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Characterization of buffalo interleukin 8 (IL-8) and its expression in endometritis



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KEYWORDS

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Abstract River buffalo (*Bubalus bubalis bubalis*) with a population over 135 million heads is an important livestock. Interleukin 8 (IL-8) is a member of the chemokine family and is an important chemoattractant for neutrophils associated with a wide variety of inflammatory diseases such as endometritis. Tissue samples from the mammary gland, uterus and ovary were obtained from river buffalo (Mediterranean type) with and without endometritis. Bacteriological examination showed the presence of both gram positive and negative in all buffalo with endometritis. RNA extraction and complementary DNA (cDNA) synthesis were conducted from all tissues. Specific primer for IL8 full coding regions was designed using known cDNA sequences of *Bubalus bubalis*, Genbank accession number AY952930.1. IL-8 gene expression was investigated in buffalo tissues. Expression of IL-8 in buffalo with endometritis was found to increase significantly over buffalo without endometritis only in the uterus ($P = 0.0159$). PCR products from uterus tissues (target organs) of buffalo with and without endometritis, were purified and sequenced. No polymorphic sites were detected in the investigated samples. IL-8 cDNA nucleotide sequences of buffalo with and without endometritis were 100% identical (accession number JX413057). Buffalo IL8 cDNAs were compared with corresponding sequences of member of subfamily Bovinae (buffalo and cattle) and subfamily Caprinae (sheep and goat). IL-8 species specific differences were identified.

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1. Introduction

Interleukins are a group of immunomodulating agents that were first seen to be expressed by leukocytes. They are produced by a wide variety of body cells. The function of the immune system depends in a large part on interleukins,

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and rare deficiencies in interleukins are associated with auto-immune diseases or immune deficiency [4].

Interleukin-8 (IL-8), the main neutrophil chemokine, was originally identified as a chemotactic factor secreted by activated leukocytes that promotes directional migration of neutrophils and T lymphocytes, has a role in acute and chronic inflammation and promotes angiogenesis [14,15]. IL-8 is an important neutrophil chemoattractant in calves with pneumonic pasteurellosis [5] and it is over expressed in invasive breast cancer cells [12]. In recent investigation of IL-8 polymorphism in buffalo with mastitis, no polymorphic sites were detected [33].

Some diseases such as metritis and endometritis are highly prevalent and lead to economic losses, due to decreased milk yield and fertility in farm animals [3,19,21]. Endometritis is an inflammatory process caused by bacterial infection causing a chronic condition in which cows fail to completely clear bacterial contaminants [20,34]. A great inflammatory response to bacterial infections occurs in cows that develop chronic endometritis and become sub-fertile [36]. Endometritis is defined by an increase in the number of polymorphonuclear leukocytes in the uterus [2,19]. In response to bacterial infection the endometrium is stimulated to produce cytokines and chemokines such as IL-8 to attract and activate neutrophils and monocytes [8,22,35] for bacterial clearance and prevention of disease [24].

The water buffalo (*Bubalus bubalis*) with a population exceeding 195 million heads [11] is an economically important livestock species in many Asian and Mediterranean countries. The buffalo in Egypt are river Buffalo (*B. bubalis bubalis*) of Mediterranean type. Egypt has the 3rd highest river buffalo population after India and Pakistan [11]. Disease is one of the major factors contributing to poor livestock productivity in developing countries. In this respect, this study aims at characterizing IL-8 gene in buffalo in comparison with other species of subfamily Bovinae and its expression in buffalo with endometritis.

2. Materials and methods

2.1. Sample collection

Three tissue samples (mammary gland, uterus and ovary) were obtained from each animal of 10 Egyptian buffalo, five with and five without endometritis, from the slaughter house. Buffalo with endometritis had signs of abnormal secretions in uterus with signs of inflammation such as swelling, redness and hardness in uterus. Two swabs from each infected uterus were separately collected in transport medium (Carry-Blair), properly labeled and sent in an ice box to the laboratory for bacterial analysis. The tissue samples were minced and frozen in liquid nitrogen.

2.2. Identification of bacteria

Collected samples were streaked onto: Blood agar plates, MacConkey agar plates as well as mannitol salt agar plates. All samples were incubated aerobically and anaerobically. Aerobic plates were incubated at 37 °C for 24 h whereas anaerobic plates were incubated in an anaerobic jar using anaerobic system (BD) at 37 °C for 48–72 h. Plates were examined for colony characters, cellular morphology and the purity of the culture. The suspected colonies were identified according to [7,13,31].

2.3. RNA extraction and cDNA synthesis

RNA was extracted from the mammary gland, uterus and ovary using PeqGold TriFast™ according to manufacturer's instructions. Synthesis of cDNA was performed using Ready-To-Go You-Prime First-Strand Beads according to manufacturer's instructions.

2.4. Primer design

Specific primers for Interleukin-8 full coding regions were designed on the bases of the *B. bubalis* sequence Genbank accession AY952930.1 using http://www.genscript.com/gi-bin/tools/primer_genscript.cgi and synthesized by Amersham Pharmacia Biotech. The designed primers were used for PCR analysis and Real time quantitative PCR (QRT-PCR) (Table 1).

For gene expression studies Large Ribosomal Protein (RPLPO) was used as endogenous control. RPLPO primers were designed based on sequence of Genbank NM 001002.3 using the primer express software (Applied Biosystems) (Table 1). RPLPO was chosen as a control because its threshold cycle (cT) values are close to those of IL-8.

2.5. Gene expression using QRT-PCR

IL8 Expression was determined in the 3 tissues (mammary gland, uterus and ovary) obtained from each animal of five Egyptian buffalo associated with endometritis disease. For each sample, cDNA is diluted 1:5 with 10 Mm Tris pH 7.5, followed by preparation of a number of new dilutions for each sample. For each sample, an appropriate amount of template is combined with 2 µl of 2X Master Mix (Bioline) and 0.1 µl of 10 µM primer set. The QRT-PCR was performed using the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Life Technologies Corporation) using default cycling conditions: 40 cycles of 95 °C for 10 min (hold) and 95 °C for 15 s (melt) and 60 °C for 1 min (anneal/extend).

Table 1 Primer sequences for PCR and qRT-PCR amplification of cDNA.

Gene	Primers sequences (forward/reverse 5'–3')	Accession no.	Annealing temperature (°C)	Reference product size (bp)
Interleukin-8	ATGACTTCCAAGCTGGCTGTT/CATGGATCTTGCTTCTCAGC	AY952930.1	60	306
RPLPO	CAACCCGAAGTGCTTGACAT/AGGCAGATGGATCAGCCA	NM 001002.3	60	109

2.6. Statistical analysis

Cycle threshold (Ct) values were obtained through the auto Ct function. Following efficiency correction, the mean threshold cycle (CT) was calculated and then normalized to the reference gene using delta (Δ) CT. The calibrator was used to carry out an additional normalization step in order to account for differences in amplification dynamics between PCR reactions between different PCR reaction plates. Changes in relative expression were calculated using the $2^{-\Delta\Delta C_t}$ method [29].

2.7. Polymerase chain reaction

Each amplification reaction (100 μ l) contained 5 μ l of buffalo tissue cDNA as a template, 0.2 mM dNTPs, 10 mM Tris, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin (W/V), 1.25 units Taq polymerase and 1 μ M of forward and reverse primers. The reaction mixture was overlaid with sterile mineral oil and was run in an MJ research PTC-100 Thermocycler. The following cycling conditions were used for 3 min. at 94 °C; 35 cycles for 1 min at 94 °C; 1 min at 60 °C; 1 min at 72 °C and a final extension for 10 min at 72 °C. Parts of the PCR products were run on a 2% agarose-gel in 1X-tris acetate buffer containing 0.8 μ l of 10 mg/ml ethidium bromide. The gels were examined under UV and photographed using Gel documentation system (Ingenius Syngene Bio Imaging).

2.8. Sequencing PCR-products

IL-8 PCR products were purified using Exo SAP-IT PCR Purification Kit (Applied Biosystems) following the manufacturer's recommended protocol. Purified products were sequenced using Big Dye TM terminator Cycle Sequencing Kit (Applied Biosystems). Nucleotide sequences were determined and protein translations of the cDNA sequences were carried out using the six frame translation analysis: (<http://searchlauncher.bcm.tmc.edu/seq-util/options/sixframe.html>).

3. Results

3.1. Bacterial analysis

Bacterial examination of uteruses of buffalo with endometritis showed the presence of bacterial contamination, buffalo with endometritis were classified into 3 groups: gram positive, gram negative and anaerobic bacteria (Table 2). Healthy buffalo did not show any sign of endometritis symptoms.

3.2. Gene expression using real time quantitative PCR (QRT-PCR)

Quantitative analysis of IL-8 expression in the uterus, mammary gland and ovary of five buffalo with and five buffalo without endometritis was carried out. In uteruses the mean $2^{-\Delta\Delta C_t}$ value in buffaloes without endometritis was 22 compared to 586 in buffalo with endometritis, indicating 26.6 number of fold difference with a significant level of $P < 0.0159$. In ovary and mammary gland there was no significant difference in IL8 expression between buffalo with and without endometritis (Fig. 1).

3.3. IL-8 gene characterization

IL-8 open reading frame was investigated in buffalo mammary gland, uterus and ovary of Egyptian buffalo with and without endometritis using IL-8 cDNA and PCR. The three tissues gave positive reaction, with the uterus showing the highest intensity. Sequence analysis was conducted for uterus samples since it is the target organ.

The amplified segment was 306 nucleotides covering the full coding region. It encoded 101 amino acids and a stop codon. Alignment of all buffalo, with and without endometritis, nucleotide sequences revealed a 100% identity. IL-8 nucleotide and amino acid sequences of Egyptian buffalo cDNA are presented in Fig. 2.

Egyptian Buffalo IL-8 open reading frame nucleotide sequence was found to be 100% identical to *B. bubalis* (gb|AY952930.1) and 98% similar with *Bos taurus* (gb|BC103310.1), where 4 substitutions were detected at nt. 81, nt. 165, nt. 222, and nt. 270 where G, C, A and A in buffalo substituted A, T, C and G in cattle, respectively. Only the variation at nt. 222 resulted in amino acid difference, Lysine (73^{Lys}) in buffalo and Asparagine (73^{Asp}) in cattle. The three other variations at nucleotide 81, 165 and 270 resulting in amino acids Arginine (27^{Arg}), Isoleucine (55^{Ile}) and Glutamine (90^{Glu}), respectively, were silent mutations.

The predicted protein from IL-8 transcript was found to have the amino acid residues that are significant for IL-8 structure and functions which included conserved ELRCXC; GPH motifs; the four highly conserved cysteine residues (Fig. 3).

Nucleotide sequence of IL8 coding region of Egyptian buffalo was aligned with other member of subfamily Bovinae [*B. bubalis* (gi|61676045|, gi|223931031| and gi|353677864|) of genus *Bubalus*; *B. taurus* (gi|31343250|, gi|56549076|, gi|73587442|, gi|161579167| and gi|1699353|); *Bos indicus* (gi|169743018|)] of genus *Bos* and members of subfamily Caprinae [*Ovis canadensis* (gi|156753152|) and *Ovis aries*

Table 2 Prevalence and typing of the isolated gram negative bacteria, gram positive bacteria and anaerobic rod bacteria from infected uteruses.

Samples	Gram positive bacteria	Gram negative bacteria	Anaerobic rod bacteria
Uterus 1	<i>S. epidermidis</i> normal	<i>E. coli</i> , <i>Klebsiella pneumonia</i>	
Uterus 2	<i>S. aureus</i> , <i>Micrococcus</i>	<i>E. coli</i>	<i>Clostridium perfringens</i>
Uterus 3	<i>Micrococcus</i> , <i>S. epidermidis</i>	<i>E. coli</i> , <i>P. vulgaris</i>	
Uterus 4	<i>Streptococcus pyogenes</i> , <i>Micrococcus</i>	<i>E. coli</i>	
Uterus 5	<i>S. epidermidis</i>	<i>E. coli</i> , <i>Klebsiella pneumonia</i> , <i>P. vulgaris</i>	<i>Clostridium perfringens</i>

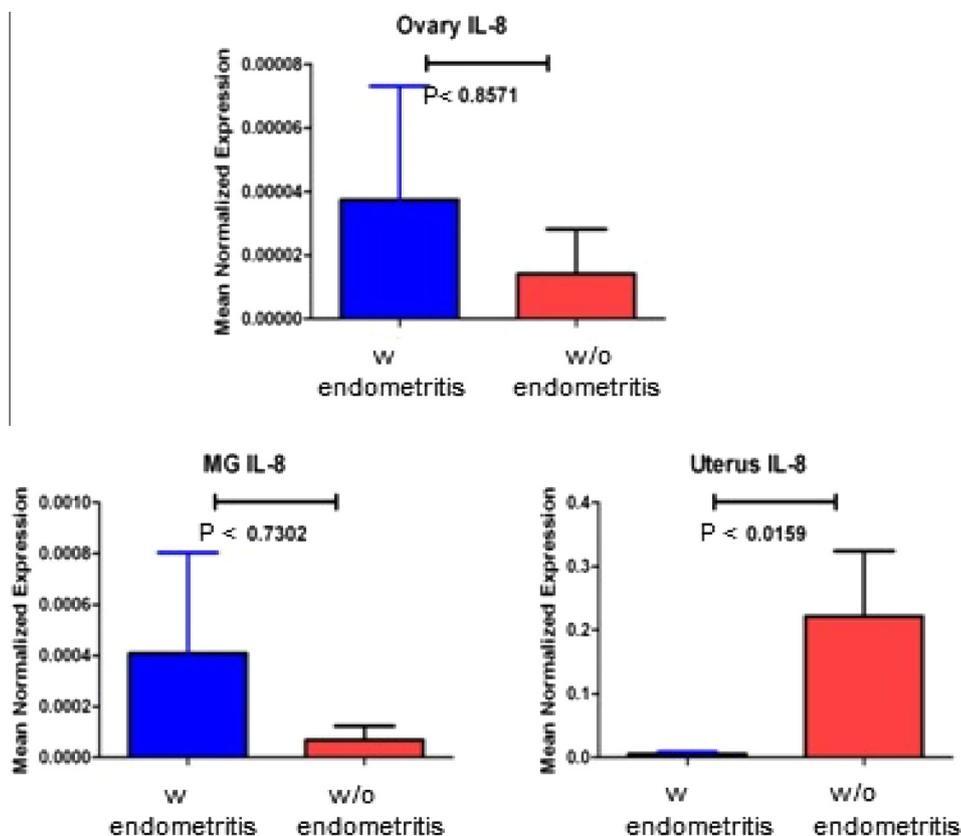


Figure 1 Mean normalized expression in IL-8 mRNA gene expression in uterus of Egyptian buffalo with (w) and without (w/o) endometritis using quantitative real time PCR. Constitutive production of IL8 full spliced transcript (306 bp) detected by RT-PCR, RPLPO is the housekeeping gene used as an internal control. Data are presented as the average \pm SD from three experiments.

ATGACTTCCAAGCTGGCTGTTGGCTCTCTTGGCAGCTTTCTGCTCTCTGCAGCTCTGTGTGAAGCTGCAGT
 TCTGTCAAGGATGAGTACAGAACTTCGATGCCAATGCATAAAAAACATTCACACCTTTCCACCCCAAAT
 TTATCAAAGAATTGAGAGTTATCGAGAGTGGGCCACACTGTGAAAATTCAGAAATCATTGTTAAGCTTACC
 AATGGAAAAGAGGCTGCTTAAACCCCAAGGAAAAGTGGGTGCAGAAGGTTGTGCAAGTATTTGTGAAGAG
 AGCTGAGAAGCAAGATCCATGA.

Figure 2 Egyptian buffalo IL-8 cDNA nucleotide sequence.

MTSKLAVALLAAFLLSAALCEAAVLSRMSTELRCQCIKTHSTPFHPKFIKELRVIESGP
H _CENSEIIVKLTNGKEVCLNPKEKWVQKVVQVFKRAEKQDP* 101

Figure 3 Buffalo IL-8 transcript showing (underlined) the conserved ELRCXC; GPH motifs; the four highly conserved cysteine residues.

(gi|427197617|, gi|463253|, gi|528775322| and gi|786590|) of genus *Ovis* and Goat: *Capra hircus* (gi|548470620|)] genus *Capra* available in GenBank database (Fig. 3). The 2 subfamilies belong to family Bovidae. The alignment showed seven nucleotide variations at nt 33(C), 183(C), 216(C), 228(G), 235(G), 272(C) and 277(T) that characterize subfamily Caprinae from subfamily Bovinae which have C, C, C, G and G at the corresponding nucleotides. These nucleotide differences resulted in three amino acid differences such as asparagine (79^{Asn}), valine (91^{Val}) and valine (93^{Val}) in subfamily Bovinae compared to aspartate (79^{Asp}), alanine (91^{Ala}) and leucine (93^{Leu}) in subfamily Caprinae in addition to 4 amino acids;

alanine (11^{Ala}), cysteine(61^{Cys}), Asparagine (72^{Asn}), Valine (76^{Val}) which are silent mutations.

The alignment showed that buffalo species has unique nucleotide sequence at nt 81(G), 165(C) and 270(A) which are different from *Bos*, *Ovis* and *Capra* species that have A, C and G at the corresponding nucleotides however these differences did not cause amino acid variations. Nucleotide 222 (C) is characteristic to *Bos* sp. since other species had A. This was reflected on the presence of a.a. 74^{Asn} in *Bos* compared to Lysine in *Bubalus*, *Ovis* and *Capra* species. The alignment showed that nucleotide 154 (C) is characteristic to *O. aries* subspecies (Figs. 4 and 5).

Egyptian buffalo	ATGACTTCCAAGCTGGTGTGCTCTCTTGGCAGCTTTCCTGCTCTCTGCAGCTCTGTGT	60
gi 61676045 B.b	60
gi 223931031 B.b	60
gi 353677864 B.b	38
gi 31343250 B.t	60
gi 56549076 B.t	60
gi 73587442 B.t	60
gi 161579167 B.t	60
gi 1699353 B.t	60
gi 169743018 B.i	60
gi 156753152 O.cC.....	60
gi 427197617 O.aC.....	60
gi 463253 O.aC.....	60
gi 528775322 O.aC.....	60
gi 786590 O.aC.....	60
gi 548470620 C.hC.....	60
Egyptian buffalo	GAAGCTGCAGTTCTGTCAAGGATGAGTACAGAACTTCGATGCCAATGCATAAAAAACAT	120
gi 61676045 B.bG.....	120
gi 223931031 B.bG.....	120
gi 353677864 B.bG.....	98
gi 31343250 B.tA.....	120
gi 56549076 B.tA.....	120
gi 73587442 B.tA.....	120
gi 161579167 B.tA.....	120
gi 1699353 B.tA.....	120
gi 169743018 B.iA.....	120
gi 156753152 O.cA.....	120
gi 427197617 O.aA.....	120
gi 463253 O.aA.....	120
gi 528775322 O.aA.....	120
gi 786590 sh O.aA.....	120
gi 548470620 C.hA.....	120
Egyptian buffalo	TCCACACCTTTCACCCCAAAATTTATCAAAGAAATGAGAGTTATCGAGAGTGGCCACAC	180
gi 61676045 B.bC.....	180
gi 223931031 B.bC.....	180
gi 353677864 B.bC.....	158
gi 31343250 B.tT.....	180
gi 56549076 B.ty.....T.....	180
gi 73587442 B.tT.....	180
gi 161579167 B.tT.....	180
gi 1699353 B.tT.....	180
gi 169743018 B.iT.....	180
gi 156753152 O.cT.....	180
gi 427197617 O.aC.....T.....	180
gi 463253 O.aC.....T.....	180
gi 528775322 O.aC.....T.....	180
gi 786590 O.aC.....T.....	180
gi 548470620 C.hT.....	180
Egyptian buffalo	TGTGAAAATTCAGAAATCATTGTTAAGCTTACCAATGGAAGAGGCTCGCTTAAACCCC	240
gi 61676045 B.b	240
gi 223931031 B.b	240
gi 353677864 B.b	218
gi 31343250 B.tC.....C.....	240
gi 56549076 B.tT.....C.....	240
gi 73587442 B.tC.....	240
gi 161579167 B.tC.....	240
gi 1699353 B.tC.....	240
gi 169743018 B.iC.....	240
gi 156753152 O.cC.....C.....G.....G.....	240
gi 427197617 O.aC.....C.....G.....G.....	240
gi 463253 O.aC.....C.....G.....G.....	240
gi 528775322 O.aC.....C.....G.....G.....	240
gi 786590 O.aC.....C.....G.....G.....	240
gi 548470620 C.hC.....C.....G.....G.....	240
Egyptian buffalo	AAGGAAAAGTGGGTGCGAAGGTTGTGCAAGTATTTGTGAGAGAGCTGAGAAGCAAGAT	300
gi 61676045 B.bA.....	300
gi 223931031 B.bA.....	300
gi 353677864 B.bA.....	263
gi 31343250 B.tG.....	300
gi 56549076 B.tG.....	300
gi 73587442 B.tG.....	300
gi 161579167 B.tG.....	300
gi 1699353 B.tG.....	300
gi 169743018 B.iG.....	300
gi 156753152 O.cG.....C.....T.....	300
gi 427197617 O.aG.....C.....T.....	300
gi 463253 O.aG.....C.....T.....	300
gi 528775322 O.aG.....C.....T.....	300
gi 786590 O.aG.....C.....T.....	300
gi 548470620 C.hG.....C.....T.....	300
Egyptian buffalo	CCATGA 306	
gi 61676045 B.b 306	
gi 223931031 B.b 306	
gi 353677864 B.b 263	
gi 31343250 B.t 305	
gi 56549076 B.t 306	
gi 73587442 B.t 306	
gi 161579167 B.t 306	
gi 1699353 B.t 306	
gi 169743018 B.i 306	
gi 156753152 O.c 306	
gi 427197617 O.a 306	
gi 463253 O.a 306	
gi 528775322 O.a 306	
gi 786590 O.a 306	
gi 548470620 C.h 305	

Figure 4 Alignment of IL-8 complete coding region nucleotide sequence between Egyptian buffalo, *Bubalus bubalis* (B.b), *Bos taurus* (B.t), *Bos indicus* (B.i), *Ovis aries* (O.a), *Ovis canadensis* (O.c) and *Capra hircus* (C.h).

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Bubalus      MTSKLAVALLAAFLLSAALCEAAVLSRMSTELRCQCIKTHSTPFHPKFIKELRVIESGPH
Bos          MTSKLAVALLAAFLLSAALCEAAVLSRMSTELRCQCIKTHSTPFHPKFIKELRVIESGPH
O.aries      MTSKLAVALLAAFLLSAALCEAAVLSRMSTELRCQCIKTHSTPFHPKFIKELRVIESGPH
O.canadensis MTSKLAVALLAAFLLSAALCEAAVLSRMSTELRCQCIKTHSTPFHPKFIKELRVIESGPH
Capra        MTSKLAVALLAAFLLSAALCEAAVLSRMSTELRCQCIKTHSTPFHPKFIKELRVIESGPH
*****

Bubalus      CENSEIIVKLTNGKEVCLNPKEKVVQKVVQVVFVKRAEKQDP
Bos          CENSEIIVKLTNGNEVCLNPKEKVVQKVVQVVFVKRAEKQDP
O.canadensis CENSEIIVKLTNGKEVCLDPKEKVVQKVVQVAFVKRAEKQDP
O.aries      CENSEIIVKLTNGKEVCLDPKEKVVQKVVQVAFVKRAEKQDP
Capra        CENSEIIVKLTNGKEVCLDPKEKVVQKVVQVAFVKRAEKQDP
*****;****;*****.*:*****

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Figure 5 Alignment of IL-8 transcripts of buffalo (*Bubalus* sp.), cattle (*Bos* sp.), sheep subspecies (*O. aries* and *O. canadensis*) and Goat (*Capra* sp.).

4. Discussion

IL-8 was detected long ago as the founding member of the chemokine superfamily. IL-8 expression was investigated in Egyptian buffalo with and without endometritis. Bacteriological examination of uteruses of buffalo with endometritis revealed the presence of *Escherichia coli*. Most studies suggested that *E. coli* is considered the most important pathogen in the genital tract of bovine [25,27,32,37]. Examination of uteruses of buffalo with endometritis also revealed the presence of *Staphylococcus* species such as *epidermidis* and *aureus*, *Streptococcus pyogenes*, *Proteus vulgaris* as well as *Micrococcus*, *Klebsiella pneumonia* and *Clostridium perfringens*. These bacteria have been reported earlier as additional flora with the major uterine pathogen [26]. Pathogenic bacteria were reported to play an important role in the process of occurrence and deterioration of endometritis [28]. Uterine bacterial contamination and uterine inflammation cause damage to the endometrium and embryo, delay ovulation, shorten or extend luteal phase after ovulation, increase time to first insemination, decrease conception rates, increase time to conception, and increase pregnancy loss [9]. It causes economic losses due to longer calving intervals, increased culling rates and the costs of treatment and extra services [17].

IL8-expression was investigated in mammary glands, uteruses and ovaries of Egyptian buffalo with and without endometritis. The results showed a significant increase (26.6-fold) in IL8 expression only in uteruses of buffalo with endometritis compared to buffalo without endometritis. No significant differences were found in IL8 expression in ovary and mammary gland. Bacterial or viral products can rapidly induce IL8 by ten- to 100-fold [23]. A significant increase in IL-8 gene expression in cows diagnosed with subclinical endometritis was reported [16]. They suggested that a lower local level of expression of pro-inflammatory cytokines in the endometrium soon after calving might impair activation of inflammation and clearance of bacteria, and lead to development of endometritis. This fact was studied in postpartum infections of the endometrium and metritis that were reported to be the common causes of delayed conception and infertility in cattle [1,9,10]. A study of different cytokines in postpartum cows with endometritis, single chemokine, such as IL-8, was found to be sufficient to monitor uterine inflammation and its expression may be useful to predict endometrial inflammation [18].

IL-8 complete cDNA nucleotide sequences of Egyptian buffalo, with and without endometritis, were 100% identical and were 100% similar to *B. bubalis* (gb|AY952930.1). In a study by Sharma et al. [33] no polymorphic sites were detected in buffalo IL-8 exon 4. However Meade et al. [30] in *in vitro* study reported the presence of promoter polymorphisms in cattle, where genetic variation in the bovine IL8 promoter that differentially regulates its expression has significant functional implications for IL8 expression *in vitro* and which may impact on susceptibility to bovine infectious disease and inflammation. IL-8 polymorphism was also reported in Chinese Holstein cattle. Chen et al. [6] identified three genotypes and reported that IL8 genotype significantly correlated with mastitis resistance. They also reported a significant association of the IL8 mutations with milk yield and milk protein yield.

Similarity of IL-8 full coding region between buffalo sequences and *B. taurus* (gb|BC103310.1) was 99% with only one a.a. difference in addition to 3 other nucleotide differences which resulted in 3 synonymous amino acids. Whereas sequence similarity of buffalo and other species of subfamily Caprinae such as sheep (*O. aries* and *O. canadensis*) and goat (*Capra hircus*) amounted to 96%. A single nucleotide in *Ovis* species differentiated between *O. aries* and *O. canadensis*. Interestingly, *C. hircus* of *Capra* sp. differs from *O. aries* at the same nucleotide.

Seven distinct nucleotide differences were found between the 2 subfamilies (Bovinae and Caprinae). These differences resulted in three asynonymous amino acids and 4 synonymous amino acids. Despite the nucleotide differences between species and subspecies of Subfamily Caprinae no amino acid differences occurred.

In this study, it is clear that the variations between healthy and diseased animals, in nucleotides and consequently in amino acids, are not correlated with endometritis. It is also clear that endometritis is associated with the uterus only and does not affect the ovaries nor the mammary glands since their gene expression was found to be non significant, where the *P* values were 0.86 and 0.73, respectively.

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