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

A subset of them are pathogenic to humans

SOURCES
Human gut
Warm –blooded animals

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

Why them?

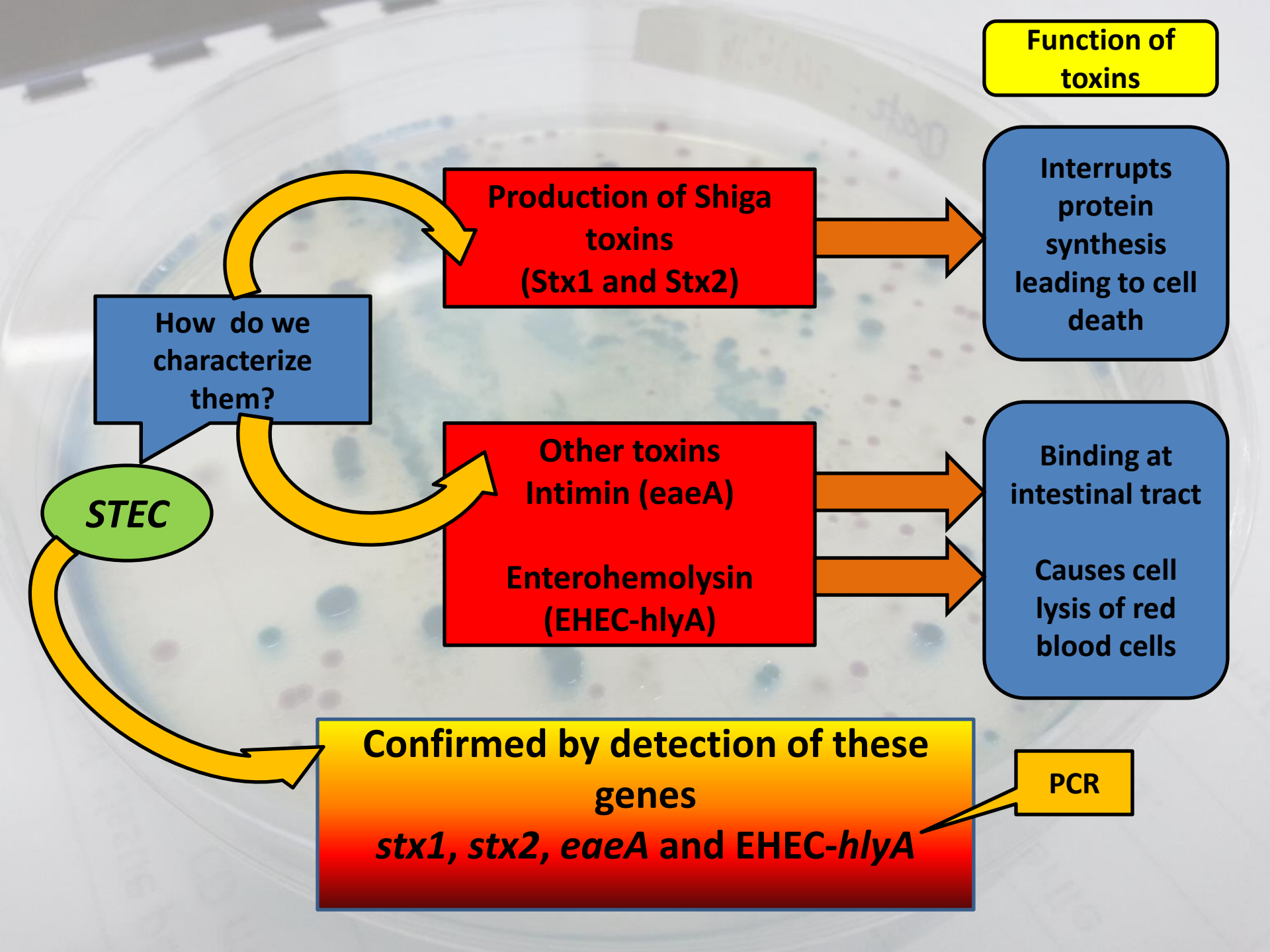
Dairy cattle
Beef cattle
Deer
Pigs

Transmission
Raw milk
Raw meat
Hamburgers
Cheese

Most dangerous
-Toxicity
-Clinical symptoms

Produces toxins

DISEASE IN HUMANS
1. Diarrhea
2. HC
3. HUS
4. TTP
5. *DEATH!!!*


Function of toxins
Production of Shiga toxins (Stx1 and Stx2)
Interrupts protein synthesis leading to cell death
How do we characterize them?
STEC
Other toxins
Intimin (eaeA)
Enterohemolysin (EHEC-hlyA)
Binding at intestinal tract
Causes cell lysis of red blood cells
Confirmed by detection of these genes
stx1, stx2, eaeA and EHEC-hlyA
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

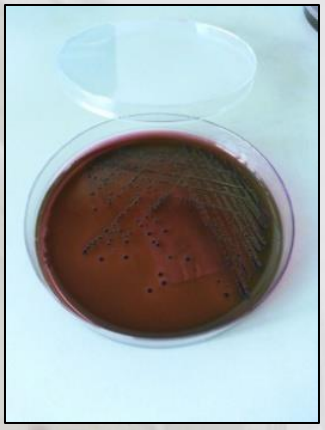
- 25-30g of composite faecal samples
- 30-40ml of bulk raw milk

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

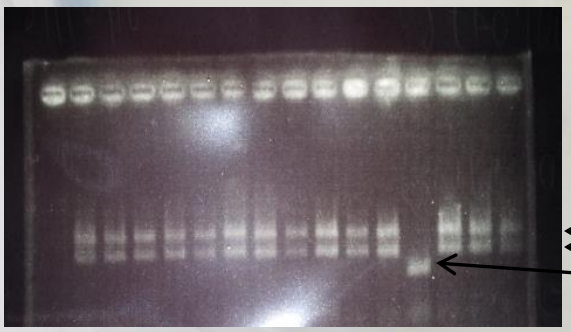
↓

Purification and Cryopreservation

↓

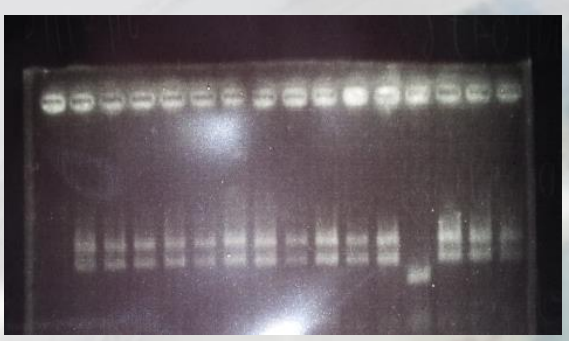
DNA extraction → PCR → Gel

stx1, *stx2*, *eaeA* and EHEC-*hlyA*



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

Methodology II



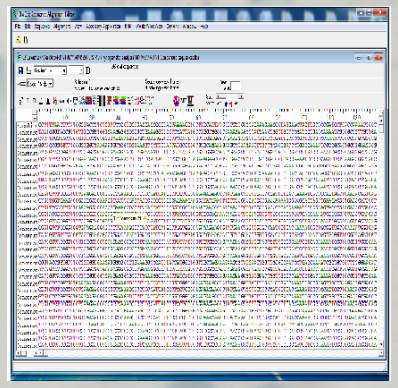
From Gel result
Select +ve isolates

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

Non-typeable isolates were *gnd*-PCR amplified

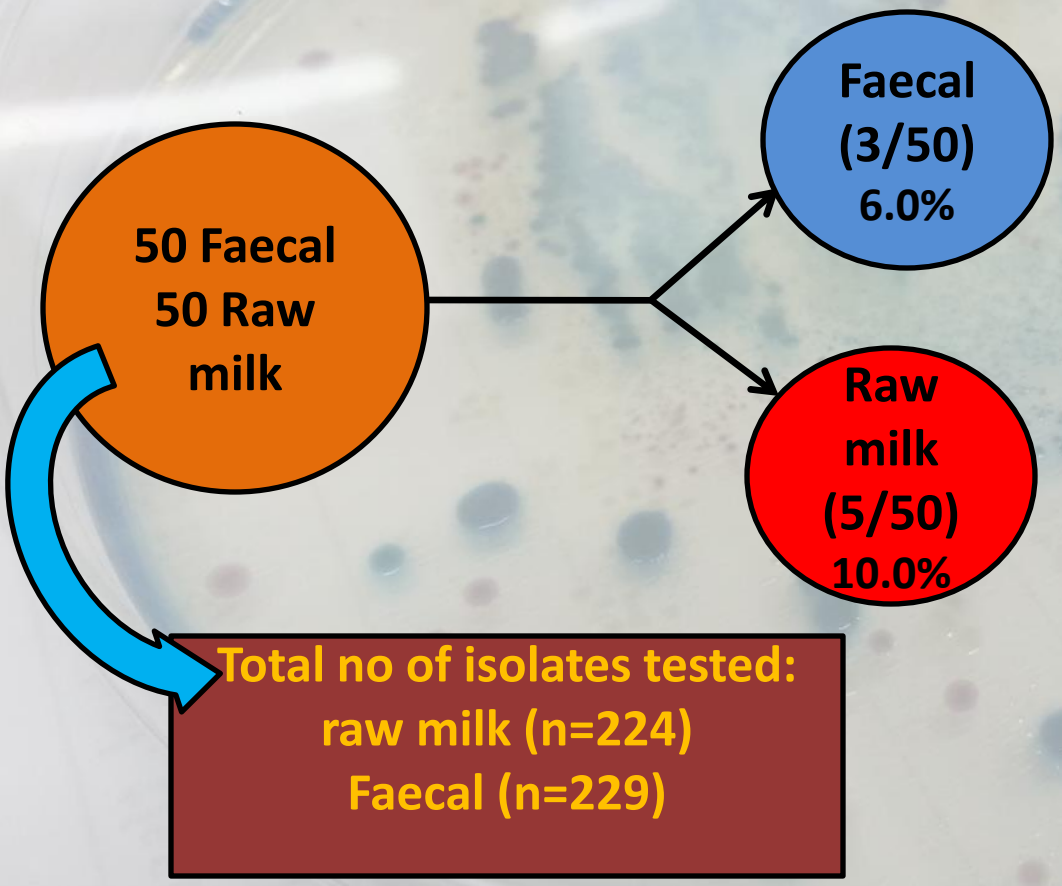
Sanger sequencing

Serogroup identification



Sequence analysis (Trimming and phylogenetic analysis

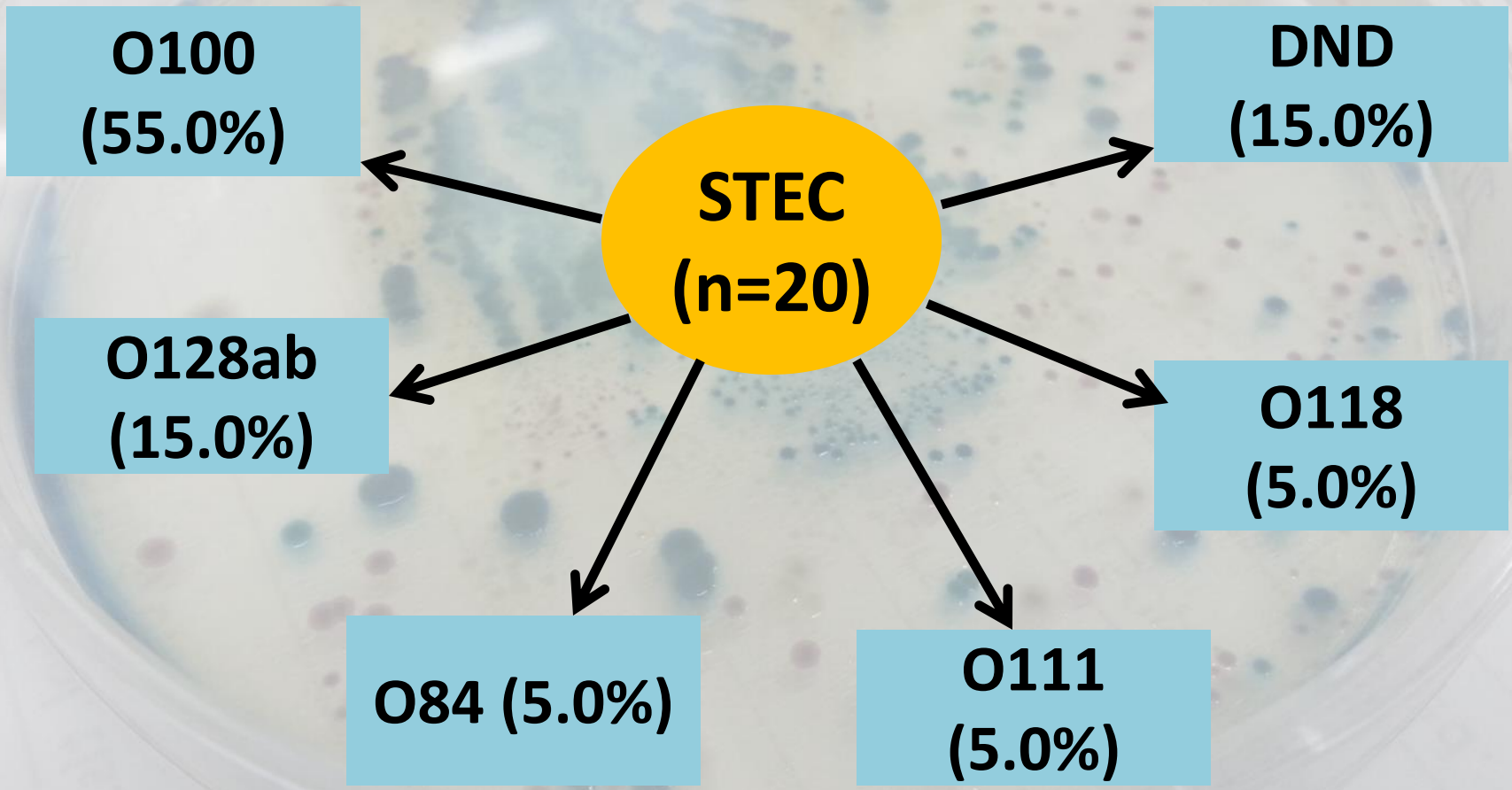
Results for Dairy cattle



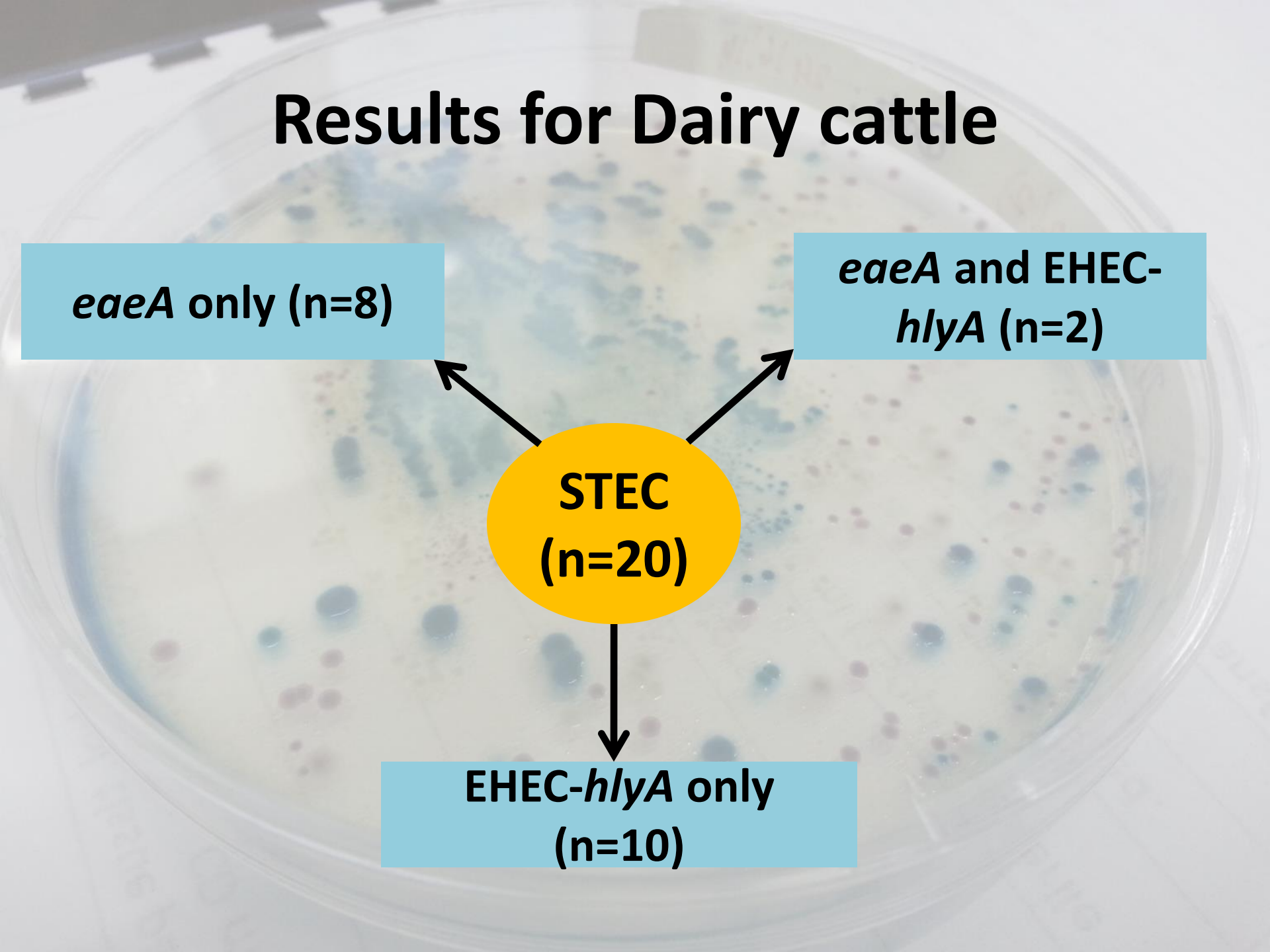
Results

- 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

Results for Dairy cattle



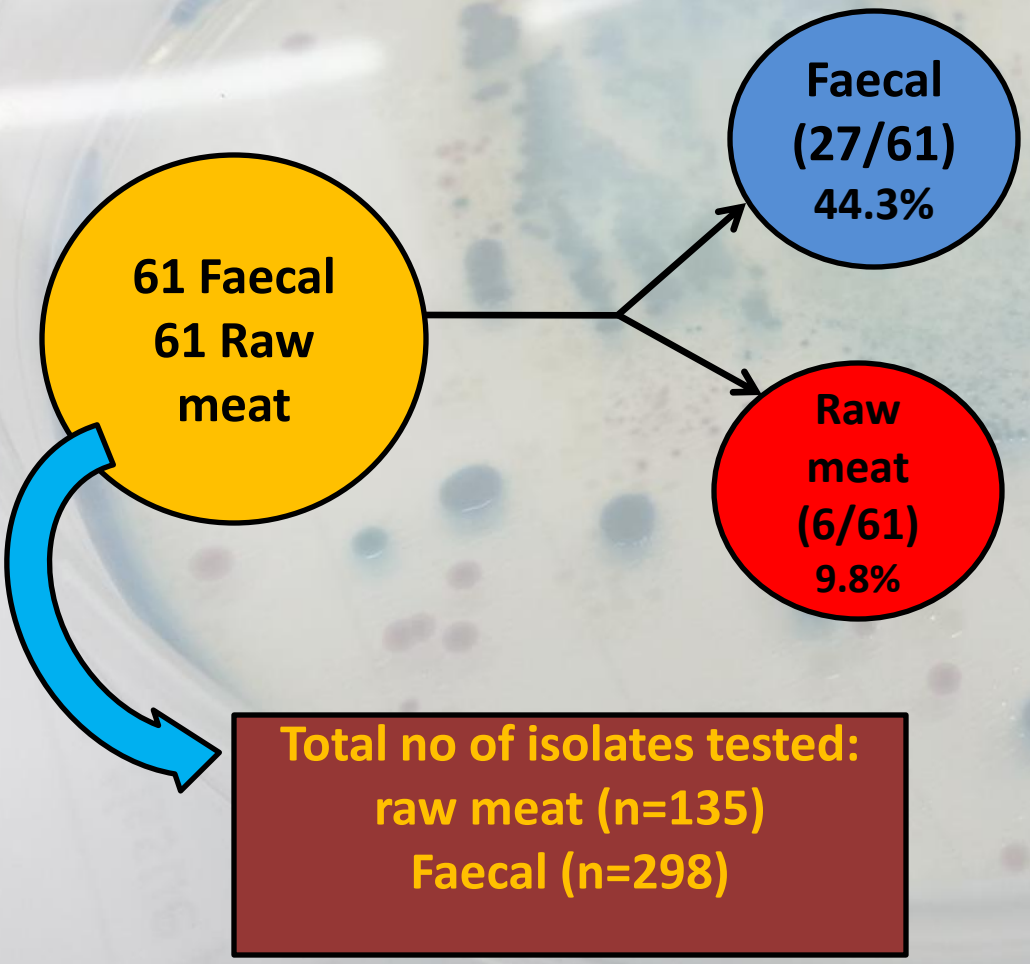
Results for Dairy cattle



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 *stx*-negative
- All isolates in the study were positive for *eae*A gene- increases risk of human infection.

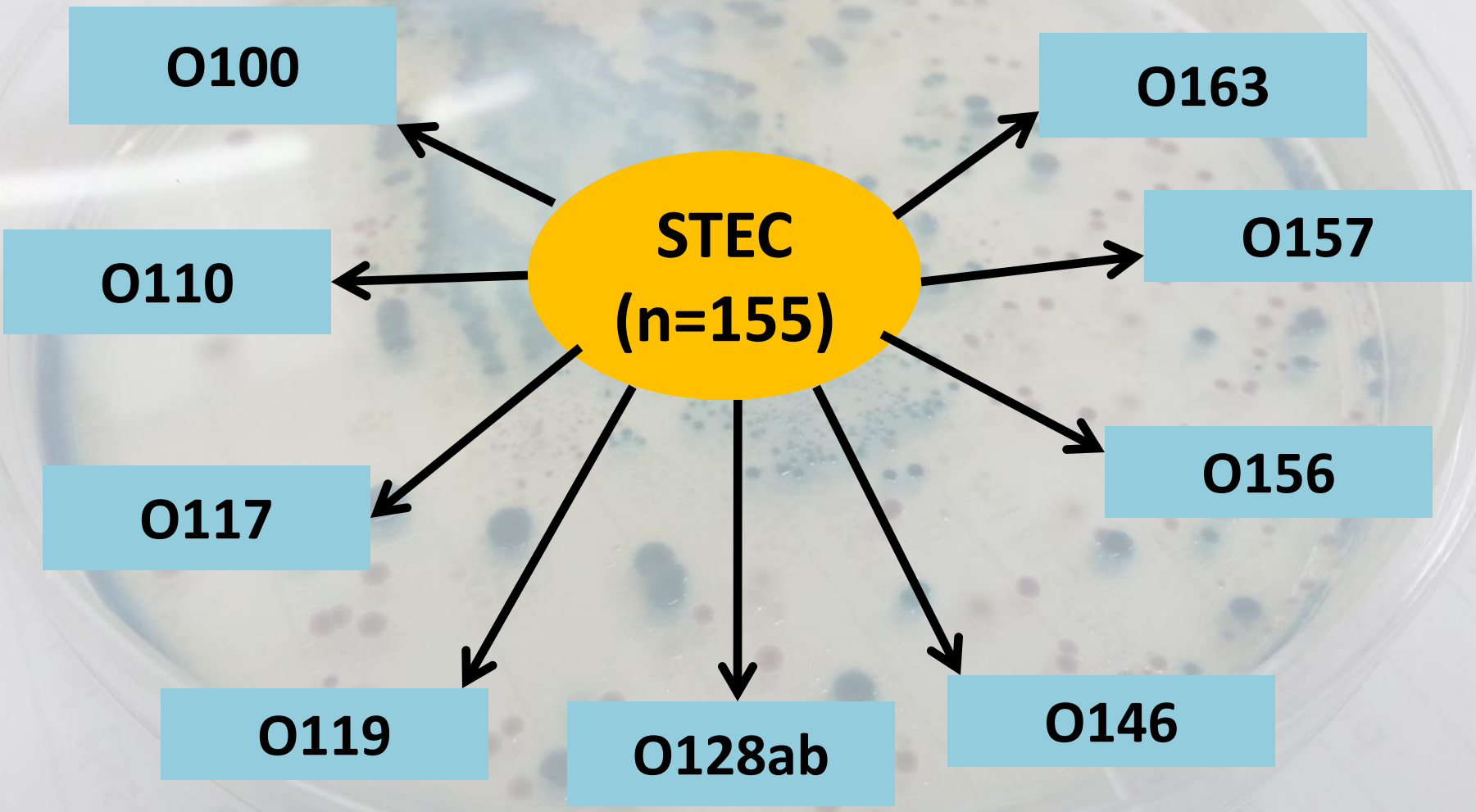
Results for Rusa deer



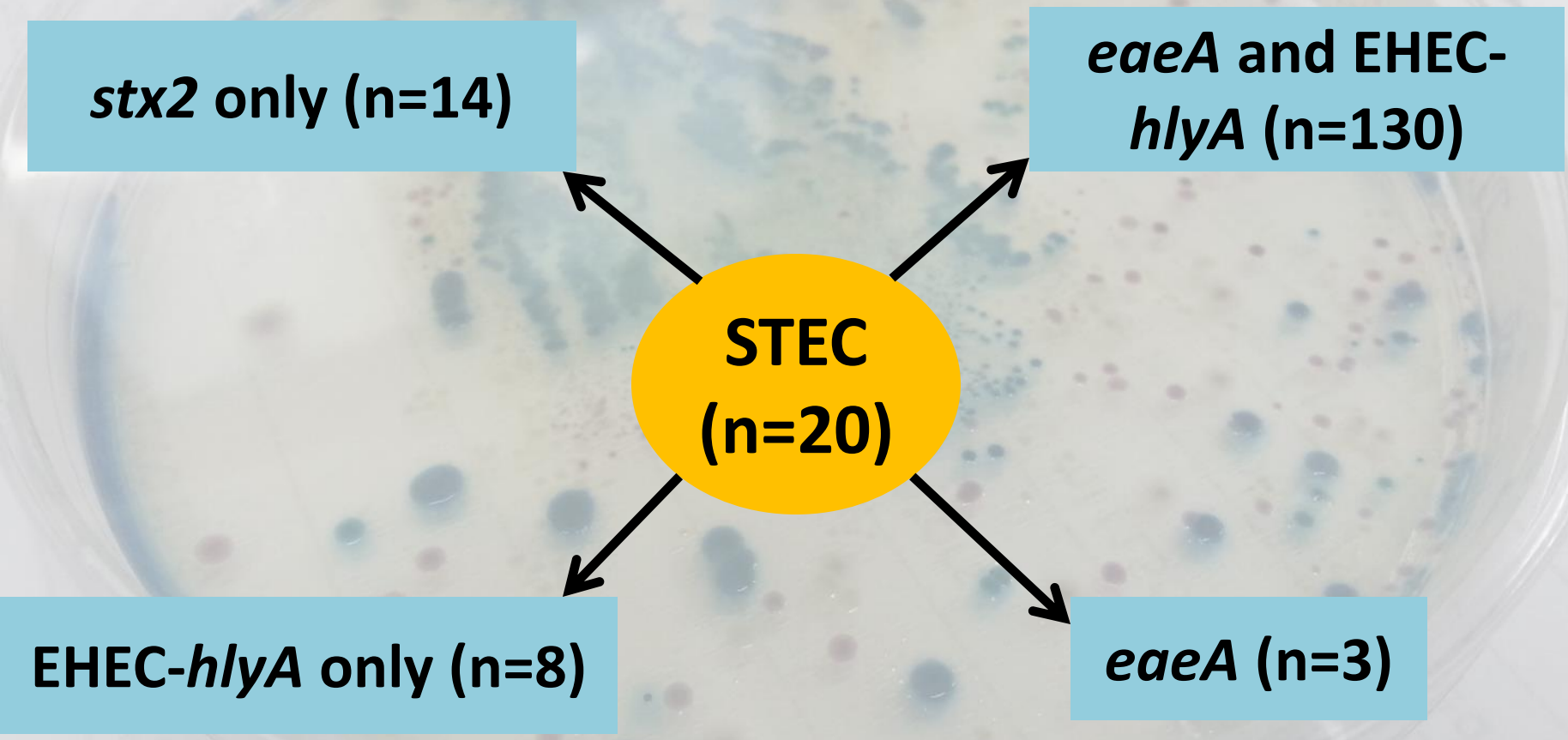
Results

- 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

Results for Rusa deer



Results for Rusa deer



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 bacteriphages)

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 livestocks


Thank you


Questions?