Microbiological diagnostics of udder infections

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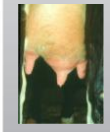
NKVet May 13-14, 2013

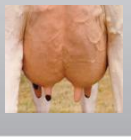
Introduction

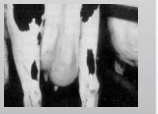
- Mastitis = in most cases associated with udder infections/intra-mammary infections (IMI)
  - Any microorganism (bacteria/fungae/algae)
  - Mainly bacteria eg
    - Staphylococci
    - Streptococci
    - Coliforms

Introduction

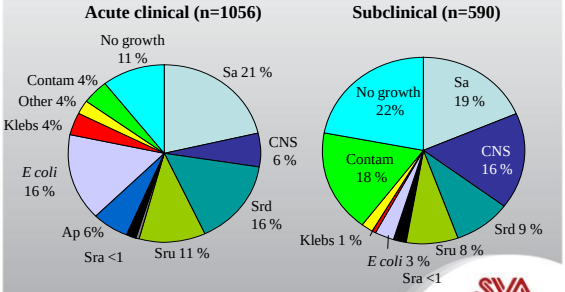
- Distribution of udder pathogens varies between
  - Countries, regions, farms
  - Clinical and subclinical cases







Example: National surveys in Sweden 2002/03 and 2008/09 – routine culturing



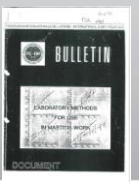
Ericsson Unnerstad et al 2009; Persson et al 2011

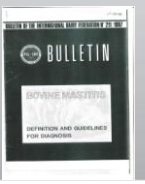
Why do we need microbiological diagnostics of udder infections?

- For treatment decisions
  - Eg clinical cases, dry-cow therapy
- To identify type of mastitis problem
  - Decisions regarding management activities, control programs etc
- To control health status
  - Eg buying/selling, breeding decisions

Microbiological diagnostics – How is it done? Guidelines

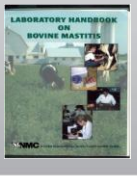
- Nordic Recommendations for the Laboratory Diagnostics of Mastitis, 1974
- International Dairy Federation, IDF
  - Laboratory Methods for use in Mastitis Work, 1981
  - Definition and Guidelines for Diagnosis, 1987





Microbiological diagnostics – How is it done? Guidelines

- NMC (National Mastitis Council)
  - Laboratory Handbook on Bovine Mastitis, 1999
  - Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determination of Milk Quality, 2004
- Laboratory accreditation (EN 17025)



Microbiological diagnostics – How is it done? Guidelines

- Guidelines are not complete
- Cover culture-dependent methods
- PCR-tests not included
- Update needed

Microbiological diagnostics - points to consider

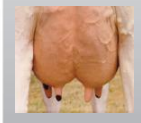
- Milk sampling
- Storage and transport of milk samples
- Investigation of growth/presence of microorganisms
  - Veterinary field lab vs commercial labs
  - Culturing vs PCR
- Interpretation of findings – making a microbiological diagnosis at the lab
- Interpretation of the results – making a decision at the cow/farm

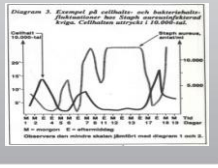
Milk sampling

- Decisions before sampling
  - Quarter/cow/herd level?
  - Type of sample? Quarter or composite sample?
    - Pros and cons, culture/PCR …
  - Which cows/quarters to sample?
    - Based on purpose, history, SCC …
  - When to sample?
    - In relation to milking, during the lactation cycle …
- The objective of the investigation decides which way to go

Milk sampling: Cow level – Quarter vs composite samples

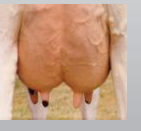
- Quarter samples = gold standard
  - Easier to sample aseptically
  - Gives the best picture of the bacterial concentration at the sampling occasion
    - Bacterial concentration varies





Milk sampling: Cow level – Quarter vs composite samples

- Cow composite sample =
  - Larger risk för contamination
  - Dilution effect



Milk sampling: Cow composite sample - manual vs test milking sampling

- Manual
  - Same routine as quarter sampling = aseptic, but collect milk from all quarters in the same tube
  - Risks
    - Must open the tube several times =< risk for contamination
    - Difficult to get the same amount from all quarters
    - Difficult to mix properly
- Via test milking equipment (PCR)
  - Easy to do
  - Risks
    - Not aseptic
      - Contamination from skin and equipment
      - Risk for carry-over between cows
    - Dilution effect
      - Subclinical quarters have lower production

Milk sampling: Composite sampling of a group of cows or a herd

- Group of cows
  - Pooling of manually taken samples
  - Risks
    - Must open the tube several times – contamination
    - May be difficult to get the same amount of milk from all cows
    - May be difficult to mix well
    - Dilution
- Herd
  - Bulk milk sample from whole or parts of herd
  - Risks
    - The sample is always contaminated from various sources
    - Dilution of milk from cows with subclinical mastitis
    - All cows not in the tank

Aseptic quarter milk sampling

- Sample a clean and dry teat of a clean and dry cow in a clean and dry barn!
  - Clean the teats if necessary using dry/wet tissues
  - Strip out some milk
  - CMT test
  - Clean the teat end with alcohol scrubs
  - Take sample aseptically using sterile milk tubes (with/without preservative)
  - Teat dipping (if suitable/needed)

Storage/transport of milk samples

- Store milk tubes chilled
- Deliver/Send milk tubes to the lab quickly
  - Store chilled at field lab
  - Transport milk tubes chilled to lab
- Risks
  - Too high temperature if not chilled
    - Abundant growth of mixed flora => not correct diagnosis
    - Overgrowth of contaminant => interpreted as pure culture => false positive sample
  - Too low temperature – freezing
    - Some pathogens can die => false negative or false positive (should have been mixed flora)

Storage/transport of milk samples

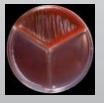
- If PCR analysis
  - Use sterile tubes with preservative (bronopol) = kills bacteria
  - No need for chilling
  - Freezing not a problem

Investigation of growth/presence of microorganisms in milk samples

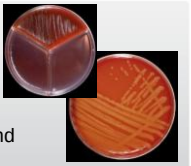
- Vet field lab vs accredited lab
- Culturing
  - Most common
  - Gold standard
- PCR (polymerase chain reaction) tests
  - Culture-indepent
  - Commerial multiplex tests available

Culturing

- Milk (10µl) is spread on
  - Non-selective agar plates (blood agar)
  - Selective agar plates (eg SELMA used at field lab)
- Incubated at 37°C (aerobic) and evaluated after
  - 18-24 h
  - 48 h

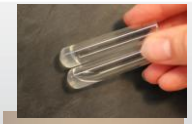


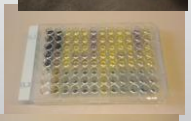
Culturing



- Evaluation of colony morphology
  - Colour, size, hemolysis…
- Evaluation of numbers of colonies and type of growth (pure, contamination)
- Growth on selective agar
- Gram-staining (microscope)
  - Size, shape (cocci/rods), chains, cluster of grapes …
- Agglutination tests (*S aureus*, streptococci)
  - OK to use with bovine isolates?

Culturing



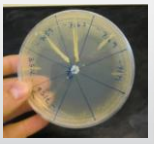


- Biochemical testing eg
  - Catalase
  - Potassium hydroxide
  - Coagulase
  - Carbohydrate fermentation
  - CAMP reaction
  - PI (PGUA/indol)
  - etc

Culturing – new techniques

- Species differentiation of bacteria
  - Matrix-Assisted Laser Desorption/Ionization – Time of Flight (MALDI-TOF) mass spectrometry
- Molecular methods identifying strains within bacterial species eg
  - PFGE
  - RAPD
  - MLST

Culturing



- Antibiotic resistance eg
  - β-lactamase production – staphylococci
    - Eg clover-leaf method
  - Microdilution method
    - Minimum inhibitory concentration (MIC)

PCR tests

- Commercial multiplex real-time PCR tests (Finnzymes Diagnostics, Finland)
- Identification of DNA
  - certain udder pathogens
  - In some tests - *bla*Z gene = β-lactamase production

PCR – How is it done?

- DNA extraction directly from milk (350 µl)
- Mix DNA extract with PCR solutions
- DNA amplification in 40 thermal cycles
  - real-time PCR instrument

Pros and cons PCR vs culturing

- Pros eg
  - Faster
  - Can detect smaller amounts of bacteria
  - Can detect killed and growth inhibited bacteria
  - Possible to use milk with bronopol
  - Can detect *Mycoplasma bovis* (difficult to culture)

- Cons eg
  - Identifies a limited number of pathogens
  - More expensive
  - Not possible to save isolates for further studies eg antimicrobial resistance, genotyping
  - Results can be difficult to interpret

Problems with interpretation of PCR results

- Staphylococci
  - If *Staphylococcus* spp are detected it can be due to presence of several species = contamination
  - If blaZ gene is detected and *Staphylococcus* spp and/or *S aureus* you can't tell which species that is resistant
- When several pathogens are detected in a sample
  - Difficult to decide if contamination or not
- PCR detects more samples with presence of bacteria than culturing
  - Interpretation? Killed or alive? True infection/contamination?
- Based on todays knowledge
  - If not aseptic samples, PCR recommended mainly at herd investigations of *Str agalactiae* and *Mycoplasma bovis*

Interpretation of findings – making a diagnosis at the lab

- Culturing
  - No growth
  - Growth of pathogen
    - Amount (1-3)
    - In pure culture
    - In mixed flora
  - Contamination

- PCR
  - No presence
  - Presence of one or more pathogens and/or *blaZ*
    - Amount (+, ++, +++)
    - Ct-values
  - Contamination?

Interpretation of the results at the cow/herd

- Combine microbiological findings with information on
  - Cow/herd history, clinical signs, SCC
  - Sampling and sample handling
  - Culturing or PCR
- Are the lab results true or false?

Interpretation of the results at the cow/herd

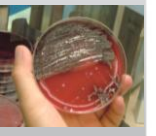
- No growth/presence of bacteria
  - True
    - Healthy udder
    - Non-infectious mastitis
    - Pathogen already eliminated
  - False
    - Pathogen needs special culturing conditions or is not included in panel
    - The pathogen concentration in milk is too low
    - Inhibiting substances
  - Take new sample? How good is one sample?

Interpretation of the results at the cow/herd

- Growth/presence of one pathogen
  - True
    - Infectious mastitis
    - Latent infection
  - False
    - If contaminated from start and sample handled/stored suboptimally can result in over-growth of one species

Interpretation of the results at the cow/herd

- Growth/presence of mixed flora/contamination
  - Culture: If 3 or more species = contamination
    - Exception if *S aureus* or *Str agalactiae* are clearly identified
  - PCR: number of species?
  - Poor sampling or sample handling/transport
  - Uncertain result - may hide an infection
  - Take new sample!



Future developments and needs

- New methods and understanding of pathogenesis
- Culture-dependent methods eg
  - Quality control (field lab/commercial lab)
  - Improvements – eg MALDI-TOF
  - Cost-efficient molecular methods for isolate differentiation within species
- Culture-independent methods eg
  - Improvements of PCR
  - New techniques to detect microorganisms in milk
- Cow-side bacteriological quick tests

Thank you for your attention!