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Acute kidney injury in dairy cattle affected with gangrenous mastitis

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Abstract
A study was carried out to identify the etiology of gangrenous mastitis in dairy cattle. A total of 615 cattle was presented with mastitis during January 2024 to March 2025. Out of 615 cattle, 19 dairy cows showed the gangrenous changes like reddish or blackish discolouration of affected quarter with brown coloured haemolytic secretion, anorexia and recumbency. The milk samples were collected from gangrene affected quarters; the bacterial culture was sequentially performed in nutrient agar, mannitol salt agar and MacConkey agar. In bacterial culture, *Staphylococcus* spp, *Klebsiella* spp, *E. coli*, *Pseudomonas* spp and *Bacillus* spp were identified based on colony morphology. These bacterial isolates were confirmed with PCR. In PCR, out of 19 isolates, 4, 2, 1 4, 3, 3 and 2 samples were positive for *Staphylococcus sciuri*, *Staphylococcus aureus*, mixed infection of *Staphylococcus sciuri* and *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. The *S. sciuri* and *B. cereus* were predominantly identified in gangrenous mastitis followed by *E. coli* and *K. Pneumoniae*. In *Klebsiella pneumoniae* infection, the gangrenous changes were observed 3 days after infection. The animals were treated with Inj. Ceftiofur sodium @ 2.2 mg/Kg IM along with supportive therapy for 7 days. The prognosis in gangrenous mastitis depends on extend of severe kidney damage. The severe kidney damage was observed in *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* infection compared to *Staphylococcus aureus and Staphylococcus sciuri* and *Bacillus cereus*. The prognosis is fair to good with respect to liveability of animals especially infected with *Staphylococcus aureus*, *Staphylococcus sciuri* and *Bacillus cereus*.

Keywords: *Escherichia coli*, *Staphylococcus aureus*, gangrene, mastitis, cattle

Introduction
Gangrenous mastitis is a serious form of mastitis in dairy cattle, most commonly caused by *Staphylococccus aureus*, *Escherichia coli* and *Clostridium perfringens* (Jubb and Kennedy 1963; Hussain *et al*., 2014) [8, 6]. The increased number of bacteria in the circulation, and expression of virulence factors enable to evade the host defence mechanism for multiplication and produce the cytotoxic toxins like leukotoxins, hemolysins and exoenzymes (Abril *et al.*, 2020) [1]. The alpha toxins released from the bacteria increases the vascular endothelial permeability and thrombosis of mammary blood vessels results in ischemia, coagulative necrosis, toxaemia and death of animals (Abubakar *et al*., 2022) [2]. The affected mammary quarters showed the blue green discoloration of affected quarter, cold to touch, swollen, firm with marked line of differentiating necrosed tissue from the healthy tissue and foul smelling brownish red (milk containing blood and pus) fluid draining from the affected quarters (Kavibharathy *et al.*, 2024) [9]. However, the enterotoxin producing *Staphylococcus aureus* did not respond to antibiotic therapy rather than non-enterotoxin producing *S. aureus* isolates (Jones and Wieneke, 1986; Hussain *et al*., 2014) [6]. The clinical signs usually observed in early lactation with or without the systemic signs of fever, anorexia, dyspnoea and toxaemia (Abubakar *et al*., 2022) [2]. There is a scarce report on sepsis associated kidney damage in animals. The present article described about the acute kidney damage in dairy cattle due to gangrenous mastitis

Materials and Methods

A total of 615 mastitis cases were presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu, Tamil Nadu from January 2024 to March 2025. Out of 615 cattle, 19 dairy cows showed the symptoms of gangrene like blackish or bluish discolouration (Figure 1 & 2) of one or more mammary quarter with foul smelling haemolytic brown coloured secretion. Apart from that the animals showed a high rectal temperature, anorexia, blisters, erythema on the affected quarter, severe respiratory distress and lateral recumbency. Some of the animals showed the gas bubbles while milking with serosanguinous secretion with intact skin. The milk samples for bacterial culture and serum samples for biochemical analysis were collected. The milk samples were inoculated in nutrient broth and incubated at 37 ^{o}C for 12-24 hours for appearance of turbidity, then broth culture was sequentially streaked in nutrient agar, mannitol salt agar, MacConkey agar, eosin methylene blue agar and cetrimide agar followed by incubation at 37 ^{o}C for 24-48 hrs. The bacteria were identified based on colony morphology in specific culture media. The biochemical parameters like blood urea nitrogen, creatinine, calcium, phosphorus and glucose levels were measured using an autoanalyzer. The animals were treated with Inj. Ceftiofur sodium @ 2.2 mg/Kg IM, Inj. Dexamethasone sodium 10 ml IM, Inj. Normal saline 2.0 litre IV, Inj. Vitamin AD$_3$E 10 ml IM for 7 days.

Results

In bacterial culture, *Staphylococcus* spp, *Escherichia coli*, *Klebsiella* spp, *Pseudomonas* spp and *Bacillus* spp were identified based on colony characters in specific culture media. The bacteria was confirmed at species level by molecular technique. In PCR, *Staphylococcus sciuri*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were confirmed using species specific primers. Out of 19 samples, 4, 2, 1 4, 3, 3 and 2 samples were positive for *Staphylococcus sciuri*, *Staphylococcus aureus*, mixed infection of *Staphylococcus sciuri* and *Staphylococcus aureus*, *Bacillus cereus* (Figure 3), *Escherichia coli* (Figure 4), *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. The *Staphylococcus sciuri*, and *Bacillus cereus* were predominantly identified in gangrenous mastitis followed by *Escherichia coli* and *Klebsiella pneumoniae* the peracute gangrenous *Escherichia coli* mastitis was predominantly observed in dairy cattle during periparturient period, whereas gangrenous form of *S. sciuri*, *S. aureus*, *B. cereus*, *K. pneumoniae* and *P. aeruginosa* were observed in early and mid-lactation periods. The gangrenous form of *P. aeruginosa* was isolated in one dairy cow with unknown history of calving and loss of suckling by calves. The serum biochemical analysis revealed elevated level of blood urea nitrogen (BUN), creatinine, normal to elevated level of phosphorus and normal to decreased level of calcium and glucose (Table 1). The biochemical alterations were varied with pathogens and severity of diseases. The blood urea nitrogen, creatinine and phosphorus levels were highly elevated but calcium and glucose levels were severely decreased in *E. coli* mastitis and moderately increased level of blood urea nitrogen, creatinine and phosphorus levels were observed in *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* infection, whereas mild elevation of BUN and creatinine and decreased level of glucose was observed in *Bacillus cereus* and *Staphylococcus aureus*, *Staphylococcus sciuri* infection. The prognosis was good in *Bacillus cereus* and *Staphylococcus aureus* and *Staphylococcus sciuri*, poor to guarded in *E. coli*, *K. pneumoniae* and *P. aeruginosa* infection.

Discussion

Gangrenous mastitis is a condition of severe inflammatory process in the mammary glands leads to septicaemia, pneumonia and toxaemia. It is caused by several microorganism, most commonly gram positive bacteria like *Staphylococcus aureus*, *Clostridium perfringens* type A (Schoonderwoerd *et al*., 1990) [15], *Bacillus cereus* (Mavangira *et al.*, 2013) and gram negative bacteria such as *Escherichia coli* (Schoonderwoerd *et al*., 1990) [15], *Klebsiella pneumoniae* (Salih and Ahmed, 2015) [14], *Pseudomonas aeruginosa* (Osborne *et al*., 1981) [11], *Klebsiella pneumoniae* (Salih and Ahmed, 2015) [14] and *Proteus mirabilis* (A1saad *et al.*, 2018). Following intramammary invasion, the bacteria liberate virulence factors (exotoxin/LPS/endotoxin/alpha toxin), which causes coagulative necrosis and thrombosis of mammary blood vessels and development of gangrene (Abubakar *et al*., 2022) [2]. The dissemination of infection and absorption of toxins into the systemic circulation leads to septicaemia, toxaemia and death. The LPS/ endotoxin triggers the immune system to release of pro-inflammatory mediators (cytokines), tumour necrosis factor alpha (TNFα), interleukin-6, kinin and kallikrein induce severe vascular changes in the blood vessels and increased the absorption of toxins in the circulation (Ribeiro *et al.*, 2008) [13]. In this study, the elevated level of blood urea nitrogen, creatinine and phosphorus may be attributed to acute kidney damage and decreased level of glucose might due to increased utilization of glucose during septicaemic condition. In severe septicaemia and endotoxic shock, there was a hypovolemia, decreased blood pressure, thrombosis of micro vessels leads to decreased renal perfusion and acute kidney injury. During sepsis, microcirculatory dysfunction occurred as a result of endothelial injury, dysfunction of autonomic nervous system, glycocalyx shedding from bacteria and activation of coagulation cascade. The shedding of glycocalyx from bacteria enhances leukocyte and platelets aggregation and potentially leads to microthrombi formation and occlusion of capillaries with a concomitant reduction in blood flow velocity leads to decreased glomerular filtration rate and elevated level of BUN and creatinine in the circulation (Peerapornratana *et al.*, 2019) [12]. In this study, the prognosis was predicted based on serum creatinine level. Prognosis was fair to good (creatinine < 3.5 mg/dl) and poor to guarded (creatinine > 5.0 mg/dl).

Table 1. Biochemical changes in gangrenous mastitis in dairy cattle

Etiology	Biochemical parameters (mg/dl)				
	BUN	Creatinine	Calcium	Phosphorus	Glucose
S. aureus & *S. sciuri*	29-39	1.65-2.71	7.4-11.9	5.29-6.11	28-56
B. cereus	75-86	3.25-3.34	9.05-10.4	5.69-8.80	20-21
E. coli	175-253	12.4-12.59	4.20-4.50	9.29-9.50	24-28
K. pneumoniae	104-124	3.74-5.50	7.80-8.90	7.70-10.97	42-46
P. aeruginosa	117-136	3.60-5.20	6.40-8.20	6.42-8.20	38-47

Fig 1: Blackish discolouration of right forequarter-*Bacillus cereus*

Fig 2: Bluish discolouration of right hindquarter-*Escherichia coli*

Fig 3: Nutrient agar-White opaque diffuse cottony colonies-*Bacillus cereus*

Fig 4: EMB agar-Greenish metallic sheen colonies-*Escherichia coli*

Conclusion

In gangrenous mastitis, animals infected with coliform organisms *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* had extreme kidney damage and death due to endotoxemia. The prognosis was fair to good in dairy cows infected with *Staphylococcus aureus*, *Staphylococcus sciuri* and *Bacillus cereus*.

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Conflict of Interest

Not available

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Reference

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