

Enrichment in Preston broth with and without growth supplement

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ISO 10272-1 - Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method

Procedure A: low numbers of (or stressed) *Campylobacter* spp. and with low level of resistant background flora, enrichment in **Bolton broth**

Procedure B: low numbers of *Campylobacter* spp. with high level of background flora (in particular ESBL-producing bacteria), enrichment in **Preston broth**

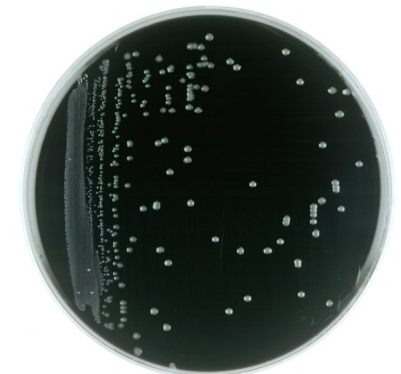
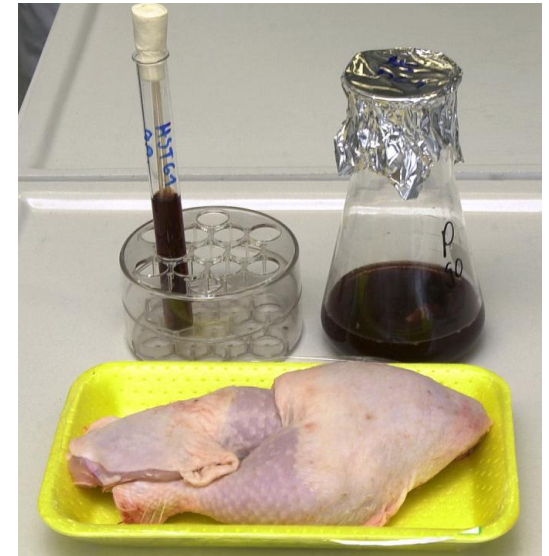
Procedure C: high number of *Campylobacter* spp., **direct streaking on mCCDA**

Preston broth:

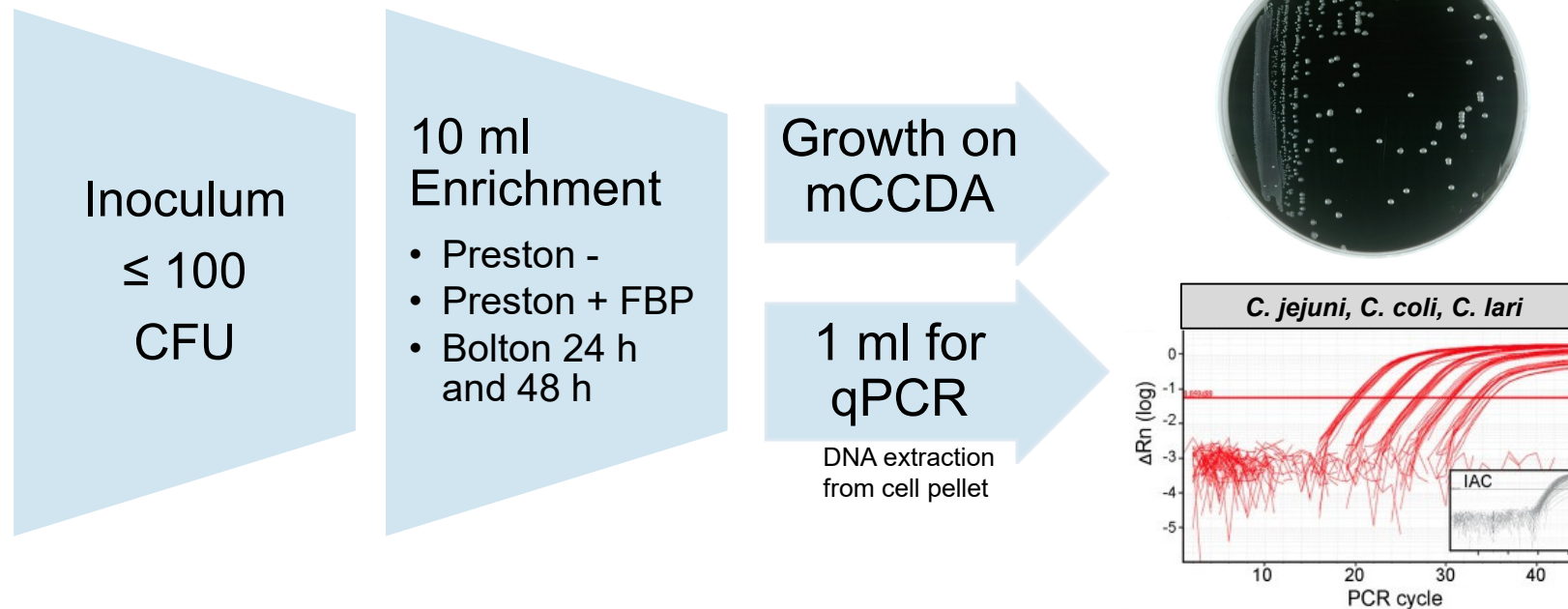
- nutrient broth
- selective antibiotics (10 mg rifampicin, 10 mg trimethoprim, 10 mg amphotericin B and 5000 IU polymyxin B per litre)
- 5 % of lysed horse blood



+ growth supplement (=FBP: 250 mg/litre of each **F**errous sulphate, Na-metab**i**sulphite, Na-**p**yruvate) – already part of ISO 17995:2019, Water quality



Enrichment of *Campylobacter* spp. in Preston broth with and without growth supplement (FBP)



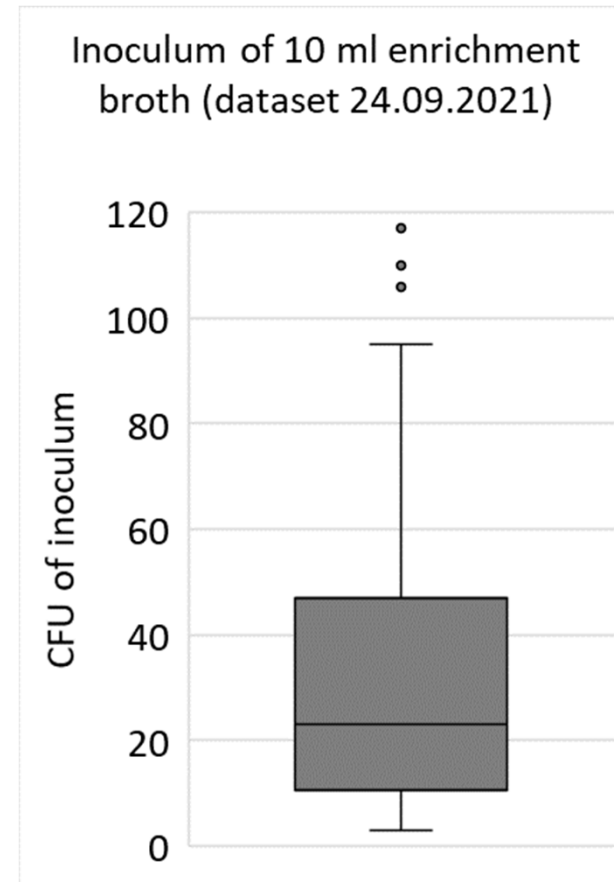
- 99 field strains (40 *C. jejuni*, 34 *C. coli*, 25 *C. lari*) from routine and strain collection
- Preston selective supplements from different companies tested

16S rRNA target (Josefsen et al. 2004)
with improved oligos (Pacholewicz et al. 2019)
→AMD1 of ISO 10272, Annex D

Confirmation of inoculum

- Start from a Columbia blood agar plate after incubation for 24 ± 2 h at $41.5 \pm 1^\circ\text{C}$
- Optical density $\text{OD}_{600\text{nm}}$ of 0.2 corresponds to $\sim 5 \times 10^8$ CFU/ml
- Inoculum was verified in duplicate on Columbia blood agar after 48 h of growth at $41.5 \pm 1^\circ\text{C}$

→ Median of inoculum: 33 CFU



Growth on mCCDA after enrichment

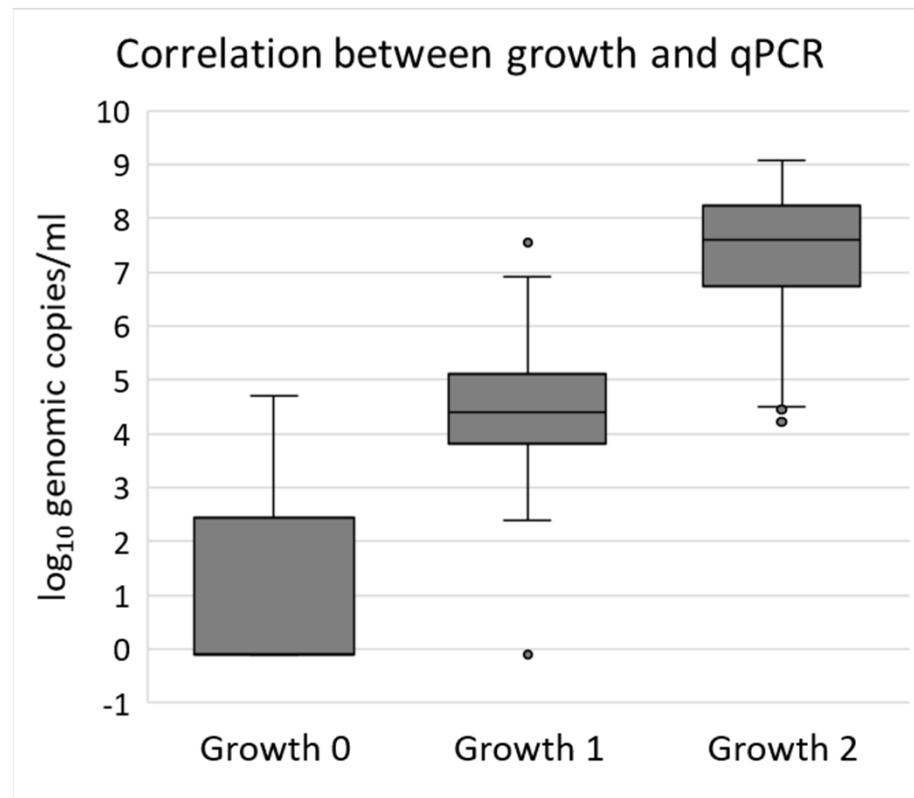
Definition:

Growth 0 = no growth at all

Growth 1 = few colonies

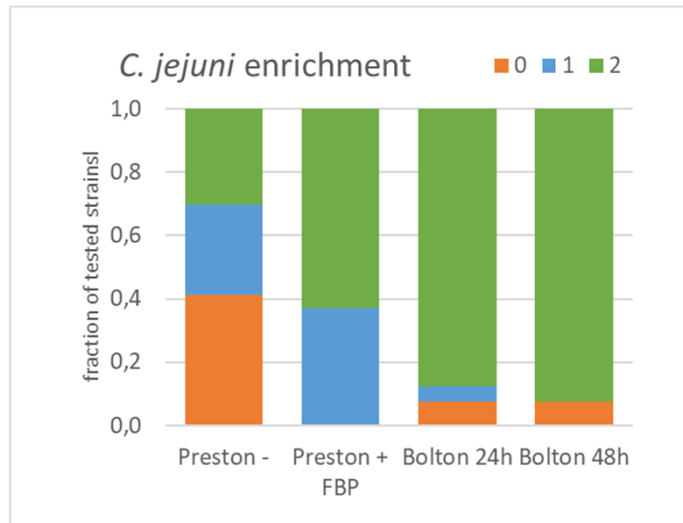
Growth 2 = lawn-like growth

„undetermined“ in qPCR = $-0.1 \log_{10}$



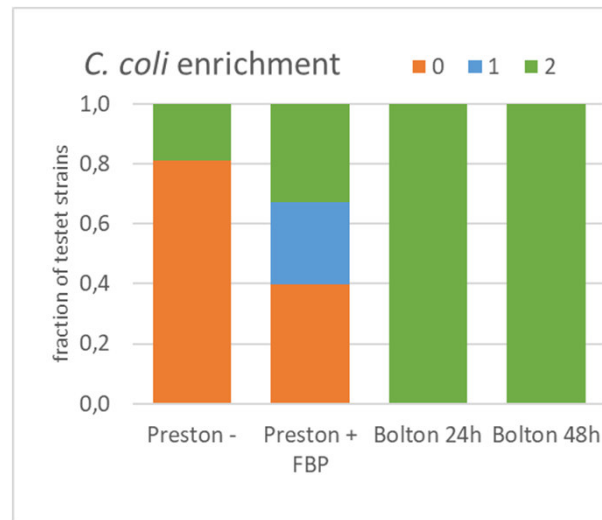
Growth after enrichment

$N_{C.jejuni} = 40$



$N_{\text{Preston}} = 73, N_{\text{Bolton}} = 40$

$N_{C.coli} = 34$



$N_{\text{Preston}} = 58, N_{\text{Bolton}} = 34$

$N_{C.lari} = 25$

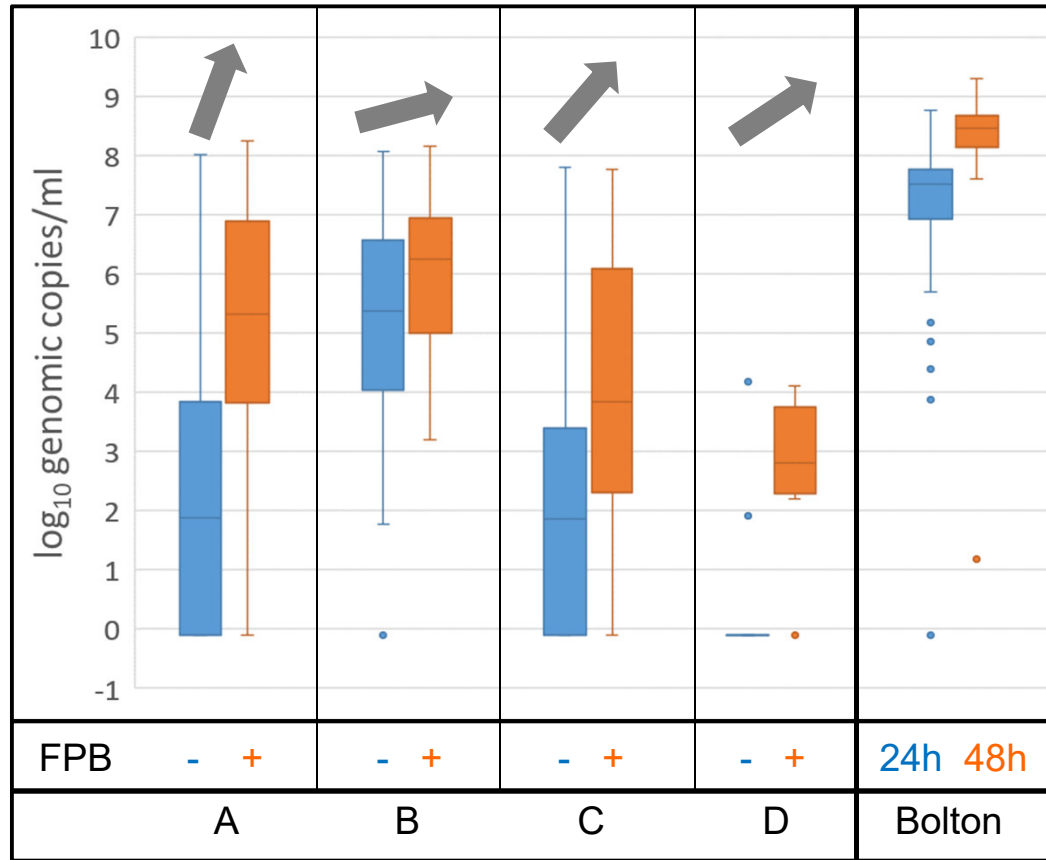


$N_{\text{Preston}} = 50, N_{\text{Bolton}} = 25$

→ Strains were from chicken, turkey, calf, pig, duck, milk and shellfish

→ Addition of FBP growth supplement improved detection of *Campylobacter* spp., in particular of *C. coli* and *C. lari*

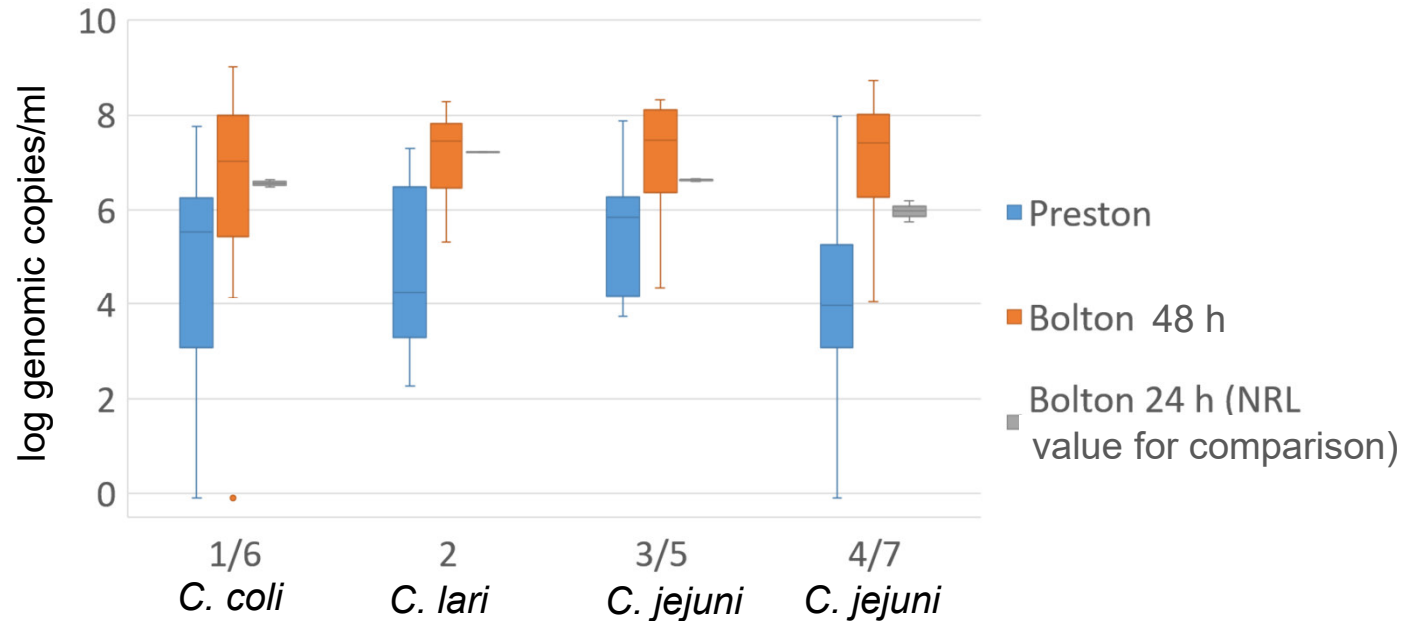
Quantification of *Campylobacter* spp. after enrichment by qPCR



Preston selective supplement of different companies (A-D)

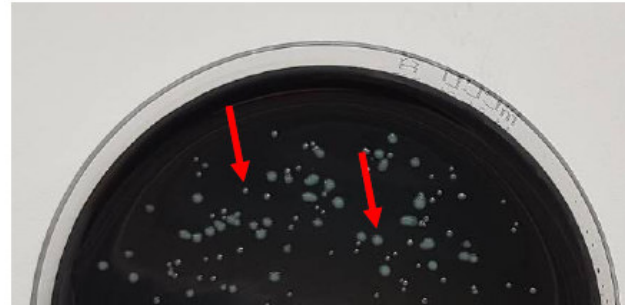
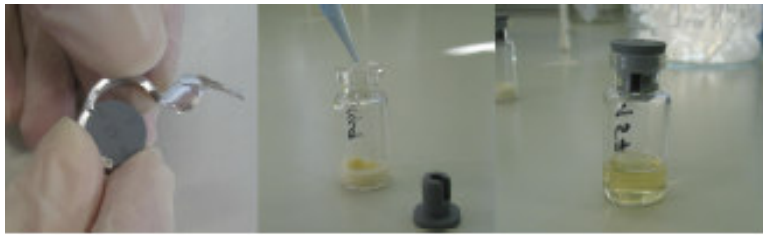
- FBP improves growth in Preston broth – seems to „buffer“ some (not all) of the toxic effects of the Preston selective supplement
- Magnitude of toxic effect and of improvement by FBP is dependent on batch of selective supplement (companies)
- Bolton enrichment is more efficient than Preston enrichment (also after 24 h)

Results of interlaboratory PT with raw milk in Germany in 2020



- Some of the federal state laboratories also performed optional Real-time PCR
- Lower median of the copy number of *Campylobacter* spp. after enrichment in Preston broth than in Bolton
- In this dataset we could not observe any correlation between batch of Preston selective supplement or addition of FBP (number of data limited)

Results of interlaboratory PT with chicken skin in Germany in 2021



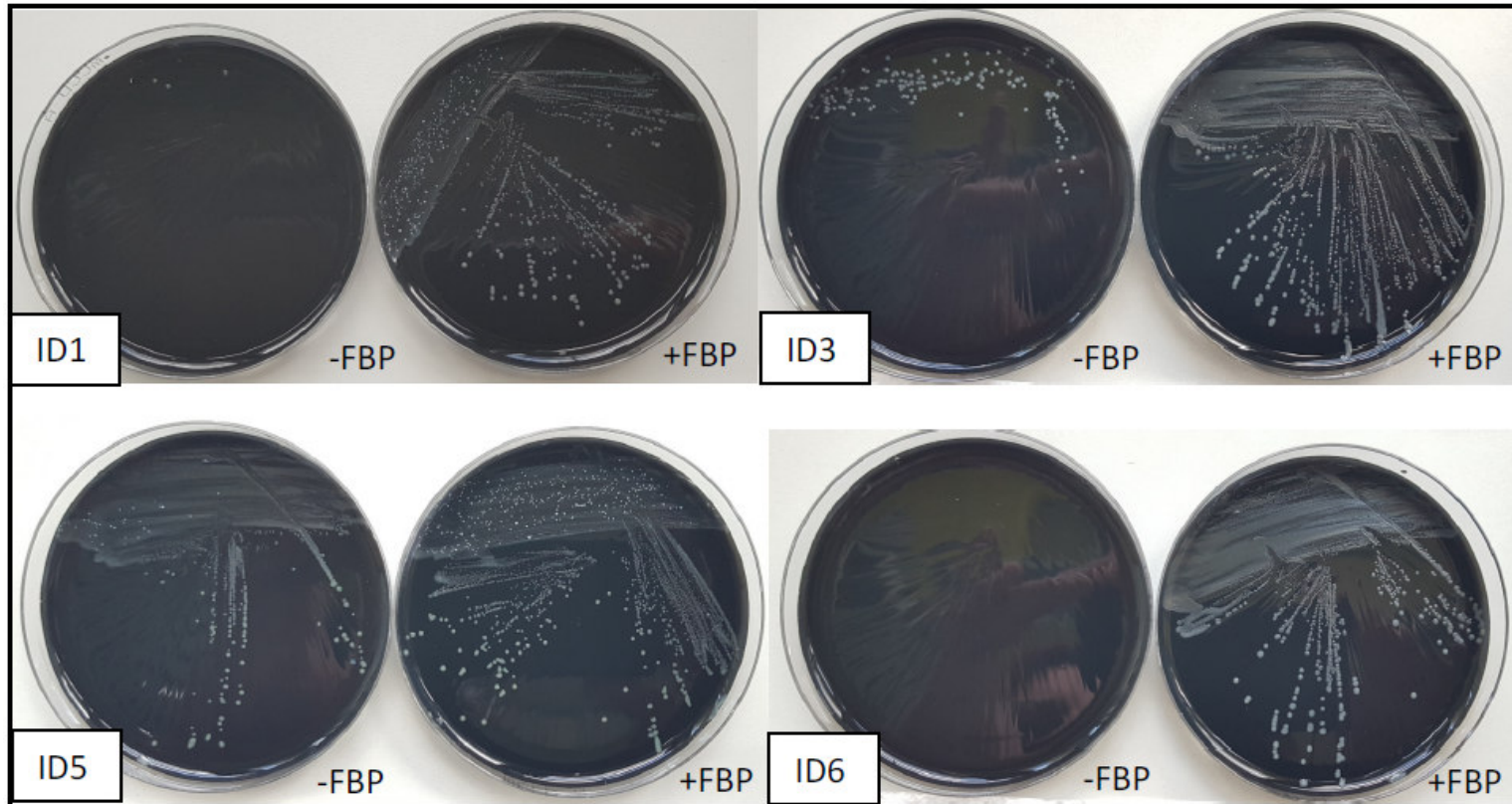
- The federal state laboratories were asked to do a parallel Preston enrichment with and without FPB

Sample ID	Species	Inoculum (log ₁₀ CFU in 10 ml sample and 90 ml enrichment broth)
1	<i>C. lari</i>	4,02
2	<i>C. jejuni</i>	2,83
3	<i>C. coli</i>	4,43
4	Blank	none
5	<i>C. jejuni</i>	3,56
6	<i>C. coli</i>	1,75

+



Pre-experiments in the NRL with and without FBP

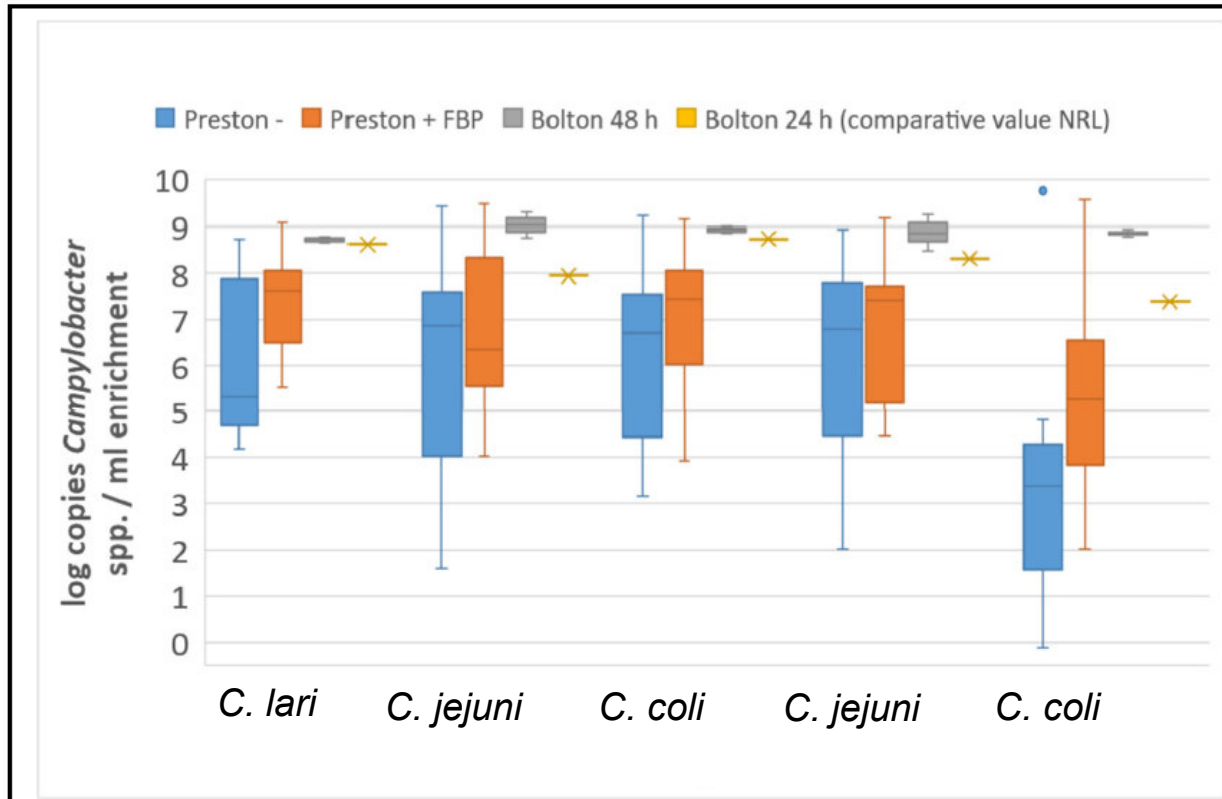


Sample ID	Species
1	<i>C. lari</i>
2	<i>C. jejuni</i>
3	<i>C. coli</i>
4	Blank
5	<i>C. jejuni</i>
6	<i>C. coli</i>

→ Improved detection in the presence of FBP for *C. lari* and *C. coli*

→ day-to-day variability in detection with same batch of selective supplement – matrix effect?

Results of interlaboratory PT with chicken skin in Germany in 2021



→ Overall improved growth in the presence of FBP, in particular for *C. lari* and low inoculum *C. coli* (last sample)

→ Bolton enrichment also after 24 h is more efficient than enrichment in Preston (even + FBP)

- FBP is recommended to be added to Preston broth – enhanced detection expected
- If samples without (or low number of) ESBL-producing background flora are expected, enrichment in Bolton broth should be favored over enrichment in Preston broth

NRL *Campylobacter* Team



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**Thank you for
your attention**