Spatiotemporal Patterns of *Mycoplasma* spp. in California Bulk Tank Milk

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Background of *Mycoplasma* spp.

- Contagious mastitis pathogens
  - *Mycoplasma bovis*
- Seasonal
  - Rain
- Transmission
  - Between cows
  - Within a cow
- Clinical and sub-clinical infections
- Bulk tank monitoring
Economics of *Mycoplasma* spp.

- **Production Losses**
  - Decreased milk production
  - Treatment costs
  - Increased culling rates

- **Culture Costs**
  - Fresh and clinical samples
  - Bulk tank samples

- **Respiratory disease in calves**
Distribution of *Mycoplasma* spp. in California

![Map showing the distribution of Mycoplasma spp. positive counties in California in 1964.](image)
Distribution of *Mycoplasma* spp. in California
Distribution of *Mycoplasma* spp. in California
# California’s Changing Dairy Industry

<table>
<thead>
<tr>
<th></th>
<th>2002</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. Dairy Cows</td>
<td>1.67 million</td>
<td>1.77 million</td>
</tr>
<tr>
<td>Total No. Dairies</td>
<td>2,153</td>
<td>1,496</td>
</tr>
<tr>
<td>Cows/Dairy</td>
<td>776</td>
<td>1,186</td>
</tr>
<tr>
<td>Milk (lbs)/Cow/Year</td>
<td>20,993</td>
<td>23,234</td>
</tr>
<tr>
<td>Milk Producing Counties</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Top 5 Dairy Producing Counties</td>
<td>Tulare, Merced, Stanislaus, Kings, San Bernardino</td>
<td>Tulare, Merced, Stanislaus, Kings, Kern</td>
</tr>
<tr>
<td>% Total CA Milk</td>
<td>67%</td>
<td>73%</td>
</tr>
</tbody>
</table>

CDFA, California Agricultural Statistics 2003, CA Dairy Statistics Annual 2013
California Bulk Tank Milk Samples: 2001 to 2014

- Estimate regional and statewide prevalence of *M. bovis*
- Evaluate for spatiotemporal clustering patterns of *M. bovis*
- Evaluate the association of temperature and rainfall patterns and region with the prevalence of *M. bovis*
Retrospective, observational study

Study Population and Sample Collection

- Bulk tank milk samples submitted for routine culture
- 3 major milk processing plants in California
- Herd number, date and county of origin

Mycoplasma culture and identification

- Milk Quality Laboratory (VMTRC, Tulare, CA)
- Direct plating and post-enrichment broth
- Incubated at 37°C in 4% CO₂ for 7 days
- Speciation using direct fluorescent-antibody staining
Map creation and statistical analysis

- Counties with submissions
  - Major dairy hub
- Prevalence data for *M. bovis*
  - One-way ANOVA with Tukey adjustment
- Daily temperature and rainfall data
  - National Oceanic and Atmospheric Association
  - Daily estimates averaged to produce monthly and annual estimates
Map Creation and Statistical Analysis Cont.

- Spatiotemporal cluster analysis
  - Bernoulli model using SatScan software
    - Maximum temporal window sizes: 1 year and 1 month
    - Maximum spatial cluster size: 100,000 Cartesian units
  - Excess cases = Observed – expected cases
  - Relative Risk = ratio of cases inside the cluster to outside the cluster
Results

Map showing milk-producing counties and dairy hub of counties with submissions in California. The map is credited to the California Dept. of Food and Agriculture, California Agricultural Statistics 2001 to 2014.
Results

65,063 bulk tank milk samples
- 2451 Dairies
- 22 Counties
- June, 2001 to Feb, 2014

12,419 samples missing county information
- Creamery 1: 157
- Creamery 2: 3,513
- Creamery 3: 8,749
<table>
<thead>
<tr>
<th>Region</th>
<th>County</th>
<th>No. Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>BUTTE</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>GLENN</td>
<td>2,671</td>
</tr>
<tr>
<td></td>
<td>HUMBOLDT</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>MARIN</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>SACRAMENTO</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>SOLANO</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>TEHAMA</td>
<td>1,117</td>
</tr>
<tr>
<td></td>
<td>YOLO</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>YUBA</td>
<td>20</td>
</tr>
<tr>
<td>N. Central Valley</td>
<td>MADERA</td>
<td>869</td>
</tr>
<tr>
<td></td>
<td>MERCED</td>
<td>3,551</td>
</tr>
<tr>
<td></td>
<td>SAN JOAQUIN</td>
<td>1,100</td>
</tr>
<tr>
<td></td>
<td>STANISLAUS</td>
<td>2,721 + 8,749</td>
</tr>
<tr>
<td>S. Central Valley</td>
<td>FRESNO</td>
<td>3,412</td>
</tr>
<tr>
<td></td>
<td>KERN</td>
<td>1,129</td>
</tr>
<tr>
<td></td>
<td>KINGS</td>
<td>9,202</td>
</tr>
<tr>
<td></td>
<td>TULARE</td>
<td>24,289</td>
</tr>
<tr>
<td>Southern</td>
<td>LOS ANGELES</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>RIVERSIDE</td>
<td>942</td>
</tr>
<tr>
<td></td>
<td>SAN BERNARDINO</td>
<td>1,011</td>
</tr>
<tr>
<td></td>
<td>SAN DIEGO</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>SANTA BARBARA</td>
<td>3</td>
</tr>
</tbody>
</table>

β 61,393 samples with region
β Northern: 4,314 samples
β N. Central Valley: 16,990 samples
β S. Central Valley: 38,032 samples
β Southern: 2,057 samples
β 3,770 samples missing location
β Excluded from analysis
Regional Dairy Demographics

Mean Number of Cows Per Dairy in Four Regions of California

- Northern: 383
- N. Central Valley: 1132
- S. Central Valley: 1976
- Southern: 968

California Dept. of Food and Ag. California Dairy Statistics Annual 2013
Overall Prevalence of M. bovis in Bulk Tank Milk Samples in Four Regions of California: 2001 to 2014

Prevalence of M. bovis

<table>
<thead>
<tr>
<th>Region</th>
<th>N</th>
<th>Prev.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern</td>
<td>4,314</td>
<td>6.03%a</td>
<td>5.32, 6.74</td>
</tr>
<tr>
<td>N. Central Valley</td>
<td>16,990</td>
<td>8.03%b</td>
<td>7.63, 8.44</td>
</tr>
<tr>
<td>S. Central Valley</td>
<td>38,032</td>
<td>8.68%b</td>
<td>8.40, 8.97</td>
</tr>
<tr>
<td>Southern</td>
<td>2,057</td>
<td>12.35%c</td>
<td>10.93, 13.77</td>
</tr>
</tbody>
</table>

Dairy Hub of Region

Means without a common subscript are significantly different. (P-value < 0.0005)
Annual Precipitation and Prevalence of *M. bovis*

**Northern**

- M. bovis Prevalence
- Precipitation (in.)
- Linear (M. bovis)

**N. Central Valley**

- M. bovis Prevalence
- Precipitation (in.)
- Linear (M. bovis)

**S. Central Valley**

- M. bovis Prevalence
- Precipitation (in.)
- Linear (M. bovis)

**Southern**

- M. bovis Prevalence
- Precipitation (in.)
- Linear (M. bovis)
Spatiotemporal *M. bovis* Clusters: 1 year window

- **Primary Cluster**
  - **Southern Region**
  - **N = 151**
  - **Obs = 46**
  - **Excess = 33**
  - **RR = 3.63**
  - **P = <0.00005**
β Spatiotemporal *M. bovis* Clusters: 1 year window

β Secondary Cluster
β N. Central Valley
β N = 550
β Obs = 96
β Excess = 50
β RR = 2.09
β P = <0.00005
Spatiotemporal *M. bovis* Clusters: 1 year window

Tertiary Cluster
- Northern Region
- N = 328
- Obs = 44
- Excess = 16
- RR = 1.59
- P = 0.035
Spatiotemporal *M. bovis* Clusters: 1 month window

- Primary Cluster
  - N. Central Valley
  - N = 23
  - Obs = 20
  - Excess = 18
  - RR = 10.34
  - P = <0.00005

Mean High Temperature and Prevalence of *M. bovis* in 1 Month Spatiotemporal Clusters

- Primary Cluster:
  - N. Central Valley
  - M. bovis Prevalence (%)
  - High Temperature (°F)

May 2002
Spatiotemporal *M. bovis* Clusters: 1 month window

- Secondary Cluster
- Southern Region
- N = 20
- Obs = 10
- Excess = 12
- RR = 5.93
- P = 0.0002
Spatiotemporal *M. bovis* Clusters: 1 month window

- **Tertiary Cluster**
  - **Northern Region**
  - $N = 28$
  - $Obs = 9$
  - **Excess = 7**
  - $RR = 3.81$
  - $P = 0.077$
### Primary 1 month spatiotemporal clusters in each year

Location of all clusters: N. Central Valley

<table>
<thead>
<tr>
<th>Year</th>
<th>Month</th>
<th>Season</th>
<th>N</th>
<th>Observed</th>
<th>Excess</th>
<th>RR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Dec</td>
<td>Winter</td>
<td>13</td>
<td>9</td>
<td>8</td>
<td>8.22</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2002</td>
<td>May</td>
<td>Summer</td>
<td>23</td>
<td>20</td>
<td>18</td>
<td>10.34</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2003</td>
<td>Mar</td>
<td>Winter</td>
<td>21</td>
<td>17</td>
<td>15</td>
<td>9.62</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2004</td>
<td>Feb</td>
<td>Winter</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>9.89</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2005</td>
<td>Sept</td>
<td>Summer</td>
<td>11</td>
<td>9</td>
<td>8</td>
<td>9.71</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2006</td>
<td>Mar</td>
<td>Winter</td>
<td>17</td>
<td>12</td>
<td>11</td>
<td>8.38</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2007</td>
<td>June</td>
<td>Summer</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>9.23</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2008</td>
<td>Aug</td>
<td>Summer</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>10.88</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2009</td>
<td>June</td>
<td>Summer</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>8.90</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2010</td>
<td>Nov</td>
<td>Winter</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>9.88</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>2011</td>
<td>Feb</td>
<td>Winter</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>11.86</td>
<td>&lt;0.0005</td>
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<tr>
<td>2012</td>
<td>May</td>
<td>Summer</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>11.86</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>
Limitations

- Herd level factors unknown
- Small sample sizes
- Counties and regions
- Choice of regions may be biased

Further analysis

- Mixed regression models
  - Further evaluate the association between season, climate, location and *M. bovis*
Conclusions

- *M. bovis* located in all regions of California
- Prevalence of *M. bovis* is declining
- Possible changes to the seasonality of *M. bovis*
  - Future studies needed to assess herd level management factors
    - Heat stress, cooling techniques, calving seasons, etc
Questions: