Jyoti Choudhary
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, RAJUVAS, Bikaner, Rajasthan, India

Nareshi Meena
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, RAJUVAS, Bikaner, Rajasthan, India

BN Shringi
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, RAJUVAS, Bikaner, Rajasthan, India

Comparative analysis of TLR6 of *Camelus dromedarius* across distant species

Jyoti Choudhary, Nareshi Meena and BN Shringi

DOI: https://doi.org/10.33545/2618060X.2024.v7.i1c.213

Abstract
Toll-like receptors (TLRs) constitute a multi-gene family of Pattern recognition receptors in vertebrate's genome. These are having a crucial role in defence mechanism against invading pathogens. Our present investigation was carried out to comparative analysis of the TLR6 of dromedary camel with twelve other species. At the amino acid level, the TLR6 dromedary camel showed highest similarity with *Camelus bactrianus* (99.88%) and *Camelus ferus* (99.76%) followed by *Bos taurus* (86.13%). While, minimum similarity was observed with *Oryctolagus cuniculus* (76.88%). On the basis of amino-acid sequence, phylogenetic tree revealed close relationship with camelid species followed by cattle, pig whereas, distantly placed were human, gorilla, and rabbit.

Keywords: Analysis, comparison, dromedary camel, TLR6

Introduction

The innate immune system employs germline-encoded pattern-recognition receptors (PRRs) which are expressed on the host immune cells that recognize conserved molecular structure on the surface of the microbial species called as pathogen-associated molecular patterns (PAMPs) (Janeway and Medzhitov, 2002; Kawasaki *et al*., 2014) [11, 13]. PAMPs include various bacterial cell wall components such as lipopolysaccharide (LPS), peptidoglycan (PGN) and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA matrix (Botos *et al*., 2011; Hickey *et al*., 2013) [4, 10]. Toll-like receptors (TLRs) were first identified, most well characterized PRRs (Beutler *et al*., 2006; Kawai and Akira, 2011) [3, 12]. TLRs are important for detection of intracellular and extracellular pathogens in the body and located in distinct cellular compartments (Mukherjee *et al*., 2014) [15]. Mammalian TLRs mainly have a role in innate immunity as they recognize specific PAMP in pathogens and activate immune system which defend itself against the harmful disease (Wald *et al.,* 2003; Rakoff-Nahoum and Medzhitov, 2009) [19, 17].

TLR6 widen the ligand capacity against pathogens by forms a heterodimer with TLR2. Target gene detect the diacylated lipoproteins lipopeptides of bacteria and mycoplasma. The mutations in TLR1, TLR2, and TLR6 leads the various autoimmune disorders. including sepsis, coronary artery disease, and intestinal inflammation (Choteau *et al*., 2017; Wang *et al*., 2020) [6, 20]. In current study, estimated evolutionary relationships of TLR6 among various species provides help to understand the role of target gene immune system of camel.

Materials and Methods
Retrieval and comparison of camel TLR6 protein sequence
Nucleotide sequence of TLR6 of single hump camel retrieve from NCBI's with accession number: MN807291.It was translated into amino acid through the Expert protein analysis system (ExPASY) software and acted as a proteomics server to analyze protein sequences**.** The percent amino acid identity of the camel TLR6 was compared with those of TLR6 genes from twelve other species through BLASTp (Basic Local Alignment Search Tool) (Altschul *et al*., 1990) [1]. (Table 1). The protein sequences of species (Table 1) also obtained from NCBI along with accession number.

Corresponding Author:
Jyoti Choudhary
Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Science, RAJUVAS, Bikaner, Rajasthan, India

Phylogenetic analysis

Multiple sequence alignment between TLR6 of dromedary camel with selected species (Table 1), by Clustalw multiple alignment program (http://www. ebi.ac.uk/ Tools/ clustalw2). Using a neighbor-joining (NJ) algorithm, a rooted phylogenetic tree was constructed based on the deduced amino acid sequences of TLR6 by using MEGA6 software (http://www. megasoftware.net) (Kumar *et al*., 2016) [14].

Results and Discussion

The amino acid sequence of TLR2 gene of dromedary camel was compared with *Camelus bactrianus*, *Camelus ferus*, cattle, buffalo, pig, horse, sheep, goat, orangutan, gorilla, human, dog and rabbit. Accession no. of sequences of these species are mentioned in table 1. At the amino acid level, it shared the highest identity with *Camelus bactrianus* (99.89%), *Camelus ferus* (99.76%) followed by cattle (86.13%). Rather than this, similarities showed in descending order with sheep, buffalo, pig, horse, goat, orangutan, gorilla, human, dog were 85.91%, 85.62%, 84.55%, 83.67%, 83.27%, 81.91%, 81.78%, 81.78% and 80.73%, respectively. Whereas, the *Oryctolagus cuniculus* (Rabbit) sequence was the least identical (76.88%) with target proteins. Amino acid homologies of targeted protein sequence in comparison to other species was above 70%. This confirms that TLR sequences are conserved in these vertebrates and share structural and functional similarities. Our results are in accordance with previous studies on TLR gene sequences which revealed a high degree of homology across species (Chang *et al.*, 2009; Raja *et al*., 2013) [5, 16].

Table 1: Comparison of amino acid sequence identity of *Camelus dromedarius* TLRs genes with other species

S. No.	Species	Amino acid similarity to camel (%)	Accession no.
1.	*Camelus dromedarius*	-	MN807291
2.	*Camelus bactrianus*	99.88	XM_010962677
3.	*Camelus ferus*	99.76	XM_032495973
4.	*Bos taurus* (Cattle)	86.13	EU746469
5.	*Ovis aries* (Sheep)	85.91	NM_001135927
6.	*Bubalus bubalis* (Buffalo)	85.62	HQ327992
7.	*Sus scrofa* (Pig)	84.55	AB085746
8.	*Equus caballus* (Horse)	83.67	NM_001257142
9.	*Capra hircus* (Goat)	83.27	NM_001285540
10.	*Pongo pygmaeus* (orangutan)	81.91	AB445656
11.	*Gorilla gorilla* (Gorilla)	81.78	NM_001279638
12.	*Homo sapiens* (Human)	81.78	DQ026421
13.	*Canis lupus familiaris* (Dog)	80.73	XM_022417243
14.	*Oryctolagus cuniculus* (Rabbit)	76.88	NM_001329084

In this study, a phylogenetic tree was constructed to demonstrate the evolutionary history of TLR6 reported in dromedary camel. Phylogeny is an effective tool for determining the evolutionary relationships among various species in terms of protein and its functionality (Hameed *et al*., 2020) [9]. The Neighbour-joining algorithm method was chosen as the tool to evaluate phylogenetic relationship of target gene among above mentioned species which was based on amino acid sequences of coding regions. Dahiya *et al.,* (2014) [7], Suthar *et al*., (2019) [18], Gahlot *et al*., (2023) [8], also conducted a phylogenetic analysis of dromedary camel TLR1 and TLR2 with different species and found close relatedness with bacterian camel, ruminants and pig. Conclusively, the current comparative phylogenetic analysis of TLR6 gene in dromedary camel give informative insights into the process of gene evolution.
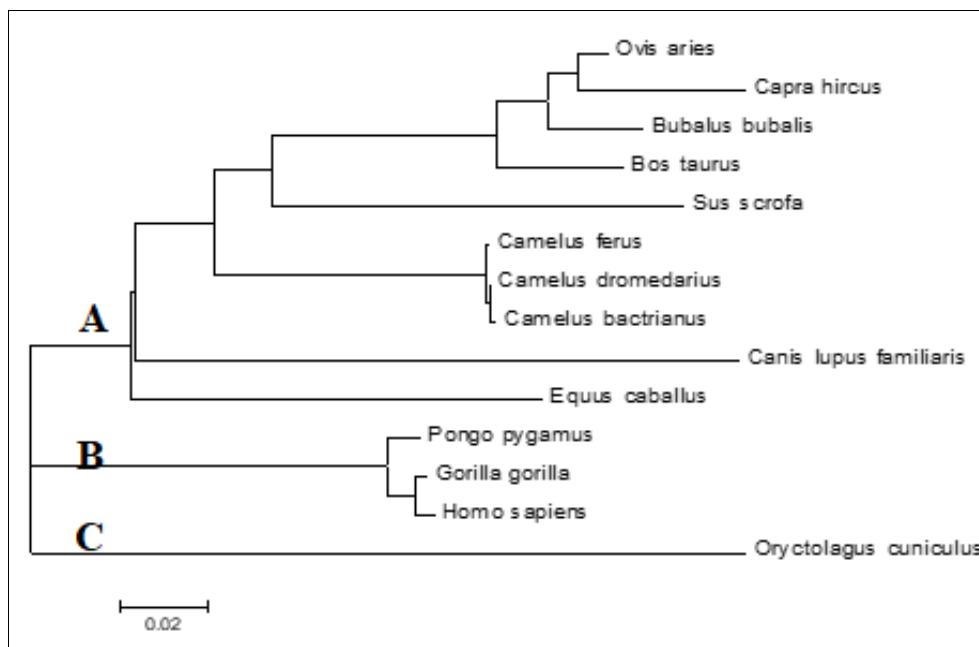

Fig 1: The species tree of TLR6 gene on the basis of amino acid sequence

As shown in Fig.1, three clades, cluster-A, cluster-B, and cluster-C were obtained by the evolutionary analysis. Cluster-A had dromedary camel, ferus camel, cattle, buffalo, sheep, goat, horse and pig. Cluster-B contained orangutan, human and gorilla whereas, rabbit belonged to the cluster-C. In this phylogram, the TLR6 of domedary camel showed a closet evolutionary relationship with bacterians camels, followed by other ruminants, pig and most distantly with rabbit (Fig. 1).

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of molecular biology. 1990 Oct 5;215(3):403-410.
2. Arif R, Ahmed S, Mustafa G. *In silico* Study to Reveal Annotation and Significant Interactions of Human Defensin with its Isoforms and their Phylogeny. Indian Journal of Pharmaceutical Sciences. 2020 May, 82(3):465-471
3. Beutler B, Jiang Z, Georgel P, Crozat K, Croker B, Rutschmann S, *et al*. Analysis of host resistance: Toll-like receptor signaling and immunity at large. Annu. Rev. Immunol. 2006 Apr; 24:353-389.
4. Botos I, Segal DM, Davies DR. The structural biology of Toll-like receptors. Structure. 2011 Apr;19(4):447-459.
5. Chang JS, Russell GC, Jann O, Glass EJ, Werling D, Haig DM, *et al*. Molecular cloning and characterization of Toll-like receptors 1-10 in sheep. Veterinary immunology and immunopathology. 2009 Jan;127(1-2):94-105.
6. Choteau L, Vancraeyneste H, Le Roy D, Dubuquoy L, Romani L, Jouault T, *et al*. Role of TLR1, TLR2 and TLR6 in the modulation of intestinal inflammation and Candida albicans elimination. Gut pathogens. 2017 Dec;9:1-3.
7. Dahiya SS, Nagarajan G, Bharti VK, Swami SK, Mehta SC, Tuteja FC, *et al*. Sequence analysis of the Toll-like receptor 2 gene of old world camels. Journal of Advanced Research. 2014 Nov;5(6):695-704.
8. Gahlot RK, Jyotsana B, Prakash V, Narnaware SD, Rathore N. Molecular characterization of toll-like receptor 2 gene in Bikaneri camels (Camelus dromedarius). The Indian Journal of Animal Sciences. 2023 Jul; 93(7):706-710.
9. Hameed U, Khalid S, Javed S, Mustafa G. 33. Importance of cytochrome oxidase subunit 1 in phylogeny reconstruction of various classes of subphylum vertebrata. Pure and Applied Biology (PAB). 2020 Aug 13;9(3):1995-2006.
10. Hickey DK, Fahey JV, Wira CR. Mouse estrous cycle regulation of vaginal versus uterine cytokines, chemokines, α-/β-defensins and TLRs. Innate immunity. 2013 Apr; 19(2):121-131.
11. Janeway Jr CA, Medzhitov R. Innate immune recognition. Annual review of immunology. 2002;20(1):197-216.
12. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity. 2011 May;34(5):637-650.
13. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Frontiers in immunology. 2014 Sep;5:461.
14. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution. 2016 Jul;33(7):1870-1874.
15. Mukherjee S, Ganguli D, Majumder PP. Global footprints of purifying selection on toll-like receptor genes primarily associated with response to bacterial infections in humans. Genome biology and evolution. 2014 Mar;6(3):551-558.
16. Raja A, Vignesh AR, Mary BA, Tirumurugaan KG, Raj GD, Kataria R, *et al*. Sequence analysis of Toll-like receptor genes 1–10 of goat (Capra hircus). Veterinary immunology and immunopathology. 2011 Apr;140(3-4):252-258.
17. Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nature reviews cancer. 2009 Jan;9(1):57-63.
18. Suthar D, Purva M, Gahlot K, Deora A, Maherchandani S. Sequence analysis of toll like receptor 1 gene of camel (*Camelus dromedarius*). Journal of Camel Practice and Research. 2019;26(1):41-47.
19. Wald D, Qin J, Zhao Z, Qian Y, Naramura M, Tian L, *et al*. SIGIRR, a negative regulator of Toll-like receptor–interleukin 1 receptor signaling. Nature immunology. 2003 Sep;4(9):920-927.
20. Wang C, Li Q, Yang H, Gao C, Du Q, Zhang C, *et al*. MMP9, CXCR1, TLR6, and MPO participant in the progression of coronary artery disease. Journal of Cellular Physiology. 2020 Nov; 235(11):8283-8292.