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Sequencing of avian pathogenic *Escherichia coli* from a colibacillosis outbreak

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Background

Avian pathogenic *Escherichia coli* (APEC) are responsible for colibacillosis in poultry. The most frequent form of avian colibacillosis is a syndromic infection that starts in the respiratory tract and is later characterized by fibrinous inflammation in internal organs and septicaemia. APEC pathotype is poorly defined, and usually strains isolated from birds suffering from colibacillosis can be very heterogenous. Recently colibacillosis has caused high mortality (over 10 % in some flocks) in Finnish broiler production although predisposing factors like viral and mycoplasma infections do not occur in commercially raised broilers.

Aim

- Systematically collect *E. coli* isolates from diseased broilers to
  - to find out if the outbreak is caused by clonal *E. coli*
  - characterize isolate properties using PCR and NGS

Materials and Methods

- During 4 week period 295 *E. coli* isolates from 40 broiler farms were collected from femoral bone marrow of birds that showed in necropsy typical signs of colibacillosis
- 3/40 of the farms were raising parent birds
- The phylogeny group, virulence factors and O1, O2, O18 and O78 serogroups of the isolates were determined using previously published PCR reactions (Clermont et al. 2000, Ewers et al. 2005 and Wang et al. 2014)
- This PCR screening showed that 84 % of the 295 isolates belonged to two different types (figure 1.)

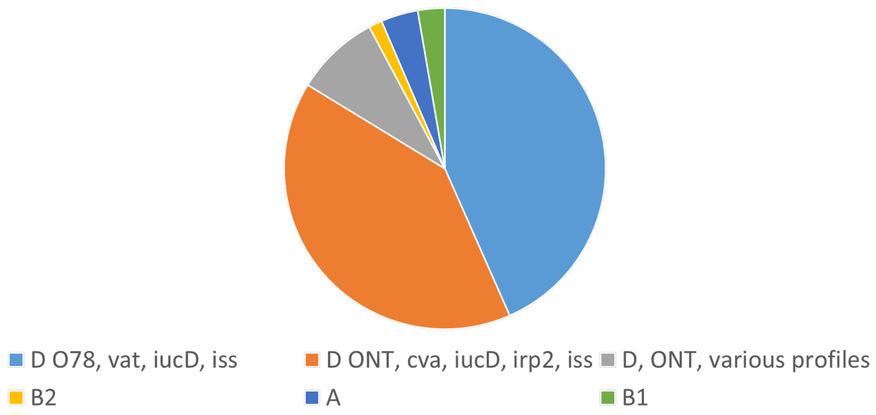

Figure 1. Distribution of the 295 E. coli isolates to different phylogroups and virotypes

- Five isolates from different farms from each of the two main group were sequenced using Illumina MiSeq platform
- The raw reads were *de novo* assembled using CLCbio's Genomics Workbench 7.5.
- Subsequently, the *de novo* assembled contigs were *in silico* MLST typed, serotyped, and virulence and antibiotic resistance genes were identified using Center for Genomic Epidemiology (DTU) programs.
- The relationship between the 10 isolates was inferred by investigating the genetic diversity in the core genome of the isolates against *E. coli* CFT073 using Burrows-Wheeler aligner
- Three publicly available ST117 E. coli genomes were included in the analysis (SEPT362, 53C and H299)
- Identification of SNPs was performed using GATK Unified Genotyper
- Filtering using NUCmer to remove positions with <10x coverage, < 90 % unambiguous variant calls, or within duplicated regions
- Purged maximum parsimony tree was made using MEGA 6.0.6.

WGS results

- All 10 isolates belonged to phylogroup D and were of *E. coli* MLST 117
- The group one isolates were of serotype O78:H4, carried the *vat, iucD* and *iss* genes (confirmed by both *in silico* analysis and specific primers) and had no antibiotic resistance genes
- The group two isolates were serotype O53:H4, carried the *cva*, *iucD*, *irp2* and *iss* genes and 3/5 had the aminoglycoside encoding resistance genes
- All group 2isolates were in silico positive for the vat gene, whereas by specific primers only one isolate was positive
- The phylogenetic analysis was based on 58 710 SNPs
- The Finnish isolates were divided into two subclades according to seropathotype:
  - subclade 1 (blue) O78, isolates E10 and E16 laying parents, E12 and E18 broilers and the most divergent isolate E9 from 6 day old parent chick
  - subclade O53 (red), all five isolates from different broiler farms

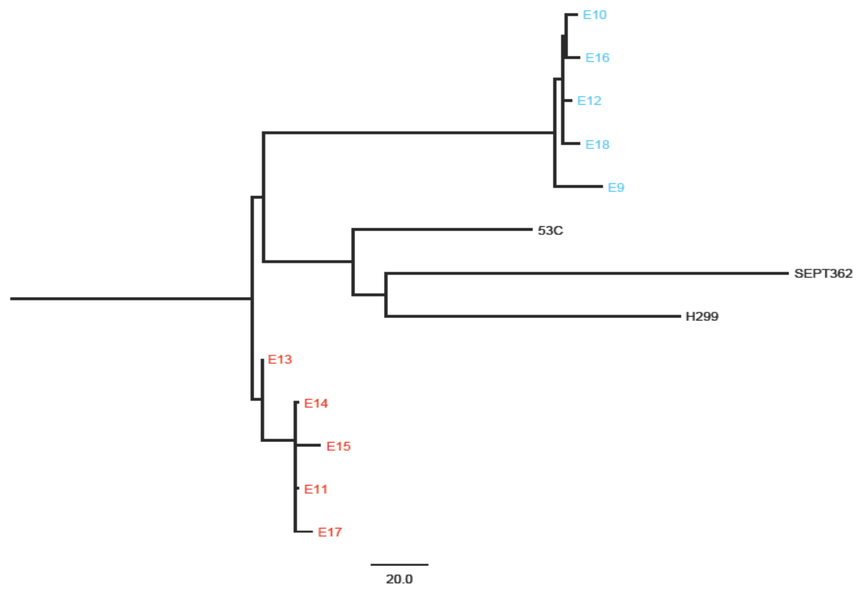

Figure 2. Magnified purged maximum-parsimony tree of all ST117 isolates based on 58,710 SNPs. and compared to E. coli CFT073. The lenght of scale bar is 20 SNPs.

Conclusions

- Colibacillosis outbreak was caused by two clones of E. coli phylogeny group D ST117 comprising 84 % of examined isolates from the outbreak
- This warrants further studies to elucidate the virulence factors (especially in the O78 clone that is now the main type in colibacillosis outbreaks in broiler farms) and develop an autogenic vaccine

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