THE PREVALENCE AND GROWTH POTENTIAL OF SOME *SALMONELLA* SPECIES IN "KITFO" AND EFFECT OF "KITFO" PREPARATION PROCESSES ON THE TEST ORGANISMS

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ABSTRACT

In an attempt to assess the microbial load and the prevalence of salmonellae in "kitfo", a total of 50 raw or "Tire kitfo" samples were collected from ten food establishments (hotels, restaurant and bars) from different areas of Addis Ababa. The microbial load in "tire kitfo" samples collected from all sources was markedly high. The highest average loads of aerobic mesophilic bacteria (8.33 Log(CFU/g)), coliforms (6.77 Log(CFU/g)), staphylococci (7.29 Log(CFU/g)), lactic acid bacteria (5.37 Log(CFU/g)), molds and yeasts (5.96 Log(CFU/g)), and aerobic spores (5.67 Log(CFU/g)) were encountered in "kitfo" from three hotels and one bar. On the other hand, the lowest average loads of aerobic mesophilic bacteria (7.30 Log(CFU/g)), coliforms (4.51 Log(CFU/g)), staphylococci (6.18 Log(CFU/g)), lactic acid bacteria (4.10 Log(CFU/g)), molds and yeasts (4.06 Log(CFU/g)), and aerobic spores (3.71 Log(CFU/g)) were found from one restaurant and two hotels. Variation within samples in counts of coliforms, staphylococci, lactic acid bacteria, molds and yeasts, and aerobic spores was significant in all or most establishments (CV > 10%). However, counts of staphylococci and lactic acid bacteria did not show marked variations among hotels, restaurant and bars. Variation within samples in the count of aerobic mesophilic bacteria in most food establishments was not significant (CV < 10%). The microbial load of slightly cooked "kitfo" ("lebleb kitfo") was found to be high, though it was less than that of raw or "tire kitfo". Variation in the counts of aerobic mesophilic bacteria, staphylococci and lactic acid bacteria in "lebleb kitfo" among ten establishments was not significant (CV < 10%), while that of coliforms, molds and yeasts, and aerobic spores was significant (CV > 10%). No microorganisms have been detected from "tibs kitfo", except very few aerobic spores. From the study of the prevalence of *Salmonella* in "kitfo" twenty one positive samples were obtained from the 50 samples collected from the above hotels, restaurant and bars. Among these positive samples of *Salmonella*, the highest number of positive samples, 5 samples (23.8%) were from each of Hotel B and Bar A, and the lowest number of positive samples (one) was from Hotel G. No *Salmonella* was isolated from "kitfo" of Hotel D, E and Restaurant A. No *Salmonella* was encountered in "lebleb" and "tibs kitfo" samples. Of the three selective enrichment broths, i.e. Selenite Broth, Thetrathionate Broth, and Rappaport-Vassiliadis Medium used for enriching the growth of *Salmonella* species, RVB was found to be superior. Of the four plating media, i.e., Salmonella-Shigella (SS)agar, MacConkey agar, Brilliant Green agar (BG) and Xylose Lysine Desoxycholate (XLD) medium, BG was found out to be superior in supporting the growth of salmonellae encountered in this work. The microbial load of swabs from raw beef was relatively low when compared to that of "kitfo". The loads of aerobic mesophilic bacteria, coliforms, staphylococci, lactic acid bacteria, molds & yeasts and aerobic spores were found to be 5.07 Log(CFU/cm^2), 2.34 Log(CFU/cm^2), 3.14 Log(CFU/cm^2), 2.63 Log(CFU/cm^2), 2.54 Log(CFU/cm^2), and 2.60 Log(CFU/cm^2), respectively. When assessing the growth potential of *Salmonella* species in "kitfo", they were found to grow well. They grew to the level of 7.0 Log(CFU/g) within 12h of incubation.

1. INTRODUCTION

Meat is the most perishable of all important foods. It contains abundant nutrients for microbial growth. The surface of meat is contaminated with a variety of microorganisms (Pelczar *et al.*, 1988; Rao and Sreenivasmuthy, 1985). When meat is cut in to pieces, more microorganisms are added to the surfaces of the exposed tissue. In the process of making hamburger,more surface area is exposed to microbial contamination of meat.

Raw meats exposed to contamination have very high total counts of microorganisms, particularly salmonellae either from infected animals or from faeces by rodents and man. The faeces of carriers are said to be more important in contaminating food than the clinically treated individuals. Consequently, food handlers that are shedding salmonellae appear to be more dangerous in the infection of humans in many countries through the contamination of meat and meat products (Ananthanarayan and paniker,1982;Cherrington,1992).

In Ethiopia meat is consumed either raw or cooked. One traditional way of preparing meat dish is "kitfo", where a chunk of lean meat is minced with "Kibe", a traditional butter containing specific types of spices. In the Gurage area and in many towns, including Addis Ababa, "kitfo" is consumed during various periods of the year.

The traditional way of mincing meat for "kitfo" preparation provides new surfaces for microbial contamination. Considering the relative unhygienic status of food handlers and the kitchen utensils used for "kitfo" preparation, it is very likely that "kitfo" may have a high microbial load and harbor *Salmonella*, which is closely associated with raw meat. In addition, the preference of a considerable number of Ethiopians for raw "kitfo" dishes makes raw "kitfo" a real health hazard.

As indicated above, meat is nutritious food and harbors a number of microorganisms including salmonellae. The capacity of growth of *Salmonella* species on meat, especially on comminuted meat such as "kitfo" is expected to be high.

The purpose of this investigation is, therefore, to evaluate the microbial load and microflora of "kitfo" as made available to the consumer,to evaluate the prevalence of *Salmonella* in “kitfo”, to examine the effect of partial and complete cooking on its microflora and to evaluate the growth potential of *Salmonella* test strains in "kitfo".

2. LITERATURE REVIEW

2.1 Taxonomy of *Salmonella*

2.1.1. General Characteristics

The genus *Salmonella* comprises Gram-negative bacteria that meet the characteristics of the family Enterobacteriaceae and the tribe salmonellae (Breed *et al.*, 1967; Krieg and Holt, 1984). Salmonellae are non-spore forming, motile, facultatively anaerobic rods (Jawetz *et al.*, 1976). Salmonellae form acid and usually gas from glucose, maltose, mannitol and dextrin. They are not able to ferment lactose, sucrose and salicin. They do not produce cytochrome oxidase and urease, but produce catalase. They are able to utilize citrate as a sole source of carbon, but do not grow on medium containing potassium cyanide. They decarboxylate lysine, arginine and ornithine (Edwards and Ewing, 1972; Jawetz *et al.* 1976; Krieg and Holt, 1984).

2.1.2. Serogroups and Serotypes

The biochemical characterisation is the preliminary identification of the *Salmonella* isolates. However, this method is of limited value. Isolates are, therefore, identified by serological tests (Prost and Riemann, 1967; Jawetz *et al.*, 1976; Collins and Lyne, 1976).

The antigens present on the cells of salmonellae include O(Somatic antigens) and H (Flagellar antigens) (Kauffmann, 1966; Jawetz *et al.*, 1976). The O(Somatic antigens) are part of the bacterial cell wall. The somatic (O) antigens are of lipopolysaccharides and provide the basis for primary isolation of salmonellae in to serogroups. There are several serogroups (A-Z) (Kauffmann, 1966). Most of the serotypes of *Salmonella* are motile with peritrichous flagella . These flagella consist of the protein (flagellin) which constitutes the flagellar (H) antigens. There are different H antigens and their identification is used for classification of salmonellae in to serotypes.

The identification of a given serotype of *Salmonella* is performed after careful analysis of the O and H antigens using polyvalent and monovalent (group specific) antisera produced against specific antigens. The primary basis for characterizing *Salmonella* isolates is serotyping (Murray *et al.*, 1995). Serotypes are divisions based on antigenic relatedness, for example, similarity of O(Somatic) antigens. Of all the isolated strains of *Salmonella* about 98% fall in to the first few groups of O, A to A4 (Freeman, 1979). Over 2000 serotypes of salmonellae have been isolated from different sources (Krieg and Holt, 1984).

2.1.3. Phage Types and Biotypes

Phage typing is also employed to identify organisms of the genus *Salmonella*. Strains found within a given serotype can be differentiated into a number of phage types by their patterns of susceptibility to lysis by members of a series of phages which have different specificities (Prost and Riemann, 1967; Mackie and

McCartney, 1989). Over 80 different phage types of *S.typhi*, 48 phage types of *S.paratyphi* B and over 200 phage types of *S.typhimurium* are distinguished (Mackie and McCartney, 1989).

Salmonellae can also be differentiated by biotyping. Strains which are found within a particular serotype can be distinguished in to a number of biotypes by the pattern of their biochemical reactions within a series of substrates. For example, *S.typhimurium* is differentiated in to over 144 different biotypes (Boyd and Hoerl, 1981; Mackie and McCartney, 1989).

2.1.4. Grouping of *Salmonella* Based on Host Adaptation

Salmonellae cause the disease salmonellosis which has world wide distribution (Prost and Riemann, 1967). In all forms of *Salmonella* infection, the organisms enter via the oral route and then clinical or subclinical infections may be produced (Jawetz *et al.*, 1976; Roth *et al.*, 1995). Size of the inoculum, the serotypes and the state of health of the host are the factors which determine *Salmonella* infection (Boyd and Hoerl, 1981; Ananthanarayan and Paniker, 1982). The number of salmonellae which causes salmonellosis after consumption of contaminated food or water depends on the serotype. For most serotypes, about $1x10^8$ cells are required to cause infection. In the case of more invasive serotypes about $1x10^2$ cells can cause salmonellosis (Boyd and Hoerl, 1981). The incubation period varies between 8 and 72 hours (for gastroenteritis) and 1 to 3 weeks (for enteric fever) and duration of illness is between 5 and 7 days (for gastroenteritis) and 3-4 weeks (for enteric fever) (Plotkin *et al.*, 1979).

The ubiquitous habits of the majority and the host specificity of a few serotypes are the main points in the natural distribution of salmonellae. The capacity to infect different animals, both warm and cold-blooded, and survive for long duration out side the host animal are the main features which make salmonellae to have a wide distribution (Buxton and Fraser, 1977; McCapes *et al.*, 1991). Salmonellae are zoonotic pathogens in that they cause infection in a wide range of hosts, both in man and other animals (Clinton *et al.*, 1981; Franco and Williams, 1991).

Considering, their host adaptation, salmonellae can be classified in to three groups.

2.1.4.1. Salmonellae Primarily Adapted to Man:- These are the salmonellae that are specific pathogens to man and cause enteric fevers (typhoid and paratyphoid). Here are *S.typhi, S.parathphi* A, B and C (Jawetz *et al.*, 1976; Boyd and Hoerl, 1981; Somerville *et al.*, 1983; Krieg and Holt, 1984; Stainer *et al.*,1989; Salyers and Whitt, 1994).

2.1.4.2. Salmonellae Primarily Adapted to Particular Animal Hosts:- These are the salmonellae which are specifically pathogenic to certain animals. These are *S.pullorum* found in poultry (a causative agent of `white diarrhoea' in chickens), *S.gallinarum* found in fowl (a causative agent of fowl typhoid), *S.abortus* ovis found in sheep (which causes abortion in sheep), *S.abortus equi* found in horses (that causes abortion in horses), *S.choleraesuis* and *S.typhisuis* found in swine (Krieg and Holt, 1984; Rao *et al.*, 1991; Roth *et al.*, 1995).

2.1.4.3 Unadapted Salmonellae:- These are salmonellae which are not host specific. In this group about 2000 serotypes are found which are pathogens of man and other animals. The well known example for this group is *S.typhimurium* (a causative agent of gastroenteritis in man) (Jawetz *et al.*, 1976; Krieg and Holt, 1984).

The explanation given above indicates that the majority of salmonellae do not show host specificity and only a few serotypes are host specific, though the host specific serotypes can also be found in unusual hosts.

2.2. Nutritional and Other Growth Requirements

Salmonellae grow readily on the usual nutrient media. They have simple nutritional requirements. Ammonium salt and glucose, pyruvate, lactate, etc. are adequate sources of nitrogen and carbon in synthetic media. The growth factors, vitamins and amino acids, are not required by many strains, but some strains of typhoid bacillus require the added tryptophan (Ananthanarayan and Paniker, 1982; Krieg and Holt, 1984; Mackie and McCartney, 1989).

Salmonellae are found in low numbers and are weak in natural samples. So resuscitation technique involving pre-enrichment in buffered peptone water appeared to be important (Morinigo *et al*, 1989). A number of culture media support the growth of salmonellae. Differential and selective media are used to isolate salmonellae from the specimens (samples) which are to be studied. These media are especially made to favour the growth of salmonellae and closely related organisms but inhibit the others (Edwards and Ewing, 1972).

The optimum temperature for the growth of salmonellae is 37°c, but growth also occurs at 43°c. They grow well with the pH range of 6-8, optimum being pH 7(Ananthanarayan and Paniker, 1982; Mackie and McCartney, 1989; D'Aoust, 1991).

Salmonellae are able to resist freezing in water and to certain chemicals such as brilliant green, sodium tertrathionate and sodium deoxycholate. Coliforms are inhibited by these compounds and therefore, salmonellae can be isolated selectively (Vanpoucke, 1990). Many species of *Salmonella* are able to tolerate selenium which is usually a very toxic element (Shrift and Boulette, 1974).

Organisms of the genus *Salmonella* are killed by heat, e.g., 1h at 55°c or 15min at 60°c (Ananthanarayan and Pankiker, 1982). These organisms are destroyed by cooking of meat, boiling and chlorination of water and pasteurization of milk (Mackie and McCartney, 1989). In dried cultures salmonellae die more quickly, but if prevented from drying cultures may survive for years.

2.3. Occurrence and Growth of *Salmonella* in Foods

2.3.1. Occurrence of *Salmonella* in Foods

Salmonellae are not only confined to animals and man. Eventhough their main habitat is the intestinal tract of man and animals, they can be spread to the surroundings where they may survive and even multiply. These microorganisms are often isolated from different foods like meats and meat products, milk and milk products, eggs and egg products (Collins and Lyne, 1976; Munoz *et al.*, 1987; Pelczar *et al.*, 1988; Mattila and Frost, 1988; Manolis *et al.*, 1991; Thorne, 1991; Kelly *et al.*, 1995; Schoeni *et al.*, 1995). The occurrence of salmonellae in bone meal and fish meal has been reported by Williams (1975) in his review of environmental conditions in salmonellosis.

Animals, eggs, meats, and meals of animal origin are found to be sources of 2473 strains in Belgium based on the isolation made from 1986 to 1990 (Pohl *et al.*, 1991). About 0.5% of cooked meat, 3% of raw pork and 12% of raw sausage meat were found to contain salmonellae as indicated by a survey of cooked and raw meats in England (Maguire *et al.*, 1993). Van Klink and Smulders (1990) found out the incidence of salmonellae to be 100% in spleens, 53% in livers, 33% in kidneys and 27% in the muscles of veal calves. In a study made by O'Toole (1995), salmonellae were found in 32 of the 100 samples of meat. In another study the *Salmonella* incidence in minced and/or pork samples was 1.8% in 112 samples from the USA, 2.0% in 1837 samples from England, 5.3%in 322 German samples, and 20.0% in

25 Canadian samples (O'Toole, 1995). Chau *et al.* (1977) reported that 55% of all pig carcasses were salmonellae positive in Hong Kong. A Total of 53 (34.4%) of 154 feral pig carcasses were contaminated with one or more serotypes of *Salmonella* (Bensink *et al.*, 1991). Here, *S. anatum*, *S.typhimurium* and *S.havana* accounted for about 70% of the total isolations. About 2.4% of the pork samples examined were contaminated with *Salmonella* (Dava *et al.*, 1988). The incidence of *Salmonella* in fresh chicken at the retail outlet was 37% as found out by an investigation carried out in Canada (Franco and Williams, 1991). The incidence of *Salmonella* on poultry carcasses, in certain cases, has been found out to be 71% (Franco and Williams,1991). In the years 1983-1988, 53% of *S.enteritidis* isolates were isolated from eggs and poultry (Fantasia *et al.*, 1991). Two hundred fourteen of 477 (45%) chicken carcasses carried one or more *Salmonella* types (Reilly *et al.*, 1991). Salmonellae were detected in 69 (24.1%) of 286 chicken meat samples,(Tokumaru *et al.*, 1991).

In Ethiopia salmonellae were isolated from the different organs of animals such as spleen consumed by man (Sahlu, 1983) and from fresh raw beef (Mogessie, 1994). Salmonellae were also isolated from undercooked eggs served after 14h of storage at room temperature Abraham *et al.*(1994) after an outbreak of food poisoning among college students.

2.3.2. Growth of *Salmonella* in Foods

It was reported that the proportion of survival of *S.enteritidis* in the eggs stored at 23°c was higher than in the eggs stored in the refrigerator after cooking by different methods (Schoeni *et al.*, 1995). The rate of survival of *Salmonella* in cooked eggs that were stored for longer periods at room temperature was higher than in those stored for shorter time (Saeed and Koon, 1993). Increase in the temperature of storage was found to result in the increase of *S.enteritidis* in the eggs (Kim *et al.*, 1989). The increase of *S. enteritidis* in the eggs stored at 4°c was less than in the eggs stored at temperatures higher than 4°c. The above points indicate that temperature and time of storage of food have effect on the survival and growth of the causative agents of salmonellosis.

Salmonella species were found to grow well in Ethiopian traditional sour milk, "ergo", where they grew to the level of 10^8 CFU/ml within 12h (Mogessie, 1993). Their growth was also found to be luxurious in Ethiopian traditional sauces, i.e., legume -based sauces or "shiro wot", vegetable-based sauces or "gomen wot" and meat-based sauces or "siga wot" (Mogessie, 1996).

2.4. Medical Importance of *Salmonella*

Salmonellae cause the disease referred to as salmonellosis. Active cases of salmonellosis are sources of contamination and transmission to other human beings and lower animals. A number of ways may be involved in the infection of one person by another. Transmission can occur directly by faecal-oral route, it can be air-born (Black *et al.*, 1960; Krieg and Holt, 1984). This transmission can take place indirectly by means of foods, towels, contaminated toilet seats, and other objects (Chapman, 1980; Gupta *et al.*, 1980). Contamination of human foods of animal origin can be brought about by salmonellae which are widely distributed in such animals. Contamination of foods such as meat and animal products can also occur during processing or handling by human carriers (Collins and Lyne, 1976; Boyd and Hoerl, 1981). Typhoid organisms are transmitted from person to person via foods and water that are contaminated by faeces. The transmission of non-typhoid *Salmonella* infections from animal to animal and then to human beings occurs directly or indirectly through the procedures of animal processing (Boyd and Hoerl, 1981; Ananthanarayan and Paniker, 1982). Contaminated foods are found to be more important than person to person transmission. Consumption of foods that have been prepared 24 to 48h before and were left standing for several hours at room temperature is the cause for the majority of salmonellosis (Boyd and Hoerl, 1981; Ananthanarayan and Paniker, 1982; Mackie and McCartney, 1989).

Young children and elderly persons are found to exhibit the highest incidence of salmonellosis. The number of young children, especially under 1 year old, with salmonellosis is the highest and at the same time mortality within this age group also appeared to be greatest (Boyd and Hoerl, 1981; Ananthanarayan and Paniker, 1982).

The mass processing of meats and other animal products which are consumed by man result in the increasing cases of salmonellosis. With 25% increase over 1976, about 27,000 cases of non-typhoid salmonellosis were reported in 1977 (Boyd and Hoerl, 1981). Out breaks of salmonellosis reached epidemic proportions in Britain in 1980's, mainly because of consuming contaminated poultry products and in 1988 about 23,000 cases of *Salmonella* infection were reported in UK (Lamabadusuriya *et al.*, 1992). In 1970s, in England, many out breaks of salmonellosis that are associated with cold cooked meats including roast pork and cooked ham and affecting at least 550 persons have been reported (Maguire *et al.*, 1993). Salmonellae were found to contribute to an average of 5-6 deaths per year in Canada (Todd, 1992). The foods involved here were those which have potential hazards, such as meat and poultry. In the United States, about 50% of out-breaks of food borne salmonellosis were caused by *S. enteritidis, S. typhimurium* and *S.heidelberg* which could be isolated from chicken ovaries and faeces (Schoeni *et al.*, 1995).

3. MATERIALS AND METHODS

3.1. Sample Collection

Fifty samples of raw "kitfo" or "Tire kitfo" were collected from 10 different hotels, bars and restaurant in Addis Ababa as made available to the consumer. The samples were collected aseptically using sterile plastic bags. Five samples were collected from each site. These sites of sample collection were: Hotel A (Lideta), Hotel B (Sidist Kilo), Hotel C,D,E and F (Arat Kilo), Hotel G(Kazanchis), Restaurant A(Giorgis), Bar A and B (Merkato). Partially cooked "kitfo ("Lebleb") and well-cooked "kitfo" ("Tibs kitfo") samples were also collected from the above sites aseptically as mentioned above. The samples of "kitfo" were collected between November 1, 1996 and February 15, 1997. The collected samples were immediately taken to the laboratory for bacteriological examination.

Swab samples were collected from an area of $100cm^2$ of solid meat bought from butchers' shops aseptically using sterile cotton swabs, and then introducing into buffered peptone water (10 ml). Samples of solid meat were collected from butcher's shop for preparation of "kitfo" in lab and determination of the growth potential of *Salmonella* species in "kitfo". The swab samples and samples of solid meat for "kitfo" preparation were collected between March 1, 1997 and March 25, 1997.

3.2. Isolation and Characterisation of Microflora

Twenty five grams of "kitfo" and 225ml of 0.1% sterile buffered peptone water were homogenized in a stomacher lab blender for 1-3 min aseptically (Beckers *et al.*, 1985; Perales and Erkiaga, 1991; Stecchini *et al.*, 1991). Appropriate dilutions in sterile 0.85% NaCl solution were plated on different plating media for microbial counts.

Aerobic Mesophilic Count

Volumes of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of plate count (PC) agar (Oxoid) plates using a bent glass rod. The media were prepared according to the instructions of the manufacturers. Colony counting was made following incubation at 37°c for 24-48 hours.

Coliform Count

Volumes of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of Violet Red Bile (VRB) agar (Oxoid) plates. The plates were incubated at 37°c for 24 hours. Purplish red colonies on VRB agar surrounded by a reddish zone of precipitated bile were counted as coliforms.

Counts of Staphylococci

Volumes of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of Mannitol Salt Agar (MSA) (Oxoid) plates. The plates were incubated at 37°c for 36 hours. Colonies were counted at the end of incubation.

Counts of Lactic Acid Bacteria

Volumes of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of Mann, Rogossa and Sharpe (MRS) agar (Oxoid) plates. Colonies were counted after incubation in anaerobic jar (Oxoid) at 37°c for 24-48 hours.

Mold and Yeast Count

Volumes of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of chloramphenicol-bramophenol blue agar. Colonies were counted after incubating the plates at 28-30°c for 4 to 5 days.

Ingredients of chlormaphenicol-bromophenol blue agar: yeast extract, 5.0g; glucose, 20.0g; chloramphenicol, 0.1g; bromophenol blue, 0.01g; agar, 15g; distilled water, 1000ml; pH, 6.0-6.4.

Spore Count

Volumes of 0.1ml of appropriate dilutions which were heat treated at 80°c in a water bath for 10 min were spread plated in duplicates on pre-dried surfaces of PC agar plates. Colonies were counted after incubation at 37°c for 24-48 hours.

After colony counting ten to twenty colonies were picked at random from different countable plates. The isolates were further purified by repeated plating and characterised in the following way.

Cell Morphology

Wet mounts were prepared from young pure cultures and examined under light microscope using oil immersion objective. The following were the morphological criteria considered in the observation.

i. Cell type:- rods, coccoid forms, cocci

ii. Cell grouping:- singles, pairs, chains, clusters

iii. Motility:- motile, non-motile

KOH-test (Test on Lipopolysaccharide)

KOH-test was made according to Gregersen (1978). The cell wall of Gram negative bacteria contains a lipopolysaccharide which is absent in Gram positive bacteria. The application of KOH dissolves the lipopolysaccharide from the cell wall which stretches when pulled with a needle.

One or two drops of 3% KOH solution were placed on a clean microscope slide (glass slide). A colony was picked from the surface of the plates with an inoculating loop. The material was stirred in the KOH solution for 5-10 seconds and the inoculating loop was then raised slowly from the mass. When the KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2cm or more. This was observed in Gram negative bacteria. When there was no slime, but a watery suspension that did not follow the loop, the reaction was negative and this was seen in Gram positive bacteria.

Catalase Test

The colonies were flooded with a 3% solution of H_2O_2. Formation of bubbles indicated a positive reaction.

Cytochrome Oxidase Test

Cytochrome oxidase was tested by the method of Kovacs (1956). Fresh preparations of reagent A and B were mixed in the ratio of 2:3 immediately before use. After flooding the colonies with the mixture, appearance of a blue color on the colonies within 30 seconds to 2 minutes indicated a positive reaction.

Reagents:- a) 1% α-naphthol in ethanol

b) 1% N, N-dimethyl-p-phenylene-diammonium chloride in water.

Oxidation-fermentation Test

Glucose metabolism was investigated by the O/F test of Hugh and Leifson (1953).

Ingredients:- peptone, 2.0g; yeast extract, 1.0g; NaC1, 5.0g; K_2HPO_4, 0.2g; glucose, 10.0g; bromothymol blue, 0.08g; agar 2.5g; distilled water, 1000ml; pH, 7.1.

The freshly prepared medium (10ml amounts in test tubes) was cooled down to about 35°c under tap water, inoculated by stabbing with a straight wire to the bottom and incubated at 37°c. Acid formation and growth regions were interpreted after 2 and 5 days.

3.3. Isolation of *Salmonella* Species

Isolation of salmonellae was carried out in the following way. To test for *Salmonella* Buffered Peptone Water (BPW) was incubated at 37°c for 16-20 hours.

3.3.1. Enrichment Broths

These appeared to be important in that they inhibit Gram positive bacteria and coliforms, permitting the rapid multiplication of salmonellae.

Selenite broth (Oxoid), Tetrathionate broth (Oxoid) and Rappaport-Vassiliadis broth (Merck) were used for enrichment. After pre-enrichment in buffered peptone water, 1ml of culture was inoculated in a tube containing 10ml of selenite broth and in to another tube containing an equal amount of Tetrathionate broth. In addition, 0.1ml of BPW was also inoculated in a tube containing 10ml of Rappapart-Vassiliadis broth. Selenite broth was incubated at 37°c and Tetrathionate broth and Rappapart-vassiliadis broth at 43°c for 24 hours in water bath (Harvey and Price, 1968; Clinton *et al.*, 1981; Beckers *et al.*, 1987)

3.3.2. Plating

It was carried out using Salmonella-Shigella (SS) agar, MacConkey agar, Brilliant green agar and Xylose lysine deoxycholate (XLD) medium (all from Oxoid). These media were prepared according to manufacturers' instruction.

Each of the above media was streaked with a loopful of culture from enrichment broths and incubated at 37°c for 18-24 hours (Xirouchaki *et al.*, 1982; Patil and Parchad, 1989; Murray *et al.*, 1995).

To check the sterility of the media uninoculated (control plates) were incubated at 37°c for 18-24 hours. Among the inoculated plates, the SS and MacConkey plates were examined for pale (uncolored) non-lactose fermenting colonies, the BG plates for red (pink) and non-mucoid colonies, and XLD plates for red and red with black centre colonies.

Using a sterile straight wire, 5 to 6 *Salmonella* looking-colonies were picked from each plate and separately inoculated into tubes containing about 3-4ml nutrient broth. These were incubated at 37°c for 18-24 hours. To check the purity of the broth inocula for biochemical tests a loopful of each broth inoculum was subcultured on nutrient agar (Oxoid) plates by streaking and incubated at 37°c for 18-24 hours. Cultural purity was confirmed by observation, Gram reaction and microscopic examination.

3.3.3. Biochemical Identification

The following biochemical tests were carried out to identify salmonellae. Using a sterile, straight wire, the broth culture was used to inoculate the following biochemical tubes.

a) Triple Sugar Iron (TSI) Agar (Oxoid) Slant:- It was prepared according to manufacturers' instruction. The butt was stabbed and the slant streaked to detect fermentation of glucose, sucrose and lactose as well as production of hydrogen sulphide.

b) Lysine Iron (LI) Agar (Oxoid) Slant:- The butt was stabbed and the slant streaked for the detection of oxidative deamination of lysine on the slant and decrboxylation of lysine in the butt.

c) Urea Agar (Oxoid) Slant:- The slant was lightly streaked to investigate hydrolysis of urea.

d) Simmons' Citrate Agar (Oxoid) Slant:- The butt was stabbed and the slant streaked to determine utilization of citrate as a sole source of carbon.

e) SIM Medium (Oxoid):- It was stabbed for the determination of hydrogen sulphide production and motility. Production of indole was also determined by adding Ehrlichs' reagent to growth in this culture medium.

f) Glucose Broth (1%):-

Ingredients:- peptone, 10g; glucose, 10g; NaC1, 5g; phenol red, 0.024g; distilled water, 1000ml; pH 7.2.

Glucose broth with inverted Durham tube was inoculated to detect fermentation and gas production from glucose.

g) Sucrose Broth (1%):-

Ingeredients:- peptone, 10g; sucrose, 10g; NaC1, 5g; phenol red; 0.024g; distilled water, 1000ml; pH 7.2.

Sucrose broth with inverted Durham tube was inoculated to detect test was carried out.

The meat which was bought from butcher's shop was cut in to small pieces using a knife (i.e., "kitfo" was prepared in the laboratory). This "kitfo" was pasteurized to kill all the salmonellae and other microflora which might be present on the meat. Then 200g of "kitfo" was inoculated with overnight cultures of *Salmonella* to give an inoculum level of about 2.0-3.0 Log(CFU/g). The inoculated "kitfo" was mixed thoroughly and left at ambient temperature for 24 hours.

Analysis of samples was done as follows. Freshly inoculated "kitfo" was sampled (20g) aseptically and was inoculated in to 180ml of 0.85% NaCl solution. This was subjected to serial dilutions. Then volumes of 0.1ml of appropriate dilutions were spread plated in duplicates, on pre-dried surfaces of MacConkey agar (Oxoid) plates using a bent glass rod to determine the initial inoculum level. Inoculated "kitfo" was sampled (20g) aseptically at 4h intervals for 16h and final count was made at 24h. Appropriate dilutions were spread-plated on MacConkey agar and incubated at 32°c for 24-48h for colony counting.

3.4. Analysis of Swabs of Solid Meats

Using sterile cotton swab $100cm^2$ surface area of solid meat was swabbed. The swab sample was inoculated in to a tube containing 10ml of sterile BPW and was shaken using Vortex mixer. To determine microbial count 1ml was taken from BPW and then subjected to serial dilution using 0.85% NaCl solution. Volumes of 0.1ml of appropriate dilutions were spread plated in duplicates on pre-dried surfaces of each of PC, VRB, MS, MRS, chloramphenicol-bromophenol blue, and PC agar plates using a bent glass rod for aerobic mesophilic count, coliform count, counts of staphylococci, counts of lactic acid bacteria, mold and yeast count and spore count, respectively. Temperatures and time of incubation of the plates for swab samples were the same as those for "kitfo" samples mentioned above.

To detect salmonellae BPW was incubated at $37^{o}c$ for 16-20 hours. After incubation 1ml of BPW was inoculated into 10ml of each of Selenite broth and Tertrathionate broth. In addition, 0.1ml of BPW was inoculated into 10ml of Rappaport-Vassiliadis broth. Temperatures and time of incubation of the broths were carried out in the same way as for "kitfo" samples. Plating and biochemical identification used to detect salmonellae in swab samples were carried out in similar ways as for "kitfo" samples indicated above.

3.5. Preparation and Inoculation of "Kitfo"

To determine the growth potential of *Salmonella* species in "kitfo", the following fermentation and gas production from sucrose.

h) Mannitol Broth (1%):-.

Ingredients:- peptone 10g; mannitol, 10g; NaCl, 5g; phenol red, 0.024g; distilled water, 1000ml; pH 7.2

This broth with inverted Durham tube was inoculated to detect fermentation and gas production from mannitol.

To check the sterility of the media for biochemical tests, all tubes with media were pre-incubated at 37°c for 18-24 hours. After inoculation all biochemical tubes were incubated at the same temperature and incubation time.

3.3.4. Serological Identification

Isolates that met the minimum biochemical profile of *Salmonella* were subjected to serological test by slide agglutination technique (Edwards and Ewing, 1972; Jawetz *et al.*, 1976). Serological identification was carried out using Difco polyvalent O antiserum and polyvalent H antiserum as follows.

A drop of antiserum was put on a sterile microscope slide. Culture from an agar slope was taken using a sterile loop and suspended in the antiserum. Then agglutination was observed.

4. RESULTS

The highest load of aerobic mesophilic bacteria was encountered from raw "kitfo" of Bar A, with the average load of 8.33 Log(CFU/g) and the lowest load from that of Restaurant A, with average load of 7.30 Log(CFU/g). The coliform load ranged between 6.77 Log(CFU/g) (Hotel B) and 4.51 Log(CFU/g) (Hotel A). "Kitfo" collected from Bar A was found to have the highest count of staphylococci with average count of 7.29 Log(CFU/g), while the lowest count was for Restaurant A (6.18 Log(CFU/g)). The count of lactic acid bacteria ranged between 5.37 Log(CFU/g) (Hotel D) and 4.10 Log(CFU/g) (Hotel A). Hotel G yielded high counts of molds and yeasts (5.96 Log(CFU/g)) where as that from Hotel A had low counts (4.06 Log(CFU/g)). "Kitfo" from Hotel B had the highest aerobic spore counts (5.67 Log(CFU/g)) and the lowest was from that of Hotel G (3.71 Log(CFU/g)) (Tables I and II).

Variations in aerobic mesophilic counts (AMC) within samples in an establishment were not significant in Hotels C,D, E and G, Restaurant A, Bar A and B (CV < 10%). Variations within samples in the other establishments were, however, significant (CV = 10.1 - 13.45%). Except two establishments, i.e., Hotel E and Bar A which have no significant variation in the counts of coliforms within samples (CV < 10%), the others have significant variation (CV = 10.32-19.29). The counts of staphylococci did not show significant variation in Hotels E and F, and Restaurant

Table I

The load of aerobic mesophilic bacteria, coliforms and staphylococci in "tire kitfo" samples from hotels, restaurant and bars

Hotels, Restaurant and Bars	Aerobic Mesophilic Bacteria					Coliforms					Staphylococci				
	Log (CFU/g)					Log (CFU/g)					Log (CFU/g)				
	Min	Max	X	S	%CV	Min	Max	X	S	%CV	Min	Max	X	S	%CV
Hotel A	5.69	8.46	7.36	0.99	13.45	3.69	5.77	4.51	0.87	19.29	5.15	7.00	6.81	0.80	11.74
Hotel B	7.00	9.39	8.31	0.84	10.10	5.23	7.39	6.77	0.76	11.22	5.68	8.43	7.21	1.10	15.25
Hotel C	6.30	8.39	7.83	0.64	8.17	4.54	7.23	6.18	0.96	15.53	5.69	8.17	6.99	0.94	13.44
Hotel D	6.93	7.94	7.32	0.34	4.64	5.50	7.30	6.39	0.66	10.32	5.68	7.60	6.42	0.67	10.43
Hotel E	7.30	7.47	7.42	0.03	0.40	5.94	6.93	6.14	0.36	5.86	6.36	7.65	6.90	0.50	7.24
Hotel F	6.92	9.39	8.10	0.96	11.85	5.00	6.38	5.72	0.59	10.31	5.84	7.14	6.83	0.48	7.02
Hotel G	7.00	8.39	7.81	0.54	6.91	4.69	6.07	5.57	0.65	11.66	5.44	7.41	6.22	0.76	12.21
Restaurant A	6.38	8.39	7.30	0.70	9.58	4.30	6.00	5.33	0.69	12.94	5.86	6.93	6.18	0.41	6.63
Bar A	7.17	9.43	8.33	0.79	9.48	4.00	5.07	4.66	0.45	9.65	5.79	8.41	7.29	1.00	13.71
Bar B	4.47	8.44	7.87	0.42	5.33	4.47	6.22	5.89	0.64	10.86	5.69	7.53	6.93	0.76	10.96

CFU, colony forming unit; X, mean; S, Standard deviation; %CV, percent coefficient of variation: Min,minimum; Max, maximum.

Table II

The load of lactic acid bacteria, molds and yeasts, and aerobic spores in "tire kitfo" samples from hotels, restaurant and bars

Hotels, Restaurant and Bars	Lactic Acid Bacteria					Molds and Yeasts					Aerobic Spores				
	Log (CFU/g)					Log (CFU/g)					Log (CFU/g)				
	Min	Max	X	S	%CV	Min	Max	X	S	%CV	Min	Max	X	S	%CV
Hotel A	4.20	5.36	4.10	0.47	11.46	3.57	4.80	4.06	0.6	14.77	3.95	5.30	4.97	0.51	10.26
Hotel B	4.39	6.44	5.05	0.83	16.43	4.00	4.90	4.46	0.33	17.71	4.46	6.39	5.67	0.79	13.93
Hotel C	4.39	6.27	5.12	0.79	15.42	3.30	4.90	4.44	0.57	19.81	4.76	4.82	4.79	0.88	18.37
Hotel D	4.07	6.46	5.37	1.13	21.04	4.00	5.25	4.62	0.63	8.65	3.11	4.07	3.92	0.40	10.20
Hotel E	4.30	6.41	5.33	0.74	13.88	4.43	6.54	5.45	0.74	12.29	4.45	6.39	5.50	0.67	12.18
Hotel F	3.74	5.44	4.78	0.64	13.38	3.46	6.39	4.50	1.05	23.33	3.62	5.13	4.93	0.64	12.98
Hotel G	4.04	6.38	5.16	0.84	16.27	4.20	6.39	5.96	0.86	14.42	3.60	3.77	3.71	0.10	2.69
Restaurant A	4.17	6.55	5.10	0.94	18.43	4.38	6.00	5.37	0.67	12.47	4.81	4.91	4.89	0.05	1.02
Bar A	3.89	6.30	4.41	0.93	21.08	3.90	5.77	4.86	0.86	17.69	5.23	5.69	5.45	0.14	2.56
Bar B	4.27	6.41	5.34	0.77	14.41	4.17	4.65	4.55	0.20	4.39	5.23	5.60	5.44	0.21	3.86

A (CV<10%), while the counts within samples of other establishments showed significant variation (CV=10.43 - 15.25%). Variations in the counts of lactic acid bacteria (LAB) within samples of the same sampling site were significant in all tested sites(CV=11.46-21.08%). Variations in the counts of molds and yeasts were significantly higher (CV=12.29-23.33%) in all establishments, except Hotel D and Bar B(CV<10%). The counts of aerobic spores within samples of an establishment showed low variation (CV<4%) in Hotel G, Restaurant A, Bar A and Bar B, while in the others variations were significant (CV=10.26-18.37%).

No significant variation was observed in mean aerobic mesophilic counts, counts of staphylococci and LAB among all establishments (CV<10%). However, mean counts of coliforms, molds and yeasts, and aerobic spores varied significantly (CV=12.6%, 11.82%, and 13.21%, respectively).

The load of microflora in partially-cooked "kitfo" ("lebleb kitfo") was found to be high, though less than that of raw or "tire kitfo". The highest loads of AMB, coliforms, staphylococci, LAB, molds and yeasts, and aerobic spores in "lebleb kitfo" were 7.90 Log(CFU/g), 5.68 Log(CFU/g), 6.96 Log(CFU/g), 4.88 Log(CFU/g), 4.90 Log(CFU/g) and 5.63 Log(CFU/g) which were collected from Hotel F, Hotel B, Bar A, Hotel D, Hotel G, and Hotel C, respectively. On the other hand, the lowest loads of the above microflora were found from that of Hotel A, Hotel A, Hotel G, Restaurant A, Restaurant A and Hotel G, respectively (Table III).

Table III

The load of microflora and aerobic spores in "lebleb kitfo" samples from hotels, restaurant and bars.

Hotels,Restaurant and Bars	Log (CFU/g)					
	AMB	Coliforms	Staph.	LAB	Molds and Yeats	Aerobic Spores
Hotel A	6.07	3.65	5.76	3.97	3.91	4.82
Hotel B	7.79	5.68	6.62	4.38	3.57	5.59
Hotel C	6.53	5.31	5.94	4.66	3.98	5.63
Hotel D	6.92	5.66	5.77	4.88	3.72	3.81
Hotel E	6.68	5.33	5.95	4.60	4.79	5.30
Hotel F	7.90	4.56	5.69	3.85	3.63	4.76
Hotel G	6.95	4.77	5.53	4.30	4.90	3.47
Restaurant A	6.76	4.55	5.74	3.67	3.51	4.35
Bar A	7.88	3.92	6.96	4.80	3.97	5.00
Bar B	6.79	4.81	6.88	4.83	3.81	5.08

AMB, aerobic mesophilic bacteria,; LAB, Lactic acid bacteria

Variations in the counts of AMB, staphylococci and LAB in "lebleb kitfo" among ten establishments were not significant (CV<10%), while that of coliforms, molds and yeasts and aerobic spores were significant (CV=14.10%, 11.3% and 14.85%, respectively).

Well-cooked "kitfo" ("Tibs kitfo") collected from all sampling sites did not display any microorganism, except for few aerobic spores.

Salmonellae were isolated from 21 of the 50 raw "kitfo" ("tire kitfo") samples collected from hotels, restaurant and bars in Addis Ababa. Out of the 21 samples with *Salmonella*, 5 samples were from each of Bar A and Hotel B, 3 samples from each of Bar B and Hotel C, 2 samples from each of Hotel A and Hotel F and 1 sample from that of Hotel G. . No *Salmonella* was recovered from "tire kitfo" samples collected from restaurant A, Hotel D and Hotel E (Table IV). No *Salmonella* was obtained from "lebleb" and "tibs kitfo" samples.

The hotels, restaurant and bars from which "kitfo" samples were collected varied from one another in their cleanliness. Hotels D and E, and Restaurant A were the cleanest sites. The kitchens where "kitfo" was prepared, kitfo preparing persons, waiters of "kitfo" and their clothings, the plates containing "kitfo" and the dining rooms were clean. Hotel G was cleaner than the rest with respect to the points mentioned above. Hotels A and F were not sufficiently clean. The kitchens were not

Table IV

Frequency of isolation of *Salmonella* from "Tire kitfo" from different

hotels,restaurant and bars

Hotels, Restaurant and Bars	No. of samples examined	No. of positive samples	% of positive samples
Hotel A	5	2	9.52
Hotel B	5	5	23.8
Hotel C	5	3	14.29
Hotel D	5	-	-
Hotel E	5	-	-
Hotel F	5	2	9.52
Hotel G	5	1	4.76
Restaurant A	5	-	-
Bar A	5	5	23.8
Bar B	5	3	14.29
All food establishments	50	21	100

clean, the waiters and their clothings were not fully clean, the plates containing "kitfo" were not thoroughly cleaned and the dining rooms had some dirts. The cleanliness of Hotel C and Bar B was poor. The kitchens were not clean and had dirt, "kitfo" preparing individuals were not clean in that the clothes they wore were dirty and the manner they handle "kitfo" was unhygienic. The same thing was true for cleanliness of the "kitfo" containing plates, waiters and the dining rooms. Hotel B and Bar A were the poorest of all establishments in their cleanliness. The kitchens where "kitfo" was prepared were very dirty. "Kitfo" preparing individuals were full of dirts (their hands and clothes were dirty) and the way they handle "kitfo" was very poor and unclean. The waiters were also careless in handling of "kitfo" and their clothes were having dirts. The dishes that contained "kitfo" and the dining rooms were also very unclean. It could be due to such variations in the status of the hotels, restaurant and bars that the prevalence of *Salmonella* in "kitfo" samples varied from one establishment to the other

The relative efficiency of the three selective enrichment media, i.e., Selenite borth, Tetrathionate broth and Rappaport-Vassiliadis broth was compared (Table V). Most of the samples with isolates , i.e., 12 samples with isolates were recovered by RVB, and TB was found to be the least in the recovery, i.e., 2 samples with isolates were recovered. Seven samples with isolates were recovered by SB.

Table V

Relative efficiency of Selenite broth, Tertrathionate broth and Rappaport-Vassiliadis broth for the recovery of *Salmonella*

Broth	No. of positive samples	% of positive samples
Selenite broth	7	33.33
Tetrathionate broth	2	9.52
Rappaport-Vassiliadis broth	12	57.14
All broths	21	100

The relative efficiency of the four culture (plating) media, i.e., Salmonella-shigella agar, MacConkey agar, Brilliant green agar and Xylose lysine deoxycholate medium was also compared. Out of the 21 samples with isolates of salmonellae, 11 were recovered from BGA, 7 from SSA, 2 from MacA, and 1 from XLD medium.

The load of microorganisms in the swab samples taken from the surfaces of solid meat was also determined. The average loads of AMB, coliforms, staphylococci, LAB, molds and yeasts and aerobic spores were 5.07 Log(CFU/cm^2), 2.34 Log(CFU/cm^2), 3.14 Log(CFU/Cm^2), 2.63 Log(CFU/cm^2), 2.54 Log(CFU/cm^2) and 2.60 Log(CFU/cm^2), respectively (Table VI).

Table VI

The load of microorganisms and aerobic spores

in the swabs of solid meats

Microorganisms and aerobic spores	Log (CFU/cm^2)
Aerobic mesophilic bacteria	5.07
Coliforms	2.34
Staphylococci	3.14
Lactic acid bacteria	2.63
Molds and yeasts	2.54
Aerobic spores	2.60

The growth potential of four *Salmonella* isolates in "kitfo" was determined. These isolates were found to grow luxuriously in "kitfo". They grew to the level of 7.0 Log(CFU/g) within 12h of incubation. Their growth was increasing from hour to hour. There was no time where growth was declined or stopped. Rapid growth was observed between 4 and 16h of incubation. Growth also continued till 24h of incubation, though the rate was not so rapid as the first 16h of incubation (Fig.1).

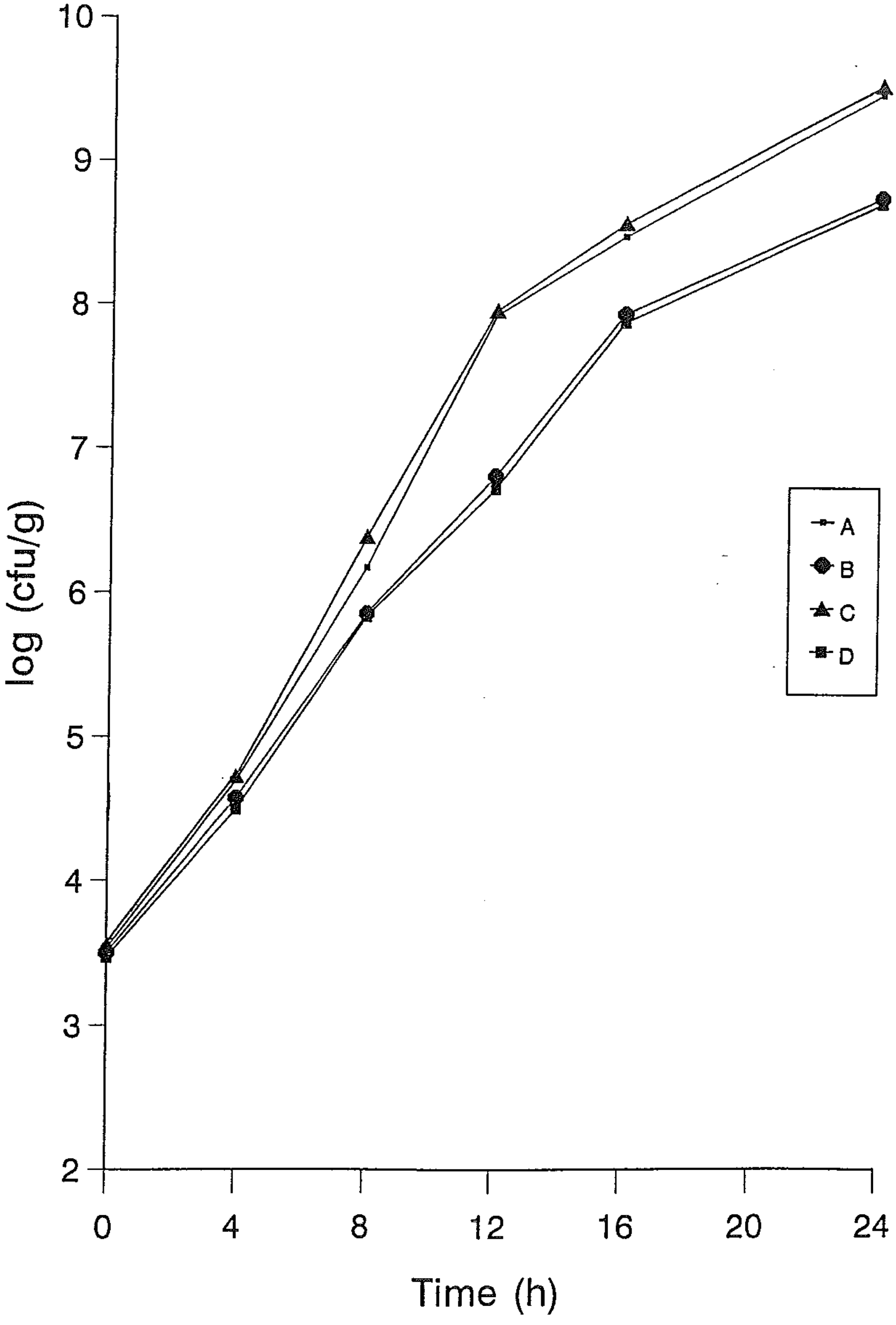

Fig. 1. Growth dynamics of four *Salmonella* test strains A,B,C, and D in 'kitfo'

5. DISCUSSION

The load of microorganisms in "tire kitfo" was found to be high. The highest load of aerobic mesophilic bacteria was encountered from "kitfo" from Bar A, with an average load of 8.33 Log(CFU/g) and the lowest from "kitfo" of Restaurant A, with an average load of 7.30 Log(CFU/g). The mean aerobic mesophilic counts (both the lowest and highest) obtained in this study were higher than the 5.0 Log(CFU/g) and 8.0 Log(CFU/g) reported by Mogessie (1994) from his study of microbial load of fresh raw beef from butchers' shops in Awassa. Coliforms were found to be high in number in raw or "tire kitfo". Their number was less than that of staphylococci, but higher than that of lactic acid bacteria in raw "kitfo" of all hotels, restaurant and bars. Staphylococci were the highest in raw "kitfo" collected from all hotels, restaurant and bars when compared with other microorganisms except that of aerobic mesophilic bacteria (count). Lactic acid bacteria were found to be less in number than both coliforms and staphylococci in all hotels, restaurant and bars. Molds and yeasts, and aerobic spores were also having high number in "tire kitfo".

The high load of microorganisms in raw "kitfo" showed that the level of contamination of "kitfo" was high. This contamination of "kitfo" could be the result of unhygienic handling process such as uncleanness of cutting knives, cutting boards, storage beans and unhygienic food handlers. The high load of microorganisms in raw "kitfo" suggested that consumption of this "kitfo" could be a health hazard. For example, the number of staphylococci was found to be high in such "kitfo" in this study. This meant that there was a chance where *Staphylococcus aureus* (known pathogen) could reach a level that initiates food intoxication.

When the load of microflora in slightly cooked "kitfo" or "lebleb kitfo" was analysed, it was found to be high, though it was less than that of raw "kitfo". It meant that consumption of "lebleb kitfo" or slightly cooked "kitfo" could also have its own health hazards. The analysis of properly cooked kitfo or "tibs kitfo" for microbial load showed that no microorganisms were encountered except for some aerobic spore formers. The absence of different microorganisms in "tibs kitfo" when compared to that of "tire" and "lebleb kitfo" indicated that consuming "tibs kitfo" is safe as it was free from pathogens such as *Salmonella* that cause disease of one sort or another.

The frequency of isolation of *Salmonella* in raw "kitfo" collected from hotels, restaurant and bars in Addis Ababa was 42%. The frequency of isolation of *Salmonella* obtained in this study was much higher than the 1.8%, 2.0%, 5.3% and 20% reported by O'Toole (1995) from USA, England, German and Canadian minced beef samples, respectively. The finding in this study of the prevalence of *Salmonella* isolates was also higher than the 9.0% reported by Mogessie (1994) from fresh raw beef samples from butchers' shops in Awassa and the 5.1% and 7% reported by Sahlu (1983) from the organs of cattles (spleen, etc.) in Addis Ababa and Dire Dawa abattoirs, respectively. The mincing process in "kitfo" preparation could have resulted in higher risks of contamination and ,thus, to the higher prelavence rate. On the other hand, the frequency of isolation of *Salmonella* obtained in this study was lower than the 55% reported by Chau *et al.* (1977) from the pig carcasses in Hong Kong and the 45% reported by Reilly *et al.* (1991) from chicken carcasses. The prevalence of *Salmonella* obtained in this study was beyond the range 0-2 to 21% reported by D'Aoust (1989) from beef carcasses, and within the range of 0.4 to 66.3% from pig carcasses and 5.0 to 79% in poultry. Pigs and poultry are, however, more exposed to *Salmonella* through their feeding habits than are cattle.

The salmonellae which were isolated from raw "kitfo" in this study could be the result of contamination of "kitfo" during the process of preparation. This is because the hotels and bars from which salmonellae were isolated were not clean and "kitfo" handlers (waiters and "kitfo" preparing persons) were not hygienic as their clothes were not clean and the way they handle "kitfo" lacked carefulness. Especially the kitchens of Bar A and Hotel B from which all of the samples were positive with *Salmonella* were hygienically poor. This meant that the possibility of contamination of "kitfo" in one way or another was high and it could be due to this point that the highest samples positive with *Salmonella* were obtained in such bar and hotel. Uncleaned utensils can harbour *Salmonella* and serve as constant source of this pathogen whenever food is processed using them. Thus, cross-contamination may be the major process by which "kitfo" in unhygienic food establishments is exposed to *Salmonella*.

As mentioned above salmonellae were recovered from many raw or "tire kitfo" samples. It is known that salmonellae are pathogens to man in that they cause disease of one sort of another. So, recovery of salmonellae from "kitfo" samples means that consumption of raw "kitfo" could have health hazards.

No *Salmonella* was obtained from "lebleb" and "tibs kitfo" samples analysed in this study. It meant that to be safe from different diseases caused by *Salmonella* species one should consume cooked "kitfo", especially "tibs kitfo" since no mciroorganisms, including *Salmonella*, have been obtained in such "kitfo", except some aerobic spore formers. Although *Salmonella* was not isolated from partially-cooked "kitfo", the high aerobic bacterial counts indicated that it is vulnerable to heat-tolerant pathogens and heat-stable toxins.

For the isolation of *Salmonella* from "kitfo" different enrichment broths, i.e., Selenite broth, Tetrathionate broth, and Rappaport-Vassiliadis broth were employed.

In this study, Tetrathionate broth was least efficient in isolating *Salmonella*. The efficiency of Selenite broth was less than Rappaport-Vassiliadis but higher than Tetrathionate. The reason that RVB appeared to be the most efficient in the isolation of *Salmonella* could be that it was a modern medium which was prepared by taking ingredients that specifically suit for the isolation of *Salmonella* from the background flora. Its inhibitory effects on the interfering competitive organisms could be higher than the other broths (Munoz *et al.*, 1987).

Of the four different differential and selective plating media, Brilliant green agar was the most efficient in *Salmonella* isolation. Brilliant green was the best medium for isolation. It is so selective that it strongly inhibits the growth of other competing microorganisms in favour of salmonellae.

The results that are obtained by different workers from previous works with regard to *Salmonella* are different. The differences in the findings of many workers could be due to the differences in the techniques employed and the culture media used. Since there is no single medium that supports the growth of all *Salmonella* serotypes, many workers use several media available at their disposal to grow *Salmonella* isolates.It is,therefore,not uncommon to find differences in the efficiency of growth by salmonellae on different media as clearly seen in this work.

The load of microflora in swabs from the surfaces of solid meat showed a lower microbial count than that of “kitfo”. Solid meat chunks have less surface area than minced meat. Consequently, the level of contamination from external sources was found to be lower since internal meat tissue is normally considered to be sterile.

No *Salmonella* was isolated from the swab samples. The absence of *Salmonella* and the decreased load of microflora from swab samples indicated that there was a great possibility of contamination of "kitfo" during the process of preparation.

The growth potential of *Salmonella* species in "kitfo" was determined and salmonellae were found to grow luxuriously. They grew to the level of 7.0 Log(CFU/g) within 12h of incubation. Their growth potential obtained in this study was less than that in "ergo" where the overnight count was 8.0 Log(CFU/ml), reported by Mogessie (1993) and also less than that in legume-based sauce or "shiro wot" where the over night count was over 10.0 Log(CFU/ml) (Mogessie, 1996). In "kitfo", the *Salmonella* species could reach infective doses within 4-8h, where less than 100,000 cells are required to initiate successful infection. This means that eating raw "kitfo" which harbours *Salmonella* species as well as "lebleb kitfo" where microbial load was high could result in disease of one sort or another. So, it is advisable not to consume "kitfo" in its raw or partially-cooked form.

RECOMMENDATIONS

In this study salmonellae were isolated in "kitfo", and their growth potential was found to be luxurious. Since raw "kitfo" as well as "lebleb kitfo" can harbour *Salmonella* species (though no *Salmonella* spp. was found in "lebleb" in this study) with good potential of growth, and salmonellae are pathogenic to man, it appears to be important to recommend the following points.

1. It is important that the kitchen where food, including "kitfo", is prepared should be clean.
2. Cutting knives, cutting boards, and storage beans should be kept clean to avoid cross contamination of "kitfo".
3. Persons who handle (prepare) "kitfo" should be clean and hygienic (their clothings and hands should be clean) and avoid faecal contamination of "kitfo".
4. Persons who are known to be carriers of salmonellae should not be involved in preparation and handling of "kitfo".
5. It is important to consume "kitfo" as soon as it is prepared and storage for long duration between preparation and consumption should be avoided.
6. It is advisable to consume cooked "kitfo" ("tibs kitfo") rather than raw and partially-cooked "kitfo" to be free from the risk of *Salmonella* infection, since proper cooking of "kitfo" can destroy or kill salmonellae (a temperature of 55^{o}c and above kills salmonellae).

REFERENCES

Abraham Assefa, Getahun Mengistu and Mogess Tiruneh (1994). *Salmonella newport*: outbreak of food poisoning among college students due to contaminated undercooked eggs. *Ethiop. Med. J.* 32(1): 1-6.

Ananthanarayan, R. and Paniker, C. K. J. (1982). *Text book of microbiology*. Sujit Mukherjee, Orient Longman Ltd. Madras: 271-285.

Beckers, H. J., Vanleusden, F. M., Meijssen, M. J. M. and Kampelmacher, E. H. (1985). Reference material for the evaluation of the standard method for the detection of salmonellae in foods and feeding stuffs. *J. Appl. Bacteriol.* 59 (6): 504-512

Beckers, H. J., Peters, R. and Pateer, P. M. (1987). Collaborative study on the isolation of *Salmonella* from reference material using selective enrichment media, prepared from individual ingredients or commercial dehydrated products. *Int. J. Food Microbiol.* 4(1): 1-11.

Bensink, J., Ekaputra, I. and Taliotis, C. (1991). The isolation of *Salmonella* from kangaroos and feral pigs processed for human consumption. *Aust. Vet. J.* 68(3): 106-107.

Black, P. H., Hunz, L. J. and Swartz, M. N. (1960). Salmonellosis - A review of some unusual aspects. *N. Eng. J. Med.* 262:811.

Boyd, R. F. and Hoerl, B. G. (1981). *Basic Medical Microbiology*, 2nd ed. Little, Brown and Company (Inc.), Boston: 391-395.

Breed, R. s., Murray, E. J. D. and Smith, N. R. (1967). *Bergys' Manual of Determinative Bacteriology*, 7th ed. Baltimore. The Williams and Wilkins Company.

Buxton, A. and Fraser, G. (1977). *Animal Microbiology*, Vol I. Blackwell Scientific publications. Oxford London Edenburgh Melbourne: 103-116.

Chapman, S. J. (1980). The occurrence of Enteric bacteria on lettuce leaves sold in local markets in penang, Malaysia. *Med. J. Mala.* 35(1): 7-8.

Chau, P. Y., Shortridge, K. F. and Houng, C. T. (1977). *Salmonella* in pig carcasses for human consumption in Hong Kong: a study of the mode of contamination. *J. Hyg.* 78: 253-260.

Cherrington, C. A., Allen, V. and Hinton, M. (1992). The influence of temperature and organic matter on the bacterial activity of short chain organic acids on salmonellae. *J.Appl. Bacteriol.* (72)6): 500-503.

Clinton, N. A., Weaver, R. W. and hidalgo, R. J. (1981). Transmission of *Salmonella typhimurium* among feedlot cattle after oral inoculation. *J.Appl. Bacteriol.* 50(1): 149-155.

Collins, C. H. and Lyne, P. (1976). *Microbiological methods*. Butter worth and co(publishers) Ltd. London: 357-375.

Dava, R. Y., Prasad, M. and Narayan, K. G. (1988). Dynamics of contamination of pork with *Salmonella* in a pork processing plant. *Ind. J. Anim. Sci.* 58((6): 663-665.

D'Aoust, J. Y. (1989). *Food Borne Bacterial Pathogens*. Boyle MP, Marcel Bekker Inc., New York: 327.

D'Aoust, J. Y. (1991). Psychrotrophy and food borne *Salmonella*. *Int. J. Food Microbiol.* 13(3): 207-216.

Edwards, P. R. and Ewing, W. A. (1972). *Identification of Enterobacteriaceae*, 3rd ed. Burgess publishing company. Minneapolis, Minnesota.

Fantasia, M., Filetici, E., Anastasio, M. P., Marcozzi, M. D., Gramenzi, M. P. and Aureli, P. (1991). Italian experience in *Salmonella enteritidis* 1978-1988: characterization of isolates from food and man. *Int. J. Food Microbiol.* 12(4): 353-362.

Franco, D. A. and Williams, C. E. (1991). Salmonellosis prevention. *J. Env. Hlth.* 53(5): 34-38.

Freeman, B. A. (1979). *Text Book of Microbiology*, 21st ed. W. B. Saunders Company. Philadelphia, London, Toronto: 518-561.

Gregersen, T. (1978). Rapid method for distinction of Gram negative from Gram positive bacteria. *Eur. J. Appl. Microbiol.* 5: 123-127.

Gupta, B. R. Singh, M. P. Verman, J. C. and Uppal, P. K. (1980). Isolates of *Salmonella* (3,10: r:-) from cases of human food poisoning. *Ind. J.Med. Res.* 71: 175-177.

Harvey, R. W. W. and Price, T. H. (1968). Elevated temperature incubation of enrichment media for isolation of salmonellas from heavily contaminated materials. *J. Hyg.* 66:377-381.

Hugh, R. and Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. *J. App. Bacteriol.* 66: 24-26.

Jawetz, E., Melnick, J. L. and Adelberg, A. (1976). *Review of Medical Microbiology*, 12th ed. LANGE Medical publications, Los Atlos, California: 208-211.

Kauffmann, F. (1966). Kauffmann-White Schema, in - *The Bacteriology of Enterobacteriaceae*. Williams and Wilkins Co., Baltimore: 149-226.

Kelly, J., Hopkin, R. and Rimsza, M.E. (1995). Rattlesnake meat ingestion and *S. arizona* infection in children: Case report and a review of literature. *Pediat. Infect. Dis. J.* 14(4): 320-322.

Kim, C. J., Emergy, D. A., Rinke, H., Nagaraja, K. V, and Halvorson, D. A. (1989). Effect of time and temperature on growth of *S. enteritidis* in experimentally inoculated eggs. *Avian Dis.* 33(4): 735-742.

Kovacs, N (1956). Identification of *Pseudomonas pyocyanea* by the oxidative reaction. *Nature*. 178: 703.

Krieg, N. R. and Holt, J. G. (1984). *Bergys' Manual of Systematic Bacteriology*, Vo.I. Williams and Wilkins, Baltimore/London: 427-491.

Lamabadusuriya, S. P., Perera, C., Devasiri, I. V., Javantha, U.K. and Chandrasiri, N. (1992). An out break of Salmonellosis following consumption of monkey meat. *J.Trop. Med Hyg*. 95:292-295.

Mackie, T. J. and McCartney, J. E. (1989). *Practical Medical Microbiology*, 125th ed. Vol.2 Churchil Living Stone, Edenberg: 456-480.

Maguire, H. C. E., Codd, A. A,, Mackay, V. E., Rowe, B. and Mitchell, E. (1993). A large out break of human salmonellosis traced to a local pig farm. *Epidemiol. Infect* 110: 239-246.

Manolis, S., Webb, G. J. W., Pinch, D., Melville, L. and Hollis, G. (1991). *Salmonella* in captive crocodiles (*Crocodylus johnstoni* and *C. porosus*). *Aust. Vet. J.* 68(3): 102-105.

Mattila, T. and Frost, A. J. (1988). The growth of potential food poisoning organisms on chicken and pork muscle surfaces. *J. App. Bacteriol.* 65: 455-461.

McCapes, R. H., Osburn, B. I. and Riemann, H. (1991). Safety of food of animal origin: Model for elimination of *Salmonella* contamination of turkey meat. *JAVMA*. 199(7): 875-880.

Mogessie Ashenafi (1993). Fate of *S.enteritidis* and *S.typhimurium* during the fermentation of "ergo" a traditional Ethiopian sour milk. *Ethiop. Med. J.* 31(2): 91-98.

Mogessie Ashenafi (1994). Microbiological flora and incidence of some food borne pathogens on fresh raw beef from butchers' shops in Awassa, Ethiopia. *Bull. Anim. Hlth. Prod. Afr.* 42: 273-277.

Mogessie Ashenafi (1996). Growth potential of some food borne pathogens in various traditional Ethiopian sauces. *Ethiop. J. Hlth. D.* 10(1): 41-45.

Morinigo, M.A., Martinez-Marzanares, E., Munoz, A., Cornax, R. Romero, P. and Borrego, J. J. (1989). Evaluation of different plating media used in isolation of salmonellas from environmental samples. *J. Appl. Bacteriol.* 66(4): 353-360.

Munoz, A., Frapolli, E., Morinigo, M. A., Borrego, J. J. and Romereo, P. (1987). Isolation of *Salmonella* using three selective enrichment broths from sausages naturally contaminated. Its relationship with other microorganisms that produce food toxi-infections. *Zbl. Bakt. Hyg.* 185: 105-11.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolken, R. H. (1995). *The Manual of Clinical Microbiology*, 6th ed. ASM Press, Washington D.C. 438-455.

O'Toole, D. K. (1995). Technical report: Microbiological quality of pork meat from local Hong Kong markets. *Wld. J. Microbiol. Biotech.* 11: 699-702.

Pelczar, M. J., Chan, E.C.S., Krieg, N. R. and Pelczar, M. R. (1988). *Microbiology*, 5th ed. McGraw-Hill Book Company, Singapore: 250-255.

Perales, I. and Erkiaga, E. (1991). Comparison between semisolid Rappaport and modified semisolid Rappaport-Vassiliadis media for the isolation of *Salmonella* species from foods and feeds. *Int. J. Food Microbiol.* 14(1): 51-58).

Plotkin, G. R., Kluge, R. and Waldamn, R. H. (1979). Gastroenteritis: ethiology, pathophysiology and clinical maniferstations. *Med.* 58(1): 95-114.

Pohl, P., Lintermans, P., Marin, M, Robaeys, G. V., Chaesseur-Libotte, M. L. and Ghysels, G. (1991). *Salmonellia* isolated from animals, meat and foodstuffs in Belgium from 1986 to 1990. *Ann. Med. Vet. J.* 135: 275-280.

Prost, E. and Riemann, H. (1967). Foodborne salmonellosis. *Ann. Reve. Microbiol.* 21: 495-528.

Roa, D. N. and Sreenivasmuthy, V. (1985). A note on microbial spoilage of sheep meat at abmient temperature. *J. Appl. Bacteriol.* 58(5): 457-460.

Rao, D. N., Nair, K. K. S., Nair, R. B. and Haleem, M.A. (1991). *Salmonella* in poultry. Poul. Adv. 24(1): 63-67.

Reilly, W. J., Oboegbulem, S. I. and Munro, D. S. (1991). The epidemiological relationship between *Salmonella* isolated from poultry meat and sewage effiuents at a long stay hospital. *Epidemiol. Infect.* 106(1): 1-10.

Roth, J. A., Bolin, C. A., Brogden, K. A., Minion, F. C. and Wannemuechler, M.J. (1995). *Virulence Mechanisms of Bacterial Pathogens*, 2nd ed. ASM press, Washington D. C.: 42-42.

Saeed, A. M. and Koon, C. W. (1993). Growth and heat resistance of *S. enteritidis* in refrigerated and abused eggs. *J. Food prot.* 56(11): 927-931.

Sahlu Ayalew (1983). A preliminary Survey of salmonellae in cattle slaughtered at the Addis Ababa and Dire Dawa abtattoirs. *M.Sc. Thesis, Addis Ababa University, Addis Ababa*: 1-17.

Salyers, A. A. and Whitt, D.D. (1994). *Bacterial pathogenesis - A molecular approach*. ASM press, Washington D.C.: 229-243.

Schoeni, J. L.; Glass, K. A., McDermott, J. L. and Wong, A.C.L. (1995). Growth and penetration of *S.enteritidis, S. heidelberg* and *S. typhimurium* in eggs. *Int. J. Food microbiol.* 24(3): 385-396.

Shrift, A. and Boulette, R. F. (1974). Form of selenium in selenite enrichment media for isolation of salmonellae. *Appl. Microbiol.* 27(4): 814-816.

Stainer, R. Y., Ingraham, J. L., Wheelis, M. L. and Painter, P. R. (1989). *General Microbiology*, 5th ed. Mammilan Education Ltd., Hong Kong: 445-460.

Stecchini, M. L., Sarais, I. and Bertoldi, M. (1991). The incidence of *Lactobacillus plantarum* culture inoculation on the fate of *Staphylococcus aureus* and *Salmonella typhimurium* in Montasio cheese. *Int. J. Food Microbiol.* 14(2): 99-110.

Thorne, G. M. (1991). *Salmonella*: the chickens and the eggs. *Clin. Microbiol. News Let.* 13(9): 65-67.

Todd, E. C. D. (1992). Food borne diseases in Canada: A 10 year summary from 1975 to 1984. *J. Food prot.* 55(2): 123-132.

Tokumaru, M., Konuma, H., Umesako, M., Konno, S. and Shinagawa, K. (1991). Rates of detection of *Salmonella* and *Campylobacter* in meats in response to the sample size and the infection level of each species. *Int. J. Food Microbiol.* 13(1): 41-46.

Van-klink, E. G. M. and Smulders, F. J. M. (1990). A comparison of different enrichment media for isolation of *S.dublin* from livers, kidneys and muscles of *Salmonella* positive veal calves. *Int. J.Food Microbiol.*10(3 and 4):177-182.

Vanpoucke, L.S. G. (1990). *Salmonella* - TEK - a rapid screening method for *Salmonella* species in food. *Appl. Env. Microbiol.* 56(4): 924-927.

Williams, B. M. (1975). Environmental consideration of salmonellosis. *Vet. Rec.* 96: 318-321.

Xirouchaki, E., Vassiliadis, P., Trichopoulos, D. and Mavromatti, C. (1982). A note on the performance of Rapport's medium, compared with Rappaport-Vassiliadis broth, in the isolation of salmonellas from meat products, after pre-enrichment. *J. Appl. Bacteriol.* 52(1): 125-127.