Harmonisation of method: isolation of *Campylobacter* from animal caecum for AMR monitoring

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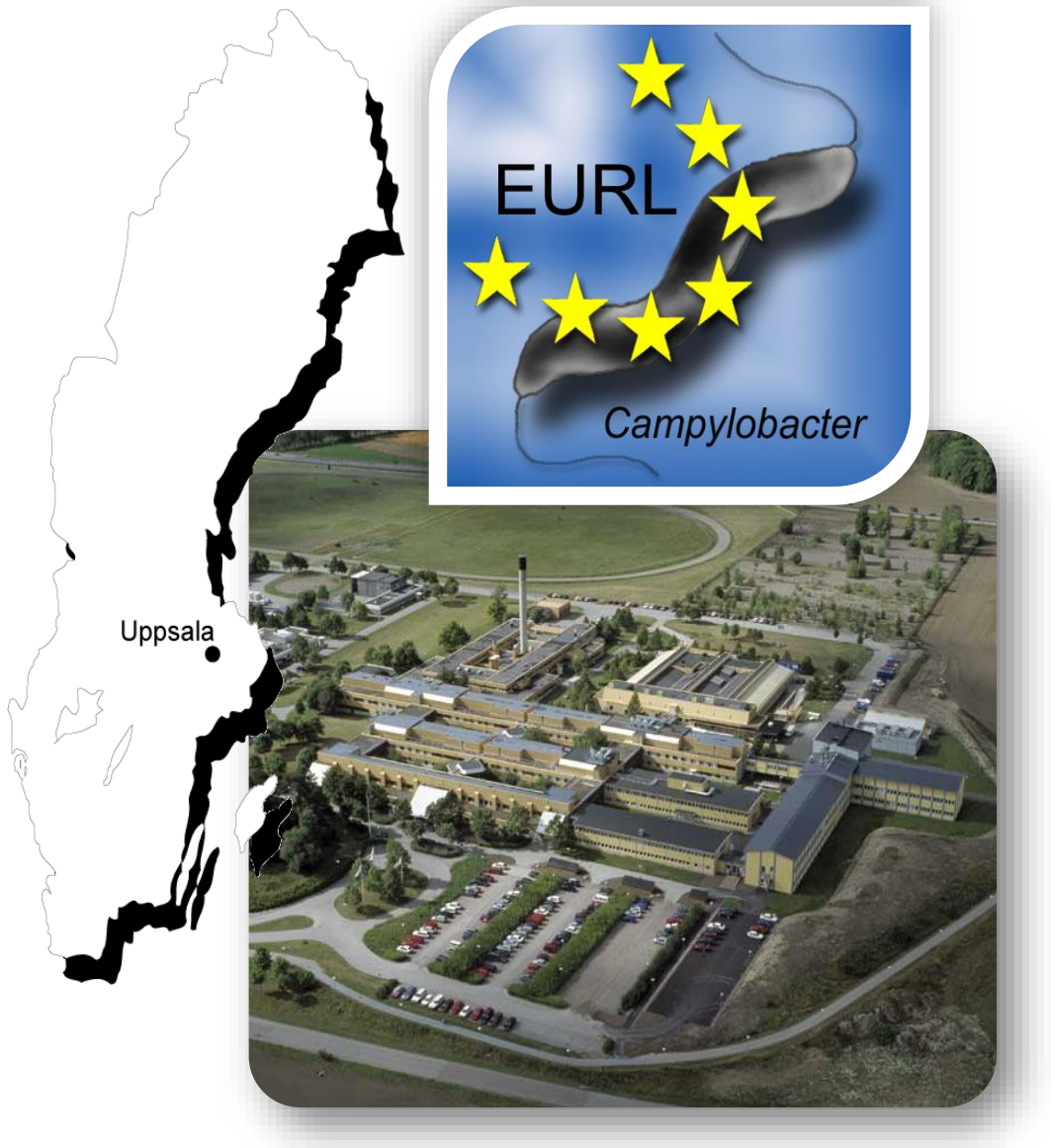
FWD AMR-RefLabCap network meeting



EURL-*Campylobacter*

- Located at the National Veterinary Institute (SVA), Uppsala, Sweden.
- Appointed *EURL-Campylobacter* 2006 by the European Commision. Now organised under DG SANTE G4 Food Hygiene
- Established according to the Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, and the tasks and duties are described in Regulation (EU) 2017/625 on official control
- Currently, 31 NRLs from MS (some MS more than one NRL) + 10 NRLs in third countries (including EFTA, candidate and potential candidate countries)
- Samples: Food and primary production
- The tasks do not include AMR (EURL-AR)

Legal bases for AMR monitoring in food and food producing animals

- **Directive 2003/99/EC** requires Member States (MS) to ensure that monitoring provides comparable data on the occurrence of antimicrobial resistance ('AMR') in zoonotic agents.
  - Also requires MS to assess the trends and sources of AMR in their territory and to transmit a report every year covering data collected in accordance with that Directive to the Commission.

- The **Commission Implementing Decision (EU) 2020/1729**, lays down detailed rules for a harmonised monitoring and reporting of AMR for the period 2021-2027 and replaces the old decision 2013/652/EC.
- In summary (for *Campylobacter*):
  - Sampling: Caeca collected at slaughter from broiler chicken and fattening pigs. Where the national production of meat is high, also from fattening turkeys and bovine animals.
  - Isolates to be tested for AMR: *C. jejuni* and *C. coli* from samples from poultry and bovine, *C. coli* from pig samples

COMMISSION IMPLEMENTING DECISION (EU) 2020/1729

A review was performed of the old implementing decision 2013/652/EC.

- For *Campylobacter*, it was considered highly desirable that the AMR monitoring would be based on harmonised methods for both isolation and antimicrobial susceptibility testing. The goal was to improve the comparability of data concerning the prevalence of *C. jejuni* and *C. coli* in the different MSs and resistance in those two species.
  - Why? Differences between *Campylobacter* isolation methods used in the MSs can affect the recovery of *Campylobacter* spp. from samples, the proportions of *C. jejuni* or *C. coli* obtained and even the diversity of the isolates recovered, including the AMR.
  - How? EFSA put together an ad hoc WG of scientific experts, working in close liaison with the EURL-AR. The WG performed a specific questionnaire survey on AMR monitoring for the NRL-ARs and made a proposal on a harmonised process of isolation of *Campylobacter* spp. within the framework of the AMR monitoring.

Questionnaire main findings concerning *Campylobacter*

In total, 27 MSs and 4 non-MSs answered the questionnaire[1].

Topics	Example of findings
Sampling procedure	Variabilities in the number of samples collected and pooled per slaughter batch, time between sampling and start of analysis, temperature for transportation of samples to the laboratories.
Detection method	Direct plating most common, but some used enrichment Variabilities in type of selective media, and if one or two medias were used
Species identification	Biochemical methods, MALDI-TOF MS, PCR metods
Number of colonies to be confirmed	Differences amongst laboratories, 1-5 colonies
Use of ISO 10272-1[2]	21 MSs reported use of ISO 10272-1 in some context, and 19 reported on accreditation for the standard

In total, 27 MSs and 4 non-MSs answered the questionnaire.

[1]EFSA technical specification. Journal 2019;17(5):5709
[2]EN ISO 10272-1:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 1: Detection method

A proposal for a harmonised protocol

EFSA technical specification. Journal 2019;17(5):5709

- Appendix N – Harmonised method for isolation, identification and storage of *Campylobacter jejuni* and/or *C. coli* from broilers, fattening turkeys, fattening pigs and calves

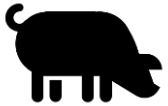
This proposal is based on ISO 10272-1:2017* but leaves some specifications to be adressed by the EURL-*Campylobacter*

- The standard does not include sampling. What is the optimal time interval between sampling and start of laboratory analysis?
- The standard leaves the possibility open to use one or two selective medias, and only specifies the use of mCCDA. Is the use of a 2nd selective medium beneficial for detection of *C. jejuni* and *C. coli*? If so, which of Preston and Butzler selective media, is in combination with mCCDA most beneficial for detection of *C. jejuni* and *C. coli?*
- According to the standard: “Select up to 5 typical or suspect *Campylobacter* colony for purification and confirmation”. How many colonies need to be picked from the selective medium/media to find both *C. jejuni* and *C. coli* if they are both present?

*EN ISO 10272-1:2017 Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 1: Detection method

Outline studies organised by the EURL-*Campylobacter* 2019-2020

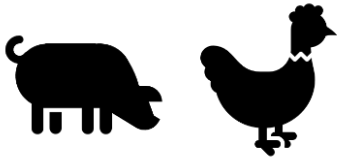
Study 1 – EURL



- **Optimal time interval between sampling and start of analysis**

Caecal contents of fattening pigs collected at 5 time points

Study 2 – NRLs & EURL



- **2nd selective medium**
- **Number of colonies to be analysed**

Caecal contents of chicken and fattening pigs.

Participating NRLs;

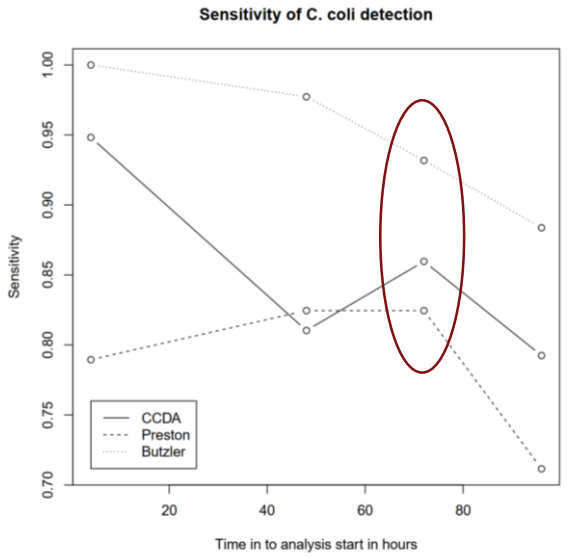
Belgium, Ireland, Italy, Rumania and Spain

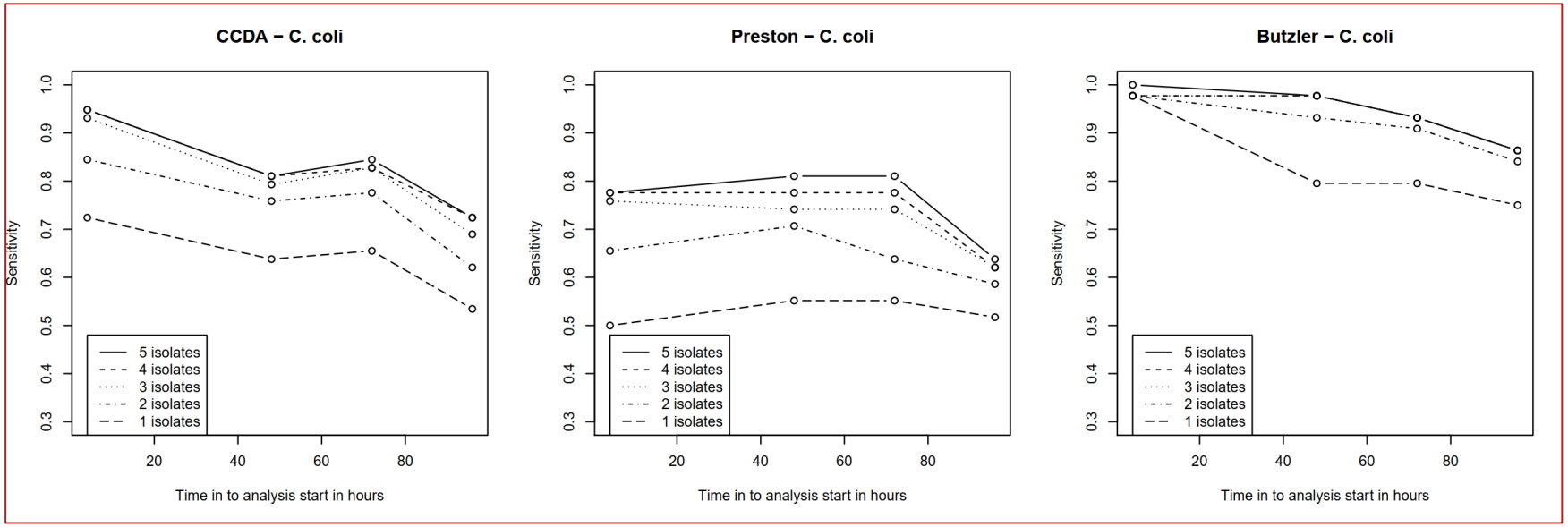
Procedure

Used the harmonised protocol (in EFSA technical specification) with some additional specifications.

- For study 1, analysis was started at 4, 48, 72 and 96 hours after sampling. For study 2, analysis was started within 72 hours after sampling.
- Plating onto 3 media: mCCDA, Preston and Butzler (all medias produced at SVA).
- From each plate, 5 typical colonies were selected for identification.
- Logistic regression (including a nested random intercept for repeated measures on each sample and the effect of the country) was used to study the association between media type, storage time, number of selected colonies and the probability of detection.

Sensitivity of the detection of *C. coli* in pig caecal samples over time – study 1

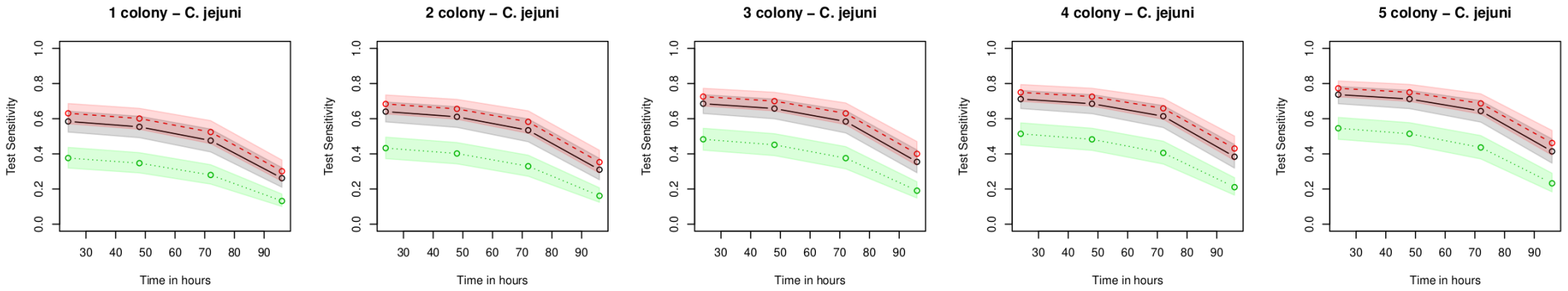




Conclusion:

- The combined results show that the biggest decrease in sensitivity of detecting *C. coli* in pig caecal samples occurs after 72 hours.
- But, using Butzler agar, and picking more than one colony, the sensitivity is still high at 96 hours after sampling.

Sensitivity of detection of *C. jejuni* in pig and chicken caecal samples - study 1 and 2



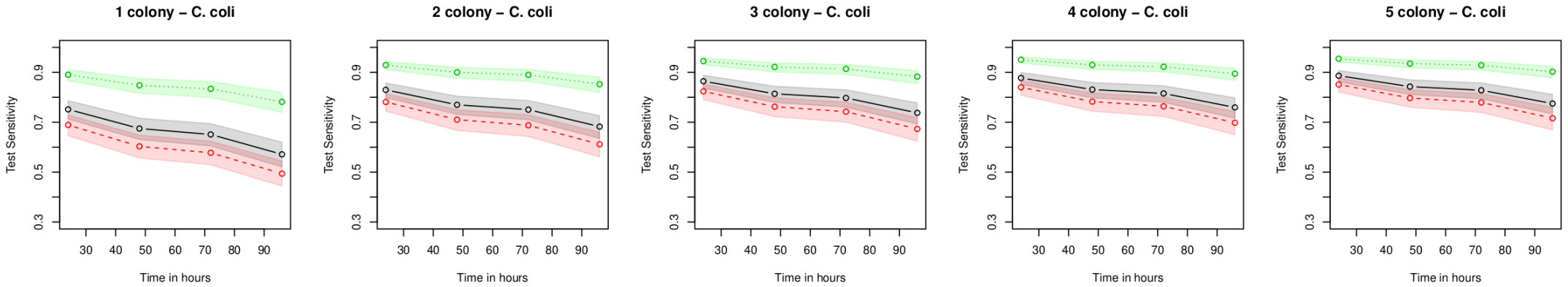
Green line = Butzler agar
Red line = Preston agar
Black line= mCCDA

Conclusions:

- The performance of Butzler was significantly lower than mCCDA in detecting *C. jejuni.*
- Picking more than 1 colony from each plate was associated with increased sensitivity in detecting *C. jejuni* and each additional colony selected consistently increased sensitivity up to five colonies*.

*Picking two (OR=1.66, CI=1.14–2.41), three (OR=2.47, CI=1.7–3.6), four (OR=3.16, CI=2.16–4.61) or five (OR=4.0, CI=2.75–5.9) colonies from each plate for confirmation was associated with increased sensitivity when compared to picking only one colony.

Sensitivity of detection of *C. coli* in pig and chicken caecal samples - study 1 and 2



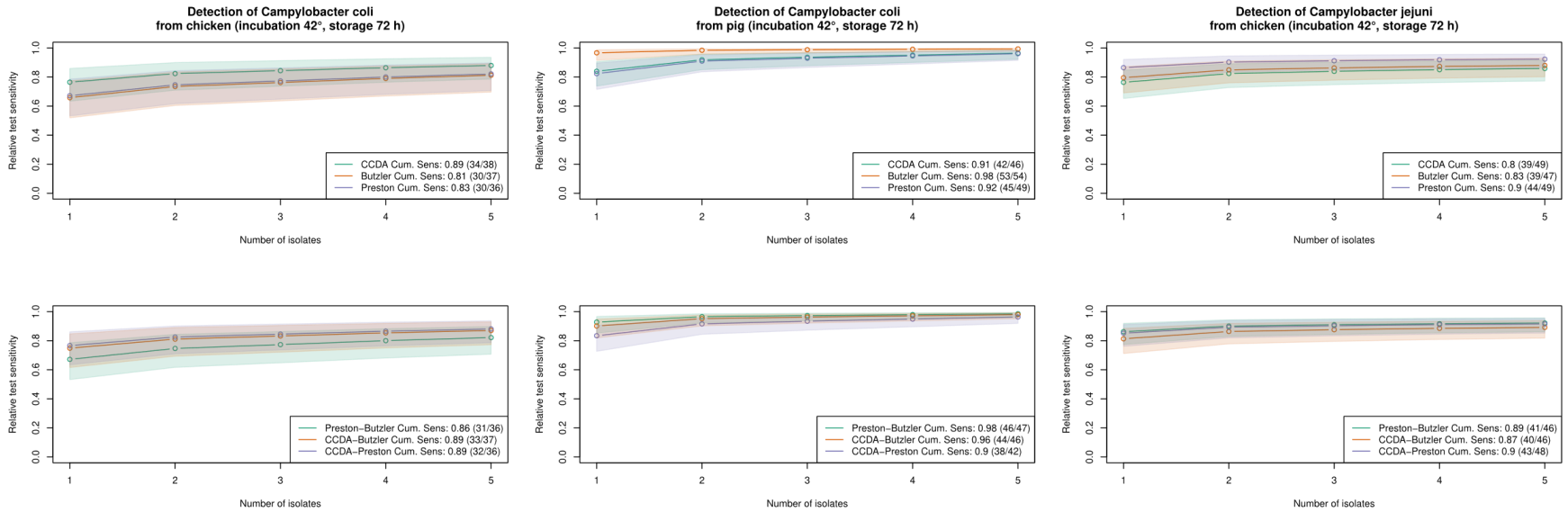
Green line = Butzler agar
Red line = Preston agar
Black line= mCCDA

Conclusions:

- The performance of Butzler was significantly higher than mCCDA in detecting *C. coli.*
- The performance of Preston was significantly lower than mCCDA in detecting *C. coli.*
- Picking more than 1 colony from each plate was associated with increased sensitivity in detecting *C. coli* and each additional colony selected consistently increased sensitivity further up to five colonies*.

*Picking two (OR = 2.19, CI = 1.69–2.82), three (OR = 3.33, CI = 2.54–4.34), four (OR = 3.99, CI = 3.04–5.24) or five (OR = 4.56, CI = 3.45–6.02) colonies from each plate for confirmation was associated with increased sensitivity when compared to picking only one colony.

Sensitivity of detection of *C. jejuni* and *C. coli* using combinations of media



Conclusions:

- The sensitivity in detecting *C. jejuni* in chicken samples and *C. coli* in pig samples is increased when using Butzler agar in combination with mCCDA compared to using only mCCDA.
- In these studies swarming cultures or overgrowth did not affect the no of typical colonies that could be picked per plate. But this can be a real issue, so using two selective plates increaes the probability of detecting *C. jejuni* and *C. coli* if one plate is non-usable.

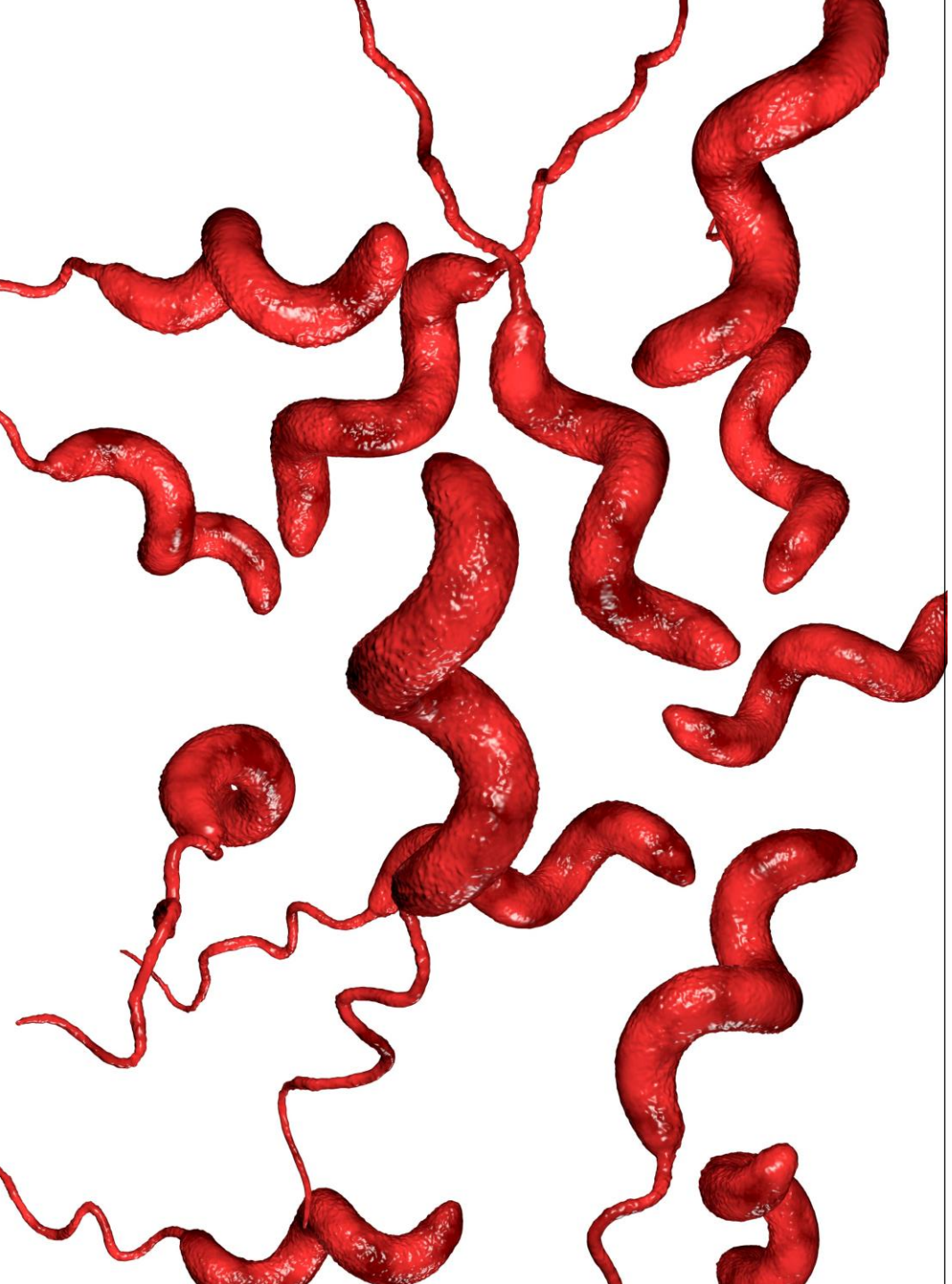
Conclusions

Specifications included into the harmonised protocol;

- Time interval between sampling and start of analysis preferably within 72 hours, but acceptable up to 96 h
- A combination of mCCDA and Butzler agar
- Select in total 4 presumtive colonies for identification from poultry and bovine samples (both *C. jejuni* and *C. coli* if present), and 2 presumptive colonies from pig samples (only *C. coli*)

The harmonised protocol and contact list to NRLs found at;

http://www.sva.se/eurl-campylobacter



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Thank you for your attention!

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