UNIVERSITY OF PRETORIA

FACULTY OF VETERINARY SCIENCE

DEPARTMENT OF PARACLINICAL SCIENCES

VETERINARY PUBLIC HEALTH SECTION

Occurrence and characterization of Shiga toxin-producing *Escherichia coli* from goats on communal rangeland in Gauteng South Africa

By

MOGAUGEDI NANCY MALAHLELA - 28556021

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science (Veterinary Science)

Veterinary Public Health Section

Department of Paraclinical Sciences

Faculty of Veterinary Sciences, University of Pretoria

Pretoria

Supervisor: **Prof Musafiri Karama**

Co-supervisor: **Dr Munyaradzi C. Marufu**

May 2022

Table of Contents

DECLARATION

I declare that this thesis is my original work and has not been submitted for any award or degree in another University.

Signed by

Mogaugedi Nancy Malahlela  Date: July 8, 2022

Approved by

Prof Musafiri Karama  Date: July 8, 2022

Dr Munyaradzi Christopher Marufu  Date: July 8, 2022

ACKNOWLEDGEMENTS

I thank God for granting me the opportunity to further my studies at the University of Pretoria. It is only through God's power that I was able to complete my studies. Thank you, Lord.

My thanks go to my supervisor **Prof. Musafiri Karama** for his guidance, mentorship, and constructive criticism throughout this research. Also, Prof Karama's faith in my capacity to carry out this research is highly appreciated. I am also very grateful to my co-supervisor **Dr Munyaradzi Christopher Marufu.**

My appreciation goes to **Mr Ali Makgato** who collected the goat samples, **Dr Thierry Youmbi Fonkui** for laboratory assistance and **Ms. Segomotso Lebogo** who tirelessly organized the procurement of all the laboratory reagents and consumables used in this research.

I am also indebted to the **National Research Foundation** (NRF), the **South African Medical Research Council** and **UNICEF-Future Africa One Health** for financial assistance and funding this research.

I am grateful to my family and friends who supported me by their continuous prayers and encouragement.

DEDICATION

To

My beloved **Father Isaiah Nkgomo Malahlela** and my mother **Ramatsimele Celia Malahlela** for their constant support through this journey.

LIST OF TABLES

Chapter 2

Chapter 3

LIST OF FIGURES

Chapter 2

Chapter 3

LIST OF ABBREVIATIONS

DAFF: Department of Agriculture, Forestry and Fisheries

DALRRD: Department of Agriculture, Land Reform and Rural Development

EAEC: enteroaggregative *Escherichia coli*

EFSA: European Food Safety Authority

HC: haemorrhagic colitis

HUS: haemolytic uremic syndrome

LB: Luria Bertani

LEE: locus of enterocyte effacement

MALDI-TOF MS: matrix-assisted laser desorption/ionization-time of flight mass Spectrometry

NGS: next-generation sequencing

NM: non-motile

NRF: National Research Foundation

O-AGC: O-antigen gene cluster

OI: O-islands

PAI: pathogenicity Islands

PCR: polymerase chain reaction

RFLP: restriction fragment length polymorphism

SRST2: short read sequence typing 2

STEC: Shiga toxin-producing *Escherichia coli*

UNICEF: The United Nations Children's Fund

VTEC: Verotoxin-producing *Escherichia coli*

WGS: whole genome sequencing

WHO: World Health Organisation

THESIS SUMMARY

Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic foodborne pathogen frequently associated with enteric disease in humans characterized by mild watery to bloody diarrhoea and severe life threating complications including haemorrhagic colitis (HC) and the haemolytic uremic syndrome (HUS) which can lead to renal failure. Ruminants including cattle, sheep and goats are the primary STEC reservoirs and sources of STEC for humans. STEC is usually transmitted to humans through consumption of contaminated foods of animal origin including meat, dairy products, water and vegetables. More than 1000 STEC serotypes have been isolated from animals, humans, the environment, and food products, while at least 400 have been linked to human diseases.

In South Africa, current data on the occurrence and characteristics of STEC in animals are scanty, although human STEC disease and outbreaks have been previously reported. Furthermore, reports on the serotypes and virulence characteristic of STEC isolates from animals remain very limited. Therefore, the main objectives of this study were to (i) investigate the occurrence of STEC in goats raised on communal rangeland in South Africa and (ii) characterise STEC by serotype, and virulence profiles. Microbiological culture and polymerase chain reaction (PCR) were used for isolation and detection of STEC in a total of 289 goat faecal samples. The faecal samples were obtained from four goat herds reared on communal rangelands in South Africa. Furthermore, PCR was used to characterise 628 goat STEC isolates by serotype (O and H antigens) and genes encoding important virulence factors or markers including Shiga toxins (*stx1*, *stx2*), intimin (*eaeA*) and hemolysin (*hlyA*).

STEC was detected in 80.2% (232/289) of faecal samples. Within herds, STEC was found in 75.3% (116/154) of goats in herd A; 90.6% (39/43) in herd B; 78.8% (41/52) in herd C and 90% (36/40) in herd D. At least 99.0% (622/628) of STEC isolates were serotyped by PCR,

which revealed 34 somatic (O) groups and 17 flagellar H types which corresponded to a total of 63 distinct O:H serotypes. The 63 serotypes included 21.3% (134/628) of isolates which belonged to four of the major seven STEC serogroups including O157:H7, 2.7% (8/289; 30/628); O157:H8 0.3% (1/289, 1/628); O157:H29, 0.3% (1/289; 1/628); O103:H8, 7.6% (22/289; 98/628), O103:H56, 0.3% (1/289; 1/628) O26:H2, 0.3% (1/289; 1/628) and O111:H8, 0.3% (1/289; 2/628). Serotypes associated with the four of the major seven STEC were recovered from 12.1% (35/289) goats. The remaining 59 serotypes were non-O157 STEC. The distribution of virulence genes among the 628 STEC isolates was as follows: 60.6% (381/628) of isolates carried *stx1*, 72.7% (457/628) possessed *stx2*, and 33.4% (210/628) were positive for both *stx1* and *stx2* concomitantly. The *eaeA* and *hlyA* genes were detected in 22.1% (139/628) and 78.0% (490/628) of isolates, respectively.

This study showed a high (80.2 %) occurrence of STEC in goats grazing on communal rangeland in South Africa. The *stx2* gene was more frequent that *stx1* among goat STEC isolates while both *stx1* and *stx2* were concurrently observed in one third of isolates. Most of the goat STEC isolates lacked *eaeA*. The *eaeA* was observed in serotypes belonging to the major STEC serogroups such as STEC O157:H7, O26:H2, O111:H8 and O103:H8 which are commonly recovered from human patients and have been associated with outbreaks of foodborne disease in human in several countries, worldwide. In addition, *eaeA* was present in a few non-O157/non-top 7 isolates including O71:H14, O108:H25 and O163:H8 isolates which have never been reported in humans or implicated in human disease or outbreaks.

In conclusion, the findings of this study suggest that goats on communal rangeland in South Africa are a reservoir and potential source of STEC serotypes that have been previously implicated in mild to severe human disease, worldwide. To the best of our knowledge, this is the first study on the presence and characteristics of STEC from goats in South Africa. Further

studies that compare human STEC with goat isolates will be needed to fully understand the role played by goat isolates in the transmission and occurrence of STEC disease in humans in South Africa.

1.0 CHAPTER I

GENERAL INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) also known as verotoxin-producing *Escherichia coli* (VTEC) has been associated with foodborne disease characterised by mild to bloody diarrhoea and severe complications including haemorrhagic colitis (HC) and the haemolytic uremic syndrome (HUS) in 5–10% infections in humans. HUS is a leading cause of acute kidney failure in humans worldwide, particularly in children and the elderly (Nataro and Kaper 1998; Karmali 2004; Tarr *et al.* 2005; Germinario *et al.* 2016). Ruminants including cattle mainly, and to a lesser extent sheep and goats are the major animal reservoirs and sources of STEC disease for humans (Hussein and Bollinger 2005; Hussein and Sakuma 2005; La Ragione *et al.* 2009; McCarthy *et al.* 2021). Undercooked food of animal origin, especially ground beef, unpasteurised milk, as well as contaminated water and vegetables are sources of STEC (EFSA, 2020).

STEC emerged for the first time as a human pathogen in 1982 when two outbreaks of foodborne disease in Oregon and Michigan, United States, were traced back to consumption of undercooked patties containing ground meat which was contaminated with *E. coli* serotype, O157:H7 (Riley *et al.* 1983). The outbreak affected 47 people who manifested painful abdominal cramps, watery and/or bloody diarrhoea.

The first case of STEC in South Africa was reported in 1990 in a surgical patient with haemorrhagic colitis which was associated with *E. coli* O157:H7 (Browning *et al.* 1990). Later, in 1992, a large outbreak of STEC O157:H7 was reported in Eswatini (formerly called Swaziland) and South Africa (Isaäcson *et al.* 1993). This outbreak resulted in 40,912 medical visits for diarrhoea, in persons over the age of five and was ascribed to consumption of water which had been contaminated with dead cattle carcases that carried STEC O157 (Isaäcson

et al. 1993; Armstrong *et al.* 1996; Effler *et al.* 2001). However, since the first outbreak of STEC disease in humans in 1992, only a few studies have been reported on the occurrence and characteristics STEC isolates from animals and humans in South Africa (Ateba and Bezuidenhout 2008; Smith *et al.* 2011; Iwu *et al.* 2016; Mainga *et al.* 2018; Karama *et al.* 2019b).

Goats are considered a reservoir of STEC, and consumption of goat-derived food products has been implicated in human STEC disease (Beutin *et al.* 1995; Islam *et al.* 2008; Oliveira *et al.* 2008; Orden *et al.* 2008; La Ragione *et al.* 2009; Schilling *et al.* 2012; Jacob *et al.* 2013a; Jacob *et al.* 2013b). Furthermore, goat-derived food products contaminated with STEC O157 have been incriminated in human STEC disease (Bielaszewska *et al.* 1997; Steen *et al.* 2001; McIntyre *et al.* 2002). In addition, contact with goats on farms, animal fairs and petting zoos has been associated with STEC disease in humans (Persad and LeJeune 2014; Conrad *et al.* 2017; Isler *et al.* 2021; Nichols *et al.* 2021).

The main virulence factors of STEC are two Shiga toxins (Stx1 and Stx2). Stx1 and Stx2-encoding genes (*stx1* and *stx2*) are carried on lysogenic bacteriophages in the chromosome of STEC (Scotland *et al.* 1983; O'Brien *et al.* 1984; Strockbine *et al.* 1988). In addition, STEC possess a number of *stx1* and *stx2* variants which play different roles in virulence and human disease severity (Boerlin *et al.* 1999; Scheutz *et al.* 2012). Intimin (*eaeA*) is another major virulence factor of STEC (Jerse *et al.* 1990; McKee and O'Brien 1996). Intimin is encoded on the locus of enterocyte effacement (LEE) in *E. coli*. Intimin is responsible for intimate adherence of STEC to intestinal epithelial cells and formation of attaching and effacing (A/E) lesions that are typical in STEC disease (Pai *et al.* 1986). Furthermore, STEC carry a number of plasmid-encoded virulence markers such as hemolysin (*hlyA*) (Brunder *et al.* 1999). Furthermore, several pathogenicity islands encoded genes have been shown to play

important roles in STEC virulence and disease pathogenesis (Karmali *et al.* 2003; Schmidt and Hensel 2004; Coombes *et al.* 2008).

In South Africa, there is paucity of research on the epidemiology and molecular risk assessment of STEC from livestock and humans. Previously, the few studies which have investigated STEC occurrence in livestock in three of the nine provinces of South Africa have reported the presence of STEC in cattle and/or beef and pigs only (beef and pork) (Ateba and Bezuidenhout 2008; Ateba and Mbewe 2011; Mainga *et al.* 2018). However, goats have never been screened for STEC or evaluated for the risk they may pose as a reservoir and source of STEC for humans. Furthermore, in most of the above-cited studies, the presence of STEC was confirmed without further serotyping or virulence characterisation of recovered STEC isolates, to fully understand the molecular epidemiology of STEC. In addition, the few studies on STEC in South Africa, have mostly investigated the presence of STEC on commercial livestock and neglected the role played by food animals on communal rangeland in food safety or as potential sources of foodborne pathogens for humans.

Communal livestock farming is one of the world oldest farming systems. According to the Department of Agriculture, Fisheries and Forestry there were around 3 million communal farmers in South Africa (DAFF 2013). Current estimates show that the goat population in South Africa is approximately 7.0 million (Meissner *et al.* 2013) of which 65% are owned by communal farmers (DALRRD 2020). Goats are ranked second to cattle as an important livestock species (Rumosa Gwaze *et al.* 2009). Communal goat farms are mostly owned by black rural small scale/smallholder subsistence non-commercial farmers who raise indigenous and crossbreed goats on tribal communal rangelands.

In south Africa, communal goats are kept for production of meat for home consumption, milk, manure, and skins (Haenlein and Ramirez 2007) and barter trade mainly (Morand-Fehr *et al.*

2004). However, a sizeable number of goats on communal land end up being sold and slaughtered in commercial abattoirs that produce meat for the country. Communal farmers sell goats occasionally when they are cash strapped, during festive seasons and traditional ceremonies, and for religious purposes, in contrast to commercial farmers who invest in goat farming as a primary or secondary business enterprise (Mahanjana and Cronjé 2000).

Aim and Objectives

The overall aim of this study was to determine the occurrence and virulence characteristics of STEC in livestock, with specific emphasis on goats raised on communal rangelands in South Africa.

Specific objectives were to:

i. Determine the occurrence of STEC in goats grazing on communal rangeland in Gauteng, South Africa.
ii. Characterise goat STEC isolates by serotype and virulence genes profiles.

The ultimate goal is to contribute to STEC surveillance and risk assessment in South Africa.

2.0 CHAPTER II

LITERATURE REVIEW

2.1 Background

Shiga toxin-producing *Escherichia coli* (STEC) also known as Verotoxin-producing *Escherichia coli* (VTEC) causes enteric disease characterised by mild to severe bloody diarrhoea in humans and complications including hemorrhagic colitis (HC) and the hemolytic uremic syndrome (HUS), a leading cause of acute kidney failure, worldwide. Small ruminants including sheep and goats are also reservoirs of STEC but to a lesser extent compared to cattle (Hussein and Bollinger 2005; La Ragione *et al.* 2009; McCarthy *et al.* 2021). Foodborne STEC is mainly transmitted to humans through consumption of undercooked foods of animal origin including undercooked meat and dairy products mainly, and via contaminated water and vegetables (Hussein and Sakuma 2005; EFSA 2011; Beutin and Martin 2012; Feng and Reddy 2013; Feng 2014; EFSA 2020; Tack *et al.* 2021). Contact with domestic animals is also considered an important risk factor for disease transmission to humans (Persad and LeJeune 2014; Nichols *et al.* 2021).

Majowicz *et al*. (2014) have estimated that STEC is globally responsible for 2,801,000 acute infections annually in humans. STEC has been reported in animals and humans in various parts of the world including Southern Africa (Browning *et al.* 1990; Isaäcson *et al.* 1993; Armstrong *et al.* 1996; Müller *et al.* 2001; Ateba and Bezuidenhout 2008; Smith *et al.* 2011; Mainga *et al.* 2018; Karama *et al.* 2019b; Karama *et al.* 2019a). STEC emerged for the first time as an important pathogen and public health concern in 1982 when two human outbreaks were traced to consumption of undercooked ground meat in the United States (Riley *et al.* 1983). The largest outbreak of STEC disease was reported in Germany in 2011 (Frank *et al.* 2011). This outbreak which affected more than 4,000 people, with 50 deaths and 800 HUS cases (~25%) was ascribed to a hybrid STEC O104:H4 strain which had combined

characteristics of STEC (capacity to produce Shiga toxin) and was an enteroaggregative *E. coli* (EAEC) (Frank *et al.* 2011). The STEC O104:H4 strain had additional virulence traits and antimicrobial resistance patterns (Frank *et al.* 2011). While current information on STEC is readily available in several countries where active surveillance of the disease is implemented, data on the association of STEC with human disease in South Africa remains limited or may be underestimated due to underreporting because of lack of active disease monitoring at national and provincial levels.

Only a few studies have reported on the occurrence and characteristics of STEC in livestock (cattle and pigs) in different provinces of South Africa: Gauteng (Mainga *et al.* 2018; Karama *et al.* 2019a) North West (Ateba and Bezuidenhout 2008) and Eastern Cape (Iwu *et al.* 2016). However, most of these reports have only investigated the presence of STEC in cattle (beef) and pigs and/or products thereof (beef and pork) (Ateba and Bezuidenhout 2008; Ateba and Mbewe 2011; Mainga *et al.* 2018). Furthermore, in most of these studies the presence of STEC was confirmed by PCR without further serotyping and virulotyping of STEC isolates.

2.2 STEC serotypes and virulence

More than 1000 STEC serotypes and thousands of genetic variants have been incriminated in human disease worldwide (WHO 1998; Bettelheim 2007; EFSA 2013; Bettelheim and Goldwater 2014; Beutin and Fach 2014). However, most human infections are commonly associated with STEC O157:H7 and a number of non-O157 STEC serotypes that belong to six serogroups, which are commonly termed “top 6” STEC: O26 (O26:H7; O26:H11), O45 (O45:H2, O45:HNT), O103 (O103:H2, O103:H21), O111(O111: NM, O111:H8), O121 (O121:H19) and O145 (O145:H28, O145:H34). Together with STEC O157 (O157:H7, O157:NM) and top 6 STEC are colloquially termed the “Big 7/Top 7” STEC. In the United States, non-O157:H7 STEC may account for up to 80% of all STEC infections (Brooks *et al.*

2005; Hughes *et al.* 2006; EFSA 2013) while the most recent EFSA report concluded that any STEC serotype can cause mild to severe illness in humans (EFSA 2020).

The main STEC virulence factors are two antigenically distinct Shiga toxins (Stx) including Stx1 and Stx2 and their subtypes (O'Brien *et al.* 1983; Scotland *et al.* 1983; Strockbine *et al.* 1988; Scheutz *et al.* 2012). The genes encoding Shiga toxins (*stx1* and *stx2*) are located on lysogenic (temperate) bacteriophages in STEC genomes (O'Brien *et al.* 1984; Huang *et al.* 1987). The following *stx1* and *stx2* encoding subtypes have been identified: *stx1a*, *stx1c*, *stx1d*, *stx1e*, *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f* and *stx2g* (Scheutz *et al.* 2012), *stx2h* (Bai *et al.* 2018), *stx2j* (EFSA 2020), *stx2i* (Lacher *et al.* 2016), *stx2k* (Yang *et al.* 2020), *stx2l* (EFSA 2020), *stx2m* (Bai *et al.* 2021). The *stx1a* and *stx2a* subtypes encode prototypic Stx1 and Stx2 (Scheutz *et al.* 2012; Melton-Celsa 2014).

Another important STEC virulence factor is intimin encoded by the *eaeA* (*E. coli* attachment effacement) (Jerse *et al.* 1990; Yu and Kaper 1992; McDaniel *et al.* 1995; McKee and O'Brien 1996; McDaniel and Kaper 1997). The *eaeA* gene is located on the locus of enterocyte effacement (LEE) which also carries a type three secretion system required for intestinal STEC colonization (Yu and Kaper 1992; Donnenberg *et al.* 1993; Tzipori *et al.* 1995). Intimin is responsible for intestinal attachment and effacement lesions, typical in STEC disease (Tzipori *et al.* 1995; Nataro and Kaper 1998). Intimin is commonly found in Big 7 STEC serotypes which are, frequently implicated in severe human disease including bloody diarrhoea, HC and HUS (Brooks *et al.* 2005; Karama *et al.* 2019b). However, several non-top 7 STEC which are *eaeA*-negative (STEC O113:H21, O91:H21/H^{-}, O128:H2/H^{-}) have also been incriminated in hemorrhagic colitis and HUS, showing that these STEC may have alternative adhesins other than *eaeA* (Karmali *et al.* 1983; Paton *et al.* 1999; Mellmann *et al.* 2009; Newton *et al.* 2009; Käppeli *et al.* 2011; EFSA 2020). In contrast, some STEC strains

which are *eaeA*-positive have been associated with mild disease (diarrhoea). Therefore, possession of *eaeA* by an STEC strain may not be essential marker for severe disease causation but just an indicator of a higher likelihood of an STEC strain to cause severe disease (EFSA, 2020).

STEC possess additional virulence markers which are plasmid-encoded and are located on a major STEC plasmid (pO157) in STEC O157:H7 including hemolysin (*hlyA*) (Schmidt *et al.* 1995), catalase peroxidase (*katP*) (Brunder *et al.* 1996), serine protease (*espP*) (Brunder *et al.* 1999) and a type two secretion system (*etpD*) (Schmidt *et al.* 1995; Brunder *et al.* 1997). Other plasmid-encoded virulence factors include a subtilase cytotoxin (*subA*) (Paton *et al.* 2004) and the STEC autoagglutinating adhesin (*saa*) (Paton *et al.* 2001). The subtilase cytotoxin has been reported in various STEC serotypes from humans, cattle, sheep, goats, and wild animals (Khaitan *et al.* 2007; Wolfson *et al.* 2009; Buvens *et al.* 2010; Irino *et al.* 2010; Orden *et al.* 2011; Sánchez *et al.* 2012; Hoang Minh *et al.* 2015; Karama *et al.* 2019b; Karama *et al.* 2019a). The *saa* gene is mostly found in *eaeA*-negative STEC isolates of human and animal origin (Jenkins *et al.* 2003; Karama *et al.* 2019b). Possession of *saa* by an STEC strain was associated with severe enteritis and systemic disease in humans (Paton *et al.* 2001; Paton *et al.* 2004).

Several functions have been ascribed to plasmid-encoded virulence proteins in the host including pore formation (HlyA), erythrocyte lysis and mucosal haemorrhage in host enterocytes (HlyA) (Schmidt *et al.* 1995); secretion of exoproteins (EtpD) (Schmidt *et al.* 1997), cleavage of coagulation factor V and immunosuppression in the host (EspP) (Brunder *et al.* 1997); and reduction of oxidative stress in host cells (KatP) (Brunder *et al.* 1996). Additional functions are: enhanced adhesion and colonization to enterocytes (Saa) (Paton *et*

al. 2001; Paton *et al.* 2004), and cytotoxicity (SubA) (Paton *et al.* 2004; Paton and Paton 2010).

Whole genome sequencing of two STEC strains which were incriminated in large outbreaks of human disease in the USA and Japan (*E. coli* O157:H7, EDL 933 and the Sakai strains) revealed at least 177 extra-clusters of large genomic DNA fragments in comparison to the *Escherichia coli* K-12 strain (Riley *et al.* 1983; Blattner *et al.* 1997; Michino *et al.* 1999; Hayashi *et al.* 2001; Perna *et al.* 2001). The genomic segments in EDL933 and Sakai strains had the characteristics of horizontally transferred DNA and were called “pathogenicity islands” (PAI). This was in reference to a term which was first used by Hacker *et al*. (1990) to describe similar DNA sequences encoding fimbrial adhesins and hemolysin virulence genes in *E. coli* isolates from human patients with urinary tract, sepsis and neonatal meningitis infections. Later, PAIs of EDL933 were designated O-islands (Perna *et al.* 2001).

Several (PAIs) (OIs) in the EDL 933 strain including OI-1, OI-43, OI-48, OI-115, OI-122, OI-140, OI-141, and OI-154 carry recognised virulence-associated genes (Karmali *et al.* 2003; Ju *et al.* 2013; Cadona *et al.* 2018). For example, OI-122-encodes marker genes which share significant similarity with recognised virulence genes in *Salmonella enterica* serovar Typhimurium virulence gene (*pagC/Z4321*) (Pulkkinen and Miller 1991), *Shigella flexneri* enterotoxin 2 (*she2T)* (*sen/Z4326*) (Nataro *et al.* 1995) and the EHEC factor for adherence (*efa1/Z4332*) (Nicholls *et al.* 2000), also termed lymphocyte inhibition factor (*lifA*) (Klapproth *et al.* 2000). OI-48 encodes an integrase, a phage, the tellurite resistance (*ter*), the IrgA homologue adhesion (*iha*) and urease (*ureC*) genes (Perna *et al*. 2001).

PAI-associated genes have been reported in human and animal STEC isolates (Karama *et al.* 2009; Buvens and Piérard 2012; Karama and Gyles 2013; Cadona *et al.* 2018; Karama *et al.* 2019b; Karama *et al.* 2019a). Possession of a number of marker genes on OI-122 (*Z4321*,

Z4326, *Z4332* and *Z4333*) and OI-43/48 (*ter*, *iha*, *ureC*) has been used for seropathotype classification of STEC (Karmali *et al.* 2003; Wickham *et al.* 2006; Konczy *et al.* 2008; EFSA 2013;2020). Seropathotype classification ranks STEC into high to low risk seropathotypes (A to E) based on the presence or absence of marker genes on OI-122 (Karmali *et al*. 2003). In addition, STEC serotypes are classified based on association with different degrees of STEC disease ranging from mild to severe illness and frequency in sporadic disease or outbreaks and/or HUS (Karmali *et al.* 2003). Seropathotype A include the most virulent and frequent STEC strains in human disease outbreaks and severe illness including STEC O157:H7 and O157:NM. Seropathotype B consists of serotypes which are infrequently implicated in severe disease and outbreaks compared to seropathotype A strains: O26:H11, O103:H2, O111:NM, O121:H19 and O145:NM (Bosilevac and Koohmaraie 2011; EFSA 2013; EFSA 2020). Seropathotype C comprises STEC serotypes which are uncommon in outbreaks but associated with severe disease: O5: NM, O91:H21, O104:H21, O113:H21, O121: NM, and O165:H25. Seropathotype D are serotypes which are rare in outbreaks and have never been incriminated in severe disease while seropathotype E are STEC serotypes which have never been implicated in human disease (Karmali *et al.* 2003). However, the European Food Safety Agency (EFSA) recently suggested that it was practically impossible to define a pathogenic STEC strain absolutely or predict which virulence factors determine STEC that are pathogenic for humans (EFSA 2013;2020). EFSA concluded that all STEC are pathogenic and able to cause diarrhoea in humans and based on which *stx* subtype a STEC strain carries, and depending on the presence or absence of *eaeA*, all STEC have the potential to cause severe disease in humans (EFSA 2020).

In addition to PAI-associated virulence genes, differences between non-pathogenic *E. coli* and STEC are based on the presence of several non-LEE encoded (*nle*) genes located on

bacteriophages or bacteriophage-like elements that are integrated in the genomes of STEC strains (Blattner *et al.* 1997; Hayashi *et al.* 2001; Asadulghani *et al.* 2009).

Non-LEE encoded (*nle*) effector genes contribute to STEC virulence (Gruenheid *et al.* 2004; Wong *et al.* 2011; Stevens and Frankel 2014) and several *nle* genes have been used as virulence markers for molecular risk assessment or STEC characterisation (Coombes *et al.* 2008). STEC that produce non-LEE effector proteins have a higher potential of causing HUS and disease outbreaks in humans (Coombes *et al.* 2008; Mellies and Lorenzen 2014). The following non-LEE-encoded genes have been found in STEC isolates from animals and humans: *nleA, nleB, nleC, nleE, nleF, nleG2-1, nleG2-3, nleG5-2, nleG6-2, nleG9, nleH1-1 and nleH1-2* (Gruenheid *et al.* 2004; Coombes *et al.* 2008; Bugarel *et al.* 2010; Bugarel *et al.* 2011; Karama and Gyles 2013; Mellies and Lorenzen 2014; Cadona *et al.* 2018; Karama *et al.* 2019b; Karama *et al.* 2019a).

The function of Nles in STEC has been demonstrated in a number of studies or inferred from their homologs in related bacteria (Gruenheid *et al.* 2004; Mundy *et al.* 2004; Wong *et al.* 2011). During infection, non-LEE effector proteins are delivered into the host cell and can be used by bacteria to subvert or inhibit various cell functions, thereby facilitating STEC multiplication, colonization, and disease causation in the host (Dean and Kenny 2009; Vossenkämper *et al.* 2011; Wong *et al.* 2011). NLEs have been associated with inhibition, disruption, and/or subversion of cellular functions in host cells by exporting proteins from the endoplasmic reticulum (EspI/NleA). Additional functions include apoptosis (NleH and NleD), disturbance of inflammatory signalling pathways (NleB, NleC, NleE and NleH) and inhibition of phagocytosis (EspJ and NleH). Furthermore, inhibition of pro-inflammatory signalling (NleB, NleC, NleD, and NleE), induction of membrane ruffling and lamellipodia formation after activating Cdc42 and Rac1 (EspT), disruption of microtubule formation (EspG2), stress fiber

formation (EspM), cell death modulation and prevention of cell detachment (NleD and Cif) have been associated with NLEs (Dean and Kenny 2009; Vossenkämper *et al.* 2011; Wong *et al.* 2011; Stevens and Frankel 2014).

2.3 Epidemiology and characteristics of Goat STEC

2.3.1 Goats as STEC reservoirs

Historically, most studies have focussed on the role of cattle and sheep in the epidemiology of STEC. However, some studies have shown that goats are a reservoir and a potential source of STEC for humans (La Ragione *et al.* 2009). STEC transmission after consumption of goats-derived food products has also been documented (Bielaszewska *et al.* 1997; Espié *et al.* 2006; Rahimi *et al.* 2012). Direct contact with goats on petting farms has also been associated with STEC disease in humans (Heuvelink *et al.* 2002; LeJeune and Davis 2004; Laughlin *et al.* 2016; Schlager *et al.* 2018; Isler *et al.* 2021; Nichols *et al.* 2021).

The prevalence of STEC in goats varies greatly in different countries, ranging from 7-100% (Mainil 1999; Vu-Khac and Cornick 2008). STEC was recovered from kids, healthy and diarrheic goats on farms, during slaughter, goat meat, milk and cheese (**Table 1**). Similar to cattle, STEC shedding in goats may be associated with age and season, with younger animals shedding less STEC in comparison to older animals (Cortés *et al.* 2005; Orden *et al.* 2008). STEC transmission routes in goats and cattle are similar. However, unlike cattle, STEC colonisation of the recto-anal junction has not been demonstrated in goats (La Ragione *et al.* 2005). Furthermore, like cattle, goats are mainly asymptomatic carriers of STEC (Beutin *et al.* 1993; Orden *et al.* 2003; Cortés *et al.* 2005; Wani *et al.* 2006; Oliveira *et al.* 2008; Vu-Khac and Cornick 2008; Mahanti *et al.* 2015; Conrad *et al.* 2017).

Although goat-derived products have been implicated in foodborne STEC disease, reports on the association of human disease with foods of goat origin are less frequent. Human

infection has been associated with drinking unpasteurised milk and eating cheese made from contaminated milk (Bielaszewska *et al.* 1997; McIntyre *et al.* 2002; Espié *et al.* 2006). Pepin et al., (1997) observed that human foodborne infections were less frequently associated with goat products in resource-poor countries where goats are raised under extensive conditions and slaughter hygiene may not be very strict, because goat meat is thoroughly boiled before consumption (Pépin *et al.* 1997).

2.3.2 Goat STEC serotypes and virulence characteristics

Similar to cattle, goats carry various STEC serogroups/serotypes (**Table 1**). Furthermore, goats are a reservoir of Big 7 STEC serogroups including O26, O45, O103, O111, O121, O145 and O157 (Vu-Khac and Cornick 2008; Schilling *et al.* 2012; Momtaz *et al.* 2013; Jacob *et al.* 2013a; Jacob *et al.* 2013b). In addition, serogroups such as O91, O113 and O128 which are also frequent in human disease in some countries, but less so compared to Big 7 STEC, have been detected in goat faeces or food products (Orden *et al.* 2003; Cortés *et al.* 2005; Schilling *et al.* 2012). Furthermore, various non-O157 STEC serogroups/serotypes including O5, O7, O15, O18, O21, O76 and O146 were frequent in goats **(Table 1**) (Beutin *et al.* 1993; Orden *et al.* 2003; Cortés *et al.* 2005; Wani *et al.* 2006; Oliveira *et al.* 2008; Mahanti *et al.* 2015).

Goat STEC isolates carry common STEC virulence-associated genes (*stx1*, *stx2, eaeA* and *hlyA*). In most studies, *stx1* was more frequent than *stx2* in goat STEC isolates (**Table 1**) (Zschöck *et al.* 2000; Orden *et al.* 2003; Wani *et al.* 2006; Schilling *et al.* 2012; Momtaz *et al.* 2013; Ndegwa *et al.* 2020). The *eaeA* gene was less frequent in goat STEC isolates (Orden *et al.* 2003; Wani *et al.* 2006; Oliveira *et al.* 2008; Vu-Khac and Cornick 2008; Ndegwa *et al.* 2020). The lower frequency of *stx2* and *eaeA* among goat STEC isolates may suggest that these isolates are most probably less virulent for humans, as possession of *stx2* and *eaeA* is

usually a predictor of higher virulence in STEC (Ostroff *et al.* 1989; Boerlin *et al.* 1999; Donohue-Rolfe *et al.* 2000; Friedrich *et al.* 2002; Ethelberg *et al.* 2004; Persson *et al.* 2007). The few studies which subtyped Shiga toxin-encoding genes reported that *stx2c* and *stx2d* were frequent subtypes among goats STEC isolates (Oliveira *et al.* 2008; Vu-Khac and Cornick 2008; Mahanti *et al.* 2015; Jajarmi *et al.* 2018).

Table 1: Occurrence, serotypes, virulence genes of goat STEC or goat-derived food products in different countries

Country	Source	Occurrence rate	Serogroup/serotypes	Major Virulence genes	Additional virulence genes		References
Germany	Dairy goats	56.1%	05:H-, 082:H8, 087:H21	*stx1, stx2,*			(Beutin *et al.* 1993)
		75.3%		*stx1, stx2, eaeA, hlyA,*			(Zschöck *et al.* 2000)
		89.3%	O113:H4, O91:H14, O157:H8, ONT:H19, ONT:H8, O145:H6, O103:H34, O145: NT, O157:H4, O157:H10, O7:H37, ONT:H4, O26:H19, ONT:H21,	*stx hlyA, eaeA*	*stx1c, stx2b*	*saa, katP, espA, espB, espC, espF, espI, espJ, espP, nleB, nleC, iha, ireA, astA, lpfA, pic, toxB, hlyE, K88*	(Schilling *et al.* 2012)
Switzerland	Petting zoo	88%					(Isler *et al.* 2021)
Spain	Healthy goat kids, replacement, and adults	47.7% Overall 75.2% Adults 35.8% Replacement 2.5% Goat kids	O5:H-, O5:H21, O7:H21, O64:H21, O76:H19, O91:H14, O110:H19, O126:H8, O128:H-, O128:H2, O145:H21, O146:H-, O146:H21, O166:H28, O174: HNT, O175:H38, ONT:H-, ONT:H2, ONT:H4, ONT:H19, ONT:H21, ONT:H28, ONT: HNT,	*stx1, stx2, ehxA*		*saa*	(Cortés *et al.* 2005)

	Healthy kids and adults' Diarrhoeic kids	16.2% Healthy goats (4.3% kids and 23.3% adults) 5.9% diarrhoeic kids	O5:H-, O18:H28, O21: HNT, O58:H21, O76: HNT, O81:H-, O81:H21, O81:H38, O128:H-, O128:H2, O128:H19, O146:H21, O156:H25, O166:H28, O173:H8, O174:H8, ONT:H-, ONT:H4, ONT:H21	*vt1, vt2, eaeA, hlyA*			(Orden *et al.* 2003)
Brazil	Healthy goats	57.5%	O174:H8, O146:H21, O5:H-, O113:H21, O43:H2, O15:H27, O87:H16, O146:H-, O17:HNT, O174:H-, O141:H14, O7:H21, O174:H21, O36:HNT, ONT:H21, ONT:25, OR:H8, OR:H16, ONT:H-, OR:HNT	*stx1, stx2, eaeA, ehxA*	*stx1c, stx2d*	*saa, iha*	(Oliveira *et al.* 2008)
Vietman	Healthy adults	31.5%	O157, O26, O145	*stx1, stx2, eaeA, hlyA*	*stx1c, stx2c, stx2d, stx2dact ,*	*saa*	(Vu-Khac and Cornick 2008)
Bangladesh	Slaughter goats	82.2%	O157, O32:H25				(Islam *et al.* 2008)
	Black Bengal goats	6.2%		*stx1, stx2, eaeA, hlyA*			(Gupta *et al.* 2016)
Czech Republic	2-6 months (chronic diarrhoea) 2-8 weeks (occasional diarrhoea	59.5% Overall 65.5% (2-6 months) 50% (2-8 weeks)	O15, O78, O128, O128:K85, K85, OX,	*stx1 hlyA, stx2, hlyA*			(Novotna *et al.* 2005)

India	Healthy nonmigratory and migratory goats (2%)	16.4%	O3, O4, O5, O6, O8, O9, O14, O15, O18, O21, O22, O23, O25, O26, O33, O35, O37, O38, O44, O49, O51, O52, O53, O60, O66, O69, O73, O75, O76, O78, O80, O81, O82, O83, O87, O88, O91, O98, O99, O101, O102, O104, O112, O113, O115, O117, O120, O127, O131, O133, O136, O145, O148, O156, O157, O158, O160, O166, O168, O172	*stx1, stx2, eaeA, hlyA*			(Wani *et al.* 2006)
	Healthy goats	14.7%	O2, O5, O20, O21, O22, O25, O41, O44, O45, O60, O71, O76, O84, O85, O87, O91, O103, O112, O113, O120, O156, O158	*stx1, stx2, eaeA, hlyA*	*stx1c stx2d stx2c*	*saa*	(Mahanti *et al.* 2015)
Iran	Goat meat and faecal sample		O113	*stx1, stx2, eaeA*	*stx1a, stx1c stx2a stx2b stx2c stx2d*		(Jajarmi *et al.* 2018)
	Healthy goats	23.9%	O113, O91, O5,	*stx1, stx2 eaeA, hlyA*		*saa, subAB, estA, Z2098, Z2099*	(Zaheri *et al.* 2020)
	Goat meat	21.9%	O157, O26, O103, O111, O45, O91, O113,	*stx1, stx2, eaeA, hlyA*			(Momtaz *et al.* 2013)
Egypt	Healthy, milk and bedding goats' samples	31.1%	O11:H2, O124	*stx1, stx2, eaeA, hlyA*			(Elsayed *et al.* 2018)

United States of America	Healthy goat kids(pre-weaned) Adults	56% Higher STEC in kids (66%) than adults (28%)	O26, O103, O146	*stx1, stx2, eaeA, hlyA*			(Ndegwa *et al.* 2020)
	Meat goats at slaughter	27.5%	O26, O45, O103, O111, O121, O145	*stx1, stx2, ,eaeA*			(Jacob *et al.* 2013b)
Nigeria	Goats and Goat meat	7.5%	O157, O111, O128,	*stx1, stx2, eaeA, hlyA*			(Ojo *et al.* 2010)
European Union	Goats	0-11.8%					(EFSA 2013)
Ethiopia	Goats	30.2%	O157:H7	*stx1, stx2, eaeA*			(Mersha *et al.* 2010)
United States of America	Goats, humans and environment		O5, O103, O157				(Nichols *et al.* 2021)
United States of America	Goats, humans and environment		O157				(Laughlin *et al.* 2016)
Greece	Raw goat milk	0.43%	O157	*stx1, stx2, eaeA, hlyA,*			(Solomakos *et al.* 2009)
Ethiopia	Goat meat	2% (*E. coli* O157:H7)	O157:H7				(Hiko *et al.* 2008)
England UK	Goats	8.9% VTEC O157	O157				(Pritchard *et al.* 2009)
Serbia	Goats	73.8%	O128				(Čobeljić *et al.* 2005)
Spain	Goat Milk and Cheese	6.85% (milk) 0% (cheese)	O157:H7, O27:H18, O91:H28, O76:H19, ONT:H21	*stx1,stx2, eaeA, ehxA*			(Rey *et al.* 2006)

USA	Goats	1.8% STEC O157:H7	O157:H7	*stx2, eaeA*			(Keen *et al.* 2006)
USA	Goats	10.1%		*stx1, eaeA, stx2*			(DebRoy and Roberts 2006)
Spain	Healthy goats		O5:H2, O5:H21, O7:H21, O18:H21, O18:H28, O58:H21, O64:H21, O76:H19, O76: HNT, O81:H2, O81:H21, O87:H38, O88:H8, O91:H14, O119:H19, O126:H8, O128:H2, O128:H19, O145:H21, O146:H21, O156:H25, O166:H28, O173:H8, O174:H8				(Horcajo *et al.* 2010)
Switzerland	Semihard and soft cheese made from goat milk		O15:H16, ONT:H9	*stx2*	*stx2g*		(Stephan *et al.* 2008)
Switzerland	Goat milk	16%	O5:H-, O76:H19, O91:H?, O113:H4, O174:H8	*stx1*, *stx2*, *hlyA*	*stx2c*, *stx2d*		(Muehlherr *et al.* 2003)
Spain	Bulk tank goats milk samples and goat farm environment		O157, O157:H7, O5:HNM, O146:H21, O106:H16, O166:H28, ONT:H4, ONT:H21	*stx1, stx2, eaeA*			(Álvarez-Suárez *et al.* 2015; Álvarez-Suárez *et al.* 2016)
Greece	Goat feces		O157:H7	*stx2*			(Dontorou *et al.* 2004)
Germany, Switzerland, and France	Goat milk and cheese		O128:H2, O146:H21, O91:H14, O178:H19, O76:H19		*Stx1c, stx2b*		(Martin and Beutin 2011)
United States of America	Goat feaces		O174:H8, ONT:H8, O161:H36, O76:H19, O5:H19, O75:H8, O39:H8, O81:H36	*stx1, stx2, ehxA*	*Stx1c, stx2d*	*saa*	(Ishii *et al.* 2007)

Switzerland	Semihard and soft cheese made from goat milk		O8:H20, O116:H28, ONT:H19, O15:H16	*stx2*	*stx2e, stx2g*		(Zweifel *et al.* 2010)

2.4 STEC O:H Serotyping

2.4.1 Phenotypic serotyping methods

2.4.1.1 Traditional O:H serotyping

Traditional (conventional) serotyping of STEC involves agglutinating specific antisera against somatic (O) and flagellar (H) surface antigens in target isolates to differentiate *E. coli* into serotypes (Ørskov and Ørskov 1984). The technique was first proposed by Kauffman in 1946 (Kauffmann 1947) after discovering that *E. coli* strains which were associated with neonatal diarrhoeal outbreaks expressed somatic (O), flagellar (H) and capsular (K) antigens (Ørskov and Ørskov 1984; Ewing 1986).

The O-antigen is determined by repeat units (10-25) of oligosaccharides (O unit) sugar residues which form part of the *E. coli* outer membrane lipopolysaccharide (LPS). O sugar residues vary structurally in arrangement and linkage at the molecular level and variation in their molecular structure is the basis for O serogrouping of *E. coli* (Guinée *et al.* 1972; Ørskov and Ørskov 1984; Ewing 1986; MacLean *et al.* 2010). Currently, there are more than 188 *Escherichia coli* O serogroups which have been discovered (Iguchi *et al.* 2015a; EFSA 2020; Iguchi *et al.* 2020). The World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*, Statens Serum Institut (SSI) in Denmark (http://www.ssi.dk/English.aspx) recognises 188 *Escherichia coli* O serogroups which are designated from O1 to O188. In this designation, O31, O47, O67, O72, O94 and O122 have been omitted while O18, O28 and O112 are duplicates (O18ab/O18ac, O28ab/O28ac, and O112ab/O112ac) (http://www.ssi.dk/English.aspx).

Somatic O antigen detection involves agglutinating boiled cultures to O antiserum (Guinée *et al.* 1972; Orskov *et al.* 1977). Boiling is carried out to eliminate heat-labile surface antigens which may be sharing epitopes. However, some O antisera may cross-react with surface

antigens of *Salmonella* and *Shigella* or other *E. coli* O groups. Furthermore, some *E. coli* strains may not react with any known O antisera because they lack an outer membrane ("rough"). In addition, variation in antisera avidity or specificity due to noncompliance to good manufacturing practices or non-uniform serum production or storage conditions can lead to false positive/negative or ambiguous O typing results (DebRoy *et al.* 2011a). *E. coli* strains that cannot be O serogrouped are designated "O nontypable" (ONT) or O untypable (OUNT) while rough *E. coli* or strains which cross-react with various sera are called "O Rough"(Ørskov and Ørskov 1984; Ewing 1986) .

Flagellar H serotyping involves agglutinating H antigens of motile isolates with H-specific antiserum (Ørskov and Ørskov 1992). Currently, there are 53 recognised H antigens, numbered 1-56. However, H13, H22 and H50 have been omitted (Ørskov *et al.* 1975; Ørskov and Ørskov 1984; Scheutz *et al.* 2004). Flagellar or H antigens are flagellin molecules which make up the surface of the flagellar filament, a single structural protein with four major domains including D0 and D1 which constitute the flagellar inner and outer tube, respectively, while D2 and D3 are surface components of the flagellar filament (Samatey *et al.* 2001). *E. coli* D0 and D1 domains are conserved whereas D2 and D3 are usually hypervariable forming the basis of H-antigen diversity and classification (Samatey *et al.* 2001). (http://www.ssi.dk/English.aspx).

Traditional H-antigen serotyping is performed in tubes or microtiter plates by subculturing *E. coli* in semi-solid motility agar to detect and select motile strains. Once motile strains are detected, well-flagellated formalin-killed cultures are reacted against different H antisera to identify the H type (Ørskov and Ørskov 1984; Ewing 1986). Traditional flagellar H typing is time-consuming compared to O typing, as *E. coli* flagella must first be induced and expressed *in vitro* through multiple passages of bacteria in tubes for a number of days or weeks. Non-

motile (NM) flagellated *E. coli* that are positive for H antigen are described as NM and strains for which the H antigen cannot be identified are termed H-non typable (HNT) (Ørskov and Ørskov 1984; Ewing 1986).

Specific combination of O and H antigens determine the "serotype" of an *E. coli* isolate. Although conventional O:H serotyping remains the "gold standard" for differentiating *E. coli*, the method presents a number of difficulties and challenges including the generation and maintenance, use of numerous antisera and cultures of standard reference *E. coli* strains. In addition, serotyping is costly, cumbersome, tedious, requires specialist resources and expertise which are only available in a few specialized reference laboratories around the world.

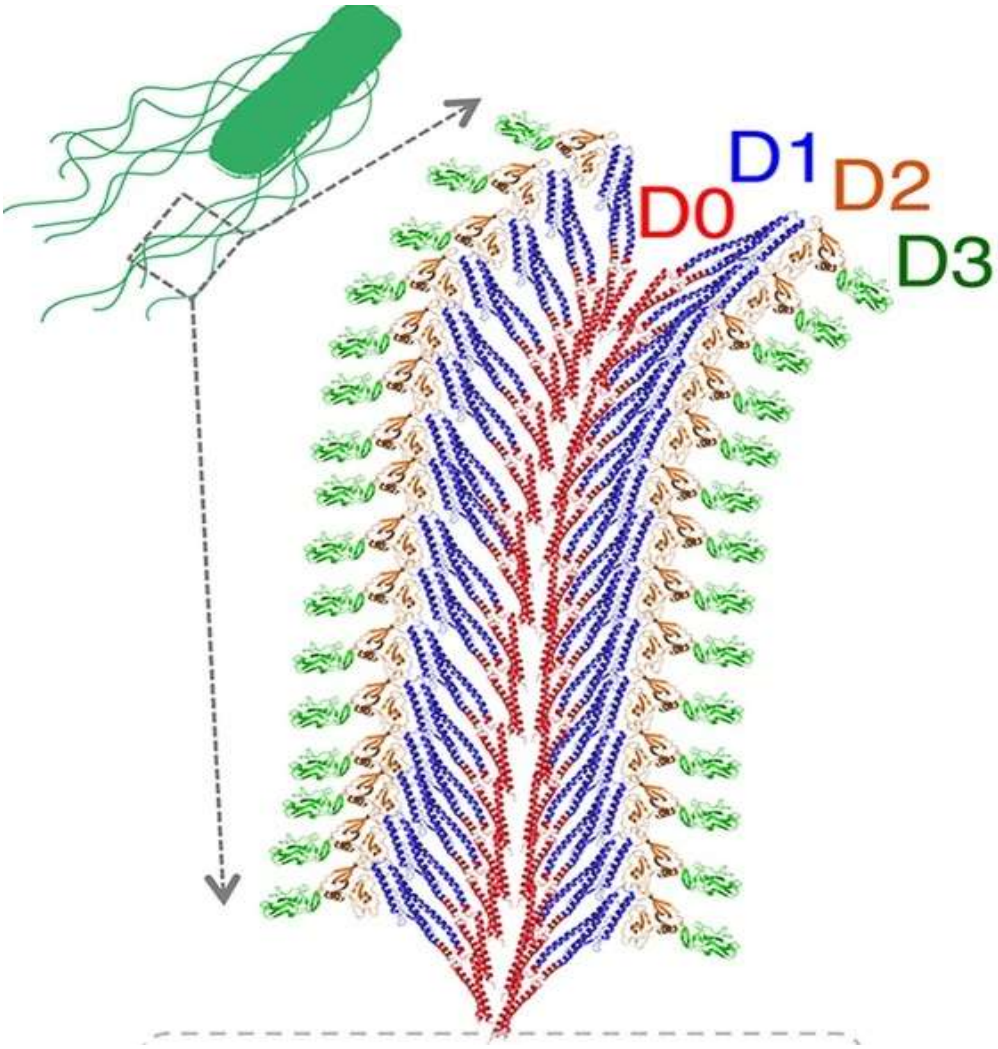
2.4.1.2. Mass spectrometry based STEC H typing

In the last decade, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a powerful, reliable simple, rapid, robust and cost-effective technique for identification of clinically relevant bacteria to genus and species level in diagnostic laboratories around the world (Kallow *et al.* 2010; Clark *et al.* 2013).

Mass spectrometry involves measurement of the ratio of the mass to charge (m/z) of chemical compounds which have been ionized into charged molecules. MALDI-TOF analysis involves preparing a sample by mixing or coating it with a solution of an organic compound which is energy-absorbent termed a matrix. The matrix dries, crystallizes and entraps the sample within by co-crystallization. A laser beam is used to ionise the sample automatically. Singly protonated ions from analytes are generated in the sample after desorption and ionization with the laser beam. When protonated ions accelerate at fixed potential, they separate from each other based on their mass-to-charge ratio (m/z). Charged analytes are usually detected

and measured by different types of mass analyzers including quadrupole time of flight (TOF) or ion trap mass analyzers, etc.

Figure 1: Flagella filament cross section view (Lu and Swartz 2016)



TOF analyzers are commonly used in microbiological applications. In MALDI-TOF analysis, the m/z ratio of an ion is determined by measuring the time required for it to travel along (length) the flight tube. Some TOF analyzers have an ion mirror incorporated at the rear end of the flight tube, to reflect ions back through the flight tube to a detector. Thus, the ion mirror not only increases the length of the flight tube but corrects minor energy differences between

ions (Yates 1998). TOF information generates a characteristic spectrum also termed peptide mass fingerprint (PMF) for different analytes in the sample.

For Identification of bacteria by MALDI-TOF MS, the PMF of an unknown bacterium is compared to a standard PMF in a database or by matching the protein mass of the unknown bacteria of interest to a PMF which has been deposited in the proteome database. For bacteria identification, ribosomal proteins (mass range m/z of 2–20 kDa) which represent about 60-70% dry weight of a bacterial cell and some housekeeping proteins are used for generating standard PMF for bacterial identification at the species level, in the database (Fagerquist *et al.* 2010; Murray 2012).

Different mass spectrometry-based approaches have been investigated and expanded to *E. coli* H-antigen/flagellar typing and compared to traditional H typing and/or whole genome sequencing serotype identification (Cheng *et al.* 2013; Cheng *et al.* 2014; Chui *et al.* 2015; Cheng *et al.* 2016b; Cheng *et al.* 2016a). Cheng *et al*. (2013), developed a membrane filtration and liquid chromatography-tandem mass spectrometry (LCMS/MS) protocol for H typing using flagellar which were extracted from 53 reference *E. coli* strains and 41 clinical isolates whose H types were known. When the PMF of the flagellar extracts were searched against the flagella antigens data base, there was 100% concordance between the identified H type by LCMS/MS and traditional H typing. It was also possible to determine the H types of nontypable isolates by traditional H typing. Cheng *et al.* (2013) concluded that LC-MS/MS was faster, relatively cost-effective with high specificity (Cheng *et al.* 2013).

Chui *et al*. (2015) designed a MALDI-TOF-based peptide mass fingerprinting approach for H typing using 61 reference *E. coli* strains which represented all the 53 H types. The protocol was tested on 85 clinical *E. coli* isolates and compared to traditional serotyping while WGS was used for resolving results that were discordant between MALDI-TOF and traditional

serotyping (Chui *et al.* 2015). With the MALDI-TOF protocol, it was possible to determine the H types of the 53 reference *E. coli* strains at 88.2% (75/85) agreement with traditional H serotyping (Chui *et al.* 2015). When the remaining 10 clinical isolates for which there was discordance between MALDI-TOF and conventional H serotyping were resolved by WGS, six agreed with MALDI-TOF and four were concordant with traditional H typing (Chui *et al.* 2015). The MALDI-TOF protocol was found to be a fast and cost-effective *E. coli* H-typing approach.

A combined mass spectrometry H typing (MS-H)-WGS O-serogrouping protocol for identification of flagellar antigens and toxins was proposed (Cheng *et al.* 2016b). The protocol was tested on 60 clinical *E. coli* and 43 additional isolates which comprised NM, HNT or ONT isolates or strains which had showed MS-H results which were inconsistent with traditional serotyping. WGS revealed that MS-H was more accurate than traditional serotyping (Cheng *et al*. 2016b). Furthermore, it was possible to identify the O antigen in 94.6% of isolates by WGS-O-serogrouping using the *wzx* and *wzy* with *wzm* and *wzt* gene pair combinations. A 78.6% O identification rate was obtained when both gene pairs were used separately (Cheng *et al*. 2016b). In addition, it was observed that 98.2% of the isolates carried toxin genes (*stx1* and *stx2*) and different virulence factors which agreed at 100% with traditional serotyping showing that the combined H typing (MS-H) with WGS O-serogrouping-toxin typing (phenotyping and genotyping) approach was a reliable *E. coli* serotyping and virulence gene characterisation method (Cheng *et al*. 2016b).

Although mass spectrometry-based flagella H typing has been found to be rapid, accurate and reliable, the high cost for purchasing different mass spectrometers/analyzers and reagents for carrying out MALDI-TOF reactions remains cost-prohibitive worldwide, particularly in developing countries. Furthermore, accessing adequate spectra databases for

analysis of mass spectrometry results can be challenging while the use of inadequate peptide spectra repositories can result in incorrect H type identification.

2.4.2. Molecular O:H Serotyping

To circumvent challenges associated with phenotypic O:H serotyping, molecular methods have been developed in the last two decades, as an alternative (**Table 2**). Most molecular serotyping protocols have used the Polymerase Chain Reaction (PCR) while other protocols have combined PCR with restriction fragment length polymorphism (PCR-RFLP) to classify *E. coli* into distinct serotypes (Coimbra *et al.* 2000; DebRoy *et al.* 2011a; Iguchi *et al.* 2015b). Furthermore, a number of microarray and whole genome sequencing-based platforms have also been developed to serotype *E. coli* (Ballmer *et al.* 2007; Lacher *et al.* 2016; Patel *et al.* 2016; Elder *et al.* 2020).

Table 2: Summary of molecular protocols for O:H serotyping

Target genes	O serogroups and H-types	Method	References
fliC	H7	PCR	(Gannon *et al.* 1997)
gnd	147 O serogroups	*rfb*-RFLP	(Coimbra *et al.* 2000)
fliC	48 H-types	H typing RFLP	(Machado *et al.* 2000)
wzx and *wzy*	O121	PCR	(Fratamico *et al.* 2003)
wzx, wzy, rfbE, wbgN, ihp1, fliC	Top 8 serogroup and H7	5'-nuclease PCR assay	(Perelle *et al.* 2004)
wzx-wzy, fliC	O26 and H11 (O:H)	mPCR	(Durso *et al.* 2005)
wzx and *wzy*	O103	Conventional and real time PCR	(Fratamico *et al.* 2005)
wzx, *wzy*, *fliC, flkA, flmA* (H54), *wbdU, wbdA, wbdH, wbdM, rfbU* (O157), *rfbE, isla29, sil-inv, sil-1, sil-2*	24 O serogroups and 47 H types	Oligonucleotide Microarray	(Ballmer *et al.* 2007)

wzx	Top 6 serogroups (without O45)	mPCR	(Monday *et al.* 2007)
wzx, wzy	O117, O126 and O146	Sequencing	(Liu *et al.* 2007)
wzx, wzy	O118 and O151	PCR assay	(Liu *et al.* 2008)
wzx and *wzy*	O145	Conventional PCR and real time PCR	(Fratamico *et al.* 2009)
gnd	O107 and O117	Sequencing	(Wang *et al.* 2009)
rfbE and *fliC*	Top 7 serogroups and H7	mPCR	(Bai *et al.* 2010; Bai *et al.* 2012)
CRISPR loci for O157, O26, O45, O103, O111, O121, O145 and O157	Top 7 serotypes	CRISPR-directed real-time PCR	(Delannoy *et al.* 2012)
wzx, wbqE, rfbE	Top7 serogroups	mPCR	(Paddock *et al.* 2012)
	Top 7 serogroups	Microbead-based Immunoassay	(Clotilde *et al*. 2013)
	13 serogroups	Luminex microbead-based Immunoassay	(Lin *et al*. 2013)
wzx, wzy	Top 7 serogroups	mPCR	(Conrad *et al.* 2014)
	11 serogroups	13-plex PCR suspension assay	(Feng *et al*. 2015)
wzx, *wzy*, *wzm*, or *wzt, orf469, rfbE*	162 O serogroups	mPCR	(Iguchi *et al.* 2015b)
wzx, wzy and *rmlA/C* (O62/O68)	O62/O68, O131, O140, O142 and O163.	PCR	(Liu *et al.* 2015)
wzx, wbqE, wbq	Top 6 serogroups	Immunomagnetic separation	(Noll *et al.* 2015a)
wzx	21 serogroups	PCR	(Sánchez *et al.* 2015)
wzy, *fliC*	14 O serogroups and 14 H-types (O:H)	PCR	(Singh *et al.* 2015)
wzx, wzy , glyc	33 novel O genotypes	mPCR	(Iguchi *et al.* 2016; Iguchi *et al.* 2020)
wzx, wbqE, wbqF, rfbE, fliC	Top 7 serogroups and H7	Real time PCR	(Shridhar *et al.* 2016)
	Top 7	BAX® System real-time PCR	(Wasilenko *et al.* 2014)
wzx, wbqE, wbqF, rfbE	Top 8 serogroups	MOL-PCR Assay	(Woods *et al.* 2016)
fliC	51 H types	mPCR	(Banjo *et al.* 2018)
Antibodies	11 Serogroups	surface plasmon resonance imaging	(Nakano *et al*. 2018)

wzx, wzy, gnd, wzm, orf469, wbdC	137 non-top-7 serogroups	mPCR	(Ludwig *et al.* 2020)

2.4.2.1 *E. coli* serogrouping by PCR

Most PCR-based protocols for *E. coli* serogrouping identify genes involved in O-antigen gene cluster biosynthesis (O-AGC) and translocation to delineate *E. coli* into different O serogroups (types (Iguchi *et al.* 2015b, DebRoy *et al.* 2018; Ludwig *et al*. 2020). Genes encoding O-antigen gene cluster biosynthesis (O-AGC) and O antigen translocation are generally variable with numerous polymorphisms among the clusters. Most genes used for serogrouping are those involved in nucleotide oligosaccharide synthesis, genes encoding for synthetases, epimerase and other proteins which are variable in their DNA sequences. However, in most O-AGC, two genes, encoding the O-antigen flippase (*wzx*) and the O-antigen polymerase (*wzy*) are unique and have been used in most PCR based *E. coli* serogrouping protocols (Liu *et al.* 1996; Samuel and Reeves 2003). The O antigen flippase unit (*wzx*) is required for O-polysaccharide transfer (Liu *et al.* 1996), the O-antigen polymerase (*wzy*) is involved in O-antigen translocation (Samuel and Reeves 2003). Other genes including those encoding the O-antigen ABC transporter permease (*wzm*) (Kido *et al.* 1995) and the ABC transporter ATP-binding (*wzt*) (Samuel and Reeves 2003) have also been used as serogrouping markers. Furthermore, PCR primers for amplifying the *wbqF* and *wbqE* genes which encode putative acetyltransferase and glycosyltransferase proteins have been also targeted for *E. coli* O serogroup delineation (Fratamico *et al.* 2003; Fratamico *et al.* 2005; Bai *et al.* 2012). Additional genes encoding a perosamine synthetase (*rfbE*), 6-phosphogluconate dehydrogenase (*gnd*), (Nasoff *et al.* 1984), a mannosyltransferase (*wbdC* and *orf469*). PCR primers for amplifying an insertion sequence (*rmlA/C*) located between genes involved in glucose-1-phosphate (*rmlA*) and dTDP-4-dehydrorhamnose 3,5-epimerase (*rmlC*)

biosynthesis of dTDP-L-rhamnose were used to identify the O62/68 serogroup (Liu *et al.* 2015; DebRoy *et al.* 2016; DebRoy *et al.* 2018), methyl and transferase genes were targeted to amplify O89/O101/O162 and the *wbrX* was for detecting O52 (DebRoy *et al.* 2018).

A number of multiplex PCR (mPCR) assays for serogrouping the major seven STEC including O157, O145, O26, O103, O121, O111 and O45 are currently available (Fratamico *et al.* 2003; Perelle *et al.* 2004; Durso *et al.* 2005; Fratamico *et al.* 2005; Monday *et al.* 2007; Fratamico *et al.* 2009; DebRoy *et al.* 2011b; Fratamico 2011; Fratamico *et al.* 2011; Madic *et al.* 2011; Anklam *et al.* 2012; Bai *et al.* 2012; Paddock *et al.* 2012; Conrad *et al.* 2014; Iguchi *et al.* 2015a; Noll *et al.* 2015a; Singh *et al.* 2015; Shridhar *et al.* 2016). Later, more PCR protocols have been developed to identify *E. coli* serogroups beyond non-big 7 STEC (DebRoy *et al.* 2011a; Paddock *et al.* 2012; Iguchi *et al.* 2015b; Iguchi *et al.* 2015a; Iguchi *et al.* 2016; Ludwig *et al.* 2020). Differences in content and sizes of genes encoding O-AGCs now form the basis of *E. coli* O-groups molecular serotyping (DebRoy *et al.* 2011a; Iguchi *et al.* 2015a; DebRoy *et al.* 2016; Ludwig *et al.* 2020).

While most of the above-mentioned PCR protocols were developed for detecting top 7 serogroups, Singh *et al*. (2015) designed mPCR protocols that to detect the seven major STEC and nine additional non-O157 STEC serogroups. Furthermore, Sanchez *et al*. (2015) developed three mPCR assays for distinguishing 21 major STEC including top 7 and a number of clinically relevant serogroups.

However, the most comprehensive PCR serotyping protocols for O serogrouping were developed and validated by Iguchi *et al*. (2015) and Ludwig *et al*. (2020). The Iguchi *et al*. (2015) protocol was able to delineate 147 serogroups using more than 20 multiplex PCR protocols (mPCR), each able to detect six to nine O serogroups (Iguchi *et al.* 2015a; Iguchi *et al.* 2016; Iguchi *et al.* 2020). Furthermore, Iguchi *et al*. (2016) were designed additional

primers to serogroup untypable and/or serologically indistinguishable *E. coli* isolates (O-serogroup untypable (ONT/OUT). The protocol resulted in the discovery of up to 14 novel O genotypes based on the amplification of O-AGCs encoding polymerase and glycosyltransferase genes sequences (Iguchi *et al.* 2016; Iguchi *et al.* 2020; Nguyen *et al.* 2021).

Ludwig *et al*. (2020) PCR platform was developed and validated on 460 STEC isolates. The protocol uses 14 mPCR assays, each including 7-12 pairs of primers and can delineate 137 STEC serogroups commonly found in cattle (Ludwig *et al.* 2020). Both the Iguchi *et al*. (2015) and Ludwig *et al*. (2020) protocols use and share previously published primers and also have included newly designed primers.

Most of the above-mentioned PCR were developed and validated using standard *E. coli* O-reference strains, which are available at the World Health Organisation Collaborating Center for Reference and Research on *Escherichia* and *Klebsiella* based at Statens Serum Institut. To check the specificity of the PCR serogrouping protocols, some studies have used previously O-serogrouped field and clinical isolates or strains obtained from *E. coli* collections in different reference laboratories. Furthermore, field isolates that belong to the Enterobacteriaceae family or other bacterial species have been used to test the specificity of the serogrouping PCR protocols: *Citrobacter freundii*, *Enterobacter cloacae*, *Hafnia alvei*, *Klebsiella pneumoniae, Salmonella enterica subsp.* Anatum, Arizona, Choleraesuis, and Typhimurium, *Shigella flexneri*, *Shigella boydii*, *Serratia marcescens*, *Yersinia enterocolitica*, *Enterococcus aerogenes*, *Enterococcus. faecalis, Lactococcus lactis*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococus aureus* and *Streptococcus pyrogenes* (DebRoy *et al.* 2018). However, a number of O-AGC target genes are genetically homologous and may cross react with closely related O antigens encoding

sequences on PCR (Wang *et al.* 2009; Iguchi *et al.* 2015b). Cross reactions on PCR were reported for serogroups O117/O107 (Wang *et al.* 2009) and O129/O135, O118/O151 (Liu *et al.* 2008). Furthermore, Iguchi et al., 2015a reported that STEC serogroups O13 was genetically identical (100%) but serologically different from O17, O44, O73, O77 and O106 whereas O50 was genetically identical to O13, O129 and O135 but serologically different from O129 and O135.

2.4.2.2 *E. coli* flagellar H typing by PCR

A number of PCR-based flagellar (H antigen) serotyping methods for *E.coli* H-type identification have been described (Gannon *et al.* 1997; Singh *et al.* 2015; Banjo *et al.* 2018). PCR flagellar typing differentiates *E. coli* and assigns isolates to various H-types based on polymorphisms in *E. coli* flagella-associated genes (*fliC*) sequences (Gannon *et al*. 1997; Bai *et al*. 2010; Singh *et al*. 2015; Banjo *et al*. 2018). Singh *et al*. (2015) developed three multiplex PCR (mPCR) protocols for detecting the 14 most common *E. coli* H antigens (Singh *et al*. 2015). Furthermore, Banjo *et al*. (2018) developed a 10 multiplex PCR scheme, comprising 51 pairs of primers for complete typing of all the 53 H-types. The Banjo *et al*. (2018) protocol was tested on H-type reference strains and 362 *E. coli* isolates. The method was found to be highly specific and accurate in distinguishing H-types. We have used both the Singh *et al*. (2015) and Banjo *et al*. (2018) protocols in our laboratory and have found them to be reliable and accurate in differentiating *E. coli* H-types. Currently, the main limitation of PCR H typing is that the technique cannot distinguish motile from nonmotile *E. coli* strains.

2.4.2.3. *E. coli* O serotyping by PCR-RFLP

A PCR-RFLP protocol for O serogrouping was developed by Coimbra *et al*. (2000) based on identifying restriction fragment length polymorphisms among *E. coli* in the *rfb* gene cluster portion of the O-polysaccharide. The RFLP protocol involves restriction digestion of the PCR

amplicon by the *Mbo*II restriction enzyme (Coimbra *et al*. 2000). Restriction digestion of the PCR amplicon resulted in a number of banding profiles that were used to distinguish *E. coli* O serogroups by gel electrophoresis. According to Coimbra *et al*. (2000), this PCR-RFLP protocol can delineate all (100%) *E. coli* serogroups. O-serogroups of clinical isolates were delineated including some rough or non-agglutinating strains. However, up to 13 O-serogroup banding profiles were shared by two or more O-serogroups (Coimbra *et al.* 2000).

2.4.2.4. PCR-RFLP *E. coli* H serotyping

Machado *et al*. (2000) and Prager *et al*. (2003) developed two PCR-RFLP protocols to delineate different H types using 182 and 220 *E. coli* isolates, respectively. Both protocol amplified the *fliC* gene using previously published primers (Fields *et al.* 1997; Machado *et al.* 2000) and digested the PCR amplicon with *Hha*I (Machado *et al.* 2000) and *Rsa*I and *Cfo*I (Prager *et al.* 2003). Machado *et al*. (2000) were able to classify the 182 isolates into 48 flagellar types of which 39 were typed unambiguously while more than one flagellar type was observed for the remaining nine H types. The Prager *et al*. (2003) protocol, assigned the 220 *E. coli* isolates to different flagellar types accurately except the H4, H17, H53, and H54 which did not show a *fliC* amplicon. There were also a few “overlaps and/or mismatches” which revealed more than one *fliC* type or conversely corresponded to more than one H type (Pager *et al*. 2003). These overlaps were ascribed to polymorphisms in the *fliC* gene sequence and/or flagellar phase variation. Moreover, it was possible to assign HNT and NM isolates to specific H types using the PCR-RFLP *fliC* protocol.

2.4.2.5. DNA microarray-based *E. coli* O:H serotyping

In addition to PCR and PCR-RFLP protocols, DNA microarray-based platforms for detecting O-AGC sequences have been developed for serogrouping STEC (Liu and Fratamico 2006; Ballmer *et al.* 2007; Bugarel *et al.* 2010; Geue *et al.* 2014). Microarray essays have also been

exploited for molecular STEC serotyping (O:H) and virulotyping concomitantly (Liu and Fratamico 2006; Bugarel *et al.* 2010; Geue *et al.* 2014; Matussek *et al.* 2017). Microarray technology involves immobilizing target *E. coli* DNA fragments (probe) within a sample of interest onto a silicon, glass or plastic chip surface and hybridizing it with DNA sequences labelled with a fluorescent dye. Complementary DNA fragments attach to target DNA spots on the chip. A fluorescent scanner is used to determine the intensity of the fluorescent dye after washing off the excess and unattached DNA fragments (Liu and Fratamico 2006; Lacher *et al.* 2014; Matussek *et al.* 2017).

Ballmer *et al*. (2007) designed and validated an arrayTube-based assay which was able to detect 24 O groups and 47 H-types correctly with high specificity and sensitivity. Furthermore, the assay identified nonmotile or nontypable *E. coli* unequivocally (Ballmer *et al.* 2007). An upgrade of the Ballmer *et al*. (2007) assay was able to identify 70 O antigen serogroups (Geue *et al.* 2014). The arrayStrip assay which allowed high throughput with full automation. When the protocol was validated on 180 reference *E. coli* isolates belonging to 70 O serogroups, there was a high degree of agreement between the microarray protocol and traditional *E. coli* serotyping and DNA-based serotyping (Geue *et al.* 2014).

In addition, a microarray-based platform for serotyping (O:H) and virulotyping *E. coli* was developed by the Food and Drug Administration (FDA-ECID, USA) to type *E. coli* isolates from various foods and predict their public health importance (Lacher *et al.* 2016; Patel *et al.* 2016). The microarray assay was designed based on *wzx, wzy* and *wzm* O alleles and tested on 103 O serogroups and the 53 recognised flagellin alleles (Patel *et al.* 2016). The array was able to identify 68 STEC O:H serotypes with high precision (Patel *et al.* 2016). A better resolution was obtained for H-nontypable or non-motile *E.coli,* and the procedure could be combined with virulotyping and single nucleotide polymorphism (SNP) resolution (Patel *et al.*

2016). The FDA-ECID array-based protocol for O:H serotyping was validated on 54 reference *E. coli* strains and found to be 94% accurate in comparison to reference data for External Quality Assurance(Patel *et al.* 2018). A 96% reproducibility was obtained when O types were performed in four additional laboratories (Patel *et al.* 2018). The protocol was was 96% reproducible and 100% accurate for H typing (Patel *et al.* 2018).

2.4.2.6. *E. coli* serotyping by whole genome sequencing

With the availability of more *E. coli* whole genome sequences (WGS) and advanced bioinformatics platforms to analyse genomes, public health laboratories are increasingly adopting whole genome sequencing (NGS) technologies to serotype *E. coli*. Currently, bioinformatics pipelines for *in silico* analysis of WGS data are being adopted by various laboratories around the world to predict *E. coli* serotypes. Platforms such as SRST2 (Short Read Sequence Typing 2) (Ingle *et al.* 2016), SerotypeFinder (Joensen *et al.* 2014), EToKi EBEis (EnteroBase *Escherichia in silico* serotyping module from EnteroBase Tool Kit), Ectyper and the Bionumerics commercial software are being used to analyse assembled genomes or raw sequence reads as standalone programs or on webservers (Inouye *et al.* 2014; Joensen *et al.* 2015; Bessonov *et al.* 2021; Zhang *et al.* 2021). Most of these bioinformatic tools can be accessed at the Center for Genomic Epidemiology, which is hosted by Denmark Technical University, (https://www.genomicepidemiology.org).

When the above-mentioned four pipelines were compared for their capacity to predict serotypes based on the sequences of 185 *E. coli* isolates that had been serotyped by traditional serotyping, a high concordance of 98-100% for O-antigens and 92-97% for H-antigens serotyping was obtained, with the ECTyper and SerotypeFinder results consistently agreeing with traditional serotyping (Bessonov *et al.* 2021). When the benchmarking assessment was extended to a collection of 6954 *E. coli* genomes that are publicly available,

to assess the performance of these bioinformatic tools on a more diverse dataset, ECTyper and SerotypeFinder had the highest concordance (Bessonov *et al.* 2021). In addition, when a serotyping validation analysis was carried out to compare Bionumerics, Ectyper and traditional serotyping on 164 *E. coli* genomes, a 91.1% and 98.2% serotype prediction accuracy was obtained, respectively with a 1.2% discordance between both pipelines (Christianson *et al.* 2018). The SerotypeFinder software predicts *E. coli* O-serogroups and H-types by searching for *E. coli* sequences encoding somatic O antigens (*wzx*, *wzy*, *wzm*, and *wzt*) and flagellin H antigens (*fliC*, *flkA*, *fllA*, *flmA and flnA*) and the search (O:H) can be combined with the SRST2 platform (Joensen *et al.* 2015; Ingle *et al.* 2016). The SRST2 platform facilitates rapid and accurate *in silico E. coli* serotype identification by directly searching for short reads that have been deposited in a curated database consisting of already known O:H serotypes. Moreover, the use of the SRST2 software has the main advantage of circumventing the need for *de novo* genome assembly which can be costly to many laboratories.

Currently NGS technologies coupled with sophisticated bioinformatics tools have been adopted as a standard, viable and cost-effective *E. coli* O:H serotyping approach in the United Sates and the United Kingdom (Dallman *et al.* 2015; Jenkins 2015; Jenkins *et al.* 2019; Ribot *et al.* 2019). A number of platforms and software for analysing sequence types combined in one single tool (plug-in) to identify O:H serotype, virulence and antimicrobial resistance genes *in silico* have been developed. Holmes *et al*. (2018) validated the use of WGS on a panel of previously characterised 150 STEC isolates at the Scottish *Escherichia coli* O157/STEC Reference Laboratory (SERL) (O:H/MLST/*eae*/Shiga toxin [*stx*]/SNP) using Public Health England (PHE) data (SnapperDB) and bioinformatics platforms. There was a high correlation of 93% to 100%, between data generated from the Scottish STEC isolates when WGS and results obtained by the PHE pipeline and BioNumerics and the two national laboratories

which were compared, showing that it was possible to standardize data among different laboratories. using the different platforms (Holmes *et al*. 2018). Lindsey *et al*. (2016) validated the use of a single workflow approach by integrating data from the Center for Genomic Epidemiology in a single plug-in to serotype STEC in combination with virulence, and antimicrobial resistance profiling using WGS and *in silico* sequence analysis. When this approach was tested on 228 *E. coli* genomes STEC, they obtained 96.1% and 96.5 % predictive rates for O and H serogrouping respectively, in comparison to traditional serotyping (Lindsey *et al.* 2016). Similarly, when this scheme was tested on 59 *E. coli* genomes, it was 95% and 100% concordant with traditional serotyping for O and H, respectively (Lindsey *et al.* 2016).

E. coli WGS serotyping is rapid and can be cost-effective and is able to serotype “O and H nontypable strains (O rough and nonmotile) and can be useful for the discovery of new serogroups (Jenkins 2015). *In silico* WGS serotype identification precludes the need for a large amount of expensive and resource-intensive antiserum and associated quality control issues. However, although WGS/*in silico* serotyping is considered reliable, rapid, and compatible with conventional serotyping, the widespread implementation of the method in many public health laboratories around the world will remain limited due the expense involved in acquiring high-cost infrastructure and equipment, and the need for expertise in genomics and bioinformatic.

2.5 References

Álvarez-Suárez ME, Otero A, García-López ML, Santos JA. Microbiological examination of bulk tank goat's milk in the Castilla y León region in Northern Spain. *J Food Prot* 78, 2227-32, 2015

Álvarez-Suárez ME, Otero A, García-López ML, Dahbi G, Blanco M, Mora A, Blanco J, Santos JA. Genetic characterization of Shiga toxin-producing *Escherichia coli* (STEC) and atypical enteropathogenic *Escherichia coli* (EPEC) isolates from goat's milk and goat farm environment. *Int J Food Microbiol* 236, 148-54, 2016

Anklam KS, Kanankege KST, Gonzales TK, Kaspar CW, Döpfer D. Rapid and reliable detection of Shiga toxin-producing *Escherichia coli* by real-time multiplex PCR. *J Food Prot* 75, 643-50, 2012

Armstrong GL, Hollingsworth J, Morris JG, Jr. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol Rev* 18, 29-51, 1996

Asadulghani M, Ogura Y, Ooka T, Itoh T, Sawaguchi A, Iguchi A, Nakayama K, Hayashi T. The defective prophage pool of *Escherichia coli* O157: prophage-prophage interactions potentiate horizontal transfer of virulence determinants. *PLoS Pathog* 5, e1000408, 2009

Ateba CN, Bezuidenhout CC. Characterisation of *Escherichia coli* O157 strains from humans, cattle and pigs in the North-West Province, South Africa. *Int J Food Microbiol* 128, 181-8, 2008

Ateba CN, Mbewe M. Detection of *Escherichia coli* O157:H7 virulence genes in isolates from beef, pork, water, human and animal species in the Northwest province, South Africa: public health implications. *Res Microbiol* 162, 240-8, 2011

Bai J, Shi X, Nagaraja TG. A multiplex PCR procedure for the detection of six major virulence genes in *Escherichia coli* O157:H7. *J Microbiol Methods* 82, 85-9, 2010

Bai J, Paddock ZD, Shi X, Li S, An B, Nagaraja TG. Applicability of a multiplex PCR to detect the seven major Shiga toxin-producing *Escherichia coli* based on genes that code for serogroup-specific O-antigens and major virulence factors in cattle feces. *Foodborne Pathog Dis* 9, 541-8, 2012

Bai X, Scheutz F, Dahlgren HM, Hedenström I, Jernberg C. Characterization of clinical *Escherichia coli* strains producing a novel Shiga toxin 2 subtype in Sweden and Denmark. *Microorganisms* 9, 2374, 2021

Bai X, Fu S, Zhang J, Fan R, Xu Y, Sun H, He X, Xu J, Xiong Y. Identification and pathogenomic analysis of an *Escherichia coli* strain producing a novel Shiga toxin 2 subtype. *Sci Rep.* 8, 6756, 2018

Ballmer K, Korczak BM, Kuhnert P, Slickers P, Enricht R, Hächler H. Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray. *J Clin Microbiol* 45, 370-9, 2007

Banjo M, Iguchi A, Seto K, Kikuchi T, Harada T, Scheutz F, Iyoda S. *Escherichia coli* H-genotyping PCR: a complete and practical platform for molecular H typing. *J Clin Microbiol* 56, 2018

Bessonov K, Laing C, Robertson J, Yong I, Ziebell K, Gannon VPJ, Nichani A, Arya G, Nash JHE, Christianson S. ECTyper: *in silico Escherichia coli* serotype and species prediction from raw and assembled whole-genome sequence data. *Microb Genom* 7, 2021

Bettelheim KA. The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Crit Rev Microbiol* 33, 67-87, 2007

Bettelheim KA, Goldwater PN. Serotypes of non-O157 Shigatoxigenic *Escherichia coli* (STEC). *Adv. Microbiol* 2014, 2014

Beutin L, Martin A. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. *J Food Prot* 75, 408-18, 2012

Beutin L, Fach P. Detection of Shiga toxin-producing *Escherichia coli* from nonhuman sources and strain typing *Microbiol. Spectr.* 2, 263-85, 2014

Beutin L, Geier D, Zimmermann S, Karch H. Virulence markers of shiga-like toxin-producing *Escherichia coli* strains originating from healthy domestic animals of different species. *J Clin Microbiol* 33, 631-5, 1995

Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J Clin Microbiol* 31, 2483, 1993

Bielaszewska M, Janda J, Bláhová K, Minaříková H, Jíková E, Karmali MA, Laubová J, Šikulová J, Preston MA, Khakhria R, Karch H, Klazarová H, Nyč O. Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. *Epidemiol Infect* 119, 299-305, 1997

Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y. The complete genome sequence of *Escherichia coli* K-12. *Science.* 277, 1453-62, 1997

Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol* 37, 497-503, 1999

Bosilevac JM, Koohmaraie M. Prevalence and characterization of non-O157 shiga toxin-producing *Escherichia coli* isolates from commercial ground beef in the United States. *Appl Environ Microbiol* 77, 2103-12, 2011

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J Infect Dis* 192, 1422-9, 2005

Browning NG, Botha JR, Sacho H, Moore PJ. *Escherichia coli* O157:H7 haemorrhagic colitis. report of the first South African case. *S Afr J Surg*. 28, 28-9, 1990

Brunder W, Schmidt H, Karch H. KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiology (Reading)* 142 (Pt 11), 3305-15, 1996

Brunder W, Schmidt H, Karch H. EspP, a novel extracellular serine protease of enterohaemorrhagic *Escherichia coli* O157:H7 cleaves human coagulation factor V. *Mol Microbiol* 24, 767-78, 1997

Brunder W, Schmidt H, Frosch M, Karch H. The large plasmids of Shiga-toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. *Microbiology* 145, 1005-14, 1999

Bugarel M, Martin A, Fach P, Beutin L. Virulence gene profiling of enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* strains: A basis for molecular risk assessment of typical and atypical EPEC strains. *BMC Microbiol* 11, 142, 2011

Bugarel M, Beutin L, Martin A, Gill A, Fach P. Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with hemorrhagic colitis and hemolytic uremic syndrome in humans. *Int J Food Microbiol* 142, 318-29, 2010

Buvens G, Piérard D. Virulence profiling and disease association of verocytotoxin-producing *Escherichia coli* O157 and non-O157 isolates in Belgium. *Foodborne Pathog Dis* 9, 530-5, 2012

Buvens G, Lauwers S, Piérard D. Prevalence of subtilase cytotoxin in verocytotoxin-producing *Escherichia coli* isolated from humans and raw meats in Belgium. *Eur J Clin Microbiol Infect Dis* 29, 1395-9, 2010

Cadona JS, Bustamante AV, González J, Sanso AM. Pathogenicity islands distribution in Non-O157 Shiga toxin-producing *Escherichia coli* (STEC). *Genes* 9, 81, 2018

Cheng K, Sloan A, McCorrister S, Peterson L, Chui H, Drebot M, Nadon C, Knox JD, Wang G. Quality evaluation of LC-MS/MS-based *E. coli* H antigen typing (MS-H) through label-free quantitative data analysis in a clinical sample setup. *Proteomics Clin Appl* 8, 963-70, 2014

Cheng K, She YM, Chui H, Domish L, Sloan A, Hernandez D, McCorrister S, Ma J, Xu B, Reimer A, Knox JD, Wang G. Mass spectrometry-based *Escherichia coli* H

Antigen/Flagella typing: validation and comparison with traditional serotyping. *Clin Chem* 62, 839-47, 2016a

Cheng K, Drebot M, McCrea J, Peterson L, Lee D, McCorrister S, Nickel R, Gerbasi A, Sloan A, Janella D, Van Domselaar G, Beniac D, Booth T, Chui L, Tabor H, Westmacott G, Gilmour M, Wang G. MS-H: a novel proteomic approach to isolate and type the *E. coli* H antigen using membrane filtration and liquid chromatography-tandem mass spectrometry (LC-MS/MS). *PLoS One* 8, e57339, 2013

Cheng K, Chui H, Domish L, Sloan A, Hernandez D, McCorrister S, Robinson A, Walker M, Peterson LA, Majcher M, Ratnam S, Haldane DJ, Bekal S, Wylie J, Chui L, Tyler S, Xu B, Reimer A, Nadon C, Knox JD, Wang G. Phenotypic H-antigen typing by mass spectrometry combined with genetic typing of H antigens, O antigens, and toxins by whole-genome sequencing enhances identification of *Escherichia coli* isolates. *J Clin Microbiol* 54, 2162-8, 2016b

Christianson S, Ziebell K, Yong I, Whiteside M, Sung S, Le K, La Rose C, Gannon V, Laing C. *In silico* serotyping of *Escherichia coli* using Ectyper. *10th International Symposium on Shiga toxin (verotoxin) producing Escherichia coli infections*. 64, 2018

Chui H, Chan M, Hernandez D, Chong P, McCorrister S, Robinson A, Walker M, Peterson LA, Ratnam S, Haldane DJ, Bekal S, Wylie J, Chui L, Westmacott G, Xu B, Drebot M, Nadon C, Knox JD, Wang G, Cheng K. Rapid, sensitive, and specific *Escherichia coli* H antigen typing by matrix-assisted laser desorption ionization-time of flight-based peptide mass fingerprinting. *J Clin Microbiol* 53, 2480-5, 2015

Clark CG, Kruczkiewicz P, Guan C, McCorrister SJ, Chong P, Wylie J, van Caeseele P, Tabor HA, Snarr P, Gilmour MW, Taboada EN, Westmacott GR. Evaluation of MALDI-TOF mass spectroscopy methods for determination of *Escherichia coli* pathotypes. *J Microbiol Methods* 94, 180-91, 2013

Clotilde LM, Bernard Ct, Salvador A, Lin A, Lauzon CR, Muldoon M, Xu Y, Lindpaintner K, Carter JM. A 7-plex microbead-based immunoassay for serotyping Shiga toxin-producing *Escherichia coli*. *J Microbiol Methods* 92, 226-30, 2013

Čobeljić M, Dimić B, Opačić D, Lepšanović Z, Stojanović V, Lazić S. The prevalence of Shiga toxin-producing *Escherichia coli* in domestic animals and food in Serbia. *Epidemiol Infect* 133, 359-66, 2005

Coimbra RS, Grimont F, Lenormand P, Burguière P, Beutin L, Grimont PAD. Identification of *Escherichia coli* O-serogroups by restriction of the amplified O-antigen gene cluster (*rfb*-RFLP). *Res Microbiol* 151, 639-54, 2000

Conrad CC, Stanford K, McAllister TA, Thomas J, Reuter T. Further development of sample preparation and detection methods for O157 and the top 6 non-O157 STEC serogroups in cattle feces. *J Microbiol Methods* 105, 22-30, 2014

Conrad CC, Stanford K, Narvaez-Bravo C, Callaway T, McAllister T. Farm fairs and petting zoos: A review of animal contact as a source of zoonotic enteric disease. *Foodborne Pathog Dis* 14, 59-73, 2017

Coombes BK, Wickham ME, Mascarenhas M, Gruenheid S, Finlay BB, Karmali MA. Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. *Appl Environ Microbiol* 74, 2153-60, 2008

Cortés C, De la Fuente R, Blanco J, Blanco M, Blanco JE, Dhabi G, Mora A, Justel P, Contreras A, Sánchez A, Corrales JC, Orden JA. Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. *Vet Microbiol* 110, 67-76, 2005

DAFF. Trend in the agricultural sector 2012.
http://www.nda.agric.za/docs/statsinfo/Trends2012.pdf,
Accessed on May 27, 2022.

Dallman TJ, Byrne L, Ashton PM, Cowley LA, Perry NT, Adak G, Petrovska L, Ellis RJ, Elson R, Underwood A, Green J, Hanage WP, Jenkins C, Grant K, Wain J. Whole-genome sequencing for national surveillance of Shiga toxin-producing *Escherichia coli* O157. *Clin Infect Dis* 61, 305-12, 2015

DALRRD. *A profile of the South African goat market value chain* 2020. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://webapps1.daff.gov.za/AmisAdmin/upload/Goat%20Market%20Value%20Chain%20Profile%202020.pdf, Accessed on May 25, 2022.

Dean P, Kenny B. The effector repertoire of enteropathogenic *E. coli*: ganging up on the host cell. *Curr Opin Microbiol* 12, 101-9, 2009

DebRoy C, Roberts E. Screening petting zoo animals for the presence of potentially pathogenic *Escherichia coli*. *J Vet Diagn Invest* 18, 597-600, 2006

DebRoy C, Roberts E, Fratamico PM. Detection of O antigens in *Escherichia coli*. *Anim Health Res Rev* 12, 169-85, 2011a

DebRoy C, Fratamico PM, Roberts E. Molecular serogrouping of *Escherichia coli*. *Anim Health Res Rev* 19, 1-16, 2018

DebRoy C, Roberts E, Valadez AM, Dudley EG, Cutter CN. Detection of Shiga toxin–producing *Escherichia coli* O26, O45, O103, O111, O113, O121, O145, and O157 serogroups by multiplex polymerase chain reaction of the *wzx* gene of the O-antigen gene cluster. *Foodborne Pathog Dis* 8, 651-2, 2011b

DebRoy C, Fratamico PM, Yan X, Baranzoni G, Liu Y, Needleman DS, Tebbs R, O'Connell CD, Allred A, Swimley M, Mwangi M, Kapur V, Raygoza Garay JA, Roberts EL, Katani R. Comparison of O-antigen gene clusters of all O-serogroups of *Escherichia coli* and proposal for adopting a new nomenclature for O-typing. *PLoS One* 11, e0147434, 2016

Delannoy S, Beutin L, Fach P. Use of clustered regularly interspaced short palindromic repeat sequence polymorphisms for specific detection of enterohemorrhagic *Escherichia coli* strains of serotypes O26:H11, O45:H2, O103:H2, O111:H8,

O121:H19, O145:H28, and O157:H7 by real-time PCR. *J Clin Microbiol* 50, 4035-40, 2012

Donnenberg M, Tacket C, James S, Losonsky G, Nataro J, Wasserman S, Kaper J, Levine M. Role of the eaeA gene in experimental enteropathogenic *Escherichia coli* infection. *J Clin Invest* 92, 1412-7, 1993

Donohue-Rolfe A, Kondova I, Oswald S, Hutto D, Tzipori S. *Escherichia coli* 0157: H7 strains that express Shiga toxin (Stx) 2 alone are more neurotropic for gnotobiotic piglets than are isotypes producing only Stx1 or both Stx1 and Stx2. *J Infect Dis* 181, 1825-9, 2000

Dontorou A, Papadopoulou C, Filioussis G, Apostolou I, Economou V, Kansouzidou A, Levidiotou S. Isolation of a rare *Escherichia coli* O157:H7 strain from farm animals in Greece. *Comp Immunol Microbiol Infect Dis* 27, 201-7, 2004

Durso LM, Bono JL, Keen JE. Molecular serotyping of *Escherichia coli* O26:H11. *Appl Environ Microbiol* 71, 4941-4, 2005

Effler E, Isaäcson M, Arntzen L, Heenan R, Canter P, Barrett T, Lee L, Mambo C, Levine W, Zaidi A. Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerg Infect Dis* 7, 812, 2001

EFSA. Shiga toxin-producing *E. coli* (STEC) O104: H4 2011 outbreaks in Europe: Taking Stock. *EFSA J* 9, 2390, 2011

EFSA. Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. *EFSA J.* 11, 3138, 2013

EFSA. Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. *EFSA J.* 18, e05967, 2020

Elder JR, Fratamico PM, Liu Y, Needleman DS, Bagi L, Tebbs R, Allred A, Siddavatam P, Suren H, Gujjula KR, DebRoy C, Dudley EG, Yan X. A Targeted sequencing assay for serotyping *Escherichia coli* using AgriSeq technology. *Front Microbiol* 11, 627997, 2020

Elsayed MSAE, Awad A, Tarabees R, Marzouk A. Virulence repertoire and antimicrobial resistance profile of Shiga toxin-producing *E.coli* isolated from sheep and goat farms from Al-Buhayra Egypt. *Pak Vet J* 38, 429-33, 2018

Espié E, Vaillant V, Mariani-Kurkdjian P, Grimont F, Martin-Schaller R, De Valk H, Vernozy-Rozand C. *Escherichia coli* O157 outbreak associated with fresh unpasteurized goats' cheese. *Epidemiol Infect* 134, 143-6, 2006

Ethelberg S, Olsen KEP, Scheutz F, Jensen C, Schiellerup P, Engberg J, Petersen AM, Olesen B, Gerner-Smidt P, Mølbak K. Virulence factors for hemolytic uremic syndrome, Denmark. *Emerg Infect Dis* 10, 842-7, 2004

Ewing WH. Edwards and Ewing's identification of Enterobacteriaceae. In: Edwards and Ewing's Identification of Enterobacteriaceae. (Elsevier) 1986

Fagerquist CK, Garbus BR, Miller WG, Williams KE, Yee E, Bates AH, Boyle S, Harden LA, Cooley MB, Mandrell RE. Rapid identification of protein biomarkers of *Escherichia coli* O157:H7 by matrix-assisted laser desorption ionization-time-of-flight-time-of-flight mass spectrometry and top-down proteomics. *Anal Chem* 82, 2717-25, 2010

Feng P. Shiga toxin-producing *Escherichia coli* (STEC) in Fresh Produce-a food safety dilemma. *Microbiol Spectr* 2, Ehec-0010-2013, 2014

Feng P, Weagant SD, Jinneman K. *Bacteriological Analytical Manual Chapter 4a Diarrheagenic Escherichia coli,* 2015 **http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070080.htm** Accessed on May 27, 2022

Feng PC, Reddy S. Prevalences of Shiga toxin subtypes and selected other virulence factors among Shiga-toxigenic *Escherichia coli* strains isolated from fresh produce. *Appl Environ Microbiol* 79, 6917-23, 2013

Fields PI, Blom K, Hughes HJ, Helsel LO, Feng P, Swaminathan B. Molecular characterization of the gene encoding H antigen in *Escherichia coli* and development of a PCR-restriction fragment length polymorphism test for identification of *E. coli* O157:H7 and O157:NM. *J Clin Microbiol* 35, 1066-70, 1997

Frank C, Werber D, Cramer JP, Askar M, Faber M, an der Heiden M, Bernard H, Fruth A, Prager R, Spode A, Wadl M, Zoufaly A, Jordan S, Kemper MJ, Follin P, Müller L, King LA, Rosner B, Buchholz U, Stark K, Krause G. Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *N Engl J Med* 365, 1771-80, 2011

Fratamico P. Detection of O antigens in *Escherichia coli*. *Article in Animal Health Research Reviews*, 2011

Fratamico PM, DebRoy C, Strobaugh J, Terence P, Chen C-Y. DNA sequence of the *Escherichia coli* O103 O antigen gene cluster and detection of enterohemorrhagic *E. coli* O103 by PCR amplification of the *wzx* and *wzy* genes. *Can J Microbiol* 51, 515-22, 2005

Fratamico PM, DebRoy C, Miyamoto T, Liu Y. PCR detection of enterohemorrhagic *Escherichia coli* O145 in food by targeting genes in the *E. coli* O145 O-antigen gene cluster and the shiga toxin 1 and shiga toxin 2 genes. *Foodborne Pathog Dis* 6, 605-11, 2009

Fratamico PM, Briggs CE, Needle D, Chen CY, DebRoy C. Sequence of the *Escherichia coli* O121 O-antigen gene cluster and detection of enterohemorrhagic *E. coli* O121 by PCR amplification of the *wzx* and *wzy* genes. *J Clin Microbiol* 41, 3379-83, 2003

Fratamico PM, Bagi LK, Cray WC, Jr., Narang N, Yan X, Medina M, Liu Y. Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 in ground beef. *Foodborne Pathog Dis* 8, 601-7, 2011

Friedrich AW, Bielaszewska M, Zhang W-L, Pulz M, Kuczius T, Ammon A, Karch H. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J Infect Dis* 185, 74-84, 2002

Gannon VPJ, D'Souza S, Graham T, King RK, Rahn K, Read S. Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. *J Clin Microbiol* 35, 656, 1997

Germinario C, Caprioli A, Giordano M, Chironna M, Gallone MS, Tafuri S, Minelli F, Maugliani A, Michelacci V, Santangelo L, Mongelli O, Montagna C, Scavia G. Community-wide outbreak of haemolytic uraemic syndrome associated with Shiga toxin 2-producing *Escherichia coli* O26:H11 in southern Italy, summer 2013. *Euro Surveill* 21, 2016

Geue L, Monecke S, Engelmann I, Braun S, Slickers P, Ehricht R. Rapid microarray-based DNA genoserotyping of *Escherichia coli*. *Microbiol Immunol* 58, 77-86, 2014

Gruenheid S, Sekirov I, Thomas NA, Deng W, O'Donnell P, Goode D, Li Y, Frey EA, Brown NF, Metalnikov P, Pawson T, Ashman K, Finlay BB. Identification and characterization of NleA, a non-LEE-encoded type III translocated virulence factor of enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* 51, 1233-49, 2004

Guinée PAM, Agterberg CM, Jansen WH. *Escherichia coli* O antigen typing by means of a mechanized microtechnique. *Appl Microbiol* 24, 127-31, 1972

Gupta MD, Das A, Islam MZ, Biswas PK. Prevalence of sorbitol non-fermenting Shiga toxin-producing *Escherichia coli* in Black Bengal goats on smallholdings. *Epidemiol Infect* 144, 2501-8, 2016

Hacker J, Bender L, Ott M, Wingender J, Lund B, Marre R, Goebel W. Deletions of chromosomal regions coding for fimbriae and hemolysins occur in vitro and in vivo in various extraintestinal *Escherichia coli* isolates. *Microb Pathog* 8, 213-25, 1990

Haenlein GFW, Ramirez RG. Potential mineral deficiencies on arid rangelands for small ruminants with special reference to Mexico. *Small Rumin Res* 68, 35-41, 2007

Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, Han C-G, Ohtsubo E, Nakayama K, Murata T. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157: H7 and genomic comparison with a laboratory strain K-12. *DNA Res* 8, 11-22, 2001

Heuvelink AE, Van Heerwaarden C, Zwartkruis-Nahuis JTM, Van Oosterom R, Edink K, Van Duynhoven YTHP, De Boer E. *Escherichia coli* O157 infection associated with a petting zoo. *Epidemiol Infect* 129, 295-302, 2002

Hiko A, Asrat D, Zewde G. Occurrence of *Escherichia coli* O157: H7 in retail raw meat products in Ethiopia. *J Infect in Dev Ctries* 2, 389-93, 2008

Hoang Minh S, Kimura E, Hoang Minh D, Honjoh Ki, Miyamoto T. Virulence characteristics of Shiga toxin-producing *Escherichia coli* from raw meats and clinical samples. *Microbiol Immunol* 59, 114-22, 2015

Horcajo P, Domínguez-Bernal G, De La Fuente R, Ruiz-Santa-Quiterai JA, Orden JA. Association of Vt1C with Verotoxin-producing *Escherichia coli* from goats and sheep. *J Vet Diagn Invest* 22, 332-4, 2010

Huang A, Friesen J, Brunton JL. Characterization of a bacteriophage that carries the genes for production of Shiga-like toxin 1 in *Escherichia coli*. *J Bacteriol* 169, 4308-12, 1987

Hughes JM, Wilson ME, Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. *Clin Infect Dis* 43, 1587-95, 2006

Hussein HS, Bollinger LM. Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. *J Food Prot* 68, 2224-41, 2005

Hussein HS, Sakuma T. Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J Dairy Sci* 88, 450-65, 2005

Iguchi A, Iyoda S, Seto K, Morita-Ishihara T, Scheutz F, Ohnishi M. *Escherichia coli* O-genotyping PCR: a comprehensive and practical platform for molecular O serogrouping. *J Clin Microbiol* 53, 2427-32, 2015a

Iguchi A, Iyoda S, Kikuchi T, Ogura Y, Katsura K, Ohnishi M, Hayashi T, Thomson NR. A complete view of the genetic diversity of the *Escherichia coli* O-antigen biosynthesis gene cluster. *DNA Res* 22, 101-7, 2015b

Iguchi A, Iyoda S, Seto K, Nishii H, Ohnishi M, Mekata H, Ogura Y, Hayashi T. Six novel O genotypes from Shiga toxin-producing *Escherichia coli*. *Front Microbiol* 7, 2016

Iguchi A, Nishii H, Seto K, Mitobe J, Lee K, Konishi N, Obata H, Kikuchi T, Iyoda S. Additional Og-typing PCR techniques targeting *Escherichia coli*-novel and *Shigella*-unique O-antigen biosynthesis gene clusters. *J Clin Microbiol* 58, 2020

Ingle DJ, Valcanis M, Kuzevski A, Tauschek M, Inouye M, Stinear T, Levine MM, Robins-Browne RM, Holt KE. *In silico* serotyping of *E. coli* from short read data identifies limited novel O-loci but extensive diversity of O:H serotype combinations within and between pathogenic lineages. *Microb Genom* 2, e000064, 2016

Inouye M, Dashnow H, Raven L-A, Schultz MB, Pope BJ, Tomita T, Zobel J, Holt KE. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med* 6, 1-16, 2014

Irino K, Vieira MA, Gomes TA, Guth BE, Naves ZV, Oliveira MG, dos Santos LF, Guirro M, Timm CD, Pigatto CP, Farah SM, Vaz TM. Subtilase cytotoxin-encoding *subAB*

operon found exclusively among Shiga toxin-producing *Escherichia coli* strains. *J Clin Microbiol* 48, 988-90, 2010

Isaäcson M, Canter P, Effler P, Arntzen L, Bomans P, Heenan R. Haemorrhagic colitis epidemic in Africa. *Lancet* 341, 961, 1993

Ishii S, Meyer KP, Sadowsky MJ. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. *Appl Environ Microbiol* 73, 5703-10, 2007

Islam MA, Mondol AS, De Boer E, Beumer RR, Zwietering MH, Talukder KA, Heuvelink AE. Prevalence and genetic characterization of shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. *Appl Environ Microbiol* 74, 5414-21, 2008

Isler M, Wissmann R, Morach M, Zurfluh K, Stephan R, Nüesch-Inderbinen M. Animal petting zoos as sources of Shiga toxin-producing *Escherichia coli*, *Salmonella* and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. *Zoonoses Public Health* 68, 79-87, 2021

Iwu CJ, Iweriebor BC, Obi LC, Okoh AI. Occurrence of non-O157 Shiga toxin-producing *Escherichia coli* in two commercial swine farms in the Eastern Cape Province, South Africa. *Comp Immunol Microbiol Infect Dis* 44, 48-53, 2016

Jacob M, Foster D, Rogers A, Balcomb C, Sanderson M. Prevalence and relatedness of *Escherichia coli* O157: H7 strains in the feces and on the hides and carcasses of US meat goats at slaughter. *Appl Environ Microbiol* 79, 4154-8, 2013a

Jacob ME, Foster DM, Rogers AT, Balcomb CC, Shi X, Nagaraja TG. Evidence of Non-O157 Shiga Toxin-Producing *Escherichia coli* in the Feces of Meat Goats at a U.S. Slaughter Plant. *J Food Prot* 76, 1626-9, 2013b

Jajarmi M, Askari Badouei M, Imani Fooladi AA, Ghanbarpour R, Ahmadi A. Pathogenic potential of Shiga toxin-producing *Escherichia coli* strains of caprine origin: Virulence genes, Shiga toxin subtypes, phylogenetic background and clonal relatedness. *BMC Vet Res* 14, 97, 2018

Jenkins C. Whole-Genome sequencing data for serotyping *Escherichia coli*-it's time for a change! *J Clin Microbiol* 53, 2402-3, 2015

Jenkins C, Dallman TJ, Grant KA. Impact of whole genome sequencing on the investigation of food-borne outbreaks of Shiga toxin-producing *Escherichia coli* serogroup O157:H7, England, 2013 to 2017. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 24, 1800346, 2019

Jenkins C, Perry NT, Cheasty T, Shaw DJ, Frankel G, Dougan G, Gunn GJ, Smith HR, Paton AW, Paton JC. Distribution of the saa gene in strains of Shiga toxin-producing *Escherichia coli* of human and bovine origins. *J Clin Microbiol* 41, 1775-8, 2003

Jerse AE, Yu J, Tall BD, Kaper JB. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci U S A* 87, 7839-43, 1990

Joensen KG, Tetzschner AMM, Iguchi A, Aarestrup FM, Scheutz F. Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. *J Clin Microbiol* 53, 2410-26, 2015

Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52, 1501-10, 2014

Ju W, Shen J, Toro M, Zhao S, Menga J. Distribution of pathogenicity islands OI-122, OI-43/48, and OI-57 and a high-pathogenicity island in Shiga toxin-producing *Escherichia coli*. *Appl Environ Microbiol* 79, 3406-12, 2013

Kallow W, Erhard M, Shah HN, Raptakis E, Welker M. MALDI-TOF MS for microbial identification: years of experimental development to an established protocol. *Mass spectrometry for microbial proteomics* 255, 276, 2010

Käppeli U, Hächler H, Giezendanner N, Beutin L, Stephan R. Human infections with non-O157 Shiga toxin-producing *Escherichia coli*, Switzerland, 2000-2009. *Emerg Infect Dis* 17, 180-5, 2011

Karama M, Gyles CL. Virulence profiling of Shiga toxin-producing *Escherichia coli* O111:NM isolates from cattle. *Appl Environ Microbiol* 79, 4164-5, 2013

Karama M, Johnson RP, Holtslander R, Gyles CL. Production of verotoxin and distribution of O islands 122 and 43/48 among verotoxin-producing *Escherichia coli* O103:H2 isolates from cattle and humans. *Appl Environ Microbiol* 75, 268-70, 2009

Karama M, Mainga AO, Cenci-Goga BT, Malahlela M, El-Ashram S, Kalake A. Molecular profiling and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* O26, O45, O103, O121, O145 and O157 isolates from cattle on cow-calf operations in South Africa. *Sci Rep* 9, 11930, 2019a

Karama M, Cenci-Goga BT, Malahlela M, Smith AM, Keddy KH, El-Ashram S, Kabiru LM, Kalake A. Virulence characteristics and antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* isolates from humans in South Africa: 2006-2013. *Toxins (Basel)* 11, 2019b

Karmali MA. Infection by Shiga toxin-producing *Escherichia coli*: an overview. *Mol Biotechnol* 26, 117-22, 2004

Karmali MA, Petric M, Steele BT, Lim C. Sporadic cases of haemolytic uremic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet.* 321, 619-20, 1983

Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, Isaac-Renton J, Clark C, Rahn K, Kaper JB. Association of genomic O island 122 of

Escherichia coli EDL 933 with Verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol* 41, 4930-40, 2003

Kauffmann F. The serology of the coli group. *J Immunol* 57, 71-100, 1947

Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg Infect Dis* 12, 780-6, 2006

Khaitan A, Jandhyala DM, Thorpe CM, Ritchie JM, Paton AW. The operon encoding SubAB, a novel cytotoxin, is present in shiga toxin-producing *Escherichia coli* isolates from the United States. *J Clin Microbiol* 45, 1374-5, 2007

Kido N, Torgov VI, Sugiyama T, Uchiya K, Sugihara H, Komatsu T, Kato N, Jann K. Expression of the O9 polysaccharide of *Escherichia coli*: sequencing of the *E. coli* O9 *rfb* gene cluster, characterization of mannosyl transferases, and evidence for an ATP-binding cassette transport system. *J Bacteriol* 177, 2178-87, 1995

Klapproth JM, Scaletsky IC, McNamara BP, Lai LC, Malstrom C, James SP, Donnenberg MS. A large toxin from pathogenic *Escherichia coli* strains that inhibits lymphocyte activation. *Infect Immun* 68, 2148-55, 2000

Konczy P, Ziebell K, Mascarenhas M, Choi A, Michaud C, Kropinski AM, Whittam TS, Wickham M, Finlay B, Karmali MA. Genomic O island 122, locus for enterocyte effacement, and the evolution of virulent verocytotoxin-producing *Escherichia coli*. *J Bacteriol* 190, 5832-40, 2008

La Ragione RM, Best A, Woodward MJ, Wales AD. *Escherichia coli* O157:H7 colonization in small domestic ruminants. *FEMS Microbiol Rev* 33, 394-410, 2009

La Ragione RM, Ahmed NM, Best A, Clifford D, Weyer U, Cooley WA, Johnson L, Pearson GR, Woodward MJ. Colonization of 8-week-old conventionally reared goats by *Escherichia coli* O157 : H7 after oral inoculation. *J Med Microbiol* 54, 485-92, 2005

Lacher DW, Gangiredla J, Jackson SA, Elkins CA, Feng PC. Novel microarray design for molecular serotyping of shiga toxin- producing *Escherichia coli* strains isolated from fresh produce. *Appl Environ Microbiol* 80, 4677-82, 2014

Lacher DW, Gangiredla J, Patel I, Elkins CA, Feng PCH. Use of the *Escherichia coli* identification microarray for characterizing the health risks of Shiga toxin-producing *Escherichia coli* isolated from foods. *J Food Prot* 79, 1656-62, 2016

Laughlin M, Gambino-Shirley K, Gacek P, Phan Q, Stevenson L, Mercante A, Mullins J, Burnworth L, Blackstock A, Razeq JH, Cartter M, Nichols M. Notes from the field: outbreak of *Escherichia coli* O157 infections associated with goat dairy farm visits-Connecticut, 2016 *MMWR. Morb Mortal Wkly Rep* 65, 1453-4, 2016

LeJeune JT, Davis MA. Outbreaks of zoonotic enteric disease associated with animal exhibits. *J Am Vet Med Assoc* 224, 1440-5, 2004

Lin A, Sultan O, Lau HK, Wong E, Hartman G, Lauzon CR. O serogroup specific real time PCR assays for the detection and identification of nine clinically relevant non-O157 STECs. *Food Microbiol* 28, 478-83, 2011

Lin A, Kase JA, Moore MM, Son I, Tran N, Clotilde LM, Jarvis K, Jones K, Kasturi K, Nabe K, Nucci M, Wagley GS, Wang F, Ge B, Hammack TS. Multilaboratory validation of a luminex microbead-based suspension array for the identification of the 11 most clinically relevant Shiga toxin-producing *Escherichia coli* O serogroups. *J Food Prot* 76, 867-70, 2013

Lindsey RL, Pouseele H, Chen JC, Strockbine NA, Carleton HA. Implementation of whole genome sequencing (WGS) for identification and characterization of Shiga toxin-producing *Escherichia coli* (STEC) in the United States. *Front Microbiol* 7, 766, 2016

Liu D, Cole RA, Reeves PR. An O-antigen processing function for Wzx (RfbX): a promising candidate for O-unit flippase. *J Bacteriol* 178, 2102-7, 1996

Liu Y, Fratamico P. *Escherichia coli* O antigen typing using DNA microarrays. *Mol Cell Probes* 20, 239-44, 2006

Liu Y, DebRoy C, Fratamico P. Sequencing and analysis of the *Escherichia coli* serogroup O117, O126, and O146 O-antigen gene clusters and development of PCR assays targeting serogroup O117-, O126-, and O146-specific DNA sequences. *Mol Cell Probes* 21, 295-302, 2007

Liu Y, Fratamico P, Debroy C, Bumbaugh AC, Allen JW. DNA sequencing and identification of serogroup-specific genes in the *Escherichia coli* O118 O antigen gene cluster and demonstration of antigenic diversity but only minor variation in DNA sequence of the O antigen clusters of *E. coli* O118 and O151. *Foodborne Pathog Dis* 5, 449-57, 2008

Liu Y, Yan X, DebRoy C, Fratamico PM, Needleman DS, Li RW, Wang W, Losada L, Brinkac L, Radune D, Toro M, Hegde N, Meng J. *Escherichia coli* O-antigen gene clusters of serogroups O62, O68, O131, O140, O142, and O163: DNA sequences and similarity between O62 and O68, and PCR-based serogrouping. *Biosensors (Basel)* 5, 51-68, 2015

Lu Y, Swartz JR. Functional properties of flagellin as a stimulator of innate immunity. *Sci Rep* 6, 18379, 2016

Ludwig JB, Shi X, Shridhar PB, Roberts EL, DebRoy C, Phebus RK, Bai J, Nagaraja TG. Multiplex PCR assays for the detection of one hundred and thirty seven serogroups of Shiga toxin-producing *Escherichia coli* associated with cattle. *Front. Cell Infect Microbiol* 10, 2020

Machado J, Grimont F, Grimont PAD. Identification of *Escherichia coli* flagellar types by restriction of the amplified *fliC* gene. *Res Microbiol* 151, 535-46, 2000

MacLean LL, Vinogradov E, Pagotto F, Farber JM, Perry MB. The structure of the O-antigen of *Cronobacter sakazakii* HPB 2855 isolate involved in a neonatal infection. *Carbohydr Res* 345, 1932-7, 2010

Madic J, Vingadassalon N, de Garam CP, Marault M, Scheutz F, Brugère H, Jamet E, Auvray F. Detection of Shiga toxin-producing *Escherichia coli* serotypes O26:H11, O103:H2, O111:H8, O145:H28, and O157:H7 in raw-milk cheeses by using multiplex real-time PCR. *Appl Environ Microbiol* 77, 2035-41, 2011

Mahanjana A, Cronje P. Factors affecting goat production in a communal farming system in the Eastern Cape region of South Africa. *S Afr J Anim Sci* 30, 149-55, 2000

Mahanti A, Samanta I, Bandyopadhyay S, Joardar SN. Molecular characterization and antibiotic susceptibility pattern of caprine Shiga toxin producing-*Escherichia coli* (STEC) isolates from India. *Iran. J. Vet. Res.* 16, 31-5, 2015

Mainga AO, Cenci-Goga BT, Malahlela MN, Tshuma T, Kalake A, Karama M. Occurrence and characterization of seven major Shiga toxin-producing *Escherichia coli* serotypes from healthy cattle on cow–calf operations in South Africa. *Zoonoses Public Health.* 65, 777-89, 2018

Mainil J. Shiga/verocytotoxins and Shiga/verotoxigenic *Escherichia coli* in animals. *Vet Res* 30, 235-57, 1999

Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, Yeung DH, Kirk MD. Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *Foodborne Pathog Dis* 11, 447-55, 2014

Martin A, Beutin L. Characteristics of Shiga toxin-producing *Escherichia coli* from meat and milk products of different origins and association with food producing animals as main contamination sources. *Int J Food Microbiol* 146, 99-104, 2011

Matussek A, Jernberg C, Einemo IM, Monecke S, Ehricht R, Engelmann I, Löfgren S, Mernelius S. Genetic makeup of Shiga toxin-producing *Escherichia coli* in relation to clinical symptoms and duration of shedding: a microarray analysis of isolates from Swedish children. *Eur J Clin Microbiol Infect Dis* 36, 1433-41, 2017

McCarthy SC, Burgess CM, Fanning S, Duffy G. An Overview of Shiga-toxin producing *Escherichia coli* carriage and prevalence in the ovine meat production chain. *Foodborne Pathog Dis* 18, 147-68, 2021

McDaniel TK, Kaper JB. A cloned pathogenicity island from enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K-12. *Mol Microbiol* 23, 399-407, 1997

McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci U S A* 92, 1664-8, 1995

McIntyre L, Fung J, Paccagnella A, Isaac-Renton J, Rockwell F, Emerson B, Preston T. *Escherichia coli* O157 outbreak associated with the ingestion of unpasteurized goat's milk in British Columbia, 2001. *Can Commun Dis Rep* 28, 6-8, 2002

McKee ML, O'Brien AD. Truncated enterohemorrhagic *Escherichia coli* (EHEC) O157: H7 intimin (EaeA) fusion proteins promote adherence of EHEC strains to HEp-2 cells. *Infect Immun* 64, 2225-33, 1996

Meissner H, Scholtz M, Palmer A. Sustainability of the South African livestock sector towards 2050 Part 1: worth and impact of the sector. *S Afr J Anim Sci* 43, 282-97, 2013

Mellies JL, Lorenzen E. Enterohemorrhagic *Escherichia coli* virulence gene regulation. *Microbiol Spectr* 2, Ehec-0004-2013, 2014

Mellmann A, Fruth A, Friedrich AW, Wieler LH, Harmsen D, Werber D, Middendorf B, Bielaszewska M, Karch H. Phylogeny and disease association of Shiga toxin-producing *Escherichia coli* O91. *Emerg Infect Dis* 15, 1474-7, 2009

Melton-Celsa AR. Shiga Toxin (Stx) Classification, structure, and function. *Microbiol spectr* 2, 10.1128/microbiolspec.EHEC-0024-2013-, 2014

Mersha G, Asrat D, Zewde BM, Kyule M. Occurrence of *Escherichia coli* O157:H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. *Lett Appl Microbiol* 50, 71-6, 2010

Michino H, Araki K, Minami S, Takaya S, Sakai N, Miyazaki M, Ono A, Yanagawa H. Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *Am J Epidemiol* 150, 787-96, 1999

Momtaz H, Safarpoor F, Rahimi E, Ezadi H, Arab R. Incidence of Shiga toxin-producing *Escherichia coli* serogroups in ruminants' meat. *Meat Sci* 95, 381-8, 2013

Monday SR, Beisaw A, Feng PCH. Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. *Mol Cell Probes* 21, 308-11, 2007

Morand-Fehr P, Boutonnet JP, Devendra C, Dubeuf JP, Haenlein GFW, Holst P, Mowlem L, Capote J. Strategy for goat farming in the 21st century. *Small Rumin Res* 51, 175-83, 2004

Muehlherr JE, Zweifel C, Corti S, Blanco JE, Stephan R. Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. *J Dairy Sci* 86, 3849-56, 2003

Müller EE, Ehlers MM, Grabow WO. The occurrence of *E. coli* O157: H7 in South African water sources intended for direct and indirect human consumption. *Water Research* 35, 3085-8, 2001

Mundy R, Jenkins C, Yu J, Smith H, Frankel G. Distribution of *espI* among clinical enterohaemorrhagic and enteropathogenic *Escherichia coli* isolates. *J Med Microbiol* 53, 1145-9, 2004

Murray PR. What is new in clinical microbiology-microbial identification by MALDI-TOF mass spectrometry: a paper from the 2011 William Beaumont hospital Symposium on molecular pathology. *J Mol Diagn* 14, 419-23, 2012

Nakano S, Nagao M, Yamasaki T, Morimura H, Hama N, Iijima Y, Shinomiya H, Tanaka M, Yamamoto M, Matsumura Y, Miyake S, Ichiyama S. Evaluation of a surface plasmon resonance imaging-based multiplex O-antigen serogrouping for *Escherichia coli* using eleven major serotypes of Shiga -toxin-producing *E. coli*. *J Infect Chemother* 24, 443-8, 2018

Nasoff MS, Baker HV, 2nd, Wolf RE, Jr. DNA sequence of the *Escherichia coli* gene, *gnd,* for 6-phosphogluconate dehydrogenase. *Gene* 27, 253-64, 1984

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 11, 142-201, 1998

Nataro JP, Seriwatana J, Fasano A, Maneval DR, Guers LD, Noriega F, Dubovsky F, Levine MM, Morris JG, Jr. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and Shigella strains. *Infect Immun* 63, 4721-8, 1995

Ndegwa E, Alahmde A, Kim C, Kaseloo P, O'Brien D. Age related differences in phylogenetic diversity, prevalence of Shiga toxins, intimin, hemolysin genes and select serogroups of *Escherichia coli* from pastured meat goats detected in a longitudinal cohort study. *BMC Vet Res* 16, 1-15, 2020

Newton HJ, Sloan J, Bulach DM, Seemann T, Allison CC, Tauschek M, Robins-Browne RM, Paton JC, Whittam TS, Paton AW, Hartland EL. Shiga toxin-producing *Escherichia coli* strains negative for locus of enterocyte effacement. *Emerg Infect Dis* 15, 372-80, 2009

Nguyen TTH, Iguchi A, Ohata R, Kawai H, Ooka T, Nakajima H, Iyoda S. Distribution of novel Og types in Shiga toxin-producing *Escherichia coli* isolated from healthy cattle. *J Clin Microbiol* 59, 2021

Nicholls L, Grant TH, Robins-Browne RM. Identification of a novel genetic locus that is required for in vitro adhesion of a clinical isolate of enterohaemorrhagic *Escherichia coli* to epithelial cells. *Mol Microbiol* 35, 275-88, 2000

Nichols MC, Gacek P, Phan Q, Gambino-Shirley KJ, Gollarza LM, Schroeder MN, Mercante A, Mullins J, Blackstock A, Laughlin ME, Olson SM, Pizzo E, Nguyen TN, Mank L, Holmes-Talbot K, McNutt A, Noel D, Muyombwe A, Razeq JH, Lis MJ, Sherman B, Kasacek W, Whitlock L, Strockbine N, Martin H, Vidyaprakash E, McCormack P, Cartter M. Agritourism and kidding season: a large outbreak of

human Shiga toxin-producing *Escherichia coli* O157 (STEC O157) infections linked to a goat dairy farm—Connecticut, 2016. *Front. Vet. Sci.* 8, 2021

Noll LW, Shridhar PB, Dewsbury DM, Shi X, Cernicchiaro N, Renter DG, Nagaraja TG. A comparison of culture- and PCR-based methods to detect six major non-O157 serogroups of Shiga Toxin-producing *Escherichia coli* in cattle feces. *PLoS One* 10, e0135446, 2015a

Novotna R, Alexa P, Hamrik J, Madanat A, Smola J, Cizek A. Isolation and characterization Shiga toxin-producing *Escherichia coli* from sheep and goats in Jordan with evidence of multiresistant serotype O157: H7. *Vet. Med. Czech* 50, 111-8, 2005

O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science.* 226, 694-6, 1984

O'Brien AO, Lively TA, Chen ME, Rothman SW, Formal SB. *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (SHIGA) like cytotoxin. *Lancet* 1, 702, 1983

Ojo OE, Ajuwape ATP, Otesile EB, Owoade AA, Oyekunle MA, Adetosoye AI. Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. *Int J Food Microbiol* 142, 214-21, 2010

Oliveira MG, Brito JR, Gomes TA, Guth BE, Vieira MA, Naves ZV, Vaz TM, Irino K. Diversity of virulence profiles of Shiga toxin-producing *Escherichia coli* serotypes in food-producing animals in Brazil. *Int J Food Microbiol* 127, 139-46, 2008

Orden J, Ruiz-Santa-Quiteria J, Blanco M, Blanco J, Mora A, Cid D, González E, Blanco J, De la Fuente R. Prevalence and characterization of Vero cytotoxin-producing *Escherichia coli* isolated from diarrhoeic and healthy sheep and goats. *Epidemiol Infect* 130, 313-21, 2003

Orden JA, Horcajo P, de la Fuente R, Ruiz-Santa-Quiteria JA, Domínguez-Bernal G, Carrión J. Subtilase cytotoxin-coding genes in verotoxin-producing *Escherichia coli* strains from sheep and goats differ from those from cattle. *Appl Environ Microbiol* 77, 8259-64, 2011

Orden JA, Cortés C, Horcajo P, De la Fuente R, Blanco JE, Mora A, López C, Blanco J, Contreras A, Sánchez A, Corrales JC, Domínguez-Bernal G. A longitudinal study of verotoxin-producing *Escherichia coli* in two dairy goat herds. *Vet Microbiol* 132, 428-34, 2008

Ørskov F, Ørskov I. Serotyping of *Escherichia coli*. In: Bergan T (ed). *Methods in Microbiology*. 43-112. Academic Press, 1984

Ørskov F, Ørskov I. *Escherichia coli* serotyping and disease in man and animals. *Can J Microbiol* 38, 699-704, 1992

Orskov I, Orskov F, Jann B, Jann K. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. *Bacteriol Rev* 41, 667-710, 1977

Ørskov I, Ørskov F, Bettelheim K, Chandler M. Two new *Escherichia coli* O antigens, O162 and O163, and one new H antigen, H56. Withdrawal of H antigen H50. *Acta Pathol Microbiol Scand B,* 83, 121-4, 1975

Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157: H7 infections. *J Infect Dis* 160, 994-8, 1989

Paddock Z, Shi X, Bai J, Nagaraja TG. Applicability of a multiplex PCR to detect O26, O45, O103, O111, O121, O145, and O157 serogroups of *Escherichia coli* in cattle feces1. *Vet Microbiol* 156, 381-8, 2012

Pai C, Kelly J, Meyers G. Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. *Infect Immun* 51, 16-23, 1986

Patel IR, Gangiredla J, Lacher DW, Mammel MK, Jackson SA, Lampel KA, Elkins CA. FDA *Escherichia coli* identification (FDA-ECID) microarray: a pangenome molecular toolbox for serotyping, virulence profiling, molecular epidemiology, and phylogeny. *Appl Environ Microbiol* 82, 3384, 2016

Patel IR, Gangiredla J, Lacher DW, Mammel MK, Bagi L, Baranzoni GM, Fratamico PM, Roberts EL, Deb RC, Lindsey RL, D VS, Martin H, Smith P, Strockbine NA, Elkins CA, Scheutz F, Feng PCH. Interlaboratory evaluation of the U.S. food and drug administration *Escherichia coli* identification microarray for profiling Shiga toxin-producing *Escherichia coli*. *J Food Prot* 81, 1275-82, 2018

Paton AW, Paton JC. *Escherichia coli* subtilase cytotoxin. *Toxins (Basel)* 2, 215-28, 2010

Paton AW, Srimanote P, Woodrow MC, Paton JC. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. *Infect Immun* 69, 6999-7009, 2001

Paton AW, Woodrow MC, Doyle RM, Lanser JA, Paton JC. Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic uremic syndrome. *J Clin Microbiol* 37, 3357-61, 1999

Paton AW, Srimanote P, Talbot UM, Wang H, Paton JC. A new family of potent AB5 cytotoxins produced by Shiga toxigenic *Escherichia coli*. *J Exp Med* 200, 35-46, 2004

Pépin M, Russo P, Pardon P. Public health hazards from small ruminant meat products in Europe. *Rev Sci Tech* 16, 415-25, 1997

Perelle S, Dilasser F, Grout J, Fach P. Detection by 5′-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157: H7,

associated with the world's most frequent clinical cases. *Mol Cell Probes* 18, 185-92, 2004

Perna NT, Plunkett G, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA. Genome sequence of enterohaemorrhagic *Escherichia coli* O157: H7. *Nature* 409, 529-33, 2001

Persad AK, LeJeune JT. Animal reservoirs of Shiga toxin-producing *Escherichia coli*. *Microbiol Spectr* 2, Ehec-0027-2014, 2014

Persson S, Olsen KEP, Ethelberg S, Scheutz F. Subtyping method for *Escherichia coli* Shiga toxin (Verocytotoxin) 2 variants and correlations to clinical manifestations. *J Clin Microbiol* 45, 2020-4, 2007

Prager R, Strutz U, Fruth A, Tschäpe H. Subtyping of pathogenic *Escherichia coli* strains using flagellar (H)-antigens: serotyping versus *fliC* polymorphisms. *Int J Med Microbiol* 292, 477-86, 2003

Pritchard GC, Smith R, Ellis-Iversen J, Cheasty T, Willshaw GA. Verocytotoxigenic *Escherichia coli* O157 in animals on public amenity premises in England and Wales, 1997 to 2007. *Vet Rec* 164, 545-9, 2009

Pulkkinen WS, Miller SI. A *Salmonella typhimurium* virulence protein is similar to a *Yersinia enterocolitica* invasion protein and a bacteriophage lambda outer membrane protein. *J Bacteriol* 173, 86-93, 1991

Rahimi E, Kazemeini HR, Salajegheh M. *Escherichia coli* O157:H7/NM prevalence in raw beef, camel, sheep, goat, and water buffalo meat in Fars and Khuzestan provinces, Iran. *Vet Res Forum* 3, 15-7, 2012

Rey J, Sánchez S, Blanco J, De Mendoza JH, De Mendoza MH, Garcia A, Gil C, Tejero N, Rubio R, Alonso J. Prevalence, serotypes and virulence genes of Shiga toxin-producing *Escherichia coli* isolated from ovine and caprine milk and other dairy products in Spain. *Int J Food Microbiol* 107, 212-7, 2006

Ribot EM, Freeman M, Hise KB, Gerner-Smidt P. PulseNet: Entering the age of next-generation sequencing. *Foodborne Pathog Dis* 16, 451-6, 2019

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype *N Engl J Med* 308, 681-5, 1983

Rumosa Gwaze F, Chimonyo M, Dzama K. Communal goat production in Southern Africa: a review. *Trop Anim Health Prod* 41, 1157-68, 2009

Samatey FA, Imada K, Nagashima S, Vonderviszt F, Kumasaka T, Yamamoto M, Namba K. Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. *Nature 2001 410:6826* 410, 331-7, 2001

Samuel G, Reeves P. Biosynthesis of O-antigens: genes and pathways involved in nucleotide sugar precursor synthesis and O-antigen assembly. *Carbohydr Res* 338, 2503-19, 2003

Sánchez S, Llorente MT, Echeita MA, Herrera-León S. Development of three multiplex PCR assays targeting the 21 most clinically relevant serogroups associated with Shiga toxin-producing *E. coli* infection in humans. *PLoS One* 10, e0117660, 2015

Sánchez S, Beristain X, Martínez R, García A, Martín C, Vidal D, Díaz-Sánchez S, Rey J, Alonso JM, Herrera-León S. Subtilase cytotoxin encoding genes are present in human, sheep and deer intimin-negative, Shiga toxin-producing *Escherichia coli* O128:H2. *Vet Microbiol* 159, 531-5, 2012

Scheutz F, Cheasty T, Woodward D, Smith HR. Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new *E. coli* O groups that include Verocytotoxin-producing *E. coli* (VTEC): O176, O177, O178, O179, O180 and O181. *APMIS* 112, 569-84, 2004

Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *J Clin Microbiol* 50, 2951-63, 2012

Schilling A-K, Hotzel H, Methner U, Sprague LD, Schmoock G, El-Adawy H, Ehricht R, Wöhr A-C, Erhard M, Geue L. Zoonotic agents in small ruminants kept on city farms in Southern Germany. *Appl Environ Microbiol.* 78, 3785-93, 2012

Schlager S, Lepuschitz S, Ruppitsch W, Ableitner O, Pietzka A, Neubauer S, Stoeger A, Lassnig H, Mikula C, Springer B. Petting zoos as sources of Shiga toxin-producing *Escherichia coli* (STEC) infections. *Int J Med Microbiol* 308, 927-32, 2018

Schmidt H, Hensel M. Pathogenicity islands in bacterial pathogenesis. *Clin Microbiol Rev* 17, 14-56, 2004

Schmidt H, Beutin L, Karch H. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157: H7 strain EDL 933. *Infect Immun* 63, 1055-61, 1995

Schmidt H, Henkel B, Karch H. A gene cluster closely related to type II secretion pathway operons of gram-negative bacteria is located on the large plasmid of enterohemorrhagic *Escherichia coli* O157 strains. *FEMS Microbiol Lett* 148, 265-72, 1997

Scotland S, Smith H, Willshaw G, Rowe B. Vero cytotoxin production in strain of *Escherichia coli* is determined by genes carried on bacteriophage. *Lancet.* 322, 216, 1983

Shridhar PB, Noll LW, Shi X, An B, Cernicchiaro N, Renter DG, Nagaraja TG, Bai J. Multiplex quantitative PCR assays for the detection and quantification of the six major non-O157 *Escherichia coli* serogroups in cattle feces. *J Food Prot* 79, 66-74, 2016

Singh P, Sha Q, Lacher DW, Del Valle J, Mosci RE, Moore JA, Scribner KT, Manning SD. Characterization of enteropathogenic and Shiga toxin-producing *Escherichia coli* in cattle and deer in a shared agroecosystem. *Front Cell Infect Microbiol* 5, 29, 2015

Smith AM, Tau NP, Sooka A, Keddy KH, Group for Enteric R, Africa MDSiS. Surveillance for enterohaemorrhagic *Escherichia coli* associated with human diarrhoea in South Africa, 2006–2009. *J Med Microbiol* 60, 681-3, 2011

Solomakos N, Govaris A, Angelidis AS, Pournaras S, Burriel AR, Kritas SK, Papageorgiou DK. Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. *Food Microbiol* 26, 865-71, 2009

Steen M, Hedin G, Hokeberg I, Eriksson E, Wallden B, Nagelhus O. EHEC from goat milk as the cause of severe diarrhoea in a young woman. *Svensk Vet Tidn* 53, 861-5, 2001

Stephan R, Schumacher S, Corti S, Krause G, Danuser J, Beutin L. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* in Swiss raw milk cheeses collected at producer level. *J Dairy Sci* 91, 2561-5, 2008

Stevens MP, Frankel GM. The locus of enterocyte effacement and associated virulence factors of enterohemorrhagic *Escherichia coli*. *Microbiol Spectr* 2, Ehec-0007-2013, 2014

Strockbine NA, Jackson M, Sung L, Holmes R, O'Brien AD. Cloning and sequencing of the genes for Shiga toxin from *Shigella dysenteriae* type 1. *J Bacteriol* 170, 1116-22, 1988

Tack DM, Kisselburgh HM, Richardson LC, Geissler A, Griffin PM, Payne DC, Gleason BL. Shiga toxin-producing *Escherichia coli* outbreaks in the United States, 2010-2017. *Microorganisms* 9, 1529, 2021

Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 365, 1073-86, 2005

Tzipori S, Gunzer F, Donnenberg MS, de Montigny L, Kaper JB, Donohue-Rolfe A. The role of the *eaeA* gene in diarrhea and neurological complications in a gnotobiotic piglet model of enterohemorrhagic *Escherichia coli* infection. *Infect Immun* 63, 3621-7, 1995

Vossenkämper A, Macdonald TT, Marchès O. Always one step ahead: how pathogenic bacteria use the type III secretion system to manipulate the intestinal mucosal immune system. *J Inflamm (Lond)* 8, 11-, 2011

Vu-Khac H, Cornick NA. Prevalence and genetic profiles of Shiga toxin-producing *Escherichia coli* strains isolated from buffaloes, cattle, and goats in central Vietnam. *Vet Microbiol* 126, 356-63, 2008

Wang Q, Perepelov AV, Feng L, Knirel YA, Li Y, Wang L. Genetic and structural analyses of *Escherichia coli* O107 and O117 O-antigens. *FEMS Immunol Med Microbiol* 55, 47-54, 2009

Wani SA, Samanta I, Munshi ZH, Bhat MA, Nishikawa Y. Shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* in healthy goats in India: occurrence and virulence properties. *J Appl Microbiol* 100, 108-13, 2006

Wasilenko JL, Fratamico PM, Sommers C, DeMarco DR, Varkey S, Rhoden K, Tice G. Detection of Shiga toxin-producing *Escherichia coli* (STEC) O157:H7, O26, O45, O103, O111, O121, and O145, and *Salmonella* in retail raw ground beef using the DuPont™ BAX® system. *Front Cell Infect Microbiol* 4, 81, 2014

WHO. *Zoonotic Non-0157 Shiga Toxin-Producing Escherichia Coli (STEC): report of a WHO scientific working group meeting, Berlin, Germany 23-26 June 1998*. 1998

Wickham ME, Lupp C, Mascarenhas M, Vazquez A, Coombes BK, Brown NF, Coburn BA, Deng W, Puente JL, Karmali MA, Finlay BB. Bacterial genetic determinants of non-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. *J Infect Dis* 194, 819-27, 2006

Wolfson JJ, Jandhyala DM, Gorczyca LA, Qadeer Z, Manning SD, Hadler J, Rudrik JT, Thorpe CM. Prevalence of the operon encoding subtilase cytotoxin in non-O157 Shiga toxin-producing *Escherichia coli* isolated from humans in the United States. *J Clin Microbiol* 47, 3058-9, 2009

Wong AR, Pearson JS, Bright MD, Munera D, Robinson KS, Lee SF, Frankel G, Hartland EL. Enteropathogenic and enterohaemorrhagic *Escherichia coli*: even more subversive elements. *Mol Microbiol* 80, 1420-38, 2011

Woods TA, Mendez HM, Ortega S, Shi X, Marx D, Bai J, Moxley RA, Nagaraja TG, Graves SW, Deshpande A. Development of 11-Plex MOL-PCR assay for the rapid screening of samples for Shiga toxin-producing *Escherichia coli*. *Front Cellr Infect Microbiol* 6, 92, 2016

Yang X, Bai X, Zhang J, Sun H, Fu S, Fan R, He X, Scheutz F, Matussek A, Xiong Y. *Escherichia coli* strains producing a novel Shiga toxin 2 subtype circulate in China. *Int J Med Microbiol* 310, 2020

Yates JR, 3rd. Mass spectrometry and the age of the proteome. *J Mass Spectrom* 33, 1-19, 1998

Yu J, Kaper JB. Cloning and characterization of the eae gene of enterohaemorrhagic *Escherichia coli* O157: H7. *Mol Microbiol* 6, 411-7, 1992

Zaheri H, Ghanbarpour R, Jajarmi M, Bagheri M, Ghanadian A, Askari Badouei M. Public health aspects of Shiga toxin-producing *Escherichia coli* (STEC) strains in sheep and goats of Bakhtiari pastoral tribe, Iran. *Trop Anim Health Prod* 52, 2721-4, 2020

Zhang X, Payne M, Kaur S, Lan R. Improved genomic identification, clustering, and serotyping of Shiga toxin-producing *Escherichia coli* using cluster/serotype-specific gene markers. *Front Cell Infect Microbiol* 11, 772574, 2021

Zschöck M, Hamann HP, Kloppert B, Wolter W. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. *Lett Appl Microbiol* 31, 203-8, 2000

Zweifel C, Giezendanner N, Corti S, Krause G, Beutin L, Danuser J, Stephan R. Characteristics of shiga toxin-producing *Escherichia coli* isolated from Swiss raw milk cheese within a 3-year monitoring program. *J Food Prot* 73, 88-91, 2010

3.0 CHAPTER III

Occurrence, serotypes and virulence characteristics of Shiga toxin-producing *Escherichia coli* isolates from goats on communal rangeland in South Africa

Chapter was published in *Toxins* 2022, *14*(5), 353;
https://doi.org/10.3390/toxins14050353 (Appendix 1)

3.1. Abstract

Shiga-toxin-producing *Escherichia coli* is a foodborne pathogen commonly associated with human disease characterized by mild or bloody diarrhoea hemorrhagic colitis and hemolytic uremic syndrome. This study investigated the occurrence of STEC in fecal samples of 289 goats in South Africa using microbiological culture and PCR. Furthermore, 628 goat STEC isolates were characterized by serotype (O:H) and major virulence factors by PCR. STEC was found in 80.2% (232/289) of goat fecal samples. Serotyping of 628 STEC isolates revealed 63 distinct serotypes including four of the major top seven STEC serogroups which were detected in 12.1% (35/289) of goats: O157:H7, 2.7% (8/289); O157:H8, 0.3%, (1/289); O157:H29, 0.3% (1/289); O103:H8, 7.6% (22/289); O103:H56, 0.3% (1/289); O26:H2, 0.3% (1/289); O111:H8, 0.3% (1/289) and 59 non-O157 STEC serotypes. Twenty-four of the sixty-three serotypes were previously associated with human disease. Virulence genes were distributed as follows: *stx1*, 60.6% (381/628); *stx2*, 72.7% (457/628); *eaeA*, 22.1% (139/628) and *hlyA*, 78.0% (490/628). Both *stx1* and *stx2* were found in 33.4% (210/628) of isolates. In conclusion, goats in South Africa are a reservoir and potential source of diverse STEC serotypes that are potentially virulent for humans. Further molecular characterization will be needed to fully assess the virulence potential of goat STEC isolates and their capacity to cause disease in humans.

Keywords: goats; STEC; serotypes; virulence; South Africa

Key Contribution: This research is a contribution towards STEC surveillance and improves our understanding of the epidemiology and virulence characteristics of goat STEC in South Africa.

3.2. Introduction

Shiga toxin-producing *E. coli* (STEC) is a foodborne pathogen commonly associated with enteric disease in humans characterized by mild watery or bloody diarrhoea, haemorrhagic colitis (HC) and the haemolytic uremic syndrome (HUS) as a complication in 5-10% of humans. According to the Foodborne Disease Burden Epidemiology Reference Group (FERG), STEC was responsible for around 2.5 million of new cases of human disease, of which 1.2 million may have been foodborne, with 3 330 HUS cases and 269 deaths, which corresponded to 27 000 Disability-Adjusted Life Years in 2010 (DALYs) (Kirk *et al.* 2015).

Domestic ruminants including cattle, sheep, and goats are the main reservoirs of STEC (Hussein 2007; La Ragione *et al.* 2009; Persad and Lejeune 2015; McCarthy *et al.* 2021). Ingestion of contaminated meat, dairy products, vegetables, and water is a risk factor for acquiring STEC infection in humans (Isaäcson *et al.* 1993; Hussein 2007; Lynch *et al.* 2009). Furthermore, contact with animals carrying STEC has also been associated with disease in humans (Schlager *et al.* 2018; Nichols *et al.* 2021).

More than 1000 different serotypes of Shiga toxin-producing *Escherichia coli* (STEC) have been described in humans, animals and the environment (EFSA 2013; Beutin and Fach 2014; Bettelheim and Goldwater 2019). STEC O157:H7 was the first serotype to be associated with a human disease outbreak and remains the most frequent strain in human illness (Riley *et al.* 1983). However, numerous non-O157 STEC serotypes have also been linked to outbreaks and severe disease in humans including HUS (Brooks *et al.* 2005; Hughes *et al.* 2006; EFSA 2013; Beutin and Fach 2014; Bettelheim and Goldwater 2019). STEC O26, O45, O103,

O111, O121 and O145 are the six most frequently incriminated non-O157 serogroups in human disease (Brooks *et al.* 2005; Hughes *et al.* 2006). Together with STEC O157, these "top 6" non-O157 serogroups are the major seven STEC serogroups, also colloquially termed "Big 7 or Top 7" STEC. Furthermore, according to the Centers for Disease Control and Prevention (CDC), at least 13 serotypes associated with top 6 serogroups including O26:H11 or nonmotile (NM); O45:H2 or NM; O103:H2, H11, H25; or NM; O111:H8 or NM; O121:H19 or H7; and O145: NM may be responsible for up to 80% of cases of human STEC disease in the United States (Brooks *et al.* 2005). In South Africa, serotypes O26:H11, O111:H8, O157:H7 and O107/O117:H7 were frequently implicated in disease between 2006–2013 (Karama *et al.* 2019b).

Bacteriophages-encoded Shiga toxins (*stx1* and *stx2*) and a number of *stx* subtypes are considered the major STEC virulence factors (Scotland *et al.* 1983; O'Brien *et al.* 1984; Strockbine *et al.* 1988; Scheutz *et al.* 2012). Identification of STEC is based on detection of one or more Shiga toxin-encoding genes (*stx1* and *stx2*) (Scotland *et al.* 1983; O'Brien *et al.* 1984; Strockbine *et al.* 1988). STEC that carry *stx2* are more frequently associated with severe disease including HUS in comparison to strains that possess s*tx2* alone or both *stx1* and *stx2* concomitantly (Ostroff *et al.* 1989; Boerlin *et al.* 1999; Donohue-Rolfe *et al.* 2000; Ethelberg *et al.* 2004; Persson *et al.* 2007).

Intimin (*eaeA*) is an additional important STEC virulence factor (Jerse *et al.* 1990; McKee and O'Brien 1996). The gene encoding intimin is located on a 35 Kb pathogenicity island in *E. coli* O157:H7 termed the locus of enterocyte effacement (LEE) (McDaniel *et al.* 1995; McDaniel and Kaper 1997). Intimin is responsible for intimate adherence of STEC to intestinal epithelial cells and formation of typical attaching and effacing (A/E) lesions in the intestine characterized by actin-rich pedestals and loss of brush border microvilli under bound bacteria

(Pai *et al.* 1986). Furthermore, STEC possess plasmid-encoded virulence markers including a hemolysin (*hly*A) and additional virulence-associated genes which are located on pathogenicity-islands (Schmidt *et al.* 1995; Schmidt and Karch 1996; Karmali *et al.* 2003; Schmidt and Hensel 2004; Coombes *et al.* 2008).

Current reports on the occurrence and characteristics of STEC in animals and humans in South Africa are scanty. Furthermore, the few studies that have reported on the occurrence of STEC in South Africa have largely investigated the presence of STEC in cattle populations (Ateba and Mbewe 2014; Mainga *et al.* 2018; Karama *et al.* 2019a) while studies on the prevalence of STEC in other ruminants including goats are lacking. Therefore, the main objectives of this study were (1) to determine the occurrence of STEC in goats raised on communal rangeland in South Africa and (2) characterize STEC by serotype (O:H) and major virulence factors (*stx1, stx2*, *eaeA*, and *hlyA*). The overall goal is to contribute to STEC monitoring and surveillance in South Africa.

3.3 Materials and methods

3.3.1 Study population and sample collection

Goat fecal samples (N=289) were obtained from four goat herds. The goat herds were located on different communal rangelands in Gauteng province, South Africa. The herds were designated using alphabetical letters: Herd A (n = 154), Herd B (n = 43), Herd C (n = 52) and Herd D (n = 40). Each herd was visited once. Refer to **Figure 2** for a map of the Gauteng province, South Africa showing the locations of the different herds (A, B, C and D) from which goat fecal samples were obtained. Fresh fecal samples were collected by rectal palpation, using a new nitrile examination glove per animal. Samples were placed in sterile specimen containers and transported in a cooler box on ice to the laboratory where they were stored at 4°C until further processing. Ethical clearance for conducting this research was obtained from

the Research Ethics and Animal Ethics Committees of the Faculty of Veterinary Science, University of Pretoria, under approval number REC110-21.

3.3.2 STEC culture

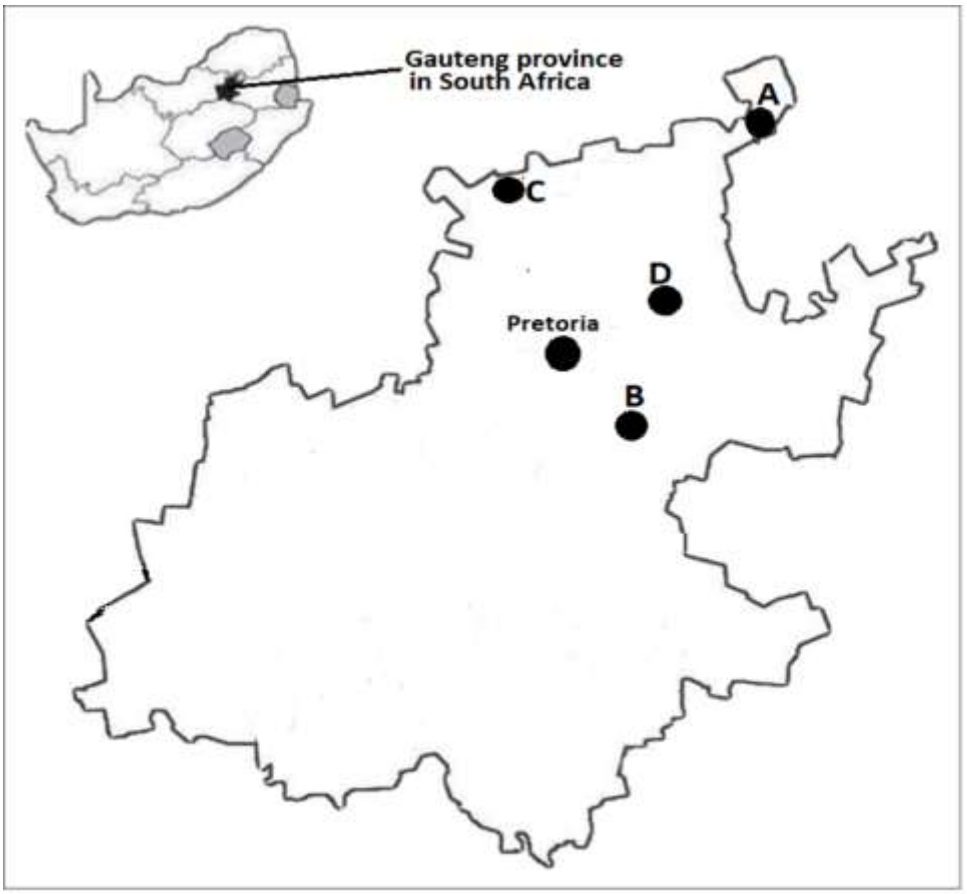
Each fecal sample (5g) was enriched at a 1:10 ratio in EC broth (CM0990, Oxoid, Basingstoke, United Kingdom) supplemented with Novobiocin (N1628, Sigma-Aldrich, St. Louis, MO) at 37 °C for 18-24 h. A 100 µl aliquot of the overnight enrichment was spread on Drigalski Lactose agar (CM0531, Oxoid, Basingstoke, United Kingdom) and CHROMagar STEC base ST162(B) containing supplement ST162(S) (CHROMagar, Paris, France, http://www.CHROMagar.com).

3.3.3 DNA extraction and STEC screening

All Drigalski Lactose and CHROMagar STEC agar petri dishes showing bacterial growth were screened for STEC by PCR (Paton and Paton 1998). Briefly, a loopful of bacterial colony sweep was collected from each Drigalski Lactose agar and CHROMagar STEC plate showing growth and suspended in 1ml of FA Buffer (223143, Becton Dickinson and Company, Sparks, MD, USA) (Mainga *et al.* 2018).

The suspension was homogenised and washed by vortexing, then centrifuged for 5 min. After centrifugation, the supernatant was discarded, and the pellet was re-suspended in FA buffer. After the second wash and centrifugation rounds, the pellet was re-suspended in 500 µl of sterile water, mixed and boiled at 100°C for 25 min. The boiled preparation was thawed on ice and stored at −20°C for further processing (Mainga *et al.* 2018). A multiplex PCR (mPCR) protocol was used to screen the DNA template for *stx1*, *stx2*, *eaeA* and *hlyA* using previously described cycling parameters and primers (Paton and Paton 1998). Briefly, each 25 µl PCR reaction mixture contained 2.5 µl of 10X Thermopol reaction buffer, 2.0 µl of 2.5 mM dNTPs

Figure 2: A map of the Gauteng province, South Africa showing the locations of the different herds (A, B, C and D) from which goat fecal samples were obtained



(deoxynucleotide triphosphate),0.25 µl of 100 mM $MgCl_2$, 0.6 µl of each primer (10 µM stock solution concentration), 1 U of Taq DNA Polymerase, and 5 µl of DNA template. DNA from *Escherichia coli* O157:H7 strain EDL933 (ATCC 43895) and sterile water were used as positive and negative PCR controls, respectively. All PCR reagents were purchased from New England BioLabs (NEB, Ipswich, MA, USA) except for the primers which were supplied by Inqaba Biotec (Pretoria, South Africa).

3.3.4 STEC Isolation and Identification

For STEC isolation and identification, colony sweeps were collected from Drigalski Lactose agar and CHROMagar plates which were positive for *stx1* and/or *stx2* on PCR and streaked

onto Drigalski Lactose agar and CHROMagar STEC to obtain single colonies. Five single colonies were purified from each plate and multiplied individually on Luria Bertani agar (REF244520, Difco Becton and Dickson & Company). Once again, DNA was extracted from purified colonies by the boiling method (Mainga *et al.* 2018). DNA from each purified colony was screened for *stx1*, *stx2* , *eaeA* and *hlyA* by PCR (Paton and Paton 1998) to verify and confirm the STEC status of each pure colony. Colonies which were positive for *stx1* and /or *stx2* were preserved at -80°C in a bacterial freezing mixture (Mainga *et al.* 2018) for further O:H serotyping.

3.3.5 STEC Serotyping

All confirmed STEC pure single colonies were serotyped (O:H) by PCR using previously described primers and cycling conditions (Iguchi *et al.* 2015; Banjo *et al.* 2018; Iguchi *et al.* 2020)**.** STEC strains which were previously serotyped by traditional serotyping at the National Microbiology Laboratory, Public Health Agency of Canada, Guelph, Ontario, Canada, and the Laboratorio de Referencia de *Escherichia coli* (LREC), Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, Spain and a number of *E. coli* O:H types in our collection (unpublished) were also used as positive controls in PCR serotyping assays. Furthermore, the following STEC isolates which were provided by the European Union Reference Laboratory for *Escherichia coli,* Istituto Superiore di Sanità, Rome Italy, were used as positive controls for serotyping the major seven STEC serogroups: STEC-C210-03 (O157), STEC-ED476 (STEC O111), STEC-C1178-04 (STEC O145), STEC-C125-06 (STEC O103) and STEC-ED745 (O26).

3.4 Results

3.4.1 STEC Occurrence

A total 289 fecal samples were collected from four goat herds on communal rangeland (Herd A, B, C, and D) in the Gauteng province of South Africa and screened for STEC. PCR

revealed that 80.2% (232/289) of goat fecal samples were positive for STEC. STEC was detected in 75.3% (116/154) of goats in herd A; 90.6% (39/43) in herd B; 78.8% (41/52) in herd C and 90% (36/40) in herd D (**Table 3** and **Figure 3**).

Table 3: Occurrence of STEC serotypes in the four goat herds (A, B, C and D)

Herds	Herd Occurrence	O Serogroups (N=34)	Serotypes (N=63)	Isolates (N= 628)	Number of Goats (N=289)
		O3 (58)	O3:H2	1	1
			O3:H11	1	1
			O3:H19	1	1
			O3:H21	55	14
A	(75.3%) 116/154	O8 (29)	O8:H14	1	1
			O8:H19	8	2
			O8:H21	5	1
			O8:H49	15	3
		O22 (3)	O22:H8	3	1
		O26 (1)	O26:H2	1	1
		O43 (50)	O43:H2	49	12
			O43:H8	1	1
		O49 (1)	O49:H11	1	1
		O54 (2)	O54:H16	1	1
			O54:H19	1	1
		O64 (1)	O64:H18	1	1
		O71 (3)	O71:H1/12	2	1
			O71:H14	1	1
		O76 (39)	O76:H2	1	1
			O76:H19	38	9

O103 (99)	O103:H8	98	22
	O103:H56	1	1
O108 (13)	O108:H19	1	1
	O108:H25	12	3
O111 (2)	O111:H8	2	1
O113 (7)	O113:H8	7	3
O146 (7)	O146:H21	7	2
O157 (30)	O157:H7	29	7
	O157:H8	1	1
O163 (1)	O163:H2	1	1
O175 (2)	O175:H7	1	1
	O175:H19	1	1
O185 (4)	O185:H8	4	3
OgN8 (4)	OgN8:H7	4	1
OgN13 (11)	OgN13:H19	9	1
	OgN13:H10	1	1
	OgN13:HNT	1	1
OgSB9 (7)	OgSB9:H2	1	1
	OgSB9:H19	6	2
OgX18 (2)	OgX18:H2	2	1
OgX25 (15)	OgX25:H8	15	3
ONT (6)	ONT:H18	1	1
	ONT:H19	1	1
	ONT:H26	1	1
	ONT:HNT	3	2

		O5 (3)	O5:H19	3	1
		O6 (14)	O6:H8	1	1
			O6:H21	1	1
			O6:H49	12	6
B	(90.6%) 39/43	O7 (1)	O7:H7	1	1
		O8 (3)	O8:H7	1	1
			O8:H8	2	2
			O8:H14	2	2
		O75 (37)	O75:H8	37	9
		O76 (8)	O76:H19	7	4
			O76:49	1	1
		O79 (1)	O79:H8	1	1
		O125 (1)	O125:H19	1	1
		O132 (1)	O132:H8	1	1
		O159 (1)	O159:H49	1	1
		O163 (1)	O163:H8	1	1
		O176 (5)	O176:H4	5	1
		OgSB9 (6)	OgSB9:H19	5	2
			OgSB9:H21	1	1
		OgX18 (1)	OgX18:H21	1	1
		OgX25 (2)	OgX25:H8	2	1
		O43 (25)	O43:H2	24	7
			O43:H8	1	1
		O76 (4)	O76:H19	4	3

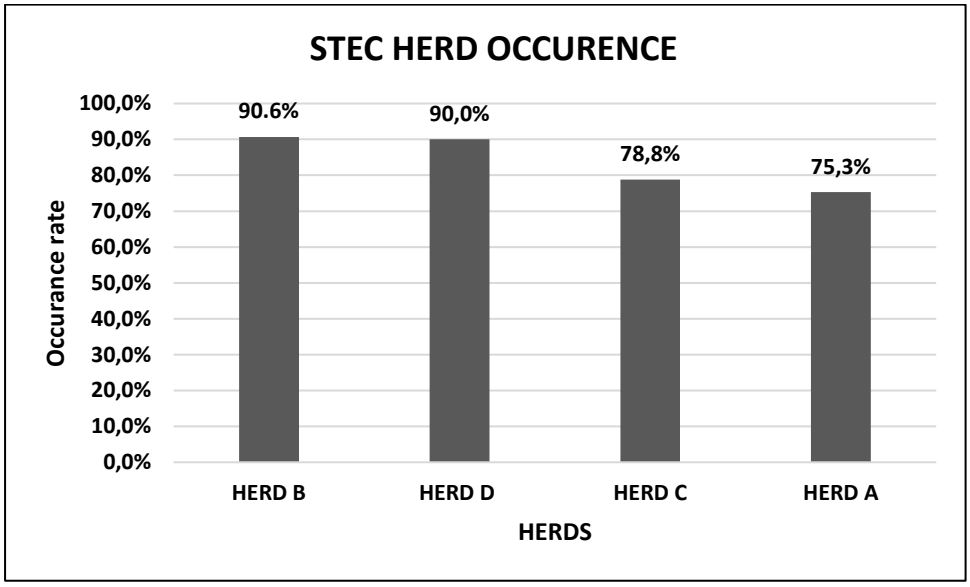
		O113 (1)	O113:H8	1	1
		O146 (11)	O146:H21	11	3
C	(78.8%) 41/52	O157 (2)	O157:H7	1	1
			O157:H29	1	1
		O174 (12)	O174:H8	12	4
		O175 (1)	O175:H21	1	1
		OgX18 (9)	OgX18:H2	9	2
		O3 (39)	O3:H21	39	9
		O8 (6)	O8:H2	1	1
			O8:H19	5	1
D	(90%) 36/40	O76 (1)	O76:H19	1	1
		O146 (13)	O146:H21	13	2
		O159 (1)	O159:H2	1	1
		O174 (4)	O174:H8	4	1
		OgX18 (15)	OgX18:H2	15	4

3.4.2 STEC O:H serotypes

At least 99% (622/628) of isolates were O:H serotypable by PCR. PCR serogrouping revealed 34 O groups and 17 H types with a total of 63 O:H distinct serotypes. Six isolates were O-untypable (ONT) (**Figure 4** and **Table 3**) and three H-untypable (HNT) (**Figure 5** and **Table 3**). The highest number of STEC serotypes was recovered from Herd A, 41 serotypes; followed by Herd B, 21 serotypes; Herd C, 10 serotypes; and Herd D, 8 serotypes. STEC O76:H19 was recovered from all herds: STEC O146:H21 and OgX18:H2 were recovered

from Herd A, C, and D. STEC O8:H14, OgSB9:H19 and OgX25:H8 were found in Herd A and B. STEC O43:H2, O43:H8, O113:H8 and O157:H7 were recovered from Herd A and C. STEC O3:H21 and O8:H19 were recovered from Herd A and D.

Figure 3: STEC occurrence within goat herds



Among the 34 O serogroups, 18 were associated with a single H type (O5:H19, O7:H7, O22:H8, O26:H2, O49:H11, O64:H18, O75:H8, O79:H8, O111:H8, O113:H8, O125:H19, O132:H8, O146:H21, O174:H8, O176:H4, O185:H8, ON8:H7, OX25:H8), and 16 O groups (O3, O6, O8, O43, O54, O71, O76, O103, O108, O157, O159, O163, O175, ON13, OSB9 and OX18) were associated with more than one H type.

The distribution of different STEC serotypes per goat herd is shown in **Table 3**. The following 17 H types were detected: H1/12, H2, H4, H7, H8, H10, H11, H14, H16, H18, H19, H21, H25, H26, H29, H49 and H56 (**Figure 5** and **Table 4**).

Among the 63 O:H distinct serotypes, 55.5%, (35/63) were each represented by a single isolate while the remaining 44.4% (28/63) were represented by more than one isolate (**Table 3**). O:H serotype combinations can be found in **Table 4**.

The six most frequent goat STEC serotypes were: O3:H21, 7.9% (23/289); O103:H8, 7.6% (22/289); O43:H2, 6.5% (19/289); O76:H19, 5.8% (17/289); O75:H8, 3.1%, (9/289); O157:H7, 2.7% (8/289). Big 7 STEC serotypes were recovered from 11.0% (32/289) of goats.

Table 4: Association between O group and H-type(s) among goat STEC isolates

O- Group	Associated H- type
O3	**H2** (1), H11 (1), H19 (1), **H21** (94)
O5	**H19** (3)
O6	H8 (1), H21 (1), **H49** (12)
O7	H7 (1)
O8	**H2** (1), **H7** (1), **H8**(2), **H14** (3), **H19**[d] (13), **H21**[d] (5), **H49** (15)
O22	**H8** (3)
O26	**H2** (1)
O43	**H2** (73), H8 (2)
O49	H11 (1)
O54	H16 (1), H19 (1)
O64	H18 (1)
O71	**H**1/**12** (2), H14 (1)
O75	**H8** (37)
O76	H2 (1), **H19** (50), H49 (1)
O79	H8 (1)
O103	H8 (98), H56 (1)
O108	H19 (1), H25 (12)
O111	**H8** (2)
O113	H8 (8)
O125	**H19** (1)
O132	H8 (1)
O146	**H21** (31)
O157	**H7** (30), **H8** (1), H29 (1)
O159	H2 (1), H49 (1)
O163	H2 (1), H8 (1)
O174	**H8** (16)
O175	H7 (1), H19 (1), **H21** (1)
O176	H4 (5)
O185	H8 (4)
ON8	H7 (4)
ON13	H10 (1), H19 (9), H- (1)
OSB9	H2 (1), H19 (11), H21(1)
OX18	H2 (26), H21 (1)
OX25	H8 (17)

a) The numbers in parentheses represent the number of goat isolates

b) Serotypes in **bold** have been associated with human disease

(Diarrhoea, Blood diarrhoea, hemorrhagic colitis, Hemolytic Uremic Syndrome) previously (reviewed by (Bettelheim and Goldwater 2019).

Big 7 STEC serotypes were distributed as follows among goats: O157:H7, 2.7% (8/289); O103:H8, 7.6% (22/289); O26:H2, 0.3% (1/289); O111:H8, 0.3% (1/289); O103:H56, 0.3% (1/289); O157:H8, 0.3% (1/289); O157:H29, 0.3% (1/289).

Figure 4: Distribution of STEC O serogroups

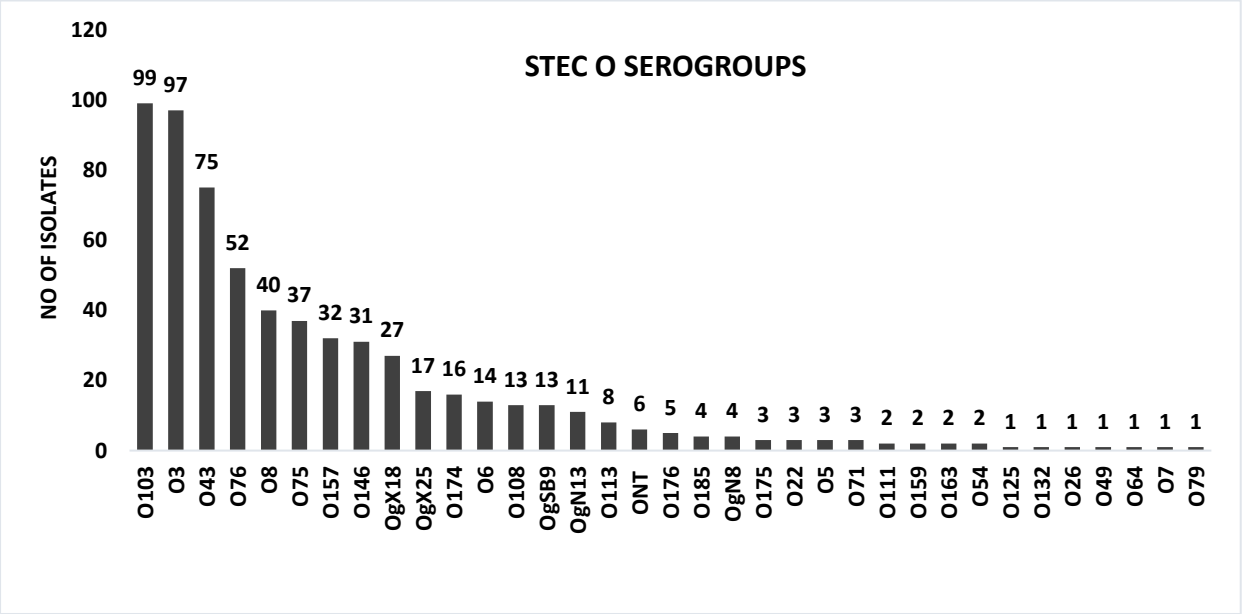
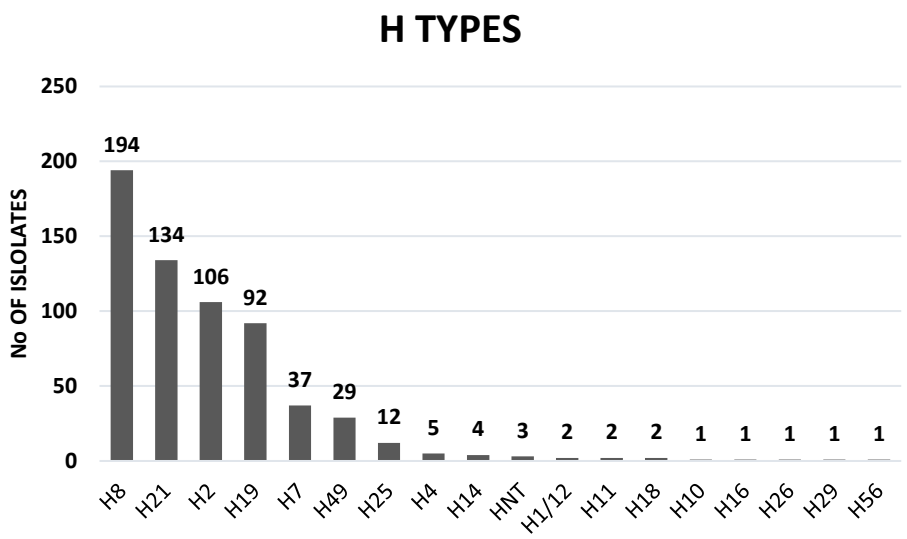


Figure 5: Distribution of STEC H-types



3.4.3 STEC virulence characteristics

The distribution of four STEC virulence genes among the 628 STEC isolates was as follows: *stx1,* 60.6% (381/628); *stx2*, 72.7% (457/628); *eaeA*, 22.1% (139/628); *hlyA*, 78.0% (490/628). Both *stx1* and *stx2* were found concomitantly in 33.4% (210/628) of isolates (**Table 5**). The following major gene combinations were observed: *stx1 stx2 hly*A, 26.9%, (169/628); *stx*2 *eaeA hlyA* 20.3%, (128/628) and *stx1 hly*A 20.0%, (126/628). STEC characteristics are depicted in **Table 5**.

Table 5. Goat STEC major virulence factors and gene combinations

Serotype	No of isolates	*stx1*	*stx2*	*eaeA*	*hlyA*	Gene combination
O3:H2	1	+	-	-	+	*stx1, hlyA*
O3:H11	1	+	+	-	-	*stx1, stx2*
O3:H19	1	+	+	-	+	*stx1, stx2, hlyA*
O3:H21	38	+	-	-	+	*stx1, hlyA*
O3:H21	39	+	+	-	+	*stx1, stx2, hlyA*

O3:H21	8	+	-	-	-	*stx1*
O3:H21	8	+	+	-	-	*stx1, stx2*
O3:H21	1	-	+	-	-	*stx2*
O5:H19	3	+	+	-	+	*stx1, stx2, hlyA*
O6:H8	1	+	+	-	+	*stx1, stx2, hlyA*
O6:H21	1	-	+	-	-	*stx2, hlyA*
O6:H49	12	-	+	-	-	*stx2*
O7:H7	1	+	+	-	+	*stx1, stx2, hlyA*
O8:H2	1	+	+	-	+	*stx1, stx2, hlyA*
O8:H7	1	-	+	-	-	*stx2*
O8:H8	2	+	+	-	+	*stx1, stx2, hlyA*
O8:H14	1	+	+	-	+	*stx1, stx2, hlyA*
O8:H14	2	-	+	-	-	*stx2*
O8:H19	5	+	+	-	-	*stx1, stx2*
O8:H19	3	+	+	-	+	*stx1, stx2, hlyA*
O8:H19	5	-	+	-	-	*stx2*
O8:H21	5	-	+	-	-	*stx2*
O8:H49	10	-	+	-	+	*stx2, hlyA*
O8:H49	5	-	+	-	-	*stx2*
O22:H8	3	-	+	-	-	*stx2*
O26:H2	1	+	-	+	+	*stx1, eaeA, hlyA*
O43:H2	15	+	+	-	-	*stx1, stx2*
O43:H2	8	+	+	-	+	*stx1, stx2, hlyA*
O43:H2	18	-	+	-	-	*stx2*

O43:H2	29	+	-	-	+	*stx1, hlyA*
O43:H2	3	-	+	-	+	*stx2, hlyA*
O43:H8	1	+	+	-	+	*stx1, stx2, hlyA*
O43:H8	1	+	-	-	+	*stx1, hlyA*
O49:H11	1	+	-	-	+	*stx1, hlyA*
O54:H16	1	+	+	-	-	*stx1, stx2*
O54:H19	1	+	+	-	-	*stx1, stx2*
O64:H18	1	+	-	-	+	*stx1, hlyA*
O71:H1/12	2	-	+	-	+	*stx2, hlyA*
O71:H14	1	-	+	+	+	*stx2, eaeA, hlyA*
O75:H8	33	+	+	-	+	*stx1, stx2, hlyA*
O75:H8	1	+	-	-	-	*stx1*
O75:H8	3	+	+	-	-	*stx1, stx2*
O76:H2	1	+	-	-	+	*stx1, hlyA*
O76:H19	36	+	-	-	+	*stx1, hlyA*
O76:H19	8	+	-	-	-	*stx1*
O76:H19	3	-	+	-	-	*stx2*
O76:H19	1	-	+	-	+	*stx2, hlyA*
O76:H19	2	+	+	-	+	*stx1, stx2, hlyA*
O76:H49	1	+	+	-	+	*stx1, stx2, hlyA*
O79:H8	1	+	+	-	+	*stx1, stx2, hlyA*
O103:H8	96	-	+	+	+	*stx2, eaeA, hlyA*
O103:H8	1	+	-	+	+	*stx1, eaeA, hlyA*
O103:H8	1	+	-	-	+	*stx1, hlyA*

O103:H56	1	+	-	-	+	*stx1, hlyA*
O108:H19	1	+	-	-	-	*stx1*
O108:H25	4	+	-	-	-	*stx1*
O108:H25	4	+	-	+	+	*stx1, eaeA, hlyA*
O108:H25	2	+	-	+	-	*stx1, eaeA,*
O108:H25	2	+	-	-	+	*stx1, hlyA*
O111:H8	2	+	-	+	+	*stx1, eaeA, hlyA*
O113:H8	7	-	+	-	+	*stx2, hlyA*
O113:H8	1	+	+	-	+	*stx1, stx2, hlyA*
O125:H19	1	+	+	-	-	*stx1, stx2*
O132:H8	1	+	-	-	-	*stx1*
O146:H21	16	-	+	-	+	*stx2, hlyA*
O146:H21	10	+	+	-	+	*stx1, stx2, hlyA*
O146:H21	5	+	-	-	-	*stx1*
O157:H7	30	-	+	+	+	*stx2, eaeA, hlyA*
O157:H8	1	-	+	+	+	*stx2, eaeA, hlyA*
O157:H29	1	-	+	-	-	*stx2*
O159:H2	1	+	+	-	+	*stx1, stx2, hlyA*
O159:H49	1	+	+	-	+	*stx1, stx2, hlyA*
O163:H2	1	+	-	-	-	*stx1*
O163:H8	1	+	-	+	+	*stx1, eaeA, hlyA*
O174:H8	13	+	+	-	+	*stx1, stx2, hlyA*
O174:H8	3	+	-	-	+	*stx1, hlyA*
O175:H7	1	+	+	-	+	*stx1, stx2, hlyA*

O175:H19	1	+	-	-	+	*stx1, hlyA*
O175:H21	1	+	+	-	+	*stx1, stx2, hlyA*
O176:H4	5	+	+	-	+	*stx1, stx2, hlyA*
O185:H8	2	+	+	-	-	*stx1, stx2*
O185:H8	2	+	+	-	+	*stx1, stx2, hlyA*
OgN8:H7	4	-	+	-	-	*stx2*
OgN13:H10	1	+	-	-	-	*stx1*
OgN13:H19	8	-	+	-	+	*stx2, hlyA*
OgN13:H19	1	-	+	-	-	*stx2*
OgN13:H-	1	+	-	-	-	*stx1*
OgSB9:H2	1	+	+	-	+	*stx1, stx2, hlyA*
OgSB9:H19	10	+	+	-	+	*stx1, stx2, hlyA*
OgSB9:H19	1	+	-	-	-	*stx1*
OgSB9:H21	1	+	+	-	+	*stx1, stx2, hlyA*
OgX18:H2	2	+	+	-	-	*stx1, stx2*
OgX18:H2	24	+	+	-	+	*stx1, stx2, hlyA*
OgX18:H21	1	+	-	-	-	*stx1*
OgX25:H8	10	-	+	-	+	*stx2, hlyA*
OgX25:H8	7	+	-	-	+	*stx1, hlyA*
ONT:H18	1	+	-	-	+	*stx1, hlyA*
ONT:H19	1	+	-	-	+	*stx1, hlyA*
ONT:H26	1	+	+	-	-	*stx1, stx2,*
ONT:H-	2	+	-	-	+	*stx1, hlyA*
ONT:H-	1	+	+	-	-	*stx1, stx2*

TOTAL	628	381	457	139	490	
% Positive		60.6	72.7	22.1	78.0	

The *eae*A gene was observed in 22.1%, (139/628) of isolates which corresponded to 12.8% (37/289) of goats which were *eaeA* positive. Of the 139 isolates that were *eaeA* positive, 131 belonged to five of the major 7 serogroups including O157:H7 (30/628 isolates, 8/289 goats), O157:H8 (1/628 isolates, 1/289 goats), O103:H8 (97/628 isolates, 22/289 goats), O26:H2 (1/628 isolate, 1/289 goats) and O111:H8 (2/628 isolates, 1/289 goats). In addition, 8 isolates (1.2%) which were non-Big 7 STEC serotypes possessed *eaeA*: O71:H14 (1/628 isolate, 1/289 goats), O108:H25 (6/628 isolates, 2/289 goats), O163:H8 (1/628 isolate, 1/289 goats) were also *eaeA*-positive (1.2%, 8/628). Most of the *eaeA* positive isolates, 92.0% (128/139) had the *stx2eaeA* genotype (O71:H14, O103:H8, 157:H7, O157:H8) while the remaining 7.9% (11/139) isolates (O26:H2, O163:H8, O103:H8, O108:H25 and O111:H8) were *stx1eaeA* positive.

3.5 Discussion

Previous reports from different countries have shown that goats are a reservoir of STEC (Zschöck *et al.* 2000; Cortés *et al.* 2005; Oliveira *et al.* 2008; Vu-Khac and Cornick 2008; La Ragione *et al.* 2009). Furthermore, contact with goats and ingestion of food products of goat origin have been associated with STEC disease in humans (Bielaszewska *et al.* 1997; La Ragione *et al.* 2009). However, published reports on the occurrence and characteristics of STEC in goats are few in comparison to cattle and sheep. Furthermore, reports on the occurrence of STEC in goats in South Africa are non-existent. This study investigated the occurrence of STEC and characterized STEC isolates in four separate goat herds in South Africa. The overall occurrence of STEC in the goat populations surveyed was 80.2% (232/289). The occurrence of STEC in this study was very high in comparison to similar

studies in Germany (Beutin *et al.* 1993; Zschöck *et al.* 2000), Brazil (57.5%) (Oliveira *et al.* 2008), Spain (47.7%) (Orden *et al.* 2003; Cortés *et al.* 2005), Vietnam (31.5%) (Vu-Khac and Cornick 2008) and Bangladesh (11.8%) (Islam *et al.* 2008) which reported STEC detection rates ranging from 11.8% to 75.3% in goats. Other reports have found STEC occurrence rates ranging from 23.9% to 89.3% in different countries; but these studies were conducted on far smaller goat sample populations (≤46) to warrant a valid comparison with the present study (Čobeljić *et al.* 2005; Novotna *et al.* 2005; Schilling *et al.* 2012; Isler *et al.* 2021).

The within-herd occurrence of STEC ranged from 75.3% (116/154) to 90.6% (39/43) which was significantly higher in comparison to similar studies in Brazil (46.7%-73.3%) (Oliveira *et al.* 2008) and Vietnam (15%-65%) (Vu-Khac and Cornick 2008). Moreover, all the four goat herds were positive for STEC, in agreement with similar reports elsewhere (Zschöck *et al.* 2000; Oliveira *et al.* 2008; Vu-Khac and Cornick 2008). However, the number of goat samples which were tested per herd in this study was “significantly” higher compared to the reports from Brazil (106) Vietnam (205) and Germany (93) which may explain why the within-herd STEC occurrence in this study was also higher. The higher occurrence of STEC in goats in this study may be ascribed to higher shedding of STEC in the goat population studied, variations in geographic locations, age (kids vs adults), goat diet (grazing or browsing versus concentrate), and management practices. Furthermore, the use of a suitable enrichment broth and two selective and sensitive STEC culture and isolation media may have increased STEC recovery (Pradel *et al.* 2000; Gouali *et al.* 2013; Wylie *et al.* 2013; Stromberg *et al.* 2015; Verhaegen *et al.* 2016; Fan *et al.* 2019).

In the present study, 99.0% of goat STEC isolates were serotypable by PCR. A total of 63 serotypes (34 O and 17 H groups) were recovered from goats. The number of serotypes detected in this study was very high compared to previous studies (Orden *et al.* 2003; Cortés

et al. 2005; Oliveira *et al.* 2008). The recovery of a very high number of serotypes may also be ascribed to the high shedding of STEC in the goat populations tested. Furthermore, the use of a sensitive, specific, accurate and reliable PCR protocol for O:H serotyping may have led to the identification of more serotypes than usually found with traditional serotyping (Iguchi *et al.* 2015; Banjo *et al.* 2018; Iguchi *et al.* 2020). Furthermore, PCR O:H serotyping has the advantage of detecting O-untypable (OUNT) and H-nontypable and/or non-motile (HNT/NM) *E. coli* isolates that carry genes encoding O:H antigens but cannot be expressed. In this study, we were able to validate the Iguchi *et al*., (2015), Iguchi *et al*. (2020) and Banjo *et al*. (2018) *E. coli* PCR serotyping (O:H) protocols which were highly discriminatory and unambiguously serotyped the large number of goats STEC isolates tested in this study (Iguchi *et al.* 2015; Banjo *et al.* 2018; Iguchi *et al.* 2020). To our knowledge, this is the most extensive serotyping of goat STEC isolates, worldwide.

Among the 63 serotypes, only 4 serotypes belonged to the major seven STEC serogroups. STEC O103:H8 (15.6%) was the most frequent Big seven STEC among goats, followed by STEC O157:H7 (4.7%), O111:H8 and STEC O26:H2. Overall, the major seven STEC serotypes accounted for 21.3% of all isolates which were serotyped, in contrast to most similar studies which never recovered major seven STEC from goats (Beutin *et al.* 1993; Bielaszewska *et al.* 1997; Orden *et al.* 2003; Cortés *et al.* 2005; Keen *et al.* 2006; Oliveira *et al.* 2008; Mersha *et al.* 2010). However, Schilling *et al*. (2012) found a higher proportion of top 7 STEC, although the recovered serotypes were those which have never been reported in human disease, in contrast to our results which showed that most of the top seven serotypes we recovered were previously incriminated in human disease outbreaks except for STEC O157:H29, O103:H8 and O103:H56.

Previously, STEC O157:H7 has been incriminated in foodborne disease after consumption of raw goat milk and home-made cheese made from raw milk (Bielaszewska *et al.* 1997; McIntyre *et al.* 2002; Espié *et al.* 2006). Furthermore, STEC O157 and STEC O103 have been incriminated in human disease after contact with goats in the USA (Nichols *et al.* 2021) while sources other than goats have been frequently associated STEC O157:H7, O111:H8 and O26:H2 with human disease worldwide including South Africa (Karama *et al.* 2019b). According to the STEC seropathotype classification, STEC O157:H7 is considered a seropathotype A strain, frequently incriminated in outbreaks and severe human disease while O111:H8 and O26:H2 are moderately implicated in outbreaks and less frequent in severe human disease, in comparison to STEC O157:H7 (Karmali *et al.* 2003). However, in this study most major seven STEC isolates were classified as STEC O103:H8. Previously, STEC O103:H8 was isolated from healthy goats and calves in China and Argentina respectively (Fernández *et al.* 2012; Bai *et al.* 2016). In addition, only one study has reported the recovery of STEC O103:H8 from patients and asymptomatic food handlers in Japan (Baba *et al.* 2019). However, the study from Japan by Baba et al (2019) never specified whether the STEC O103:H8 isolate was from patients or asymptomatic food handlers (Baba *et al.* 2019). Therefore, although O103:H8 is classified as a major STEC (serogroup), its importance as a human pathogen remains unclear as there are no reports until now, which have unequivocally associated this STEC serotype with human disease.

The remaining 59 serotypes were non-O157, of which 24 have been previously incriminated in mild to severe human disease worldwide including South Africa, Europe, North America and Asia (Hong *et al.* 2009; EFSA 2013; Beutin and Fach 2014; Bettelheim and Goldwater 2019; Karama *et al.* 2019b). The recovery of STEC serotypes which have been associated with mild to severe human disease is evidence that goats are a reservoir and a potential source of these highly pathogenic STEC strains in South Africa.

Highly diverse and farm specific STEC serotypes were observed in individual goat herds except for STEC O76:H19 which was the serotype shared among the four goat herds surveyed while STEC O146:H21 and OgX18:H2 were recorded in three herds. Overall, the highly diverse and farm specific serotypes are most likely a reflection of the fact that the four herds were situated in geographically separate and distant areas from each other to allow isolate interchange between herds.

Regarding the virulence characteristics of the STEC isolates under study, *stx2* was more frequent that *stx1* among goat STEC in contrast to similar studies which have shown that *stx1* is predominant among goat STEC isolates (Zschöck *et al.* 2000; Orden *et al.* 2003; Cortés *et al.* 2005; Novotna *et al.* 2005; Wani *et al.* 2006; Vu-Khac and Cornick 2008; Horcajo *et al.* 2010; Mahanti *et al.* 2015; Jajarmi *et al.* 2018). However, our findings agree with a study by Oliveira *et al*. (2008) which reported that *stx2* was more prevalent in goat STEC isolates. Reports on clinical STEC have suggested that *stx2* positive isolates are more virulent and frequently incriminated in severe human disease including hemorrhagic colitis and hemolytic uremic syndrome in comparison to STEC isolates carrying *stx1* or both *stx1* and *stx2* (Ostroff *et al.* 1989; Boerlin *et al.* 1999; Donohue-Rolfe *et al.* 2000; Ethelberg *et al.* 2004; Persson *et al.* 2007).

The *hlyA* gene was present in 78.0% (490/628) of goat STEC isolates, consistent with previous reports which have shown similar rates in goat STEC elsewhere (Beutin *et al.* 1993; Zschöck *et al.* 2000; Cortés *et al.* 2005) . However, lower rates of *hlyA* ranging from 35%-60.9% have also been reported (Orden *et al.* 2003; Oliveira *et al.* 2008; Vu-Khac and Cornick 2008). The *hlyA* gene encodes a pore-forming hemolysin which lyses human erythrocytes with subsequent release of iron from heme, a chemical needed for STEC growth and survival in the intestine. Previously, the presence and expression of *hlyA* has been associated with

severe STEC disease in humans including HC and HUS (Schmidt and Karch 1996). However, STEC that were *hlyA* negative have also been incriminated in severe disease including bloody diarrhoea, HC and HUS, thereby suggesting that the pathogenic role of *hlyA* in STEC remains uncertain (Ethelberg *et al.* 2004).

Most of the goat STEC were *eaeA* negative except for top seven STEC (22.1%) including O157:H7, O26:H2, O111:H8 and O103:H8 and a few (0.7%) non-O157/non-top seven isolates: O71:H14, O108:H25 and O163:H8, in agreement with previous studies which have shown that *eaeA* is not common among goat STEC (Zschöck *et al.* 2000; Orden *et al.* 2003; Oliveira *et al.* 2008). The presence of *eaeA* in goat top seven STEC isolates is of clinical significance as *eaeA* is considered an important STEC adhesin and marker of high virulence and potential to cause severe disease (HC and HUS) in humans (EFSA 2020), especially when accompanied with *stx2* (Ethelberg *et al.* 2004). However, in some cases, *eaeA*-negative serotypes (O91:H21 and O113:H21) STEC have also been associated with severe disease thereby suggesting that other virulence or unknown host factors may influence disease severity (Karmali *et al.* 1985; Paton *et al.* 1999; EFSA 2020). The absence of *eaeA* may indicate that goat STEC are less virulent and may also explain why goat STEC are rarely incriminated in human disease worldwide. Of particular interest were *eaeA* positive goat isolates which belonged to serotypes O103:H8, O71:H14, O108:H25 and O163:H8 but have never been associated with human disease or outbreaks. These isolates will be worth monitoring closely as possession of *eaeA* may be indicative of higher virulence potential and likelihood to cause severe disease in humans.

3.6 Conclusions

Historically, studies on the presence of STEC in goats are very few compared to cattle which are considered the main STEC reservoir. This study is the first report on the presence of

STEC in goats in South Africa. The findings of this study show that goats carry a diverse range of STEC serotypes, some of which have been previously incriminated in mild to severe enteric disease in humans. Collectively, these findings suggest that goats grazing on communal rangeland in South Africa are a reservoir and potential source of STEC for humans in South Africa. Further molecular characterization of goat STEC isolates will be needed in the future to fully assess the virulence potential of goat STEC and capacity to cause disease in humans. In addition, studies that compare STEC isolates from goats and humans will be necessary to fully understand the role played by goats as a source of STEC human disease in South Africa. Data from this study will be useful for understanding the epidemiology of STEC in animals and formulating policies aimed at preventing and controlling zoonotic or foodborne diseases along the food chain.

3.7 References

Ateba CN, Mbewe M. Genotypic characterization of *Escherichia coli* O157: H7 isolates from different sources in the North-West province, South Africa, using enterobacterial repetitive intergenic consensus PCR analysis. *Int J Mol Sci* 15, 9735-47, 2014

Baba H, Kanamori H, Kudo H, Kuroki Y, Higashi S, Oka K, Takahashi M, Yoshida M, Oshima K, Aoyagi T, Tokuda K, Kaku M. Genomic analysis of Shiga toxin-producing *Escherichia coli* from patients and asymptomatic food handlers in Japan. *PLoS One* 14, e0225340, 2019

Bai X, Hu B, Xu Y, Sun H, Zhao A, Ba P, Fu S, Fan R, Jin Y, Wang H, Guo Q, Xu X, Lu S, Xiong Y. Molecular and phylogenetic characterization of non-O157 Shiga toxin-producing *Escherichia coli* strains in China. *Front. Cell. Infect. Microbiol.* 6, 2016

Banjo M, Iguchi A, Seto K, Kikuchi T, Harada T, Scheutz F, Iyoda S. *Escherichia coli* H-genotyping PCR: a complete and practical platform for molecular H typing. *J Clin Microbio* 56, e00190-18, 2018

Bettelheim KA, Goldwater PN. A Compendium of Shigatoxigenic *Escherichia coli*: origins, bacteriological and clinical data on the serogroups, serotypes and untypeable strains of *E. coli* reported between 1980 and 2017, excluding O157: H7. *Adv Microbiol* 9, 905-9, 2019

Beutin L, Fach P. Detection of Shiga toxin-producing *Escherichia coli* from nonhuman sources and strain typing *Microbiol. Spectr.* 2, 263-85, 2014

Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J Clin Microbiol* 31, 2483, 1993

Bielaszewska M, Janda J, Bláhová K, Minaříková H, Jíková E, Karmali MA, Laubová J, Šikulová J, Preston MA, Khakhria R, Karch H, Klazarová H, Nyč O. Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. *Epidemiol Infect* 119, 299-305, 1997

Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol* 37, 497-503, 1999

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J Infect Dis* 192, 1422-9, 2005

Čobeljić M, Dimić B, Opačić D, Lepšanović Z, Stojanović V, Lazić S. The prevalence of Shiga toxin-producing *Escherichia coli* in domestic animals and food in Serbia. *Epidemiol Infect* 133, 359-66, 2005

Coombes BK, Wickham ME, Mascarenhas M, Gruenheid S, Finlay BB, Karmali MA. Molecular analysis as an aid to assess the public health risk of non-O157 shiga toxin-producing *Escherichia coli* strains. *Appl Environ Microbiol* 74, 2153-60, 2008

Cortés C, De la Fuente R, Blanco J, Blanco M, Blanco JE, Dhabi G, Mora A, Justel P, Contreras A, Sánchez A, Corrales JC, Orden JA. Serotypes, virulence genes and intimin types of Verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. *Vet Microbiol* 110, 67-76, 2005

Donohue-Rolfe A, Kondova I, Oswald S, Hutto D, Tzipori S. *Escherichia coli* 0157: H7 strains that express Shiga toxin (Stx) 2 alone are more neurotropic for gnotobiotic piglets than are isotypes producing only Stx1 or both Stx1 and Stx2. *J Infect Dis* 181, 1825-9, 2000

EFSA. Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. *EFSA J.* 11, 3138, 2013

EFSA. Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. *EFSA J.* 18, e05967, 2020

Espié E, Vaillant V, Mariani-Kurkdjian P, Grimont F, Martin-Schaller R, De Valk H, Vernozy-Rozand C. *Escherichia coli* O157 outbreak associated with fresh unpasteurized goats' cheese. *Epidemiol Infect* 134, 143-6, 2006

Ethelberg S, Olsen KEP, Scheutz F, Jensen C, Schiellerup P, Engberg J, Petersen AM, Olesen B, Gerner-Smidt P, Mølbak K. Virulence Factors for hemolytic uremic syndrome, Denmark. *Emerg Infect Dis* 10, 842-7, 2004

Fan R, Shao K, Yang X, Bai X, Fu S, Sun H, Xu Y, Wang H, Li Q, Hu B, Zhang J, Xiong Y. High prevalence of non-O157 Shiga toxin-producing *Escherichia coli* in beef cattle detected by combining four selective agars. *BMC Microbiol* 19, 213, 2019

Fernández D, Sanz M, Parma A, Padola N. Characterization of Shiga toxin-producing *Escherichia coli* isolated from newborn, milk-fed, and growing calves in Argentina. *J Dairy Sci* 95, 5340-3, 2012

Gouali M, Ruckly C, Carle I, Lejay-Collin M, Weill FX. Evaluation of CHROMagar STEC and STEC O104 chromogenic agar media for detection of Shiga toxin-producing *Escherichia coli* in stool specimens. *J Clin Microbiol* 51, 894-900, 2013

Hong S, Oh K-H, Cho S-H, Kim J-C, Park M-S, Lim H-S, Lee B-K. Asymptomatic healthy slaughterhouse workers in South Korea carrying Shiga toxin-producing *Escherichia coli*. *FEMS Immunol Med Microbiol* 56, 41-7, 2009

Horcajo P, Domínguez-Bernal G, De La Fuente R, Ruiz-Santa-Quiterai JA, Orden JA. Association of Vt1C with Verotoxin-producing *Escherichia coli* from goats and sheep. *J Vet Diagn Invest* 22, 332-4, 2010

Hughes JM, Wilson ME, Johnson KE, Thorpe CM, Sears CL. The Emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. *Clin Infect Dis* 43, 1587-95, 2006

Hussein HS. Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J Anim Sci* 85, E63-E72, 2007

Iguchi A, Iyoda S, Seto K, Morita-Ishihara T, Scheutz F, Ohnishi M. *Escherichia coli* O-genotyping PCR: a comprehensive and practical platform for molecular O serogrouping. *J Clin Microbiol* 53, 2427-32, 2015

Iguchi A, Nishii H, Seto K, Mitobe J, Lee K, Konishi N, Obata H, Kikuchi T, Iyoda S. Additional Og-typing PCR techniques targeting *Escherichia coli*-novel and Shigella-unique O-antigen biosynthesis gene clusters. *J Clin Microbiol* 58, 2020

Isaäcson M, Canter P, Effler P, Arntzen L, Bomans P, Heenan R. Haemorrhagic colitis epidemic in Africa. *Lancet* 341, 961, 1993

Islam MA, Mondol AS, De Boer E, Beumer RR, Zwietering MH, Talukder KA, Heuvelink AE. Prevalence and genetic characterization of shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. *Appl Environ Microbiol* 74, 5414-21, 2008

Isler M, Wissmann R, Morach M, Zurfluh K, Stephan R, Nüesch-Inderbinen M. Animal petting zoos as sources of Shiga toxin-producing *Escherichia coli*, *Salmonella* and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. *Zoonoses Public Health* 68, 79-87, 2021

Jajarmi M, Askari Badouei M, Imani Fooladi AA, Ghanbarpour R, Ahmadi A. Pathogenic potential of Shiga toxin-producing *Escherichia coli* strains of caprine origin: Virulence genes, Shiga toxin subtypes, phylogenetic background and clonal relatedness. *BMC Vet Res* 14, 97, 2018

Jerse AE, Yu J, Tll BD, Kaper JB. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc. Natl. Acad. Sci. U.S.A.* 87, 7839-43, 1990

Karama M, Mainga AO, Cenci-Goga BT, Malahlela M, El-Ashram S, Kalake A. Molecular profiling and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* O26, O45, O103, O121, O145 and O157 isolates from cattle on cow-calf operations in South Africa. *Sci Rep* 9, 2019a

Karama M, Cenci-Goga BT, Malahlela M, Smith AM, Keddy KH, El-Ashram S, Kabiru LM, Kalake A. Virulence characteristics and antimicrobial resistance profiles of shiga toxin-producing *Escherichia coli* isolates from humans in South Africa: 2006–2013. *Toxins (Basel)* 11, 424, 2019b

Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis* 151, 775-82, 1985

Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, Isaac-Renton J, Clark C, Rahn K, Kaper JB. Association of genomic O island 122 of *Escherichia coli* EDL 933 with Verocytotoxin-producing *Escherichia coli*

seropathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol* 41, 4930-40, 2003

Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg Infect Dis* 12, 780-6, 2006

Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleesschauwer B, Döpfer D, Fazil A, Fischer-Walker CL, Hald T. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS Med* 12, e1001921, 2015

La Ragione RM, Best A, Woodward MJ, Wales AD. *Escherichia coli* O157:H7 colonization in small domestic ruminants. *FEMS Microbiol Rev* 33, 394-410, 2009

Lynch MF, Tauxe RV, Hedberg CW. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. *Epidemiol Infect* 137, 307-15, 2009

Mahanti A, Samanta I, Bandyopadhyay S, Joardar SN. Molecular characterization and antibiotic susceptibility pattern of caprine Shiga toxin producing-*Escherichia coli* (STEC) isolates from India. *Iran. J. Vet. Res.* 16, 31-5, 2015

Mainga AO, Cenci-Goga BT, Malahlela MN, Tshuma T, Kalake A, Karama M. Occurrence and characterization of seven major Shiga toxin-producing *Escherichia coli* serotypes from healthy cattle on cow–calf operations in South Africa. *Zoonoses Public Health.* 65, 777-89, 2018

McCarthy SC, Burgess CM, Fanning S, Duffy G. An Overview of Shiga-Toxin Producing *Escherichia coli* Carriage and Prevalence in the Ovine Meat Production Chain. *Foodborne Pathog Dis* 18, 147-68, 2021

McDaniel TK, Kaper JB. A cloned pathogenicity island from enteropathogenic *Escherichia coli* confers the attaching and effacing phenotype on *E. coli* K-12. *Mol Microbiol* 23, 399-407, 1997

McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc. Natl. Acad. Sci. U.S.A.* 92, 1664-8, 1995

McIntyre L, Fung J, Paccagnella A, Isaac-Renton J, Rockwell F, Emerson B, Preston T. *Escherichia coli* O157 outbreak associated with the ingestion of unpasteurized goat's milk in British Columbia, 2001. *Can Commun Dis Rep* 28, 6-8, 2002

McKee ML, O'Brien AD. Truncated enterohemorrhagic *Escherichia coli* (EHEC) O157: H7 intimin (EaeA) fusion proteins promote adherence of EHEC strains to HEp-2 cells. *Infect Immun* 64, 2225-33, 1996

Mersha G, Asrat D, Zewde BM, Kyule M. Occurrence of *Escherichia coli* O157:H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. *Lett Appl Microbiol* 50, 71-6, 2010

Nichols MC, Gacek P, Phan Q, Gambino-Shirley KJ, Gollarza LM, Schroeder MN, Mercante A, Mullins J, Blackstock A, Laughlin ME, Olson SM, Pizzo E, Nguyen TN, Mank L, Holmes-Talbot K, McNutt A, Noel D, Muyombwe A, Razeq JH, Lis MJ, Sherman B, Kasacek W, Whitlock L, Strockbine N, Martin H, Vidyaprakash E, McCormack P, Cartter M. Agritourism and kidding season: a large outbreak of human Shiga toxin-producing *Escherichia coli* O157 (STEC O157) infections linked to a goat dairy farm—Connecticut, 2016. *Front. Vet. Sci.* 8, 2021

Novotna R, Alexa P, Hamrik J, Madanat A, Smola J, Cizek A. Isolation and characterization Shiga toxin-producing *Escherichia coli* from sheep and goats in Jordan with evidence of multiresistant serotype O157: H7. *Vet. Med. Czech* 50, 111-8, 2005

O'Brien AD, Newland JW, Miller SF, Holmes RK, Smith HW, Formal SB. Shiga-like toxin-converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. *Science.* 226, 694-6, 1984

Oliveira MG, Brito JR, Gomes TA, Guth BE, Vieira MA, Naves ZV, Vaz TM, Irino K. Diversity of virulence profiles of Shiga toxin-producing *Escherichia coli* serotypes in food-producing animals in Brazil. *Int J Food Microbiol* 127, 139-46, 2008

Orden J, Ruiz-Santa-Quiteria J, Blanco M, Blanco J, Mora A, Cid D, González E, Blanco J, De la Fuente R. Prevalence and characterization of Vero cytotoxin-producing *Escherichia coli* isolated from diarrhoeic and healthy sheep and goats. *Epidemiol Infect* 130, 313-21, 2003

Ostroff SM, Tarr PI, Neill MA, Lewis JH, Hargrett-Bean N, Kobayashi JM. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157: H7 infections. *J Infect Dis* 160, 994-8, 1989

Pai C, Kelly J, Meyers G. Experimental infection of infant rabbits with Verotoxin-producing *Escherichia coli*. *Infect Immun* 51, 16-23, 1986

Paton AW, Paton JC. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx*1, *stx2*, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfb*O111, and rfbO157. *J Clin Microbiol* 36, 598-602, 1998

Paton AW, Woodrow MC, Doyle RM, Lanser JA, Paton JC. Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic- uremic syndrome. *J Clin Microbiol* 37, 3357-61, 1999

Persad AK, Lejeune JT. Animal reservoirs of Shiga toxin-producing *Escherichia coli*. *Enterohemorrhagic Escherichia coli and Other Shiga toxin-producing E. coli*, Washington, DC, USA, pp 211-30. American Society of Microbiology, 2015

Persson S, Olsen KEP, Ethelberg S, Scheutz F. Subtyping method for *Escherichia coli* Shiga toxin (Verocytotoxin) 2 variants and correlations to clinical manifestations. *J Clin Microbiol* 45, 2020-4, 2007

Pradel N, Livrelli V, De Champs C, Palcoux JB, Reynaud A, Scheutz F, Sirot J, Joly B, Forestier C. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. *J Clin Microbiol* 38, 1023-31, 2000

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype *N Engl J Med* 308, 681-5, 1983

Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *J Clin Microbiol* 50, 2951-63, 2012

Schilling A-K, Hotzel H, Methner U, Sprague LD, Schmoock G, El-Adawy H, Ehricht R, Wöhr A-C, Erhard M, Geue L. Zoonotic agents in small ruminants kept on city farms in Southern Germany. *Appl Environ Microbiol.* 78, 3785-93, 2012

Schlager S, Lepuschitz S, Ruppitsch W, Ableitner O, Pietzka A, Neubauer S, Stoeger A, Lassnig H, Mikula C, Springer B. Petting zoos as sources of Shiga toxin-producing *Escherichia coli* (STEC) infections. *Int J Med Microbiol* 308, 927-32, 2018

Schmidt H, Karch H. Enterohemolytic phenotypes and genotypes of Shiga toxin-producing *Escherichia coli* O111 strains from patients with diarrhea and hemolytic-uremic syndrome. *J Clin Microbiol* 34, 2364-7, 1996

Schmidt H, Hensel M. Pathogenicity islands in bacterial pathogenesis. *Clin Microbiol Rev* 17, 14-56, 2004

Schmidt H, Beutin L, Karch H. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157: H7 strain EDL 933. *Infect Immun* 63, 1055-61, 1995

Scotland S, Smith H, Willshaw G, Rowe B. Vero cytotoxin production in strain of *Escherichia coli* is determined by genes carried on bacteriophage. *Lancet.* 322, 216, 1983

Strockbine NA, Jackson M, Sung L, Holmes R, O'Brien AD. Cloning and sequencing of the genes for Shiga toxin from *Shigella dysenteriae* type 1. *J Bacteriol* 170, 1116-22, 1988

Stromberg ZR, Lewis GL, Marx DB, Moxley RA. Comparison of enrichment broths for supporting growth of Shiga toxin-producing *Escherichia coli*. *Curr Microbiol* 71, 214-9, 2015

Verhaegen B, Van Damme I, Heyndrickx M, Botteldoorn N, Elhadidy M, Verstraete K, Dierick K, Denayer S, De Zutter L, De Reu K. Evaluation of detection methods for non-O157 Shiga toxin-producing *Escherichia coli* from food. *Int J Food Microbiol* 219, 64-70, 2016

Vu-Khac H, Cornick NA. Prevalence and genetic profiles of Shiga toxin-producing *Escherichia coli* strains isolated from buffaloes, cattle, and goats in central Vietnam. *Vet Microbiol* 126, 356-63, 2008

Wani SA, Samanta I, Munshi ZH, Bhat MA, Nishikawa Y. Shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* in healthy goats in India: occurrence and virulence properties. *J Appl Microbiol* 100, 108-13, 2006

Wylie JL, Van Caeseele P, Gilmour MW, Sitter D, Guttek C, Giercke S. Evaluation of a new chromogenic agar medium for detection of Shiga toxin-producing *Escherichia coli* (STEC) and relative prevalences of O157 and non-O157 STEC in Manitoba, Canada. *J Clin Microbiol* 51, 466-71, 2013

Zschöck M, Hamann HP, Kloppert B, Wolter W. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. *Lett Appl Microbiol* 31, 203-8, 2000

4.0 CHAPTER IV

General Discussion

Shiga toxin-producing *E. coli* emerged in 1982 in the United States as an enteric foodborne pathogen characterised by diarrhoea, haemorrhagic colitis and the hemolytic uremic syndrome, a common cause of kidney failure in 5-10% of diseased humans. Domestic ruminants including goats are considered reservoirs of STEC in different parts of the world (Gyles 2007). However, current studies on the epidemiology and virulence characteristics of goat STEC in South Africa are lacking. In this study, 289 faecal samples were collected from goats and screened for STEC. Furthermore, 628 goat STEC isolates were characterised by serotype and virulence genes including *stx1*, *stx2*, *eaeA* and *hlyA,* to assess the potential of goat STEC to cause disease in humans.

The findings of this study revealed that STEC was present in 80.2% of goats surveyed. The in-herd occurrence of STEC ranged from 75.3%-90.6%. This occurrence rate was higher compared to similar studies which have previously searched for STEC in goat populations (Beutin *et al.* 1993; Zschöck *et al.* 2000; Orden *et al.* 2003; Cortés *et al.* 2005; Islam *et al.* 2008; Oliveira *et al.* 2008; Vu-Khac and Cornick 2008). The high occurrence rate of STEC in goats may be ascribed to differences in methods used to isolate and identify STEC, geographic locations, and variations in the age of animals surveyed and management practices.

Furthermore, the 628 goat isolates which were serotyped belonged to 63 different serotypes which corresponded to a total of 34 somatic O serogroups and 17 flagellar H types. Among the 63 serotypes, 24 have been previously linked to human disease worldwide, including four which were “Big 7” STEC (EFSA 2013; Beutin and Fach 2014; Bettelheim and Goldwater 2019). However, Big 7 STEC were less frequent in this study in comparison to other non-

O157/non-Big 7 STEC, in agreement with previous reports which have suggested that Big 7 STEC are uncommon in goats (Bielaszewska *et al.* 1997; Keen *et al.* 2006; Mersha *et al.* 2010). Big seven serotypes included O157:H7, O157:H8, O157:H29, O26:H2, O111:H8, O103:H8 O103:H56. Big 7 STEC serotypes such as O157:H7, O26:H2 and O111:H8 are commonly incriminated in severe human illness worldwide including South Africa (Brooks *et al.* 2005; Johnson *et al.* 2006; Karama *et al.* 2019).

In addition, it was possible to serotype 99% (622/628) of goat STEC isolates by PCR (Iguchi *et al.* 2015; Iguchi *et al.* 2016; Banjo *et al.* 2018; Iguchi *et al.* 2020), suggesting that the PCR serotyping platforms used in this study were reliable and accurate in identifying STEC serotypes. To the author's knowledge, this is the first study which has serotyped the largest collection of goat STEC isolates worldwide.

Virulence gene profiling showed that more goat STEC isolates possessed *stx2* than *stx1* genes, consistent with a study conducted in Brazil (Oliveira *et al.* 2008). However, most studies have reported a higher prevalence of *stx1* than *stx2* among goat isolates (Zschöck *et al.* 2000; Orden *et al.* 2003; Cortés *et al.* 2005; Wani *et al.* 2006; Vu-Khac and Cornick 2008; Horcajo *et al.* 2010). Most STEC isolates in the present study lacked *eaeA*, agreeing with most studies which have shown that goat STEC isolates lack *eaeA* (Zschöck *et al.* 2000; Orden *et al.* 2003; Oliveira *et al.* 2008). The *eaeA* gene was mainly found in Big 7 STEC serotypes including O157:H7, O157:H8, O26:H2, O111:H8 and O103:H8 which are common in human disease (except O103:H8) and a few non-Big 7 STEC isolates such as O71:H14, O108:H25 and O163:H8 which have never been linked to human disease. The lack of *eaeA* suggests that goat STEC may be less virulent and therefore only capable of causing mild disease. In addition, the importance of Big 7 STEC such as O157:H29 (*eaeA*-negative); O103:H8 (*eaeA*-positive) and O103:H56 (*eaeA*-negative) remains unclear because these serotypes have never been implicated in human disease. However, we suggest that the

presence of STEC O103:H8 isolates in goats which carried *eaeA* and were mostly *stx2* positive will be worth monitoring because *eaeA* together with *stx2* is indicative of high virulence potential for humans (EFSA 2020).

4.1 Conclusions

This study provides preliminary data on the presence of STEC in goats in South Africa. To the author's knowledge, this is the first study reporting on occurrence and characteristics of STEC isolates from goats on communal rangeland in South Africa. The findings of this study showed a high occurrence of STEC which suggests that goats grazing on communal rangeland are a reservoir and may be a source of disease for humans in South Africa. Furthermore, sixty-three distinct STEC serotypes were identified including major STEC O157:H7, O26:H2 and O111:H8 which are among the seven major serotypes commonly implicated in human disease worldwide including South Africa. Most STEC isolates possessed *stx1, stx2, hlyA* but lacked *eaeA*, an important virulence factor in human disease. This study is an important contribution to STEC monitoring and surveillance in South Africa.

4.2 Recommendations

Further studies on STEC will be needed to characterise the goat STEC isolates in detail and fully evaluate their potential to cause disease in humans. In addition, molecular risk assessment studies comparing goat and human STEC isolates will be necessary to assess to what extent goats raised on communal rangelands may be a source of STEC disease for humans in South Africa. Data from this study will be useful for formulating policies aimed at preventing and controlling foodborne pathogens including STEC along the food chain.

4.3 References

Banjo M, Iguchi A, Seto K, Kikuchi T, Harada T, Scheutz F, Iyoda S. *Escherichia coli* H-genotyping PCR: a complete and practical platform for molecular H typing. *J Clin Microbiol* 56, 2018

Bettelheim KA, Goldwater PN. A Compendium of Shigatoxigenic *Escherichia coli*: origins, bacteriological and clinical data on the serogroups, serotypes and untypeable strains of *E. coli* reported between 1980 and 2017, excluding O157: H7. *Adv Microbiol* 9, 905-9, 2019

Beutin L, Fach P. Detection of Shiga toxin-producing *Escherichia coli* from nonhuman sources and strain typing *Microbiol. Spectr.* 2, 263-85, 2014

Beutin L, Geier D, Steinruck H, Zimmermann S, Scheutz F. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J Clin Microbiol* 31, 2483, 1993

Bielaszewska M, Janda J, Bláhová K, Minaříková H, Jíková E, Karmali MA, Laubová J, Šikulová J, Preston MA, Khakhria R, Karch H, Klazarová H, Nyč O. Human *Escherichia coli* O157:H7 infection associated with the consumption of unpasteurized goat's milk. *Epidemiol Infect* 119, 299-305, 1997

Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, Strockbine NA. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J Infect Dis* 192, 1422-9, 2005

Cortés C, De la Fuente R, Blanco J, Blanco M, Blanco JE, Dhabi G, Mora A, Justel P, Contreras A, Sánchez A, Corrales JC, Orden JA. Serotypes, virulence genes and intimin types of verotoxin-producing *Escherichia coli* and enteropathogenic *E. coli* isolated from healthy dairy goats in Spain. *Vet Microbiol* 110, 67-76, 2005

EFSA. Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. *EFSA J* 11, 3138, 2013

EFSA. Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC. *EFSA J.* 18, e05967, 2020

Gyles C. Shiga toxin-producing *Escherichia coli*: an overview. *J Anim Sci* 85, E45-E62, 2007

Horcajo P, Domínguez-Bernal G, De La Fuente R, Ruiz-Santa-Quiterai JA, Orden JA. Association of Vt1C with Verotoxin-producing *Escherichia coli* from goats and sheep. *J Vet Diagn Invest* 22, 332-4, 2010

Iguchi A, Iyoda S, Seto K, Morita-Ishihara T, Scheutz F, Ohnishi M. *Escherichia coli* O-genotyping PCR: a comprehensive and practical platform for molecular O serogrouping. *J Clin Microbiol* 53, 2427-32, 2015

Iguchi A, Iyoda S, Seto K, Nishii H, Ohnishi M, Mekata H, Ogura Y, Hayashi T. Six Novel O genotypes from Shiga toxin-producing *Escherichia coli*. *Front Microbiol* 7, 2016

Iguchi A, Nishii H, Seto K, Mitobe J, Lee K, Konishi N, Obata H, Kikuchi T, Iyoda S. Additional Og-typing PCR techniques targeting *Escherichia coli*-novel and Shigella-unique O-antigen biosynthesis gene clusters. *J Clin Microbiol* 58, 2020

Islam MA, Mondol AS, De Boer E, Beumer RR, Zwietering MH, Talukder KA, Heuvelink AE. Prevalence and genetic characterization of shiga toxin-producing *Escherichia coli* isolates from slaughtered animals in Bangladesh. *Appl Environ Microbiol* 74, 5414-21, 2008

Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. *Clin Infect Dis* 43, 1587-95, 2006

Karama M, Cenci-Goga BT, Malahlela M, Smith AM, Keddy KH, El-Ashram S, Kabiru LM, Kalake A. Virulence characteristics and antimicrobial resistance profiles of shiga toxin-producing *Escherichia coli* isolates from humans in South Africa: 2006–2013. *Toxins (Basel)* 11, 424, 2019

Keen JE, Wittum TE, Dunn JR, Bono JL, Durso LM. Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg Infect Dis* 12, 780-6, 2006

Mersha G, Asrat D, Zewde BM, Kyule M. Occurrence of *Escherichia coli* O157:H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. *Lett Appl Microbiol* 50, 71-6, 2010

Oliveira MG, Brito JR, Gomes TA, Guth BE, Vieira MA, Naves ZV, Vaz TM, Irino K. Diversity of virulence profiles of Shiga toxin-producing *Escherichia coli* serotypes in food-producing animals in Brazil. *Int J Food Microbiol* 127, 139-46, 2008

Orden J, Ruiz-Santa-Quiteria J, Blanco M, Blanco J, Mora A, Cid D, González E, Blanco J, De la Fuente R. Prevalence and characterization of Vero cytotoxin-producing *Escherichia coli* isolated from diarrhoeic and healthy sheep and goats. *Epidemiol Infect* 130, 313-21, 2003

Vu-Khac H, Cornick NA. Prevalence and genetic profiles of Shiga toxin-producing *Escherichia coli* strains isolated from buffaloes, cattle, and goats in central Vietnam. *Vet Microbiol* 126, 356-63, 2008

Wani SA, Samanta I, Munshi ZH, Bhat MA, Nishikawa Y. Shiga toxin-producing *Escherichia coli* and enteropathogenic *Escherichia coli* in healthy goats in India: occurrence and virulence properties. *J Appl Microbiol* 100, 108-13, 2006

Zschöck M, Hamann HP, Kloppert B, Wolter W. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. *Lett Appl Microbiol* 31, 203-8, 2000