



## INTRODUCTION

The zoonotic pathogen STEC O157:H7 emerged during the 1980s as a causative agent of foodborne outbreaks associated with haemorrhagic colitis and haemolytic uremic syndrome which can be fatal. STEC O157:H7 is endemic in cattle populations worldwide, and food contamination can occur domestically and non-domestically posing food safety challenges.

We investigated an emerging lineage causing outbreaks of STEC O157:H7 associated with raw produce, identified and quantified the domestic and non-domestic reservoirs and quantified case exposures across the population.

The aim of this study was two fold; 1) To describe the population structure of lineage IIc and estimate the contribution of a domestic reservoir to clinical disease 2) To ascertain whether cases in lineage IIc are more likely to have a fresh produce exposure than cases from other lineages of STEC O157:H7.

## METHODS

All human STEC O157:H7 lineage IIc (n=925) isolates from June 2015 to December 2020 in England were whole genome sequenced and the population structure described. Ancestral state reconstruction using case travel information defined domestic and non-domestic clades and transmission dynamics. Exposures for cases infected with isolates from domestic clades were assessed using multinomial univariate and multivariable regression.

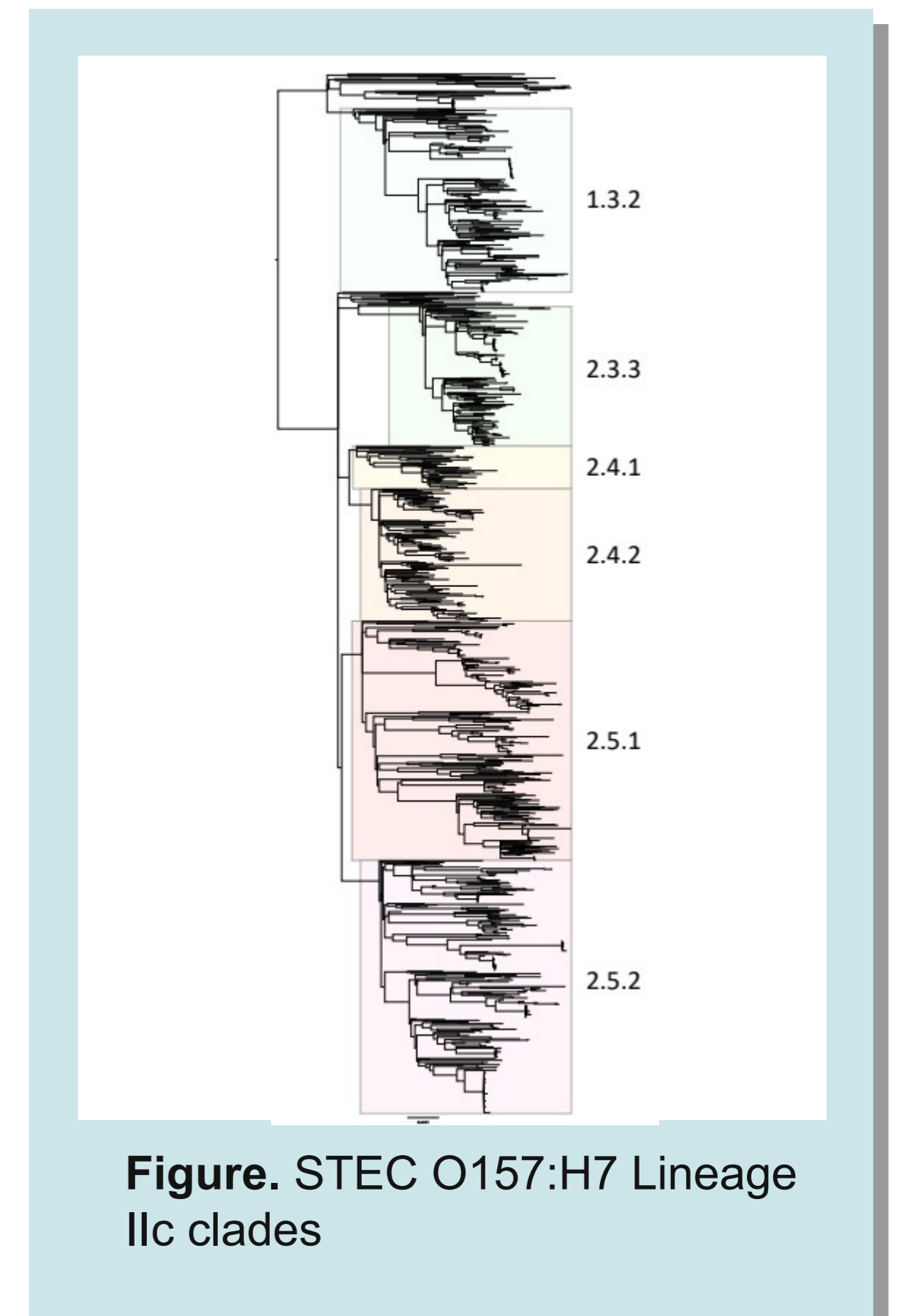


Figure. STEC O157:H7 Lineage IIc clades

## RESULTS

Lineage IIc emerged 50 years ago, and subsequent clonal expansions have resolved into 6 major extant clades, Figure 1.

We defined two UK domestic clades that emerged in the last 30 years and 4 non-domestic clades comprising isolates infecting/transmitted to English cases either via international travel or consumption of imported food.

Transmission rates between domestic and non-domestic strains is shown in Figure 2.

- Throughout the study period non-domestic clades contributed approximately twice the number of infection as those from domestic clades
- Cases infected with domestic IIc clade strains reported more frequent exposure to fresh produce (raw vegetables, p-value 0.015 and pre-packaged salad, p-value 0.017), animal contacts (dogs p-value 0.082, cattle p-value 0.0056) and farm visits (p-value 0.0091) than other STEC O157:H7 cases
- A multivariable multinomial model identified major risk factors for infection within the domestic clades to be pre-packaged salad (Clade 2.3.3, relative risk ratio, RRR 1.44, p-value 0.10) and visiting farms (Clade - 2.5.2, RRR 2.10, p-value 0.002), Table.

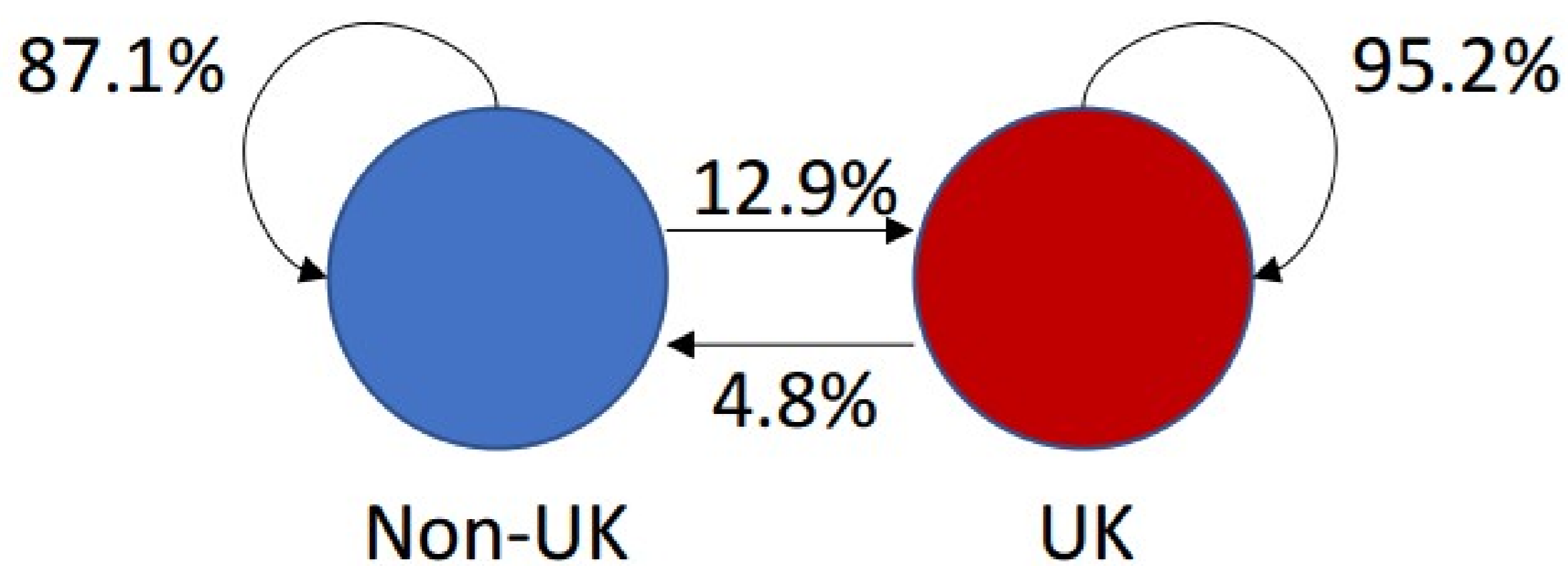


Figure 2. Transmission rate between domestic and non-domestic states as inferred from the ancestral state reconstruction.

| Variable  | clade 2.3.3, Relative risk ratio (95 % CI), Wald p-value | clade 2.5.2, Relative risk ratio (95 % CI), Wald p-value | Overall p-value of the model |
|---|--|--|------------------------------|
| Prepackaged salad                                 | 1.44 (0.93 – 2.24), 0.10                                 | 0.96 (0.61 – 1.49), 0.84                                 | 0.25                         |
| Contact to cattle                                 | 1.69 (0.79 – 3.62), 0.17                                 | 1.53 (0.76 – 3.08), 0.24                                 | 0.25                         |
| Visiting farm                                     | 1.44 (0.82 – 2.53), 0.20                                 | 2.10 (1.30 – 3.38), 0.002                                | 0.0089                       |
| <b>Adjustment variables</b>                       |  |  |                              |
| Traveling outside UK                              | 0.070 (0.022 – 0.22), <0.001                             | 0.18 (0.088 – 0.38), <0.001                              | <0.001                       |
| Index of multiple deprivation (divided by 10,000) | 1.19 (0.95 – 1.48), 0.14                                 | 0.90 (0.73 – 1.09), 0.28                                 | 0.16                         |
| Age, <5 years                                     | 0.61 (0.33 – 1.11), 0.10                                 | 0.56 (0.33 – 0.97), 0.037                                | 0.027                        |
| Year, 2016  | 1.17 (0.37 – 3.75), 0.79                                 | 0.71 (0.36 – 1.39), 0.32                                 |                              |
| 2017  | 2.98 (1.00 – 8.87), 0.049                                | 0.67 (0.33 – 1.36), 0.26                                 |                              |
| 2018  | 5.87 (2.06 – 16.76), 0.001                               | 0.55 (0.27 – 1.16), 0.12                                 |                              |
| 2019  | 2.53 (0.82 – 7.83), 0.11                                 | 0.86 (0.42 – 1.74), 0.67                                 |                              |
| 2020  | 3.07 (0.98 – 9.62), 0.054                                | 1.97 (1.02 – 3.80), 0.043                                | <0.001                       |

Table. Multivariable multinomial regression model of major risk factors for domestic clades of STEC O157:H7 Lineage IIc (clades 2.3.3 and 2.5.2)

## DISCUSSION

Lineage IIc has emerged as the most prevalent lineage of STEC O157:H7 in England with a sizeable domestic reservoir, and human infection is associated with consumption of contaminated fresh produce.

Patients infected with lineage IIc predominantly presented with severe clinical outcomes, including bloody diarrhoea in about 80 % of the cases, although progression to HUS was rare (<1 %). This clinical picture is explained by the almost ubiquitous presence (>95 %) of *stx1a* and the rare occurrence of *stx2a* in the population.

The collection of routine, detailed exposure data on case patients integrated with high resolution microbiological typing enables powerful reframing of our understanding of foodborne disease risk within a one health context.

## CONCLUSIONS

- We have robustly identified and quantified the role of a domestic reservoir in infections of STEC O157:H7 lineage IIc in England.
- Exploiting case exposure data, collected during routine enhanced surveillance, using a fit-for-purpose statistical model, we identified pre-packed salad and direct contact with cattle as significant exposure risks causing human infections.
- Whilst currently the acquisition of *stx2a* by lineage IIc strains is a rare event, the integration of Stx2a-encoding phage has occurred at various locations across the phylogeny, highlighting the potential for the lineage IIc to increase in pathogenicity.
- Collecting detailed exposure data on sporadic as well as outbreak cases and marrying that to high resolution microbiological typing afforded by WGS provides powerful analysis that can be used to reframe our understanding of foodborne disease risk within a one health context.

## ACKNOWLEDGEMENTS

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