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Title

Shiga-toxigenic *Escherichia coli* from Animal Food Sources in Mauritius: prevalence, serogroup diversity and virulence profiles

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Highlights

- ~~Cattle, pigs and deer are colonized by STEC, with cattle acting as the main reservoir (representing most diverse virulence patterns and serogroup diversity).~~
- Cattle, rusa deer and pigs are colonized by STEC, with cattle as the principal reservoir
- ~~28.4% (74/261) of animal source foods samples were contaminated by STEC, of which retail beef establishments accounted for 24.1% (63/261).~~
- Retail beef accounted for over 85% of the 74 STEC-positive food samples
- ~~73.7% (28/38) of recovered O-serogroups were previously linked to human diseases such as HUS or bloody diarrhea.~~
- 73.7% (28/38) of O-serogroups were previously linked to HUS or bloody diarrhea
- ~~Four EHEC-7 strains (O26, O103, O145 and O157) and eight non EHEC-7 serovars (O91, O100, O104, O110, O117, O146, O177 and ONT) are of higher importance given their outbreaks history and/or multiple virulence profiles.~~
- Two rarely documented serovars (O117 and O119) were recovered in rusa deer
- 38.5% of STEC strains had multiple toxigenic profiles involving *stx2* and/or ~~*eaeA*~~ *eae*.

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Abstract

Shiga-toxigenic *Escherichia coli* (STEC) are important human pathogens associated with diarrhea and in some cases haemorrhagic colitis. Contaminated food derived from cattle and wildlife species are often associated with disease outbreaks. In this study, we report the prevalence, serogroup diversity and virulence profiles of STEC strains derived from cattle, rusa deer and pig. Of the 422 samples analyzed, STEC were detected in ~~34% (17/50)~~ 40% (80/200) of

cattle ~~intestinal tracts~~, ~~31.5% (35/111)~~ 27.0% (33/122) of deer ~~of animal faeces~~ and ~~28.4% (74/261)~~ 13.0% (13/100) of pigs. ~~Animal Source Foods (ASF) sampled.~~ STEC isolates belonged to 38 O-serogroups whereby 5.2% (24/462) of the isolates belonged to clinically important EHEC-7 serogroups: O26 (n=2), O103 (n=1), O145 (n=3) and O157 (n=18). Fourteen serogroups (O26, O51, O84, O91, O100, O104, O110, O117, O145, O146, O156, O157, O177 and ONT) displayed multiple virulence profiles. We also identified two serovars (O117 and O119) in deer which are not well-documented in epidemiological surveys. 73.7% (28/38) of recovered O-serogroups are known to be associated with serious human illnesses including haemolytic uremic syndrome (HUS) and bloody diarrhea. STEC isolates harboring single genotypes *stx1, stx2, ~~eaeA~~ eae* and *hlyA* accounted for 3.0% (14/462), 9.1% (42/462), 47.6% (220/462) and 1.7% (8/462) of all STEC isolates screened, respectively. Virulence combinations *stx1* and *stx2* were harbored by 1.3% of isolates while strains with genetic profiles ~~*eaeA*~~ *eae* /*hlyA* were the second most prevalent amongst STEC isolates. The full known virulent genotypes (*stx2*/~~*eaeA*~~ *eae*, *stx1*/*stx2*/~~*eaeA*~~ *eae*, *stx1*/*stx2*/*hlyA* and *stx2*/~~*eaeA*~~ *eae* /*hlyA*) were present in 22 of the 462 STEC strains. A total of 10 different virulence patterns were recovered amongst animal species. Phylogeny of the ~~gnd~~ *gnd* gene showed that amongst STEC strains, serovar O100 outlied the main cluster. Fourteen (n=14) different sequence types (STs) were identified from a panel of twenty (n=20) STEC isolates. One of the isolate (PG007B) possessed a unique ST (*adk* 10, *fumC* 693,*gyrB* 4, *icd* 1, *mdh* 8, *purA* 8, *recA* 2) that could not be assigned using MLST databases. None of the ST's recovered in deer were observed in domestic species. Our findings shows that food associated animals found on the tropical island of Mauritius carry a diversity of STEC strains with many serovars known to be associated with human disease. This report

indicates that increased awareness, surveillance and hygienic attention at critical stages of the human food chain are warranted.

Keywords: *Escherichia coli*, Food safety, public health, domestic and wildlife animals, surveillance, Indian Ocean islands

Introduction

Escherichia coli is a harmless gut commensal but also a versatile pathogen of humans estimated to cause more than two million deaths annually (Nataro and Kaper, 1998). Shiga-toxigenic *Escherichia coli* (STEC) are recognized globally as foodborne pathogens with varied clinical manifestation ranging from non-bloody diarrhea to more severe conditions such as haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombocytopenic purpura (TTP) (Karmali *et al*., 2010). These syndromes are directly related to the prevalence of virulence genes (Anonymous, 2011). STEC pathovars may possess a potent combination of virulence factors: Shiga toxins (*stx1* and *stx2*), intimin and enterohemolysin (encoded by ~~*eaeA*~~ *eae* and ~~EHEC-*hlyA*~~ *hlyA*, respectively) that contribute to its low infective dose, cytotoxicity effects and general disease severity. Attachment and effacing (A/E) lesions resulting from expression of ~~*eaeA*~~ *eae*, disruption of eukaryotic red blood cells by ~~EHEC-*hlyA*~~ *hlyA* along with the repression of protein synthesis effects via binding to its receptor globotriaosylceramide (Gb3) from Shiga toxins are the main characteristics associated with STEC virulence (Bosivelac and Koohmaraie, 2011, Paton and Paton, 1998a, Schmidt *et al*., 1995). These virulence factors found in single or multiple combinations constitute the virulence profile for a particular pathovar.

STEC, like other *E. coli* strains are classified based on the highly immunogenic O-somatic antigens whose biosynthetic pathway depends on the highly variable O-antigen gene cluster (O-AGC) generally flanked between the *gnd* and *galF* chromosomal genes (DebRoy *et al*., 2016). Pathogenic STEC strains have been shown to belong to a broad range of O serogroups (Johnson *et al*., 2006; Tozzoli and Scheutz, 2014). Seven serogroups (O26, O45, O103, O111, O121, O145 and O157) are referred collectively to as EHEC-7 and are indicated as globally pandemic and predominant in clinical cases (Dewey-Mattia *et al*., 2016). There is also increased evidence that non-EHEC-7 strains are linked to clinical cases (Johnson *et al*., 2006). ~~STEC are classified into five seropathotypes (A-E) based on disease frequency, relative incidence, and association with severe disease such as HUS and HC (Karmali *et al*., 2003). STEC has been classified into five seropathotypes (A-E) based on disease frequency, relative incidence, and association with severe disease such as HUS and HC (Karmali *et al*., 2003). In addition, a more recent analysis (ESFA, 2013) suggests that serogroups of group I (O157, O26, O103, O145, O111, O104) in combination with *stx* and *eae* or *aaiC* and *aggR* should be regarded as HUS-associated serotypes (HAS) and considered as high risk of diarrhea and HUS, whereas other seropathotypes D and E with the same gene combinations are potential risk for diarrhea but currently unknown for HUS.~~

All STEC strains, irrespective of their O serogroups are now classified as pathogenic in humans, capable of causing either mild diarrhoea or severe illnesses such as HUS or HC, depending on the presence of additional aggravating/colonization factors such as *eae* (EFSA, 2020). The latest pathogenic assessment report also defines that no single or multiple combinations of virulence factors (including *stx*-subtypes) can be used as a predictor for clinical outcome.

Domestic animals, particularly cattle, are regarded as natural reservoirs of STEC (Caprioli *et al*., 2005). Wildlife animals such as deer and non-ruminant species such as pigs are also STEC carriers (Bessone *et al*., 2017; Cha *et al*., 2018; Díaz-Sanchez *et al*., 2012; Rounds *et al*., 2012) and have previously been linked to outbreaks (Keene *et al*., 1997; Trotz-Williams *et al*., 2012). As a consequence, STEC can contaminate food intended for human consumption (Caprioli *et al*., 2005). In most cases, water/food consumption is the predominant vehicle of transmission which can take place at any step of the "farm-to-fork" process (EUFIC, 2006). In this context, it is essential for epidemiologists to characterize ~~characterization of~~ STEC strains from Animal Source Foods (ASF) ~~based on a 'farm-to-fork' approach is most useful~~ to set food safety priorities and public health policies. In a previous study, we showed that STEC were detected in 25.3%, 10.0% and 32.0% of faeces, raw milk and raw meat samples of bovine origin (Thierry *et al*., 2018). Less is known, however, about the epidemiology of STEC from other important food producing animals such as deer and pigs.

~~With the goal of understanding the overall risk of STEC associated with Animal Food Sources (AFS), we determined serogroup diversity and virulence profile abundance of STEC strains circulating in cattle, deer and pigs. To confirm the role of cattle as a STEC reservoir, we collected additional samples for STEC isolation. We then compared this data with previous findings to confirm the hypothesis that STEC strains could be widespread amongst livestock animals and therefore constitute a public health challenge. We further investigated whether sero-pathovars (O130 and O139; *stx1*/*stx2*/EHEC *hlyA*) documented earlier were prevalent amongst~~

~~other livestock in similar climatic zones. We suggest that data generated should correlate to previously published public health information.~~

The main objective of the current study was to examine the public health risk potential of STEC associated with ASF by assessing the prevalence, serogroup diversity and virulence profile abundance of STEC strains circulating in the animal (cattle, deer and pigs) supply chain on the island of Mauritius. ~~We collected additional samples for STEC isolation to further examine the role of cattle as a STEC reservoir and potential transmission dynamics of most dangerous sero-pathovars (O130 and O139; *stx1*/*stx2*/EHEC-*hlyA hlyA*) documented earlier. From this dataset, a comparative analysis was done against previous findings to assess the hypothesis that STEC strains could be widespread amongst livestock animals. We suspect that data generated should correlate to a broad range of pathogenic serovars previously associated with clinical cases.~~

Materials and Methods

Description of study population

Mauritius is geographically situated around 890 km East of Madagascar and forms part of the Mascarene Islands (Figure 1a). The island possesses a livestock production system primarily composed of poultry, cattle, pig, goats, sheep and deer which are classified into three production systems: intensive, semi-intensive and backyard/extensive (FAO, 2007). The actual livestock of Mauritius consists of some 6,447 cattle (excluding imports), 21,235 pigs and 65,000-70,000 deer (Defimedia, 2016a; MAIFS, 2016; Roger *et al*., 2009).

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Sample collection

From 2015-2017, a total of 422 samples were collected from cattle, rusa deer and pigs (Figure S1). For cattle, intestinal tract contents (25-30ml) were collected from the Mauritius Meat Authority (MMA) abattoir while retail raw beef samples (25-100g) were purchased from 15 retail outlets (six different municipal markets and nine villages) (Figure 1b). For each pig carcass presented for slaughter at the MMA abattoir, faecal (25-30g) and raw meat (25-100g) samples were collected. Similarly, for deer, faecal (25-30g) and raw meat (25-100g) samples were collected from deer carcasses after the evisceration process at hunter check-in stations of three different *chassés* (Figure 1b). Depending on the nature and consistency of samples collected, specimens were either placed into separate sterile 50 ml stool containers or sterile zip 'n' seal bags and were immediately placed on ice and transported to the laboratory where they were processed within 24 hours.

Isolation and characterization of STEC

The microbiological cultural and molecular-based approaches were adapted from Thierry *et al*. (2018). This consisted of an *E. coli* enrichment step in a 1:10 sample/broth ratio consisting of modified Tryptic Soy Broth (mTSB, Oxoid CM0989, Basingstoke, United Kingdom), after which a portion was cultured onto CHROMagar STEC (CHROMagar, Paris, France). After incubation at 37°C for 24h, up to five pink-mauve colonies (characteristic of presumptive STEC) were further plated on Eosine Methylene Blue (EMB, Oxoid CM0069, Basingstoke, United Kingdom) agar as an *E. coli* confirmatory test. Isolates were purified on nutrient agar (NA, Oxoid CM0003, Basingstoke, United Kingdom) and were kept at 4°C for further analysis. DNA was extracted from presumptive STEC strains using the boiling method (heat treatment of cells

for 10 min at 100°C followed by immediate cooling on ice for 5 min), after which supernatant was collected and used as DNA template in polymerase chain reaction (PCR) methods using previously reported primers associated with major virulence genes (Paton and Paton, 1998a). Oligonucleotide sequences and primer names used for amplification of *stx1*, *stx2*, ~~*eaeA*~~ *eae* and ~~EHEC-*hlyA*~~ *hlyA* are listed in supporting information Table S1. *E. coli* O157:H7 EDL 933 was used as a positive control for the multiplex PCR assay. STEC were characterized by positive amplification of one, two, three or all of the targeted genes (*stx1*, *stx2*, ~~*eaeA*~~ *eae* and ~~EHEC-*hlyA*~~ *hlyA*). The genotypic profiles of STEC isolates were identified by running an agarose gel electrophoresis after the end of the multiplex PCR reaction. Isolates that were PCR-confirmed as STEC were further characterized using the 6-phosphogluconate dehydrogenase *gnd* gene PCR assay for sequence-based serogrouping (Gilmour *et al*., 2007).

Sequencing, phylogenetic, mlst and statistical analysis

A sample was confirmed as STEC positive if at least one STEC isolate was recovered. Statistical analysis was carried out using WINPEPI program for epidemiologists (PEPI 4.0). Consensus sequences of the *gnd* genes were generated from both *gnd*-F and *gnd*-R fasta files using Bioedit v.7.2.5 (Hall, 1999). Once all consensus sequences were generated, comparative analysis of each sequence was performed through an online *E. coli* database (*E. coli* O Typer: https://www.corefacility.ca/ecoli_typer/) for eventual determination of serogroup. Before phylogenetic analysis of the *gnd* gene, multiple sequence alignment of the *gnd* gene (643 bp in length) was generated using the online server MAFFT (http://mafft.cbrc.jp/alignment/software). Phylogenetic analyses of the *gnd* gene was done using the maximum parsimony method (with options: heuristic search, tree bisection-reconnection swapping algorithm, gaps treated as

missing, excluding non-informative sites) using PAUP* (test version 4.0a162; Swofford, 2002) as previously described (Thierry *et al*., 2018). The analysis was composed of 397 sequences, subdivided into 393 screened STEC isolates, three STEC references sequences (O157:H7, O26:H11 and O121:H19) and one outgroup (*Serratia marcescens* WW4). Supporting values for the branching topology were calculated via a 1000-bootstrap approach implemented in PAUP. The resulting phylogenetic tree was visualized, edited and annotated using Interactive tree of Life (iTOL) v3 (http://itol.embl.de) (Letunic and Bork, 2016). A panel of twenty (n=20) STEC isolates: cattle (n=8); deer (n=6); pig (n=6) were subjected to sequence types (STs) targeting seven of the housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*). STs were identified using Multi Locus Sequence Types (MLST 2.0; with options: *Escherichia coli*#1) (Larsen *et al*., 2012) and *E. coli* MLST database available at Enterobase v.1.1.2 (http://enterobase.warwick.ac.uk/)

Results

Prevalence and distribution of STEC amongst livestock

From the 422 samples collected, 30% (126/422) were found to be STEC-positive as represented in Table 1. Using *E. coli* isolates as the epidemiological unit and as a bacterial monitoring indicator for estimation of STEC prevalence, we found that 37.8% [(462/1,221): $CI_{99\%}$: 34.3–41.5] of all the *E. coli* isolates screened were classified as STEC (after bacteriological analysis and virulence PCR assay). In terms of ASF, we found that raw meat from cattle origin had the

highest prevalence of STEC (74.6%; $CI_{99\%}$, 67.3% to 81.0%) followed by raw meat from rusa deer (24.4%; $CI_{99\%}$, 15.6% to 35.2%) and pigs (14.3%; $CI_{99\%}$, 7.2% to 24.4%).

Serogroup diversity and distribution of STEC amongst livestock

Of the 462 STEC isolates characterized, representing 126 samples, 38 different O-antigen serogroups were identified (Table 2). Further classification and distribution showed that STEC serogroups were most heterogeneous in cattle (n=32), followed by rusa deer (n=10) and pigs (n=5), of which eight serogroups (identified by **) were shared amongst livestock. Interestingly, only serogroup O157 was shared amongst all three livestock. A number of serogroups were specific to deer (O110, O119, O128ab and O163), pigs (O130) and cattle (25 other serogroups). Twelve isolates were non-typeable (ONT) and in 68 of the 462 STEC strains, the O antigens could not be fully sequenced and were denoted by DND.

Serogroup frequency

The number of STEC strains identified as belonging to a particular serogroup is displayed in Table 2. 67.5% (312/462) isolates were classified into 14 serogroups. The most frequent O groups with frequencies (f > 20 isolates) were O119 (n=50), O128ab (n=37), O91 (n=34), O76 (n=29), O100 (n=25), O117 (n=25) and O146 (n=21). The remaining serogroups with frequencies ($f \geq 10$ isolates) were O157 (n=18), O174 (n=16), O15 (n=12), O156 (n=12), ONT (n=12), O104 (n=11) and O154 (n=10). Unlike O157 (n=18), other clinically important serogroups O26 (n=2), O103 (n=1) and O145 (n=3) were less frequent.

Virulence profiles of STEC isolates and distribution amongst livestock

From the multiplex PCR assay, fourteen isolates carried only the *stx1* virulence marker tested. Overall, 61.5% (284/462) of isolates possessed single virulence genotypes (*stx1* only, *stx2* only, ~~*eaeA*~~ *eae* only and *hlyA* only) while the remaining isolates were grouped into six multiple virulence combinations. The most frequent profiles recorded were ~~*eaeA*~~ *eae* (220 strains), ~~*eaeA*~~ *eae* /*hlyA* (150 strains), *stx2* (42 strains) and *stx1*/*stx2*/-~~*eaeA*~~ *eae* (17 strains) (Table 2). A total of ten different virulence patterns were recovered throughout this study. The patterns were non-uniformly distributed amongst the three livestock, whereby cattle possessed 8 of the 10 virulence patterns and rusa deer and pigs possessed fewer patterns (Table 3).

Multiple virulence profiles of serogroups

We also found that different virulence gene profiles were detected among strains of the same serogroup; for instance, O26 (n=2 strains) displayed two different virulence profiles: ~~*eaeA*~~ *eae* (n=1 strain) and ~~*eaeA*~~ *eae* /*hlyA* (n=1 strain). In all, these multiple profiles (identified by *) were displayed by 14 serogroups (O26, O51, O84, O91, O100, O104, O110, O117, O145, O146, O156, O157, O177 and ONT). Amongst these serogroups, isolates from O91 and O157 displayed the highest virulence profiles (n=5), followed by O146 (n=4), O100 and ONT (n=3 profiles). Of those 68 strains that were unsuccessfully characterized for their O serogroup, seven virulence profiles were observed (Table 2). Detailed serogroup-virulotype combinations of the 462 isolates are shown in Table S2.

Distribution of virulence factors amongst positive samples

Three animal sources actually accounted for 84.9% of the 126 STEC-positive samples, where cattle meat had a 50% contribution (63/126) (Figure 2). In terms of virulence proportions amongst these positive samples, 8.7% (11/126), 20.7% (26/126), 86.4% (109/126) and 32.5% (41/126) had *stx1, stx2, ~~eaeA~~ eae* and the *hlyA* genes, respectively. Further evaluation showed that the *stx1* gene was principally recovered from cattle meat (10/126, 7.9%) and was absent both from cattle intestinal and deer sources. The *stx2* gene was mainly associated to cattle meat (11/126, 8.7%), deer faeces (6/126, 4.8%) and pig faeces (5/126, 4.0%) and to a lower extent to pig meat (2.4%) and deer meat (0.8%). The intimin (*~~eaeA~~ eae*) gene was detected in 86.4% of the positive samples. Cattle meat and deer faecal samples had high percentages of *~~eaeA~~ eae* genes compared to other samples. Interestingly, only *~~eaeA~~ eae* was recovered from cattle intestinal tract samples (identified by *). Compared to cattle meat (46%), the *~~eaeA~~ eae* gene was less frequent in pig sources and in deer meat (1.6% - 3.1%). The *hlyA* gene was detected in four of the six sources analyzed, and was prevalent across deer faeces (19.8%; 25/126), cattle meat (7.9%) and deer meat (4.0%) and to a much less extent in pig faeces (0.8%).

Phylogeny of the gnd gene

For the phylogenetic analysis of the *gnd* gene sequences, only informative sites were considered. On basis of this criterion, 236 base alleles were identified as parsimony-informative. The analysis of the 396 *gnd* gene sequences with PAUP showed that isolates sharing similar base alleles clustered together. Overall, the tree topology classified 77.1% (303/393) of the isolates into 21 major serogroups that we identified via different colors (Figure 3). PAUP generated an important parsimonious clade at the beginning of the phylogram. One side of the clade was

composed of *gnd* gene sequences from 25 isolates of pig and deer origin. These 25 taxa were genetically distant to the other side of the clade and was well supported by maximum parsimony bootstrap value (>75%).The other side of the clade accounted for 97.4% (37/38) of the O-serogroups. They were composed of *gnd* sequences from the remaining 371 isolates and were more diverse and had significant branching levels generating multiple clades and sub-clades.

MLST

Of the 20 STEC isolates analyzed, 14 different ST's were identified: ST16 (n=1), ST20 (n=3), ST101 (n=1), ST212 (n=1), ST295 (n=1), ST297 (n=2), ST300 (n=2), ST328 (n=1), ST738 (n=2), ST765 (n=1), ST793 (n=1), ST1632 (n=1), ST1788 (n=1) and ST8355 (n=1) (Table 3b). One of the isolate (PG007B) had a unique ST (*adk* 10, *fumC* 693,*gyrB* 4, *icd* 1, *mdh* 8, *purA* 8, *recA* 2) that could not be assigned using either MLST 2.0 or EnteroBase v.1.1.2. Sequence types in cattle isolates were more diverse (n=8) when compared to pigs (n=4) and deer (n=5). In terms of distribution, ST20 and ST297 were observed in cattle and pigs. None of the ST's recovered in deer were observed in domestic species.

Discussion

Nearly a quarter of African countries have reported isolation of STEC O157:H7 either from humans, animals, food or the environment (Lupindu, 2018). There are few reports, though, describing the isolation of STEC other than STEC O157:H7 in the South-western Indian Ocean region (Bumunang *et al*., 2019; Randremanana *et al*., 2012; Thierry *et al*., 2018). In this present study, STEC was detected and isolated from all three ASF's, namely cattle, deer and pigs. The prevalence of STEC ranged from 34% (17/50) to 42% (63/150), 9.8% (6/61) to 44.3% (27/61),

10% (5/50) to 16% (8/50) for cattle, rusa deer and pigs, respectively (Table 1). Cattle are well-known reservoirs of STEC and a wide range of STEC prevalence has been reported worldwide in ruminants, particularly in beef cattle (Hussein, 2005a; Hussein, 2007). For beef in Mauritius, prevalence rates (32%; Thierry *et al*., 2018) and presently 42% (63/150) were consistent with percentage reports published by Llorente *et al*., 2014 (36.1%) and Magwedere *et al*., 2013 (35.3%) but were ~~inferior~~ lower to that reported in retail markets of Argentina (52.2%; Brusa *et al*., 2012), country with the highest incidence of HUS-confirmed cases (Rivas *et al*., 2003). STEC were also bacteriological detectable in the contents of the intestine post-slaughter with an isolation rate of 34%, a result indicating that ~~high carriage animal at the abattoir increases the risk of meat contamination during the slaughtering process~~ this section is importantly involved in the colonization of STEC.

This is the first study to report the prevalence of STEC in rusa deer and pigs in Mauritius. Epidemiological studies involving STEC in deer is relatively new and so is the increasing number of reports on STEC in game meat. The high occurrence of STEC in deer faeces (44.3%; 27/61) was also reported by Kistler and Mauro (56%) (2011). Similar high rates of carriage were also identified in Germany (42%) and Spain (23.9%), respectively (Eggert *et al*., 2013; Sánchez *et al*., 2009). Such broad range of STEC isolation from deer is most probably associated with ecological interactions since deer studied herein is neither known nor observed to share pasture with domestic animals. The isolation rate recorded in venison was within range (5.9-22%) previously reported across Asia (Asakura *et al*., 1998; Fukuyama *et al*., 1999), USA (Rounds *et al*., 2012) and Europe (Díaz-Sánchez *et al*., 2012; Piérard *et al*., 1997; Thoms, 1999). Besides ruminants, non-ruminant species such as pigs are known to shed STEC at a similar rate as cattle (Borie *et al*., 1997; Johnsen *et al*., 2001; Nakazawa and Akiba, 1999) and relatively high

prevalence of STEC (65.3-68.3%) has been recently been reported in finishing pigs in the USA (Tseng *et al*., 2015; Cha *et al*., 2018). In pigs, studies conducted on healthy swine across South Africa (Ateba and Mbewe, 2011), Peru (Rivera *et al*., 2012), China (Meng *et al*., 2014) and USA (Tseng *et al*., 2015; Cha *et al*., 2018), showed high variation in isolation rates of STEC (0-68.3%). Comparing our results with those published elsewhere, the prevalence in pigs is relatively low (16%) and this result may be related to some farm management practices, although this has not been investigated herein. The occurrence of STEC in pork (10%) was higher compared to data reported from Czech Republic (4.6%; Skočková *et al*., 2017) but significantly lower to those from Hubei Province of China (41.3%; Khan *et al*., 2018), the country with the highest pork consumption.

Also, similar to our prior study, cattle showed high diversity among serovars (Table 2). We identified 32 distinct serovars and a comparative analysis with the 2018 survey revealed that eighteen serovars (O2, O15, O21, O26, O38, O51, O84, O91, O104, O117, O139, O142, O145, O146, O153, O154, O157 and O174) have already been reported in cattle (Thierry *et al*., 2018). The bacteriological detection of thirteen additional serovars (O1, O5, O6, O7, O8, O46, O55, O76, O103, O113, O156, O177 and O179) indicates that STEC is more diverse within cattle. As shown in Table 2, clinically significant serovars O26, O145 and O103 were only detected among cattle isolates. Among the total 38 distinct serovars identified herein, only *E. coli* O157 was found in cattle, deer and pigs. *E. coli* O157 is the most extensively investigated serovar worldwide due to the important relationship towards public health and is known to be a geographically disseminated clone (Kim, Nietfeldt and Benson, 1999). In addition to EHEC-7, isolates belonging to serovars O91, O103, O111, O113, O121, O128, and O145 as well as O104

are of significant public health concern (Bielaszewska *et al*., 2011). In this study, 73.7% (28/38) of the serovars found were previously associated with HUS or bloody diarrhea (Johnson *et al*., 2006; Tozzoli and Scheutz, 2014). Non-typeable strains are frequently found in cattle (Oliviera *et al*., 2008). In this study, non-typeable strains (2.4%; 11/462) were mostly isolated from cattle, with one strain isolated in deer. Two serovars, O91 and O76 were most prevalent among cattle. However, this observation is contrary to our previous study on cattle, whereby serovar O100 have been found as recurrent (Thierry *et al*., 2018). The varying prevalence of STEC and serovars observed from both surveys emphasizes the importance of adopting a longitudinal sampling approach.

In this study, deer harboured 50% less serovar compared to cattle (Table 2). In contrast to a large majority of studies focusing on STEC O157:H7 in deer (see Jay-Russell, 2013, for review), this study focused on both STEC sub-populations. Comparing our data with studies on deer elsewhere, only four serovars (O110, O128ab, O146 and ONT) were previously reported in food products in countries such as Germany (Miko *et al*., 2009; Martin and Beutin, 2011). Similarly, those serovars were also commonly associated with STEC infections in Germany during the surveillance period 1998-2006 (Miko *et al*., 2009). Other STEC serovars O146 and O128 have been described as usual colonizers of large game animals (Sánchez *et al.*, 2009; Martin & Beutin, 2011; Mora *et al.*, 2012). In the present study, 14 and 37 isolates belonged to these serovars, respectively. Martin and Beutin (2011) also described the presence of ONT in deer and indicatives that such strains are geographical disseminated amongst wildlife animals and in game meat.

Three serovars, O117, O119 and O128ab were most prevalent and accounted for more than 71% of the ten serovars found in deer. The prevalent serovars, O117 and O119, however, appears to be poorly documented in deer. We identified a single report describing O117 as a cause of diarrhea in deer fawns (Kramer *et al*., 1971). As shown in Table 3, O156 and O163 were also detected among deer. To the best of our knowledge, there are no reports in the literature on the occurrence of O119, O156 and O163 in deer suggesting that the diversity in cervid such as deer is more diverse than previously reported. Excluding O157, *E. coli* O100 was the only serovar that was shared by deer and pigs while four serovars (O117, O146, O156 and ONT) were shared by deer and cattle. Present data suggests some degree of spill-over between reservoir livestock-wildlife animals. For instance, wild boar has been identified in Europe as STEC-carriers and could play a role in the dissemination of STEC (Miko *et al*., 2009; Sánchez *et al*., 2009).

The highest typeability was seen in STEC isolates of pig origin (100%; 5/5). Typically, only two serovars (O100 and O157) were most frequent in pigs and represented 68.4% of the dataset (Table 2). The occurrence of *E. coli* O157 in pig is not uncommon but raises public health ~~issues~~ concerns~~, given clinical history~~. Serogroup O100 has been frequently identified from domestic pigs in other studies (Martin and Beutin, 2011; Cha *et al*., 2018) and was also recovered in deer herein. Serovar O100 was also reported as a cause of bloody diarrhea (Bockemuhl, Aleksic and Karch, 1992). The other serovars, O15 and O174 were shared by cattle and pigs, suggesting that this serovar can colonize different animal reservoirs. In addition, O174, the fourth serovar in pigs has been isolated from sheep (Blanco *et al*., 2004) and has been associated with non-bloody diarrhea, bloody diarrhea and HUS (Ethelberg *et al*., 2004; Eklund, 2005; Rivas *et al*., 2006).

Together, these results demonstrate multiple potential sources for zoonotic transmission to humans. Serovar O130 was detected in one pig. Unlike cattle, this result was unexpected given that pigs are transient colonizers of *E. coli* O130 (Gannon *et al*., 1988; Chapman *et al*., 2006). *E. coli* O130 has previously been isolated from cattle (Thierry *et al*., 2018) and has been linked to HUS (Elliott *et al*., 2001). Since isolates from both hosts were likely to be of the same clone as they shared similar sero-pathotpyes (O130, *stx1/stx2/*~~EHEC *hlyA*~~ *hlyA*), our hypothesis of *in-situ* transmission between species in similar climatic zones seems to be supported.

STEC are etiologic agents and the clinical symptomology and pathology is closely associated to virulence genes (Grauke *et al.*, 2002; Law, 2000). In particular, STEC strains carrying ~~*eaeA*~~ *eae* and *stx2* genes are well established and commonly recovered from patients with diarrhea or HUS (Boerlin *et al*., 1999; Ethelberg *et al*., 2004; Caprioli *et al*., 2005). The intimin gene (*eae*) was highly abundant (84.6%) as represented in Table 3, proof that the locus of enterocyte effacement (LEE) is widely distributed. In cattle, strains carrying *eae* were mostly found in the intestines, which is in agreement with Etcheverría and Padola (2013). Cattle are known as ~~perfect~~ asymptomatic reservoirs of STEC since the intestinal epithelial cells are devoid of *stx* receptors, elements essential for systemic disease.

Although the majority of the isolates were *eae*+, those harboring *stx1* and/or *stx2* prevailed (15.4%, 71/462), and more than 4.5% in cattle carried either *eae* or ~~EHEC *hlyA*~~ *hlyA* as additional virulence gene (Table 3). It is interesting to note that while all the four virulence genes were detected, none of the STEC isolates possessed all four virulence genes altogether. The fact

that cattle had the most diverse virulence profiles (80% of all combinations, Table 3), with 60% *stx*'s-containing genes suggests that *stx*-encoding phages are widely spread in the cattle environment. Overall, 38.5% (178/462) of all STEC strains examined had multiple virulence genes. The most dangerous virulence profiles associated with HUS were detected in multiple isolates: *stx2*/~~*eaeA*~~ *eae* serovar ONT, *stx1*/*stx2*/~~*eaeA*~~ *eae* serovars O91 and 146, *stx2*/~~*eaeA*~~ *eae* /~~EHEC~~ ~~*hlyA*~~ *hlyA* serovar O157 and *stx1*/*stx2*/ ~~EHEC~~ ~~*hlyA*~~ *hlyA* serovar O130. The genotypic profiles *stx2* and ~~*eaeA*~~ *eae* /~~EHEC~~ ~~*hlyA*~~ *hlyA* were the most frequent in STEC from deer which is in agreement with a previous study characterizing *stx* in deer (Miko *et al*., 2009). The synergy between ~~*eaeA*~~ *eae* and ~~EHEC~~ ~~*hlyA*~~ *hlyA* genes were previously reported in deer (Boerlin *et al*., 1999; Adwan *et al*., 2002).The present data reinforces the role of wild species, especially deer, as reservoirs of potentially pathogenic STEC strains. Like deer, *stx2* was the most frequently reported from pigs. The relative occurrence of *stx2* is not uncommon as associations between *stx2* subtype *stx2e* with STEC are documented in pigs (Fratamico *et al*., 2004; Zweifel *et al*., 2006). Unlike cattle, pigs can be STEC-sensitive, which can results in edema disease in post-weaning and young finishing pigs.

STEC isolates were genetically diverse as supported by phylogeny of the *gnd* gene and typing analysis of some strains via MLST. Phylogeny revealed that serovar O100 was excluded from the main cluster as shown in Figure 3. Over 33% additional polymorphic sites were detected in this cluster and BLAST analysis had a sequence identity 99.07-100% to strain NCTC9100 (serovar O100) GenBank Accession No (LR134239.1) (Table S3). In 1994, Nelson and Selander described that several *gnd* sequences were imported from *Citrobacter* or *Klebsiella* to *E. coli*, which seems to explain this atypical *gnd* allelic profile observed herein. To assess temporal and

spatially separated relationship amongst *E. coli* strains, some isolates were further assayed via MLST. Recently, 95 different ST's were reported with FDA STEC regulated foods (Gonzalez-Escalona and Kase, 2018).

Interestingly, 6 out of the 14 ST's detected were previously associated with human pathogenicity: ST16, ST20, ST101, ST295, ST297 and ST738~~, after data comparison~~. The non-identification of one ST (in the case of PG007B, serovar O100) demonstrates that new allelic types are yet to be identified and typed. Some ST's were identified in two or more strains, of which two (ST20 and ST297) were shared in cattle and pigs isolates (Table 3b).Conversely, none of the ST's in deer was shared between domestic animals. ST300 was recently identified in cattle and camel samples (Geue *et al*., 2017; Baschera *et al*., 2019) while ST765 was lately recovered in cattle (Barth *et al*., 2016). The remaining of the ST's: ST212, ST328, ST793, ST1632, ST1788 and ST8355 appears to be poorly documented in livestock and human clinical reports, requesting further investigations.

~~This study, using the sensitivity of CHROMagar STEC and patterns of STEC diversity revealed that STEC from bovine, deer and porcine food sources displayed heterogeneity both in terms of their virulence combinations and serovars. In summary, the prevalence of STEC in cattle, rusa deer and pigs were determined at 37.8%, most of which were non-EHEC7 serovars. However, 24 isolates were classified as clinically important serovars (O26, O103, O145 and O157), with *stx2* as the predominant *stx* gene and *eaeA* as most prevalent virulence factors. Also, significant differences were observed in prevalence and serovars by animal species, which suggests that~~

environmental factors might be involved. These results altogether highlights the possibility of these animals as a source to human infections.

Our investigation focused on the overall risk of STEC associated with AFS's in Mauritius. The sensitivity of CHROMagar STEC and patterns of STEC diversity in terms of abundance and richness were used to evaluate prevailing STEC pathovars. In this study, 126 samples were positive for STEC and 462 strains were isolated. The occurrence of STEC in beef (42%), venison (9.8%) and pork (10%) were consistent with a comprehensive range of reports published elsewhere (Brusa *et al*., 2012; Díaz-Sánchez *et al*., 2012; Khan *et al*., 2018; Llorente *et al*., 2014; Magwedere *et al*., 2013; Skočková *et al*., 2017). ASF's are primary vehicles of STEC and are thus widely associated with epidemic clinical outbreaks; however, in the case of beef, the STEC contamination level, confirmed via isolation (74.6%) is threefold and fivefold higher than that reported in venison and pork (Table 1). As a consequence, beef represented the most important high-risk food. Additionally, an increase (19%) in the microbial contamination level of STEC from 55.6% in carcasses (Thierry *et al*., 2018) to 74.6% in retail markets indicates that beef contamination rises either during transfer of carcasses and/or during the processing to prepare same for the retail markets.

STEC are serologically diverse and differ in terms of serogroup richness and serogroup frequency across animal species. In this study, nearly 85% of the serogroups were associated

with cattle while deer and pigs harboured 50% less serovars (Table 2). Of the three EHEC-7 serovars detected, O26 and O145 were exclusively isolated from cattle. Conversely, *E. coli* O157, the most extensively investigated serovar worldwide was isolated from all species. The considerable variations observed for *E. coli* O157 (domestic: n=17 vs. deer: n=1), leads us to speculate that such disparity maybe related to cross-species transmission amongst domestic animals. Together with our previous national survey, similar patterns in terms of non-EHEC-7 predominance were observed. Our data, assessing intraspecies diversity confirms cattle as a principal reservoir to both STEC subpopulations. Deer studied herein is neither known nor observed to share pasture with domestic animals, which suggests that the inter-specific overlap, as a surrogate for the transmissibility of STEC is driven by ecological interactions. It appears moreover (based on shared serogroups frequency) that deer have a tendency to act as maintenance hosts (deer: n=58 vs. domestic: n=37 isolates). Whether deer behave as maintenance hosts or spill-over hosts require further epidemiological surveys on larger sample sizes and from different *chassés*.

During the screening process, we recovered another EHEC-7 serovar (O103) in cattle and two serogroups (O117 and O119) which are poorly documented in deer. There is only one report that we are aware of that refers to O117 as a cause of diarrhea in deer fawns (Kramer *et al*., 1971). To the best of our knowledge, there are no reports in the literature on the occurrence of O119 in deer and this is most likely related to the large majority of studies focused on O157 (see Jay-Russell, 2013, for review). *E. coli* O130, which was isolated from a pig (n=1) has previously been isolated from cattle (Thierry *et al*., 2018). Unlike cattle, pigs are transient colonizers of *E. coli* O130 (Gannon *et al*., 1988; Chapman *et al*., 2006). Since isolates from both hosts were likely to

be of the same clone as they shared similar sero-pathotpyes (O130, *stx1/stx2/*EHEC-*hlyA*), this supports our hypothesis of *in-situ* transmission between species in similar climatic zones.

STEC is the primary cause of HUS (Majowicz *et al*., 2014). In this study, 73.7% (28/38) of the serovars found were previously associated with HUS or bloody diarrhea (Johnson *et al*., 2006; Tozzoli and Scheutz, 2014). STEC serogroups most frequently associated with cattle, deer and pigs (n=14) were reported in their food products elsewhere (Colello *et al*., 2016; Hussein, 2007; Hussein and Sakuma, 2005; Martin and Beutin, 2011; Miko *et al*., 2009). Altogether, 71.4% of those frequent serovars correlates to a panel of non-O157 STEC serovars described globally amongst patients with STEC infections (Johnson *et al*., 2006). Such source of information confirms ASF's to act as receptacle of STEC and carry serovars commonly linked to human illnesses. Our data suggests that additional EHEC-7 is likely to be present in cattle while pathogenic recovered in deer may be an important cause of gastrointestinal infections in deer fawns, and perhaps humans.

In the present study, the *gnd* gene was used as a proxy to determine the O-somatic richness of STEC pathovars via *E. coli* O Typer, a web-based tool. Of greater interest is the identification of 12 non-typeable (ONT) strains (Table 2) and serovar O100 that was excluded from the main cluster (Figure 3). In recent years, several studies have evaluated the appearance of new emerging STEC serogroups (Geue *et al*., 2017; Iguchi *et al*., 2016), evidence of the dynamic genome of pathotypes. Over 33% additional polymorphic sites were detected in the serovar O100. BLAST analysis showed that these *gnd* sequences had a sequence identity 99.07-

100% to strain NCTC9100 (serovar O100) GenBank Accession No (LR134239.1) (Table S3). The most likely explanation for the atypical *gnd* allelic profile in serovar O100 comes from Nelson and Selander (1994), which mention that several *gnd* sequences from were imported from *Citrobacter* or *Klebsiella*. Consequently, it can be postulated that these additional *gnd* variants (alleles) were probably a result of horizontal transfer or a result of the diversifying selection in the O-ACG cluster. Based on these evidences, *E. coli* O Typer needs to be regularly updated to keep its parallel applicability during screening and outbreak investigations.

STEC are etiologic agents and the clinical symptomology and pathology is closely associated to virulence genes (Grauke *et al*., 2002; Law, 2000). In this work, the toxigenic diversity, assessed independently of serovars, revealed that five virulence profiles were predominant and accounted for over 95% of isolates (Table 2). Consistent with our previous findings (Thierry *et al*., 2018), single genotypic profiles were most frequently recovered and none of the isolates possessed all four-known virulence genes. Whilst the predominance of certain profiles represents a cause for concern, newly discovered genetic profiles (*stx1*/*stx2*; *stx2*/*eaeA* and *stx1*/*stx2*/*eaeA*) suggest that additional profiles are yet to be identified. The differences in the virulence patterns between species in this study (Table 3) should be interpreted cautiously due to disparity in the sample size. Further studies are thus warranted to examine whether differences observed herein between species are real.

A large majority of STEC are able to colonize intestinal tract with a characteristic (A/E) cytopathology. This ability is encoded on the locus of enterocyte effacement (LEE) genomic

island, which is linked to superior fitness and encodes a panel of virulence genes including intimin (*eaeA*). The high abundance of *eaeA* (84.6%) reflects the dissemination of this genomic island and is therefore likely to be associated with large outbreaks (Cobbold and Desmarchelier, 2001). Similarly, a high abundance of the plasmid-encoded enterohemolysin observed here describes important plasmid acquisition/exchange across pathogenic *E. coli*, which is cause for concern since it is associated with diarrheal illnesses (Beutin *et al*., 1989). Whether the *stx*-negative strains that contained *eaeA* or EHEC *hlyA* putative virulence marker genes were EHEC-like (those losing *stx* genes) strains should be further investigated. With the exception of *eaeA*/*hlyA*, we found that STEC isolates with multiple virulence typically carries *stx2*. In general, *stx2* are 1000 times more cytotoxic than *stx1*. STEC strains from patients suffering severe disease such as HC or HUS are frequently *stx2* and *eaeA* positive and many also carry the *hlyA* gene (Caprioli *et al*., 2005; Friedrich *et al*., 2002). In more general terms, *stx2* multiple toxigenic profiles attest of the higher pathogenic potential to cause severe diseases in humans.

Several studies corroborate the absence of *stx1* in deer (Asakura *et al*., 1998; Díaz-Sanchez *et al*., 2012). The precise reason for *stx1* negative strains (Figure 2) is difficult to ascertain due to the relatively small sample size and the intrinsic factors that drives *stx*-encoding bacteriophages. Franklin and colleagues (2013) found that *stx2* was combined with *eaeA* and *hlyA* genes in wild cervid faeces. Here, we found that *stx2* occurred independently in deer isolates while *eaeA* and *hlyA* genes were in synergy (by equal proportion) as observed in various studies (Adwan *et al*., 2002; Boerlin *et al*., 1998). The lack of Gb3 receptors, essential for *stx* receptivity, however, explains the absence of *stx1* and *stx2* in cattle gastrointestinal tract (Priumboom-Brees *et al*., 2000). Previous studies confirmed that the specific colonization site for STEC (*stx*'s-positive)

occur at the recto-anal junction (Low *et al*., 2005; Naylor *et al*., 2003). The relative occurrence of *stx2* in pigs is of particular interest given that associations between *stx2* (subtype *stx2e*) are documented as a cause of HUS in humans and edema disease amongst pigs (da Silva *et al*., 2001; Gyles and Fairbrother, 2010; Muniesa *et al*., 2000; Thomas *et al*., 1994).

STEC are a leading cause of human infections, with an annual global incidence of 2,801,000 cases (Majowicz *et al*., 2014). In Mauritius, little is known about STEC infections. The "extrapolation" made by Majowicz *et al*. (2014) for members of the Mascarene Islands attests to the lack of data in the Indian Ocean region. Thus, these data are the first to assess such parameters in deer and pigs to enhance understanding of STEC epidemiology across cattle, deer and pigs in this region. Since our surveys were mainly cross-sectional studies and only involved a limited number of virulence genes, we are not able to make direct comparison of the prevalence of STEC and specific serogroups across time since such type of information are still unavailable here. Although these STEC serovars (O15, O76, O91, O100, O104, O117, O119, O128ab, O146, O154, O156 and O174) are not included in standard international regulations, surveillance is recommended at least in the region and neighboring islands.

Conclusion

This study investigated the occurrence of STEC in food-producing animals present on the island of Mauritius where tourism constitutes a significant portion of the Gross Domestic Product (GDP) (Ministry of Tourism, 2018). Mauritius and similar locales globally can ill afford to have

~~a high incidence of diarrheagenic disease. Since STEC were recovered in 37.8% of the *E. coli* isolates screened, ASF's present in Mauritius and most likely other South Western Indian Ocean (SWIO) islands present a sufficient risk to suggest that national and regional surveillance system needs to be revised and focused on Good Hygienic Practices (GHP), Good Manufacturing Practices (GMP), and training of personnel dealing with ASF, in particular small food vendors and suppliers. In view of the results obtained along with the intentions of the governments to boost the tourism industry in SWIO islands, we believe that urgent preventive measures, backed by political awareness will help strengthen the existing food safety standards and initiate further research to evaluate the clinical impact of STEC in tropical regions. Such initiative would also help other SWIO islands to develop and/or improve their public health surveillance systems, given the persistence of STEC globally.~~

The epidemiological data presented in this study indicates that STEC serovars, some of which are classified as clinically important are circulating in cattle, rusa deer and pigs present on the island of Mauritius. Although no STEC outbreak cases have been reported in Mauritius, further epidemiological surveys and risk factor analysis related to these animal source foods are thus required to elucidate the role of these animals as reservoirs of STEC and assess the importance as a public health threat. This is the first report documenting the virulence of STEC isolates from rusa deer and pigs on the island of Mauritius.

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Conflict of interest

The authors confirm that there is no conflict of interest in relation to the above study.

Author's contributions

Design of study: SILT, SJS, YJF, JEG; Coordination of study: SJS; Sample and laboratory investigations: SILT; Writing of initial manuscript: SILT; Reviewing of manuscript: SJS, YJF and JEG; Editing of manuscript: SILT. All co-authors reviewed and approved the final manuscript.

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IJFM Tables

Table 1: Summary of bacteriological results from 422 livestock (beef cattle, rusa deer and pigs) samples collected and analyzed.

	Bacteriological results for livestock samples			
Livestock's	**Samples**	**N_{+ve}/N_T [a]**	**$N_{STEC}/N_{screened}$ (%) [b]**	**99% Confidence interval**
Beef cattle	**Intestinal tract**	(17/50)	58/200 = 29.0%	21.1 - 38.0
	Raw meat	(63/150)	211/283 = 74.6%	67.3 - 81.0
Rusa deer	**Faeces**	(27/61)	122/298 = 40.9%	33.6 - 48.5
	Raw meat	(6/61)	33/135 = 24.4%	15.6 - 35.2
Pigs	**Faeces**	(8/50)	21/186 = 11.3%	6.1 - 18.6
	Raw meat	(5/50)	17/119 = 14.3%	7.2 - 24.4
	Total	(126/422)	462/1,221 = 37.8%	34.3 - 41.5

a- Number of STEC positive samples/total number of samples collected.

b- Number of confirmed STEC isolates after PCR/ total number of presumptive STEC isolates screened from EMB agar (along with 99% Confidence interval).

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Table 2: Serogroups and virulence profiles of 462 STEC isolates recovered from cattle, rusa deer and pigs of Mauritius

Serogroups	Virulence profiles (No. of strains with genotype)	Sources (isolates)						Total No. of strains
		Cattle		Rusa deer		Pigs		
		CM	CI	DM	DF	PM	PF	
O1	*eaeA* (3)	3						3
O2	*eaeA* (6)	6						6
O5	*eaeA* (2)	2						2
O6	*eaeA* (1)	1						1
O7	*eaeA* (9)	9						9
O8	*eaeA* (2)		2					2
O15**	*eaeA* (12)	6				3	3	12
O21	*eaeA* (3)	1	2					3
O26	*eaeA* (1), *eaeA/hly*A (1)*	1	1					2
O38	*eaeA* (8)	1	7					8
O46	*eaeA* (3)	1	2					3
O51	*eaeA* (4), *eaeA/hly*A (2)*	2	4					6
O55	*eaeA* (1)		1					1
O76	*eaeA* (29)	28	1					29
O84	*stx1* (4), *eaeA* (1)*	5						5
O91	*stx1* (1), *stx2* (1), *eaeA* (20), *stx1/stx2* (4), *stx1/stx2/eaeA* (8)*	30	4					34
O100**	*stx2* (15), *hly*A (8), *eaeA/hly*A (2)*			2	8	4	11	25
O103	*eaeA/hly*A (1)	1						1
O104	*eaeA* (8), *stx1* (3)*	7	4					11
O110	*eaeA* (2), *eaeA/hly*A (3)*			5				5
O113	*eaeA* (2)	2						2
O117**	*eaeA* (1), *eaeA/hly*A (24)*	1		23	1			25
O119	*eaeA/hly*A (50)			46	4			50
O128ab	*eaeA/hly*A (37)			24	13			37
O130	*stx1/stx2/hly*A (1)						1	1
O139	*eaeA* (3)	2	1					3
O142	*eaeA* (2)	1	1					2
O145	*eaeA* (2), *eaeA/hly*A (1)*	2	1					3
O146**	*stx1* (1), *stx2* (14), *eaeA* (4), *stx1/stx2/eaeA* (2)*	4	3	9	5			21
O153	*eaeA* (1)		1					1
O154	*eaeA* (10)	9	1					10
O156**	*eaeA* (2), *eaeA/hly*A (10)*	2	1	7	2			12
O157**	*stx2* (11), *eaeA* (3), *stx1/stx2* (1), *eaeA/hly*A (1), *stx2/eaeA/hly*A (2)*	6		1		6	5	18
O163	*eaeA/hly*A (1)			1				1
O174**	*eaeA* (16)		11			4	1	16
O177	*eaeA* (2), *eaeA/hly*A (6)*	8						8
O179	*eaeA* (4)	4						4
ONT[a]**	*eaeA* (9), *stx2/eaeA* (1), *eaeA/hly*A (2)*	8	3	1				12
DND[b]	*stx1* (5), *stx2* (1), *eaeA* (48), *eaeA/hly*A (5), *stx1/stx2* (1), *stx2/eaeA* (1), *stx1/stx2/eaeA* (7)*	58	7	3				68
Total (38)	<u>*Single:*</u> *stx1* (14), *stx2* (42) ,*eaeA* (220), *hly*A (8) <u>*Multiple*</u>*: stx1/stx2* (6), *stx2/eaeA* (2), *eaeA/hly*A (150), *stx1/stx2/eaeA* (17), *stx1/stx2/hly*A (1), *stx2/eaeA/hly*A (2)	211	58	122	33	17	21	462

ONT[a], O nontypeable

DND[b], serogroups that was not determined.

CM, cattle meat; CI, cattle intestinal; DM, deer meat; DF, deer faeces; PM, pig meat; PF, pig faeces.

*, multiple virulence combinations (with number of isolates) for a a specific serogroup.

**, serogroups shared amongst livestock.

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Table 3(a): Virulence gene patterns observed amongst the 462 STEC strains isolated from cattle, rusa deer and pigs of Mauritius

Virulence patterns no	Virulence patterns/Livestock			Genotypes	No. of isolates positive for genotypes (%)
	Cattle	Rusa deer	Pigs		
1	✓	-	-	***stx1***	14 (3.0)
2	✓	✓	✓	***stx2***	42 (9.1)
3	✓	✓	✓	***eaeA***	220 (47.6)
4	-	✓	-	**EHEC-*hly*A**	8 (1.7)
5	✓	-	-	***stx1/stx2***	6 (1.3)
6	✓	-	-	***stx2/eaeA***	2 (0.4)
7	✓	-	-	***stx1/ stx2/eaeA***	17 (3.7)
8	✓	✓	-	***eaeA*/ EHEC-*hly*A**	150 (32.5)
9	-	-	✓	***stx1/stx2*/ EHEC-*hly*A**	1 (0.2)
10	✓	-	-	***stx2/eaeA*/ EHEC-*hly*A**	2 (0.4)
Total (n= 10)	(n= 8)	(n= 4)	(n= 3)		462 (100)

Table 3(b): Animal source, reference ID, serovar and MLST results from 20 STEC strains isolated from cattle, rusa deer and pigs of Mauritius

Source	Reference ID	Serovar	MLST	Reference
Cattle	**DC021E**	O26	ST20	Thierry *et al*. (2018)
Cattle	**DC028B**	O15	ST8355	
Cattle	**DC047D**	OUT	ST328	
Cattle	**DC060D**	O130	ST297	
Cattle	**DC065E**	O26	ST212	
Cattle	**DC092A**	O165	ST1632	
Cattle	**DC100B**	O111	ST16	
Cattle	**DC127B**	O51	ST295	
Pigs	**PG007B**	O100	Unknown*	This study
Pigs	**PG012B**	O174	ST20	
Pigs	**PG021A**	O15	ST793	
Pigs	**PG051C**	O174	ST20	
Pigs	**PG066B**	O157	ST1788	
Pigs	**PG086A**	O130	ST297	
Rusa deer	**RD009B**	O146	ST738	This study
Rusa deer	**RD023B**	O100	ST101	
Rusa deer	**RD025B**	O119	ST300	
Rusa deer	**RD066A**	O128ab	ST765	
Rusa deer	**RD067B**	O117	ST300	
Rusa deer	**RD069A**	O146	ST738	

* Unknown MLST

Figure 1a_1b Geographical location of places

Figure 2 Distribution of virulence factors amongst samples

Figure 3 Phylogeny of STEC isolates

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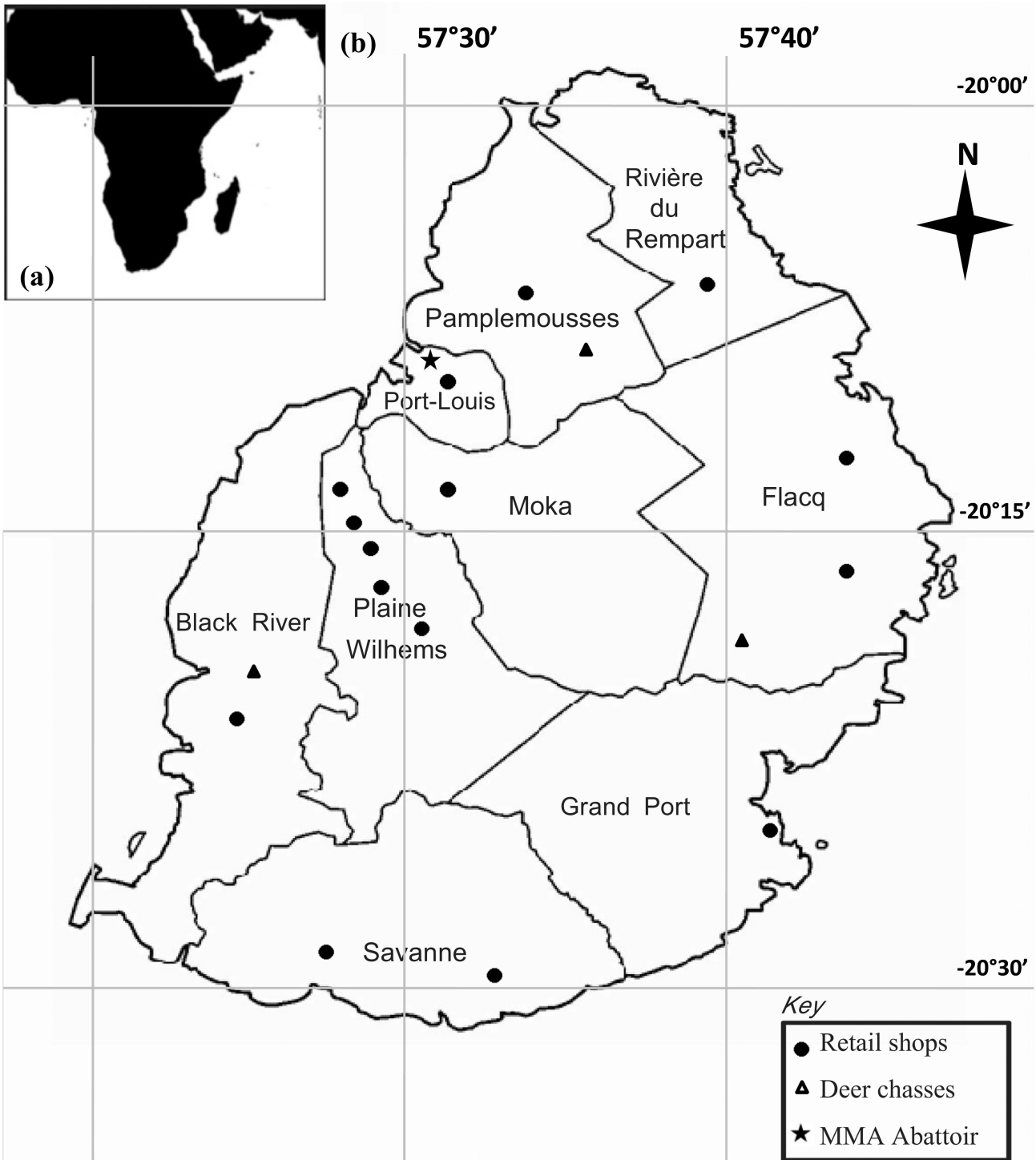
(a)
(b)
57°30’
57°40’
-20°00’
-20°15’
-20°30’
N
Rivière
du
Rempart
Pamplemousses
Port-Louis
Moka
Flacq
Black River
Plaine
Wilhems
Grand Port
Savanne
Key
Retail shops
Deer chasses
MMA Abattoir

Figure 1

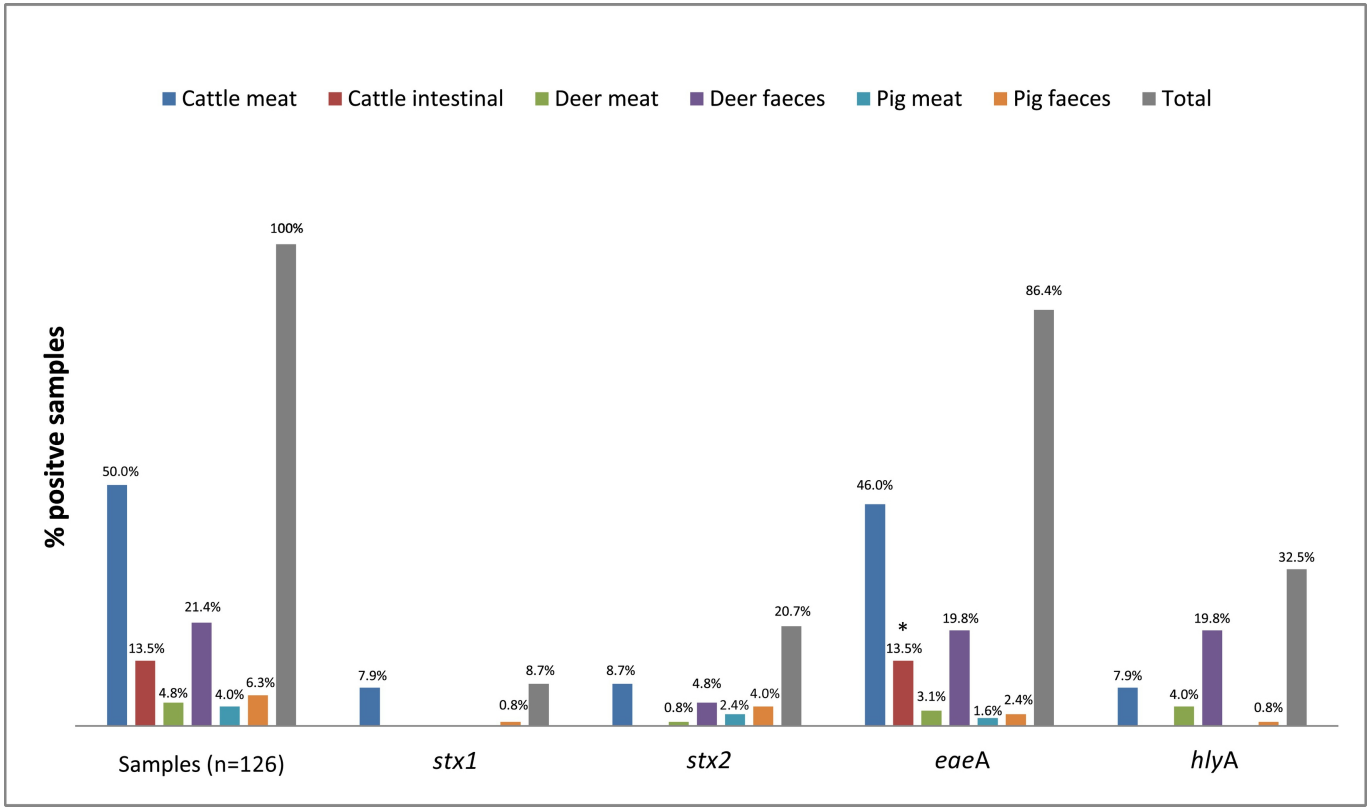
Cattle meat
Cattle intestinal
Deer meat
Deer faeces
Pig meat
Pig faeces
Total
% positve samples
50.0%
13.5%
4.8%
21.4%
4.0%
6.3%
100%
7.9%
0.8%
8.7%
8.7%
0.8%
4.8%
2.4%
4.0%
20.7%
46.0%
*
13.5%
3.1%
19.8%
1.6%
2.4%
86.4%
7.9%
4.0%
19.8%
0.8%
32.5%
Samples (n=126)
stx1
stx2
eaeA
hlyA

Figure 2

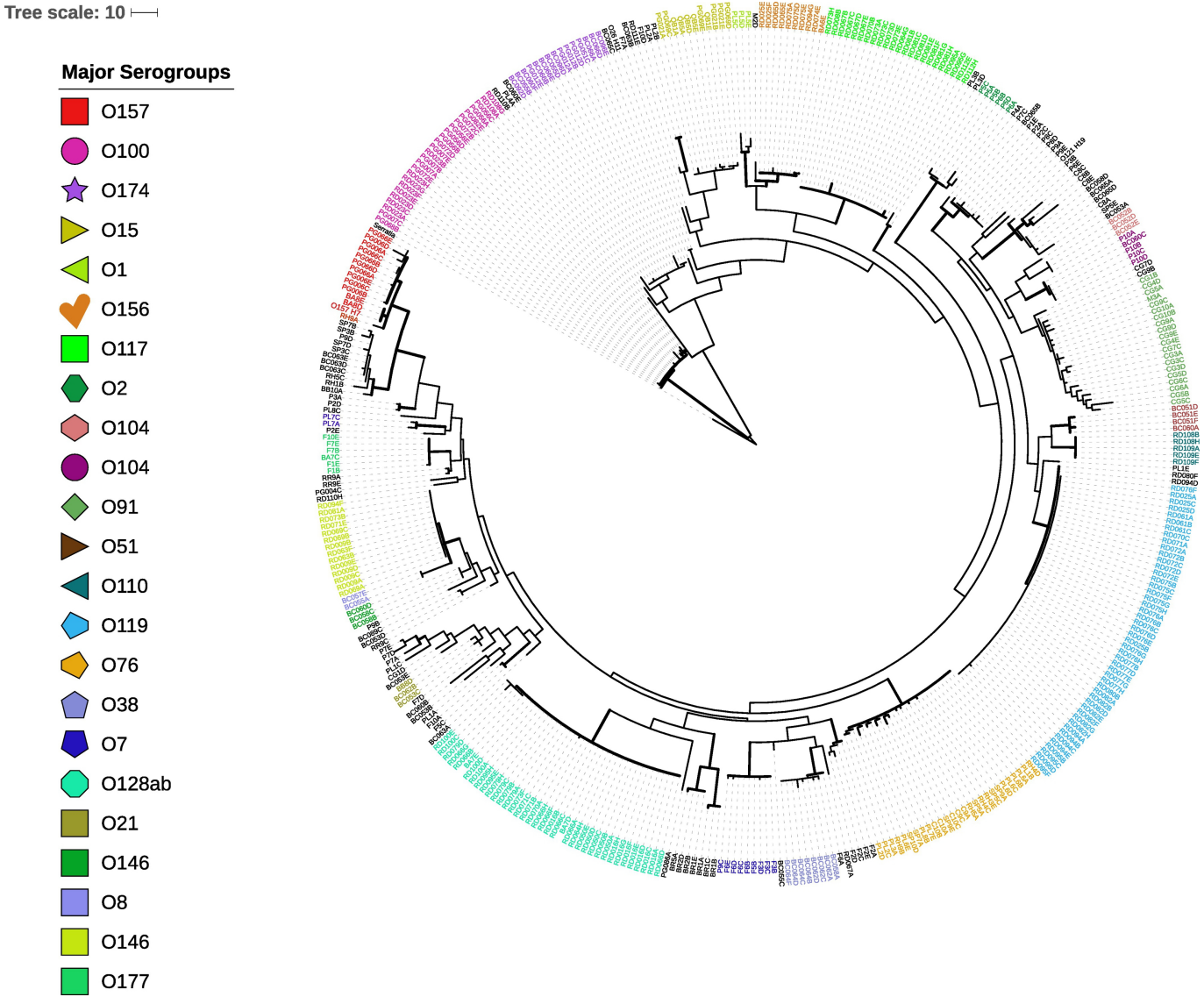
Tree scale: 10
Major Serogroups
O157
O100
O174
O15
O1
O156
O117
O2
O104
O104
O91
O51
O110
O119
O76
O38
O7
O128ab
O21
O146
O8
O146
O177

Figure 3