Milk Examination

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Objectives

At the end of the sessions students will able to determine and explain

- What mastitis mean and different forms and causes of mastitis
- Different methods of diagnosis of mastitis
  - Physical examination of milk
  - Chemical examination of milk
  - Microscopic and bacteriological examination of milk

Introduction

- Mastitis is Inflammation of mammary gland accompanied by physical and pathological changes of udder and physical, chemical and bacteriological changes of milk with or without systemic reaction.
- Mastitis continues to be the most costly disease of dairy animals.
- Mastitis is among the factors contributing to reduced milk production In Ethiopia (Biffa et al., 2015), and it is among the most important diseases in dairy animals with worldwide distribution (Zhao and Lacasse, 2007).

- The most common mastitis pathogens are found either in the udder as contagious pathogens or in the animal surroundings such as bedding and manure soil as environmental pathogens.
- Among the contagious pathogens, the most common are *Staphylococcus aureus and Streptococcus agalactiae.*
- These are spread from infected to clean udders during the milking process through contaminated milker's hands, cloth towels used to wash or dry udder of more than one animal and possibly by flies.

- The most common environmental pathogens, bacteria are *Strepcococcus uberis*, *Str. dysgalactiae*, *Coliforms such as E. coli and* Klebsiella.
- Transmission of these pathogens may occur during milking but primarily between milkings.
- Coliform infections are usually associated with unsanitary environment, while Klebsiella are found in sawdust that contains bark or soil.
- Environmental pathogens are most often responsible for the clinical cases.
- Approximately 70-80% of Coliform infections are manifested by abnormal milk, udder swelling and systemic disturbances such as high fever, swollen quarters, watery milk and depressed appetite.

Forms of Mastitis

1. Subclinical Mastitis

- Most prevalent and costly form of mastitis
- Animal does not exhibit typical mastitis symptoms
  - No change in udder appearance
  - No observable changes in milk
- Milk production will be lowered, but likely overlooked
- Can only be detected with individual cow somatic cell count (SCC) or microbiological culture
- Infected cows serve as reservoirs and can infect other cows

2. Clinical Mastitis

- Severity depends on bacteria causing infection
- Symptoms may include:
  - Abnormal milk (Flakes or clots, Discolored or watery)
  - Abnormal udder (swelling, heat, hard or sensitive to touch) may be slight to absent
- Drop in milk production

a) Acute Mastitis

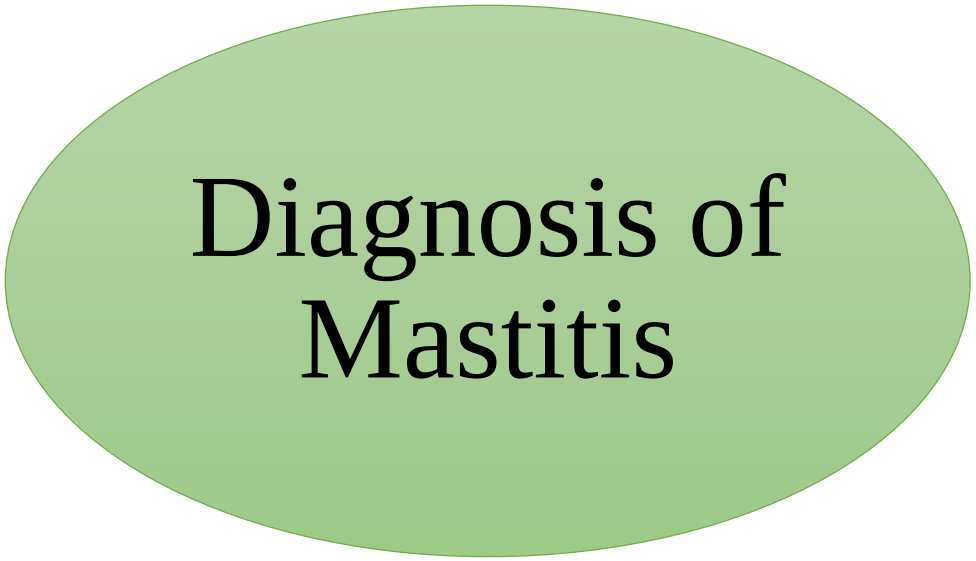
Depending on the course

- Has Sudden onset of symptoms
- Symptoms may include:
  - Fever, Off-feed and off-water, Rapid pulse, Weakness,
  - Udder may be red, swollen, hard and/or painful to touch
  - Grossly abnormal milk
  - Dramatic drop in milk production
- Can be caused by coliforms, pseudomonas and other bacteria

b) Chronic Mastitis

- Has long duration (months to years)
- Symptoms vary depending on phase of infection
  - Cows may remain subclinically infected indefinitely
  - Cows may alter between clinical and subclinical mastitis
- Multiple infections in the same quarter within a lactation or into the next lactation are a sign of chronic mastitis

- Therefore, knowledge of routine physical examination of udder together with diagnostic screening tests for early detection (i.e. during sub clinical form) of mastitis and proper treatment of affected animals.



Case history

Clinical examination

- Animal
- Udder
- Teat

Milk examination

I. Case history

- In this part we need to ask the owner about

1. Current amount of milk: to detect hypogalactia (decrease of milk production) or agalactia (absence of milk production).

2. Milking Technique

a) Hand milking → Contaminated hand→ entrance of microorganism inside udder causing mastitis

b) Milking machine
   - Contaminated machine → entrance of microorganism inside udder causing mastitis.
   - Faulty pressure of machine→ excessive suction→ teat injury and deformity → causing mastitis

3. Ask about milking hygiene including washing, disinfection of udder and teat dipping before milking so entrance of microorganism inside udder causing mastitis

4. Ask about type of food → high protein and estrogen → damage of secretory cells → decrease milk production

5. Number and course of previous lactation as milk production is normally small in first lactation but reach to peak in 4-8 lactation

6. Ask about previous mastitis and treatment

II. Clinical Examination

a) **Examination of animal:** determine animal temperature, pulsation, respiration, superfacial mucous membranes and superfacial lymph nodes. To:

1. Presence or absence of systemic reaction to avoid septicemia and bacteremia.
2. Detect specific signs for specific diseases

b) Examination of udder

1. **Inspection:** observe udder from rear, front and sides
   - Normally: udder appear symmetric, one mass, without any abnormalities and hind quarters larger than fore quarter
   - Abnormally: Swelling in one or more quarters or atrophy and Presence of vesicle, ulcers, scar and wound as in cases of FMD, LSD, Pox

2. Palpation:

- Normally
  - Udder appear as fine grains or spongy when free from milk,
  - No cardinal signs of inflammation,
  - Pliable skin under hand and
  - Supramammary lymph node not felt.
- Abnormally
  - Coarse grains and spongy when contain milk due to clotted milk in case of chronic inflammation,
  - Cardinal signs of inflammation,
  - Not pliable udder with adherence to underlying tissue and
  - Enlarged and inflamed supramammary lymph node

c) Examination of teat

1. Presence of fibrous cord at the base of teat or at teat opening
2. Presence of fibrous cord within teat canal
3. Dilated and obstructed teat opening

III. Milk examination

1. Physical examination

A. Color: Normally is whitish yellow in cow and White in buffalo

- *Physiological Discoloration*
  - Yellow: ration contain high carotene, Colostrum, Some breeds as Jersey cow
  - Blood stained: high producing animal
- *Pathological Discoloration*
  - Red: Dicumarol toxicity, Leptospirosis, Staphylococcosis
  - Green: Corynebacterium
  - Yellow: Most of bacterial Mastitis

B. Odor

- **Normally:** milky odor
- Abnormally
  - Fetid (Putrefied) odor: Gangrenous Mastitis, Dry cow Mastitis
  - Fecal odor: Coliform Mastitis
  - Acetone odor: Ketosis
  - Rancid odor: Milking in bad ventilated place or leaving milk for 2 days in environment after milking
  - Antiseptic odor: excessive dipping of teat

C. Consistency

- Normally
  - Milky consistency
  - In dry period milk turns too watery.
- Abnormally:
  - Watery milk (decrease consistency): Streptococcal mastitis
  - Increase consistency: Corynebacterial mastitis

2. Chemical examination

i. pH measurements:

- The PH of mid lactation cow milk falls between 6.4 and 6.8 with 6.6 (slightly acidic) the most usual value at 25 oC.
- The pH is lower (6.0) in colostrums and higher (up to 7.5) in case of mastitis than in normal milk of mid lactation.
- During **mastitis** become alkaline due to high sodium, chloride and bicarbonate and low casein, lactose and fat
- Physiological alkaline during dry period
- **Detection by**: *pH paper, pH meter, Bromothymol blue test and Bromocresol purple tests*

✓ Bromothymol Blue (BTB) Test:

- This is a pH indicator test, after adding the milk with Bromothymol blue, different colors are developed due to changes in pH of milk.
- For this test BTB card test papers may be prepared from what man filter paper No. 1. The diagnostic card can be prepared by adding one drop of BTB test solution (Bromothymol blue-1.6g in 100 ml ethanol) at 4 different spots on the paper and indicates left fore (LF), left hind (LH), right fore (RF) and right hind (RH).

- One drop of suspected milk has to be put directly on the indicator spot and observed changes in color are scored as follows :
  - Pale green indicates normal quarter and +, ++ and +++ (according to change in color from moderate green to dark blue green).
- The disadvantage of this test is that cow in later stages of lactation may give false positive reaction.

✓ Bromocresol Purple Test:

- This test is also pH indicator test.
- Take 2-3 drops of 0.9% bromocresol purple solution in a test tube and add 3 ml of freshly drawn milk.
  - Normal milk appears as yellow (Pale grayish purple) while mastitis milk will appear as blue or purple.

ii. Chloride Test:

- This test denotes presence of increased quantity of chloride in mastitis milk.
- In normal milk, chloride level varies from 0.08 to 0.14%.
- There exists an inverse relationship between the concentration of lactose and sodium chloride which depends on the stage of lactation.
- Davis (1999) reported that in mastitis there is decreased amount of lactose and increased amount of sodium chloride to maintain the normal milk pressure hence during inflammation (mastitis) there is increase in the chloride content (0.14%).

- **Procedure:**
  - Solution:
    - Solution A: Silver nitrate 1.34 g Distilled water-1000 ml
    - Solution B: Potassium Chromate 10 g Distilled water- 1000 ml
- Take 1 ml of milk and add 5 ml silver nitrate solution (solution A) followed by two drops of potassium chromate solution (Solution B). Observe for the change of color.
- Development of yellow color indicates the chloride level > 0.14 % i.e. quarter is positive for mastitis and appearance of brownish red color indicates chloride level < 0.14 % i.e. negative to mastitis.
- This test is more sensitive than the Bromothymol test

iii. Somatic cell count (SCC):

- The determination of milk SCC is widely used to monitor udder health and the milk quality.
- The higher the SCC, denotes raw milk contamination with pathogens and antibiotic residues, milk produced under poor standards of hygiene and from unhealthy animals.
- The elevated SCC consist primarily of leucocytes which include macrophages, lymphocytes and neutrophils (WBCs + Sloughed epithelial cells).
- During inflammation, major increase in SCC is because of the influx of neutrophils into milk and at this time over 90% of the cells may be PMN leukreytes.

- The dairy cow milk has a natural level of 100,000-150,000 somatic cells/ml and higher SCC indicates secretory disturbance.
- Milk from normal uninfected quarters generally contain below 200,000 somatic cells /ml. A value of SCC above 300,000 is abnormal and an indication of inflammation in the udder.

- **Factors affecting SCC:**
    1. Age: old > Young (Heifer)
    2. Seasonal incidence: Summer > Winter **:** Afternoon > Morning
    3. Frequency of lactation: ↑ frequency ➔ ↑ SCC
    4. Period of post-calving and Late stage of pregnancy: ↑ SCC (Physiologically)
    5. Hygienic Measures: ↓ Hygienic Measures➔ ↑ SCC
- A negative relationship generally exists between SCC and the milk yield.
- ***N.B****: SCC can be used as control program*

- **Methods of SC Counting**

a) Direct Method: Direct Microscopic Counting or using Direct Somatic Cell Counter

b) Indirect Method: Using Chemical Reaction:

1. California Mastitis Test (Schalm's Test – CMT)
2. Modified white Side Test (MWST)
3. Surf Filed Mastitis Test

1. California Mastitis Test (Schalm's Test – CMT)

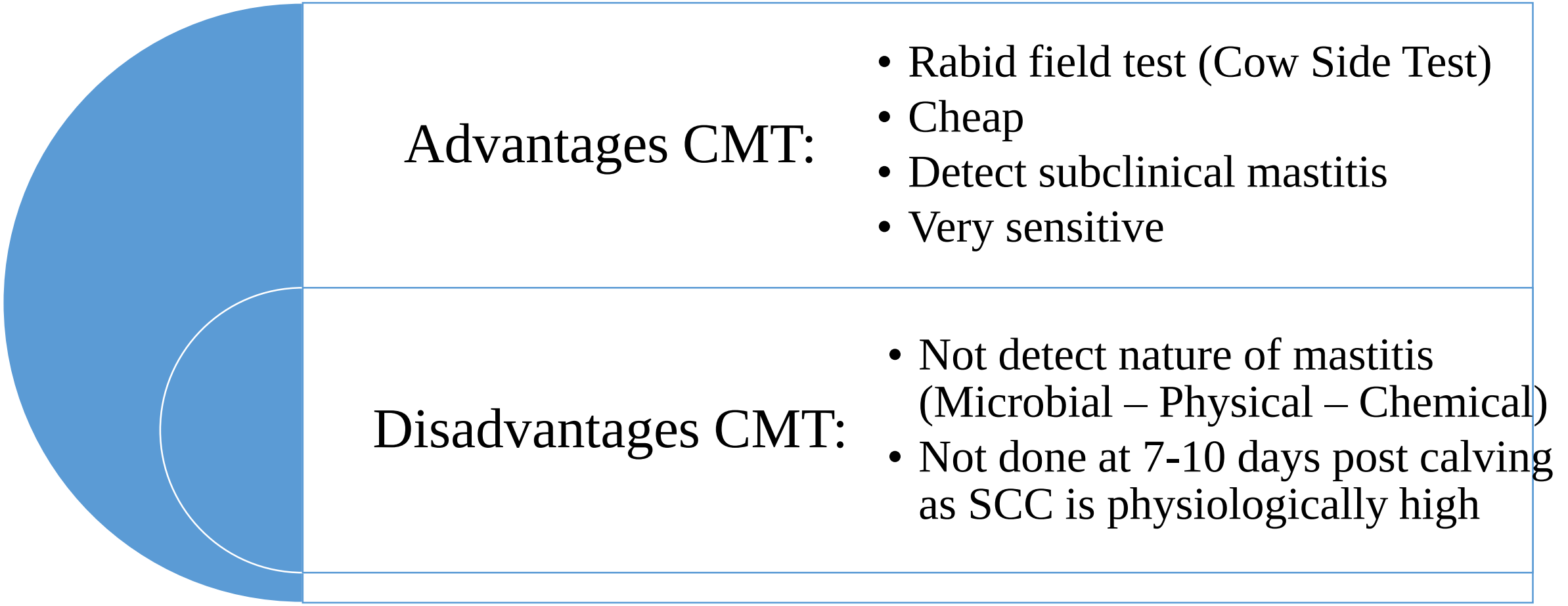
- California mastitis test is a simple, inexpensive and rapid screening test for mastitis.
- The test is based on the increase in number of leukocytes and alkalinity of the mastitis milk.
    - These changes are due to inflammatory exudation and increased contents of basic salts during inflammation.
- Principle: it depend on reaction between anionic surface of Schalm's reagent and DNA Liberated from somatic cell leading to precipitation and gel formation and according to amount of PPT number of Somatic Cells can be detected.

- Procedures:
  - On the paddle put equal amount of milk from and Schalm's reagent (each well represent one quarter of udder) –
  - Gentle rotatory movement for 10 sec

- **NB:** milk sample must be examined by CMT after milking by 2 hrs (loss viability) as so if examination will be delayed preserve sample using formalin 40% (1 ml can preserve up to 160-180 ml milk)

Results and Interpretation:

CMT Score	Description	Interpretation
N (Negative)	No Change	Healthy quarter
T (Trace)	Slime formed which disappeared with continuous movement of paddle	Sub Clinical mastitis
1(Weak)	Distinct slime, but no gel formation	Sub clinical mastitis
2(Distinct Positive)	Viscous with gel formation , which adhered to the margin	Severe mastitis infection
3(Strong Positive)	the gel formation with convex projection, the gel did not dislodge after swirling movement of the paddle	Severe mastitis infection


Advantages CMT:
• Rabid field test (Cow Side Test)
• Cheap
• Detect subclinical mastitis
• Very sensitive
Disadvantages CMT:
• Not detect nature of mastitis (Microbial – Physical – Chemical)
• Not done at 7-10 days post calving as SCC is physiologically high

2. Modified white Side Test (MWST)

- Principle: depend on reaction between NaOH and DNA Liberated from destructed somatic cells → absorb solid and Fat from milk → leading to precipitation and gel formation
- Procedures: on clean glass slide put 5 drops of tested milk + 2 drops of NaOH 4% then mix well until 15-20 sec
- Result: Precipitation and Gel Formation → + Mastitis
  - Negative samples were entirely free of precipitate.
- Adv. And Dis. Similar to CMT

- The amount of precipitate formed was graded from slight to thick viscid mass and was considered to be indicative of the degree of irritation in the udder.

Grading	Description
Mixture remains opaque and free of particles	(-) Negative
Fine dispersed particles on close inspection	(+/-) Trace A
definite thickening and mixture separates into a milky whey and white particles	(+) Distinct Positive
Mixture thickens immediately and follows glass rod	(++) strong positive

3. Surf Filed Mastitis Test:

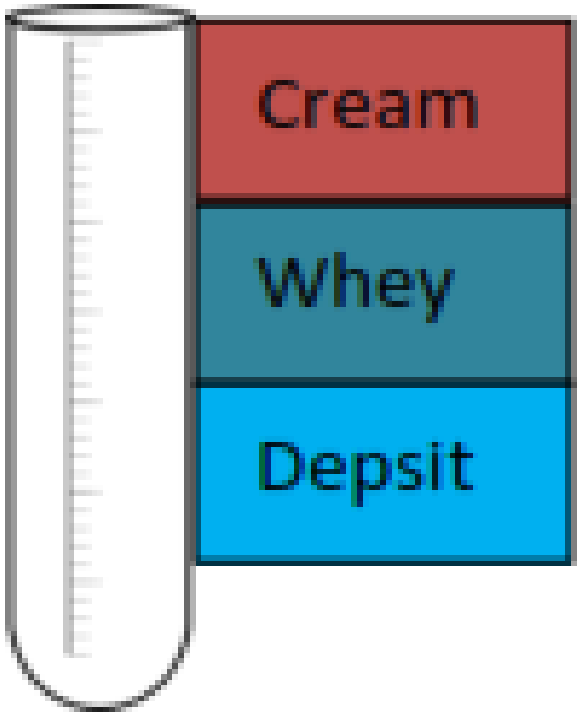
- The advantage of this test is that the readily available house hold surf (detergent) is used as reagent.
- The principle of this test is the reaction of somatic cell's DNA with detergent (Surf) and leads to the formation of gel of varying degree depending upon the number of somatic cells in the milk.
- 3% Surf solution-Dissolve 3 g of surf in 100 ml of clean tap water or 6 teaspoonful of house hold detergent surf in 500 ml of clean tap water. The test solution is table for 6months at room temperature

- **Procedure:**
  - Take equal quantity of 3% reagent and milk in the paddle or container.
  - The mixture is swirled for about 1 minute and then examined visually for the presence of small floccules and gel.
  - If floccules or gel is formed it indicates the presence of intramamary infection. In the absence of any floccules or gel, sample is negative

iv. Electrical conductivity:

- Electrical conductivity of milk has been considered as a possible index of mastitis.
- The specific conductance of milk reflects its concentration and activity of ions and is of the order of 0.004 to 0.005 Siemens or $ohms^{-1}cm^{-1}$ (4-5$mScm^{-1}$) at 25o C.
- Higher values represent mastitis infection in which there is an increase in the concentration of sodium and chloride in milk (high level of electrolytes).
- The test can be effectively carried out in a laboratory with the aid of an electronic digital conductivity meter or in the field with the help of a portable pocket type conductivity meter.

3. Bacteriological examination



- Aim of the bacteriological examination is :
  - Isolation and Identification of microorganism
  - Culture and Sensitivity test to detect drug of choice
- Sampling
  - Sample Preparation: incubation at 37 oC overnight 18 hrs then centrifugation at 3000 rpm/ 20min → 3 layers formed (Cream for TB; Whey for Mycoplasma; and Deposit for others)

- **Laboratory Procedures:**

a. Direct smear: staining by giemsa, gram, ziehl neelsen, according to suspected microorganism

b. Culturing on specific media: sabouraud dextrose agar (Mycotic), baired parker media (Staph.), edward's media (Strept.), Macconkey (enterobacteriaceae)

c. Culture and Sensitivity test (C & S): colony → brain heart infusion broth → incubate 37 oC 18 hrs → mueller hinton agar→Dispense ABX on agar → measure inhibition zone for each Antibiotic and according to these inhibition zones select drug of choice

• Headlines for Treatment of mastitis

1. Detoxification of Toxins:

- Physical Detoxification: milking every 2 hrs – cold fomentation
- Chemical Detoxification: Diuretics (increase excretion of toxins), Fluid therapy (Dilute toxins) and anti-inflammatory (decrease toxin spread)

2. Elimination of microorganism (ABX)

- Systemic at: Systemic reaction to avoid Septicemia and Bacteremia – badly swollen udder – help local treatment of udder
- Local by intramammary infusions: disposable plastic syringe contain standard dose of AB dispensed on oil or aqueous phase with or without anti-inflammatory


QUESTIONS