



# Population genetics of antibiotic-resistant Campylobacter jejuni in broilers in Finland – Vertical transmission, fact or fiction?

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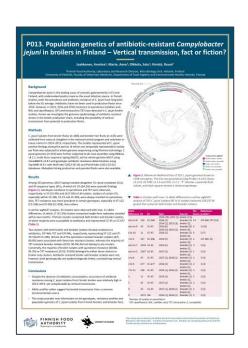
**EURL-Campylobacter workshop** 

**NRL-Finland** 





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Master's Thesis (in Finnish): http://hdl.handle.net/10138/596233

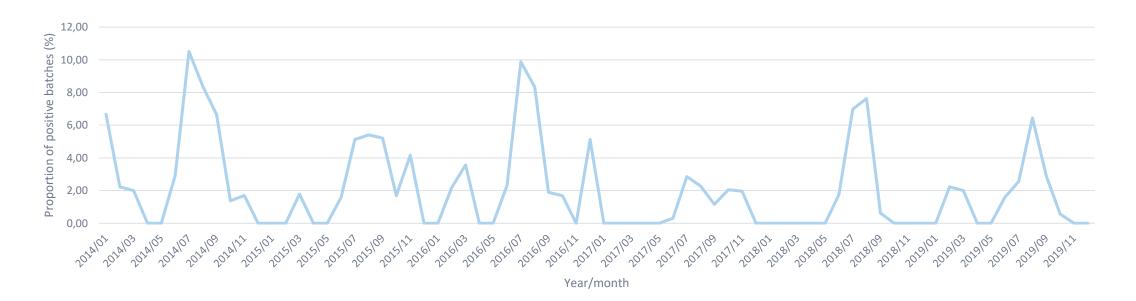


# Background: thermotolerant *Campylobacter* sp. in broilers in Finland

# Prevalence of *Campylobacter* sp. in broilers (caeca) at slaughter



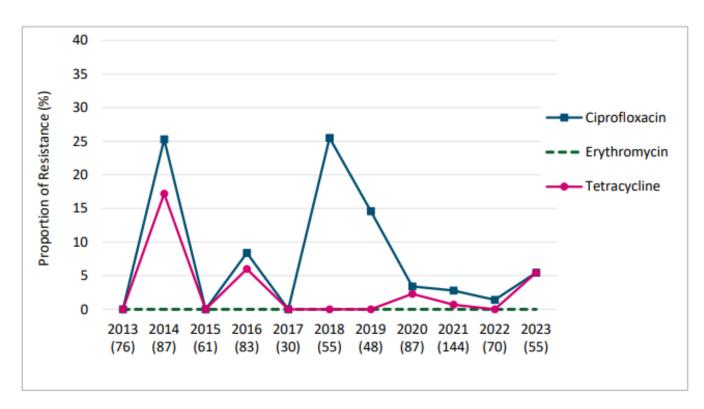
- National control program (since 2004)
  - Every slaughtered batch tested for Campylobacter (ISO 10272-1) in June–Oct, sample in Nov–May
  - Combined caecal sample of 10 birds per slaughtered batch/flock
  - Phenotypic antibiotic resistance tested on one isolate per each positive flock on MIC panels (FINRES-Vet)
  - On average, 6.5% of slaughtered broiler batches positive for Campylobacter sp. in June–Oct (92.1% C. jejuni)
  - Highest prevalence in July–Aug (10.5%)



# Proportion of antibiotic-resistant *C. jejuni* isolates from broilers at slaughter



- Antibiotic resistance has long been below the EU average
  - Production flocks have not been treated since 2010 (ETT.fi)
- Before 2013, less than 4% of the isolates were resistant to quinolones or tetracycline (TET)
- After 2013, quinolone (CIP) and TET resistance peaked in certain years



The number of isolates tested each year are in brackets.

Source: FINRES-Vet, 2024.



## **Breeder hens (parental flocks)**

- Annually, 3–20% of the parental flocks had been treated with antibiotics, highest proportion in 2014 (ETT.fi)
  - Penicillin, but also amoxicillin and oxytetracycline
  - Quinolones had not been used
- Also in 2013, the import practises and origin countries of grandparental flocks changed
- Voluntary own-check sampling of every slaughtered batch until 2023
  - Almost all flocks positive for Campylobacter
  - No (phenotypic) antibiotic resistance testing



# The study



### **Aims**

• Genotypes, resistance profiles and population genetics of *C. jejuni* from Finnish broilers and breeder hens

• Whether the antibiotic-resistant *C. jejuni* could have transmitted vertically from parental to production flocks or not



#### **Materials**

- *C. jejuni* isolates (n=333) from broiler production flocks (n=188) and parental flocks (n=145) in 2014–2019
  - One temporally representative isolate per flock
  - Represented annually 34–74% of Campylobacter positive production flocks
  - Represented all positive parental flocks
- Metadata linking production and parental flocks





- Whole-genome sequencing (Illumina)
- Genome assembly and quality control, MLST
  - INNUca v4.2.2
- Ad hoc whole genome MLST
  - chewBBACA v2.8.5
- Genotypic antibiotic resistance determination
  - StarAMR v0.9.1 with ResFinder (2022-05-24) and PointFinder (2021-02-01) databases



## **Antimicrobial susceptibility results**

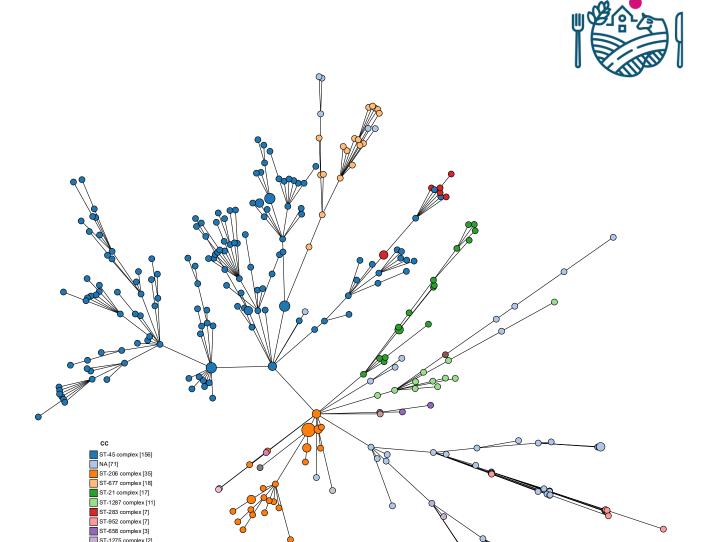
- Higher resistance rates to quinolones among broiler than breeder isolates
- Tetracycline resistance evenly in both generations
- No genotypic resistance to other tested antibiotics

	Broiler isolates, phenotypic resistance	Broiler isolates, genotypic resistance <sup>1</sup>	Breeder isolates, genotypic resistance
Ciprofloxacin (Quinolones)	44/185 (23.8%)		
Nalidixic Acid (Quinolones)	52/185 (28.1%)		
Tetracycline	15/185 (8.1%)		
gyrA T86I or T86A (Quinolones)		48/188 (25.5%)	5/145 (3.4%)
tet(O) or tet(O/32/O) (Tetracycline)		16/188 (8.5%)	10/145 (6.9%)

<sup>&</sup>lt;sup>1</sup>97.7%–100.0% concordance with the phenotypic results

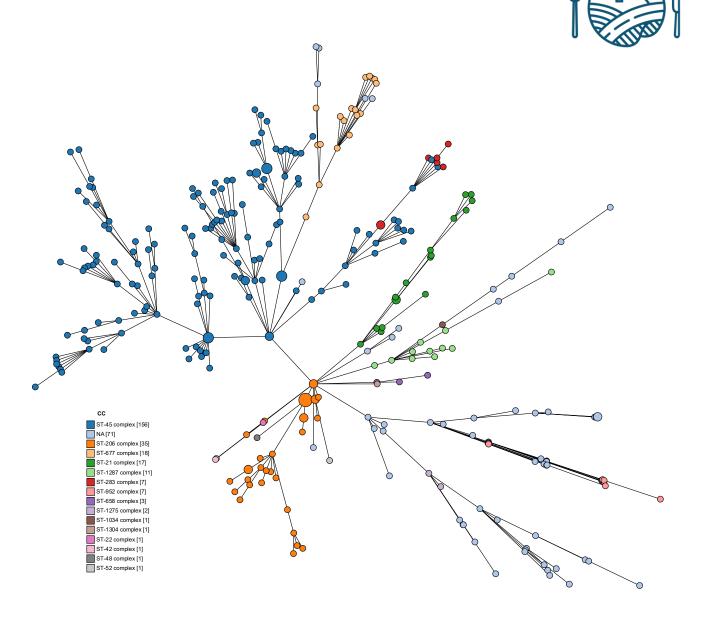


- 95 sequence types (STs) and 15 clonal complexes (CCs)
- 61 STs (64.2%) represented by single isolates
- Same STs between broiler (57.5%) and breeder (61.5%) isolates



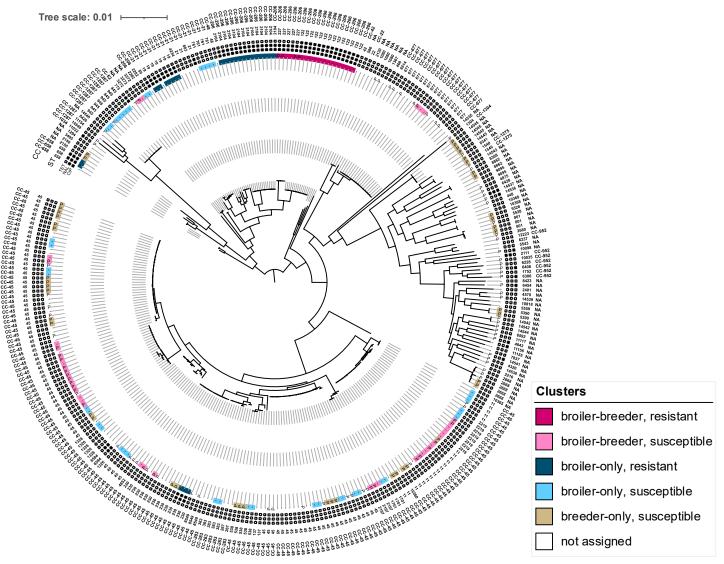


- CC-45 was the most prevalent CC
  - 51.0% of breeder isolates and 43.6% of broiler isolates
  - Only CC isolated every year in both generations
  - Mainly susceptible genotype
- ST-45 also the most prevalent ST in human infections in Finland for several years



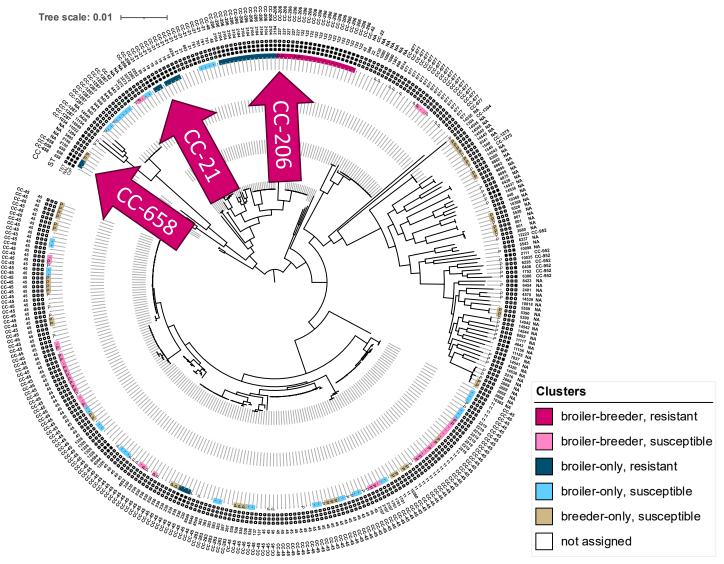


- Antibiotic resistance was associated with certain sequence types
  - Quinolone resistance: CC-206, CC-21, CC-658
  - Tetracycline resistance: CC-206, CC-658





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  - Tetracycline resistance:
    CC-206, CC-658





## Clusters (ad hoc wgMLST)

- With max. 11 allelic differences, 54 clusters (2–14 isolates in each cluster)
  - 31.5% single-farm replicates sampled within a two-month period
  - Often same *C. jejuni* clone in different rearing halls of the same farm
- 13 clusters comprised both broiler and breeder isolates
  - 2 resistant clusters (CC-206): CIP-NAL-TET (14 isolates) and CIP-NAL (5 isolates)
  - 80.0% of quinolone-resistant breeder isolates were associated with these resistant clusters, whereas 90.0% of TET-resistant breeder isolates were sporadic findings
  - 83.3% of quinolone-resistant broiler isolates and 93.8% of TET-resistant broiler isolates belonged to these clusters or broiler-only clusters



#### **Resistant clusters**

Clusters with max. 11 allelic differences in *ad hoc* wgMLST analysis of 333 *C. jejuni* isolates (95 % of isolates harbored 1292/3728 genes) that comprise antibiotic-resistant isolates

No.	Allele difference	ST	СС	Year <sup>1</sup>	Source <sup>1</sup>	Resistance profile <sup>1</sup>	No. farms	Distance of farms <sup>2</sup>
1	zero to 2	2104	CC-206	2018 (13), 2019 (1)	broiler (14)	CIP-NAL (14)	6	Within 50 km
2	zero to 8	122	CC-206	2014 (10), 2015 (1), 2016 (3)	broiler (13), breeder (1)	CIP-NAL-TET (14)	13	Within 60 km. Same region <sup>3</sup> and partly same farms as in cluster 1
3	1 to 5	227	CC-206	2014 (5)	broiler (2), breeder (3)	CIP-NAL (5)	2	40 km
4	6 to 11	19	CC-21	2019 (4)	broiler (4)	CIP-NAL (4)	2	12 km
5	1 to 6	230	CC-45	2016 (3)	broiler (3)	CIP-NAL (3)	2	200 km. One farm in the region of clusters 1 and 2
6	2	19	CC-21	2019 (2)	broiler (2)	CIP-NAL (2)	1	Same farm as in cluster 2
7	4	658	CC-658	2016 (2)	broiler (2)	CIP-NAL-TET (2)	1	Same farm as in cluster 1

<sup>&</sup>lt;sup>1</sup>Number of isolates in parenthesis.

<sup>&</sup>lt;sup>2</sup>All farms located within 310 km.

<sup>&</sup>lt;sup>3</sup>Region of Finland where animal production is concentrated: broilers, cattle, fur animals.



#### **Vertical transmission**

 No epidemiological linkage was observed between antibiotic-resistant broiler and breeder isolates

- Some uncertainty remains
  - Most broiler flocks had animals originating from several parental flocks
  - Metadata was missing for many of the broiler flocks
  - Only one temporally representative isolate per flock was sequenced → representative of the whole diversity?



### **Conclusions**

• Genotypically diverse *C. jejuni* populations with overlaps between broiler and breeder isolates

• Unlikely vertical transmission of antibiotic-resistant *C. jejuni* → rather horizontal transmission from a common (unknown) environmental source

### **ACKNOWLEDGMENTS**





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WALTER EHRSTRÖMIN SÄÄTIÖ





# QUESTIONS?

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# FINNISH FOOD AUTHORITY

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