Streptococcus agalactiae an increasing problem in Scandinavia

Report from Denmark,
Jørgen Katholm, Danish Cattle Federation

S. agalactiae is CAMP positive

PREVALENCE OF S. AGALACTIAE IN OTHER COUNTRIES

Germany 2001 - 2002 (80 herds 32 cows tested)
29% of herds
Tenhagen B.A. et al 2006

Canada
Prince Edward Island (all 258 dairy herds Bulk tank sampled for 3 weeks)
1.6% (4 herds were positive at least once)

Belgium (Flanders)
1087 herds -1 cross sectional sampling of all dairy herds in 3 years
5.3% (41 herds - in 28 herds only 1 quarter)
Piepers S. et al 2007

Holland 1996 - 1999 (3542 dairy farmers 46015 cows SCC > 250.000)
1.5 % infected cows
Poelarends J.J. et al 2001

USA - Wisconsin 1994 -2001 (77172 samples send to laboratory)
Decrease from 8,1% to 3%
Makoveg and Ruegg 2003

USA – New York and Pensylvania (108.312 cows)
10.1%
Wilson et al 1997

Finland
1988 – 493 randomly selected farms 4.403 cows 17.111 quarters
1995 – 238 randomly selected farms 2.648 cows 10.337 quarters
2001 – 216 randomly selected farms 3.282 cows 12.661 quarters
1988 0.78 % of quarters
1995 0.12% of quarters
2001 0.02% (in total 3 quarters infected)

PREVALENCE OF S. AGALACTIAE IN OTHER COUNTRIES

Finland 1988 – 493 randomly selected farms 4.403 cows 17.111 quarters
1995 – 238 randomly selected farms 2.648 cows 10.337 quarters
2001 – 216 randomly selected farms 3.282 cows 12.661 quarters

1988
0.78 % of quarters
1995
0.12% of quarters
2001
0.02% (in total 3 quarters infected)

5 Streptococcus agalactiae in Danish dairy herds 9 June 2010
6 Streptococcus agalactiae in Danish dairy herds 9 June 2010
Eradication investigations
40 old infected herds april 1974 Jensen N.E. 1976

Not clean 5 Herds

In total there has been

4,85 herd investigations

In the 35 old herds that were cleaned of the infection

Conclusions Jensen N.E. 1976

It is still not a problem to clean herds for the infection
The problem is the unexplained new infections

Conclusions Andersen and Huda 1995

The bulk tank test can find

1 infected cow in a 200 cow herd

High infection rate
Also in a period with intensified eradication program

Report Dansih Cattle 2004 Pedersen, Nielsen and Jepsen 2004

Period from 1989 – 2003
1976 new infected herds
Quarterinvestigation of 56,133 cows
Prevalence of infected cows
average 10% positive cows
in 37% of herds less than 5% infected cows
in 24.9 % of herds between 5 and 10% infected cows
in 1.2% of herds more than 50% of cows infected

Report Dansih Cattle 2004 Pedersen, Nielsen and Jepsen 2004

CMT investigation of 8268 S. agalactiae positive cows
CMT 1 2136 25.8%  
CMT 2 973 11.8%  
CMT 3 988 11.9%  
CMT 4 1507 18.2%  
CMT 5 2664 32.2%
Milk yield and bacterial infection

Schukken et al 2009

Cows infected with

- CNS + 0.45 kg/day sd 0.12 p<0.001
- S. agalactiae - 3.6 kg/day sd 0.12 p<0.001
- Streptococcus spp - 1.6 kg/day sd 0.18 p<0.001
- S. aureus - 1.8 kg/day sd 0.18 p<0.001

352,614 records from 4200 hole herd mastitis screening

Streptococcus agalactiae and SCC

Y. H. Schukken et al 2009

Heifers
- S. agalactiae

Cows
- Staf a.
- CNS
- Coryne bovis
- Negative

Herd size in Denmark 1999-2009

Number of AMS herds in Denmark

Yearly test of bulk milk from all herds

Yearly since 1995. From 1963-1995 different intervals

Card with barcode to start inload of milk
Sampling device at truck
VM OVP valve, company VM Tarm A/S
First 30-40 l, no sampling
Thereafter 1.5 ml 40 times at interval depending on last herd milk load
Total sample 60 ml
Cleaning of device with pressure air
Label from farm with barcode
Stored on ice until testing

Culture of bulk milk sample

120 µl mixed with 12 ml streptococci
selective media deep culture
- Polymyxin B, neomycin, fucidin
Suspected colonies
- Haemolytic
- Aesculin negative
CAMP reaction
- I.e. agglutination
**B-register**

Herds is tested at yearly screening (120µl)
1. if negative                      Free
   if positive
2. new sample (120µl + 500µl)
   if positive                      B-herd
   if negative
3. new sample
   if positive                      B-herd
   if negative

Clear of register
Neg. 4 BTMS more than 1 month apart Free
Neg. individual sampling of all quarters Free


**Herds in B-register**
2 June 2010, 344 herds

**Incidence of Danish dairy herds in the B-register per year per 1,000 herds**

**Cleared herds per year in percentage of the number of herds in the B-register as of 31 December**

**Percentage of milking herds in B-register in relation to herd size**
Percent herds in B register according to milking system and AMS producer

<table>
<thead>
<tr>
<th>Herd type</th>
<th>Number of herds</th>
<th>% herds in B register</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lely</td>
<td>401</td>
<td>10.2%</td>
</tr>
<tr>
<td>DeLaval</td>
<td>392</td>
<td>4.6%</td>
</tr>
<tr>
<td>Other AMS</td>
<td>67</td>
<td>9.0%</td>
</tr>
<tr>
<td>Konvensional</td>
<td>3391</td>
<td>5.7%</td>
</tr>
<tr>
<td>Total</td>
<td>4251</td>
<td>6.1%</td>
</tr>
</tbody>
</table>

Data on the 21 December 2009

Number of cows and producer of AMS is taken into account in this logistic regression with the two factors in an additive model.

Questionnaire to 233 milking herds in B-register as of 2nd July 2008. Owner answers in % from 77 herds (33%).

Questionnaire to 233 milking herds in B-register as of 2nd July 2008. Veterinary answer in % from 82 herds (35%).
Questionnaire to farmers and vets from 233 herds in B-register 2nd July 2008

Replies from farmers 33%, vets 35%

Farmers

Clinical problems 21
Increase in BMSCC 29
No use of PMTP 25

Veterinarians

Culture of Str. agalactiae in the herd 22
No use of DCT 26
Eradication programme 12

Trade patterns among infected herds 2000 – 2nd July 2008

Number of infected dairy herds 233
No introduction of cattle 24
- 17 free since 1991
Only introduction of cattle from free herds 104
Introduction of cattle from B-register herds 105

S. agalactiae serotyping of 21 isolates 2008

Knowledge before trial. N E Jensen

From 1976-1979 serotype III, 46%
NT, 3%

From 1976 – 1979 reduction most herds with serotype III

Types other than type III do not seem to be spread as easily as type III in new-infected herds

Around 50% of introduction of GBS in Danish dairy herds may be of human origin

Affection of the general condition of the cow is seen more often in GBS mastitis cases of human origin
Human strains have tendency to relatively fast spontaneous elimination

Selection of herds

Acute infection in Herds
- 16 farmers replied to a questionnaire that the infection causes more clinical mastitis in the herd
- 15 were positive in February 2009

Cronically infected herds
- Since 2000, 13 herds out of 233 herds in the Danish B-register had no more than one of the yearly testings negative
- 10 were positive in February 2009
Isolates in Danish dairy cows is of human origin

<table>
<thead>
<tr>
<th>ST</th>
<th>%</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24%</td>
<td>6 of 25</td>
</tr>
<tr>
<td>19</td>
<td>8%</td>
<td>2 of 25</td>
</tr>
<tr>
<td>23</td>
<td>20%</td>
<td>5 of 25</td>
</tr>
<tr>
<td>67</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

Is ST-17 common in Danish dairy herds: No

Is clinical appearance due to difference in serotype or MLST: Maybe

Is MLST found in Danish dairy herds different from human types: No

**Use of Bulk tank milk for mastitis surveillance**

**Good quality milk - Culture**

- Total bacterial count: < 5,000
- Termoflie: < 175
- Coliform: < 20
- Pseudomonas: < 500
- Str. uberis: < 200
- Total stafilococal number: < 200
- Staf. aureus: < 10
- SCC: < 150,000

**Ability of bulk milk culture for estimating Streptococcus agalactiae prevalence in Danish dairy herds**

- Figure: Scatter plot showing correlation between prevalence and bulk milk culture results.
Comparison of PCR and culture
4258 Bulk tank samples

<table>
<thead>
<tr>
<th>Test for B-strep</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>177 (12)</td>
</tr>
<tr>
<td>Negative</td>
<td>133 **(35)</td>
</tr>
</tbody>
</table>

Positive 177 (12) * 11 herds in B-register 9 only one positive

** 28 herds in B-register

Numbers in paraphrase is herds between 37 og 39,9

Distribution of bacteria in 4258 bulk tank samples from all Danish dairy herds in 2009
Percent herds with NoCt, median, mean and standard deviation of herds with a Ct value

<table>
<thead>
<tr>
<th>Bakteria</th>
<th>% NoCt</th>
<th>Median</th>
<th>Mean rest</th>
<th>± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staf aureus</td>
<td>9</td>
<td>32,4</td>
<td>32,5</td>
<td>2,91</td>
</tr>
<tr>
<td>Staf spp</td>
<td>0</td>
<td>29,8</td>
<td>29,8</td>
<td>2,07</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>22</td>
<td>34,8</td>
<td>34,8</td>
<td>2,65</td>
</tr>
<tr>
<td>Str. agalactia (B)</td>
<td>93</td>
<td>31,5</td>
<td>31,5</td>
<td>4,80</td>
</tr>
<tr>
<td>Str. dys</td>
<td>14</td>
<td>31,6</td>
<td>31,6</td>
<td>3,18</td>
</tr>
<tr>
<td>Str. uberis</td>
<td>5</td>
<td>30,3</td>
<td>30,3</td>
<td>3,44</td>
</tr>
<tr>
<td>C. bovis</td>
<td>10</td>
<td>33,5</td>
<td>33,7</td>
<td>1,67</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>22</td>
<td>33,7</td>
<td>33,6</td>
<td>2,84</td>
</tr>
<tr>
<td>E. coli</td>
<td>39</td>
<td>35,8</td>
<td>35,2</td>
<td>3,54</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>87</td>
<td>38,5</td>
<td>38,8</td>
<td>3,54</td>
</tr>
<tr>
<td>S. macescens</td>
<td>98</td>
<td>37,8</td>
<td>37,0</td>
<td>2,96</td>
</tr>
<tr>
<td>A.pyo/P. ind</td>
<td>37</td>
<td>35,7</td>
<td>35,2</td>
<td>2,95</td>
</tr>
</tbody>
</table>

Dansk Kvæg

PCR results 4258 bulk tank samples

<table>
<thead>
<tr>
<th>Bakteria</th>
<th>Lowest</th>
<th>Median</th>
<th>Fraktil 10</th>
<th>Fraktil 25</th>
<th>Fraktil 75</th>
<th>Fraktil 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staf aureus</td>
<td>19,5</td>
<td>32,4</td>
<td>28,9</td>
<td>30,5</td>
<td>34,4</td>
<td>36,3</td>
</tr>
<tr>
<td>Staf spp</td>
<td>17,7</td>
<td>29,8</td>
<td>27,3</td>
<td>28,6</td>
<td>31,0</td>
<td>32,1</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>22,2</td>
<td>34,8</td>
<td>31,5</td>
<td>33,1</td>
<td>36,6</td>
<td>38,4</td>
</tr>
<tr>
<td>Str. agalactia (B)</td>
<td>17,3</td>
<td>31,5</td>
<td>25,7</td>
<td>28,5</td>
<td>35,1</td>
<td>37,9</td>
</tr>
<tr>
<td>Str. dys</td>
<td>15,9</td>
<td>31,8</td>
<td>27,7</td>
<td>29,6</td>
<td>33,6</td>
<td>35,6</td>
</tr>
<tr>
<td>Str. uberis</td>
<td>13,9</td>
<td>30,3</td>
<td>26,0</td>
<td>28,1</td>
<td>32,4</td>
<td>34,8</td>
</tr>
<tr>
<td>C. bovis</td>
<td>24,5</td>
<td>33,5</td>
<td>31,9</td>
<td>32,6</td>
<td>34,5</td>
<td>35,8</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>20,8</td>
<td>33,7</td>
<td>30,0</td>
<td>31,9</td>
<td>35,6</td>
<td>37,3</td>
</tr>
<tr>
<td>E. coli</td>
<td>17,6</td>
<td>35,8</td>
<td>30,4</td>
<td>33,2</td>
<td>38,1</td>
<td>39,2</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>18,9</td>
<td>36,5</td>
<td>31,3</td>
<td>33,7</td>
<td>38,6</td>
<td>39,6</td>
</tr>
<tr>
<td>S. macescens</td>
<td>25,4</td>
<td>37,8</td>
<td>33,8</td>
<td>36,4</td>
<td>38,9</td>
<td>39,4</td>
</tr>
<tr>
<td>A.pyo/P. ind</td>
<td>18,5</td>
<td>35,7</td>
<td>31,8</td>
<td>33,9</td>
<td>37,2</td>
<td>38,5</td>
</tr>
</tbody>
</table>

Dansk Kvæg
### Distribution of bacteria in 30 bulk tank samples from all Faroe Island dairy herds in 2009

<table>
<thead>
<tr>
<th>Bakteria</th>
<th>% NoCt</th>
<th>Median</th>
<th>Mean rest</th>
<th>± sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staf aureus</td>
<td>7</td>
<td>33,61</td>
<td>33,61</td>
<td>2,92</td>
</tr>
<tr>
<td>Staf spp</td>
<td>0</td>
<td>31,51</td>
<td>31,4</td>
<td>2,56</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>40</td>
<td>36,71</td>
<td>36,35</td>
<td>2,49</td>
</tr>
<tr>
<td>Str. agalactia (B)</td>
<td>77</td>
<td>33,45</td>
<td>31,75</td>
<td>2,82</td>
</tr>
<tr>
<td>Str. uberis</td>
<td>100</td>
<td>32,28</td>
<td>32,77</td>
<td>1,82</td>
</tr>
<tr>
<td>C. bovis</td>
<td>43</td>
<td>34,4</td>
<td>34,9</td>
<td>1,66</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>43</td>
<td>36,93</td>
<td>36,59</td>
<td>1,97</td>
</tr>
<tr>
<td>E. coli</td>
<td>97</td>
<td>38,72</td>
<td>37,72</td>
<td>-</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>97</td>
<td>39,7</td>
<td>39,7</td>
<td>-</td>
</tr>
<tr>
<td>S. maesencens</td>
<td>100</td>
<td>37,31</td>
<td>36,54</td>
<td>2,20</td>
</tr>
</tbody>
</table>

Percent herds with NoCt, median, mean and standard deviation of herds with a Ct value.

### Culture on composite samples and PCR on yield control samples

<table>
<thead>
<tr>
<th>Test for B-strep.</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>38</td>
</tr>
<tr>
<td>Negative</td>
<td>93</td>
</tr>
</tbody>
</table>

3 herds 442 samples

<table>
<thead>
<tr>
<th>Herd (1)</th>
<th>2013 (3 – NoCt) and 2710 (8 – NoCt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd (2)</td>
<td>2232 (2-NoCt), 2334 (34-15,67), 2361 (345-NoCt), 2249 (237-45), 2305 (5 – NoCt), 2475 (28 – 27.11)</td>
</tr>
</tbody>
</table>

### Streptococcus agalactia eradication 19 jan 2009

23 Culture positive 63 PCR positive of 181/183 prøver
Infected beginning of 2009 after expansion
After around 6 months prevalence 34% (I/S T)

KJ at the edge of giving up (prevalence 61%)

KJ number of cow treated for mastitis

Mastitis, cases and treatments herd 1.
Segregation 19 Jan 2009

Infected beginning of 2009 after expansion
After around 6 months prevalence 34% (I/S T)

SCC

Tested

Culling and treatment

Infected beginning of 2009 after expansion
After around 6 months prevalence 34% (I/S T)
### Status of known eradications

<table>
<thead>
<tr>
<th>Chr</th>
<th>Date of eradicating</th>
<th>Animals</th>
<th>Culture</th>
<th>PCR pos</th>
<th>Prevalence</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>19/8/09</td>
<td>303</td>
<td>281</td>
<td>95</td>
<td>34</td>
<td>26/4 10</td>
<td>33.9</td>
</tr>
<tr>
<td>5</td>
<td>11/9/09</td>
<td>166</td>
<td>27</td>
<td>7</td>
<td>4</td>
<td>26/4 10</td>
<td>33.9</td>
</tr>
<tr>
<td>6</td>
<td>15/10/09</td>
<td>175</td>
<td>159</td>
<td>29=13</td>
<td>26</td>
<td>26/5 10</td>
<td>21.6</td>
</tr>
<tr>
<td>7</td>
<td>10/11/09</td>
<td>125</td>
<td>107</td>
<td>8=3</td>
<td>10</td>
<td>26/5 10</td>
<td>27.3</td>
</tr>
<tr>
<td>8</td>
<td>16/12/09</td>
<td>181</td>
<td>149</td>
<td>79=12</td>
<td>61</td>
<td>26/5 10</td>
<td>NA</td>
</tr>
<tr>
<td>9</td>
<td>5/1/10</td>
<td>103</td>
<td>94</td>
<td>17=3</td>
<td>21</td>
<td>26/4 10</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>1/10/09</td>
<td>50</td>
<td>48</td>
<td>1</td>
<td>2</td>
<td>26/5 10</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>15/2/10</td>
<td>42</td>
<td>Pool</td>
<td>3 pool 3 cows</td>
<td>7</td>
<td>26/5 10</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>2/2/10</td>
<td>75</td>
<td>68</td>
<td>0=2</td>
<td>3</td>
<td>10/4 10</td>
<td>NA</td>
</tr>
<tr>
<td>13</td>
<td>1/2/10</td>
<td>75</td>
<td>75</td>
<td>16</td>
<td>21</td>
<td>10/4 10</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>3/3/10</td>
<td>301</td>
<td>Pool</td>
<td>15</td>
<td>5</td>
<td>10/4 10</td>
<td>NA</td>
</tr>
<tr>
<td>15</td>
<td>3/3/10</td>
<td>105</td>
<td>102</td>
<td>9</td>
<td>9</td>
<td>26/4 10</td>
<td>NA</td>
</tr>
<tr>
<td>16</td>
<td>3/3/10</td>
<td>105</td>
<td>Pool</td>
<td>4</td>
<td>4</td>
<td>26/5 10</td>
<td>NA</td>
</tr>
<tr>
<td>17</td>
<td>20/4/10</td>
<td>105</td>
<td>105</td>
<td>4</td>
<td>4</td>
<td>26/5 10</td>
<td>NA</td>
</tr>
</tbody>
</table>

Thank to the Danish Milk Levy Fund for funding.