



16S rRNA Sequence Indicates Presence of Unculturable Bacteria in Postpartum Uterus of Buffalo

Shabaz Hussain Dar¹, Salauddin Qureshi², Muthu Sankar³ Sanjeev Mehrotra¹, Manas K Patra¹, Sanjay K Singh¹, Harendra Kumar¹ and Narayanan Krishnaswamy

¹Division of Animal Reproduction, ²Division of Bacteriology and Mycology, ³Division of Bacteriology and Mycology
Indian Council of Agricultural Research (ICAR)-Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly 243 122 Uttar Pradesh, India

ABSTRACT

Bacterial contamination of the uterus is inevitable during the first three weeks of calving in the cow. The potential importance of unculturable bacteria in the establishment of postpartum endometritis is reported in the cow; however, existence of unculturable bacteria is not known in the postpartum uterus of the buffalo. Accordingly, sterile endometrial swab was collected in peptone water on day 20-21 post-calving from Murrah buffalo (n=12) without vaginal contamination and bacterial gDNA was isolated. Using gene specific primers, 16S rRNA was amplified by PCR. The purified PCR product was sequenced and was compared in Ribosomal database project (RDBP). Genetic distance was calculated using multiple alignments with ClustalW model in MegAlign module of Lasergene Package. The phylogenetic relationship was analyzed using the software MEGA 5.1 by neighbourhood joining method. The results revealed that invariably every sample contained uncharacterized bacteria (100%); however, no new species of was found. The clinical significance of uncharacterized bacteria in the establishment of postpartum endometritis needs to be determined in the buffalo.

Key words: metagenomic, 16S rRNA, bacteria, postpartum uterus, buffalo.

How to cite :- Dar, S. H., Qureshi, S., Sankar, M., Mehrotra, S., Patra, M. K., Singh, S. K., Kumar, H., & Krishnaswamy, N. (2023). 16S rRNA Sequence Indicates Presence of Unculturable Bacteria in Postpartum Uterus of Buffalo. *The Indian Journal of Animal Reproduction*, 44(2), 90–92. 10.48165/ijar.2023.44.02.17

INTRODUCTION

During the first three weeks of postpartum (PP), the uterus is infected because of the ascending bacteria through patent cervix. The physiological inflammatory process of uterine

involution clears the uterus off the infection and persistence of infection results in PP endometritis that is reported to be 20 % in the buffalo (Rao and Sreemannarayana, 1983). Classical bacteriological studies revealed that the composition of the flora, bacterial load, and their virulent factors

^{*}Corresponding author.

E-mail address: vetnanny@gmail.com (Narayanan Krishnaswamy)

Received 23-05-2023; Accepted 30-11-2023

Copyright @ Journal of Extension Systems (acspublisher.com/journals/index.php/ijar)

are dynamic till three-week PP in the cow (Olson et al., 1984; Bicalho et al., 2012) and influence the subsequent onset of subclinical endometritis and ovarian function (Williams, 2007). Molecular biological approaches such as terminal restriction length polymorphism (T-RFLP) (Elkjaera et al., 2013) and metagenomic pyrosequencing of 16S ribosomal RNA (16S rRNA) gene demonstrated that the early PP bovine uterus contains unculturable bacteria (Machado et al., 2012). 16S rRNA is a component of the 30S small subunit of prokaryotic ribosomes which are used in reconstructing phylogenies as the sequence is highly conserved (Patel, 2001). One of the most potential uses of 16S rRNA gene sequence informatics is to identify genus and species of isolates that do not fit into recognized biochemical profiles like *Sneathia sp* as demonstrated in the uterus of post-partum dairy cow (Machado et al., 2012). Using 16S rRNA, we report that the early PP buffalo uterus harbours unculturable bacteria.

MATERIALS AND METHODS

Source of Experimental material

Apparently normal postpartum Murrah buffaloes (n=12; parity; 1 – 3), maintained at the dairy farm, LPM section of IVRI were used for the study. Through calving register, the experimental buffaloes were identified and a sampling calendar was prepared to collect endometrial swab on 20-21dpp as described elsewhere (Dar et al., 2015) and brought to laboratory in peptone water. Bacterial gDNA was isolated (Geneaid™ DNA Isolation Kits) and the concentration as well as purity were assessed using Nano-Drop ND-1000 spectrophotometer at wave length 260 and 280

nm that ranged 1.8-2.0. As one sample was lost, the final data represents n=11.

PCR of bacterial 16S rRNA gene

Published primers were used in the study (Register and Yersin, 2005). The PCR mixture was prepared as per manufacturer's protocol. The cycling conditions include: 95°C for 3 min (initial denaturation) and 35 cycles at 95°C for 30 sec (denaturation), 53°C for 30 s (annealing), 72°C for 30 sec (extension) and 72°C for 10 min (final extension). The amplicons were resolved on 1% agarose and the product was sliced from the gel and purified (GeneJET Gel Extraction Kit, Thermo Scientific, USA). The PCR products were ligated with the vector pTZ-57R/T and transformed into *E. coli* cells. Recombinant clones, represented as white colonies, were lysed and used as templates for PCR amplification of bacterial 16S rRNA gene using specific primers. Following confirmation of the product, sequencing was done by outsourcing. Briefly, the samples were labelled and shipped for commercial sequencing (Xcelris genomics limited, Ahmedabad -380015 Gujarat). Sequences for 16S rRNA gene were analysed and compared in Ribosomal database project (RDBP). Genetic distance of the sequences generated in the present study was calculated using multiple alignments with ClustalW model in MegAlign module of Lasergene Package (DNASTAR, Inc, USA) using references sequences obtained from NCBI. Sequences were aligned using ClustalW software; the phylogenetic relationship was analyzed using the software MEGA 5.1 by neighbourhood joining method.

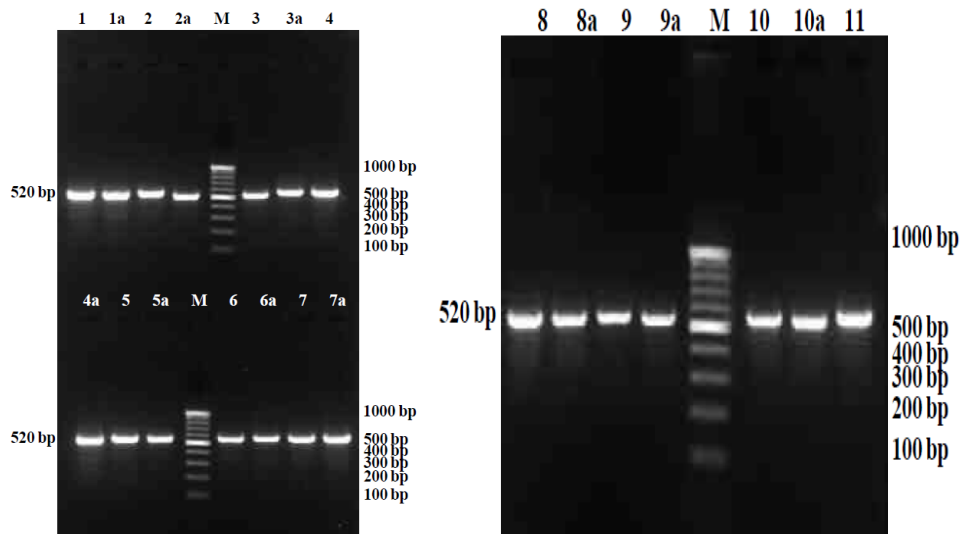


Fig. 1: Colony PCR of 16S rRNA gene (lane 1 – 11 positive)

RESULTS AND DISCUSSION

Colony PCR of transformed clones confirmed the presence of 16S rRNA (520 bp) in each sample (Figure 1) and the RDBP sequence results (n=11) is presented in Table 1.

Table 1: RDBP based 16S rRNA sequence analysis (n=11)

Bacteria (Family/Genus)	No. of positive	Percent
<i>Escherchia/Shigella</i>	7	64
<i>Bacillus</i>	2	18
<i>Streptococcus</i>	1	9
<i>Actinobacterium</i>	1	9
Uncharacterized bacteria	11	100

The predominant culturable bacterial genus present on day 20-21 PP was *Escherchia/Shigella* (64%), which is in accordance with the earlier reports in the cow (Sheldon and Dobson, 2004) and buffalo (Ajevar et al., 2014). Presence of *Bacillus sp* (18%), *Streptococcus sp* (9%), and *Truperalla sp* (9%) in the buffalo uterus is also reported (Onnureddy et al., 2013). The striking finding is that invariably every sample contained uncharacterized bacteria (100%), which is in conformity with the results in the PP cow (Machado et al., 2012). New species of bacteria were not reported in this study. Metagenomic analysis of the PP bovine uterus using 16S rRNA revealed that the *Gammaproteobacteria* was predominant in healthy cows while *Fusobacteria* was dominant in metritis cows (Santos et al., 2011). The teleological and biological significance of uncharacterized bacteria in the PP buffalo uterus need to be determined.

CONCLUSIONS

Analysis of 16s rRNA sequence revealed that the uterus of the buffalo contains uncharacterized bacteria by day 20-21 PP.

CONFLICT OF INTEREST

None

REFERENCES

- Ajevar, G., Muthu, S., Sarkar, M., Kumar, H., Das, G. K., and Krishnaswamy, N. (2014). Transcriptional profile of endometrial TLR4 and 5 genes during the estrous cycle and uterine infection in the buffalo (*Bubalus bubalis*). *Vet. Res. Commun.*, **38**(2): 171-176.
- Bicalho, M. L. S., Machado, V. S., Oikonomou, G., Gilbert, R. O. and Bicalho, R. C. (2012). Association between virulence factors of *Escherichia coli*, *Fusobacterium necrophorum*, and *Arcanobacterium pyogenes* and uterine diseases of dairy cows. *Vet. Microbiol.*, **157**(1): 125-131.
- Dar, S. H., Chaudhary, G. R., Mehrotra, S., Qureshi, S. D., Sankar, M., Jan, M. H. and Narayanan, K. (2015). Association of *Escherichia coli* and *Fusobacterium necrophorum* with subclinical endometritis in postpartum Murrah buffalo. *Indian J. Vet. Pathol.*, **39**(4): 311-315.
- Elkjæra, K., Anckera, M. L., Gustafsson, H., Friggens, N. C., Waldmann, A., Mølbak, L. and Callesen, H. (2013). Uterine bacterial flora in postpartum Danish Holstein dairy cows determined using DNA-based fingerprinting: Correlation to uterine condition and calving management. *Anim. Reprod. Sci.*, **138**(1-2): 39- 48
- Machado, V. S., Oikonomou, G., Bicalho, M. L. S., Knauer, W. A., Gilbert, R. and Bicalho, R. C. (2012). Investigation of postpartum dairy cows' uterine microbial diversity using metagenomic pyrosequencing of the 16S rRNA gene. *Vet. Microbiol.*, **159**(3-4): 460 - 469.
- Olson, J. D., Ball, L., Mortimer, R. G., Farin, P. W., Adney, W. S., and Huffman, E. M. (1984). Aspects of bacteriology and endocrinology of cows with pyometra and retained fetal membranes. *Am. J. Vet. Res.*, **45**(11): 2251-2255.
- Onnureddy, K., Vengalrao, Y., Mohanty, T. K., and Singh, D. (2013). Metagenomic Analysis of Uterine Microbiota in Postpartum Normal and Endometritic Water Buffaloes (*Bubalus bubalis*). *J Buffalo Sci.*, **2**(3): 124.
- Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Mol. Diagn.*, **6**: 313-321.
- Rao, A. V. N. and Sreemannarayana, O. (1983). Clinical analysis of reproductive failure among female buffaloes (*Bubalus bubalis*) under village management in Andhra Pradesh. *Theriogenology*, **18**(4): 403-411.
- Register, K., B. and Yersin, A., G. (2005). Analytical verification of a PCR for identification of *Bordetella avium*. *J. Clin. Microbiol.*, **43**(11): 5567-5573.
- Santos, T.M., Gilbert, R.O. and Bicalho, R.C. (2011). Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. *J. Dairy Sci.*, **94**(1): 291-302.
- Sheldon, I. M. and Dobson, H. (2004). Postpartum uterine health in cattle. *Anim Reprod Sci.*, **82**(7): 295-306.
- Williams, E. J., Fischer, D. P., Noakes, D. E., England, G. C., Rycroft, A., Dobson, H., and Sheldon, I. M. (2007). The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. *Theriogenology*, **68**(4): 549-559.