



Detecting *Campylobacter* in the food chain— An Olympian Challenge!

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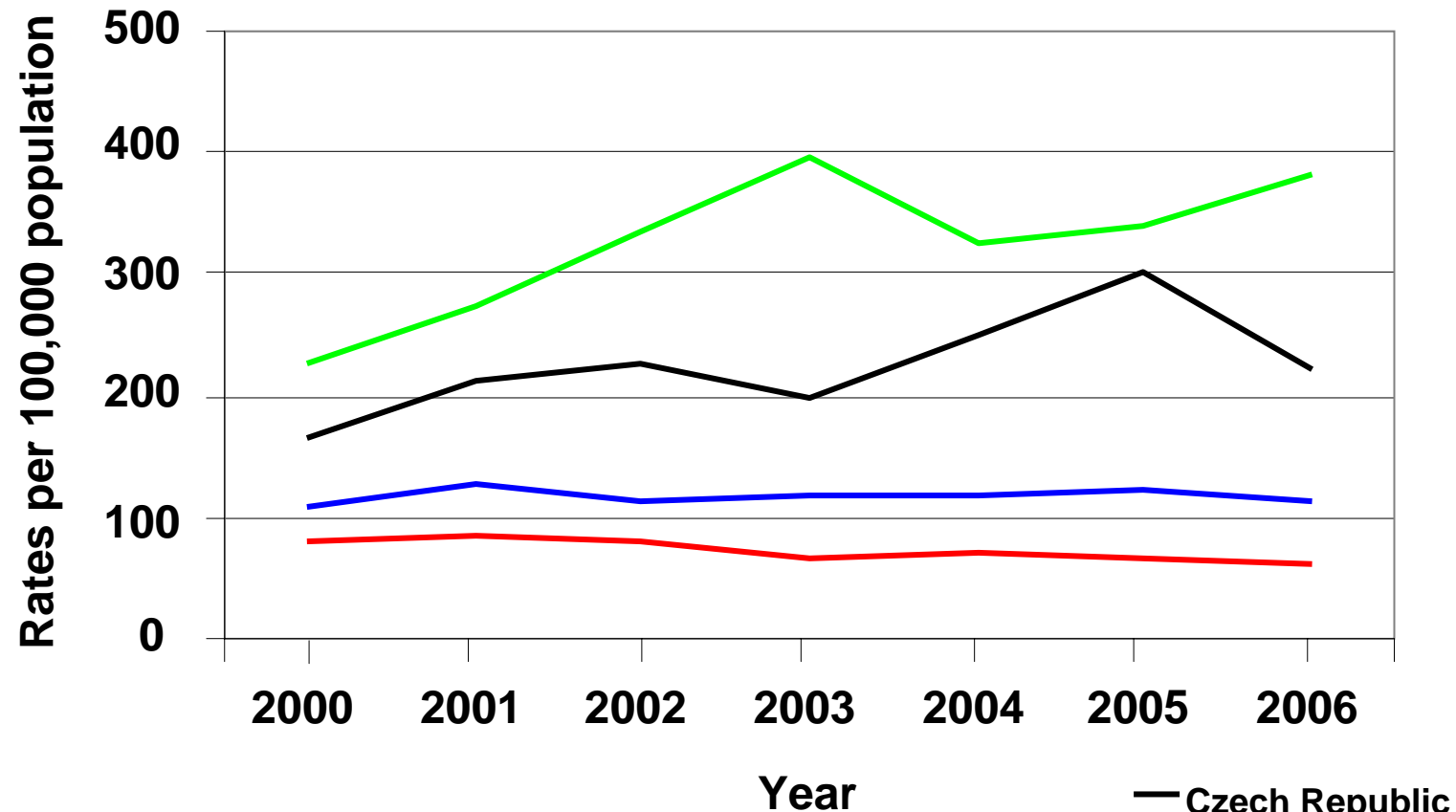
Christchurch, New Zealand

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protecting people and their environment through science

Campylobacter rates per 100,000 population overseas.

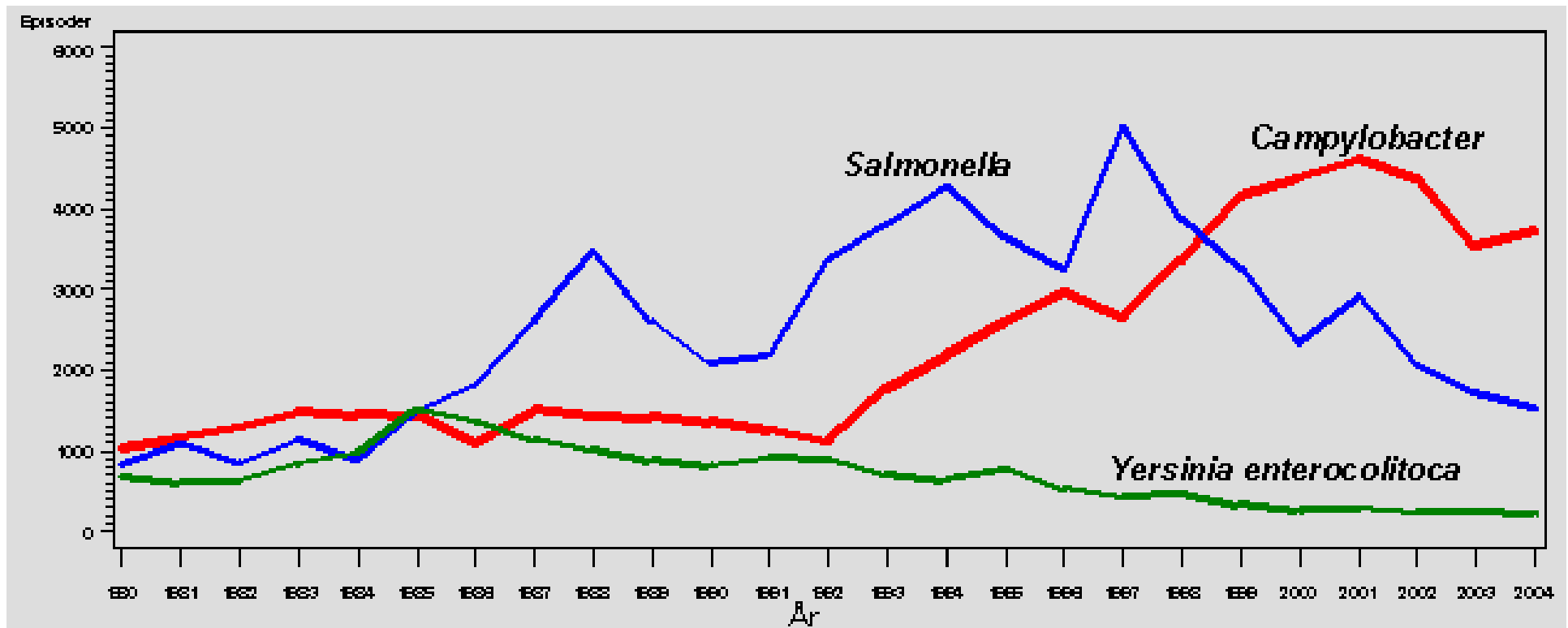


Data derived from EFSA annual reports (Europe); ESR Annual reports (New Zealand); OzFoodNet (Australia, excluding New South Wales)

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Denmark – the best case scenario?!



Human cases of common zoonoses in Denmark 1980-2004

The distribution of campylobacters: **EVERYWHERE!**

(Chickens, ducks, geese, turkeys, ostriches, cattle, deer, pigs, dogs, cats, sparrows, terns, seals, penguins, reptiles...)

(and their products...we eat most of them!)

(and their environments...)

Detecting campylobacters in foods is vital for public health protection, evaluation of intervention strategies and identification of outbreaks

Basic difficulties in isolation

- **Specific atmospheric requirements**
- **Relatively slow growing**
- **Competitive flora**
- **Low numbers, stressed condition (not clinical samples)**

The atmosphere for growth

- **Most Campylobacters are microaerophilic – require 3-6% O₂ in the atmosphere for growth**
- **Too little, growth is impaired or does not occur**
- **Too much and the bacteria are killed**
- **Some species (not thermophilic species) require H₂ for growth; all species are enhanced with H₂**
- **Problem: H₂ and O₂ is an explosive mixture – getting the balance correct is a challenge**

Creating the atmosphere



The perfect atmosphere?

- **EC project “Campycheck” – aimed to develop methods for all Campylobacter species**
- **3 year project**
- **Independent laboratories suggested that a gas composition of 3% O₂, 7% H₂, 10% CO₂, 80% N₂ would support growth of all 17 campylobacter species tested**

Commonly used enrichment media

Medium	Nutrient base	Antibiotics	Authors	Remarks
Preston	Nutrient broth, lysed blood	Trim, PolyB, Rif, Cyclohex	Bolton & Robertson 1982	Modified Bolton et al 1984; ISO
Exeter	Nutrient broth, FBP, lysed blood	Trim, Cefo, Colistin, Vanco, Rif, Amphot.	Humphrey 1986	Aerobic incubation; UK HPA protocol
Bolton	Peptones, yeast extract, lysed blood	Cefo, Vanco, Cyclohex	Bolton 1995	US FDA protocol
C.E.B.	Brucella broth	Cefo, Trim, 5-FU	Martin et al 1983	
Park & Sanders	Brucella broth, lysed blood	Cefo, Trim, Vanco, Ampho	Park and Sanders 1981	ISO; aerobic incubation modification

A summary of selective media

Table 3
Formulation of liquid and solid selective media for thermophilic campylobacters (concentrations in mg per litre unless otherwise stated)

Medium name/ reference. Other details	Dual medium	Antioxygen system	Cephalo- sporins	Trimetho- prim	Polymyxin B or Colistin (C) in μ	Vanco- mycin or Teico- planin (T)	Rifam- picin	Novo- bincin	5-Fluoro- uracil or Na-deoxy- cholate (D)	Bacitracin in μ	Anti- fungals
Dekeyser A ¹	TA	15% S			10000			5		25000	50 CY
Skirrow A ^{2a}	BAB	7% LH		5	2500	10					
Blaser A ³	BA	10% S		5	2500	10		2			
Butzler A ⁴	TB	10% S	15 cthin		10000 (C)			5		25000	50 CY
CAMPY-BAP A ⁵	BA	10% S	15 cthin	5	2500	10					2 AM
Blaser-Wang A ⁶	BAB	7% LH	15 cthin	5	2500	10					2 AM
C-2 A ⁷	BA	5% H/FBP	5 cthin	5	8000	10					
C-3 A ⁷	BA	5% H/FBP	1 cthin	5	2500	10					
Park (1981) B ⁸	BB	-		4	8000	8					
BU40 B ⁹	TB	10% S	15 cthin		40000 (C)			5		25000	50 CY
Rosef B ^{10a}	NB	-		5	2500	10					
Preston A ^{11a}	NB	5% LH		10	5000		10				100 CY
Preston B ^{11b}	NB	5% LH/FRP		10	5000		10				100 CY
Doyle-Roman B ^{12,c}	BB	7% LH		5	20000	15					50 CY
Christopher B + A ¹³	BB	0.5g P	15 cthin	5	2500	10					2 AM
FBP-AM B/A ¹⁴	BB/A	FBP (B) 5% DH (A)	15 cthin	5	2500	10					2 AM
Lander B ¹⁵	VI	5g C		10	50000	40					100 CY
Park-Stankiewicz B ^{16,d}	BB	2g F, 0.25g B, 0.5g P	30 cthin	4	3 mg (C)	8			100		100 CY
Butzler medium Virion A ¹⁷	CA	5-7% S	15 czone		10000 (C)		10				2 AM
Butzler medium Oxoid A ¹⁸	CA	5-7% S	15 crolin		10000 (C)	5				25000	50 CY
Modified Park (1981) B ¹⁹	BB	FBP		7.5	5000	15					
CEB B ²⁰	BB	-	32 czone	32					333		
VTP-FBP B ²¹	BB	FBP, 5% LH		10	5000	20					
CAK A ²²	GA	7% H	15 cthin								1 AM
Rosef-Kapperud A ²³	GA, IVs	-	15 cthin		10000 (C)						25000 μ NY
ATB ^{24,e}	T, YE, NaCl	FBP, HT	6.25 codin		20000		25				
CCD A ^{25,f}	NB	4g C, 0.25g F, 0.25g P	10 czolin						1000 (D)		
CCD B ^{25,f}	NB	4g C, 0.25g FBP	10 caofin						1000 (D)		

Corry et al., 1995

A summary of selective media

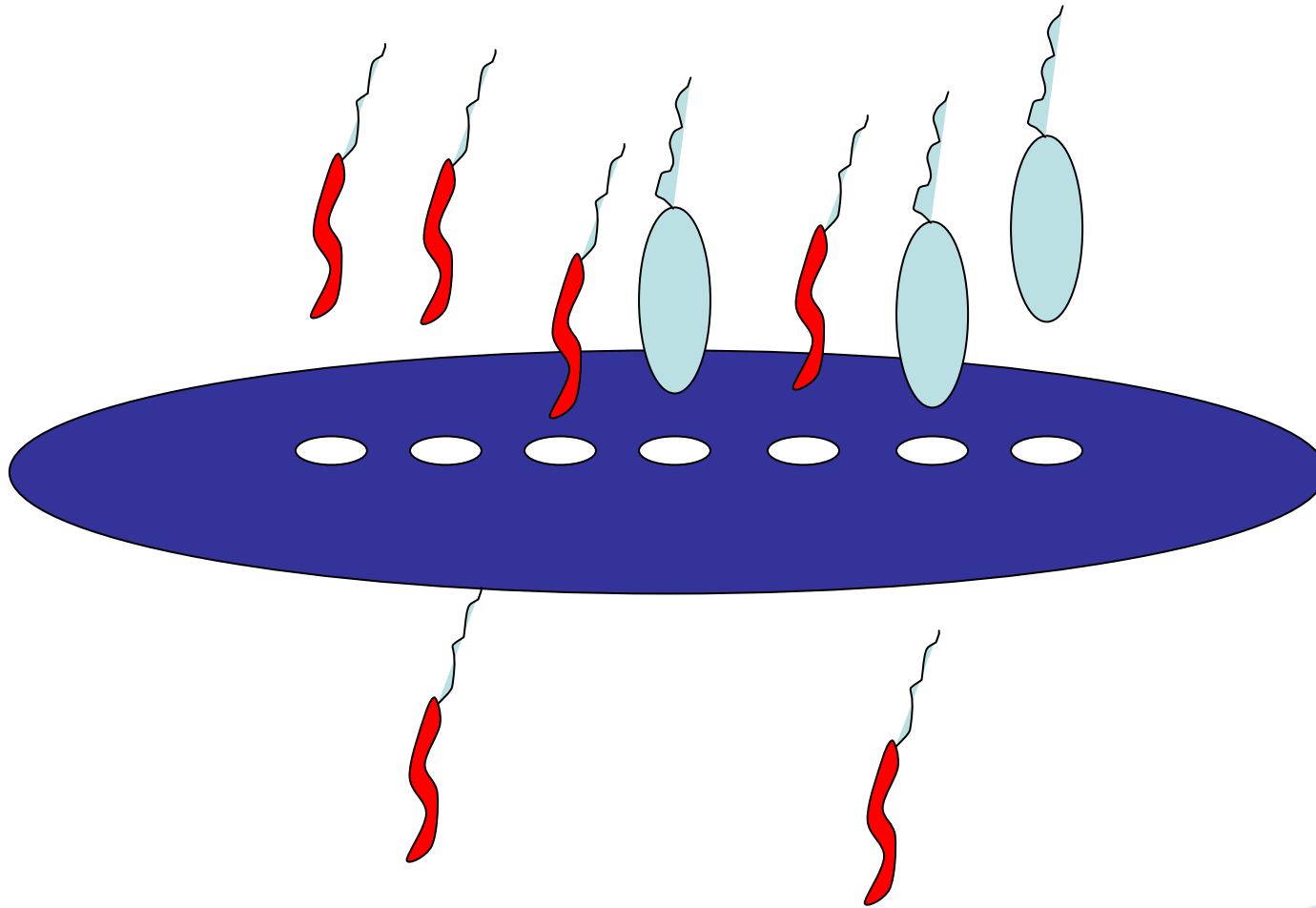
mCCD A ^{29f}	NB	4g C, 0.25g F, 0.25g P	32 zone				1000 (D)	10 AM
Doyle-Raman B ^{27,4}	BB							
Steele-McDermott ²⁸	-	10% S + FBP	5	5	2.9 mg (C)	10		
Waterman A ²⁹	BAB	5% DH	5	5	2500	10		100 CY
Waterman B ²⁹	TB	5% DH	20	20	10000			
Virion ³⁰	CA	5-7% S	30 zone					10
Exeter A + B ^{31,h}	NB	5% LH, 0.5g F, 0.2g B, 0.2g P	15 zone	10	4 mg (C)	10 ⁵	10 ⁵	1 AM
Karmali A ³²	CA	4g C, 0.32g HT, 0.1g P	32 zone					
CAR B/A ³³	BA/BE	FBP, 3% LH	32 zone				10	2.5 AM
Exeter A + B ³⁴	NB	FBP, 5% LH	15 zone		4 mg		10	2 AM
SSM B + A ³⁵	MHB + 4g/l A	-	30 zone	50				
Bolton ³⁶	A	5% LH, 0.1g HE, 0.5g P, 0.5g B	20 zone			20		50 CY
Park-Sanders B ³⁷	BB	0.25 P, 1g Na citrate, 5% LH	32 zone **	10		10		100 CY **
Hunt-Rudle B ³⁸	NB, YE	FBP, 5% LH	15 zone ***	12.5		10		100 CY or 2 AM 200 CY
Campy Cefex A ³⁹	BA	0.5g F, 0.2g B, 0.5g P, 5% LH	33 zone					
CATA ^{40f}	NB ^f	4g C	8 zone		4 (D)		1000 (D)	10 AM

Corry et al., 1995

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Physical separation – the filter method



Sampling strategies for foods

- Rinse
- Swab
- Blend
- *The best approach needs to be assessed on a case-by-case basis*
- *The fresher, the better – transport media may help (several available)*

What is used?

- 2001 – EC-sponsored survey of methods used in 1014 clinical laboratories in 10 European union countries (Takkinen et al., unpublished data)
- Eight different media used
- Statistical treatment of the results showed the following factors associated with increased recovery rate:
 - Use of mCCDA
 - Preparation of media in-house
 - Participation in an external quality assurance scheme

NO COMPARABLE DATA FOR FOOD LABORATORIES

The standards

- **FDA - Enrichment/preenrichment – Bolton**
- **Agars – mCCDA or Abeyta-Hunt-Bark**
- **Atmosphere – Gaspak, Gas replacement**

- **NKML - Enrichment/preenrichment – Preston or Park-Sanders**
- **Agars – mCCDA, 2nd of own choice**
- **Atmosphere – “microaerobic”**

- **NKML proposed ISO/CEN standard - 10273**

...And the problems

- FDA method – 2001. Many developments since.
- NKML – what was the basis for choosing mCCDA?
- Why two enrichment broths?
- Evaluation – 14 laboratories, 7 EU countries – substantive variation (Rosenquist et al. 2007):
- **“We suggest that the poor detection of low numbers, the underestimation in milk samples, and the large variation between laboratories can be explained by the general difficulties in handling *Campylobacter*. “**
- **“The sensitivity...was significantly lower for *C. coli* compared to *C. jejuni*”**

May I suggest...

- ...that the use of different enrichment broths may have an influence?
- ...that the different composition of microaerobic atmospheres may have an influence?
- ...that alternative isolation media could be better?
- ...that the LOD of 25 CFU/g may prove a limiting factor?
- ...that the substantive difference in method sensitivity for *C. coli* is of concern, and may also suggest deficiencies for other strains of *C. jejuni*?*
- *The validation study used spiked samples with one strain of each species

So what about PCR for *Campylobacter*?

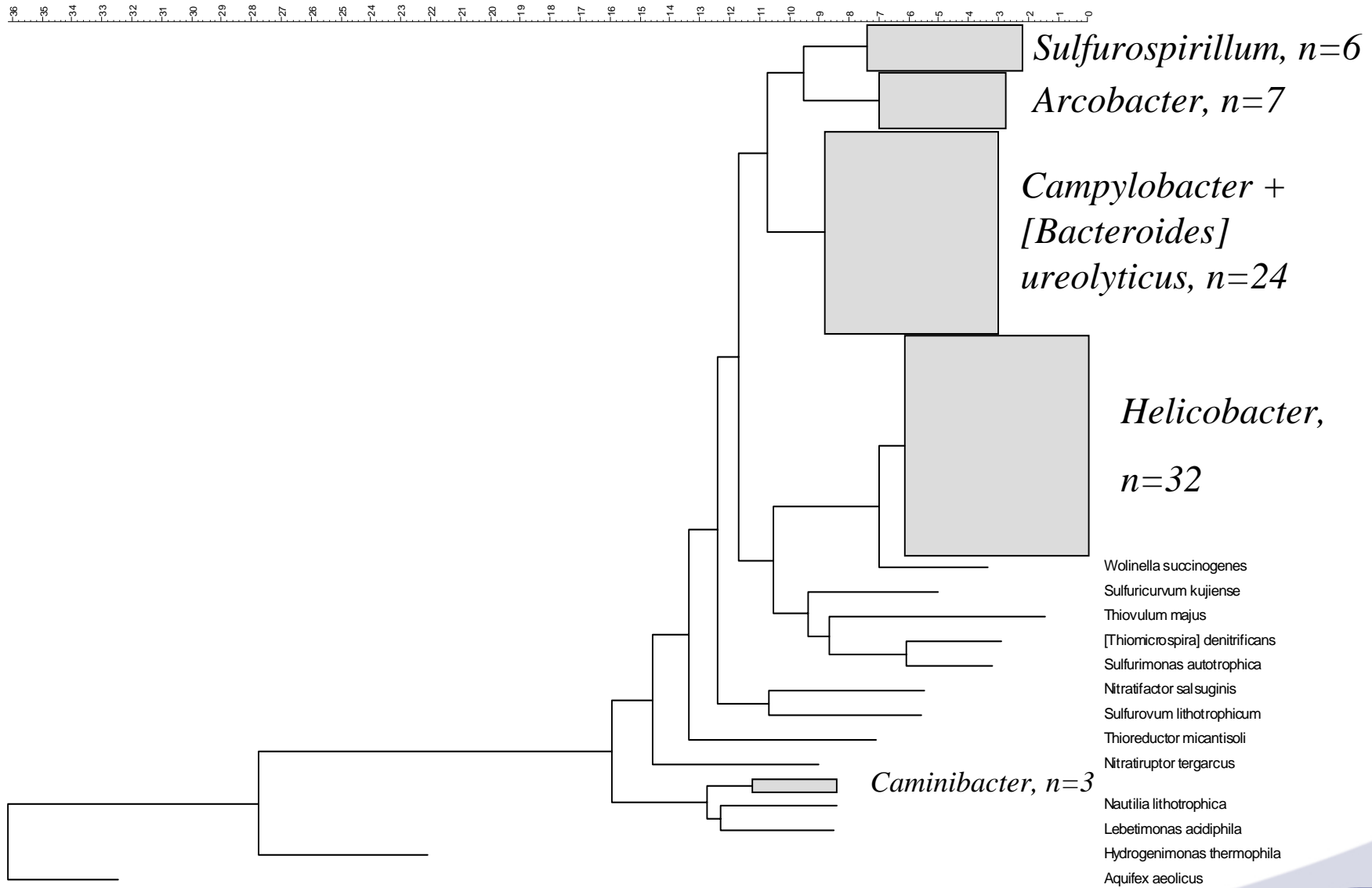
- Many, many assays described with different primer sets
- Genus, species, thermophilic-specific
- Verification of assay specificity heavily dependant on parameters used – identity and number of strains
- Other variables: thermocyclers, inhibitors + facilitators, real-time/conventional, limit of detection/cell concentration methods, nested, semi-nested or not...

Comparing *C. jejuni/coli* PCR tests

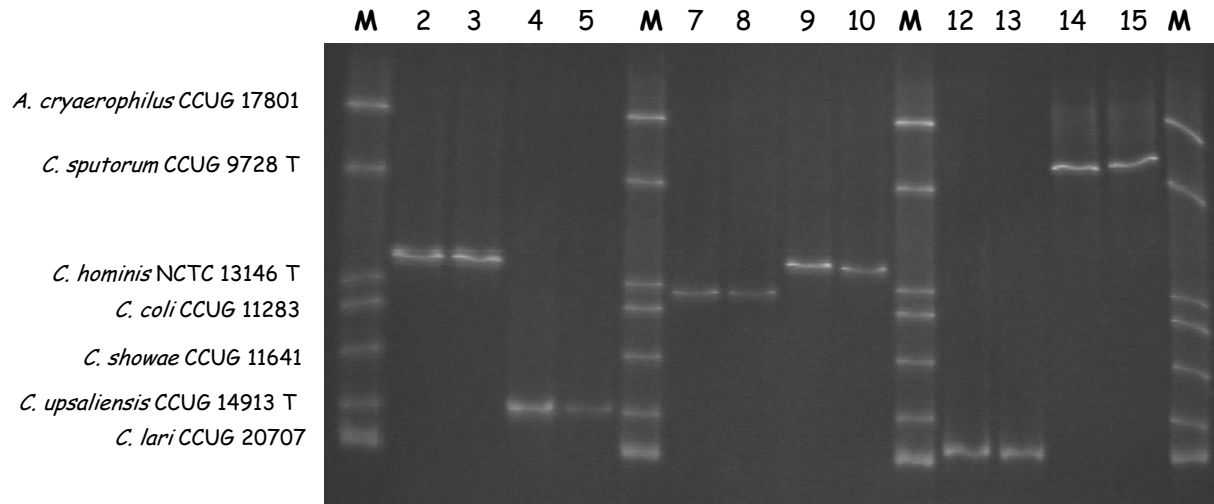
	<i>C. jejuni</i>							<i>C. coli</i>				
Assay no.	1	2	3	4	5	6	7	8	9	10	11	12*
Target	23S rRNA	Random	<i>mcpA</i>	<i>ceuE</i>	Random	Random	<i>hipO</i>	Random	Random	<i>asp</i>	<i>ceuE</i>	23S rRNA
Ref. no.	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[5]	[6]	[7]	[4]	[1]
Sensitivity	100	92	100	88	100	93	91	100	100	100	100	100
Specificity	84	92	90	98	86	100	100	100	100	100	100	0

Conclusion: Polyphasic strategy for discriminating these species recommended (On & Jordan 2003. J. Clin. Microbiol. 41: 330)

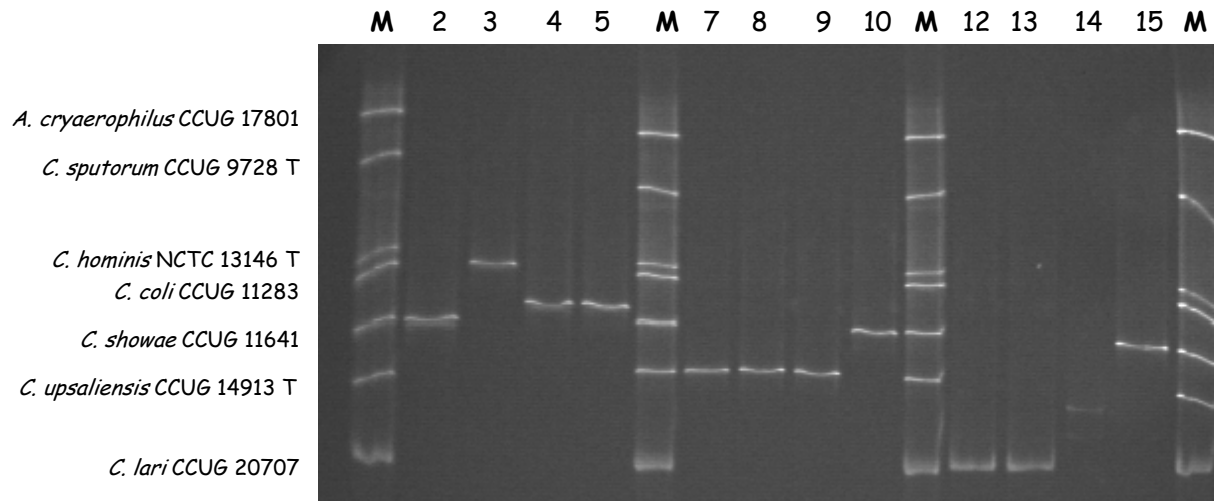
And now for the bad news....!



PCR-DGGE analysis: a culture-independant detection / identification method for Epsilonbacteria (Petersen et al., 2007)



- 2 *H. bilis* LMG 18386
- 3 *H. bilis* LMG 18443
- 4 *H. hepaticus* 60/031003
- 5 *H. hepaticus* CCUG 33637
- 7 *H. pametensis* CCUG 26255 T
- 8 *H. pametensis* CCUG 29257
- 9 *H. rodentium* 2718
- 10 *H. rodentium* 95-1707 T
- 12 *H. salomonis* Mini
- 13 *H. salomonis* Inkinen=CCUG 37845 T
- 14 *H. winghamensis* ATCC BAA-430 T
- 15 *H. winghamensis* ATCC BAA-432



- 2 *C. hyointestinalis* sub. *hyo* CCUG 14169
- 3 *C. hyointestinalis* sub. *hyo* SVS 3035
- 4 *C. hyointestinalis* sub. *lawsonii* CHY 5
- 5 *C. hyointestinalis* sub. *lawsonii* CHY 7
- 7 *C. jejuni* subs. *doylei* CCUG 24567
- 8. *C. jejuni* subs. *jejuni* CCUG 11284
- 9 *C. jejuni* subs. *jejuni* NCTC 11168
- 10 *C. lanienae* NCTC 13004
- 12 *C. lari* CCUG 23947
- 13 *C. lari* CCUG 20707
- 14 *C. mucosalis* CCUG 6822
- 15 *C. rectus* CCUG 20446

MoniQA: a call to arms

- The influence of the extensive range of variables for campylobacter isolation protocols for foods has not been authoritatively addressed
- This may explain the variation between laboratories using or trialling standard methods
- Ditto for PCR approaches!
- **There is every justification for the MoniQA network to initiate a project (survey or lab-based) that addresses these important data gaps**
- The issue of non-enteropathogenic species continues to be an area of relevant, but ultimately unknown importance