Section: Biology and Microbiology

COW'S MILK SAFETY: CONTAMINATION RISKS AND THE NEED FOR MASTITIS CONTROL

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Mastitis, especially subclinical, remains a major issue affecting milk productivity and quality in cows, goats, ewes, and rabbits [1, 2]. It affects 7–80% of cows [3]. and leads to economic losses due to lower milk yield, culling (30–35%), and treatment costs. Contaminated milk poses health risks, and antibiotic treatment leaves residues that affect milk safety [4].

Mastitis results from infections, environmental stress, and poor farm hygiene [5]. The disease peaks after childbirth (33–89%), during reproductive disorders (25–60%), and at peak lactation (22–52%). Infections mainly enter the mammary gland via the teat canal (galactogenic route), with streptococci and staphylococci causing up to 90% of cases [6]. The disease is more prevalent in autumn, winter, and spring (72%) than in summer (7–14%).

Clinical mastitis manifests as serous (2–26%), catarrhal (3–46%), purulent-catarrhal (2–71%), and other forms. Subclinical mastitis, present in 32–90% of lactating females, is harder to diagnose without laboratory tests [7]. Diagnosis involves physical-chemical (Whiteside test), cytological (somatic cell count), biochemical (enzyme activity), and bacteriological methods [8]. Screening for contagious pathogens helps in herd monitoring [9].

Successful mastitis control depends on rapid diagnosis and appropriate treatment. Milk examination determines bacterial contamination and antibiotic resistance [10]. Increased somatic cells (>100,000/cm³) indicate infection and tissue breakdown, often correlating with bacterial contamination.

To ensure accurate bacteriological examination of milk, samples should be collected at least 14 days after antibiotic useor according to the drug's elimination period. Proper aseptic techniques are essential to prevent contamination during sampling.

Before collecting milk, the udder, lower abdominal wall, and inner thighs should be washed with soap and disinfected with 70% alcohol. The first milk spurts are discarded, and 10–15 cm³ is collected in a sterile container, which is then sealed, cooled, and sent to the laboratory within 4 hours at ≤8°C.

In rabbits and small animals, postmortem mastitis diagnosis is performed to control infection or in cases of ineffective treatment. Affected mammary gland tissues are collected under aseptic conditions and delivered to the lab within 4–6 hours, kept under cold storage.

Mastitis in animals is commonly caused by *Staphylococcus aureus*, a leading pathogen in clinical cases. Samples are cultured on blood agar, Baird Parker agar, mannitol salt agar, and yolk-salt agar at 37°C for 24–48 hours.

Hemolysis Types:

β-hemolysis (transparent zone) – pathogenic *Staphylococcus aureus*.

α-hemolysis (opaque zone) – less common.

γ-hemolysis (no hemolysis) – non-pathogenic strains.

Staphylococcus confirmation assumes Gram staining, Gram-positive cocci in clusters or tetrads identified. A positive catalase reaction accompanied with gas bubbles in hydrogen peroxide. Anaerobic Glucose Fermentation differentiates *staphylococci* from *micrococci*. One should use rabbit blood plasma to confirm *Staph. aureus*. A positive test forms a stable clot. Phosphatase activitycan be evaluated. Cultures are incubated on phenolphthalein phosphate medium. Upon exposure to ammonia vapors, pathogenic strains turn pink, while non-pathogenic strains remain yellow.

Mastitis-causing *Staphylococcus aureus* produces DNAse, an enzyme linked to enterotoxigenicity. Cultures are grown on DNAse medium in Petri dishes. After 24 hours at 37°C, hydrochloric acid is added, and a clear zone around colonies indicates a positive reaction.

To assess lipolytic enzyme production, cultures are grown on tributyrin agar. Lipolytic bacteria create a clear zone in the yellow cloudy medium, showing tributyrin degradation.

Biological pathogenicity test can be made. *Staphylococcus* cultures (0.5–1.0 cm^3) are injected into white mice. If 2–3 mice die within 3 days, the strain is considered pathogenic. Or cultures are grown in milk for 48–120 hours at 37°C; given orally to mice, hamsters, guinea pigs, or kittens. Positive reaction: vomiting within 30–60 minutes, sometimes followed by diarrhea.

Quantitative Assessment of *Staphylococcus aureus* assumes use of selective media: samples are grown on blood agar with novocaine, Baird Parker agar, or DNA-novocaine agar. Milk samples are diluted 1:100 in a sterile saline solution.

Colony counting formula: (No. of colonies) × 10 × dilution factor (100).

Example: If 15 CFU are counted, then 15 × 10 × 100 = 15,000 microbial cells per cm^3 of milk.

Identification of *Staphylococcus aureus*: on blood agar → hemolysis zone indicates *S. aureus*. On DNA-novocaine agar → large round colonies with clear boundaries appear under hydrochloric acid.

These methods ensure accurate detection, differentiation, and quantification of *Staphylococcus aureus* in milk samples, aiding in the diagnosis and management of mastitis in animals.

The milk of cows and goats suffering from mastitis most often contains staphylococci of the species *St. aureus (Staphylococcus aureus), St. haemolyticus (hemolytic Staphylococcus), St. epidermidis (epidermal Staphylococcus)*.

Staphylococcus species *St. saprophuticus* is the causative agent of mastitis and can be detected in the milk of both healthy and sick cows due to its contamination. Hence, the determination of pathogenicity in white mice takes place. Staphylococci of the species *St. aureus*, possessing pathogenicity enzymes: lecithinase, hemolytic activity, plasma coagulation property and/or DNAse activity, and decomposing mannitol and maltose in anaerobic conditions, are classified as causative agents of mastitis.

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