



## Probiotics Intervention for Mitigation of Uterine Infection in Dairy Animals -An Update

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### ABSTRACT

Probiotic bacteria are an important subject to explore when it comes to inhibiting infectious bacteria, also commercially available for its usage in a variety of sectors including animals and humans. Postpartum uterine infections including metritis and endometritis are common in buffaloes and cow a major problem dairy industry, as it is associated with reduced fertility and decreased milk yield. To address this issue, the present review discusses the role of probiotics in the treatment of infectious uterus and its implications in the reproductive efficiency of dairy animals. Probiotic administration in the host has proven to improve health status by competing for the nutrient utilization of infectious microbes. The antibiotic activity of probiotic bacteria is also known to reduce stress on the host. Application of the probiotic strains has been proposed as an alternative to prevent uterine infection and inflammation. Hence, this review article summarizes the effects of probiotics on metritis and endometritis in bovines. And the current studies on probiotics with their effects on the uterus include their immunomodulatory effects on the animal host.

### Introduction

India is a home to 192.5 million cattle and 109.9 million buffaloes. Dairy industry is the backbone of the Indian rural economy and contributes to the food and nutritional wellbeing of communities in India. Milk production in the country increased from 143.3 million tonnes in 2014-15 to 187.7 million tonnes during 2019-20 with 394 g per capita availability of milk. To keep the dairy industry sustainable,

it is necessary to have optimum reproduction. The production pressure, reduced metabolic turnover, and increased load of non-specific uterine/genital infections during postpartum are responsible for financial losses to the farmers. Postpartum metritis, clinical, and sub-clinical endometritis require immediate veterinary attention, as it causes production losses, increased open days and inter-calving period, and end up with increased odds of having animals culled from the herds.

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The uterus of postpartum cow is usually contaminated (different from infected) with a range of microorganisms like *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, *Prevotella* spp. etc (Sheldon et al., 2017), but this is not consistently associated with clinical disease. Many studies demonstrated that microbial communities and inflammatory responses differ between healthy and metritis cows during early postpartum. Infection during postpartum implies the adhesion of pathogenic organisms to the mucosa, colonization or penetration of the epithelium, and/or the release of bacterial toxins that lead to the establishment of uterine diseases. Depending on the immune response of the cows/buffaloes and the species and number (load or challenge) of the bacteria, a uterine disease will be develops. The development of uterine disease is a balance between the response of the host defence system, environmental microbial load, and virulence of the microbes. During the transition period, dairy cows undergo immunosuppression, which is associated with impaired leukocyte functions (Sheldon et al., 2006; LeBlanc, 2008). Several investigators have reported that although the phagocytic activity of neutrophils remains high, their bactericidal capacity is weakened, especially after parturition (Sheldon et al., 2014; Bradford et al., 2015). In addition, blood concentrations of IgG and IgM reach the lowest at calving which is suggestive of a decrease in the local bactericidal activity (Deng et al., 2015<sup>a</sup>). The state of immunosuppression in transition cows is associated with a high incidence of bacterial infections especially of the uterus (metritis) and mammary gland (mastitis), rendering cows more vulnerable to periparturient diseases. Acute metritis is inflammation of the uterus which occurs during 21 days after calving with greater prevalence.

During the first seven days in milk, symptoms do include a fever of more than 39.5° C, red-brown watery foul-smelling vaginal discharge, dullness, and anorexia (Sheldon et al., 2006). To address this issue, a number of research groups and professionals have tried various single and/or combination of broad-spectrum antibiotics. Several researchers attempted to solve the problem by developing antibiotic SOPs. However, when it comes to polymicrobial infections, these antibiotics or antibiotic protocols produce varying or limited results (67 to 77 %, Drillich et al., 2001). Further, this antibiotic approach also leads to anti-bacterial resistance and is a public health issue worldwide (Deng et al., 2015a, Deng et al., 2015b). Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial drug that was originally effective for the treatment of infections caused by it. Many alternatives of antimicrobials were explored including Ethno veterinary medicine medicines, immune modulators, hormone therapy or combinations. Recently probiotics isolated from the

reproductive tract were explored to evaluate their performance in non-specific infectious diseases of the uterus during the postpartum period.

## Probiotics

The term “probiotic” is derived from the Greek terms “pro” and “bios,” and it signifies “for life.” Mechnikov, who discovered that some bacteria may have a favourable influence on the gut flora, is thought to have proposed the first concept of probiotics in 1907. The term “probiotic” was most likely coined by Ferdin and Vergin (1995), who compared the negative effects of antibiotics and other antimicrobial agents on the gut microbiota with the good effects of chosen bacteria in the classic 1954 study (Vergin, 1954) entitled “Anti- und Probiotika” (probable probiotics). Over the period of time, the definition of probiotics was largely modified and the World Health Organization (WHO) defines probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” Similarly, in May 2002, the Food and Agriculture Organization (FAO) and WHO issued the guidelines for the evaluation of probiotics in food. The definition was endorsed by International Scientific Association for Probiotics and Prebiotics (ISAPP).

The use of probiotics is more prevalent for gut health and alimentary tract and mostly in humans. The use of probiotics in humans is much more defined and practiced than in veterinary. The use of probiotics in veterinary practice especially for gut health is developing but for reproductive health, it is still in infancy. During the early nineties, culturable methods were there to identify and explore new bacteria/probiotics, however, during the last two decades; scientists have started using culture-independent tools to identify microbiota. This approach could explore much about probiotic selection. Further many bioinformatics tools which could help in Human Microbiome Project (HMP) and integrative HMP (iHMP) funded by the National Institute of Health (NIH), have explored microbiome profiling of gut, vagina, oral, and skin communities. Both projects were planned to unravel microbes from above anatomical sites. HMP revealed that microbiota changes during the establishment of diseases could be a potential biomarker for the diagnostic purpose.

The human vaginal microbiome (Smith and Raval, 2017; Gajer et al., 2012) and buffalo and cow vaginal microbiome (Patel et al., 2018; Dhama et al., 2019; Mahesh et al., 2020; 2021; Gohil et al., 2021) confirmed that vaginal microbiota comprises a diverse array of beneficial microbes and opportunistic pathogens that inhibit vaginal milieu. Our reports suggest that cow or buffalo vag-

inal microbiota are much diverse than the human vagina (Mahesh et al., 2021; Gohil et al., 2021). For a long it was believed that the reproductive environment is sterile, however, a recent reports suggested the presence of bacteria during the estrous cycle and even during pregnancy (Patel et al., 2018; Dhama et al., 2019). It is well established that there are good and bad bacteria in the body (Seay et al., 2021).

An anatomical barrier like vulva, vagina, and cervix prevents environmental bacteria, however, windows like estrus and especially parturition allow environmental contamination and opportunistic bacteria to establish disease pathogenesis and change the reproductive milieu. This phenomenon causes subfertility/infertility in cows and buffaloes. Jeon and Galvão (2018) demonstrated that blood can transmit the infection to the reproductive tract during early postpartum. Further, it is also established that pathogenic bacteria alter ovarian growth, folliculogenesis, oogenesis, lower conception, and reduce reproductive efficiency. Firmicutes, Bacteroides, and Proteobacteria are the three common bacterial phyla present in the reproductive tract of the cattle and buffaloes (Rosales and Ametaj, 2021; Mahesh et al., 2021). The presence of uterine disease-causing genera *Trueperella* spp., *Acinetobacter* spp., *Fusobacteria* spp., *Proteus* spp., *Prevotella* spp., and *Peptostreptococcus* spp. was confirmed by Moore et al. (2017).

Uterine involution, uterine contractions, innate and adaptive immunity, and regeneration rate of the endometrium clear the pathogens during the first three to four weeks postpartum (Sheldon et al., 2017). Sheldon et al. (2017) also reported that 20-30% of early postpartum cows persist with non-specific postpartum infection. This pathogenic infection causes reproductive dysbiosis. It reflects on the vaginal and uterine microbial community. Multiple studies demonstrated its association with gynaecological diseases.

## Therapeutics

Many reviews and studies described the use of therapeutics like antimicrobial compounds, hormones, antibacterial, ethnoveterinary medicines, or its combinations with different efficiencies (Drillich et al., 2001; Rosales and Ametaj, 2021) to cure non-specific infections.

Antimicrobial use in non-specific uterine diseases is much more prevalent; however, there are several associated issues. It includes *in vitro* drug sensitivity test before antimicrobial usage, potential to be excreted through milk and milk withdrawal period. Anti-microbial resistance disrupts reproductive microbiota, increases production cost and affects economic consequences of the farm. Further

many researchers combined antimicrobial with hormonal therapy (Oxytocin, Estrogens, and PGF<sub>2</sub>α) and also tried to develop antibiotic SOPs with and without the combination of hormones to solve the problem. Considering polymicrobial infection, however, these antibiotics or antibiotic protocols have varying or limited results (Drillich et al., 2001). Although mixed responses were reported, other studies also tried unconventional therapies like the use of NSAIDs, granulocyte colony-stimulating factor, interleukin-8 (IL-8), etc. to modulate immune response during early postpartum. Hence, many groups including ours evaluated alternatives for antimicrobials.

## Probiotics and Reproductive Efficiency

The knowledge advancement in human microbiota has accelerated the pace of new ventures in live biotherapeutics using beneficial microorganisms. Infection of *Clostridioides difficile* was cured with fecal microbiota transplantation. Similarly, vaginal microbiota transplantation could be effective in treating problematic vaginal infections. Successful cure of vaginosis in women with probiotic treatment encouraged many research groups in the animal world also.

Wang et al. (2013) isolated LABs from the mucus of healthy multiparous dairy cows' vaginal tracts and delivered them intravaginally to transition dairy cows near calving. A total of 82 cows were randomly assigned to one of two experimental groups (41 cows per group). *Lactobacillus sakei*, *Pediococcus acidilactici* FUA 3140, and *Pediococcus acidilactici* FUA 3138 were given 1 mL of a mixture of three lactobacilli at 10<sup>10</sup>-10<sup>12</sup> CFU of LAB/wk/cow to the treatment group, whereas the control group received 1 mL of sterile skim milk. Six treatments were administered intra-vaginally at weeks 2 and 1 prior to parturition and at weeks 1 to 4 postpartum on a weekly basis. The findings of this study demonstrated for the first time in dairy cows that intra-vaginal probiotics could decrease the occurrence of purulent vaginal discharges (PVD; Ametaj et al., 2014), lower plasma haptoglobin, a marker of uterine infection, and increased milk yield from multiparous cows by 3 kg/d for the first 50 DIM.

Gartner et al. (2014) isolated and characterized *L. ruminis*, *L. buchneri*, and *L. amylovorus* from cow uterus. They found that isolated *Lactobacillus* spp. induces interleukin (IL) IL1A, IL6, IL8, and PGF2 in endometrial cells when compared to control cells. This study discovered that isolated *Lactobacillus* spp. has immunomodulatory properties.

Genís et al. (2017) randomly assigned 100 cows equally to three groups and, treated those either with carrier alone (sterile skim milk), where, two doses of LAB only prepar-

tum, one dose of the carrier (treatment 1), or two doses of LAB prepartum, and one dose of the carrier at calving (treatment 2) or two doses of LAB prepartum, and one dose of LAB after calving (treatment 3). The two treatment groups received the same LAB combination (consisting of *L. sakei* FUA 3089, *P. acidilactici* FUA 3138, and *P. acidilactici* FUA 3140 with a cell count of  $10^8$ - $10^9$  CFU/dose). As per the observations, cows given LAB had a decreased incidence of metritis and systemic lipopolysaccharide-binding protein (LBP), as well as lower systemic serum amyloid A (SAA) and increased vaginal mucus sIgA. Furthermore, data from the same study revealed that intra-vaginal LAB expedited uterine involution, as seen by smaller uterine horns and bodies in treated cows 14 days after delivery. Genís et al. (2017) confirmed that intra-vaginal LABs increased milk output and feed efficiency in treated cows. In the same study, LAB-treated cows had higher concentrations of IgG and lower concentrations of Hp in the colostrum (*i.e.*, 3 DIM) compared with cows that were left untreated. Interestingly, intra-vaginal LAB lowered concentrations of NEFA in the serum of the treated cows.

A high concentration of NEFA levels in the blood has been linked to a negative energy balance and fatty liver during calving (Ametaj et al., 2005). Surprisingly, the incidence of uterine infections was reduced by 50% in the organically managed Jersey herd. For the first 56 days after treatment, all dairy cows treated with intra-vaginal LAB produced 4.0 kg more milk per day than untreated cows. In a similar study, Genís et al. (2017) discovered that employing a mix of three commercially available LAB, intra-vaginal probiotics reduced the prevalence of uterine infections by 60%. In the same study, they investigated the impact of LAB in preventing *E. coli* infection of endometrial cells *in vitro*. In the presence of *Pediococcus acidilactici*, *E. coli* infection was reduced by 83 %, *Lactobacillus sakei* reduced endometrial cell infection by 87 %, and *Lactobacillus rhamnosus* and *Lactobacillus reuteri* reduced *E. coli* infection by 38 %, and 78 %, respectively.

Our group working on similar lines have isolated *Pediococcus pentosaceus* and *L. plantarum*, from the vagina of cows and buffaloes. All three strains were challenged for *in vitro* probiotic efficiency tests such as gas production from glucose, pH tolerance, growth at different temperatures, free from virulence genes, catalase-negative and oxidase tests, motility, indole producing, etc.

Above probiotic strains were then tested *in vivo* under field conditions. For this, 80 postpartum buffaloes ( $45 \pm 11$  DIM) with endometritis were randomly allocated to three groups. In Group 1 animals (control) 40 ml of normal saline was infused intra-vaginally as placebo, while in treatment Groups 2 and 3, 40 ml ( $1 \times 10^8$  CFU/ml)

of *Pediococcus pentosaceus* and *L. plantarum*, respectively, were offered. The treatment groups expressed estrus  $6 \pm 2$  days earlier than the placebo treatment. The treatment of probiotics in our study, which is in progress demonstrated a reduced service period.

## Conclusions

Postpartum non-specific uterine infection is common in dairy cows and buffaloes in India. Anti-microbial, disinfectants, hormones, ethnoveterinary medicines, or a combination of these compounds to cure non-specific uterine infection were reported with varying successes. Further antimicrobials cause antimicrobial drug resistance. Hence, the alternative therapeutic approach needs to explore. Probiotics are showing a promising alternative, however, more research is warranted in this field, especially route of administration, dose, consortia of probiotics, mechanism of action of probiotics, and interaction with non-specific uterine infections and archaea.

## Competing Interest

Authors declare none.

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