HARMONISED PROTOCOL FOR DETECTION OF CAMPYLOBACTER FOR MONITORING OF ANTIMICROBIAL RESISTANCE

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EURL-Campylobacter

BACKGROUND

- Monitoring of antimicrobial resistance (AMR) directive 2003/99/EC
  - COM decision 2013/652/EC
    - details rules for monitoring and reporting of AMR
    - valid from 2014 to the end of 2020
  - A review of the implementing legislation on AMR
    - EFSA was requested to update technical specification on
      - harmonised monitoring of AMR
        - *Salmonella, Campylobacter,* indicator *E. coli* and *Enterococcus* transmitted via food
        - MRSA in animals and food
      - randomised sampling for harmonised monitoring of AMR
    - EFSA Journal 2019;17(5):5709, 124 pp. doi:10.2903/j.efsa.2019.5709

MONITORING OF CAMPYLOBACTER

- Decision 2013/652/EC
  - Monitoring every 2nd year
  - Animal species
    - Broilers, turkeys*
      - Matrix: caecal contents
      - *C. jejuni* mandatory
      - *C. coli* voluntary
  - Target number of isolates
    - 170
    - 85
      - if production < 100 000 tonnes/year

- Proposal EFSA + COM
  - Monitoring every 2nd year
  - Animal species
    - Broilers, turkeys*, cattle < 1 year*, slaughter pigs
      - Matrix: caecal contents
      - More prevalent species *C.jejuni/C. coli,* for pigs *C. coli*
  - Target number of isolates
    - 170
    - 85
      - if production < 100 000 tonnes/year

*production > 10 000 tonnes/year

CAMPYLOBACTER SURVEY

- A questionnaire performed by the EURL-AMR and EFSA on laboratory routines on detection of microbes used for AMR monitoring in June 2018

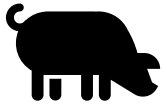
MAIN FINDINGS OF THE QUESTIONNAIRE STUDY

Topics	Findings
Sampling procedure	Variabilities in the number of samples collected and pooled (1-10) per slaughter batch. In 8 MSs, a caecal sample from one broiler, in 15 MSs, a pool of caeca from 10 birds/slaughter batch
Detection method	Direct plating, without enrichment in 23 MSs Two selective media in 16 MSs Karmali, Preston, blood agar, CFA, CASA or Skirrow used as second selective medium
Over weekend culturing	Differences between laboratories in the procedures used for cultures over weekend periods
Species identification	MALDI-TOF or PCR (Denis et al 1999) most common Microscopy and/or oxidase, catalase, hippurate, indoxyl acetate tests
Number of colonies	Variabilities between laboratories, 1-5 colonies
Use of ISO 10272-1	Most (n=21 MSs) used ISO 20272-1, 19 MSs accredited for the method

AIMS

- To investigate the optimal time interval between sampling and start of laboratory analysis
- To define a 2nd selective medium for detection of *C. jejuni* and *C. coli* in addition to mCCDA
- To assess the number of colonies (1 to 5) to be confirmed from the selective medium

OUTLINE



- Study 1 – EURL
  - Pig caeca
  - Optimal time interval between sampling and start of analysis
  - 2nd selective medium
  - Number of colonies to be picked and confirmed

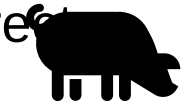
- Study 2 – NRLs & EURL
  - Pig and chicken caeca
  - 2nd selective medium
  - Number of colonies to be picked and confirmed

STUDY 1 - SAMPLING

- 15 pigs from one slaughter batch sampled at an abattoir in Uppsala at five time points
  - September 16, 2019
  - October 14, 2019
  - November 10, 2019
  - December 2, 2019
  - June 9, 2020
- Caecal contents aseptically collected and placed in a clean jar
- Transport to the laboratory of the EURL-Campylobacter within 2 h

STUDY 1 – LABORATORY ANALYSIS

- Analysis according to ISO 10272:2017- part 1, procedure C by using direct plating except for

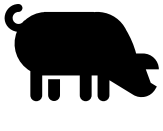


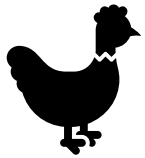
  - plating onto 3 media: mCCDA (Oxoid), Preston (in-house) and Butzler (Oxoid)

  - incubation at 37 °C for 48 h (samplings 1-4), sampling 5 incubated at 41.5 °C for 48 h

- Confirmation MALDI-TOF

STUDY 2 - SAMPLING





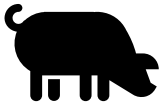
- 20 pigs from one slaughter batch sampled at an abattoir in Italy, Spain and Sweden in June 2020

- 20 samples, caeca of 10 chicken per slaughter batch pooled to one, sampled at an abattoir in Belgium, Ireland, Italy, Romania and Spain in June-July 2020

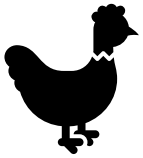
- Transport to the laboratory of the NRL-*Campylobacter* in IT, ES, BE, IE, RO and the EURL-*Campylobacter*, respectively, within 72 h

STUDY 2 – LABORATORY ANALYSIS

- Analysis according to ISO 10272:2017- part 1, procedure C using direct plating except for
  - plating onto 3 media: mCCDA (Oxoid), Preston (in-house) and Butzler (Oxoid)



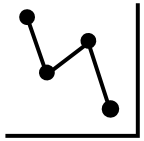
- Selective media provided by the EURL-Campylobacter to all participating laboratories
  - dispatched on June 8, arrival on June 9
  - dispatched on June 22, arrival on June 23 or 24



- Confirmation by MALDI-TOF (3 laboratories) or PCR* (3 laboratories)

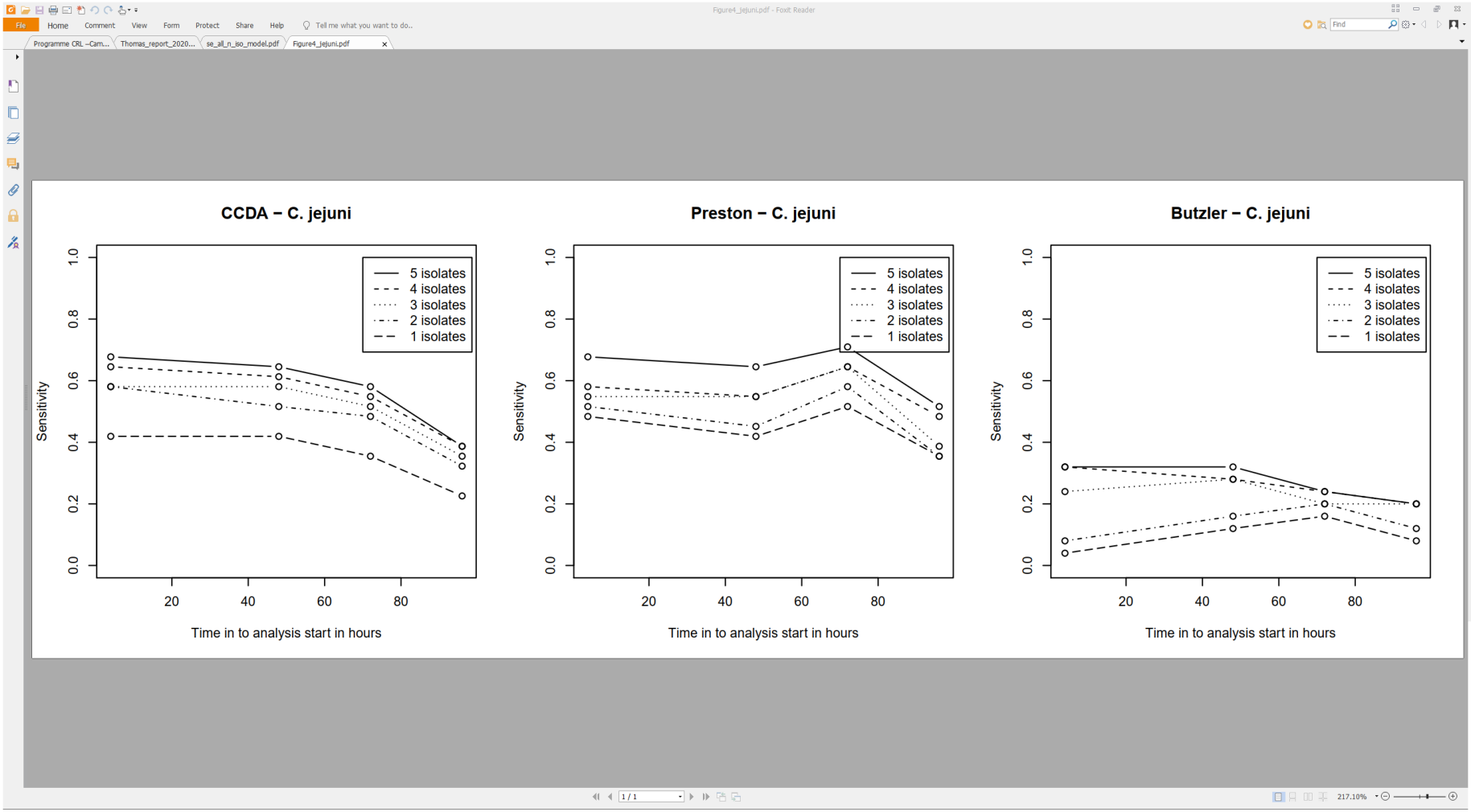
*Denis et al.1999, Wang et al. 2002

STATISTICAL ANALYSIS

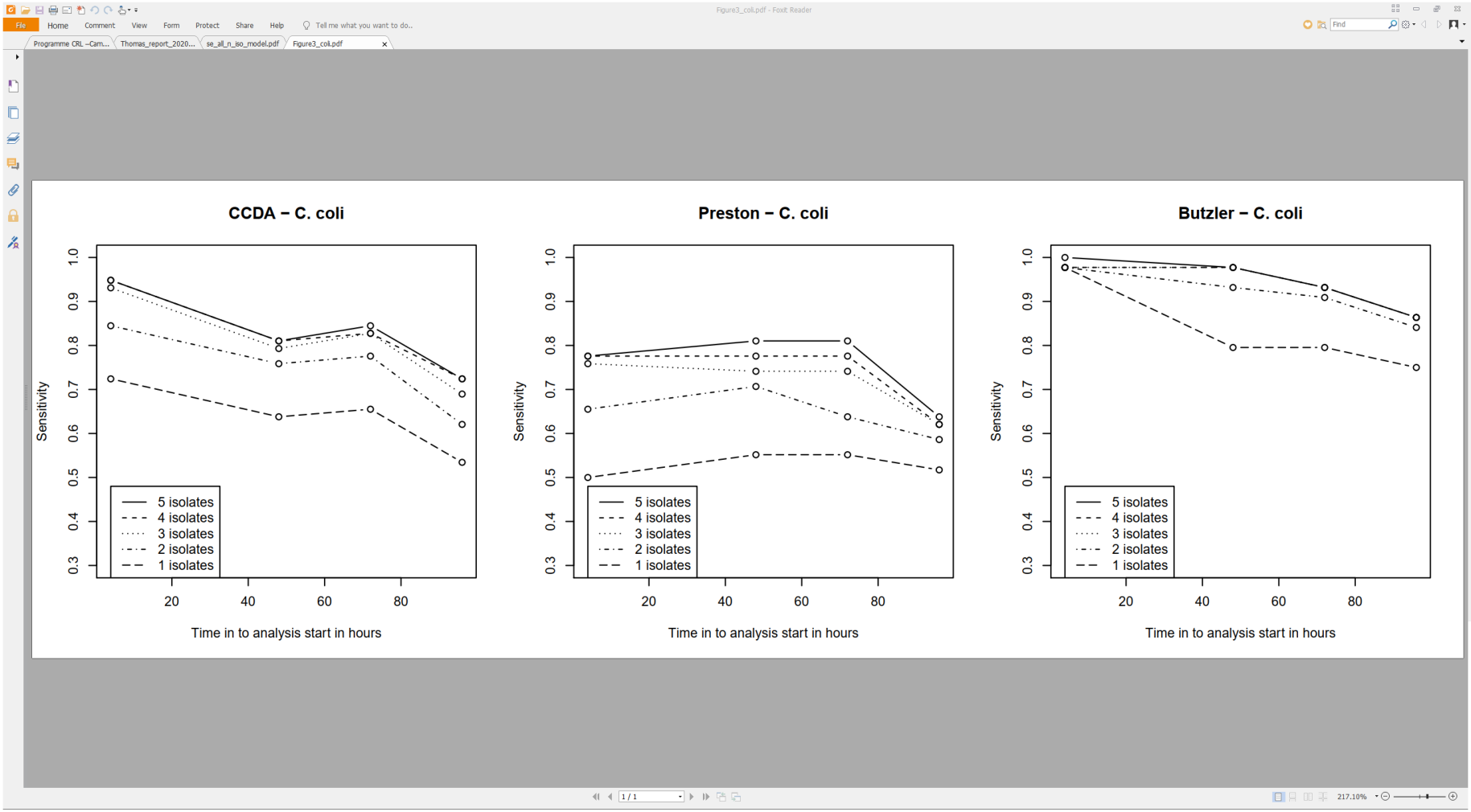


- A positive sample
  - *C. jejuni* or *C. coli* was detected in any of the 60 isolates (12 plates * 5 colonies) from a given sample
- A negative sample
  - No *C. jejuni* or *C. coli* detected
- Association between media type, storage time, number of selected colonies and the probability of detection tested using logistic regression including a nested random intercept for repeated measures on each sample and the effect of the country
- A pair-wise comparison of the selective media in progress
- Data analysis performed in R version 4.0.2

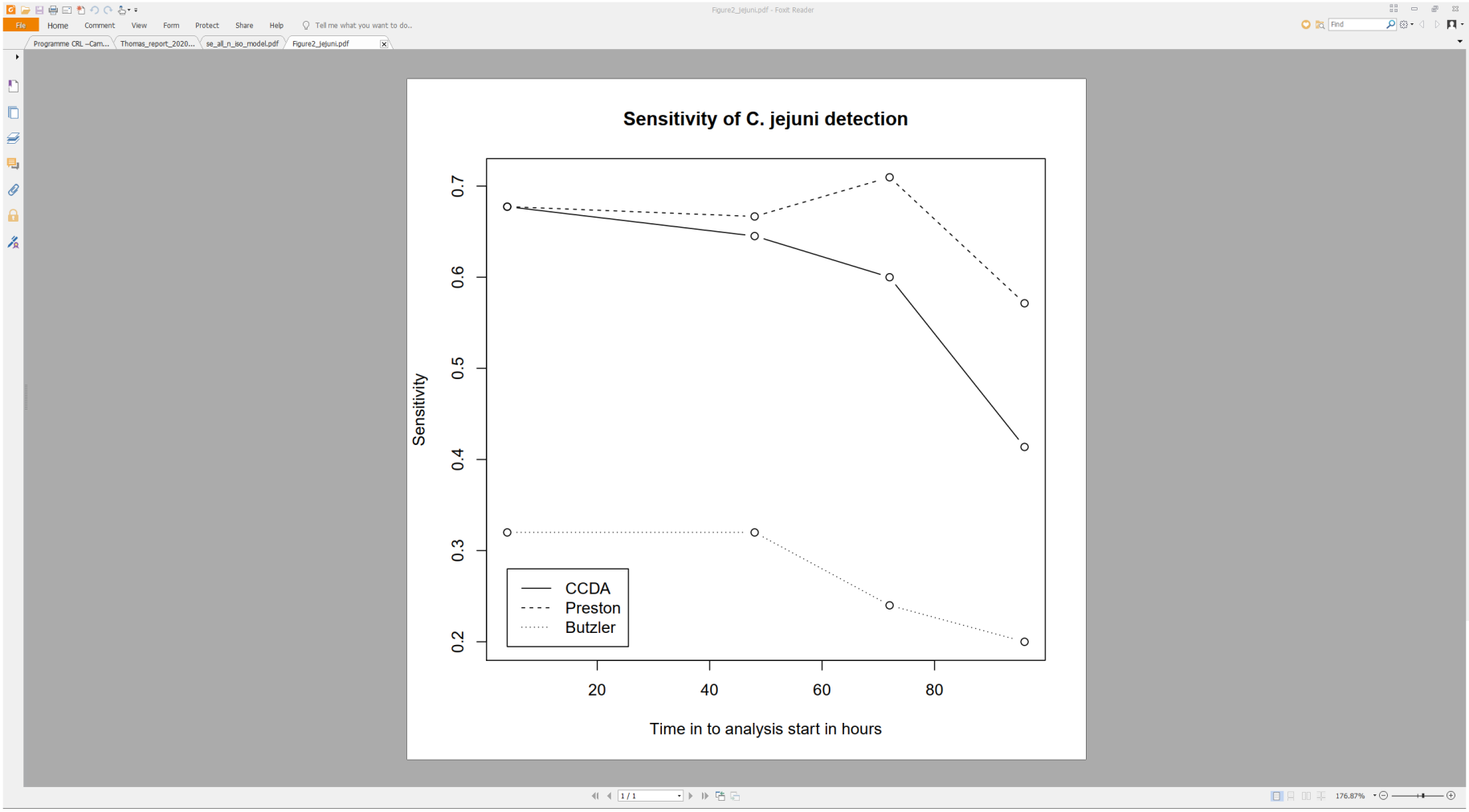
DETECTION OF *C. JEJUNI* FROM PORCINE CAECAL SAMPLES – STUDY 1



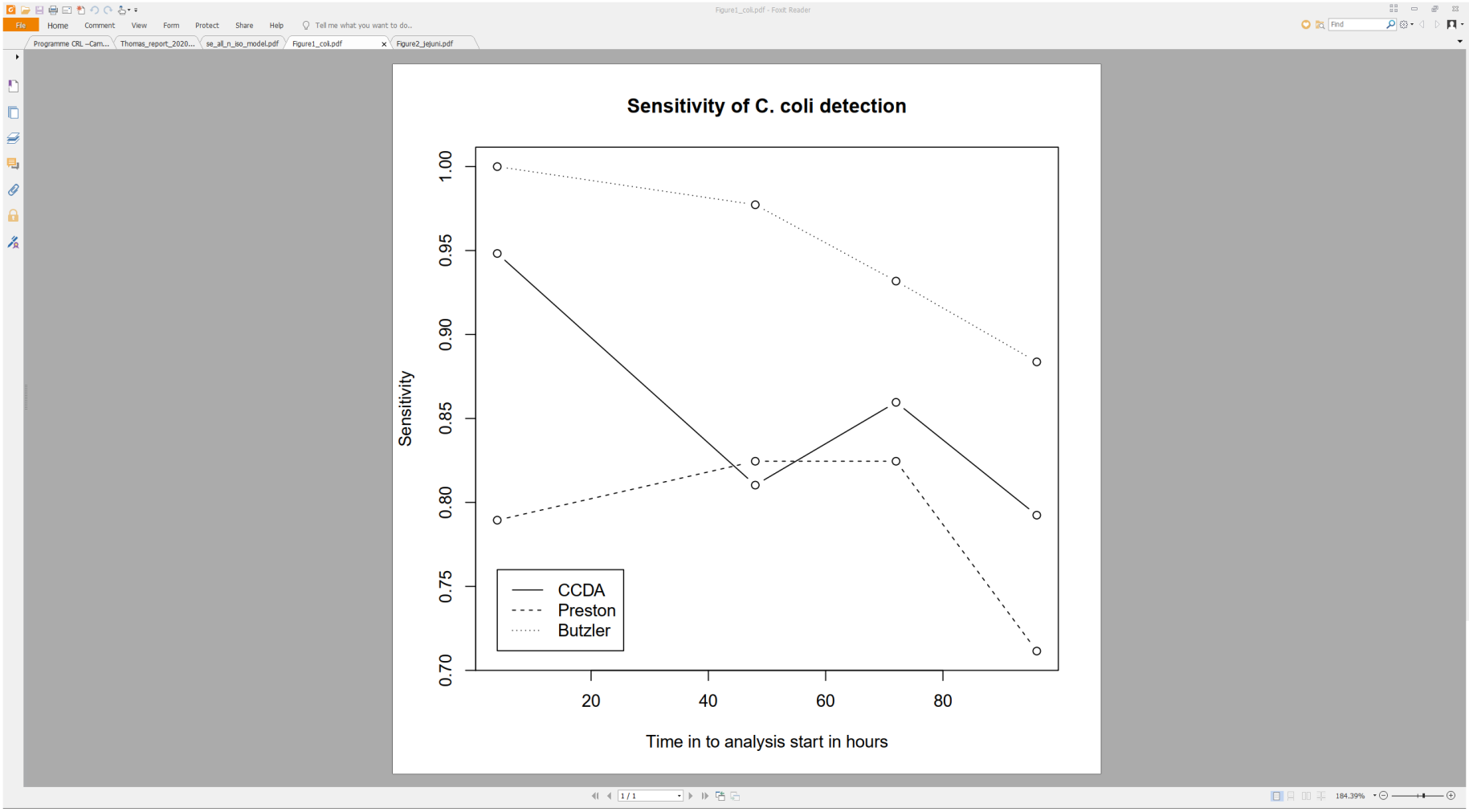
DETECTION OF *C. COLI* FROM PORCINE CAECAL SAMPLES – STUDY 1



SENSITIVITY OF THE DETECTION OF *C. JEJUNI* FROM PORCINE SAMPLES –STUDY 1



SENSITIVITY OF THE DETECTION OF *C. COLI* IN PORCINE CAECAL SAMPLES – STUDY 1



SPECIES DISTRIBUTION- STUDY 2

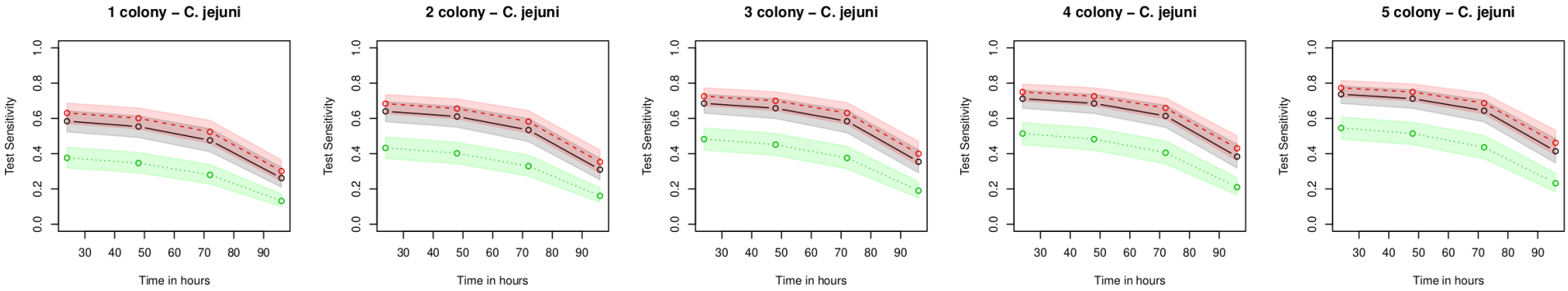
Chicken samples

- *Campylobacter* spp. detected in 73 of the 100 samples tested
- *C. jejuni* most common among the isolates (54.6%, n=547)
- *C. coli* (43.9%, n=478)
- Other *Campylobacter* (0.10%, n=1)

Pig samples

- *Campylobacter* spp. detected in 55 of the 60 samples tested
- *C. coli* dominated among the isolates (92.6%, n=586)
- *C. lari* (6.3%, n=40)
- *C. jejuni* (1.1%, n=7)

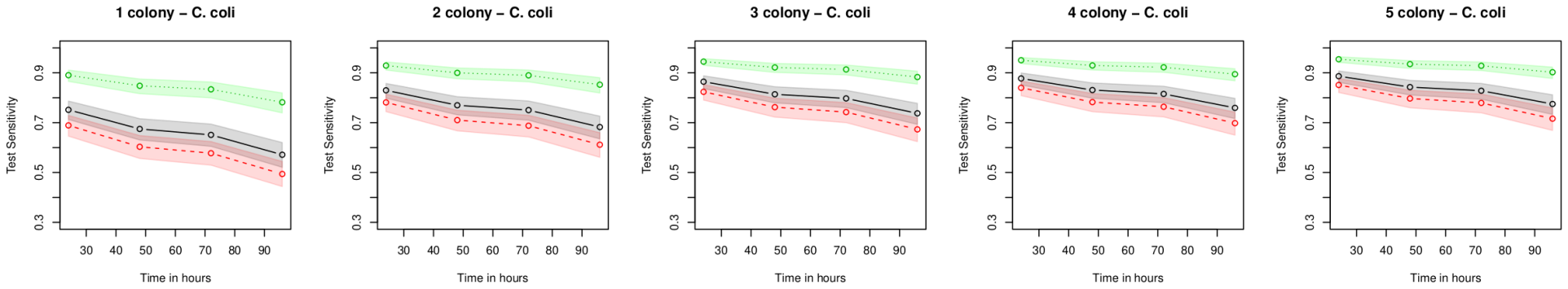
DETECTION OF *C. JEJUNI* IN PORCINE AND CHICKEN CAECAL SAMPLES WHEN 1-5 COLONIES WERE PICKED AND CONFIRMED



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

Data compiled from studies 1 and 2

DETECTION OF *C. COLI* IN PORCINE AND CHICKEN CAECAL SAMPLES WHEN 1-5 COLONIES WERE PICKED AND CONFIRMED



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

Data compiled from studies 1 and 2

CONCLUSIONS

- Time interval between sampling and start of analysis preferably shorter than 96 h
- A combination of mCCDA and Butzler seems to be most optimal for detection of *C. jejuni* and *C. coli*
- 3 presumtive colonies could be confirmed per selective medium
- A pair-wise comparison is in progress

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Questions?

Comments!