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

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Notified chlamydial cases in Sweden 1997-2009

Men
Women

“Catch up”
nvCT
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


- **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)
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%)  
- Sörmland 30%  
  Nov-Dec 2006
- Örebro 39%  
  Oct-Dec 2006
- Borås 26%  
  Feb-Mar 2007
- Halland 24%  
- Kalmar 20%  
  Dec 2006–Jan 2007
- Skåne 24%  
  Nov 2006–March 2007

BD counties - 1/3 (7-19%)  
- Dalarna 64%  
  Dec 2006–March 2007
- Norrbotten 10%  
  Oct 2006–Feb 2007
- Uppsala 19%  
  Nov 2006–Feb 2007
- Jönköping 14%  
  Dec 2006–Jan 2007
- Blekinge 7%  
  Nov 2006–Feb 2007

Herrmann, et al. EID. 2008
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


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Microbiology. 2010; 156: 1394-1404

(Comparison to all available *C. trachomatis* genomes, and relevant wild type strains of different serovars, incl. clinic and epidemiology)
<table>
<thead>
<tr>
<th>Strain</th>
<th>nvCT (Sweden2)</th>
<th>UW-3/CX</th>
<th>TZ1A828/OT</th>
<th>Jali20</th>
<th>HAR-13</th>
<th>434/BU</th>
<th>UCH-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biovar</td>
<td>Trachoma</td>
<td>Trachoma</td>
<td>Trachoma</td>
<td>Trachoma</td>
<td>Trachoma</td>
<td>Trachoma</td>
<td>Trachoma</td>
</tr>
<tr>
<td>Origin</td>
<td>Sweden</td>
<td>USA</td>
<td>Tanzania</td>
<td>The Gambia</td>
<td>Saudi Arabia</td>
<td>USA</td>
<td>England</td>
</tr>
<tr>
<td>Chromosome (bp)</td>
<td>1,042,839</td>
<td>1,042,519</td>
<td>1,044,282</td>
<td>1,044,352</td>
<td>1,044,459</td>
<td>1,038,842</td>
<td>1,038,869</td>
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<tr>
<td>G+C content (%)</td>
<td>41.3</td>
<td>41.3</td>
<td>41.3</td>
<td>41.3</td>
<td>41.3</td>
<td>41.3</td>
<td>41.3</td>
</tr>
<tr>
<td>Predicted CDSs</td>
<td>889</td>
<td>894</td>
<td>879</td>
<td>875</td>
<td>920</td>
<td>889</td>
<td>889</td>
</tr>
<tr>
<td>Coding density (%)</td>
<td>89</td>
<td>90</td>
<td>89</td>
<td>89</td>
<td>90</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>rRNA operon</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>tRNA operon</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Average gene size (bp)</td>
<td>1056</td>
<td>1050</td>
<td>1051</td>
<td>1056</td>
<td>1032</td>
<td>1052</td>
<td>1052</td>
</tr>
<tr>
<td>No. pseudogenes</td>
<td>14</td>
<td>5</td>
<td>14</td>
<td>18</td>
<td>8</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Plasmid size (bp)</td>
<td>7,169</td>
<td>7,493</td>
<td>7,502</td>
<td>7,506</td>
<td>7,510</td>
<td>7,499</td>
<td>7,500</td>
</tr>
</tbody>
</table>

LGV, lymphogranuloma venereum; ND, not yet determined; CDS, coding sequence

a These genomes have been annotated elsewhere, thus predicted CDSs and pseudogene no. may not be comparable.
b Pseudogene no. do not include cytotoxin gene fragments.
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 chlamydiace

- **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**!

Unemo, et al. Microbiology. 2010
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)!

Unemo, et al. Microbiology. 2010
Growth characteristics (development cycle, inclusion formation and morphology) in BGMK cells using transmission electron microscopy (TEM) ⇒ no obvious differences!

Unemo, et al. Microbiology. 2010
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**!

Unemo, et al. Microbiology. 2010
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) $\Rightarrow$ 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!

Unemo, et al. Microbiology. 2010
Unaltered biological fitness further confirmed by examination of genovar distributions in Örebro County, Sweden 1999-2000 vs. 2006

Jurstrand, et al. STI. 2009
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.”

Unemo, et al. Microbiology. 2010
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)?

NAATs used for Chlamydia trachomatis diagnostics

- Cobas Amplicor (Roche)
- Cobas TaqMan (Roche)
- RealTime (Abbott)
- BD ProbeTec (BD)
- Aptima (Gen-Probe)
- Nanogen Q-PCR (Nanogen)
- artus (Qiagen)
- In-house PCR

Percentage (%) of participants (jan-06, jan-07, jan-08, jan-09)
**Do we know how widespread nvCT is?**

- Few recent publications!


- 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. Tidskrift Nor Legeforen. 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!