Kafkas Univ Vet Fak Derg
18 (5): 791-798, 2012
DOI:10.9775/kvfd.2012.6446

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Uterine Microbiology and Histopathology in Repeat Breeder Anatolian Water Buffaloes: An Abattoir Study

Oktay YILMAZ * Yahya KUYUCUOĞLU ** Alper SEVİMLİ ***
Ebubekir YAZICI * Mehmet UÇAR *

* Department of Obstetrics and Gynaecology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKIYE
** Department of Microbiology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKIYE
*** Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, TR-03200 Afyonkarahisar - TURKIYE

Makale Kodu (Article Code): KVFD-2012-6446

Summary

The objective of the study was to determine the bacterial contaminants causing endometritis in cycling Anatolian water buffalo cows at Afyonkarahisar province Turkey. Data were collected from the genital tracts of 71 cycling pluriparious slaughtered water buffalo cows that had not conceived after three or more services following observed oestrus with no abnormal vaginal discharge presented. Animals were divided into two groups as luteal and follicular phase. Swabs for bacteriology and tissues for histopathology were collected from the *cornu uteri* of each buffalo. Totally 76% of buffaloes were found to have any histopathological changes, whereas mild endometrial inflammatory changes were commonly found from the remainders. The microorganisms most prevalent in the uterine lumen were *Escherichia coli*, *Staphylococcus aureus, Candida* spp. and *Trueperella pyogenes* in both healthy uteri and those presenting with mild endometritis. Most of the isolates were recovered from luteal phase. The uteruses of buffalo cows affected with endometritis defined by histopathology showed no bacterial isolation in follicular phase of the oestrous cycle. It is suggested that *E. coli* and *T. pyogenes* existing in normal uterus might be the most non-specific uterine pathogens that were associated with endometritis in Anatolian water buffaloes.

Keywords: *Water Buffaloe, Repeat Breeder, Endometritis*

Repeat Breeder Anadolu Nehir Mandalarında Uterus Mikrobiyolojisi ve Histopatolojisi: Bir Mezbaha Çalışması

Özet

Sunulan çalışmada, Türkiye'nin Afyonkarahisar ilinde yaşayan siklik Anadolu nehir mandası ineklerinde endometritise neden olan bakteriyel kontaminantların tespit edilmesi amaçlandı. Anormal vaginal akıntı olmaksızın üç veya daha fazla sayıda çiftleştirildiği halde gebe kalmamış, 71 adet siklik puliripar manda ineğinin genital kanalı değerlendirilmek üzere toplandı. Hayvanlar luteal ve folliküler evre olacak şekilde iki gruba ayrıldı. Bakteriyoloji ve histopatoloji için örnekler her mandanın kornu uterisinden toplandı. Toplamda bütün mandaların %76'sında herhangi bir histopatolojik değişiklik bulunmazken, diğer hayvanlarda sıklıkla hafif formda yangısel değişiklikler izlendi. Sağlıklı ve hafif endometritisli uteruslardan sıklıkla *Escherichia coli*, *Staphylococcus aureus*, *Candida* spp. ve *Trueperella pyogenes* izole edildi. İzolatlar çoğunlukla luteal evreden elde edildi. Histopatolojik olarak endometritis teşhisi konulan folliküler evredeki manda uteruslarında ise herhangi bir izolata rastlanmadı. Sonuç olarak, sağlıklı uteruslarda da tespit edilen *E. coli* ve *T. pyogenes* izolatlarının, Anadolu nehir mandalarında endometritis ile ilişkili spesifik olmayan uterus patojenleri olabileceği ileri sürülmektedir.

Anahtar sözcükler: *Nehir Mandası, Repeat Breeder, Endometritis*

INTRODUCTION

Poor reproductive efficiency and extended calving intervals are the major problems faced by buffalo breeders [1,2]. Moreover, postpartum inflammation of uterus is one of the most important conditions in water buffaloes [3]. Bacterial

İletişim (Correspondence)
+90 272 2149309
oktayyilmaz@aku.edu.tr

contamination of the water buffalo uterus during postpartum period delays the cervical and uterine involution, the occurrence of first postpartum oestrus and extends the service period [4]. Endometritis is one of the uterine inflammations affecting fertility and has different incidence rates such as 33.2% in Iranian local breed water buffalo cows [5], 22.4% in Egyptian buffalo cows [6], 47.9% in Baghdad buffaloes [7] and 43.3% in Basra buffalo cows [8]. Azawi et al.[9] suggested that poor hygiene, vaginal stimulation for milk let down and wallowing might be the contributing factors causing uterine infections in water buffaloes compared to cows. There is also a relation between hormonal status and uterine defence mechanism. It is known that the uterus is resistant to infection when progesterone (P4) concentrations are basal and it is susceptible when P4 concentrations are increased [10].

Calving of Anatolian buffaloes occurs in a seasonal pattern [11-13]. Anatolian buffaloes are considered to have a low reproductive performance and an increased average length of calving interval [11]. Beside, endometritis may prolong the service period as well as causing infertility in Anatolian buffaloes. There can be clear mucus, clear mucus with flakes of pus or mucopurulent discharge on vaginal examination of buffalo cows suffering from endometritis [14]. On the other hand, endometritis occurred by inflammation of the uterus in the absence of purulent or mucopurulent uterine discharge detectable in the vagina should be also taken into consideration. The identification of microorganism associated with endometritis and its effect on reproductive efficiency in Anatolian buffaloes has not been reported. Moreover, endometritis might be a reason for culling in Anatolian water buffaloes. Therefore, the present study aimed to investigate the microbiological and histological environment of uterus in cycling Anatolian water buffaloes conceived with no abnormal vaginal discharge over three months after calving.

MATERIAL and METHODS

Sampling of Genital Tracts of Animals

All data of the genital tracts of 71 cycling pluriparious water buffaloes were obtained over a one-year period from abattoirs in Afyonkarahisar, Turkey. Before slaughter, age, weight and anamnesis of the animals were recorded. Following slaughtering, the genital tracts were separated from the carcass with the help of scissors and forceps, then wrapped in steril polythene bags and taken to the department laboratory.

Blood Sampling and Progesterone Determination

Prior to slaughter, blood samples were taken from vena jugularis into serum test tubes. The tubes were centrifuged at 3.000 rpm for 10 minutes. Sera were transferred in Eppendorf tubes and were kept in a freezer at –20°C for later estimation of P4 concentrations to confirm that water buffaloes had normal ovarian function. Serum P4 concentrations were determined by electrochemiluminiscence immunoassay (ECLIA) using a commercial test kit (Elecsys Progesterone II, Roche Diagnostics GmbH, Germany) in an immunologic test analyser (cobas e 601, Roche Diagnostics GmbH, Germany).

Classifying of Ovaries and Grouping of Animals

Each ovary was examined to determine whether the cow was cycling, according to the gross appearance of the luteal tissue [15-17] and serum P4 concentrations [15,16]. In this study, the length of oestrous cycle was accepted as 21 days. Briefly, metestrous (days 1-4) describes the interval between ovulation and the time when the epithelium is growing over the rupture point of the former follicle. In early diestrous (days 5-10), the CL is fully developed with visible vascularization around its periphery, the apex is red or brown and the rest is greyish. In late diestrous (days 11-17), CL has red or brown colour at the beginning of the stage, whereas the entire CL is bright red or grey at the end. In proestrous and oestrous (days 18-21), regressed CL appears small, hard and bright [15]. Animals had luteal tissue and serum P4 concentration more than or equal to1 ng/ml were accepted as in luteal phase, whereas animals had regressed CL and serum P4 concentration less than 1 ng/ml were accepted as in follicular phase of the oestrous cycle. Therefore, animals were divided into two groups as being either in luteal phase (metestrous, early and late diestrous) and follicular phase (proestrous or oestrus). When no luteal tissue or an abnormal vaginal discharge was found, these cows were excluded from the study.

Morphological Examination

After sampling for bacterial investigation, morphological examination was performed by inspection of the genital tract following the incision from the external os of the cervix and to the tip of the uterine horns. The character of any mucus present was noted and uteruses presenting non-clear mucus or gross lesions of endometritis were excluded from the study.

Histopathology

Full thickness tissue samples were obtained from both uterine horns and placed in 10% phosphate buffered formaldehyde solution. After 24 h, tissue samples were then processed in paraffin, sectioned to a width of 5 µm and stained routinely with haematoxylin and eosin [18]. Histologically, the endometrium was classified as negative (no evidence associated with inflammation), mild (with mild infiltration of polymorphonuclear leucocytes (PMNs), lymphocytes and plasma cells and fibrocytes with little evidence of cystic glandular degeneration or vascular changes), moderate (with some lymphocytes infiltration, moderate peri-glandular and perivascular fibrosis, and some cystic glandular degeneration), severe (with severe infiltration of poly-morphonuclear leucocytes (PMNs), and mononuclear (MNs) cells severe glandular fibrosis or atrophy and fibrosis) as described by Jubb et al.[19]. Genital tracts of buffaloes were categorized as either healthy (normal) or inflammed (with

endometritis in different forms).

Uterine Swab Collection and Bacteriology

For bacteriological investigation, the external surface of the both uterine horns was firstly cleaned with polyvinyl iodine, a 1.5 cm incision made aseptically with a sterile surgical blade and a swab sampled from lumens of both uterine horns via the one incision site in department laboratory. Swabs were transferred to Stuart Transport Medium (Unipath, Basingstoke, UK) and immediately transported to the microbiology laboratory at 4°C for bacteriological culture on sheep Blood and MacConkey Agars (Oxoid Ltd., Hampshire, England) at 37°C aerobically for 48 h. Identification of bacteria was based on the tests previously described by Holt et al.[20] and Quinn et al.[21].

Statistical Analysis

Chi-square test was conducted to the distribution of types of bacteria isolated from healthy and inflammed uteruses of water buffaloes, and under the luteal and follicular phases. All statistical analysis was performed with the SPSS software for windows (15.0). Statistical significance was declared at $P<0.05$.

RESULTS

In the present study, the history of the animals has been collected from the owners. All buffaloes had not conceived after three or more services to a standing heat with no abnormal vaginal discharge and had calved around 137±17 days, aged 5-7 years and weighing 450-550 kg. No buffalo cows had any mucopurulent, purulent or cloudy vaginal discharge, either noted by the owner or during vaginal inspection. Six, 17, 34 and 14 water buffaloes were determined as being at metestrous (1.01±0.11 ng/ml), early diestrous (5.63±2.52 ng/ml), late diestrous (7.86±3.71) and follicular phase (0.30±0.19 ng/ml) based on serum P4 concentrations. Thus, among 71 water buffaloes, 57 buffaloes were in luteal phase, while 14 buffaloes were in follicular phase.

Histopathology

A total of 54 water buffaloes (76%) were found to have any histopathological changes, whereas mild endometrial inflammatory changes were found in 11 uterine horns *(Fig. 1B),* moderate changes were found in four uterine horns *(Fig. 1C),* and severe endometritis were determined in two uterine horns *(Fig. 1D)*.

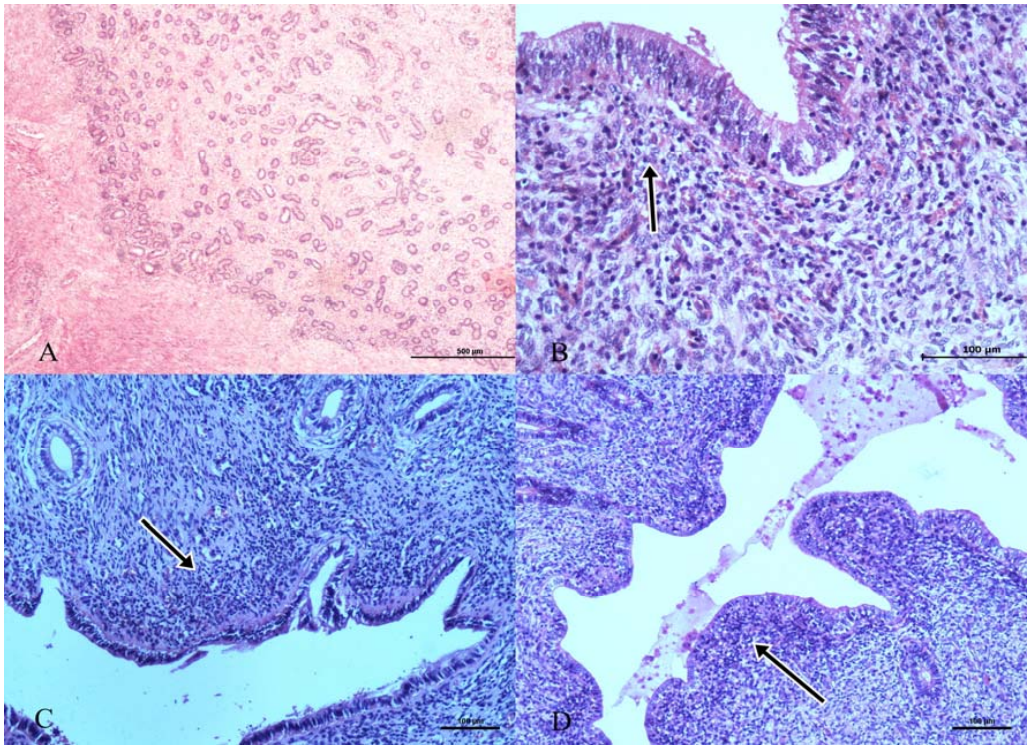

Fig 1. Endometrium of healthy uterus (A), mild inflammatory changes in endometrium, mild form (B); infiltration of polymorphonuclear leucocytes (PMNs), lymphocytes and plasma cells, and fibrocytes with little evidence of cystic glandular degeneration or vascular changes (arrow), the moderate form (C); moderate periglandular and perivascular fibrosis, and some cystic glandular degeneration (arrow) and the severe form (D) severe infiltration of PMNs (arrow), and mononuclear (MNs) cells, severe glandular fibrosis or atrophy and fibrosis (Magnification; x 4, A; x 20, B; x 10, C; x 10, D)

Şekil 1. Sağlıklı uterus endometriumu (A), hafif forma ait endometrial yangısal değişiklikler (B); polimorfnükleer lökosit infiltrasyonu (PMN), lenfositler ve plazma hücreleri, kistik glanduler dejenerasyon ve vaskuler değişiklikler (ok) ile birlikte fibrositler, orta form (C); orta şiddette periglanduler ve perivaskuler fibrozis ve bazı kistik glanduler dejenerasyon (ok), şiddetli form (D); şiddetli PMN infiltrasyonu (ok) ve mononükleer (MN) hücreler, çok sayıda glanduler fibrozis ve atrofi ile birlikte fibrozis (Büyütme; x 4, A; x 20, B; x 10, C; x 10, D)

Microbiological Investigation

No microorganisms were isolated from 29 healthy and nine inflammed uterine horns. Regarding the bacteriological findings, 12 different types of bacteria were isolated from the healthy uteruses of 54 water buffaloes, while only six types of bacteria were isolated from the inflammed uteruses of 17 water buffaloes. The most prevalent isolates in the healthy uteruses of water buffaloes were *Escherichia coli* (27.78%), *Staphylococcus aureus* (14.81%), *Candida spp.* (12.96%) and *Trueperella pyogenes* (11.11%), whereas the most prevalent microorganisms in the uteruses of water buffaloes with endometritis were *E. coli* (25%), *T. pyogenes* (16.67%), *S. aureus* (16.67%), *Candida spp.* (16.67%) and *Bacillus spp.* (16.67%) *(Table 1)*. The bacteria isolated most commonly in mild form of histopathological changes were *E. coli*, *S. aureus* and *Bacillus spp.,* whereas *E. coli* and *T. pyogenes* were isolated from the remainders *(Table 2)*. There was no significant differences

Table 1. *The prevalence of bacteria recovered from healthy and inflammed uterus of water buffaloes*
Tablo 1. *Sağlıklı ve yangılı manda ineği uterusundan elde edilen bakterilerin prevalansı*

Bacterial Isolates	Normal		Endometritis	
	Number	%	Number	%
Escherichia coli	15	27.78	3	25.00
Staphylococcus aureus	8	14.81	2	16.67
Candida spp.	7	12.96	2	16.67
Trueperella pyogenes	6	11.11	2	16.67
Staphylococcus epidermidis	5	9.26	1	8.33
Bacillus spp.	2	3.70	2	16.67
Proteus spp.	3	5.56	--	--
Pseudomonas aeruginosa	1	1.85	--	--
Streptococcus spp.	3	5.56	--	--
Pasteurella spp.	1	1.85	--	--
Streptococcus pneumonia	2	3.70	--	--
Streptococcus agalactia	1	1.85	--	--
Total	54	100.0	12	100.0
Isolate/sample	54/25		12/8	
No bacterial isolate	29		9	
There was no significant differences among types of isolated bacteria either from healthy or inflammed uteruses of water buffaloes (P>0.05)				

Table 2. *The prevalence of bacterial isolates recovered from the inflammed uterus of water buffaloes with histopathological changes*
Tablo 2. *Histopatolojik değişikliklerle birlikte seyreden yangılı manda ineği uterusundan elde edilen izolatların prevalansı*

Bacterial Isolates	Histopatological Changes					
	Mild		Moderate		Severe	
	No	%	No	%	No	%
Escherichia coli	2	22.22	1	50.00	--	--
Staphylococcus aureus	2	22.22	--	--	--	--
Candida spp.	2	22.22	--	--	--	--
Trueperella pyogenes	--	--	1	50.00	1	100.0
Staphylococcus epidermidis	1	11.11	--	--	--	--
Bacillus spp.	2	22.22	--	--	--	--
Proteus spp.	--	--	--	--	--	--
Pseudomonas aeruginosa	--	--	--	--	--	--
Streptococcus spp.	--	--	--	--	--	--
Pasteurella spp.	--	--	--	--	--	--
Streptococcus pneumonia	--	--	--	--	--	--
Streptococcus agalactia	--	--	--	--	--	--
Total	9	100.0	2	100.0	1	100.0
Isolate/sample	9/5		2/2		1/1	
No bacterial isolate	6		2		1	

among types of isolated bacteria either from healthy or inflammed uteruses of water buffaloes.

T. pyogenes, S. epidermidis and *Bacillus spp.* were the bacteria isolated from single isolates from the inflammed uteruses. The total percentage of single isolates found in inflammed uteruses was 25% (3/12), whereas 75% (9/12) of isolates found in inflammed uteruses were mixed. Mixed isolates were the majority of bacterial isolates (93%, 50/54) in healthy uterine horns and *E. coli* was the most prevalent agent found in mixed isolates in both healthy (30%) and inflammed (33%) uterine horns. Moreover, *S. aureus, Candida spp.* and *T. Pyogenes* were also isolated from mixed isolates of all buffalo cows *(Table 3)*.

According to the phase of the oestrous cycle of the animals, there was no significant difference for the isolation of bacteria either from luteal or follicular phases. On the other hand, most of the isolates recovered from the normal and inflammed uteruses of water buffaloes originated from luteal phase of the oestrous cycle. The uteruses of water buffaloes affected with endometritis showed no bacterial isolation in follicular phase of the oestrous cycle *(Table 4)*.

DISCUSSION

Pubertas and first conception ages vary among buffalo breeds all over the world. Water buffaloes reach puberty at 15-18 month-old age and first conception age is around 24-36 month [22]. It is reported that first oestrous age of Anatolian water buffaloes is 917.7±103.3 days (33 months on average) under the appropriate breeding, management and climate conditions [23]. In the present study, it was recorded that the age of slaughtered animals varied between 5-7 years old and the calving interval was around 137±17 days. Therefore, it is suggested that extension of calving interval of non-pregnant buffaloes due to uterine diseases might be a culling reason for buffalo breeders in Afyonkarahisar province.

Repeat breeder cows with subclinical endometritis determined by uterine lavage technique, based on non-pregnancy at 190 ± 40 days postpartum [24] support the suggestion that water buffaloes could suffer from endometritis in the absence of bacterial agents at 137±17 days postpartum. Moreover, the high prevalence of *E. coli*, *S. aureus* and *Bacillus spp.* seems to be related to the mild type of endometritis in the present study. The moderate and severe uterine inflammatory changes were associated with *T. pyogenes* infection. This finding is supported by studies carried on experimentally induced endometritis in water buffaloes [25]. Similar results were obtained in cows by Del Vecchio et al.[26] and in water buffaloes by Azawi et al.[14]. In the present study, *T. pyogenes, E. coli, Bacillus spp.* and *S. aureus* were the most prevalent isolates that were isolated from buffalo cows with endometritis evaluated histopathologically in the absence of vaginal discharge. It was reported that *T. pyogenes, E. coli, Proteus vulgaris, Pseudomonas aeruginosa* and *S. aureus* were the most prevalent isolates in water buffaloes had clear mucus with flakes of pus and mucopurulent discharge [14]. It is suggested that these bacteria might be the effective agents that may cause endometritis in the course of time. Although endometritis usually results from bacterial infection, the role of fungi in the uterine infections is also recognised [27]. The demonstration of *Candida spp.* in normal uteruses (seven isolates in a total of 54 isolates) and inflammed uteruses

Table 3. *Prevalence of bacteria as single or mixed isolates recovered from the uterus of water buffaloes*
Tablo 3. *Manda ineği uterusundan tek veya miks olarak izole edilen bakterilerin prevalansı*

Bacterial Isolates	Normal				Endometritis			
	Single		Mixed		Single		Mixed	
	No	%	No	%	No	%	No	%
Escherichia coli	--	--	15	30.00	--	--	3	33.33
Staphylococcus aureus	2	50.00	6	12.00	--	--	2	22.22
Candida spp.	--	--	7	14.00	--	--	2	22.22
Trueperella pyogenes	--	--	6	12.00	1	33.33	1	11.11
Staphylococcus epidermidis	--	--	5	10.00	1	33.33	--	--
Bacillus spp.	--	--	2	4.00	1	33.33	1	11.11
Proteus spp.	1	25.00	2	4.00	--	--	--	--
Pseudomonas aeruginosa	--	--	1	2.00	--	--	--	--
Streptococcus spp.	--	--	3	6.00	--	--	--	--
Pasteurella spp.	--	--	1	2.00	--	--	--	--
Streptococcus pneumonia	1	25.00	1	2.00	--	--	--	--
Streptococcus agalactia	--	--	1	2.00	--	--	--	--
Total	4	100.0	50	100.0	3	100.0	9	100.0

There was no significant difference between single and mixed isolates recovered from healthy and inflammed uteruses of water buffaloes (P>0.05)

Table 4. *Bacteria recovered from healthy and inflammed uteruses of water buffaloes at luteal or follicular phases of oestrous cycle. N: Normal uterus, E: Endometritis*

Tablo 4. *Östrüs siklusunun luteal ve folliküler evrelerindeki sağlıklı ve yangılı manda ineği uteruslarından elde edilen bakteriler. N: Normal uterus, E: Endometritis*

Bacterial Isolates	Luteal Phase		Follicular Phase	
	N	E	N	E
Escherichia coli	13	3	2	--
Staphylococcus aureus	7	2	1	
Candida spp.	6	2	1	
Trueperella pyogenes	6	2	--	--
Staphylococcus epidermidis	3	1	2	
Bacillus spp.	2	2	--	--
Proteus spp.	2	--	1	--
Pseudomonas aeruginosa	1	--	--	--
Streptococcus spp.	2	--	1	--
Pasteurella spp.	--	--	1	--
Streptococcus pneumonia	--	--	2	--
Streptococcus agalactia	1	--	--	--
Total	43	12	11	--
Isolate/sample	43/21	12/7	11/4	--
No bacterial isolate	22	7	8	2

There was no significant difference among types of isolated bacteria from uteruses of water buffaloes at different stages of oestrous cycle (P>0.05)

(two isolates in a total of nine isolates) by bacteriology suggested that the pathogen might be implicated in the aetiology of endometritis in water buffalo.

Introducing of bacteria into the uterus and genital tract following dystocia with assisted parturition and retained placenta is one of the major factor affecting postpartum period causing uterine infection [3,28,29]. *E. coli* mainly presents and dominates the uterus of dairy cows within first days after calving [30] as well as the uterus of buffaloes after 6 h of calving in assisted and unassisted parturition [3]. The frequency of bacterial isolation from the uterus of healthy water buffaloes as shown in the results of the present study *(Table 1)* suggested that *E. coli* was the most prevalent bacteria associated with normal microbiological profile in Anatolian water buffalo uterus in accordance with Azawi et al.[14] who found the same result in cycling Iraqi buffalo cows. *E. coli* (30%) was also the most prevalent agent found in only mixed isolates from healthy and inflammed buffalo cows. Moreover, mixed isolates were the majority of bacterial isolates (92%, 50/54) in normal uteruses of water buffaloes. *E. coli* is needed to damage the endometrium enabling absorption of endotoxins and a damaged epithelium is usually required to establish infection [31,32]. Therefore, it can be suggested that endometritis occurs as a result of contamination by resources including *E. coli* which favours the development of uterine infection by other bacteria. This microorganism can suppress the defence mechanism of the uterus itself and facilitate other organisms to participitate in the infection. *S. aureus* and *T. pyogenes* were the bacteria which were isolated from healthy uterus of water buffaloes in the present study. Although *T. pyogenes* is known as a pathogenic microorganism, isolation of *T. pyogenes* from healthy water buffaloes in the present study was similar with the finding of Azawi et al. [3]. This finding may indicate that this microorganism needs the uterine endometrium to damage to penetrate and colonize the uterus.

T. pyogenes, *S. epidermidis* and *Bacillus spp*. were the most prevalent bacteria found as single isolates in the inflammed uterus and the total percentage of single isolates was 25%, whereas the prevalence of mixed isolates was 75%. Most bacterial isolates were recovered as mixed isolates from uterine tissue with mild histopathological changes. This was not consistent with the results of Azawi et al.[28] who found that the major bacterial isolates were recovered from severe and moderate forms during the metritis period between post partum 30-40 days. This inconsistent finding might be due to the postpartum days of the animals (over 100 days) in the present study. When a uterus becomes infected and unable to overcome organisms, the organisms proliferate, inflammatory cells infiltrate the endometrium and the infection process initiates [33,34]. The reason of the majority of mild forms in the present study may be associated with water buffaloes that fail to resolve the endometrial inflammation by the beginning of the breeding period.

It has been shown that post partum cows that received intrauterine infusions of *T. pyogenes* and *E. coli* after the onset of luteal function and at the P4 dominance had begun to increase developed uterine infections [26]. In addition, high estradiol concentrations that occur at oestrus and parturition

regulate the immune function by changing in the number of circulating white blood cells, increasing blood supply to the uterus and causing an increase in the quantity of vaginal mucus as a protective physical barrier [29]. Nevertheless, there was no significant difference as compared to isolation of bacteria from luteal and follicular stages of oestrous cycle in the presented study. On the other hand, follicular phase appears to be particularly beneficial to the resolution of uterine infection, as presented in this study which the inflammed uterus of water buffaloes showed no bacterial isolation in follicular phase of the oestrous cycle. Moreover, the isolation of *T. pyogenes* and *E. coli* from both normal and inflammed uteruses from luteal phase as presented in the study indicated that progesterone seems to suppress uterine immune defences. In the presented study, it may suggest that *T. pyogenes* and *E. coli* could cause histopathological changes not only during postpartum period but also after postpartum period, when the uterus was under the immunosuppressive effect of P4.

The reason of decreasing water buffaloe breeding in Turkey [35] may be also originated from reproductive problems rather than management. Moreover, no literature is available on the uterine bacteriology of the uteruses from either healthy Anatolian water buffaloes or those affected by endometritis. Histopathologically evaluated endometritis in the absence of any abnormal mucus could indicate that uterine cytology should be taken into consideration on the diagnosis of endometritis at over three months after calving in buffalo cows.

In conclusion, our results indicated that *E. coli* was mainly present in normal uteruses from cyclic water buffaloes and might favour the colonization of other bacteria in the uterine lumen of buffaloes. Mild form of endometritis in the absence of vaginal discharge should be taken into consideration as an infertility reason for buffalo reproduction. Despite a small number of samples related to endometritis, it could be suggested that *E. coli* and *T. pyogenes* might be the most non-specific uterine pathogens that associated with endometritis. Further studies using numerous animals are needed to clarify the effect of uterine infection and related bacterial agents on reproductive performance of Anatolian water buffaloes *in vivo*.

REFERENCES

1. Singh J, Alanda AS, Adams GP: The reproductive pattern and efficiency of female buffaloes. *Anim Reprod Sci,* 61, 593-604, 2000.

2. El-Wishy AB: The postpartum buffalo II. Acyclicity and anestrous. *Anim Reprod Sci*, 97, 216-236, 2007.

3. Azawi OI, Rahawy MA, Hadad JJ: Bacterial isolates associated with dystocia and retained placenta in Iraqi buffaloes. *Reprod Dom Anim*, 43, 286-292, 2008.

4. Usmani RH, Ahmad N, Shafiq P, Mirza MA: Effect of subclinical uterine infection on cervical and uterine involution, estrous activity and fertility in postpartum buffaloes. *Theriogenology*, 55, 563-571, 2001.

5. Moghami SM, Saiyari M, Mayatri M, Sharma RN: Pathology of uterus in buffaloe slaughtered in Ahwaz (Iran) abattoir. *Buffalo J,* 12, 213-218, 1996.

6. Ghanem M, Shalaby AH, Sharawy S, Saleh N: Factors leading to endometritis in Egypt with special reference to reproductive performance. *J Reprod Sci*, 48, 371-375, 2002.

7. Alwan AF, Abdul-Hammed AN, Khammas DJ: A macroscopical study of abnormal genitalia of Iraqi female buffaloes. *Iraqi J Vet Sci*, 14, 129-132, 2001.

8. Al-Fahad TA, Alwan AF, Ibraheem NS: Histological and morphological study of abnormal cases of female reproductive system in Iraqi buffaloes. *Iraqi J Vet Sci*, 18, 109-115, 2004.

9. Azawi OI: Clinical, bacteriological and pathological studies of uterine infections in Iraqi buffaloes. PhD Thesis, College of Veterinary Medicine, University of Baghdad, Iraq, pp. 122-170, 2006.

10. Lewis GS: Steroidal regulation of uterine resistance to bacteria infection in livestock. *Reprod Biol Endocrinol*, 1, 117-125, 2003.

11. Aksoy M, Kaya A, Uçar M, Lehimcioglu N, Tekeli T: Effect of seasonal conditions on oestrus occurrence and postpartum period in Anatolian water buffaloes. *Dtsch tierärztl Wschr*, 109, 416-418, 2002.

12. Ucar M, Küçükkebabçi M, Çelebi M, Akalin HN: Murrah mandalarında postpartum anöstrus sorununun rektal muayene ve süt progesteron testi ile belirlenerek sorunlu hayvanlarda PRID+PMSG hormon tedavisinin fertiliteye etkisinin araştırılması. *Turk J Vet Anim Sci*, 26, 1389-1393, 2002.

13. Uçar M, Küçükkebabçi M, Gündoğan M, Uğuz C, Saban E: Reproductive performance of Anatolian water buffaloes during postpartum period. *Indian Vet J*, 81, 784-786, 2004.

14. Azawi OI, Omran SN, Hadad JJ: A study of endometritis causing repeat breeding of cycling Iraqi buffalo cows. *Reprod Dom Anim*, 43, 735-743, 2008.

15. Ali A, Abdel-Razek AK, Abdel-Ghaffar S, Glatzel PS: Ovarian follicular dynamics in buffalo cows *(Bubalus bubalis). Reprod Dom Anim*, 38, 214-218, 2003.

16. Uçar M, Yılmaz O, Özenç E, Kahraman E: Anadolu Nehir Mandalarında folliküler dinamikler. *III. Veteriner Jinekoloji Kongresi, Antalya, 23-26 Ekim,* s. 32-33, 2008.

17. Yilmaz O, Yazici E, Ucar M: Measurements of some genital tract organ size in Anatolian Water buffalos. *Kafkas Univ Vet Fak Derg,* 17 (6): 1057, 2011.

18. Lee G, Luna HT: Manual of histological staining methods of the armed forces institute of pathology, 3rd ed. 235-236, McGraw-Hill Books Co., New York, 1968.

19. Jubb KVF, Kennedy PC, Palmer N: Pathology of Domestic Animals. 3rd ed. Academic Press, 1985.

20. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST: Bergey's manuel of determinative Bacteriology. 9th ed. Lippincott Williams and Wilkins, Philadelphia, 2000.

21. Quinn PJ, Carter ME, Markey BK, Carter GR: Clinical veterinary microbiology, 2nd ed. 21-618, Mosby Wolfe Publishing, 1999.

22. Gordon IR: Reproductive Technologies in Farm Animals. Cambridge, MA, USA: CABI Publishing, p 24. http://site.ebrary.com/lib/afyon/Doc?id=10073619&ppg=38, 2005, accessed on 10.04.2012.

23. Gündoğan M, Uçar M, Tekerli M: The effect of climatic conditions and birth weight on age at first estrus in Anatolian Water Buffaloes. *Hay Araş Derg*, 13, 5-8, 2003.

24. Salasel B, Mokhtari A, Taktaz T: Prevalance, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. *Theriogenology*, 74, 1271-1278, 2010.

25. Chaudhery SK, Gupta RC, Uppal RP: Administration of experimentally induced metritis condition in buffaloes. *Theriogenology*, 28, 961-969, 1987.

26. DelVecchio RP, Matsas DJ, Inzana TJ, Sponenberg DP, Lewis GS: Effect of intrauterine bacterial infusion and subsequent endometritis on PGF2a metabolite concentration in postpartum beef cows. *J Anim Sci*, 70, 3158-3162, 1992.

27. Petit T, Spergser J, Rosengarten R, Aurich J: Prevalance of potentially pathogenic bacteria as genital pathogens in dairy cattle. *Reprod Dom Anim*, 44, 88-91, 2009.

28. Azawi OI, Omran SN, Hadad JJ: A study on postpartum metritis in

Iraqi buffalo cows: bacterial causes and treatment. *Reprod Dom Anim*, 43, 556-565, 2008.

29. Azawi OI: Uterine infection in buffalo cows: A review. *Buffalo Bulletin*, 29, 154-171, 2010.

30. Huszenicza G, Fodor M, Gags M, Kucsar MM, Dohmen MJW, Varmos M, Porkolas L, Kegel T, Bartyik J, Lohuis JACM, Janos S: Uterine bacteriology, resumption of cyclic ovarian activity and fertility in postpartum cows kept in large-scale dairy herd. *Reprod Domest Anim*, 34, 237-245, 1999.

31. Sheldon IM, Dobson H: Postpartum uterine health in cattle. *Anim Reprod Sci*, 82, 295-306, 2004.

32. Sheldon IM, Bushnell M, Montgomery J, Rycroft AN: Minimum inhibitory concentration of some antimicrobial drugs against bacteria causing uterine infection in cattle. *Vet Rec*, 155, 383-387, 2004.

33. Sheldon IM, Lewis SL, LeBlanc S, Gilbert RO: Defining postpartum uterine disease in cattle. *Theriogenology*, 65, 1516, 2006.

34. Dhaliwal GS, Murray RD, Woldehiwet Z: Some aspects of immunology of the bovine uterus related to treatments for endometritis. *Anim Reprod Sci*, 67, 135-152, 2001.

35. Sarıözkan S: Türkiye'de manda yetiştiriciliğinin önemi. *Kafkas Univ Vet Fak Derg*, 17 (1): 163-166, 2011.