Dairy cattle and Rusa deer (*Rusa Timorensis*): Potential sources of Shiga-toxigenic *Escherichia coli* (STEC) of clinical significance to Mauritians

Ian Thierry, Y Jaufeerally-Fakim, J Gannon and S Santchurn

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Introduction

E. coli

HARMLESS bacteria

SOURCES
Human gut
Warm-blooded animals

Six groups
1. EPEC
2. ETEC
3. EAggEC
4. EIEC
5. DAEC
6. EHEC/STEC

Diseases in humans
1. Diarrhea
2. HC
3. HUS
4. TTP
5. DEATH!!!

Transmissions
Raw milk
Raw meat
Hamburgers
Cheese

Why them?

Dairy cattle
Beef cattle
Deer
Pigs

Most dangerous
- Toxicity
- Clinical symptoms

Produces toxins
STEC

Production of Shiga toxins (Stx1 and Stx2)

How do we characterize them?

Other toxins
- Intimin (eaeA)
- Enterohemolysin (EHEC-hlyA)

Confirmed by detection of these genes
- stx1
- stx2
- eaeA
- EHEC-hlyA

Function of toxins
- Intermits protein synthesis leading to cell death
- Binding at intestinal track
- Causes cell lysis of red blood cells

PCR
Objectives

1. To estimate the prevalence of STEC in faeces and raw milk derived from five dairy farms in Mauritius
2. To estimate the prevalence of STEC in faeces and raw meat derived from Rusa deer at slaughtering houses in Mauritius

Sampling

Five (n=5) dairy farms were selected
Collection of samples
- 25-30g of composite faecal samples
- 30-40ml of bulk raw milk

Three (n=3) extensive deer farms were sampled
Collection of samples
- 25-100g of raw meat
- 25-30g faecal samples
Methodology I

Samples → Enrichment → CHROMagar

STEC → EMB agar

Purification and Cryopreservation

Gel

EHEC-hlyA (534bp)
eaeA (384bp)
stx2 (255bp)

PCR

stx1, stx2, eaeA and EHEC-hlyA

DNA extraction
Methodology II

From Gel result
Select +ve isolates

Sequence analysis (Trimming and phylogenetic analysis)

PCR for serogroup primers (O26, O45, O103, O111, O145 and O157)

Non-typeable isolates were gnd-PCR amplified

Serogroup identification

Sanger sequencing
Results for Dairy cattle

Results

• 4/5 farms were +ve for STEC

• Overall Prevalence of 8.0% (8/100)

• 20 STEC isolates were recovered:
  • 5 from faecal
  • 15 from raw milk

Total no of isolates tested:
raw milk (n=224)
Faecal (n=229)

50 Faecal
50 Raw milk

Faecal
(3/50)
6.0%

Raw milk
(5/50)
10.0%
Results for Dairy cattle

- O100 (55.0%)
- O128ab (15.0%)
- O84 (5.0%)
- O111 (5.0%)
- O118 (5.0%)
- DND (15.0%)
Results for Dairy cattle

- eaeA only (n=8)
- EHEC- hlyA only (n=10)
- eaeA and EHEC- hlyA (n=2)
- STEC (n=20)
Results for Dairy cattle

- STEC prevalence was slightly higher in raw milk (10.0%; 5/50) as compared to faeces (6.0%; 3/50).
- All the strains were \textit{stx}-negative.
- All isolates in the study were positive for \textit{eaeA} gene-increases risk of human infection.
Results for Rusa deer

Results

- All three farms were +ve for STEC

- Overall Prevalence of 27.0% (33/122)

- 155 STEC isolates were recovered:
  - 122 from faecal
  - 33 from raw meat

Total no of isolates tested:
- raw meat (n=135)
- Faecal (n=298)
Results for Rusa deer

STEC (n=155)

- O100
- O110
- O117
- O119
- O128ab
- O146
- O156
- O157
- O163
Results for Rusa deer

- **stx2 only** (n=14)
- **EHEC-hlyA only** (n=8)
- **eaeA and EHEC-hlyA** (n=130)
- **eaeA** (n=3)

STEC (n=20)
Results of Rusa deer

• The overall STEC prevalence in deer was 27.0% (33/122), being significantly higher in faeces (27/61).
• Nine (n=9) serogroups were recovered.
• O157 was confirmed by PCR but could not be isolated.
Discussion I

Dairy cattle

• STEC cannot be easily detected in bulk raw milk since their number is often very low in the raw milk
• One farm that was previously known to be STEC-positive in 2014 was found to be STEC-negative at the time of sampling-asymptomatic carriers of STEC
• All the strains were stx-negative (suggest low interaction between stx-encoding bacteriophages)
Discussion II
Dairy cattle

• Results obtained from this study, in addition to a previous study in 2014 demonstrated that there exist other STEC serogroups that have not been screened yet (e.g. O84 and O118) at farm-level.

• The discovery of two new serogroups (O84 and O118) raises questions concerning the serogroup diversity existing in dairy cattle and possible emergence of new STEC pathotypes.
Discussion I
Rusa deer

- Serogroup O146 was found to possess stx2 only, which has been proved to be 1000 more toxic than stx1
- 84.5% of STEC strains harbored both intimin (eaeA) and enterohemolysin (EHEC-hlyA) (131/155) revealing their potential to cause human pathogenicity
Discussion II
Rusa deer

• “Spill-over” and “spill-back” transmission, together with the geographical expansion of human/wildlife interactions increases the risk of STEC transmission and the emergence of infectious diseases in both humans and wildlife animals.
Discussion

The risk of STEC being transmitted into raw milk (6.0%) and raw deer meat (10.0%) is real.

Implies that good hygienic practices at both farm level and consumer level is of prime importance.
Future work

• To estimate the prevalence of STEC in faeces and raw meat derived from pigs in Mauritius
• To estimate the prevalence of STEC in faeces and raw meat derived from beef cattle in Mauritius
• To characterize STEC isolates based on molecular O serogrouping
• To compare STEC isolates from the different livestock species
Thank you
Questions?