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RELATIONSHIP BETWEEN THE TYPES OF BACTERIA ISOLATED FROM POSTPARTUM UTERI AND THE CELL-MEDIATED IMMUNITY IN DAIRY COWS

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ABSTRACT

The main aim of the present study was to evaluate the relationship between the isolated bacteria from the uterus and the immune status of dairy cows during the postpartum period.

Uterine swabs were collected from 41 Holstein-Friesian cows with normal calving and 5 cows with retained fetal membranes (RFM) at 7-11 days, 17-25 days and 27-38 days postpartum. After swabbing, blood samples were collected from tail veins of 34 cows at 7-11 days, 30 cows at 17-25 days and 29 at 27-38 days postpartum for carrying

out lymphocyte stimulation test. The collected swabs were cultured and the isolated bacteria were categorized according their expected pathogenic potential within the uterus into: pathogenic, less pathogenic and non-pathogenic bacteria.

*During the examined postpartum periods, a total of 108 uterine swabs were collected from 46 cows. With the exception of a swab from a cow with normal calving at 17-25 days postpartum, all the uterine swabs were bacteriologically positive. One hundred-sixty five and 23 isolates were recovered from cows with normal calving and cows with RFM, respectively during the examined postpartum periods. The isolated less pathogenic bacteria were Enterococcus spp. and Histophilus somni, pathogenic were Trueperella (T.) (Arcanobacterium) pyogenes and Escherichia (E.) coli and non pathogenic were Bacillus subtilis, Corynebacterium spp., Pseudomonas (P.) aeruginosa, Proteus spp., Bacillus spp.**,** Micrococcus spp. and Streptococcus spp.*

Regarding the results of lymphocytes stimulation test, 57% of cows were healthy and 37.6% were healthy with lower immunosuppression. On the other hand, low percentage of cows (5.4%) was immunosuppressed. Of 159 bacterial isolates, 90 (57%) were isolated from healthy cows, 62 (39%) from lower immunosuppressed cows and 7 (4 %) from immunosuppressed. Of 46 pathogenic bacterial isolates, 28 (61%) were isolated from healthy cows and 18 (39%) from lower immunosuppressed cows. Of 89 less pathogenic bacterial isolates, 49 (55 %) were isolated from immunohealthy cows, 34 (38 %) from lower immunosuppressed and 6 (7%) from immunosuppressed. Of 24 non pathogenic isolates, 13 (54%) were isolated from healthy cows, 10 (42%) from lower immunosuppressed and 1 (4%) from immunosuppressed. In conclusion, the presence of pathogenic and less pathogenic bacteria might stimulate the cellular immune response in dairy cows during the postpartum period.

INTRODUCTION

The postpartum period in cattle is characterized by an increased risk of uterine infection due to persistence of cervical dilation for several days **(Sheldon, 2004)**. After parturition aerobic and anaerobic bacteria, including *Escherichia* (*E.) coli, Trueperella* (*T.*) (*Arcanobacterium) pyogenes, Pseudomonas (P.) aeruginosa, Pasteurella multocida, Staphylococcus aureus, Streptococcus uberis, Clostridium spp., Prevotella spp.* and *Fusobacterium spp*. invade the uterus ***(Griffin et al., 1974; Olson et al., 1984; Bretzlaff, 1987 and Noakes et al., 1991)***. However, *E. coli, T. pyogenes, Fusobacterium necrophorum* and *Prevotella melaninogenicus* are commonly associated with uterine disease ***(Sheldon et al., 2002)***. The outcomes of bacterial infection of postpartum uterus are clinical metritis, clinical endometritis, pyometra and subclinical endometritis ***(Sheldon et al., 2006)***. These diseases may delay the complete regeneration of the endometrium and disturb the resumption of cyclic ovarian function resulting in postponement of the first insemination, increasing the number of inseminations per conception and thus prolonging the calving interval and decreasing the calving rate ***(Hussain and Daniel, 1991)***. The frequency of isolation of each bacterium species from the postpartum uterus of cows is variable among studies and postpartum days and among cows with normal or abnormal calving ***(Griffin et al., 1974, Hussien et al., 1990, Takács et al, 1990, Noakes et al., 1991, Huszenicza et al., 1999, Dohmen et al., 2000 and Kocamuftuoglu and Vural, 2008)***.

Although immune responses progressively eliminate the microbes, up to 40% of the cows have bacterial infection, 3 weeks after calving ***(Sheldon et al., 2008)***. The cellular immune response has a predominant role in the uterine defense mechanism against infection compared to the limited humeral immune response ***(Mestecky et al., 2005)***. Lymphocytes and antigen-presenting macrophages play an important role in the recognition and processing of foreign antigen, including pathogenic bacteria which invade the uterus ***(Leung et al., 2000).*** Measurement of blood lymphocytic proliferation has been used to indicate the functional status of the immune system ***(Mallard et al., 1998). Ramadan et al. (1997)*** confirmed that intrauterine inoculation of pathogenic bacteria (*E. coli* and *A. pyogenes*) resulted in an increase in the uterine defense mechanism, regardless of the stage of the estrous cycle when it has been introduced. On the other hand, it has been reported that none of ewes that received intrauterine infusions of *T. pyogenes* and *E. coli* during estrus developed uterine infections, but all of the ewes that received *T. pyogenes* and *E. coli* infusions during the luteal phase of the estrous cycle developed uterine infections ***(Seals et al., 2003)***.

The aims of the present study were to isolate and identify the bacteria from uteri of cows after normal calving and in cows with retained fetal membranes, evaluating the antibacterial susceptibility of the isolated bacteria and the relationship between the isolated bacteria from the uterus and the immune status of the body during the postpartum period of dairy cows.

MATERIAL AND METHODS

2.1. Animals:

Forty-six pluriparous Holstein-Friesian cows belonging to a dairy farm (Agroproduct Company, Pàpa, Hungary) were used in the present study during the period from February to July 2010. Forty-one cows had calved normally and five cows had suffered from retained fetal membranes. Fetal membranes were considered retained if they were not expelled within 24 hours of calving ***(Kelton et al., 1998).*** The cows were examined during the period between 7-11 days, 17-25 days and 27-38 days postpartum. Some cows (n=17) were missed at the sampling periods of 17-25 and 27-38 days postpartum. After swabbing, a clean lubricated gloved hand was inserted through the vulva of each cow into the vagina and withdraw the mucus content of the vagina for examination the odor and the character of this mucus to ensure that the cow has normal postpartum or suffer from postpartum disorders.

2.2. Sample collection:

2.2.1. *Uterine swab samples*:

After the vulva of the animal had been cleaned using dry paper towel, a transcervical guarded swab (Equi.Vet®, Catalog #: 290955, Denmark) was collected from the uterine body of each cow during the period of 7-11 days, 17-25 days and 27-38 days postpartum as described by ***Noakes et al. (1989)***. By manipulation of the cervix via the rectum, the instrument was advanced into the body of the uterus. The sterile swab

was then pushed out of its protective sheath and pressed against the mucosa of one of the uterine horns. The swab was drawn back into its protective sheath and pulled out of the genital tract. The swab was transferred to a bijou bottle containing ***Stuart's (1959)*** transport medium (Biolab ZRt, Hungary).

2.2.2. *Blood samples*:

After uterine swabbing, blood samples (5 ml) were withdrawn from the tail vein of 34 cows at 7-11 days, 30 cows at 17-25 days and 29 at 27-38 days postpartum into heparinised vacutainer tubes (S-Monovette®, Sarstedt AG & Co., D-51588 Numbrecht, Germany) for carrying out lymphocyte stimulation test.

2.3. Isolation and identification of the bacteria:

The swabs were cultured within 3 hours after collection aerobically at 37°C for 24-48 hours on sheep blood agar (agar base, Biolab ZRt) and MacConky agar media (Biolab ZRt). The cultured plates were then examined for bacterial growth. Different colonies were picked up and purified by subculturing on blood agar ***(Quinn et al., 2000)***. The suspected bacteria were identified by standard procedures according to ***Quinn et al. (2000)*.** The isolated bacteria were categorized according their expected pathogenic potential within the uterus into: a) pathogenic bacteria that frequently cause endometritis, b) less pathogenic bacteria that rarely cause endometritis and c) non-pathogenic bacteria, which are not recognized as uterine pathogen ***(Sheldon et al., 2004b and Jadon et al., 2005)*** (Table 1).

Table (1): Categorization of the isolated bacteria based on their potential pathogenicity ***(Sheldon et al., 2004b and Jadon et al., 2005)***

Bacterial categories		
Pathogenic bacteria	**Less pathogenic**	**Non-pathogenic**
T. pyogenes	*Enterococcus* spp.	*Corynebacterium* spp., *Micrococcus* spp.
E. coli	*Histophilus (H.) somni*	*Proteus* spp. *Streptococcus* spp., *Pseudomonas (P.) aeruginosa*, *Bacillus* spp., *Bacillus (B.) cereus*, *B. subtilis*

2.4. Immunological test (lymphocyte stimulation assay):

The cellular immunity was monitored by lymphocyte stimulation assay ***(Iwata and Inoue, 1993)***. Peripheral blood lymphocytes (PBL) were isolated by density gradient centrifugation (400x *g* for 15 minutes) using Ficoll-Paque (Pharmacia) according to standard protocols. The reactivity of the cells was tested by lymphocyte blastogenesis with PHA and Con A mitogens. The number of viable PBLs was determined by trypan blue exclusion in a haemocytometer. The cells were diluted in DMEM supplemented with antibiotics and 10% fetal bovine serum. Cells were plated at $1x10^5$ cells/well density into 96 well plates, 4 wells (100µl each) for each mitogen. The cultures were incubated for 4 days at 37ºC under 5% CO2 tension. Blastogenesis was measured by a colorimetric assay ***(Hussain et al., 1993)*** using MTT as a reagent. Twenty µl of MTT (5 mg/ml) was added to each well and incubated for 4 hours. The microtiter plates were centrifuged (1400x *g*, 10 minutes at room temperature) and the untransformed MTT was removed. The optical density was measured by microplate photometer at 570 nm and 630 nm after dissolving the crystalline formazan product with 100 µl of DMSO

mixed with 0.01 N HCl. Absorbance of the product at 630 nm was subtracted from the absorbance at 570 nm to calculate total dye conversion. The OD value of a negative control sample was set at 0 and a positive control (EL-4 continous mouse T lymphocyte cell line) OD was 100. Results of stimulation of samples expressed as the percentage of the positive control OD. Based on lymphocytes stimulation test, the animals were classified into immunohealthy, healthy with lower immunosuppression and immunosuppressed if the percentage lymphocyte stimulation were ≥ 31%, 16 to 30 % and 0 to 15 %, respectively ***(Tuboly, 2012,*** Personal Communication).

RESULTS

3.1. Number of bacteria positive swabs in cows with normal calving and with retained fetal membranes during postpartum period:

During the examined postpartum periods, a total of 108 uterine swabs were collected from 46 cows. Ninety-seven uterine swabs were collected from 41 cows with normal calving and 11 uterine swabs were collected from 5 cows with retained fetal membranes. With the exception of a swab from a cow with normal calving at 17-25 days postpartum, all the uterine swabs were bacteriologically positive (Table 2).

3.2. The average number of isolates/ swab collected from cows during postpartum period:

A total of 165 bacterial isolates were identified from 97 uterine swabs collected from cows with normal calving and 23 bacterial isolates were identified from 11 uterine swabs collected from cows with retained

fetal membranes during the examined postpartum periods. The average number of isolates per swab increased with increasing days postpartum reaching the highest were at 27-38 days in both cows with normal calving and with retained fetal membranes. The average number of isolates per swab was higher (2.09 vs. 1.70) in cows with retained fetal membranes than in cows with normal calving (Table 3).

Table (2): Number of bacteria positive swabs in cows with normal calving and cows with retained fetal membranes (RFM) during postpartum period

Days postpartum	*Normal parturition*			*RFM*		
	n	**Positive**	**Negative**	**n**	**Positive**	**Negative**
7-11 (*n*=46)	41	41	0	5	5	0
17-25 (*n*=33)	30	29	1	3	3	0
27- 38 (*n*=29)	26	26	0	3	3	0
Total No. of swabs (n=108)	**97**	**96**	**1**	**11**	**11**	**0**

n= number of cows (swabs)

Table (3): The average number of isolates per swab collected from cows with normal calving (NC) and cows with retained fetal membranes (RFM)

Days postpartum	*Number of isolated bacteria*		*Number of animals (swab)*		*Isolates per swab*	
	NC	**RFM**	**NC**	**RFM**	**NC**	**RFM**
7-11	65	8	41	5	1.59	1.60
17-25	51	7	30	3	1.70	2.33
27- 38	49	8	26	3	1.88	2.67
Total	**165**	**23**	**97**	**11**	**1.70**	**2.09**

3.3. The frequency and types of isolated bacteria from cows with normal calving:

Of 165 isolated bacteria, 58.1% (96/165) were less pathogenic bacteria, 27.9% (46/ 165) were pathogenic bacteria and 13.9% (23/165) were non pathogenic bacteria. The isolated less pathogenic bacteria during the examined postpartum periods were *Enterococcus* spp. (73/165; 44.2%) and *H. somni* (23/165; 13.9%). In addition, the isolated pathogenic bacteria were *T. pyogenes* (26/165; 15.8%) and *E. coli* (20/165; 12.1%). The isolated non pathogenic bacteria were *B. subtilis* (12/165; 7.3%), *Corynebacterium* spp., (3/165; 1.8%), *P. aeruginosa* (3/165; 1.8%), *Proteus* spp. (2/165; 1.2%), *Bacillus* spp. (1/165; 0.6%), *Micrococcus* spp. (1/165; 0.6%) and *Streptococcus* spp. (1/165; 0.6%).

The frequencies of isolation of *Enterococcus* spp. were nearly constant during the examined periods. In contrast, the frequency of isolated *H. somni* increased with increasing days postpartum. *T. pyogenes* was isolated in highest frequency during 7-11 days postpartum then decrease gradually during the other examined periods. While the frequency of isolation of *E. coli* increased at 17-25 days postpartum and then there was a little decrease at 27-38 days postpartum (Table 4).

3.4. The frequency and types of isolated bacteria from cows with retained fetal membranes:

Of 23 bacterial isolates identified from cows with retained fetal membranes 43.4% (10/23) were less pathogenic bacteria, 39.1% (9/23) were pathogenic and 17.4% (4/23) were non pathogenic bacteria. The isolated less pathogenic bacteria during the examined postpartum period

were *Enterococcus* spp. (9/23; 39.1%) and *H. somni* (1/23; 4.3%). The isolated pathogenic bacteria were *E. coli* (5/23; 21.7%) and *T. pyogenes* (4/23; 17.4). The isolated non pathogenic bacteria were *B. subtilis* (2/23; 8.7%) followed by *B. cereus* (1/23; 4.3%) and *Proteus* spp. (1/23; 4.3%) (Table 5).

Table (4): The frequency and types of isolated bacteria from cows with normal calving.

Categories of isolated bacteria	**Days postpartum**			**Total isolates (*n*=165)**
	7-11 (*n*=65)	**17-25 (*n*=51)**	**27-38 (*n*=49)**	
Pathogenic				
T. pyogenes	16 (24.6%)	8 (15.7 %)	2 (4.1%)	**26 (15.8%)**
E. coli	7 (10.8 %)	7 (13.7 %)	6 (12.2 %)	**20 (12.1%)**
Total	**23 (35.4%)**	**15 (29.4%)**	**8 (16.3%)**	**46 (27.9%)**
Less pathogenic				
Enterococcus spp.	29 (44.6 %)	22 (43.1 %)	22(44.9%)	**73 (44.2%)**
H. somni	6 (9.2 %)	7 (13.7 %)	10 (20.4%)	**23 (13.9%)**
Total	**35 (53.8%)**	**29 (56.8%)**	**32 (65.3%)**	**96 (58.1%)**
Non pathogenic				
Corynebacterium spp.	2 (3 %)	0	1 (2.1 %)	**3 (1.8%)**
Proteus spp.	2 (3 %)	0	0	**2 (1.2%)**
Streptococcus spp.	1 (1.5 %)	0	0	**1 (0.6%)**
P. aeruginosa	1 (1.5 %)	2 (4 %)	0	**3 (1.8%)**
B. subtilis	1 (1.5 %)	4 (8 %)	7 (14.3%)	**12 (7.3%)**
Bacillus spp.	0	1 (2%)	0	**1 (0.6%)**
Micrococcus spp.	0	-	1 (2 %)	**1 (0.6%)**
Total	**7 (10.7%)**	**7 (14%)**	**9 (18.4%)**	**23 (13.9%)**

n= numbers of isolates.

Table (5): The frequency and types of isolated bacteria from cows with retained fetal membranes.

Categories of isolated bacteria	Days postpartum			Total (*n*=23)
	7-11 (*n* =8)	17-25 (*n* =7)	27-38 (*n* =8)	
Pathogenic				
T. pyogenes	1(12.5 %)	2 (28.6%)	1 (12.5%)	4 (17.4)
E. coli	2 (25 %)	1 (14.3%)	2 (25%)	5 (21.7%)
Total	**3 (37.5%)**	**3 (42.9%)**	**3 (37.5%)**	**9 (39.1%)**
Less pathogenic				
Enterococcus spp.	4 (50 %)	4 (57 %)	1 (12.5%)	9 (39.1%)
H. somni	0	0	1 (12.5%)	1 (4.3%)
Total	**4 (50%)**	**4 (57%)**	**2 (25%)**	**10 (43.4%)**
Non-pathogenic				
Proteus spp.	1 (12.5%)	0	0	1 (4.3%)
B. subtilis	0	0	2 (25%)	2 (8.7%)
B. cereus	0	0	1 (12.5%)	1 (4.3%)
Total	**1 (12.5%)**	**0**	**3 (37.5%)**	**4 (17.4%)**

n= numbers of isolates.

3.5. Cell-mediated immunity in postpartum cows:

3.5.1. *Response of lymphocyte to mitogens*:

With advancing in the postpartum days, the percentage of the lymphocyte stimulation to PHA and Con A increased from 31.21±12.38 at 7-11 days postpartum to 35.4±10.5 at 17-25 days postpartum. Thereafter, there was a little decrease in this response (33.62±10.68) at 27-38 days postpartum. The response of the lymphocyte to Con A was higher than that of PHA (Table 6).

Table (6): Mean (± S.D.) percentage of lymphocyte stimulation to PHA + Con A during the postpartum periods.

Days p.p.	Lymphocyte stimulation to mitogens		
	PHA	Con A	PHA+ Con A
7-11	30.33±12.30 (34)	32.09±12.93 (34)	31.21±12.38 (34)
17-25	33.8±10.5 (30)	37.1±10.5 (30)	35.4±10.5 (30)
27- 38	32.62±10.77 (29)	34.62±11.24 (29)	33.62±10.68 (29)

Numbers within parenthesis are numbers of cows.

3.5.2. *Immunological status of cows during the postpartum periods*:

The highest percentage of the cows was immune healthy followed by healthy with lower immunosuppression, while, the least percentage of cows was immunosuppressed (Table 7).

3.5.3. *Relationship between categories of isolated bacteria and the immune status of cows during the postpartum periods:*

Most of bacterial isolates were from healthy cows, followed by lower immunosuppressed cows, while the least bacterial isolates were from immunosuppressed cows. Also most of pathogenic, less pathogenic and non pathogenic bacterial isolates were isolated from immune-healthy cows (Table 8).

Table (7): The immune status of the cows during the postpartum periods according to lymphocyte stimulation test.

Days postpartum	*healthy*	*Healthy with immunosuppression*	*Immunosuppression*
7-11 (*n*=34)	18 (53%)	12 (35.3%)	4 (11.8%)
17-25 (*n*=30)	19 (63.3%)	10 (33.3%)	1 (3.3%)
27- 38 (*n*=29)	16 (55.2%)	13 (44.8%)	0 (0%)
Total (*n*=93)	**53/93 (57%)**	**35/93 (37.6%)**	**5/93 (5.4%)**

n= number of animals. Immunosuppression= 0-15%, healthy but lower immunosuppression= 16-30% and healthy ≥ 31%.

Table (8): Distribution of bacterial categories among cows according to their immune status during the examined postpartum periods.

Categories of bacteria	*Healthy*	*Healthy with lower immunosuppression*	*Immunosuppression*
Pathogenic (*n*=46)	28 (61%)	18 (39%)	0 (0%)
Less pathogenic (*n*=89)	49 (55 %)	34 (38 %)	6 (7%)
Non pathogenic (*n*=24)	13 (54%)	10 (42%)	1 (4%)
Total isolates (*n*=159)	**90 (57%)**	**62 (39%)**	**7 (4 %)**

3.5.4. *Distribution of pathogenic bacteria in each category of immune status of cows during post partum periods*:

In both healthy and lower immunosuppressed cows, the frequency of isolation of *T. pyogenes* decreased with advancing the postpartum periods, while the frequency of isolation of *E. coli* was constant in healthy cows or increased at 17-25 days then decreased at 27-38 days in lower immunosuppressed cows. Neither *T. pyogenes* nor *E. coli* were isolated in immunosuppressed cows (Table 9).

3.5.5. *Distribution of less pathogenic bacteria in each category of immune status of cows during postpartum periods*:

In both healthy and lower immunosuppressed cows, there was a fluctuation in the frequency of isolation of *Enterococcus* spp. during the examined postpartum periods. While *H. somni* increased in frequency with advancing the postpartum periods. In immunosuppressed cows, low frequencies of *Enterococcus* spp. and *H. somni* were isolated (Table 10).

3.6.6. *Distribution of non-pathogenic bacteria in each category of immune status of cows during post partum periods*:

With one exception, all isolates of non pathogenic bacteria were isolated from healthy (13 isolates) and lower immunosuppressed cows (10 isolates) (Table 11). Out of 13 isolates of non-pathogenic in healthy cows, 5 were isolated with pathogenic bacteria, 4 were isolated with less pathogenic bacteria and 4 were isolated alone.

Table (9): Distribution of pathogenic bacteria (*T. pyogenes* and *E. coli*) among different immune status categories of cows during the post partum periods.

Days postpartum	*Healthy*		*Healthy with lower immunosuppression*		*Immunosuppression*	
	T. pyogenes	*E. coli*	*T. pyogenes*	*E. coli*	*T. pyogenes*	*E. coli*
7-11 (*n*=20)	9 (45%)	4 (20%)	6 (30%)	1 (5%)	0 (0%)	0 (0%)
17-25 (*n*=16)	5 (31.25%)	4 (25%)	3 (18.75%)	4 (25%)	0 (0%)	0 (0%)
27-38 (*n*=10)	2 (20%)	4 (40%)	1 (10%)	3 (30%)	0 (0%)	0 (0%)
Total no of isolates (*n*=46)	**16 (34.8%)**	**12 (26.1%)**	**10 (21.7%)**	**8 (17.4%)**	**0 (0%)**	**0 (0%)**

Table (10): Distribution of less pathogenic bacteria *(Enterococcus* spp. and *H. somni*) among different immune status categories of cows during the post partum periods.

Days postpartum	*Isolated bacteria*	*Healthy*	*Healthy with lower immunosuppression*	*Immuno-suppression*
7-11 (*n*=29)	*Enterococcus* spp.	13 (44.8%)	8 (27.6%)	4 (1.4%)
	H. somni	2 (7%)	1 (3.4%)	1 (3.4%)
17-25 (*n*=31)	*Enterococcus* spp.	16 (55.2%)	7 (24.1%)	1 (3.4%)
	H. somni	2 (7%)	3 (10.3%)	0 (0%)
27- 38 (*n*=12)	*Enterococcus* spp.	10 (32.3%)	11 (35.5%)	0 (0%)
	H. somni	6 (19.4%)	4 (13%)	0 (0%)

Table (11): Distribution of non-pathogenic bacteria among different immune status categories of cow during the post partum periods

Days postpartum	*Isolated bacteria*	*Healthy*	*Healthy with lower immunosuppression*	*Immuno-suppression*
7-11 (*n*=5)	*Proteus* spp.	1 (20%)	0 (0%)	0 (0%)
	P. aeruginosa	0 (0%)	1 (20%)	0 (0%)
	Streptococcus spp.	0 (0%)	1 (20%)	0 (0%)
	Corynebacterium spp.	0 (0%)	1 (20%)	1(20%)
17-25 (*n*=7)	*B. subtilis*	2 (28.6%)	2 (28.6%)	0 (0%)
	P. aeruginosa	2 (28.6%)	0 (0%)	0 (0%)
	Bacillus spp.	1 (14.3%)	0 (0%)	0 (0%)
27-38 (*n*=12)	*B. subtilis*	5 (41.7%)	4 (33.3%)	0 (0%)
	B. cereus	0 (0%)	1 (8.3%)	0 (0%)
	Corynebacterium spp.	1 (8.3%)	0 (0%)	0 (0%)
	Micrococcus spp.	1 (8.3%)	0 (0%)	0 (0%)

DISCUSSION

Bacterial contamination of the uterine lumen is common in cattle after parturition; this contamination doesn't always imply a disease ***(Sheldon et al., 2008).*** The results of the present study showed that with the exception of a cow with normal calving at 17-25 days postpartum, uteri of all cows were contaminated with different bacterial species throughout the examined postpartum periods. In contrast, ***Griffin et al. (1974), Sheldon et al. (2004a)*** and ***Williams et al. (2005)*** recorded that 80-100% of cows have bacterial contamination of the uterus in the first 2 weeks postpartum followed by rapid decrease in the percentage of contaminated uteri during the subsequent five weeks postpartum. This difference in the prevalence of bacterial infection might be attributed to the difference in hygienic conditions among studied farms. Regarding cows with retained fetal membranes (RFM), all the collected uterine swabs were bacteriologically positive. Similar finding was recently reported in a study carried out in Egypt ***(Amer et al., 2010).***

In the present study, the average number of isolates per swab increased with increasing days postpartum days in cows with normal calving and retained fetal membranes. In contrast, other studies reported that the average number of isolates per swab was high during early postpartum, followed by a progressive decline at the end of postpartum period ***(Hussien et al., 1990 and Jadon et al., 2005).*** This difference in the average number of isolates per swabs may be attributed to the difference in hygienic conditions among farms**.** In cows with RFM, the average number of isolates per swab (2.09) was higher than that in cows

with normal calving (1.7). Similar findings were reported by ***Hussain et al. (1990) and Jadon et al. (2005)***. The cervix of cows with RFM might have remained open for longer period leading to a greater chance for bacterial invasion ***(Hussain et al., 1990)***.

Different types of bacteria were isolated from cows with normal parturition including *T. pyogenes, E. coli, Enterococcus* spp., *H. somni, Corynebacterium* spp., *Proteus* spp., *Streptococcus* spp., *P. aeruginosa, B. subtilis, Bacillus* spp., *Micrococcus* spp. Similar observations were obtained in other studies ***(Griffin et al., 1974, Bonnett et al., 1991, Sheldon et al., 2004b and Williams et al., 2005).*** In cows with normal calving***,*** *Enterococcus* spp.***,*** *T. pyogenes, H. somni* and *E. coli* were the predominant isolated bacteria in our study. However, ***Hussain et al. (1990)*** reported that *E. coli, Streptococcus* spp. and *Proteus* spp. were the most frequently isolated bacteria from bovine uterus.

Regarding the less pathogenic bacteria, *Enterococcus* spp. was isolated in highest frequency in our study (44.2%). While, ***Williams et al. (2005)*** isolated it in frequency of 12.2%. *H. somni* was isolated in the present study in 13.9%, while ***Bonnett et al. (1991)*** isolated it in 1.2 % from cows. *H. somni* is a gram negative cocobacilli and it is known to be a common component of the normal bacterial flora of the female genital tract, can remain in the vagina for long periods without clinical signs ***(Yaeger and Holler, 2007).***

Regarding the pathogenic bacteria, *T. pyogenes* was isolated in 15.8% of cows in the present study. Higher frequencies (35%) were reported by ***Huszenicza et al. (1999) and Griffin et al. (1974)*** (33.3%**)**,

but lower frequencies (10%) were reported by ***Noakes et al. (1991) and Kocamuftuoglu and Vural (2008)*** **(**7.7%**)** in cows with normal calving. In the present study *T. pyogenes* was isolated in highest frequency during 7-11 days postpartum then decrease gradually during the examined periods. These results are in accordance with the results reported by ***Griffin et al. (1974)*** who found that *T. pyogenes* was the most frequently isolated bacteria until 21 days postpartum then the organism was rare in its isolation in cows with normal calving.

In the present study, *E. coli* was isolated in 12.1% of all isolates between 7-38 days postpartum. Similar results (12.5%**)** were obtained by ***Noakes et al. (1991).*** On the other hand, higher frequencies of *E. coli* were recorded ***by Kocamuftuoglu and Vural (2008) (84.6%), Takács et al. (1990) (55.1%) and Dohman et al. (2000)*** (33%).

Regarding the non-pathogenic bacteria, the frequency of their isolation in our study were 7.3% for *B. subtilis,* 1.8% for *Corynebacterium* spp. and *P. aeruginosa*, 1.2% for *Proteus* spp. and 0.6% for *Streptococcus* spp., *Bacillus* spp. and *Micrococcus* spp. between 7-38 days postpartum. Higher frequencies of isolation of these bacteria were recorded in other studies ***(Griffin et al., 1974, Hussain et al., 1990, Huszenicza et al., 1999, Dohman et al., 2000 and Kocamuftuoglu and Vural, 2008).*** The decline in the number of non-pathogenic bacteria after the second week postpartum in the present study may be due to the effect of continuous flushing of the uterus, caused by myometrial contraction and immune defense mechanism of the uterus ***(Hussain et al. 1990).***

In the present study, the most prevalent bacterial isolates from cows with RFM were the less pathogenic *Enterococcus* spp. (39.1%), followed by the pathogenic *E. coli* (21.7%) and *T. pyogenes* (17.4%). Similar results were recorded by ***Dohman et al., 2000*** *and* ***Kocamuftuoglu and Vural (2008).*** However, higher frequencies of isolation of the pathogenic bacteria were recorded by ***Dohman et al. (2000)*** and ***Kocamuftuoglu and Vural (2008).*** Also, results of the present study showed the highest frequency of *E. coli* during 7-11 days postpartum and highest frequency of *T. pyogenes* at 17-25 days postpartum. This result was in accordance with the result reported by ***Dohman et al. (2000)*** who found a positive relationship between the presence of *E. coli* at early postpartum and *T. pyogenes* at later postpartum in cows with REF due to the presence of *E. coli* and lipopolysaccharide (LPS) in lochia which may favor the development of uterine infection by *T. pyogenes* in early postpartum period. The high frequency of *E. coli* at 27-38 days postpartum is attributed to recontamination of the uterus as reported by ***Griffin et al. (1974)*** *and* ***Sheldon et al. (2002)***.

Only the non pathogenic bacteria (*Proteus* spp.; 4.3%, *B. subtilis;* 8.7% and *B. cereus;* 4.3%) were isolated from cows with retained placenta in lower frequencies than in cows with normal parturition. Similar result was recorded by ***Luginbuhl and Kupfer (1980)*** who found that the non pathogenic bacteria disappeared more quickly from the uterus of cattle after difficult calving than after normal calving.

In cows with RFM, the isolated pathogenic and less pathogenic bacteria were the same of that isolated from cows with normal parturition. This result is in accordance with ***Kaczmarowski et al. (2004)***

who found that the same species of bacteria can be isolated from the uterus of both cows with normal calving and retained fetal membranes.

Our study recorded an increase in the mean percentage of stimulation of lymphocyte to mitogens from 31.21±12.38 at 7-11 days postpartum to 35.4±10.5 at 17-25 days postpartum. This result is supported by ***Kehrli et al. (1989)*** who recorded that the peripheral blood lymphocyte decreased from two weeks pre-partum and then began to increase at two weeks post partum in Holstein cows. In addition, ***Saad et al. (1989)*** recorded a steady decline in lymphocyte response of Swedish red and white cows to mitogen from three week pre-partum to two or three weeks postpartum. The little decrease in the lymphocyte response at 27-38 days postpartum observed in our study is similar to that reported by ***Kashiwawazaki et al. (1985)*** who reported a decrease in lymphocyte activity in multiparious dairy cows twice within 40 days after calving: the first one occurred within 10 days and the second one was around 30 days postpartum. ***Wells et al. (1977) and Kashiwawazaki et al. (1985)*** suggested that the stress and the increased level of serum corticosteroid might be related to the decreased blastogenic response of lymphocyte in the parturient period.

High percentage of pathogenic bacteria (61%) was isolated from healthy cows indicating that the pathogenic bacteria might have stimulated the cellular immune response. This explanation is supported by the results of ***Ramadan et al. (1997)*** who demonstrated that intrauterine inoculation of pathogenic bacteria (*E. coli* and *T. pyogenes*) resulted in an increase in PGF2α concentration which is an important component of uterine defense mechanism, regardless of the stage of the

cycle when bacteria introduced. In addition, ***Seals et al. (2003)*** reported that PGF2α stimulated lymphocyte proliferation; consequently, the cows might have developed higher lymphocyte activity against *T. pyogenes* and *E. coli* ***(Ramadan et al., 1997).*** The isolation of non-pathogenic bacteria from healthy and lower immunosuppressed cows in our study is attributed to its association with pathogenic and less pathogenic bacteria. In conclusion, the presence of pathogenic and less pathogenic bacteria might stimulate the cellular immune response in dairy cows during the postpartum period.

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