THE BACTERIAL STATUS OF FOODSTUFFS AS INDICATOR OF POTENTIAL FOOD-BORNE DISEASES IN BOTSHABELO, SECTION M

HELEN VAN DER WESTHUIZEN

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DECLARATION OF INDEPENDENT WORK

I, HELEN VAN DER WESTHUIZEN, do hereby declare that this research project submitted for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent work that has not been submitted before to any institution by me or anyone else as part of any qualification.

.....dvanderwesthuizen.....
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OPSOMMING

Botshabelo, Seksie M word gekenmerk deur 'n hoë voorkoms van diarree, wanvoeding en ander voedsel- en melkverwante siektes. Sestig huise is daarom met behulp van 'n steekproef geselekteer om aan die studie deel te neem. Die doel van die projek was om organismegroepe te isoleer en te kwantifiseer, gepaardgaande met moontlike voedsel bronne wat 'n bydrae kan lewer tot hierdie onreëlmatighede in Seksie M. Mikro-organismes wat bestudeer is, het ingesluit totale mesofiele organismes, aerobe en anaerobe spoorvormers, koliforme, *Escherichia coli*, lede van die familie *Enterobacteriaceae*, giste en skimmels. Verteenwoordigende monsters van melk asook voorbereide voedsel is versamel. Vraelyste met betrekking tot sosio-ekonomiese status, waar die inwoners hulle voedsel en melk aankoop, asook die beskikbaarheid van water, elektrisiteit en sanitêre geriewe, is verwerk. Aanvullende inligting is ook verkry van die plaaslike owerheid en kliniek.

Twee gemeenskapswerkers is opgelei in die metodes van monsterneming en die invul van vraelyste. Dit is versamel oor 'n tydperk van 6 weke, 10 huisgesinne per week. Daar is gevind dat die higiëniese kwaliteit van melk aansienlik laer was as diè van voedsel, met die voorkoms van *E. coli* so hoog as 10^5 kve/ml. 'n Hoë voorkoms van spoorvormers het verder gedui op swak of onvoldoende pasteurisasie. Ondanks die feit dat voedsel laer mikrobiologiese waardes as melk getoon het, het dit steeds die toegelate standaard van 100 kve/cm^2 volgens die Suid-Afrikaanse wetgewing oorskry. Die voorkoms van mikro-organismes in voedsel het gewissel van 10^1 tot 10^7 kve/cm^2. In beide die voedsel en melk monsters het die voorkoms van mikro-organismes 'n oneweredige verspreiding van een huishouding na 'n ander getoon.

Ten einde die verwantskappe tussen indikator-organismes (totale tellings, koliforme, *E. coli*) met ander organismes in voedsel en melk te bepaal, is korrelasies bereken tussen mikro-organismes, voedsel en melk. Die tradisionele indikator-organismes het egter swak verwantskappe met mekaar getoon. Die *Enterobacteriaceae* het betekenisvol gekorreleer (r = 0.634) met die totale mesofiele organismes. 'n Sterk korrelasie is gevind tussen die mikro-organisme inhoud van voedsel en melk (r = 0.991). Dit is 'n sterk aanduiding dat swak hantering van voedsel en melk deur die verbruiker, eerder as swak voedsel- en melk kwaliteit van die verskaffer, die hoofoorsaak van kontaminasie is. Hierdie resultate, tesame met die gelyksoortige vingerafdruk-patrone van organismes in voedsel en melk, ondersteun hierdie voorstel. Die voorkoms van voedsel- en melkgedraagde siektes kan dus in 'n groot mate verminder word deur goeie higiëniese praktyke tuis.

SUMMARY

Botshabelo, Section M, is characterised by a high incidence of diarrhoea, malnutrition and other food- and milk-related diseases. For this reason, 60 households were randomly selected in the area to partake in the study. The aim of the project was to isolate and quantify organism groups concomitant with possible food sources that might contribute to these irregularities in Section M. Micro-organisms studied included total mesophilic organisms, aerobic and anaerobic spore formers, coliforms, *Escherichia coli*, members of the family *Enterobacteriaceae*, yeasts and moulds. Representative samples were collected from milk and prepared food, together with questionnaires covering the socio-economic status, food and milk purchases, as well as the availability of water, electricity and sanitation. Additional information was gathered from the local council and clinic.

Two community workers were educated on the methods of sampling and the completion of questionnaires. These were collected over a period of 6 weeks, covering 10 households per week. The hygienic quality of milk was found to be considerably lower than that of food with the occurrence of *E. coli* as high as 10^5 cfu/ml. A high occurrence of spore formers, furthermore, indicated poor or inappropriate pasteurisation. Despite the fact that food showed lower microbiological values than milk, the standard of 100 cfu/cm^2 set in South African legislation was, however, exceeded. The incidence of micro-organisms in food varied from 10^1cfu/cm^2 to 10^7 cfu/cm^2 in the sampled households. In both the food and milk samples, the microbial incidence showed an uneven distribution from one household to another.

To establish comparisons between indicator organisms (total counts, coliforms and *E. coli*) with the other organisms isolated in food and milk, correlations were calculated between

micro-organisms, food and milk. The traditional indicator organisms, however, showed poor relationships with each other. The *Enterobacteriaceae* correlated positively (r = 0.634) with the total mesophilic organisms. A strong correlation was found between the microbial incidence of food and milk (r = 0.991), which suggested that poor handling of food and milk by the consumer was the main source of contamination, rather than poor food and milk quality supplied by the suppliers. These results, together with similar fingerprint patterns of organisms in food and milk supported this proposal. The possibility of food- and milk-borne diseases can thus be reduced by good hygiene practices at home.

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CHAPTER 1

INTRODUCTION

Helen van der Westhuizen

1. INTRODUCTORY REMARKS

As early as 1866, William Clendenin wrote in the Cincinnati Daily Gazette of July 23: "Before erecting statues, buildings, opera houses and art galleries, and buying expensive pictures, towns should be relieved of bad odours and fermenting pestilence. Good privies are far higher signs of civilisation than grand palaces and fine art galleries" (Blaser *et al.*, 1995). Although more than a 100 years have since passed, in today's terms this means ensuring a healthy community, health programs should be focussed on environmental hygiene, sanitation, the alleviation of poverty as well as proper education to the public (Stewart, 1978). This aspect has been amplified by the WHO (World Health Organisation) defining health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (Stewart, 1978).

The WHO has recognised the importance of safe food and water supply in human health and included the concept of safe water and basic sanitation in the Comprehensive Primary Health Care-document in 1978, recognising that the use of contaminated water and poor personal hygiene measures could lead to cross-contamination of food (Molbak *et al.*, 1989; WHO, 1995). As a result of this, the period between 1980 and 1990 was declared the International Drinking Water Supply and Sanitation Decade (Molbak *et al.*, 1989). However, despite all the improvements in general good housekeeping, education and medical care in South Africa, registrated deaths during 1988 due to food- and milk-borne diseases were an alarming 6 693 (SALUS, 1993).

Many of the diseases that occur in communities are related to lifestyle. For example, the main causes of death in Western Europe are cardiovasculary diseases, neoplastic diseases and degenerative conditions, whereas in Africa, transmittable diseases including parasitic infections

cause most of the deaths (De Haan, 1988). The people most affected by unsafe food are the poor who suffer from a lack of food and under-nutrition (WHO, 1997). Food-borne diseases are a major cause of mortality and morbidity in Europe and America, second only to respiratory infections (Notermans and Hoogenboom-Verdegaal, 1992; Savarino and Bourgeois, 1993; Blaser *et al.*, 1995). Statistics from the United States Food and Drug Administration have shown that there are about 81 million cases of food-borne diseases and more than 10 000 preventable deaths from food-borne diseases in America each year (Blaser *et al.*, 1995). Furthermore, human rights violations and inappropriate socio-economic policies have resulted in many populations becoming nutritionally vulnerable (WHO, 1992).

In general, increased disease and death-rates are caused by a lack of sanitation, inadequate facilities for the removal and treatment of sewage, insufficient and/or polluted food and water supplies and the presence of flies and insect vectors (De Haan, 1988). Improvements in food and water hygiene, through the provision of clean and safe water as well as latrines, should therefore reduce the risk of infectious diarrhoea, a food-borne disease. This statement has been emphasised in a study conducted by Imong *et al.* (1995), where a hygiene intervention program, coupled with improved water supplies and sanitation measures, led to the reduction in the incidence of gastro-enteritis.

2. AN OVERVIEW OF FOOD- AND MILK-BORNE DISEASES

Food-borne diseases have been known since biblical times and even before Christ. In Leviticus 11:39 Moses commanded the Israelites not to eat meat from animals afflicted with wasting diseases and in 434-354 BC, Xenophon described honey poisoning from *Andromela*, a genus of rhododendron (Morse *et al.*, 1994). More recently, the health problem posed by food-borne diseases was highlighted as an agenda of international concern by the WHO and

the Food and Agricultural Organisation (FAO) in 1984. The problem regarding food-borne diseases is two-fold as there is a world-wide increase in reported incidences of food-borne diseases, as well as an increase in new food-borne pathogens (Ehiri, 1995). According to Notermans and Hoogenboom-Verdegaal (1992), food-borne diseases are still the most common causes of disease in the world as a result of the ingestion of food and drink (mostly milk) contaminated with micro-organisms or their toxins, and may be toxic or infectious by nature (Musaiger, 1996). To cause food poisoning a number of factors must be present, for example, the numbers of pathogenic organisms must be high enough to cause disease and secondly the food must be physically and chemically suitable for proliferation of these organisms. The temperature must, furthermore, be optimal for rapid reproduction and enough time must elapse between contamination and consumption of the food to allow the organisms to multiply (Blaser *et al.*, 1995; Collins *et al.*, 1995).

2.1 Food-borne diseases

There are many water- and food-associated diseases known to man, with cholera probably the most common. Cholera outbreaks in the 1850's in England, and the 1890's in Germany and the United States, gave rise to the onset of the sanitary revolution (Blaser *et al.*, 1995). The essence of this revolution was the commitment of communities to long-term investments in the delivery of safe water and sewage systems for sanitary conditions. During this time the food handling practices also improved as the food production and food-serving industries went through a slow process of improvement in food safety. The investments made in the sanitary revolution had a major impact on industrialised nations. The solution for countries that still carry the burden of cholera and other diarrhoeal diseases, therefore lies in their own sanitary revolution. However, it is often argued that resources that are necessary to address these problems, are not available (Blaser *et al.*, 1995).

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Apart from cholera, the main diseases that have been shown to occur in localised outbreaks are typhoid and paratyphoid fevers as well as shigellosis. *Salmonella typhi* and *S. paratyphi* are the causative agents for typhoid and paratyphoid fever respectively, while *Shigella* spp. are responsible for shigellosis (Jacob, 1989). Spore-forming organisms that have been incriminated in food-borne diseases are *Bacillus* spp. and *Clostridium perfringens* (Kramer and Gilbert, 1989; Labbe, 1989; Johnson, 1990[1,2]; Jay, 1992; Hobbs and Roberts, 1993; Wrigley, 1994). *Escherichia coli*, *Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus* have also been involved in food-borne disease outbreaks (D'Aoust, 1989; Doyle and Padhye, 1989; Wachsmuth and Morris, 1989; Bergdoll, 1990; Doyle and Cliver, 1990[1,2]; Jay, 1992; Hobbs and Roberts, 1993; Martin and Myers, 1994; Maurelli and Lampel, 1994; Neill *et al.*, 1994; Ziprin, 1994). Other examples of food-borne organisms include *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Listeria monocytogenes* and *Yersinia enterocolitica* (Lovett, 1989; Schiemann, 1989; Stern and Kazmi, 1989; Twedt, 1989; Bahk and Marth, 1990; Doyle, 1990; Doyle and Cliver, 1990[3,4]; Jay, 1992; Hobbs and Roberts, 1993; Chai and Pace, 1994; Donnelly, 1994; Feng and Weagant, 1994; Franco and Williams, 1994; Schultz and Smith, 1994).

Food-borne botulism is another severe food-borne disease, caused by the ingestion of food contaminated with a pre-formed botulinum neurotoxin. The organism responsible for botulism is *C. botulinum* (Hauschild, 1989; Sugiyama, 1990; Jay, 1992; Dodds, 1994). Reports of botulism are, however, fairly rare and misdiagnosed in many cases. Argentina is the only country in the Southern Hemisphere that reported a substantial number of botulism outbreaks, while African countries like Chad, Egypt, Kenya, Madagascar and Zimbabwe also reported

cases of botulism (Dodds, 1994). Other food-borne diseases include Giardiasis (*Giardia lamblia*), Norwalk Virus infection (Norwalk Virus), Hepatitis A (Hepatitis A virus) and Trichinellosis (*Trichinella spiralis*, Jacob, 1989; Bishai and Sears, 1993).

Alkanahl and Gasim (1993) conducted a study in the period from 1982-1986 on the incidence of food-borne diseases in the eastern parts of Saudi Arabia. In their results it was concluded that the food service establishments and workers homes (homes similar to the households in Botshabelo, Section M where this study was conducted, also with improper cooking and refrigeration facilities) ranked highly in the incidents and cases of food-borne diseases. The most frequently reported foods associated with food-borne incidents were dairy foods, meat and chicken, of which insufficient cooking and improper storage of foodstuffs were suggested as being the main contributing factors to these incidents (Alkanahl and Gasim, 1993).

The consumption of contaminated fruit and vegetables has also led to a number of food-borne diseases, especially during summer months (Desai, 1987; Beuchat, 1995). Fresh fruit and vegetables are an essential part of the diet of people around the world, thus families grow vegetables and fruits where land is available, mostly in the backyard. Pathogenic micro-organisms from domestic waste as well as animals contaminate these foodstuffs at various stages during growth (Beuchat, 1995). In a study by Abdelnoor *et al.* (1983) on washed and unwashed vegetables and fruit, the authors isolated a variety of microflora of the genera *Enterobacter*, *Citrobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Providencia*, *Escherichia*, *Staphylococcus* and *Salmonella*. Many of these organisms are either pathogens or opportunistic pathogens.

Other than micro-organisms being causative agents, food-borne disease can also result from heavy metal poisoning of food. This syndrome is often associated with acidic foods or carbonated beverages that have been stored in metal containers (Bishai and Sears, 1993).

2.2 Milk-borne diseases

In South Africa, the staple food of about 90 % of residents of disadvantaged, marginalised urban settlements include milk and porridge or milk and bread. Although milk that leaves the udder of a healthy cow contains very few micro-organisms, it becomes contaminated with bacteria and other chemicals at various stages (Banwart, 1989; Philips and Griffiths, 1990). Bacteria may contaminate milk during handling and milking, for example, from the cow, milker, extraneous dirt or unclean water (Stewart, 1978; Rohde, 1985; Banwart, 1989; Roberts, 1991). The threat of diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is vast.

Many of the micro-organisms present in milk cause serious zoonotic diseases. These diseases are transmitted between animals and humans and are common in developing countries where public health and proper food hygiene practices are lacking (Foster, 1990). General infections often transmitted by milk include typhoid fever, diphtheria, scarlet fever and mastitis-related entero-toxaemia, while the most severe zoonoses transmitted via milk are probably tuberculosis and brucellosis (Foster, 1990). Tuberculosis is a communicable disease caused by *Mycobacterium tuberculosis* and has been a plague of humans and animals since earliest history (Stiles, 1989). Brucellosis, also known as undulant fever, is caused by *Brucella melintensis*, *B. abortus* and *B. suis* through the consumption of contaminated raw or inadequately pasteurised milk (Banwart, 1989; Stiles, 1989; Halling and Young, 1994). These

are, however, heat sensitive and are normally destroyed by proper pasteurisation (Banwart, 1989).

Other bacteria generally associated with milk include the genera *Pseudomonas*, *Escherichia*, *Salmonella*, *Shigella*, *Micrococcus*, *Staphylococcus* as well as *Lactobacillus* and *Lactococcus*. Thermoduric organisms like *Bacillus* and *Clostridium* are also present in milk, since their spores gain access to milk and dairy products through air, water, food and fodder (Cousins and Bramley, 1981; Gilmour and Rowe, 1981). The incidence of *B. cereus* intoxication from milk is not common, since the products become organoleptically unacceptable before it reaches the toxic stage (Roberts, 1991). Although *C. perfringens*, like *B. cereus*, is able to withstand pasteurisation, refrigeration temperatures inhibit the germination of spores produced by this organism. An outbreak of *C. perfringens* food poisoning occurred in the United Kingdom in 1980, but was attributed to contamination from milk powder (Philips and Griffiths, 1990).

Inadequate pasteurisation and post-pasteurisation contamination result in most of the reported *Salmonella* outbreaks associated with pasteurised milk (Philips and Griffiths, 1990). This is illustrated by annual outbreaks of food poisoning caused by *Salmonella* and *C. jejuni* in Whales and England (Hobbs and Roberts, 1993; Levick, 1997). Another outbreak of salmonellosis occurred in Illinois, USA, and surrounding states where *S. paratyphi* contamination of low-fat pasteurised milk caused 10 000 confirmed cases of salmonellosis in less than one month in 1985 (Philips and Griffiths, 1990; Todd, 1991). Cross contamination between raw and pasteurised milk also led to a *Salmonella* outbreak in the mid-western area of the United States, while *S. dublin* and *S. typhimurium* have been reported to be responsible for food poisoning outbreaks through the consumption of raw milk (Gilmour and Rowe, 1981; Savarino and Bourgeois, 1993).

Apart from *Salmonella* being present in milk, dairy cattle may also harbour *E. coli* 0157:H7. This organism has several characteristics, such as being able to survive refrigeration temperatures and, unlike most pathogens, being able to grow slowly at 5 °C. Refrigerated dairy products contaminated after pasteurisation consequently can support the growth of this organism. Fortunately, adequate pasteurisation or cooking destroys *E. coli* 0157:H7 (Duncan and Hackney, 1994). The presence of *E. coli* in milk is used as indicator of faecal contamination of the product, and although not all *E. coli* strains are entero-pathogenic, it should be regarded as pathogenic with regard to public health (Kruger *et al.*, 1986; Simango *et al.*, 1992).

The absence of adequate refrigeration as well as storage of milk at ambient temperatures allows the growth of bacteria (Rohde, 1985; Collins *et al.*, 1995). At ambient temperatures, members of the genus *Lactococcus* dominate the bacterial growth, later being replaced by lactobacilli and coliform bacilli. All of these organisms ferment lactose in milk and increase the lactic acid content, which consequently leads to the souring of milk (Collins *et al.*, 1995). While the souring of milk is not always sensorially acceptable, the increase in acid prevents the multiplication of pathogenic organisms for example *Salmonellae*, *St. aureus* and *E. coli* organisms (Collins *et al.*, 1995). Souring of milk is, thus a practise often utilised amongst South African rural inhabitants as a method of preservation. *E. coli*, however, can withstand pH-values of as low as 3.7, thus fermented products with no additional heat processing, could still pose a serious health risk (Duncan and Hackney, 1994).

The importance of good temperature control was illustrated in an outbreak of staphylococcal food poisoning in chocolate milk. Investigations into this matter revealed that the milk was kept for several hours at high temperatures prior to pasteurisation and although no

Staphylococci were present in the pasteurised milk, SEA (*Staphylococci* enterotoxin A) was detected. This enterotoxin is a relatively heat stable enterotoxin produced by *Staphylococci* and survived the pasteurisation process, indicating that *Staphylococci* had grown in the milk prior to pasteurisation (Bergdoll, 1990; Cliver, 1990).

3. DIARRHOEAL-RELATED DISEASES

Gastro-enteritis is a world-wide phenomenon and a term used for a variety of syndromes and states of diseases (Van der Berg, 1989). Symptoms of this group of diseases include nausea, vomiting and diarrhoea (Stewart, 1978; Jacob, 1989; Bishai and Sears, 1993). The gastro-enteritis syndrome results from the disruption of the digestive process giving rise to vomiting and diarrhoea in an attempt to remove the offending agent, while severe vomiting and diarrhoea could result in dehydration and depletion of serum electrolytes (Stewart, 1978). Diarrhoea, which is one of the most common symptoms associated with food- and milk-borne diseases, can be defined as the malabsorption of salt and water, resulting in a loss of fluid from the body or watery stools. This loss of fluid from the body could be a determining factor in the cause of death (Frischman, 1991; Eley, 1992).

Diarrhoeal diseases are caused by a variety of factors (Henry *et al.*, 1990). Possible candidates for food- and milk-borne diseases such as dysentery, gastro-enteritis and cholera are individuals who do not have access to clean water supplies and good sanitation, since these factors influence the hygienic quality and safety of food and milk. This includes people living in refugee camps and shanty towns, because the availability of the necessary facilities does not exist here. Refugee camps and shanty towns are, furthermore, often overcrowded, thus contributing to the rapid spread of diarrhoeal diseases (SALUS, 1993). In developing countries, particularly in rural and farming communities, where the standard of public

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sanitation and water supplies are low and hygienic practices are substandard, the incidence of diarrhoea is normally high (Nyatoti, 1997). Househam (1985) stated that the incidence of diarrhoea could be related to socio-economic conditions rather than climate. Thus, poverty is a stronger factor than the place of residence.

Inter-person transmission of diarrhoea is closely related to the hygiene practices of a community. The relationship between diarrhoea and improper washing of the hands has been documented by Blaser *et al.* (1995). In a study conducted in Bangladesh, a definite relationship between the incidence of diarrhoea and the state of water and sanitation facilities was reported (Henry, *et al.*, 1990). It was suggested that the impact of hand washing might depend on the level of sanitary facilities and hygiene. The disposal of faecal material also played a vital role in the control of diarrhoeal diseases, since the stools of infected persons contained large numbers of entero-pathogens. The misuse of clothing acted as a role player in the spreading of pathogenic organisms (Blaser *et al.*, 1995). Feeding utensils such as baby bottles, cups, spoons and dirty containers have, furthermore, been found to be frequently contaminated with faecal bacteria including *E. coli*, *Salmonella*, *Shigella* and *S. aureus*, even after being "cleaned" (Blaser *et al.*, 1995).

The incidence of diarrhoea is, furthermore, significantly affected by climate. Diarrhoeal diseases in South Africa have a summer peak and a winter trough, which is a result of an increase in the environmental temperature and contamination by bacteria such as *E. coli* (Househam, 1985; SALUS, 1993). In South Africa there is a major incidence of diarrhoea in the coloured and black communities, while among the white population, it is an insignificant medical problem. In developed countries like Europe and North America diarrhoea is, on the other hand, a winter disease caused by a rotavirus (Househam, 1985).

The problem that faces many developed and developing countries is the high mortality rate among infants and children, with diarrhoea and malnutrition as determining factors (Wittman and Hansen, 1965; Tswana *et al.*, 1990; Tumwine and Mackenzie, 1992; SALUS, 1993; Pant *et al.*, 1996). During 1993, diarrhoea was responsible for 3 million childhood deaths in developing countries, while parallel studies have showed that 35-56 % of deaths among children in developing countries is related to prolonged diarrhoea (Frischman, 1991; WHO, 1995). Diarrhoeal diseases are more likely to be persistent in two groups of infants, namely the very young and the malnourished (Frischman, 1991). Indirectly, malnutrition has led to a high mortality rate among children, since they are very susceptible to diseases and have a low immunity platform (Dykes, 1994). It was thus noted that up to 3 million children might die annually from dehydration and malnutrition as a result of prolonged diarrhoea (SALUS, 1993). Figure 1 represents the various components that play a role in the development of inadequate dietary intake and disease, resulting in malnutrition and death. The framework helps to identify the potential problem areas and also to clarify the objective of actions to be taken (UNICEF, 1990).

In non-fatal cases, diarrhoea has a negative effect on child growth and development. Statistics have shown that the average African child will suffer from 4.9 attacks of diarrhoea annually (SALUS, 1993). If it is kept in mind that each attack lasts up to 6 days, it can be presumed that every child will have diarrhoea and lose weight for more than one month of each year (SALUS, 1993). In South Africa, the incidence of diarrhoea, for instance in Cape Town, is very high in relation to other countries, since more than 6 000 patients are admitted annually at the Red Cross Children's Memorial Hospital, suffering from diarrhoea (Desai, 1987). However, most of the diarrhoeal diseases are self-limiting and will last for only a few days. A study conducted in India, reported that only 5 % of diarrhoeal diseases lasted longer than 14

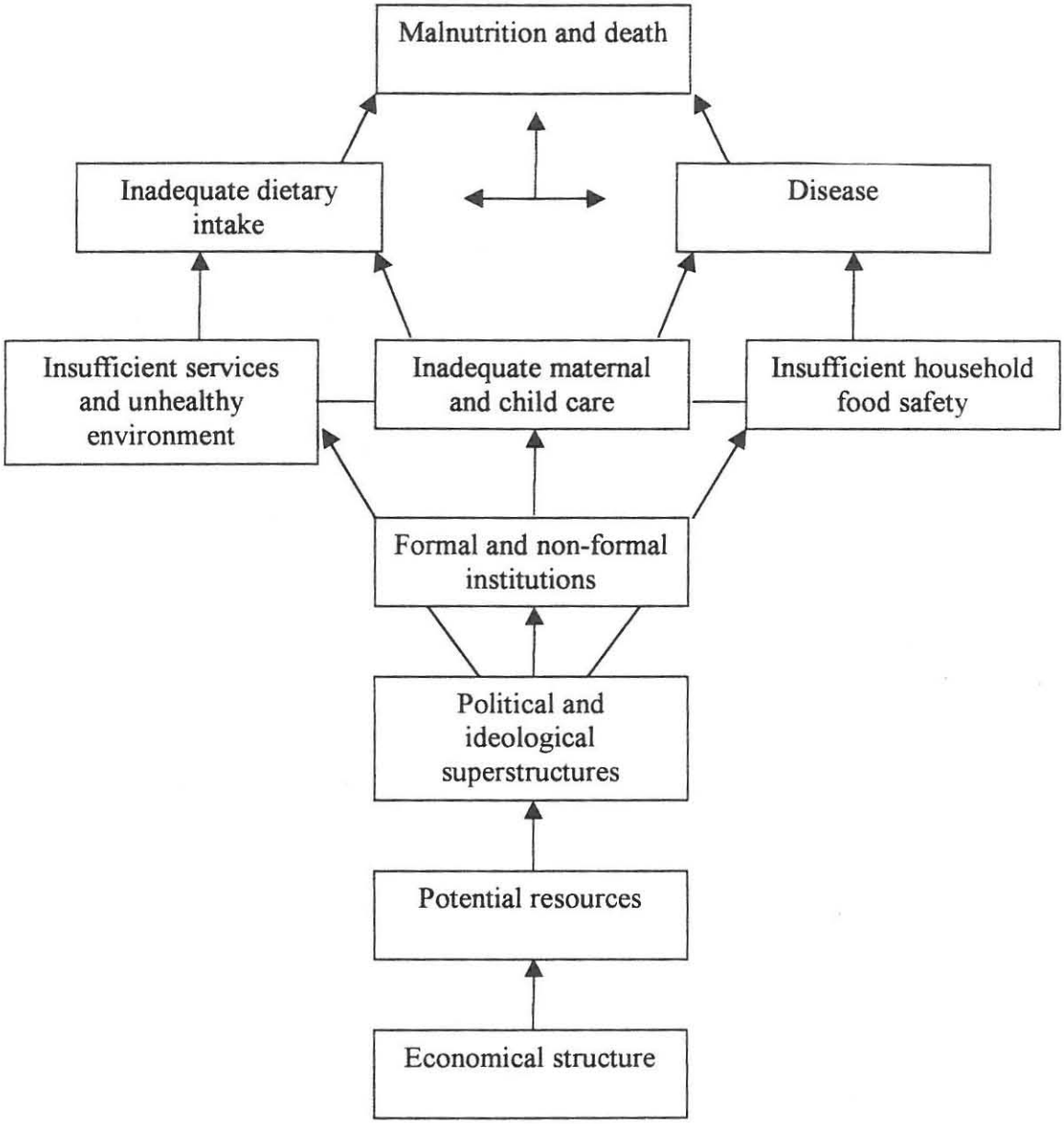

Fig. 1 The conceptual framework of determinants influencing the occurrence of malnutrition and diarrhoea (UNICEF, 1990)

days. Of these, the mortality rate was 14 %, compared with the 0.7 % mortality rate for acute diarrhoeal episodes (Frischman, 1991). The manifestations of the disease and the incubation periods of the disease are influenced by the inoculum size of the organisms, vehicles of infection, virulence properties of the organisms and host factors (Blaser *et al.*, 1995).

In spite of the common occurrence of diarrhoea and other food- and milk-borne diseases, the extent of their real impact on the community health is unknown. This is due to the fact that only a small proportion of cases is reported and even fewer cases investigated. This under-reporting of food-borne diseases and lack of knowledge of the health impact of these diseases are serious problems and may be responsible for the low priority given to food safety in many countries (Notermans and Hoogenboom-Verdegaal, 1992; Abdussalam and Käferstein, 1994).

4. BACKGROUND ON THE CAUSATIVE AGENTS OF FOOD-BORNE DISEASES IN SOUTH AFRICA

Clostridium perfringens is a common food poisoning agent (Andersson *et al.*, 1995). This organism is an anaerobic spore-former found in the excreta of humans and animals, in raw meat and poultry as well as in soil and dust (Jacob, 1989; Eley, 1992; Jay, 1992; Reed, 1994[1]). Food poisoning by *C. perfringens* is caused by a heat labile enterotoxin (Jay, 1992; Andersson *et al.*, 1995). The incubation period ranges from 8-24 hours with symptoms of abdominal pain and diarrhoea (Jacob, 1989; Eley, 1992; Jay, 1992). Most outbreaks occur when meat is pre-cooked or cooled slowly and then reheated (Jacob, 1989). *C. botulinum*, on the other hand, causes botulism through a toxin that affects the nervous system and often causes fatal illness. The incubation period is 12-96 hours with symptoms such as dizziness, headache,

tiredness and double vision. This organism is especially dangerous in canned and vacuum-packed foods (Jacob, 1989).

Bacillus cereus is also a spore-forming organism, but aerobic. It is widespread in the environment (in soil and dust, Jacob, 1989; Bishai and Sears, 1993; Reed, 1994[2]). A wide variety of food has been associated with outbreaks of *B. cereus*-related food-borne diseases (Jacob, 1989). *B. cereus* is responsible for a diarrhoeal syndrome caused by an enterotoxin as well as an emetic syndrome associated with an emetic toxin. The diarrhoeal syndrome is characterised by an incubation period of 8-16 hours followed by abdominal pains, cramps and diarrhoea similar to that caused by *C. perfringens*. The emetic syndrome is similar to that caused by *S. aureus*, with nausea and vomiting and an onset time of less than 1-5 hours (Jackson, 1991; Eley, 1992; Bishai and Sears, 1993; Reed, 1994[2]).

Escherichia coli belongs to the coliform group of bacteria (Jay, 1992). Human disease associated with *E. coli* can be divided into two broad categories: 1) those associated with intestinal infections and 2) those associated with extra-intestinal infections. The intestinal *E. coli* can further be sub-divided into five types, namely entero-pathogenic *E. coli* (EPEC), entero-toxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), entero-haemorrhagic *E. coli* (EHEC) and entero-adherent *E. coli* (EAEC). EPEC is the main enteric pathogen in developing countries (Molenda, 1994, Fagundes-Neto *et al.*, 1996). Some strains of *E. coli* can cause gastro-enteritis in adults and children (Jacob, 1989). The bacteria may be present in water, human excreta and many raw foods. It is passed to cooked foods by means of contaminated hands, surfaces, containers and other equipment. The incubation period is 12-72 hours with symptoms of abdominal pain, fever, vomiting and diarrhoea (Jacob, 1989).

Salmonella and *Shigella* are classified under the family *Enterobacteriaceae* (Le Minor, 1984; Rowe and Gross, 1984; Hobbs and Roberts, 1993). *Salmonella* occurs in animals and reaches food directly or indirectly through water polluted by sewage, animal or human excreta (Jacob, 1989; Collins *et al.*, 1995). The incubation period for salmonellosis can be 6-72 hours, but is usually 12-36 hours (Jacob, 1989; Doyle and Cliver, 1990[2]). Symptoms include diarrhoea, abdominal pain, chills, fever, vomiting, dehydration and headache (Jacob, 1989; Doyle and Cliver, 1990[2]). Shigellosis, caused by *Shigella* spp., is also known as the "filth disease", because of its association with poor personal hygiene and sanitation (Banwart, 1989; Eley, 1992). Symptoms associated with this disease include diarrhoea accompanied by fever, nausea and sometimes vomiting and cramps with an incubation period of 1-7 days (Jacob, 1989; Eley, 1992). The most common source of infection is person-to-person or faecal-oral transmission (Bishai and Sears, 1993).

Food-borne illness due to staphylococcus is caused by the ingestion of food contaminated by a toxin produced by *S. aureus* (Jacob, 1989; Reed, 1993). The incubation period is 4-6 hours with resulting nausea, vomiting, abdominal pain, prostration and subnormal temperature (Jacob, 1989; Jay, 1992; Reed, 1993; Collins *et al.*, 1995). The origin of these organisms is essentially the skin of man and animals, however, it can colonise food-processing equipment causing continuous contamination of food (Notermans and Hoogenboom-Verdegaal, 1992). Foods frequently involved with staphylococcal food poisoning include protein foods, salty foods as well as foods that are handled extensively (Reed, 1993; Ollinger-Snyder and Matthews, 1996).

Poultry, meat, milk and water have been implicated in incidents of *Campylobacter* infection. Sources of this organism include birds and household animals. Less common is

person-to-person spread. Environmental contamination, particularly among the poorer sectors of the population where domestic cattle, fowl and people are often housed together, appears to be important in the spread of infection. The incubation of disease is 3-5 days lasting for 1-10 days. Onset of disease is sudden with abdominal cramps and foul smelling, bile-stained stools (Jacob, 1989). Listeriosis, caused by *Listeria*, is relatively uncommon and produces mild fever with an incubation period of one to several weeks. *Listeria monocytogenes* is widely distributed in the environment and several food commodities have been implicated in transmission, including milk and dairy products, raw meat products, poultry, seafood, vegetables and salads (Jacob, 1989).

5. THE LOCAL SITUATION

5.1 South Africa

South Africa is facing rapid growth, especially in townships. People are moving from rural areas to the cities in their thousands looking for work and better living standards, since rapid urbanisation has led to problems such as provision of shelter, employment, education and health care (Myrdal *et al.*, 1994). This tendency can be referred to as "micro-urbanisation", which is a term used to describe the contrasts and migration of rural people from more dispersed and agricultural settlements to more concentrated populations still in the interior countryside (Mendoza *et al.*, 1996). These are generally referred to as marginalised urban settlements or MUS. Unfortunately for these people, the infrastructure of these areas is underdeveloped or non-existent, leaving them to cope with the available facilities.

Because of the overcrowding conditions, people are exposed to a variety of health risks. Poverty, low educational standards, unemployment, the under-use and lack of medical services, contribute to the high disease and mortality rates (De Haan, 1988). Socio-economic factors

like family income and occupation, socio-economic scales, together with over-crowding, large families, low maternal age, lack of access to improved water and sanitation as well as the absence of the mother are all risk factors for diarrhoea (Blaser *et al.*, 1995). All the factors mentioned are valid in South African townships and squatter-areas.

The policies of the government of the day are aimed at primary health care, with emphasis on the prevention of diseases and the elimination of diseases, rather than the treatment of the symptoms. However, the steps that are taken in primary health care, are over-shadowed by the lack of and gaps in the infrastructure of food preparation and storage, water and sanitation (Blaser *et al.*, 1995). Public health advances on several fronts have had tremendous impact on improving diminishing food quality and environmental sanitation (Savarino and Bourgeois, 1993). A large number of the South African population, however, still does not have access to primary health care. Therefore, information to the public regarding safe food preparation and handling should be emphasised.

5.2 The study area (Botshabelo, Section M)

Botshabelo, where this study was conducted, is a typical rural community, situated more or less 50 km South-East of Bloemfontein in the Free State province, South Africa (Fig. 2). Section M's socio-economic structure varies from slum-areas to high-income groups and is characterised by inefficient sanitary facilities, relying on pit latrines or the bucket system for waste disposal. Only cold water is available outside the house. Furthermore, electricity is unavailable to almost all of the homes, with no cooling facilities or hot water. Figures 3-5 show a number of scenarios of the mentioned area, including overall layout, food vendors and domestic environment. Energy for cooking purposes is mainly supplied by means of gas stoves, while candles or gaslights are the main source of light and heat is supplied by means of

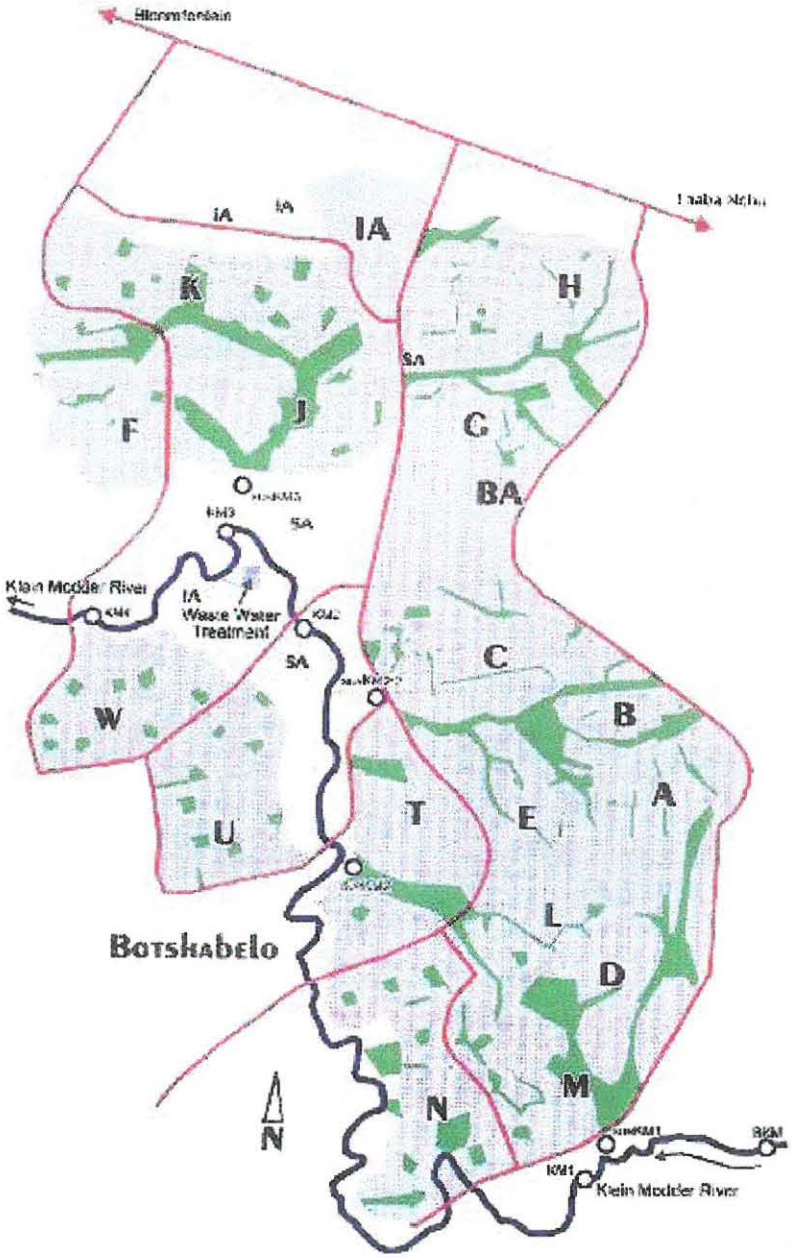

Fig. 2 A map of the sampling area showing the lay-out of Botshabelo as well as the various sections, including Section M (The area is situated in the Free State Province, about 30 minutes' drive from the city of Bloemfontein).

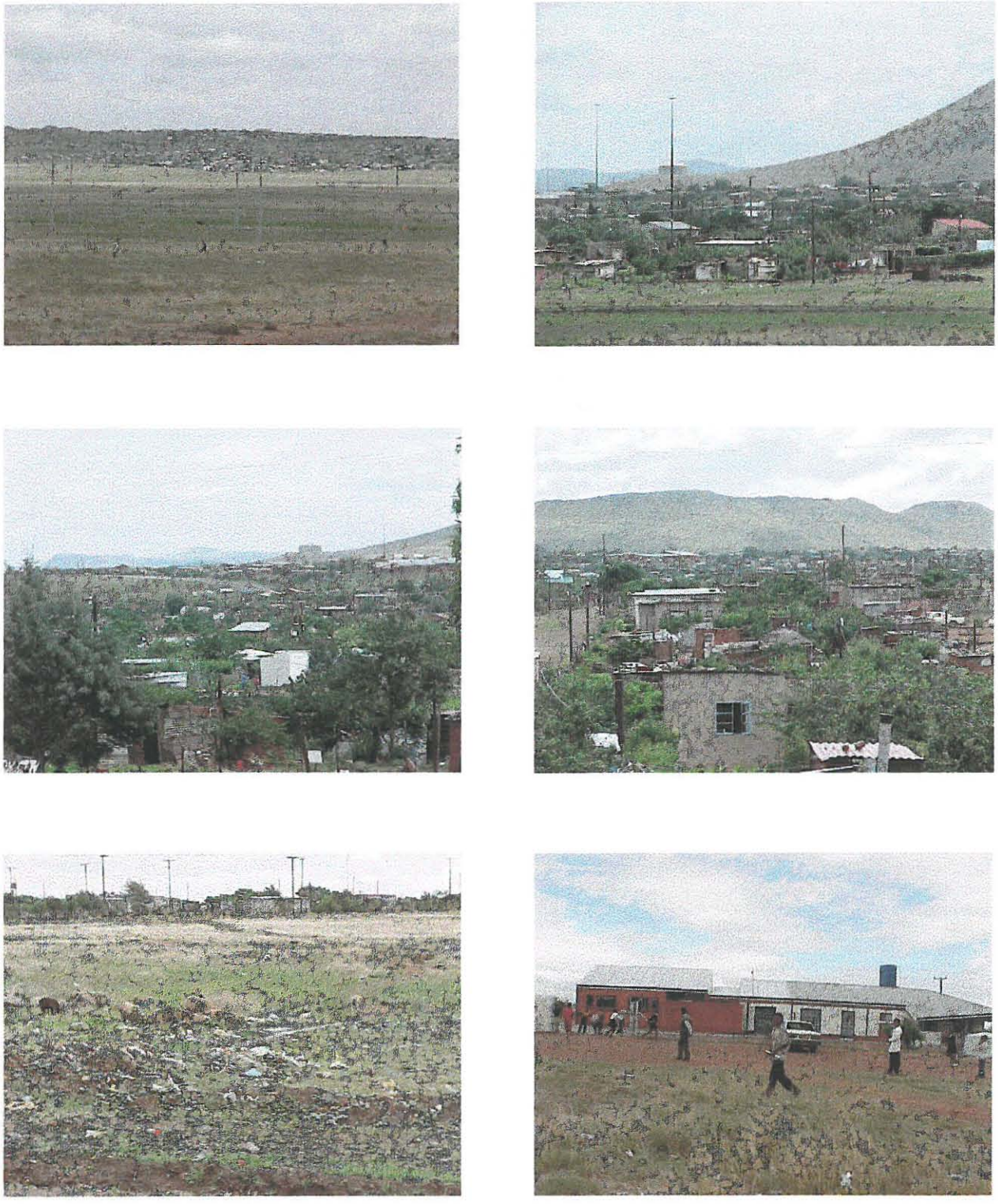

Fig. 3 Images of Botshabelo and the area of Section M, showing the lay-out of the settlement and basic infrastructure. The picture on the bottom, right corner shows the clinic and nutrition centre at Section M.

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Fig.4 A number of images representing aspects of food and milk supply, food handling and vendor-infrastructure in Botshabelo and the area of Section M.

Fig. 5 Images of the domestic environment of Section M, showing some of the common household practices as well as the sanitation facilities.

coal or paraffin. The average house is either made from zinc or mud and is divided into a kitchen and a bedroom - only a few are privileged to live in brick houses. The number of children in a family varies from 4-8, with most of the children under the age of 6 years. The residents' general education level is grade 7, resulting in unemployment or jobs with low income, as they do not have the tuition and training to apply for jobs with better salaries.

Most of the inhabitants are commuters that travel from Botshabelo to Bloemfontein on a daily basis. On average one of the parents has a part-time job, or both are jobless. It is very rare for both parents to have jobs. The general income of the community is thus very low, with most of the inhabitants living practically on the bread line. The mothers are mostly working during the day to provide an extra income, leaving the children on their own or in the care of older children, leading to a general lack of education and general hygiene. Most of the residents buy their bread and milk from a vendor. When affordable, meat is mostly bought from vendors or tuck shops and occasionally from butchers. The quality of the products obtained from these suppliers is highly suspect, and numerous studies are currently being conducted on the microbiological integrity of these products. Fruit and vegetables are either grown by the residents themselves or bought from a nearby farmer. Clinic records of the residents in Section M, Botshabelo, are unsatisfactory and alarming, as they are incomplete, and although the incidence of disease is high, people do not seem to visit the clinic regularly. Most of the people visiting the clinic are mothers with babies.

6. AIM OF THE STUDY

When considering the situation of food hygiene in South African marginalised urban settlements, it must be concluded that the socio-economic environment has a direct influence on the quality of food and milk consumed. The lack of knowledge about basic food hygiene,

the insufficient health services and unhealthy environment lead to inadequate dietary intake and disease from hazardous foodstuffs. The inadequate dietary intake, on the other hand, leads to lack of resistance to disease. All of these conditions could lead to malnutrition and death.

In this study the aim was to investigate the microbial populations associated with foodstuffs and milk in the area of Section M, Botshabelo. We further aimed to show that socio-economic conditions and proliferation of selected microbial groups could be used as indices of the prevalence of diarrhoea and other food- and milk-borne related illnesses. General housekeeping activities and privileges were also monitored with the aim to make suggestions for the safer handling of foodstuffs in order to reduce the occurrence of food-borne disease amongst residents. Regarding the changing circumstances in South Africa and the emphasis on primary health care, this study can be seen as a project aimed at assisting the people to help themselves, and ultimately be of help in the upliftment of rural areas.

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CHAPTER 2

A SURVEY OF THE MICROBIOLOGICAL QUALITY OF MILK IN THE RURAL COMMUNITY OF BOTSHABELO, SECTION M

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A survey of the microbiological quality of milk in the rural community of Botshabelo, Section M

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ABSTRACT

Milk samples from 60 randomly selected households in Botshabelo, Section M were collected and analysed for the microbiological content. In addition, information was gathered from the Botshabelo Local District Council and clinic pertaining to the residents' knowledge on nutrition as well as their socio-economic status. From the results it was deduced that the total mesophilic and coliform organisms by far exceeded the standards stipulated in national legislation. Moreover, the hygienic quality of the milk was found to be unacceptable with total aerobic counts and coliform counts as high as 8.6 x 10^8 cfu/ml, while the Escherichia coli counts were around 10^5 cfu/ml. The aerobic and anaerobic spore-forming bacteria showed a presence of 86.6 % and 56.6 % respectively. The data showed that the general food-hygiene related infrastructure of the community was very poor, confirming relationships between socio-economic status and household hygiene. The alarmingly high microbial counts could, furthermore, definitely point to milk as a sure cause of food-related infection in the region.

Key words: Milk, microbial quality, milk-borne diseases

INTRODUCTION

Quality control of milk is considered essential to the health and welfare of a community. However, people from low-income groups have shown very little concern as to whether food and drink are good or detrimental to their health, their only concern being to buy enough food to keep them from starvation (Fox and Cameron, 1989). In Western societies, practices generally used to kerb microbial proliferation in milk include pasteurisation and refrigeration. These practices however, are not utilised by these low-income groups, due to lack of infrastructure and funds (Rohde, 1985; Collins *et al.*, 1995). To add to this problem, health and hygiene in South Africa have been characterised by a series of changes since the Milk Board, acting as watchdog over the quality of milk, has recently been deregulated. This was done as part of the decentralisation of the South African dairy industry in an attempt to promote free market trade (Coetzee, 1998, Director: Irene Research Institute, Pretoria, *Personal Interview*). Since the deregulation, however, a decline in the milk quality of informal milk suppliers, including street vendors in rural areas and informal settlements, has been observed although milk quality in urban areas is to a large extent still intact. The lack of knowledge concerning the dangers involved, the lack of basic infrastructure as well as poor housekeeping techniques can all be possible causes for this decline in milk quality.

Being a nutritionally, balanced foodstuff that contains few organisms when it leaves the udder, milk gets contaminated at various stages be it from the cow, milker, extraneous dirt or unclean water (Stewart, 1978; Rohde, 1985; Banwart, 1989; Philips and Griffiths, 1990). The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable (Foster, 1990). Microflora generally associated with milk and milk spoilage are coryneforms, micrococci and lactococci, and

include the genera *Pseudomonas*, *Brucella*, *Escherichia*, *Salmonella*, *Shigella* as well as *Bacillus* and *Clostridium* (Gilmour and Rowe, 1981; Cousins and Bramley, 1981; Dommett, 1992). Furthermore, the health hazards posed by milk-borne zoonotic diseases like brucellosis, tuberculosis and mastitis-related entero-toxaemia are well known (Hobbs and Roberts, 1993). Although the proliferation of micro-organisms in milk is considered undesirable, the excessive growth of micro-organisms in milk could result in the souring of milk, which in turn prevents the multiplication of putrefactive organisms (Collins *et al.*, 1995).

The fears voiced by the environmental health sector in South Africa have been confirmed in a recent national survey on milk that showed that only 25 % of the milk produced by both formal and informal farmers conformed to the national standard (Keller, 1995, Irene Research Institute, Pretoria, *Personal Interview*). In order to address this emerging problem, it is necessary to assess the extent of milk hygiene in the mentioned informal settlements and investigate ways to rectify these problem areas by identifying the parameters that influence milk quality. In this study, we investigated the microbiological status of milk in a randomly selected area of a rural settlement in the Free State Province by enumerating a variety of indicator organisms in the milk. We selected an area (Section M) that is relatively homogeneous with regard to infrastructure and general milk hygiene. In addition, we evaluated these results as a possible source of milk-borne infection among the residents of Botshabelo, Section M. Measures to rectify the hygienic status of milk were also taken into consideration.

MATERIALS AND METHODS

Sampling protocol

Sixty households were randomly selected for sampling with the aid of the Department of Biostatistics, University of the Orange Free State, South Africa. In addition, data was obtained from the Local District Council and clinic in Botshabelo, Section M, pertaining to aspects of information on food-suppliers and basic infrastructure, giving insight into the relationships between general infrastructure and milk hygiene in the selected area. Two community workers were trained to collect the samples from each household. Sampling was performed early in the morning from the milk containers on a weekly basis over a period of 6 weeks (10 households per week) with the aid of sterile sampling bags (Whirl-Pack, NASCO). The samples were kept on ice to restrict microbial multiplication and were transported to the laboratory for analysis.

Microbiological analysis

Serial dilutions were prepared with the use of Peptone buffer (BIOLAB). The streak plate method was used to quantify the various microbial groups (Herbert, 1990). For the enumeration of members of the family *Enterobacteriaceae*, Violet Red Bile Glucose agar (VRBG, OXOID) was used (Christen *et al.*, 1993), while the detection and enumeration of yeasts and moulds were done by means of Potato Dextrose Agar (PDA, MERCK, Frank *et al.*, 1993). The pH of this medium was adjusted to 4.5 with lactic acid to restrict bacterial growth. Plate Count Agar (PCA, MERCK) was used to isolate aerobic mesophiles as well as the aerobic and anaerobic spore formers in the milk (Houghtby *et al.*, 1993). Petrifilm™ (3M) was used to enumerate the coliforms and *E. coli* (Matner *et al.*, 1990). To analyse the spore-forming organisms, the samples were exposed to 80 °C for 10 minutes to destroy

vegetative cells and transferred to the PCA agar plates. The anaerobic spore-forming organisms were incubated in anaerobic flasks with Anaerocult A (MERCK) and Anaerotest (MERCK) to indicate an anaerobic atmosphere.

The aerobic spore-forming organisms and the PDA agar plates were incubated for 12-18 hours (Labotec), while the Petrifilm^{TM}, PCA agar plates, VRBG agar plates and the anaerobic spore-forming PCA agar plates were incubated at 35 °C for a period of 48 hours. A colony counter (Gerber) was used for enumeration. Micro-organisms belonging to the family *Enterobacteriaceae* produced round, purple colonies, that were 1-2 mm in diameter and surrounded by purple haloes (Mossel *et al.*, 1986). *E. coli* was characterised by blue colonies associated with gas bubbles, whereas coliforms were characterised by red colonies with gas bubbles.

RESULTS AND DISCUSSION

Socio-economic infrastructure

In Table 1 the information on milk supply to Botshabelo, Section M, is shown (data obtained from the local clinic and District Council). Purchases from a tuck shop in the area were higher than that of the local supermarkets of Botshabelo or the nearby city of Bloemfontein. Only 19 % of the residents bought their milk from the local supermarket and this could be attributed partly to the distance of the supermarket from Section M. Possible reasons for the non-purchase of milk from the Bloemfontein supermarkets could be the lack of sufficient transport facilities from Bloemfontein to Botshabelo, as well as lack of space to keep the goods on the bus. Another reason may be the odd hours that the people work, resulting in the situation that they do not have enough time to purchase milk from a supermarket. In most

Table 1 Utilisation of milk suppliers in Botshabelo, Section M, in terms of percentage purchases.

Sources	Purchases (%)
Botshabelo supermarket	19
Tuck shop	79.5
Other	0.8
Direct from farmer (street vendor)	-
Self generating	-
Bloemfontein supermarkets	-

instances people have to travel long distances from work to the bus stop, using several taxis and buses before eventually reaching their homes. Hence, it would be very inconvenient to purchase milk from supermarkets in Bloemfontein. The percentage consumption of milk from tuck shops was found to be high, namely 79.5 % (Table 1), probably as a result of lower prices offered at the tuck shop. Apart from the economic advantages, the frequent usage of tuck shops could probably be attributed to these outlets being situated in close proximity to the customers, in this instance, Section M. Only 0.8 % of the milk was obtained from other sources, including individuals selling milk from their homes as an extra income. No milk was found to be self-generated or bought directly from small-scale farmers.

Patterns of microbial groups isolated from milk

The distribution of the various microbial groups found in milk from Botshabelo, Section M, is shown in Figure 1. The total aerobic mesophilic counts (Fig. 1 (A)) varied considerably from one household to another, however, the samples from household numbers 1-4, 11-23 and 54-60 showed a different pattern. A logical conclusion could thus be that these households bought their milk from the same supplier and probably used similar methods of cleaning and household sanitation. When considering the total aerobic mesophilic organisms, high numbers were found over the entire sampling range with only few variations compared to the anaerobic spore-forming bacteria (Fig.1 (C)) that showed a relatively uneven growth distribution. The *E. coli* counts (Fig. 1 (E)) occurred in high numbers in sample numbers 11-23 and 51, while the occurrence of *E. coli* in the remaining households were almost undetectable. When evaluated visually, the patterns of coliforms of households 11-23 and 50-60 (Fig. 4) were comparable with the total mesophilic counts. This observation confirmed the relationship between infrastructure and level of housekeeping. With regard to household numbers 11-23 the aerobic spore-forming bacteria (Fig.1 (B)), coliforms (Fig.1 (D)), *E.* coli

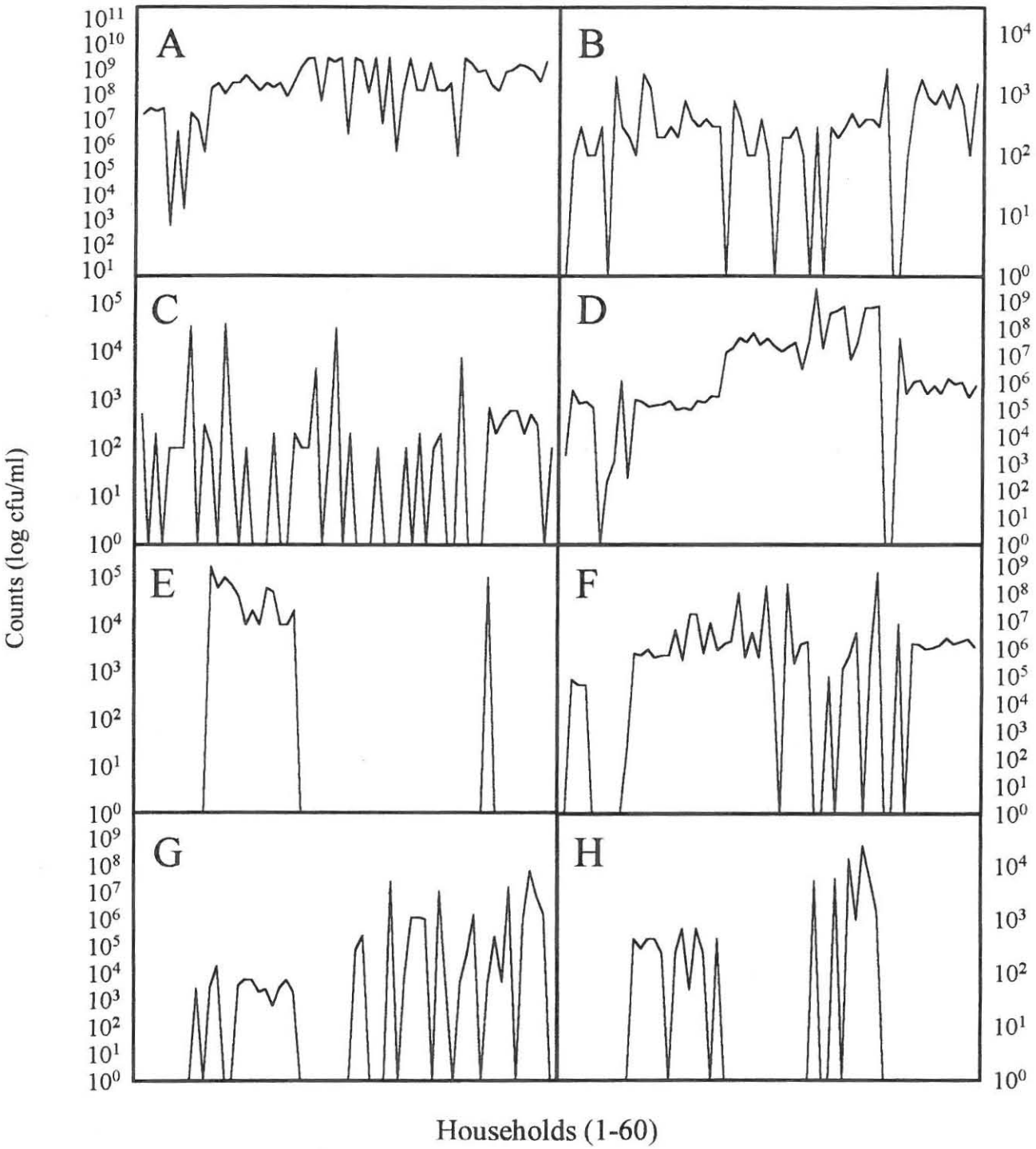

Fig. 1 Patterns of microbial groups found in milk from households in Section M, Botshabelo (A: total aerobic mesophiles; B: aerobic spore-forming bacteria; C: anaerobic spore-forming bacteria; D: coliforms; E: *E. coli*; F: *Enterobacteriaceae*; G: Yeasts; H: Moulds)

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(Fig.1 (E)), *Enterobacteriaceae* (Fig. 1 (F)) and moulds (Fig. 1 (H)) were more evenly dispersed than in other households. The fluctuations between the various milk samples can be attributed to the level of household hygiene and the time and temperature of storage, since these factors influence microbial growth in milk considerably. Comparing the organism counts of the aerobic spore-forming bacteria (Fig. 1 (B)) with the anaerobic spore-forming bacteria (Fig. 1 (C)), the variations were more pronounced in the case of the aerobic spore-forming bacteria than the anaerobic spore-forming bacteria. Coliforms (Fig. 1 (D)), *E. coli* (Fig. 1 (E)) and *Enterobacteriaceae* (Fig. 1 (F)), indicative of faecal contamination, varied considerably after reaching alarmingly high numbers.

The percentage occurrence of the various microbial groups in the sampled households is presented in Table 2. The results showed that the greater number of samples had a total mesophilic count between the10^6-10^7 cfu/ml range (28.3 %), followed by the 10^8-10^9 cfu/ml interval (21.6 %), the 10^9-10^{10} cfu/ml interval (18.3 %) and the 10^7-10^8 cfu/ml interval (16.6 %) respectively. These high counts could be regarded as representative of the quality of milk samples from residences in Section M. With regard to the aerobic and anaerobic spore-forming bacteria, quantities of 13.3 % (aerobic) and 43.3 % (anaerobic) occurred in the 10^0-10^1 cfu/ml range. The relatively high counts in the range of 10^2-10^3 cfu/ml (73.3 % for aerobic and 48.3 % for anaerobic spore-forming organisms) were quite notable. In the 10^3-10^4 cfu/ml range a 13.3 % occurrence of aerobic and 3.3 % for anaerobic spore formers were found (Table 2). No spore-forming bacteria were found in the ranges 10^5-10^6 to 10^9-10^{10} cfu/ml, whereas 5 % anaerobic spore formers were found in the 10^4-10^5 cfu/ml range (Table 2). On average, more aerobic spore-forming bacteria were present than anaerobic spore formers. It has been shown that milk-borne spore forming microflora belong mainly to the genera *Bacillus* and *Clostridium*, which are able to withstand extreme environmental

Table 2 The microbial groups associated with milk in the Botshabelo, Section M region, expressed as percentage (%) occurrences.

Occurrence	Total[a]	A.sf.[b]	An.sf.[c]	Coli[d]	*E. coli*	Enterobact.[e]	Yeasts	Moulds
10^0-10^1	1.6	13.3	43.3	5	76.6	23.3	46.6	70
10^1-10^2	-	-	-	-	-	-	-	1.6
10^2-10^3	1.6	73.3	48.3	3.3	-	1.6	1.6	16.6
10^3-10^4	1.6	13.3	3.3	3.3	-	-	25	8.3
10^4-10^5	-	-	5	-	18.3	5	5	3.3
10^5-10^6	10	-	-	40	5	26.6	3.3	-
10^6-10^7	28.3	-	-	11.6	-	33.3	11.6	-
10^7-10^8	16.6	-	-	26.6	-	3.3	6.6	-
10^8-10^9	21.6	-	-	10	-	6.6	-	-
10^9-10^{10}	18.3	-	-	-	-	-	-	-

[a]Total mesophilic counts, [b]Aerobic spore forming bacteria, [c]Anaerobic spore forming bacteria, [d]Coliforms, [e]members of the family *Enterobacteriaceae*

conditions and high temperatures (Jay, 1992). Because of these characteristics, as well as their ability to grow fast, spores are likely to survive the pasteurisation process and produce sensorially unacceptable products (Andersson *et al.*, 1995). *Clostridium* spp., for instance, ferment lactose and lactate to butyric acid, while *Bacillus* spp. are able to produce enzymes resulting in the production of sweet curdling and bitty cream (Sutherland, 1986; Dommett, 1992; Becker *et al.*, 1994; Andersson *et al.*, 1995). The high counts of aerobic and anaerobic spore formers may therefore indicate the degree of pasteurisation of the milk, and could equally be indicative of the degree of contamination. Apart from spoilage, *B. cereus* is also responsible for diarrhoeal and emetic syndromes (Eley, 1992; Reed, 1994), whereas *C. perfringens* is the causative agent of food poisoning, producing severe heat labile enterotoxins (Andersson *et al.*, 1995).

The presence of coliforms could be used as indicators of the hygienic standard of milk. Since *E. coli* and *Enterobacteriaceae* were found in milk consumed by the residents of Bothsabelo they can therefore serve as indicators of faecal contamination (Banwart, 1989; Eley, 1992; Jay, 1992; Simango *et al.*, 1992). The coliform numbers were found to be acceptable in only a small number of households (5 % in the 10^0-10^1 cfu/ml range, Table 2). It is unfortunately not known whether the milk sampled was pasteurised or not. Samples from localities 6, 47 and 48 were the only ones found to conform to national legislation allowing a coliform count of 10 cfu/ml for pasteurised milk and 20 cfu/ml for raw milk (South Africa, 1997). In general, coliform organisms were found to be present in high quantities (40 % in the 10^5-10^6 cfu/ml interval, 11.6 % in the 10^6-10^7 cfu/ml interval and 26.6 % in the 10^7-10^8 cfu/ml interval). Regarding *E. coli*, national legislation stipulates that no *E. coli* is allowed in pasteurised milk (South Africa, 1997). It was thus alarming to find high *E. coli* counts in a number of households (18.3 % in the 10^4-10^5 cfu/ml range, Table 2). The 76.6 % incidence of

E. coli, found in the range of 10^0-10^1 cfu/ml, could be deemed satisfactory, as these organisms were either absent or present in negligible quantities. Surprisingly, the coliforms were present in small quantities (5 %), compared to high numbers of *E. coli* found in the 10^0-10^1 cfu/ml range. In comparison the coliforms were present at 40 % in the 10^5-10^6 cfu/ml range, whereas *E. coli* amounted to 5 % in this range (Table 2). These results suggest that there does not necessarily exist a strong relationship between the number of total coliforms and *E. coli* in heavily contaminated milk. High counts of *E. coli* could, for example, appear to be concomitant with low numbers of coliforms, whereas the reverse could also be true. *E. coli* is responsible for causing travelers' diarrhoea and other food-borne related outbreaks through the ingestion of contaminated foodstuffs. It also causes severe bloody diarrhoea, otherwise known as haemorrhagic colitis through *E. coli* serotype 0157:H7 (Doyle and Padhye, 1989; Jay, 1992; Neill, 1994).

The *Enterobacteriaceae* counts varied considerably, with the highest occurrence between 10^6-10^7 cfu/ml (33.3 %) followed by 10^5-10^6 cfu/ml (26.6 %, Table 2). Since it is known that a variety of the genera included under the family *Enterobacteriaceae* are pathogenic, it could be suggested that only 23.3 % of the samples (12 in the 10^0-10^1 cfu/ml interval) corresponded with standards set in legislation, stating that pasteurised milk is not allowed to have any pathogens (South Africa, 1997). Pathogenic members of the family *Enterobacteriaceae* are represented by organisms such as *Salmonella* and *Shigella* and are found in the intestines of humans and animals (Le Minor, 1984; Rowe and Gross, 1984; Collins *et al.*, 1995). These organisms could be responsible for various diseases among humans, including typhoid fever caused by *S. typhi*, paratyphoid fever caused by *S. paratyphi* and bacillary dysentery caused by *Shigella* through the ingestion of contaminated foodstuffs (Jay, 1992; Maurelli and Lampel, 1994).

Columns eight and nine (Table 2) show the fungi counts, represented by yeasts and moulds isolated from the milk in Section M. The moulds showed higher counts (70 %) in the 10^0-10^1 cfu/ml range than the yeasts (46.6 %). In the higher ranges the yeasts in general had higher counts than the moulds, especially in the 10^3-10^4 cfu/ml and 10^6-10^7 cfu/ml intervals, where the presence of yeasts were 25 % and 11.6 % respectively, whereas the numbers of moulds were 8.3 % and 0 %. Although high counts of yeasts and moulds were found, their growth in milk is rather uncommon, as the pH in milk is neutral, causing bacteria to predominate (Frazier and Westhoff, 1988; Pitt and Hocking, 1997). However, the growth of lactic acid bacteria in milk, could reduce the pH aiding fungal growth (Banwart, 1989; Pitt and Hocking, 1997). The potential risks of accessive amounts of fungi lie in their ability to produce mycotoxins that could be fatal, as well as sensorial degradation of milk (Banwart, 1989; Jacob, 1989). The most common mould found in milk, *Geotrichum candidum*, have been reported to proliferate in milk due to unclean processing lines, whereas the growth of the yeast *Cladosporium butyri* in milk is considered undesirable due to its ability to cause rancidity (Pitt and Hocking, 1997).

The composition of the microbial population generally found milk in Section M, as represented by the mean counts, is shown in Figure 2. In cases where the initial microbial load of milk was high, various hazardous handling and storage practises would have triggered and enhanced the proliferation of different microbial genera (Andersson *et al.*, 1995). The total aerobic mesophilic counts yielded an average of 7.19×10^8 cfu/ml (Fig. 2), reflecting the high average counts of the other organisms that were isolated. The average count of *E. coli* was 1.22×10^4 cfu/ml (Fig.2), which exceeded the standard set in legislation by far, since raw or pasteurised milk is not allowed to contain any *E. coli* in 1.0 ml of milk. Because *E. coli* is used as an indicator of faecal contamination, it can be concluded that the milk sampled was

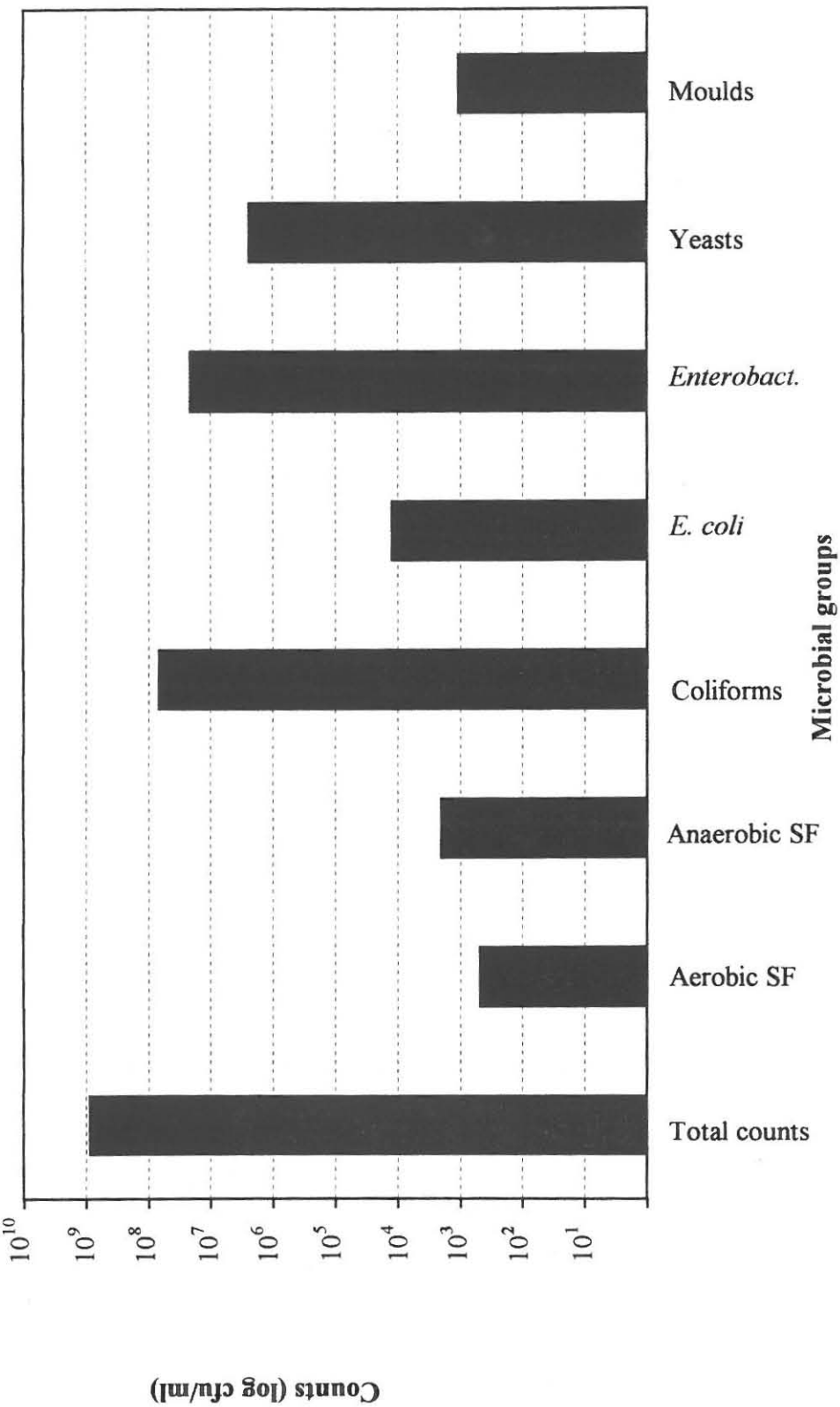

Fig.2 Quantities of selected micro-organisms isolated from milk sampled in Botshabelo, Section M, area (SF: spore formers)

contaminated with faecal material to a considerable extent. Together with the coliforms ($6.17x10^7$ cfu/ml, fig. 2) and *Enterobacteriaceae*, that consists of various organisms including *Salmonella* and *Shigella*, this could be indicative of the numbers of pathogens. *Enterobacteriaceae* had an average count of $2.07x10^7$ cfu/ml (Fig. 2), which also exceeded the standard (South Africa, 1997). The yeasts showed an average of $2.36x10^6$ cfu/ml in contrast with the $1.06x10^5$ cfu/ml average number of moulds (Fig. 2). This phenomenon could be the result of the wider distribution of the unicellular form of the yeasts compared to the mycelial form of the moulds in liquids (Banwart, 1989; Pitt and Hocking, 1997). Additionally, the growth of yeasts have been shown to be very common in dairy products, due to their ability to grow at low temperatures, the production of proteolytic and lipolytic enzymes, their ability to ferment lactose and to assimilate lactic acids (Pitt and Hocking, 1997).

The minimum and maximum values of some microbial groups varied extensively. The total mesophilic counts for example ranged from almost undetectable quantities to a maximum value of $3x10^9$ cfu/ml. A large variation between the minimum and maximum values ($1x10^1$ cfu/ml and $6x10^8$ cfu/ml) was shown for the members of the family *Enterobacteriaceae*. The aerobic and anaerobic spore-forming bacteria as well as *E. coli* had a smaller difference between the minimum and maximum values. These micro-organisms ranged from almost undetectable quantities to $2.7x10^3$ cfu/ml (aerobic spore formers), $3.71x10^4$ cfu/ml (anaerobic spore formers) and $1.7x10^5$ cfu/ml for *E. coli* respectively. The minimum counts for yeasts were as low as $1x10^1$ cfu/ml, while the maximum value was $6.85x10^7$ cfu/ml, whereas the moulds had a minimum value of $1x10^0$ cfu/ml and a maximum of $2.5x10^4$ cfu/ml.

Prevalence of micro-organisms with regard to existing legislation

The numbers of total aerobic mesophiles in comparison to the national guideline are shown in Figure 3. Milk from a mere two households (numbers 5 and 7) complied with the national guideline of 50 000 cfu/ml (South Africa, 1997), while some other households showed total mesophilic counts as high as $1x10^9$ cfu/ml.

Figure 4 represents the coliform counts compared with the national guideline. In South Africa, raw milk for consumption is not supposed to have more than 20 cfu/ml (South Africa, 1997). As it is not known whether the milk used in Section M is pasteurised, a standard of 10 cfu/ml or 20 cfu/ml may be acceptable. However, according to the results found in this study, all samples exceeded this standard, with the exception of 3 localities (5 %). Taking into consideration the standards set in legislation for total mesophilic counts, only 3 % (2 houses out of a total of 60) complied, while house numbers 5 and 7 exceeded the standards for coliforms in milk, as shown in Figure 4.

Based on the high microbial counts observed in this study, it can be concluded that the milk quality definitely poses a health risk in the study area. It is suggested that the high counts for the various micro-organisms are mainly due to: 1) poor quality milk supplied by local street vendors, which proved to be the most popular suppliers amongst residents of the M Section, 2) lack of basic infrastructure for storage and handling, and 3) ignorance of the residents about the fundamental aspects of good and safe housekeeping. Lack of electricity, lack of proper sanitation and inadequate water supply can further lead to the proliferation of contaminants in the milk. Because of the lack of electricity in Section M and the consequent absence of refrigeration facilities, the milk is kept mostly at ambient temperatures giving rise to ample possibilities for microbial growth. *Bacillus* species have, amongst others, been

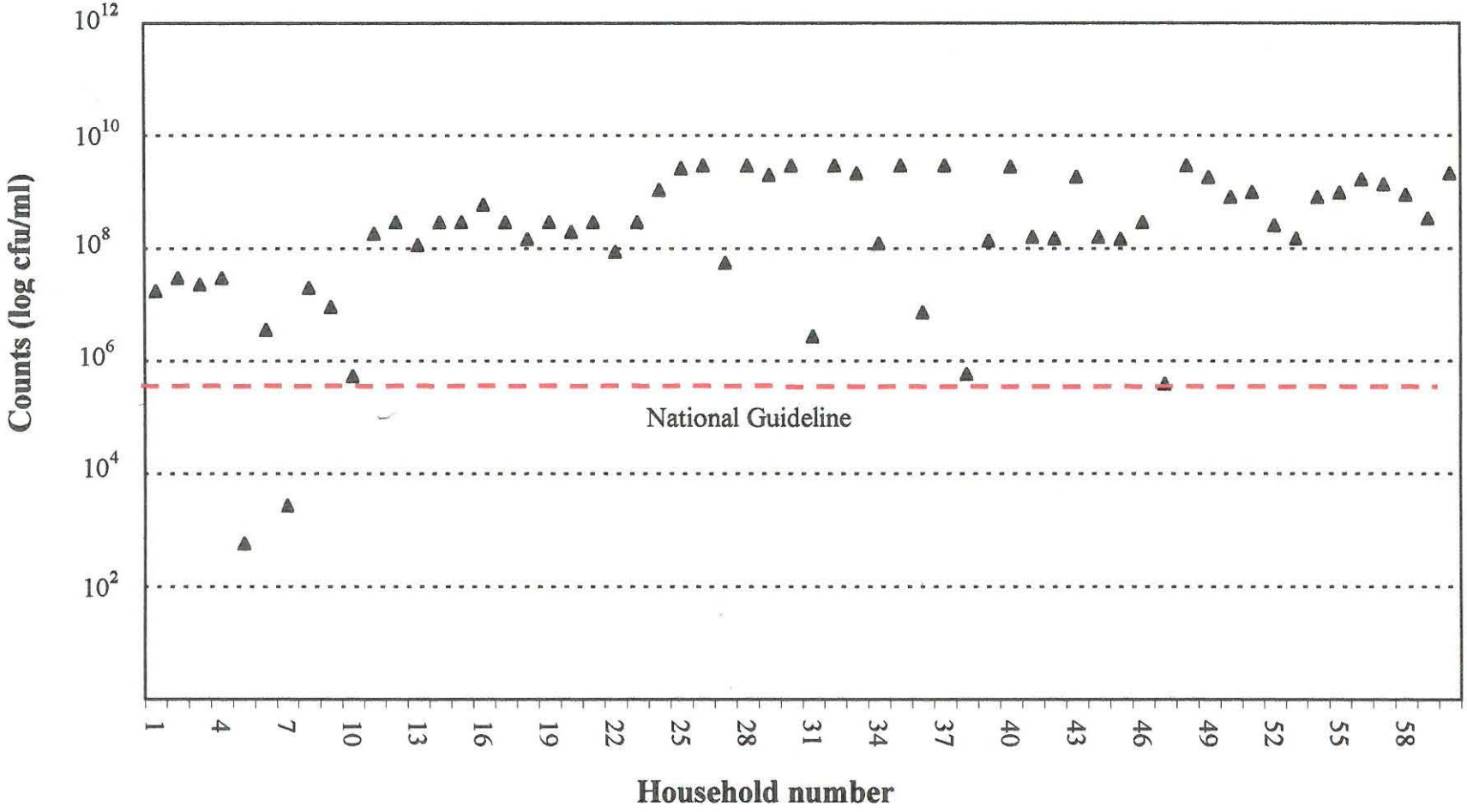

Fig. 3 The patterns of total mesophilic organisms in milk from selected households of Botshabelo, Section M, compared with the national guideline as stipulated in legislation.

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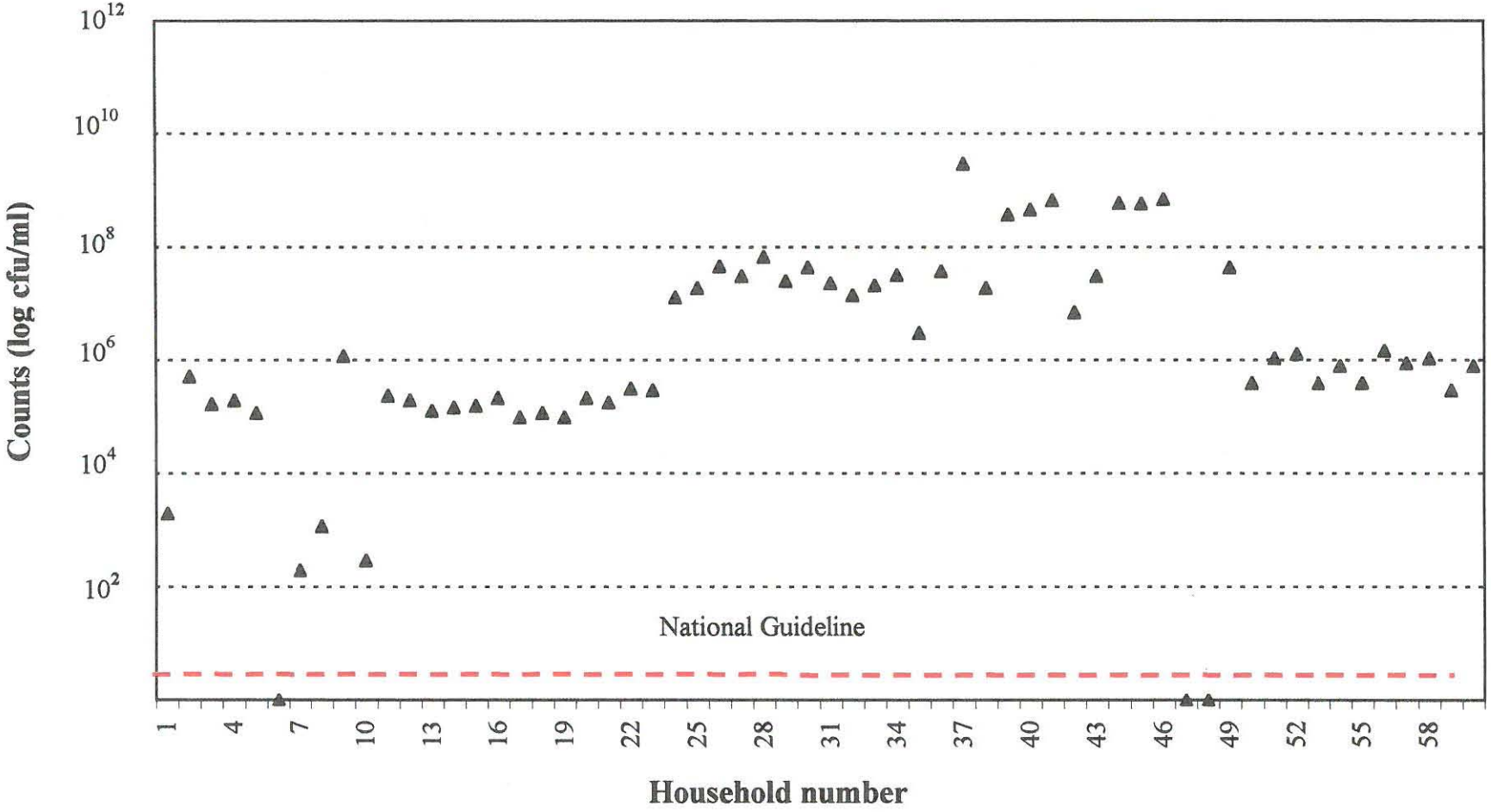

Fig. 4 The patterns of coliform bacteria in milk from selected households of Botshabelo, Section M, compared with the national guideline as stipulated in legislation.

known to increase rapidly when the storage temperature is elevated from 6-8 °C, and keeping in mind that the ambient temperatures in the households are well above 8 °C, it can be expected that the numbers will increase drastically (Dommett, 1992; Andersson *et al.*, 1995). The low education and economical standard of residents living in Botshabelo, Section M, are conducive to the poor hygienic standard in the homes. These people also lacked consumer awareness, since the residents were not aware of the risks involved with contaminated milk.

The high incidence of the organisms referred to above could, furthermore, be due to faulty milking techniques, poor personal hygiene, poor housekeeping techniques as well as poor health of the supplier herds. Therefore, research with regard to the milk quality and milking protocols of small-scale farmers in the Botshabelo area, supplying milk to the local vendors and supermarkets, should be conducted. Apart from educating the consumers and housewives, the small-scale farmers and street vendors should be educated on the basic principles of good manufacturing practice (GMP). As a result of the inadequate education of consumers regarding the proper cleaning and handling of containers, containers may be washed with water polluted with animal or human waste. In addition to this, organisms may gain access to milk through various sources, for example faecal contamination from cows infected with *Campylobacter* spp. or *Salmonella* spp. (Hobbs and Roberts, 1993), whereas *Staphylococcus aureus* may be present in raw milk from mastitic cows (Tranter, 1991). Inadequate pasteurisation, post-pasteurisation contamination or cross-contamination between raw and pasteurised milk may be sources of the pathogen *Salmonella* in pasteurised milk (Philips and Griffiths, 1990; Todd, 1991).

The ideal would have been proper electricity and water supplies to each household, but since funds to rectify this are lacking, education as to the optimal use of the available resources is

the most probable solution. This would include, amongst others, educating residents on buying safe milk from vendors, looking for sensorial indicators of poor or spoiled milk, keeping milk in clean and well-washed containers, using milk promptly and heating milk prior to usage. The lack of a electricity supply to Botshabelo, Section M, can be partly overcome by keeping milk and other easily perishable products in a cool area and by covering it. To address the ignorance of residents concerning their state of health, however, people should also be informed about the risks involved in consuming contaminated milk, as contaminated milk is, apart from gastro-enteric infections, also a vector of serious zoonotic diseases (Stiles, 1989; Foster, 1990).

This study attempted to identify the general hygienic condition of milk and to determine the organisms that are found in a rural community such as Botshabelo, Section M. Since the emphasis on the upliftment of the community has been one of the primary goals of the government, further research on the possible sources of the various contaminants is recommended. These results should give a more comprehensive overview of possible diseases involved as well as an indication of the sources of these organisms that could in turn be addressed. Similar studies are also advised for parallel settlements in South Africa.

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CHAPTER 3

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(Article submitted for publication in journal: *South African Journal of Public Health*)

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The interactions between microbial populations and food preparation infrastructure in a rural settlement

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ABSTRACT

Coliforms, Escherichia coli, spore-forming bacteria, total mesophilic aerobes, fungi and members of the family Enterobacteriaceae were analysed in 60 randomly selected households in Botshabelo, Section M. This was done to determine the quality of foodstuffs consumed by the residents living in this area. In addition, information was gathered by means of questionnaires on some aspects of the communities' nutrition, socio-economic and health status. High numbers of total aerobic organisms (up to 10^6 cfu/cm^2) were associated with foodstuffs, while the aerobic spore-forming bacteria and yeasts were present at 63 % and 66 % respectively. The anaerobic spore-forming bacteria, coliforms and E. coli represented 61.6 %, 78.3 % and 96.6 % respectively in the 10^0-10^1 cfu/cm^2 interval. The largest percentage of Enterobacteriaceae (65 %) and moulds (97 %) were found in the 10^0-10^1 cfu/cm^2 range. From the quantitative investigation it was found that residents had a low monthly income, lacked facilities and had a mean education level of Grade 7. Results, furthermore, showed that the microbiological quality of food in numerous households in the study-area is cause for concern. It is suggested that definite relationships exist between the household hygiene practices and socio-economic status of the area.

Key words: Food, microbial quality, food-borne diseases

INTRODUCTION

South Africa had a population growth of 23 % from 1978 to 1996 (30 million to 37 million), resulting in a need for more food (Stewart, 1978; Moeketsi, 6 October, 1998, *Personal Interview*, Bloemfontein). Together with the high population growth, South Africa is also characterised by a climate of unemployment, poverty and illiteracy amongst the biggest part of the population. These conditions have created the opportunity for street vendors, lacking the basic facilities for safe food preparation and storage as well as sanitation facilities, including clean water, to exploit the food market. Furthermore, the South African national legislation (South Africa, 1987) has not yet presented clear guidelines for street-vended food, nor does the government have the necessary infrastructure to enforce this legislation. Currently, however, new legislation is drafted to address this problem. Meanwhile, because of the low cost of foodstuffs sold by these street vendors, as well as the traditional and ethical preferences of the residents, large quantities of food with suspect microbiological and nutritional attributes are made available to the community (Ghuliani and Kaul, 1995).

Food has been recognised by the World Health Organisation (WHO) as an important source of pathogens and toxic chemicals, and one of the main vectors of disease, including gastro-enteritis and diarrhoea (Stewart, 1978; Ehiri, 1995; WHO, 1997). Diarrhoea, nausea, vomiting and abdominal pain are clinical features of several communicable diseases transmitted through food, of which acute diarrhoea is one of the main health problems in developing countries (Jacob, 1989; Mendoza *et al.*, 1996). In third-world, disadvantaged communities, the most common food-borne diseases have been shown to be typhoid and paratyphoid fever, cholera, shigellosis, Hepatitis A, Giardiasis and Trichinellosis (Jacob, 1989). These diseases, caused by the ingestion of contaminated food or water, may be toxic

or infectious by nature (Notermans and Hoogenboom-Verdegaal, 1992; Musaiger, 1996). The causative agents of food-borne diseases may be biological or chemical, of which biological agents have been found to be the most common by far, especially *Staphylococcus aureus*, *Salmonella* and *Shigella* spp., *E. coli*, *Bacillus cereus*, *Clostridium perfringens* and *C. botulinum* (Jay, 1992; Notermans and Hoogenboom-Verdegaal, 1992; Hobbs and Roberts, 1993).

To ensure that food contamination is restricted to the absolute minimum, food hygiene must cover all aspects of food production, preparation, storing and serving, together with aspects such as the hygienic quality of equipment and work surfaces, waste disposal, and fly and vermin control (Stewart, 1978). Understandably, these concepts and means are well out of grasp of the typical South African rural community, therefore, the people most affected by unsafe food, are the poor who have been shown to suffer from a lack of food and under-nutrition (WHO, 1997). According to Dykes (1994) it was ascertained that up to 70 % of all diagnosed diarrhoea cases amongst children are transmitted by food. To add to this, the risk of food poisoning and food-borne diseases among infants and children are often neglected due to the under-reporting of such cases (Musaiger, 1996). Desai (1987) reported that in Cape Town, South Africa, 6 000 patients have been admitted to hospital suffering from food-borne diseases. Taking into account the economic situation, the scenario has probably worsened since these reports in 1987.

The aim of this study was to firstly assess the magnitude and composition of microbial contaminants in the foodstuffs of a representative area of Bothsabelo, Section M. Secondly, a qualitative survey was conducted on the basic infrastructure, socio-economic and health aspects of the mentioned community. This study, therefore, attempted to cast light on the

aspects of food hygiene as well as draw some conclusions on the relationships between household hygiene, health and socio-economic status.

MATERIALS AND METHODS

Qualitative analysis

With the assistance of the Department of Food, Clothing and Hotel Management (Technikon Free State) as well as the Department of Biostatistics (University of the Orange Free State), sixty households were randomly selected and questionnaires compiled that covered aspects of housing infrastructure, electricity and water supply, sanitation, food supplies and transport (Appendix A). The questionnaires were completed on an interview basis using two community volunteers. Prior to interviewing, the volunteers were trained in the various aspects of qualitative analysis with respect to questionnaire completion. These included comforting, introduction to the study and re-testing aspects.

Sampling protocol

The mentioned community workers were utilised to collect microbial samples from each of the selected households. The surface-swab method (Harrigan and McCance, 1976) was used to sample the plates used by the family directly after breakfast, as this was the meal most of the family members attended. Samples were collected over a period of 6 weeks (10 households per week). As in the case of the questionnaires, the community workers were trained in advance in the technique of the surface-swab method. Samples were collected directly after sampling and transported to the laboratory at 0-5 °C to restrict undesirable multiplication of micro-organisms.

Analysis of data

The streak-plate method was used to quantify the various microbial groups (Herbert, 1990). The enumeration of total aerobic mesophiles, aerobic spore-forming bacteria and anaerobic spore-forming bacteria was done by means of Plate Count Agar (PCA, MERCK, Houghtby *et al.*, 1993). For the detection of members of the family *Enterobacteriaceae*, Violet Red Bile Glucose agar (VRBG, OXOID) was used (Christen *et al.*, 1993). The isolation of fungi was done by means of Potato Dextrose Agar (PDA, MERCK) with pH 4.5 (Frank *et al.*, 1993). E. *coli* was isolated on Petrifilm™ (3M, Matner *et al.*, 1990). To analyse the spore-forming organisms, the samples were treated at 80 °C for 10 minutes to destroy vegetative cells, and then transferred to PCA. The anaerobic spore-forming organisms were incubated in anaerobic jars (Anaerocult A and Anaerotest, MERCK).

All plates were incubated at 35 °C (Labotec low-temperature incubator) for a period of 48 hours, whereas the aerobic spore-forming micro-organisms and PDA plates were incubated for 12-18 hours. All experiments were done in triplicate.

RESULTS AND DISCUSSION

Qualitative data

For the purpose of this study, the socio-economic data probably of most consequence to food hygiene were the following aspects: supply, preparation of food, transport and sanitation. Table 1 illustrates the extent of purchases of food by residents in Botshabelo, Section M. The largest group of residents living in Botshabelo, Section M, bought their bread, fruit, vegetables and meat from a tuck shop in the area. This may be attributed mainly to the convenience of the location of tuck shops, as there is no proper formal transport to the nearest

Table 1 Food supplies and purchase information of the average household in Botshabelo, Section M.

Sources	Bread (%)	Fruit & vegetables (%)	Meat (%)
Botshabelo Supermarket	19	30.3	41
Tuck shop	79.5	58.2	50.8
Other	0.8	-	-
Direct from farmer (street vendor)	-	3.3	-
Self generating	-	6.6	-
Butchery	-	-	7.4
Bloemfontein Supermarket	-	1.6	0.8

supermarket in Botshabelo. Because of the general lack of knowledge concerning the potential dangers of food-borne illness, residents are ignorant on the aspects of food supply and whether they buy from a tuck shop or the supermarket. The economical profile of the inhabitants may also determine the vendor. Since people do not have the means to pay for higher quality food, they will often settle for food of lesser quality. Only 7.4 % of the people bought their meat from a butcher, 50.8 % from a tuck shop and 41 % bought from a supermarket in Botshabelo (Table 1). This again can be linked to ignorance and the general economic status. The butcheries are further away from Section M, causing people to rather buy from the tuck shop or the supermarket as they either do not have or cannot afford the transport to go to the butchery. The low purchase of fruit, vegetables and meat from Bloemfontein supermarkets, were presumably due to overcrowded buses and the distance of the supermarket from Section M. To add to this, commuters are mostly pressed for time at the end of the workday. The purchase of fruit and vegetables from a farmer (3.3 %) as well as from self-generating sources (6.6 %), could be ascribed to small yards and the lack of water supply in some cases, inhibiting the production of fruit and vegetables by the residents themselves.

According to results shown in Table 2, it was evident that none of the households in Botshabelo, Section M, had a supply of hot water, whereas a mere 3.3 % of the homes was equipped with indoor cold water. All the homes had, however, a cold water tap in the yard. This lack of proper water provision inside the homes presents a potential health risk as illustrated in a study conducted by Myrdal *et al.* (1994), reporting that 9.5 % of diarrhoea cases occurred in homes with indoor water supplies, whilst 14 % of the diarrhoea cases occurred in homes with water provided outside. With regard to the electricity supply, only 3.3 % of the homes were supplied with electricity (Table 2). The absence of electricity in the

Table 2 The socio-economic profile of households with relation to food hygiene and food preparation in the Botshabelo, Section M area.

Parameter	%
Warm water	-
Cold water in the homes	3.3
Stove	2.5
Fridge	0.8
Electricity	3.3
Electrical appliances	0.8
Gas	0.8
Primus stoves	94.3
Coal	0.8
Open fire	3.3

homes has the consequence that no cooling facilities are available. This influences the food quality considerably, as alternative methods have to be utilised to store and keep perishable foodstuffs. Storing of perishable foodstuffs at ambient temperatures have been shown to enhance the proliferation of hazardous, pathogenic microflora (Jay, 1992; Ollinger-Snyder and Matthews, 1996). Many of these pathogens occur amongst the microbial groups analysed in this study (for example *Enterobacteriaceae*). Molbak *et al.* (1989) reported that the high cost of heat-energy resulted in fewer freshly prepared foods per day, causing food to be prepared in bulk, be consumed cold or reheated for only short periods. Sinell (1995) noted that temperature abuse has been recognised to be a primary factor in triggering the onset of food-borne diseases.

Microbial populations

The patterns of micro-organisms found in foodstuffs of households in Section M are represented in Figure 1. The households varied considerably from one other, with little uniformity amongst them. The *E. coli* (Fig. 1 (E)) counts from households 23 and 25 corresponded with the remaining microbial groups found in these households, indicating the hygienic quality of foodstuffs prepared and consumed by residents living in these households. Furthermore, the occurrence of the aerobic spore-forming bacteria (Fig. 1 (B)) did not correspond with the anaerobic spore-forming bacteria (Fig. 1 (C)). The total plate counts of all the households were 10^3 in excess of per square centimeter of the plate, except for households 7, 26 and 34 (Fig. 1 (A)). These 3 households also had lower counts with regard to anaerobic spore-forming bacteria (Fig. 1 (C)), coliforms (Fig. 1 (D)), *E. coli* (Fig. 1 (E)), *Enterobacteriaceae* (Fig. 1 (F)), yeasts (Fig. 1 (G)) and moulds (Fig. 1 (H)). When the yeasts and moulds were compared, a peak occurrence was detected in the households 11 and 12 (Figs. 1 (G and H)). Households 58-60 showed no coliforms, *E. coli* or *Enterobacteriaceae*,

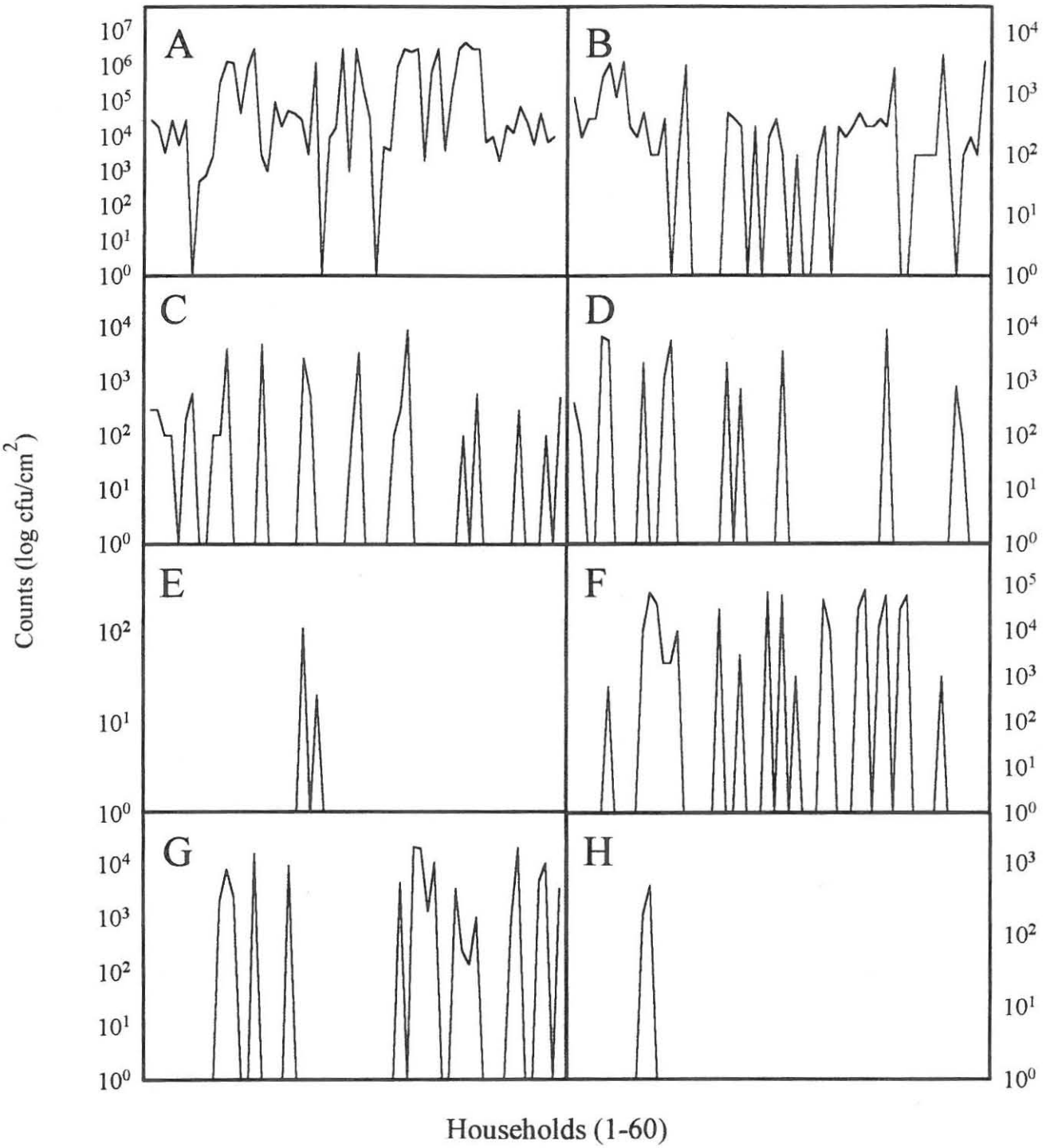

Fig. 1 Patterns of microbial groups found in foodstuffs from households in Section M, Botshabelo (A: total arobic mesophiles; B: aerobic spore-forming bacteria; C: anarobic spore-forming bacteria; D: coliforms; E: *E coli*; F: *Enterobacteriaceae*; G: Yeasts; H: Molds)

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while the total aerobic mesophilic organisms, aerobic and anaerobic spore-forming bacteria, yeasts and moulds varied from $1x10^0$-$1x10^4$ cfu/cm^2.

In Table 3 the relative occurrence of micro-organisms in each household is shown as percentiles. The highest counts with regard to the aerobic and anaerobic spore formers, coliforms, *Enterobacteriaceae*, yeasts and moulds occurred in the range of 10^0-10^1 cfu/cm^2. However, with regard to the total mesophilic organisms, the largest percentage (43.3 %) occurred in the 10^4-10^5 cfu/cm^2 range with very few (5 %) at the 10^0-10^1 cfu/cm^2 range (Table 3). These results suggested that, although some microflora was detected in small amounts, the aerobic mesophiles were present in high numbers. The relatively high presence of aerobic and anaerobic spore-forming bacteria, (63.3 % and 28.3 % in the range of 10^2-10^3 cfu/cm^2) could indicate insufficient cooking or preparation practices, again perhaps due to poor socio-economic circumstances and insufficient preparation. These practices may allow members of the spore-forming genera *Bacillus* and *Clostridium* to survive.

The yeast counts were higher than the mould counts (3.3 % in the 10^2-10^3 cfu/cm^2 range, 18.3 % in the 10^3-10^4 cfu/cm^2 range and 10 % in the 10^4-10^5 cfu/cm^2 range), except in the range of 10^0-10^1 cfu/cm^2, where the moulds had a 97 % occurrence compared to the 66.6 % of the yeasts (Table 3). Excessive fungal growth in food could lead to various effects, for example the softening of the product, off-flavours, odours and changes in appearance of foodstuffs. In addition, moulds may produce mycotoxins in food that render it unsafe and in some cases even fatal (Marth, 1990). *Rhizopus* (bread mould) is a very common food spoilage organism and is known to cause spoilage in many foodstuffs (Frazier, 1988). In general, spoilage by yeasts is not cause for much concern, as spoilage by these organisms generally result in innocuous off-flavours and tastes due to fermentation. Spoilage yeasts are

Table 3 Occurrence (%) of micro-organisms in households of the Section M area.

Occurrence	Total[a]	A.sf.[b]	An.sf[c]	Coli[d]	*E. coli*	Enterobact.[e]	Yeasts	Moulds
10^{-0}-10^{1}	5	25	61.6	78.3	96.6	65	66.6	97
10^{1}-10^{2}	-	-	-	-	1.6	-	-	-
10^{2}-10^{3}	10	63.3	28.3	8.3	1.6	1.6	3.3	3
10^{3}-10^{4}	26.6	11.6	8.3	13.3	-	8.3	18.3	-
10^{4}-10^{5}	43.3	-	-	-	-	25	10	-
10^{5}-10^{6}	10	-	-	-	-	-	-	-
10^{6}-10^{7}	5	-	-	-	-	-	-	-

[a]Total mesophilic organisms, [b]Aerobic spore forming bacteria, [c]Anaerobic spore forming bacteria, [d]Coliforms, [e]members of the family *Enterobacteriaceae*

known to proliferate quickly in a variety of foodstuffs and include species of the genera *Sacharomyces*, *Zygosacharomyces*, *Torula* and *Candida* (Jay, 1992). Most yeasts require more moisture than moulds and their growth is favoured by a pH of 4-4.5 (Frazier, 1988).

In Figure 2 the mean counts of the various organisms are shown. The aerobic and anaerobic spore formers had relatively similar numbers on the plates ($5.48x10^2$ and $4.68x10^2$ cfu/cm^2 respectively). The total mesophilic counts were $1.71x10^5$ cfu/cm^2 (fig. 2), which exceeded the standards for surfaces by far (South Africa, 1987). The coliforms, *E. coli* and members of the family *Enterobacteriaceae* had values of $6.31x10^3$ cfu/cm^2, $2.1x10^2$ cfu/cm^2 and $1.0x10^4$ cfu/cm^2 respectively (Fig. 2). The presence of these organisms could indicate faecal contamination of the food (Eley, 1992; Simango *et al.*, 1992). The yeast counts were $2.33x10^3$ cfu/cm^2 and the moulds $1.2x10^2$ cfu/cm^2.

The minimum and maximum values for each of the organisms varied considerably. Total mesophilic plate counts varied, for example, from none detected to a maximum of 1.33×10^6 cfu/cm^2. Both the aerobic and anaerobic spore formers had maximum values of $4.4x10^3$ cfu/cm^2 and $8.9x10^3$ cfu/cm^2 respectively compared to an absence in some localities. Alarmingly, however, the high *E. coli* counts had a maximum of $1.1x10^3$ cfu/cm^2 (minimum 0 cfu/cm^2), while the coliforms had a maximum of $8.8x10^3$ cfu/cm^2 (minimum 0 cfu/cm^2) and members of the family *Enterobacteriaceae* a maximum of $8x10^4$ cfu/cm^2 (minimum 0 cfu/cm^2). The maximum counts of moulds were $5x10^2$ cfu/cm^2 and the minimum 1 cfu/cm^2, while the minimum count for yeasts were 1 cfu/cm^2 and the maximum $2x10^4$ cfu/cm^2.

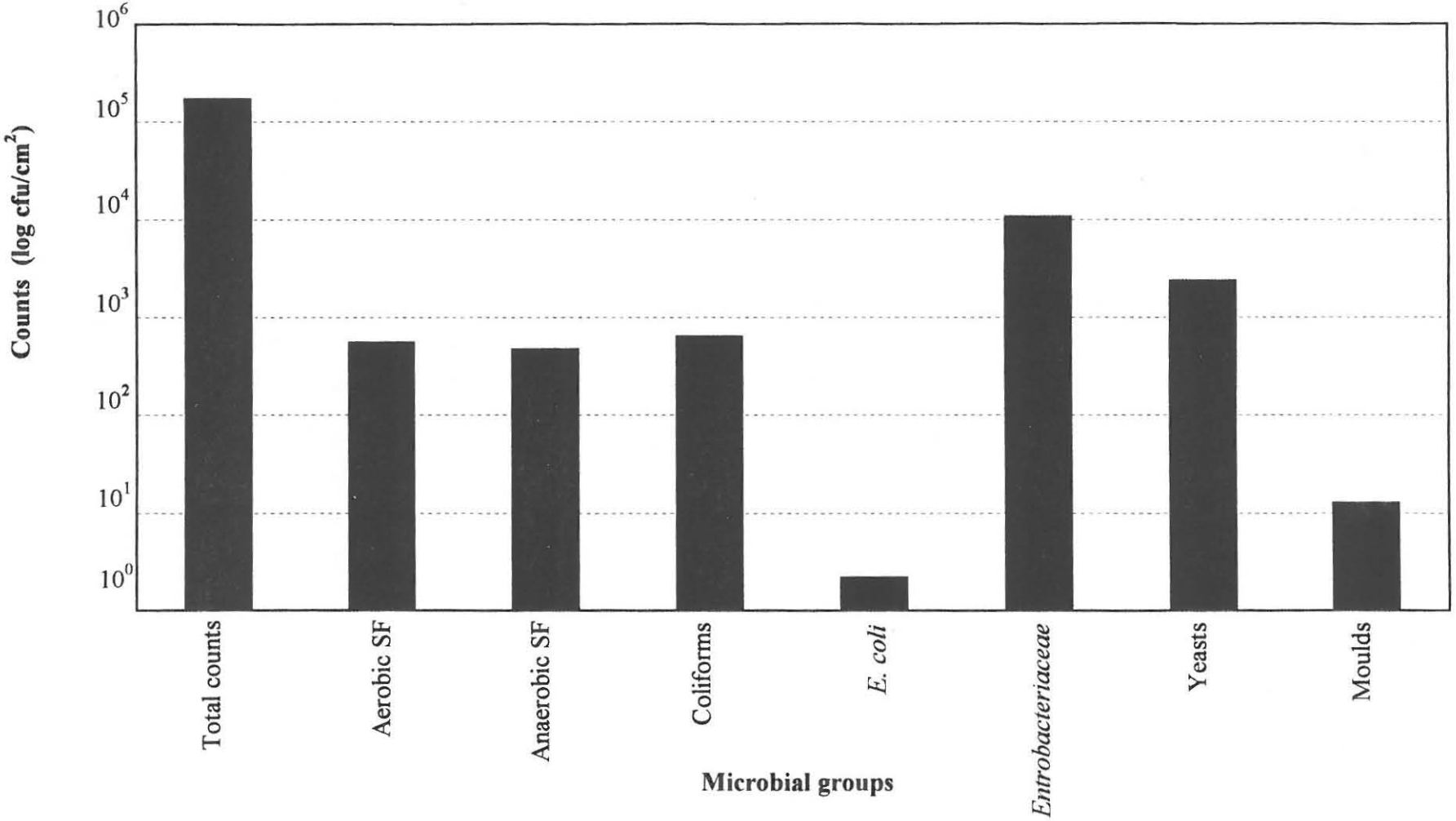

Fig. 2 The average of the various organism groups isolated from foodstuffs in Botshabelo, Section M (SF: spore-forming bacteria)

Conformance with national legislation

The growth of total mesophiles in food samples from the households compared with the national guideline stipulated in legislation (South Africa, 1987) is shown in Figure 3. The national standard for total counts on any food-related surface, including equipment or utensils, is 100 cfu/cm^2 (South Africa, 1987). In Figure 3, none of the selected households complied with these standards, except for numbers 7, 26 and 34, which amounts to 3 % of the selected households. Counts found in most of the households were between 10^3 and 10^6 cfu/cm^2 and even higher (Fig.3, Table 3). The fact that 3 % of the households could maintain an acceptable standard of hygiene under such abhorrent circumstances, shows that the possibility to improve the general conditions through proper education exists.

The reason for the high microbial counts in some samples can be attributed to improper cooking, handling and storage of food, together with the lack of education regarding food hygiene, sanitation and other socio-economic related hazards. These conditions enhance the spread of organisms and may serve as potential sources of food-borne diseases. These are common problems in urban slum areas (Molbak *et al.*, 1989), such as Botshabelo, Section M. Abdussalam and Käferstein (1996) stated that people who do not cook their food adequately might suffer from food-borne diseases. This may also be true in Section M. The residents leave for work at 6:30 in the morning only to be back at 18:30 in the evening, thus leaving them little time to prepare food well. Very often, food is thus prepared far in advance, stored and reheated. The prolonged storage of food increases the risk of contamination by household members, animals or insects and could take place when foodstuffs are left at room temperature to cool down, when left overnight or when it is allowed to cool down slowly (Agbodaze and Owusu, 1989; Molbak *et al.*, 1989; Reed, 1993; Nyatoti *et al.*, 1997). Ghuliani and Kaul (1995) stated that direct and cross-contamination of food were high in poor

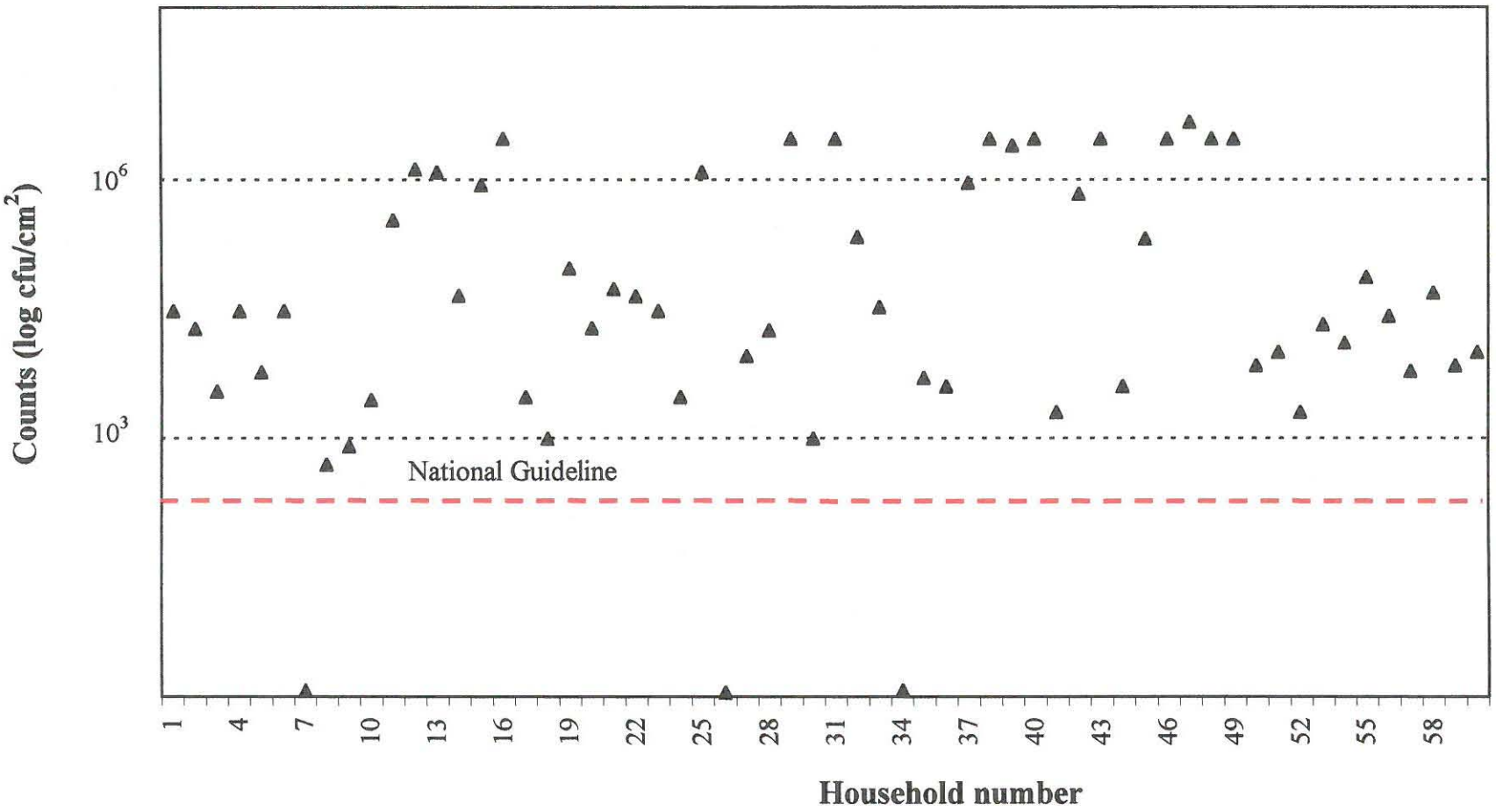

Fig. 3 The patterns of total mesophilic organisms in food from selected households of Botshabelo, Section M, compared with the national guideline as stipulated in legislation.

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communities with unsatisfactory environmental conditions, careless customs and traditions typical to Bothsabelo, Section M. It is obviously a cross-cultural problem, as these people do not follow the lifestyle of the black races of previous centuries, but have to adapt to a Western lifestyle without having the amenities to make it work. Traditionally, street vendors have been particular regarding the safety of their products. Nowadays, however, even these small-scale entrepreneurs are lowering standards due to the pressure of commercialism, bringing with it the neglect of basic, traditional standards.

According to these results, it can be concluded that food prepared and consumed by residents living in Bothsabelo, Section M, has a very short shelf-life due to the high presence of organisms and poor hygienic standards. Furthermore, results suggest that the poor hygienic conditions have a strong relationship with the socio-economic profile and infrastructure of the area. As a result, the food can cause severe food-borne disease amongst the consumers. To rectify the standard of food preparation, people need to be educated on the proper cooking and holding methods, as well as the temperatures at which pathogenic organisms can be found as well as ways to inhibit their growth by manipulating these temperatures. The situation can also be aided by educating people to eat more foodstuffs with inherent keeping ability like sour porridge and sour milk.

In preventing the cross-contamination of foodstuffs by the food handler, the community must, furthermore, be informed about proper personal hygiene and the risks involved with food-borne diseases. The cleaning of equipment cannot be done properly if the water used is contaminated with micro-organisms. To improve the sanitary conditions, proper water facilities should be installed together with electricity supplies to ensure proper cooking and cooling. Unfortunately, all of these proposals cannot be realised because of the reality of the

weak economic situation that is complicating the existing problems of the vast numbers and population growth in the area. An effective education program could, however, be launched, addressing the public on basic hygiene as well as more traditional cooking, preservation and eating habits.

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APPENDIX A

Central University of Technology, Free State

Technikon Free State

SOCIO-ECONOMIC QUESTIONNAIRE

FAMILY NO. ☐☐☐ **1-3**

1. HOUSING:

1.1 Are you (the family) a owner or a tenant? 1= owner 2= tenant ☐ **4**

1.2 How many families including your own, are on the plot? ☐ **5**

1.3 Room division

		Number
Kitchen	☐	**6**
Dining room	☐	**7**
Sittingroom	☐	**8**
Dining-sittingroom	☐	**9**
Bedroom	☐	**10**
Bathroom with toilet	☐	**11**
Bathroom without toilet	☐	**12**
Toilet outside	☐	**13**
Other _____	☐	**14**

1.4 Structure of the house

1 = Sement house 2 = Tin house

3 = Brick house 4 = Mud ☐ **15**

Courtesy of Department of Food, Clothing and Hotel Management

2. ELECTRICITY AND WATER SUPPLY

2.1 Do you have:

a)	Electricity?	☐	16
b)	Electric-appliances (e.g. toaster, kettle)?	☐	17
c)	Deep-freeze?	☐	18
d)	Fridge?	☐	19
e)	Stove?	☐	20
f)	Cold water?	☐	21
g)	Warm water?	☐	22

1 = Yes in house

2 = Yes, somewhere else on premises

3 = No

2.2 What kind of fuel do you use most often for light?

1 = Electricity

2 = Gas

3 = Oil

4 = Paraffin

5 = Coal

6 = Wood

7 = Other _____ ☐ 23

2.3 What kind of fuel do you use most often for heat?

1 = Electricity

2 = Gas

3 = Oil

4 = Paraffin

5 = Coal

6 = Wood

7 = Other _____ ☐ 24

2.4 How do you prepare food most often?

1 = Electric stove / plates

2 = Gas stove

3 = Primus

4 = Stove / wood / coal

5 = Open fire outside

6 = Other _____ ☐ 25

3. SANITATION

3.1 Type of toilet (in the case of 2 take the best)

1 = Well toilet

2 = Bucket toilet

3 = Flush toilet inside

4 = Flush toilet outside

5 = None

6 = Other _____ ☐ 26

4. EXPENSES

4.1 Where does the family most often buy:

Milk / bread? ☐ 27

Fruit / vegetables? ☐ 28

Meat? ☐ 29

Sweets? ☐ 30

Groceries (such as tinned food)? ☐ 31

1 = Café / Supermarket in Botshabelo

2 = Supermarket in Bloemfontein

3 = Farmer

4 = Vender / Tuck shop

5 = Other

5. TRANSPORT

5.1 Does your family have its own transport?

1 = Yes ☐ 32

2 = No

5.2 What kind of transport does your family use most often?

1 = Bus

2 = Train

3 = Taxi

4 = Own car

5 = Walk

6 = Bicycle

7 = Other _____ ☐ 33

CHAPTER 4

CONVENTIONAL INDICATOR BACTERIA AND OTHER MICROFLORA AS INDICES OF HYGIENE IN A MARGINALISED URBAN SETTLEMENT

(Article submitted for publication in journal: *Epidemiology and Infection*)

Helen van der Westhuizen

Conventional indicator bacteria and other microflora as indices of hygiene in a marginalised urban settlement

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ABSTRACT

The relationships amongst a variety of micro-organisms isolated from food and milk in Section M, Botshabelo, were investigated. The data were compared with the patterns of indicator organisms in an attempt to evaluate these classical hygiene indicators. The software program Sigma Plot was, furthermore, used to create "fingerprints" of the microbial content of a typical household in Section M. The conventional indicators (Total counts, coliforms and Escherichia coli) showed poor correlations with other micro-organisms, except for the relationships between Enterobacteriaceae and total mesophilic organisms in food (r =0.634). In addition, the microbial status of the food and milk samples was compared, resulting in a correlation of 0.991. This high correlation strongly suggests that the microbiological quality of foodstuffs and milk in the average household, is a consequence of poor household hygiene, rather than poor quality products from the supplier. This compared well with the fingerprints of milk and food. In general, higher organism counts were found in milk than in food.

Key words: Food, milk, correlation, indicators

INTRODUCTION

Already during the 1890's *E. coli* was used as indicator of faecal contamination in water, and since then it has been used extensively in the food industry (Frazier and Westhoff, 1988; Banwart, 1989). Later coliforms and members of the family *Enterobacteriaceae* have also been included as indicators of food safety and hygiene (Hartman *et al.*, 1986; Frazier and Westhoff, 1988; Banwart, 1989; Jay, 1992). Other indicator bacteria include, amongst others, *Staphylococcus aureus* and *Clostridium* species. The presence of large numbers of *S. aureus* is, for example, an indication of a potential hazard due to staphylococcal enterotoxin as well as questionable sanitation (Frazier, 1988; Banwart, 1989). *Clostridium* species have been used as they can give an indication of both faecal contamination as well as the presence of spore-forming bacteria (Banwart, 1989).

An indicator organism can be defined as an organism that, when found at specified levels, provides a warning of a safety or spoilage hazard (Jay, 1994). These organisms are used to indicate levels of hazardous, detrimental food-spoilage organisms, as they are easier to isolate and quantify, and also survive better in the foodstuffs than the actual pathogen (Jay, 1992). The functions of indicator organisms include the determination of the microbial integrity of foodstuffs as well as the sanitary conditions of food processing, production and storage (Banwart, 1989). Organisms should meet certain criteria of quality or hygiene to act as an indicator. Therefore it must be present and detectable in foods of which the quality is to be assessed and its growth and numbers should have a direct negative correlation with the product quality. It should, furthermore, be easily detectable and enumerated as well as clearly distinguishable from other organisms. Indicator organisms should be enumerable within a

short period of time and its growth should not be affected adversely by other components of the food flora (Banwart, 1989; Jay, 1992).

Micro-organisms are, however, more often employed to assess food safety and sanitation than quality. Food safety indicators should therefore, apart from the above-mentioned criteria, also comply with other criteria. First of all, it must have a history of association with the pathogen which presence it should indicate, therefore being present when the pathogen is present and absent when foods are free of the pathogen. Furthermore, its numbers, growth requirements and growth rate should be equal to those of the pathogen. In addition, food safety indicators' die-off time should be parallel to that of the pathogen (Banwart, 1989; Jay, 1992). When the concept of faecal indicators was applied to food safety, some additional criteria were stressed. Firstly the bacteria should be specific to the intestinal environment. Secondly its occurrence in faeces should be in sufficient numbers to make detection in high dilutions possible, and thirdly it should have a resistance to the extra-enteral environment. These indicator organisms should finally be relatively easily detectable when present in low numbers (Banwart, 1989; Jay, 1992). Faecal coliforms, eg. *E. coli* are more closely related to faecal contamination than other coliforms (Banwart, 1989).

In South Africa, food legislation has stipulated total counts, coliforms and *E. coli* as indicators of microbial proliferation and faecal contamination, in other words of overall hygiene (South Africa, 1997). However, these standards have traditionally been enforced in the more formal and commercial sectors. With recent awareness about community health, as well as reports that food-related disease is one of the foremost killers in informal settlements, a few questions have arisen regarding the above-mentioned indicators. For example, the question arose whether these indicators are effective and reliable indices of food and milk contamination in

informal settlements, where many products of suspect origin are consumed. Another question would be what the relationships are between these organisms and other common food-borne microflora, and also whether these indicators, together with other microflora could be utilised to establish fingerprints of the microbial status of food and milk in a typical South African marginalised urban settlement.

The aim of this study was, therefore, to investigate the relationship between food- and milk contamination in Section M, Botshabelo, in order to determine whether the handling of these commodities are related to general household hygiene. It was also attempted to establish relationships between traditional indicator bacteria and other microflora isolated from food and milk in order to evaluate the applicability of the indicators used in local legislation. Microbial fingerprinting was suggested as index of the microbial composition of the food from a typical household in Section M.

MATERIALS AND METHODS

Two community workers were trained in the basic methodologies of microbiological sampling of surfaces and liquids. These workers collected, on a weekly basis over a period of six weeks, samples from 60 randomly selected households in Section M. The samples were collected in the morning after breakfast, as this was the meal attended by most of the family members. The samples were collected aseptically and labelled clearly with the house numbers. After collection the samples were kept below 5 °C and transported to the laboratory for analysis.

Analysis of data

All experiments were done in triplicate. Serial dilutions were prepared with peptone buffer (BIOLAB), while the streak-plate method was used to isolate the various microbial groups (Herbert, 1990). Aerobic mesophiles as well as aerobic and anaerobic spore-forming bacteria were enumerated on Plate Count Agar (PCA, MERCK, Houghtby *et al.*, 1993). For the enumeration of members of the family *Enterobacteriaceae*, Violet Red Bile Glucose agar (VRBG, OXOID) was used (Christen *et al.*, 1993). The detection of yeasts and moulds were done by means of Potato Dextrose Agar (PDA, MERCK). The pH of this medium was adjusted to 4.5 with lactic acid to restrict bacterial growth (Frank *et al.*, 1993). Petrifilm™ (3M) was used for coliforms and *E. coli* (Matner *et al.*, 1990). For isolation and enumeration of the spore-forming bacteria, samples were exposed to 80 °C for 10 minutes to destroy the vegetative cells, and were then transferred to PCA agar plates. The anaerobic spore-forming organisms were incubated in anaerobic jars with Anaerocult A (MERCK) and Anaerotest (MERCK). The Petrifilm™, PCA agar plates, VRBG agar plates together with the aerobic spore formers were incubated at 35 °C (Labotec) for a period of 48 hours, while the aerobic spore-forming bacteria and the PDA agar plates were incubated for 12-18 hours. The colonies were counted with a colony counter (Gerber Instruments). Blue colonies with gas bubbles were an indication of *E. coli*, whereas red colonies with gas bubbles were an indication of coliforms. Members of the family *Enterobacteriaceae* growing on VRBG agar produced round, purple colonies, 1-2 mm in diameter surrounded by purple haloes (Mossel *et al.*, 1986).

Statistical analysis

Statistical correlation between the variables was done with the SPSS-4 for Unix and Solo Statistical Systems (BMDP, Ratkowsky, 1983), while the fingerprints were compiled using Sigma Plot for Windows (Ver. 3).

RESULTS AND DISCUSSION

Relationships between indicator bacteria and other microflora

Food

The correlation values of the various organisms with total mesophilic organisms, coliforms and *E. coli* are shown in Table 1. The total counts showed a weak correlation with the anaerobic spore formers (r = 0.249) and almost no relationship with the aerobic spore formers and moulds (r = -0.115 and r = 0.049 respectively). These results indicate that the patterns of these organisms had no or a negligible influence on one another. The correlation between the *Enterobacteriaceae* and the total counts was, however, higher (r = 0.634). The growth patterns of *Enterobacteriaceae* could therefore be indicative of those of the total counts. There was no meaningful correlation noted between the coliforms (r = 0.148) and *E. coli* (r = -0.062) as compared with the total mesophilic organisms in the food (Table 1). Suprisingly, the coliform organisms correlated very weakly with both *E. coli* (r = 0.115) and the *Enterobacteriaceae* (r = 0.167), suggesting that these organisms did not have such strong links with one another as generally believed. *E. coli* and *Enterobacteriaceae* also did not show any particular relationships regarding their patterns in the different sampling points (r = -0.071).

Milk

As was the case with the patterns of the various organisms in households of the selected area, no significant correlations could be found between the indicator organisms and any of the other groups in milk (Table 1). According to these results it could perhaps be concluded that the *Enterobacteriaceae* be included as possible indicator of the hygiene of food in the

Table 1 The various organism groups sampled in milk and food from Botshabelo, Section M, in correlation with total mesophilic organisms, total coliforms and *E. coli*

FOOD

	Total[a]	**Coli**[b]	***E. coli***	**A.sf.**[c]	**An.sf.**[d]	**Entero-bact.**[e]	**Yeasts**	**Moulds**
Total[a]		0.148	-0.062	-0.115	0.259	0.634	0.249	0.049
Coli[b]	0.148		0.115	0.166	0.014	0.167	-0.0004	-0.147
E. coli	-0.062	0.155		-0.011	0.182	-0.071	-0.067	-0.025

MILK

	Total[a]	**Coli**[b]	***E. coli***	**A.sf.**[c]	**An.sf.**[d]	**Entero-bact.**[e]	**Yeasts**	**Moulds**
Total[a]		0.214	0.175	0.094	0.022	0.111	0.147	-0.039
Coli[b]	0.214		-0.109	0.084	-0.068	0.144	0.307	0.339
E. coli	0.175	-0.109		0.091	0.172	-0.077	-0.095	-0.075

[a]Total mesophilic organisms, [b]coliforms, [c]aerobic spore forming bacteria, [d]anaerobic spore forming bacteria, [e]members of the family *Enterobacteriaceae*

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sampling area. This would collaborate with Banwart (1989) who reported that the entire family *Enterobacteriaceae* could be used as an indicator group. However, this same author further stated that their direct relationship to faecal contamination or potential pathogens is still questioned, as some occur as saprophytes in nature, some are plant parasites, many are found in the intestines and others are potential pathogens (Banwart, 1989). Banwart (1989), furthermore, said that the survival of *E. coli* is similar to that of *Salmonellae* in many food products. However, many pathogens may persist after *E. coli* is destroyed. On the other hand, the presence of *E. coli* does not mean that enteric pathogens are also present. Jay (1994) has stated that coliforms are not good indicators of *Salmonella*. This might be indicative of the assumption that the presence of coliforms had no effect and no correlation on the presence of *E. coli* and *Enterobacteriaceae* (Table 1).

Relationships between food and milk

Figure 1 compares the various micro-organisms sampled in food and milk with each other. The relationship between the organisms isolated from the food and milk was found to be extremely high ($r = 0.991$), suggesting that poor household hygiene was reflected in all the commodities used in a specific household. Furthermore, this high correlation indicated that the hygienic quality of these foodstuffs was not as much a result of the poor food quality from the supplier but handling by the consumer. The problem, therefore, seems to lie with the consumer, suggesting that the foodstuffs might be of a more acceptable quality when bought from the supplier, but that inadequate handling by the consumer causes contamination of the product. The socio-economic status of residents might also contribute to the poor hygienic quality of food and milk, as they lack basic facilities such as sanitation, warm water and

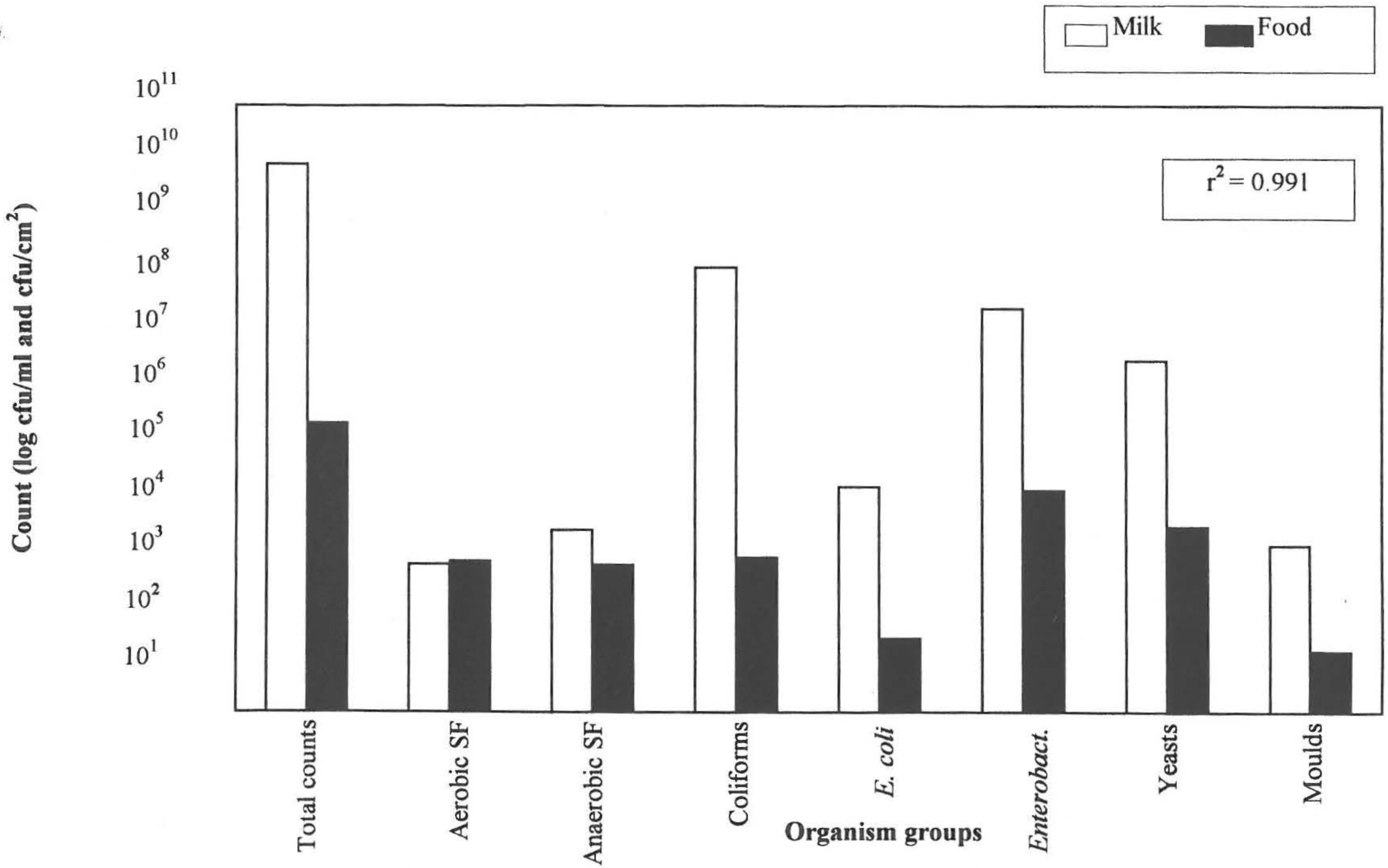

Fig. 1 The relationship between organism groups sampled in milk and food in Botshabelo, Section M (SF = spore formers; *Enterobact.* = *Enterobacteriaceae*)

electricity. These findings could thus be significant, as it re-iterates the need for basic facilities and education on good housekeeping and basic hygiene.

The results Figure 1 showed that the foodstuffs had considerably lower counts for all the micro-organisms than the milk (apart from aerobic spore-forming bacteria). One must however, keep in mind that the fact that the food is cooked probably explains the similarities in the counts for aerobic spore-forming bacteria, which could survive the cooking process. In general, it was expected that milk would show more contaminants, taking into account that it is a highly perishable product. The fact that food and milk, in this study, was compared, served the purpose of indicating the most probable risks of food-borne illness from the commodities ingested by residents of Section M. When considering the high number of microbial counts as displayed in Figure 1, especially for groups containing many pathogenic species like *Enterobacteriaceae*, it could be concluded that milk presented the highest risk.

Microbial indices and fingerprints

A profile of the microbial groups in milk and food found in a household of a typical rural settlement, is shown in Figures 2 A and B. By using visual representations as depicted in Figure 2, an attempt was made to establish a "fingerprint" of the microbiological composition of foodstuffs and milk used by residents of a typical household of a South African rural settlement. By comparing such fingerprints to other settlements, as well as to the formal and developed sectors, conclusions can be drawn regarding the overall hygiene, food-borne hazards and even the socio-economic status and education levels of these sectors. One would, for example expect parallel curves from a more intact environment to be more uniform than the uneven distribution of Figures 2 A and B. By linking such fingerprints to, for example, the food-borne disease profile of information gathered from local clinics, one should be able

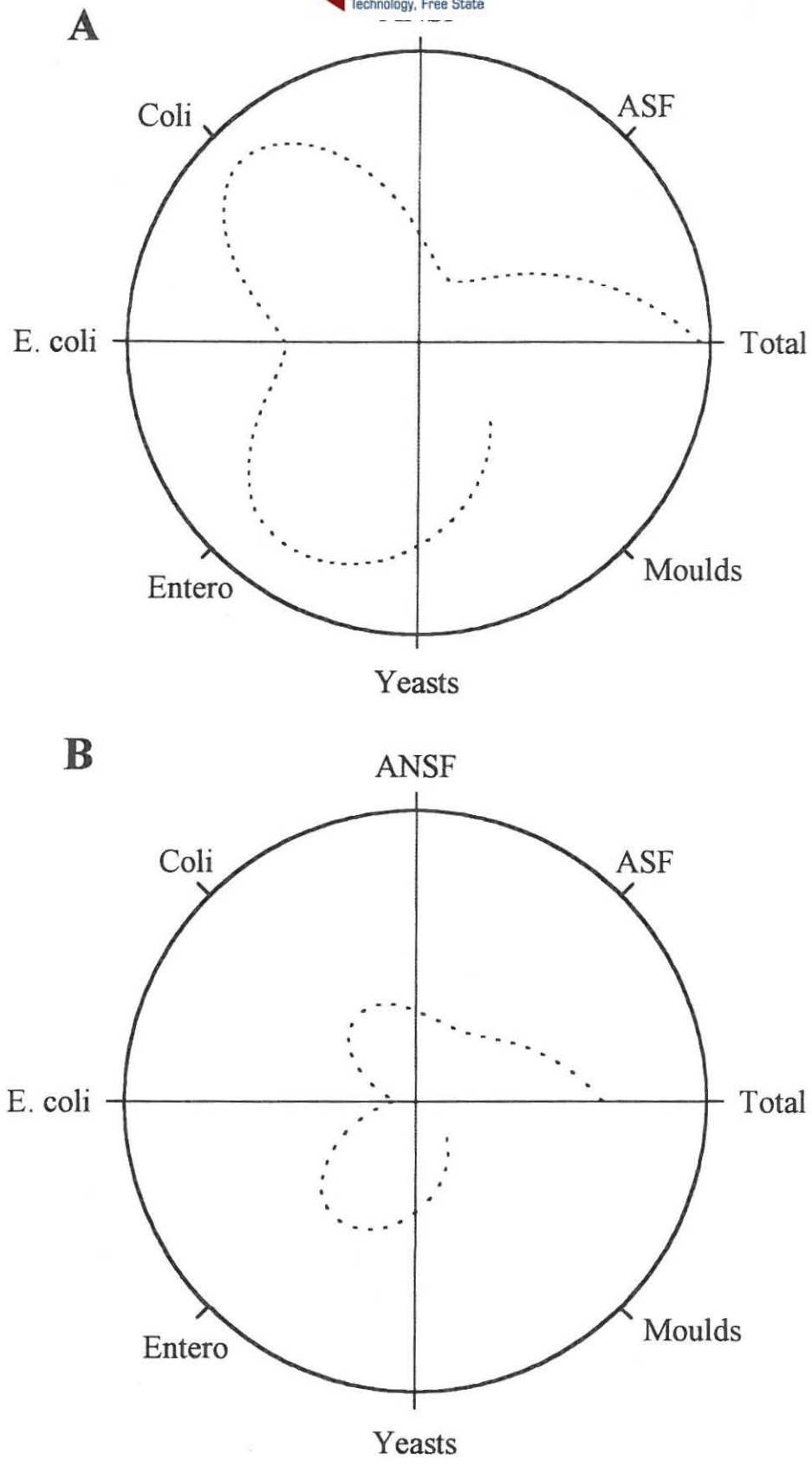

Fig. 2 Polar plot "fingerprints" of the microbial populations of milk (A) and foodstuffs (B) in Botshabelo, Section M.(Total: total mesophilc counts; ASF: aerobic spore formers; ANSF: anaerobic spore formers; Coli: coliforms; *E.coli*: faecal coliforms; Entero: members of the *Enterobacteriaceae*)

to quantify the hazards and risks posed by the food and drink of a specific community. Figure 2 confirmed earlier reports that the coliforms and members of the *Enterobacteriaceae* pose the biggest risk with regard to prevalence in the food and milk. The fact that the curves for milk and food revealed the same trend indicates the similarity in handling procedures regarding these food groups.

It can be concluded that the reliance on *Enterobacteriaceae* as indicator organisms should be done with great care, since they have a wide distribution in nature and are not confined to only the intestines of humans and animals. Therefore, the use of the well-known indicator organisms (total counts, coliforms and *E. coli*) should not be discarded. However, results found in this study posed the question as to whether the street enforcement of food legislation based on these classical indicators, is justified. The correlation found in this study between organisms isolated from food and milk confirmed that contamination took place at home, rather than at the supplier, as the consumers did not buy food and milk from the same supplier. The lack of facilities (cooling, warm water and electricity) in households of Section M probably contributed to the microbial status of the food and milk. It is suggested that, although the counts in the food were lower than that of milk, it was still very high (up to 10^5 cfu/cm^2, Fig. 1), and definite measures should be taken to rectify these shortcomings. Steps to be taken should include training in food- and milk-borne diseases and the risks involved; the proper use of detergents, emphasis on personal hygiene and sufficient provision of heat in order to kill pathogenic organisms.

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CHAPTER 5

CONCLUSIONS

Helen van der Westhuizen

CONCLUSIONS

South Africa is characterised by a mixture of cultures, races, beliefs, norms, traditions and socio-economic standards, thereby acquiring the label "The Rainbow Nation". This aspect, however, makes it difficult to determine whether South Africa is a developed or developing country. The majority of South Africans lack proper education and have low socio-economic living conditions, therefore, suggesting that South Africa is a developing country rather than developed. Over the past view years marginalised urban settlements have increased, resulting in poor hygienic conditions and giving rise to a variety of diseases, of which many originate from food and drink. Botshabelo, Section M, is a typical example of such a settlement. The aim of this study was to cast light on the microbiological status of the food and drink in the area, so as to draw some conclusions on the relationships between household hygiene and the risk of contracting food- and milk-borne diseases.

Milk and food hygiene

The incidence of micro-organisms in milk was alarmingly high with counts of 10^9 cfu/ml for the total mesophilic organisms and 10^5 cfu/ml for *Escherichia coli*. The family *Enterobacteriaceae* ranged between intervals of 10^1 and 10^8 cfu/ ml. All of these organisms could contain pathogens, suggesting that milk consumed in M Section is a potential risk factor in contracting milk-borne diseases. Although contaminated to a considerable extent, samples from food had, in general, lower microbial counts than milk. The microbial incidence in food showed counts of up to 10^7 cfu/cm^2 for total mesophilic organisms. Bearing these high counts in mind and the fact that samples of food and milk, almost without exception, exceeded South African legislation standards, the health risks posed by these commodities are alarming.

With regard to the information gathered from questionnaires, as well as the microbiological data, the fundamental causes of poor quality food can be outlined as follows: 1) lack of electricity, 2) lack of proper water and sanitation facilities, 3) lack of education and 4) poor housekeeping techniques. The lack of electricity supply leads to lack of cooling of milk and food, which in turn, results in the proliferation of undesirable organisms. Warm prepared food can therefore not be cooled down quickly and is left on the table or shelf to cool down. This slow decline in temperature supports the growth of organisms. Lacking provision of warm water inside the homes could aggravate contamination of food and milk, since the residents are forced to carry water in buckets over long distances and use only cold water for washing purposes. This inconvenience consequently leads to the total omission of washing hands and propagates poor personal hygiene.

It emanated from this study that a lack in education is concomitant with poor housekeeping. Apart from not being aware of the risks involved with food- and milk-borne diseases, it also leads to ignorance concerning safe handling of food and the risks involved. Commuting between Botshabelo and Bloemfontein is another factor that leaves little time for food preparation, causing food to be prepared far in advance and very seldom reheated properly. Organisms that might have grown since preparation are, therefore, not destroyed. Neglect in covering food and milk attracts flies and other insects, leading to contamination by these vectors as well as dust.

Indicator organisms

The use of traditional indicator organisms (total counts, coliforms and *E. coli*) was not found to be as effective in indicating the occurrence of other organisms. In this study it was found that *Enterobacteriaceae* showed sufficient relationships with, amongst others, the total

mesophilic organisms, thus it could be suggested that the use of *Enterobacteriaceae* might also be considered as indicator organisms. When the microbial contamination of food and milk were compared, a strong correlation of 0.991 was found, suggesting that handling by the consumer rather than the supplier is indicative of the microbial status of food and milk. In addition, microbial fingerprints of the food and milk compared well with each other, supporting the correlation and giving good indication of the microbiological patterns of the food in this region.

Probably the first step in addressing the problem, would be a community education program that addresses all the aspects pertaining to poor food quality. When such a program is launched it should include both family members and suppliers. In general, the elements of such community awareness programs should include the following: aspects of keeping food at correct temperatures (warm and cold), aspects of personal hygiene, aspects of washing procedures and aspects of good housekeeping. Examples of such practices might include washing food commodities prior to use, and preparing smaller amounts of food and properly reheating it. Other precautionary measures that can be stressed in an education program, include keeping food and milk covered in a cool place, reducing microbial growth. Milk can, furthermore, be soured. Apart from being a favoured delicacy by many in the South African milieu, the low pH value of the product inhibits the growth of spoiling bacteria. In addition to the intervention program, it must be emphasised that water used for washing purposes should be kept clean. It should also be stressed to the consumer and suppliers that cross-contamination between fresh and cooked foods can be prevented by keeping these items separate. Children can, furthermore, already be educated at school-level regarding some of these aspects. The risks and consequences of food- and milk-borne diseases should also be stressed to the public, emphasising the importance of safe handling of food and milk.

Since the availability of healthy and nutritious foods is a basic right of all people, special attention should be given to the suppliers, especially street vendors, as they are the main suppliers of food and milk to Section M. National legislation is currently being prepared with the focus on HACCP (Hazard Analysis and Critical Control Points), and the identification of possible contamination areas in order to rectify them. As street vendors form a part of the food supply sector, they should be educated in and guided according the rules stipulated by legislation. To ensure that all components of the food supply sector comply with legislation, Environmental Health Officers (EHO's) should be trained in aspects of HACCP and the implementation thereof amongst both formal food suppliers and street vendors. The EHO, therefore, plays an important role in the upliftment of the community due to his/her important input in intervention programs as well as the enforcement of national legislation.

A diagrammatic representation of the role that this study, as well as similar studies can play in future as part of community upliftment and development in proposed in Figure 1. The key problem, highlighted in Chapter 1 of this study, was the high incidence of disease and malnutrition in the study area. However, many marginalised urban settlements in South Africa are characterised by the same situation. By identifying the sources of disease and analysing the microbiological content, solutions and recommendations can be made to rectify the problem. As a result, the health and welfare of the community should improve. However, this is a time consuming, and in many cases also an expensive exercise, that cannot be brought about in one day.

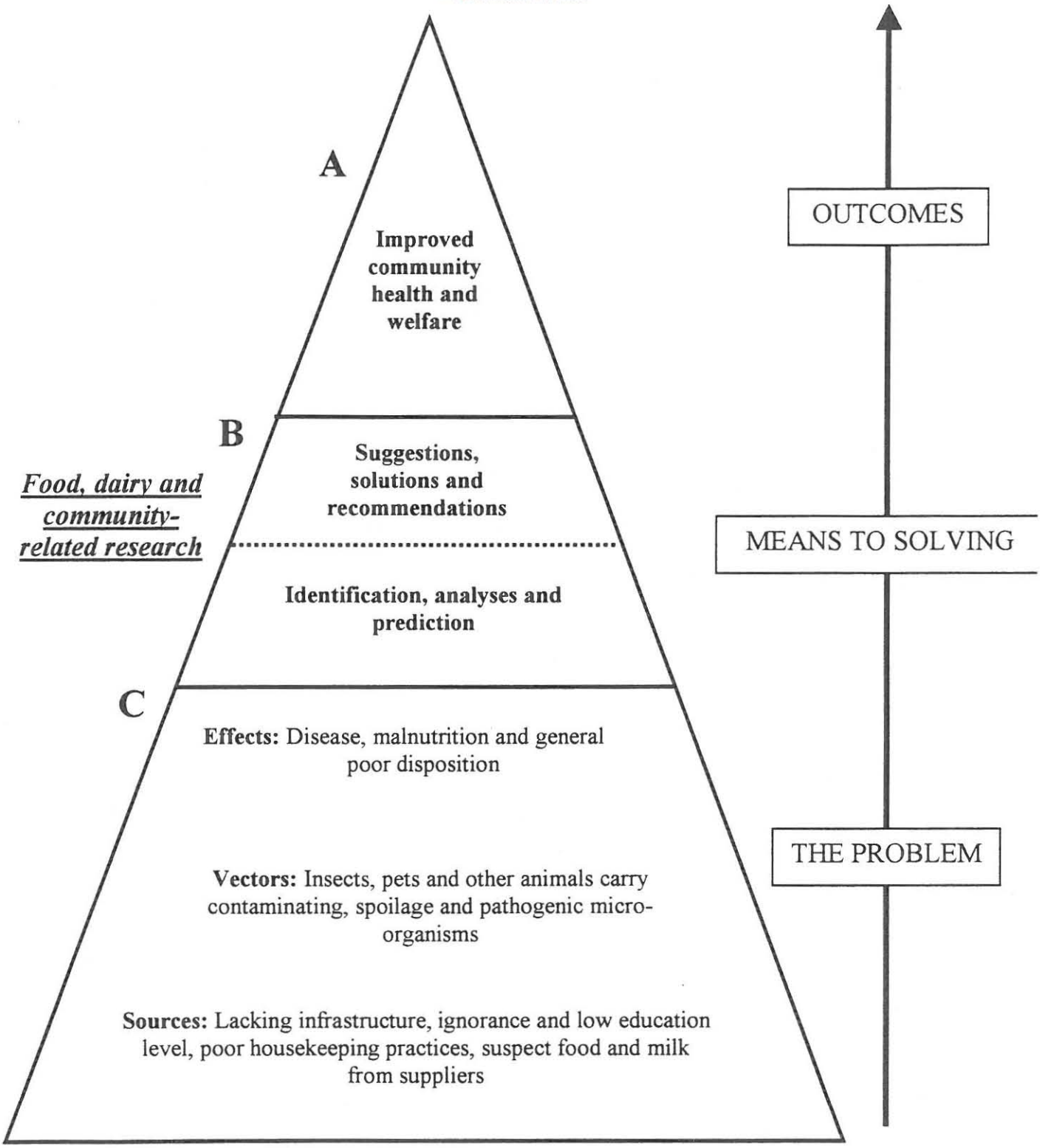

Fig. 1 A proposed flow diagram of the impact of food hygiene related research on the health and welfare of communities like Botshabelo. Section B, of the pyramid indicates the work done in this study, in an attempt to address the problems identified in section C of the diagram. By analysing the microbial populations involved, together with a questionnaire study, attempts were made to establish typical representations of the situation with regard to food hygiene in the area. Although some suggestions are made to solve the problems, more research is necessary to fully address all the problem-areas.

Future research

With reference to this study, further research aspects might include the following:

- Launching a food-hygiene intervention program addressing key aspects such as safe food preparation and basic hygiene of which the effectiveness could be verified by analysis of food and milk.
- Identification of causative micro-organisms of food- and milk-borne diseases by taking samples of patients' stools and identifying to species level. This should allow solution of the specific source of a disease and elimination thereof.
- Inclusion of more foodstuffs and possible risk factors to identify the disease with the objective of determining corrective measures.
- Estimation of the extent of microbial hygiene in other areas of South Africa, including larger sample sizes.