Molecular Epidemiology: Lessons Learnt and Application to Streptococcus agalatiae

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Mastitis Control – Herd Level
Contagious mastitis: 5-Point Plan
Environmental mastitis: Pressure vs. Resistance

Mastitis Control – Cow Level
Host-adapted
“Knows how to behave”
Reduce duration: treatment, culling

Opportunistic
“Does not know how to behave”
Reduce new cases: prevention

Table 1: Description of characteristics of host-adapted and non-host-adapted ecotypes of Streptococcus agalatiae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Host-adapted</th>
<th>Non-host-adapted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain heterogeneity</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Manifestation</td>
<td>Subclinical</td>
<td>Clinical</td>
</tr>
<tr>
<td>Source</td>
<td>Soil</td>
<td>Soil</td>
</tr>
<tr>
<td>Incubation in infected animals</td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>Resistance to treatment of mastitis cases</td>
<td>Poor</td>
<td>Improved</td>
</tr>
</tbody>
</table>

Cow ↔ Herd
• What happens at cow level affects what happens at herd level

Strain Typing (Fingerprinting)

Strain Heterogeneity
Klebsiella from soil
Strep. uberis from soil

Many different strains in the environment
Many different strains present in environmental mastitis cases
Applications and Insights

- What happens in the cow
  - Transient and persistent infections
  - Treatment outcome
- Introduction of bacteria
  - Animals
  - Other sources
- What happens in the herd
  - Contagious
  - Environmental
  - Point source
  - Host-to-host

Repeated Clinical Mastitis (E. coli)

- Scenario 1
  - Cow becomes infected, CM
  - Cow clears infection
  - Cows become infected, CM
  - Cow clears infection
  - Etc.
- Failure of prevention
  - Multiple infections, multiple strains

- Scenario 2
  - Cow becomes infected, CM
  - Clinical signs disappear
  - Clinical signs come back, CM
  - Clinical signs disappear
  - Etc.
- Failure of cure
  - Single infection, single strain

It looks the same, but the cause and solution are different!

Persistent E. coli Infections

- Clinical mastitis in lactation due to infection in dry period
- Multiple clinical cases within a single quarter
  - 47% persistent infection – cow never cured, treatment failure
  - 53% cure and new infection – cow is susceptible to new infection
- If you don’t have access to strain typing, use culture or SCC data

Treatment Outcome (Strep. uberis)

- Opportunistic Strep. uberis
- Host-adapted Strep. uberis

Pathogen Introduction – Cattle

- One-year study with 4 samplings in 15 herds
- More animals purchased, more strains introduced
- May apply to expanding herds in Nordic countries
- Could apply to Staph. aureus, Strep. agalactiae, Mycoplasma
**Transmission**

- Multiple strains in lactating cows
- Different strains in pre-calving heifers – not milked yet
- Transient, severe (heifer) or mild (cow) clinical mastitis
- Not controlled by 5-point plan

**Staph. aureus Transmission**

<table>
<thead>
<tr>
<th>Type</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1</td>
<td>B1</td>
<td>1</td>
<td>C1</td>
<td>1</td>
<td>D1</td>
</tr>
<tr>
<td>A2</td>
<td>1</td>
<td>B2</td>
<td>2</td>
<td>C2</td>
<td>1</td>
<td>D2</td>
</tr>
<tr>
<td>A3</td>
<td>1</td>
<td>B3</td>
<td>1</td>
<td>C3</td>
<td>1</td>
<td>D3</td>
</tr>
<tr>
<td>A4</td>
<td>1</td>
<td>B4</td>
<td>1</td>
<td>C4</td>
<td>1</td>
<td>D4</td>
</tr>
<tr>
<td>A5</td>
<td>1</td>
<td>B5</td>
<td>1</td>
<td>C5</td>
<td>1</td>
<td>D5</td>
</tr>
</tbody>
</table>

**Strep. uberis Transmission**

- One strain affecting multiple lactating cows
- Spread via liners of milking machine demonstrated
- PMTD and treatment/segregation of infected animals
- Ignore the 5-point plan at your peril!
Contagious or Point Source?

Klebsiella mastitis

- One strain in most cows
- Contagious or point source
- Isolated from liners
  - Multiple strains
  - Isolated from bedding
  - One dominant strain

"Contagious via the Environment"

Streptococcus agalactiae?

MRSA transmission - PFGE

- 1978 - MRSA mastitis in dairy cattle in Belgium - milkers thought to be the source
- 2007 - MRSA mastitis in dairy cattle in Hungary - milker and cattle share same strain

MRSA - MLST

- Multi-locus sequence typing
- Host-association of clusters
- Major human clusters
- Major bovine clusters
  - CC97
  - CC151
  - CC479
- MRSA in cattle and milker: ST1
  - <0.004% among 258 cattle isolates
  - 1.3% among 4771 human isolates
  - Probably human MRSA in cattle

Paradigm shift?
**Strep. agalactiae Host Species**

- **People**
  - Asymptomatic carriage
  - Invasive disease in neonates and infants
  - Emerging disease in non-pregnant adults
- **Cattle**
  - Intramammary infection
- **Fish**
  - Septicaemia, encephalitis, mortality
  - Emerging disease in aquaculture (tilapia)

**Strep. agalactiae (GBS) in People**

- Asymptomatic carriage
  - 20 to 35% of men and women
  - Gastro-intestinal and genito-urinary tract
  - Sexual transmission
- Emerging disease
  - Elderly and immunocompromised patients
  - Increases reported in USA, Norway, Denmark
- Risk factors
  - Sexual activity
  - Consumption of tilapia fish
  - Exposure to cattle

**Multi Locus Sequence Typing**

- Allelic profile: 3-5-2-1-7-18-4
- Sequence type: ST246

**MLST Strep. agalactiae**

- Amplification of selected targets
- Comparison with database for allele assignment
- Combination of alleles into allelic profile and assignment of ST

**Human Strep. agalactiae**

- Capsule type frequency (%) 1a to 6a, NT
- Capsule phenotype frequency (%) 1a to 6a, NT

**S. agalactiae Evolution — Bovine to Human?**

- Hyperinvasive Neonatal Group B Streptococcus Has Arisen from a Bovine Ancestor

References:

- Dogan et al., J Clin Microbiol. 2005;43(12):5899-906
**S. agalactiae Evolution – Human to Bovine?**

- **Genome of S. agalactiae isolate from bovine milk**
- **Comparison against genomes of human isolates (+ NEM316)**
- "Bovine specific" genes concentrated in 8 regions
- Regions are remnants of mobile genetic elements
- Some regions shared with other mastitis pathogens

**Cattle-Specific Genes?**

- Poor specificity
- Poor sensitivity

**Mastitis Specific Genes?**

- Most human S. agalactiae does not utilize lactose
- Most bovine S. agalactiae does utilize lactose
- Different mastitis pathogen species share similar genes

**Human S. agalactiae in Cows?**

- Human isolate causes clinical mastitis of short duration in cow (Jensen, 1982)
- Occasional clinical S. agalactiae in low SCC (<150,000 cells/ml) herd (Barkema, 1998)
- Low S. agalactiae count in bulk tank milk not always linked to a positive cow (Andersen, 2002)
- S. agalactiae in bulk tank milk, but not in any cow milk sample (QMPS, 2003)
- S. agalactiae in one cow in long-term closed herd (QMPS, 2002)
- Occasional, clinical, tetracyclin-resistant S. agalactiae in low SCC herd (Sears, 2003)

**Multi-host Strain Typing: S. agalactiae**

- ST67 74%
- ST103 20%
- ST1 25%
- ST23 20%

- **n = 98, UK**
- **n = 191, Denmark**

**Richards et al., Infect Genet Evol. 2011;11(6):1263-75**

**Finch and Martin, J Appl Bacteriol. 1984;57(2):273-8**

**Bisharat et al., J Clin Microbiol. 2004;42:2161**
**Lactose Fermentation – PCR vs phenotype**

<table>
<thead>
<tr>
<th>ST</th>
<th>lacR positive</th>
<th>lacR negative</th>
<th>lactose fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>7</td>
<td>yes</td>
</tr>
<tr>
<td>23</td>
<td>5</td>
<td>5</td>
<td>yes</td>
</tr>
<tr>
<td>103</td>
<td>0</td>
<td>9</td>
<td>yes</td>
</tr>
</tbody>
</table>

**Strep. agalactiae From Finland**

- **Human isolates**
  - Carriage and disease (n = 46)
  - 13 STs, no new STs
- **Bovine isolates**
  - Cow/quarter milk (n = 63)
  - 13 STs, 5 new STs
- **Shared across hosts**
  - Minority of STs (n = 3)
  - Majority of isolates (n = 59)

**Host and Country Comparisons**

- Human isolates Finland (carriage or clinical, n = 46)
- Bovine isolates Finland (individual milk samples, n = 63)
- Bovine isolates Denmark (bulk tank samples, n = 377)
Host and Country Comparisons

- ST1 common across countries and species
- ST12 found in Finland across species
- ST19 largely limited to people
- ST23 not found in cattle in Finland
- ST61 only found in cattle in Finland
- ST103 found in cattle across countries

Strain-Specific Epidemiology

- More likely to cause clinical mastitis
- Shed at lower levels from quarters
- Less likely to spread within herd
- Introduced into herds by people
- More likely to cause subclinical mastitis
- Shed at higher levels from quarter
- More likely to spread within herd
- Move from herd to herd via cows

→ Strain-specific within-herd prevalence?
→ Contact tracing between positive herds?
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  - More likely to spread within herd
  - Move from herd to herd via cows

→ Strain-specific within-herd prevalence?  DK → No?
→ Contact tracing between positive herds?

### Strep. agalactiae Transmission - knowledge gaps -

- Milking
- Cattle - infection
- Host - infection
- Human - infection
- Cattle - carrier
- Human - carrier
- Environment (faeces)
- Effluent
- Spread contact

### The 5-Point Plan

1. Good milking machine and technique
2. Teat disinfection
3. Treat clinical cases
4. Cull chronic cases
5. Routine dry cow treatment with antimicrobials

- Denmark: conventional milking machine 5.7%, AMS 10%
- Nordic countries: judicious use of antimicrobials, selective dry cow treatment

### Conclusions

- *Strep. agalactiae* is more common in people than in cattle
- Host-adaptation of *Strep. agalactiae* is not absolute
- Transmission between host species seems possible
- Time course of events or directionality difficult to prove
- Patterns differ between countries, mechanism unknown
- WGS may provide more insight
- Little is known about *S. agalactiae* in faeces and environment
- Epidemiology = host + pathogen + management

### Acknowledgements

- Ulrike Lyhs
- Laura Kulkas
- Jørgen Katholm
- Christian Delannoy
- David Rodgers
- Jordan Mitchell
- Michael Churakov