

Full Length Research Paper

Partial characterization of three β -defensin gene transcripts in river buffalo and cattle

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In this study, the tracheal tissues from Egyptian river buffalo and cattle were screened for the presence of three bovine β -defensin gene transcripts. Three primer pairs were designed on the basis of published *Bos taurus* sequences for partial amplification of β -defensin 4, β -defensin 10 and β -defensin 11 complementary DNA (cDNA). The amplified cDNA products of the three genes in both buffalo and cattle were sequenced. The sequences were analyzed to verify gene identity and to identify differences with the corresponding buffalo (*Bubalus bubalis bubalis*) and/or cattle (*Bos taurus*) β -defensin mRNAs published sequences in the GenBank database. β -Defensin 4 and β -defensin 10 primer pairs amplified cDNA sequences in buffalo and cattle that corresponded to those mRNAs of the two genes in GenBank database with nucleotide percentage homology of 83 and 84% for β -defensin 4, and 87 and 90% for β -defensin10, respectively. The translated protein sequences obtained for buffalo and cattle showed protein percentage similarity of 86 and 81% for β -defensin 4, and 87.5 and 87% for β -defensin 10 with the corresponding proteins of *B. bubalis bubalis* and/or *B. taurus* in GenBank database. On the other hand, cDNA sequences amplified by β -defensin 11 primer pair in both buffalo and cattle corresponded more to lingual antimicrobial peptide (LAP) mRNAs of *B. bubalis bubalis* and *B. taurus* (94 and 82% nucleotide similarity and 92 and 77% translated-protein similarity) rather than β -defensin 11 mRNA of *B. taurus* (68 and 66% nucleotide similarity and 74 and 65.5% translated-protein similarity).

Key words: β -Defensin 4, β -defensin 10, β -defensin 11, lingual antimicrobial peptide (LAP), river buffalo, cattle.

INTRODUCTION

Antimicrobial peptides are important components of natural immunity and have been described for and isolated from plants, insects, fishes and mammals (Broekaert et al., 1995; Jin et al., 2010). They have been classified in several different families on the basis of their structural features, antimicrobial properties, and expression patterns (Boman, 1995). Defensins and cathelicidins can be considered the most important antimicrobial peptides, whose main function is to provide a first line of defense against bacterial, fungal, and viral infections both at epithelial surfaces and in phagocytic cells (Ganz and Lehrer, 1994; Lehrer and Ganz, 1999; Zhao et al., 2009).

Defensins and defensin-like molecules comprise a

diverse group of cationic antimicrobial peptides. Vertebrate defensins are classified as α -, β -, θ -defensins, according to the gene structure as well as to the placement and connectivity of the six cysteine residues in their sequence. The β -defensins represent a major branch of defensins family. They have the capacity to modulate both innate and adaptive responses to infection and inflammation (Ganz and Lehrer, 1994). Beside their antimicrobial activity, β -defensins have chemo-attractant activity for dendritic and T cells (Yang et al., 1999).

The first isolated β -defensin was from bovine respiratory tract and was named tracheal antimicrobial peptide (TAP) (Diamond et al., 1991). In addition to TAP, 16 more bovine β -defensin peptides have been isolated which include 13 bovine neutrophil β -defensins (β -defensins 1-13) (Selsted et al., 1993; Roosen et al., 2004), lingual antimicrobial peptide (LAP), putative bovine β -defensin (BBD), enteric β -defensin

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Table 1. Sequences of the tested primers.

Gene	Primers sequence (forward/reverse)	<i>Bos taurus</i> accession number	Annealing temperature (°C)	Reference product size (bp)
Bovine neutrophil defensin 4	β- CCTCTTCCTGGTCCTGTCTG GTTTCTGACTCCGCATTGG	AF014107	60	209
Bovine neutrophil defensin 10	β- CTCCTGCTCCTCTTGGTGG CCTGCAGCATTTTACTCGG	AJ567990 AJ567991	59	165
Bovine neutrophil defensin 11	β- CTCCTCGCGCTCCTCTTCC TACCACGACCTGCAGCATTTTA C	AJ567992 AJ567993	61.5	164

(EBD) (Schonwetter et al., 1995; Tarver et al., 1998; Luenser et al., 2005) and the sperm associated antigen 11 (SPAG11) (Avellar et al., 2007). The expression of bovine β -defensin peptides seems to be tissue specific where TAP is predominantly in the trachea (Diamond et al., 1991) and EBD in the intestinal epithelia (Tarver et al., 1998). Neutrophil β -defensins (β -defensins 1-13) are expressed in neutrophils of different tissues (Selsted et al., 1993). Because of the importance of the neutrophil β -defensins in innate immune response in general, neutrophil β -defensin genes of farm animals have attracted considerable attention. Molecular analysis of β -defensins is of great importance as a first step to study its evolutionary relationship and biological role. Neutrophil β -defensin genes have been characterized in many domestic animals such as cattle, sheep, goat and pig (Mahoney et al., 1995; Zhao et al., 1999; Abou Mossallam et al., 2008).

In this study, partial molecular characterization of three β -defensin gene transcripts has been performed in two farm animals reared in Egypt: River buffalo (*Bubalus bubalis bubalis*) and cattle (*Bos primigenius Taurus*, widely known as *Bos taurus*) which belong to two genera of subfamily *Bovinae*.

MATERIALS AND METHODS

Samples collection, RNA extraction and complementary DNA (cDNA) synthesis

Tracheal tissue samples from healthy Egyptian river buffalo and native cattle (referred to hereafter as buffalo and cattle) were obtained at the slaughter house for RNA extraction. Total RNA was extracted from each sample using PeqGold TriFast™ according to the manufacturer's instructions. Synthesis of cDNA was performed using Ready-To-Go You-Prime First-Strand Beads according to the manufacturer's instructions.

Polymerase chain reaction (PCR)

Specific primers (Table 1) for partial amplification of β -defensin 4, 10 and 11 cDNA were designed using known sequences of *B.*

taurus DNA published in database. Sequences of primers were determined using the software for primer design (http://www.genscript.com/gi-bin/tools/primer_genscript.cgi). The primers were synthesized by Amersham Pharmacia Biotech.

Each amplification reaction (100 μ l) contained 5 μ l of buffalo or cattle first-strand tracheal cDNA, 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 primers. The reaction mixture was overlaid with sterile mineral oil and was run in an MJ research PTC-100 Thermocycler. The reaction mixture was cycled once for 3 min at 94°C followed by 30 cycles for 1 min at 94°C, 2 min at the appropriate annealing temperature for each primer pair and 2 min at 72°C. Finally, the reaction mixture was cycled for 10 min at 72°C. Parts of the reaction products were electrophoresed on 2% agarose 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.

cDNA sequencing

PCR products were purified using Exo SAP-IT PCR Purification Kit (Applied Biosystems) following the manufacturer's recommended protocol. cDNAs amplified segments were sequenced using Big Dye TM terminator Cycle Sequencing Kit (Applied Biosystems). Nucleotide sequences were determined using ABI3700 automated DNA-sequencer (Applied Biosystems).

In silico analyses

In this study, tracheal cDNAs of buffalo and cattle were used to amplify β -defensin 4, β -defensin 10 and β -defensin 11. PCR amplified products were sequenced. Pairwise alignment of each of the amplified cDNAs and the corresponding *B. bubalis bubalis* and/or *B. taurus* mRNA sequences in GenBank database were carried out using CLUSTAL W program (Gasteiger et al., 2003). Protein translations of the determined cDNA sequences were carried out using the six frame translation analysis (<http://searchlauncher.bcm.tmc.edu/seq-util/options/sixframe.html>). CLUSTAL W program was also used in pair wise alignment of each of the translated proteins and the corresponding protein sequence of *B. bubalis bubalis* and/or *Bos taurus* in GenBank database.

RESULTS

β -Defensin 4 amplified products of both buffalo and cattle

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Buffalo      CCTCTTCCTGGTCCTGTCTGCTGGGTCAGGATTTACTCAAGGAGTAGGAAATCCTGTAAG 60
AY392452.1  CCTCTTCGTGGTCCTGTCTTCTGTGTCAGGATTTACTCAAGGAATAAGAAGCCCTCTAAG 60
*****
Buffalo      CTGTGCTAGGAATAAAGGCATCTGTGTGCCGAGCAGGTGCCCTGGAAACATGAGACAGAT 120
AY392452.1  CTGCCGTGGGAATAGAGGCGTCTGTTTGCCGATCAGGTGCCCTGGAAGACTGAGACAGAT 120
*** * *****
Buffalo      TGGCACCTGCCTCGGCCCCCGAGTAAAATGC-GCAT 155
AY392452.1  TGGCACCTGTTTCGGGCCCCGAGTACCATGCTGCA- 155
*****

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Figure 1a. Pair wise nucleotide alignment of buffalo cDNA sequence (155 bp) and *B. bubalis bubalis* β-defensin 4 mRNA (accession number: AY392452.1). "*" = Identical.

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Buffalo      LFLVLSAGSGFTQGVGNPVS CARNKGICVPSRCPGNMRQIGTCLGPPVKCA 51
AY392452.1  L FVVLSSVSGFTQGI RSP LSCRGNRGVCLPI RCPGR L RQIGTCFGRVPCC 51
**:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*:*

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Figure 1b. Pairwise comparison of the translated-protein from buffalo cDNA sequence and *B. bubalis bubalis* β-defensin 4 translated-protein (accession number: AY392452.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

were sequenced. Partial cDNA sequences of β-defensin 4 in buffalo (155 bp) and in cattle (186 bp) were submitted to GenBank with accession numbers AB299981.1 and AB297970.1, respectively. Pairwise nucleotide alignment of buffalo cDNA sequence and *B. bubalis bubalis* β-defensin 4 mRNA published in GenBank database (accession number: AY392452.1) showed 83% nucleotide similarity (Figure 1a). The buffalo translated-protein showed 63% identical amino acids (a.a.), 23% functionally equivalent a.a., and 14% functionally different a.a. (Figure 1b) with published *B. bubalis bubalis* β-defensin 4 translated-protein (accession number: AY392452.1).

Alignment of cattle β-defensin 4 cDNA sequence and *B. taurus* β-defensin 4 mRNA published in GenBank database (accession number: XM_002706761.1) showed 84% nucleotide similarity (Figure 2a). The cattle translated-protein showed 76% identical amino acids, 5% functionally equivalent a.a., 12% functionally different a.a. in addition to 7% (4 a.a.) insertions at the end of the translated a.a. polypeptide chain of the investigated cattle protein (Figure 2b) with *B. taurus* β-defensin 4 translated-protein (accession number: XM_002706761.1).

The primer pair of β-defensin 10 amplified tracheal cDNAs segments in both buffalo and cattle. Partial cDNA sequences of β-defensin 10 in buffalo (146 bp) and in cattle (135 bp) were determined. Buffalo sequence was submitted to GenBank (accession number: GQ231527.1). Buffalo β-defensin 10 cDNA sequence was aligned with *B. taurus* β-defensin 10 mRNA published in database (accession number: NM_001115084.1) since no β-defensin 10 mRNA

sequence for *B. bubalis bubalis* is available in the database. Alignment results showed 87% nucleotide similarity (Figure 3a) whereas, the buffalo translated-protein showed 75% identical amino acids, 12.5% a.a. replacements with equivalent functions, and 12.5% a.a. replacements with different functions (Figure 3b) with *B. taurus* β-defensin 10 translated-protein (accession number: NM_001115084.1).

In case of the cattle, β-defensin 10 cDNA sequence aligned with *B. taurus* β-defensin 10 mRNA (accession number: NM_001115084.1); the results indicate that the percentage of similarity between the nucleotide sequences was 90% (Figure 4a). Pairwise alignment of cattle β-defensin 10 and *B. taurus* (accession number: NM_001115084.1) translated-proteins showed 82% identical a.a., 5% functionally equivalent a.a., and 13% functionally different a.a. (Figure 4b).

β-Defensin 11- primer pair amplified tracheal cDNAs segments in both buffalo and cattle. Partial cDNA sequences of the amplified segments in buffalo (147) and in cattle (146) were determined. Nucleotide sequences of buffalo and cattle were aligned with the expected *B. taurus* β-defensin 11 mRNA sequence deduced from DNA sequences of exon 1 (accession number: AJ567992.1) and exon 2 (accession number: AJ567993.1) since no *B. bubalis bubalis* or *B. taurus* β-defensin 11 mRNA sequence are available in database. Pairwise alignment of buffalo and cattle obtained sequences with the expected *B. taurus* β-defensin 11 mRNA sequence resulted in 68 and 66% nucleotide similarity, respectively (Figures 5a and 6a).

Alignment of buffalo translated-protein and *B. taurus* β-

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Cattle          CCTCTTCTGGTCTGTCTGCTGGGTCAGGATTTACTCAAAGAGTAAGAAATCCTCAAAG 60
XM_002706761.1 CCTCTTCTGGTCTGTCTGCTGGGTCAGGATTTACTCAAAGAGTAAGAAATCCTCAAAG 60
*****

Cattle          CTGCCGTTGGAATATGGGTGTCTGTATTCCGATCTCGTTCCTTGTTAACATGAGACAGAT 120
XM_002706761.1 CTGCCGTTGGAATATGGGTGTCTGTATTCCGTTCTGTGCCGTGTAGGCATGAGACAGAT 120
*****

Cattle          TGGCACCTGTTTCGGGCCCGAGTAGACAAGCTTCATGATCAGAGCGAATTATAAAAAGTA 180
XM_002706761.1 TGGCACCTGTTTCGGGCCCGAGTAC-CATGCTGCA-----GGAGGTAGTAAAAGAAGGC 174
*****

Cattle          AAA-----GTC 186
XM_002706761.1 AAACACACGGTC 186
***          ***
    
```

Figure 2a. Pairwise nucleotide alignment of cattle cDNA sequence (186 bp) and *B. taurus* β -defensin 4 mRNA (accession number: XM_002706761.1). "*" = Identical.

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Cattle          LFLVLSAGSGFTQVRVNPQSCRWNMGVCIPISFLVNMQRIGTCFGRVVDKLDQSEL 57
XM_002706761.1 LFLVLSAGSGFTQVRVNPQSCRWNMGVCIPFLCRVGMQRIGTCFGRVPCRR---- 53
*****
                :      * .*****
    
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Figure 2b. Pairwise comparison of the translated-protein from cattle cDNA sequence and *B. taurus* β -defensin 4 translated-protein (accession number: XM_002706761.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

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Buffalo         CTCCTGCTCCTCTTGGTGGTCTGTCTTCTGGGTCAGGATTTACTCAAGGAGTAGGAAGT 60
NM_001115084.1 CTCCTGCTCCTCTTGGTGGTCTGTCTTCTGGGTCAGGATTTACTCAAGGAGTAAGAAGT 60
*****

Buffalo         AATCTAACCTGCTGGCGGAATAGAGGCATCTGTTCAGCTGAAGCAGG-GTCCTGTACGCAT 119
NM_001115084.1 TATCTAAGCTGCTGGGGGAATAGAGGCATCTGTTTGCTTAA-CAGGTGCCCTGGACGCAT 119
*****

Buffalo         GAATCAGATAGGCACCTGCTTCGGGTT 146
NM_001115084.1 GAGACAGATTGGCACCTGTTTAGCGCC 146
**  *****  *****  * * * *
    
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Figure 3a. Pairwise nucleotide alignment of buffalo cDNA sequence (146 bp) and *B. taurus* β -defensin 10 mRNA (accession number: NM_001115084.1). "*" = Identical.

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Buffalo         LLLLLLVVLSSSGSGFTQGVGSNLTCWRNRGICQLKQGPVRMNQIGTCFG 48
NM_001115084.1 LLLLLLVVLSSSGSGFTQGVRSYLSCWGNRGICLLNRCPPGRMRQIGTCLA 48
*****
                * * : ** ***** * : : * * * .***** : .
    
```

Figure 3b. Pairwise comparison of the translated-protein from buffalo cDNA sequence and *B. taurus* β -defensin 10 translated-protein (accession number: NM_001115084.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

defensin 11 translated-protein showed 57% identical amino acids, 17% functionally equivalent a.a., 11% functionally different a.a., and 15% a.a. insertions or deletions (Figure 5b) whereas, alignment of cattle translated-protein and *B. taurus* β -defensin 11 translated-protein showed 48% identical amino acids, 17.5% functionally equivalent a.a., 19% functionally different a.a., and 15.5%

a.a. insertions or deletions (Figure 6b).

12 inserted nucleotides (Figures 5a and 6a) and consequently four amino acids insertions in the translated-proteins (Figures 5b and 6b), of both buffalo and cattle investigated sequences, were found to occur at the same positions.

Because of the low similarity between the obtained

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Cattle          CTCCTGCTCCTCTTGGTGGTCCTGTCTTCTGGGTCAGGATTTACTCAAGGAGTAAGAAGT 60
NM_001115084.1 CTCCTGCTCCTCTTGGTGGTCCTGTCTTCTGGGTCAGGATTTACTCAAGGAGTAAGAAGT 60
                *****

Cattle          TATCTAAGCTGCTGGGGGAATAGAGGCATCTGTCTGCTGAGCAGGT-TCCTGTACGCAT 119
NM_001115084.1 TATCTAAGCTGCTGGGGGAATAGAGGCATCTGTTTGTCTTAA-CAGGTGCCCTGGACGCAT 119
                ***** ** *

Cattle          GAGTCAGATAGAAGGG 135
NM_001115084.1 GAGACAGATTGGCACC 135
                *** ***** *
    
```

Figure 4a. Pairwise nucleotide alignment of cattle cDNA sequence (135 bp) and *B. taurus* β -defensin 10 mRNA (accession number: NM_001115084.1). "*" = Identical.

```

Cattle          LLLLLVVLSSGSGFTQGVRSYLSCWGNRGICQLKQVPVRMSQIEG 45
NM_001115084.1 LLLLLVVLSSGSGFTQGVRSYLSCWGNRGICLLNRCPPGRMRQIGT 45
                ***** *: : * * *
    
```

Figure 4b. Pairwise comparison of the translated-protein from cattle cDNA sequence and *Bos taurus* β -defensin 10 translated-protein (accession number: NM_001115084.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

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Buffalo        CTCCTCGCGCTCCTCTTCTGGTCTGTCTGCTGGGTCAGGATTTACTCAAGGAGTAGGA 60
Bos11mRNA      CTCCTCGCGCTCCTCTTCTGGTCTGTCTGCTGGGTCAGGA-----ATAAGT 48
                ***** ** *

Buffalo        AATCCTGTAAGCTGTGCTAGGAATAAAGGCATCTGTGTGCCGAGCAGGTGCCCTGGAAAC 120
Bos11mRNA      GGTCTCTAAGCTGCCGTAGGAATGGAGGCGTCTGTATTCCGATCAGGTGCCCTGGACCC 108
                **** ***** ***** ***** * **** *****

Buffalo        ATGAGACAGATG---ATCTGCCACGATTAC----- 147
Bos11mRNA      ATGAGACAGATTGGCACCTGTTTCGGGCGCCAGTAAAA 147
                ***** * *** ** *
    
```

Figure 5a. Pairwise nucleotide alignment of buffalo cDNA sequence (147 bp) and *Bos taurus* β -defensin 11 (Bos11 mRNA) (deduced from genomic sequences accession numbers AJ567992.1 and AJ567993.1). "*" = Identical.

```

Buffalo        LLALLFLVLSAGSGFTQGVGNPVSCARNKGICVPSRCPGNMRQM-ICHDY--- 49
Bos11protein   LLALLFLVLSAGS----GISGPLSCRRNGGVCIPIRCPGPMRQIGTCFGRPVK 49
                ***** *: . * : * * * * : * : * * * * * : * .
    
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Figure 5b. Pairwise comparison of the translated-protein from buffalo cDNA sequence and *B. taurus* β -defensin 11 (Bos11) translated-protein (predicted from genomic sequences accession numbers AJ567992.1 and AJ567993.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

nucleotide sequences of buffalo and cattle and the nucleotide sequence of *B. taurus* β -defensin 11 mRNA, a blast analysis (www.NCBI.nlm.nih.gov/blast/) was run on

both buffalo and cattle obtained sequences. The highest percentage of alignment was found to be with lingual antimicrobial peptide (LAP) mRNA for both species.

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Cattle      CCCCTCGCGCTTCTCTTCTGGTCTGTTTGCTTGGTCAGGATTTACTCAAGGAGTAAGA 60
Bos11mRNA  CTCTCTCGCGCTCCTCTTCTGGTCTGTCTGTGGGTCAGGA-----ATAAGT 48
*          *          *          *          *          *          *          *          *          *
          *          *          *          *          *          *          *          *          *          *

Cattle      AATCCTCTAAGCTGTGGTAGGAATAAAGGCATCTGTGTGCCGATCAAGTGCTCTGTAAAC 120
Bos11mRNA  GGTCTCTAAGCTGCCGTAGGAATGGAGGCGTCTGTATTCCGATCAGGTGCCCTGGACCC 108
          *          *          *          *          *          *          *          *          *          *
          *          *          *          *          *          *          *          *          *          *

Cattle      ATGAGTCAGAGCGTAGCCAACCCT-----CC----- 146
Bos11mRNA  ATGAGACAGATTGGCACCTGTTTCGGGCGCCAGTAAA 146
          *          *          *          *          *          *          *          *          *          *
          *          *          *          *          *          *          *          *          *          *

```

Figure 6a. Pairwise nucleotide alignment of cattle cDNA sequence (146 bp) and *B. taurus* β -defensin 11 (Bos11 mRNA) (deduced from genomic sequences accession numbers AJ567992.1 and AJ567993.1). "*" = Identical.

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Cattle      PLALLFLVLFWSGFTQGVRNPLSCGRNKGICVPIKCSVNMSQS---VANP- 48
Bos11protein LLALLFLVLSAGS----GISGPLSCRRNGGVCIPIRCPGPMRQIGTCFGRPV 48
          *          *          *          *          *          *          *          *          *          *
          *          *          *          *          *          *          *          *          *          *

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Figure 6b. Pairwise comparison of the translated-protein from cattle cDNA sequence and *B. taurus* β -defensin 11 (Bos11) translated-protein (predicted from genomic sequences accession numbers AJ567992.1 and AJ567993.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

Buffalo sequence nucleotide similarity was 94% (Figure 7a) compared to the published *B. bubalis bubalis* LAP mRNA (accession number DQ458768.1). Pairwise alignment of buffalo translated-protein and *B. bubalis bubalis* LAP translated-protein (accession number DQ458768.1) showed 88% identical amino acids, 4% functionally equivalent a.a., 4% functionally different a.a., and 4% insertion and a deletion (Figure 7b).

Cattle sequence nucleotide similarity was 82% (Figure 8a) compared to *B. taurus* LAP mRNA (accession number NM_203435.3). Pairwise alignment of cattle translated-protein and *B. taurus* published LAP translated-protein (accession number NM_203435.3) showed 67% identical amino acids, 10% functionally equivalent a.a., and 23% functionally different a.a. (Figure 8b).

DISCUSSION

β -Defensins is the largest class of the defensin family. Their transcripts have been found in many tissues including the tracheal tissues of humans and animals (Bagnicka et al., 2010).

Tracheal tissues from Egyptian river buffalo and native cattle were screened for the presence of three bovine β -defensin gene transcripts. Three primer pairs were designed on the basis of published cattle (*B. taurus*) sequences of β -defensin 4, β -defensin 10 and β -defensin 11. Primers designed from cattle sequences have been successfully used in buffalo and usually amplify segments of the same gene with a high percentage in sequence similarity. They have been used extensively in developing the river buffalo gene map because of the

genetic conservation between cattle and buffalo, not only at the cytogenetic level (Di Meo et al., 2008) but also at the molecular level (El Nahas et al., 2001), since, they both belong to subfamily *Bovinae*.

The three β -defensins amplified cDNA products obtained in both buffalo and cattle were sequenced to verify identity and to identify differences with the corresponding buffalo (*B. bubalis bubalis*) and/or cattle (*B. taurus*) β -defensin mRNAs published sequences in the GenBank database. β -Defensin 4 and β -defensin 10 primer pairs amplified cDNA sequences in buffalo and cattle that corresponded to those mRNAs of the two genes in GenBank database with nucleotide percentage homology of 83 and 84% for β -defensin 4 and 87 and 90% for β -defensin10, respectively. The translated-protein sequences obtained for buffalo and cattle showed 63 and 76%, and 75 and 82% identical amino acids with the translated-protein sequences of β -defensin 4 and β -defensin 10, respectively, in *B. bubalis bubalis* and/or *B. taurus*.

On the other hand, β -defensin11 primer pair amplified cDNA sequences in buffalo and cattle with nucleotide percentage homology of 68 and 66%, respectively with *B. taurus* β -defensin 11 mRNA sequence. The translated-protein sequences obtained for buffalo and cattle showed only 57 and 48% identical amino acids with *B. taurus* β -defensin11 translated-protein. Because of the low nucleotide similarity between the obtained sequences in buffalo and cattle using β -defensin11 primer pair and the sequence of *B. taurus* β -defensin 11 mRNA, blast analysis was carried out to determine the nature of the amplified segments. The results show that the amplified sequences in buffalo and cattle aligned with the published mRNA sequence of lingual antimicrobial

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Buffalo          CTCCTCGCGCTCCTCTTCCTGGTCTGTCTGCTGGGTCAGGATTTACTCAAGGAGTAGGA 60
DQ458768.1      CTCCTCGCGCTCCTCTTCCTGGTCTGTCTGCTGGGTCAGGATTTACTCAAGGAGTAGGA 60
*****

Buffalo          AATCCTGTAAGCTGTGCTAGGAATAAAGGCATCTGTGTGCCGAGCAGGTGCCCTGGAAAC 120
DQ458768.1      AATCCTGTAAGCTGTGCTAGGAATAAAGGCATCTGTGTGCCGAGCAGGTGCCCTGGAAAC 120
*****

Buffalo          ATGAGACAGATG--ATCTGCCACGATTAC 147
DQ458768.1      ATGAGACAGATTGGCACCTGCCTCGG---C 147
*****      *   *****   *
    
```

Figure 7a. Pairwise nucleotide alignment of buffalo cDNA sequence (147 bp) and *B. bubalis bubalis* LAP mRNA (accession number: DQ458768.1). "*" = Identical.

```

Buffalo          LLALLFLVLSAGSGFTQGVGNPVSCARNKGICVPSRCPGNMRQM- ICHDY 49
DQ458768.1      LLALLFLVLSAGSGFTQGVGNPVSCARNKGICVPSRCPGNMRQIGTCLG- 49
*****: *
    
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Figure 7b. Pairwise comparison of the translated-protein from buffalo cDNA sequence and *B. bubalis bubalis* LAP translated-protein (accession number: DQ458768.1). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

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Cattle           CCCCTCGCGCTTCTCTTCCTGGTCCTGTTTGCTTGGTCAGGATTTACTCAAGGAGTAAGA 60
NM_203435.3      CTCCTTGCCTCCTCTTCCTGGTCCTGTCTGCTGGGTCAGGATTTACTCAAGGAGTAAGA 60
* ** * ***** *****

Cattle           AATCCTCTAAGCTGTGGTAGGAATAAAGGCATCTGTGTGCCGATCAAGTGCTCTGTAAC 120
NM_203435.3      AATCTCAAAGCTGCCGTAGGAATAAAGGCATCTGTGTGCCGATCAGGTGCCCTGGAAGC 120
*** ** ***** *****

Cattle           ATGAGTCAGAGCGTAGCCAACCCTCC 146
NM_203435.3      ATGAGACAGATTGGCACCTGTCTCGG 146
***** ***** * ** *
    
```

Figure 8a. Pairwise nucleotide alignment of cattle cDNA sequence (146 bp) and *B. taurus* LAP mRNA (accession number: NM_203435.3). "*" = Identical

```

Cattle           PLALLFLVLFWSGFTQGVNRNPLSCGRNKGICVPIKCSVNMSQSVANP 48
NM_203435.3      LLALLFLVLSAGSGFTQGVNRNSQSCRNKGICVPIRCPGSMRQIGTCL 48
***** * ***** . ** *****:*. .* * :
    
```

Figure 8b. Pairwise comparison of the translated-protein from cattle cDNA sequence and *B. taurus* LAP translated-protein (accession number: NM_203435.3). "*" = Identical, ":", "." = conserved and semi-conserved replacements with functionally equivalent amino acids, respectively.

peptide (LAP) of *B. bubalis bubalis* and *B. taurus*. The percentages of nucleotide homology between buffalo and cattle cDNA sequences and published LAP mRNA sequences of *B. bubalis bubalis* and *B. taurus* were found to be 94 and 82%, respectively whereas the translated-protein sequences of buffalo and cattle showed 88 and 67% identical amino acids with the published protein sequences of LAP in *B. bubalis bubalis* and *B. taurus*, respectively. The aforementioned results indicate that β -defensin 11 primer pair amplified LAP cDNA segments in

both Egyptian buffalo and cattle tracheal tissues rather than β -defensin11 segments as would be expected. LAP has been previously detected in tracheal tissue by Russell et al. (1996).

It is worth mentioning however that, the forward primer of β -defensin 11 aligned completely with the LAP mRNA of *B. bubalis bubalis* and *B. taurus* (accession numbers: DQ458768.1 and NM_203435.3), as for the reverse primer although four nucleotides at the 5' end mismatched LAP mRNA of the both species (the same

two above accession numbers); they will not affect amplification of LAP cDNA segment, since non-specific binding that occurs at the 5' end of the primer does not necessarily adversely affect amplifications (Yuryev, 2007).

The nucleotide differences detected in this study between the β -defensins cDNAs of Egyptian buffalo and cattle and their corresponding mRNA sequences of *B. bubalis bubalis* and *B. taurus* in GenBank database resulted in differences between the translated protein sequences of Egyptian buffalo and cattle and their corresponding translated protein sequences published in GenBank database. Some amino acids differences were due to conserved and semi-conserved replacements with functionally equivalent amino acids. Conservation of protein sequences is indicated by the presence of identical amino acid residues at analogous parts of proteins while conservation of protein structures is indicated by the presence of functionally equivalent, though not necessarily identical, amino acid residues and structures between analogous parts of proteins (Thompson et al., 1997). This means that amino acids replacements with functionally equivalent ones in the established protein sequence does not affect the protein function (Thompson et al., 1997; Mongkoltharuk et al., 2011). Subsequently, by adding the percentage of functionally equivalent amino acids to the percentage of identical amino acids, the percentages of protein similarities between buffalo and cattle protein sequences and their corresponding published sequences would be 86 and 81% for β -defensin 4 and 87.5 and 87% for β -defensin 10 and 92 and 77% for LAP, respectively.

The other detected differences in the translated protein sequences were due to amino acids replacements with functionally different ones, insertion or deletion. Replacements with functionally different amino acids, insertion or deletion in the established protein sequence may disturb the characteristic helical hydrogen bonding pattern, or some other interactions, which may modify the overall 3D structure of the protein. Such modifications may affect the protein function, and may even result in the loss of function (Mongkoltharuk et al., 2011; Zhang, 2008). One of the consequences of the principle of preservation of the integrity of the tertiary protein structure is the observation that insertions and deletions are most often found in regions between secondary structure elements, and loop regions. Insertions and deletions within secondary structure elements may simply affect the structure and function of a protein to a degree that cannot be tolerated by evolution (Mongkoltharuk et al., 2011; Zhang, 2008).

Conclusion

This study indicate that β -defensin 4 and β -defensin 10 primer pairs amplified cDNA sequences in Egyptian

buffalo and cattle that corresponded to those mRNAs of the two genes in GenBank database with nucleotide percentage homology of 83 and 84% for β -defensin 4 and 87 and 90% for β -defensin10, respectively. On the other hand, β -defensin 11 primer pair amplified cDNA sequences in both buffalo and cattle that corresponded more to lingual antimicrobial peptide (LAP) mRNAs of *B. bubalis bubalis* and *B. taurus* (94 and 82% nucleotide similarity) rather than β -defensin 11 mRNA of *B. taurus* (68 and 66% nucleotide similarity). The nucleotide differences detected in this study between the β -defensins cDNAs of Egyptian buffalo and cattle and their corresponding mRNA sequences in GenBank database, resulted in consequent differences between the translated protein sequences.

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