Diagnostic Dilemmas
How to Understand Mastitis
Diagnostic Results from Labs, Farms and PCR Tests

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Mastitis

- Bacterial infection of the udder
- 99% occurs when bacterial exposure at teat end exceeds ability of immune defenses of cow
- Subclinical mastitis
  - Milk appears normal but contains excessive numbers of inflammatory cells
- Clinical mastitis
  - Visual abnormalities of milk
  - Definition varies among farms

Practical Questions

- When we detect inflammation does that mean that infection is present?
  - Do we need to use an antibiotic?
- When bacteria are identified in a milk sample, does that mean that they are the cause of an infection?
  - Do we need to use an antibiotic?
- What about all these new tests?
  - How do PCR tests compare to traditional culture?

We Detect Mastitis Based on:
Results of the immune response
NOT the moment of INFECTION

We are detecting inflammation
NOT INFECTION!

To Control Mastitis The Cause of Mastitis Must be Known

- Mastitis is a bacterial disease
- Different bacteria
  - Infect different parts of the udder
  - Have different reservoirs
  - Require different treatments
  - Have different rates of spontaneous cure

Objective

Compare and Contrast Culture Methods versus PCR testing for Control of Mastitis

What are the Options for Detecting Bacteria in Milk?

- Microbiological Culture
  - Submission to a reference laboratory
  - On farm culture
  - In Vet Clinic Culture
- Use of PCR Testing
Practical Aspects of Using Culture Data

- Obtaining a useful sample
- Using the right lab test
- Evaluating the results
- Making management decisions

It is Easy to Find Bacteria in Milk
But...they Aren't All From Mastitis....

- Mastitis is almost always caused by a single type of bacteria
- When >2 types of bacteria are recovered the milk sample is almost useless
- Proper sampling MUST be performed
  - Train & evaluate
- Take a ¾ sample
  - After prep
  - Before attach

Sources & of Bacteria in Milk

- There are multiple ways that bacteria can get into milk
  - Contamination during sampling
  - Poor sample handling
  - Teat skin microflora
  - Streak canal microflora
  - Etc., Etc..
- Sampling procedures are even MORE important when PCR testing is done

What Happens in the Lab?
Culturing

- In most culture labs
  - The objective is to rapidly identify likely mastitis pathogens
- Most lab methods are simple
- A small droplet of milk is placed on growth media
- The inoculated plate is allowed time to grow

Laboratory Procedures for Milk Samples

- Collecting Milk for Culture
  - Wear gloves or wash hands
  - Use a sterile container
    - Not reused
  - Predip & dry the teat
  - Use alcohol to THROUGHLY scrub the teat end
  - Take the sample without cross contamination

Laboratory Supplies

Inoculating Growth Media

Sources & of Bacteria in Milk

- The plate is observed for growth
- Different methods are used to identify the bacteria
  - Gram stain
  - Characteristics of the bacteria

Incubation & Identification

- Incubate 24-48 hours
- Look at Colonies & apply Gram Stain
- Examine using a microscope
Perform a Variety of Other Tests

- Phenotypic tests
  - Appearance of bacteria on plates
  - Growth on specific medias
  - Reactions with enzymes (catalase, coagulase etc.)
  - Biochemical reactions
    - Fermentation of sugars
    - Motility etc...
- Compared with “typical” characteristics of the bacteria are known
- A Best guess is made relative to ID
  - Confidence level is assigned
    - 75% confidence is often the cutpoint

So...what can we expect from on-farm culture systems???

OFC are about 80% Accurate and Should be Used to Direct Treatment Decisions
but Farmers Need a Backup Lab for Diagnosis of Specific Bacteria

What is Different about an OFC or Vet Clinical Culture LAB?

- Goal is different
  - The same level of accuracy is not required
- Want to rapidly arrive at a bacteriological diagnosis of mastitis
- Use of selective medias to make a rapid diagnosis
  - Look at the colonies on the plate
- OFC using selective media cannot identify most bacteria to species level

PCR Tests Currently Used In Diagnosis of Mastitis

- Commercial or proprietary PCR tests
  - Used to detect bacterial DNA

Principle of On Farm Culturing

- Use laboratory shortcuts to arrive at a fast, presumptive diagnosis
  - Don’t treat with antibiotics until the diagnosis is made
- Use of selective medias to make a rapid diagnosis
  - Laboratory “shortcuts”

Typical Decisions

- Treat or No Treat (TNT)
  - Gram + versus Gram neg or no growth
  - Chronic
  - Staph aureus
- Treat with Gram negative spectrum drug
- Alter the duration of treatment

PCR = Polymerase Chain Reaction

- Based on concept that nucleus of bacterial cells contain DNA with unique sequences of nucleotides
- Nucleotides are building blocks of DNA
  - Adenine, cytosine, guanine, thymine
- PCR tests identify bacteria that
  - Have known nucleotide sequences that are in a master library
    - “Primer”
  - And are in the particular PCR mix
    - You have to know what you are looking for and include the primer in the test mix
How is PCR Used for Mastitis Diagnosis?

- **Pathoproof©**
  - PCR test that can ID DNA in milk from up to 16 potential mastitis pathogens
- Can potentially find DNA from about 90% of the pathogens that cause mastitis in WI herds
- Unable to identify about 10-15% of organisms that cause mastitis in WI
  - Wide diversity of opportunistic organisms
  - Primer not in mix

Detection of DNA Does NOT Equal Infection

- Finding 1 colony of bacteria does NOT equal infection
  - Usually require 300-500 cfu/ml
- Finding some types of bacteria does NOT equal infection
  - Most Bacillus
- No one knows how to interpret the recovery of DNA from Milk samples
- Possible sources of DNA in milk
  - Contamination
    - Milk meters
    - Teat skin
    - Equipment
    - Hands
    - Teat canal
    - Dirt
  - Part of bacteria killed as part of a successful immune response

Use of PCR on Field Collected Milk Samples Koskinen et al., J Dairy Sci, 2010

- Study conducted in Finland & Holland
- 1,000 quarter milk samples collected using **ASEPTIC** collection methods
  - 780 Clinical cases
  - 220 from Healthy cows
- Bacterial culture performed in labs using standard techniques
  - >3 colony types were considered contaminated
  - Few colonies of Bacillus, etc. were ignored
- PCR performed using Pathoproof®
Bacteria Found in Culture Negative Samples

- Number of bacterial DNA identifications in culture negative samples
  - 1 species: 68%
  - 2 species: 23%
  - >2 species: 9%
- CNS & C Bovis were most common

**PCR tests detect Bacillus (n = 4)**

CNS 172 >1 Bacillus

Yeast

uberis 137 10

D PCR tests result in a large proportion of multiple bacterial isolations from milk

- Decision making for treatment, segregation and culling based on PCR tests is unknown

**What About PCR for Bulk Tanks?**

- Bulk tank culturing is useful for
  - Detection of contagious pathogens
  - Monitoring hygiene
  - Interpretation of bacterial DNA in bulk milk is completely unknown
  - No science to guide decision making

**Comparison of PCR and Culture Results in 780 Clinical Cases**

<table>
<thead>
<tr>
<th>Bacteria Found</th>
<th>Culture Positive</th>
<th>False Neg. Positive</th>
<th>Culture Negative</th>
<th>False Neg. Positive</th>
</tr>
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<tbody>
<tr>
<td>PCR Pos.</td>
<td>PCR Neg.</td>
<td>PCR Pos.</td>
<td>PCR Neg.</td>
<td></td>
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<td>--------</td>
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<td>A Pyogenes</td>
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<td>50</td>
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<td>8</td>
<td>67</td>
<td>636</td>
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<tr>
<td>Strep uberis</td>
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<td>10</td>
<td>137</td>
<td>560</td>
</tr>
</tbody>
</table>

**When You Have PCR Results ALWAYS Look at the Cow History**

- When PCR indicates Staph aureus, Strep ag or M bovis:
  - Does the cow have a history of high SCC?
  - Does the cow have a history of recurrent clinical cases?
  - There are many sources of environmental bacteria
  - Usefulness of PCR testing for these organisms is unknown

**Bacteria Found in Culture & not using PCR**

**Clinical Cases**

- Of 780 cases (6%)
  - Bacillus (n = 10)
  - Enterobacter (n = 3)
  - Gram neg. rods (n = 6)
  - Lactococcus (n = 4)
  - Proteus (n = 1)
  - Pseudomoans (n = 2)
  - Strep bovis (n = 3)
  - Strep spp (n = 8)
  - Yeast (n = 7)

**Subclinical Cases**

- Of 46 cases (20%)
  - Bacillus (n = 3)
  - Gram neg rods (n = 2)
  - Strep spp (n = 3)
  - Yeast (n = 1)

**Take Home Point**

- PCR tests detect Bacterial DNA from both dead and live bacteria
- When PCR tests are used for mastitis the milk sample must be collected aseptically
- PCR tests result in a large proportion of multiple bacterial isolations from milk
  - Decision making for treatment, segregation and culling based on PCR tests is unknown

When using PCR testing always refer to the cow history to help make decisions
Culturing or Other Tests

- Diagnostic tests are only cost effective when the result of the test will be used to make a management decision that increases profits
  - Treatment
  - Culling
  - Segregation
  - Disease prevention

Take Home Message

- The use of molecular methods will increase as the methods get cheaper
- Just like other diagnostic tests
  - Value of test is based on the value of the intervention
- Molecular tests give us different information than we have previously used
  - Need to understand
    - how the tests work
    - Strengths & weaknesses

Final Conclusion

- Always:
  - know why you are performing a test
  - Know how to use the results
  - Combine results with medical history of the animal to make an intervention decision

For more information: http://milkquality.wisc.edu or....