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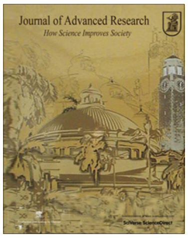

SHORT COMMUNICATION

Sequence analysis of the Toll-like receptor 2 gene of old world camels

Shyam S. Dahiya [a],*, Govindasamy Nagarajan [a], Vijay K. Bharti [b], Shelesh K. Swami [a], Sharat C. Mehta [a], Fateh C. Tuteja [a], Shirish D. Narnaware [a], Nitin V. Patil [a]

[a] *National Research Centre on Camel, Post Bag No. 7, Jorbeer, Bikaner 334 001, Rajasthan, India*
[b] *DIHAR, DRDO, Ministry of Defence, C/O-56 APO, Leh, 901205, Jammu & Kashmir, India*

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ABSTRACT

The Toll-like receptor 2 (TLR2) gene of old world camels (*Camelus dromedarius* and *Camelus bactrianus*) was cloned and sequenced. The TLR2 gene of the dromedary camel had the highest nucleotide and amino acid identity with pig, i.e., 66.8% and 59.6%, respectively. Similarly, the TLR2 gene of the Bactrian camel also had the highest nucleotide and amino acid identity with pig, i.e., 85.7% and 81.4%, respectively. Dromedary and Bactrian camels shared 77.9% nucleotide and 73.6% amino acid identity with each other. Interestingly, the amidation motif is present in camel (Dromedary and Bactrian) TLR2 only, and the TIR domain is absent in Dromedary camel TLR2. This is the first report of the TLR2 gene sequence of Dromedary and Bactrian camels.

Introduction

Old world camels (*Camelus dromedarius* and *Camelus bactrianus*) have acquired many special abilities and attributes to survive in harsh environments, including cold or hot, arid regions with poor grazing, including deserts or semidesert areas. The camel is less susceptible to many diseases that affect other livestock species, such as brucellosis [1]. Species-specific viral infections, such as camelpox [2] and contagious ecthyma [3], have been reported in Indian Dromedary camels. Camels are susceptible to foot and mouth disease, but no naturally occurring cases seem to occur [4].

Innate immunity is an evolutionarily conserved form of host defense present in invertebrate as well as vertebrate organisms. Activation of innate immunity initiates subsequent adaptive immune responses. The ability to recognize microorganisms depends in part on a family of cell surface transmembrane receptors known as the Toll-like receptors (TLRs). TLRs are among a growing number of receptors that recognize pathogen-associated molecular patterns (PAMPs) as infectious non-self-ligands and, in response, activate an inflammatory cascade that includes recruitment of dendritic cells, the most potent antigen-presenting cells of innate immunity [5]. Although most TLRs appear to function as homodimers, TLR2 forms heterodimers with TLR1 or TLR6, each dimer

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* Corresponding author. Tel.: +91 151 2230183; fax: +91 151 2231213.
E-mail address: dshsing04@gmail.com (S.S. Dahiya).

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having different ligand specificity, thus increasing its binding repertoire [6]. Individual TLRs trigger specific biological responses. The TLR2–TLR1 heterodimer recognizes triacylated lipopeptides from Gram negative bacteria and mycoplasmas, whereas the TLR2–TLR6 heterodimer recognizes diacylated lipopeptides from Gram positive bacteria and mycoplasmas. TLR2 is also involved in the recognition of viral components such as human cytomegalovirus [7]. Considering the extremely diversified and peculiar features of camels with respect to their tolerance to a variety of climatic conditions and pathogens, the role of innate immunity in camels should not be overlooked. In this study, we sequenced the TLR2 gene of both Dromedary and Bactrian camels to better understand the history of their evolution and to provide a resource for research into the immune system of camels.

Material and methods

All animal experiments were performed according to protocols approved by the institutional committee for use and care of animals (Animal ethical clearance No. 354/ÇPCSEA, National Research Centre on Camel, Bikaner, India). Total RNA was extracted from peripheral blood mononuclear cells (PBMCs), and cDNA was prepared using a standard procedure [8] from Dromedary camels maintained at NRCC, Bikaner, India, and Bactrian camels from the State Government Farm, Leh, India. To amplify the TLR2 of Dromedary and Bactrian camel, blood samples were collected by jugular vein puncture, and PBMCs were isolated by density-gradient centrifugation using Histopaque-1077 (Sigma–Aldrich). PBMCs were cultured in RPMI 1640 medium (Gibco BRL) containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 ng/mL streptomycin. Cells (1×10^7/mL) were grown in 6-well plates and stimulated with 5 μg/mL lipopolysaccharide (LPS) for 12 h. Total RNA was isolated from LPS-stimulated PBMCs using the RNA isolation kit (Bangalore Genie). An aliquot of total RNA (5 μg) was reverse-transcribed using the Easyscript First Strand cDNA Synthesis Kit (Applied Biological Materials) in a 20 μL volume reaction. TLR2 cDNA was amplified by PCR using primers designed on the basis of TLR2 nucleotide sequence of the pig (*Sus scrofa*; GenBank GQ304753). Cycling conditions for PCR were 35 cycles at 94 °C for 60 s, 57 °C for 60 s, and 72 °C for 150 s, followed by a final extension at 72 °C for 10 min. Amplified PCR products were separated on 1% agarose gels containing 10 mg/mL ethidium bromide and visualized under ultraviolet light. Purified PCR products were cloned into pGEM-T easy vector (Promega), and the resultant plasmids were transformed into *Escherichia coli* DH5α. Positive clones were confirmed by colony PCR and restriction analysis with *Eco*RI and then sequenced in both directions using universal T7 and SP6 primers at Delhi University, India. The sequences were submitted to GenBank, and the assigned accession numbers are JQ979305 (Dromedary camel) and JX453495 (Bactrian camel). The nucleotide and deduced amino acid sequences were analyzed using the BLAST program (NCBI). The resultant nucleotide and amino acid sequences of TLR2 gene from the Dromedary and Bactrian camel were assembled and analyzed with those of fifteen animal species published earlier in the GenBank (Tables 1 and 2) using BioEdit Version 7.0.9. These sequences were compared in Clustal W, and the Phylogenetic tree was constructed in MEGA4 by neighbor-joining method [9]. The functional motifs such as Prokaryotic membrane lipoprotein lipid attachment site, RGD (motif), and amidation of the gene products were predicted by using the computer software Generunner version 3.05 (hastings Software Inc. Hastings, NY, USA; http://www.generunner.net). The secondary structure of the TLR2 amino acid sequence is analyzed by protean program of DNASTAR software.

Results and discussion

TLR2 cDNA sequences of Dromedary and Bactrian camel were translated into amino acid sequences using EditSeq (DNA STAR). The ORF of Dromedary camel was found to be 1857 bp long encoding for 618 amino acid with molecular weight of 692.72 kDa. The ORF of Bactrian camel has same length of 2358 bp as that of *S. scrofa* (GQ304753) encoding

Table 1 Details of TLR-2 gene of *Camelus dromedarius* nucleotide and amino acid identity with TLR-2 of other species.

Host	Country and year of isolation	NCBI accession number	% Identity with *Camelus dromedarius*	
			Nucleotide	Amino acid
Camelus dromedarius	India, 2012	JQ979305	–	–
Camelus bactrianus	India, 2012	JX453495	77.9	73.6
Antidorcas marsupialis	UK, 2008	EU580538	66.0	57.5
Bos taurus	UK, 2006	AY634629	66.2	58.0
Boselaphus tragocamelus	India, 2011	DQ286731	66.1	57.5
Bubalus bubalis	India, 2007	EU178742	66.2	57.8
Canis lupus familiaris	Japan, 2009	AB189639	64.5	56.9
Capra hircus	India, 2011	DQ872435	66.3	57.8
Capra ibex	UK, 2008	EU580540	66.4	58.7
Cervus nippon	China, 2011	HQ260631	66.4	58.7
Equus caballus	NA	NM_001081796	66.6	59.3
Giraffa camelopardalis	UK, 2008	EU580542	66.5	59.1
Gorilla gorilla	Japan, 2008	AB445627	64.4	56.0
Homo sapiens	France, 2009	DQ012266	64.4	56.0
Ovis aries	UK, 2009	AM981300	66.2	58.4
Pongo pygmaeus	Japan, 2008	AB445628	64.5	56.0
Sus scrofa	Japan, 2003	AB085935	66.8	59.6

Table 2 Details of TLR-2 gene of *Camelus bactrianus* nucleotide and amino acid identity with TLR-2 of other species.

Host	Country and year of isolation	NCBI accession number	% Identity with *Camelus bactrianus*	
			Nucleotide	Amino acid
Camelus bactrianus	India, 2012	JQ979305	–	–
Camelus dromedarius	India, 2012	JX453495	77.9	73.6
Antidorcas marsupialis	UK, 2008	EU580538	85.1	79.8
Bos taurus	UK, 2006	AY634629	85.2	80.7
Boselaphus tragocamelus	India, 2011	DQ286731	85.3	79.8
Bubalus bubalis	India, 2007	EU178742	85.4	80.6
Canis lupus familiaris	Japan, 2009	AB189639	83.1	78.0
Capra hircus	India, 2011	DQ872435	85.1	80.1
Capra ibex	UK, 2008	EU580540	85.3	81.0
Cervus nippon	China, 2011	HQ260631	85.4	80.6
Equus caballus	NA	NM_001081796	85.6	81.2
Giraffa camelopardalis	UK, 2008	EU580542	85.4	80.5
Gorilla gorilla	Japan, 2008	AB445627	82.9	77.4
Homo sapiens	France, 2009	DQ012266	82.9	77.4
Ovis aries	UK, 2009	AM981300	84.9	80.2
Pongo pygmaeus	Japan, 2008	AB445628	83.1	77.5
Sus scrofa	Japan, 2003	AB085935	85.7	81.4

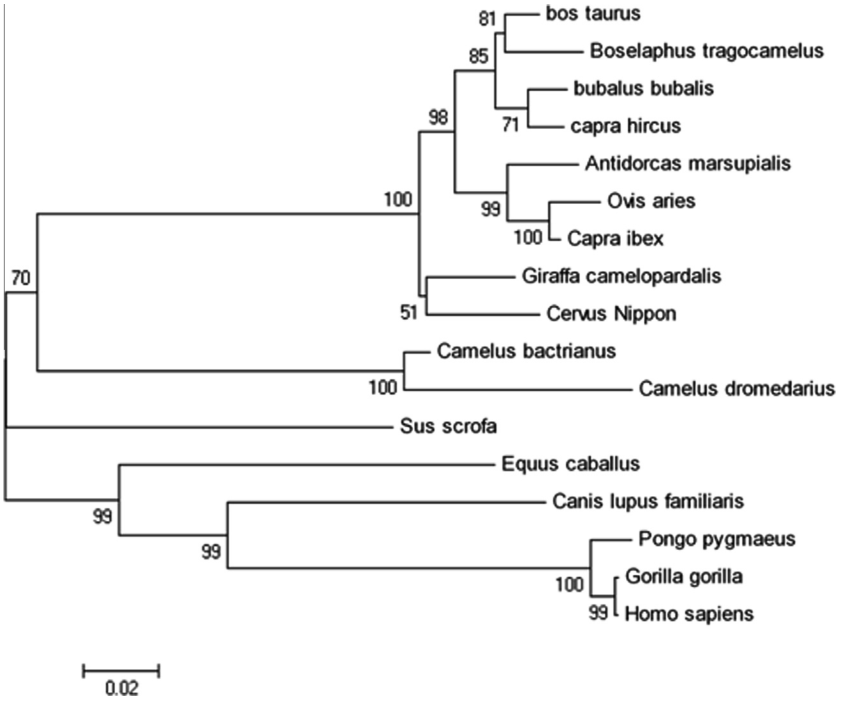
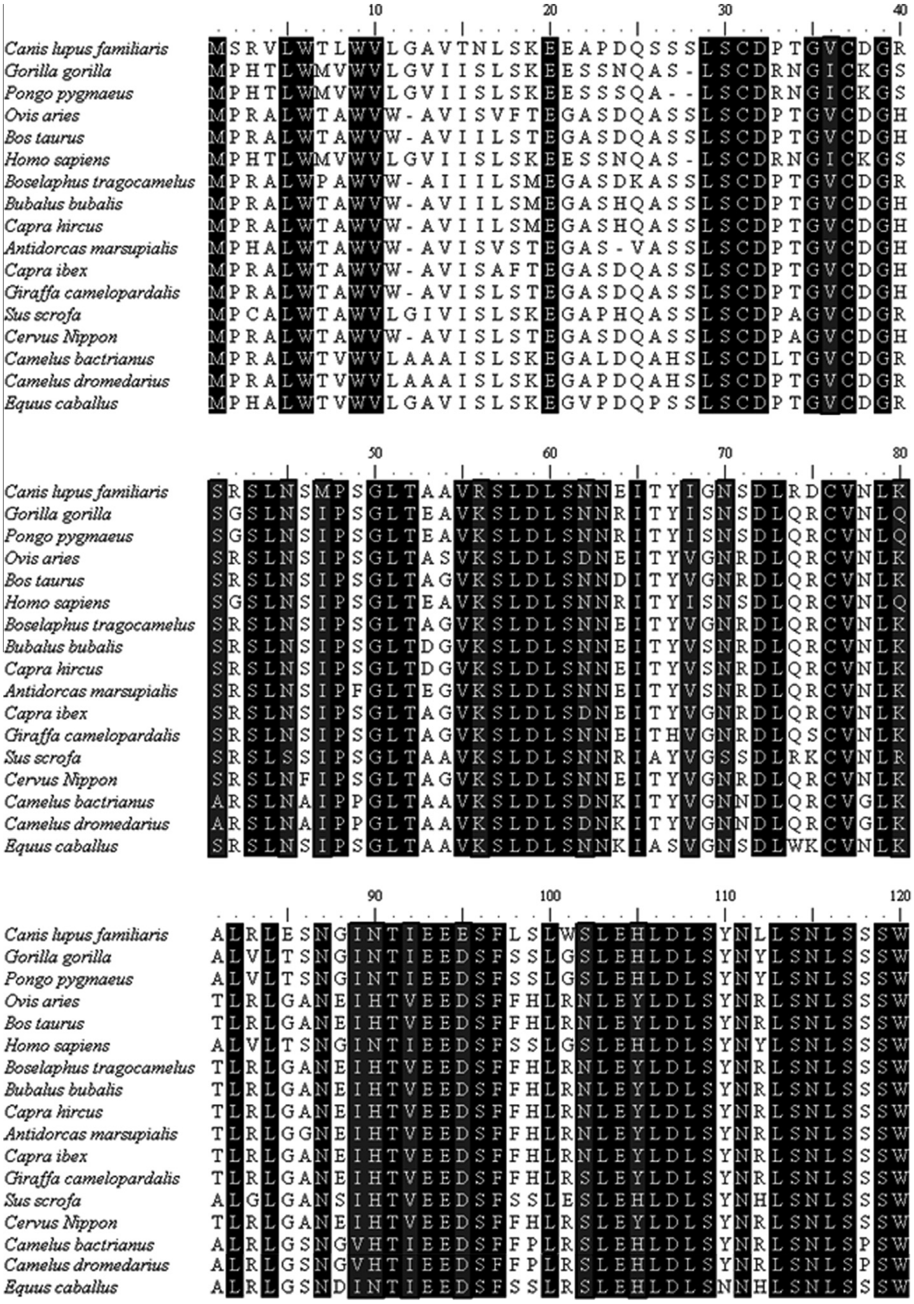


Fig. 1 Phylogenetic tree based on amino acid sequences of TLR2 gene of *Camelus dromedarius*. The tree was constructed by neighbor-joining method using Mega 4 (Molecular Evolutionary Genetics Analysis) software with bootstrap values calculated for 1000 replicates. Horizontal distances are proportional to the genetic distances. Vertical distances are arbitrary. The numbers at each branch represent bootstrap values (1000 replicates). The scale bar at the bottom measures the nucleotide distance.

785 amino acid but molecular weight of 898.27 kDa as compared to 894.99 kDa of *S. scrofa*.

Among the TLR2 gene of different species, TLR2 gene of the Dromedary camel had the highest nucleotide identity with pig (66.8%), compared to 64.4–66.6% with cattle, sheep, horse, goat, human, and other species of animals (Table 1). At the amino acid level, the predicted TLR2 protein of the Dromedary camel had highest identity with pig (59.6%), compared to 56–59.3% identity with cattle, sheep, horse, goat, human, and other species of animals. The TLR2 gene of the Bactrian camel had the highest nucleotide identity with pig (85.7%) and 82.9–85.6% with cattle, sheep, horse, goat,



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                              10        20        30        40
Canis lupus familiaris      MSRVLWTLWVLGAVTNLSKEEAPDQSSSLSCDPTGVCDGR
Gorilla gorilla             MPHTLWMVWVLGVIISLSKEESSNQAS-LSCDRNGICKGS
Pongo pygmaeus              MPHTLWMVWVLGVIISLSKEESSSQA--LSCDRNGICKGS
Ovis aries                  MPRALWTAWVW-AVISVFTEGASDQASSLSCDPTGVCDGH
Bos taurus                  MPRALWTAWVW-AVIILSTEGASDQASSLSCDPTGVCDGH
Homo sapiens                MPHTLWMVWVLGVIISLSKEESSNQAS-LSCDRNGICKGS
Boselaphus tragocamelus     MPRALWPAWVW-AIIILSMEGASDKASSLSCDPTGVCDGR
Bubalus bubalis             MPRALWTAWVW-AVIILSMEGASHQASSLSCDPTGVCDGH
Capra hircus                MPRALWTAWVW-AVIILSMEGASHQASSLSCDPTGVCDGH
Antidorcas marsupialis      MPHALWTAWVW-AVISVSTEGAS-VASSLSCDPTGVCDGH
Capra ibex                  MPRALWTAWVW-AVISAFTEGASDQASSLSCDPTGVCDGH
Giraffa camelopardalis      MPRALWTAWVW-AVISLSTEGASDQASSLSCDPTGVCDGH
Sus scrofa                  MPCALWTAWVLGIVISLSKEGAPHQASSLSCDPAGVCDGR
Cervus Nippon               MPRALWTAWVW-AVISLSTEGASDQASSLSCDPAGVCDGH
Camelus bactrianus          MPRALWTVWVLAAAISLSKEGALDQAHSLSCDLTGVCDGR
Camelus dromedarius         MPRALWTVWVLAAAISLSKEGAPDQAHSLSCDPTGVCDGR
Equus caballus              MPHALWTVWVLGAVISLSKEGVPDQPSSLSCDPTGVCDGR

                              50        60        70        80
Canis lupus familiaris      SRSLNSMPSGLTAAVRSLDLSNNEITYIGNSDLRDCVNLK
Gorilla gorilla             SGSLNSIPSGLTEAVKSLDLSNNRITYISNSDLQRCVNLQ
Pongo pygmaeus              SGSLNSIPSGLTEAVKSLDLSNNRITYISNSDLQRCVNLQ
Ovis aries                  SRSLNSIPSGLTASVKSLDLSDNEITYVGNRDLQRCVNLK
Bos taurus                  SRSLNSIPSGLTAGVKSLDLSNNDITYVGNRDLQRCVNLK
Homo sapiens                SGSLNSIPSGLTEAVKSLDLSNNRITYISNSDLQRCVNLQ
Boselaphus tragocamelus     SRSLNSIPSGLTAGVKSLDLSNNEITYVGNRDLQRCVNLK
Bubalus bubalis             SRSLNSIPSGLTDGVKSLDLSNNEITYVSNRDLQRCVNLK
Capra hircus                SRSLNSIPSGLTDGVKSLDLSNNEITYVSNRDLQRCVNLK
Antidorcas marsupialis      SRSLNSIPFGLTEGVKSLDLSNNEITYVSNRDLQRCVNLK
Capra ibex                  SRSLNSIPSGLTAGVKSLDLSDNEITYVGNRDLQRCVNLK
Giraffa camelopardalis      SRSLNSIPSGLTAGVKSLDLSNNEITHVGNRDLQSCVNLK
Sus scrofa                  SRSLSSIPSGLTAAVKSLDLSNNRIAYVGSSDLRKCVNLR
Cervus Nippon               SRSLNFIPSGLTAGVKSLDLSNNEITYVGNRDLQRCVNLK
Camelus bactrianus          ARSLNAIPPGLTAAVKSLDLSDNKITYVGNNDLQRCVGLK
Camelus dromedarius         ARSLNAIPPGLTAAVKSLDLSDNKITYVGNNDLQRCVGLK
Equus caballus              SRSLNSIPSGLTAAVKSLDLSNNKIASVGNSDLWKCVNLK

                              90        100       110       120
Canis lupus familiaris      ALRLESNGINTIEEESFLSLWSLEHLDLSYNLLSNLSSSW
Gorilla gorilla             ALVLTSNGINTIEEDSFSSLGSLEHLDLSYNYLSNLSSSW
Pongo pygmaeus              ALVLTSNGINTIEEDSFSSLGSLEHLDLSYNYLSNLSSSW
Ovis aries                  TLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Bos taurus                  TLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Homo sapiens                ALVLTSNGINTIEEDSFSSLGSLEHLDLSYNYLSNLSSSW
Boselaphus tragocamelus     TLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Bubalus bubalis             TLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Capra hircus                TLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Antidorcas marsupialis      TLRLGGNEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Capra ibex                  TLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSW
Giraffa camelopardalis      TLRLGANEIHTVEEDSFFHLRSLEYLDLSYNRLSNLSSSW
Sus scrofa                  ALGLGANSIHTVEEDSFSSLESLEHLDLSYNHLSNLSSSW
Cervus Nippon               TLRLGANEIHTVEEDSFFHLRSLEYLDLSYNRLSNLSSSW
Camelus bactrianus          ALRLGSNGVHTIEEDSFFPLRSLEHLDLSYNRLSNLSPSW
Camelus dromedarius         ALRLGSNGVHTIEEDSFFPLRSLEHLDLSYNRLSNLSPSW
Equus caballus              ALRLGSNDINTIEEDSFSSLRSLEHLDLSNNHLSNLSSSW
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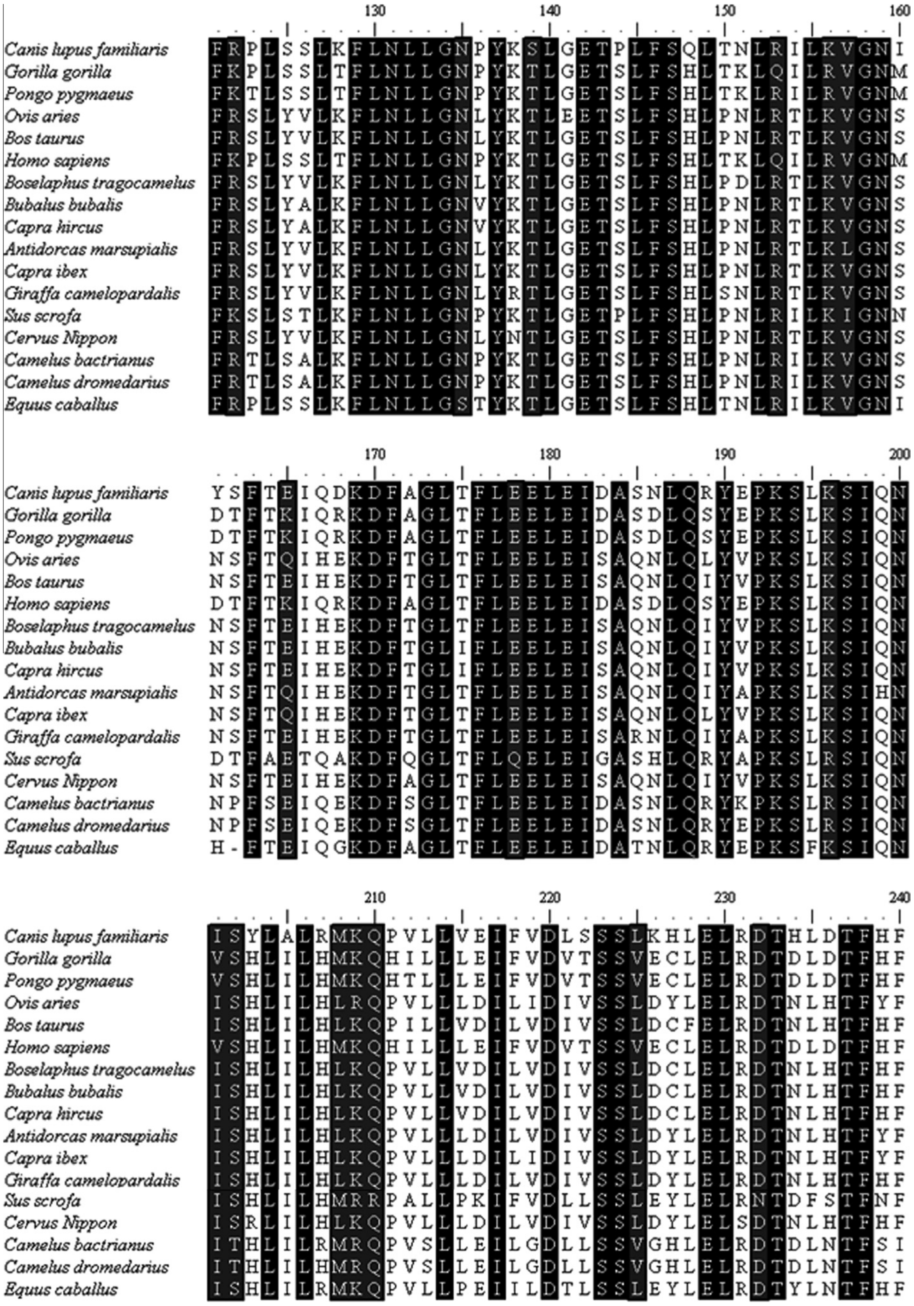
Fig. 2 Alignment of amino acid sequences of TLR2 encoding gene of Dromedary and Bactrian camel with TLR2 of other species, using the software BioEdit Version 7.0.9. Star indicates the position of amidation motif in Dromedary and Bactrian camel TLR2. Arrow denotes the RGD motifs in Bactrian and pig TLR2 and triangle denotes the position of Prokaryotic membrane lipoprotein lipid attachment site in Dromedary camel TLR2. Shaded areas indicate the conserved amino acids in the protein described.

human, and other species of animals (Table 2). At the amino acid level, the predicted TLR2 protein of the Bactrian camel had the highest identity with pigs (81.4%), compared to 77.4–81.2% with cattle, sheep, horse, goat, human, and other species of animals. Dromedary and Bactrian camels shared 77.9% nucleotide and 73.6% amino acid identity with each other. Homologues of human TLRs 1–10 are present in both cattle and sheep, with > 95% and 83–90% nucleotide sequence identity to the corresponding human reference sequences, respectively, while the degree of conservation of amino acid sequences between homologous ruminant and human TLRs is 84–97% [10]. Phylogenetic analysis of 17 TLR2 nucleotide sequences from different species using the MEGA4 program showed that the TLR2 sequence of Dromedary and Bactrian camels cluster together (Fig. 1).

The ORF of both, i.e., Dromedary and Bactrian camel TLR2 has one amidation motif at position 572 (marked with star symbol in Fig. 2). One RGD motif, i.e., cell attachment



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                                   130       140       150       160
Canis lupus familiaris       FRPLSSLKFLNLLGNPYKSLGETPLFSQLTNLRILKVGNI
Gorilla gorilla              FKPLSSLTFLNLLGNPYKTLGETSLFSHLTKLQILRVGNM
Pongo pygmaeus               FKTLSSLTFLNLLGNPYKTLGETSLFSHLTKLRILRVGNM
Ovis aries                   FRSLYVLKFLNLLGNLYKTLEETSLFSHLPNLRTLKVGNS
Bos taurus                   FRSLYVLKFLNLLGNLYKTLGETSLFSHLPNLRTLKVGNS
Homo sapiens                 FKPLSSLTFLNLLGNPYKTLGETSLFSHLTKLQILRVGNM
Boselaphus tragocamelus      FRSLYVLKFLNLLGNLYKTLGETSLFSHLPDLRTLKVGNS
Bubalus bubalis              FRSLYALKFLNLLGNVYKTLGETSLFSHLPNLRTLKVGNS
Capra hircus                 FRSLYALKFLNLLGNVYKTLGETSLFSHLPNLRTLKVGNS
Antidorcas marsupialis       FRSLYVLKFLNLLGNLYKTLGETSLFSHLPNLRTLKLGNS
Capra ibex                   FRSLYVLKFLNLLGNLYKTLGETSLFSHLPNLRTLKVGNS
Giraffa camelopardalis       FRSLYVLKFLNLLGNLYRTLGETSLFSHLSNLRTLKVGNS
Sus scrofa                   FKSLSTLKFLNLLGNPYKTLGETPLFSHLPNLRILKIGNN
Cervus Nippon                FRSLYVLKFLNLLGNLYNTLGETSLFSHLPNLRTLKVGNS
Camelus bactrianus           FRTLSALKFLNLLGNPYKTLGETSLFSHLPNLRILKVGNS
Camelus dromedarius          FRTLSALKFLNLLGNPYKTLGETSLFSHLPNLRILKVGNS
Equus caballus               FRPLSSLKFLNLLGSTYKTLGETSLFSHLTNLRILKVGNI

                                   170       180       190       200
Canis lupus familiaris       YSFTEIQDKDFAGLTFLEELEIDASNLQRYEPKSLKSIQN
Gorilla gorilla              DTFTKIQRKDFAGLTFLEELEIDASDLQSYEPKSLKSIQN
Pongo pygmaeus               DTFTKIQRKDFAGLTFLEELEIDASDLQSYEPKSLKSIQN
Ovis aries                   NSFTQIHEKDFTGLTFLEELEISAQNLQLYVPKSLKSIQN
Bos taurus                   NSFTEIHEKDFTGLTFLEELEISAQNLQIYVPKSLKSIQN
Homo sapiens                 DTFTKIQRKDFAGLTFLEELEIDASDLQSYEPKSLKSIQN
Boselaphus tragocamelus      NSFTEIHEKDFTGLTFLEELEISAQNLQIYVPKSLKSIQN
Bubalus bubalis              NSFTEIHEKDFTGLIFLEELEISAQNLQIYVPKSLKSIQN
Capra hircus                 NSFTEIHEKDFTGLIFLEELEISAQNLQIYVPKSLKSIQN
Antidorcas marsupialis       NSFTQIHEKDFTGLTFLEELEISAQNLQIYAPKSLKSIHN
Capra ibex                   NSFTQIHEKDFTGLTFLEELEISAQNLQLYVPKSLKSIQN
Giraffa camelopardalis       NSFTEIHEKDFTGLTFLEELEISARNLQIYAPKSLKSIQN
Sus scrofa                   DTFAETQAKDFQGLTFLQELEIGASHLQRYAPKSLRSIQN
Cervus Nippon                NSFTEIHEKDFAGLTFLEELEISAQNLQIYVPKSLKSIQN
Camelus bactrianus           NPFSEIQEKDFSGLTFLEELEIDASNLQRYKPKSLRSIQN
Camelus dromedarius          NPFSEIQEKDFSGLTFLEELEIDASNLQRYEPKSLRSIQN
Equus caballus               H-FTEIQGKDFAGLTFLEELEIDATNLQRYEPKSFKSIQN

                                   210       220       230       240
Canis lupus familiaris       ISYLALRMKQPVLLVEIFVDLSSSLKHLELRDTHLDTFHF
Gorilla gorilla              VSHLILHMKQHILLLEIFVDVTSSVECLELRDTDLDTFHF
Pongo pygmaeus               VSHLILHMKQHTLLLEIFVDVTSSVECLELRDTDLDTFHF
Ovis aries                   ISHLILHLRQPVLLLDILIDIVSSLDYLELRDTNLHTFYF
Bos taurus                   ISHLILHLKQPILLVDILVDIVSSLDCFELRDTNLHTFHF
Homo sapiens                 VSHLILHMKQHILLLEIFVDVTSSVECLELRDTDLDTFHF
Boselaphus tragocamelus      ISHLILHLKQPVLLVDILVDIVSSLDCLELRDTNLHTFHF
Bubalus bubalis              ISHLILHLKQPVLLVDILVDIVSSLDCLELRDTNLHTFHF
Capra hircus                 ISHLILHLKQPVLLVDILVDIVSSLDCLELRDTNLHTFHF
Antidorcas marsupialis       ISHLILHLKQPVLLLDILVDIVSSLDYLELRDTNLHTFYF
Capra ibex                   ISHLILHLKQPVLLLDILIDIVSSLDYLELRDTNLHTFYF
Giraffa camelopardalis       ISHLILHLKQPVLLLDILVDIVSSLDYLELRDTNLHTFHF
Sus scrofa                   ISHLILHMRRPALLPKIFVDLLSSLEYLELRNTDFSTFNF
Cervus Nippon                ISRLILHLKQPVLLLDILVDIVSSLDYLELSDTNLHTFHF
Camelus bactrianus           ITHLILRMRQPVSLLEILGDLLSSVGHLELRDTDLNTFSI
Camelus dromedarius          ITHLILHMRQPVSLLEILGDLLSSVGHLELRDTDLNTFSI
Equus caballus               ISHLILRMKQPVLLPEIILDTLSSLEYLELRDTYLNTFHF
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Fig 2. *(continued)*

sequence is present at position 637 and 293 in Bactrian and pig TLR2, respectively (marked with arrow symbol in Fig. 2). A Prokaryotic membrane lipoprotein lipid attachment site is present only in Dromedary camel at position 606 (marked with a triangle symbol at position 678 in Fig. 2), which is absent in all other 16 TLR2 sequences considered for analysis in the present study. It is interesting to observe that TLR2 of Dromedary camel lacks a TIR domain and a transmembrane region whereas both of these are present in Bactrian camel. These motifs may be responsible for imparting the specific biological activity to the TLR2 receptors, which further needs to be investigated experimentally so as to provide insight into their role in resistance to bacterial and viral pathogens.

The secondary structure of the TLR2 amino acid sequence is analyzed by protean program of DNASTAR software. Secondary structure analysis of TLR protein sequence of *C. dromedarius* showed higher propensity of beta sheet as compare to alpha helix. On the other hand, TLR protein sequence of *C.* bactrianus showed higher propensity of alpha helix as

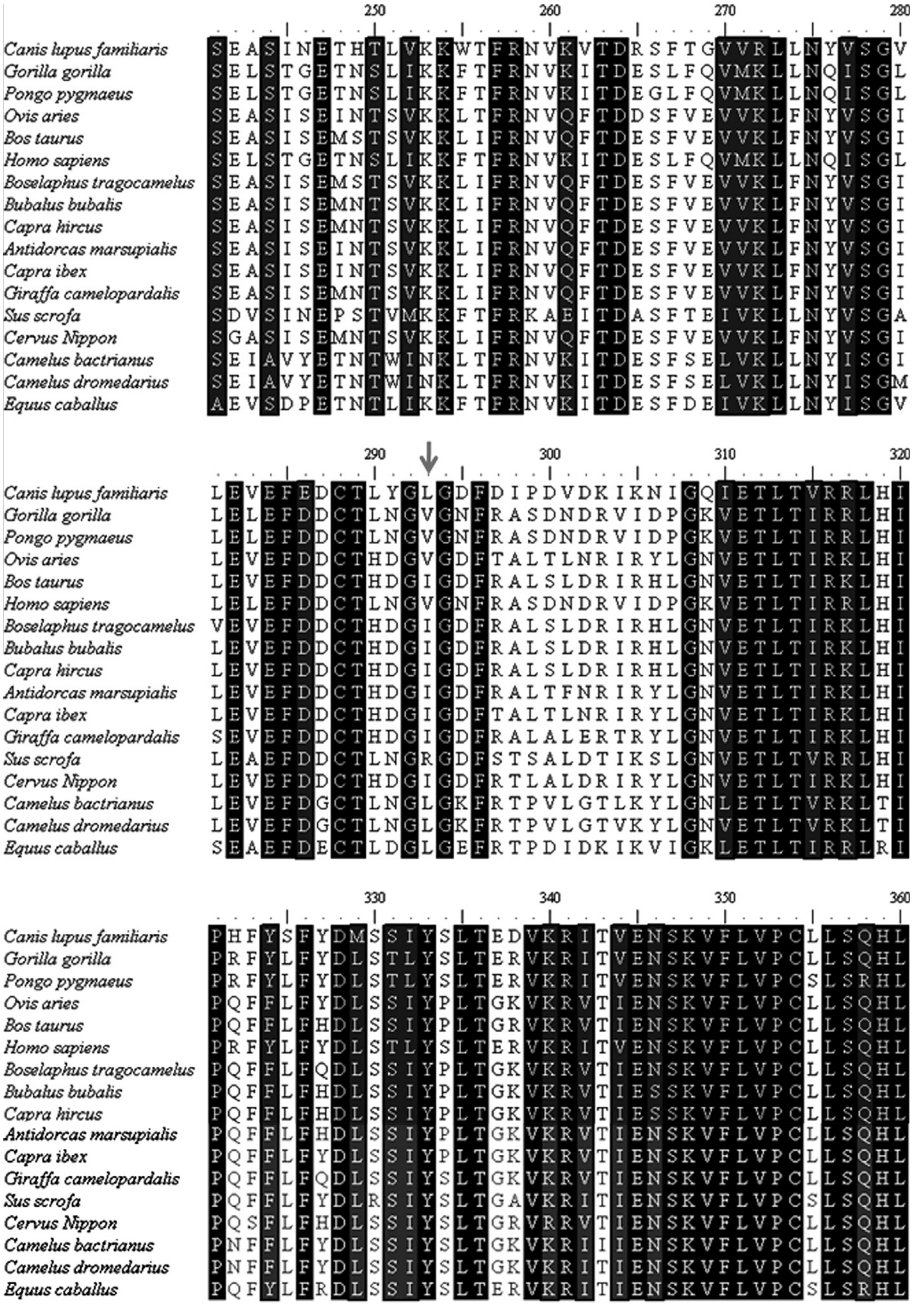

Fig 2. *(continued)*

compare to beta sheet. Both the TLR showed higher antigenicity index toward the C terminus.

Conclusions

This is the first report of the TLR2 gene sequence in Dromedary and Bactrian camels, and this information may be useful for studies of evolutionary lineages, phylogenetic analysis, and immune functions associated with bacterial infection of camels.

Conflict of interest

The authors have declared no conflict of interest.

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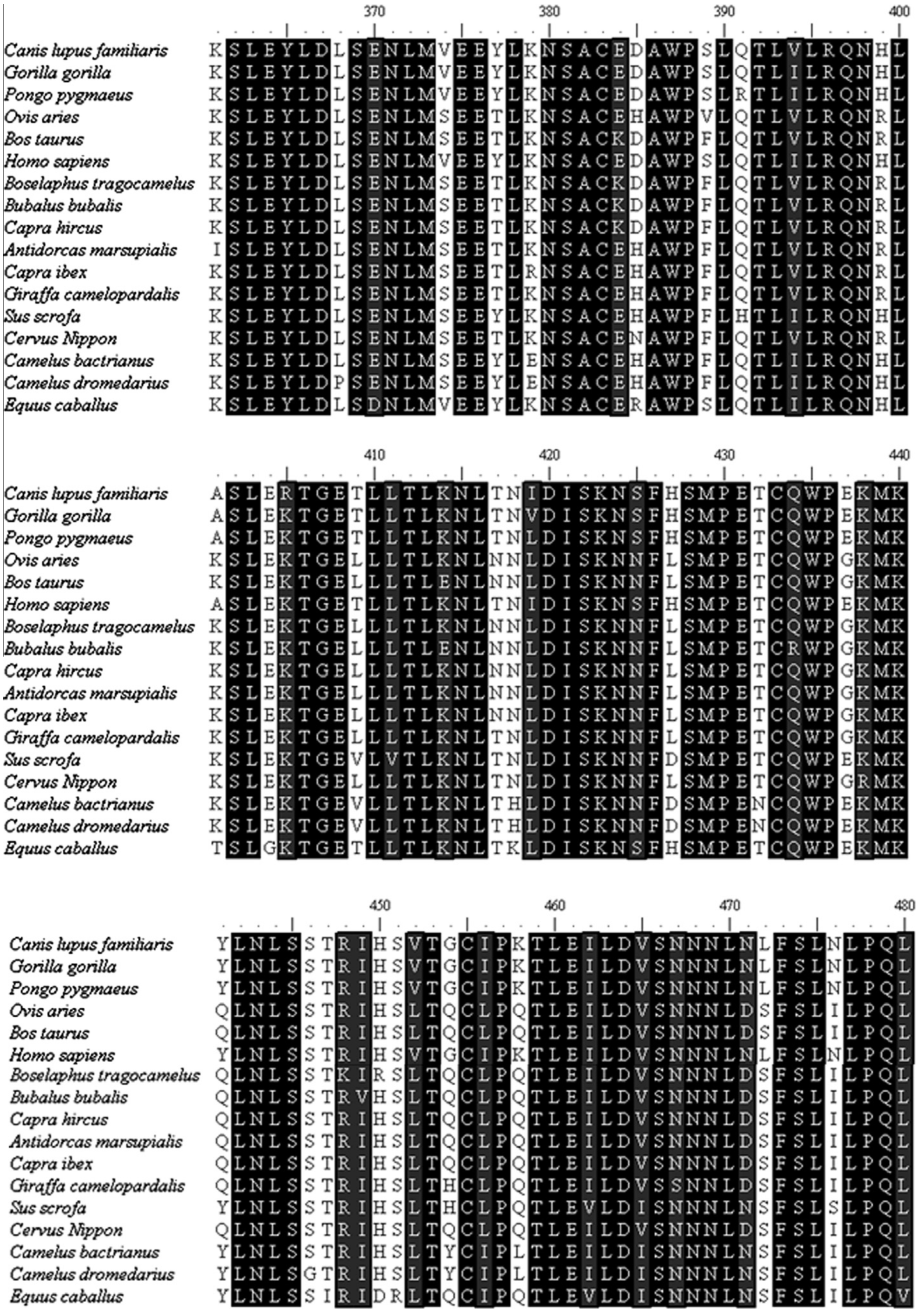

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                                 370       380       390       400
Canis lupus familiaris    KSLEYLDLSENLMVEEYLKNSACEDAWPSLQTLVLRQNHL
Gorilla gorilla           KSLEYLDLSENLMVEEYLKNSACEDAWPSLQTLILRQNHL
Pongo pygmaeus            KSLEYLDLSENLMVEEYLKNSACEDAWPSLRTLILRQNHL
Ovis aries                KSLEYLDLSENLMSEETLKNSACEHAWPVLQTLVLRQNRL
Bos taurus                KSLEYLDLSENLMSEETLKNSACKDAWPFLQTLVLRQNRL
Homo sapiens              KSLEYLDLSENLMVEEYLKNSACEDAWPSLQTLILRQNHL
Boselaphus tragocamelus   KSLEYLDLSENLMSEETLKNSACKDAWPFLQTLVLRQNRL
Bubalus bubalis           KSLEYLDLSENLMSEETLKNSACKDAWPFLQTLVLRQNRL
Capra hircus              KSLEYLDLSENLMSEETLKNSACKDAWPFLQTLVLRQNRL
Antidorcas marsupialis    ISLEYLDLSENLMSEETLKNSACEHAWPFLQTLVLRQNRL
Capra ibex                KSLEYLDLSENLMSEETLRNSACEHAWPFLQTLVLRQNRL
Giraffa camelopardalis    KSLEYLDLSENLMSEETLKNSACEHAWPFLQTLVLRQNRL
Sus scrofa                KSLEYLDLSENLMSEEYLKNSACEHAWPFLHTLILRQNHL
Cervus Nippon             KSLEYLDLSENLMSEETLKNSACENAWPFLQTLVLRQNRL
Camelus bactrianus        KSLEYLDLSENLMSEEYLENSACEHAWPFLQTLILRQNHL
Camelus dromedarius       KSLEYLDPSENLMSEEYLENSACEHAWPFLQTLILRQNHL
Equus caballus            KSLEYLDLSDNLMVEEYLKNSACERAWPSLQTLILRQNHL

                                 410       420       430       440
Canis lupus familiaris    ASLERTGETLLTLKNLTNIDISKNSFHSMPETCQWPEKMK
Gorilla gorilla           ASLEKTGETLLTLKNLTNVDISKNSFHSMPETCQWPEKMK
Pongo pygmaeus            ASLEKTGETLLTLKNLTNLDISKNSFHSMPETCQWPEKMK
Ovis aries                KSLEKTGELLLTLKNLNNLDISKNNFLSMPETCQWPGKMK
Bos taurus                KSLEKTGELLLTLENLNNLDISKNNFLSMPETCQWPGKMK
Homo sapiens              ASLEKTGETLLTLKNLTNIDISKNSFHSMPETCQWPEKMK
Boselaphus tragocamelus   KSLEKTGELLLTLKNLNNLDISKNNFLSMPETCQWPGKMK
Bubalus bubalis           KSLEKTGELLLTLENLNNLDISKNNFLSMPETCRWPGKMK
Capra hircus              KSLEKTGELLLTLKNLNNLDISKNNFLSMPETCQWPGKMK
Antidorcas marsupialis    KSLEKTGELLLTLKNLNNLDISKNNFLSMPETCQWPGKMK
Capra ibex                KSLEKTGELLLTLKNLNNLDISKNNFLSMPETCQWPGKMK
Giraffa camelopardalis    KSLEKTGELLLTLKNLTNLDISKNNFLSMPETCQWPGKMK
Sus scrofa                KSLEKTGEVLVTLKNLTNLDISKNNFDSMPETCQWPEKMK
Cervus Nippon             KSLEKTGELLLTLKNLTNLDISKNNFLSMPETCQWPGRMK
Camelus bactrianus        KSLEKTGEVLLTLKNLTHLDISKNNFDSMPENCQWPEKMK
Camelus dromedarius       KSLEKTGEVLLTLKNLTHLDISKNNFDSMPENCQWPEKMK
Equus caballus            TSLGKTGETLLTLKNLTKLDISKNSFHSMPETCQWPEKMK

                                 450       460       470       480
Canis lupus familiaris    YLNLSSTRIHSVTGCIPKTLEILDVSNNNLNLFSLNLPQL
Gorilla gorilla           YLNLSSTRIHSVTGCIPKTLEILDVSNNNLNLFSLNLPQL
Pongo pygmaeus            YLNLSSTRIHSVTGCIPKTLEILDVSNNNLNLFSLNLPQL
Ovis aries                QLNLSSTRIHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Bos taurus                QLNLSSTRIHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Homo sapiens              YLNLSSTRIHSVTGCIPKTLEILDVSNNNLNLFSLNLPQL
Boselaphus tragocamelus   QLNLSSTKIRSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Bubalus bubalis           QLNLSSTRVHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Capra hircus              QLNLSSTRIHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Antidorcas marsupialis    QLNLSSTRIHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Capra ibex                QLNLSSTRIHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Giraffa camelopardalis    QLNLSSTRIHSLTHCLPQTLEILDVSSNNLDSFSLILPQL
Sus scrofa                YLNLSSTRIHSLTHCLPQTLEVLDISNNNLNSFSLSLPQL
Cervus Nippon             QLNLSSTRIHSLTQCLPQTLEILDVSNNNLDSFSLILPQL
Camelus bactrianus        YLNLSSTRIHSLTYCIPLTLEILDISNNNLNSFSLILPQL
Camelus dromedarius       YLNLSGTRIHSLTYCIPLTLEILDISNNNLNSFSLILPQL
Equus caballus            YLNLSSIRIDRLTQCIPQTLEVLDISNNNLNSFSLILPQV
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Fig 2. *(continued)*

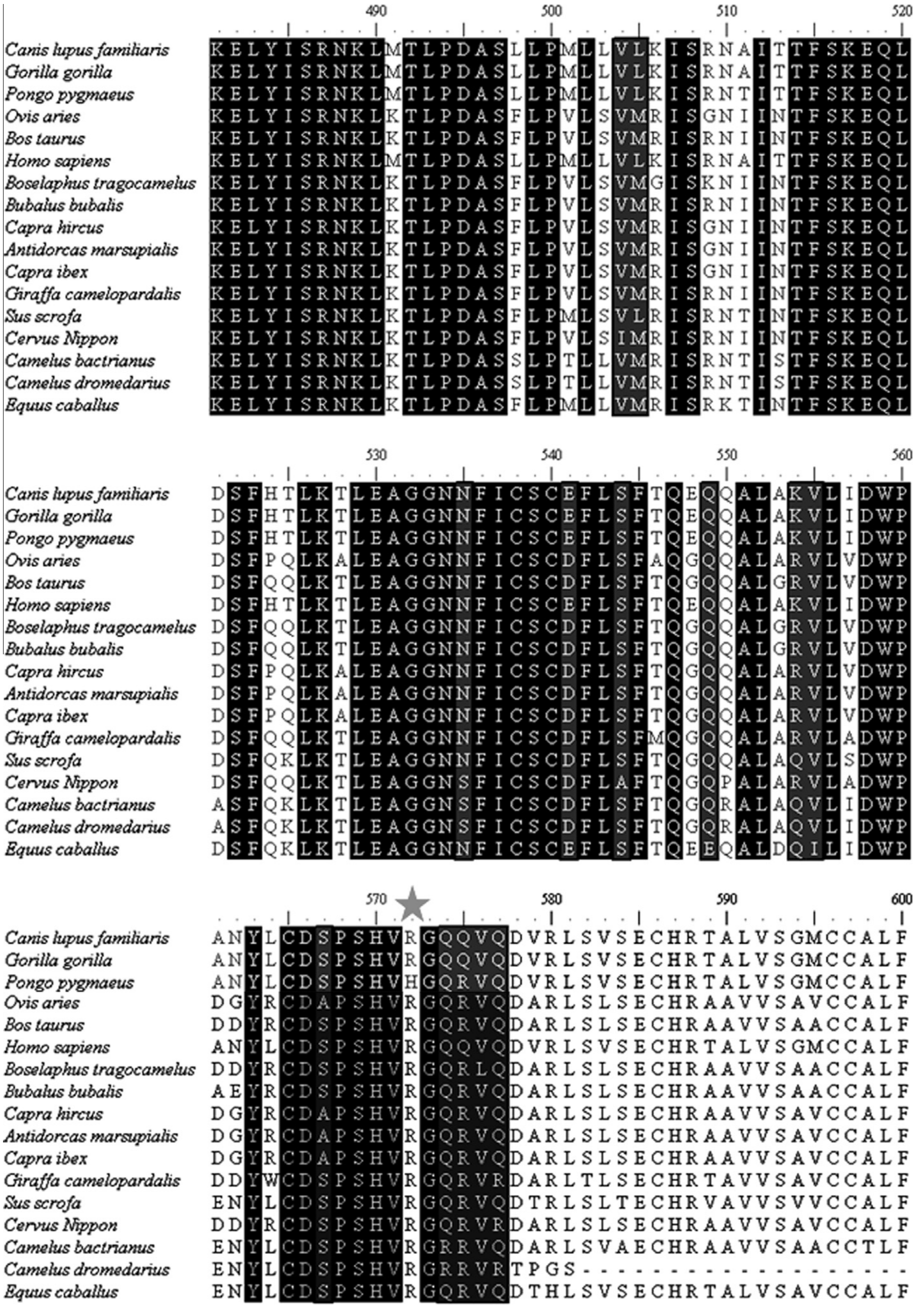
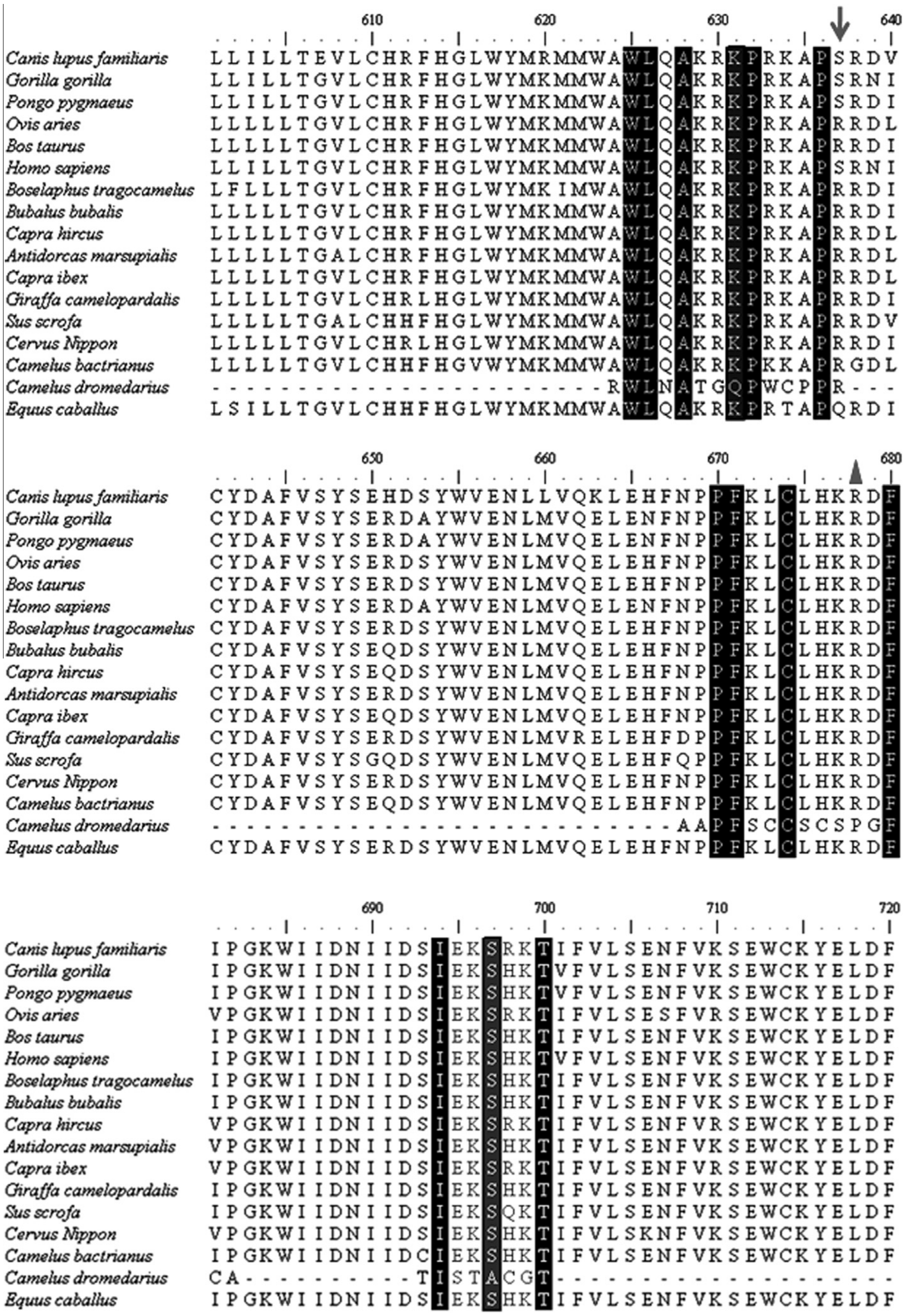


Fig 2. *(continued)*



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                                 610       620       630       640
                        ....|....|....|....|....|....|....|....|
Canis lupus familiaris  LLILLTEVLCHRFHGLWYMRMMWAWLQAKRKPRKAPSRDV
Gorilla gorilla         LLILLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPSRNI
Pongo pygmaeus          LLILLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPSRDI
Ovis aries              LLLLLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPRRDL
Bos taurus              LLLLLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPRRDI
Homo sapiens            LLILLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPSRNI
Boselaphus tragocamelus LFLLLTGVLCHRFHGLWYMKIMWAWLQAKRKPRKAPRRDI
Bubalus bubalis         LLLLLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPRRDI
Capra hircus            LLLLLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPRRDL
Antidorcas marsupialis  LLLLLTGALCHRFHGLWYMKMMWAWLQAKRKPRKAPRRDL
Capra ibex              LLLLLTGVLCHRFHGLWYMKMMWAWLQAKRKPRKAPRRDL
Giraffa camelopardalis  LLLLLTGVLCHRLHGLWYMKMMWAWLQAKRKPRKAPRRDI
Sus scrofa              LLLLLTGALCHHFHGLWYMKMMWAWLQAKRKPRKAPRRDV
Cervus Nippon           LLLLLTGVLCHRLHGLWYMKMMWAWLQAKRKPRKAPRRDI
Camelus bactrianus      LLLLLTGVLCHHFHGVWYMKMMWAWLQAKRKPKKAPRGDL
Camelus dromedarius     -----------------------RWLNATGQPWCPPR---
Equus caballus          LSILLTGVLCHHFHGLWYMKMMWAWLQAKRKPRTAPQRDI

                                 650       660       670       680
                        ....|....|....|....|....|....|....|....|
Canis lupus familiaris  CYDAFVSYSEHDSYWVENLLVQKLEHFNPPFKLCLHKRDF
Gorilla gorilla         CYDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDF
Pongo pygmaeus          CYDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDF
Ovis aries              CYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDF
Bos taurus              CYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDF
Homo sapiens            CYDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDF
Boselaphus tragocamelus CYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDF
Bubalus bubalis         CYDAFVSYSEQDSYWVENLMVQELEHFNPPFKLCLHKRDF
Capra hircus            CYDAFVSYSEQDSYWVENLMVQELEHFNPPFKLCLHKRDF
Antidorcas marsupialis  CYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDF
Capra ibex              CYDAFVSYSEQDSYWVENLMVQELEHFNPPFKLCLHKRDF
Giraffa camelopardalis  CYDAFVSYSERDSYWVENLMVRELEHFDPPFKLCLHKRDF
Sus scrofa              CYDAFVSYSGQDSYWVENLMVQELEHFQPPFKLCLHKRDF
Cervus Nippon           CYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDF
Camelus bactrianus      CYDAFVSYSEQDSYWVENLMVQELEHFNPPFKLCLHKRDF
Camelus dromedarius     ---------------------------AAPFSCCSCSPGF
Equus caballus          CYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDF

                                 690       700       710       720
                        ....|....|....|....|....|....|....|....|
Canis lupus familiaris  IPGKWIIDNIIDSIEKSRKTIFVLSENFVKSEWCKYELDF
Gorilla gorilla         IPGKWIIDNIIDSIEKSHKTVFVLSENFVKSEWCKYELDF
Pongo pygmaeus          IPGKWIIDNIIDSIEKSHKTVFVLSENFVKSEWCKYELDF
Ovis aries              VPGKWIIDNIIDSIEKSRKTIFVLSESFVRSEWCKYELDF
Bos taurus              IPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDF
Homo sapiens            IPGKWIIDNIIDSIEKSHKTVFVLSENFVKSEWCKYELDF
Boselaphus tragocamelus IPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDF
Bubalus bubalis         IPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDF
Capra hircus            VPGKWIIDNIIDSIEKSRKTIFVLSENFVRSEWCKYELDF
Antidorcas marsupialis  VPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDF
Capra ibex              VPGKWIIDNIIDSIEKSRKTIFVLSENFVRSEWCKYELDF
Giraffa camelopardalis  IPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDF
Sus scrofa              IPGKWIIDNIIDSIEKSQKTIFVLSENFVKSEWCKYELDF
Cervus Nippon           VPGKWIIDNIIDSIEKSHKTIFVLSKNFVKSEWCKYELDF
Camelus bactrianus      IPGKWIIDNIIDCIEKSHKTIFVLSENFVKSEWCKYELDF
Camelus dromedarius     CA----------TISTACGT--------------------
Equus caballus          IPGKWIIDNIIDSIEKSHKTIFVLSENFVKSEWCKYELDF
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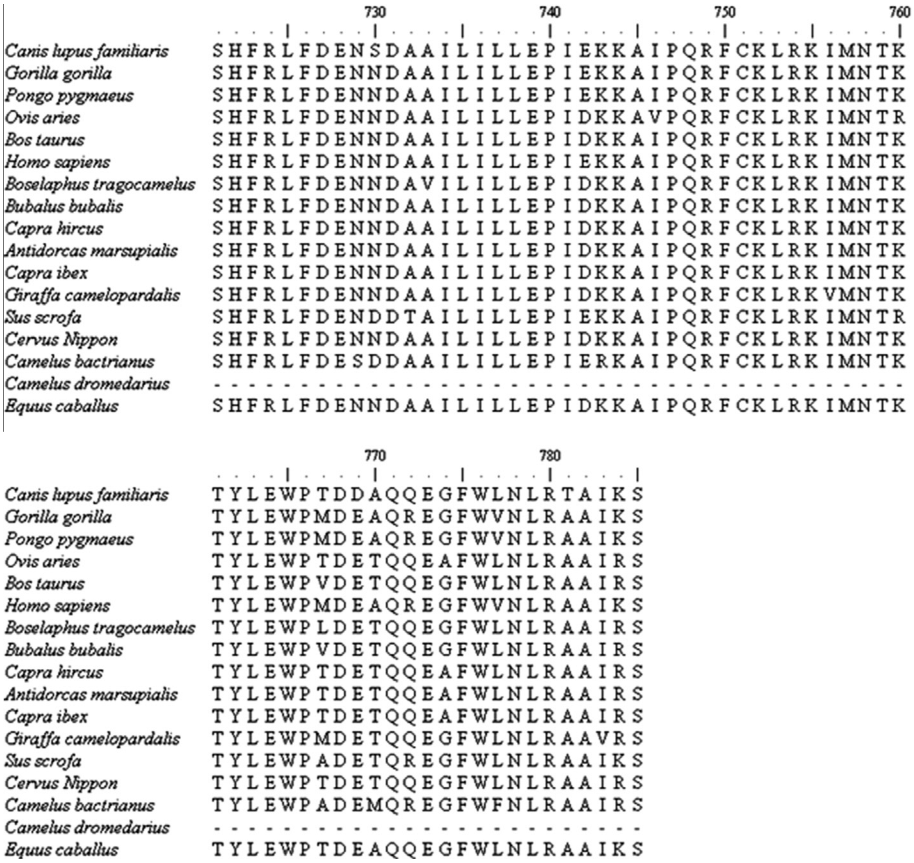
Fig 2. *(continued)*



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                                     730       740       750       760
                            ....|....|....|....|....|....|....|....|
Canis lupus familiaris      SHFRLFDENSDAAILILLEPIEKKAIPQRFCKLRKIMNTK
Gorilla gorilla             SHFRLFDENNDAAILILLEPIEKKAIPQRFCKLRKIMNTK
Pongo pygmaeus              SHFRLFDENNDAAILILLEPIEKKAIPQRFCKLRKIMNTK
Ovis aries                  SHFRLFDENNDAAILILLEPIDKKAVPQRFCKLRKIMNTR
Bos taurus                  SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK
Homo sapiens                SHFRLFDENNDAAILILLEPIEKKAIPQRFCKLRKIMNTK
Boselaphus tragocamelus     SHFRLFDENNDAVILILLEPIDKKAIPQRFCKLRKIMNTK
Bubalus bubalis             SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK
Capra hircus                SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK
Antidorcas marsupialis      SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK
Capra ibex                  SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK
Giraffa camelopardalis      SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKVMNTK
Sus scrofa                  SHFRLFDENDDTAILILLEPIEKKAIPQRFCKLRKIMNTR
Cervus Nippon               SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK
Camelus bactrianus          SHFRLFDESDDAAILILLEPIERKAIPQRFCKLRKIMNTK
Camelus dromedarius         ----------------------------------------
Equus caballus              SHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTK

                                     770       780
                            ....|....|....|....|....|
Canis lupus familiaris      TYLEWPTDDAQQEGFWLNLRTAIKS
Gorilla gorilla             TYLEWPMDEAQREGFWVNLRAAIKS
Pongo pygmaeus              TYLEWPMDEAQREGFWVNLRAAIKS
Ovis aries                  TYLEWPTDETQQEAFWLNLRAAIRS
Bos taurus                  TYLEWPVDETQQEGFWLNLRAAIRS
Homo sapiens                TYLEWPMDEAQREGFWVNLRAAIKS
Boselaphus tragocamelus     TYLEWPLDETQQEGFWLNLRAAIRS
Bubalus bubalis             TYLEWPVDETQQEGFWLNLRAAIRS
Capra hircus                TYLEWPTDETQQEAFWLNLRAAIRS
Antidorcas marsupialis      TYLEWPTDETQQEAFWLNLRAAIRS
Capra ibex                  TYLEWPTDETQQEAFWLNLRAAIRS
Giraffa camelopardalis      TYLEWPMDETQQEGFWLNLRAAVRS
Sus scrofa                  TYLEWPADETQREGFWLNLRAAIKS
Cervus Nippon               TYLEWPTDETQQEGFWLNLRAAIRS
Camelus bactrianus          TYLEWPADEMQREGFWFNLRAAIRS
Camelus dromedarius         -------------------------
Equus caballus              TYLEWPTDEAQQEGFWLNLRAAIKS
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Fig 2. *(continued)*

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