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Characterization of Cathlelicidin gene in river buffalo

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Abstract

Egyptian river buffalo tissues such as lung, trachea, muscle, intestine, liver, mammary gland, ovary, colon, testis, tongue, kidney, lymph node and blood, were screened for the presence of Cathlelicidin (Cathl) gene transcripts. Based on published *Bubalus bubalis* Cathl cDNA, primer pairs were designed to amplify a mRNA segment of 500bp that includes the full coding region (444bp). Cathl was expressed in trachea, lung, mammary gland, ovary, lymph node and liver. Alignment of the 500bp nucleotide full spliced Cathl mRNA of buffalo trachea and lung revealed two SNPs at nt-52(C/T) and at nt-179(C/A), of the coding region (sequences were submitted to NCBI/ GenBank with accession numbers KC354786 and AB675411, respectively). The nt-52 SNP results in two codons *C*TG and *T*TG which were both translated to Leucine, a silent mutation. SNP at nt-179 results in the two codons GAC/GA*A* translated to glutamic acid in trachea versus aspartic acid in lung which is a conserved amino acids substitution. Variations in amino acids of Cathl cDNA between Egyptian buffalo and Indian buffalo, which are also of the river type, have been investigated. The extent of intron retention in Cathlelicidin has been investigated in Egyptian buffalo Cathl cDNA, since intron-I retention has been reported previously. A set of 3 primer pairs were designed from *Bos taurus* DNA to amplify segments that cover parts of every two successive exons. The first primer pair amplified a 248 bp segment in lung. However it amplified a 353 bp segment in other tissues including the trachea which revealed the presence of intron-I (105 bp). A second primer pair, designed to amplify a 275bp in cattle DNA, amplified a fragment of 275bp in cDNA of buffalo lung, mammary gland, trachea, ovary, tongue, colon, testis, intestine, liver and blood; revealing the retention of intron-II (131bp). However retention of intron III in Egyptian buffalo was not verified. The results indicate that Cathl occurs in Egyptian buffalo in two mRNA variants: a fully spliced and introns-retaining isoforms.

Keywords: Cathlelicidin, River buffalo, NCBI (AB675411 and KC354786), Intron retention

INTRODUCTION

Cathlelicidins are a family of antimicrobial peptides acting as multifunctional effector molecules in innate immunity. Cathlelicidins range in size from 12 to 80 amino acid residues and cover a wide range of structures [11]. They are most abundantly present in the granules of phagocytic cells and also to a lesser extent in many other cell types such as mucosal epithelial cells and skin keratinocytes [31; 28 and 29]. In addition, Cathlelicidin is present in the mouth, tongue, esophagus, submucosal glands of the airway, and lung surface fluid [2] and is expressed in the genitourinary tract where it can function as a host defense barrier [24].

Cathlelicidins show marked structural diversity, they are stored in cells in an unprocessed form in which each sequence is joined to a fairly conserved N-terminal prosequence known as the "Cathlelin" domain [29].They comprise a family of mammalian proteins containing a C-terminal cationic antimicrobial domain that becomes active after being freed from the N-terminal Cathlelin portion of the holoprotein [29]. Most Cathlelicidin precursors are proteolytically cleaved when activated. Cleavage releases the Cathlelin domain and the C-terminal mature peptides with antimicrobial activities.

The unprocessed or differentially processed forms are often found in the biological fluids [28 and 29].

Cathlelicidins display broad spectrum activity and rapid killing against both Gram-positive and Gram-negative bacteria [24 and 22]. They also show inhibitory activity against certain fungi (e.g. *Candida albicans*), parasites (e.g. *Cryptosporidium parvum*) [27] and herpes simplex virus (HSV) [14]. Cathlelicidin selectively alter innate immunity in response to infection. It can alter T-cell-dependent activation of the humoral response *in vivo* and thus modulate the activities of both B and T lymphocytes [26]. Cathlelicidin preferentially promote the apoptosis of infected airway epithelium [20] and required for NK cell-mediated suppression of tumor Growth [4]. Production of Cathlelicidin is suppressed by nicotinic acetylcholine system that is activated during stress, thus increasing host susceptibility to pathogen [20].

Cathlelicidin gene contains IV exons (198; 108; 72 and 56bp) with III introns (102; 136 and 586bp), the first 3 exons comprise N-terminal signal peptide, a highly conserved prosequence and Cathlelin prodomain, while the fourth exon encodes the processing site and variable C-terminal antimicrobial peptide [30].

Cathlelicidin-4, popularly known as indolicidin, which is one of the seven bovine Cathlelicidins, was first discovered in the cytoplasmic granules of bovine neutrophils [8]. Thirty Cathlelicidin members have been found in mammalians [19]. While humans and mice each possess a single Cathlelicidin, other species such as cattle and pigs express many different Cathlelicidins [21].

In 2006, Das *et al.* [7] cloned and characterized Cathlelicidin cDNA sequence of *Bubalus bubalis* homologous to *Bos taurus* Cathlelicidin-4. Abou Mossallam [1], in her study on partial Cathlelicidin-4 in river buffalo, reported intron-I retention in trachea, mammary gland, ovary, colon, testis and intestine tissues but not in lung, tongue, muscle, liver, or blood. The present study is conducted to characterize full Cathlelicidin coding region and to further investigate intron retention in river buffalo mRNA.

MATERIALS AND METHODS

RNA Isolation and First-strand cDNA synthesis

Different tissue samples were obtained from six native river buffalo at the slaughter house. Total RNAs were isolated from the frozen tissues (lung, trachea, muscle, intestine, liver, mammary gland, ovary, colon, testis, tongue, kidney, lymph node and blood) using Trifast reagent (Peqlab, Biotechnologie GmbH) according to the manufacturer's instructions **[13].**

Complementary DNAs (cDNAs) were synthesized using the total RNAs obtained from the different tissues with the Ready-To-Go RT-PCR kit (Amersham Biosciences) according to the instructions of the manufacturer. The first-strand cDNA synthesis was prepared with 2 µg of total RNA using reverse transcriptase (FPLC pure) and the oligo (dT) primer. No PCR products were detected in the absence of reverse transcriptase, which indicated the lack of contaminating genomic DNA.

Primer design

Primer pairs were designed using cDNA sequences of *Bos taurus* and *Bubalis bubalis* published in database with different accession numbers. The sequences of the primers were determined using Primer 3 software at *http://www.genome.wi.mit.edu* **[17].** The primers were synthesized by Amersham Pharmacia Biotech.

Polymerase chain reaction (PCR)

PCR was performed using MJ research PTC-100 thermocycler using 1 cycle for 3 min. at 94^{o}C, followed by 30 cycles for 1 min. at 94^{o}C, 2 min. at 60-63^{o}C and 2 min. at 72^{o}C and finally 1 cycle for 10 min. at 72^{o}C. The reaction products were electrophoresed on 1.5% agarose in 1X- Tris acetate buffer (TAE) containing 0.8 µl of 10 mg/ml ethidium bromide.

Sequence analysis

PCR products were purified for sequenced using GeneJET™ PCR Purification Kit (Fermentas #K0701) and sequenced by Bioneer, ABI 3730XL DNA analyser. Sequence analysis and alignments were carried out using CLUSTAL-W **[9].**

RESULTS

Egyptian buffalo-Cathl cDNA has been investigated. A primer pair was designed to amplify the full Cathl cDNA (500 bp) in buffalo (Acc. # EF050453) [Table 1]. cDNAs from different tissues such as lung, mammary gland, trachea, ovary, tongue, kidney, lymph node, liver and blood have been tested. Positive PCR reaction occurred in trachea, lung, mammary gland, ovary, lymph node and liver. It was negative in tongue, kidney and blood as seen in Fig.1.

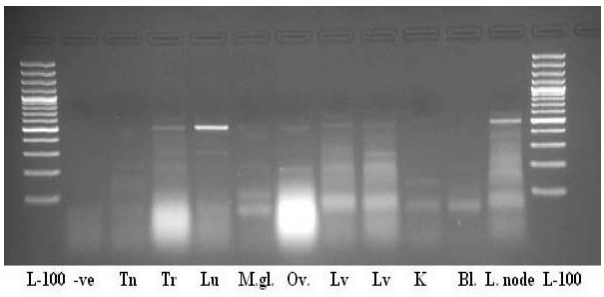

Fig. 1. Cathl cDNA amplification in different tissues of Egyptian buffalo. L-100: ladder (100 bp), -ve: negative control, Tn: tongue, Tr: trachea, Lu: lung, M.gl: mammary gland, Ov: ovary, Lv: liver, K: kidney, Bl: blood and L.node: lymph node.

Table 1. DNA sequences of the investigated primers

Primers	Sequence	Accession #	Expected size (bp)
Full Seq.	CATGCAGACCCAGAGGGCCA ATCAGACACTTAGGACTCTTCCCCG	EF050453 Bubalus bubalis mRNA	500
Part of Exon I-II	GTGGTCGCTGTGGCTACTGCTGC CTCCGCGGGCTGCTGAATCG	NW_001494125 BTA22	248
Part of Exon II-III	AATGAAGATCTGGGCACTCG GTGACTGTCCCCACACACTG	NW_001494125 BTA22	275
Part of Exon III-IV	ACAGTGTGTGGGGACAGTCA CCATTTCCAGGGTAGGATGA	NW_001494125 BTA22	681

Lung and trachea amplicons were sequenced and the nucleotide and amino acids sequences were submitted to NCBI/ GenBank with the accession numbers AB675411 and KC354786, respectively. Alignment of nucleotide sequences of full spliced Cathl mRNA of buffalo trachea and lung revealed the presence of two SNPs at nt-52(C/T) and at nt-179(C/A), of the coding region, respectively. The nt-52 SNP results in two codons CTG and TTG which were both translated to Leucine, a silent mutation. SNP at nt-179 results in the two codons GAC/GAA translated to glutamic acid in trachea versus aspartic acid in lung which is a conserved amino acid substitution.

Egyptian buffalo cDNAs were compared with riverine Indian buffalo cDNAs from bone marrow (Das et al., 2006, accession number AJ812216.1), reproductive tract cells (accession number EF050453) and myeloid cells (accession number DQ832666) [Fig. 2].

The translated amino acid sequences, presented in Fig.3, revealed that Indian buffalo reproductive Cathl sequence was 100% identical with Egyptian buffalo lung. Indian buffalo myeloid cells Cathl sequence showed 8 amino acid differences from Egyptian buffalo lung. The first variation was amino acid 15 *(Leu15Pro)*. It occurs in the signal peptide region (first 29 amino acid of coding region). Five amino acids variations occurred in the Cathelin prosequence domain: three conserved amino acids variations were detected at amino acid 61*(Glu61Asp)*, 93*(Ala93Thr)* and 95*(Gln95Arg)*, in addition to two variations at amino acid 68*(Glu68Ala)* and 126*(Ala126Glu)*. The two left amino acid variations occurred at 139*(Arg139Pro)* and 146*(Val146Phe)* in mature protein region (the last 18 amino acids of the coding region).

On the other hand, Indian buffalo bone marrow-Cathl main sequence-differences, from other buffaloes, lies in the mature peptide region. The mature peptide region comprises only 14 amino acids whereas in other buffalo sequences they were 18amino acids. Eight of 14 amino acids of the mature peptide in Indian buffalo bone marrow sequence were found to be different from other Indian and Egyptian buffalo sequences as seen in Fig. 3.

A previous report on Cathlelicidin intron-I retention in Egyptian buffalo **[1]** has directed us to investigate the extent of introns retention. To cover the whole gene, a set of 3 primer pairs, designed from *Bos taurus* DNA to amplify segments that cover parts of every two successive Exons (Table 1), has been used. The first primer pair was designed to cover parts of Exon-I and Exon-II and to amplify a PCR fragment of 248 bp in cDNA of cattle (Table 1).The results showed that in buffalo cDNA a 248bp segment was amplified in the lung sample (spliced normal form), whereas, a 353bp was amplified in a trachea sample, in addition to mammary gland, ovary, colon, testis, intestine and blood with different levels of expression as seen in Fig. 4. In order to check if intron III can also be retained, a third primer was designed to cover parts of Exon-III and Exon-IV and expected to amplify a segment of 681 bp in *Bos taurus* DNA. No PCR reaction occurred in buffalo cDNA. Several attempts were tried using newly designed primers, all attempts failed. PCR products of the 248bp and 353bp amplicons were sequenced. Alignment of the sequences with cDNA and DNA of *Bos taurus* showed that the 353bp buffalo trachea amplicon has retained intron-I (105bp).

A second primer was designed to cover parts of Exon-II and Exon-III and to amplify a PCR fragment of 275 bp in *Bos taurus* DNA. Buffalo tissues cDNAs of lung, ovary, colon, testis, tongue, intestine and trachea expressed a 275bp fragment (Fig. 5) indicating retention of intron II (131bp). Fig. 6 shows the deduced segment of the two intron-retained fragments and its alignment with bovine Cathl DNA.

DISCUSSION

Full river buffalo Cathl coding sequence (CDS) has been identified and has been compared with Indian buffalo CDS sequences from bone marrow **(**accession number AJ812216.1**);** reproductive tract cells (accession number EF050453) and myeloid cells (accession number DQ832666). Variation between river and Indian buffalo from different organs has been identified. One main difference between Indian buffalo bone marrow full Cathl sequence **[7]** on one hand, river buffalo Cathl full sequence of lung and trachea (the present results), and Cathl mRNA full sequence of Indian buffalo reproductive tract and myeloid cells on the other hand, is the deletion of 4 amino acid in mature peptide as seen in Fig. 6. It was interesting to find that cattle mRNA mature peptide (accession number NM_174827.2) also lacks these 4 amino acids. Amino acids variations between the different tissues may reflect their special needs of specific antimicrobial peptides. Lung and trachea, being equally exposed to the same environmental factors, may produce the same protein.

The present results indicate that Cathl has been expressed at different levels in river buffalo tissues. **Wang *et al.* [25]** has reported that the level of expression of a gene may be tissue specific. The results also show that Cathl occurs in river buffalo in two variants of mRNA: a fully spliced and introns-retained isoforms. Tissue specificity of intron retention has been reported by Wang et al. **[25].** We have proved in this study that retention of Cathl intron-I and intron-II has occurred in some buffalo tissues. However, we could not verify if intron-III may also be retained or not. When a PCR was conducted using primer pairs that cover parts of exons-III and IV, no reaction could be detected. This might be attributed to the mutations detected in buffalo exon-IV sequence when compared with *Bos taurus* DNA (BTA22 accession number NW_001494125).

In higher eukaryotes, it was known that the dominant form of gene expression results from constitutive splicing of intronic sequences from RNA. Most genes produce mRNA transcripts that contain several introns. Intron-retained mRNAs are thought to be prevented from being transported to the cytoplasm due to the formation of the spliceosome complex. The latter, commits the pre-mRNA to the splicing pathway **[12]**. However, intron retention, as a form of alternative splicing, has been observed to occur during the developmental control of Drosophila genes in addition to a number of other different genes such as bovine growth hormone, platelet-derived growth factor A chain, and in the transcripts encoding fibronectin **[15]**. Bovine genes show fewer alternative splicing (21%) events compared to human (68%) and mouse (57%) genes **[6]**. Alternative splicing is a major mechanism for generating functional and evolutionary diversity of proteins in mammals (reviewed by **Gelfand, [10]**). It allows for generation of novel proteins without sacrificing old ones and if a new isoform proves to be beneficial, its fraction increases by subtle regulatory changes [**18]**. Mis-spliced forms are real transcripts which could have potential regulatory functions **[5].**

In conclusion, the results of this study indicate that Cathl gene may occur in river buffalo in two mRNA variants: a fully spliced and introns-retained isoform, which may be used to produce different functional proteins, where the

intronic retention may lead to more efficient gene expression. Cathlelicidin plays an important role in controlling intracellular survival of mycobacteria. Overexpression of Cathlelicidin in macrophages significantly reduces the intracellular survival of mycobacteria relative to control cells **[23].** On the other hand, knowledge of the multiple functions and structures of Cathlelicidin provide a new lead in the design of potent antimicrobial peptides with therapeutic application. Cathlelicidin gene may be a good candidate antibiotic for preventing disease outbreaks in domestic livestock and a starting point for the development of novel synthetic antimicrobial agents for these animals.

```
Egy. lung          -----atgcagacccagagggccagcctctcgttggggcggtggtcaccgtggcttttgc
Egy. trachea       --------.....................................................c...
Ind. reprod.tract  ggacc.....................................................
Ind. bone marrow   ggacc.....................................................t...........
Ind. myeloid cells ------------------------------------.....t......a....
                                                      ********** ******  ***

Egy. lung          tgctggggcttgtggtgtcctcgaccagtgcccaggacctcagctacagggaggccgtgc
Egy. trachea.      ............................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   .......c..a.....ac.....g....c.....................................
Ind. myeloid cells .............................c.........................a.......
                   ******* ** *****  ***** **** ********************** *******

Egy. lung          ttcgtgctgtggatcagctcaatgagcggtcctcagaagctaatctctaccgcctcctgg
Egy. trachea       ............................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   ............................................................
Ind. myeloid cells .................................t...........................
                   ******************************** ****************************

Egy. lung          agctagacccgcctcccaaggatgatgcagatctgggcactcgaaagcctgtgagcttca
Egy. trachea       .......a....................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   t.........a....t.....................................................
Ind. myeloid cells .......a..a................a.....................................
                    ****** ** **** ********** *******************************

Egy. lung          cggtgaaggagactgtgtgccccaggacgactcagcagcccacggagcggtgtgacttca
Egy. trachea       ............................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   .........................................tg......a...........
Ind. myeloid cells .........................................tg......a...........
                   ************************************  ****** ***********

Egy. Lung          aggaggaagggcgggtgaagcagtgtgtggggacagtcaccctggacccgtccaatgacc
Egy. trachea       ............................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   .....a.............a.....................................
Ind. myeloid cells ............................................................
                   ***** ************* ****************************************

Egy. lung          agtttgacctaaactgtaatgagctccagagtgtcaggatacgctttccatggccatggc
Egy. trachea       ............................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   ......................c.............g.cc...c..ggat..t..t.c..t
Ind. myeloid cells .......................c.....................................
                   ********************** ************* *  *** **    ** ** * **

Egy. Lung          catggccatggtggcgcagattccgaggttgatggacaagaactgttggatcccgagccc
Egy. trachea       ............................................................
Ind. reprod.tract  ............................................................
Ind. bone marrow   g------------..ttttc..ta........g...g..t.g..a.ca.....t.....t
Ind. myeloid cells g...................g.....................................t
                               **      *  ******** *** ** * ** *  ***** *****

Egy. lung          ggggaagagtcctaagtgtctgat----------------------
Egy. trachea       ........................----------------------
Ind. reprod.tract  ........................ttgttcagattc-----------
Ind. bone marrow   ...........tagt..c.gatt.gttcagattcgggcttctggaca
Ind. myeloid cells ..............g.....ccggatcactagtgaattcg-----
                   ***********    *  *
```

Fig. 2. Nucleotide alignment of full Cath. cDNA sequences of Egyptian buffalo (Egy) lung and trachea with Indian (Ind) buffalo bone marrow (accession number AJ812216.1); reproductive tract (accession number EF050453) and myeloid cells (accession number DQ832666) sequences. *=identical

```
Egy. lung          MQTQRASLSLGRWSPWLLLLGLVVSSTSAQDLSYREAVLRAVDQLNERSSEANLYRLLEL
Ind. reprod.tract  MQTQRASLSLGRWSPWLLLLGLVVSSTSAQDLSYREAVLRAVDQLNERSSEANLYRLLEL
Ind. bone marrow   MQTQRASLSLGRWSLWLLLLGLVVPSASAQDLSYREAVLRAVDQLNERSSEANLYRLLVL
Ind. Myeloid cells ----------RWSLWLLLLGLVVSSTSAQDLSYREAVLRAVDQLNERSSEANLYRLLEL
                              *** ********* *:****************************** *

Egy. lung          DPPPKDDADLGTRKPVSFTVKETVCPRTTQQPTERCDFKEEGRVKQCVGTVTLDPSNDQF
Ind. reprod.tract  DPPPKDDADLGTRKPVSFTVKETVCPRTTQQPTERCDFKEEGRVKQCVGTVTLDPSNDQF
Ind. bone marrow   DPPLKDDADLGTRKPVSFTVKETVCPRTTQQPAEQCDFKEKGRVKQCVGTVTLDPSNDQF
Ind. Myeloid cells EPPPKDDEDLGTRKPVSFTVKETVCPRTTQQPAEQCDFKEEGRVKQCVGTVTLDPSNDQF
                   :** *** ************************:*:*****:******************

Egy. lung          DLNCNELQSVRIRFPWPWPWPWWRRFRG
Ind. reprod.tract  DLNCNELQSVRIRFPWPWPWPWWRRFRG
Ind. bone marrow   DLNCNALQSVGL----PWILLRWLFFRG
Ind. Myeloid cells DLNCNALQSVRIRFPWPWRWPWWRRVRG
                   ***** **** :    **    *   **
```

Fig.3: Amino acid Alignment of Cath. cDNA of Egyptian (Egy.) buffalo lung with Indian (Ind.) buffalo bone marrow (accession number AJ812216.1); reproductive tract (accession number EF050453) and myeloid cells (accession number DQ832666) sequences. Signal peptide region (first 29 amino acid) is in Italics; the mature peptide (18 amino acid) is underlined and the Cathelin prosequence domain is between the signal peptide and mature peptide regions

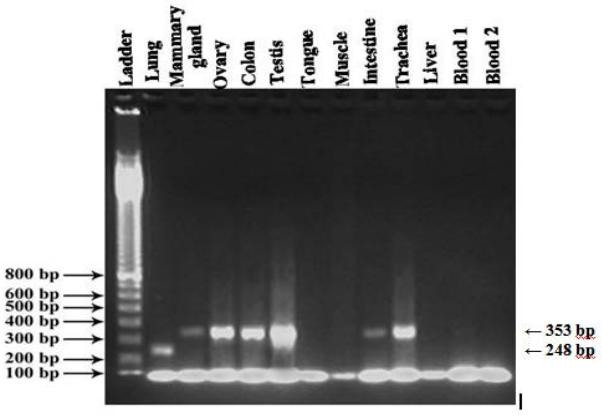

Fig. 4. Amplified PCR fragments of Cathl gene, using primer pair covering parts of Exon-I and Exon-II, in different tissues of Egyptian

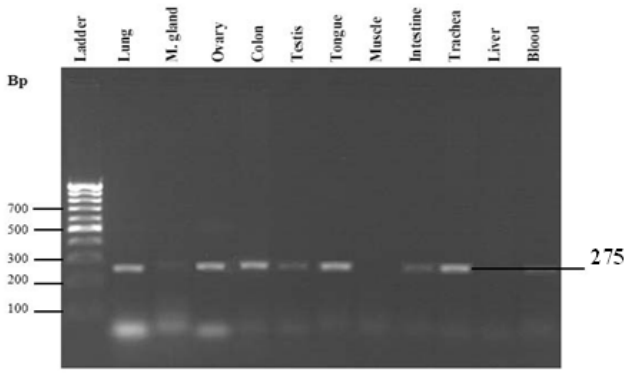

Fig. 5. Amplified PCR fragments of Cathl gene, using primer pair covering parts of Exon-II and Exon-III, in different tissues of river buffalo. The 275 arrow indicates a fragment retaining intron-II.

```
BTA22               ATGCAAACCCAGAGGGCCAGCCTCTCACTGGGGCGGTGGTCACTGTGGCTACTGCTGCTG 60
Cathl ded.seg.      ---------------------------------TGTGGTCGCTGTGGCTACTGCTGCCG 26
                                                     ****** *************** *

BTA22               GGACTAGTGGTGCCCTCGGCCAGCGCCCAAGCCCTCAGCTACAGGGAGGCCGTGCTTCGT 120
Cathl ded. Seg.     GGACTAGTGGTGCCCTCGGCCAGCGCCCAAGCCCTCAGCTACAGGGAGGCCGTGCTTCGT 86
                    ************************************************************

BTA22               GCTGTGGATCAGCTCAATGAGCTGTCCTCAGAAGCTAATCTCTACCGCCTCCTGGAGCTA 180
Cathl ded.seg.      GCTGTGGATCAGCTCAATGAGCTGTCCTCAGAAGCTAATCTCTACCGCCTCCTGGAGCTA 146
                    ************************************************************

BTA22               GACCCACCTCCCAAGGATGTGAGTTGGGGAGGGGACTGTCTAGGTGAGGGGCAGGGAGAC 240
Cathl ded.seg.      GACCCACCTCCCAAGGATGTGAGTTGGGGAGGGGACTGTCTAGGTGAGGGGCAGGGAGAA 206
                    ************************************************************

BTA22               AGA-TCAGAGAAGGAAAAATGAGCCTGAACCCAGTTTCCCCCGCGCTTTAATCCGTC-AC 298
Cathl ded. Seg.     AAAATCAGAGAAGGAAAAATGAGCCTGAACCCA-TCTCTTACATATCCCAAAAAACTGAT 265
                    * * ***************************** * **   *       **       *

BTA22               CAGAATGAAGATCTGGGCACTCGAAAGCCTGTGAGCTTCACGGTGAAGGAGACTGTGTGC 358
Cathl ded. Seg.     TGTGATGAAGATCTGGGCACTCGAAAGCCTGTGAGCTTCACGGTGAAGGAGACTGTGTGC 325
                        ************************************************************

BTA22               CCCAG-GACGATT-CAGCAGCCCGCGGAGCAGTGTGACTTCAAGGAGAAAGGGGTGAGGC 416
Cathl ded. Seg.     CCCAGAGACGACTACAGCAGCCCGCGGAGCGGTGTGACTTCAAGGAGGAAGGGGTGAGGC 385
                    ***** ***** * ***************** ***************** ************

BTA22               TGGGGGCTTGGGGTCAATGTTTCCCAGGGAGCTGAACAGGGAGCTTCTGGGAAGGTTTTC 476
Cathl ded. Seg.     TGGGGGTTGGGGGTCAATGTTTCCCAGGGAGCTGAACAGGGAGCTTCTGGGAAGGTTTCC 445
                    ****** * ************************************************** *

BTA22               TGTCTCTGGGGTGAGGCTGGGAGGTTATGGCCAAGGGGATTCCAGTTTGACCTTGAGCCT 536
Cathl ded. Seg.     TGTCTCTGGGGTGAGGCTGGGAGGTTATNGCCAAGGGGATGCCAGTTTGACCTTGAGCCT 505
                    **************************** *********** ******************

BTA22               CTCCTTCCAGCGGGTGAAACAGTGTGTGGGGACAGTCACCCTGGACCCATCCAATGACCA 596
Cathl ded. Seg.     CTCCTTCCAGCGGGTGAAACAGTGTGTGGGGACAGTCA---------------------- 543
                    ************************************

BTA22               GTTTGACCTAAACTGTAATGAGGTGAGTGGTCCCTTCTGGACTGGGGGGGTTCTAGGGAA 656
Cathl ded. Seg.     ------------------------------------------------------------

BTA22               GATAGTGTGTGGAACATCCTCTGTACCAATGACCCGCTGTCCCATCCAGGGCAGACAGAG 716
Cathl ded. Seg.     ------------------------------------------------------------
```

Fig. 6. Egyptian buffalo deduced segment of intron-I and intron-II retained fragments (Cathl ded. Seg.) and its alignment with bovine Cathl DNA (BTA22). Purple and underlined sequences: forward and reverse primers covering parts of Exon-I and II, respectively. Red and underlined sequences: forward and reverse primers covering parts of Exon-II and III, respectively. Sequences in blue: retained introns.

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