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 Case B HUS in a close area

Epidemiological link with a farm (bovine)

Atypical STEC cases:

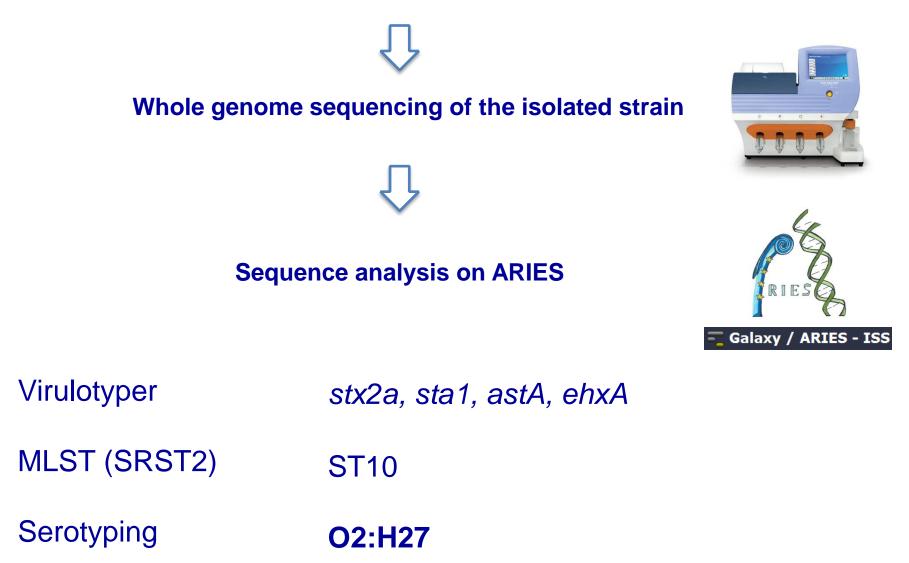
stx2-positive, *eae*-negative, *aggR/aaiC*-negative, *saa* & *tia*-negative, *subAB*-ne gative Serogroup not identified (no top-13)

Cluster of cases?













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

PFGE identical to ED1169 (family of case A) Agglutination with antisierum vs O2

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



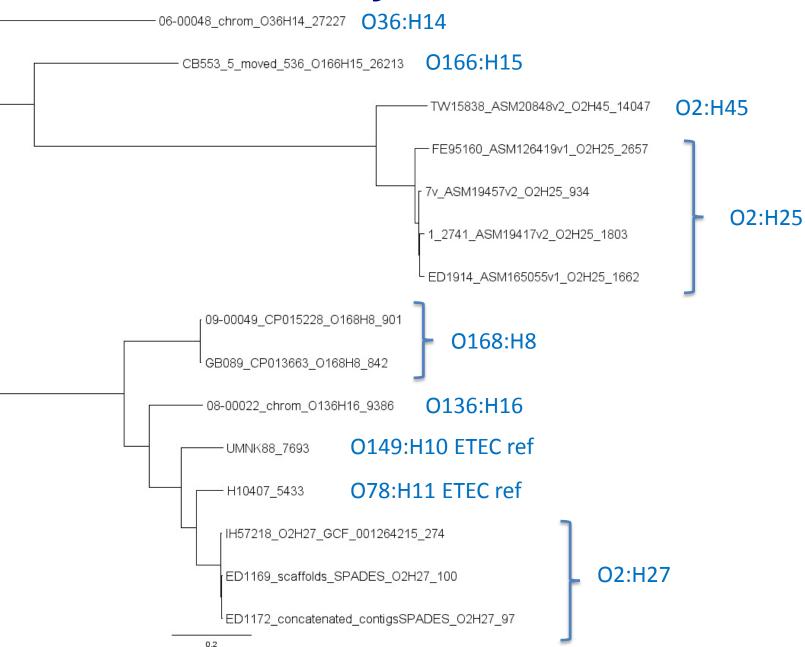
Development of an ELISA test to identify the presence of anti-O2 antibodies

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-STEC**



stx2a bacteriophage

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Integration site: wrbA

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





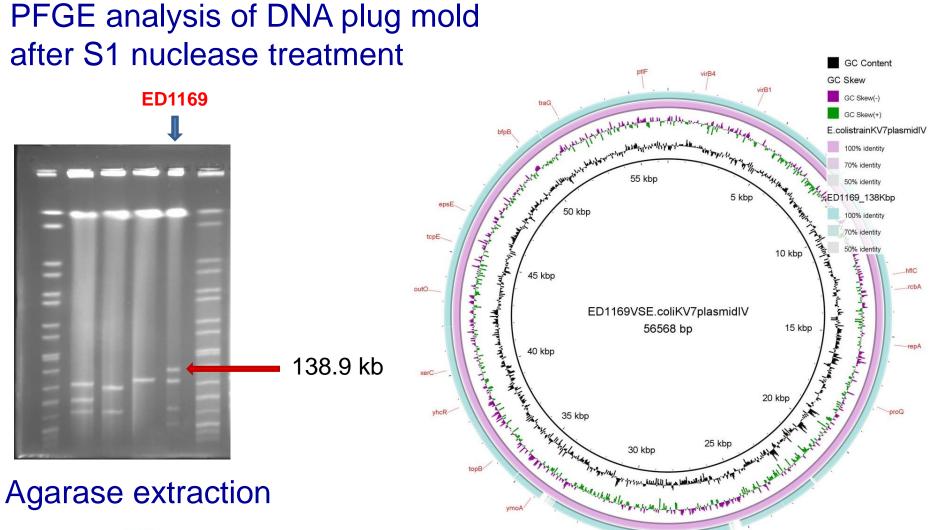
Different origin of replication from other ETEC-STEC

	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	02	H25	IncFIB(AP001918)
IH57218	02	H27	IncFII_1
ED1172	02	H27	Incl2
ED1169	O2	H27	Incl2

- *sta1* Heat-stable enterotoxin (STp) (96%)
- astA EAST1 enterotoxin
- *ehxA* Enterohaemolysin (subtype D with differences)
- **faeG K88** Fimbrial protein with differences

Conjugative plasmid

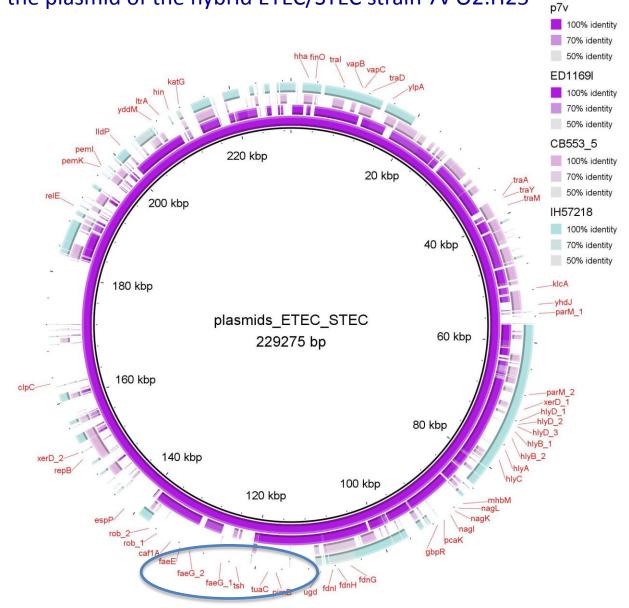
Plasmid analysis





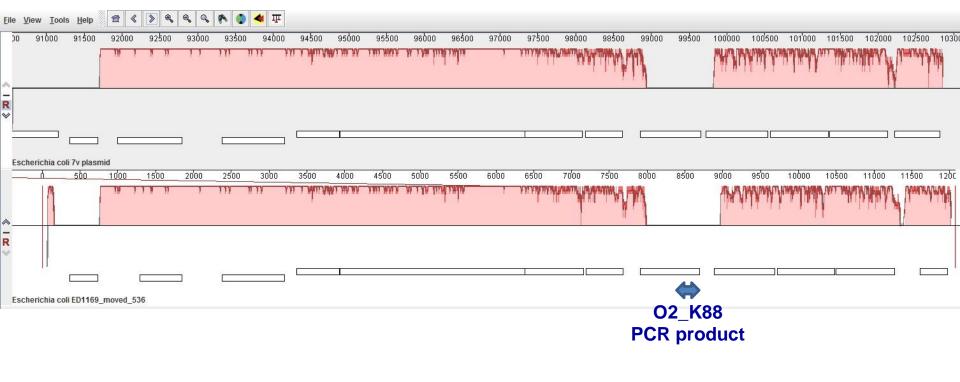
Partial sequence, covering the whole plasmid IV of *E. coli* KV7 Type IV secretion system

Comparison with the plasmid of the hybrid ETEC/STEC strain 7v O2:H25

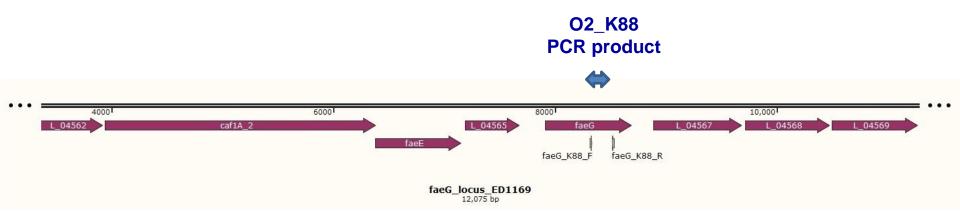


Different plasmid. Differences in the area coding the fimbriae involved in colonization

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



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





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Thank you for your attention!



