

# NGS BASED *FLAA* TYPING OF *CAMPYLOBACTER* STRAINS

## CAMPREVENT

REDUCTION OF *CAMPYLOBACTER* IN BROILER FLOCKS:  
IDENTIFICATION OF RISK FACTORS  
AND EVALUATION OF THE EFFECT OF INCREASED BIOSECURITY  
AND PROTECTIVE ROLE OF THE MICROBIOTA IN *CAMPYLOBACTER* FREE FLOCKS

# CONTEXT

- broiler industry as a source of human campylobacteriosis cases
  - vertical transmission not the problem
    - reduction strategies
  - biosafety management

# AIM & QUESTIONS

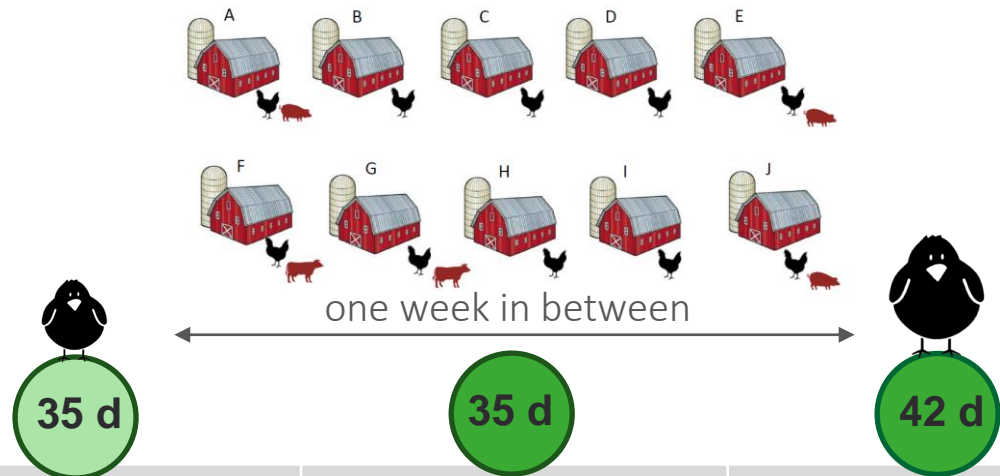
- how does *Campylobacter* enter the poultry house ?
  - identify possible contamination sources
  - identify possible transmission routes
- what are possible biosecurity management solutions ?






thinning ?

= broiler strains ?

FlaA typing ?

# SAMPLING & METHODS



Before thinning	Thinning	After thinning
Screening 19 broiler flocks (from 10 farms) on <i>Campylobacter</i> spp.		
house 1: 50 caecal droppings	truck surface 	identical sampling as before thinning
house 2: overshoes	truck wheels 	
	forklift 	
	catching crew (boots and gloves) 	
	containers 	

direct plating and enrichment

*Campylobacter* specific PCR

PFGE (SmaI)

+

FlaA RFLP (DdeI)




+

(PFGE (KpnI))

BioNumerics software  
> combined strain type (e.g. 1.1)

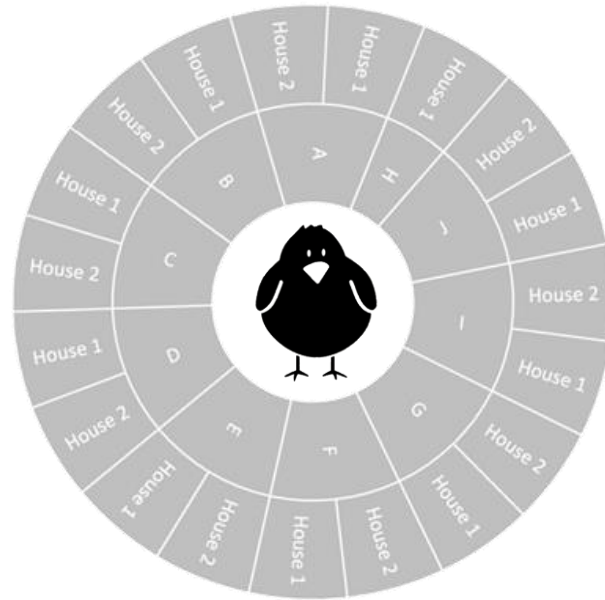
FlaA NGS




# RESULTS

-  free of *Campylobacter* spp.
-  colonized / contaminated with *C. jejuni*
-  colonized / contaminated with *C. jejuni* but no link was found

before thinning

RESULTS

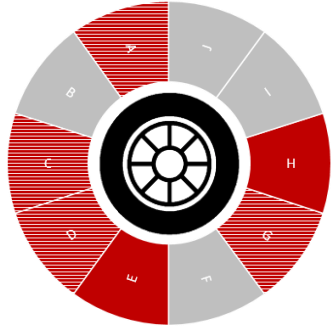


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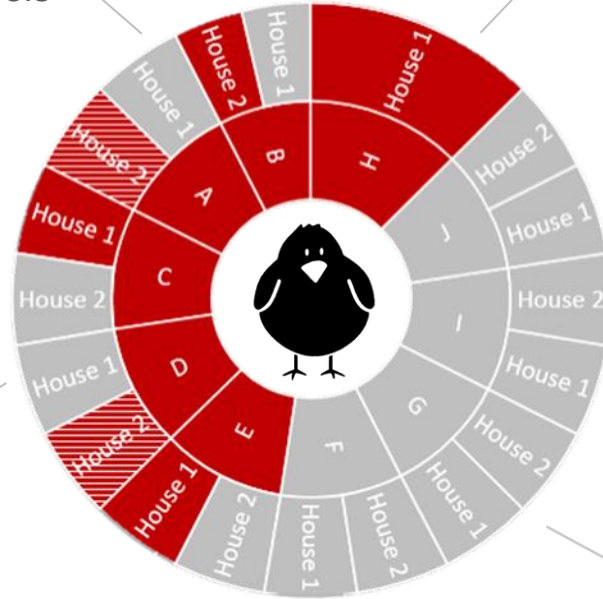
RESULTS



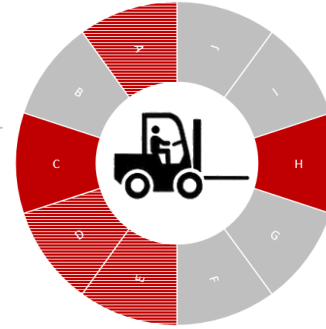
60%  
truck wheels



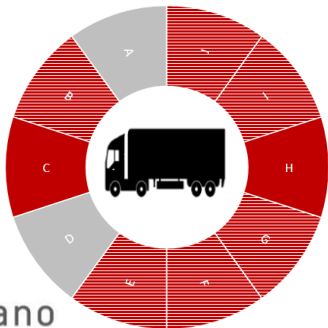
10%  
catching crew



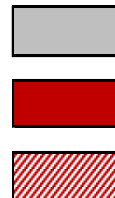
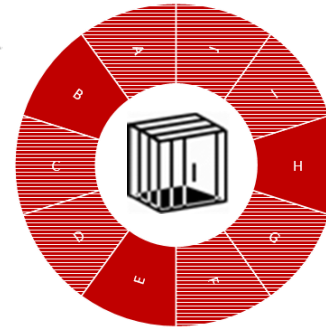
50%  
forklift



80%  
truck surface



100%  
containers



free of *Campylobacter* spp.

colonized / contaminated with *C. jejuni*

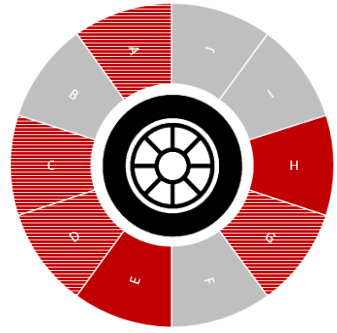
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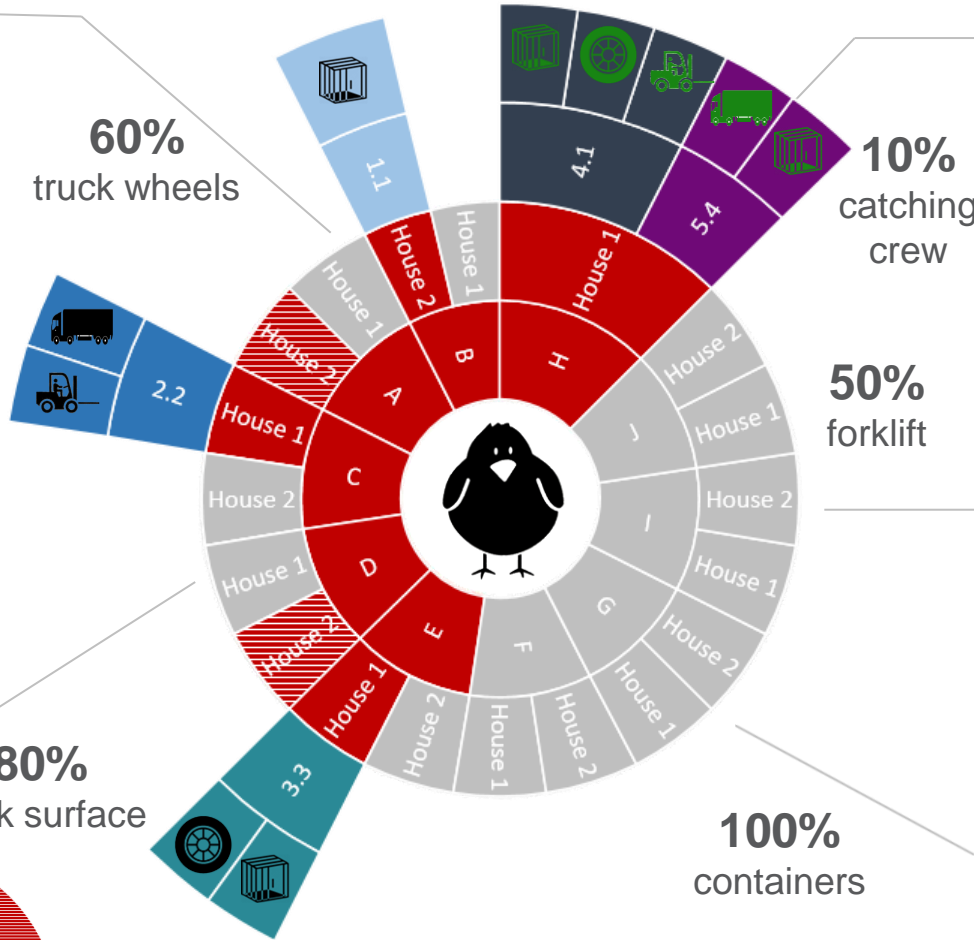
thinning

after thinning

RESULTS



60%  
truck wheels

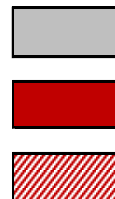


10%  
catching crew

50%  
forklift

80%  
truck surface

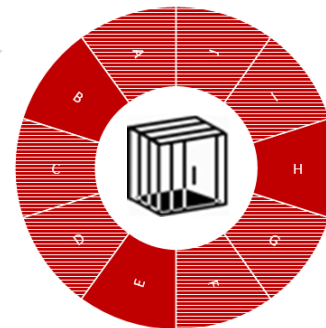
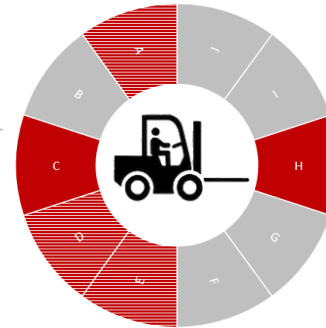
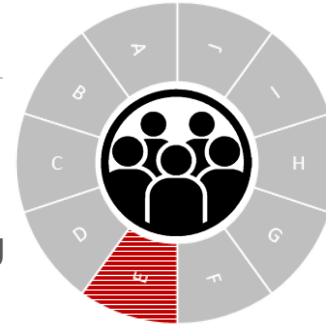
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DNA extraction

FlaA-NGS



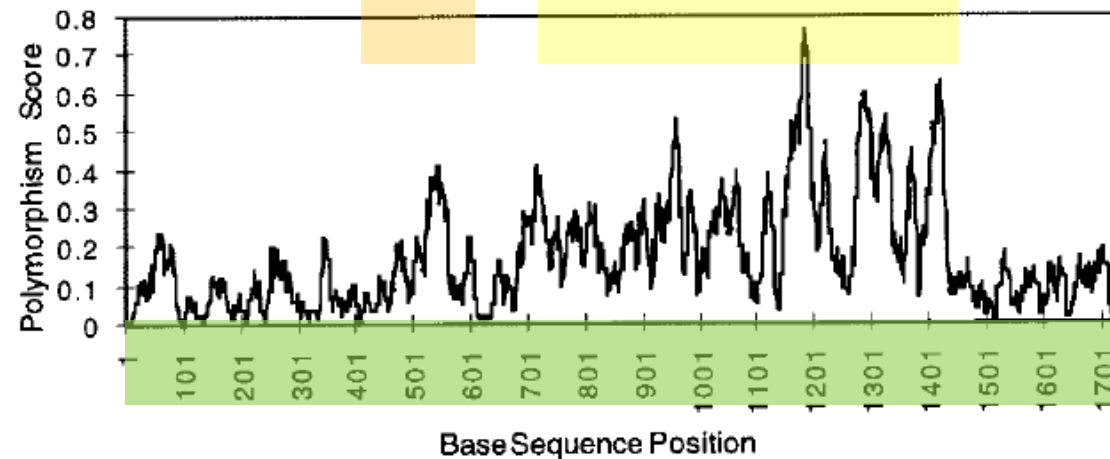
# FLAGELLIN A (FLAA) SEQUENCING

- FlaA gene amplicon sequencing
  - 1764 bp
  - SVR region
  - *Campylobacter* sub-species determination ?

450 – 600: shorter high variability +  
flanked by conservative regions: 267 bp

**SVR**

700 – 1450: high variability



# FLAGELLIN A (FLAA) SEQUENCING

NCBI/ENA:

- > 505 isolates: cgMLST analysis (1.343 loci)
- > 1<sup>st</sup> criterion: > 95 % loci present in the genome
- > 2<sup>nd</sup> criterion: < 95 % of called loci are identical
- > 468 isolates: 219 *C. jejuni* + 249 *C. coli*

extract flaA gene sequence + SVR sequence

*In silico* PCR (Meinersmann et al, 1997):

- 288 no SVR amplification
- 228 multiple hits through genome
- 60 with > 6 mismatches in F and R primers

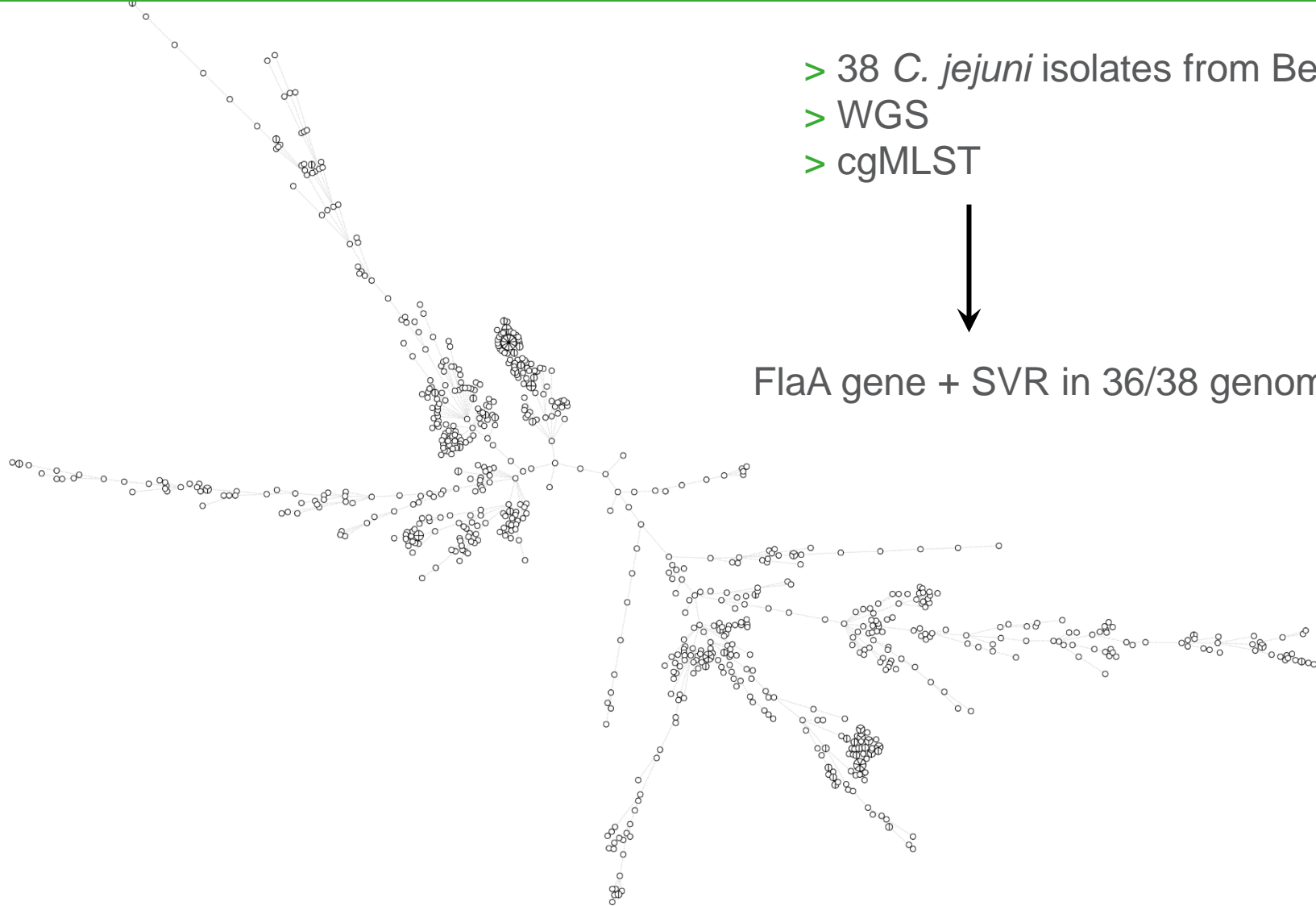
207 reference genomes: FlaA gene not detected

# FLAGELLIN A (FLAA) SEQUENCING

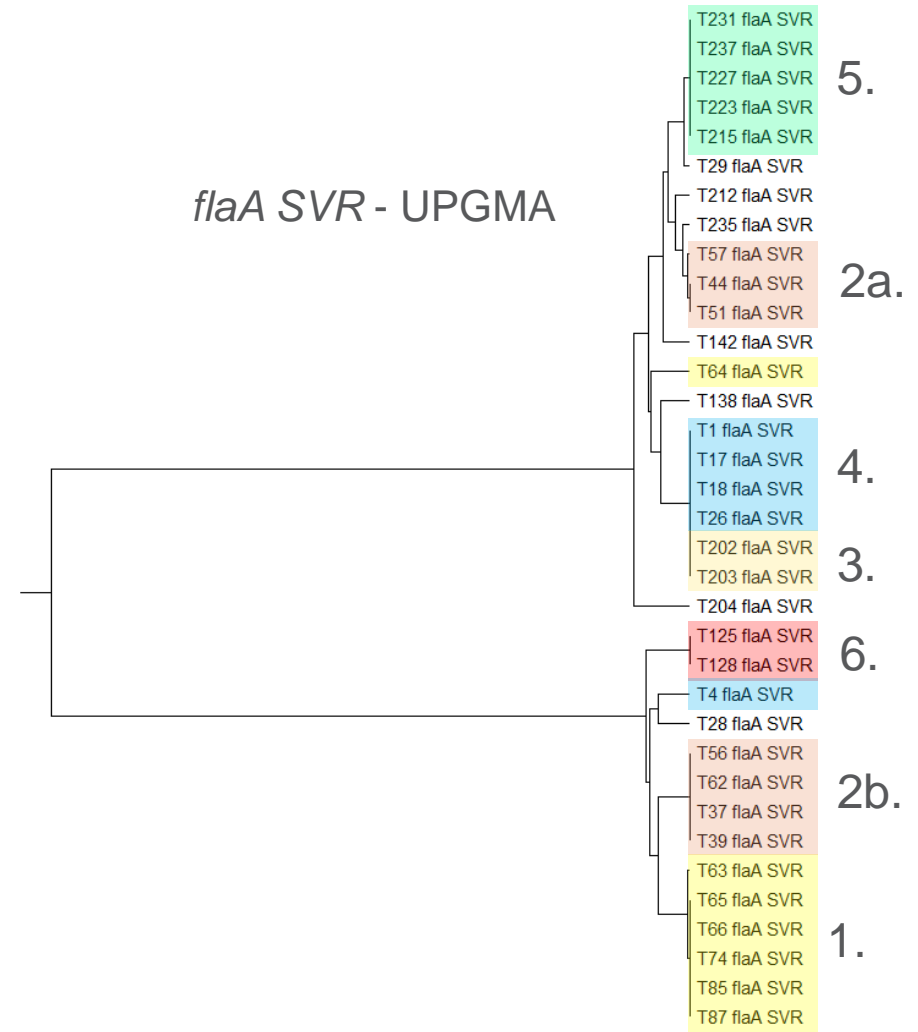
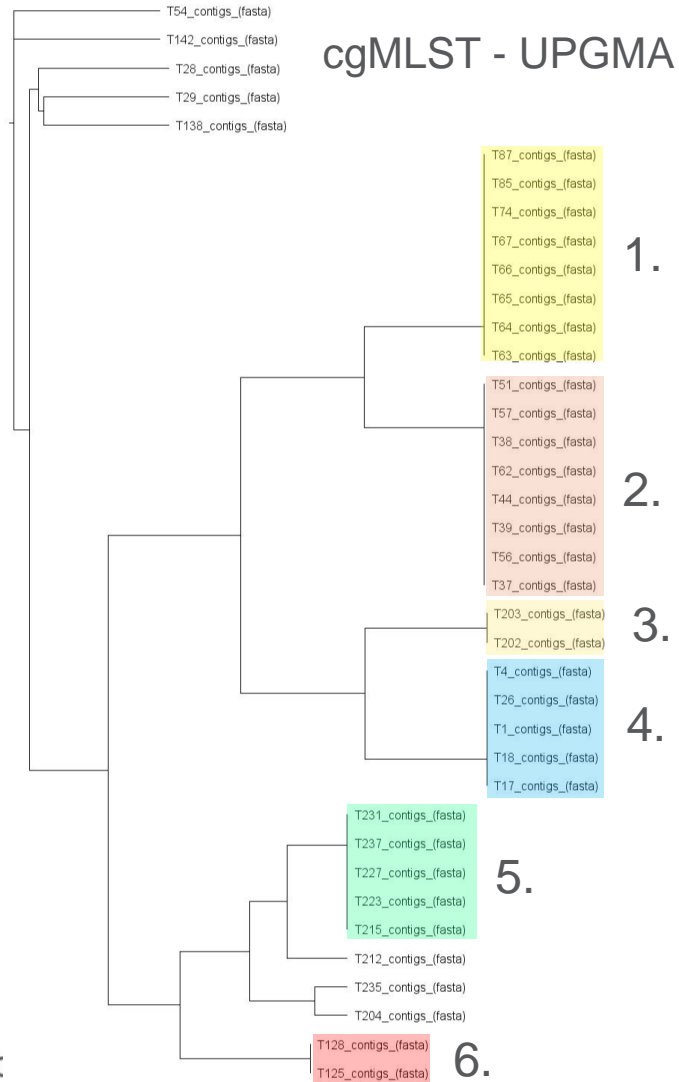
- > 38 *C. jejuni* isolates from Belgian slaughterhouses
- > WGS
- > cgMLST



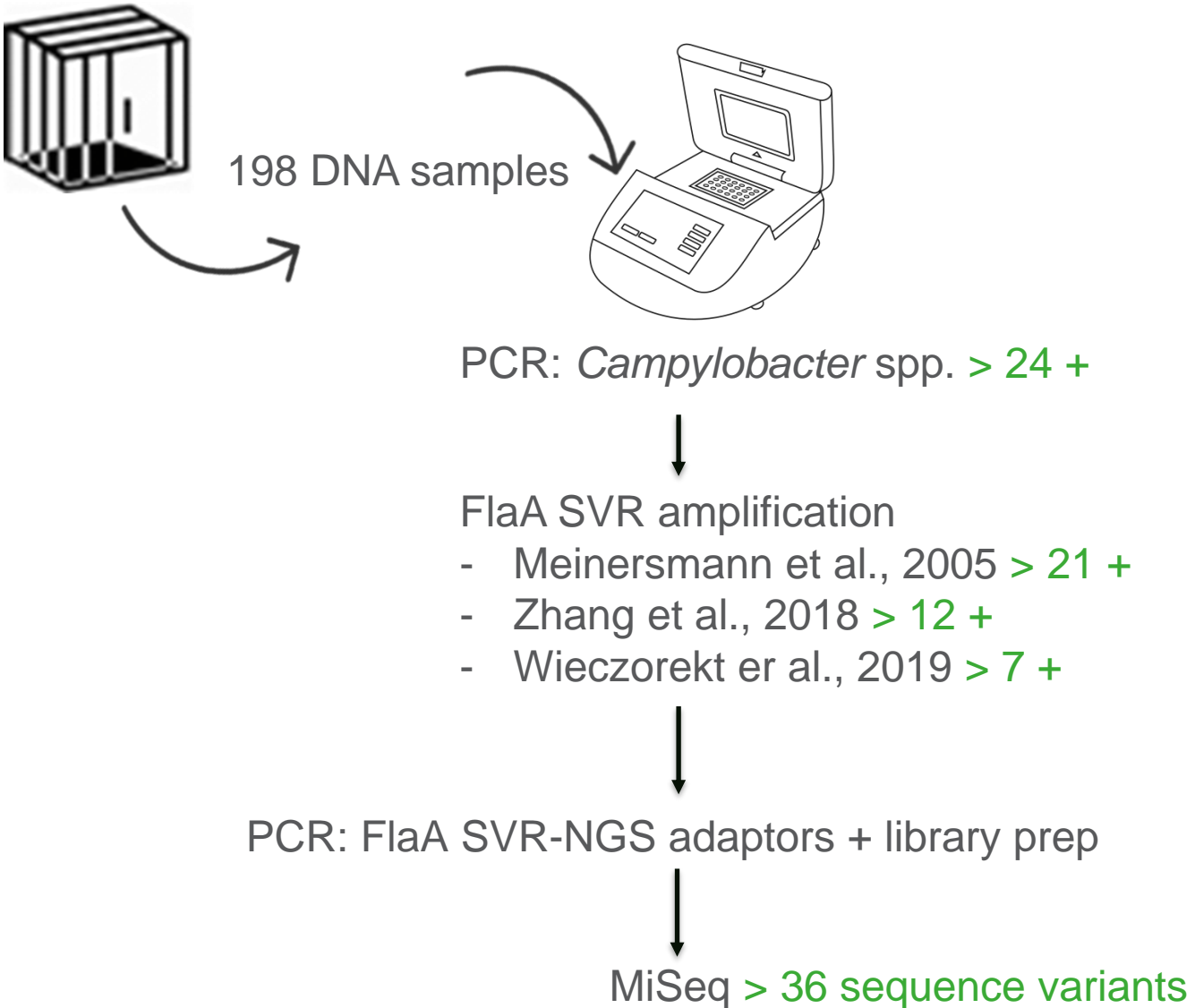
FlaA gene + SVR in 36/38 genomes



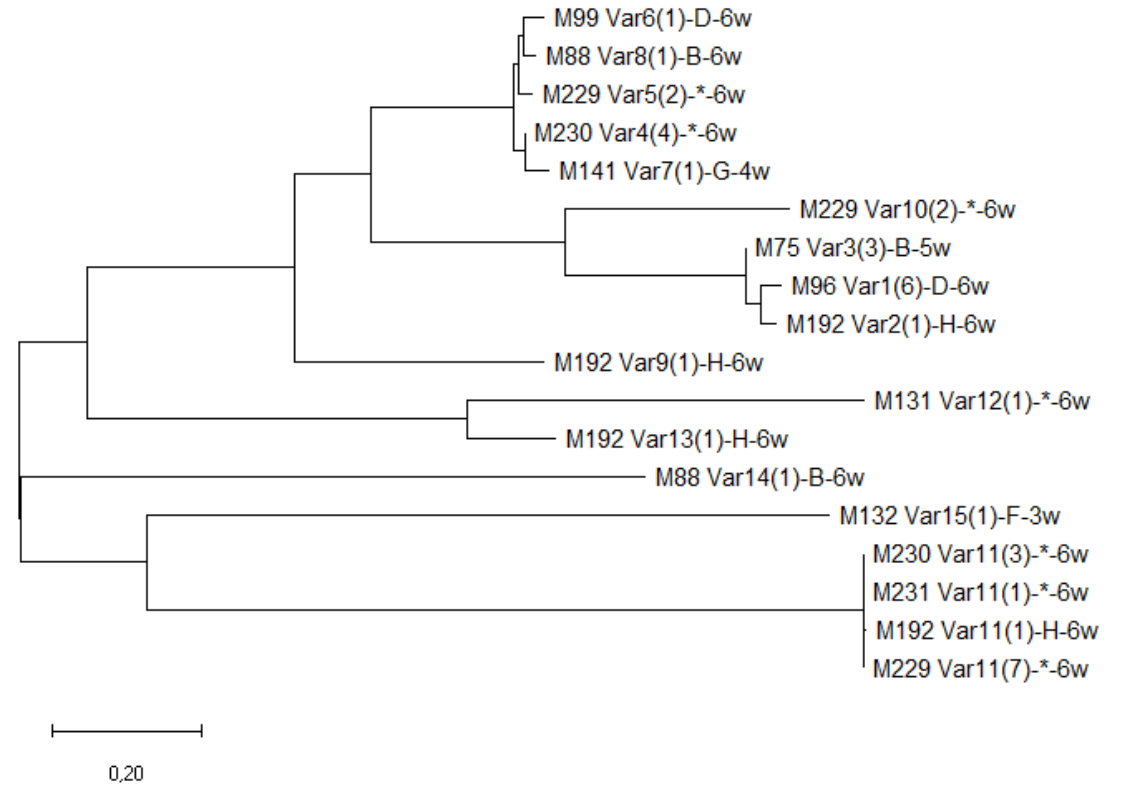
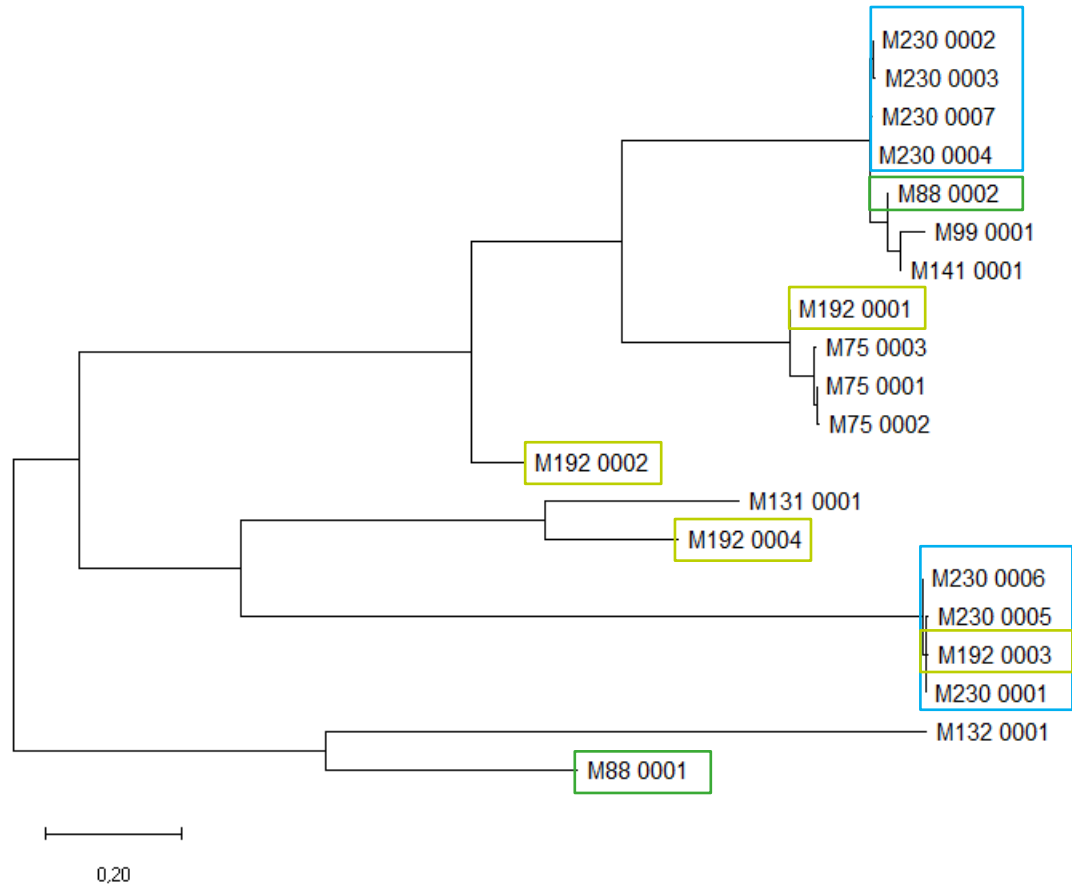
# FLAGELLIN A (FLAA) SEQUENCING – DISCRIMINATIVE POWER



# FLAGELLIN A (FLAA) SEQUENCING – METAGENOMIC APPROACH



# FLAGELLIN A (FLAA) SEQUENCING - METAGENOMICS



# FLAGELLIN A (FLAA) SEQUENCING - CONCLUSIONS

- SVR sequencing has a high discriminatory power
  - culture independent subtyping possible
  - less sensitive than conventional microbiology
- FlaA gene seems not to be present in all *Campylobacter* isolates

# THANK YOU

Karolien Hertogs  
Geertrui Rasschaert  
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