

# Clinical Intervention of Puerperal Metritis in Indian Water Buffaloes (*Bubalus bubalis*) in Meerut, Uttar Pradesh

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## ABSTRACT

Puerperal metritis is an inflammation of animal uterus around one month of calving. This condition is usually caused by bacterial infection of uterus and considered as one of the most economically important diseases

in Indian water buffaloes. The present study was conducted to determine the clinical interventions, clinical signs, bacteriological examination and evaluation of the treatment efficacy of postpartum metritis in Indian water buffaloes. A total of 18 buffaloes with clinical signs of metritis and calving of approximately 1 month were selected for the study and examined clinically including gross external inspection, transrectal

palpation of the cervix, uterus, and ovaries. Uterine discharges from the uterine lumen of each buffalo were collected for isolation of bacteria. Uterine discharge samples were cultured on general growth media and selective media for bacterial isolation. Bacteria were identified based on colony, morphological, biochemical characteristics, and confirmed by PCR based amplification of specific genes. The most prevalent bacteria in uterine discharge were *Escherichia coli* and *Staphylococcus aureus*. Antibiogram revealed that most of the bacterial isolates were sensitive to cefaperazone and gentamicin with sensitivity of 88.88% and 83.33%, respectively. Buffaloes were treated with intrauterine infusion of 2% povidone-iodine and systemically with cefaperazone and hormonal treatment (cloprostenol). Buffaloes with puerperal metritis showed good clinical recovery, uterine involution with this treatment and conceived after artificial insemination.

## INTRODUCTION

Postpartum uterine diseases, caused by infectious pathogenic bacteria, are highly prevalent in animals all around the world (Dahiya *et al.*, 2018). Puerperal metritis, an inflammation of uterus around 1 month of calving is economically important condition in Indian water buffaloes and causes significant economic losses to dairy industry in terms of decreased milk production, extra expenditures involved in treatment of this condition and, delay in uterine involution, which ultimately leads to poor reproductive efficiency in dairy animals (Sheldon *et al.*, 2006; Lof *et al.*, 2014; Lima *et al.*, 2019). The puerperal metritis are clinically characterized by pyrexia, inappetence or anorexia, decrease in milk production, and fetid discharge from vulva of animals (Azawi *et al.*, 2007). The animal, which is affected by puerperal metritis, may cause significant economic losses to dairy sector because of chronic nature of uterine infection leading to prolonged intercalving interval and culling of the animal (Melendez *et al.*, 2004; Karimi *et al.*, 2004; Azawi *et al.*, 2007).

After calving in animals, there is involution of the uterus and discharge of lochia. Entry of pathogenic bacteria in the uterus either due to systemic infection or due to extreme stretching of uterus, trauma in genital tissues during the calving, or dystocia or obstetrical manipulation or unhygienic conditions, etc, leads to puerperal metritis (Königsson *et al.*, 2002; Noakes *et al.*, 2002). To get the success in treatment of uterine infection, focus should be on removal of pathogen, evacuation of uterine fluids and early involution of the uterus (Noakes *et al.*, 2002; Azawi *et al.*, 2008).

Effectiveness of treatment depends on selection of the antibiotic drug to remove the pathogen from uterine fluid, while uterine evacuation depends on use of hormones (Lewis, 1997). The antibiotic used in the uterine infection should be broad spectrum and remain active in uterine environment. If there are chances of degradation of antibiotics in uterine environment, then systemic use of antibiotics are indicated (Amiridis *et al.*, 2003; Azawi *et al.*, 2008). The present study was designed to determine the clinical interventions, clinical signs, bacteriological examination, and evaluation of the treatment efficacy of postpartum metritis in Indian water buffaloes.

## MATERIALS AND METHODS

### Animals, Clinical Examination and Sample Collection

The study was conducted during October 2019 to March 2020 on lactating Indian water buffalo of Murrah breed in and around Meerut, Uttar Pradesh, India. Buffaloes, calved recently (approximately 1 month), aged 5-8 years and infected with puerperal metritis were selected for the study. All the animals were maintained indoors either in commercial dairy farm or in backyard farming with milking twice a day.

Buffaloes were fed balanced nutritional ration such as green fodder, straw, and concentrate including mineral mixture. The animals were examined clinically, including general health condition, rectal temperature, pulse rate, respiration rate, inappetance or

appetite, and presence of any discharge on the genitalia, perineum, or tail. The conditions of cervix, uterus, oviducts, and ovaries were examined per-rectally. Buffaloes showing pyrexia along with non-retractable uterus and fetid vulval discharge were included in this study.

After clinical examination and confirmation of puerperal metritis, samples were collected for bacteriological examination. For sample collections, the vulval lips were parted and sterile sheath along with artificial insemination gun was inserted into vagina and fixed into the cervix. The uterine discharge was sucked using sterile disposable syringe and sealed syringes with discharge were transported to the laboratory under cold conditions (4°C) for further processing. In the present study, a total of 18 Indian water buffaloes were included with puerperal metritis.

### Laboratory Examination

The uterine discharge was cultured and incubated at 37°C for 24 hrs aerobically on Tryptose soya broth (TSB), and subsequently streaked over MacConkey Lactose agar (MLA), Mannitol Salt agar (MSA). The pink color colonies over MLA were transferred to Eosin Methylene Blue agar (EMB), and incubated at 37°C for 24 hrs. Bacteria were identified on the basis of colony characteristics (pink color colonies over MLA, yellow color colonies over MSA, and metallic sheen over EMB), staining (Gram positive or negative), morphology (cocci, rod or coccobacilli), and biochemical characteristics (catalase, coagulase, oxidase, indole production, methyl red reduction, voges-proskauer, citrate production, and sugar utilization).

The bacterial isolates primitively identified based on cultural, morphological and biochemical tests were confirmed by PCR based amplification and sequencing of PCR products. The DNA was isolated as per the instruction of manufacturer using GeneJET Genomic DNA Purification Kit (#K0721, Thermo Fisher Scientific, USA). The amplification was performed as per the primers and protocols previously described for E.

coli (Anbazhagan *et al.*, 2010) and *Staphylococcus aureus* (Brakstad *et al.*, 1992).

The susceptibility to antimicrobial drugs of bacteria was tested by disc diffusion method according to the standards of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2017). The study included a panel of antibacterial drugs namely Ciprofloxacin, Gentamicin, Amoxicillin, Cloxacillin, Enrofloxacin, Penicillin, Amikacin, Oxytetracycline, Cefoperazone, Metronidazole, Levofloxacin, Cephalexin and Ceftriaxone (Himedia, India) based on previous reports and recommendations of clinicians for the treatment of clinical metritis.

### Treatment and Follow-Up Examination

Buffaloes with puerperal metritis were subjected to the treatment regimen using systemic cefoperazone antibiotics (intramuscular route) based on drug sensitivity, a hormonal preparations using cloprostenol 500 µg intramuscularly as a single dose and intrauterine administration of 30 ml of 2% povidone-iodine administration for 3 days. In the present study, no buffaloes were selected in control group due to threat to life in puerperal metritis cases. After 14 days of treatment, buffaloes were examined clinically. Absence of pus discharge from genitalia, uterine involution at follow up examination was considered as clinical cure. These buffaloes were given rest for subsequent estrous and then subjected to artificial insemination. The pregnancy was confirmed by per rectal examination on 3rd month of conception.

### RESULTS AND DISCUSSION

On clinical examination, all the buffaloes affected with puerperal metritis showed pyrexia (rectal temperature ranged  $103.6 \pm 0.22$  °F), Pulse rate ( $110.4 \pm 6.66$  / min), respiration rate ( $46.2 \pm 3.8$ /min), inappetance or anorexia, depression, painful straining. Vaginal examination showed watery fetid white or brownish discharge. Rectal examination of the genital tracts of buffaloes revealed that 88.89% (16/18) with puerperal metritis had enlarged cervical diameter (7.5 cm or more) and enlarged atonic uterine horns. Previous studies (Azawi *et al.*, 2007;

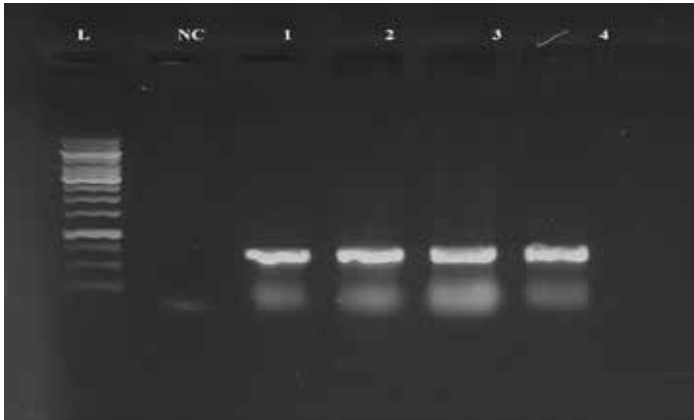
**Figure 1:** PCR assay for detection of *E. coli*

Lane L: 250 bp DNA ladder

Lane NC: Negative Control

Lane 1: Positive control (*E. coli*)

Lane 2-4: Sample (Uterine Discharge)



Hehenberger *et al.*, 2015) also reported similar clinical signs in case of uterine infections in dairy animals.

On bacteriological examination, the most prevalent bacteria in uterine discharge were *Escherichia coli* (12/18) and *Staphylococcus aureus* (8/18) (Figure 1 and 2). Different bacteria like *Archanobacterium pyogenes*, *Escherichia coli*, *Staphylococcus* spp., *Streptococcus* spp., *Fusobacterium necrophorum*, and *Prevotella melaninogenicus* species and *Proteus* spp. etc have been previously isolated from uterine infection of animals (Lohuis *et al.*, 1994; Williams *et al.*, 2005; Azawi *et al.*, 2008; Ordell *et al.*, 2016; Dahiya *et al.*, 2018). Similarly, in the present study, *Escherichia coli* and *Staphylococcus aureus* are the main bacteria isolated from the puerperal metritis with the prevalence of 66.7 and 44.4%, respectively. Previous studies suggested that puerperal metritis in buffaloes usually occur due to ascending infection by the non-specific environmental organisms (Azawi *et al.*, 2008).

During the parturition, the

broken physical barrier of the uterus, the dilated cervix and relaxed vulva allow bacteria to invade the uterine lumen (Paisley *et al.*, 1986). Periparturient conditions like dystocia, prolapse of uterus or vagina, retention of foetal membranes further aggravated the condition as these may diminish the ability of uterus to eliminate the contaminants pathogens (Azawi *et al.*, 2007). Azawi *et al.*, 2007 reported that *E. coli* is the main contaminant in uterus after parturition and could penetrate and

colonize the endometrium of uterus.

*In vitro* antibiotic sensitivity test revealed cefoperazone and gentamicin with highest sensitivity against the bacteria isolated from puerperal metritis in buffaloes, and

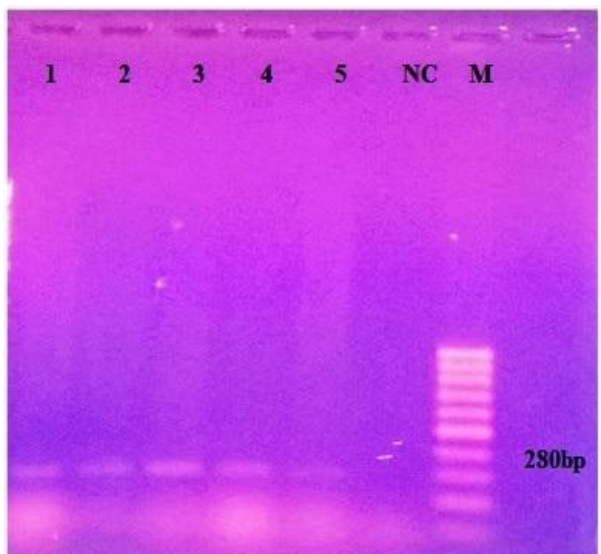
**Figure 2:** Amplification of *nun* gene for detection of *Staphylococcus aureus*

Lane M: 100 bp DNA ladder

Lane NC: Negative Control

Lane 1: Positive control (*Staphylococcus aureus*)

Lane 2-5: Sample (Uterine Discharge)



the most of bacterial isolates were sensitive to cefoperazone and gentamicin with sensitivity of 88.88% and 83.33%, respectively. Treatment results showed significant clinical cure in buffaloes affected with puerperal metritis using intrauterine infusion of 30 ml of 2% povidone-iodine and 10 mg/kg body weight of cefoperazone systemically for 3 days, with cloprostenol 500 µg intramuscularly as a single dose.

Out of 18 buffaloes under study, 17 (94.44%) produced clear secretions with no fetid smell after 14 days post-treatment and subsequent estrous, while 01 (5.55%) did not respond to the treatment and continue to produce purulent discharge. Previous studies suggested the use of penicillins (Ordell *et al.*, 2016), cetiofur (Mc Laughlin *et al.*, 2012; Haimerl *et al.*, 2017), and cefapirin (Hehenberger *et al.*, 2015) as antimicrobial drugs for the treatment of uterine infection in dairy animals. Selection of antibiotic drugs for the treatment of puerperal metritis requires the knowledge of antibiotic sensitivity patterns of commonly isolated bacteria from uterine infections (Azawi *et al.*, 2008; Haimerl *et al.*, 2017). This may further help in the prediction of clinical outcome of the antimicrobial treatment and reduction in development of antibiotic drug resistance due to unnecessary use of antibiotics that are ineffective or less effective.

The clinical recovery in buffaloes suffering with puerperal metritis after treatment with intrauterine infusion with 2% povidone-iodine and systemic injections of cefoperazone was seen by comparing the uterine fetid discharge and improvement of uterine involution pre- and post-treatment. In systemic use of antibiotics, higher concentrations throughout the genital tract may be achieved in comparison to intrauterine therapy of cefoperazone (Haimerl *et al.*, 2017). All buffaloes subjected to treatment were followed in subsequent estrous for the examination of uterine discharges for its color, consistency and pH revealed clear discharges with normal pH. These were provided rest of one estrous and then al-

lowed for artificial insemination (AI). Out of 17 buffaloes 9 (52.9%) conceived on first insemination and 6 (35.3%) conceived on subsequent insemination. One conceived on 3rd consecutive artificial insemination. This variation may be attributed to time of AI and managemental practices as some were maintained by individuals.

The post parturient period is very crucial for maintaining the reproductive efficiency of buffaloes. For optimization of milk production, a short calving to conception interval is important, which can be achieved by early uterine involution and the reestablishment of the ovarian function (Lof *et al.*, 2014; Dahiya *et al.*, 2018). The results indicated that use of single dose of Cloprostenol injection with antibiotics in Indian water buffalo affected with puerperal metritis helped in achieving clinical cure and uterine involution. Although, the present study was conducted on few numbers of buffaloes as pilot study, hence, more studies on a large number of animals are required to assure the usefulness of cloprostenol and antibiotic treatment of puerperal metritis in buffaloes.

## CONCLUSION

*Escherichia coli* and *Staphylococcus aureus* were the most common bacterial pathogen isolated from cases of puerperal metritis in the Indian water buffalo with prevalence of 66.7 and 44.4%, respectively. Intrauterine infusion of 2% povidone-iodine and systemic injection of cefoperazone and single use of cloprostenol positively affected a cure and uterine involution in buffaloes with puerperal metritis.

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## Competing Interests

The authors declare that they have no competing interests.

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