

Mastitis Infections and Mastitis Pathogens



-mast
mid 19th century: from
Greek *mastos* 'breast' or 'mammary'
-itis
forming names of inflammatory diseases

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Quality Milk Production Services



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Cornell University
College of Veterinary Medicine

Animal Health Diagnostic Center

Objectives

- **Bovine immune systems**
- **Mastitis and the infection process**
- **Acute and chronic mastitis infections**
- **Facts about mastitis pathogens**



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Immune Systems

- **Innate**
 - Anatomical / physical
 - Humoral
 - Complement, Lactoferrin,...
 - Cellular
 - Macrophages & Neutrophils (somatic cells)
 - Bacteria-specific receptors (PRR)
 - i.e. LPS receptors
- **Adaptive**
 - Antibody-dependent immunity
 - Memory-driven cellular response



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Cellular Immune System Somatic Cells in Milk

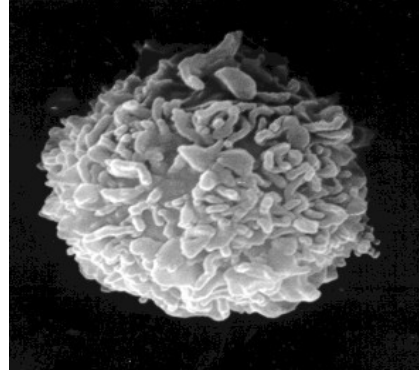
Cell type	Normal milk	Mastitis milk
Total cells	< 100,000	>> 250,000
Leucocytes	>85%	>99%
Macrophages	35%	99-100%
PMNs	25%	
Lymphocytes	25%	
Epithelial cells	<15%	<1%



Somatic Cells in milk



Macrophages (Mφ)



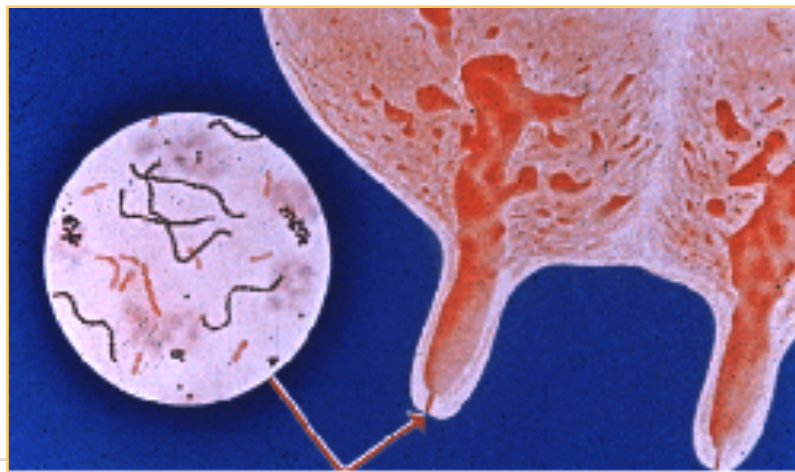
Neutrophils or PolyMorphoNuclear cells (PMNs)



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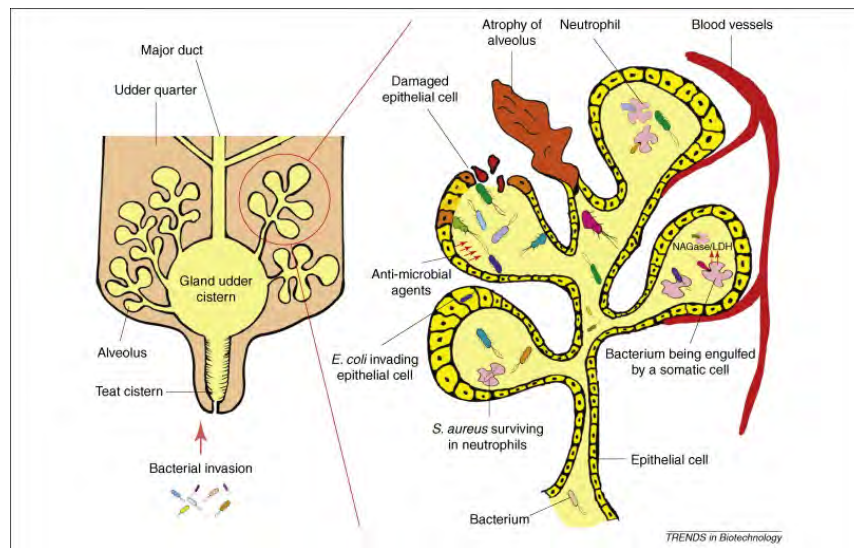
Mastitis Infections How Do They start?



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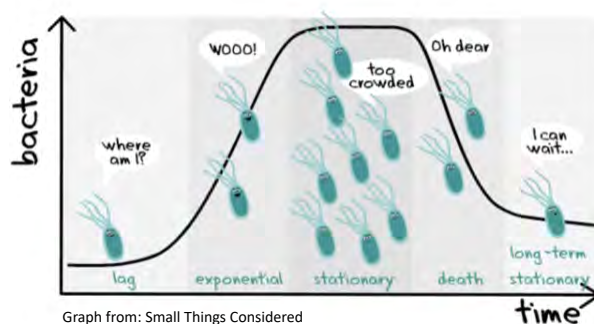


Intramammary Infections (IMI)



Udder diagram from www.cell.com Trends in Biotechnology

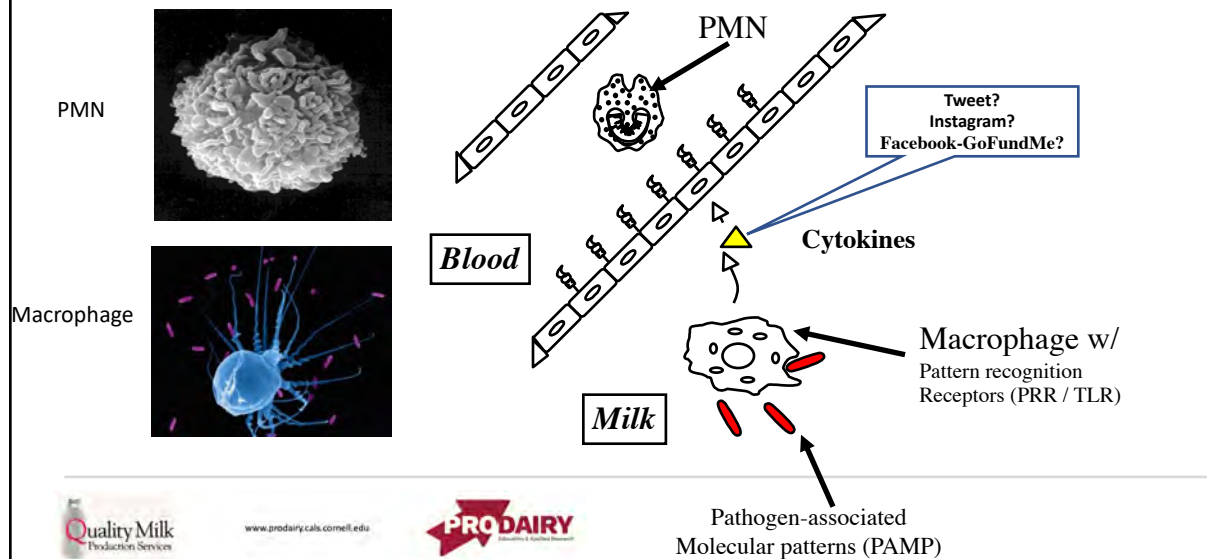
Mastitis Infections Bacterial Growth



Escherichia coli

Binary fission shown at
1760 times normal speed

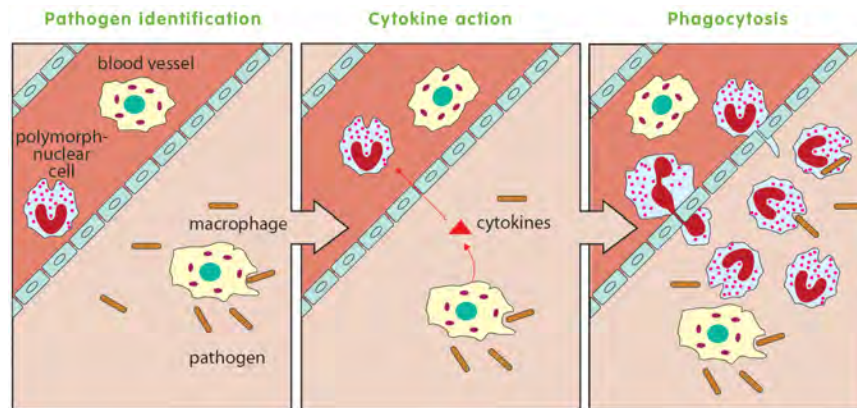
Macrophages recognize pathogens



PMN Chemotaxis Somatic cell movement into tissue

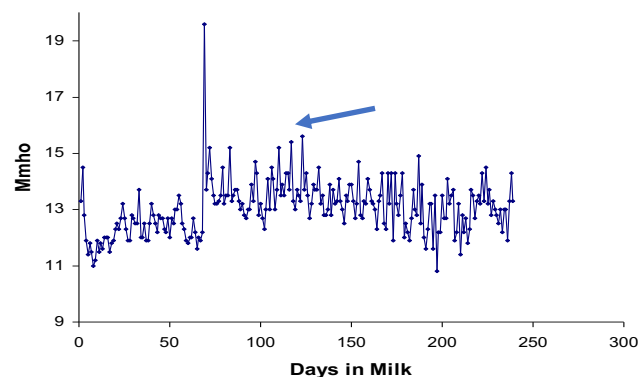


Recruitment and migration of somatic cells



Electrical Conductivity

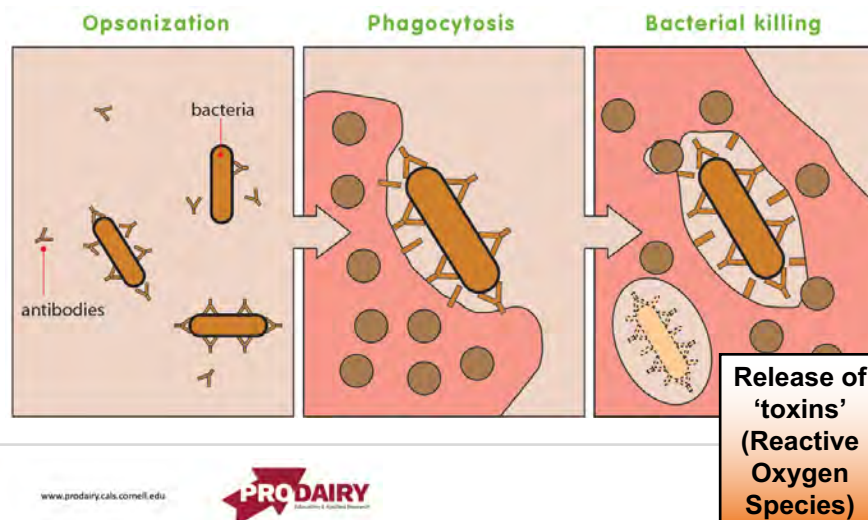
Further investigation – clinical signs, CMT, SCC
are necessary, but signal can be very helpful

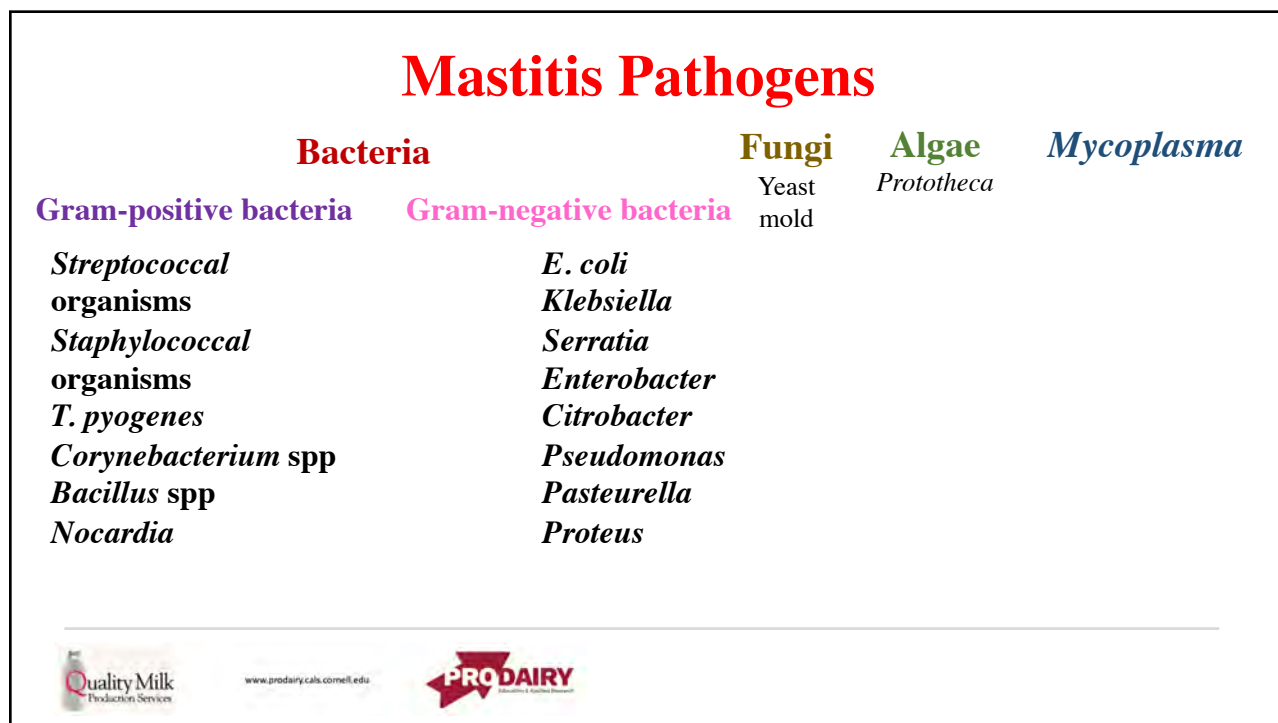
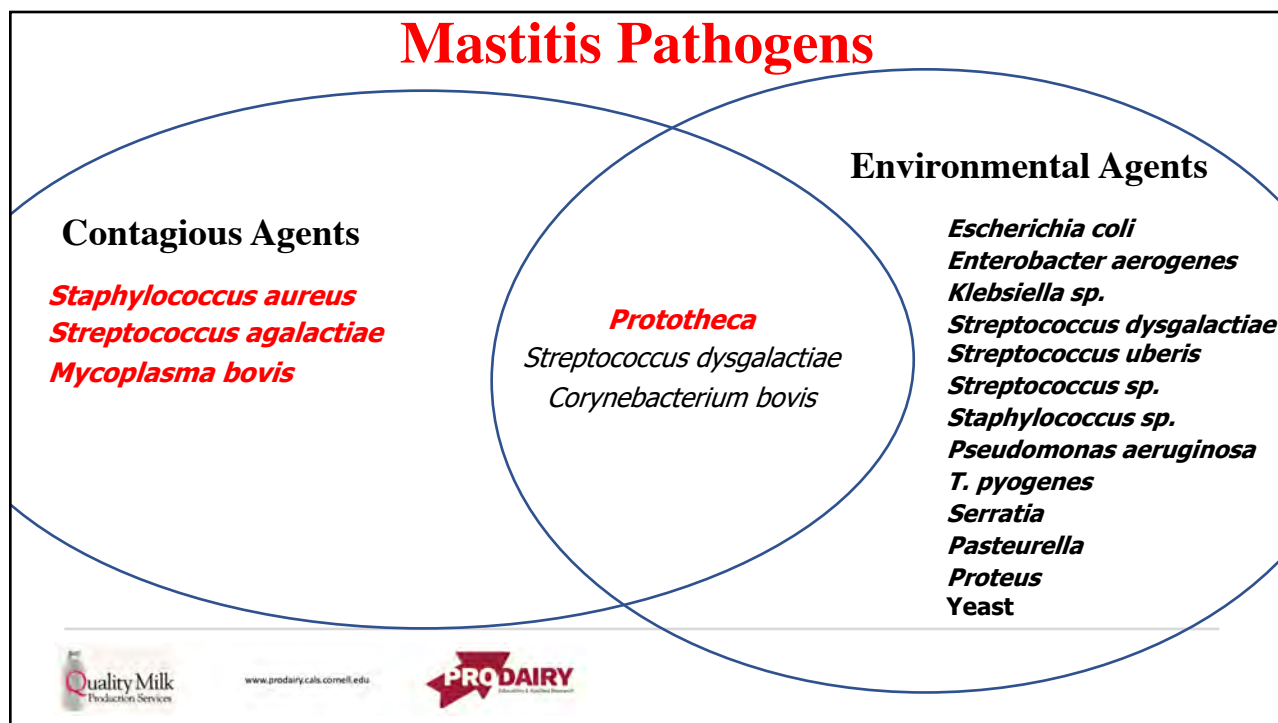


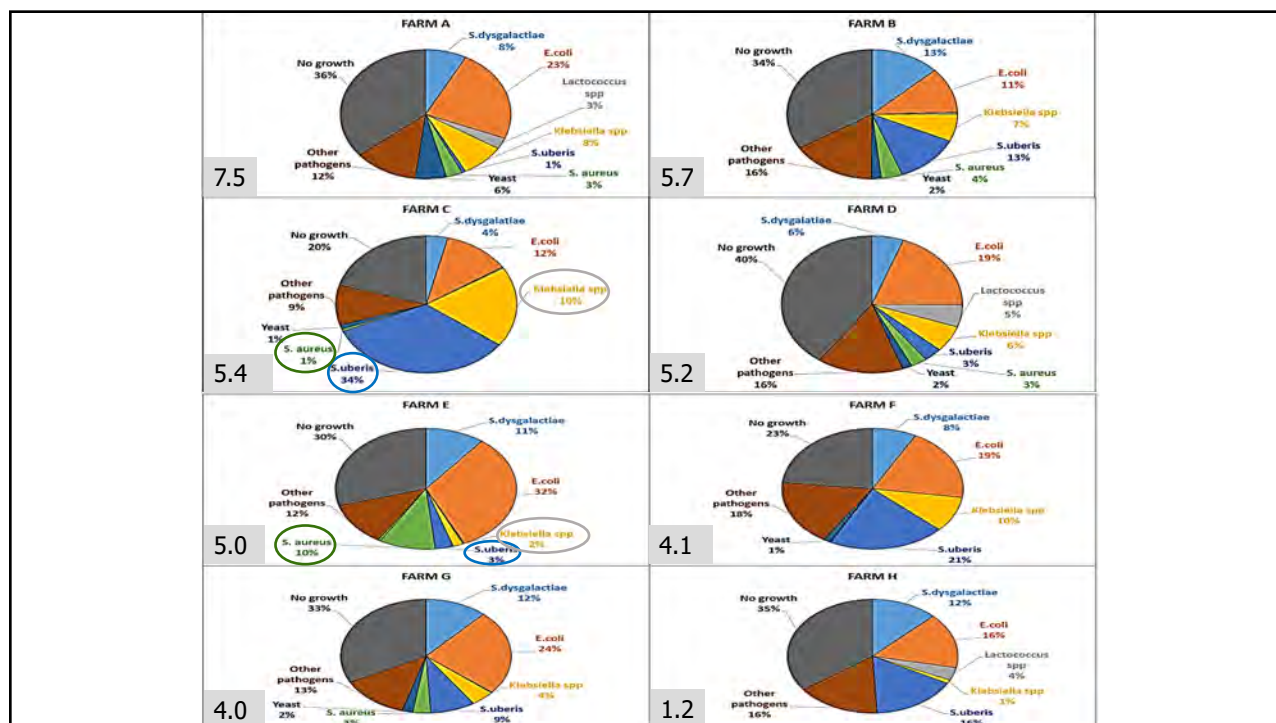
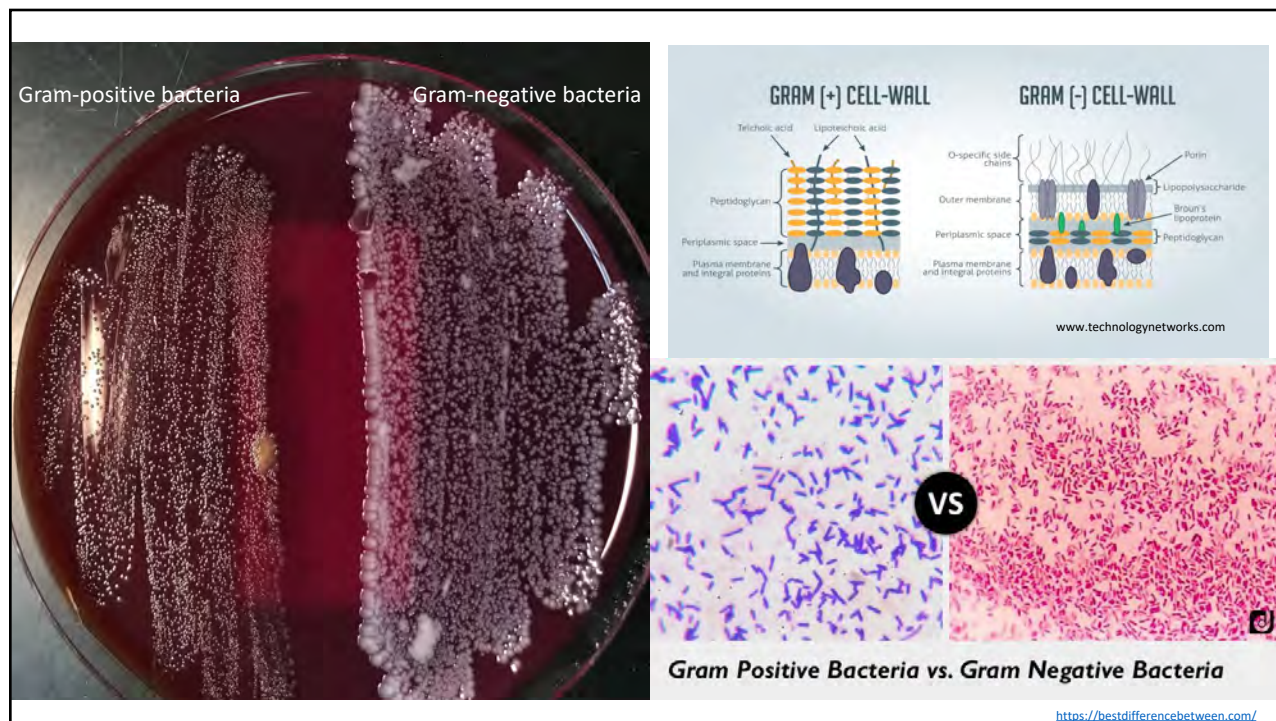
Somatic cell chasing *Staphylococcus aureus*



Phagocytosis and killing bacteria (Gobbling up bacteria)







“What do you mean its negative?”

- Mastitis detection based on results of immune response, not the act of infection
- Clinical signs are inflammation, not infection
- At least 20-30% of milk samples will be culture negative if they are properly collected



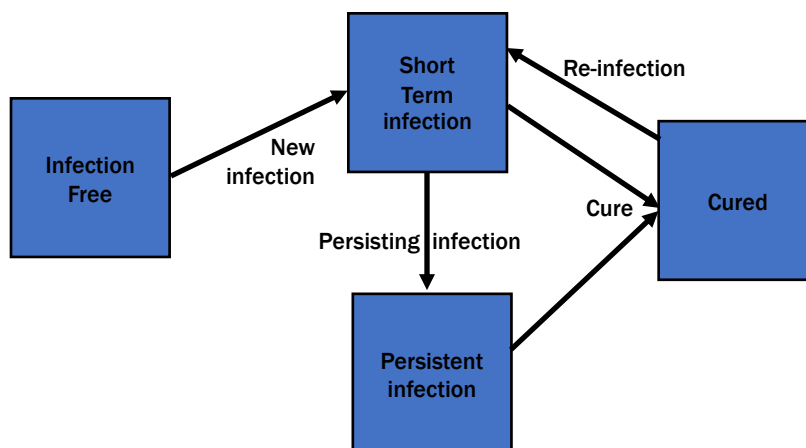
If something grows from all of them...Sampling was not correct



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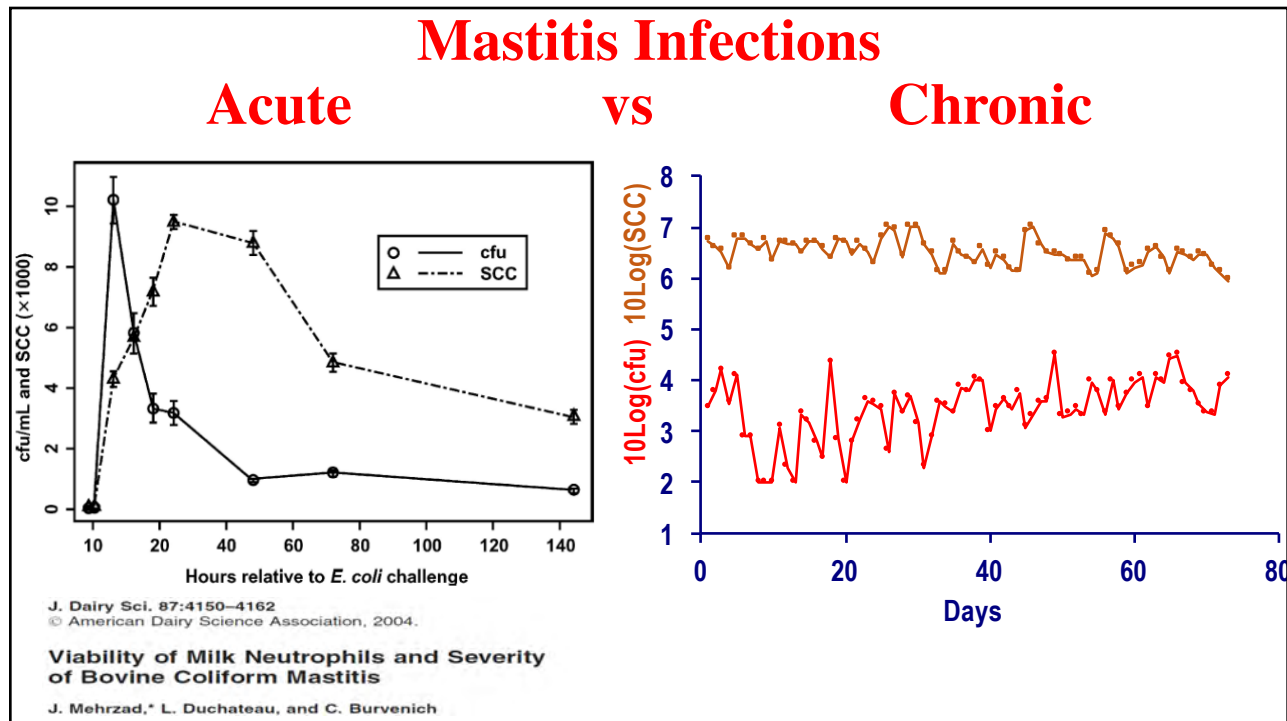
Infection Dynamics



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Barlow et al. PVM 2009



Chronic Mastitis Infections

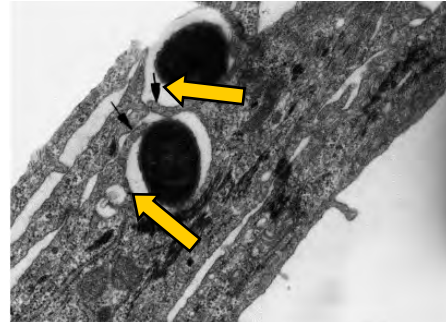


Chronic Mastitis Infections

Escherichia coli



Escherichia coli
attached to udder epithelial cell



Escherichia coli
invaded into udder epithelial cell

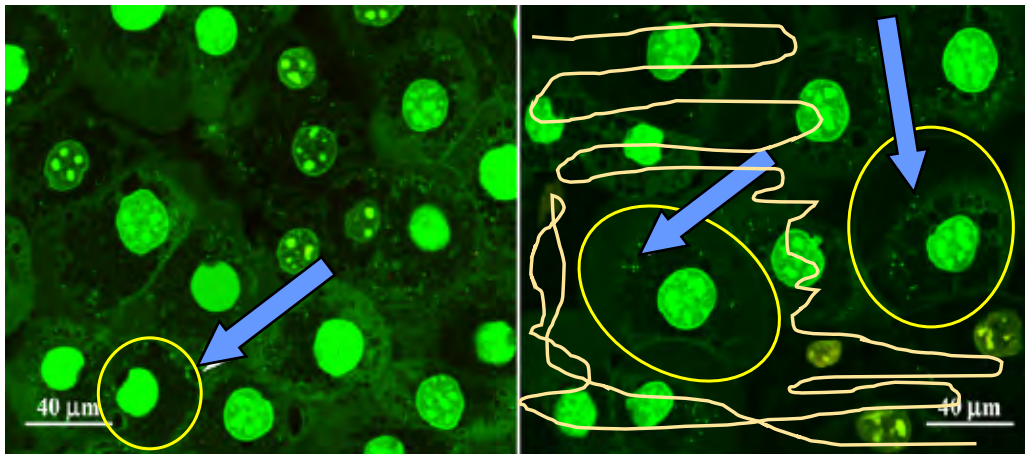


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(From Döpfer et al., 2000, Veterinary Microbiology)

Chronic Mastitis Infections

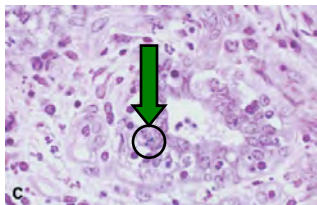
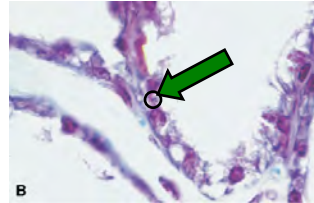


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Tamilselvan et al., 2006

Progression of Infections

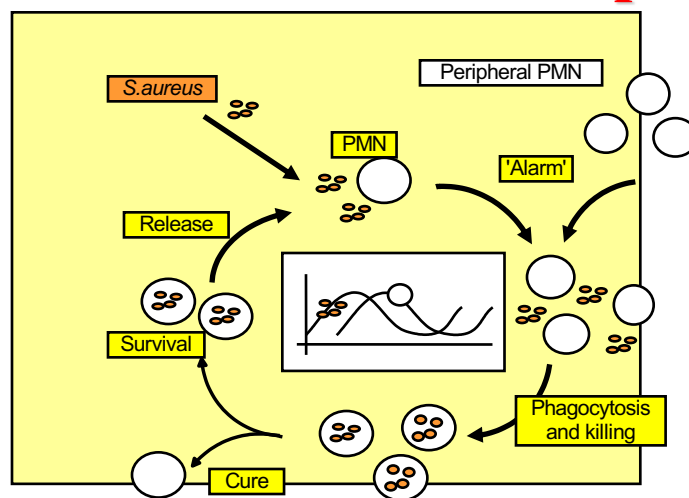


Staphylococcus aureus

- A. in alveoli
- B. in udder epithelium
- C. in connective tissue
- D. beyond cure.....

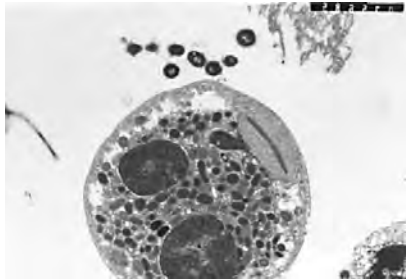
(From Hensen et al., 2001, Journal of Dairy Science)

Chronic Infections Intracellular Survival of Staph aureus

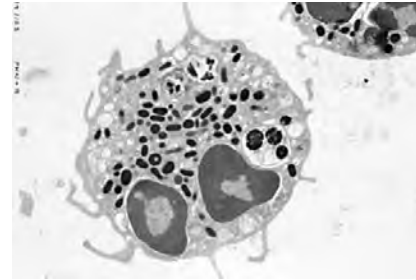


Chronic Infections *Streptococcus uberis*

Inactivation of PMNs



Inactivated PMN
Co-culture with *S. uberis*



Activated PMN



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Prevent Mastitis and Improve Immune Response

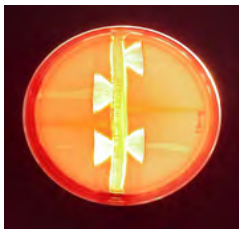
- Identify pathogens
- Monitor (KPI, milking equipment and parlor function)
- Hygiene
 - Teat-ends
 - Animal (dry, transition, lactating)
 - Environment
- Teat-end quality:
 - Teat-end scoring
 - Interaction of teat ends and milking machine
 - Teat-end cleanliness
- Preventing negative energy balance and other diseases



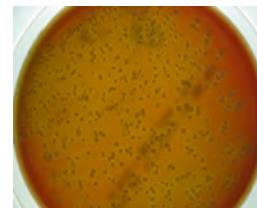
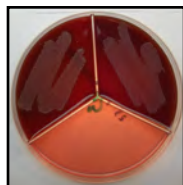
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Mastitis Pathogens Streptococcus and Streptococcus-like Organisms



CAMP Test



Streptococcus dysgalactiae



Lactococcus lactis



Streptococcus uberis



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Mastitis Pathogens *Streptococcus agalactiae*



Characteristics

- Highly contagious
- Subclinical in appearance
- Obligate parasite of the udder
- Can be eliminated from herd
- Associated with herd expansion
- Treatment during lactation is both effective and economically feasible
- Positively identify with CAMP test

Mastitis

- Subclinical - no clinical signs
 - Primarily infects cistern and duct system
- Individual high SCC (>1,000,000/ml)
 - Produces an irritant which inflames the gland.
- High bacteria counts
- Very low spontaneous cure rate
- Subsequent inflammation destroys secretory tissues
 - Decreased milk production

Management

- Milk *Strep. agalactiae* infected cows last until confirmed infection free.
- Pre-dipping is advised
- Post milking teat dipping is a MUST
- Effective dry cow therapy
- Monitor BTM frequently

Control

- Need to resample the herd and repeat treatments until eliminated from all quarters on all cows
- Cull of cows with chronic infections that do not respond to treatment

Mastitis Pathogens ***Streptococcus dysgalactiae***

Characteristics

- Colonies are small, moist, convex, and translucent
- most are non-hemolytic or have a greenish hemolysis
- Gram stain: Gram-positive cocci in chains
- Lancefield group C
- Esculin negative

Management

- Recommend intramammary therapy
 - Reduces SCC and risk of contagious spread
 - Focus on younger cows
- High quality pre and post dip

Mastitis

- Can act as both environmental and contagious
 - Smith and Hogan, 1995
- More likely to results in clinical mastitis
- Can be found in milk, bedding, manure, flies and other organic matter
- Causes high SCCs
- Infection will reduce milk production

Control

- Some repeatability of strains across farms
 - Over 60% had more than one cow/strain
- Common environmental source?
- Trade of infected animals?
- Flies spreading locally?
- More virulent strains?

Mastitis Pathogens ***Streptococcus uberis***

Characteristics

- Considered environmental but may spread contagiously
- Infection can occur at any stage of lactation or during dry period
- Colonies are small, moist, convex, and translucent
- most have a greenish hemolysis or are non-hemolytic
- Gram stain: Gram-positive cocci in chains

Mastitis

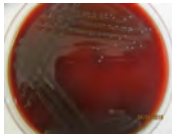
- Can cause CM and SCM
- Duration of infection may vary
- Longer duration may increase risk of contagious spread
- *S. uberis* may be cultured out of liners after infected cows are milked
- 80%+ of infected cows had >1000 cfu/ml when infected
- Infections detected at calving were shorter than ≥ 7 DIM

Therapy/Management

- Cures:
 - Spontaneous in 14/54 and 10/30 qtrs
 - After treatment in 14/54 and 6/30 qtrs
- Segregation of infected animals
- Post dipping (appears to have reduced transmission parameters)

Control

- Environmental
 - Good at replicating in environment.
 - Make sure cows are clean and dry
 - Keep bedding clean
 - Discuss lime addition as an option (short lived)
 - Evaluate fresh cows and dry cow housing
 - Make sure that transition cow management is appropriate
- Contagious
 - Pre and post dip
 - Milk problem cows last



Mastitis Pathogens *Lactococcus*

Characteristics

- Still learning about *Lactococcus* and mastitis
- Environmental pathogen?
- *Lactococcus* not typically identified with standard testing
- Gram-positive cocci in chains
- Colonies are small (1 to 3 mm diameter), moist, convex, and semi-translucent; most are non-hemolytic or have a partial (greenish) hemolysis.

Mastitis

- *Lactococcus lactis* present in “clusters” and associated with clinical mastitis and high SCC
- Higher risk of animals leaving herd due to chronic high SCC than *Streptococcus* infections
- *Lactococcus garvieae*
 - Subclinical mastitis in water buffalo and cows, Spain
 - Subclinical mastitis in cows, Belgium
 - Subclinical mastitis in water buffalo, Brazil
- *Lactococcus lactis*
 - Chronic mastitis in NY, MN
 - Clinical and subclinical mastitis samples

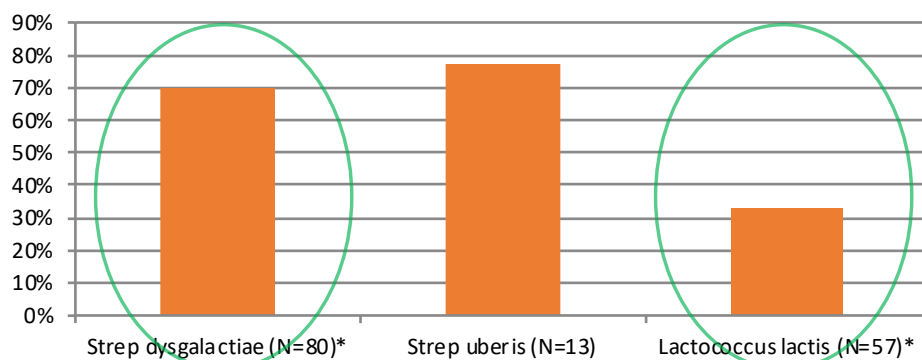
Management

- Culture
- Milk infected animals last
- Effective treatment questionable

Control

- Prevention through proper milking procedures
- Effective pre- and post- dipping
- Good animal hygiene
- Environmental management

Percent SCC resolution after treatment— individual quarters/clinical mastitis samples



* $P < 0.001$

Resolution defined as SCC under 200,000 cells/ml, 15-45 days after sampling (at least 5 days after treatment), regardless of product used or duration. Does not take additional mastitis events into account



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Mastitis Pathogens

Staphylococcus aureus and Staphylococcus species

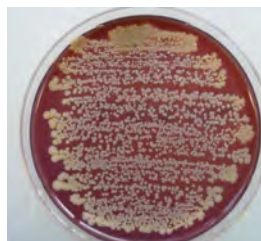
Organisms



Staphylococcus aureus



Coagulase Test



Staphylococcus species



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Mastitis Pathogens

Staphylococcus aureus

Characteristics

- Most common contagious mastitis pathogen (USA)
- Primary source for new infection is the udder
- Environmental strains & sources exist
- Herd Eradication is difficult
- Control is possible (<5% herd)
- Chronic, subclinical infections
- Teat end lesions (hyperkeratosis) and chapped teat skin increase the risk for infection

Mastitis

- Often characterized by high and fluctuating SCC
- Subclinical infections most prevalent.
- Significant reduction in milk production
- Deep seated infection of alveolar tissue
- Duct systems blocked with inflammatory debris
- Abscess and scar tissue formation
- Poor antibiotic penetration
- Periodic shedders

Management



- Prevention over treatment
- Application of a precise and consistent milking procedures designed to minimize contagious transmission must be in place before herd control systems are initiated.
- Milk positive animals last or with separate unit
- Cull/segregate

Control

- Prominent identification and control by segregation of infected cows.
- All milkers should wear gloves while milking.
- Single-use towel to clean and dry teats.
- Forestripping.
- Use of pre and post milking teat disinfectants of known efficacy.
- Establishment of a milking sequence to reduce infection risk.

Mastitis Pathogens

Staphylococcus species (coagulase-negative Staphylococcus)

Characteristics

- “Minor” mastitis pathogen
- Species level identification
 - Access to technology
 - 54 species, 10 identified as causing mastitis in dairy cattle-dominant bovine IMI CNS species:
 - S. chromogenes*
 - S. epidermidis*
 - S. haemolyticus*
- Suggested protection against infection with major pathogens

Mastitis

- Subclinical mastitis-common
- Clinical mastitis -rare
- Higher prevalence of Staph. spp. in heifers (compared to cows)
- Outcomes
 - Milk production- mixed results
 - Increase in SCC Causing SCC 200k-500k cells/mL

Management


- Responsive to Abx — need to treat?
- Monitor

Control


- Be critical of cultures with few numbers of CNS (perhaps no need for tx)
- Encourage management changes (environment, post-dip, sealants) to troubleshoot
- Stay posted for more updates on CNS/ANS

Mastitis Pathogens


Coliform Organisms




Klebsiella pneumoniae



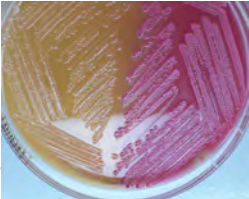
Serratia species




Enterobacter species




E.coli

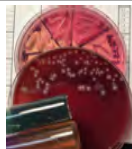


MacConkey agar

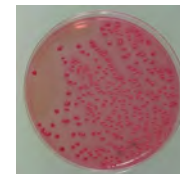


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Mastitis Pathogens *Escherichia coli*



Characteristics

- Gram negative 'coliform' bacteria
- Causes mild to severe clinical mastitis
- Can cause severe, acute clinical mastitis with toxic shock and sepsis especially in fresh cows.
- Some strains cause persistent intramammary infections and chronic mastitis.
- Typical environmental microorganism. Common sources of *E. coli* include manure, bedding and soil.

Mastitis

- Clinical mastitis
- Severity determined by cow's immune system
- High incidence in early lactation
- High production losses
- Mild to moderate cases: no benefit of treatment
- Vaccine reduces severity
- Control through udder health management

Management

- Work towards excellent milking procedures and parlor hygiene
- Clean and dry facilities
- Excellent dry off procedures
- Monitor susceptible groups:
 - Fresh cows
 - Dry cows
 - Heifers
- In case of contagious spread create a separate group and milk last or backflush units

Control



Mastitis Pathogens *Klebsiella*



Characteristics

- Gram-negative coliform bacteria
- Causes mild to severe clinical mastitis
- Can cause severe, acute clinical mastitis with toxic shock and sepsis especially in fresh cows.
- Bedding: shavings and sawdust
- Originates from live trees, soil
- *Klebsiella pneumoniae* in 81% of fecal samples
- Shedding is intermittent
- Pattern is random

Mastitis

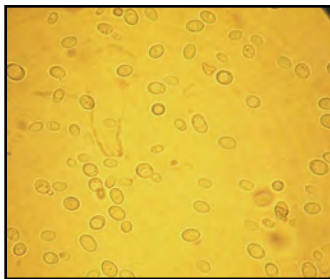
- Severe cases: death
- Clinical cases:
 - production loss 15 lbs/d for heifers, 22 lbs/d for cows
- Chronic cases:
 - repeated clinical cases
 - high SCC, even when there are no clots/flakes
 - culling

Management

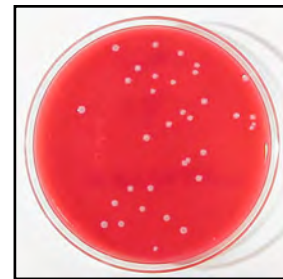
- Treatment and cure:
 - 34-37% cure for *Klebsiella* vs. 71-81% for *E. coli*
 - treatment often limited effect

Control

- Analysis of isolates from milking equipment?
 - Replaced rubber liners
 - Increase in milking vacuum
- Contagious transmission?
 - Known infected animals segregated or banded and unit was flushed after milking
- Environment/animal hygiene
 - Increase bedding frequency
 - Increase alley scraping
 - Monitored with hygiene scoring



Mastitis Pathogens Yeast



Characteristics

- Sources include contaminated multi-dose medication, contaminated syringe, bedding, soil, decaying matter, poor aseptic technique during intramammary treatment

Mastitis

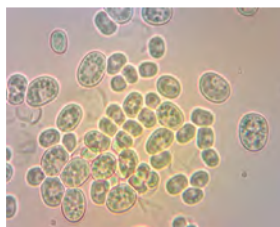
- Decreased milk
- Swelling of udder
- Thick, yellow or flaky secretions
- Symptoms intensify after treatment

Management

- Avoid antibiotic therapy
- Strip out affected quarters
- Immune system clears infection

Control

- Use proper techniques when treating performing intramammary infusions.
- Avoid treatment with multi-dose bottles



Mastitis Pathogens *Prototheca*



Characteristics

- Unicellular
- Colorless
- Chlorophyll-lacking
- Algae
- Environmental/contagious

Mastitis

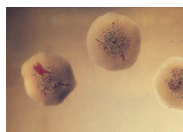
- Chronic non-responsive mastitis
- Can cause subclinical and clinical mastitis
- Sheds intermittently

Management

- Because it is algae-like, resistant to antibiotics
- Milk last or with a separate unit
- Consider culling if only a few infected animals identified
- Consider creating a “Prototheca/Staph” aureus group if many

Control

- Some resistance to dairy disinfectants-biofilm formation
- Diagnostics (most important management tool)
- Culture-high and low SCC, clinical and subclinical
- Environmental cultures?
- Eliminate exposure to wet areas with manure and decaying organic matter
- Clearly identify infected animals



Mastitis Pathogens *Mycoplasma*



Characteristics

- Becoming more prevalent (LARGE HERDS) Often associated with herd expansion
- Spread via milking equipment or via the respiratory tract in poorly ventilated barns
- Hospital pen management
- Cows with persistent infections are often intermittent shedders
- Association between mastitis and respiratory disease in calves and heifers

Mastitis

- More than one quarter affected
- Drop in milk production
- High somatic cell counts
- Mastitis unresponsive to treatment
- Damages secretory tissue, produces fibrosis with abscesses and enlarged supramammary lymph nodes
- Respiratory infections, joint disease, ear infections in calves

Management

- Culture –individual animals, herd
- Treatment not effective
- Animals with mastitis milked with a separate unit. Disinfect unit between cows with hot water and a disinfectant.
- Screen chronic high SCC cows
- Screen fresh animals after 24 hours (key is no colostrum sample). Animals moved to the lactating pens as soon as a negative culture results are available.
- Cull/segregate positive animals

Control

- Screen animals with chronic mastitis and/or high SCC but negative culture results, purchased animals, heifers returning from heifer raising, and animals with respiratory disease. Keep segregated until negative culture results are available.
- Develop a continuous monitoring program

Questions?

