



Detection of *Escherichia coli* in Postpartum Clinical Endometritis of Dairy Cattle by PCR

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ABSTRACT

Background: *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Trueperella pyogenes* (*T. pyogenes*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Bacillus* spp. and *Fusobacterium necrophorum* (*F. necrophorum*) are the common bacterial agents isolated from postpartum endometritis in dairy cattle. The objective of the present study was to determine the association of *E. coli* with postpartum endometritis by polymerase chain reaction (PCR).

Methods: The present study was conducted from September 2019 to January 2020 at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, Kerala and organised dairy farms in Wayanad district, Kerala. Endometrial cytobrush samples (n = 36) were collected by cytobrush technique at 28 to 35 days postpartum (dpp). 16S rRNA gene was employed for the identification of *E. coli* by PCR.

Result: Upon evaluating endometrial cytological smear, 26 animals were positive for endometritis with more than 5% polymorphonuclear (PMN) cells. The prevalence of endometritis was 72.22 per cent (26/36). *E. coli* was detected in 12 samples by PCR. The study suggested PCR as a highly sensitive method for the identification of *E. coli* associated with endometritis in postpartum dairy cows.

Key words: Bovine clinical endometritis, *E. coli*, PCR, Post-partum.

INTRODUCTION

Postpartum uterus of more than 80% of dairy cattle during the first two weeks after calving will invariably be contaminated with bacteria (Sheldon *et al.*, 2008). Most of the cows eliminate these uterine pathogens during subsequent four to five weeks. Failure to eliminate this contamination leads to uterine infection (Sheldon *et al.*, 2006). These organisms *in-utero* cause histological changes to the endometrium, delays uterine involution, perturb pituitary LH secretion, alters ovulation and increases calving to first service interval and reduces the conception rate.

SE is the endometrial inflammation without any clinical signs. The presence of PMN cells is a good indicator of endometrial inflammation (Kasimanickam *et al.*, 2004). Diagnosis of SE was made based on the presence of PMN cell per cent in cytological smear and various authors proposed different threshold values (4 to 25%) for the diagnosis of SE (Kasimanickam *et al.*, 2004; Hammon *et al.*, 2006; Lopdell *et al.*, 2011; Lima *et al.*, 2014). Endometrial cytology is more accurate and reliable diagnostic method for the detection of SE. Various techniques *viz.* uterine lavage (Barlund *et al.*, 2008), cytobrush (Kasimanickam *et al.*, 2005; Dubuc *et al.*, 2010; Lee *et al.*, 2018) and cytotape technique (Pascottini *et al.*, 2015) are employed for the collection of endometrial samples. The prevalence of clinical endometritis (CE) varied from 15-20% (LeBlanc *et al.*, 2002; William *et al.*, 2005; Hammon *et al.*, 2006) and subclinical endometritis (SE) varies from 17-53% (Dourey *et al.*, 2011; Madoz *et al.*, 2014; Singh *et al.*, 2016).

Most common bacterial agents associated with postpartum endometritis are *E. coli*, *T. pyogenes*, *F. necrophorum*,

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P. melaninogenicus, *Staphylococcus aureus*, *Manheimia haemolytica*, *Pasturella multocida* and *K. pneumoniae*. Among them, *Escherichia coli* and *T. pyogenes* are considered as common pathogenic organisms associated with postpartum endometritis (Williams *et al.*, 2005 and Parmar, 2021). *E. coli* is a normal inhabitant of the gastrointestinal tract and plays a pivotal role in mammalian health as a commensal organism. However, the presence of *E. coli* *in-utero* during early postpartum period favours the growth of other bacteria and increases the severity of endometritis (Dohmen *et al.*, 2000; Williams *et al.*, 2005).

The diagnostic methods, at present, for the detection of endometritis is the nature, colour and consistency of discharge and isolation and identification of organisms by routine bacteriology and biochemical tests. Even though culture method is considered as gold standard for

identification of bacterial organism, they are laborious and time consuming. So, in recent years, PCR has proven as a culture-independent and sensitive technique for the identification microorganisms (Liu *et al.*, 2009; Bicalho *et al.*, 2012; Guo *et al.*, 2012) associated with uterine infection.

The objective of the present study was the identification of *E. coli* associated with endometritis in postpartum dairy cows.

MATERIALS AND METHODS

The study was carried out during September 2019 to January 2020 at Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Pookode, Kerala and Organised Dairy Farms in Wayanad District, Kerala. The endometrial cytobrush samples (n=36) were collected by cytobrush technique at 28 to 35 days postpartum (dpp). Animals with postpartum complications like RFM, metritis and those received antibiotic treatment within 20 days postpartum were excluded from the study.

Endometrial sample collection

Human cytobrush (steri UNO®) was modified to 5 cm length and heat fixed to previously sharpened stylet. The modified cytobrush assembly was introduced into AI sheath and covered with a sanitary plastic sleeve to prevent contamination from vagina. The cytobrush assembly was sterilised in laminar airflow cabinet for 15 min. Each cow was restrained properly and the perineal area was thoroughly cleaned with soap and water and wiped dry. Modified cytobrush assembly was introduced into the cranial vagina and sanitary sleeve was punctured at the level of external os and the assembly was introduced into the uterus. Once inside the uterus, the stylet was pushed forward to expose the cytobrush, which was gently rotated clockwise over the sides of uterine wall. After sample collection, the cytobrush was retracted into AI sheath within the uterus itself and withdrawn from the genital tract. Then the cytobrush was detached from the assembly,

transferred to a screw cap vial and transported to laboratory on ice.

Cytological smear was prepared by gentle rotation of the cytobrush over clean grease free slide and fixed with methanol (99 per cent) for 2 to 3 min. The slide was stained by modified Wright-Giemsa stain (Pascottini *et al.*, 2015) using standard staining procedure and were evaluated for the presence of PMN cells. The smears were observed under 400 x magnification of a light microscope (Magnus research microscope®, Magnus Opto Systems India Pvt. Ltd). A total of 100 cells were counted from each slide, including endometrial cells, PMN cells and lymphocytes. The samples with more than five per cent PMN cells were considered as positive for SE (Madoz *et al.*, 2014).

DNA extraction from cytobrush

In the laboratory, 2 ml of brain heart infusion broth was added to the screw cap vial containing cytobrush and incubated at 37°C for 6 hrs. Centrifuged at 10000 rpm for 10 min. Supernatant was discarded and the pellet was transferred to a 2 ml microtube. Bacterial DNA was extracted from endometrial cytobrush samples by Genomic DNA extraction mini kit (PureLink®, Thermo Fisher Scientific Corporation, USA) as per manufacturer's instructions.

Identification of *E.coli* by PCR

The 340bp 16SrRNA gene was employed for amplification of *E.coli* using 5' -GTT AAT ACC TTT GCT CAT TGA-3' and 5' -ACC AGG GTA TCT AAT CCT GTT-3' as forward and reverse primer, respectively (Aghamiri *et al.*, 2014).

Standard culture of *E.coli* was used to optimise the PCR assay. 25µl PCR reaction mixture was formulated (Table 1) and performed as per the protocol (Table 2).

Agarose gel electrophoresis

Agarose gel was prepared by measuring 0.5g agarose gel powder in a conical flask and dissolved in 40 ml Tris borate ethylene diamine tetra acetate buffer (TBE buffer, 1X). After cooling to 50°C, 0.50µg/ml concentration of ethidium bromide was added and cast on gel tray by fixing acrylic combs and allowed for solidification. The 10µl DNA samples were mixed with 2.5µl of 6X loading buffer and the first well was loaded with 5µl of 100 bp DNA ladder (Bangalore Genei, India). The electrophoresis was performed at 5V/cm of gel until the bromophenol blue gel progressed to more than two-third the length of the gel. The products were visualized in UV Transilluminator (Bio-rad Laboratories, USA) and documented in Gel-documentation system (Bio-rad Laboratories, USA).

Table 1: *E. coli* reaction mixture for detection of 16S rRNA gene.

Components	Quantity (µl)
Dream taq hot start PCR master mix (2X)	12.5
Forward primer (10pmol/ µl)	1.00
Reverse primer (10pmol/ µl)	1.00
Bacterial DNA	3.00
Nuclease free water	8.50
Total volume	25.00

Table 2: *E. coli* PCR program by using 16S rRNA gene.

Stage	Steps	Temperature (°C)	Duration	No. of cycles
1	Initial denaturation	95	10 min	
2	Denaturation	94	30 s	
	Annealing	59	30 s	30
3	Extension	72	1 min	
	Final extension	72	5 min	

RESULTS AND DISCUSSION

Twenty-six samples (26/36, 72.22%) were positive for postpartum SE with more than 5% PMN cell as threshold value. Dourey *et al.* (2011), Madoz *et al.* (2014) and Singh *et al.* (2016) reported a lower prevalence of SE than the present study. The mean \pm SE percent of PMN cell was 9.94 ± 1.75 . Different diagnostic techniques were proposed for the collection of endometrial sample (Kasimanickam *et al.*, 2004; Barlund *et al.*, 2008; Westermann *et al.*, 2010; Pascottini *et al.*, 2015). Singh *et al.* (2016) collected endometrial cytological samples for the diagnosis of SE in repeat breeder cow and observed that cytobrush techniques as more reliable cow side technique for diagnosis of SE. Madoz *et al.* (2014) proposed a global threshold value of five per cent PMN cells at 21 to 62 dpp for the diagnosis of SE in postpartum cows.

Bacterial DNA was directly isolated from the cytobrush samples by PCR (Sun *et al.*, 2011; Bicalho *et al.* 2012). Present study detected *E. coli* in 46.15% (12/26) of the samples positive for SE by PCR (Fig 1). Aghamiri *et al.* (2014) detected *E. coli* by employing 16S rRNA in 6.65% (4/61) cows at 28 to 35 dpp. *E. coli* and *T. pyogenes* are pre-dominant organisms associated with endometritis (Dohmen *et al.*, 2000). Presence of *E. coli* during early postpartum period made the endometrium susceptible for infection with other gram-negative bacteria in late postpartum period. As the postpartum period progress, the population of *E. coli* decreases while that of *T. pyogenes* increases (Williams *et al.*, 2007). Presence of *E. coli* favoured the growth of other bacterial organisms in late postpartum period by damaging endometrium. Presence of *E. coli* at 7 to 10 dpp were 3.7 times more likely to have postpartum metritis when

compared to healthy cows (Kassé *et al.*, 2016). Molecular characterisation of *E. coli* detected *fimH*, 16S rRNA, *hlyA*, *cdt*, *kpsMII*, *ibeA* and *astA* as virulent genes of *E. coli* associated with uterine infection and among them *fimH* was the most prevalent gene (Bicalho *et al.*, 2010). Hence, early diagnosis and timely treatment is necessary to combat uterine infections during initial dpp. PCR technique was sensitive, faster and culture-independent for the diagnosis of *E. coli* associated with postpartum endometritis in dairy cow.

CONCLUSION

E. coli had high degree of correlation with endometritis and 16S rRNA gene can be used for the detection of *E. coli* in endometritis of postpartum dairy cows. This technique is faster and culture-independent for the diagnosis of *E. coli*.

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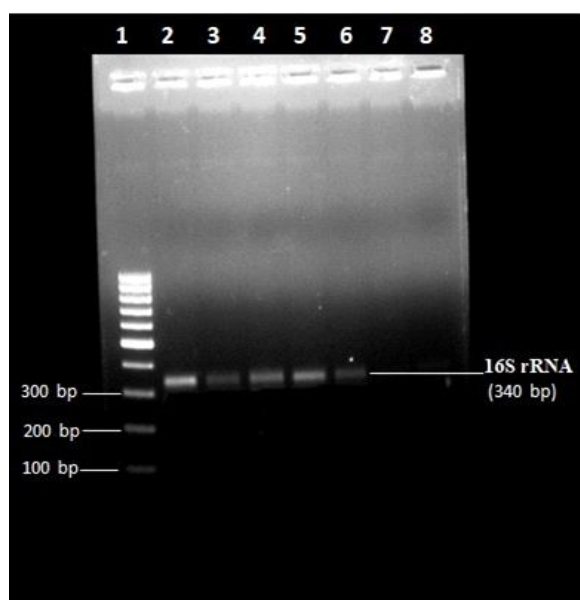


Fig 1: PCR product.

Lane 1 - 100 bp DNA ladder marker.
Lane 2, 3, 4, 5 and 6 - *E. coli* (16S rRNA) at 340bp.

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