https://dergipark.org.tr/tr/pub/ataunivbd
2021; 16(1): 32-40
Research Article/Araştırma Makalesi
DOI: 10.17094/ataunivbd.845646

Atatürk Üniversitesi Veteriner Bilimleri Dergisi
Atatürk University Journal of Veterinary Sciences

Pathogenic Bacteria Present in the Lochia First 10−Day Postpartum Prolongs Days Open in Dairy Cows

Mehmet Cemal ADIGUZEL[1a], Seyda CENGIZ[1b]✉, Mehmet CENGIZ[2c], Armagan HAYIRLI[3d]

1. Atatürk University, Faculty of Veterinary Medicine, Department of Microbiology, Erzurum, TURKEY.
2. Atatürk University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Erzurum, TURKEY.
3. Atatürk University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disorders, Erzurum, TURKEY.

ORCID: ORCID: 0000-0002-2385-9649[a], ORCID: 0000-0002-1273-2941[b], ORCID: 0000-0001-9913-3468[c], ORCID: 0000-0002-4446-0848[d]

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
23.12.2020	19.02.2021	26.04.2021

Bu makaleye atıfta bulunmak için/To cite this article:
Adiguzel MC, Cengiz S, Cengiz M, Hayirli A: Pathogenic Bacteria Present in the Lochia First 10−Day Postpartum Prolongs Days Open in Dairy Cows. Atatürk University J. Vet. Sci., 16(1): 32-40, 2021. DOI: 10.17094/ataunivbd.845646

Abstract: The aims of this study were to evaluate the antibiotic resistance profiles and virulence genes of bacteria (*Escherichia coli*, *Trueperella pyogenes*, *Prevotella melaninogenica*, and *Fusobacterium necrophorum*) isolated from lochia during early postpartum and their effects on days open. The study was conducted on isolates, which were obtained from uterine discharges in the first 10 days of postpartum period, from 36 multiparous Simmental cows. The virulence genes of the isolates were investigated by polymerase chain reaction. The antimicrobial susceptibilities of the isolates were examined by the disc diffusion method. *T. pyogenes* (n = 97) and *E. coli* (n = 47) were revealed to carry the virulence gene (name of the gene), but not *F. necrophorum* (n = 16) and *P. melaninogenica* (n = 15). All isolates tested in this study were significantly sensitive to beta−lactam and tetracycline. *E. coli* and *T. pyogenes* were susceptible to aminoglycoside antibiotics, while *F. necrophorum* and *P. melaninogenica* were resistant to them. *E. coli* and *F. necrophorum* were susceptible to trimethoprim−sulfamethoxazole while *T. pyogenes* and *P. melaninogenica* were resistant. Those bacteria were predominant in cows with a prolonged days open (P<0.05). The uterine bacteria are responsible for prolonged days open and beta−lactam−derived antibiotics could be used as the first choice in the treatment of fresh cow uterine infections.

Keywords: Antimicrobial resistance, Bacteria, Cows, Days open, Uterus inflammation.

Doğum Sonrası İlk On Günde Loşyada Bulunan Patojen Bakteriler Süt Sığırlarında Boş Gün Süresini Uzatır

Öz: Bu çalışmanın amacı, postpartum erken dönemde loşyadan izole edilen bakterilerin (*Escherichia coli*, *Trueperella pyogenes*, *Prevotella melaninogenica* ve *Fusobacterium necrophorum*) antibiyotik direnç profili, virülans geni ve bunların ineklerde yeniden gebe kalma süresi üzerindeki etkilerini değerlendirmektir. Çalışma, birden fazla doğum yapmış 36 Simental inekten postpartum ilk 10 gündeki uterus akıntısından elde edilen izolatlar üzerinde gerçekleştirildi. Kültür ve biyokimyasal özellikleri belirlenen izolatların virülans genleri polimeraz zincir reaksiyonu ile incelendi. İzolatların antimikrobiyal duyarlılıkları disk difüzyon yöntemi ile saptandı. Çalışmadaki izole edilen *T. pyogenes* (n = 97) ve *E. coli*'nin (n = 47) virülans geni taşıdığı, ancak *F. necrophorum* (n = 16) ve *P. melaninogenica* (n = 15)'nın taşımadığı belirlendi. Tüm izolatlar beta − laktam antibiyotiklere ve tetrasikline karşı önemli derecede duyarlıydı. *E. coli ve T. pyogenes*, bu çalışmada aminoglikozid antibiyotiklere duyarlı iken *F. necrophorum* ve *P. melaninogenica* dirençliydi. *E. coli* ve *F. necrophorum* trimetoprim − sülfametoksazole duyarlı iken *T. pyogenes* ve *P. melaninogenica* dirençliydi. Ayrıca, *E. coli*, *T. pyogenes*, *P. melaninogenica* ve *F. necrophorum*'un yeniden gebe kalma süresi uzayan hayvanlarda öne çıkan bakteriler idi (P<0.05). Bu bulgular, uterus bakterilerinin yeniden gebe kalma süresinin uzamasına neden olduğunu ve uterus infeksiyonlarının tedavisinde ilk seçenek olarak beta − laktam türevi antibiyotiklerin kullanılabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Antimikrobiyal direnç, Bakteri, İnek, Uterus inflamasyonu, Yeniden gebe kalma süresi.

✉ Seyda Cengiz
Atatürk University, Faculty of Veterinary Medicine, Department of Microbiology, Erzurum, TURKEY.
e-mail: seydacengiz@atauni.edu.tr

INTRODUCTION

Inflammations of the uterus can occur as a result of negative pressure during and after normal-difficult parturitions or postpartum health problems (*e.g.*, retained placenta, uterine prolapse, and metabolic diseases). Improper technical aid may also cause uterine contamination during delivery, as well. The microorganisms that can reach the endometrium produce toxins by colonizing the epithelial layer of the uterus (1). Uterine infections divide into three groups: metritis, subclinical endometritis, and clinical endometritis. Metritis is characterized by an anomalously enlarged uterus, fever, fetid odor, and red−brownish or purulent vaginal discharge within 21 days after parturition (2). As the natural barriers of the cervix, vagina and vulva are accessed at the beginning of calving, the entry of environmental bacteria into the uterine lumen is facilitated. However, postpartum uterine infection does not occur in all cows (3,4). The risk factors for uterine infection could also contribute, which are related to the immune system, ration, and bacteria type as well as their virulence and load (1).

Although the reduction in the size of the uterus occurs in 20−25 days after parturition, involution of the whole uterus ends in 47−50 days. The uterine caruncles are usually detached by 12 days and an area of uterus decidua is shed by 8 days after parturition. Lochia is a uterine discharge, a shaped viscous flow and generally containing shed decidua, blood, exudate, fetal membrane, and liquid. Lochia discharges present different colors and textures during 13−18 days after parturition by myometrial contraction. The lochia contains blood and exudates that favor the growth of pathogenic bacteria in the uterus (5,6).

The uterus is colonized by more than thirty-five different bacterial species during parturition. *Escherichia coli* (*E. coli*), *Trueperella pyogenes* (*T. pyogenes*), *Prevotella melaninogenica* (*P. melaninogenica*), and *Fusobacterium necrophorum* (*F. necrophorum*) are the primary agents involved in the uterus infection (3-5). They are related to heavily purulence and odor in the vagina, leading to abolishment of dominant follicle growth and estradiol production as well as a decreased rate of conception rate (2). A study conducted in dairy cows demonstrated that six virulence genes (*fimH*, *astA*, *cdt*, *kpsMII*, *ibeA*, and *hlyA*) in *E. coli* were related to metritis and endometritis (7). Among these, the fimbrial subunit (*fimH*) is the most common one (8). Virulence factors such as pyolysin (*plo*), collagen−binding protein (*CbpA*), and *fimA* increase pathogenicity of the *T. pyogenes*. In another study involving Holstein dairy cows, it was investigated the relationship between virulence genes and metritis incidence (9). They detected *plo* gene in all *T. pyogenes* strains related to metritis, while one strain carried *cbpA* gene. The major virulence factor of *F. necrophorum* is leukotoxin gene (*lktA*) (10,11). The *phyA* gene encodes hemolysis factor of *P. melaninogenica* (12).

This study was conducted to evaluate the antibiotic resistance profiles and virulence genes of bacteria isolated from the lochia (*E. coli*, *T. pyogenes*, *P. melaninogenica*, and *F. necrophorum*) during early postpartum and their involvement in days open.

MATERIAL and METHODS

Animals

The study was performed between May 2016 and May 2017 at the breeding cattle farm, Erzurum, Turkey, upon the approval and permission of the Ethics Committee of Ataturk University Faculty of Veterinary Medicine, Experimental Animals Production and Research Center (Approval Number: 2017/91). The isolates were obtained from the uterine discharge (n=216) on days 0, 2, 4, 6, 8, and 10 postpartum, from 36 multiparous Simmental cows. No antibiotic treatment was applied before and during sampling. None of the cows had dystocia, puerperal metritis, and retained fetal membranes.

Samples and Isolation

Placental samples were collected on day 0. Lochial discharge samples were collected from the cranial part of the uterus under aseptic conditions on days 2, 4, 6, 8, and 10 (13). In brief, perineum area of the cow was disinfected with 70% ethanol. The plastic infusion pipette was manipulated through the cervix into the uterus, 40 ml sterile saline solution was administered inside, and then the liquid (5−15 ml) immediately aspirated again. All samples were put in the 50 ml sterile plastic tubes, labeled and immediately shipped in the icebox to the laboratory. Each sample was streaked onto two sheep blood Columbia agar (5% v/v) and MacConkey agar directly, then incubated microaerobically, anaerobically, and aerobically at 37°C for 24 hours. The Gram characteristics, hemolysis, and biochemical properties of the colonies were identified by using routine phenotypic identification biochemical pattern (4,14), then pure cultures on sheep blood Columbia agar were stored in glycerol stocks at −80°C until further use.

Detection of Virulence Genes by PCR

Genomic DNA was extracted from bacterial isolates using DNA extraction kit (PureLink™ Genomic DNA Mini Kit, Invitrogen, Cleveland, USA) according to the manufacturer's instructions. Each PCR amplification was performed in a volume of 25 µl containing 2.5 µl 10X PCR buffer, 1.5 mM $MgCl_2$, 2.5mM µl dNTP mix (2.5 mM each of deoxynucleotide triphosphate), 1 µl forward and reverse primer (10 pmol), 0.2 µl Taq polymerase (5 U/µl, Thermo Scientific, Cleveland, USA), 2 µl target DNA, and up of distilled water. The PCR conditions were 35 cycles of amplification at 94°C for 2 min, 94°C for 30s, 57−63°C for 30s, 72°C for 40s, and 5 minutes at 72°C (Table 1). The PCR products were separated in agarose gel electrophoresis for 30 minutes by using ethidium bromide. *E. coli* ATCC 25922 DNA was used as a quality control strain (7,15).

Table 1. PCR primers and reaction conditions.
Tablo 1. PCR'da kullanılan primerler ve reaksiyon koşulları.

Target genes		Primer sequence (5'–3')	Annealing (°C)	bp	References
T. pyogenes	*plo*	F − GGCCCGAATGTCACCGC R − AACTCCGCCTCTAGCGC	55	270	12
E. coli	*fimH*	F −TGCAGAACGGATAAGCCGTGG R −GCAGTCACCTGCCCTCCGGTA	63	508	21
F. necrophorum	*lktA*	F −AATCGGAGTAGTAGGTTCTG R −CTTTGGTAACTGCCACTGC	60	401	35
P. melaninogenica	*phyA*	F−CGTCATGAAGGAGATTGG R− ATAGAACCGTCAACGCTC	54	389	34

plo : pyolysin gene, *fimH* : fimbrial subunit, *lktA* : leukotoxin gene, *phyA* : hemolysis factor gene

Antimicrobial Assay

Antimicrobial susceptibilities test was performed on Mueller Hinton agar, including defibrinated sheep blood (5%) and Mueller Hinton agar without sheep blood by the disk diffusion method (16). The antimicrobial discs (Oxoid, Hampshire, UK) tested were amoxicillin clavulanic acid (30 µg), sulbactam ampicillin (20 µg), trimethoprim−sulfamethoxazole (25 µg), gentamicin (30 µg), streptomycin (10 µg), oxytetracycline (30 µg), tetracycline (30 µg), cephalothin (30µg), ceftazidime (30 µg), and cefoxitin (30 µg). *E. coli* ATCC 25922 was used as a quality control strain.

The strains tested in this study were recorded as resistant, intermediate, and susceptible according to the inhibition zone diameter (17). The plates for *E. coli* and *T. pyogenes* plates were incubated at 37°C for 24 hours and those for the anaerobic plates were incubated at 37°C for 24 hours after 96 hrs. Isolates resistant to three or more antimicrobial classes were defined as multidrug resistance (MDR) isolate (18).

Statistical Analysis

Days open is one of the most relevant determinants of reproductive success in dairy herds. Data were subjected to descriptive statistics. Bacterial load in lochia was an independent variable ascribing day open in regression approach using SAS (Statistical Analysis System) software (SAS Institute, Inc., Cary, North Carolina, USA). P values <0.05 were evaluated statistically significant for analyses.

RESULTS

In this study *E. coli* (n=47), *T. pyogenes* (n=97), *F. necrophorum* (n=16), and *P. melaninogenica* (n=15) were isolated from uterine discharges (n=216) on days 0, 2, 4, 6, 8, and 10 postpartum, from 36 multiparous Simmental cows. The prevalence of isolates and their effect on days open were summarized in Table 2. The total number of isolates in each sample increased as days open prolonged linearly (P<0.05) (Figure 1). Bacteriological analysis revealed 21.8% (47/216) *E. coli*, 44.9% (97/216) *T. pyogenes*, 7.4% (16/216) *F. necrophorum*, and 6.9% (15/216) *P. melaninogenica*. All *T. pyogenes* (100.0%) and *E. coli* (100.0%) strains carried virulence gene *plo* and *fimH*, respectively, while none of *P. melaninogenica* and *F. necrophorum* isolates carried them.

Table 2. Days open for cows according to the frequency of the isolates.
Tablo 2. İzolatların dağılımına göre ineklerdeki açık gün sayıları.

Sample ID	Days open	Sampling days and isolated strains					
		0	2	4	6	8	10
4	105	PM	–	TP+FN	TP	TP	TP
9	140	EC+PM	–	–	–	TP	TP+PM
15	102	–	TP	EC+TP	EC+TP	EC+TP	TP
20	87	–	FN	–	PM	TP+FN	FN
28	143	–	–	–	–	PM	PM
32	126	–	–	PM	TP+PM	TP+PM	PM
33	59	–	–	–	–	–	–
40	93	–	–	–	–	–	–
43	233	–	EC	EC	EC+TP	EC+TP	EC
46	50	–	–	–	–	–	–
48	95	–	–	–	–	–	–
49	348	–	TP	TP	TP	TP	TP
55	88	–	–	–	TP	TP	TP
56	55	–	TP+FN	TP+FN	TP+FN	TP+FN	TP
65	158	–	–	TP	TP	TP	TP
69	107	EC	EC	EC+TP	EC+TP	EC+TP	EC+TP
81	303	FN	EC+TP	EC+TP	EC+TP	EC+TP	EC+TP+PM
85	83	–	–	–	–	–	EC
516	183	–	–	FN	–	–	–
520	92	–	–	–	–	–	–
601	58	FN		PM		TP	–
602	96	–	–	–	–	–	–
608	57	–	–	TP	TP	TP+PM	TP+PM
609	58	–	TP+FN	EC+TP	TP	TP	TP
610	57	EC+TP	EC+TP	EC+TP	EC+TP	EC+TP	EC+TP
701	104	EC+FN	FN	EC+TP	TP	TP	–
702	118	EC	EC+TP	TP	EC+TP	EC+TP+FN	TP
703	59	–	EC+TP	EC+TP	EC+TP	EC+TP	TP
704	59	–	TP	TP	TP	TP	TP
705	65	–	EC+PM	EC+TP	TP	TP	TP
706	69	–	–	TP	TP	–	–
708	48	–	EC+TP	EC+TP	EC+TP	EC+TP	EC+TP
709	116	–	–	TP	TP	TP	TP
710	161	–	–	TP	TP	TP	TP
711	58	–	EC	–	EC+TP	–	–
712	58	FN	–	TP	–	–	–

EC: *E. coli*; TP: *T. pyogenes*, FN: *F. necrophorum*; PM: *P. melaninogenica*

Overall, all isolates were sensitive to β−lactam (amoxicillin clavulanic acid, cefoxitin, ceftazidime, cephalothin, sulbactam ampicillin) and tetracycline (oxytetracycline, tetracycline) antibiotics. *E. coli* and *T. pyogenes* were susceptible to aminoglycoside antibiotics (gentamicin, streptomycin) according to antimicrobial susceptibility test. However, all *F. necrophorum* and *P. melaninogenica*, which were isolated in the first 10 days of the postpartum period, were resistant to aminoglycoside antibiotics tested in this study (100.0%). *E. coli* and *F. necrophorum* were susceptible to trimethoprim−sulfamethoxazole 83.0% and 87.5%, respectively, whereas the phenotypic resistance for the same antibiotic was detected in *T. pyogenes* (91.8%) and *P. melaninogenica* (86.7%) isolates tested in this study (Table 3).

Table 3. Antimicrobial resistance rate of isolates.
Tablo 3. İzolatların antimikrobiyal dirençlerinin dağılımı.

Antibiotics	*T. pyogenes* (n=97) (%)			*E. coli* (n=47) (%)			*F. necrophorum* (n=16) (%)			*P. melaninogenica* (n=15) (%)		
	S	I	R	S	I	R	S	I	R	S	I	R
AMC	99.0	–	1.0	80.9	8.5	10.6	100.0	–	–	100.0	–	–
SAM	100.0	–	–	89.4	2.1	8.5	100.0	–	–	100.0	–	–
CF	96.9	–	3.1	42.6	12.8	44.7	100.0	–	–	93.3	–	6.7
CAZ	89.7	–	10.3	100.0	–	–	100.0	–	–	86.7	–	13.3
FOX	95.9	1.0	3.1	100.0	–	–	87.5	–	12.5	93.3	–	6.7
CN	64.9	13.4	21.6	87.2	6.4	6.4	87.5	–	12.5	–	–	100.0
S	85.6	1.0	13.4	40.4	4.3	55.3	–	–	100.0	–	–	100.0
SXT	8.2	–	91.8	83.0	–	17.0	87.5	–	12.5	13.3	–	86.7
TE	70.1	24.7	5.2	76.6	–	23.4	68.8	25.0	6.3	93.3	–	6.7
OT	75.3	22.7	2.1	76.6	–	23.4	68.8	25.0	6.3	93.3	–	6.7

AMC: Amoxicillin clavulanic acid, FOX: Cefoxitin, CAZ: Ceftazidime, CF: Cephalothin, CN: Gentamicin, OT: Oxytetracycline, S: Streptomycin, SAM: Sulbactam ampicillin, TE: Tetracycline, SXT: Trimethoprim−sulfamethoxazole, S: Susceptible, I: Intermediate, R: Resistance

DISCUSSION and CONCLUSION

Uterine infections (*e.g.*, endometritis, metritis, and pyometra) can result in prolonged postpartum re−conception and culling of the cow from the herd, as well as infertility (1). *E. coli*, *T. pyogenes*, *P. melaninogenica*, and *F. necrophorum* are the major bacteria associated with uterine infections (3,4,10). These bacteria determine the degree of the purulent or fetid materials in the uterus and inflammation (2,7). In addition, the presence of lipopolysaccharide and *E. coli* in lochia in the early postpartum period provides a suitable environment for the development of *T. pyogenes* and Gram−negative anaerobic bacteria in later phases (19). We isolated *E. coli* (21.8%), *T. pyogenes* (44.9%), *F. necrophorum* (7.4%), and *P. melaninogenica* (6.9%), which were the main targeted bacteria in this study. Similarly, the studies have also reported bacteria including *Staphylococcus aureus*, *Streptococcus* spp., *Bacillus* spp., and *Acinetobacter calcoaceticus* except those bacteria (20,21,22).

The characteristic color and odor of the uterine discharges were determined by the endometritis score, which were highly correlated with *T. pyogenes* in the uterus (4). A study in lactating Holstein cows showed that *T. pyogenes* was responsible for the likelihood of clinical endometritis and reproductive disorders, furthermore, prolonged days open in cows with purulent vaginal discharge and cytological endometritis (23). A higher rate of clinical endometritis was reported in *T. pyogenes* positive cows (24,25). In addition, researchers have detected that the probability of having mucopurulent or purulent vaginal discharge increases in the highly contaminated cows by *T. pyogenes* and anaerobic bacteria (26,27). Similarly, Piersanti et al. (28) observed that the cows with moderate or severe endometritis had higher persistence and prevalence of *T. pyogenes*, in another study, *T. pyogenes* was also the most frequently isolated bacterium from uterus biopsy (29). Despite a strong relationship between *T. pyogenes* and endometritis, in a study reported that only 41% of cows with purulent vaginal discharge were positive for *T. pyogenes* in the bacteriological culture (30).

α−hemolytic *Streptococcus* spp. was strongly associated with ~~a~~ lower reproductive performance compared to *T. pyogenes* (31). *E. coli*, *T. pyogenes*, *F. necrophorum*, and *Prevotella* spp. isolated on postpartum in the first seven days have been shown to affect estradiol concentration with the first and second dominant follicle growth (32). A positive correlation has been obtained between the time of *T. pyogenes* presence in the uterus, the days open and the calving interval in the first estrus (1,10). The results in this study ascertain an etiological role of *T. pyogenes* in endometritis and subsequent reproductive disorders.

The possibility of contamination with *F. necrophorum* in the first 10 days of the postpartum period was associated with *E. coli* strains to carry *fimH* gene (7). Another study on buffalo reported that *E. coli* and *T. pyogenes* were the most important causative agents of uterus infections during the postpartum period. However, the authors did not investigate the *fimH* gene (33). Researchers proved that there was a relationship between *E. coli* in the first two days of the postpartum period and intrauterine endotoxin, also between *T. pyogenes* in the first 14 days of the postpartum period and Gram−negative bacterial load (19). Moreover, they found that the metritis ratio at the first 8−10 days of lactation was lower in *E. coli* positive cows carrying the fimH gene compared to negative strains. Additionally, they reported that more than 90% of *E. coli* positive cows carrying the *fimH* gene on the first three days of lactation were infected with *F. necrophorum* in seven days, and the existence of *F. necrophorum* posed a crucial contamination factor for *T. pyogenes*. These findings confirm that Gram−negative anaerobic bacteria are affected by the appropriate environment created by the previous *E. coli* colonization in the uterine infection. In the present study, none of *F. necrophorum* and *P. melaninogenica* were detected to harbor *lktA* and *phyA* genes, which were major virulence factors for each of them based on a previous study (34). It may be related to non−invasive strains, which may account for the disparity detection frequency observed in this study.

From some samples in this study, more than one agent was isolated in various combinations at the same time. As reported in previous studies, (7,35) mixed isolates can cause an infection in a longer and more severe course of disease development. In many studies examining the physiological status of the uterus in the postpartum period, mild or subclinical metritis tends to heal spontaneously regardless of the causative agent in the weeks after calving (36,37). Therefore, it is thought that so many etiological factors of postpartum uterine disorders and can change in uterine microbial population during lactation in cows. Besides, these bacteria in the blood and feces as evidence of the transported of uterine pathogens through the blood from the intestine to the uterus (38).

In agreement with previous studies (4,21,35), the isolated bacteria were susceptible to β−lactam and tetracycline group of antibiotics. Sharma et al. (20) and Takamtha et al. (21) reported that their Gram−negative and Gram−positive bacterial isolates were highly susceptible to gentamicin. *E. coli* and *T. pyogenes* isolates were susceptible to gentamicin whereas *F. necrophorum* and *P. melaninogenica* were resistant (100%). This finding was considered to be due to the limited activity of gentamicin on anaerobic bacteria (27). On the other hand, it was thought that it was due to the limited and controlled use of antibiotics in the farm where the sampling was performed in order to determine the low antimicrobial resistance to β−lactam and tetracycline antibiotics, which are the first choice, especially in the treatment of uterine infections.

In summary, *E. coli* and *T. pyogenes* initiate uterus infection. *E. coli*, which colonizes endometrium after parturition, creates a conducive environment for anaerobic bacteria, including *F. necrophorum*. Predominance of these bacteria in the uterus within the first 10 days after parturition was associated with prolonged days open. They were sensitive to beta−lactam−derived antibiotics, which should be the first choice of antibiotics for treatment.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgement

This work was supported by the Scientific Research Projects Coordination Unit of Atatürk University [grant numbers THD−2017−6207 and TSG-2017-6412].

REFERENCES

1. Sheldon IM., Lewis GS., LeBlanc S., Gilbert RO., 2006. Defining postpartum uterine disease in cattle. Theriogenology, 65, 1516-1530.
2. McDougall S., Hussein H., Aberdein D., Buckle K., Roche J., Burke C., Mitchell M., Meier S., 2011. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. Theriogenology, 76, 229−240.
3. Pascottini OB., Van Schyndel SJ., Spricigo JW., Rousseau J., Weese JS., LeBlanc SJ., 2020. Dynamics of uterine microbiota in postpartum dairy cows with clinical or subclinical endometritis. Sci Rep, 10, 12353.
4. Pohl A., Lübke-Becker A., Heuwieser W., 2018. Minimum inhibitory concentrations of frequently used antibiotics against *Escherichia coli* and *Trueperella pyogenes* isolated from uteri of postpartum dairy cows. J Dairy Sci, 101, 1355-1364.
5. LeBlanc SJ., 2008. Postpartum uterine disease and dairy herd reproductive performance: A review. Vet J, 176, 102-114.
6. Sheldon IM., Price SB., Cronin J., Gilbert RO., Gadsby JE., 2009. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reprod Domest Anim, 44, 1−9.
7. de Cassia Bicudo L., Oba E., Bicudo SD., da Silva Leite D., Siqueira AK., de Souza Monobe MM., Nogueira M., de Figueiredo Pantoja JC., Listoni FJ., Ribeiro MG., 2019. Virulence factors and phylogenetic group profile of uterine *Escherichia coli* in early postpartum of high-producing dairy cows. Anim Prod Sci, 59, 1898-1905.
8. Moreno E., Planells I., Prats G., Planes, AM., Moreno G., Andreu A., 2005. Comparative study of *Escherichia coli* virulence determinants in strains causing urinary tract bacteremia versus strains causing pyelonephritis and other sources of bacteremia. Diagn Microbiol Infect Dis, 53, 93-99.
9. Santos TM., Gilbert RO., Bicalho RC., 2011. Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. J Dairy Sci, 94, 291-302.
10. Williams EJ., Fischer DP., Pfeiffer DU., England GC., Noakes DE., Dobson H., Sheldon IM., 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology, 63, 102-117.
11. Zhou H., Bennett G., Hickford JG., 2009. Variation in *Fusobacterium necrophorum* strains present on the hooves of footrot infected sheep, goats and cattle. Vet Microbiol, 135, 363-367.
12. Yoshida A., Tachibana M., Ansai T., Takehara T., 2005. Multiplex polymerase chain reaction assay for simultaneous detection of black−pigmented *Prevotella* species in oral specimens. Oral Microbiol Immun, 20, 43-46.
13. Gilbert RO., Shin ST., Guard CL., Erb HN., Frajblat

M., 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology, 64, 1879−1888.
14. Winn WC., Allen S., Hall GS., Janda W., 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Lippincott Williams & Wilkins, Baltimore.
15. Ertaş HB., Kılıç A., Özbey G., Muz A., 2005. Isolation of *Arcanobacterium* (*Actinomyces*) pyogenes from abscessed cattle kidney and identification by PCR. Turk J Vet Anim Sci, 29, 455-459.
16. Clinical & Laboratory Standards Institute 2006. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; proposed guideline. M45−P. CLSI, 6-7.
17. Clinical & Laboratory Standards Institute 2015. Performance standards for antimicrobial susceptibility testing; twenty−fifth informational supplement. M100−S25. CLSI, 35.
18. Adiguzel MC., Diren Sigirci B., Celik B., Kahraman BB., Metiner K., Ikiz S., Bagcigil AF., Ak S., Ozgur NY., 2018. Phenotypic and genotypic examination of antimicrobial resistance in thermophilic *Campylobacter* species isolated from poultry in Turkey. J Vet Res, 62, 463-468.
19. Dohmen MJ., Joop K., Sturk A., Bols, PEJ., Lohuis JACM., 2000. Relationship between intra−uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. Theriogenology, 54, 1019−1032.
20. Sharma A., Singh M., Kumar P., Sharma A., Kashyap A., Neelam IB., Sharma A., Chaudhary N., Sharma P., 2017. Bacterial isolation, culture sensitivity test, endometrial cytology of postpartum cows and assessment of their reproductive performance. Int J Curr Microbiol Appl Sci, 6, 519-527.
21. Takamtha A., Phanaratkitti V., Adirekkiet O., Panyapornwitaya V., Boonyayatra S., Kraeusukol K., 2013. Prevalence of isolated bacteria from clinical endometritis uterine and antimicrobial susceptibility in postpartum dairy cows. Chiang Mai V J, 11, 237-245.
22. Chen H., Fu K., Pang B., Wang J., Li H., Jiang Z., Feng Y., Tian W., Cao R., 2020. Determination of uterine bacterial community in postpartum dairy cows with metritis based on 16S rDNA sequencing. Vet Anim Sci, 20, 100102.
23. Bicalho ML., Lima FS., Machado VS., Bicalho MLS., Lima FS., Machado VS., Meira JREB., Ganda EK., Foditsch C., Bicalho RC., Gilbert R O., 2016. Associations among *Trueperella pyogenes*, endometritis diagnosis, and pregnancy outcomes in dairy cows. Theriogenology, 85, 267-274.
24. Brick TA., Schuenemann GM., Bas S., Daniels JB., Pinto CR., Rings DM., Rajala-Schultz PJ., 2012. Effect of intrauterine dextrose or antibiotic therapy on reproductive performance of lactating dairy cows diagnosed with clinical endometritis. J Dairy Sci, 95, 1894-1905.
25. Appiah MO., Wang J., Lu W., 2020. Microflora in the reproductive tract of cattle: A review (Running Title: The Microflora and Bovine Reproductive Tract). Agriculture, 10, 232.
26. Lima FS., 2020. Recent advances and future directions for uterine diseases diagnosis, pathogenesis, and management in dairy cows. Anim Reprod, 17, e20200063.
27. Papich MG., 2002. Saunders Handbook of Veterinary Drugs. Elsevier, North Carolina.
28. Piersanti RL., Zimpel R., Molinari PC., Dickson MJ., Ma Z., Jeong KC., Santos JE., Sheldon IM., Bromfield JJ., 2019. A model of clinical endometritis in Holstein heifers using pathogenic *Escherichia coli* and *Trueperella pyogenes*. J Dairy Sci, 102, 2686-2697.
29. Galvao KN., Bicalho RC., Jeon SJ., 2019. Symposium review: The uterine microbiome associated with the development of uterine disease in dairy cows. J Dairy Sci, 102, 11786-11797.

30. Galvao KN., Greco LF., Vilela JM., Filho MFS., Santos JEP., 2009. Effect of intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows. J Dairy Sci, 92, 1532-1542.
31. Sens A., Heuwieser W., 2013. Presence of *Escherichia coli*, *Trueperella pyogenes*, α−hemolytic streptococci, and coagulase−negative staphylococci and prevalence of subclinical endometritis. J Dairy Sci, 96, 6347-6354.
32. Sheldon IM., Noakes DE., Rycroft AN., Pfeiffer DU., Dobson H., 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction, 123, 837-845.
33. Azawi OI., 2008. Postpartum uterine infection in cattle. Anim Reprod Sci, 105, 187-208.
34. Staton GJ., Crosby−Durrani H., Roberts G., Gilbert RO., Gadsby JE., 2020. Novel ulcerative leg lesions in yearling lambs: Clinical features, microbiology and histopathology. Vet Microbiol, 247, 108790.
35. Brodzki P., Bochniarz M., Brodzki A., Wrona Z., Wawron W., 2014. *Trueperella pyogenes* and *Escherichia coli* as an etiological factor of endometritis in cows and the susceptibility of these bacteria to selected antibiotics. Pol J Vet Sci, 17, 657-664.
36. Gautam G., Nakao T., Koike K., Long ST., Yusuf M., Ranasinghe RMSBK., Hayashi A., 2010. Spontaneous recovery or persistence of postpartum endometritis and risk factors for its persistence in Holstein cows. Theriogenology, 73, 168-179.
37. Green MP., Ledgard AM., Beaumont SE., Berg MC., McNatty KP., Peterson AJ., Back PJ., 2011. Long−term alteration of follicular steroid concentrations in relation to subclinical endometritis in postpartum dairy cows. Anim Sci J, 89, 3551-3560.
38. Jeon SJ., Cunha F., Vieira−Neto A., Bicalho RC., Lima S., Bicalho ML., Galvao KN., 2017. Blood as a route of transmission of uterine pathogens from the gut to the uterus in cows. Microbiome, 5, 109.