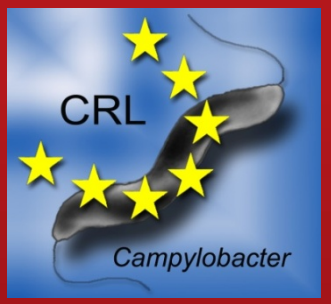


CRL - *Campylobacter*

Community Reference Laboratory for *Campylobacter*
National Veterinary Institute, SVA

Eva Olsson Engvall

SVA – An Authority under the Swedish Ministry of Agriculture

Specialist areas and Reference laboratory functions at SVA

- Virology
- Parasitology
- Bacteriology
- Chemistry
- Pathology
- Immunbiology
- Antibiotic studies
- Feed studies
- Epidemiology
- Animal species specialists

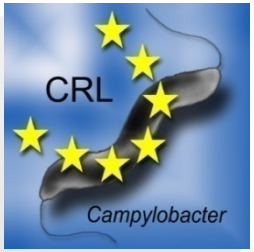
National Reference Laboratory (NRL) for numerous diseases and pathogens, e.g. *Campylobacter* in animals and feed, avian influenza and *Salmonella*.

EU's reference laboratory (CRL) for *Campylobacter*.

OIE Collaborating Centre and

OIE reference laboratory.

SVA as CRL- *Campylobacter* since 1 July 2006



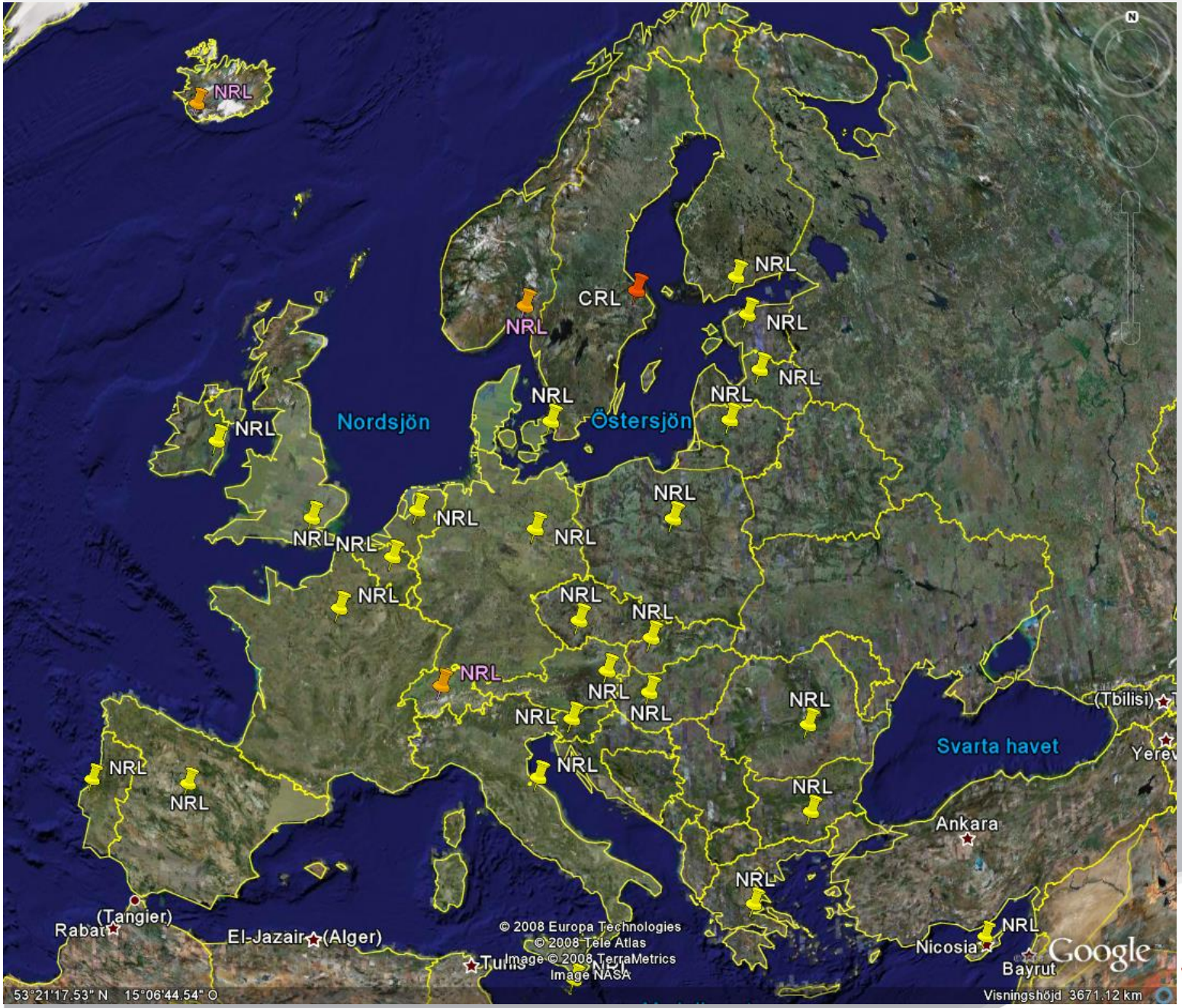
- The legal basis:

Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents

Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and welfare rules

Network of reference laboratories:

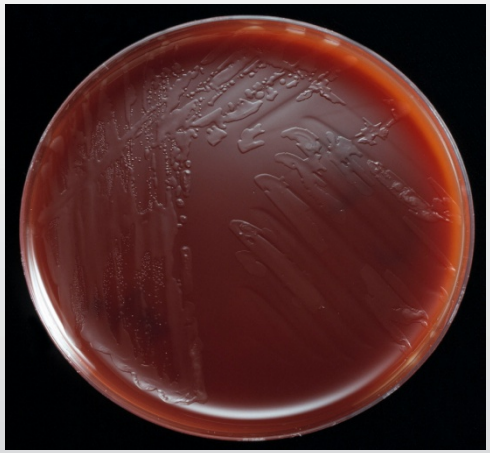
- CRL plus at least 35 NRLs- *Campylobacter*


NRL
CRL
Nordsjön
Östersjön
Svarta havet
(Tbilisi)
Ankara
Nicosia
Bayrut
Rabat
(Tangier)
El-Jazair
(Alger)
© 2008 Europa Technologies
© 2008 Tele Atlas
Image © 2008 TerraMetrics
Image NASA
Google
53°21'17.53" N 15°06'44.54" O
Visningshöjd 3671.12 km

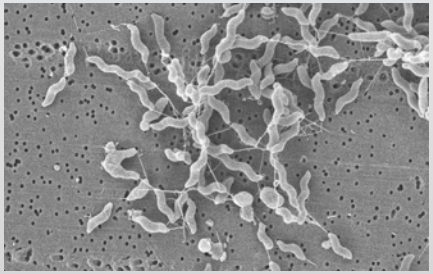
The real basis...

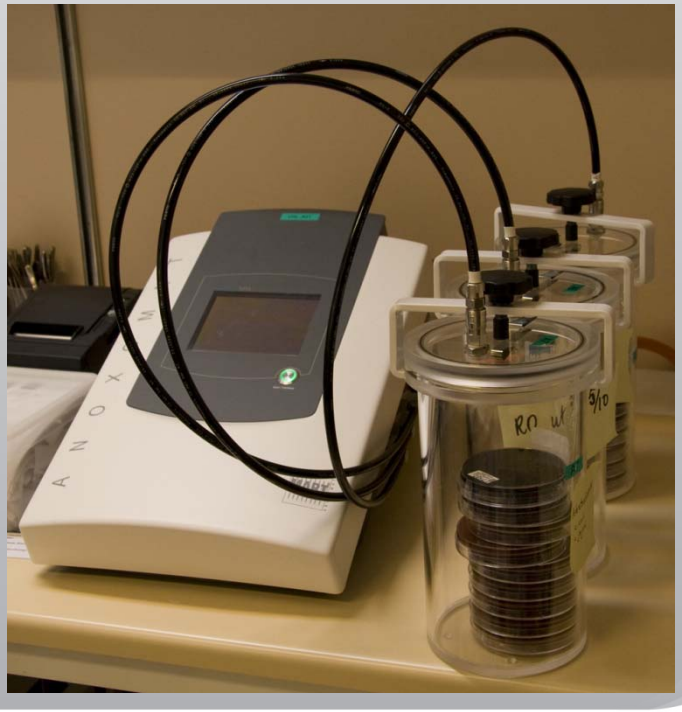
- Campylobacteriosis, the most frequently reported zoonotic disease in humans,

  ~200 000 laboratory confirmed human cases reported each year in EU
- Sporadic and/or small outbreaks
- Mainly food-borne transmission
- Poultry, significant source of human campylobacteriosis



Campylobacter
- the bacterium





CRL- *Campylobacter* activities

- Questionnaire (1)
- Workshops (3)
- Training course(1)
- Ring tests (4-5)
- Study visits from MS (7 people)
- Assistance to Commission, EFSA, NRLs
- EFSA wgs, ISO/CEN wgs
- R&D, incl networks eg MVN, CampEc
- Communication, incl webpage (via www.sva.se)
- Analysis of baseline survey isolates

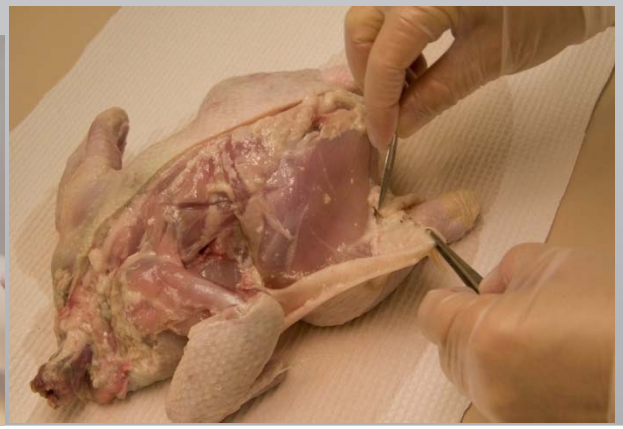
EU baseline survey

- Commission Decision 2007/516/EC:
- *Campylobacter* prevalence in broiler flocks: **caecum samples**
- Antimicrobial resistence in *Campylobacter* isolates from broiler flocks
- *Campylobacter* prevalence in broiler carcasses + enumeration: **broiler carcasses**
- (*Salmonella* prevalence in broiler carcasses)

- Methods for *Campylobacter* analyses:
  ISO 10272 -1 och 2: 2006

A training course was organized by request….

Training course in 2007



EU survey on the prevalence of *Campylobacter* in broilers, 2008

Commission Decision of 19 July 2007

Annex I, Part C 2.3. and Part D 2.5.1.
For quality assurance, a proportion of *Campylobacter* spp. isolates with a maximum of 8 isolates from caecum samples and a maximum of 8 isolates from carcass samples shall be sent to the CRL-*Campylobacter* for confirmation and species identification.

I.e., a maximum of 416 (432) isolates could be sent to CRL for analysis

Submission, collection, tests, and documentation of baseline isolates at the CRL

CRL prepared a ”strain submission form” and a test protocol
Isolates submitted by regular airmail or by courier
Email contact between CRL and NRLs
Isolates grown immediately when arrived at CRL
Subcultured, phenotyped asap, genotyped when time allowed
Stored at -70ºC
Results registered and sent back to NRL when pheno- and genotyping ready
All information registered in excelfile (”database”)

Strain submission form

Baseline study of *Campylobacter* in broilers and broiler carcasses 2008
Strain submission to CRL - *Campylobacter*

To be filled in by NRL:

Isolate ID: _____ Date of isolation: _____

Institute: _____ Country: _____

Contact person: _____

Species identification: _____

Method of species identification: _____

Origin of isolate: ☐ Caecum ☐ Carcass

Additional remarks: _____

To be filled in by CRL:

Arrival date**:** _____

CRL-Camp ID no.: _____

Species identification by

Phenotyping: _____

Genotyping: _____

Final results and comments: _____

Additional remarks: _____

Contact person:
Eva Olsson Engvall
CRL-*Campylobacter*
National Veterinary Institute
SE-751 89 Uppsala
Sweden
eva.olsson@sva.se

Phenotyping (ISO 10272-1:2006)

For confirmation:

Morphology and motility

Growth at 25ºC (microaerobic)

Growth at 41,5ºC (aerobic)

Detection of oxidase

For species identification:

Detection of catalase

Detection of hippurate hydrolysis

Detection of indoxyl acetate hydrolysis

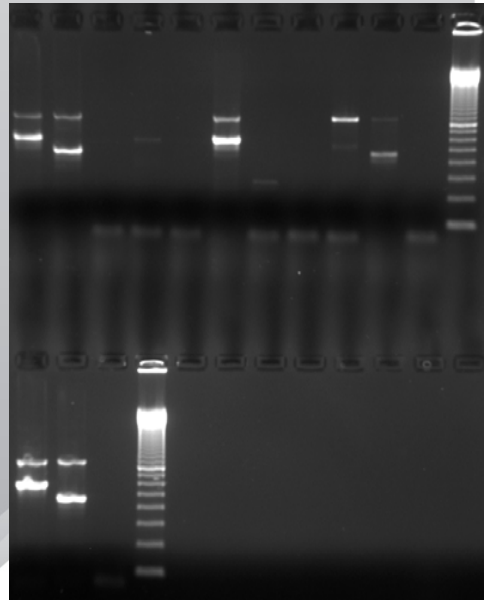
(Detection of sensitivity to nalidixic acid and cephalothin)

Genotyping by PCR assays

All isolates were tested by the multiplex PCR described by Denis et al 1999

Target genes for primers:

- 16S rRNA 857 bp (*C. jejuni, C. coli*)
- *mapA* 589 bp (*C. jejuni*)
- *ceuE* 462 bp (*C. coli*)



Other PCR assays that were used:

Fermér and Engvall 1999: PCR/RFLP, 23S rRNA gene for thermophilic *Campylobacter* spp, digestion by two enzymes (*Alu*I and *Tsp*I). Identifies and discriminates between *C. jejuni*, *C. coli*, *C.lari* and *C. upsaliensis*.

Marshall et al 1999: PCR/RFLP for identification of *Campylobacter*, *Arcobacter* and *Helicobacter*

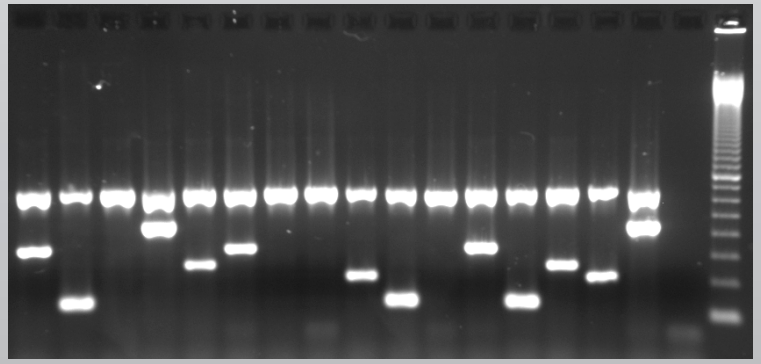
The additional PCRs were used for isolates that gave conflicting (or no) results with the PCR by Denis et al.

Multiplex PCR by Wang et al. 2002

Sample no.	Species ID by Multiplex PCR	DNA
1	*C. jejuni*	*C. jejuni*
2	*C. coli*	*C. coli*
3	Negative	*C. hyointestinalis*
4	*C. fetus fetus*	*C. fetus fetus*
5	*C. lari*	*C. lari*
6	*C. jejuni*	*C. jejuni*
7	Negative	*C. mucosalils*
8	Negative	*A. butzleri*
9	*C. upsaliensis*	*C. upsaliensis*
10	*C. coli*	*C. coli*
11	Negative	*H. pullorum*
Ref. 1	*C. jejuni*	*C. jejuni*
Ref. 2	*C. coli*	*C. coli*
Ref. 3	*C. lari*	*C. lari*
Ref. 4	*C. upsaliensis*	*C. upsaliensis*
Ref. 5	*C. fetus fetus*	*C. fetus fetus*

PCR-amplified products:

	23 S	Target gene:
C. jejuni	650 bp	323 bp *hipO*
C. coli	650 bp	126 bp *glyA*
C. lari	650 bp	251 bp *glyA*
C. upsaliensis	650 bp	204 bp *glyA*
C. fetus fetus	650 bp	435 bp *sapB2*



Results – strains submitted in 2008

- 348 isolates submitted to CRL from 24 MS and 2 non- MS (CH and NO)
- 22 submissions could not be analysed, the Campylobacter were dead/did not grow/contaminated at arrival

Of the 326 isolates:

149 from caecum samples

177 from carcass samples

Results – strains submitted in 2008 species id

Caecum samples:

C. jejuni – 85

C. coli – 58

Other – 6 (*C. lari, H. pullorum*, mixed *C.j* and *C. c*)

Carcass samples:

C. jejuni - 118

C. coli - 56

Other – 3 (*A. butzleri*, mixed *C.j* and *C. c)*

Results – strains submitted in 2008 conflicting results

- Isolates submitted as *C. jejuni* were *C. coli* or vice versa
- Isolates submitted as *C*. spp were *C. j* or *C. c*
- One isolate submitted as *C. upsaliensis*, was *C. coli*, one submitted as *C. coli* and one as unknown, were *H. pullorum*
- Mixtures of *C.jejuni* and *C. coli*
- **NB ~90% of NRL identifications corresponded with CRL results!**

Conclusions, consequenses, questions

- **The majority of isolates were correctly species identified**
- **The vast majority were C. jejuni and C. coli (97%)**
- *C. jejuni* / *C. coli* – misidentifications – and mixtures thereof - significance for testing antimicrobial resistance? For epidemiological conclusions?
- Significance of dead/contaminated samples?
- How were submitted isolates selected?

Isolates submitted in 2008+ 2009

- More isolates from the 2008 baseline survey have been submitted in January-March 2009
- In all, 417 isolates (at least), from 28 countries (26 MS and 2 non-EU countries) will be analysed from the baseline survey
- Will send out results as soon as possible…

CRL activities in 2009

- Preparing ring test using freeze-dried cultures with different concentrations. Will be sent out 11 May
- Workshop 5-7 October
- Assistance to EC, EFSA, NRLs, Communication, R&D, missions, etc etc
- Further analyses of baseline isolates?
- Strain characterization by standardized PFGE and MLST protocols planned

CRL- *Campylobacter* team

- 10 persons from different departments at SVA work for the CRL
- Part time – full time
- Other people involved, belong to ’core facilities’

Eva Olsson Engvall
Ingrid Hansson
Elina Lahti
Gunilla Lindgren
Boel Harbom
Linda Svensson
Ninni Pudas
Ivar Vågsholm
Linda Hallenberg
Bo Sundqvist