Isolation and detection of Shiga-toxigenic *Escherichia coli* (STEC) in beef meat reveals public health implications for Mauritians

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Project dans le cadre du plan stratégique 2015-2020, Axe 3 : Sûreté des aliments et contrôle de la qualité

• Informer et protéger les consommateurs des dangers sanitaires liés aux aliments
• Surveillance des pathogènes
• Détection des *E.coli* STEC dans les viandes de bovin, cerf, et porc de l’Ile Maurice 2014-2017
  – Cerf (échantillonnages dans les chassés)
  – Bovin et porc (échantillonnages a l’abattoir)
Introduction

E. coli

Non-pathogenic

Pathogenic

EPEC
ETEC
EIEC
DAEC
EAggEC
EHEC/STEC

Warm-blooded animals

Cattle
Pig
Deer

As a consequence ⇒ enter food chain (faeces excreted/gut content/hides)

Food contamination
Most dangerous!!
Why?

STEC

“Public health priority”
WHO since 1998

Low infective dose (<10 cells)
Acid resistant (stomach)
Specific colonization (Gb3/Gb4 receptors)
Mechanism of infection (TTSS)

Toxigenic properties

• Virulence genes (multiples) ($stx1$, $stx2$) – repress protein synthesis
• Intimin ($eaeA$): Attachment and effacement lesion
• Enterohemolysin ($EHEC-hlyA$): disrupt red blood cells
• Other virulence factors

Pathology
Severe diarrhea
Bloody diarrhea
Renal failure
CNS
Death

Typing: O-antigen => Seven are considered as globally pandemic (O26, O45, O103, O111, O121, O145 and O157)
• As EHEC-7 or Big “Seven” STEC
Rationale

• The oceanic island lacks adequate surveillance systems

• In Mauritius, beef is the second most consumed animal source food (5,000 tonnes: 2,000t fresh) after poultry (46,000t) (MAIFS, 2016). All cattle are slaughtered at the MMA

• Previous study showed that STEC were present in raw meat collected at MMA slaughterhouse-level (Thierry et al., 2018)

• Are STEC present in retail beef? => most important/unaddressed section of the local food chain
Aims

- Prevalence of STEC in beef meat at retail-level
- Serogroup diversity and virulence profiles of STEC strains
- Consumption of beef meat = or ≠ risk with respect to STEC infections
Materials and Methods

Sampling

150 samples (10/outlet)
15 locations (6 Urban, 9 rural)

Isolation

Enrichment: mTSB broth
Selective medium: CHROMagar STEC
Max 5 isolates/sample

Molecular characterization

DNA extraction of presumptive STEC

Confirmation of STEC by PCR
Targeted genes: stx1, stx2, eaeA and hlyA
(Paton and Paton, 1998a)

Serogrouping of confirmed STEC by sequencing of the gnd gene
1. Here, our results confirm that STEC are present at retail-level.
2. Prevalence (42%) was higher than that reported at slaughterhouse-level (32%) (Thierry et al., 2018).

=> Contamination (slaughter-to-retail) positive => (14/15)

Figure 1: Prevalence of STEC from beef samples collected in the 15 outlets.
Results: Serogroup diversity

- Presence of pandemic serogroups (Pub. health)
- STEC isolates were serologically diverse, with serogroup richness varying from 1 to 11 amongst outlets => High molecular diversification at the molecular level

- 153 strains were serogrouped
- 58 strains were not determined (DND)

Figure 2: Diversity of serogroups recovered from beef samples at each of the 15 outlets
1. Possible presence of other serogroups
   (Emergence of new seropathotypes: The case of E. coli O104 in Germany, 2011)
2. O91 => (7/15 outlets) O76 => (4/15 outlets)
3. O91 was previously reported to cause HUS while O76 lead to bloody diarrhea (Johnson et al., 2006)

Figure 3: Frequency of STEC serogroups from the 211 STEC isolates
Results: Virulence profiles

1. Clearly documented observed clinical symptoms => linked to presence of virulence determinants.
2. None possessed all four virulence genes.
3. Risk of emergence of STEC possessing all four virulence genes.
4. High prevalence of eaeA (associated with superior fitness and increased capacity to bind to epithelial cells).

Table 1: Virulence profiles of 211 STEC isolates

- **stx1 only**: 14
- **stx2 only**: 2
- **eaeA only**: 149
- **stx1/stx2**: 6
- **stx2/eaeA**: 2
- **eaeA/hlyA**: 19
- **stx1/stx2/eaeA**: 17
- **stx2/eaeA/hlyA**: 2

Total: 211
## Results: Serogroup/virulence

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<tr>
<th>Serogroup</th>
<th>eaeA</th>
<th>stx1</th>
<th>stx2</th>
<th>stx1/stx2</th>
<th>eaeA/hlyA</th>
<th>stx2/eaeA</th>
<th>stx1/stx2/eaeA</th>
<th>stx2/eaeA/hlyA</th>
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### Selection of 10 STEC serogroups + vir. profiles

1. Of the 28 serogroups, 21 were previously linked to STEC clinical cases (either HUS or bloody diarrhea)

2. Presence of non-typeable (OUT) suggests presence of new O antigens (adaptation by pathogens to colonize host)

3. Association with stx2/eaeA = HUS cases

4. O157, O91, O146, OUT => Higher likelihood to cause HUS

5. O76, O84, O91, OUT => Higher likelihood to cause HUS
• STEC were confirmed from 42% (63/150) of samples screened
• Beef represents a potential mode of transmission of STEC to consumers
• How to prevent STEC infections?
  – Appropriate GHP (Good Hygienic Practices) at slaughterhouse-level and at retail-level
  – Proper cooking temperatures (> 63°C)
  – Good hygiene
• Assess their clinical impact of STEC in SWIO islands
• Future work: Compare STEC isolates recovered from deer, pigs and cattle
Publications


*(In progress)*

Thierry, S. I. L., Jaufeerally-Fakim, Y., Gannon, J. E., Santchurn, S. J. Virulence factor profiles and serogroup classification of Shiga-toxigenic *Escherichia coli* from cattle, rusa deer and pigs of Mauritius
References


