

Atypical STEC strain from HUS: an hybrid ETEC-STEC

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Epidemiological investigation on an HUS case

Case A

HUS with neurological complications

Epidemiological link with a farm
(bovine)

Case B

HUS in a close area

Atypical STEC cases:

stx2-positive, *eae*-negative, *aggR/aaiC*-negative, *saa* & *tia*-negative, *subAB*-negative

Serogroup not identified (no top-13)

Cluster of cases?



Isolation of a STEC strain from a person of case A family (ED1169)



Whole genome sequencing of the isolated strain



Sequence analysis on ARIES



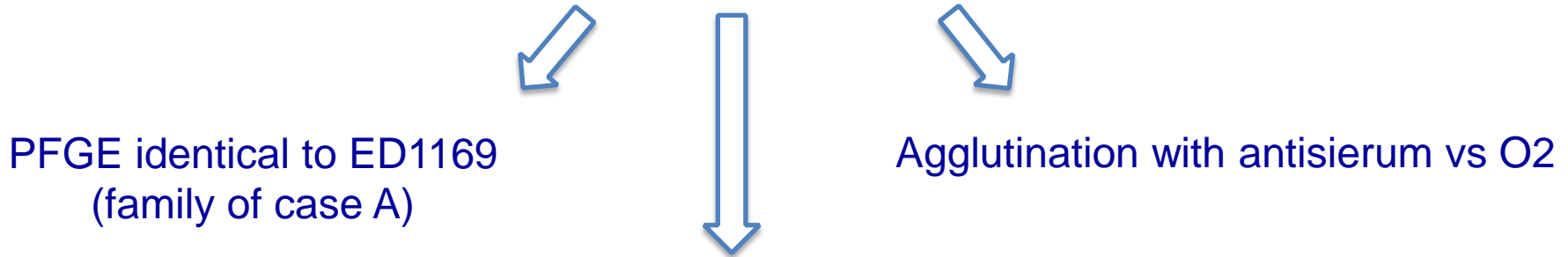
Galaxy / ARIES - ISS

Virulotyper *stx2a, sta1, astA, ehxA*

MLST (SRST2) ST10

Serotyping **O2:H27**

**Isolation of a STEC strain from faeces
of animals of the farm linked to case A
(ED1172)**



Whole genome sequencing and confirmation
of the same characteristics identified for
ED1169



**Development of an ELISA test to identify the presence of
anti-O2 antibodies**

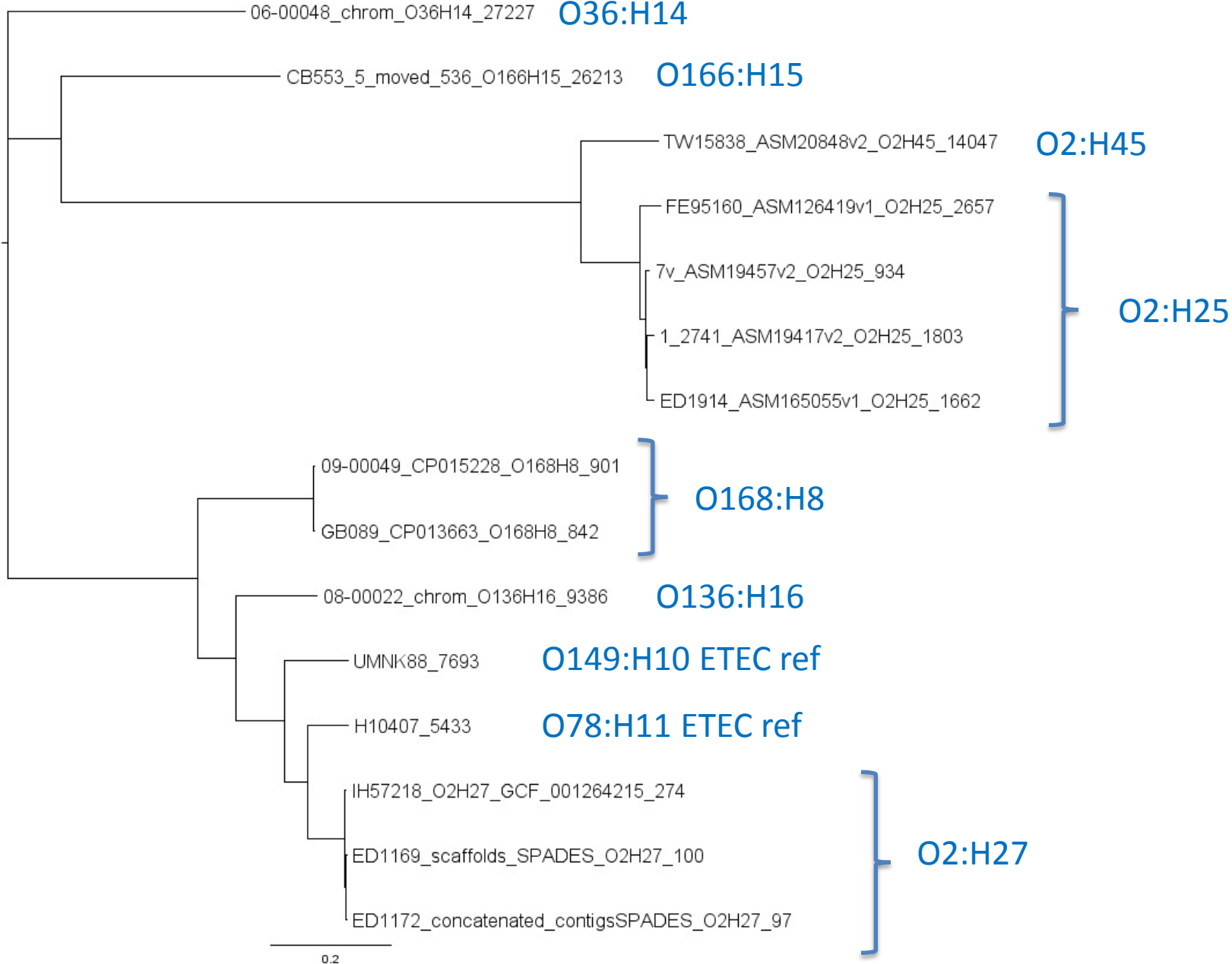
Case A positive

Case B negative

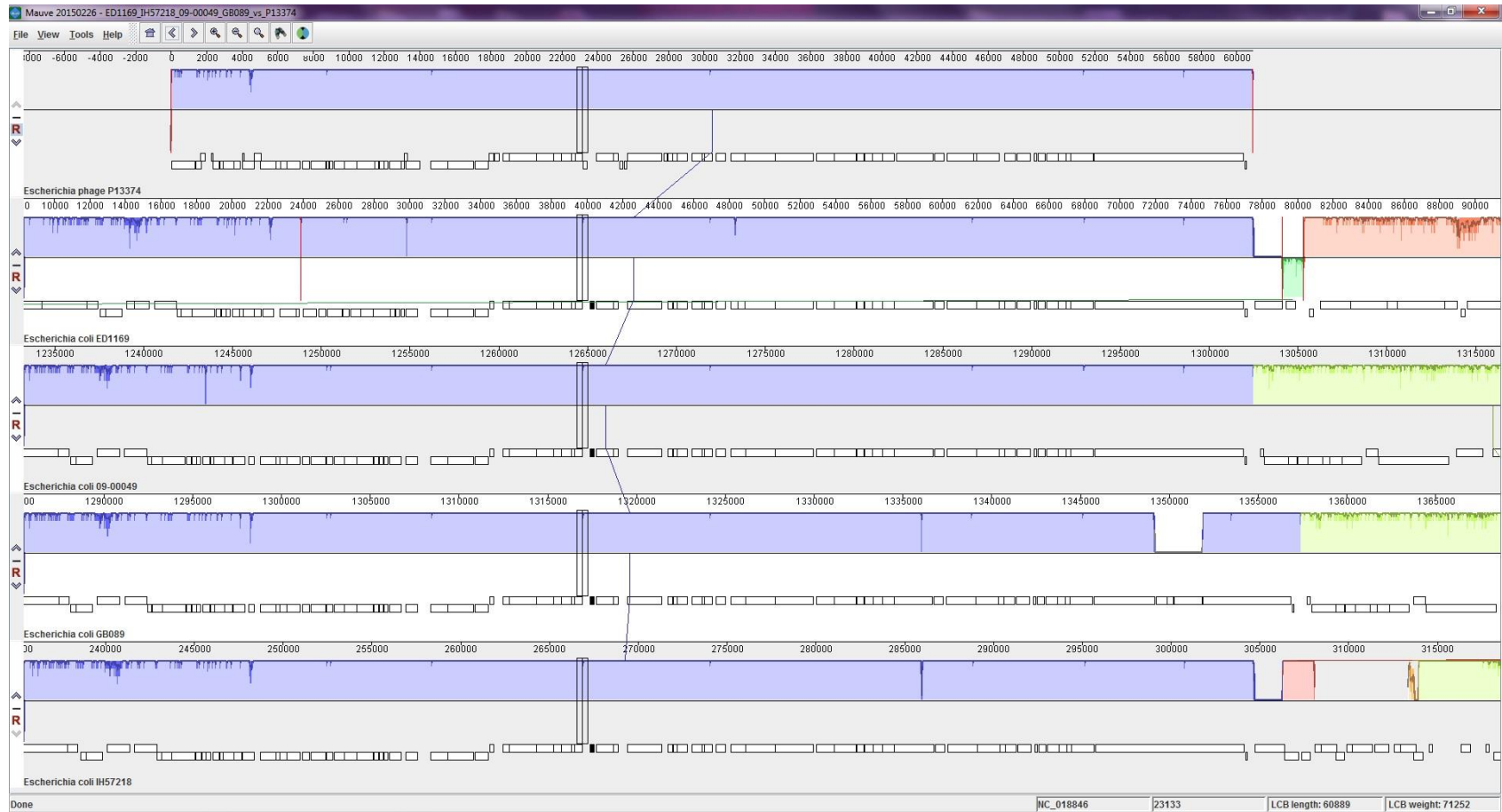
**Confirmation of the link
between case A and the
farm**

**Exclusion of a link
between case A and
case B**

WGS analysis of ETEC-STECS



stx2a bacteriophage



Integration site: *wrbA*

Identical to P13374 (identified in O104:H4 and in other STEC from HUS)

Different origin of replication from other ETEC-STEC

	Serogroup	H-type	Plasmid replicon
06-00048_plasmid_pCFSAN004178P_02	O36	H14	IncFIB(AP001918)
08-00022_plasmid_pCFSAN004179G	O136	H16	IncFIB(AP001918)
CB553_5	O166	H15	IncFIB(AP001918)
GB089plasmid_pCFSAN004181P	O168	H8	IncFIB(AP001918)
09-00049_plasmid_pCFSAN004180G	O168	H8	IncFIB(AP001918)
TW15838_ASM20848v2	O2	H45	IncFIB(AP001918)
plasmid_p7v	O2	H25	IncFIB(AP001918)
ED1914_ASM165055v1	O2	H25	IncFIB(AP001918)
1_2741_ASM19417v2	O2	H25	IncFIB(AP001918)
FE95160	O2	H25	IncFIB(AP001918)
IH57218	O2	H27	IncFII_1
● ED1172	O2	H27	IncI2
● ED1169	O2	H27	IncI2

Plasmidborne virulence genes in ED1169 and ED1172

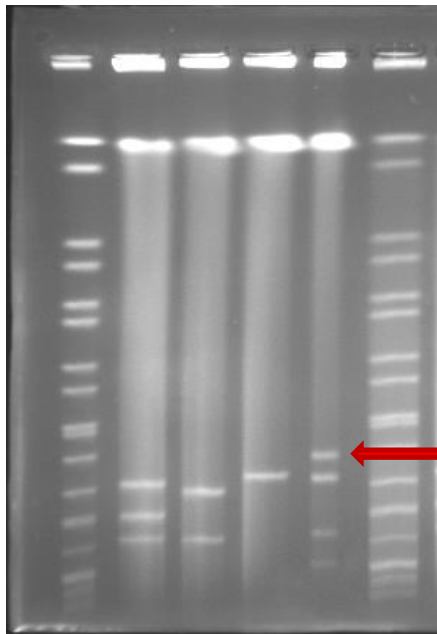
<i>sta1</i>	Heat-stable enterotoxin (STp) (96%)
<i>astA</i>	EAST1 enterotoxin
<i>ehxA</i>	Enterohaemolysin (subtype D – with differences)
<i>faeG K88</i>	Fimbrial protein – with differences

Conjugative plasmid

Plasmid analysis

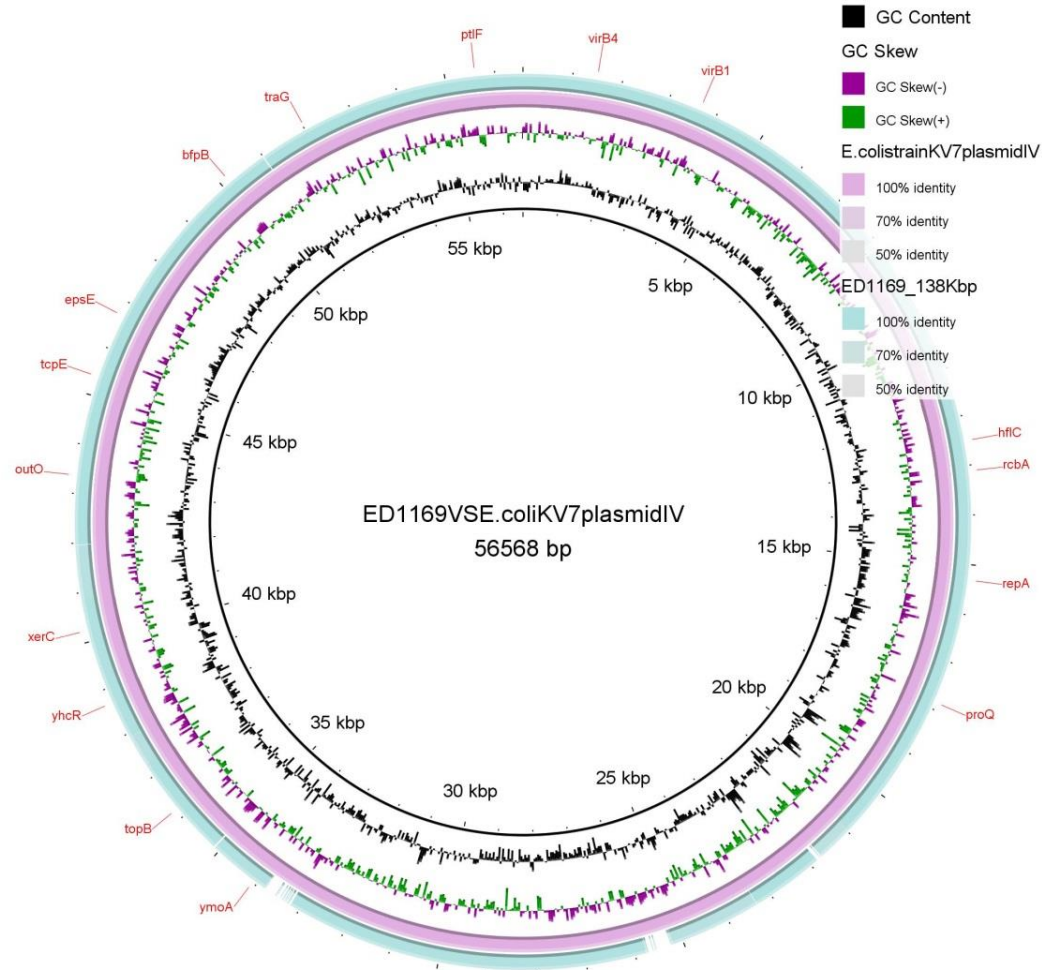
PFGE analysis of DNA plug mold after S1 nuclease treatment

ED1169



138.9 kb

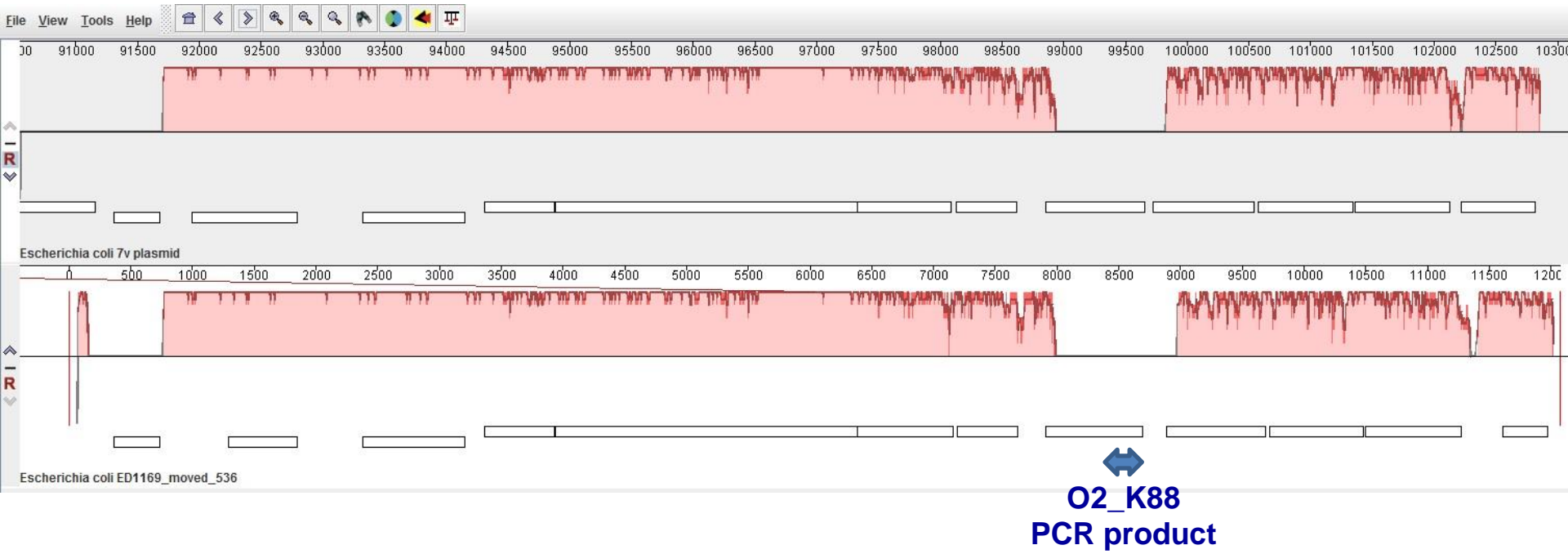
Agarase extraction



Partial sequence, covering the whole plasmid IV of *E. coli* KV7
Type IV secretion system

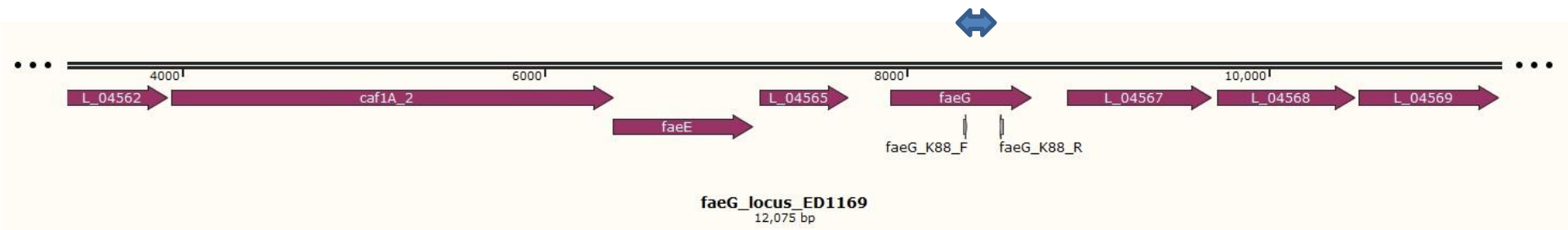
Differences in *faeG* gene

FaeG is the fimbrial component K88 responsible for the binding specificity on the host cells



Development of a primer pair targeting the O2-specific *faeG* allele

O2_K88
PCR product



Isolation of a STEC strain from a person of case B family

stx2-positive, *eae*-negative, *aggR/aaiC*-negative, *saa* & *tia*-negative,

subAB-negative

Negative for the O2-specific *faeG* allele

O174 serogroup

Two «atypical» STEC cases in the same geographical area. Epidemiological cluster excluded thanks to the use of whole genome sequencing coupled with classical methods

Conclusions

NGS can be used to tackle different aspects of food safety

- Investigation of cases of infections through NGS can connect the cases to sources
- Investigation of cases of infections through NGS can exclude correlations among cases
- NGS can be used to design rapid specific tests to type bacterial isolates (phenotypical and genotypical tests)

NGS represents an invaluable tool to enhance surveillance and control of food-borne diseases

Conclusions

Characterization of an atypical strain causing HUS:

- O2:H27
 - *stx2a*
 - STp - differences with the ref sequence
 - K88-like colonization factor
 - *ehxA* type D
- } Hybrid plasmid similar to p7v

The findings support the hypothesis (Lorenz 2016) that plasmids harbouring *ehxA* subtype D represent novel virulence plasmids evolved from a different evolutionary lineage than the plasmids carrying the other *ehxA* subtypes.

STEC strains harbouring plasmids from this family, although rare and belonging to rare serogroups, can be associated with severe disease

Acknowledgements



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Thank you for your attention!

