

nvCT?



The amazing story of the Swedish new variant of *Chlamydia trachomatis* (nvCT)

Magnus Unemo

PhD, Assoc. Professor, WHO Collaborator

Swedish Reference Laboratory for Pathogenic Neisseria

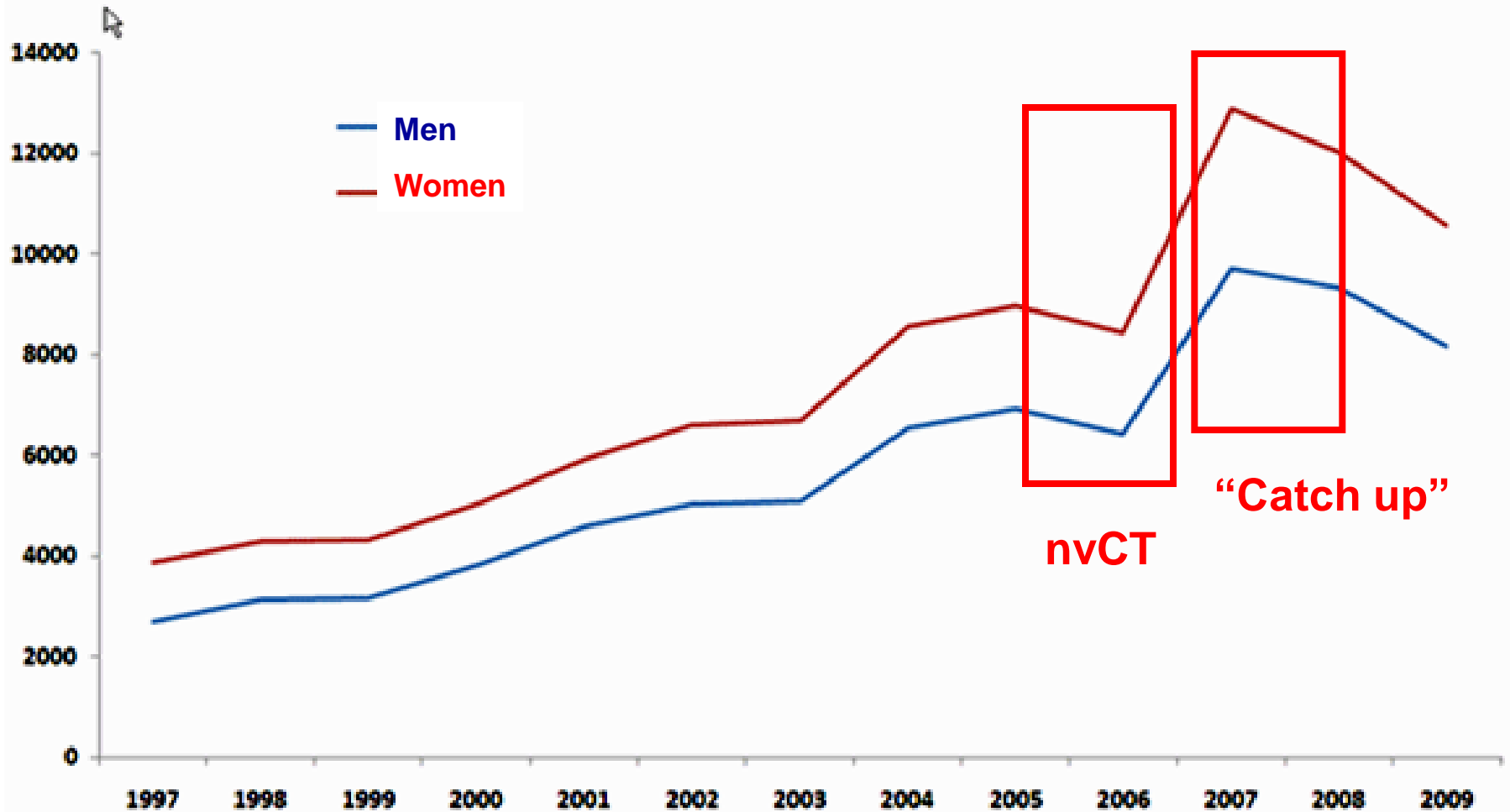
Department of Laboratory Medicine, Microbiology



Örebro University Hospital

Sweden

Notified chlamydial cases in Sweden 1997-2009



Halland County, Sweden

(Torvald Ripa detected nvCT!)

October 2006

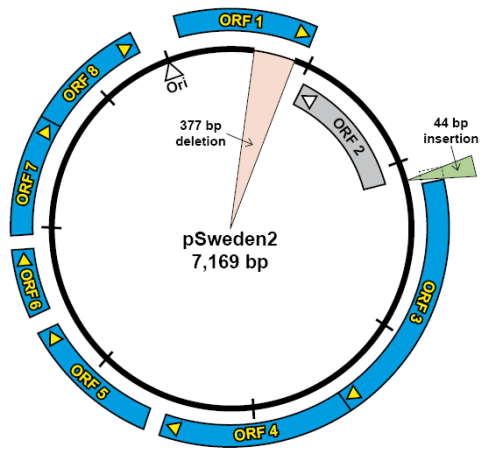
(Ripa T, Nilsson P. Euro Surveill. 2006)

- **spring 2006, a 25% decrease** of genital chlamydial infection
- **mutated *C. trachomatis*** (10%) was undetected using RealTime CT/NG test (Abbott Laboratories)
- **377 bp deletion in ORF1 of the cryptic plasmid**, containing the **targets** for the diagnostic **systems from Abbott Laboratories and Roche Diagnostics** (Amplicor/Cobas Amplicor/TaqMan CT/NG test).

(Ripa T, Nilsson P. STD. 2007)

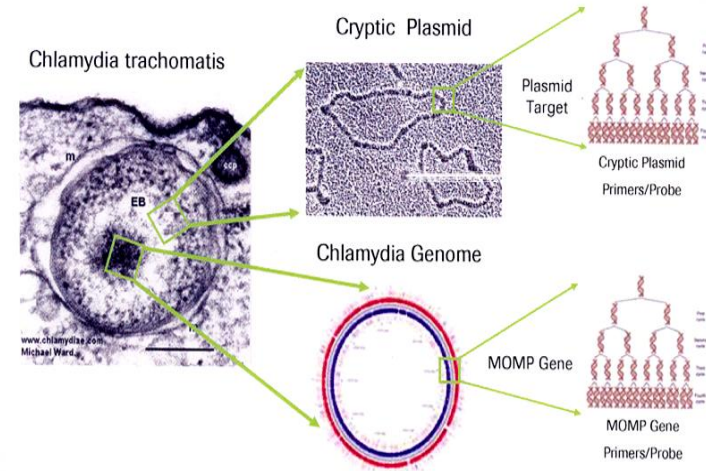
Emergent situation

- **Clonal origin stated**, using *ompA* sequencing, multilocus sequence typing (MLST), and variable number of tandem repeats (VNTR) (Unemo, et al. Euro Surveill. 2007; Pedersen, et al. CMI. 2008; Herrmann, et al. EID. 2008; Jurstrand, et al. STI. 2009)
- **Many non-BD laboratories: LightMix 480HT RT PCR** (*ompA*, quantitative, inhibition control) after evaluation (Unemo, et al. Euro Surveill. 2007)
- **Some non-BD laboratories: BD ProbeTec ET or artus** (Qiagen)



nvCT plasmid characterised
 (Seth-Smith, et al. BMC Genomics. 2009)

CTM CT V2.0 MASTER MIX TARGETS



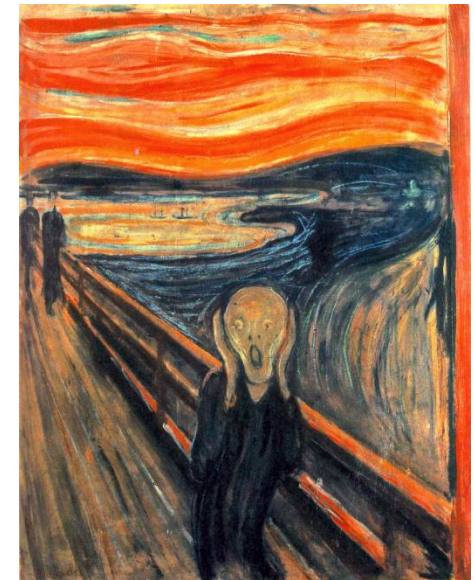
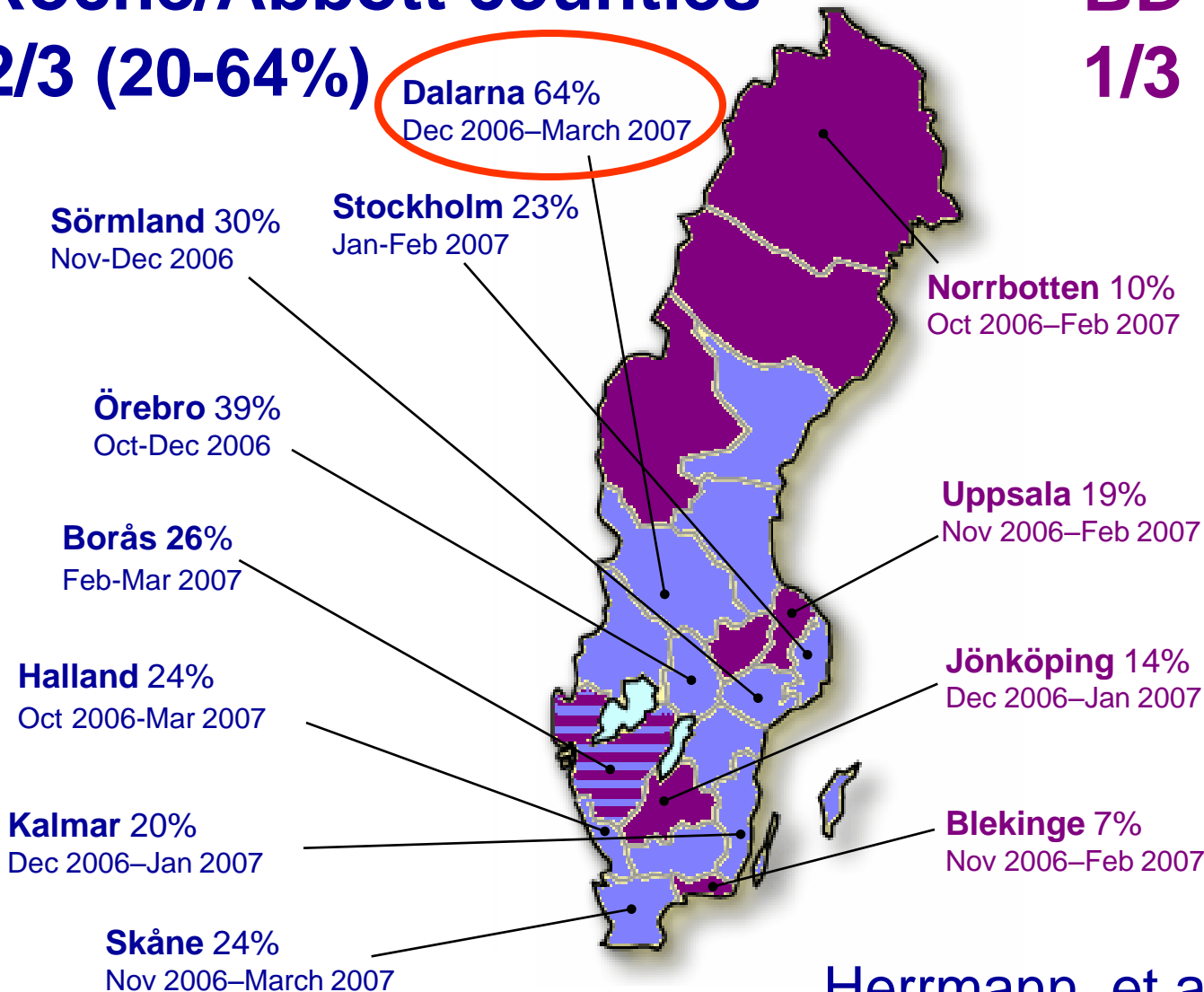
Dual-target assays developed

- **EU certified dual-target assays** from Abbott (January 2008; 2 × plasmid target) and Roche (June 2008; plasmid + additional *ompA* target)
- **emergent solution** (LightMix 480HT RT PCR [*ompA*]) ⇒ compared to CTM CT V2.0 (Roche) **10% false negatives** (Hadad, et al. STI. 2009)!

Proportions of nvCT in Sweden 2006-2007

**Roche/Abbott counties-
2/3 (20-64%)**

**BD counties-
1/3 (7-19%)**



The Scream
(Edvard Munch)

Considerations at that time

- **Proportions remained high** (despite new dual-target NAATs) **but mainly decreasing** in Sweden (Hadad, et al. STI. 2009; Klint, et al. CMI. 2011)
- The **rapid nationwide spread** of nvCT in Sweden (but not in other countries)
- **One study:** nvCT caused **asymptomatic infection in women more common** than wild type CT (wtCT; Bjartling, et al. STD. 2009)
- **Plasmid alterations** in nvCT, **live plasmid-free *C. trachomatis* exceedingly rare** (unable to accumulate glycogen) and **plasmid a virulence factor** (Carlson, et al. Infect Immun. 2008)



Altered biological fitness and alterations
in other genes + diagnostic selective advantage???

The Swedish new variant of *Chlamydia trachomatis*: genome sequence, morphology, cell tropism and phenotypic characterization

**Unemo M, Seth-Smith H, Cutcliffe L, Skilton R, Barlow D, Goulding D,
Persson K, Harris S, Kelly A, Bjartling C, Fredlund H, Olcén P,
Thomson N, Clarke I**

Örebro University Hospital, Örebro, Sweden

The Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Malmö University Hospital, Malmö, Sweden

Southampton General Hospital, Southampton, United Kingdom

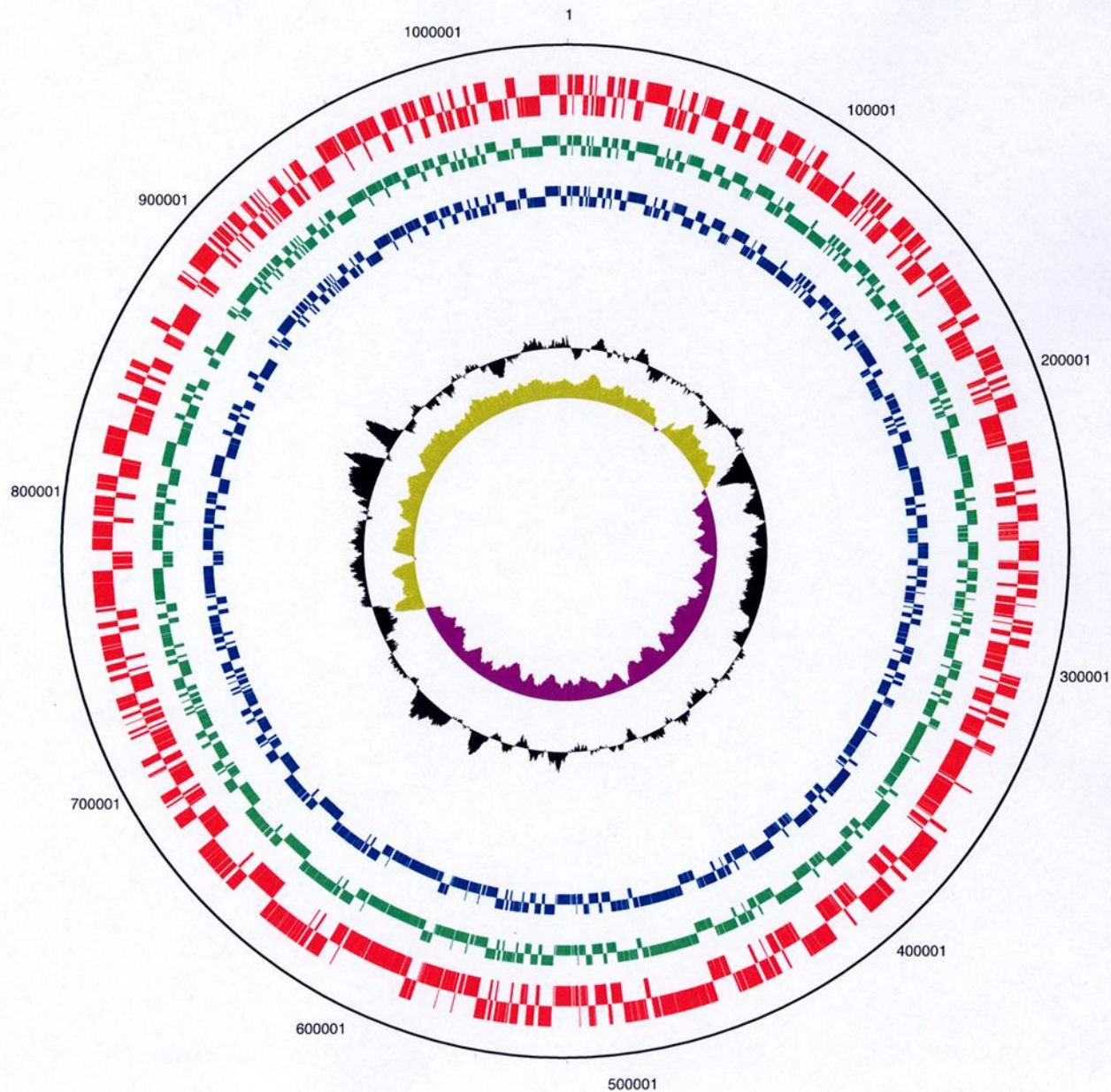
Microbiology. 2010; 156: 1394-1404

(Comparison to all available *C. trachomatis* genomes, and relevant wild type strains of different serovars, incl. clinic and epidemiology)

General features of available CT genomes (A, B, D, L2, L2b)

Strain	nvCT (Sweden2)	UW-3/CX	TZ1A828/OT	Jali20	HAR-13	434/BU	UCH-1
Serovar	E	D	B	B	A	L2	L2b
Biovar	Trachoma (genitotropic)	Trachoma (genitotropic)	Trachoma (oculotropic)	Trachoma (oculotropic)	Trachoma (oculotropic)	LGV	LGV
Origin	Sweden	USA	Tanzania	The Gambia	Saudi Arabia	USA	England
Genome accession	ND	EMBL: AE001273	EMBL: FM872307	EMBL: FM872307	EMBL: CP000051	EMBL: AM884176	EMBL: AM884177
Chromosome (bp)	1,042,839	1,042,519	1,044,282	1,044,352	1,044,459	1,038,842	1,038,869
G+C content (%)	41.3	41.3	41.3	41.3	41.3	41.3	41.3
Predicted CDSs	889	894	879	875	920	889	889
Coding density (%)	89	90	89	89	90	89	89
rRNA operon	2	2	2	2	2	2	2
tRNA operon	37	37	37	37	37	37	37
Average gene size (bp)	1056	1050	1051	1056	1032	1052	1052
No. pseudogenes	14	5	14	18	8	15	15
Plasmid size (bp)	7,169	7,493	7,502	7,506	7,510	7,499	7,500

Comparison with all available CT genomes (A, B, D, L2, L2b)

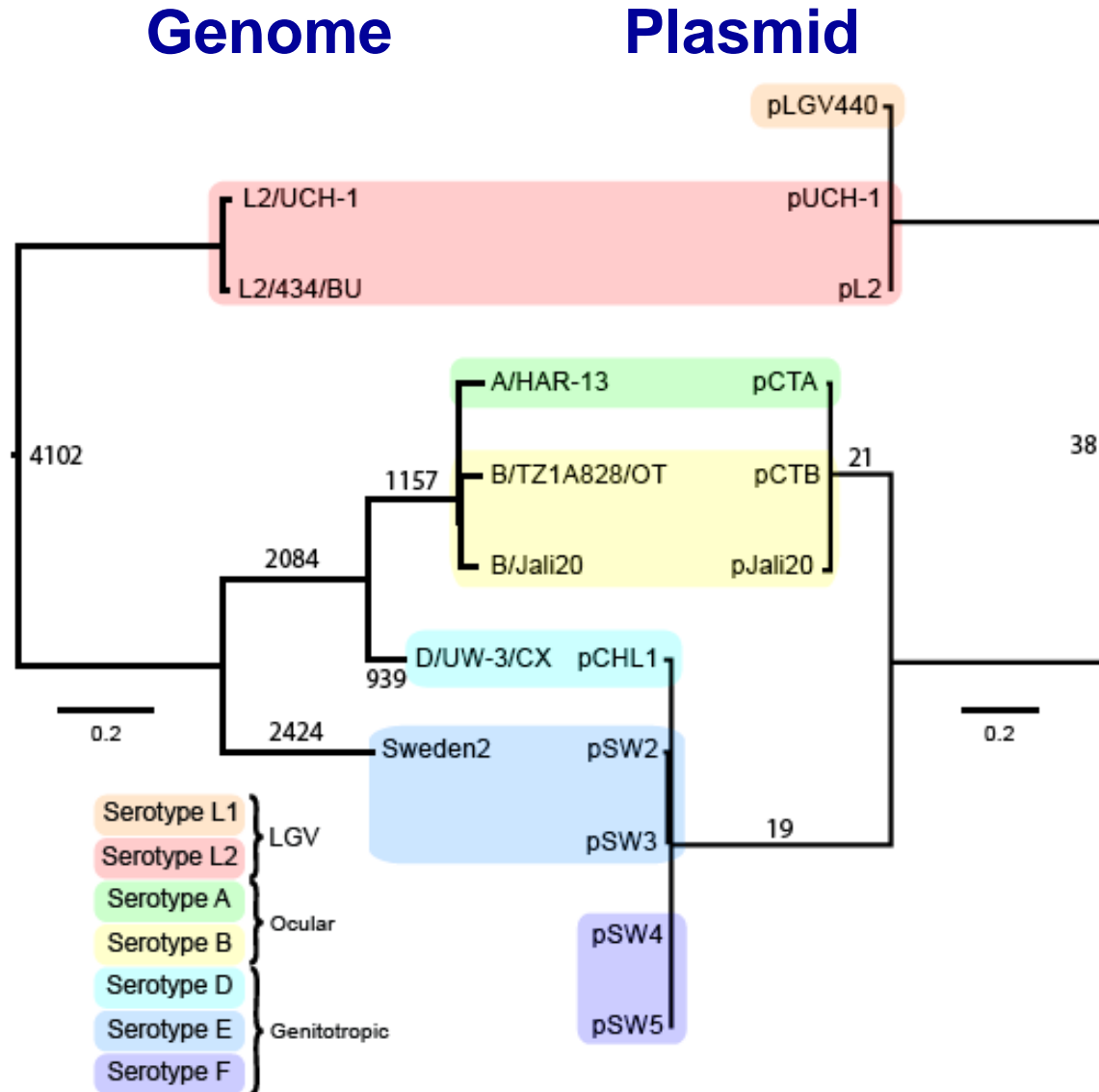


Genome comparisons

- **nvCT genome** consisted of **1,042,839 bp**, comprised a **high level of sequence identity** to, and was **syntenic with all available genomes** from *C. trachomatis* of other serovars!
- **No whole gene differences** was found, and only 5896 SNPs compared to the most closely related genome D/UW-3)!
- **Most of the genetic polymorphisms was in the plasticity zone**, comprises most of the variations in chlamydiae
- nvCT had **14 (six unique) pseudogenes and additional sequence variations within coding sequences (CDSs)**, e.g. *hctB*, *tarp*, and *ompA*, however, none indicates any altered biological fitness!
- Plasmid **copy number unaltered!**

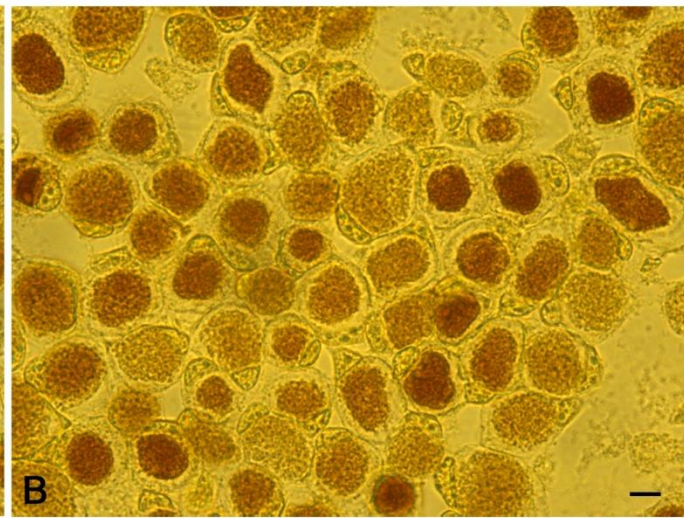
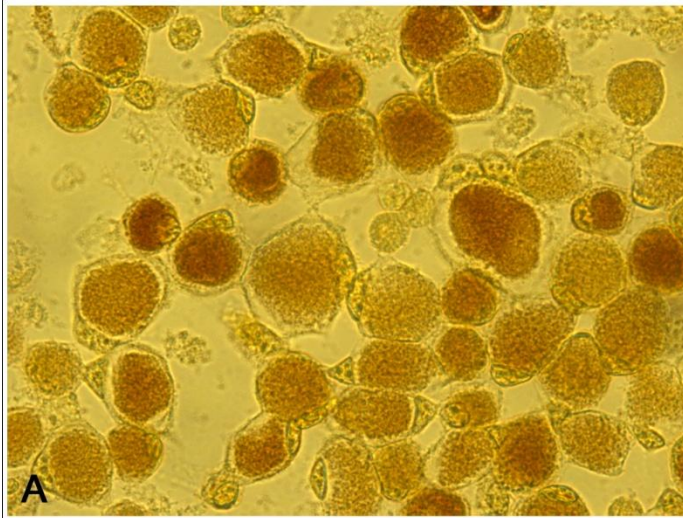
Phylogenetic relationships within *C. trachomatis*

– plasmid co-evolves with cognate genome, i.e. plasmid likely not especially mobile (Seth-Smith. BMC Gen. 2009; Unemo. Microbiology. 2010)



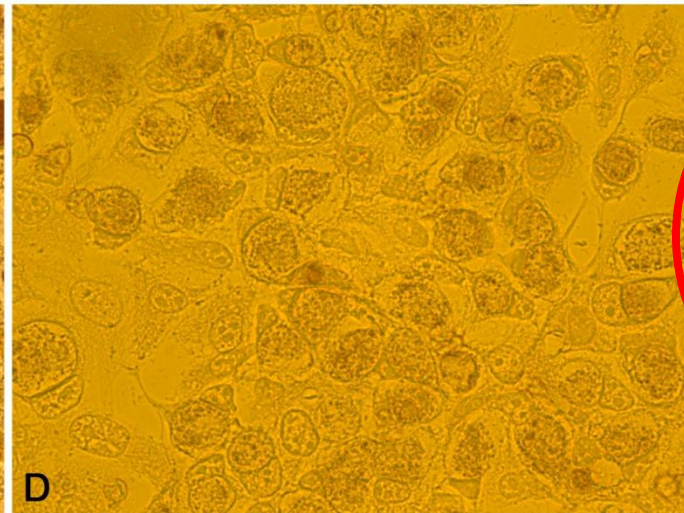
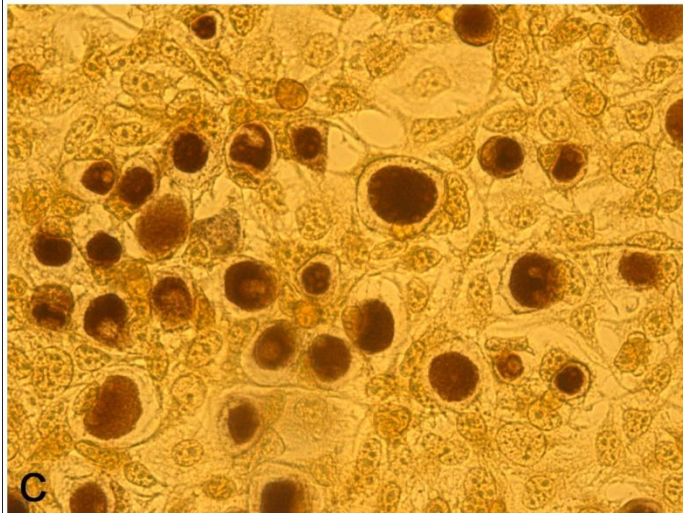
Glycogen accumulation (iodine staining) ⇒ No differences between nvCT, and Bour or Sweden3 (wtCT E)!

nvCT
+



Sweden3
(wtCT E)
+

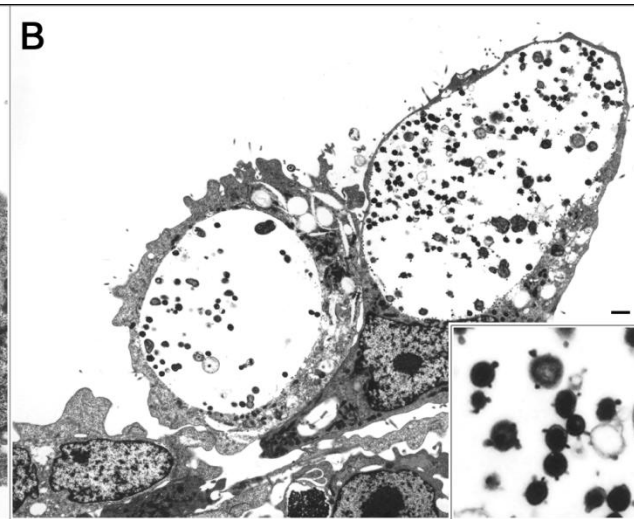
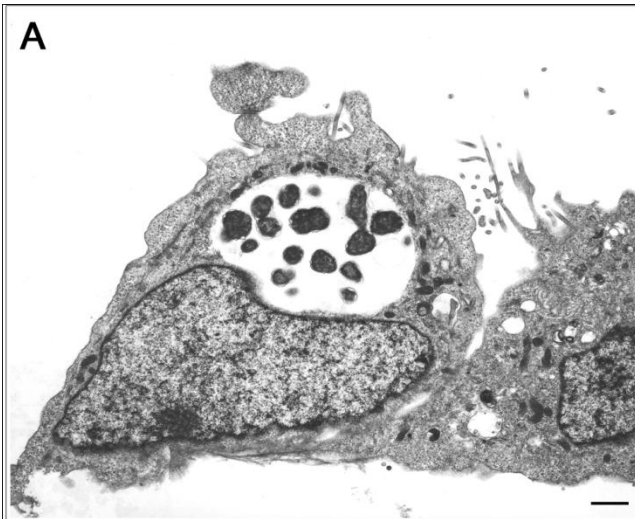
Bour
(wtCT E)
+



C599
(plasmid
free E)
-

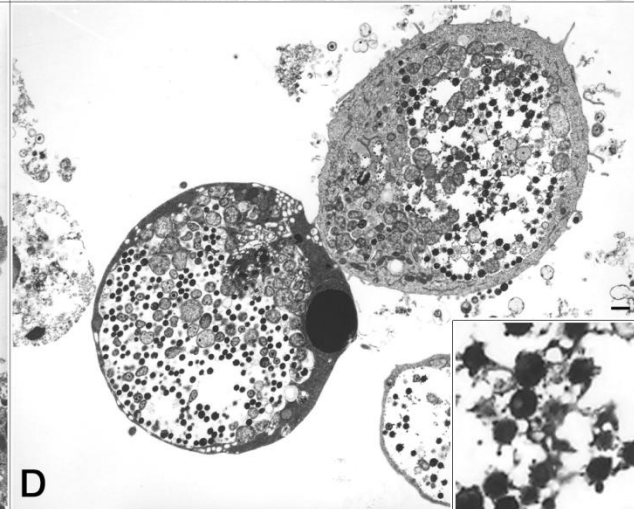
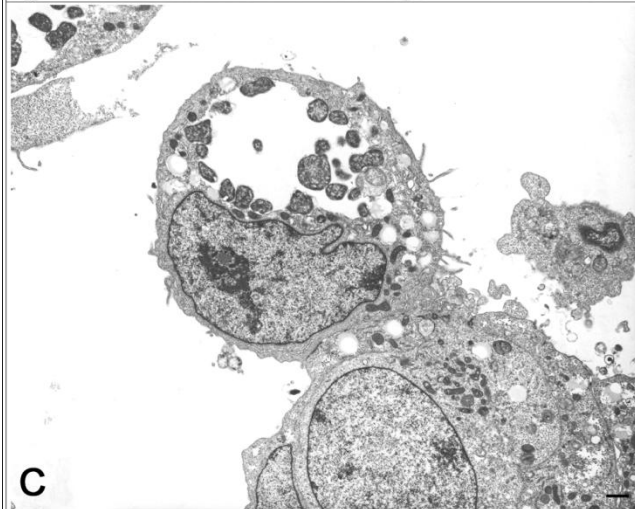
Growth characteristics (development cycle, inclusion formation and morphology) in BGMK cells using transmission electronmicroscopy (TEM) ⇒ no obvious differences!

**nvCT
24 h
Mid.**



**nvCT
48 h
Mature**

**Bour
(wtCT E)
24 h
Mid.**



**Bour
(wtCT E)
48 h
Mature**

Additional phenotypic characteristics

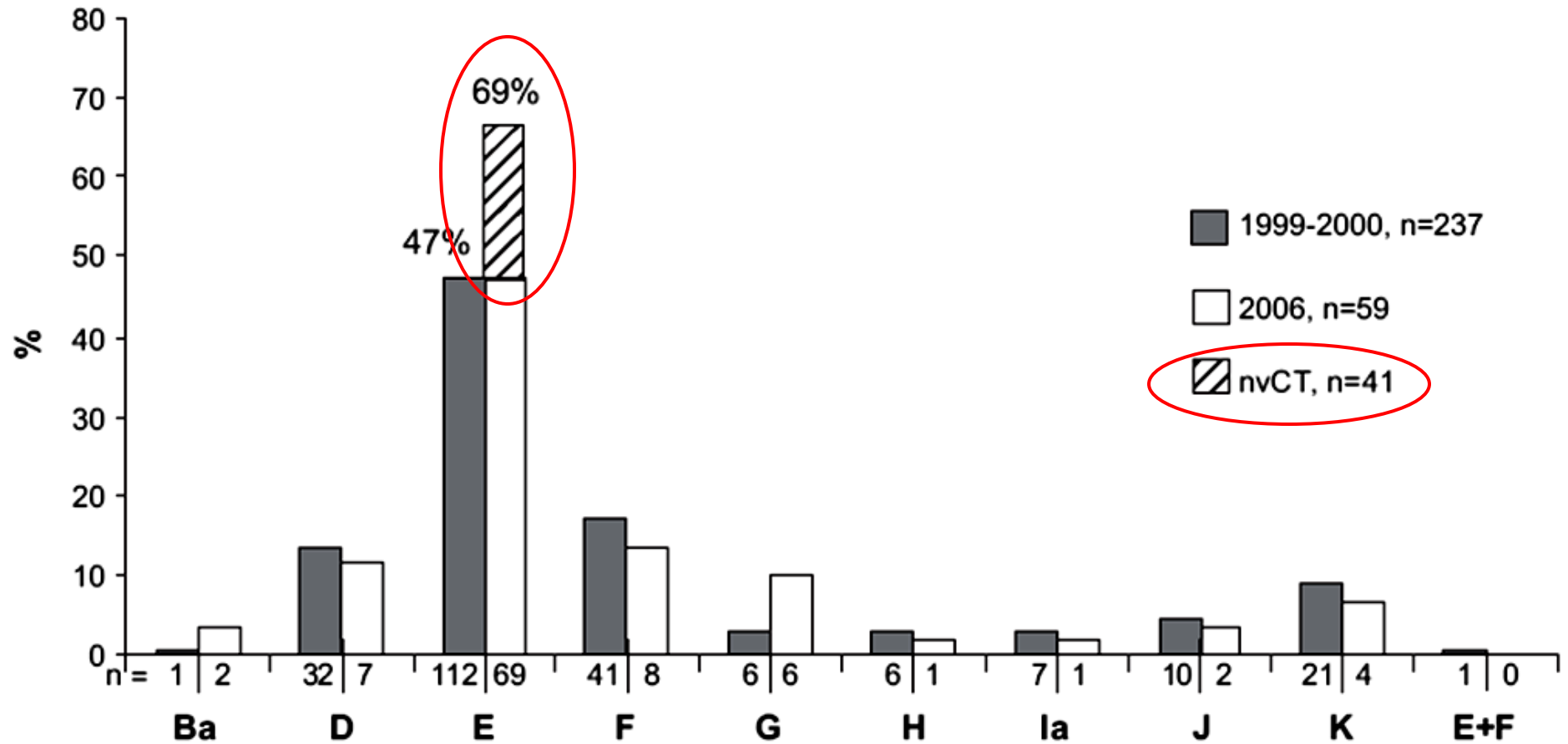
(compared to wild type CT (E) strains)

- **Growth characteristics** examined using quantitative real-time PCR and phase contrast microscopy ⇒ **no obvious differences!**
- **Growth characteristics** examined using high quality digital time lapse video photomicroscopy ⇒ **no obvious differences!**
- **Cell tropism** and **glycogen accumulation** in Hep2, McCoy, BGMK, Vero and 293A cell lines ⇒ **no major differences!**
- MICs of tetracycline (0.25 mg/l), erythromycin (0.25 mg/l), and ciprofloxacin (0.25 mg/l) low – **no antimicrobial resistance of nvCT or obvious differences!**

Conclusions

- **nvCT has unaltered biological fitness!**
 - A. **No major polymorphisms in the genome**, genes for central metabolism, development cycle, virulence, etc., were conserved!
 - B. **Growth and other phenotypic characteristics (7 assays) ⇒ no main differences from wild type CT!**
- Supported by the **similarities of nvCT and wtCT infections**, epidemiology and clinic!
- The **rapid nationwide transmission of nvCT** in Sweden was only **due to the strong diagnostic selective advantage and introduction into a high-frequency transmitting population!**

Unaltered biological fitness further confirmed by examination of genovar distributions in Örebro County, Sweden 1999-2000 vs. 2006



Emergence of nvCT – HOW

(when no biological selection)?

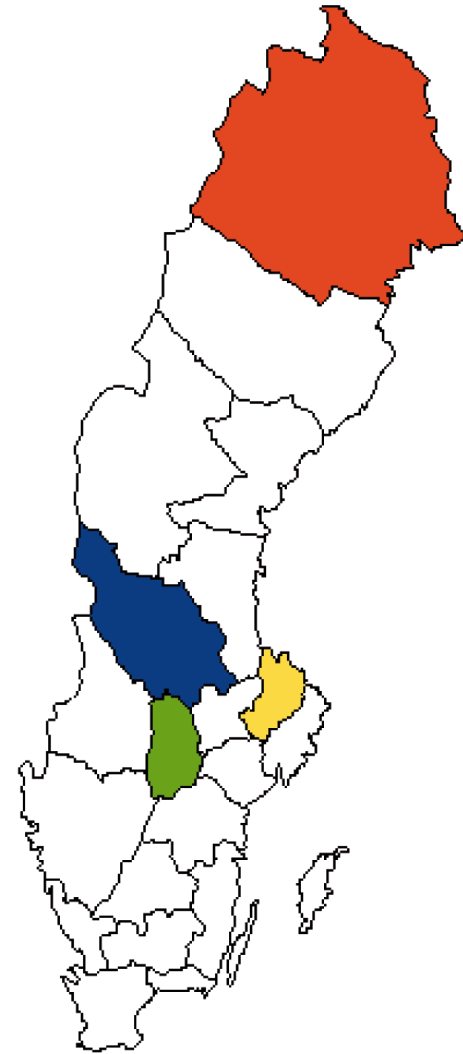
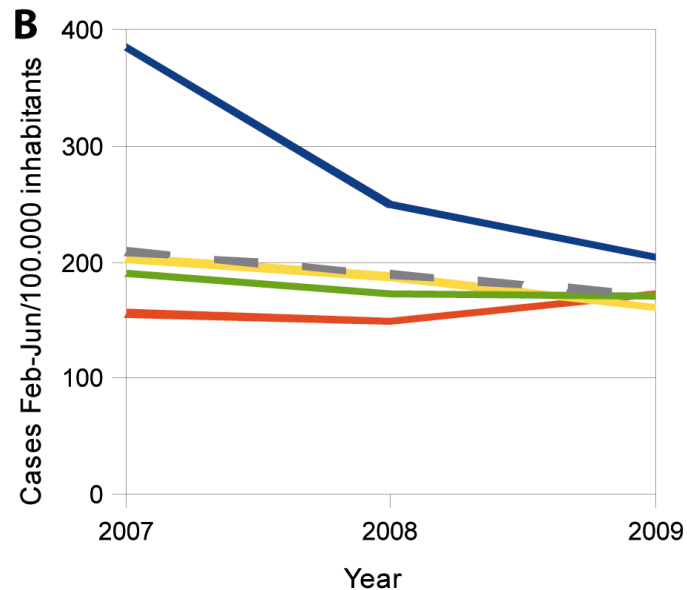
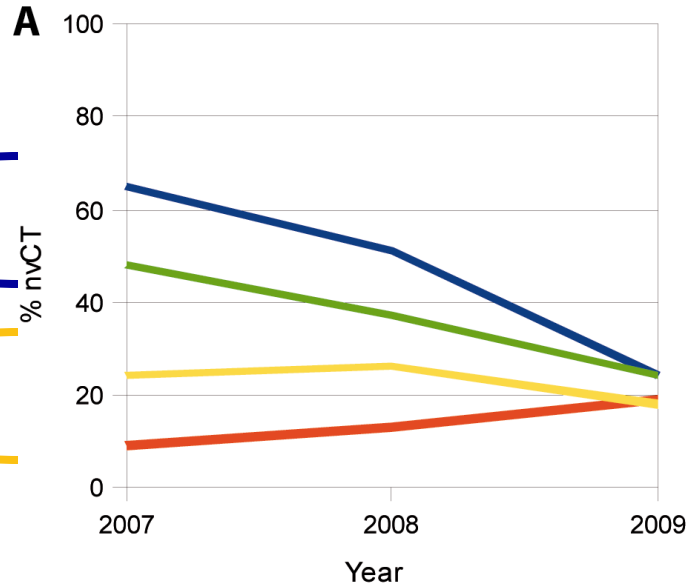
- **Most plausible explanation for the emergence of the nvCT?**
“...due to a **recent single genetic event**, which appears to be **neutral with regard to biological fitness**. It is likely to have **occurred within a single bacterial cell that clonally expanded and initially existed as a coinfection** together with the wild type parent/progenitor *C. trachomatis*. The nvCt and progenitor were **initially transmitted simultaneously**; **however, at some time, the nvCT was able to separate from the progenitor and cause a single clonal infection, by chance rather than by out-competition of the progenitor** due to increased biological fitness. The nvCT was then **rapidly and widely transmitted due to the strong diagnostic selective advantage**. Accordingly, nvCT escaped detection (treatment and contact tracing) and thus could spread rapidly, especially in high-frequency transmitting populations.”

Epidemiology of the nvCT – the 4 Swedish Counties study (Klint, et al. CMI. 2011) – Equilibrium?- Very soon new data!

nvCT proportions in:

2 Roche Counties

2 BD Counties

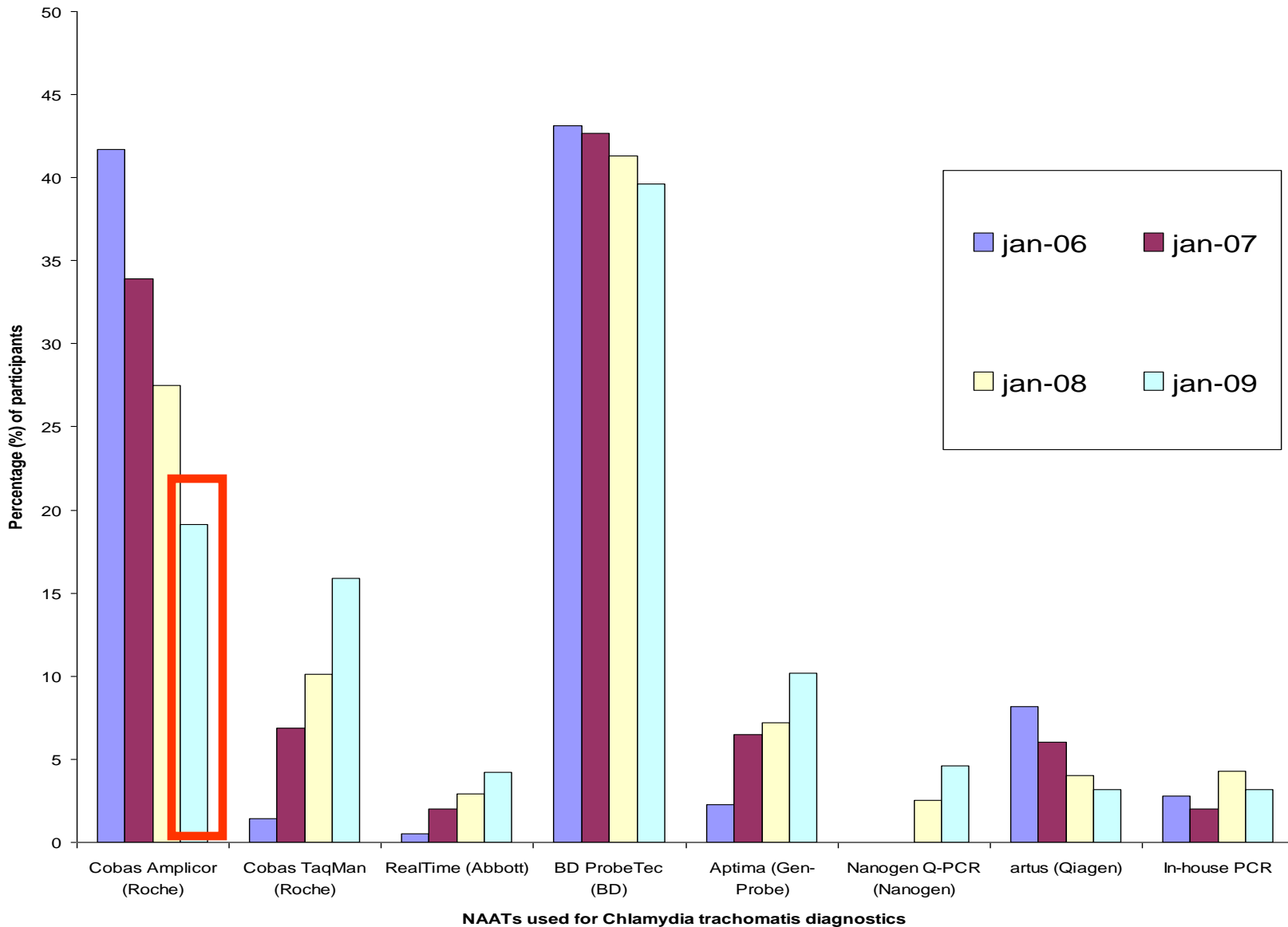


County CT incidence

Presence of nvCT internationally

- Initially only **sporadic cases outside the Nordic countries**, e.g. in Ireland, Scotland and France (Lynagh, et al. Epi-Insight. 2007; Health Protection Scotland. HPS Weekly Report 42(2008/39); de Barbeyrac, et al. Euro Surveill. 2007)
- **Early EU surveillance** (ESSTI and ECDC; Savage, et al. Euro Surveill. 2007) and **international studies** found **none/few additional nvCT**
- **nvCT spread** (Europe and globally)?

UK NEQAS scheme (2009, 283 labs. in 22 countries) for molecular detection of *C. trachomatis* 2006-2009 (Unemo, et al. Euro Surveill. 2009)



Do we know how widespread nvCT is?

- Few recent publications!
- Many European laboratories can still not detect nvCT (Unemo, et al. Euro Surveill. 2009; Reischl, et al. Euro Surveill. 2009)!
- Laboratories that can detect it do not know it!
- Early transmission in several countries (nvCT increased 1% - 3.2% in Oslo, Norway [Jan 2007-June 2008], Reinton, et al. Tidsskrift Nor Lægeforen. 2010)?

Remaining considerations?

- Will nvCT reach equilibrium with wtCT or be eradicated in Sweden?
- Presence of nvCT beyond the Nordic countries (early or late spread in other countries; **Swedish sex tourism?**)?
- Increased knowledge of the **sexual networks** needed?
- Increased knowledge regarding **CT strain populations** (previously and presently) needed?
- **Is nvCT clonal**, probably, however is *ompA* sequencing, MLST and VNTR enough for such statement?
- **Improved genetic typing** methods are crucial!

Lessons learned are numerous!

- **Monitor and ANALYSE incidence**, locally, nationally and internationally, and **timely alert unexplained significant declines** (nvCT or other mutants)!
- Frequent participation in appropriate **external quality assessments systems (EQAS)** is crucial!
- **EQAS** should ideally include **temporally, geographically, phenotypically and genetically diverse strains, different methods, and divergent populations!**
- Laboratories using **Amplicor CT/NG, Cobas Amplicor CT/NG, and *in house* NAATs** targeting the nvCT deletion are encouraged to **change diagnostic method!**

- Ideally, **multi-target assays, detecting essential conserved non-cryptic multicopy species-specific genes/RNA** (for most pathogens?), ought to be used!
- **Several assays** should be available and used **on a national level!**
- **Frequent surveillance and evaluations** of diagnostic methods, strategies, and guidelines worldwide are crucial!
- **Response plans for** similar situations are essential!
- **Unique opportunity** to get further insight in the epidemiology and transmission of CT (and other STIs?)!

Thank you for your attention!

