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Management of clinical mastitis in a water buffalo associated with enteropathogenic strain of *Escherichia coli*: A case report

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Abstract
A seven-year-old murrah buffalo weighing approximate 380 kg was presented to the Referral Veterinary Polyclinic of ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar with the complaint of sudden drop in milk yield, anorexia, red and swollen udder, yellow watery milk for past three days and history of calving six days back. Physical examination of udder revealed heat, pain on palpation, edema, red and swollen udder. The changes in milk included alkaline pH and yellow watery color of milk. On laboratory examination of milk, score for california mastitis test was +++ and somatic cell count was found to be 4.00 $\times 10^5$ cells/ml of milk. Hematological investigation revealed neutrophilic leukocytosis. On culture and isolation, the causative agent was isolated and identified to be *Escherichia coli*. Antibiotic sensitivity test revealed that *Escherichia coli* was found more sensitive to enrofloxacin, cefoperazone sublactum, gentamicin and moxifloxacin. Molecular confirmation for *E. coli* was done by PCR for the presence of eaeA gene. The therapeutic management of buffalo was done by using parenteral enrofloxacin, flunixin meglumine, multivitamin, intramammary cefoperazone, topical herbal gel and oral vitamin E and inorganic selenium supplementation. This case study deals with the management of clinical mastitis in buffalo associated with enteropathogenic strain of *Escherichia coli.*

Keywords: Udder, california mastitis test, *Escherichia coli*, somatic cell, eaeA gene

1. Introduction
For the majority of people in the world, dairy products, particularly milk, are among their most important food sources. It is necessary to increase the average milk yield per cow due to the rising demand for dairy products worldwide. Mastitis is the major problem impacting on high milk yield and cause huge losses to dairy industries (Lucy, 2001) [7]. Mastitis derives its traditional meaning from the Greek term "Matos", meaning breast or udder, and the suffix "itis," meaning inflammation (Kebede & Tilahun, 2023) [5]. Mastitis is an inflammatory condition of the udder. It is an international issue that is characterized by pathological alterations in the glandular tissue of the udder and physical, chemical, and microbiological changes in the milk (Khan & Muhammad, 2005) [6]. Three main elements combine to cause this complicated and multifaqctorial disease: the animal, the pathogen, and the environmental and management factor (Radostits *et al*., 2007) [10]. Based on the level of inflammation mastitis is classified as clinical, subclinical and chronic intramammary inflammation (Cheng & Han, 2020) [4]. Clinical mastitis characterized by changes in color of milk, red and swollen udder, heat and pain on palpation of udder ((Qadri *et al*., 2015) [9]. Clinical mastitis was higher in cattle (20%) compared to buffaloes (11%), whereas subclinical was higher in buffaloes (66%) than cattle (53%) (Ali *et al*., 2021) [1]. One of the most common pathogens isolated from clinical mastitis is *Escherichia coli*; it is an environmental pathogen. It was more severe than other bacterial causes and commonly observed in early lactation and during the housing period, resulting in sub-acute to per-acute inflammation; it can cause necrosis of the mammary

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gland epithelium in both severe experimental and severe clinical *Escherichia coli* mastitis that occurs naturally (Bradley and Green, 2001) [3]. The present paper deals with the management of clinical mastitis in buffalo associated with enteropathogenic strain of *Escherichia coli.*

2. Case Presentation

A seven-year-old murrah buffalo weighing approximate 380.00 kg was brought to the Referral Veterinary Polyclinic of ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar with the history of sudden drop in milk yield, anorexia, yellow watery milk for past three days and history of calving six days back. Clinical examination of udder revealed heat, pain on palpation, edema, red and swollen udder (Figure 1). The changes in milk included alkaline pH and yellow watery color of milk (Figure 2). Score for california mastitis test was +++ and somatic cell count was found to be 4×10^5 cells/ml of milk. Hematological analysis revealed neutrophilic leukocytosis.

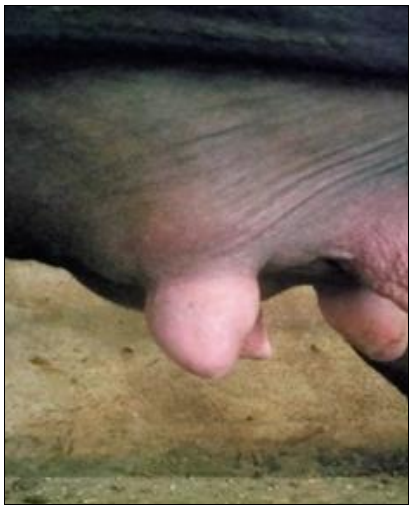

Fig 1: Red and swollen udder.

Figure. 2: Yellow watery color of milk.

Milk sample was collected in sterile syringe. Collected milk Samples were centrifuged for 20 minutes at 3000 rpm after being incubated at 37 °C for 24 hours. The sediment was streaked on Nutrient agar, MacConkey agar, and Eosin-methylene blue (EMB) agar, and the supernatant fluid was discarded. After 24 hours of incubation at 37 °C, the inoculated agar plates were checked for bacterial growth (Mahmoud *et al*., 2015) [2]. *Escherichia coli* colonies grow on EMB agar with a green metallic sheen (Figure 3). Thus, on culture and isolation, the causative agent was isolated and identified to be *Escherichia coli.*

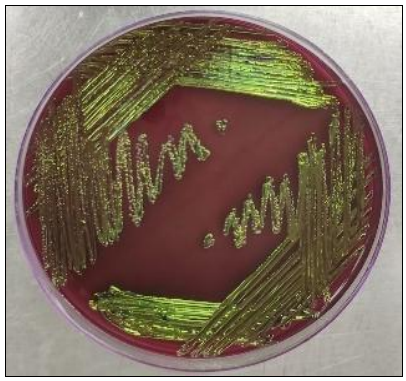

Fig 3: Green metallic sheen on EMB agar plate.

Molecular confirmation for *E. coli* was done by PCR for the presence of eaeA gene. In order to identify the nucleotide sequence of the eaeA gene, the oligonucleotide primers for PCR were synthesized in accordance with Marashifard *et al*. (2019) [8]. PCR identification of eaeA gene give positive reaction at 400 bp (Figure 4). Based on the above information, the animal was suspected to be infected with clinical mastitis caused by enteropathogenic strain of *Escherichia coli.*

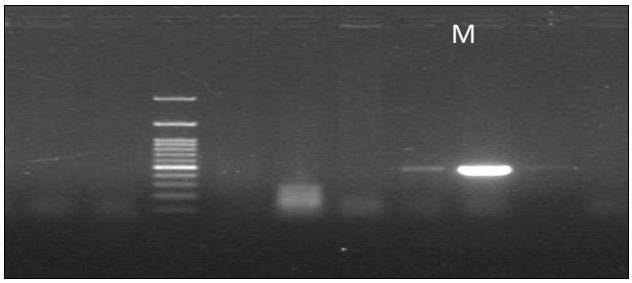

Fig 4: eaeA gene was detected in *E. coli* isolate by using PCR amplification and measured to be 400 kb in length. Lane-M represent ladder, Lane-2 denotes positive control for *E coli*, Lane-1 denotes positive for eaeA gene of *E. coli.*

Antibiotic sensitivity testing done on muller-hinton agar plates by using Kirby bauer disk diffusion method. ABST revealed that *Escherichia coli* was found more sensitive to Enrofloxacin, cefoperazone sublactum, gentamicin and moxifloxacin.

3. Results and Discussion

In accordance with the results of an antibiotic sensitivity test, the affected buffalo was treated with inj. enrofloxacin at the dose rate of 5.00 mg/kg body weight intramuscular once in a day for five days, inj. flunixin was given at the dose rate of 2.2 mg/kg body weight intramuscularly once in a day for three days, intramammary cefoperazone was given at the dose rate of one tube per affected quarter after complete milking, inj pheniramine maleate at the total dose of 10 ml per animal intramuscularly once in a day for three days, along with supportive therapy of vitamin E at the dose rate of 500 IU/day through oral route for five days, inorganic Selenium at the dose rate of 6 mg/day orally for five days, topical herbal gel therapy and oral probiotic to maintain gut udder axis. Post treatment follow up of buffalo has done after five days and complete remission of clinical sign was noticed (Figure 5); the milk was white in color, transparent, and devoid of clots or flakes.

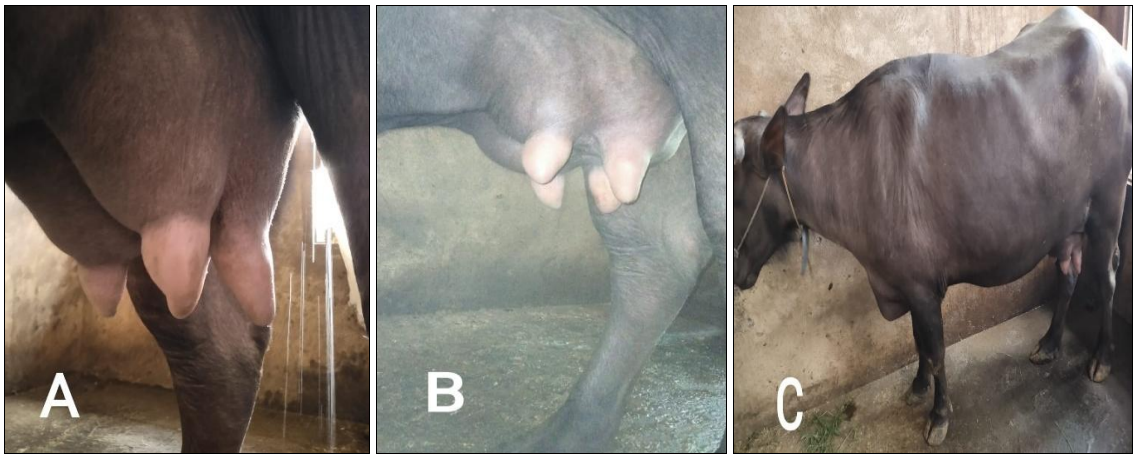

Fig 5: A, B and C shows udder of buffalo after complete recovery from clinical mastitis.

The milk sample was found negative for CMT, SCC and for culture. The milk pH was found in normal range. Mastitis is caused by environmental pathogen and contagious pathogen. Environmental pathogen includes *Escherichia coli*, *Strepococcus dysgalactiae*, *Strepococcus uberis*, *Klebsiella pneumoniae*, *Pseudomonas and Staphylococcus aureus*, *Strepococcus agalactiae*, *Mycoplasma bovis*, *Corynebacterium bovis* are the contagious pathogens. Apart from these, there are some myocotic mastitogens *Aspergillus fumigatus* and *Candida albicans.* The antibiotic sensitivity test for the isolate showed high sensitivity for enrofloxacin, cefoperazone sublactum, gentamicin and moxifloxacin, while found resistant for oxytetracycline and ampicillin. Pheniramine maleate used for inhibition of histamine release and meloxicam was given as an anti-inflammatory drug. Topical herbal gel, oral vitamin E and inorganic selenium used as supportive therapy. Intravenous fluid therapy was done for dilution of toxins produced by bacteria. Owner was advised to disinfect the animal shed and teat before milking. So antibiotic therapy along with supportive therapy help in quick recovery from clinical mastitis.

4. Acknowledgement
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5. Conflict of Interest
Author has disclosed no possible conflict of interest.

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