

Genomic analysis of *E. coli* strains isolated from calf diarrhoea in Denmark

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Calf diarrhoea

- Important cause of mortality in young calves
- Most severe in the first weeks of life
- Multiple factors associated with the disease
 - Pathogens (in Denmark)
 - *E. coli*, *Clostridium perfringens*, *Salmonella*
 - BoCoV, *Rotavirus*,
 - *Cryptosporidium parvum* and *Eimeria* spp
 - Management
 - Insufficient passive transfer of immunity
 - Hygiene

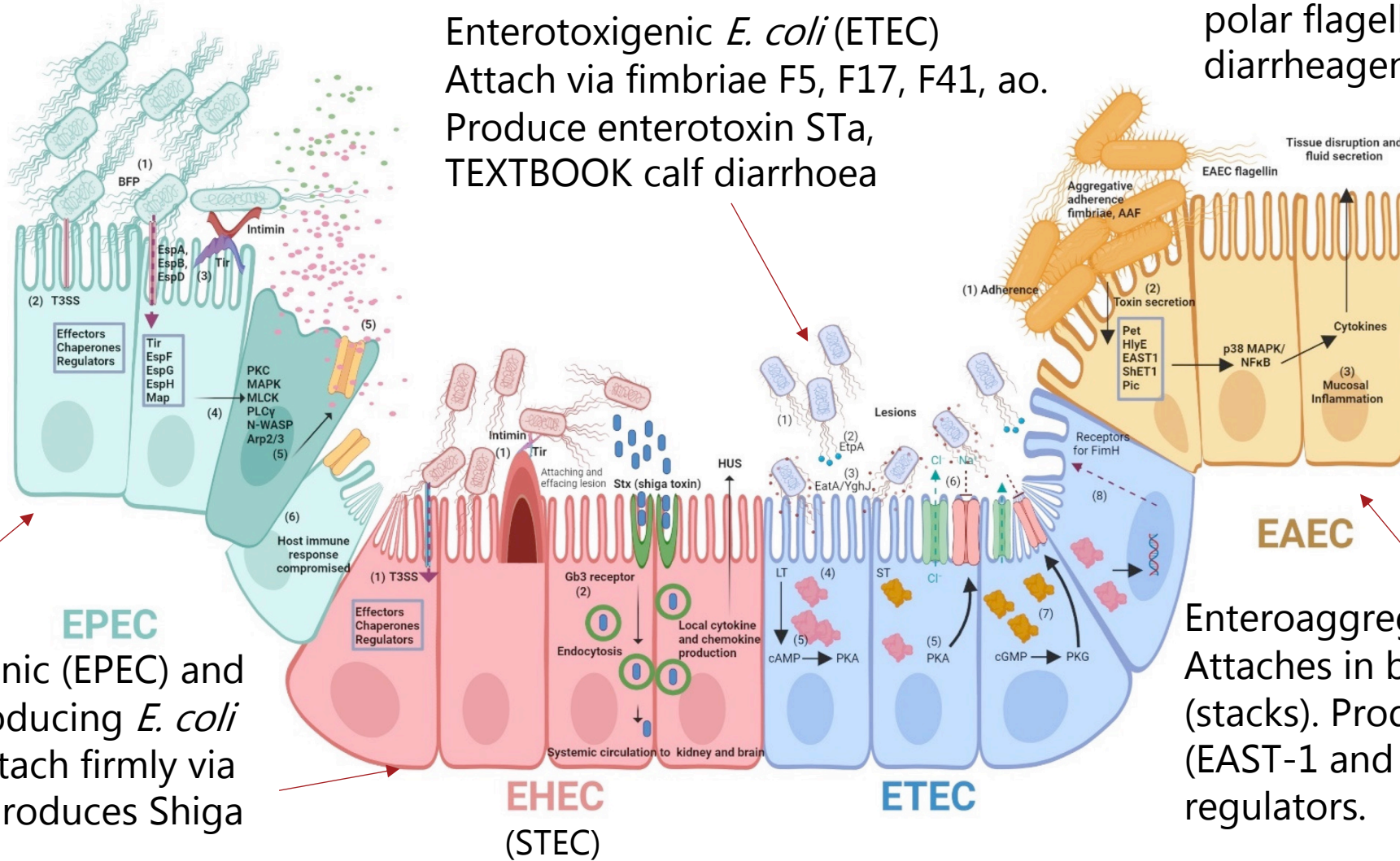
Focus of today



Pathogenic *E. coli* cause diarrhoea in different ways

Enterotoxigenic *E. coli* (ETEC)
 Attach via fimbriae F5, F17, F41, ao.
 Produce enterotoxin STa,
 TEXTBOOK calf diarrhoea

Adherent-invasive *E. coli* (AIEC). Adheres with long polar flagella. Can both be diarrheagenic and invasive



EPEC
 Enteropathogenic (EPEC) and Shiga toxin producing *E. coli* (STEC). Both attach firmly via intimin. STEC produces Shiga like toxins

Diffusely adhering *E. coli* A lot like EAEC, but no EAST-1.

EAEC
 Enteroaggregative *E. coli* (EAEC). Attaches in bundles via fimbriae (stacks). Produces enterotoxin (EAST-1 and different signal regulators).

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ORIGINAL ARTICLE



Evaluation of a novel multiplex qPCR method for rapid detection and quantification of pathogens associated with calf diarrhoea

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118 samples from calves with diarrhoea
 Zero positive for *E. coli* F5 by PCR

Diagnostic support to
 SEGES diagnostic lab.
 (Kjellerup) when *E. coli*
 detected

65 samples from calves with diarrhoea
 Zero positive for *E. coli* F5 by PCR

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Effect of feeding dairy calves with milk fermented with selected probiotic strains on occurrence of diarrhoea, carriage of pathogenic and zoonotic microorganisms and growth performance

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25 samples from calves with diarrhoea
 Three positive for *E. coli* F5+toxine by PCR

Strains also kindly obtained from

Investigation of the current causes of diarrhoea in calves in Denmark.



Landbrugets Veterinære
Konsulenttjeneste

Results reported with permission

DNA sequencing from *E. coli* isolates from calf diarrhea samples

- 65 *E.coli* isolates
 - 40 from CEVA+LVK investigation (25 massive growth of *E. coli*; 9 no other pathogen detected; 10 *C. perfringens* only pathogen)
 - 25 from SEGES+GUDP project (from calves where *E. coli* was likely the relevant pathogen)
- Sequenced by MiSeq (Illumina), Coverage of 30 (cut-off)
- Raw files were trimmed (trimmomatic) and assembled (spades)
- Assembled genomes were used to find virulence genes and antimicrobial resistance genes (Center for Genomic Epidemiology – database)

Strains from SEGES (25)

N	Serotype	ST-Type	Pathotype(s)*
6	O101:H9	10	5 ETEC (F5+STa), 1 F5-tox negative
2	O26:H11	21	EHEC (STEC)
5	O25:H28 + 3 other serotypes	58	2 EAEC, 3 putative AIEC (long polar flagella)
4	O8:H9 and H17	88	2 putative DAEC/EAEC (afa-genes, long polar flagella, EAST-1 gene), 1 putative AIEC, 1 hybrid.
4	O119:H4	117	4 putative DAEC/EAEC (long polar flagella, EAST-1 gene), 2 putative AIEC (long polar flagella)
4	The rest different types	Different types	1 EHEC (STEC), 2 putative EAEC, 1 unassigned

Strains from CEVA+LVK investigation (40)

*Best hit based on virulence gene profile. Hybrid types indicated

N	Serotype	ST-Type	Pathotype(s)*
5	O101:H9	10	2 F5+,tox negative, 1 putative DAEC/EAEC, 2 unassigned
2	O26:H11	21	1 EHEC (STEC), 1 aEPEC
2	O99:H33	34	unassigned
5	O8:H25 (2) + two other types	58	1 EAEC/DAEC, 4 unassigned
2	O15:H6, H18	69	AEEC/DAEC
9	O6, O8, O86 different flagella types	88	6 putative DAEC/EAEC, 2 putative AIEC
3	O9:H9, H30	540	2 EAEC/DAEC, 1 unassigned
12	Different O and H types	Different ST types	F17-tox neg, 4 EAEC/DAEC, 7 unassigned

Conclusions

- F5 ETEC is not commonly detected in Denmark
- ETEC (in general) found in few samples.
 - ETEC positive samples in CEVA/LVK investigation were from calves up to 8 days of age.
- ETEC, DAEC, EAEC, STEC, AIEC associated genes were found in the *E. coli* strains isolated from calf diarrhoea (cave not all samples can be said to contain an *E. coli* strains that has caused diarrhoea).

Change in pathotypes of diarrhoeagenic *E. coli* from calves?



- 2024 project ("Mælkeafgiftsfonden") headed by Rikke Heideman Olsen, Department of Veterinary and Animal Sciences, University of Copenhagen
- Collaboration with LVK (Kenneth Krogh and Birgitta Svensmark)
- Collaboration with SEGES (Henrik Læssøe Martin)
- Aims to determine which pathotypes are present in Danish calves to be able to adjust diagnostic methods and vaccine strategies

Future work

- Collect more *E.coli* from calves with diarrhoea (LVK)
- Isolate and characterize strains by NGS
- Characterize strains by phenotypic methods (cell culture interaction, among other methods) to confirm type
- Direct sequencing for detection and recommendation for new PCR methods
- Investigate reservoir in herds (SEGES)

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- Maja Albrechtsen, CEVA and Birgitta Svensmark, LVK for sending samples and data
- Annette Sønderholm Juul, SEGES laboratory for sending strains
- Jibril Adurahmann for analysis of sequences from CEVA/LVK study