

Prevalence and characterization of shiga toxin-producing escherichia coli from go...

[Parts of the World, Africa](#)



Title: Prevalence and characterization of shiga toxin-producing Escherichia coli from goats in South Africa

Study rationale

STEC remain being the most important foodborne pathogen because of its high prevalence around the globe especially in America, Canada and other countries including South Africa. It causes diarrheal infections characterised by abdominal cramps and often lead to bloody diarrhoea. Severity of this pathogen causes complications illness such as Hemolytic Uremic Syndrome (HUS) and Thrombotic Thrombocytopenic Purpura (TTP).

Centers for Disease Control and Prevention (CDC) have estimated that E. coli O157: H7 causes 73, 000 cases and 61 deaths while non-O157: H7 causes 30 deaths and 36000 infections every year in the United States (Mead et al, . 1999). There is not enough data about STEC in goats. About 91. 5% of STEC infections were confirmed in New Zealand and found that they were caused by serotype O157: H7. According to World Health Organization (WHO) it was discovered that environmental exposure factors lead to differences in microbial loading. Recent outbreaks showed some STEC infections caused by non-O157: H7 serotypes.

Literature review

Shiga toxin-producing Escherichia coli (STEC) which is also known as Vero toxin producing Escherichia coli is the most important foodborne pathogen that mainly causes diarrhoea in humans and further lead to severe complications such as Haemolytic Uremic Syndrome(HUS) and Haemorrhagic colitis(HC)(Karmali, 1989). Consumption of contaminated food such as

undercooked meat or poultry, unpasteurised milk and cheese is known to be the source of infected strains. Those domestic animals that are unable to digest plant material because they lack cellulase serve as reservoirs for the bacteria because they colonise the intestines and survives in faeces and soil (Maule, 2000, DuPont, 2007).

STEC produce two shiga toxins that are very similar to those produced by bacteria *Shigella dysenteriae*. Shiga toxin 1 and toxin 2 are encoded by two genes namely *stx1* and *stx2*. These two toxins are not distinguishable from the toxin produced by *Shigella dysenteriae*, shiga toxin 2(*stx2*) has a 56% amino acid homology to shiga toxin 1(*stx1*), so they differ at the protein level. Shiga toxin-Producing *Escherichia coli* has been reported that it has caused about 2, 801, 000 acute illness globally annually among those, about 809, 000 cases in those of 0-4 years of age, 554, 000 in those between 5 and 15 years, 974, 000 in those 16-59 years and lastly 464, 000 in those at most 60 years. The estimation of STEC lead to 3890 cases of HUS and 230 deaths and among which 10 200 cases of STEC infections occur in Africa with an incidence rate of 1. 4 cases of 100 000 person-years and STEC O157: H7 continues to remain the most important foodborne in food security (Majowicz et al, . 2013). STEC O157: H7 has been isolated from all parts of Africa including Tanzania, Ethiopia and Kenya.

In 2006 more than 7% was reported in patients with diarrhoea in east African country Tanzania. The prevalence of 4. 7% STEC O157: H7 was isolated in Ethiopia from goat and sheep. In South Africa the first human infection case was reported in 1990 in Johannesburg (Browning et al., 1990) Mohammad et

al. (2008) found that out of 110 fecal (n= 110) samples collected from goats, cows (n= 139) and buffalo (n= 174) in Bangladesh the prevalence of STEC was 10. 0% while 11. 3% had both stx1 and stx2, only 3. 6% (n= 4) had stx1 and 7. 3% (n= 8) had stx2 for goats. All STEC positives contained eaeA (100%) and hlyA (100%) encoding genes. Those that were STEC O157 were isolated from 9. 1% of the goats and 71 STEC non-O157 strains were also isolated from 60 stx positive samples from 0. 9% of the goats.

Though STEC O157 strains were high in buffalos (14. 4%) but they were isolated more in goats (9. 1%) than in cattle (7. 2%). The non-O157 STEC isolates belonged to 36 different Serogroups (O antigen) and 52 O: H serotypes. About 78. 9% of Non-O157 STEC isolates were positive for stx1 which showed more prevalence of stx1 than STEC O157. This was the first evidence to show that slaughtered animals goats in Bangladesh are reservoirs for STEC and virulent STEC strain O157. Hypothesis: H1-Goat are reservoirs for STEC H0- Goats are not reservoir for STEC.

Bacterial culture

About 5g of faecal sample will be enriched into 50ml tube containing 45ml of sterilized EC broth from Oxoid supplemented with 20ml/L Novobiocin. The enrichment broth with faecal samples is then incubated in a shaker at 37°C for 24hours. 100µl of the enriched broth was spread on Drigalski Lactose agar (CM0531, Oxoid, United Kingdom) and Chrome Agar STEC (<http://www.chromagar.com>) media using a sterile swab and incubated at 37°C under aerobic conditions for overnight. DNA bacterial extractionGrowth of yellow colonies on Drigalski lactose agar were scooped with a sterile bacterial loop

and suspended in a sterile 1.5 ml Eppendorf tube containing 1000 µl of FA buffer.

The suspended bacteria in FA buffer were mixed using vortex and centrifuged at 12,000 rpm for 5 minutes to obtain a pellet. The supernatant obtained was discarded and again suspended in 1 ml of sterile FA buffer, vortex and centrifuged. In the last wash the supernatant was discarded and resuspended in 500 µl of sterile distilled and boiled for 25 minutes on a heating block to lyse the cells. The lysate was cooled on ice for 15 minutes and later stored at -20°C or centrifuged and supernatant was used as a template for Polymerase chain reaction (PCR). Subculture and reconfirmation After screening for stx1, stx2, eaeA and hlyA, positive plates from both Drigalski Lactose agar and CHROMagar were streaked aseptically onto CHROMagar and Drigalski Lactose agar to obtain single colonies and incubated for 18-24 hours at 37°C.

Five single colonies were picked from each plate and multiplied and purified on Luria Bertani agar and incubated at 37°C for 18-24 hours. After incubation the DNA is extracted from pure colonies, and a multiplex PCR (Paton and Paton, 1998) was used to confirm STEC from pure colonies. Pure single colonies confirmed for STEC were stored at -80°C in sterile cryovials containing a freezing mixture of Brain Heart Infusion broth (70%) and glycerol (30%) for further processing. Identification of STEC and O antigen by multiplex PCR Each PCR assay was carried in 25 µl reaction mixture containing 10X Thermopol buffer, 2.0 µl deoxynucleotide triphosphates (dNTPs, 200 µM each, New England, NEB, USA), 0.6 µl of each (Integrated DNA Technologies

San Diego USA), 0.25 µl Taq DNA Polymerase (5U/µl, New England Bio Labs® Inc.), and sterile water to 25 µl.

Finally, 5 µl DNA template was added in each reaction mix. E. coli strain ATCC 25922 (EDL933) DNA was used as a positive control while sterile water was used for negative control. This mixture was placed in the thermal cycler with the following conditions: 35 cycles each containing of 1 minute of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. The PCR was done under the laminar flow hood. A 2% (w/v) agarose gel was prepared in 1X TAE (Tris -Acetate-Ethylenediamine tetra acetic acid, pH 8) buffer and each well of the gel was loaded with 12 µl of PCR product mixed with 3 µl loading dye (6X, New England, NEB, USA).

The samples were run in 1X running buffer (TAE) in constant voltage of 100V and 100 bp DNA ladder as a molecular weight marker (New England, NEB, USA). Agarose gel was stained in 0.05 mg/ml ethidium bromide for 15 minutes followed by excessive wash with water and visualized under ultraviolet (UV) light with Gel Doc system (Bio-Rad, USA). Frozen pure STEC culture were streaked on Violet Red Bile agar (Oxoid, UK) and incubated at 37°C overnight, pink single colonies were picked and multiplied by a subculture on Luria Bertani (LB) agar (Difco Becton and Dickson & Company) then incubated overnight at 37°C. DNA was extracted from STEC single colonies by the boiling method described before and reconfirmation of STEC (stx1, stx2, eaeA and hlyA) using multiplex PCR (Paton and Paton, 1998). All

STEC positive isolates were detected for E. coli serogroups (O antigen) described in table 1 using PCR.