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

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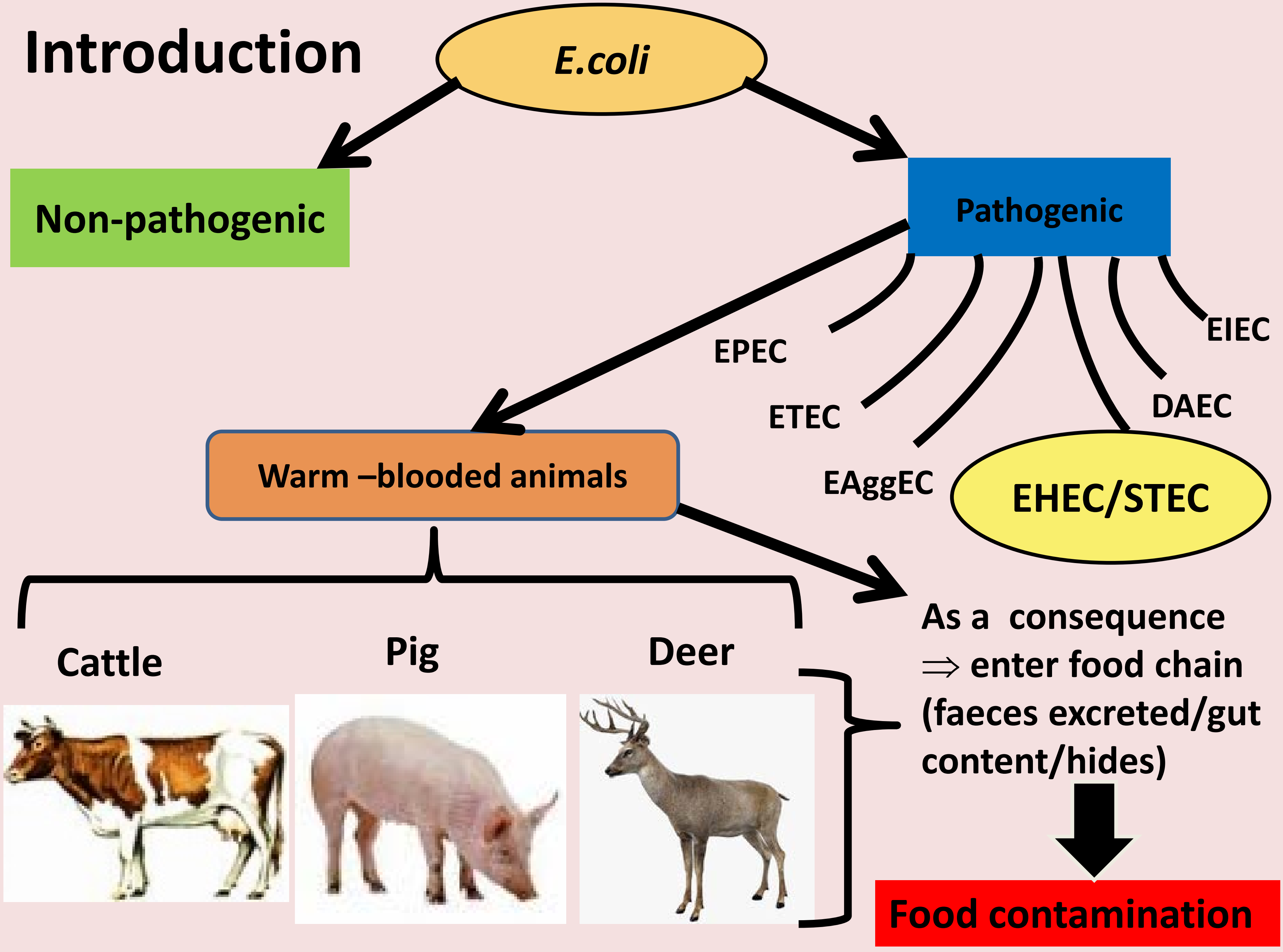
Project dans le cadre du plan stratégique 2015-2020, Axe 3 : Sûreté des aliments et contrôle de la qualité

- **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



STEC

Most dangerous!!
Why?

"Public health priority" WHO since 1998

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

Pathology

Severe diarrhea
Bloody diarrhea
Renal failure
CNS
Death

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**

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

Results: Prevalence

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)

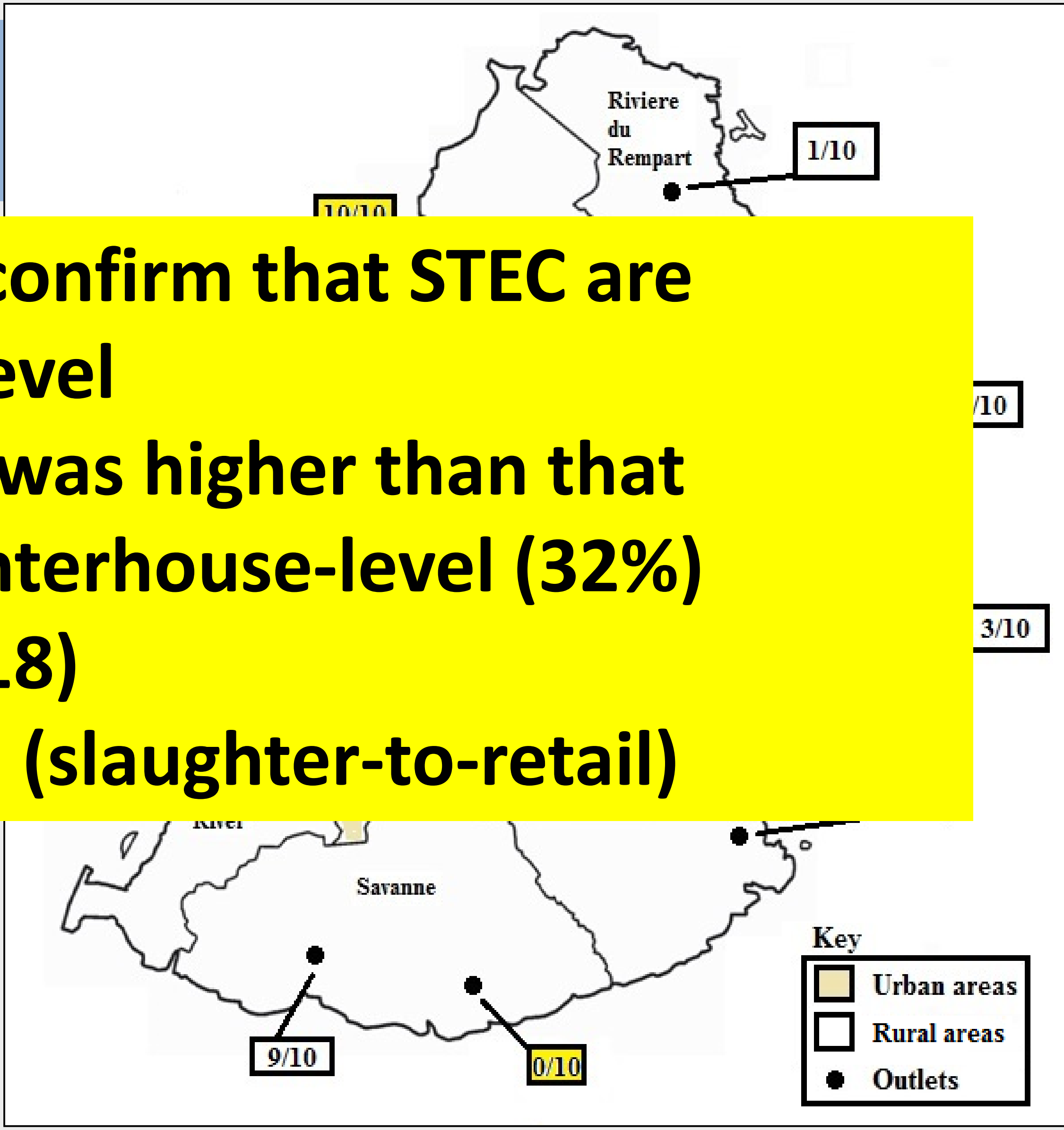


Figure 1: Prevalence of STEC from beef samples collected in the 15 outlets

Results: Serogroup diversity

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-

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

serogrouped

- 58 strains were not determined (DND)

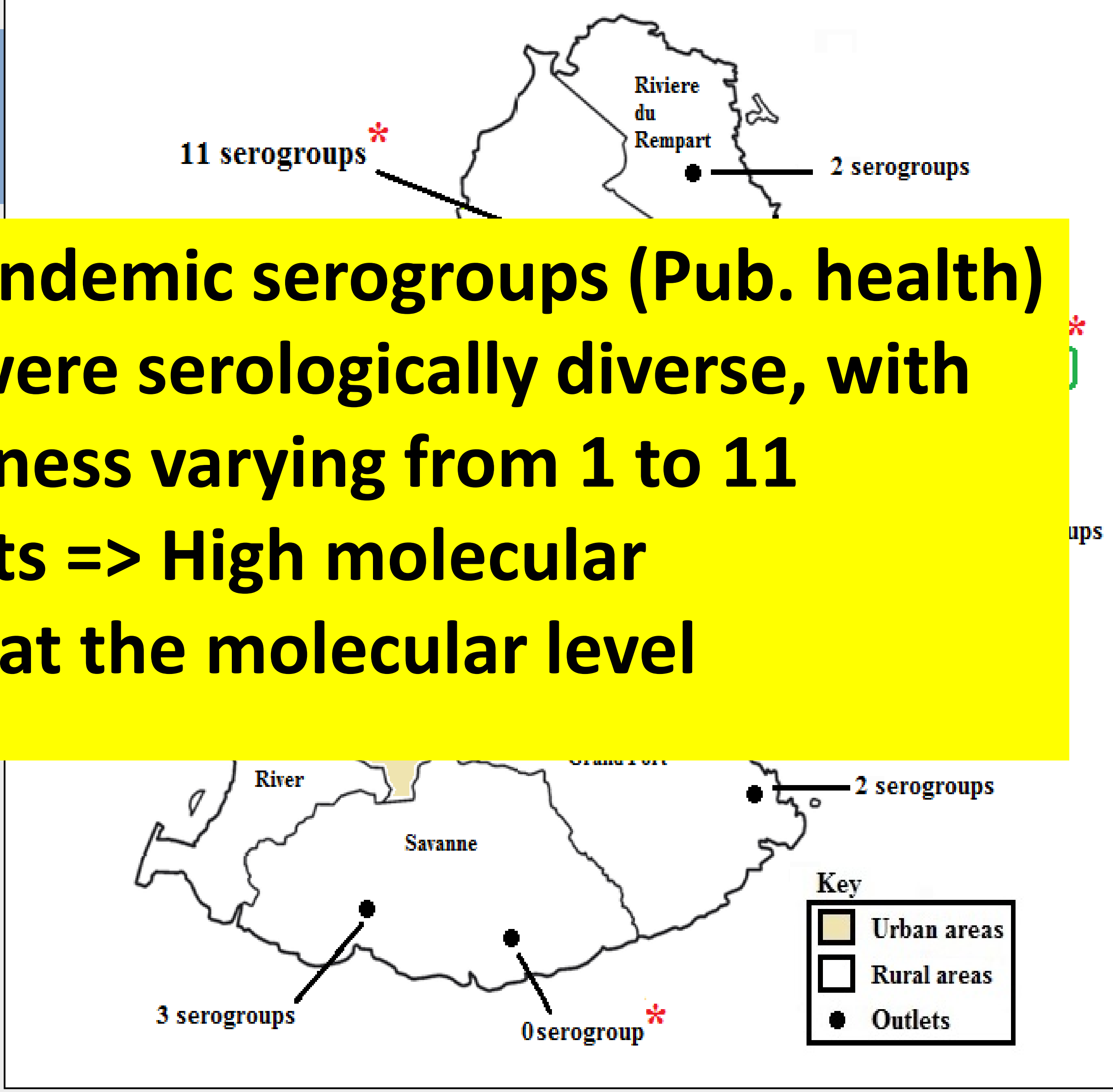
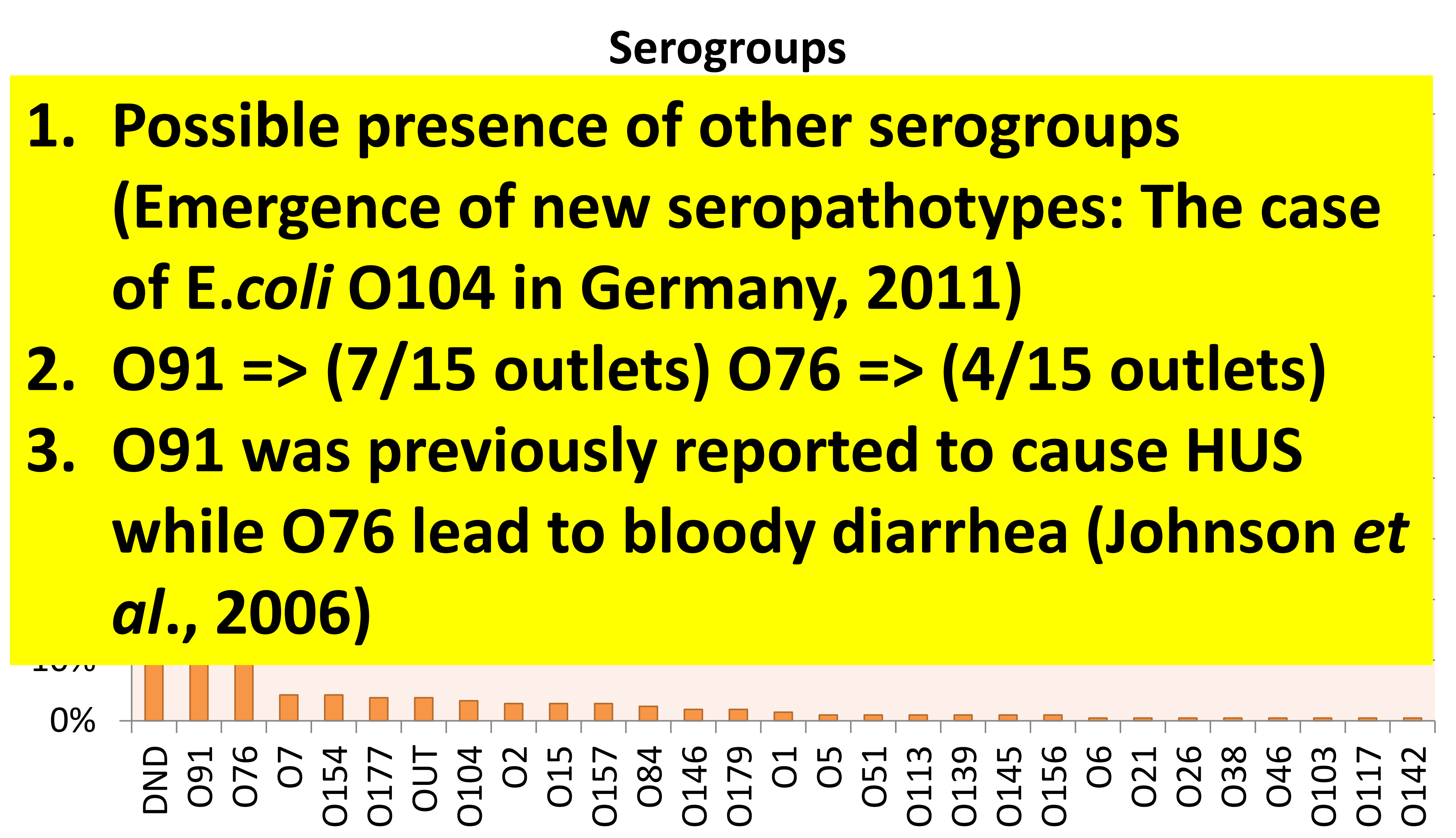


Figure 2: Diversity of serogroups recovered from beef samples at each of the 15 outlets

Results: Serogroup diversity

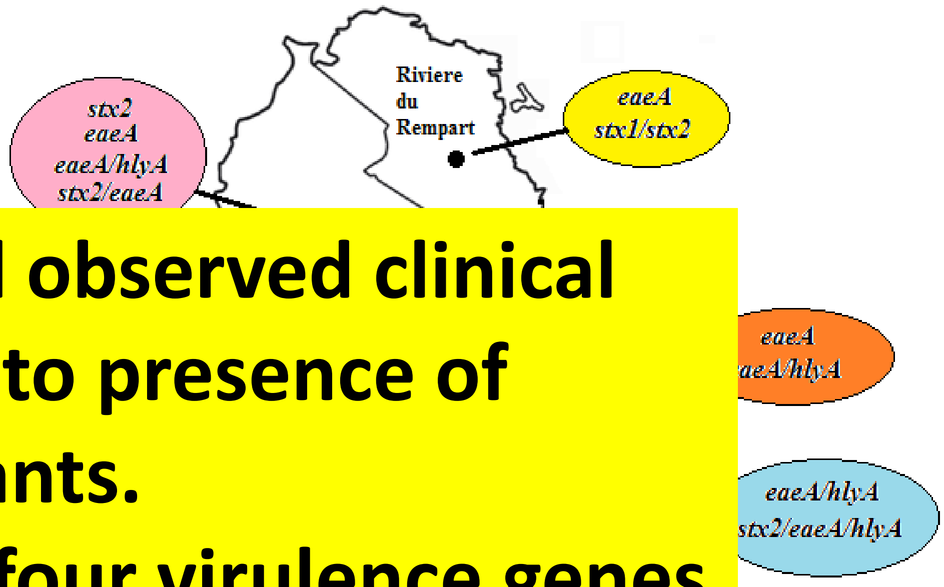


1. **Possible presence of other serogroups (Emergence of new seropathotypes: The case of E.*coli* O104 in Germany, 2011)**
2. **O91 => (7/15 outlets) O76 => (4/15 outlets)**
3. **O91 was previously reported to cause HUS while O76 lead to bloody diarrhea (Johnson *et al*., 2006)**

Figure 3: Frequency of STEC serogroups from the 211 STEC isolates

Results: Virulence profiles

Vir
stx1 o
stx2 o
eaeA
stx1/s
stx2/e
eaeA/
stx1/s
stx2/e
Total



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 … isolates

… ered beef samples from the 15 outlets

Results: Serogroup/virulence

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**

O146	+	+					+	
OUT	+				+	+		

Conclusion

- **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

1. Thierry, S. I. L., Santchurn, S. J., Jaufeerally-Fakim, Y and Gannon, J. E (2014) Prevalence of Shiga-toxigenic *Escherichia coli* in Mauritian Dairy cattle. *REMVT* 67(3): 87-140.
2. Thierry, S. I. L., Jaufeerally-Fakim, Y., Gannon, J. E., Santchurn, S. J. (2018) Shiga-toxigenic *Escherichia coli* of cattle origin represents a surveillance priority and an important human health threat to public and travelers of the Indian Ocean islands. *J Food Saf* 38 (3), e12454. https://doi.org/10.1111/jfs.12454

(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

- Johnson, K. E., Thorpe, C. M., Sears, C. L. (2006) The Emerging Clinical Importance of Non-O157 Shiga Toxin--Producing *Escherichia coli*. *Clin. Infect. Dis*.43, 1587–1595. https://doi.org/10.1086/509573
- MAIFS (2016). Strategic Plan for Year 2016-2020. Available online: http://agriculture.govmu.org/English/Documents/Book%20Final.pdf (Date accessed: 21 October 2018)
- Majowicz, S. E., Scallan, E., Jones-Bitton, A., Sargeant, J. M., Stapleton, J., Angulo, F.J., Yeung, D. H., Kirk, M. D. (2014) Global Incidence of Human Shiga Toxin–Producing *Escherichia coli* Infections and Deaths: A Systematic Review and Knowledge Synthesis. *Foodborne Pathogens and Disease* 11, 447–455. https://doi.org/10.1089/fpd.2013.1704
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