

## Molecular characterization of bovine intrauterine *Escherichia coli* isolates in rat model

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### ABSTRACT

The present study was undertaken to characterize the virulence factors of *Escherichia coli* isolated from bovine clinical endometritis, and comparison of uterine pathology induced by different phylogroups of *E. coli* in a rat model. Forty *E. coli* isolates assigned to different phylogroups, that is, *A*, *B1*, *C*, *D*, *E*, *F*, and *clad1* were used. Phenotypic detection using Congo red binding, biofilm and haemolysin production observed 77% (n=31), 55% (n=22) and 68% (n=28) positive isolates, respectively. Multiplex PCR detection of 11 VFs genes, viz; *Pap*, *Sfa*, *Afa*, *Hly*, *Cnf1*, *Cnf2*, *eaEA*, *F41*, *Sta*, *csgA*, and *csgD*, found only one positive isolate each for *pap* and *csgD* while 3 isolates for *F41* and 8 isolates for *csgA* were positive. Endometritis was induced in diestrus rats using 4 phylogroups of *E. coli*. Expression for *COX-2* and *iNOS* inflammatory marker genes was observed, but there was no statistical significant difference of expression among the phylogroups. On the basis of the present findings, it may inferred that different phylogroups of *E. coli* exhibit different virulence characteristics, but do not differ significantly in their ability to cause uterine disease in rats.

**Key words:** *E. coli*; phylogroups; Congo red; biofilm; endometritis, rat

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### Introduction

Postpartum uterine diseases, viz. endometritis, metritis, and pyometra, are a significant concern for dairy farmers for economic reasons. Endometritis in dairy cows is mainly classified into two categories, clinical and subclinical, and the clinical form is defined by the presence of

purulent or mucopurulent uterine discharges, detectable externally or in the anterior vagina, with the association of systemic signs of illness. The prevalence of endometritis is very common in India but varies considerably among studies, with a high prevalence rate of 38.54% to a low prevalence

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of 12% (RAMAN and BAWA, 1977; GAHLOT et al., 2017). Several uterine pathogens have been associated with endometritis, including *Escherichia coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, and *Prevotella species*, but *E. coli* is described as the main pathogen (ARORA et al., 2000). Pathogenic *E. coli* are classified into different pathotypes, viz. commensal, intestinal pathogenic, and extraintestinal. Intra uterine *E. coli* (IUEC) is newly described extraintestinal pathogen associated with endometritis in dairy animals, possessing the genes encoding virulence factors (VFs), such as *cdt*, *astA*, *ibeA*, *hlyA*, *hlyE*, *fyuA*, and *fimH* (BICALHO et al., 2010). The population structure of *E. coli* is predominantly clonal, and strains can be classified into one of eight phylogenetic groups: A, B1, B2, C, D, E, F, and cryptic clade I, depending on the presence of *chuA* (outer membrane hemin receptor gene), *yjaA* (uncharacterized protein) and *TspE4.C2* genes (anonymous DNA fragment) (CLERMONT et al., 2013). The relationship between the phylogenetic groups with a repertoire of the virulence profile for *E. coli* in endometritis remains unclear. Moreover, the differences in the pathogenic potentials of important phylogroups of *E. coli* in the development of endometritis, need to be investigated. The present study aimed to investigate the virulence determinants in different phylogroups of *E. coli* isolated from clinical cases of bovine endometritis, and to compare the pathogenic potential of different phylogroups in a rat model.

## Materials and methods

**Bacterial isolates.** *E. coli* bacterial isolates ( $n=40$ ) from dairy animals with a history of clinical endometritis were used. The isolates were checked for cultural, morphological and biochemical characters.

**Phenotypic detection of virulence determinants.** The isolates were tested for Congo red binding, haemolysin and biofilm production assays.

**Congo red binding.** The Congo red binding ability of isolates was analysed as per BERKHOFF and VINAL (1986). The isolates were streaked on Tryptic soy agar, supplemented with 0.03% Congo

red dye, and incubated at 37°C for 24-72 h. Positive reactions showed intensely orange or brick red colonies.

**Haemolysin assay.** The isolates were streaked on blood agar plates containing 5% sheep RBC. The plates were incubated at 37°C for 24 h and observed for a clear zone around the colonies for  $\beta$ -haemolysis.

**Biofilm formation.** The detection of biofilm production was performed by the microtiter plate method (O'TOOLE et al., 2000) using Luria-Bertani broth with M63 minimal media, and staining with 0.1% crystal violet. Quantification was done at 600 nm in a microplate reader (Thermo Fisher Scientific, USA). The isolates were categorized as mild, moderate and high biofilm producers, as per MATHUR et al. (2006).

**Multiplex-PCR for detection of virulence genes.** Multiplex-PCR was used to detect the virulence genes *Pap*, *Sfa*, *Afa*, *Hly*, *Cnf1*, *eaeA*, *F41*, *Sta*, *csgA*, *csgD* in all *E. coli* isolates. Bacterial DNA was isolated by the snap chill method (ALEXOPOULOU et al., 2006). 25  $\mu$ L of PCR reaction mixture was prepared by adding 2.5  $\mu$ L template DNA, 12.5  $\mu$ L dream taq master mix, 1  $\mu$ L of each primer (10 pmol), making up to the final volume with NFW. PCR amplification was carried out in Techne TC-500 thermo cycler (Bibby Scientific, United Kingdom). The primers used are given in Table 1.

Multiplex 1 was used to target *Pap*, *Sfa*, *Afa*, *Hly*, *Cnf1* genes, while multiplex 2 and multiplex 3 genes were used to target *eaeA*, *F41*, *Sta* and *csgA*, *csgD*, respectively. *Cnf2* was targeted by uniplex PCR. The thermal cycling conditions included initial denaturation at 94°C for 5 min, final extension at 72°C for 3 min, and hold at 4°C for all. For multiplex 1, we used 30 cycles of amplification with denaturation at 94°C for 2 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min; for multiplex 2, 25 cycles of amplification with denaturation at 94°C for 30 sec, annealing at 50°C for 45 sec, extension at 72°C for 40 sec; for multiplex 3, 30 cycles of amplification with denaturation at 94°C for 1 min, annealing at 63°C for 45 sec, and extension at 72°C for 3 min; and for

*Cnf2*, 25 cycles of amplification with denaturation at 94°C for 1 min, annealing at 52°C for 1 min, and extension at 72°C for 1 min. PCR products were analyzed in 1.5% agarose gel electrophoresis, as

described by SAMBROOK and RUSSEL (2001), and visualized under a UV Gel documentation system (Uvitec Ltd, UK).

Table 1: Primer sequences used for virulence gene detection of *E. coli* isolates

Gene	Primer Sequence	Amplicon Size	Reference
<i>Pap</i>	F5'-GCAACAGCAACGCTGGTTGCATCAT-3' R5'-AGAGAGAGCCACTCTTATACGGACA-3'	336	NEAMATI et al. (2015)
<i>Sfa</i>	F5'-CTCCGGAGAACTGGGTGCATCTTAC-3' R5'-CGGAGGAGTAATTACAAACCTGGCA-3'	410	TIBA et al. (2008)
<i>Afa</i>	F5'-GCTGGGCAGCAAACCTGATAACTCTC-3' R5'-CATCAAGCTGTTTGTTCGTCGCCCG-3'	150	TAJBAKHSI et al. (2016)
<i>Hly</i>	F5'-AACAAGGATAAGCACTGTTCTGGCT-3' R5'-ACCATATAAGCGGTCATTCCCGTCA-3'	1177	TIBA et al. (2008)
<i>Cnf1</i>	F5'-AAGATGGAGTTTCCTATGCAGGAG-3' R5'-CATTCAGAGTCCTGCCCTCATTATT-3'	498	YAMAMOTO et al. (1995)
<i>Cnf2</i>	F5'-ACTGAAGAAGAAGCGTGGAATA-3' R5'-ATAAGTTGAGCCGAGCGAGG-3'	654	KAIPAINEN et al. (2002)
<i>eae-A</i>	F5'-ATATCCGTTTTAATGGCTATCT-3' R5'-AATCTTCTGCGTACTGTGTTCA-3'	425	FRANCK et al. (1998)
<i>F41</i>	F5'-GCATCAGCGGCAGTATCT-3' R5'-GTCCCTAGCTCAGTATTATCACCT-3'	380	OK et al. (2009)
<i>Sta</i>	F5'-GCTAATGTTGGCAATTTTTATTTCTGTA-3' R5'-AGGATTACAACAAAGTTCACAGCAGTAA-3'	190	OK et al. (2009)
<i>csgA</i>	F5'-CAGCAATCGTATTCTCCGGTA-3' R5'-CGTTGTTACCAAAGCCCAACC-3'	408	SILVA et al. (2009)
<i>csgD</i>	F5'-TTATCGCCTGAGGTTATCGTTT-3' R5'-TAAATCTTCTTTCAGGCGACA-3'	601	SILVA et al. (2009)

*Endometritis and real-time expression study.* *E. coli* isolates belonging to four different phylogroups (AGRAWAL et al., 2021) were used.

*Laboratory animals and induction of endometritis.* The study was approved for using adult female wistar rats by the Committee for IAEC as per the guidelines of the Govt. of India. Rats

in diestrus phase were divided into five groups. Group 1 served as a negative control and Groups 2, 3, 4 and 5 as treatment groups. Xylazine @ 10 mg/kg body weight and ketamine @ 80 mg/kg body weight were used for anaesthesia. Treatment groups were inoculated with *E. coli* culture ( $1 \times 10^6$  CFU/mL) into the lumen of both uterine horns. All

the animals were humanely sacrificed on the 10<sup>th</sup> day post inoculation and uterine samples collected in RNAlater were stored at -80°C.

*cDNA synthesis.* cDNA synthesis was carried out using total RNA extracted from uterine samples by

a Trizol® reagent. A Revertaid® first strand cDNA synthesis kit was used following the manufacturer's instructions. The polymerase chain reaction was standardized for COX-2, GAPDH and iNOS genes using the gene specific primers given in Table 2.

Table 2: Primer sequences used to study expression of inflammatory genes

Gene	Primer Sequence	Amplicon Size	Reference
Cox-2 Fwd	5'AAAGCCTCGTCCAGATGCTA 3'	249 bp	BARBEIRO et al. (2016)
Cox-2 Rev	5'ATGCTGGCTGTCTTCGTAGG 3'		
iNOS Fwd	5'CCAACCTGCAGGTCTTCGATG3'	257 bp	McADAMet al. (2012)
iNOS Rev	5'GTCGATGCACAACGGGTGAAC3'		
GAPDH Fwd	5'AAGGCTGAGAACGGGAAACT 3'	101 bp	TURCHETTI et al. (2015)
GAPDH Rev	5'TACTCAGCACCAGCATCACC 3'		

*Real-time quantitative PCR (qPCR).* Real-time PCR was performed for quantification of *COX-2* and *iNOS* gene expression. 2 X SYBR Green master mix (Thermo Fischer Scientific, USA) was used in a CFX-96 Thermal Cycler (BioRad, USA). The 20 µL reaction mixture consisted of 10 µL SYBR Green master mix, 0.5 µL of each primer (10 pmol), 1 µL of cDNA, and NFW to make up the final volume. The reaction was carried at 95°C for 2 min, followed by 40 cycles of amplification with denaturation at 95°C for 15 sec, annealing at 56.6°C (COX-2), 62°C (iNOS), 60°C for GAPDH for 15 sec and extension at 72°C for 1 min each. For each gene, a dissociation curve was generated at temperatures from 60°C up to 95°C. The results were expressed as threshold cycle values ( $C_T$ ). The formula used to calculate the fold change in gene expression was “fold change =  $2^{-\Delta\Delta C_T}$ ” (where  $\Delta\Delta C_T = [(C_{T \text{ target gene}} - C_{T \text{ ref}}) \text{ treatment} - (C_{T \text{ target gene}} - C_{T \text{ ref}}) \text{ control}]$  as described previously by LIVAK and SCHMITTGEN (2001).

*Statistical analysis.* Statistical analysis was performed using Graph-Pad Prism Software (GraphPad Software, Inc, USA). One-way analysis of variance (ANOVA) was employed, followed by Tukey Post hoc analysis, to determine statistically significant differences between the mean values of each group. Values with  $P < 0.05$  were considered significant.

## Results

Out of 40 *E. coli* isolates confirmed for typical *E. coli* characteristics, 77% (n=31) were positive on Congo red assay, 68% (n=28) isolates were found to be β-haemolytic and 55% (n=22) were positive for biofilm production. Among all the *E. coli* isolates, 27.27% (n=6) were observed to be mild, while 36.36% (n=8) isolates each were moderate and high biofilm producers.

The phylogroup wise analysis of *E. coli* isolates for phenotypic virulent determinants is presented in Table 3.

Table 3. Phenotypic detection of virulence determinants in different phylogroups of *E. coli*

Test	<i>E. coli</i> Phylogroups							
	A	B1	C	D	E	F	CladI	Untypable (UT)
	(n=9)	(n=9)	(n=3)	(n=8)	(n=2)	(n=3)	(n=1)	(n=5)
Congo red binding	5(55.5%)	9(100%)	3(100%)	5(62.5%)	2(100%)	2(66.6%)	1(100%)	4(80%)
Haemolysin	6(66.6%)	8(88.8%)	2(66.6%)	3(37.5 %)	2(100%)	3(100%)	1(100%)	3(60%)
Biofilm	3(33.3%)	8(88.8%)	2(66.6%)	4(50 %)	1(50%)	2(66.6%)	1(100%)	1(20%)
Median score / isolate	1.5	2.7	2.3	1.5	2.5	2.3	3	1.6

Out of the 11 virulence factors (VFs) tested, only 4 genes were detected, viz. *Pap*, *F41*, *csgA*, and *csgD* (Fig. 1-3). The genes encoding curli fimbriae were present in 8 isolates. The *Pap* gene

was amplified in only one isolate. In the present study, PCR failed to detect genes coding for *Cnf1*, *Cnf2*, *Afa*, *eaeA*, *Stx*, *Sfa*, and *Hly* genes.

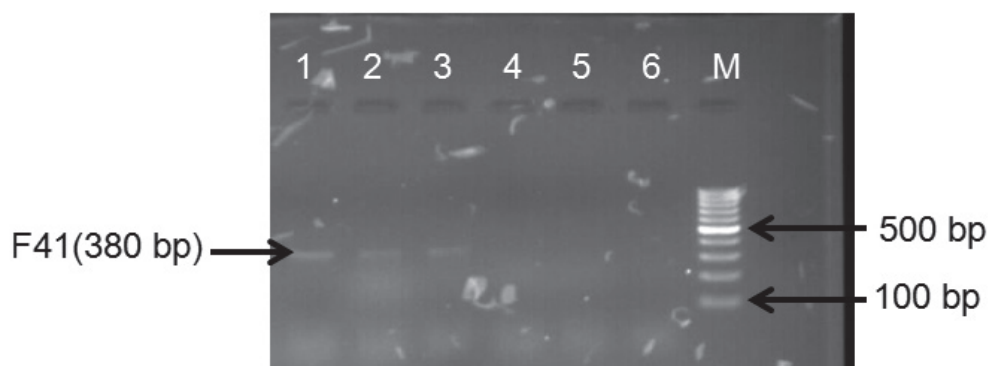


Fig. 1. Multiplex PCR for *eaeA*, *F41* and *Stx* genes  
Lane M: 100 bp DNA ladder; Lane 1-3: An amplicon of 380 bp for *F41* gene.

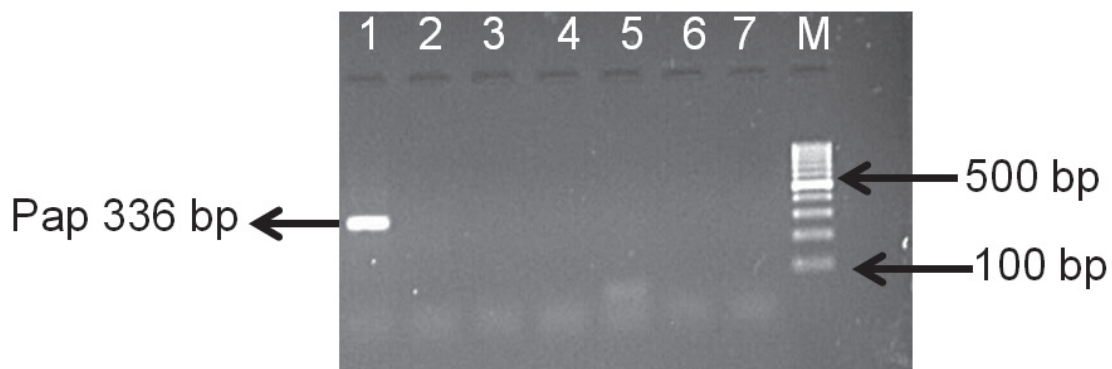


Fig. 2. Multiplex PCR for *Pap*, *Sfa*, *Afa*, *Hly* and *Cnf1* genes  
Lane M: 100 bp DNA ladder; Lane 1: An amplicon of 336 bp for the *Pap* gene.

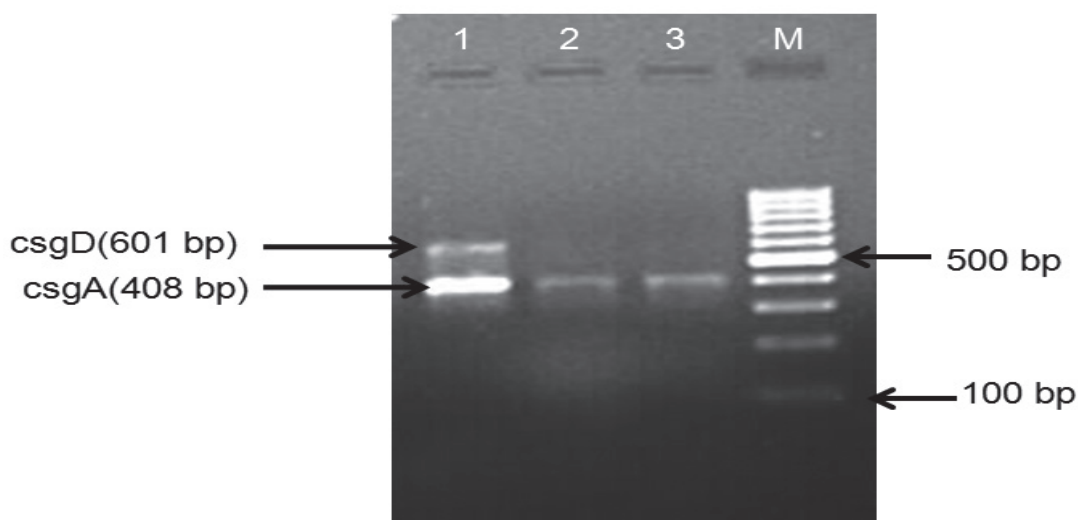


Fig. 3. Multiplex PCR for the *csgA* and *csgD* genes  
Lane M: 100 bp DNA ladder; Lane 1: An amplicon of 601 bp for the *csgD* gene;  
Lane 1-3: An amplicon of 408 bp for the *csgA* gene.

In the RT-qPCR analysis of *COX-2* and *iNOS*, there was up-regulated expression in either of the genes (Fig. 4-5). A fold change, ranging from 1.86 to 8.68 for *COX-2* and 9.41 to 1294.13 for

*iNOS* mRNA expressions, was observed. However, the differences were statistically non-significant between the experimental groups.



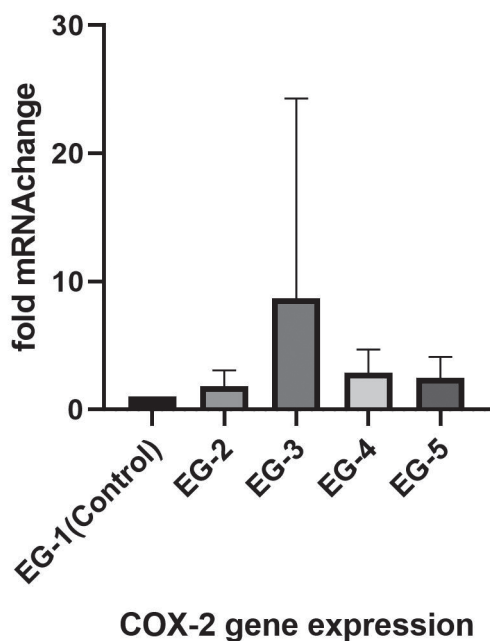


Fig. 4. Mean fold change of *COX-2* gene expression in different phylogroups of *E. coli*

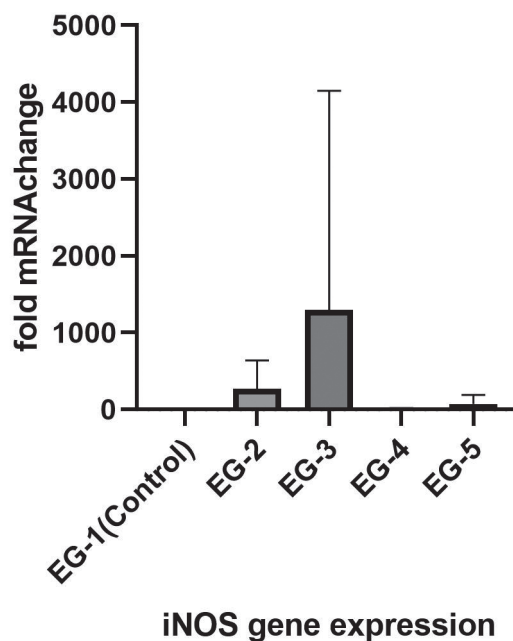


Fig. 5. Mean fold change of *iNOS* gene expression in different phylogroups of *E. coli*

## Discussion

CR has been used to select curli-producing bacteria. These curli fimbriae enhance bacterial adherence to mammalian host cells and mediate invasion of epithelial cells (WANG et al., 2006). Out of 40 *E. coli* isolates confirmed for typical *E. coli* characteristics, 77% (n=31) were positive on Congo red assay. RAHEEL et al. (2020) reported that 46.6% of the recovered *E. coli* uterine isolates were phenotypically positive on Congo red binding assay.

In the present study, 55% (n=22) of the *E. coli* isolates were positive for biofilm production, which was similar to an earlier study of 60% in repeat breeder cows (AHMADI et al., 2017). Further, biofilm producing isolates were characterized as mild, moderate and high biofilm producers, on the basis of the mean OD value observed in optical reading. Among all the *E. coli* isolates, 27.27% (n=6) were observed to be mild, while 36.36% (n=8) isolates each were moderate and high biofilm

producers. BAKHTIARI et al. (2018) reported moderate biofilm production in 53.3% isolates, while 16.6% of *E. coli* isolates were strong biofilm producers. In another study, 18.5 % of the strains had a strong capacity, while 40.7 % had a moderate to weak capacity (FERNANDES et al., 2011), and in another study 40% had a strong capacity, 12% had a moderate capacity, and 4% had a weak one (MILANOV et al., 2015). The ability of *E. coli* to form a biofilm may promote their persistence and tolerance to antimicrobial agents.

In present study, 68% (n=28) isolates were found to be  $\beta$ -haemolytic. This finding is similar to that described by RESENDE et al. (2007) who reported 69.8% isolates from cow vaginas produced hemolysin. Contrary to this study, most (90%) bovine *E. coli* strains isolated from the uterus showed non-hemolytic colony morphology. To identify bacterial genes that are important for establishing endometritis, 11 virulence genes were

examined in *E. coli* strains related to adhesion, invasion and blood hemolysis. Out of the 11 virulence factors (VFs) tested, only 4 genes were detected, viz. *Pap*, *F41*, *csgA*, and *csgD*. The genes encoding curli fimbriae were present in 8 isolates. The *Pap* gene was amplified in only one isolate. RESENDE et al. (2007) examined 71 *E. coli* isolates from cow's vaginal swabs and observed that none of the isolates were positive for the *Pap* gene. In the present study, PCR failed to detect genes coding for *Cnf1*, *Cnf2*, *Afa*, *eaeA*, *Sta*, *Sfa*, and *Hly* genes, and thus the endometritis isolates lacked the common pathogenicity genes associated with virulence of IUEC. However, their potential role in postpartum uterine infection cannot be overlooked due to the already broken epithelial barrier in the postpartum uterus, giving open access to bacteria into other endometrial cell components.

In RT-qPCR analysis of *COX-2* and *iNOS*, there was up-regulated expression in both the genes. A fold change ranging from 1.86 to 8.68 for *COX-2* and 9.41 to 1294.13 for *iNOS* mRNA expressions was observed. However, the differences were statistically non-significant between the experimental groups. *COX-2* is the primary isoenzyme involved in the endometrial production of prostaglandins and is responsible for the endometrial production of *PGE2* in the bovine endometrium during the estrous cycle (AROSH et al., 2002). JANA et al. (2009) observed up regulation of *COX-2* in cultured *E. coli* infected bovine endometrial tissue. Similarly, Nitric oxide (NO) is also an inflammatory mediator that causes smooth muscle relaxation and mediates cytoimmunity and inflammation toxicity. Inducible *iNOS* is responsible for a high, stable level of nitric oxide, and is expressed in response to pro-inflammatory factors.

### Conclusions

The present study found that intrauterine *E. coli* isolates from bovine clinical endometritis possess different phenotypic and genotypic virulent determinants which aids in the establishment of uterine diseases in dairy animals. It was also inferred that the different phylogroups of *E. coli* studied

may exhibit several virulence characteristics for the induction of endometritis in dairy animals, but do not differ significantly between the different phylogroups in their ability to cause uterine disease in the rat model.

### Conflict of Interest

The authors have no conflict of interest

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### References

- AGRAWAL, S., A. P. SINGH, R. SINGH, R. SAIKIA, S. CHOUDHURY, A. SHUKLA, S. N. PRABHU, J. AGRAWAL (2021): Molecular characterization of extended-spectrum-beta-lactamase-producing *Escherichia coli* isolated from postpartum uterine infection in dairy cattle in India. *Vet. World* 14, 200-209.  
DOI: 10.14202/vetworld.2021.200-209
- AHMADI, M. R., A. DERAKHSHANDEH, S. SHIRIAN, Y. DANESHOD, L. M. ANSARI, S. NAZIFI (2017): Detection of bacterial biofilm in uterine of repeat breeder dairy cows. *Asian Pac. J. Reprod.* 6, 136.
- ALEXOPOULOU, K., A. FOKA, E. PETINAKI, E. JELASTOPULU, G. DIMITRACOPOULOS, I. SPILIOPOULOU (2006): Comparison of two commercial methods with PCR restriction fragment length polymorphism of the *tuf* gene in the identification of coagulase-negative staphylococci. *Lett. Appl. Microbiol.* 43, 450-454.  
DOI: 10.1111/j.1472-765X.2006.01964.x
- ARORA, A. K., J. SINGH, G. R. PANGAONKAR, A. S. NANDA (2000): Bacteriological studies on genital tract in repeat breeder bovines. *Int. J. Animal Sci.* 15, 205-207.
- AROSH, J. A., J. PARENT, P. CHAPDELAIN, J. SIROIS, M. A. FORTIER (2002): Expression of Cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. *Biol. Reprod.* 67, 161-169.  
DOI: 10.1095/biolreprod67.1.161
- BAKHTIARI, M. N., S. GOORANINEZHAD, M. KARAMI (2018): Biofilm producing ability of bovine extraintestinal pathogenic *Escherichia coli* and its correlation with attachment factors. *Jundishapur J. Health Sci.* 10, 77130.
- BARBEIRO, D. F., M. K. KOIKE, A. M. M. COELHO, F. P. Da SILVA, M. C. C. MACHADO (2016): Intestinal barrier dysfunction and increased *COX-2* gene expression in the



- gut of elderly rats with acute pancreatitis. *Pancreatology* 16, 52-56.  
DOI:10.1016/j.pan.2015.10.012
- BERKHOF, H. A., A. C. VINAL (1986): Congo red medium to distinguish between invasive and non-invasive *Escherichia coli* pathogenic for poultry. *Avian Dis.* 30, 117-121.
- BICALHO, R. C., V. S. MACHADO, M. L. S. BICALHO, R. O. GILBERT, A. G. V. TEIXEIRA, L. S. CAIXETA, R. V. V. PEREIRA (2010): Molecular and epidemiological characterization of bovine intrauterine *Escherichia coli*. *J. Dairy Sci.* 93, 5818-5830.  
DOI: 10.3168/jds.2010-3550
- CLERMONT, O., J. K. CHRISTENSON, E. DENAMUR, D. M. GORDON (2013): The Clermont *Escherichia coli* phylo-typing method revisited improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Reprod.* 5, 58-65.  
DOI: 10.1111/1758-2229.12019
- FERNANDES, J. B. C., L. G. ZANARDO, N. N. GALVÃO, I. A. CARVALHO, L. A. NERO, M. A. S. MOREIRA (2011): *Escherichia coli* from clinical mastitis serotypes and virulence factors. *J. Vet. Diagn. Invest.* 23, 1146-1152.  
DOI: 10.1177/1040638711425581
- FRANCK, S. M., B. T. BOSWORTH, H. W. MOON (1998): Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin producing *Escherichia coli* strains from calves. *J. Clin. Microbiol.* 36, 1795-1797.  
DOI: 10.1128/JCM.36.6.1795-1797.1998
- GAHLOT, S. C., S. KUMAR, A. KUMARESAN, S. CHAND, R. K. BAITHALU, S. LATHIKA, T. K. PATBANDHA, S. S. LATHWAL, T. K. MOHANTY (2017): Efficiency of uterine fluid cytology in the diagnosis of subclinical endometritis in the water buffalo (*Bubalus bubalis*). *Reprod. Domest. Anim.* 52, 513-516.  
DOI: 10.1111/rda.12899
- JANA, B., A. KOZŁOWSKA, M. KOSZYKOWSKA, M. MAJEWSKI (2009): Expression of cyclo-oxygenase-2 in the inflammatory changed porcine uterus. *Pol. J. Vet. Sci.* 12, 1-8.
- KAIPAINEN, T., T. POHJANVIRTA, N. Y. SHPIGET, A. SHWINMER, S. PVORALA, S. PELKONEN (2002): Virulence factor of *Escherichia coli* isolated from bovine clinical mastitis. *Vet. Microbiol.* 85, 37-46.  
DOI: 10.1016/s0378-1135(01)00483-7
- LIVAK, K. J., T. D. SCHMITTGEN (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-ΔΔCt</sup> Method. *Methods* 25, 402-408.  
DOI: 10.1006/meth.2001.1262
- MATHUR, T., S. SINGHAL, S. KHAN, D. J. UPADHYAY, T. FATMA, A. RATTAN (2006): Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. *Indian J. Med. Microbiol.* 24, 25-29.  
DOI: 10.4103/0255-0857.19890
- McADAM, E., H. N. HABOUBI, G. FORRESTER (2012): Inducible nitric oxide synthase (iNOS) and nitric oxide (NO) are important mediators of reflux-induced cell signalling in esophageal cells. *Carcinogenesis* 33, 2035-2043.
- MILANOV, D., B. PRUNIĆ, M. VELHNER, D. TODOROVIĆ, V. POLAČEK (2015): Investigation of biofilm formation and phylogenetic typing of *Escherichia coli* strains isolated from milk of cows with mastitis. *Acta Vet.-Beograd* 65, 202-216.
- NEAMATI, F., F. FIROOZEH, M. SAFFARI, M. ZIBAEI (2015): Virulence genes and antimicrobial resistance pattern in uropathogenic *Escherichia coli* isolated from hospitalized patients in Kashan Iran Jundishapur. *J. Microbiol.* 8, e17514.  
DOI: 10.5812/jjm.17514
- OK, M., L. GULER, K. TURGUT, U. OK, I. SEN, I. K. GUNDUZ, M. F. BIRDANE, H. GUZELBEKTES (2009): The studies on the aetiology of diarrhoea in neonatal calves and determination of virulence gene markers of *Escherichia coli* strains by multiplex PCR. *Zoonoses Public Health* 56, 94-101.  
DOI: 10.1111/j.1863-2378.2008.01156.x
- O'TOOLE, G., H. B. KAPLAN, R. KOLTER (2000): Biofilm formation as microbial development. *Annu. Rev. Microbiol.* 54, 49-79.  
DOI: 10.1146/annurev.micro.54.1.49
- RAHEEL, I. A. E. R., W. H. HASSAN, S. S. R. SALEM, H. S. H. SALAM (2020): Biofilm forming potentiality of *Escherichia coli* isolated from bovine endometritis and their antibiotic resistance profiles. *J. Adv. Vet. Anim. Res.* 7, 442-451.  
DOI: 10.5455/javar.2020.g440
- RAMAN, S. R. P., S. J. S. BAWA (1977): Incidence of pre and post partum reproductive disorders in buffaloes. *Haryana Vet.* 16, 99-101.
- RESENDE, D., E. SANTO, C. MACEDO, J. M. MARIN (2007): Prevalence of virulence factors in *Escherichia coli* strains isolated from the genital tract of healthy cows. *Arq. Bras. Med. Vet. Zootec.* 59, 564-568.  
DOI:10.1590/S0102-09352007000300003
- SAMBROOK, J., D. RUSSELL (2001): *Molecular Cloning: A Laboratory Manual* 3rd ed. Cold Spring Harbor Laboratory Press.
- SILVA, E., S. LEITÃO, T. TENREIRO, C. POMBA, T. NUNES, L. B. L. da COSTA, L. MATEUS (2009): Genomic and phenotypic characterization of *Escherichia coli* isolates recovered from the uterus of puerperal dairy cows. *J. Dairy Sci.* 92, 6000-6010.  
DOI: 10.3168/jds.2009-2358

- TAJBAKSH, E., P. AHMADI, E. ABEDPOUR-DEHKORDI, N. ARBAB-SOLEIMANI, F. KHAMESIPOUR (2016): Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. *Antimicrob. Resist. Infect. Control* 5, 11.  
DOI: 10.1186/s13756-016-0109-4
- TIBA, M. R., T. YANO, D. DASILVALEITE (2008): Genotypic characterization of virulence factors in *Escherichia coli* strains from patients with cystitis. *Rev. Inst. Med. Trop. Sao Paulo*. 50, 255-260.  
DOI: 10.1590/s0036-46652008000500001
- TURCHETTI, A. P., L. F. COSTA, E. L. ROMÃO, R. T. FUJIWARA, T. A. PAIXÃO, R. L. SANTOS (2015): Transcription of innate immunity genes and cytokine secretion by canine macrophages resistant or susceptible to intracellular survival of *Leishmania infantum*. *Vet. Immunol. Immunopathol.* 163, 67-76.  
DOI: 10.1016/j.vetimm.2014.11.010
- WANG, X., M. ROCHON, A. LAMPROKOSTOPOULOU, H. LÜNSDORF, M. NIMTZ, U. RÖMLING (2006): Impact of biofilm matrix components on interaction of commensal *Escherichia coli* with the gastrointestinal cell line HT-29. *Cell. Mol. Life Sci.* 63, 2352-2363.  
DOI: 10.1007/s00018-006-6222-4
- YAMAMOTO, S., A. TERAJ, K. YURI, H. KURAZONO, Y. TAKEDA, O. YOSHIDA (1995): Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immunol. Med. Microbiol.* 12, 85-90.  
DOI: 10.1111/j.1574-695X.1995.tb00179.x

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**KEYANG, D., R. SINGH, A. P. SINGH, S. AGRAWAL, S. CHOUDHURY: Model štakora za molekularnu karakterizaciju bakterije *Escherichia coli* izoliranu iz uterusa goveda. *Vet. arhiv* 93, 581-590 2023.**

#### SAŽETAK

Ovo je istraživanje provedeno kako bi se opisali čimbenici virulencije bakterije *Escherichia coli* izolirane iz goveda s kliničkim endometritisom, te kako bi se u modelu štakora provela usporedba uterine patologije uzrokovane različitim filogenetskim skupinama *E. coli*. Upotrijebljeno je ukupno četrdeset izolata *E. coli* raspoređenih u različite filogenetske skupine: *A*, *B1*, *C*, *D*, *E*, *F*, i *clad1*. Fenotipskom detekcijom s pomoću Kongo bojila za crvenilo, uočeno je 77% (n=31) pozitivnih izolata, na temelju sposobnosti stvaranja biofilma uočeno je 55% (n=22) pozitivnih izolata, a na temelju proizvodnje hemolizina 68% (n=28) pozitivnih izolata. Primjenom Multiplex PCR-a za 11 VFs gena – *Pap*, *Sfa*, *Afa*, *Hly*, *Cnf1*, *Cnf2*, *eaeA*, *F41*, *Sta*, *csgA* i *csgD* – pronađen je po jedan izolat pozitivan na *Pap* i *csgD*, 3 izolata pozitivna na *F41* i 8 izolata pozitivnih na *csgA*. Upotrebom četiriju filogenetskih skupina bakterije *E. coli*, endometritis je induciran u štakora u fazi diestrusa. Uočena je ekspresija upalnih genskih markera za *COX-2* i *iNOS*, ali nije bilo statistički znakovite razlike među filogenetskim skupinama. Na temelju dosadašnjih nalaza može se zaključiti da različite filogenetske skupine *E. coli* pokazuju različite značajke virulencije, ali se ne razlikuju znakovito u svojoj sposobnosti da uzrokuju bolest maternice u štakora.

**Ključne riječi:** *E. coli*; filogenetske skupine; Kongo crvenilo; biofilm; endometritis; štakor

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