The Incidence, Growth and Survival of Diarrhoeagenic *Escherichia coli* in South African Meat Products

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The Incidence, Growth and Survival of Diarrhoeagenic *Escherichia coli* in South African Meat Products

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This thesis is dedicated to my family

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LIST OF ABBREVIATIONS

ACMSF	Advisory Committee on the Microbiological Safety of Food, United Kingdom
AMP	Adenosine monophosphate
AOAC	Association of Official Analytical Chemists
APC	Aerobic plate count
APHA	American Public Health Association
ATCC	American Type Culture Collection, Rockville, Maryland
A_w	Water activity
BC	Before Christ
BHI	Brain heart infusion broth
BPW	Buffered peptone water
B-P	Boerewors without preservative
B+P	Boerewors with preservative
cfu	Colony forming units
ºC	Degrees Celcius
DAEC	Diffuse adhering *Escherichia coli*
DEC	Diarrhoeagenic *Escherichia coli*
DNA	Deoxyribonucleic acid
E	*Escherichia*
EAF	Enteropathogenic adherence factor
EAggEC	Enteroaggregative *Escherichia coli*
edn	Edition
Ed(s)	Editor(s)
eg	For example
E-Hly	Enterohaemolysin
EHEC	Enterohaemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EPEC	Enteropathogenic *Escherichia coli*
et al.	(*et alii*) and others
etc	(*et cetera*) and so forth
ETEC	Enterotoxigenic *Escherichia coli*

Fig	Figure
g	gram
GB3	Globotriaosyl ceramide
GRAS	Generally recognised as safe
H	Flagella antigen
HC	Haemorrhagic colitis
HI:NP	High inoculum, without preservative
HI:WP	High inoculum, with preservative
HN(0)	High inoculum (7.5 log cfu/g)
HUS	Haemolytic uremic syndrome
kg	Kilogram
K	Capsule antigen
LI:NP	Low inoculum, without preservative
LI:WP	Low inoculum, with preservative
LN(0)	Low inoculum (3.5 log cfu/g)
log	Log_{10}
LT	Heat-labile enterotoxin
min	Minute
mg	Milligram
ml	Millilitre
mm	Millimetre
MUG	4-Methyl-umbelliferyl-ß-D-glucuronide
NCSS	Number Cruncher Statistical Systems, Kaysville, Utah, USA.
NCTC	National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom
ND	Not detected
O	Somatic antigen
OMP	Outer membrane protein
pp	Page(s)
sec	Second
SLT	Shiga-like toxin
SLTEC	Shiga-like toxin-producing *Escherichia coli*
spp	Species
SPCA	Standard plate count agar

ST	Heat-stable enterotoxin
STEC	Shiga toxin-producing *Escherichia coli*
Stx	Shiga toxin
™	Trade mark
TTP	Thrombotic thrombocytopaenic purpura
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
US$	United States dollar
VT	Verocytotoxin
VTEC	Verocytotoxin-producing *Escherichia coli*
w/v	Weight per volume
£	United Kingdom pound
¥	Japanese Yen

CHAPTER 1

INTRODUCTION

Virulent strains of *Escherichia coli* which cause diarrhoea are referred to as diarrhoeagenic *E. coli* (DEC). They were first isolated by Escherich in 1885 from stools of infants with enteritis (Bell, 2002). Castellani and Chalmers later described the genus *Escherichia* as part of the tribe *Escherichiae*, belonging to the family *Enterobacteriaceae* (Wilshaw et al., 2000). *Escherichia coli* are predominant normal inhabitants of the intestinal tracts of humans and other mammals (Doyle et al., 1997; Wilshaw et al., 2000). Cattle are the most important reservoir for DEC and carcass contamination mainly occurs during the hide removal process and evisceration (Bonardi et al., 2001). Foods of bovine origin, especially ground beef products, have been identified as major vehicles of transmission in most outbreaks (Doyle et al., 1997).

Most *E. coli* strains are harmless commensals but others are pathogenic. Differentiation of the pathogenic strains from the commensal ones was accomplished on the basis of virulence properties, mechanisms of pathogenicity, clinical syndromes and serotyping of distinct "O" (somatic), "H" (flagella) and "K" (capsule) antigens (Doyle et al., 1997, Wilshaw et al., 2000). The pathogenic strains may further be classified into virotypes which include enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC)*,* enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffuse adhering *E. coli* (DAEC) and verocytotoxin producing *E. coli* (VTEC) also referred to as Shiga toxin-producing *E. coli* (STEC; Bell, 2002; Enteropathogenic Resource Integration Centre, 2004).

Enteropathogenic *E. coli* cause a watery diarrhoea accompanied by vomiting and fever in children under the age of three (Wilshaw et al., 2000). Enteroinvasive *E. coli* cause *Shigella*-like dysentery (bacillary diarrhoea; Harris, 2001) which is acute and watery at first accompanied by fever and abdominal cramps. The diarrhoea can worsen leading to bloody and mucoid stools (Wilshaw et al., 2000). Enterotoxigenic *E. coli* cause "traveller's diarrhoea" characterised by a watery stool, abdominal cramps, fever, malaise and vomiting

(Harris, 2001). Enteroaggregative *E. coli* cause persistent watery diarrhoea accompanied by vomiting, dehydration and abdominal pain in children (Wilshaw et al., 2000; Harris, 2001). Diffuse adhering *E. coli* cause childhood diarrhoea and they have been associated with diarrhoea in children in Mexico (Doyle et al., 1997). Verocytotoxin producing *E. coli* were first described in 1977 by Konowalchuk and his co-workers (Wilshaw et al., 2000). They were recognised as significant causative agents of haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP). Verocytotoxin producing *E. coli* illness can be fatal especially to children, the elderly, the pregnant and the immunocompromised (Acheson, 2000; Wilshaw et al., 2000; Bell, 2002; Pruett et al., 2002).

Escherichia coli O157:H7 is the predominant VTEC and it was first associated with severe gastroenteritis in 1982 when it caused two major outbreaks of HUS from the same restaurant chain in Oregon and Michigan in the United States of America (Riley et al., 1983; Harris, 2001). Numerous worldwide outbreaks occurred in subsequent years (Harris, 2001; Dontorou et al., 2003; Cagney et al., 2004). Fatal cases were recorded in some of the outbreaks. A multi-state outbreak in the United States of America in 1993 affected 731 people with 178 hospitalisations, 56 HUS cases and 4 deaths (Doyle et al., 1997). In Sakai City, Japan, 5 727 people were infected in 1996. There were more than 100 HUS cases and three deaths (Wilshaw et al., 2000). In Africa, *E. coli* O157:H7, was first isolated from a man in Johannesburg in 1990 (Browning et al., 1990). Since then, some incidences have been reported across the continent. Germani et al. (1997) reported two severe outbreaks in 1996 in Central African Republic involving 290 cases and two fatalities. Zebu cattle meat was suspected as the vehicle of transmission. In Cameroon, 11 patients were infected in 1998 (Germani et al., 1998). Paquet et al. (1993) reported more than 20 000 cases in Mozambican refugee camps in Central and Southern regions of Malawi. Isaacson et al. (1993) reported an outbreak in South Africa and Swaziland in which thousands were infected and some deaths recorded.

This led to more research on the growth and survival of the predominant VTEC O157 in ground beef products to elucidate its behaviour in those foods under specified conditions (Tamplin, 2002). It has been demonstrated that *E. coli* O157:H7 is cryotolerant (Doyle and Schoeni, 1984) and that temperature abuse at 8 ℃ can result in growth of the pathogen leading to greater risk of infection (Palumbo et al., 1997).

There is a lack of information on the incidence of DEC in minced beef and boerewors in South Africa, and on the growth, survival and thermal inactivation of *E. coli* O157:H7 in boerewors. The aims of this study will, therefore, be to:

- Determine the incidence of DEC in minced beef and boerewors in the Bloemfontein District of South Africa;
- Investigate the growth and survival of *E. coli* O157:H7 at low and high inoculum levels in boerewors with and without sulphur dioxide preservative followed by storage at 0°C, 4°C and 10°C.
- Determine the thermal inactivation end point of *E. coli* O157:H7 in boerewors.

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CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Millions of people become ill from microbiological food-related diseases each year, though many cases are not reported (Wallace et al., 2000). It is difficult to obtain accurate estimates of the incidence of microbiological food-related diseases. Based on population studies it has been estimated that 76 million cases of food-related diseases may occur each year in the United States of America (USA), resulting in 325 000 hospitalisations and 5 000 deaths (Mead et al., 1999; Ranson et al., 2002). Extrapolating the USA data to the rest of the world would mean that up to one third of the population in developed countries are affected by microbiological food-related disease each year, while the problem is likely to be even more widespread in developing countries (Kaferstein and Abdussalam, 1999).

The global increases in foodborne diseases pose major challenges to health delivery and consequently economic wellbeing of all nations, particularly the developing countries. Global figures show that an estimated 1.5 billion episodes of diarrhoeal diseases are recorded every year in children below five years of age and 70% of these are linked to foodborne pathogens of which diarrhoeagenic *Escherichia coli* are significant causative agents. This figure only represents about 10% of the actual cases, as shown by recurring outbreaks and underreporting estimates, since many cases of foodborne disease resolve spontaneously and they will never reach the health system, and since only a fraction of the cases reaching the health system, will actually go through to laboratory confirmation (Plaut, 2000; Parirenyatwa, 2004). These diseases impose a substantial burden on healthcare systems and markedly reduce economic productivity resulting in loss of income. An estimate of medical costs and productivity losses in the USA due to foodborne disease is in the range of US$6.6 billion – US$37.1 billion per annum per 250 million population (Butzby and Roberts, 1997).

Garbutt (1997) reported an estimated cost of US$4 800 million per annum per 23 million population in the USA and £1 billion per annum for the UK population. In Japan, foodborne diseases caused by *E. coli* O157:H7 from elementary school lunches affected 268 persons in 1996 at Iwate prefecture. The cost of the outbreak was estimated at ¥82 686 000.00 (Abe et al., 2002).

There is currently a lack of information on the occurrence and economic impact of foodborne diseases in South Africa. Although there are no statistics available, it cannot be inferred that potential problems do not exist. The South African Department of National Health and Population Development requires notification only when 5 or more cases of foodborne disease are simultaneously reported at a physician or medical institution (Vorster, 1992). The causative agents are usually not identified. This could be due to late or incomplete laboratory investigations. In some instances, the responsible pathogen may have escaped detection even after thorough laboratory investigations either because the available laboratory techniques may be unable to detect the pathogen, or the pathogen may not be recognised as a cause of foodborne disease. Moreover, it is rare for suspected food vehicles to be available for microbiological examination. Hence many associations between diseases and their vehicles of transmission have been based on epidemiological data. Thus, the food vehicle of transmission and causative agents are not known in most cases (Schlundt, 2002).

In most countries there is an urgent need for a holistic approach to food safety issues incorporating better data collection covering food, animal human and environmental aspects throughout the food continuum from farm to patient. Food safety is an important part of public health linking health to agriculture and other food production sectors. Developments in food production and new control philosophies have positively contributed to food safety systems in most developed countries. Nevertheless, a number of food-borne microbiological diseases still remain dominant with some pathogens causing increased incidences over the last decades (Schlundt, 2002).

Diarrhoea is the commonest symptom of food-related illness, but other serious consequences include kidney and liver failure, brain and neural disorders and even death. Reactive arthritis and paralysis are some of the long-term complications of food-related disease. Diarrhoea

caused by *Escherichia coli* (*E. coli*) is probably the most important cause of children's diarrhoea in developing countries (Schlundt, 2002). Apart from diarrhoeal disease, *E. coli* commonly cause non-food related diseases such as urinary tract infections and a range of extra-intestinal diseases (Sussman, 1997).

2.2 Food-related disease

The term *food-related disease* is used to denote any disease that arises because consumed food was contaminated by micro-organisms or their metabolites. These pathogens have the potential for global spread and are sustained by complex epidemiological factors, including the shift to highly intensive livestock production systems in combination with high-volume and high speed processing facilities (Tauxe, 1997; Ranson et al., 2002). Harrigan and Park (1991) recognise three classes of food-related diseases, namely foodborne disease, infection-type food poisoning.

2.2.1 Foodborne disease

The term *foodborne disease* is used for any disease that arises from the contamination of food by disease-producing agents that cannot multiply, or at any rate have not multiplied, on or in the incriminated food (Garbutt, 1997). Many diseases have the potential to be transferred through food from one human being or animal to another human being without the organism having grown on the food to increase its number. A clear distinction is difficult to get between this means of spread giving rise to a "foodborne disease" and the means of spread which requires growth on the food to occur giving rise to an "infection-type food poisoning." This is due to factors such as the nature of food, the amount of the initial contamination of the food and the sensitivity of the individual eating the food that may all affect the outcome (Harrigan and Park, 1991).

Organisms that are thought that they never or only rarely grow on foods include viruses (such as the genus *Enterovirus,* eg. *Poliovirus*), the rickettsiae, prions, protozoa and parasites (Harrigan and Park, 1991). Micro-organisms such as *Salmonella* spp., *Shigella* spp., *Vibrio*

cholerae and *E. coli* are capable of growing on foods under normal conditions. However, if the food is initially heavily contaminated, these organisms cause disease so that further growth is not required, or the food would be of a type that protects the pathogens from the acid barrier of the stomach so that a smaller dose than usual is infective (Harrigan and Park, 1991). Sometimes the pathogens such as verotoxin-producing *E. coli* O157:H7 and *Shigella* spp. naturally have a low infective dose (Garbutt, 1997; Byrne et al., 2002; Samelis et al., 2002).

2.2.2 Infection-type food poisoning

The term *food poisoning* is defined as any disease that results because microorganisms have grown on the incriminated food before it is ingested (Harrigan and Park, 1991). Infection type-food poisoning is caused by the ingestion of live organisms that have grown on the food to produce a sufficiently large population to constitute an infective dose (Harrigan and Park, 1991; Garbutt, 1997).

Multiplication of microorganisms in food to give an infective dose is linked to outbreaks of disease caused by *Salmonella* spp., *Listeria monocytogenes, Shigella* spp., *E. coli, Yersinia enterocolitica, Vibrio parahaemoliticus, Vibrio cholerae, Aeromonas* spp. and *Clostridium perfringens* (Garbutt, 1997).

2.2.3 Intoxication-type food poisoning

Intoxication-type food poisoning is caused by the growth of microorganisms in a food producing a metabolite that is toxic to the consumer. Examples of intoxication-type food poisoning include intoxications by *Staphylococcus aureus, Clostridium botulinum* and *Bacillus cereus.* Other diseases included in this class are scombrotoxicosis, diflagenolate poisoning, food-associated mycotoxicoses such as ergotism, and "yellow rice disease" (Harrigan and Park, 1991, Garbutt, 1997; South African Department of Health, 2000).

2.3 *Escherichia coli*

The organism *Bacterium coli commune* was discovered in 1885 by Dr. Theodor Escherich during his work on bacteria in stools of infants with enteritis (Bell, 2002). It has been recognised as an important cause of food-related disease virtually since its discovery and it is now known as *Escherichia coli* (*E. coli*). *Escherichia coli* strains belong to the coliform group of microorganisms which are a common part of the normal facultative anaerobic microflora of the intestinal tracts of most mammals including humans. These flagellated gut flora are mainly found in the colon (Wilshaw et al., 2000)*.* Coliforms include all the aerobic and facultatively anaerobic, Gram negative, non-spore forming, rod shaped bacteria which ferment lactose with gas formation within 48 hours at 35°C (American Public Health Association, 1971). *Escherichia coli* belong to the genus *Escherichia* which in turn is part of the tribe *Escherichiae* belonging to the family *Enterobacteriaceae.* The genus *Escherichia* contains four other species besides *E. coli.* They are *E. hermanii, E. fergusonii, E. vulneris* and *E. blattae. Escherichia blattae* were isolated from cockroaches while *E. hermanii, E. fergusonii* and *E. vulneris* were all isolated from both intestinal and extra-intestinal human sources (Wilshaw et al., 2000). The vast majority of serotypes of *E. coli* are non-pathogenic to humans and other warm-blooded animals. There are, however, some serotypes that, if present in the body, can cause health problems. It is therefore of clinical importance to be able to differentiate between various serotypes of *E. coli.* Bacterial serotypes are defined by antibodies in the serum of the patients or animals that identify the specific type of antigen presented by the bacteria. There are three major surface antigens that enable serotyping of *E. coli*. "Types" of antigens are designated by letters. Numbers refer to known "subtypes"of antigens which can be differentiated by use of specific antibodies and thus used to identify bacterial serotypes. The "O" antigens are somatic cell wall phospholipid-polysaccharide complexes. The "H" antigens are components of the flagella (Doyle et al., 1997; Wilshaw et al., 2000). They are heat labile protein antigens found in flagellin, the protein that constitutes the flagella of motile *E. coli* (Wilshaw et al., 2000). The "K" antigens are the surface or capsular antigens that are acidic polysaccharides (Doyle et al., 1997). They were originally further divided into three classes: A, B and L. Only the A type K antigens are now considered to be important for typing antigens. They are mainly associated with pathogenic strains of *E. coli* that cause extra-intestinal infections and not those associated with diarrhoeal

disease (Wilshaw et al., 2000). Currently, it is only considered necessary to determine the O and H antigens to serotype strains of *E. coli* associated with diarrhoeal disease.

Pathogenic *E. coli* which cause diarrhoea are known as diarrheagenic *E. coli* (DEC). The pathogenic strains of *E. coli* are collectively known as enteropathogenic or enterovirulent *E. coli* (EEC) (Kornacki and Marth, 1982). They are categorised into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O:H serogroups (O'Brien and Holmes, 1987; Doyle et al., 1997). These groups include (Bell, 2002):

- Enteropathogenic *E. coli* (EPEC)
- Enteroinvasive *E. coli* (EIEC)
- Enterotoxigenic *E. coli* (ETEC)
- Enteroaggregative *E. coli* (EAggEC)
- Diffuse-adhering *E. coli* (DAEC)
- Verotoxin-producing *E. coli* (VTEC) or Shiga-toxin producing *E. coli* (STEC) (Includes Enterohaemorrhagic *E. coli* (EHEC).

The disease characteristics, mechanisms of pathogenesis, and epidemiology of these groups will now be discussed in more detail with special emphasis on VTEC/STEC, since this study focused on these organisms.

2.3.1 Enteropathogenic *Escherichia coli* (EPEC)

Enteropathogenic *E. coli* cause a watery diarrhoea accompanied by vomiting and fever. Though this diarrhoea is often self-limiting, it can cause protracted chronic enteritis leading to wasting and failure to thrive. Enteropathogenic *E. coli* are not routinely screened for in adults with diarrhoea because they are usually associated with infants and young children under 3 years of age (Wilshaw et al., 2000). Enteropathogenic *E. coli* strains are mainly spread by the direct faecal-oral route (Mossel et al., 1995).

2.3.1.1 EPEC mechanisms of pathogenesis

Donnenberg et al. (1989) defined EPEC as "the diarrhoeagenic *E.coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related to either heat-labile enterotoxins, heat-stable enterotoxins or *Shigella*-like invasiveness. However they induce attaching and effacing (AE) lesions in cells to which they adhere and can invade epithelial cells." The adherence of EPEC to intestinal mucosa rather than toxin production, is the important mechanism in their pathogenisis (Wilshaw et al., 2000). The "classical" EPEC adhere to Hep-2 cells forming a localised pattern of attachment. The EPEC adherence factor (EAF) plasmid is necessary for the full expression of adherence to Hep-2 cells. Chromosomal virulence factors are also required in addition to EAF plasmid. Intimin, an outer membrane protein (OMP), encoded by the chromosomal *eaeA* gene enables intimate attachment to epithelial cells. The response of the affected epithelial cells includes protein phosphorylation, calcium influx and the rearrangement of cytoskeletal components under the adherent EPEC. Consequently there is reduced absorptive capacity of the brush border and a stimulation of intestinal secretion (Wilshaw et al., 2000).

2.3.1.2 EPEC epidemiology

Infantile enteritis outbreaks were observed with increasing frequency during the 1940's. The serotype scheme was first used for epidemiological studies for EPEC in the late 1940's (Wilshaw et al., 2000). Outbreaks were mainly during winter compared with "summer diarrhoea" outbreaks that had been the annual scourge of infants in Europe and the USA until the 1930's. From the 1930's through to the 1960's infantile enteritis had become widespread globally as indicated by studies in England, Scotland, Northern Ireland, Eire, Canada, Indonesia and the USA (Gross et al., 1985). *Escherichia coli* serotypes O55 and O111 were implicated as the causative agents for nursery school outbreaks in London and Aberdeen in the 1940's (Gross, 1990). Serotypes O114:H2, O119:H6, O127:H6, O128:H2 and O142:H6 were isolated from infected infants in North America and Europe during the 1950's and up to the early 1970's in the United Kingdom (UK). These outbreaks had a high attack rate

characterised by high mortality rate, which could exceed 50%. Babies less than 6 months of age, especially those that were bottle-fed, had the highest rates of infection. Transmission occurred by the faecal-oral route facilitated by contaminated formula feeds and feeding equipment. Since the 1970's, EPEC epidemiology in developed countries was significantly less. The rarity of infantile diarrhoea has been attributed to improvements in hospital hygiene, improved preparation and formulation of milk feeds and better nursery management to avoid cross-infection. However, sporadic cases still occur. In 1994, England and Wales are reported to have had 387 EPEC cases among children less than 3 years old (Wilshaw et al., 2000).

Enteropathogenic *E. coli* diarrhoea among adults in developed countries is difficult to evaluate since EPEC are normally screened for in stools from children up to 3 years of age. Some outbreaks involving adults have, however, been reported. In Finland *E. coli* O111 was incriminated as the diarrhoeagenic *E. coli* serotype that caused a large outbreak of diarrhoea in 650 children and adults (Viljanen et al., 1990).

Enteropathogenic *E. coli* strains remain a major cause of infantile diarrhoea in developing countries or where the standards of hygiene are poor. The incidence of EPEC diarrhoea is highest in warm seasons. Outbreaks are community or institutional based as well as sporadic infections (Gomes, 1989; Kain et al., 1991). The reservoir of EPEC strains is the human gastro-intestinal tract and there is no evidence for zoonotic infections (Wilshaw et al., 2000). Generally the infection period is from the onset of weaning to the age of three via the faecal-oral route by contamination of weaning foods by contaminated water supplies.

2.3.2 Enteroinvasive *Escherichia coli* (EIEC)

Enteroinvasive *E. coli* diarrhoea often clinically resembles bacillary diarrhoea, caused by *Shigella* (Levine and Elderman, 1984; Gross, 1990). Enteroinvasive *E. coli* resemble *Shigella* biochemically: anaerogenic, non-lactose fermenting and lysine decarboxylase negative. The diarrhoea is non-bloody, acute and watery at first, accompanied by fever and abdominal

cramps (Doyle et al., 1997). It may progress to the colonic phase where stool becomes bloody and mucoid.

2.3.2.1 EIEC mechanisms of pathogenesis

Enteroinvasive *E. coli* like *Shigella*, invade the colonic epithelial cells. There is intracellular growth, intracellular movement and cell to cell spread without entry into the extracellular medium (Wilshaw et al., 2000). This results in the host cell's death leading to non-bloody diarrhoea and dysentery similar to that caused by *Shigella* species. They produce neither heat-stable nor heat-labile enterotoxins (Flowers et al., 1992). The invasive capacity of EIEC, as for *Shigella* species, is associated with the presence of a large plasmid that encodes several outer membrane proteins involved in invasiveness (Doyle et al., 1997).

2.3.2.2 EIEC epidemiology

Strains of EIEC were first identified in allied troops suffering from dysentery in the Mediterranean Sea during the Second World War (Ewing and Gravatti, according to Wilshaw et al., 2000). Worldwide outbreaks have been reported among school children in UK hospitals and institutions in the USA, Australia and Czechoslovakia. Imported French cheese contaminated with *E. coli* was linked directly with a large outbreak of infection in the USA (Wilshaw et al., 2000). Humans are a major reservoir of EIEC and the serotypes most frequently associated with illness include O28ac, O29, O112, O124, O136, O143, O144, O152, O164 and O167. Among these serogroups, O124 is commonly encountered (Doyle et al., 1997). Sporadic cases of EIEC infection appear to be rare in the developed world, although incidence may be underestimated because of the difficulty most clinical laboratories would have in identifying the organisms. Like EPEC and ETEC strains, EIEC are endemic in many developing countries. Enteroinvasive *E. coli* are similar to *Shigella* and there is no evidence that they are zoonotic. The human gut appears to be their reservoir and children between 3 and 5 years of age are most at risk. Enteroinvasive *E. coli* also cause travellers' diarrhoea (Wanger et al., 1988).

2.3.3 Enterotoxigenic *Escherichia coli* (ETEC)

ETEC diarrhoea is watery, accompanied by abdominal cramps, fever, malaise and vomiting. Some ETEC strains produce a severe form of infection characterized by "rice water stools" resembling the diarrhoea caused by *Vibrio cholerae* (Wilshaw et al., 2000). ETEC are a major cause of infant diarrhoea in developing countries and they are a significant cause of death (Rea and Fle mming, 1994). They were also implicated as the causative agents for traveller's diarrhoea or gastro-enteritis (Hitchins et al., 1992; Doyle et al., 1997).

2.3.3.1 ETEC mechanisms of pathogenesis

Enteropathogenic *E. coli* colonise the proximal small intestines by fimbrial colonisation factors and produce either heat-labile (LT) or heat-stable (ST) enterotoxins or both. The enterotoxins elicit fluid accumulation and diarrhoeal response (Flowers et al., 1992). There are two types of LT enterotoxin; LT? and LT??. The heat-labile toxin, LT?, is closely related in its structure, function and antigenicity to cholera toxin (CT) produced by strains of *Vibrio cholerae*. An increase in the level of cyclic adenosine 5-monophosphate (cAMP) results in electrolyte disturbances with increased secretion of chlorine from crypt cells and impaired absorption of sodium chloride by the cells at the tips of the villi. Water follows the electrolytes resulting in profuse watery diarrhoea. There are two major types of ST toxins; ST? (STa) and ST?? (STb). The heat-stable toxin, ST?, acts on guanylate cyclase leading to excess of cGMP (not cAMP) with resultant fluid secretion in the colon. The ST ?? is found only in porcine strains and does not alter intracellular levels of either cAMP or cGMP (Wilshaw et al., 2000).

2.3.3.2 ETEC epidemiology

Like EPEC infections, ETEC infections are not an important cause of diarrhoea in the developed world in both children and adults (Gross, 1990). However, some infantile diarrhoea outbreaks occurred in England, Scotland and the USA, and incriminated serogroups O6, O78 and O159 as the causative agents. The sources and routes of

transmission were not elucidated, though cross-infection was very important. There were outbreaks associated with contaminated well water in Japan. Food outbreaks associated with contaminated turkey, imported French cheese and salad vegetables occurred in England, Japan and USA. Infected food handlers were also implicated as vehicles for transmission for some outbreaks (Wilshaw et al., 2000; Hillers et al., 2003).

Gross et al. (1985) reported that like EPEC, ETEC strains are a significant cause of infantile diarrhoea in regions of poor hygiene especially in the tropics. They are significant causes of death (Rea and Flemming, 1994). Children up to two years are particularly infected and the diarrhoea declines in older children and adults due to progressive development of immunity. The infective dose of ETEC may be significantly lowered by the development of clinical malnutrition brought about by diarrhoea of other aetiology (Wilshaw et al., 2000). Contaminated weaning foods and latrine contaminated unprotected water supplies are some of the vehicles of transmission. Unlike EPEC, ETEC are zoonotic since pathogenic strains shed from healthy livestock, including pigs and cattle, can contaminate the environment. Asymptomatic human carriers form the principal reservoir of ETEC strains (Rea and Flemming, 1994). Enterotoxigenic *E. coli* have been shown to cause "travellers' diarrhoea" or gastroenteritis especially among travellers from the temperate regions with good sanitation and hygiene visiting tropical countries (Echeverria et al., 1981; Hitchins et al., 1992; Doyle et al., 1997).

2.3.4 Enteroaggregative *Escherichia coli* (EAggEC)

Enteroaggregative *Escherichia coli* infections usually result in persistent watery diarrhoea with vomiting, dehydration and abdominal pain in some patients. This lasts for more than 14 days. Bloody diarrhoea and fever have been described in children infected with EAggEC.

2.3.4.1 EAggEC mechanisms of pathogenesis

Enteroaggregative *E. coli* possess fimbriae (fibrils) on their surfaces that are responsible for their ability to produce a characteristic pattern of aggregative adherence on HE-p2 cells.

They appear like bricks stacked on the surface of the HE-p2 cells (Doyle et al., 1997). Mechanisms of pathogenicity have not been clearly elucidated (Wilshaw et al., 2000).

2.3.4.2 EAggEC epidemiology

EAggEC are one of the newer *E. coli* groups (Wilshaw et al., 2000) and more epidemiological information is needed to elucidate its significance as an agent of diarrhoeal disease (Doyle et al., 1997). Persistent diarrhoea has been reported mainly in developing countries (Savarino, 1993), however, Wanke et al. (1991) reported a worldwide incidence of EaggEC persistent diarrhoea in infants and children. Serotypes O44:H18, O111ab:H25 and O126:H27 were some of the EAggEC strains that have been linked to sporadic cases of diarrhoea in Britain. Strains of serotype O44:H18 resulted in outbreaks of infection among young children and the elderly in Britain and in 1994 food-borne EAggEC were linked to several outbreaks of gastroenteritis (Wilshaw et al., 2000).

2.3.5 Diffuse -adhering *Escherichia coli* (DAEC)

DAEC diarrhoea is characterized by mucus containing watery stools with some fever and vomiting (Wilshaw et al., 2000).

2.3.5.1 DAEC mechanisms of pathogenisis

The adhesion among DAEC is heterogenous, and genes encoding diffuse adherence may be plasmid or chromosomally encoded. They are identified by a characteristic diffuse pattern of adherence to HE-p2 or HeLa cell lines (Doyle et al., 1997). Mechanisms of pathogenicity have not been clearly elucidated (Wilshaw et al., 2000).

2.3.5.2 DAEC epidemiology

Diffuse-adhering *E. coli*, like EAggEC, are one of the newer *E. coli* groups. They have been associated with diarrhoea in children in Mexico (Doyle et al., 1997). Children between 4 and 5 years of age have a greater risk of infection with DAEC than infants (Wilshaw et al., 2000)

2.3.6 Verocytotoxin-producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC)

Verocytotoxin-producing *E. coli* are described as significant causative agents for haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) (Karmali et al., 1983; Wachsmuth, 1994). Haemorrhagic colitis is characterised by abdominal pain and watery diarrhoea, followed by bloody or non-bloody diarrhoea usually without fever. This may then lead to the development of HUS characterised by microangiopathic haemolytic anaemia and thrombocytopaenia, followed by acute renal failure. Haemolytic uremic syndrome is mostly common among young children although it occurs in all age groups. The spectrum of disease caused by VTEC also includes thrombotic thrombocytopenic purpura (TTP). Fever and a low platelet count associated with thrombi formation that gives rise to severe neurological disorders constitute the clinical features of HUS (Wilshaw et al., 2000; Garbutt, 1997).

2.3.6.1 VTEC nomenclature

Strains of *E. coli* isolated from cattle that produce potent cytotoxins were first reported in 1977 (Bell, 2002). They were described as verotoxin-producing *E. coli* (VTEC), because the cytotoxins are active on the African green monkey kidney (vero) cells in tissue culture. It was then discovered that the toxins are similar to shiga toxins produced by *Shigella dysenteriae* Type 1 and so the toxins were also known as shiga-like toxins (SLT) while the organisms also became known as "Shiga-like toxin producing" *E. coli* (SLTEC) or "Shiga-toxin producing" *E. coli* (STEC; Johnson et al., 1983; Karmali, 1989). The VT-producing *E. coli* (or SLT-producing *E. coli*) have been renamed after the prototype of the family Stx (shiga

toxins; Calderwood et al., 1996). Verocytotoxin-producing *E. coli* contain subgroups of *E. coli* with many properties and virulence factors. One subgroup of VTEC is enterohaemorrhagic *E. coli* (EHEC) with *E. coli* O157:H7 as the predominant serotype (Doyle et al., 1997; Restaino et al., 2001; Bell, 2002).

Enterohaemorrhagic *E. coli* are one of the greatest microbiological challenges to hit the food industry since the scourge of botulism about 80 years ago. This is because they (Bell, 2002):

- Are highly infectious
- Have a very low infectious dose (100 to 200 or even <10 cells in susceptible consumers) (Byrne et al., 2002; Samelis et al., 2002)
- Cause serious acute illness
- Cause serious long term sequelae, especially kidney failure
- Are naturally occurring in cattle (and other animals) and hence are also in the soil
- Are not clinically apparent in infected cattle and other animals
- Occur globally.

2.3.6.2 *E. coli* O157:H7

This is the most predominant VTEC serotype and has a 3 to 4 days incubation period. It causes the following symptoms:

- Inflammation of the colon giving rise to diarrhoea and abdominal pain with blood appearing in stool (haemorrhagic colitis, HC).
- Renal failure due to blood clots in the kidney tubules and damage may be permanent (haemolytic uremic syndrome, HUS). Haemolytic uremic syndrome is particularly serious in children in which severe and permanent kidney damage can lead to death or the requirement of a kidney transplant.
- Internal bleeding due to lack of blood platelets resulting in brain damage can occur in serious cases (thrombotic thrombocytopenic purpura, TTP).

Recovery from the disease usually takes about 2 to 9 days. *Escherichia coli* O157:H7 has a significant mortality rate among children, the elderly, the pregnant and the immunocompromised. Its infective dose is thought to be as low as 10 to 100 organisms. The

pathogen appears to compete well with natural microflora in foods, particularly at temperatures approaching the optimum (Garbutt, 1997).

Most strains of *E. coli* 0157:H7 possess several characteristics that are uncommon to most other *E. coli* such as:

- Inability to grow well, if at all, at temperatures $\geq 44.5^{\circ}C$
- Inability to ferment sorbitol within 24 hours
- Inability to produce β-glucuronidase (implying that it cannot hydrolyse 4-methyl-umbelliferyl-D-glucuronide, MUG)
- Possession of an attaching and effacing (*eae*) gene
- Carriage of a 60-Mda plasmid
- Expression of an uncommon 5 000 - 8 000 molecular weight outer membrane protein (OMP) (Padhye and Doyle, 1991)
- Unique tolerance of acidic environments (Feng, 1995; Berry and Foegeding, 1997; Bollman et al., 2001; Iu et al., 2001)
- **Thermal sensitivity/inactivation.** Thermal sensitivity studies have shown that *E. coli* 0157:H7 has no unusual resistance to heat (Doyle and Schoeni, 1984). Pasteurisation of milk ($72^{\circ}C$ for 16.2 seconds) and proper heating of foods of animal origin to an internal temperature of $68.3^{\circ}C$ is effective against *E. coli* O157:H7 and is an important critical control point to ensure inactivation of the pathogen (Doyle et al., 1997).
- **Antibiotic resistance.** Kim et al. (1994) reported that there is a trend towards increased *E. coli* 0157:H7 resistance to antibiotics such as streptomycin and tetracycline.

2.3.6.3 VTEC mechanisms of pathogenesis

All VTEC produce factors that are cytotoxic to the African Green Monkey kidney (vero) cells called verotoxins (VTs) or shiga-like toxins (SLTs) or shiga toxins (Stx) because the SLT-1 toxin of *E. coli* closely resembles the shiga toxin of *Shigella dysenteriae* Type 1

(Johnson et al., 1983; Karmali, 1989). There are 2 major types of VT (Stx): VT1 (Stx1) and VT2 (Stx2). Strains of *E. coli* O157:H7 produce a variant toxin known as VT2c, together with VT2. Verotoxins are LT proteins that, like shiga toxins, cause an irreversible inhibition of protein synthesis in eukaryotic cells (Wilshaw et al., 2000). Verotoxins comprise of one A subunit and five B subunits. They bind specifically to the glycolipid globotriaosyl ceramide (GB3). Verotoxins elicit fluid accumulation in ileal loops. Apart from VT production, some VTEC, like classical EPEC, have the ability to attach and efface the microvilli of the intestines. Strains belonging to serotypes O157, O5, O26, O111 possess this property. Another putative virulence factor of VTEC is enterohaemolysin (E-Hly) production. E-Hly is produced virtually by all O157 VTEC and strains of several other serogroups (Levine, 1987).

2.3.6.4 VTEC epidemiology

Unlike EPEC, ETEC and EIEC infections, diarrhoea caused by VTEC has been recognised as an emerging problem especially in North America, Europe and Japan. Karmali et al. (1983) were the first to establish the association between VTEC and HUS. *Escherichia coli* O157:H7 is the most predominant serotype and it was first identified as a human pathogen in 1982 (Hengge-Aronis, 1996; Doyle et al., 1997; Johnsen et al., 2001; Schlundt, 2002). There have been few investigations of the incidence of non-O157 VTEC mainly because of lack of simple tests for the primary isolation of these organisms. While there is great emphasis placed on the predominant VTEC O157:H7 especially in the USA, other countries around the world have had significant problems with non-O157:H7 VTEC. Various serotypes of non-O157:H7 VTEC were associated with HUS in Canada in 1985 (Karmali et al., 1983). Verocytotoxin-producing *E. coli* serotype O111:NM was responsible for a large outbreak of intestinal disease and 23 cases of HUS in Australia in 1995 (Paton et al., 1996). *Escherichia coli* O104:H21 was responsible for an outbreak in the USA in 1994 that involved 11 confirmed cases and 7 suspected cases most of which had bloody diarrhoea. Over 50 children were infected with VTEC O111 in the summer of 1999 at a camp in Texas (Acheson, 2000). Surveys of diarrhoeal stools in Canada, Germany and Belgium indicated that 0.7%, 6.6% and 1.0%, respectively, contained non-O157 VTEC (Wilshaw et al., 2000). Some 10% to 30% of HUS in Germany, Italy and the UK resulted from non-O157 VTEC infection. In Italy and France, HUS resulted from *E. coli* O111:H- (Caprioli et al., 1997). Non-O157 VTEC

infections suddenly increased in Italy and Germany in 1996 and serotypes O103 and O26 were incriminated as the causative agents (Bell, 2002).

Reilly (1998) reported a high incidence of *E. coli* O157:H7 infection and HUS for the geographical regions of Argentina, Scotland and Northern America. Brazil, Chile and Thailand had less frequent VTEC infections in humans than those of EPEC and ETEC (Rosa et al., 1998). Enterohaemorrhagic *E. coli* infections have in more recent years gained in notoriety because of the severity of the infections and several reports have noted that O157 VTEC are a major cause of haemorrhagic colitis (Karmali et al., 1983; Feng, 1995; Doyle et al., 1997). Wilshaw et al. (2000) reported that many studies in North America and Europe have shown that O157 VTEC are more commonly isolated from patients with HUS than are VTEC from other serogroups. The reported rates of isolation ranged from 19% to more than 60%. Approximately 10% of patients infected with O157 VTEC in England and Wales develop HUS.

The first reported community outbreak of infection with O157 VTEC occurred in the USA in 1982 in Oregon and Michigan (Riley et al., 1983). They were linked to the consumption of ground beef sandwiches from the same restaurant chain and O157 VTEC were isolated from the frozen patties. A very large outbreak occurred in Western USA in 1993 and involved consumption of hamburgers from multiple outlets of a restaurant chain. There were 732 cases of infection. Fifty-five patients developed HUS and four fatalities were recorded (Feng, 1995). Another community outbreak in Missouri, USA, occurred in 1989. It affected 243 people including two cases of HUS and four fatalities. It is likely that the source of the organism in this case was contaminated municipal water supply after pipe fractures (Wilshaw et al., 2000).

The first isolation of O157 VTEC from a beef burger in the UK was associated with a small community outbreak in Wales (Wilshaw et al., 1994). A larger outbreak linked to consumption of beef burgers from a restaurant chain were linked epidemiologically to O157 VTEC infection in 1991. There were 1 087 confirmed cases of O157 VTEC infection in England and Wales in 1997. This was the highest annual total for the pathogen on record and represented an overall incidence of 2.09 per 100 000 population (Institute of Food Research,

2003). In this geographical region, confirmed cases of O157 VTEC infection increased 20 fold between 1985 and 1998. Regional variations in incidence for 1997 showed highest incidences at Trent (3.25 per 100 000 population), South Western (3.09 per 100 000 population) and Yorkshire (3.01 per 100 000 population). Lowest incidences were recorded in the Thames region where South Western Thames had an incident of 0.92 per 100 000 population (Institute of Food Research, 2003). Scotland was the hardest hit recording highest rates between 1990 and 1994 in the Grampian and the Borders. The rates increased from between 4 and 10 per 100 000 (1990 to 1994) in the Borders to 21.7 per 100 000 (1996) without any large recorded outbreaks. The increases may be due, in part, to improved isolation techniques and better ascertainment; increased awareness following large outbreaks in Central Scotland in November 1996 and February 1997, which involved about 501 confirmed cases and 21 elderly people died; as well as a true increase in O157 VTEC infections (Wilshaw et al., 2000).

Other geographical regions like Argentina have a current annual incidence of HUS in children under 5 years of age averaging 7.8 per 100 000 population. Verocytotoxin-producing *E. coli* have been incriminated as the causative agents in about 70% of the cases (Wilshaw et al., 2000). The largest foodborne outbreak occurred in Japan in 1996. More than 9 000 cases were reported in several different prefectures between the end of May and September. More than 5 000 people were infected in Sakai City in July (Watanabe et al., 1996; Chang et al., 2000).

It is thought that only in the order of 10% of cases are actually reported in developed countries and the figure may be as little as 1% in developing countries (Warriss, 2000). Thus, relatively little is known about the African continent. Sporadic cases have been reported from Egypt, Cameroon, Malawi and Central African Republic (Kaddu-Mulindwa et al., 2001). Hamburgers and dairy products were implicated in an *E. coli* O157:H7 outbreak in Egypt in 1994. In a follow-up survey, the pathogen was detected in 6% of unpasteurised milk samples (World Health Organisation Press Release 58, 1997). Verocytotoxin-producing *E. coli* O157:H7 were isolated from faeces of patients and incriminated cattle in South Africa and Swaziland where several thousands of people were infected following consumption of surface water contaminated with *E.coli* O157:NM (Isaacson et al., 1993; Kaddu-Mulindwa et

al., 2001). Diarrhoeagenic *E. coli* serotypes that were most frequently isolated from faecal samples of patients with diarrhoea in the Bloemfontein area of South Africa in 1998 included O18, O26, O55, O111, O119, O126, O86, O114, O125, O127, O128, O44, O112, O124 and O142 (Kruger, according to Greyling, 1998).

2.4 Growth and survival of diarrhoeagenic *E. coli*

The general growth parameters for all *E. coli* include a minimum temperature of $7^{o}C$ to $8^{o}C$ and an optimum temperature of $35^{o}C$ to $40^{o}C$; a minimum pH of 4.4 and an optimum pH of 6 to 7; a minimum water activity of 0.95 with 0.995 as the optimum. There is no detailed information on the effect of environmental parameters on the growth and survival of diarrhoeagenic *E. coli* other than VTEC. Previous studies used various mixtures comprising ETEC, EPEC and EIEC. Publications on EaggEC and DAEC growth and survival are currently not available (Wilshaw et al., 2000).

Growth of a 10 strain mixture of EPEC, ETEC and EIEC in brain heart infusion broth was inhibited by sodium chloride at 8% (w/v) or higher irrespective of other factors. In 6% (w/v) sodium chloride or higher, growth occurred between pH 6.8 and 5.6 at temperatures in the range $15^{o}C$ to $35^{o}C$ but not at $10^{o}C$. When compared to a mixture of *Salmonella* strains, the *E. coli* mixture showed better survival. The individual behaviour of EPEC, ETEC and EIEC was, however, not determined (Wilshaw et al., 2000). A mixture of ETEC and EIEC strains grew by 1 to 3 $\log_{10}$ units in skim milk at $21^{o}C$ and $32^{o}C$ in the presence of various levels of lactic acid starter culture. After 6 to 9 hours growth of the *E. coli* strains was completely inhibited by lactic acid bacteria (Frank and Marth, 1977). Arnold and Kaspar (1995) reported a 26% survival rate after 1 hour for EPEC strain of serotype O127:H6 following inoculation of 10^{4} cfu/ml into an artificial gastric fluid at pH 1.5.

Most information on the growth and survival of VTEC relates specifically to *E. coli* O157:H7. Since *E. coli* O157:H7 first came to prominence in 1982, it has been associated with many foodborne outbreaks bringing this serotype to the forefront of food safety concerns and this justified research. Acidification is one of the methods commonly used in

the food industry to control the growth and survival of spoilage-causing and pathogenic microorganisms. However, microorganisms exposed to moderately acidic environment may develop cells with increased resistance and longer survival times when transferred to a more acidic environment (acid adaptation response). *Escherichia coli* O157:H7 can survive and be protected in acidic environments such as apple cider (pH of about 4.0; Uljas and Ingham, 1998). Cheng et al. (2003) reported that acid adaptation in tryptic soy broth increased acid tolerance of *E. coli* O157:H7 strains tested and was dependent on strain, acid adaptation time and pH of the challenge. Test organisms, regardless of strains, exhibited most pronounced acid adaptation response after adaptation for 4 hours at pH 3.0, followed by pH 4.0 and lastly pH 5.0.

Tamplin (2002) reported that both the exponential growth rate and maximum population density of single and multiple strains of *E. coli* O157:H7 increased with decreasing fat levels in ground beef. The addition of 4% sodium lactate in beef burger patty formulations can reduce the risks posed to consumers due to *E. coli* O157:H7 contamination by, firstly; reducing pathogen survival during freezing and frozen storage of the uncooked product; and, secondly, by increasing the susceptibility of the pathogen to heat during normal cooking processes (Byrne et al., 2002). In South African fresh sausage formulations, sulphur dioxide is usually used for preservation of colour and odour and as a microbial preservative. Until recently, the use of sulphur dioxide has been generally recognised as safe (GRAS) but investigations showed that some asthma patients were placed at high risk by relatively small amounts of sulphites (Ough, 1993).

2.5 Some reservoirs of diarrhoeagenic *E. coli*

Cattle are the principal reservoir for VTEC and the highest prevalence of the organisms is found in weaned calves and during the warm seasons. Prevalence estimates vary, but it appears that a substantial percentage of both dairy and beef feedlots have infected animals (Wells et al., 1991; Hancock et al., 1994; Zhao et al., 1995; Chapman et al., 1997; Johnsen et al., 2001). In Italy, values of faecal carriage of VTEC by cattle had a prevalence ranging from 0% to 13.1% depending on the nutritional status of cattle examined, the season and

sampling criteria (Bonardi et al., 2001). Johnsen et al. (2001) reported a herd prevalence of VTEC O157 of 0.35% and an animal prevalence of 0.19% in cattle herds from the South West part of Norway. In the USA, the prevalence in cattle is reported to range from below 2% to around 45% at animal level and from 4.5% to above 70% at herd level (Wells et al., 1991; Hancock et al., 1994; Zhao et al., 1995; Faith et al., 1996; Elder et al., 2000). *Escherichia coli* O157:H7 does not cause diarrhoea or apparent illness in animals (Doyle et al., 1997).

Poultry is not a primary source of *E. coli* O157:H7 but chicks can be readily colonised by small populations of *E. coli* O157:H7 and continue to be long time shedders making it possible that chickens and hens can serve as vehicles of *E. coli* O157:H7 (Schoeni and Doyle, 1994). Sheep have also been identified as transient carriers of *E. coli* O157:H7 particularly during summer months (Kudva et al., 1996; Chapman et al., 1997). Humans are also a reservoir for *E. coli* O157:H7. Several outbreaks have occurred in day-care settings in which the pathogen was spread by person-to-person contact (Spika et al., 1986). Secondary transmission by humans can further amplify a foodborne outbreak. Human faecal excretion of the pathogen can last for weeks but usually not more than 13-21 days following the onset of symptoms (Doyle et al., 1997).

2.6 Diarrhoeagenic *Escherichia coli* (DEC) vehicles for transmission

Outbreaks of diarrhoeagenic *E. coli* infections have been associated with a variety of foods including raw milk, raw vegetables, ground beef, pork, lamb, poultry and contaminated water supplies (Cheville et al., 1996; Hengge-Aronis, 1996; Berry and Foegeding, 1997). Highly acidic foods such as apple cider, mayonnaise, yoghurt, fruits and salad vegetables have been major sources of O157 VTEC food-borne outbreaks (Feng, 1995; Hengge-Aronis, 1996; Berry and Foegeding, 1997; Iu et al., 2001; Li et al., 2001). Verocytotoxin-producing *E. coli* in milk, especially unpasteurised milk and milk products such as cheese prepared from raw cow's milk are important vehicles of transmission that were implicated in outbreaks of both O157 VTEC and non-O157 VTEC infections in North America, England, Czech Republic

and Scotland (Wilshaw et al., 2000). Both drinking and recreational water can serve as vehicles for transmitting darrhoeagenic *E. coli* infections (Feng, 1995). From 1982 to 1997, more than 100 outbreaks of infection from *E. coli* O157:H7 were reported in the USA. In cases where the vehicle of infection was identified, 52% were from foods derived from cattle, 16% person to person spread, 14% fruit and vegetable produce, 12% water and 5% other foods (World Health Organisation, 1997). Since meat and meat products have been widely implicated as vehicles of transmission of DEC and this study focussed on the products of minced beef and boerewors, aspects of the microbial contamination of meat and processed meat products will now be discussed in more detail.

2.6.1 Meat

Meat is defined as those animal tissues which are suitable for use as food (Forrest et al., 1975). In practice the definition is restricted to a few dozen of the 3 000 mammalian species. Apart from the muscle tissues, it often includes organs such as liver, kidney and brains (Lawrie, 1968). All processed products which might be prepared from these tissues are included in this definition. Meat can be categorised as follows (Forrest et al., 1975):

1. Red meat
2. Poultry meat
3. Sea foods
4. Game meat

"Red" meat is the largest category in terms of volume of consumption. The most common types include beef, veal, mutton and lamb. Horse, goat, eland, llama, camel, water buffalo and rabbit are also commonly consumed in many countries. Poultry meat is the flesh of domestic birds and includes that of chickens, turkeys, ducks, geese and guinea fowl. Sea foods are the flesh of aquatic organisms such as fish, clams, lobsters, oysters and crabs. In terms of consumption, fish make the largest group of sea foods. Game meat is made up of flesh of all non-domesticated animals.

Meat is one of the most nutritious foods. It is an excellent source of high quality proteins. It also provides large amounts of minerals and vitamins of the B series, notably vitamin B_{12} (Forrest et al., 1975). Meat consumption is often directly related to economic status of a country or individual. As a nation industrialises and improves its economic position, its meat consumption increases; and as people rise in economic status, they tend to demand greater and higher quality of meat products (Forrest et al., 1975).

2.6.1.1 Microbial contamination of meat

The organisms which spoil meat may gain access through infection of the living animal (endogenous disease) or by contamination of the meat post mortem (exogenous disease) (Lawrie, 1968). Foodborne pathogens in meat products are generally associated with contamination by gut contents and faeces of livestock. During dressing operations, transfer of micro-organisms can occur from both the gut and the hide.

Normal microflora include *Staphylococcus, Micrococcus, Pseudomonas*, yeasts and moulds. This can also include any soil microbes, particularly if the animal is wet or muddy at the time of slaughter. If the intestines are pierced before evisceration in abattoirs, intestinal contents can contaminate muscle. Most contamination comes from the hide removal process (Sutherland and Varnam, 1982; Bonardi et al., 2001; James, 2002). The deboning process involves extensive handling and exposure to surfaces that are more susceptible to contamination (Nel et al., 2004).

Hog processing is one of the cleanest. The carcass is singed to remove hair, then scalded for 1 to 2 minutes in boiling water or steam spray. Only microbes deeper in the skin can be protected. The carcass is then chilled immediately. Contamination can then come from knives, workers hands, clothing and other aspects of the processing environment (Forrest et al., 1975). Poultry feathers increase contamination particularly with psychrotrophic *Acinetobacter* and *Moraxella*. Increased contamination occurs with overcrowding. Generally birds are not fed for several hours before slaughter to decrease intestinal contents. Flapping of wings during bleeding can increase contamination. Carcasses are routinely scalded (60°C

to 63°C). Psychrotrophs would be especially reduced in number by this process which is needed to remove feathers. Defeathering creates aerosols producing contamination to knives, equipment etc. Spray washing carcass after defeathering and evisceration decreases numbers (Forrest et al., 1975).

Red meat is a terrific growth medium for spoilage and pathogenic microbes: a high A_w of 0.99, 15% to 22% protein, 2.5 to 37% fat, 0% to 1.2% carbohydrate, adequate amounts of smaller molecular weight organic phosphates and other minerals (1.2% to 3.5% wet weight) and a pH 5.4 to 5.6 (down from pH 7 before slaughter). *Escherichia coli* and *S. aureus* are frequent contaminants of meat in South Africa. A study by Vorster et al. (1994) revealed that *E. coli* was found in 74.5% while *S. aureus* was found in 23.4% of the ground beef samples. *Moraxella* and *Acinetobacter* tolerate the low pH. Ground meats spoil fastest. They have the highest microbial titres. This may be due to interaction of several factors such as lower quality meat being used and also the meat may be butchered and left unrefrigerated for a long time before grinding. Grinding ruptures the cells releasing soluble nutrients for bacteria. Bacteria that were once restricted to the surface of the meat get mixed in with the inside mass. The outside remains aerobic and develops the aerobic bacteria: Pseudomonads, *Acinetobacter* and *Moraxella,* while the inside becomes rapidly anaerobic and develops anaerobes like lactobacilli (Sutherland and Varnam, 1982). Other enterics and *Aeromonas* can occur here more often than any other meat (Lawrie, 1968). Psychrotrophs also further contaminate meat in cutters and cutting rooms from working surfaces and the air. If the temperature is allowed to rise above chill, the growth rate of psychrotrophic spoilage flora will increase significantly and the numbers of these organisms rise above the level expected under the conditions of good process control (Garbutt, 1997).

2.6.2 Processed meat products

Processed meat products are generally classified into three groups on the basis of the size of particles of meat used. Products within a group are linked by a common technology and manufacturing procedure (Hugo, 2004).

The first group are the whole muscle products in which the muscle tissue is still clearly visible and defined in the finished product. Products belonging to this group include ham, shoulder ham and bacon or beef silverside (pressed beef). Ground meat products make up the second group. In this group the muscle structure has undergone some degree of comminution such as mincing, dicing or chopping. Therefore, the muscle structure is no longer recognisable in its fibrous form, but becomes particulate in nature. Products belonging to this group include salami, fresh sausages, frikadels (meat balls) and hamburger patties. Prepared dishes such as goulash meat, corned beef and bolognaise mince are also included in this group. Emulsified meat products make up the third group. In this group the muscle tissue has been chopped up to such an extent that it is no longer recognisable in its fibrous nature or the particle state. This group consists of products such as frankfurters, polonies, viennas, liver spread and pastes, and all the various loaf products such as pimento and olive loaves (Hugo, 2004).

2.6.2.1 Ground meat products

Ground meat products are classified as group 2 products. They cover a wide range varying from the dried fermented salami which takes several weeks to produce, to fresh sausage products and to the very popular champion of the modern day concept of "fast foods" namely the beef-burger. The typical South African sausage investigated in this thesis, the boerewors, is also classified under group 2 products. Group 2 products themselves differ considerably in properties such as flavour, texture and shelf life. One common property is that they are all manufactured from muscle meat which has been ground, minced or chopped to produce a particle-type texture as opposed to the fibrous-type texture of whole muscle products (group 1), and the finely ground and emulsified products (group 3) in which the particle state is no longer recognisable. The differences among the group 2 products are achieved through the production steps which follow the grinding/mincing process. The simplest form of the ground meat products is pure minced meat. It could comprise of one or more species of meat but no salt, spices or other ingredients are added. It is also not packaged. It is simply sold loose over the counter. In their more complex forms, ground meat products make use of bacterial fermentation, drying processes, humidity controls, spices, flavours, other ingredients and additives, pre-cooking, sophisticated forming and packaging processes.

Stringent quality assurance systems are employed to monitor these production processes (Hugo, 2004).

2.6.2.1.1 Boerewors

The manufacture of sausages by man began over two thousand years ago and they played an important part in man's diet since then. The modern word "sausage" is derived from the Latin word "salsus," which means "salted" as a means of preservation (Oscar Enterprises, 1998). From as early as 900 B.C., sausages have been regarded as convenience foods and each country developed its own characteristic sausage during the middle ages. These developments were determined by climate, discovery of new spices and preferences of inhabitants of certain areas. For example, Bologna sausage originated in the town of Bologna in Northern Italy, Lyons sausage from Lyons in France, Berliner sausage from Berlin in Germany and in England they became associated with county's such as Berkshire and Wiltshire (Steyn, according to Hugo, 1992; Oscar Enterprises, 1998).

Boerewors is a very traditional South African sausage inherited from the country's pioneering forefathers during the seventeenth and eighteenth centuries. The self-sufficient South African farming community used to combine minced beef and cubed pork fat with salt, pepper and various other spices especially coriander for preservation and flavour purposes. Originally, the meat was filled into clean cattle, hog or sheep intestine casings using a horn. Fresh boerewors were prepared by grilling and consumed as part of breakfast or supper. During the pioneers trek through the hinterland, large quantities of sausage, called "wors," would be made during their stopover and that which could not be eaten would be hung to dry and taken along for sustenance as they continued with their explorations. In the decades that followed, this particular type of "wors" gradually evolved and the term "boerewors" became entrenched in South African culture (Anon., 2004; Hugo, 1992). Boerewors is a very popular product in South Africa and 2 497 metric tonnes were consumed in 1999 (Hugo, 2004).

The manufacture of boerewors is currently governed by a statute (government regulation No. R2718, 1990) to guard against cheap imitations which were brought into the market from the 1960's onwards by experimenting entrepreneurs (Hugo, 1992).

2.7 CONCLUSIONS

The severity of illness caused by diarrhoeagenic *E. coli* infection bring these organisms to the forefront of food safety concerns and justifies research to determine food processing strategies that reduce the risk. The food industry and regulatory agencies require tools, including microbiological models that permit the prediction of diarrhoeagenic *E. coli,* especially *E. coli* O157:H7, behaviour under various food production steps, product storage and different food preparation scenarios in order to devise interventions to reduce the impact of this disease on public health.

Although a variety of foods have been implicated in diarrhoeagenic *E. coli*-associated illness, most outbreaks have been associated with the consumption of raw or undercooked foods of bovine origin. Given the prevailing high rates of infection that have been reported worldwide and the epidemiological data suggesting a link with bovine products, particularly the group 2 (ground meat) products and the unavailability of published data on the incidence, growth and survival of diarrhoeagenic *E. coli* in African meat products in general, and in South African ground meat products in particular, this project was aimed at the determination of the incidence of diarrhoeagenic *E. coli* in Bloemfontein District's ground (minced) beef, and boerewors (a typical South African fresh sausage), the serotyping of *E. coli* isolates recovered and the determination of the growth and survival of *E. coli* O157:H7 in boerewors during storage and heat treatment.

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CHAPTER 3

The incidence of diarrhoeagenic *Escherichia coli* in minced beef and boerewors

Abstract

A survey of diarrhoeagenic *E. coli* (DEC) was carried out on 21 minced beef and 21 boerewors (a traditional South African fresh sausage) samples purchased from 30% of butcheries in the Bloemfontein District of South Africa. The samples were cultivated on standard plate count agar for aerobic plate counts and chromocult coliform agar for coliform, *E. coli*, *Salmonella enteritidis*, and *Shigella sonnei* counts without enrichment. Diarrhoeagenic *E. coli* were isolated after enrichment in MacConkey broth followed by cultivation on chromocult coliform agar. The resultant *E. coli* colonies were selected and serotyped using the slide agglutination test for DEC. Enrichment enabled optimal recovery rates for *E. coli* and coliforms while *Salmonella enteritidis* and *Shigella sonnei* recovery rates decreased for both minced beef and boerewors. Eight (38.10%) minced beef samples were positive for DEC. The 8 positive samples consisted of 5 (23.81%) EPEC and 1 each (4.76%) of EIEC, EAggEC, and VTEC. A total of 100 isolates were serotyped (5 from each sample) and these comprised of 6 (6%) EPEC serotypes ($3 \times$ O18; $1 \times$ O125; and $1 \times$ O142), and 1 (1%) each of EIEC ($1 \times$ O128$_{ac}$), EAggEC ($1 \times$ O44) and VTEC ($1 \times$ O26). Boerewors had 6 (28.57%) samples that were positive for DEC. EPEC and VTEC were the virotypes that were isolated at 23.81% (5/21) and 4.76% (1/21) respectively. A total of 105 isolates were serotyped (5 from each sample) and these comprised of 7 (6.66%) EPEC ($3 \times$ O18, $2 \times$ O125, $1 \times$ O55 and $1 \times$ O142); and 1 (0.95%) VTEC ($1 \times$ O26). EPEC were the most frequent virotype while VTEC were the most virulent virotype recovered from both products. No *E. coli* O157:H7 were recovered. High levels of contaminants such as *Salmonella enteritidis* in both products could indicate poor hygiene practices especially at the abattoirs and/or butcheries.

Keywords: Diarrhoeagenic *E. coli*; Serotype; Minced beef; boerewors; South Africa

3.1 INTRODUCTION

Infection with strains of diarrhoeagenic *E. coli* can result in asymptomatic infection or a number of ailments such as mild diarrhoea and very severe diseases like haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP) (Doyle et al., 1997; Tarr et al., 1999; Park et al., 1999; Wilshaw et al., 2000). Diarrhoeagenic *E. coli* (DEC), strains of *E. coli* with the potential to cause enteric disease, were differentiated in the late 1940's from the avirulent strains by serotyping of the somatic "O" antigens (Wilshaw et al., 2000). This led to the division of *E. coli* species into potentially pathogenic and commensal strains.

At least 6 classes of DEC are recognised. These include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAggEC) and verocytotoxin-producing *E. coli* (VTEC). Enteropathogenic *E. coli* infections result in infantile diarrhoea (Wilshaw et al., 2000) while EIEC cause illness which resembles bacillary diarrhoea caused by *Shigellae* (Levine and Elderman, 1984; Gross, 1990). Diffuse adhering *E. coli* infections result in watery stools containing mucus (Doyle et al., 1997) while EAggEC infections induce a persistent watery diarrhoea accompanied by vomiting, dehydration and abdominal pain (Wilshaw et al., 2000). Verocytotoxin-producing *E. coli* are the most virulent virotype and infection with them leads to bloody and non-bloody diarrhoea, HC, HUS and TTP (Wachsmuth, 1994; Acheson, 2000). An important DEC, *E. coli* O157:H7, is ranked among the 13 major foodborne pathogens (Hillers et al., 2003) and the life-threatening complications, especially among the young, elderly, pregnant and immunocompromised, arising from its infection are a cause for concern.

According to Mead et al. (1999), more than 200 known pathogens, as well as some pathogens not yet identified, can be transmitted through food. Since their first association with disease in 1982 following two disease outbreaks in the USA, when *E. coli* O157:H7 caused haemorrhagic colitis (Acheson, 2000), VTEC have been implicated in high profile outbreaks

mostly linked to foods of bovine origin, especially the ground beef products (Riley et al., 1983; Bell et al., 1994; Dontorou et al., 2003).

Cattle are regarded as the main reservoir for *E. coli* O157:H7 (Elder et al., 2000). Apart from foods of bovine origin, other foods have also been implicated as vehicles for the transmission of *E. coli* O157:H7. These include apple and orange juices (Besser et al., 1993; Just and Daeschel, 2003), mayonnaise (Weagant et al., 1994) and deer (Keene et al., according to Dontorou et al., 2003).

A number of studies worldwide have reported on the incidence of *E. coli* O157:H7 in minced beef and ground beef products. Heuvelink et al. (1999) reported that 6 out of 571 samples analysed in the Netherlands (1.1%) were positive for *E. coli* O157:H7. In France, a study by Vernozy-Rozand et al. (2002) reported a low 0.12% (4/3450) *E. coli* O157:H7 prevalence in minced beef. A study by Tarr et al. (1999) in Seattle, USA, found no *E. coli* O157:H7 from 1 400 ground beef samples surveyed. In contrast, Samadpour et al. (2002) reported that 16.8% (50/296) of the samples analysed in Washington State, USA, were positive for *E. coli* O157:H7. Acheson (2000) reported that 5 (15.6%) of ground beef sampled from Boston, New York, Chicago and Cincinnati in the USA, were positive for VTEC of the following serotypes: O133:Hu, O22:NM, O82:H8, O8:H9 and O13:Hu.

Very little is known about the prevalence of DEC in foods in Africa but some outbreaks have been reported. Following an *E. coli* O157:H7 outbreak in which hamburgers and dairy products were implicated as vehicles of transmission in Egypt in 1994, a survey detected *E. coli* O157:H7 in 6% of unpasteurised milk samples (World Health Organisation Press Release 58, 1997). Cattle-contaminated surface drinking water was implicated as the vehicle of transmission in a huge outbreak of *E. coli* O157:H7 infection that occurred in South Africa and Swaziland in 1993 affecting thousands of people (Isaacson et al., 1993). Serotypes O18, O26, O55, O111, O119, O126, O86, O114, O125, O127, O128, O44, O112, O124 and O142 were frequently isolated from diarrhoea patients in the Bloemfontein district of South Africa (Kruger, according to Greyling, 1998).

Given the importance of diarrhoea to the community and the link of ground beef products as vehicles of transmission of DEC, this study was carried out with the aim to determine the incidence of DEC in minced beef and boerewors (a traditional South African fresh sausage) in the Bloemfontein district of South Africa.

3.2 MATERIALS AND METHODS

3.2.1 Sampling procedure and preparation

A list of all butcheries in the Bloemfontein district was obtained and the butcheries were classified according to their demographic area (north, south, east, west and central). Using the random number generation analysis tool of Microsoft Excel™, 21 (30%) of the butcheries were randomly selected. Each butchery was sampled once, 300g of minced beef and 300g of boerewors being purchased from each of the selected butcheries and transported in a cooler box at 4 ^{o}C. Three butcheries were sampled once a week (Tuesdays) at 09:00 hrs. All samples were analysed immediately upon arrival at the laboratory. The samples were prepared according to Harrigan (1998). A stomacher (Lab Blender 400, Art Medical Equipment) was used to homogenise the samples.

3.2.2 Aerobic plate count (APC)

The homogenised samples were serially diluted (1:10) in 0.1% buffered peptone water (BPW) and spread plated (0.1 ml aliquots) in duplicate on standard plate count agar (SPCA; Oxoid CM463). The plates were incubated at 32 ^{o}C for 72 hours.

3.2.3 Presumptive coliform, *E. coli, Salmonella enteritidis* and *Shigella sonnei* counts

The samples were serially diluted (1:10) in 0.1% BPW and spread plated in duplicate (0.1 ml aliquots) on chromocult coliform agar (Merck 1.10426). The plates were incubated at 37 ℃ for 24 hours. Presumptive colonies were identified according to manufacturer's instructions and enumerated. Salmon to red colonies and dark blue to violet ones gave the total coliform count. Dark blue to violet colonies which were indole positive gave the *E. coli* count. Light blue to turquoise, indole negative and fluorescent (uv light at 366nm) colonies were enumerated as *Shigella sonnei*, and colourless, indole negative non-fluorescent colonies were counted as *Salmonella enteritidis*.

3.2.4 Isolation of *E. coli* for serotyping

The samples (25g) were weighed into sterile stomacher bags (Nasco Whirl-Pak™) and homogenised for 2 minutes in 225 ml of MacConkey broth (Difco 0020-01) using a stomacher (Lab Blender 400, Art Medical Equipment). The homogenised samples were then incubated at 37 ℃ for 18 hours for enrichment (Restaino et al., 2001; Flores et al., 2004; Koohmaraie, M., 2004, Personal Communication; Sharma et al., 2004). Serial dilutions (1:10) were made in 0.1% BPW followed by selective and differential spread plating in duplicate on chromocult coliform agar (Merck 1.10426). The plates were incubated at 37 ℃ for 24 hours.

3.2.4.1 Determination of serological groups

Typical *E. coli* colonies (5 from each plate) were selected using Harrison's disc method (Harrison, according to Harrigan, 1998). Each of the presumptive *E. coli* colonies were streaked on MacConkey agar (Difco 0020-01) and incubated for 18 hours at 37 ℃. The isolates were further streaked on nutrient agar (Oxoid CM3) and incubated at 37 ℃ for 18 hours. Resultant colonies were serotyped according to the slide agglutination test for

diarrhoeagenic *E. coli* (Mast Assure™; Mast Diagnostics). The antisera used are classified in Table 3.1 according to their virotypes. No antisera for the diffuse adhering *E. coli* (DAEC) were available.

Table 3.1

Classification of the antisera used according to their virotypes

EPEC	ETEC	EIEC	VTEC	EAggEC
O18	O20	O28$_{ac}$	O111	O44
O26	O128	O112$_{ac}$	O157	O86$_a$
O55		0124		O111
O111				
O114				
O119				
O125				
O126				
O127				
O142				

EPEC: Enteropathogenic *E. coli*

ETEC: Enterotoxigenic *E. coli*

EIEC: Enteroinvasive *E. coli*

VTEC: Verotoxic *E. coli*

EAggEC: Enteroaggregative *E. coli*

3.2.5 Water activity (A_w) determination

A_w was measured using 3.5g of sample using a Novasina Thermoconstanter TH 200 water activity meter.

3.2.6 pH determination

Homogenates were prepared by blending 10g of sample in 100ml of distilled water (Koniecko, 1985) using an Ultra Turrax T25, blender. The homogenates were filtered using a Whatman number 1 filter paper. The pH of the filtrate was measured with a Hanna Instruments, Model HI 8915 ATC, pH meter with a glass electrode (Metrohm 100). The pH meter was standardised using pH 7 and pH 4 buffer solutions (BDH Laboratory Supplies).

3.2.7 Statistical analysis

Differences in location of product were determined using an analysis of variance (ANOVA) using the NCSS (2004) statistical package. Pearson correlation analysis was carried out between A_w, pH, APC, coliforms, *E. coli*, *Salmonella enteritidis* and *Shigella sonnei* using the NCSS (2004) statistical package. The recovery rate for coliforms, *E. coli*, *Salmonella enteritidis* and *Shigella sonnei* was calculated as follows:

Recovery rate = <u>Number of positive samples</u> × 100

                        Total number of samples

3.3 RESULTS AND DISCUSSIONS

3.3.1 Minced beef organism recoveries without enrichment

The mean APC for the minced beef on SPCA are presented in Table 3.2. The mean APC for all the 21 minced beef samples analysed was 6.04 log cfu/g. This ranged from a minimum of 4.35 log cfu/g (sample M52) to a maximum of 7.37 log cfu/g (sample M1). The Pearson correlation analysis revealed that there was no significant correlation ($p<0.05$) between A_w and APC recoveries (Table 3.3). There was, however, a significant correlation ($p<0.05$) between APC and pH (Table 3.3). An analysis of variance revealed that there were no significant differences ($p<0.05$) between APC recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

The South African Foodstuffs, Cosmetics and Disinfectant Act (Act Number 54 of 1972) and regulations made under the act and amended to 3 May 2002 under "Regulations Governing Microbial Standards for Foodstuffs and Related Matters," regulation Number R692, 16 May 1997 as amended, does not specify any microbial quality limits for minced beef, boerewors, raw meat or processed beef/pork products. A self-regulatory Good Manufacturing Practices regime applies to the manufactures. The maximum APC recommended quality limit for raw minced meat at the "best before" date in Sweden is 8 log cfu/g (Shapton and Shapton, 1991). Thus according to the Swedish recommended quality limits, all the minced beef analysed satisfied the requirements for good quality. The mean APC of 6.04 log cfu/g was considerably higher and out of the range compared to earlier studies in the United States of America. A mean APC incidence of 3.90 log cfu/g was reported by the United States Department of Agriculture (USDA) after conducting a nationwide raw ground beef survey from 1993 to 1994 (USDA according to Pruett et al., 2002). A study by Pruett et al. (2002) reported very low levels of aerobic mesophiles relative to generally expected levels for ground beef products. They reported an APC range of <2.00 log cfu/g to 2.85 log cfu/g in frozen beef patties produced over an 8-hour shift.

Table 3. 2

Comparison of non-enriched minced beef and boerewors presumptive organism recoveries

Minced beef organism recoveries (Mean Log cfu/g)						Boerewors organism recoveries (Mean Log cfu/g)					
Sample code	APC	Coliforms	*E. coli*	*Salmonella enteritidis*	*Shigella sonnei*	Sample Code	APC	Coliforms	*E. coli*	*Salmonella enteritidis*	*Shigella sonnei*
M1	7.37	3.30	1.30	5.13	2.45	B1	5.94	2.30	0.00	4.46	3.20
M2	7.09	4.10	0.00	5.09	3.00	B2	5.88	3.08	0.00	4.01	2.30
M4	5.70	3.68	0.00	4.39	0.00	B4	5.83	2.53	1.78	2.38	1.30
M5	5.54	3.62	2.08	2.64	0.00	B5	4.89	2.30	0.00	2.80	0.00
M14	6.18	3.86	0.00	3.91	2.78	B14	5.93	3.62	3.15	2.82	2.78
M16	6.14	4.01	0.00	3.68	0.00	B16	5.36	1.78	0.00	3.12	0.00
M26	5.55	2.68	0.00	3.09	2.30	B26	5.50	3.59	2.45	2.11	2.56
M28	5.12	3.50	0.00	3.51	1.90	B28	6.89	3.05	1.30	3.39	2.00
M29	7.02	2.08	0.00	5.37	0.00	B29	6.08	4.03	3.20	4.10	0.00
M30	6.55	4.06	2.55	5.53	2.20	B30	6.73	4.20	0.00	4.63	3.00
M32	6.60	4.71	0.00	2.30	3.00	B32	6.46	2.94	0.00	4.34	1.78
M35	6.09	3.26	2.60	3.66	0.00	B35	5.30	4.19	1.30	3.62	2.30
M46	6.18	3.99	0.00	3.98	2.60	B46	6.02	2.77	0.00	3.44	0.00
M48	6.88	2.30	0.00	2.80	0.00	B48	4.55	1.30	0.00	2.80	0.00
M52	4.35	3.41	2.58	3.31	0.00	B52	6.13	4.22	2.60	4.19	0.00
M53	4.67	2.48	0.00	2.62	0.00	B53	4.68	2.30	2.00	2.99	0.00
M59	5.29	3.15	2.45	2.83	2.00	B59	5.00	3.26	3.00	4.38	0.00
M64	6.59	2.34	1.90	2.71	0.00	B64	6.70	3.37	1.90	3.20	1.60
M65	5.89	4.15	3.66	3.64	2.30	B65	5.54	3.81	3.78	3.34	2.30
M67	5.99	4.96	2.60	3.90	2.30	B67	6.06	3.08	0.00	3.98	0.00
M68	6.03	3.15	0.00	3.88	0.00	B68	6.41	3.00	0.00	4.49	0.00
% Recovery	100	100	42.86	100	52.38	% Recovery	100	100	52.38	100	52.38

APC: Aerobic plate count

N = 21 for each of minced beef and boerewors samples

Table 3.3

Pearson correlation analysis for non-enriched minced beef

	A_w	Significance	pH	Significance
Aerobic plate count	0.18	NS	0.51	*
Coliforms	0.10	NS	0.01	NS
Escherichia coli	0.25	NS	0.10	NS
Salmonella enteritidis	-0.03	NS	0.44	NS
Shigella sonnei	0.11	NS	0.02	*

NS: Not significant

*:   Significant (p<0.05)

n = 21

The minced beef mean coliform counts on chromocult coliform agar are shown in Table 3.2. The recovery rate for the coliforms was 100% (21 out of the 21 samples surveyed) with a mean count of 3.47 log cfu/g ranging from a minimum of 2.08 log cfu/g (sample M29) to a maximum of 4.96 log cfu/g (sample M67). The Pearson correlation analysis showed that there were no significant correlations (p<0.05) between coliform counts, A_w and pH (Table 3.3). An analysis of variance revealed that there were no significant differences (p<0.05) between coliform recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

All the samples investigated satisfy the Swedish recommended quality regulation for coliforms which stipulates a maximum limit of 5 log cfu/g if an incubation temperature of 37⁰C was used (Shapton and Shapton, 1991). In a nationwide raw ground beef survey on levels of indicator organisms conducted from 1993 to 1994, the USDA reported a mean coliform count of 1.98 log cfu/g (USDA, according to Pruett et al., 2002). This was 1.49 log cfu/g less than the findings of this survey. In contrast to the findings of this survey, Pruett et al. (2002) reported a very low coliform range of <1.00 log cfu/g in frozen ground beef patties produced over an 8 hour shift. Possible limitations for the direct comparison of these studies were, among others, the ground beef product type, materials and methods used and format of the studies.

The recovery of *E. coli* on chromocult coliform agar is depicted in Table 3.2. The recovery rate for *E. coli* was 42.86% (9 out of the 21 samples tested). The mean *E. coli* count on chromocult coliform agar was 1.03 log cfu/g ranging from <2.00 log cfu/g (sample M2, M4, M14, M16, M26, M28, M29, M32, M46, M48, M53 and M68) to 3.66 (sample M65) log cfu/g. The USDA reported a higher mean *E. coli* Biotype I count of 1.73 log cfu/g after a nationwide raw ground beef survey conducted from 1993 to 1994 (USDA, according to Pruett et al., 2002). A very low *E. coli* range of <1.00 log cfu/g was reported by Pruett et al. (2002). The Pearson correlation analysis showed that there were no significant correlations ($p < 0.05$) between *E. coli* counts, A_w and pH (Table 3.3). An analysis of variance revealed that there were no significant differences ($p < 0.05$) between *E. coli* recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

Presumptive *Salmonella enteritidis* had a 100% recovery rate (all 21 samples surveyed were positive) on chromocult coliform agar with a mean of 3.71 log cfu/g ranging from 2.30 log cfu/g (sample M32) to 5.53 log cfu/g (sample M30; Table 3.2). The Pearson correlation analysis showed that there was no significant correlation ($p < 0.05$) between presumptive *Salmonella enteritidis* counts and A_w and pH ($p < 0.05$; Table 3.3). An analysis of variance revealed that there were no significant differences ($p < 0.05$) between presumptive *Salmonella enteritidis* recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District. All the minced beef samples analysed did not

satisfy the Swedish recommended quality limit for *Salmonella* spp. which stipulates that a product is unsuitable for consumption if it contains *Salmonella* spp. in 10g (Shapton and Shapton, 1991).

Presumptive *Shigella sonnei* had a recovery rate of 52.38% (11 out of the 21 samples surveyed) on chromocult coliform agar with a mean of 1.13 log cfu/g ranging from <2.00 log cfu/g (samples M4, M5, M16, M29, M35, M48, M52, M53, M64 and M68) to 3.00 log cfu/g (sample M2; Table 3.2). The Pearson correlation analysis showed that there were no significant correlations ($p<0.05$) between presumptive *Shigella sonnei* counts, A_w (Table 3.3). There was, however, a significant correlation ($p<0.05$) between presumptive *Shigella sonnei* counts and pH (Table 3.3). An analysis of variance revealed that there were no significant differences ($p<0.05$) between presumptive *Shigella sonnei* recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

3.3.2 Boerewors organism recoveries without enrichment

The mean APC for boerewors on SPCA are shown in Table 3.2. All the boerewors samples analysed gave a mean of 5.81 log cfu/g on SPCA ranging from 4.55 log cfu/g (sample B48) to 6.89 log cfu/g (sample B28). The Pearson correlation analysis showed that there was no significant correlation ($p<0.05$) between APC and A_w while the correlation between APC and pH was signifcant (Table 3.4). An analysis of variance revealed that there were no significant differences ($p<0.05$) between APC recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District. The recoveries satisfied the acceptable recommended fresh sausage quality limit at the "best before" date for Ohio State which recommends a maximum APC of 7.18 log cfu/g (Shapton and Shapton, 1991). However, according to Brazil's recommended APC quality limit of 6.00 log cfu/g for fresh sausages at "best before" date, 42.86% (9 out of the 21) of samples surveyed do not satisfy the requirements for good quality (Shapton and Shapton, 1991). Even though boerewors gave lower APCs, there was no significant difference ($p<0.05$) between the boerewors and the minced beef counts.

The total coliform counts for the boerewors samples on chromocult coliform agar are shown in Table 2. The recovery rate for the coliforms was 100% (21 out of the 21 samples surveyed) which was the same as that obtained for minced beef. Boerewors, compared to the minced beef, gave a lower mean count of 3.08 log cfu/g ranging from 1.30 log cfu/g (sample B48) to 4.22 log cfu/g (sample B52). However, this difference was not significant ($p < 0.05$). The Pearson correlation analysis showed that there were no significant correlations ($p < 0.05$) between coliform counts, A_w and pH (Table 3.4). An analysis of variance revealed that there were no significant differences ($p < 0.05$) between coliform recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District. According to the Ohio State quality limit guidelines for coliforms which recommends a maximum coliform count of 4.7 log cfu/g, 100% (21 out of 21) of the samples analysed were suitable for consumption (Shapton and Shapton, 1991).

Table 3.4

Pearson correlation analysis for non-enriched boerewors

	A_w	Significance	pH	Significance
APC	-0.18	NS	0.51	*
Coliforms	-0.06	NS	0.40	NS
Escherichia coli	-0.13	NS	0.10	NS
Salmonella enteritidis	0.24	NS	0.13	NS
Shigella sonnei	-0.08	NS	0.50	*

NS: Not significant

*: Significant ($p < 0.05$)

The recovery rate for *E. coli* in boerewors on chromocult coliform agar was 47.62% (10 out of the 21 samples surveyed). The mean *E. coli* count was 1.26 log cfu/g ranging from <2.00 log cfu/g (samples B1, B2, B5, B16, B30, B32, B46, B48, B67 and B68) to 3.78 log cfu/g (sample B65; Table 3.2). The mean recovery of *E. coli* was higher for boerewors compared to minced beef but the difference was not significant (p<0.05). The Pearson correlation analysis showed that there were no significant correlations (p<0.05) between *E. coli* counts, A_w and pH (Table 3.4). An analysis of variance revealed that there were no significant differences (p<0.05) between *E. coli* recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District. According to Brazil's recommended *E. coli* quality limit of 2.00 log cfu/g for fresh sausages at "best before" date (Shapton and Shapton, 1991), 28.57% (6 out of 21) of the samples were not suitable for consumption.

The recovery of presumptive *Salmonella enteritidis* from boerewors on chromocult coliform agar was 100% (21 out of the 21 samples surveyed; Table 3.2), which was equal to that observed for minced beef. The mean count was 3.55 log cfu/g with a range of 2.11 log cfu/g (sample B26) to 4.63 log cfu/g (sample B30). This was slightly lower compared to the recovery from minced beef. However, the differences were not significant (p<0.05). The Pearson correlation analysis showed that there were no significant correlations (p<0.05) between presumptive *Salmonella enteritidis* counts, A_w and pH (Table 3.4). An analysis of variance revealed that there were no significant differences (p<0.05) between presumptive *Salmonella enteritidis* recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District. None of the samples analysed were suitable for consumption according to Brazil's acceptable *Salmonella* spp. fresh sausage quality limit of 0 cfu in 25g (Shapton and Shapton, 1991).

The recovery of presumptive *Shigella sonnei* from boerewors on chromocult coliform agar is shown in Table 3.2. The presumptive *Shigella sonnei* recovery rate was 52.38% (11 out of 21) of the samples investigated. This was the same as the recovery rate obtained for presumptive *Shigella sonnei* in minced beef. A slightly higher mean of 1.20 log cfu/g was observed for boerewors compared to minced beef and this ranged from <2.00 log cfu/g

(samples B5, B16, B29, B46, B48, B52, B53, B59, B67 and B68) to 3.20 log cfu/g (sample B1). The Pearson correlation analysis showed that there was no significant correlation ($p < 0.05$) between presumptive *Shigella sonnei* counts and A_w. However, the correlation between presumptive *Shigella sonnei* and pH was significant (Table 3.4). An analysis of variance revealed that there were no significant differences ($p < 0.05$) between presumptive *Shigella sonnei* recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District.

3.3.3 Minced beef organism recoveries after enrichment

Table 3.5 shows the organism recoveries from minced beef and boerewors after enrichment. The minced beef enriched recovery of 100% (21 out of the 21 samples surveyed) for coliforms on chromocult coliform agar was equal to that obtained without enrichment (Tables 3.2 and 3.5). The mean coliform count was 11.70 log cfu/g ranging from 9.25 log cfu/g (sample M29) to 13.37 log cfu/g (sample M67; Table 3.5). The Pearson correlation analysis showed that there were no significant correlations ($p < 0.05$) between coliform counts, A_w and pH (Table 3.6). An analysis of variance revealed that there were no significant differences ($p < 0.05$) between coliform recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

The minced beef mean *E. coli* count on chromocult coliform agar after enrichment was 10.84 log cfu/g with a range of <2.00 log cfu/g (sample M32) to 12.89 log cfu/g (sample M28; Table 3.5). The enriched recovery rate was 95.24% (20 out 21) of the samples compared to the non-enriched recovery rate of 42.86%. Only sample number M32 (4.76%) was free from *E. coli* after enrichment. The Pearson correlation analysis showed that there were no significant correlations ($p < 0.05$) between *E. coli* counts, A_w and pH (Table 3.6). An analysis of variance revealed that there were no significant differences ($p < 0.05$) between *E. coli* recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

Table 3.5

Comparison of enriched minced beef and boerewors presumptive organism recoveries

Minced beef organism recoveries (Mean Log cfu/g)					Boerewors organism recoveries (Mean Log cfu/g)				
Sample code	Coliforms	*E. coli*	*Salmonella enteritidis*	*Shigella sonnei*	Sample Code	Coliforms	*E. coli*	*Salmonella enteritidis*	*Shigella sonnei*
M1	9.92	9.81	8.95	0.00	B1	10.38	10.15	10.34	0.00
M2	12.60	11.77	11.60	0.00	B2	9.68	9.53	9.50	8.00
M4	11.72	11.48	10.30	0.00	B4	11.56	11.51	0.00	0.00
M5	11.73	11.67	11.40	0.00	B5	12.32	8.48	11.59	0.00
M14	10.52	9.00	9.78	0.00	B14	13.53	13.39	12.30	0.00
M16	12.90	11.78	12.49	0.00	B16	12.90	11.78	12.49	0.00
M26	12.17	12.02	11.49	0.00	B26	9.94	9.26	9.63	0.00
M28	13.05	12.89	11.53	0.00	B28	11.83	11.60	11.70	0.00
M29	9.25	9.25	7.60	0.00	B29	9.09	9.09	8.51	0.00
M30	10.66	10.63	9.30	0.00	B30	12.63	11.60	11.95	11.00
M32	11.11	0.00	11.84	0.00	B32	12.13	11.74	11.14	11.61
M35	12.07	12.06	10.48	0.00	B35	12.79	12.60	12.08	0.00
M46	10.82	10.11	10.90	10.48	B46	8.40	8.32	8.26	0.00
M48	11.51	11.46	11.51	0.00	B48	13.45	13.45	12.88	0.00
M52	11.83	11.60	1078	0.00	B52	11.90	11.89	10.70	0.00
M53	12.13	11.74	10.60	10.00	B53	12.80	12.63	0.00	12.15
M59	12.78	12.77	11.30	0.00	B59	13.40	13.40	0.00	0.00
M64	12.82	12.11	12.51	0.00	B64	12.08	11.90	11.29	0.00
M65	9.82	9.78	0.00	8.26	B65	10.95	10.94	0.00	9.00
M67	13.37	12.77	12.75	0.00	B67	13.40	13.30	12.49	12.04
M68	12.95	12.83	12.41	11.00	B68	13.29	12.66	13.58	0.00
% Recovery	100	95.24	95.24	19.05	% Recovery	100	100	80.95	28.57

n = 21 for each of minced beef and boerewors

Table 3.6

Pearson correlation analysis for enriched minced beef

	A_w	Significance	pH	Significance
Coliforms	0.06	NS	-0.43	NS
Escherichia coli	0.01	NS	-0.04	NS
Salmonella enteritidis	-0.05	NS	-0.20	NS
Shigella sonnei	0.25	NS	-0.26	NS

NS: Not significant

N = 21

Presumptive *Salmonella enteritidis* recovery rate from minced beef on chromocult coliform agar declined slightly to 95.24% (20 out of the 21 samples analysed) compared to the non-enriched recovery rate which was at 100% (Tables 3.2 and 3.5). The mean presumptive *Salmonella enteritidis* recovery was 9.91 log cfu/g with a range of <2.00 log cfu/g (sample M65) to 12.75 log cfu/g (sample M67; Table 3.5). The Pearson correlation analysis showed that there were no significant correlations ($p<0.05$) between presumptive *Salmonella enteritidis* counts, A_w and pH (Table 3.6). An analysis of variance revealed that there were no significant differences ($p<0.05$) between presumptive *Salmonella enteritidis* recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

The enriched recoveries of presumptive *Shigella sonnei* in minced beef on chromocult coliform agar are depicted in Table 3.5. The mean presumptive *Shigella sonnei* recovery was

1.89 log cfu/g with a range of <2.00 log cfu/g (samples M1, M2, M4, M5, M14, M16, M26, M28, M29, M30, M35, M48, M52, M59, M64 and M67) to 11.00 log cfu/g (sample M68). The enriched recovery rate for presumptive *Shigella sonnei* decreased dramatically to 14.29% (3 out of 21) of the samples tested compared to the obtained non-enriched recovery rate of 52.38%. The Pearson correlation analysis showed that there were no significant correlations ($p<0.05$) between presumptive *Shigella sonnei* counts, A_w and pH (Table 3.6). An analysis of variance revealed that there were no significant differences ($p<0.05$) between presumptive *Shigella sonnei* recoveries from minced beef and between various regions (North, South, East, West, Central) of the Bloemfontein District.

The decreases noted for the recovery rates and mean counts for *Salmonella enteritidis* and *Shigella sonnei* after enrichment were possibly due to increased competition from the more successful coliforms and *E. coli*. For *E. coli*, notable increases were observed in both recovery rate and mean count. Coliforms gave a sustained recovery rate of 100% and higher mean count. The general decrease in presumptive *Salmonella enteritidis* and presumptive *Shigella sonnei* recoveries with concomitant increases in *E. coli* and coliform recoveries could also be attributed to the type of medium used, viz: chromocult coliform agar (Merck 1.10426). Chromocult coliform agar selectively supports the growth of all coliforms but not all enterobacteriaceae. It facilitates rapid colony growth and even repair of sublethally injured coliforms due to the presence of peptones, pyruvate, sorbitol and phosphate buffer. It also contains tergitol® 7 which does not have any negative effects on the growth of coliform bacteria but inhibits the growth of Gram positive bacteria as well as some Gram negative bacteria (Merck, 2003).

3.3.4 Boerewors organism recoveries after enrichment

The boerewors mean coliform count on chromocult coliform agar after enrichment was 11.83 log cfu/g ranging from 8.40 log cfu/g (sample B46) to 13.53 log cfu/g (sample B14; Table 3.5). The coliform recovery rate was 100% (21 out of 21) of the samples surveyed and this was equal to the recovery rate obtained from boerewors without enrichment. The Pearson correlation analysis showed that there was no significant correlation ($p<0.05$) between coliform counts and A_w while the correlation between coliforms and pH was significant

(Table 3.7). An analysis of variance revealed that there were no significant differences (p<0.05) between coliform recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District.

The boerewors recoveries of *E. coli* on chromocult coliform agar after enrichment are shown in Table 3.5. The enriched mean *E. coli* count was 11.39 log cfu/g with a range of 8.32 log cfu/g (sample B46) to 13.45 log cfu/g (sample B48). The recovery rate was 100% (21 out of 21) of the samples tested and this was notably higher compared to a recovery rate of 52.34% obtained without enrichment. The Pearson correlation analysis showed that there were no significant correlations (p<0.05) between *E. coli* counts, A_w and pH (Table 3.7). An analysis of variance revealed that there were no significant differences (p<0.05) between *E. coli* recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District.

Table 3.7

Pearson correlation analysis for enriched boerewors

	A_w	Significance	pH	Significance
Coliforms	0.25	NS	-0.52	*
Escherichia coli	0.26	NS	-0.36	NS
Salmonella enteritidis	0.06	NS	-0.07	NS
Shigella sonnei	-0.03	NS	-0.16	NS

NS   Not significant

*   Significant (p<0.005)

n = 21

The enriched count for presumptive *Salmonella enteritidis* on chromocult coliform agar in boerewors gave a mean of 9.07 log cfu/g ranging from <2.00 log cfu/g (samples B4, B53, B59 and B65) to 13.58 log cfu/g (sample B68; Table 3.5). There was a decline in the recovery rate for presumptive *Salmonella enteritidis* from 100% before enrichment to 90.48% (19 out of 21 samples surveyed) after enrichment. This trend was similar to that obtained from minced beef before and after enrichment. The Pearson correlation analysis showed that there were no significant correlations ($p<0.05$) between presumptive *Salmonella enteritidis* counts, A_w and pH (Table 3.7). An analysis of variance revealed that there were no significant differences ($p<0.05$) between presumptive *Salmonella enteritidis* recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District.

The recoveries of presumptive *Shigella sonnei* in boerewors after enrichment are shown in Table 3.5. The mean recovery obtained was 2.85 log cfu/g ranging from <2.00 log cfu/g (samples B1, B4, B5, B14, B16, B26, B28, B29, B35, B46, B48, B52, B59, B64 and B68) to 12.15 log cfu/g (sample B53). The recovery rate was 28.57% (6 out of 21 samples surveyed) and this was notably lower compared to the recovery rate of 52.38% obtained without enrichment. Poor recovery of presumptive *Shigella sonnei* after enrichment compared to its recovery without enrichment was also observed in minced beef samples. The Pearson correlation analysis showed that there were no significant correlations ($p<0.05$) between presumptive *Shigella sonnei* counts, A_w and pH (Table 3.7). An analysis of variance revealed that there were no significant differences ($p<0.05$) between presumptive *Shigella sonnei* recoveries from boerewors and between various regions (North, South, East, West, Central) of the Bloemfontein District.

A study by Bonardi et al. (2001) on the faecal carriage of verocytotoxin-producing *E. coli* O157:H7 and carcass contamination in cattle at slaughter in Northern Italy concluded that hygiene standards and good management practices are extremely important to contain the rate of carcass contamination when VTEC O157 infected cattle are presented to the slaughter line. Measures such as these are designed to prevent or reduce carcass contamination that may occur from either contaminated hides or the leakage of faecal material during intestine

removal, cross contamination between carcasses that are adjacent and in contact in the slaughter line and use of contaminated machinery and tools (Grau, 1987; Ayres, 1995; Elder et al., 2000). The high levels of contaminating organisms such as presumptive *Salmonella enteritidis* in both the minced beef and boerewors found in this study could therefore be indicative of poor hygiene practices as well as poor management practices (Nel et al., 2004). Areas that should be managed critically would include the farm, where animal exposure to pathogens should be curbed, the abattoir, where hygienic evisceration is critical, the transportation of the carcasses to meat factories and butcheries, where the cold chain must still be maintained, through to the manufacture of the products. A trained workforce is also vital.

3.3.5 The incidence of diarrhoeagenic *E. coli* serology groups in minced beef

The incidence of DEC in minced beef is summarised in Table 3.8. Minced beef gave a DEC incidence of 38.10% (8 out of 21 samples tested). Enteropathogenic *E. coli*, EIEC, EAggEC and VTEC were the 4 virotypes that were isolated. Enteropathogenic *E. coli* were the most dominant virotype and had a prevalence value of 23.81% (5 out of 21) of the samples surveyed. Serotypes O18, O114, O125 and O142 were isolated. Serotype O28$_{ac}$ was the EIEC serotype isolated, serotype O44 was the EAggEC serotype isolated and serotype O26 was the VTEC serotype isolated. Each of the EIEC, EAggEC and VTEC gave an incidence of 4.76% (1 out of 21) of the samples surveyed.

The incidence of non-VTEC DEC is highly under reported while some studies have investigated the prevalence of VTEC in various foods (Suthienkul et al., 1990; Samadpour et al., 1994; Rogerie et al., 2001). The morbidity and mortality associated with VTEC infections in contrast to infections with EPEC, EIEC, EAggEC and DAEC led to the recognition of VTEC as an emerging problem justifying more research particularly in North America, Europe and Japan (Wilshaw et al., 2000).

Table 3.8

Incidence of diarrhoeagenic *E. coli* in 21 minced beef and 21 boerewors samples

	Minced beef recoveries		**Boerewors recoveries**	
Virotype	No. of positive samples	% positive of total sampled	No. of positive samples	% positive of total sampled
EPEC	5	23.81	5	23.81
EIEC	1	4.76	0	0
EAggEC	1	4.76	0	0
VTEC	1	4.76	1	4.76
Total	8	38.10	6	28.57

Although *E. coli* O157:H7 appears to be the most frequently encountered VTEC, the general consensus is that there may be a higher level of underreporting for the other serotypes of VTEC mainly because of the difficulties in their isolation and identification (Karch et al., 1999; Smidt et al., 1999; Nishikawa et al., 2002; Samadpour et al., 2002).

In this study, the 4.76% prevalence of VTEC obtained was higher but comparable to that of the 3.70% reported by Made and Stark, according to Samadpour et al. (2002). On the other hand, Samadpour et al. (1994) found a high VTEC prevalence of 23.00% in fresh retail beef samples from grocery stores in Seattle, Washington.

The reasons for the variations in prevalence rates might stem from the actual differences in the prevalence of VTEC in the various study locations and times, the detection methods employed and the quantity of the samples analysed. Most reported outbreaks of enterohaemorrhagic *E. coli* infection were caused by *E. coli* O157:H7 strains. This may suggest that this serovar is more virulent or more transmissible than other serotypes (Snyder, 1998). *Escherichia coli* O157:H7 was not isolated in this study from either minced beef or boerewors. This result is similar to findings by most studies. Although beef products have been widely implicated as vehicles of *E. coli* O157:H7 infection, a number of studies worldwide have either failed to find the organism or have reported a very low prevalence of the organism in retail raw meat samples (Lindqvist et al., 1998; Little and de Louvois, 1998; Tarr et al., 1999, Chapman et al., 2001).

Examination of beef minced meat samples for *E. coli* O157:H7 in Denmark revealed that 0.3% of the 1 584 samples examined were contaminated with the organism (Boel et al., 1997). Heuvelink et al. (1999) reported that 1.1% of the 571 samples of raw minced beef samples examined in The Netherlands were contaminated with *E. coli* O157:H7. A study in Switzerland by Fantelli and Stephan (2001) found no *E. coli* O157:H7 from 211 minced raw beef samples tested. Dontorou et al. (2003) also found no *E. coli* O157:H7 from 64 raw minced beef samples obtained from supermarkets, retail shops and National Army canteens in Greece over a 2 year period (2000 to 2001). Results from another study in Northern Greece reported by Kansouzidou et al. (1994) were all negative for *E. coli* O157:H7 from

187 faecal specimens from calves, heifers and poultry, and 87 fresh and frozen minced meat samples.

Table 3.9 outlines the minced beef recovery frequencies based on five *E. coli* colonies randomly selected from chromocult coliform agar plates obtained from each sample using Harrison's disc method (Harrison, according to Harrigan, 1998). A total of 100 isolates were serotyped and EPEC had the highest frequency of 6%. Of these six EPEC isolates, three reacted with antiserum O18, one reacted with antiserum O114, one reacted with antiserum O125 and one reacted with antiserum O142.

Enteroinvassive *E. coli*, EAggEC, and VTEC each had a frequency of 1%. The EIEC isolate reacted with antiserum O28$_{ac}$, the EAggEC isolate reacted with antiserum O44 and the VTEC isolate reacted with antiserum O26. The available antisera were not reactive to 91% of the isolates that were typed.

3.3.6 Incidence of diarrhoeagenic *E. coli* serology groups in boerewors

The incidence of DEC in boerewors is shown in Table 3.8. Boerewors had a DEC incidence of 28.57% (6 out of the 21 samples tested). The virotypes isolated were EPEC and VTEC. Enteropathogenic *E. coli* prevalence was 23.81% (5 out of the 21 samples tested) and serotypes recovered were O18, O55, O125 and O142. Serovar O26 was the VTEC serotype isolated and had a prevalence of 4.76% (1 out of the 21 samples tested). This result was similar to that of a study carried out in Argentina by Chinen et al. (2001). They reported that 4.80% of the 83 fresh sausages tested and 3.30% of the 30 dry sausages tested were positive for VTEC O157.

In contrast, Dontorou et al. (2003) reported that 1.30% of the 75 Greek traditional fresh sausages tested were positive for VTEC O157. Another study in France revealed that 0.40% of the 250 pork sausage samples tested were positive for VTEC O157 (Vernozy-Rozand et al., 1997).

Table 3.9

Recovery frequencies of *E. coli* serovars from minced beef isolated from agar plates using Harrison's disc.

Virotype	Antisera	Number of positive samples	% positive of total sampled
EPEC	O18	3	3
	O114	1	1
	O125	1	1
	O142	1	1
	O55	0	0
	O111	0	0
	O119	0	0
	O126	0	0
	$O127_a$	0	0
ETEC	O20	0	0
	0128	0	0
EIEC	$O28_{ac}$	1	1
	$O112_{ac}$	0	0
	O124	0	0
VTEC	O26	1	1
	O111	0	0
	O157	0	0
EAggEC	O44	1	1
	086_{ac}	0	0
	O111	0	0
Untypable isolates*		91	91

*   Isolates that were not reactive to available antisera

n = 100

Serotype O26 and O111 are some of the enterohaemorrhagic *E. coli* (EHEC) serotypes that have emerged as significant causes of human diseases over the past 20 years associated with fresh sausages and other ground meat products although the prototype, VTEC O157:H7, has been associated with important foodborne outbreaks worldwide (Sharma, 2002). Consequently there was increased interest in *E. coli* O157:H7 leading to more research.

However, apart from *E. coli* O157:H7, there is insufficient laboratory surveillance data collected for other VTEC and other DEC, principally because appropriate detection methods are not available in most diagnostic laboratories (Nishikawa et al., 2002). Generic *E. coli* was isolated from all the 21 boerewors samples tested.

The diarrhoeagenic *E. coli* recovery frequencies for boerewors based on 5 isolates randomly selected from chromocult coliform agar plates obtained from each sample using Harrison's disc method (Harrison, according to Harrigan, 1998) are shown in Table 3.10. One hundred and five isolates were serotyped and EPEC gave the highest frequency of 6.66% (7 out of the 105 isolates tested). This was a similar but slightly higher frequency compared to that of 6.00% obtained as the highest frequency from the raw, fresh minced beef samples. Of the seven EPEC isolates, three reacted with antiserum O18, two reacted with antiserum O125, one reacted with antiserum O55 and one reacted with antiserum O142. VTEC had a frequency of 0.95% (1 out of the 105 isolates tested) and this reacted with antiserum O26. The available antisera was not reactive to 92.38% (97 out of 105) isolates tested. In this study, no trend could be discerned relating to the recoveries of indicator organisms to the incidence of VTEC or other non-VTEC diarrhoeagenic *E. coli*.

Escherichia coli O125 was recovered from both samples M1 and B1. *Escherichia coli* O18 was recovered from samples M30 and B30 as well as samples M35 and B35 (Table 3.10). The reason for this might stem from the fact that samples M1 and B1 were purchased from the same butchery and so were samples M30 and B30 followed by samples M35 and B35 (Table 3.11). The fresh meat used by the butcheries may have been contaminated from the abattoirs (e.g. during evisceration) or within the butcheries (e.g. through use of contaminated equipment).

Table 3.10

Recovery frequencies of *E. coli* serovars from boerewors isolated from agar plates using Harrison's disc

Virotype	Antisera	Number of positive samples	% Positive of total sampled
EPEC	O18	3	2.86
	O114	0	0
	O125	2	1.90
	O142	1	0.95
	O55	1	0.95
	O111	0	0
	O119	0	0
	O126	0	0
	O127$_a$	0	0
ETEC	O20	0	0
	0128	0	0
EIEC	O28$_{ac}$	0	0
	O112$_{ac}$	0	0
	O124	0	0
VTEC	O26	1	0.95
	O111	0	0
	O157	0	0
EAggEC	O44	0	0
	086$_{ac}$	0	0
	O111	0	0
Untypable isolates*		97	92.38

* Isolates that were not reactive to available antisera

n = 105

Table 3.11

Serotypes isolated from each sample and geographic sampling areas in Bloemfontein District

	NORTH				SOUTH							EAST	WEST				CENTRAL				
Butchery	1	2	4	5	14	16	26	28	29	30	32	35	46	48	52	53	59	64	65	67	68
Minced beef	O125	-	-	-	O18	O26	-	-	-	O18	-	O18	O142 O114	-	O28	-	O44	-	-	-	-
Boerewors	O142 O125	-	-	-	-	-	-	-	-	O18	-	O18 O125	O26	-	-	-	O18	-	O55	-	-

n = 21 for each of minced beef and boerewors

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Cattle carcass contamination with *E. coli* from intestinal contents during slaughter is one of the most common ways in which human food becomes contaminated (Yilmaz et al., 2002; Hancock et al., 1997; Karch et al., 1999, Park et al., 1999). The manufacture of boerewors using the contaminated meat could have led to a direct transfer of the contaminants from the beef to the fresh sausage. Verocytotoxin-producing *E. coli* O26 was recovered from samples from the southern and western areas of the Bloemfontein District (Table 3.11).

3.4 CONCLUSIONS

It can be concluded that the incidence of diarrhoeagenic *E. coli* in Bloemfontein District's minced beef is 38.10% and 28.57% in boerewors. A wider range of virotypes (EPEC, EIEC, EaggEC and VTEC) was recovered from minced beef compared to boerewors (EPEC and VTEC). This may be attributed to the presence of humectants, organic acids and sulphur dioxide preservative in boerewors formulations. Enteropathogenic *E. coli* were predominant in both products and hence, incriminating minced beef and boerewors as possible major vehicles contributing to the dominance of EPEC in dia rrhoea patients from the Bloemfontein District.

In both minced beef and boerewors without enrichment, increased pH resulted in significantly ($p<0.05$) higher APC and *Shigella sonnei* recoveries while the correlations between pH and recoveries of coliforms, *E. coli* and *Salmonella enteritidis* were not significant ($p<0.05$). The recoveries of coliforms, *E. coli*, *Salmonella enteritidis* and *Shigella sonnei* in minced beef after enrichment were not significantly ($p<0.05$) correlated to both A_w and pH. In boerewors after enrichment, the correlation between pH and coliform recoveries was significant ($p<0.05$). However, no significant ($p<0.05$) correlations were established between A_w and coliform, *E. coli*, *Salmonella enteritidis,* and *Shigella sonnei* recoveries. There were no significant ($p<0.05$) differences that were established between APC, coliform, *E. coli*, *Salmonella enteritidis* and *Shigella sonnei* recoveries from both enriched and non-enriched minced beef and boerewors, and between various regions (North, South, East, West and Central) of the Bloemfontein District. The high levels of organisms like *Salmonella*

enteritidis, are an indication of poor hygiene along the food supply chain either at the abattoir, meat factory or butchery levels.

Enteropathogenic *E. coli* O18 was the serotype most frequently recovered from both minced beef and boerewors while VTEC O26 was the most virulent serotype recovered from both products. The incidence of VTEC was very low (4.76%) in both minced beef and boerewors. No VTEC O157 were recovered and this should provide some reassurance to producers, retailers and consumers. However, even though the VTEC incidence is very low, possible contamination of the odd product still exists and presents a potential for causing future foodborne diarrhoea outbreaks. Monitoring of food production, processing and retail establishments and cross-contamination control strategies should, therefore, be introduced by the medical, veterinary and consumer authorities to safeguard public health. It can also be concluded that there is a very low incidence of EIEC and DAEC.

It was also found that an enrichment step should be included when screening for DEC on chromocult™ chromogenic agar (Merck 1.10426) to increase the chances of recovering pathogens especially DEC and coliforms.

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CHAPTER 4

The growth, survival, and thermal inactivation of *Escherichia coli* O157:H7 in boerewors

Abstract

This study investigated the growth and survival of *Escherichia coli* O157:H7 inoculated into boerewors models with and without sulphur dioxide preservative at a low (3.5 log cfu/g) and high (7.5 log cfu/g) inoculum followed by storage at $0\,^{o}C$, $4\,^{o}C$ and $10\,^{o}C$ for 10 days. The pathogen's thermal inactivation at $50\,^{o}C$, $60\,^{o}C$, $65\,^{o}C$ and $70\,^{o}C$ was also evaluated after inoculation of the boerewors model with sulphur dioxide preservative to 7.00 log cfu/g. Boerewors without preservative at both low and high inoculum had significantly (p<0.001) higher recoveries at all temperatures compared to boerewors with preservative. The low and high inocula with preservative had significant (p<0.001) reductions in recoveries at $0\,^{o}C$, declining to below detectable limits at days 8 and 10 respectively. At $4\,^{o}C$, the low and high inocula with preservative recoveries declined significantly (p<0.001) to below detectable limits and to 1.0l log cfu/g respectively at day 10. At $10\,^{o}C$, significant (p<0.001) increases of 1.85 log cfu/g and 2.35 log cfu/g respectively were observed from day 0 to day 10. At $0\,^{o}C$ the low and high inocula without preservative treatments had declines in recoveries of 1.21 log cfu/g and 1.45 log cfu/g respectively and insignificant (p<0.001) increases of 0.19 log cfu/g and 0.26 log cfu/g respectively at $4\,^{o}C$ while significant (p<0.001) increases of 1.97 log cfu/g and 1.84 log cfu/g respectively were noted at $10\,^{o}C$ from day 0 to day 10. The combination of sulphur dioxide preservative and low temperature ($0\,^{o}C$ and $4\,^{o}C$) demonstrated the best efficacy against the survival of *E. coli* O157:H7. Thermal inactivation end point temperatures of *Escherichia coli* O157:H7 inoculated into boerewors with preservative at 7.00 log cfu/g was 60 minutes at $60\,^{o}C$, 80 seconds at $65\,^{o}C$ and 60 seconds at $70\,^{o}C$. This study demonstrated that *E. coli* O157:H7 can survive in boerewors with and without preservative and is more sensitive to heat treatment at $70\,^{o}C$.

Keywords: Escherichia coli O157:H7, preservative, boerewors, inoculum size, temperature

4.1 INTRODUCTION

Escherichia coli O157:H7 was first associated with illness in 1982 (Doyle et al., 1997). Since then the pathogen has gained in notoriety due to a number of outbreaks which have occurred worldwide (Byrne et al., 2002a). The pathogen is an aetiological agent of haemorrhagic colitis, haemolytic uremic syndrome and thrombotic thrombocytopaenic purpura (Karmali et al., 1983). It is estimated that in the United States of America, the number of *E. coli* O157:H7 foodborne illness cases are 62 500 per annum including 10 800 hospitalisations and 50 deaths (Mead et al., 1999).

Although a number of foods such as lettuce (Li et al., 2001), unpasteurised apple cider (Besser et al., 1993), drinking water (Isaacson et al., 1993) and yoghurt (Bachrouri et al., 2002) have been associated with some *E. coli* O157:H7 outbreaks, most of them have been associated with the consumption of foods of bovine origin such as ground beef and raw milk (Griffin and Tauxe, 1991). In the United States of America, about 50% of the foodborne outbreaks for the period 1982 to 1994 in which the vehicle of transmission was identified, were associated with the consumption of ground beef products (Meng and Doyle, 1998).

Growth and survival studies of *Escherichia coli* O157:H7 in specific ground beef products is important for the prediction of the pathogen's behaviour in those foods since broth based models for the prediction of *Escherichia coli* O157:H7 growth may be compromised when the models are applied to the ground beef medium (Tamplin, 2002). General growth parameters for all *E. coli* include a minimum temperature of 7 $^{\circ}$C to 8 $^{\circ}$C, an optimum temperature of of 35 $^{\circ}$C to 40 $^{\circ}$C, a minimum pH of 4.4 and an optimum pH of 6.0 to 7.0 and a minimum water activity of 0.935 and an optimum of 0.995 (Wilshaw et al., 2000). Palumbo et al. (1997) observed the growth of *Escherichia coli* O157:H7 in ground beef stored at 8 $^{\circ}$C when there was little or no background flora. It has been shown that *Escherichia coli* O157:H7 can survive in frozen ground beef patties for 9 months with little decline in numbers (Doyle and Schoeni, 1984). Therefore, contamination of beef by the pathogen poses significant risks that make it imperative for consumers to ensure adequate cooking.

Several researchers have shown that *Escherichia coli* O157:H7 does not posses an unusual heat resistance and D-values at 50°C to 64°C ranging from 92.67 to 0.16 minutes have been reported (Doyle and Schoeni, 1984; Line et al., 1991; Ahmed et al., 1995). Murphy et al. (2004) reported D-values at 55°C to 70°C ranging from 23.23 to 0.03 minutes in ground and formulated beef/turkey. The United Kingdom's Advisory Committee on the Microbiological Safety of Food (ACMSF) recommended that minced beef burgers should be heated to an internal temperature of 70°C for 2 minutes (ACMSF, according to Byrne et al., 2002a).

The pathogen has a very low infective dose of about 10 to 100 organisms (Garbutt, 1997) and some consumers frequently subject food to inadequate refrigeration and heating leading to increased chances of infection and outbreaks. The addition of antimicrobials in food improves the safety of the food by providing additional hurdles to reduce or eliminate pathogens (Byrne et al., 2002b)

Boerewors is a very popular South African traditional fresh sausage that may be manufactured with or without sulphur dioxide preservative and for which there is no information available on the growth, survival and thermal inactivation of contaminating *Escherichia coli* O157:H7. *Escherichia coli* O157:H7 was chosen for the growth and survival as well as thermal inactivation studies because of the severity with which it causes illness and the high frequency at which it has been associated with ground beef disease outbreaks. Therefore, the aims of this study were to investigate the growth and survival of *Escherichia coli* O157:H7 in boerewors with and without sulphur dioxide preservative at 0°C, 4°C and 10°C as well as the determination of the pathogen's thermal inactivation end point.

4.2 MATERIALS AND METHODS

4.2.1 Preparation of boerewors models

Boerewors models were manufactured in the meat laboratory of the Department of Microbial, Biochemical and Food Biotechnology at the University of the Free State

following typical industrial procedures. Table 4.1 shows the formulation used for the manufacture. Fresh meat (beef [70/30] and pork [50/50]), was purchased from a butchery in the Bloemfontein District. The models consisted of 90% total meat content formulated to contain 30% fat and 60% lean meat. The meat was cubed, frozen and stored overnight at -18 ^{o}C in oxygen permeable polythene bags. It was then tempered to +4 ^{o}C. The crumbs, spices, Worcester sauce and vinegar were mixed in 2.4% ice water and left to stand for 5 minutes to allow for hydration.

Table 4.1

Formulation used in the manufacture of boerewors models (Hugo, 2004)

Ingredient	%
Beef (70/30)	60
Pork (50/50)	30
Vinegar	1.5
Water	2.4 + 1*
Rusk	2
Spice mixture	2.7
Worcester sauce	0.4
Total	**100**
Sodium chloride	1.26%
Sulphur dioxide **	450 mg/kg
Ascorbic Acid:	62.308 mg/kg

*   1% water was added with the organism at the spiking stage

**   Sulphur dioxide was added as sodium metabisulphite

The spice mixture consisted of coriander (24.37%), monosodium glutamate (6.50%), black pepper (6.00%), nutmeg (5.00%), cloves (4.00%), thyme (1.00%), sodium chloride (46.55%), sodium metabisulphite (2.63%), ascorbic acid (0.23%) and dextrose (3.72%). It was thoroughly hand mixed with the cubed meat and minced through a 4.5 mm plate. The boerewors with preservative spice mixture had 450 mg/kg sulphur dioxide added as sodium metabisulphite while the boerewors without preservative spice mixture was formulated without the addition of the sulphur dioxide. The boerewors was packaged as 99g into sterile stomacher bags (Whirl-Pak™) sealed with masking tape and frozen stored at -12 ^{o}C until required.

4.2.2 Microbial analysis

4.2.2.1 Test for the presence of *E. coli* O157:H7

Screening for *E. coli* O157:H7 was done separately for the beef (70/30), pork (50/50) and the finished boerewors without preservative and boerewors with preservative. Boerewors samples for microbial analysis were taken immediately after manufacture of the models and analysed within 30 minutes.

The sample (10g) was weighed into a sterile stomacher bag (Whirl-Pak™) and homogenised in 90 ml of MacConkey broth (Difco 0020-01) using a stomacher (Lab Blender 400, ART Medical Equipment). The mixture was aseptically transferred to sterile 100 ml Schott bottles and enriched by incubating for 18 hours at 37 ^{o}C. Serial dilutions were made followed by spread plating of 0.1 ml aliquots in duplicate on fluorocult *E. coli* O157:H7 agar (Merck 1.04036). The plates were incubated at 37 ^{o}C for 24 hours. The above procedure was repeated once and mean values were calculated from the data obtained from duplicate experiments.

4.2.2.2 Other microbial counts

Random duplicate samples of each of the boerewors without and boerewors with preservative were taken from the two bulk batches. The samples were prepared according to Harrigan (1998). A stomacher (Lab Blender 400, Art Medical Equipment) was used to homogenise the samples. The homogenised samples were serially diluted (1:10) in 0.1% buffered peptone water (BPW) and spread plated (0.1 ml aliquots) in duplicate on the following media: Standard plate count agar (SPCA; Oxoid CM463) for aerobic plate counts, and incubated at 32 $^{\circ}$C for 72 hours; Chromocult coliform agar (Merck 1.10426) for coliforms, and incubated at 37 $^{\circ}$C for 24 hours; Potato dextrose agar (Bioloab, Merck ART No. C100) for yeasts and moulds, and incubated at 25 $^{\circ}$C for 7 days. Mean values were calculated from 4 plate readings obtained from duplicate experiments.

4.2.3 Chemical analysis

4.2.3.1 pH

Homogenates were prepared by blending 10g of sample in 100 ml of distilled water (Koniecko, 1985) using an Ultra Turrax T25 laboratory blender. The homogenates were filtered using a Whatman number 1 filter paper. The pH of the filtrate was measured with a Hanna Instruments, Model HI 8915 ATC, pH meter with a glass electrode (Metrohm 100). The pH meter was standardised using pH 7 and pH 4 buffer solutions (BDH Laboratory Supplies). Mean pH values were calculated using two readings from duplicate samples.

4.2.3.2 Water activity (A $_w$) determination

A_w was measured using 3.5g of sample using a Novasina Thermoconstanter TH 200 water activity meter. Mean A_w values were calculated using two readings from duplicate samples.

4.2.3.3 Protein determination

Protein (% total nitrogen multiplied by a factor of 6.25) was determined in 0.5g sample using the Kjeldahl analytical method (AOAC, 2000). The mean protein (%) values were calculated using data obtained from three replicates.

4.2.4 Experimental design

Fig. 4.1 outlines the design of the experiment. It consisted of a 2×2×3×6 factorial. Boerewors without preservative and boerewors with preservative were the two product formulations used. Each of the two formulations was spiked with *E. coli* O157:H7 to low (3.5 log cfu/g) and high (7.5 log cfu/g) inoculum levels. The spiked samples were stored at 0 $^{\circ}$C, 4 $^{\circ}$C and 10 $^{\circ}$C for 10 days.

4.2.5 Preparation of *E. coli* O157:H7 inoculum

Escherichia coli O157:H7 (ATCC 43895) on a nutrient agar slope (Oxoid CM3) was streaked out on fluorocult *E. coli* O157:H7 agar plates (Merck 1.04036) and incubated for 24 hours at 37 $^{\circ}$C. Colonies were confirmed by serotyping using the slide agglutination test for *E. coli* O157:H7 on heat killed organisms (Mast Assure™; Mast Diagnostics). A loopful of culture was suspended in 10 ml brain heart infusion (BHI) broth (Biolab C114) and incubated at 37 $^{\circ}$C for 24 hours. The resultant culture (3 ml aliquot) was added to 300 ml BHI broth and incubated at 37 $^{\circ}$C for 18 hours. The culture was centrifuged at 3 000g for 10 minutes at 4 $^{\circ}$C using a Beckman J2-21 centrifuge. The supernatant was decanted and the bacterial cell pellet was washed once with 20 ml of 0.1% buffered peptone water (BPW). The pellet was then resuspended in 0.1% BPW to McFarland density number 1. Counts were determined to about 10^5 cfu/ml for the low inoculum and about 10^9 cfu/ml for the high inoculum.
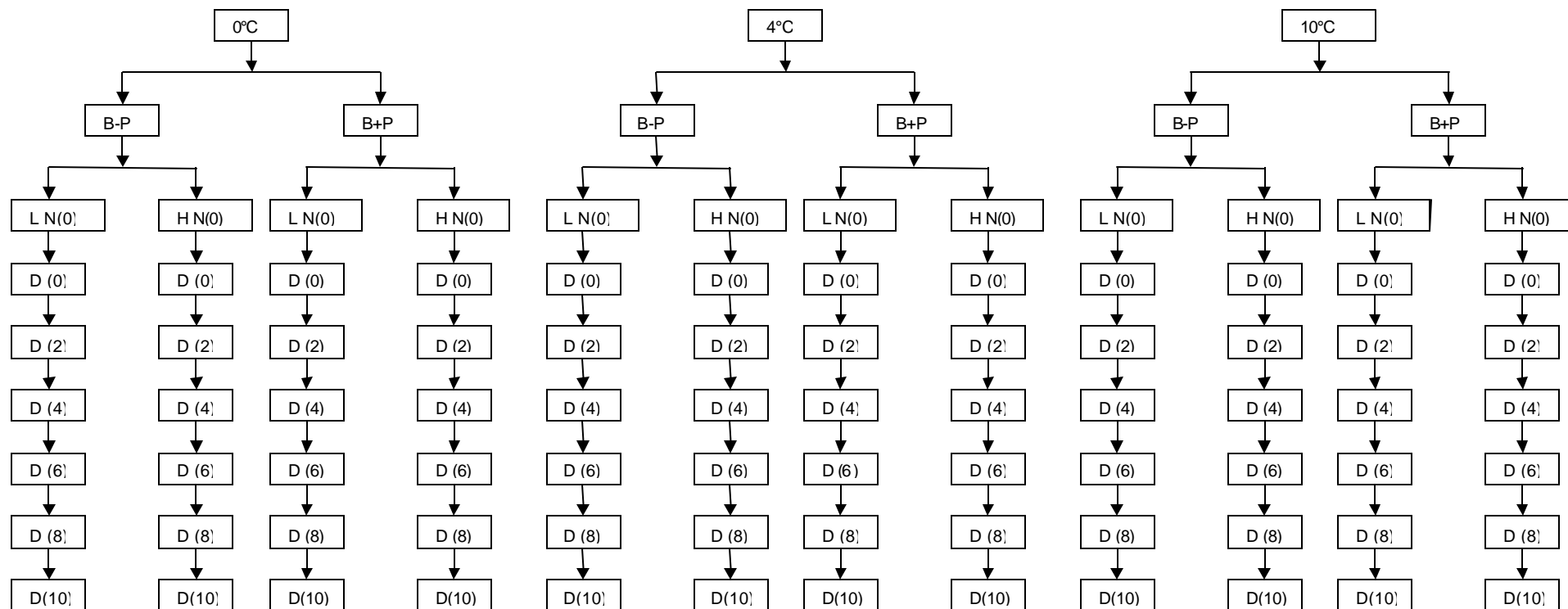
B-P   Boerewors without preservative

B+P   Boerewors with preservative

L N(0)   Low inoculum (3.5 log cfu/g)

H N(0)   High inoculum (7.5 log cfu/g)

D   Day

Fig. 4.1. Outlay of the experimental design

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Each of the 72×99g of boerewors samples without and samples with preservative in stomacher bags (Nasco Whirl-Pak™) was inoculated with 1 ml of low inoculum suspension to give a final organism concentration of about 10^3 cfu/g while each of the 72×99g boerewors without and boerewors with preservative was inoculated with 1 ml of high inoculum to give a final concentration of 10^7 cfu/g. The addition of 1 ml of the suspension allowed for raising the total amount of water added to the formulation to 3.4% without affecting the overall water activity of the boerewors. The stomacher bag contents were hand mixed for 30 seconds and homogenised for 4 minutes using a stomacher (Lab Blender 400, Art Medical Equipment). Serial dilutions were made in 0.1% BPW followed by spread plating in duplicate of 0.1 ml aliquots on fluorocult *E. coli* O157:H7 agar (Merck 1.04036).

All the above investigations were repeated once. An average of 8 plate readings per sampling point were used to represent the mean number of organisms recovered.

4.2.6 Determination of *E. coli* O157:H7 thermal inactivation end point

The sample used was boerewors with sulphite preservative prepared according to the procedure described previously in section 4.2.1 of this chapter. The inoculum was prepared according to the procedure described previously in section 4.2.5 of this chapter. The inoculum at McFarland density number 1 had counts determined to be about 10^9 cfu/ml.

The processing temperatures were 50 °C, 60 °C, 65 °C and 70 °C. For each of the four temperatures, 2×99g samples in stomacher bags (Nasco Whirl-Pak™) were spiked with 1 ml of the inoculum to give a final organism concentration of about 10^7 cfu/g. The stomacher bag contents were hand mixed for 30 seconds and homogenised for 4 minutes using a stomacher (Lab Blender 400, Art Medical Equipment). The samples were further packaged aseptically in 10g amounts into stomacher bags (Nasco Whirl-Pak). Three representative stomacher bags were fitted with thermometers embedded into the sample to allow for temperature monitoring. The bags were sealed using masking tape before being submerged in a water bath (Memmert, 854, Germany).

The water bath was first allowed to stabilise at the desired temperature ($\pm 1^{o}$C) and the samples were placed into the water bath with the level of the water being above the sample level inside the stomacher bags. The processing times were measured from the time the core temperature of the test substrate reached the target temperature (<3 minutes).

Once the desired temperature and exposure time were reached, duplicate samples were withdrawn at regular intervals during the heat treatment and immediately cooled to 0 ^{o}C (<5 minutes) in ice water. Samples were withdrawn as follows: at 0, 10, 20, 25, 30, 45, 60, 90 and 120 minutes for samples treated at 50 ^{o}C and 60 ^{o}C; and at 0, 20, 30, 40, 60, 80, 90 and 120 seconds for samples treated at 65 ^{o}C and 70 ^{o}C.

The cooled samples were homogenised for 2 minutes in 90 ml of MacConkey broth (Difco 0020-01) using a stomacher (Lab Blender 400, Art Medical Equipment) and enriched for 24 hours at 37 ^{o}C. The enriched samples were serially diluted (1:10) in 0.1% BPW and surface plated in duplicate (0.1 ml aliquots) on fluorocult *E. coli* O157:H7 agar (Merck 1.04036). The plates were incubated at 37 ^{o}C for 24 hours. Typical *E. coli* O157:H7 colonies were enumerated. Confirmation of *E. coli* O157:H7 colonies was done using the slide agglutination test (Mast Assure™; Mast Diagnostics).

4.2.7 Statistical analysis

The microbial counts obtained were transformed to log cfu/g and subjected to analysis of variance (ANOVA; NCSS, 2004). The interactions between storage temperature $\times$ time and inoculum size $\times$ level of preservative $\times$ time were further investigated by means of the Tukey-Kramer multiple comparison test (NCSS, 2004).

4.3 RESULTS AND DISCUSSION

4.3.1 Quality of the manufactured boerewors models

No *E. coli* O157:H7 was recovered from the pork (50/50), beef (70/30), boerewors without and boerewors with preservative. Table 4.2 shows the quality of the boerewors models prepared with and without sulphite preservative. Boerewors without preservative gave a mean *E. coli* count of 1.75×10^2 cfu/g. This was higher than the *E. coli* count obtained for boerewors with preservative which gave a mean count of 1.00×10^2 cfu/g.

According to Brazil's recommended quality criteria for finished products, boerewors with preservative satisfied the requirement for good quality which stipulates a maximum *E. coli* count of 1.00×10^2 cfu/g while the boerewors without preservative failed marginally to satisfy the acceptable quality range (Shapton and Shapton, 1991).

Boerewors without preservative gave a mean APC of 2.88×10^5 cfu/g. This was higher compared to that obtained from boerewors with preservative which had a mean APC of 8.15×10^4 cfu/g (Table 4.2). Both models were suitable for consumption according to Brazil's recommended quality limit of 1.00×10^6 cfu/g for APC (Shapton and Shapton, 1991). The mean APC's for both boerewors with and without preservative were similar to the range of 1.00×10^5 cfu/g to 3.16×10^6 cfu/g observed by Ajjarapu and Shelef (1999) in ground beef at retail outlets. However, Coleman et al. (2003) reported a lower APC range of 5.01×10^3 cfu/g to 1.26×10^4 cfu/g for fresh ground beef sampled at a grinder.

The mean coliform counts of 4.67×10^2 cfu/g and 1.13×10^2 cfu/g were obtained for boerewors without and with preservative respectively (Table 4.2). Even though boerewors with preservative had a lower count compared to boerewors without preservative, neither product satisfied Brazil's recommended coliform maximum quality limit of 1.00×10^2 cfu/g (Shapton and Shapton, 1991).

Table 4.2
Quality of pork/beef boerewors without preservative and boerewors with preservative

	Boerewors without preservative (cfu/g)	Boerewors with preservative (cfu/g)
E. coli O157:H7	ND	ND
Generic *E. coli*	1.75×10^2	1.00×10^2
Aerobic plate count	2.88×10^5	8.15×10^4
Coliforms	4.67×10^2	1.13×10^2
Yeasts	4.28×10^5	5.78×10^4
Moulds	ND	ND
Protein	16.53%	16.82%
Fat	30%	30%
Salt	1.26%	1.26%
pH	5.41	5.34
Water activity	0.94	0.95

ND   Not detected
-       Not tested
Counts for boerewors with and without preservative are the means of four samples analysed in duplicate

Boerewors without preservative gave a higher yeast count of 4.28×10^5 cfu/g compared to that obtained from boerewors with preservative which had a mean count of 5.78×10^4 cfu/g. Both products failed dismally in satisfying Brazil's recommended 1.00×10^2 cfu/g maximum limit for yeasts (Shapton and Shapton, 1991). No moulds were recovered from both models which satisfied Brazil's recommended 1.00×10^2 cfu/g maximum quality limit for moulds (Shapton and Shapton, 1991).

Boerewors with and without preservative models had a protein content of 16.53% and 16.82% respectively (Table 4.2). During preparation of sausage emulsions, meat proteins serve to encapsulate or emulsify fat and to bind water both of which are important for a stable product (Rust, 1987). The protein content created favourable conditions for the survival and growth of *E. coli* O157:H7. Proteins exert a cryoprotective effect by forming hydrogen bonds with microbial proteins, thus stabilising and protecting them against denaturation (Bollman et al., 2001). Both models were formulated to a 30% fat content in compliance with the South African "Regulations Governing the Composition and Labelling of Raw Boerewors, Raw Species Sausages and Raw Mixed-Species Sausage" published under Government Notice Number R.2718 of 23 November, 1990 in terms of the Foodstuffs, Cosmetics and Disinfectants Act, (Act Number 54 of 1972). Tamplin (2002) reported that the growth profile of *E. coli* O157:H7 in irradiated ground beef with fat levels of 5%, 12% and 16% showed increased exponential growth rate and maximum population density with decreasing fat levels. *Escherichia coli* O157:H7 does not possess unusual resistance to heat but a high fat content can increase its heat resistance (Bell, 2002). The boerewors models had a salt content of 1.26% which allows for the growth of *E. coli* O157:H7 since it is inhibited by a sodium chloride concentration above 9% (Garbutt, 1997). The boerewors without and with preservative models had pH values of 5.41 and 5.34, and water activities of 0.94 and 0.95 respectively. Both pH levels were suitable for growth of *E. coli* O157:H7. The pathogen can also survive in acidic foods such as apple cider and salad dressing (Cheng, 2003). *Escherichia coli* O157:H7 is only inhibited at a water activity lower than 0.93 (Garbutt, 1997).

4.3.2 Growth and survival of *E. coli* O157:H7

Table 4.3 shows the analysis of variance for the treatments of storage time, storage temperature, inoculum level and preservative as well as the interactions among these treatments in relation to *E. coli* O157:H7 and indigenous generic *E. coli* recoveries. All the treatments and interactions were significant (p<0.001) for *E. coli* O157:H7. The interactions of time × inoculum level and temperature × time were not significant (p<0.001) for generic *E. coli*. However, the interactions between time × preservative × inoculum and temperature × time × preservative × inoculum were significant (p<0.005) while the remaining interactions

between time × temperature, time × preservative, temperature × preservative, inoculum × preservative and temperature × time × preservative, were significant for generic *E. coli* (p<0.001).

Table 4.3

Analysis of variance for various treatments and interactions

Treatment	*E. coli* O157:H7	Generic *E. coli*
Storage time	*	*
Storage temperature	*	*
Inoculum level	*	*
Preservative	*	*
Interactions		
Time × temperature	*	*
Time × inoculum	*	NS
Time × preservative	*	*
Temperature × inoculum	*	NS
Temperature × preservative	*	*
Inoculum × preservative	*	*
Temperature × time ×preservative	*	*
Temperture × time ×inoculum	*	NS
Temperature × preservative × inoculum	*	NS
Time × preservative ×inoculum	*	**
Temperature × time × preservative × inoculum	*	**

* Significant at p<0.001

** Significant at p<0.05

NS Not significant

n = 288

4.3.2.1 Effect of the various treatments at different temperatures

4.3.2.1.1 Temperature 0 $^{\circ}$C

Fig. 4.2 shows the survival of *E. coli* O157:H7 inoculated into boerewors without and with preservative at low inoculum (3.5 log cfu/g) and a high inoculum (7.5log cfu/g) and stored at 0 $^{\circ}$C for 10 days. For both low and high inoculum levels, *E. coli* O157:H7 recoveries at day 0 were less for the boerewors with preservative models compared to boerewors without preservative models by a mean of 1.96 log cfu/g for and 2.1 log cfu/g respectively. This suggested that the significant lethal component was the sulphur dioxide preservative which had an immediate impact on the survival of the *E. coli* O157:H7. Sulphur dioxide is used at a concentration of 450 mg/kg to inhibit enterobacteria in high pH products like sausages (Garbutt, 1997). Sulphur dioxide and metabisulphite work best in acid environments. Sodium metabisulphite produces sulphur dioxide in an acidic environment outside the cell. The sulphur dioxide enters the cell by diffusion and it reacts with water to form sulphite ions:

$$Na_2S_2O_5 \; ? \;\; SO_2 \; ? \;\; \text{\small DIFFUSION INTO CELL} \; ? \;\; SO_2 + H_2O \; ? \;\; HSO_3^- + H^- \; ? \;\; SO_3^{2-} + 2H^+$$

The sulphite ions produced inside the cell are highly reactive reducing agents since they possess a lone pair of electrons. They form bonds with a wide range of cellular compounds and they are also capable of reacting with oxygen producing free radicals (Garbutt, 1997). Sulphite ions interfere with energy production systems, intermediate metabolism, protein synthesis, DNA replication and activities of the cell membrane. They are capable of forming disulphide bonds that result in conformational changes in enzymes. In yeasts, sulphite ions destroy enzyme cofactors resulting in inhibition of fermentation. The primary reason for the use of sulphite appears to be its antioxidant properties which result in preservation of colour but its slowing down or prevention of growth of potential pathogens makes meats safer and preserves their odour (Ough, 1993).
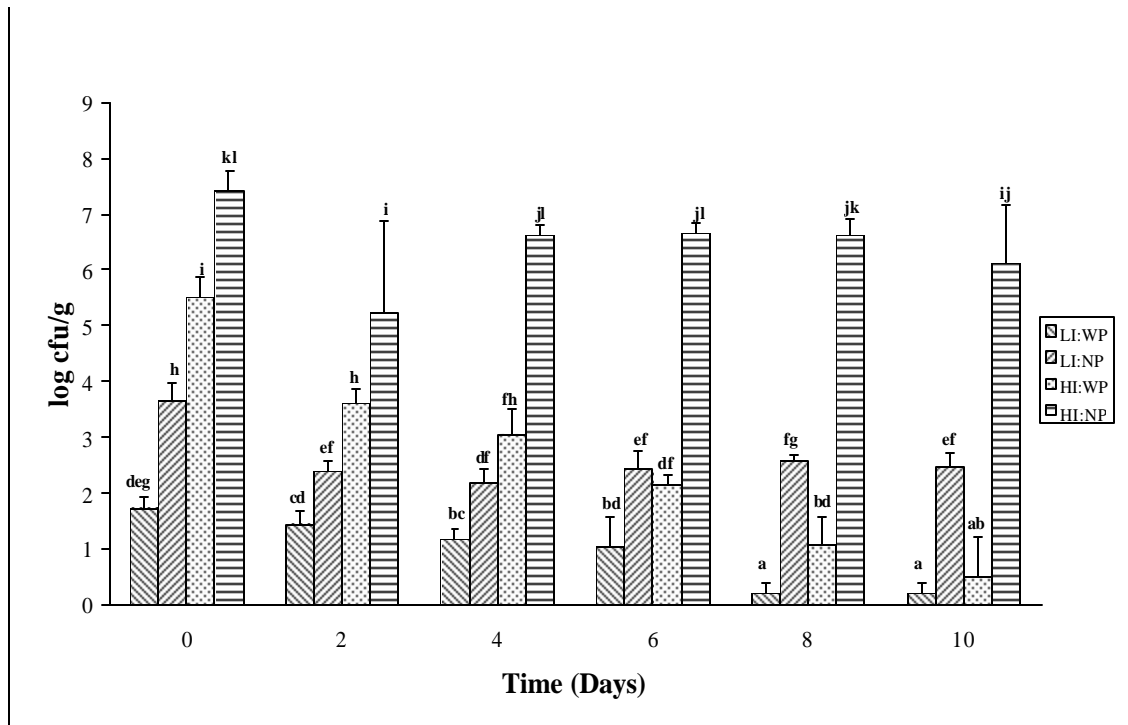
Fig. 4.2. Survival of *Escherichia coli* O157:H7 inoculated into boerewors with and without preservative at a low inoculum (3.5 log cfu/g) and a high inoculum (7.5 log cfu/g) and stored at 0°C for 10 days. LI:WP, low inoculum with preservative; LI:NP, low inoculum without preservative; HI:WP, high inoculum with preservative; HI:NP: high inoculum without preservative. Results with different superscripts are significantly different (p<0.001). Bars represent standard deviations. n = 288.

The treatments of low inoculum without and with preservative and high inoculum without and with preservative had significant reductions (p<1.001) in surviving *E. coli* O157:H7 of 1.32 log cfu/g, 0.28 log cfu/g, 0.94 log cfu/g and 1.95 log cfu/g respectively by day 2. The reductions observed in this study could be attributed to the presence of microbial hurdles incorporated in the boerewors models. The hurdle technology uses a combination of suboptimal growth conditions, sometimes in combination with chemical preservatives, in which each factor on its own is insufficient to prevent the growth of spoilage organisms or pathogens but the combination gives effective control (Garbutt, 1997). Notable microbial hurdles in the boerewors without preservative were vinegar (1.5%), ascorbic acid (62.308

mg/kg) and sodium chloride (1.26%) leading to reduced pH (5.41) and water activity (0.94). In addition to that, boerewors with preservative contained sulphur dioxide at 450 mg/kg as a chemical preservative.

From day 4 to day 10, recoveries from both low inoculum and high inoculum without preservative remained mostly at the same levels of mean log cfu/g of 2.52 and 6.76 respectively. This is in agreement with Doyle and Schoeni (1984) who showed that *E. coli* O157:H7 strain 932 was able to survive in frozen ground beef patties for nine months with little decline in numbers. There was an overall decline in cell numbers for both the low and high inocula without preservative which stood at 1.21 log cfu/g and 1.44 log cfu/g respectively over the 10 day storage period. Similar results were obtained by Ajjarapu and Shelef (1999) who reported approximately 1 log cfu/g reduction for *E. coli* O157:H7 in ground beef stored at 2 ^{o}C for 18 days. A study by Ansay et al. (1999) found a slightly higher decrease of 1.9 log cfu/g after inoculating ground beef patties with a four strain mixture of *E. coli* O157:H7 and storage at 2 ^{o}C for 4 weeks. In contrast, Barckocy-Gallagher et al. (2002) reported small but significant losses in cell numbers by day 14 at 1 ^{o}C with reduction of 0.3 log cfu/g and 0.6 log cfu/g for two of the six *E. coli* O157:H7 strains studied. Recoveries for the remaining four strains remained generally constant throughout the 14 day storage period.

There was a steady decline in *E. coli* O157:H7 numbers for the low inoculum with preservative model while that for the high inoculum with preservative model was more dramatic. Both decreases were significant (p<0.001; Table 4.3). Counts fell to below detectable limits at days 8 and 10 for the low and high inocula with preservative models respectively. The effect of preservative on high inoculum compared to low inoculum is similar to observations by Graves-Delmore et al. (1998) and Pohlman et al. (2002) who reported that decontamination treatments were more effective in reducing bacterial numbers when the initial contamination levels were high.

Direct comparison of the results obtained in this study with literature is not possible because of the types of ground meat products, media and methods, storage time and temperature, and different strains investigated.

4.3.2.1.2 Temperature 4 $^{\circ}$C

Figure 4.3 shows the survival of *E. coli* O157:H7 inoculated into boerewors without and with preservative at low and high inoculum and stored at 4 $^{\circ}$C for 10 days. A similar trend was observed during the first 2 days at the storage temperature of 4 $^{\circ}$C as for the storage temperature of 0 $^{\circ}$C. For both low and high inoculum, recoveries of *E. coli* O157:H7 at day 0 were significantly less (p<0.001; Fig. 4.3) by a mean of 2.17 log cfu/g for the boerewors with preservative models compared to boerewors without preservative. This also suggested that the preservative had an immediate lethal effect on *E. coli* O157:H7 survival. The treatments of low inoculum without and with preservative and high inoculum without and with preservative had significant reductions (p<0.001) in surviving *E. coli* O157:H7 of 1.24 log cfu/g, 1.36 log cfu/g, 1.40 log cfu/g and 0.65 log cfu/g respectively by day 2. These reductions could be attributed to the presence of the microbial hurdles of vinegar, sodium chloride and low storage temperature and sulphur dioxide.

After day 2 the low and high inocula without preservative increased significantly by 1.43 log cfu/g to 3.61 log cfu/g and by 1.66 log cfu/g to 7.70 log cfu/g respectively by day 10. The apparent increase in counts at 4 $^{\circ}$C may be due to an increase in number of original cells regaining viability. Smaller increases were obtained by Barkocy-Gallagher et al. (2002). They investigated the fate of field-isolated *E. coli* O157:H7 in ground beef at different storage temperatures and reported slight increases in cell numbers of *E. coli* O157:H7 strains 114AC1, 299AB3 and ATCC 43895 in ground beef stored at 4 $^{\circ}$C but the increases did not reflect any meaningful growth. The low and high inoculum without preservative had marginally higher recoveries at day 10 compared to day 0 by 0.19 log cfu/g and 0.26 log cfu/g respectively. These increases were not significant (p<0.001) and did not represent growth, defined as more than 1 log cfu/g increase in numbers by 250 hours of incubation (Buchanan et al., 1993).

Escherichia coli O157:H7 counts for boerewors with preservative inoculated at low inoculum declined to below detectable limits at day 10 while those inoculated at high inoculum declined significantly (p<0.001) to a mean of 1.06 log cfu/g at day 10 implying that, in the presence of preservative and at low temperature, contamination at a lower level is inactivated in fewer days compared to higher contamination levels.
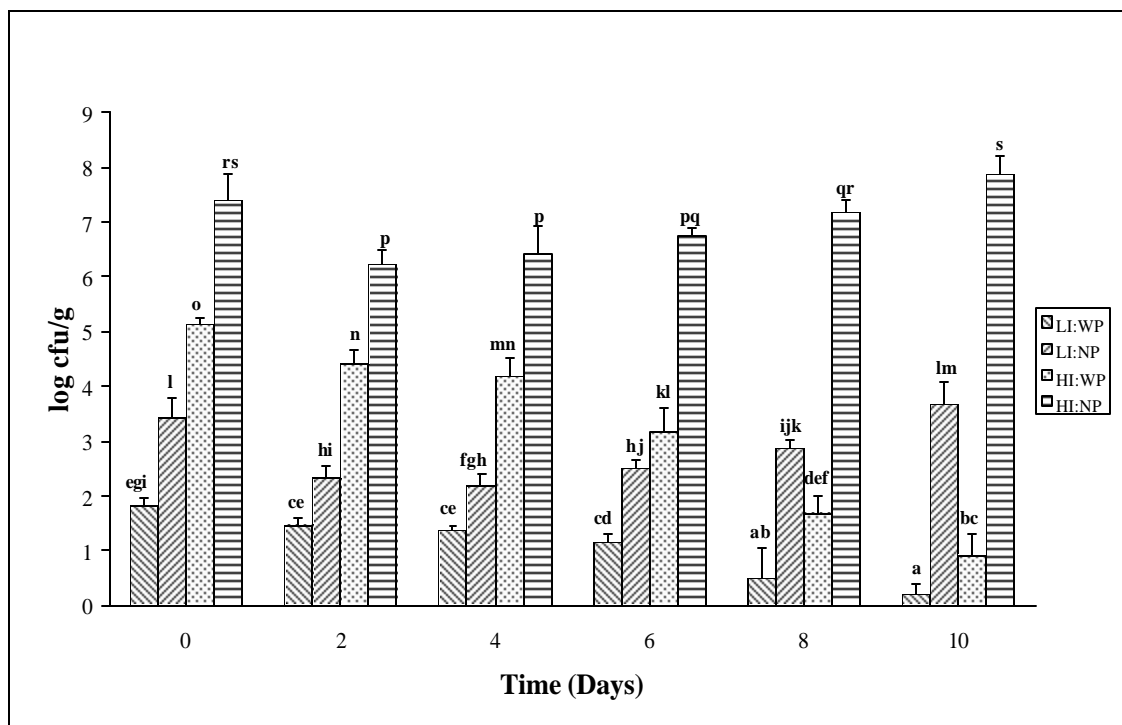
Fig. 4.3. Survival of *Escherichia coli* O157:H7 inoculated into boerewors with and without preservative at a low inoculum (3.5 log cfu/g) and a high inoculum (7.5 log cfu/g) and stored at 4°C for 10 days. LI:WP, low inoculum with preservative; LI:NP, low inoculum without preservative; HI:WP, high inoculum with preservative; HI:NP: high inoculum without preservative. Results with different superscripts are significantly different (p<0.001). Bars represent standard deviations. n = 288.

4.3.2.1.3 Temperature 10°C

The growth and survival of *E. coli* O157:H7 in boerewors without and with preservative at low and high inoculum stored at 10 ^{o}C is depicted in Fig. 4.4. A similar trend was observed at 10 ^{o}C compared to the trends observed at 0°C and 4 ^{o}C at day 0. For both boerewors with low and high inoculum, *E. coli* O157:H7 recoveries were significantly less (p<0.001) by a

mean of 2.11 log cfu/g for the boerewors with preservative models compared to the boerewors without preservative models.

Similar to the results obtained at 0 $^{\circ}$C and 4 $^{\circ}$C on the second day of storage, decreases of 1.25 log cfu/g, 1.09 log cfu/g, 0.30 log cfu/g and 0.89 log cfu/g were observed for low and high inoculum without preservative and low and high inoculum with preservative stored at 10 $^{\circ}$C respectively. These results were similar to those obtained by Barkocy-Gallagher et al. (2002). They examined the fate of field isolated *E. coli* O157:H7 in ground beef (without preservative) at different storage temperatures over a 14 day period. They reported most of the loss in cell viability within the first day at an incubation temperature of 10 $^{\circ}$C.
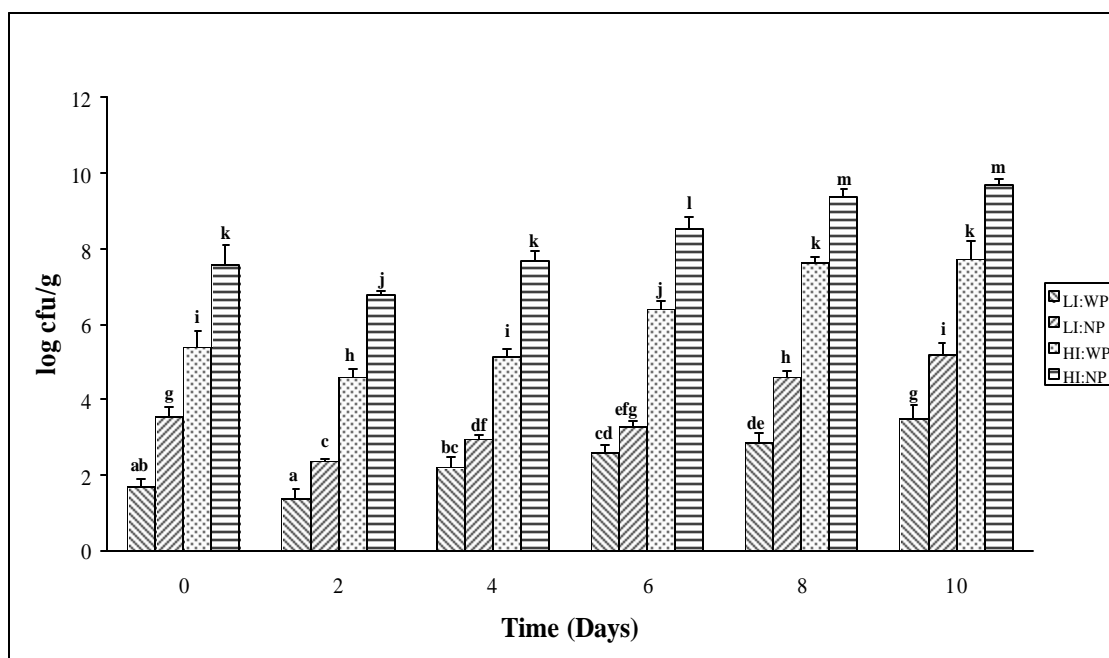
Fig. 4.4. Survival of *Escherichia coli* O157:H7 inoculated into boerewors with and without preservative at a low inoculum (3.5 log cfu/g) and a high inoculum (7.5 log cfu/g) and stored at 10 $^{\circ}$C for 10 days. LI:WP, low inoculum with preservative; LI:NP, low inoculum without preservative; HI:WP, high inoculum with preservative; HI:NP: high inoculum without preservative. Results with different superscripts are significantly different (p<0.001). Bars represent standard deviations. n = 288.

All the boerewors treatments studied at 10 ^{o}C resulted in significant (p<0.001) increases in recoveries at day 10 compared to day 0 by 1.65 log cfu/g, 1.85 log cfu/g and 2.35 log cfu/g respectively. Barkocy-Gallagher et al. (2002) also reported that six *E. coli* O157:H7 strains inoculated into ground beef and stored at 7 ^{o}C for 14 days, all demonstrated significant and substantial growth by day 14. One of the six *E. coli* O157:H7 strains (strain 114AC1) demonstrated significant growth before day 7. Variations in strain to strain increases was, however, not significant.

A study by Palumbo et al. (1997) on *E. coli* strains 1558 and 1772 were inoculated into ground beef (with starting background flora equal to the starting count of *E. coli*) and held at 10 ^{o}C, 12 ^{o}C and 15 ^{o}C. Both strains increased at all three of the temperatures after 14 days. Growth of *E. coli* O157:H7 in other foods at comparatively low temperatures has been reported. Abdul-Raouf et al. (1993) reported growth of the pathogen in lettuce and cucumber stored at 12 ^{o}C while Zhao et al. (1993) observed growth at 8 ^{o}C of the organism in apple cider, an acidic product that was earlier generally regarded as safe from VTEC O157. Kasrazadeh and Genigeorgis (1995) reported a minimum growth temperature for *E. coli* O157:H7 of 10 ^{o}C at pH 6.6 in Hispanic type cheese (Queso Fresco) stored under vacuum at temperatures ranging from 8 ^{o}C to 30 ^{o}C.

4.3.2.2 Effect of temperature on various treatments

4.3.2.2.1 Low inoculum without preservative

Fig. 4.5 shows the effect of temperature (0 ^{o}C, 4 ^{o}C and 10 ^{o}C) on the growth and survival of *E. coli* O157:H7 inoculated at a low inoculum in boerewors without preservative. The mean initial recovery for all three of the temperatures was 3.65 log cfu/g and it declined significantly (p<0.001) to 2.38 log cfu/g at day 2. The effect of temperature was more pronounced from day 2 to day 10.

The organism only maintained viability at 0 ^{o}C and had a mean of 2.52 log cfu/g at day 10. This was 1.21 log cfu/g lower than the recovery obtained at day 0.

An analysis of variance showed that there were no significant differences among days 2, 4, 6, 8 and 10 recoveries at 0°C. However, there was a significant (p<0.001) decline of 1.32 log cfu/g between day 0 and day 2 recoveries. There were no significant differences (p<0.001) among day 0 counts at 0 °C, 4 °C and 10 °C as well as among day 2 counts at the three storage temperatures.

There was a significant decline (p<0.001) of 1.24 log cfu/g at 4 °C between day 0 and day 2 counts. Counts from days 2, 4, and 6 did not differ significantly from each other but there was a significant (p<0.001) increase in counts from day 8 to day 10. The recovery of 3.8 log cfu/g at day 10 did not differ significantly (p<0.001) from day 0 recovery.
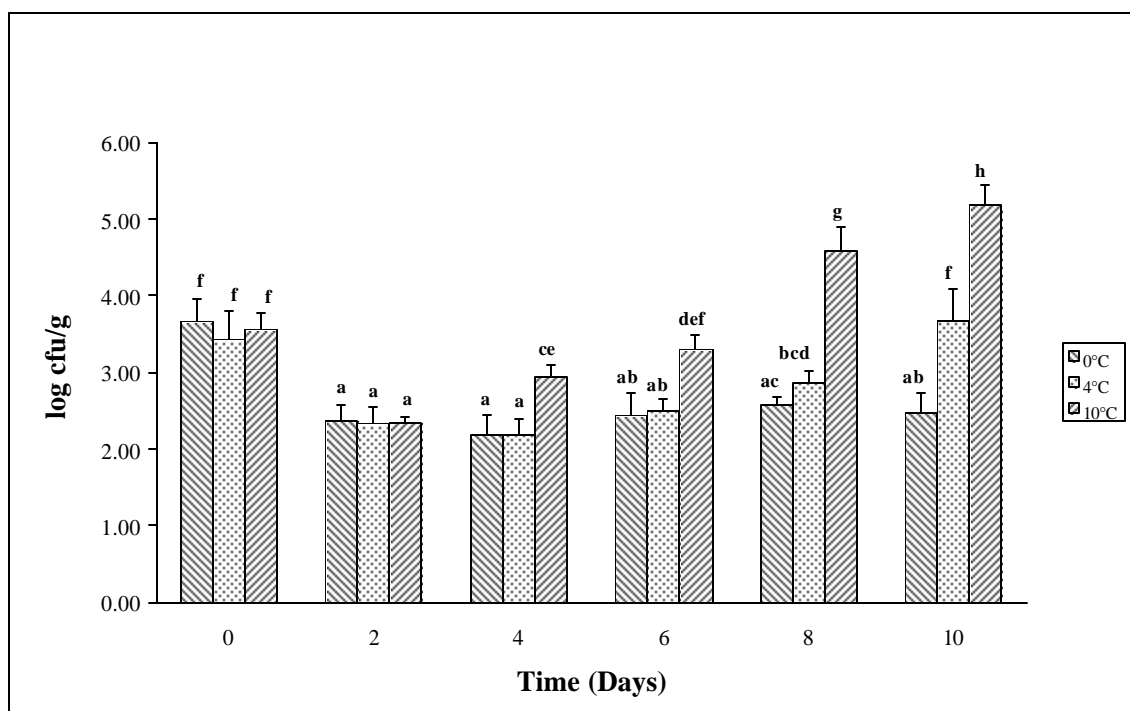

Fig. 4.5. The effect of storage temperature (0 °C, 4 °C and 10 °C) on the growth and survival of *E. coli* O157:H7 in boerewors without preservative at a low inoculum level (3.5 log cfu/g). Results with different superscripts are significantly different (p<0.001). Bars represent standard deviations. n = 288.

At 10 $^{\circ}$C, the recovery of 3.61 log cfu/g at day 0 declined significantly (p<0.001) by 1.25 log cfu/g at day 2. There was a significant (p<0.001) increase of 0.64 log cfu/g from day 2 to day 4 but even though there was an increase of 0.32 log cfu/g from day 4 to day 6, this was not significant (p<0.001). Days 8 and 10 had increased recoveries of 4.65 log cfu/g and 5.26 log cfu/g respectively and these differed significantly (p<0.001) between each other and also between day 0 recoveries.

4.3.2.2.2 Low inoculum with preservative

In contrast, the effect of temperature (0°C, 4°C and 10 $^{\circ}$C) on the growth and survival of *E. coli* O157:H7 in boerewors with preservative inoculated at a low inoculum resulted in reduced counts throughout the 10 day storage period (Fig. 4.6). The mean recovery for all three temperatures compared to the mean recovery obtained for low inoculum without preservative at day 0 declined significantly by 1.87 log cfu/g to 1.78 log cfu/g. Mean recoveries further declined by 0.31 log cfu/g to 1.47 log cfu/g at day 2. This decrease was not significant (p<0.001). Further decreases of 0.20 log cfu/g and 0.10 log cfu/g were noted at 0 $^{\circ}$C and 4 $^{\circ}$C respectively but the differences were not significant (p<0.001). Thereafter recoveries at 0°C and 4 $^{\circ}$C declined significantly (p<0.001) to below detectable limits at day 8 and day 10 respectively. Recoveries at 10 $^{\circ}$C increased steadily from day 4 to day 10. There was no significant difference in counts between days 4 and 6, and 6 and 8. Day 10 gave a mean count of 3.59 log cfu/g and it differed significantly from all the other days.

4.3.2.2.3 High inoculum without preservative

Fig. 4.7 depicts the effect of temperature (0°C, 4°C and 10 $^{\circ}$C) on the growth and survival of *E. coli* O157:H7 inoculated in boerewors without preservative at a high inoculum. The mean recovery of *E. coli* O157:H7 for all three of the temperatures at day 0 was 7.76 log cfu/g and this decreased slightly by 1.14 log cfu/g to 6.62 log cfu/g at day 2. There was a consistent but marginal decline at 0 $^{\circ}$C leading to a mean recovery of 6.28 log cfu/g at day 10. The recoveries from day 0 to day 8 gave differences that were not significant (p<0.001) but day 10 recovery was significantly lower (p<0.001) compared to days 0 to 8. *Escherichia coli* O157:H7 counts at 4 $^{\circ}$C increased slightly to a mean of 7.96 log cfu/g at day 10. This was 0.26 log cfu/g more than the day 0 recovery but the difference was not significant.

Fig. 4.6. The effect of storage temperature (0 ℃, 4 ℃ and 10 ℃) on the growth and survival of *E. coli* O157:H7 in boerewors with preservative at a low inoculum level (3.5 log cfu/g). Results with different superscripts are significantly different (p<0.001). Bars represent standard deviations. n = 288.

Recoveries of the organism at 10 ℃ increased to a mean of 9.72 log cfu/g by day 10. This increase of 1.85 log cfu/g compared to day 0 recovery was significant (p<0.001) and represented actual growth at this temperature. This is in agreement with other researchers who showed that *E. coli* O157:H7 is capable of growth at 10 ℃ in broth models (Palumbo et al., 1995; Coleman et al., 2002), as well as in ground beef models (Palumbo et al., 1997; Barkocy-Gallagher et al., 2002; Tamplin, 2002).
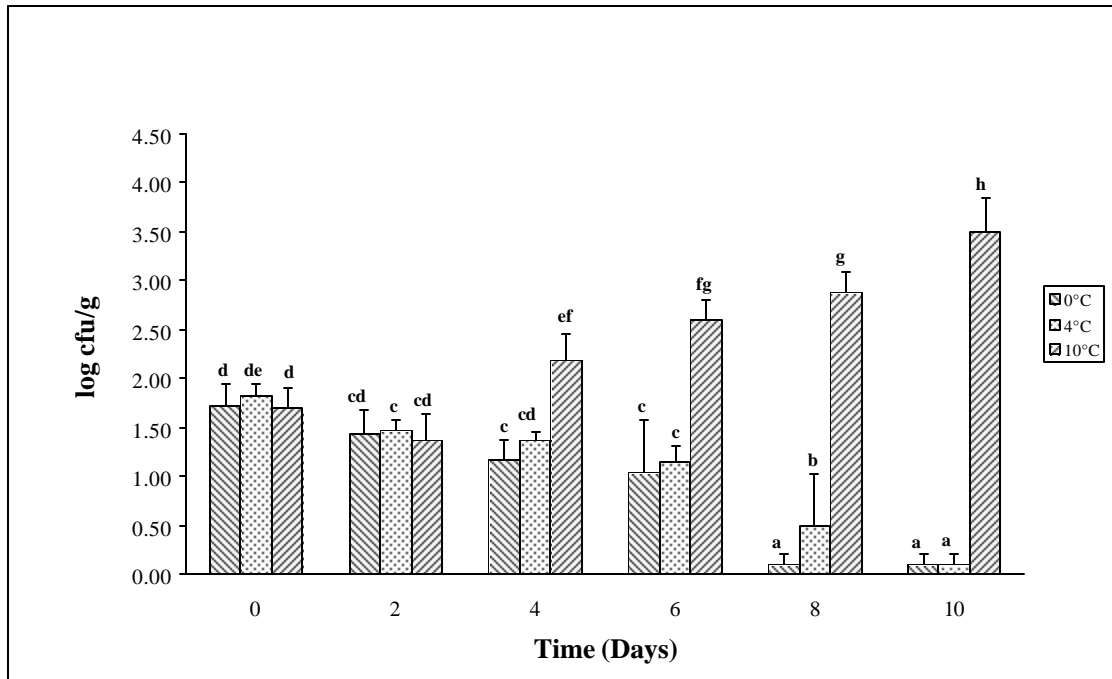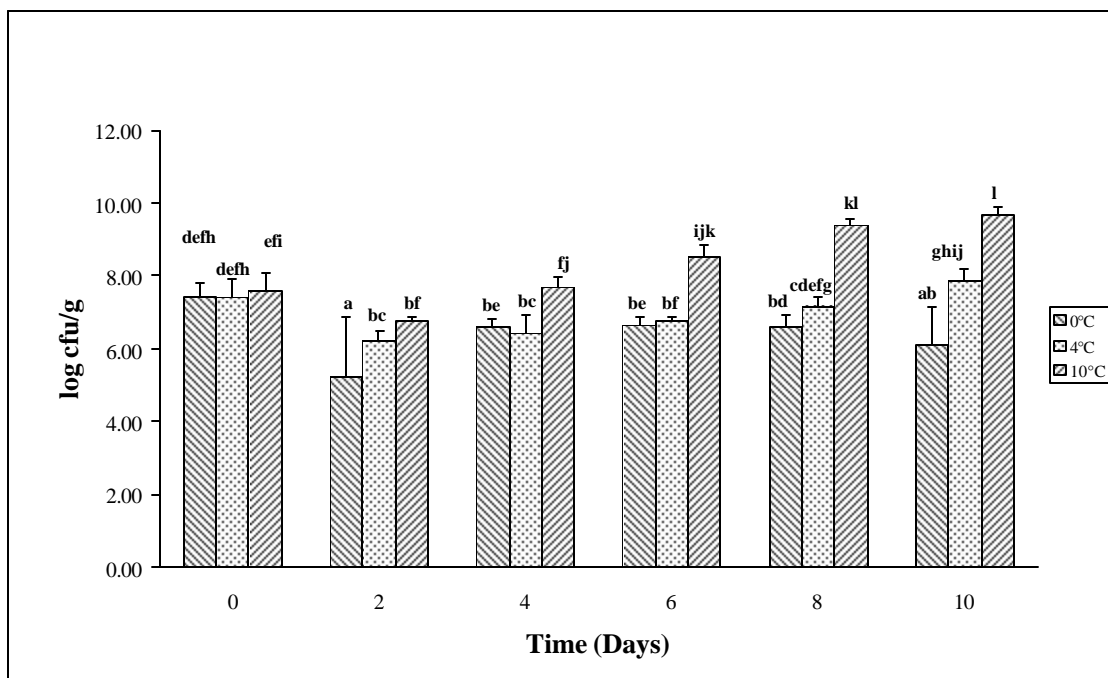
Fig. 4.7. The effect of storage temperature (0 °C, 4 °C and 10 °C) on the growth and survival of *E. coli* O157:H7 in boerewors without preservative at a high inoculum level (7.5 log cfu/g). Results with different superscripts are significantly different (p<0.001). Bars represent standard deviations. n = 288.

4.3.2.2.4 High inoculum with preservative

Fig. 4.8 shows the effect of temperature (0 °C, 4 °C and 10 °C) on the growth and survival of *E. coli* O157:H7 inoculated in boerewors with preservative at a high inoculum. The mean recovery of the organism at day 0 for all three of the temperatures was 5.43 log cfu/g. There was a consistent and significant (p<0.001) decline in counts at 0 °C and 4 °C resulting in below detectable limits and 1.01 log cfu/g respectively at day 10. In contrast, the organism's recovery at 10 °C increased significantly (p<0.001) to a mean of 7.88 log cfu/g. This affirmed that elevated temperatures cause *E. coli* O157:H7 to multiply in boerewors even in the presence of preservative. Alternatively, the combination of low temperatures (= 4 °C) and sulphur dioxide preservative provides microbial hurdles which are sufficient to arrest the growth and inactivate *E. coli* O157:H7 in boerewors.
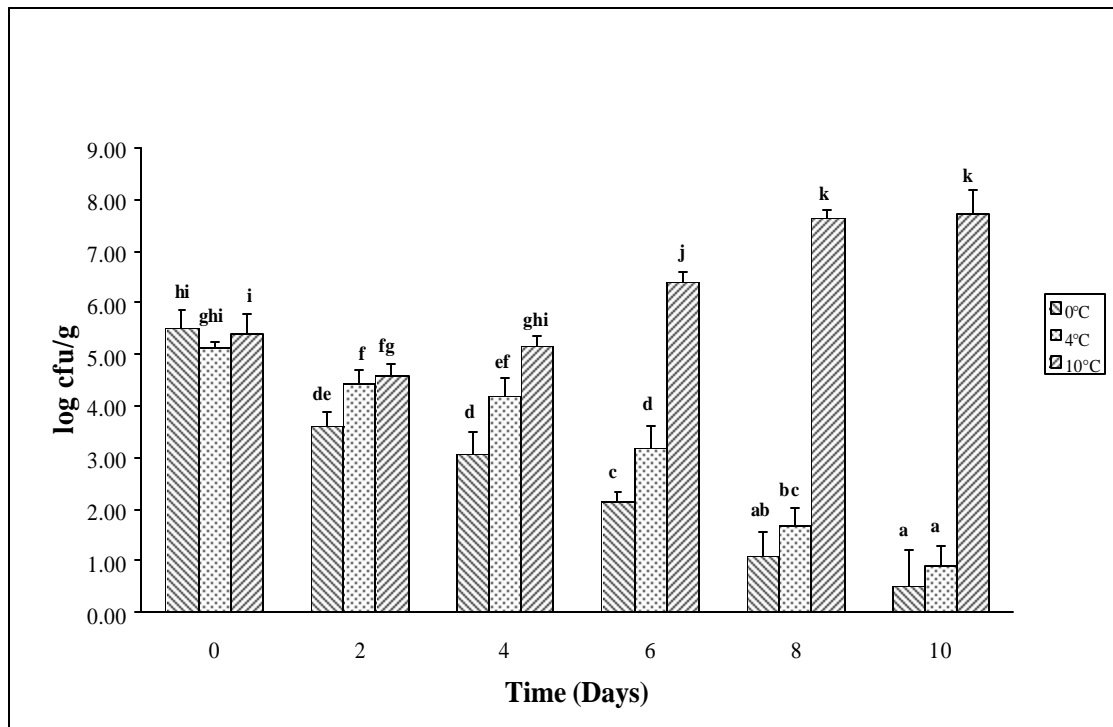
Fig. 4.8. The effect of storage temperature (0 °C, 4 °C and 10 °C) on the growth and survival of *E. coli* O157:H7 in boerewors with preservative at a high inoculum level (7.5 log cfu/g). Results with different superscripts are significantly different (p<0.001). Bars represent standard deviation. n = 288.

Even though this study focussed on the growth and survival of *E. coli* O157:H7 in boerewors, indigenous generic *E. coli* were also investigated and will now be briefly discussed.

4.3.3 The growth and survival of indigenous generic *E. coli*

The survival of indigenous generic *E. coli* which contaminated the boerewors models without and with preservative, and which were inoculated at a low and a high inoculum with *E. coli* O157:H7 and stored at 0 °C, 4°C and 10 °C is shown in Table 4.4. The mean recoveries of *E. coli* at day 0 from boerewors without preservative inoculated with a low *E. coli* O157:H7 inoculum were 1.62 log cfu/g, 2.65 log cfu/g and 1.48 log cfu/g at 0 °C, 4 °C and 10 °C

respectively. At day 10, recoveries at 0°C and 4°C decreased by 0.61 log cfu/g and 1.12 log cfu/g to 1.01 log cfu/g and 1.53 log cfu/g respectively while the recovery at 10 °C increased steadily by 1.32 log cfu/g to 2.80 log cfu/g.

The mean recoveries of *E. coli* at day 0 from boerewors without preservative inoculated with a high *E. coli* O157:H7 inoculum were 1.48 log cfu/g, 1.40 log cfu/g and 1.44 log cfu/g at 0 °C, 4 °C and 10 °C respectively. At day 10, the recovery at 0°C declined by 0.48 log cfu/g to 1.00 log cfu/g and the recovery at 4°C increased marginally by 0.11 log cfu/g to 1.51 log cfu/g while the recovery at 10°C increased by 0.86 log cfu/g to 2.30 log cfu/g.

Higher storage temperature (10°C) resulted in higher *E. coli* recoveries at day 10 compared to day 0 for both low and high inoculum without preservative treatments. There was a steady decline in *E. coli* recoveries at 0°C and 4°C for both treatments with increased storage time. At 10 °C, both treatments had a slight decrease in *E. coli* recoveries at day 2 compared to day 0, followed by a gradual increase in recoveries from day 4 to fay 10.

Boerewors without preservative spiked with *E. coli* O157:H7 at a low initial inoculum level mainly gave higher *E. coli* recoveries compared to boerewors without preservative spiked with *E. coli* O157:H7 at a high inoculum level. The reason for that might stem from the fact that bacterial growth at suboptimal conditions, such as low temperature and low pH, has been shown to be dependent on initial density and communication between cells (Kaprelyants and Kell, 1996). Inoculating the boerewors with a higher initial inoculum of *E. coli* O157:H7 could have resulted in a higher number of *E. coli* O157:H7 cells that were in the appropriate physiological state to multiply (Baranyi, 1998). This could have led to the quorum sensing phenomenon and faster *E. coli* O157:H7 growth. Quorum sensing was described as the theory that links regulation of gene expression to changes in bacterial cell densities and subsequently coordinated behaviour based on threshold densities of the population of the bacterial cells (Surette et al., according to Coleman et al., 2003). The faster growing high inoculum *E. coli* O157:H7 population could then have exerted the Jameson effect on *E. coli* more than the *E. coli* O157:H7 population at low inoculum level. The Jameson effect was described as an ecological phenomenon that leads to the inhibition of slow growing organisms by the total density of all the microbial populations present in the food matrix (Ross et al., 2000).

Table 4.4
Generic *Escherichia coli* counts (log cfu/g ± standard deviation)

Day	Low inoculum, without preservative			High inoculum, without preservative			Low inoculum, with preservative			High inoculum, with preservative		
	0°C	4°C	10°C	0°C	4°C	10°C	0°C	4°C	10°C	0°C	4°C	10°C
0	1.62 ± 1.32	2.65 ± 2.83	1.48 ± 0.88	1.48 ± 0.85	1.40 ± 1.00	1.44 ± 1.37	ND	ND	ND	ND	0.33 ± 0.50	ND
2	1.38 ± 0.87	1.35 ± 0.95	1.33 ± 1.00	1.24 ± 0.55	1.30 ± 0.73	1.35 ± 1.10	3.18 ± 0.50	ND	ND	ND	ND	ND
4	1.35 ± 1.07	1.24 ± 0.85	1.48 ± 1.03	1.18 ± 1.00	1.27 ± 0.92	1.35 ± 0.85	ND	ND	ND	ND	ND	3.18 ± 0.50
6	1.42 ± 1.48	1.27 ± 1.00	1.72 ± 1.22	1.30 ± 1.01	1.27 ± 0.92	1.63 ± 1.55	ND	ND	ND	ND	ND	ND
8	1.05 ± 0.55	1.40 ± 1.08	2.01 ± 1.66	1.18 ± 0.85	1.30 ± 1.03	1.81 ± 1.42	ND	ND	ND	ND	ND	ND
10	1.01 ± 0.71	1.53 ± 1.23	2.80 ± 2.50	1.00 ± 0.50	1.51 ± 1.30	2.30 ± 2.17	ND	ND	ND	ND	ND	ND

ND   Not detected
n = 288.

Escherichia coli was generally not recovered from boerewors with preservative models inoculated with a low *E. coli* O157:H7 inoculum at all three storage temperatures except for day 2 which had a recovery of 3.18 log cfu/g. Similar observations were made for the boerewors with preservative model inoculated with a high inoculum except for day 0 at 4℃ and day 6 at 10 ℃ which had recoveries of 0.33 log cfu/g and 3.18 log cfu/g respectively. The general inactivation of the *E. coli* could be attributed largely to the presence of sulphur dioxide preservative which was very effective against the organism.

4.3.4 Thermal inactivation end point of *E. coli* O157:H7 in boerewors

Figure 4.9A shows the thermal inactivation curves for *E. coli* O157:H7 in boerewors with preservative which was cooked at 50 ℃ and 60 ℃ for 120 minutes. The data are the means of four plate readings from duplicate samples at each sampling point. *Escherichia coli* O157:H7 was recovered throughout the 120 minute cooking time at 50 ℃. The mean recovery of the organism after enrichment was 9.76 log cfu/g at time 0 and this marginally declined to 9.62 log cfu/g after 120 minutes. Enriched recoveries at 60 ℃ were generally similar to those obtained at 50 ℃ in the first 45 minutes of the heat treatment. Cooking at 60 ℃ was, however, sufficient to inactivate the pathogen after 60 minutes.

The inactivation of *E. coli* O157:H7 at 60 ℃ required 15 more minutes compared to the Advisory Committee on the Microbiological Safety of Food (ACMSF) recommended safety equivalent treatment of 60 ℃ for 45 minutes (ACMSF, according to Byrne et al., 2002a). This might stem from the observation that even though thermal inactivation of microorganisms was traditionally described as log-linear in nature, deviations from linear declines in log numbers with time have been reported as initial lag periods or shouldered death curves and tailing (Juneja et al., 1997; Jiang et al., 2003). Sub-populations of more persistent bacteria that decline at slower rates are sometimes observed but at present the actual reason for variability in death kinetics is not known (Juneja et al., 1997).

Thermal inactivation curves for *E. coli* O157:H7 in boerewors with preservative cooked at 65 ℃ and 70 ℃ for 120 seconds are shown in Fig. 4.9B. The data are the means of 4 plate readings from duplicate samples at each sampling point.
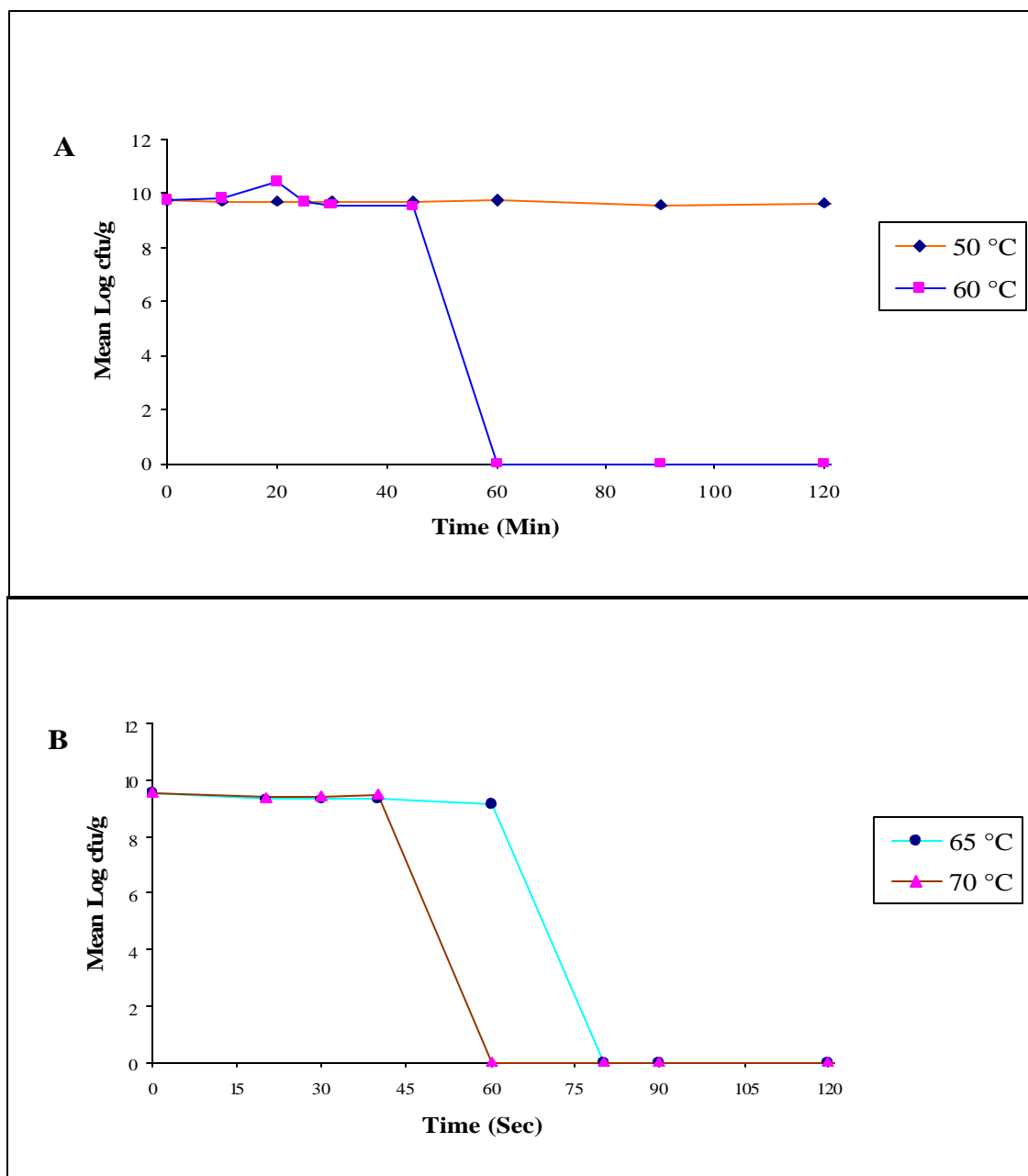
Fig 4.9. Thermal inactivation curves for *E. coli* O157:H7 inoculated at 7 log cfu/g in boerewors with preservative: (A) 50 $^{\circ}$C and 60 $^{\circ}$C; (B) 65 $^{\circ}$C and 70 $^{\circ}$C. The data are the means of four plate readings from duplicate samples at each sampling point.

The pathogen was completely inactivated after 80 seconds and 60 seconds of thermal processing at 65 $^{\circ}$C and 70 $^{\circ}$C respectively. Since the pathogen was inactivated after 1 minute at 70 $^{\circ}$C it complied with safety recommendations by the ACMSF which recommends that

minced beef and minced beef products should be heated to an internal temperature of 70 $^{\circ}$C for 2 minutes or an equivalent treatment that will result in a 6-D reduction in *E. coli* O157:H7 counts (ACMSF according to Byrne et al., 2002a; Jiang et al., 2003).

The apparent increased sensitivity to heat treatment of the *E. coli* O157:H7 in boerewors was also evident at 65 $^{\circ}$C where only 80 seconds were sufficient to inactivate the pathogen. The ACMSF recommended a heat treatment of 10 minutes at this temperature (ACMSF according to Byrne et al., 2002a). Apart from the fact that several studies have shown that *E. coli* O157:H7 does not possess unusual heat resistance (Doyle and Schoeni, 1984; Line et al., 1991; Ahmed et al., 1995), the boerewors formulation in terms of humectants and sulphur dioxide content could have made the organism more susceptible to heat treatment. In a study by Byrne et al. (2002a), *E. coli* O157:H7 showed decreased heat resistance in beef burger formulations and they concluded that product formulations should be a critical consideration for a holistic approach to the development and application of cooking processes which can inactivate the pathogen.

4.4 CONCLUSIONS

It can be concluded that *E. coli* O157:H7 can survive in boerewors. The lower the temperature, the fewer the days that the organism survived. The smaller the inoculum size, the fewer the number of days that were needed to inactivate *E. coli* O157:H7 to below detectable limits at 0 $^{\circ}$C and 4 $^{\circ}$C in the presence of preservative. In the absence of preservative, there was little change in recoveries at 0 $^{\circ}$C and 4 $^{\circ}$C inspite of inoculum size and the pathogen survived throughout the 10 days of storage. At 10 $^{\circ}$C, both inoculum levels had significant ($p<0.001$) increases in recoveries by day 10 despite the presence or absence of preservative.

The high fat content (30%) of the boerewors models did not inhibit the growth of the pathogen, possibly due to the high protein content which may have exerted a cryoprotective effect on the *E. coli* O157:H7 since proteins form hydrogen bonds with microbial proteins which stabilise and protect them against denaturation.

Sulphur dioxide added to boerewors at 450 mg/kg as sodium metabisulphite is effective against *E. coli* O157:H7 at low temperatures of 0ºC and 4ºC. The best temperature to store boerewors was 0ºC.

Temperature abuse at 10 ^{o}C allows the growth of the pathogen despite the presence of preservative and this leads to increased chances of infection. Since low *E. coli* O157:H7 inoculum levels generally resulted in increased counts for indigenous generic *E. coli* it can be concluded that higher levels of *E. coli* O157:H7 have an inhibitory effect on the levels of background *E. coli*. The preservative is very effective against background *E. coli* in boerewors at low densities and stored at or below 10 ^{o}C.

An ideal situation would be to produce a product that is completely free of *E. coli* O157:H7 contamination. While producers try to prevent contamination of products by *E. coli* O157:H7, the consumer has ultimate control through thermal inactivation during cooking. Since this study established that *E. coli* O157:H7 in boerewors can be inactivated by cooking it at 70 ^{o}C for 60 seconds, the recommended ACMSF time and temperature regime of 70 ^{o}C for 2 minutes is sufficient to make boerewors safe from the pathogen. Other temperature/time end point regimes for the organism in boerewors are 60 ^{o}C for 60 minutes and 65 ^{o}C for 80 seconds. The thermal inactivation end point regime of 70 ^{o}C for 1 minute is recommended for the South African consumer because of its efficacy in a short cooking time.

It can also be concluded that *E. coli* O157:H7 in boerewors has increased susceptibility to heat treatment at 70 ^{o}C while at 60 ^{o}C it is more resistant requiring 60 minutes for total inactivation.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Although most *E. coli* strains are harmless commensals, others are pathogenic. Virulent strains were first classified according to their serotypes in the late 1940's (Wilshaw et al., 2000). Infection with diarrhoeagenic *E. coli* (DEC) results in a spectrum of illnesses which includes infantile enteritis caused by EPEC; bacillary diarrhoea caused by EIEC; traveller's diarrhoea caused by ETEC; persistent watery diarrhoea caused by EAggEC; mucus containing watery diarrhoea caused by DAEC; haemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP) caused by VTEC. Some VTEC cases are fatal (Doyle et al., 1997; Garbutt, 1997). Huge economic losses are incurred by individuals and industries because of these illnesses (Garbutt, 1997; Abe et al., 2002). Sources of DEC include pigs, sheep, poultry, dogs, cats, humans and cattle. Cattle are the primary reservoir of DEC (Bell, 2002). Diarrhoeagenic *E. coli* are tramsmitted via person-to-person contact, person-to-animal contact, contaminated water and food. Their major route of transmission is oral through the consumption of ground beef products (Bell, 2002, Meng and Doyle, 1998).

A survey conducted in this study indicated that minced beef was contaminated with more DEC (38.10%) compared to boerewors (28.57%). A wider range of virotypes (EPEC, EIEC, EAggEC, and VTEC) was recovered from minced beef compared to boerewors (EPEC and VTEC). This may be attributed to the presence of humectants, organic acids and sulphur dioxide preservative in boerewors formulations. Consistent with Bell (2002), DEC proved to be widely distributed and there were no significant differences ($p<0.05$) that were established between DEC recoveries from both enriched and non-enriched minced beef and boerewors, and between various regions (North, South, East, West and Central) of the Bloemfontein District. Generic *E. coli,* presumptive *Salmonella enteritidis* and presumptive *Shigella sonnei* were also widely distributed. The occurrence of these organisms is an indication of poor hygiene along the food supply chain (Nel et al., 2004). Improved standards of hygiene and

implementation of programmes such as the Hazard Analysis Critical Control Point (HACCP) can go a long way in reducing product microbial load thus improving its quality and shelf-life.

Enteropathogenic *E. coli* were the most frequently isolated virotype in both minced beef and boerewors possibly indicating that the two products are the main vehicles of transmission contributing to the dominance of EPEC in patients in the Bloemfontein District. Verocytotoxin-producing *E. coli* were the most virulent virotype and they had a low recovery rate of 4.76%. The most virulent serotype isolated was *E. coli* O26. *Escherichia coli* O157:H7 was not recovered from both products. This should be reassuring to producers, retailers and consumers.

In both minced beef and boerewors without enrichment, the positive correlations between increased pH and presumptive *Salmonella enteritidis*, APC, presumptive *Shigella sonnei*, and coliform recoveries resulted in significantly ($p < 0.05$) higher recoveries of the organisms while the correlations between pH and recoveries of coliforms, *E. coli* and presumptive *Salmonella enteritidis* were not significant ($p < 0.05$). The recoveries of coliforms, *E. coli*, presumptive *Salmonella enteritidis* and presumptive *Shigella sonnei* in minced beef after enrichment were not significantly ($p < 0.05$) correlated to either A_w and pH. In boerewors after enrichment, the correlation between pH and coliform recoveries was significant ($p < 0.05$). However, no significant ($p < 0.05$) correlations were established between A_w and coliform, *E. coli,* presumptive *Salmonella enteritidis,* and presumptive *Shigella sonnei* recoveries. There were no significant ($p < 0.05$) differences that were established between APC, coliform, *E. coli*, presumptive *Salmonella enteritidis* and presumptive *Shigella sonnei* recoveries from both enriched and non-enriched minced beef and boerewors, and between various regions (North, South, East, West and Central) of the Bloemfontein District. The high levels of organisms like presumptive *Salmonella enteritidis*, are an indication of poor hygiene along the food supply chain.

It was also found that an enrichment step is necessary for the recovery of DEC that would otherwise escape detection since they generally occur in low numbers.

Observations from chapter 4 revealed that sulphur dioxide (450 mg/kg) was effective against *E. coli* O157:H7 at refrigeration temperatures (0 $^{\circ}$C and 4 $^{\circ}$C). The synergy between preservative and low temperature does not only reduce pathogen numbers making the boerewors safer, but it also helps to maintain its fresh colour and aroma (Ough, 1993). The pathogen was inactivated in fewer days when it was at a low inoculum size compared to a high inoculum size. This is good news in terms of food safety given that *E. coli* O157:H7 naturally occurs in low mumbers making it possible for the preservative to inactivate it as long as the storage temperature is kept low. This also offers a possible explanation that even if boerewors consumption (sometimes undercooked) is very high in South Africa, there are no reported outbreaks in which it was implicated as the vehicle of transmission.

Increased temperature was shown to encourage the growth of *E. coli* O157:H7 in boerewors with and without preservative and hence it is imperative for consumers to ensure that their fridges are in good working order to reduce the hazards posed by the pathogen's proliferation.

This study revealed that, in agreement with other researchers, *E. coli* O157:H7 does not have unusual heat resistance in boerewors thus providing consumers with an easier task to thermally inactivate the pathogen during cooking (Doyle and Schoeni, 1984). Boerewors is a very complex food system. The boerewors with and without preservative models had a protein content of 16.82% and 16.53% respectively. Proteins exert a cryoprotective effect and provide a medium which is conducive for the survival and growth of microorganisms (Bollman et al., 2001). Both models had a 30% fat and 2% rusk content. Tamplin (2002) reported that a decrease in fat content resulted in an increase in the exponential growth rate and maximum population density of *E. coli* O157:H7. A study by Oteiza et al. (2003), revealed that an increase in fat and starch content resulted in increased heat resistance by *E. coli* O157:H7 in "morcilla," a Spanish and Argentinian type of blood-link sausage.

Boerewors with and without preservative had pH values of 5.34 and 5.41, and A_w values of 0.95 and 0.94. According to Byrne et al. (2002), a reduction in pH causes conditions for pathogen survival to be less favourable while increased heat resistance was reported in food systems with low A_w (Kaur et al., 1998). Total inactivation of possible *E. coli* O157:H7

contaminants cannot be over emphasized since the organism has a very low infective dose (Samelis et al., 2002). The pathogen was more sensitive to heat treatment at 70 ^{o}C requiring 60 seconds for inactivation. The combined effect of boerewors ingredients and additives on the growth and survival of *E. coli* O157:H7 could have been dominated by the effect of protein content leading to the observed survival of the pathogen in the models, and the effect of preservative, vinegar and ascorbic acid content leading to a reduction in the pathogen's heat resistance. Observations from the thermal inactivation studies will be beneficial to boerewors consumers in ensuring appropriate cooking temperatures and exposure times to totally inactivate *E. coli* O157:H7.

Future research may include:

- Incidence of DEC on cattle hides, faecal samples and carcasses in abattoirs;

- Exploration of the efficacy of alternative antimicrobials to sulphur dioxide such as organic acids;

- Effect of other additives, singly or in synergy, on the sensitivity of DEC to heat treatment;

- Effect of sublethal injury of DEC on the expression of various pathogenic genes.

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CHAPTER 6

SUMMARY

Escherichia coli that cause diarrhoeal disease are classified into virotypes based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O:H serotypes. These virotypes include enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), diffuse adhering *E. coli* (DAEC) and verocytotoxin-producing *E. coli* (VTEC) also known as Shiga toxin-producing *E. coli* (STEC) which includes the most virulent subgroup, the enterohaemorrhagic *E. coli* (EHEC). The most predominant EHEC is *E. coli* O157:H7. Cattle are the principal reservoir for diarrhoeagenic *E. coli* (DEC) and ground beef products are the major vehicle for their transmission.

The aim of this study was to determine the incidence of DEC in minced beef and boerewors and to investigate the growth, survival and thermal inactivation of *E. coli* O157:H7 in boerewors. In the first part of this study, a survey was conducted on 30% of butcheries in the Bloemfontein District and isolated virotypes were identified by serotyping. More minced beef samples were positive for DEC (38.10%) compared to boerewors samples (28.57%). Enteropathogenic *E. coli* were the most frequently isolated virotype while VTEC were the most virulent virotype recovered from both samples, hence highlighting the potential of ground meat products as vehicles of transmission of DEC. An enrichment step enabled optimal recovery of DEC on chromocult™ chromogenic agar (Merck 1.10426).

In the second part of this study, boerewors with and without sulphur dioxide preservative were manufactured following a typical commercial procedure and inoculated with *E. coli* O157:H7 at low (3.5 log cfu/g) and high (7.5 log cfu/g) inoculum size, and stored at $0\,^{o}C$, $4\,^{o}C$ and $10\,^{o}C$ followed by evaluation of growth and survival every 48 hours for 10 days. Boerewors with preservative had significantly lower recoveries compared to boerewors without preservative at all the three storage temperatures. The low and high inocula with preservative had significant ($p<0.001$) reductions in recoveries at $0\,^{o}C$, declining to below

detectable limits at days 8 and 10 respectively. At 4 ^{o}C, the low and high inocula with preservative recoveries declined significantly (p<0.001) to below detectable limits and to 1.01 log cfu/g respectively at day 10. At 10 ^{o}C, significant (p<0.001) increases of 1.85 log cfu/g and 2.35 log cfu/g respectively were observed from day 0 to day 10. At 0°C, the low and high inocula without preservative treatments had declines in recoveries of 1.21 log cfu/g and 1.45 log cfu/g respectively. Insignificant (p<0.001) increases of 0.19 log cfu/g and 0.26 log cfu/g respectively were noted at 4°C while significant (p<0.001) increases of 1.97 log cfu/g and 1.84 log cfu/g respectively were noted at 10 ^{o}C from day 0 to day 10. The combination of sulphur dioxide preservative and low temperature (0 ^{o}C and 4 ^{o}C) demonstrated the best efficacy against the survival of *E. coli* O157:H7.

It was established that thermal inactivation end point temperatures of *E. coli* O157:H7 inoculated into boerewors with preservative at 7.00 log cfu/g, was 60 minutes at 60 ^{o}C, 80 seconds at 65 ^{o}C and 60 seconds at 70 ^{o}C. This study demonstrated that *E. coli* O157:H7 can survive in boerewors with and without preservative, stored at 0°C, 4°C and 10 ^{o}C and is more sensitive to heat treatment at 70°C.

Keywords: Escherichia coli O157:H7, preservative, boerewors, inoculum size, temperature, diarrhoeagenic *E. coli*, serotype.

CHAPTER 7

OPSOMMING

Escherichia coli wat diarree veroorsaak word in verotipes verdeel op grond van hul virulente eienskappe, meganismes van patogenisiteit, kliniese simptome en spesifieke O:H serotipes. Hierdie verotipes sluit die volgende in: enteropatogeniese *E. coli* (EPEC), enteroindringende *E. coli* (EIEC), enterotoksigeniese *E. coli* (ETEC), enteroaggregerende *E. coli* (EaggEC), diffuus-adherende *E. coli* (DAEC) en verositotoksien-produserende *E. coli* (VTEC) ook bekend as Shiga-toksienproduserende *E. coli* (STEC) wat die mees virulente subgroep, die enterohemorhargiese *E. coli* (EHEC) insluit. Die mees prominente EHEC is *E. coli* O157:H7. Beeste is die hoof bron van diarheageniese *E. coli* (DEC) en gemaalde beesvleisprodukte is die hoof oordragingsvektor.

Die doel van hierdie studie was om die voorkoms van DEC in gemaalde beesvleisprodukte en boerewors te bepaal en om die groei, oorlewing en termiese inaktivering van *E. coli* O157:H7 in boerewors te ondersoek. 'n Opname is uitgevoer op 30% van slaghuise in die Bloemfontein distrik en geïsoleerde verotipes is deur middel van serotipering geïdentifiseer. Meer gemaalde beesvleis monsters was positief vir DEC (38.10%) in vergelyking met boerewors monsters (28.57%). Enteropatogeniese *E. coli* was die mees geïsoleerde verotipe, terwyl VTEC die mees virulente verotipe uit beide monsters was. Dit onderstreep die potensiaal van gemaalde beesvleisprodukte as oordragingsvektore van DEC.

Boerewors met en sonder swaeldioksied as preserveermiddel is daarna vervaardig volgens 'n tipiese kommersiële prosedure en geïnokuleer met *E. coli* O157:H7 by 'n lae (3.5 log kve/g) en hoë (7.5 log kve/g) inokulum vlak en opgeberg by 0 ^{o}C, 4 ^{o}C en 10 ^{o}C gevolg deur evaluering van die groei en oorlewing elke 48 uur vir 10 dae. Boerewors met preserveermiddel het betekenisvolle laer herwinnings gehad as boerewors sonder preserveermiddel by al drie die opbergingstemperature. Die lae en hoë inokula met preserveermiddel het betekenisvolle (p<0.001) afnames in herwinnings by 0 ^{o}C gehad, met 'n afname tot onder opspoorbare limiete op dae 8 en 10 respektiewelik. By 4 ^{o}C het die lae en hoë inokula met preserveermiddel se herwinnings betekenisvol (p<0.001) afgeneem tot onder

opspoorbare limiete en tot 1.01 log kve/g respektiewelik op dag 10. By 10 ^{o}C, is betekenisvolle (p<0.001) toenames van 1.85 log kve/g en 2.35 log kve/g respektiewelik waargeneem vanaf dag 0 tot dag 10. By 0 ^{o}C het die lae en hoë inokula sonder preserveermiddel afnames in herwinnings gehad met 1.21 log kve/g en 1.45 log kve/g respektiewelik. Nie-betekenisvolle (p<0.001) toenames van 0.19 log kve/g en 0.26 log kve/g is respektiewelik by 4 ^{o}C waargeneem, terwyl betekenisvolle (p<0.001) toenames van 1.97 log kve/g en 1.84 log kve/ g respektiewelik by 10 ^{o}C vanaf dag 0 tot 10 waargeneem is. Die kombinasie van swaeldioksied preserveermiddel en lae temperatuur (0 ^{o}C en 4 ^{o}C) het die beste effektiwiteit teen *E. coli* O157:H7 oorlewing getoon.

Daar is verder aangetoon dat termiese inaktivering eindpunt temperature van *E. coli* O157:H7 wat in boerewors met preserveermiddel geïnokuleer is teen 7.00 log kve/g, 60 minute by 60 ^{o}C, 80 sekondes by 65 ^{o}C en 60 sekondes by 70 ^{o}C was. Hierdie studie toon dus aan dat *E. coli* O157:H7 kan oorleef in boerewors met preserveermiddel, opgeberg by 0 ^{o}C, 4 ^{o}C and 10 ^{o}C en dat dit meer sensitief is vir hittebehandeling by 70 ^{o}C.

Sleutelwoorde: *Escherichia coli* O157:H7, preserveermiddel, boerewors, inokulum grootte, temperatuur, diarrheageniese *E. coli*, serotipe.