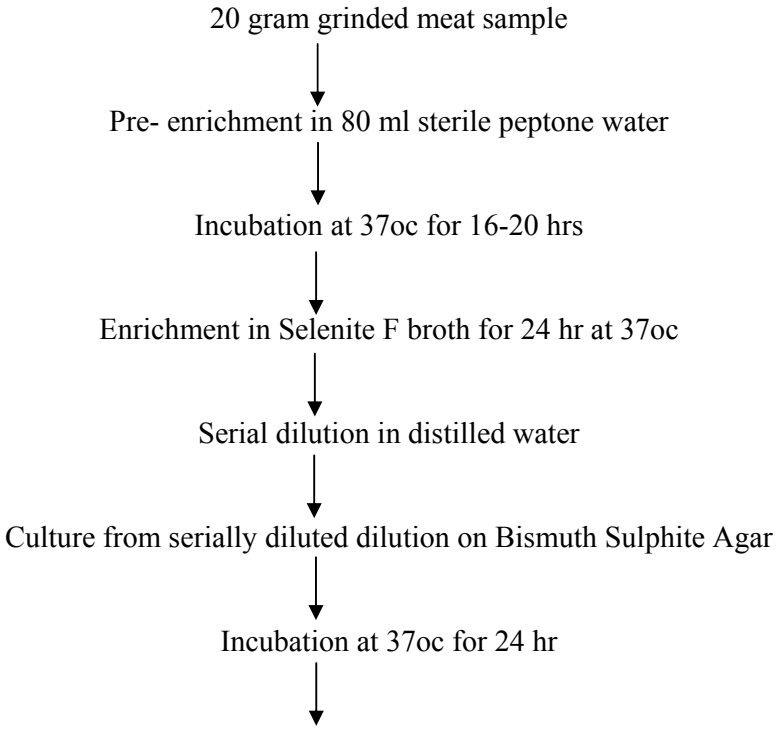

Figure 3.6.5 Flow chart for detection of Salmonellae species

3.6.6. *Shigella* detection

Shigella was detected according to Harrigan and McCane (1979).

3.7. Study of Sanitary condition of meat shops and Personal Hygiene

A questionnaire (See Appendix A) was prepared to study *s*anitary condition of meat shops and Personal hygiene of butcher. Questionnaire was compiled along with the study of that area. The data related to the sanitary problems of that area around sampling site was collected and situational analysis conducted.

3.8. Data Analysis

Of the six parameters analyzed except two (*Salmonella* and *Shigella*) were statistically analyzed. The raw data were statistical processed for significant difference by ANOVA (Two factors without replication) in the computer using Data Analysis feature of Microsoft Office. LSD testing was done according to Gomez and Gomez 1983.

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Analysis of Meat samples

The results of microbiological analysis of meat samples obtained from ten different locations of Dharan are presented in Table 4.1.

Table 4.1 Microbiological analysis of meat

unit: *cfu/gm*

Source/Site	TPC	TC	*E. coli*	*S.aureus*	*Salmonella*	*Shigella*
A	$1.8x10^7$	$9.7x10^3$	$3.6x10^2$	$3.1x10^3$	+	+
B	$2.6x10^7$	$3.9x10^3$	$2.8x10^2$	$3.1x10^3$	+	+
C	$2.2x10^6$	$1.0 x10^3$	$1.5x10^2$	$6.0x10^3$	-	+
D	$3.8x10^7$	$4.2x10^4$	$2.3x10^3$	$1.7x10^4$	+	+
E	$4.6x10^7$	$3.1x10^4$	$2.9x10^3$	$2.6x10^3$	+	+
F	$3.7x10^6$	$5.8x10^3$	$1.2x10^2$	$4.9x10^3$	+	+
G	$9.6x10^7$	$6.4x10^4$	$5.2x10^3$	$1.2x10^4$	+	+
H	$5.3x10^7$	$2.7x10^4$	$1.6x10^3$	$4.1x10^4$	+	+
I	$4.1x10^6$	$2.6x10^3$	$4.7x10^2$	$4.2x10^3$	-	+
J	$7.2x10^7$	$1.9x10^4$	$3.5x10^3$	$2.8x10^3$	+	+
Average	$3.59x10^7$	$2.06x10^4$	$1.68x10^3$	$9.67x10^3$		

(Note:

TPC = Total Plate Count, TC = Total Coliforms, *E. coli = Escherichia coli, S. aureus = Staphylococcus aureus,* ND = Not detected + = Positive and - = Negative)

Meat and meat products are favorable growth media for microorganisms including many pathogens because of their high nutritive value. The increasing population, urbanization, and modernization of the Dharan are also responsible for the pollution. The impact of pollution is also on various food borne diseases due to contamination by various pathogenic bacteria. Microorganisms set into the meat and meat products by water, unclean utensils, knives, unscientific slaughtering practices and cruel handling methods,

besides, environmental contamination and handling of meat in its preparation and sales. Due to lack of scientific methods of storage and due to lack of knowledge of microorganisms, many types of microorganisms introduce into the meat. Once microorganisms are introduced into the meat, they multiply rapidly and reach levels sufficient to produce infections or intoxications depending upon the types of invasion.

The number of microbes in the meat and meat products at any given time depends on its handling, storage condition, storage temperature and length of time it has been kept. The contaminating organisms may include those responsible for food borne illness. But the number or dose of organisms necessary to infect or to produce sufficient toxin to cause symptoms not only varies with the species and kind of organisms but also varies with the resistance of the person who consumed the meat and its products. Even though the microbial population in the meat does not cause food borne disease, certain microbial contamination is an indicator of poor sanitary practice in the processing and storage of meat. Meat is generally checked for the presence of indicator organisms such as *E. coli* and coliforms to indicate the possible contamination with viscera or fecal material (Brown and Baird-Parker, 1982).

Table 4.1 shows the average, maximum and minimum values for total plate count of meat samples. Average value was found to be $3.59x10^7$Cfu/g with the maximum value of $9.6x10^7$cfu/gm and minimum value $2.2x10^6$Cfu/g. The average *coliform*, *E. coli* and *staphylococcus aureus* counts were 2.06×10^4, 1.69×10^3 and $9.67x10^3$cfu/g respectively. Except sample C and I, 80% samples were found to be *salmonella* positive whereas all the samples (100%) were found to be *Shigella* positive.

The microbiological condition of fresh buffalo meat of local market can be assumed to be heavily contaminated with spoilage and pathogenic organisms, keeping in mind the unhygienic slaughtering conditions and lack of microbiological standards regarding meat in Nepal. This can be further demonstrated by following studies.

In the previous study of Munankami (1998) counts of $1.4x10^5$, $1.3x10^5$ and $5.6x10^4$ cfu/gm total *Enterobacteriaceae*, total *Coliform* and pathogenic *Staphylococci* count in the buffalo meat purchased from the local market of Dharan have been reported. *Salmonella* was also reported to be present in the meat.

Taking the case of Kathmandu valley a few earlier studies have reports as following. Karki (1995) found the total bacterial and total *coliform* count of buffalo meat $1.1x10^3$ to $1.8x10^5$ Cfu/gm and $3.0x10^1$ to $6x10^4$Cfu/g respectively from the samples of different

locations of Kathmandu valley. Prasai (2000) has reported the total viable count, total Coliform count and total *Staphylococcus aureus* count of buffalo meat of different places of Kathmandu in the range $1.1x10^6$ to $2.9x10^7$, $1.6x10^5$ to $1.1x10^6$ and 0 to $4.2x10^4$ cfu/g respectively. It shows that meat hygiene is very poor in Nepal as a whole.

Taking the reference of microbial standards of Europe and United States (Appendix A) the average total plate count was found to be higher than the inspected German quality meat standards referred for cutting and packaging plant which is less than 5.0×10^6/g. It was also greater than the Oregon state microbiological standard for fresh meat i.e. 5×10^6/g. The average total coliform count of the analyzed sample was also found beyond the EU microbiological standard of cut meat for retail sale and further processing i.e. 5×10^3/g.

The *E. coli* count of the market sample of buffalo meat of Dharan was also found to be higher than the Oregon state microbiological standards of maximum 50/g. The average *Staphylococcus aureus* count of the samples collected from the market was found higher than the maximum limit of 5×10^3/g of EU microbiological standards of cut meat for retail sale and further processing. EU standards for meat require *Salmonella* negative in 25 gm (Anonymous, 2003), but *Salmonella* was detected in most of the samples.

Raw meat containing large numbers of bacteria does not present a health hazard but it should be viewed as having been produced unhygienically or poorly stored or contaminated during processing and it poses particular risk if it is eaten raw (Brown and Baird-Parker, 1982).

There are reports that count of 10^5/g or less of *Staphylococcus aureus* wouldn't be expected to result in enterotoxin production. A food contaminated with a few toxin forming staphylococci is also not a danger to the consumer. Minor and Marth (1971) have shown that counts must be 10^7-10^8/g for detectable enterotoxin production.

The risk of *Salmonella* contamination to other foods and subsequent multiplication remains, even when the particular food in question is unable to support the growth. It is therefore undesirable in a food because other foods may be contaminated and even low numbers may cause illness (Corry, 1976). Although one or few typhoid organisms are found to be sufficient to cause illness in human, it is believed that much higher number are required to cause food poisoning.

4.2. Analysis of swab samples of chopping cart

Results of analysis of swab samples of different chopping cart of meat shops are presented in Table 4.2

Table 4.2 Microbiological analysis of Chopping cart swabs

unit: *cfu/cm*^{2}

Source/Site	TPC	TC	*E. coli*	*S.aureus*	*Salmonella*	*Shigella*
A	$3.3x10^4$	$1.0x10^2$	ND	$2.0x10^2$	+	+
B	$4.9x10^4$	$1.5x10^3$	ND	$9.0x10^2$	-	+
C	$5.6x10^4$	$2.3x10^2$	$1.0X10^2$	$3.0x10^2$	-	-
D	$3.5x10^3$	$1.7x10^2$	$3.0x10^1$	$5.0x10^2$	-	+
E	$6.5x10^4$	$2.0x10^2$	ND	$2.0x10^2$	-	-
F	$4.3x10^3$	$2.4x10^3$	$6.0x10^2$	ND	-	-
G	$3.7x10^4$	$1.4x10^3$	$2.5x10^2$	$1.3x10^3$	+	+
H	$3.8x10^3$	$2.0x10^3$	ND	$2.1x10^3$	-	+
I	$6.2x10^3$	$5.0x10^2$	ND	$3.0x10^2$	-	-
J	$5.7x10^4$	$2.6x10^3$	ND	$4.0x10^2$	-	-
Average	$3.15x10^4$	$1.11x10^3$	$9.8x10^1$	$6.2x10^2$		

Note: TPC = Total Plate Count, TC = Total Coliforms, *E. coli* = *Escherichia coli, S. aureus* = *Staphylococcus aureus,* ND = Not detected + = Positive and - = Negative

Meat demands strict hygiene during slaughter and further processing. It is an ideal culture medium for many microorganisms because it is high in moisture, rich in nitrogenous foods of various degree of complexity and plentifully supplied with minerals and accessory growth factors. Also it usually has some fermentable carbohydrate and is at a favorable pH for most microorganisms (Bacus and Brown, 1981).

The contamination of meat and meat products by bacteria is attributed to the exposure of meat to different sources of microbial contamination including contact with hide, viscera, mucous secretion, hands and clothing of personnel, water used for washing carcass and even air in the processing and storage area (Frazier and Westhoff, 1997).

4.3. Analysis of swab samples of Knife

Results of analysis of swab samples of knife of different meat shops of Dharan are presented in Table 4.3

Table 4.3 Microbiological analysis of knife swabs

unit: *cfu/cm*^{2}

Source/Site	TPC	TC	*E. coli*	*S.aureus*	*Salmonella*	*Shigella*
A	$4.1x10^3$	$3.8x10^3$	$1.9x10^2$	ND	-	-
B	$2.9x10^3$	$1.7x10^3$	$3.0x10^2$	$1.7x10^2$	+	-
C	$5.2x10^3$	$6.0x10^2$	ND	$2.0x10^2$	-	-
D	$3.9x10^3$	$2.5x10^3$	$2.1x10^2$	$3.1x\ 10^2$	-	+
E	$2.7x10^3$	$3.0x10^2$	$1.4x10^2$	$1.2\ x10^2$	-	-
F	$2.2x10^3$	$9.0x10^2$	ND	ND	-	-
G	$3.1x10^3$	$1.2x10^3$	$2.3x10^2$	$3.0x10^2$	+	+
H	$3.5x10^3$	$5.0x10^2$	$3.0x10^1$	ND	-	-
I	$2.8x10^3$	$1.1x10^3$	$4.1x10^2$	$5.0x10^2$	+	-
J	$4.3x10^3$	$5.0x10^2$	$1.5x10^2$	$2.3x10^2$	-	-
Average	$3.47x10^3$	$1.31x10^3$	$1.66x10^2$	$1.83x10^2$		

Note: TPC = Total Plate Count, TC = Total Coliforms, *E. coli* = *Escherichia coli, S. aureus* = *Staphylococcus aureus,* ND = Not detected + = Positive and - = Negative

Table 4.2, Table 4.3 and Table 4.4 show the average value for total plate count of Chopping cart, knives and palms of butchers were found to be $3.15x10^4$, $3.47x10^3$ and $2.01x10^4$cfu/cm^2 respectively. The average coliform, *E. coli* and *staphylococcus aureus* counts of chopping cart were found to be $1.11x10^3$, $9.8x\ 10^1$ and $6.2x10^2$cfu/cm^2. The average coliform, *E. coli* and *staphylococcus aureus* counts of chopping knives were found to be $1.31x10^3$, $1.66x\ 10^2$ and $2.83x10^2$cfu/cm^2. The average coliform, *E. coli* and *Staphylococcus aureus* counts of the palms of butchers were found to be $1.95x10^3$, $1.66x\ 10^2$ and $1.77x10^2$cfu/cm^2.

In case of *Salmonella* two samples(A and G) of chopping cart, three samples (B, G and I) of Knives and three swab samples(A, E and H) of hands of butchers were found to be positive. Out of ten swab samples five samples (A, B, D, G and H) of chopping cart,

three samples (D, G and J) of Knives and two samples (A and J) of hands were detected for *Shigella*.

4.4. Analysis of swab samples of Hands

Results of analysis of swab samples of palms of the butchers of Dharan are presented in Table 4.4

Table 4.4 Microbiological analysis of Hand swabs

unit: *cfu/cm*^{2}

Source/Site	TPC	TC	*E. coli*	*S.aureus*	*Salmonella*	*Shigella*
A	$2.8x10^4$	$3.1x10^3$	$2.5x10^2$	$4.0x10^2$	+	+
B	$3.5x10^4$	$2.9x10^3$	$2.3x10^2$	$2.1x10^2$	-	-
C	$1.7x10^4$	$1.1x10^3$	$3.8x10^2$	$3.0x10^2$	-	-
D	$3.6x10^3$	$5.0x10^2$	ND	$2.0x10^1$	-	-
E	$4.1x10^4$	$4.3x10^3$	$1.7x10^2$	$1.8x10^2$	+	-
F	$2.6x10^3$	$1.0x10^2$	ND	ND	-	-
G	$1.9x10^4$	$7.0x10^2$	$9.0x10^1$	$2.1x10^2$	-	-
H	$3.2x10^4$	$2.8x10^3$	$3.1x10^2$	$3.0x10^2$	+	-
I	$1.8x10^3$	$3.0x10^2$	ND	ND	-	-
J	$2.1x10^4$	$3.7x\ 10^3$	$2.3x10^2$	$1.5x\ 10^2$	+	+`
Average	$2.01x10^4$	$1.95x10^3$	$1.66x10^2$	$1.77x10^2$		

(Note: TPC = Total Plate Count, TC = Total Coliforms, *E. coli* = *Escherichia coli, S. aureus* = *Staphylococcus aureus,* ND = Not detected + = Positive and - = Negative)

High numbers of Coliforms on these sources indicate inadequate cleaning, unsanitary handling and post processing contamination from dirty atmosphere around shops.

Fecal coliforms group possess sanitary significance. Because of recent involvement of *E. coli* in several cases of food poisoning, it indicates presence of other pathogenic flora. As an indicator of hygiene and sanitary quality, *E coli* count suggests consumers are at greater risk of being food poisoned. So, Tools used for preparing meat can be said to be highly unhygienic and can be claimed as main spreader of these organisms.

Staphylococcus aureus is ubiquitous organism whose normal habitat is skin, skin gland and mucous membranes of warm blooded animals. The current trend of production practices may enhance the growth through knives and hands (Bacus and Brown, 1981).

Salmonella can reach meat from animal excreta at time of slaughter, from human excreta or from water polluted by animal or human sewage. They are brought into shops in raw meat via hands, surfaces, utensils and other equipment (Roberts, 1982).

From the statistical analysis, ANOVA two factors without replication (Appendix D), no significant difference was found at $p<0.05$ among the sampling sites for the parameters TPC, Total Coliforms, *E. coli* and *S. aureus*. This can be further evidenced by the survey result also. Such results can be directly correlated with the sampling sites keeping in mind that sampling sites were found to be in the same condition and same type of atmosphere. The way by which animals are handled, slaughtered and meat sold were observed to be the same.

But significant difference at $P<0.05$ was found among the sample type viz. meat sample and swab samples of knife, chopping block and hands of butchers for the parameters TPC, total Coliforms, *E. coli* and *S. aureus*. From the LSD table sample types meat and swabs of knives, meat and swabs of chopping cart, meat and swabs of hands were significantly different ($P<0.05$) to each other in terms of TPC, total Coliforms, *E. coli* and *S. aureus*. But swabs of knives, swabs of chopping cart and swabs of hands were not significantly different at 5% level of significance level to each other in terms of TPC, total Coliforms, *E. coli* and *S. aureus*.

Meat sample covers whole microbial load of primary and secondary contamination from the sample taken whereas other sample(swabs) analyzed only contained secondary contamination, so the result can be attributed as such. The possible reasons behind this may be due to the difference among the cleaning and sanitizing habits. Frequency of cleaning was found to be different among the butchers.

The data presented on the Tables 4.1, 4.2, 4.3 and 4.4 showed the higher number of microbes on meat samples which might be due to other contaminating sources also. The cross contamination from these sources could not be ignored. Selling of intestinal and respiratory tract along with the meat and handling by same man with same cutting knives can spread the *Coliforms* and other microbes. The prevalence of *Salmonella* and *Shigella* in knives, chopping blocks and hands signify that they provide main vector for its distribution.

Microbiological analysis showed heavy contamination of knives, chopping blocks and hands. Because of varied sources, the kinds of microorganisms likely to contaminate meat are many. This directly reflects highly polluted and unhygienic condition of meat being sold on local market of Dharan.

4.5. Survey on the sanitary condition of meat shop

From the survey with the help of questionnaire (Appendix A) of the entire buffalo meat shops and interview with butchers suggest about the unhygienic and unscientific method of handling, lack of sanitation and knowledge of microorganisms resulting in higher number of contamination. The detailed survey finding is given in Appendix B.

4.5.1. Sanitary condition of meat/shop

It was found that all the meat sellers control the flies manually. None was found using any type of chemical to get rid of the flies. 54.84 % of the butchers clean the shop daily while 16.13 % of the butchers clean their shop only 2-4 times a week. Further it was found that 29.03 % of the meat handlers clean their shop and shop periphery once a week.

74.12 % of the shops used water for cleaning, 22.58 % used soap or detergent powder as sanitizing agent. Few (3.22%) used cloth for the cleaning purpose. 90.32 % butchers did not use apron.

All the 31 butchers of Dharan cleaned chopping block by scrapping with knife. 26 out of 31 butchers cleaned their knives before use while 5 butchers (16.13 %) denied any cleaning of knives before processing.

From the survey, sanitary condition of the shop was found satisfactory (64.52%). 16.13 % of the shops were found dirty and 19.35 % of the shops were found and observed to be well cleaned.

4.5.2. Selling condition

22.58% of the shops were found to have metal wire fence around shop while 77.42% of the shops did not have metal wire fencing to protect the meat from dogs and rodents. Only 9.67% sold meat on cemented platform. 45.16% sold on the wooden table while 32.26% used carpet and 12.9% used tin plate for serving meat in the shop.

4.5.3. Storage of Meat/Leftovers

When asked about leftovers, it was learnt that 29.03% kept the meat in refrigeration while 35.49% said they would sell the meat following day. 29.03% left meat as it is and only 6.45 % said they dump the leftovers. 93.55 % shops did not use refrigerator for leftovers while rest 6.45 % used refrigerator for storing the leftovers.

4.5.4. Knowledge about Zoonoses

All the butchers responded that they did not examine the animal for diseases before slaughter. 64.52% of the butchers were unaware of zoonoses while 22.58 % had the knowledge that meat was a prominent source of disease. 12.9% did not have any idea about it.

4.5.5. Knowledge about Acts and Regulations

29.03 % were found to be familiar and 70.97 % denied having any idea about the Meat Act. 48.39 % of the butchers felt the necessity of slaughterhouse.

4.5.6. Facility found in shops

Rickshaw (41.94%) was found to be most prominent transportation vehicle. Only 9.67% used hand cart for transporting meat from slaughter site. 25.81 % used four-wheel for the purpose and 22.58 % used self carrying option.

32.26 % of the shops had nearly located toilet whereas 67.74 % shops had no toileting facility. The entire 31 butcher utilized tap/tank water for the further processing of meat.

4.5.7. Management of solid wastes

32.26 % sold the hide on slaughter site. 6.45 % sold placing along with meat and 3.22% sold the meat without dehiding. Among them, 58.06 % informed they do not deal the hide.

Feet and shanks of the slaughtered animal were found selling alongside with the meat on 32.26% of the shops while 29.03% sold them away from shop. 38.71 % shops were found not dealing with the feet and shanks of slaughtered animal.19.35 % of the shops were found to sell viscera with the meat while 51.61 % informed dealing them on distant

place from the meat sold and 29.03 % said they do not deal with such items. Among them, 45.16% used same knife for the meat and viscera.

The survey showed that the hygiene around meat shop to be quite unsatisfactory. The atmosphere around shop was found dirty of 16.13 % and not so good of 64.52%. Flies were manually controlled, no special attention were paid to control the flies. Although, 7 out of 31 shops have metal wire boxes they were found to be non-functional. 90.32 % of butchers do not use apron while preparing meat to sell.

Besides, the butchers and sellers were seemed to be ignorant about the basics of the handling and sanitation measures. 17 out of 31 meat shop owners reported having cleaned their shop daily. From the survey it was found that 23 out of 31 butchers utilize the tap/tank water to clean the shop while only 22.58 % were using soap to sanitize the shop and there was also a cloth cleaning system (3.22%) of the shop. Scrapping of wooden chopping cart was the only one system of cleaning found in Dharan meat market. Only 26 out of 31 meat handlers used to clean the knives before each use. Butchers (45.16%) utilize the wooden table for the meat serving purposes. Only three out of 31 shops used cement made platform for selling meat. Although 29.03 % of butchers informed storing of leftovers in the refrigeration unit, 93.55% informed not possessing of refrigeration unit.

21 meat shops of buffalo meat in Dharan were found to be without toilet in their near proximity. This is why they frequently toilet around open place near the shops and contaminate meat as only 54.84% shops have hand sanitizing soap/ detergent on their shop.

The contamination of meat by equipment begins with the slaughtering the animal. During the slaughtering operation, the equipment used comes in contact with maximum of animal surfaces. When the animal is cut and served to consumer equipments such as knives, cutting blocks and the seller's hand are the main sources for the cross contamination of the meat. The microflora thus gets transferred to cut meat surfaces by the knives. 45.16% used same knife for meat and viscera. 18 shops informed they do not handle skin of slaughtered animal and there were only 14 shops taking meat slaughtered by other butchers. 3.45% informed they sold the hide with the meat otherwise 32.26% sold the hide on slaughter site. There is a maximum chance of contamination with the feet and shanks of slaughtered animals as 32.26% sold the legs placing it nearly of the meat while only 29.03% sold the feet and shanks placing on the farther side of meat. 51.61% sold the

visceral content on farther side of shop but there is a chance of contamination by flies as no protective coverings on the meat shops were found.

Therefore, the cleanliness of the utensils, knife and other contact surfaces are equally responsible for the poor hygiene quality of the marketed meat in Dharan.

Butchers of Dharan were found to be unknown about the zoonoses and Meat Acts. Practice of ante-mortem inspection was not found among the butchers. 20 out 31 butchers informed meat is not a source of diseases for the human and only 12.9 % denied having any idea about zoonoses. 22 out of 31 informed they are not familiar with the meat Acts and Regulations. 51.61% do not felt necessity of animal slaughterhouse.

Survey findings are comparable to study of Joshi and Olesen (1999). The majority of butchers (64.9%) lack awareness of meat borne diseases and 14.29% of meat shops had refrigeration facility whereas transportation of meat by local butchers was done with rickshaws.

CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Hygiene quality of buffalo meat marketed in Dharan was assessed by enumerating the microbial load of the meat samples and by questionnaire survey on shops and butchers. Randomly chosen ten places were used to take meat samples.

Following conclusions can be drawn from the research work:

1. All the buffalo meat samples were found to contain higher microbial load than prescribed standards of Europe and United States.
2. The bacterial counts of meat samples were found to be high which might be due to poor sanitary condition of meat shop, handlers and slaughtering premises.
3. Presence of *E. coli* indicated that the meat might be contaminated by the visceral content.
4. Except two meat samples all were found to contain *Salmonella*
5. Thus the study showed that degree of contamination is dependent upon the hygienic condition of those localities and the way of handling, cutting and preparing meat.
6. All the findings of survey suggest about the unhygienic and unscientific method of handling, lack of sanitation and knowledge of micro organisms resulting higher number of contamination.
7. The sanitary condition of meat, seller and shop need to be improved.

The overall quality of buffalo meat marketed in Dharan was found to be quite unsatisfactory when compared with the standards given for cut meat and meat for retail sale of United States and Europe (Appendix C). When considered to the developing country like Nepal, where no microbiological standards are found regarding meat and level of awareness, facility and infrastructure for the meat processing premises are on the lower level, the profile of microbes could be considered as not safe and there is strict need of awareness campaign. The most contaminating source for the meat was found to be cutting knives, chopping blocks and hands of butchers. The cleaning of meat shop regularly with great attention can check the further contamination from those sources also.

Regular visit of shops by concerned authority and consumers can make butchers think more about sanitation and the quality of meat which he is selling.

5.2. Recommendations

The buffalo meat samples of Dharan were found to contain high counts of micro organisms. The hygiene quality was unsatisfactory. The findings imply that people of these areas need to be careful about the quality.

To improve the bacteriological quality of the raw meat some well-known, simple techniques that can be recommended are:

1. Slaughter of animals in properly constructed hygienic surroundings by improved and humane method
2. Proper zoning plans with provisions of clean water places to dispose of wastes-blood, gut content, bones etc
3. Animal slaughterhouse and meat inspection act and other acts concerning meat commodity should be fairly implemented for better quality and disease free meat, and for standardization of meat handlers and their facility
4. Regular checks on meat quality by concerned authority need to be strictly implemented for public health protection.
5. Transportation of animals prior to slaughter should be less stressful and short distances should be utilized.
6. Effective and adequate sanitation facility (Wash basins with soap/detergent powder, Toilets, Sanitized towels, etc) should be available on the meat shop and its premises.
7. Proper utilization of by-products should be managed.
8. Management of excreta as manure should be promoted.
9. Training programs on humane method should be conducted to upgrade the small firms as well as butchers ensuring that they are more aware of their responsibilities to the public.
10. Consumer awareness campaign should be arranged to promote the good products.

CHAPTER VI

SUMMARY

The raw buffalo meat sampled from ten different places was examined for the enumeration of total plate count, total Coliforms, *E. coli* and *Staphylococcus aureus*. *Salmonella* and *shigella* were also checked for their presence and absence.

Swabbed samples of Chopping carts, Knives and Palms of Butchers were also studied for the above mentioned micro organisms.

The average value for total plate count of the analyzed meat sample was found to $3.59x10^7$cfu/g. The average coliform, *E. coli* and *staphylococcus aureus* counts were $2.06x10^4$, $1.69x10^3$ and $9.67x10^3$cfu/g respectively. Except two samples C and I, 80% samples were found to be *salmonella* positive where as all the meat samples (100%) were found to be *Shigella* positive.

The average value for total plate count of Chopping cart, knives and palms of butchers were found to be $3.15x10^4$, $3.47x10^3$ and $2.01x10^4$cfu/cm^2 respectively. The average coliform, *E. coli* and S*taphylococcus aureus* counts of chopping cart were found to be $1.11x10^3$, $9.8x\ 10^1$ and $6.2x10^2$cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of chopping knives were found to be $1.31x10^3$, $1.66x\ 10^2$ and $2.83x10^2$cfu/cm^2. The average Coliform, *E. coli* and *Staphylococcus aureus* counts of the palms of butchers were found to be $1.95x10^3$, $1.66x\ 10^2$ and $1.77x10^2$cfu/cm^2.

In case of *Salmonella* two samples of chopping cart, three samples of Knives and three swab samples of hands of butchers were found to be positive. Out of ten swab samples five samples of chopping cart, three samples of Knives and two samples of hands were detected for *Shigella.*

Thus, from the work it is observed that bacterial contamination of the meat samples is dependent on the micro flora of possible contaminating sources. The examined contaminating source also showed heavy population of micro organisms.

From the survey it was cleared that buffalo are slaughtered unhygienically and unscientifically. The methods of slaughtering animal and serving meat need to be upgraded. Personal hygiene of the butchers needs high improvements through awareness campaign. Waste disposal places should be clearly allocated. There is need of strict enforcement of Animal slaughterhouse and meat inspection act and education about sanitation.

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APPENDICES

Appendix A

Questionnaire used for Survey of Sanitary condition of meat shop

Name: Address:

Signature: Sample Code:

1. Where do you place the meat in the shop?
   a. Cemented place
   b. Wooden table
   c. Tin plate
   d. Carpet
2. Do you have metal wire fence around the shop?
   a. Yes
   b. No
3. What do you do with the hide of the animal?
   a. Selling on the slaughter site
   b. Selling alongside of meat
   c. Do not handle
   d. Selling with hide
4. How do you sell the feet and shanks of the slaughtered animal?
   a. Selling alongside of meat
   b. Selling far from meat
   c. Do not handle
5. Do you use apron while processing?
   a. Yes
   b. No
6. Where do you sell the viscera?
   a. Remain attached with meat
   b. Outside the shop
   c. Do not handle
7. How do you control flies in your shop?
   a. Chemically
   b. Manually
   c. Do nothing

8. How often do you clean the shop on a week?
    a. Seven
    b. Two - four
    c. One
    d. Zero
9. What do you use while cleaning?
    a. Water
    b. Soap/ Detergent powder
    c. Cloth
10. What is done with the leftovers?
    a. Refrigeration
    b. Selling next day
    c. Left as it is
    d. Dispose off
11. How do you clean the chopping block?
    a. Scrapping
    b. By water
    c. Do not clean
12. Is equipment used to process meat cleaned and/or sanitized before each use?
    a. Yes
    b. No
13. Is the slaughtering animal examined before killing?
    a. Yes
    b. No
14. How is the sanitary condition in the shop?
    a. Well cleaned
    b. Dirty
    c. Satisfactory
15. Do you have refrigerator in the shop?
    a. Yes
    b. No

16. Are soaps and wash basins provided in the shop?
    a. Yes
    b. No
17. Is there toilet near the shop?
    a. Yes
    b. No
18. From which source do you use water?
    a. Tap/Tank
    b. River
19. Do you think slaughterhouse is necessary for slaughtering?
    a. Yes
    b. No
20. How do you transport meat from the slaughtering place?
    a. Car
    b. Handcart
    c. Rickshaw
    d. Carrying
21. Are separate knives used for meat and intestines?
    a. Yes
    b. No
22. What is your opinion in "Meat is a source of disease for human being"?
    a. Yes
    b. No
    c. No idea
23. Are you familiar with the Slaughterhouse and Meat Inspection act?
    a. Yes
    b. No
24. How do you rate the meat you are selling?
    a. Well
    b. Satisfactory
    c. Not good

Appendix B

Table B.1. Detail output of Survey

Total no of respondents = 31

Survey Question no.	No. of Respondents			
	a	b	c	d
1	3	14	4	10
2	7	24	-	-
3	10	2	18	1
4	10	9	12	-
5	3	28	-	-
6	6	16	9	-
7	0	31	-	-
8	17	5	9	-
9	23	7	0	1
10	9	11	9	2
11	31	0	-	-
12	26	5	-	-
13	0	31	-	-
14	6	5	20	-
15	2	29	-	-
16	17	14	-	-
17	10	21	-	-
18	22	9	-	-
19	15	16	-	-
20	8	3	13	7
21	14	17	-	-
22	7	20	4	-
23	9	22	-	-
24	18	13	-	-

Appendix C

Microbiological Standards

1. Guidelines for Total Plate Count in Meat and Meat Products

Product	TPC Max
Fresh Meat (Cut and Packaging meat)	5xlog 6/sq.cm or g
Separated Meat	5xlog 6/ g

2. Inspected German Quality Meat

≤ log 4/ g or sq.cm. in freshly slaughtered meat
≤ 5xlog6/g or sq. cm. in cutting and packaging plant

Danish Quality Assurance Warranty

≤ log 4/ sq. cm. in freshly slaughtered meat

3. EU microbiological standards of cut meat for retail sale and further processing

Bacteria	M	m
coliform bacteria n=5, c=2	5 x log 3/g	5 x log 2/g
Staph. aureus n=5, c=2	5 x log 3/g	5 x log 2/g
Salmonella n=5, c=0	not detectable in 1g	

4. Oregon State Microbiological Standard

Total Plate Count	max. 5 x 10^6/g
E. Coli	max. 50/g

(Note:
M = maximum limit; beyond which meat is not acceptable, and applies the following:
M = 10m while counting in solid medium
M = 30m while counting in liquid medium
m = limit, at and under which meat is acceptable
n = number of replicates
c = number of replicates, the count of which lies between m and M.)

(Source: Anonymous, 2003)

Appendix D

Table D.1. ANOVA Two factor without replication for TPC

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Sampling Site	2.23057×10^{15}	9	2.47841×10^{14}	1.000903602	2.250131477
Sample Type	9.6562×10^{15}	3	3.21873×10^{15}	12.99880663*	2.960351321
Error	6.68568×10^{15}	27	2.47618×10^{14}		
Total	1.85724×10^{16}	39			

* Since there is significant difference between the sample type for the variate TPC, LSD testing is necessary.

LSD testing to analyze difference between average values in terms of TPC.
LSD = 14426455

Sample Type	Average	Difference of Average	Comments
Meat	35900000	M-K = 35896530	>LSD
Swabs of Knives	3470	M-C = 35868520	>LSD
Swabs of Chopping Cart	31480	M-H = 35879900	>LSD
Swabs of Hands	20100	C-K = 28010	<LSD
		C-H = 11380	<LSD
		H-K = 16630	<LSD

Here,
M = Meat,
K = Swabs of Knives,
C = Swabs of Chopping cart and
H = Swabs of Hands

From the LSD table sample types meat and swabs of knives, meat and swabs of chopping cart, meat and swabs of hands are significantly different ($P<0.05$) to each other in terms of TPC. But swabs of knives, swabs of chopping cart and swabs of hands are not significantly different ($P<0.05$) to each other in terms of TPC.

Table D.2. ANOVA Two factor without replication for Total Coliforms

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Sampling Site	954386200	9	106042911.1	0.985461547	2.250131477
SampleType	2752354750	3	917451583.3	8.52591887*	2.960351321
Error	2905398600	27	107607355.6		
Total	6612139550	39			

* Since there is significant difference between the sample type for the variate total Coliforms, LSD testing is necessary.

LSD testing to analyze difference between average values in terms of total Coliforms.

LSD = 9510.204

Sample Type	Average	Difference of Average	Comments
Meat	20600	M-K = 19290	>LSD
Swabs of Knives	1310	M-C = 19490	>LSD
Swabs of Chopping Cart	1110	M-H = 18650	>LSD
Swabs of Hands	1950	K-C = 200	<LSD
		H-K = 640	<LSD
		H-C = 840	<LSD

Here,
M = Meat,
K = Swabs of Knives,
C = Swabs of Chopping cart and
H = Swabs of Hands

From the LSD table sample types meat and swabs of knives, meat and swabs of chopping cart, meat and swabs of hands are significantly different ($P<0.05$) to each other in terms of total Coliforms. But swabs of knives, swabs of chopping cart and swabs of hands are not significantly different ($P<0.05$) to each other in terms of total Coliforms.

Table D.3. ANOVA Two factor without replication for *E. coli*

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Sampling Site	6748290	9	749810	0.944243343	2.250132525
Sample Type	17925790	3	5975263.333	7.524709764*	2.960348411
Error	21440310	27	794085.5556		
Total	46114390	39			

* Since there is significant difference between the sample type for the variate *E. coli*, LSD testing is necessary.

LSD testing to analyze difference between average values in terms of *E. coli*.
LSD = 816.9632

Sample Type	Average	Difference of Average	Comments
Meat	1688	M-K = 1522	>LSD
Swabs of Knives	166	M-C = 1590	>LSD
Swabs of Chopping Cart	98	M-H = 1522	>LSD
Swabs of Hands	166	K-C = 68	<LSD
		K-H = 0	<LSD
		H-C = 68	<LSD

Here,
M = Meat,
K = Swabs of Knives,
C = Swabs of Chopping cart and
H = Swabs of Hands

From the LSD table sample types meat and swabs of knives, meat and swabs of chopping cart, meat and swabs of hands are significantly different ($P<0.05$) to each other in terms of *E. coli*. But swabs of knives, swabs of chopping cart and swabs of hands are not significantly different ($P<0.05$) to each other in terms of E. *coli.*

Table D.4. ANOVA Two factor without replication for *S. aureus*

Source of Variation	*SS*	*df*	*MS*	*F*	*F crit*
Sampling Site	352127700	9	39125300	1.120953473	2.250132525
Sample Type	656024930	3	218674976.7	6.265114247*	2.960348411
Error	942396920	27	34903589.63		
Total	1950549550	39			

* Since there is significant difference between the sample type for the variate *S. aureus*, LSD testing is necessary.

LSD testing to analyze difference between average values in terms of *S. aureus*.
LSD = 5416.315

Sample Type	Average	Difference of Average	Comments
Meat	9670	M-K = 9487	>LSD
Swabs of Knives	183	M-C = 9050	>LSD
Swabs of Chopping Cart	620	M-H = 9493	>LSD
Swabs of Hands	177	K-C = 437	<LSD
		H-K = 443	<LSD
		H-C = 06	<LSD

Here,
M = Meat,
K = Swabs of Knives,
C = Swabs of Chopping cart and
H = Swabs of Hands

From the LSD table sample types meat and swabs of knives, meat and swabs of chopping cart, meat and swabs of hands are significantly different ($P<0.05$) to each other in terms of *S. aureus*. But swabs of knives, swabs of chopping cart and swabs of hands are not significantly different ($P<0.05$) to each other in terms of *S. aureus.*

Appendix E

Table E.1. Name of Buffalo meat shops of Dharan

S.N.	Owner	Address	Remarks
1	Ganesh Khadgi	Dharan-3, Sadan Bazar	*
2	Kumar Sahi	Dharan-3, Sadan Bazar	*
3	Lal Kaji Shahee	Dharan-3, New Palika Bazar	*
4	Nashima Khatun	Dharan-3, Shanti Path	***
5	Bimal Khadgi	Dharan-3, Kailash Path	***
6	Ganesh Khadgi	Dharan-6, New Road Line	**
7	Mohankaji Khadgi	Dharan-7, below Traffic Chowk	*
8	Kamal Sahi	Dharan-7, In front of Himalaya Pump	****
9	Chandra Prasad Shrestha	Dharan-7, below Traffic Chowk	**
10	Lal Kaji Khadgi	Dharan-7, Inside Ram mini Market	**
11	Allaudin Ansari	Dharan-8, Near Atal Pump	***
12	Lal Kaji Khadgi	Dharan-9, Old Palika Bazar	**
13	Tara Sahi	Dharan-9, Old Palika Bazar	**
14	Madina Khatun	Dharan-9, Gautam Path	***
15	Jainuf Khatun	Dharan-9, Gautam Path	***
16	Ambika Devi Sahi	Dharan-10, In front of Ganga Mill	**
17	Chandra Prasad Shrestha	Dharan-10, Ramailo Chowk	*
18	Tara Sahi	Dharan-10, Ramailo Chowk	*
19	Shyam Kaji Sahi	Dharan-10, Ramilo Chowk	*
20	Allaudin Ansari	Dharan-11, Manglabare Chowk	***
21	Ambika Devi Sahi	Dharan-11, Kalyan Chowk	**
22	Kamal Sahi	Dharan-12, Chatara Line	****
23	Iliyas Ansari	Dharan-13, Zero Point	*
24	Shyam Kaji Sahi	Dharan-13, Zero Point	**
25	Mohan Kaji Khadgi	Dharan-15, Shyam Chowk	**
26	Mohan Kaji Khadgi	Dharan-15, Pindeswor Chowk	**
27	Shiv Ganga Khadgi	Dharan-15, Shyam Chowk	****
28	Taiyab Ansari	Dharan-15, Everest Line	**
29	Iliyas Ansari	Dharan-16, Jana Path	**
30	Dambar Bahadur Rai	Dharan-17, Railway Line	**
31	Jahir Ansari	Dharan-17, Railway Line	**

Source: Personal Communication

Note:

* = Slaughters Buffalo daily
** = Takes Meat from Others
*** = Slaughters Buffalo twice a week
**** = Slaughters Buffalo once a week

Appendix F
Major Market channels for Buffalo Meat.

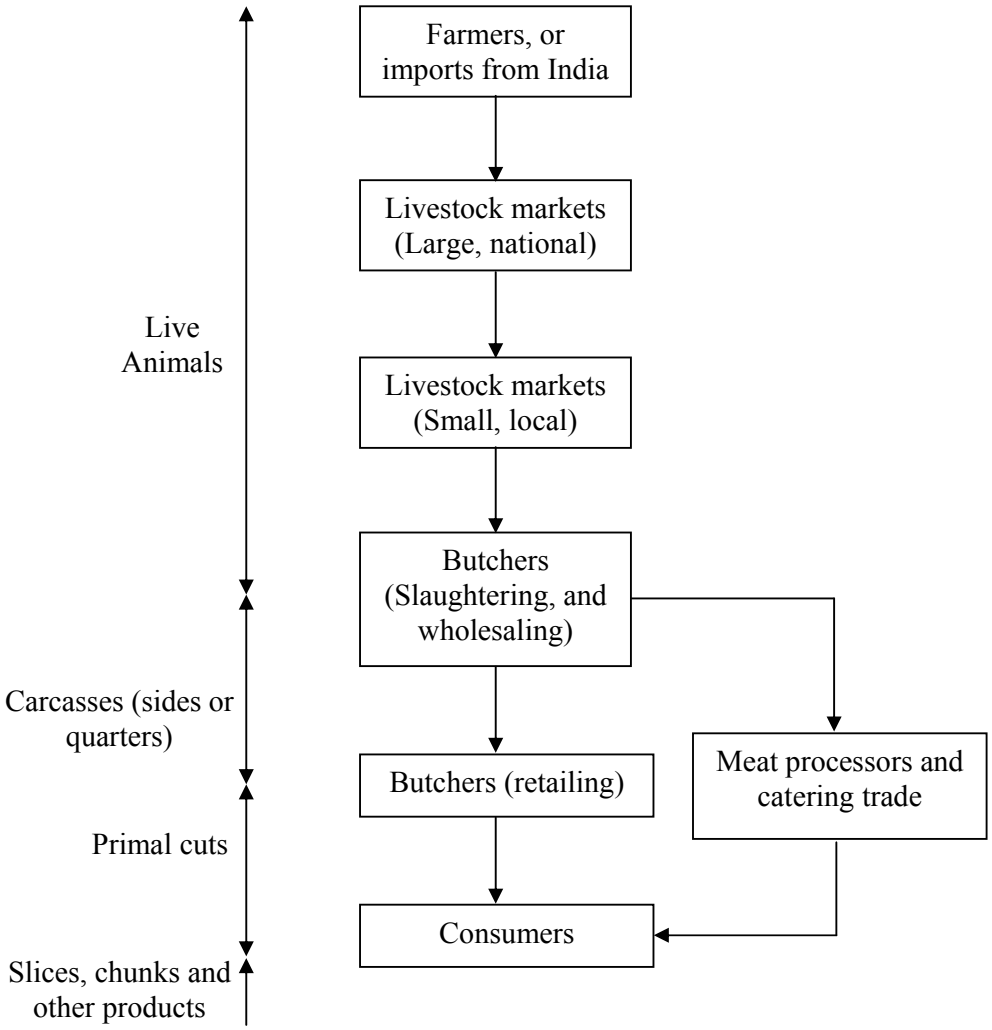


Figure F.1 Major Market channels for Buffalo meat

Source: Anonymous, 2002

Appendix G

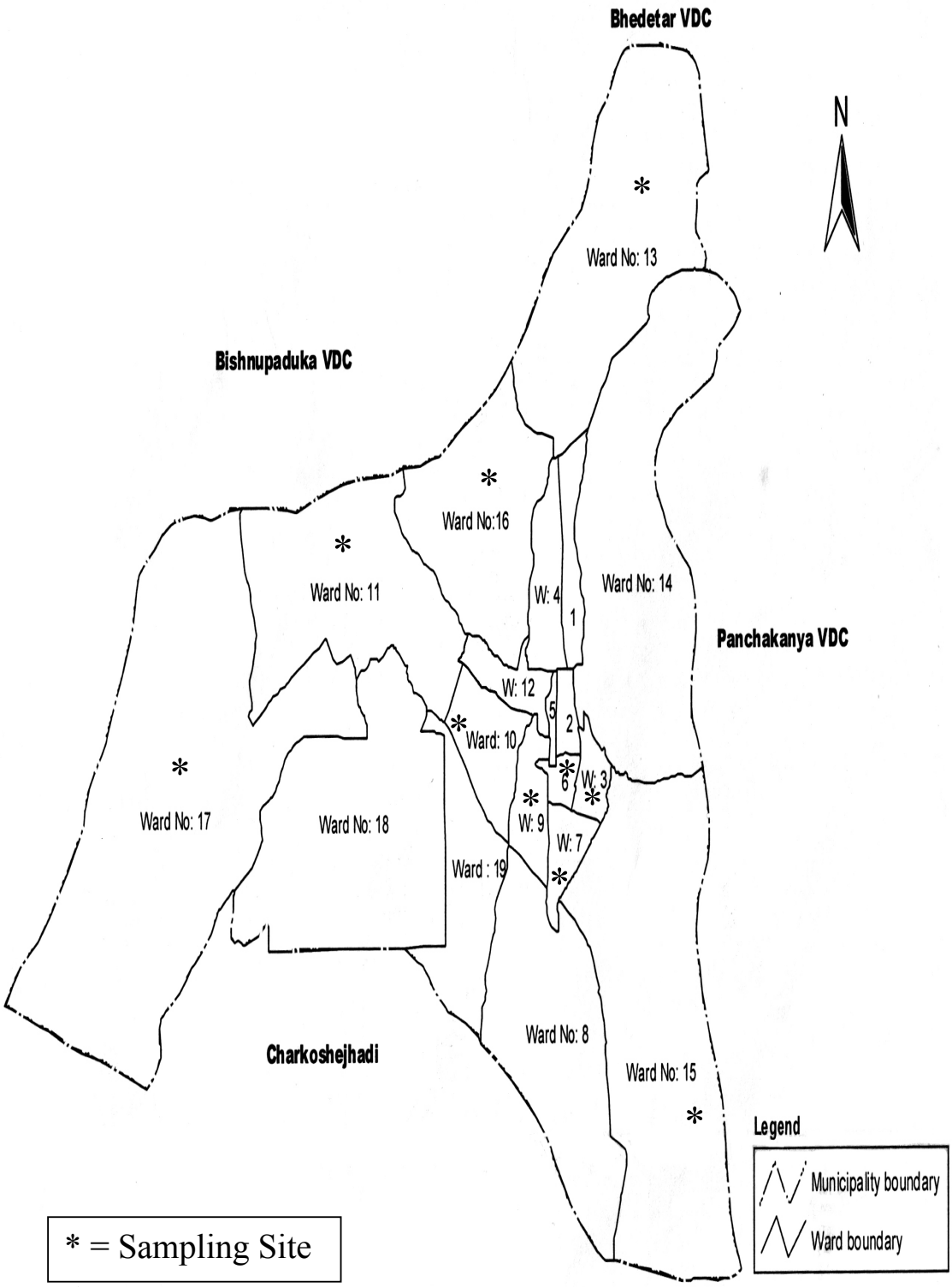
Table G.1. Parameters of importance for some pathogens for health concern

Organisms	Parameters for development			
		Minimum	Optimum	Maximum
Bacillus cereus	Temperature	10°c	28-35°c	50°c
	pH	4.9	-	9.3
	Salt	Inhibited by 10%		
	a_w	0.92		
Clostridium botulinum				
a) Proteolytic strains	Temperature	12°c	30-40°c	48°c
	pH	4.6	7.0	9.0
	Salt	Inhibited by 5% at 35°c and pH 5.2		
	a_w	0.91		
b) Non-proteolytic strains	Temperature	3.3°c (type E)		
		4°c (type F)	25-37°c	45°c
		5°c (type B)		
	pH	5.0	6.5-7.0	9.0
	NaCl	Inhibited by 3.5% at 3. 0c and pH 5.2		
	a_w	0.91		
Cl. Perfringens	Temperature	12°c	43-45°c	50°c
	pH	5.0	6-7.5	8.3
	NaCl	Inhibited by 6%		
	a_w	0.95		
Salmonella spp	Temperature	5.1°c	37°c	45-47°c
	pH	5.4	6.5-7.5	9.0
	Salt	Inhibited at >8%		
	a_w	Limited at 0.95 and below		
Staphylococcus aureus	Temperature	11°c	37°c	48°c
	pH	4.0	6-7	9.8-10
	a_w	0.86	0.98	0.99
	Parameters for toxin production			
	Temperature	10°c	40-45°c	48°c
	pH	Limited below 5.0	7-8	9.6
	a_w	0.85	0.98	0.99

Source: Shapton & Shapton, 1991

Appendix H

Map of Sampling Area



Appendix I

Photograph showing Meat shops of Dharan