ISSN 0971-6777 (Print)
ISSN 2277-8934 (Online)

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com • www.indianjournals.com

Volume 27 August 2020 Number 2

In This Issue

JOURNAL OF CAMEL PRACTICE AND RESEARCH

CAMEL PUBLISHING HOUSE
Bikaner - 334001, INDIA

Manuscripts and other related correspondence may be made to :

Dr. T.K. Gahlot
Editor, Journal of Camel Practice and Research
67, Gandhi Nagar West
Near Lalgarh Palace
Bikaner-334001, INDIA

Phone : 0091-151-2527029 (R)
Mobile : 0091-9414137029

Email : tkcamelvet@yahoo.com
Website : www.camelsandcamelids.com • www.tkgahlotcamelvet.com • www.indianjournals.com

Subscription : Annual subscribers are advised to send the subscription for the year 2020 and onwards in favour of **"Camel Publishing House"** Bikaner. Renewals should invariably be done before April every year so that the number of subscribers may be ascertained before the next issue of the Journal of Camel Practice and Research (JCPR) is published.

SUBSCRIPTION RATE - 2020

ANNUAL	Rs. 4500/- or US $ 450

Note : *Subscription in Rupees is applicable to Indian subscribers only.*

Publisher : The **Journal of Camel Practice and Research** (Triannual) is published by the **"Camel Publishing House"** 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner-334001, India. Phone : 0091-151-2527029, email: tkcamelvet@yahoo.com

Cover Design: Dromedary camels running for life in Australia

Courtesy: T.K. Gahlot

Printer: Sankhla Printers, Vinayak Shikhar, Near Polytechnic College, Bikaner-334003, India.
Phone: 0091 - 151 - 2242023

CONTENTS

Volume 27 **August 2020** **Number 2**

INTERNATIONAL YEAR OF CAMELIDS, 2024

Origin of International Year of Camelids is an old story which clicked in the year 2017. Seventy-second session Agenda item 19 Resolution adopted by the United Nations General Assembly on 20 December 2017 [on the report of the Second Committee (A/72/420)] decides to declare 2024 the International Year of Camelids. Agenda noted that camelids are strictly herbivorous, even-toed ungulate mammals that first appeared in America 45 million years ago, and that there are six living species of camelids, namely, dromedary camels, Bactrian camels, llamas, alpacas, vicuñas and guanacos, in North Africa, South-West and Central Asia, Oceania and South America. Agenda further noted that camelids constitute the main means of subsistence for millions of poor families that live in the most hostile ecosystems on the planet, and that they contribute to the fight against hunger, the eradication of extreme poverty, the empowerment of women and the sustainable use of terrestrial ecosystems. Agenda encourages all Member States, the United Nations system and all other actors to take advantage of the International Year to promote awareness among the public of the economic and cultural importance of camelids and to foster the consumption of the goods produced from these mammals, including edible goods, in order to contribute to the eradication of hunger, food insecurity and malnutrition. It has requested the Food and Agriculture Organisation of the United Nations to inform the General Assembly at its eightieth session regarding the implementation of the present resolution, including an evaluation of the International Year.

As a part of education programme of http://www.texascamelcorps.com, Doug Baum is hosting an online maiden camel conference of the year 2020 entitled, "Southwest Camel Conference" on 28 October. There will be many online speakers in the conference and local participants. Congrats to Doug Baum for taking this unique initiative.

Current issue has contributions from the camelid scientists of Turkey, Egypt, UAE, Saudi Arabia, China and India. It contains papers on dromedaries, bactrians and llama. Those based on Bactrian camel research are Proteome profile of the hump and camel milk alleviates alcohol induced liver injury in mice. A case report on llama is about endocardial fibroelastosis. Remaining manuscripts are on dromedaries which include bioinformatics of pyrimidine metabolism in camels and *Trypanosoma evansi:* uridine 5′-diphosphate (UDP) metabolic pathways and targeting ATP diphosphatase, Biomarkers of infection and inflammation, Investigation on biochemical parameters of cerebrospinal fluid in neurological disorders, *Klebsiella oxytoca* isolated from nostrils of dromedary camels: resistance pattern and pehX gene based genotyping, Deoxyuridine 5-monophosphate (dUMP) metabolising enzyme and the bifunctional dihydrofolate reductase-thymidylate synthase in camels and *Trypanosoma evansi,* Enzymatic and antioxidant activity of camel milk fermented with different strains of lactic acid bacteria, genetic polymorphism at κ-casein gene in indian camel breeds, Middle east respiratory syndrome (MERS) in an adult dromedary camel, haematological studies during late pregnant and early lactation stage in Jaisalmeri camels, In-vitro capacitation of spermatozoa as assessed by chlortetracycline staining. It has regular columns of news and instructions to contributors also.

It is believed that owing to the lockdown in various countries due to COVID 19 threat, the camelid research was also affected or delayed but hopefully now it will resume faster and results will soon appear in form of new manuscripts for publication in the Journal of Camel Practice and Research.

With my best wishes to all the camelid scientists and practitioners to stay safe and healthy during the COVID 19 pandemic.

(Dr. T.K. Gahlot)
Editor

DOI : 10.5958/2277-8934.2020.00021.1 Vol 27 No 2, p 141-144

PROTEOME PROFILE OF THE HUMP OF BACTRIAN CAMEL

Bin Yang[1], Shan Bao[2], Ming Yong[3], Lihui Chen[1], Huar Bao[1], Sharku[4], Shi Liu[5] and Demtu Er[1*]

[1]College of Veterinary Medicine, Inner Mongolia Agricultural University, Key Laboratory of Clinical Diagnosis and Treatment Technology in Animal Disease, Ministry of Agriculture and Rural Affairs, P.R. China, Hohhot, 010011, China
[2]Animal Husbandry Workstation of Agriculture and Animal Husbandry Bureau of Bayannur city, Xinhua west street,
[3]Veterinary Administration Bureau of Bayannur city, Xinhua west street, Linhe district, Bayannur city, 015000,
[4]Agriculture and Animal Husbandry Integrated Service Centre of Yinggen Sumu, Alxa Left Banner, 750323,
[5]Animal Disease Prevention and Control Centre of Alxa Left Banner, Bayanhot, 750300,
Inner Mongolia Autonomous Region, P.R. China

ABSTRACT

Proteome profile of hump of Bactrian camel was identified using a shotgun proteomic approach. GO annotation and KEGG were predicted using bioinformatic tools. As a result, a total of 1077 proteins were identified. We found that the hump of Bactrian camel is equipped with a variety of functional proteins related to cellular process, metabolic process, binding, catalytic activity, cell, cell part and organelle. Three hundred and one different pathways in the hump of Bactrian camel were identified by KEGG analysis. Most of the pathways were associated with signal transduction pathways, metabolic pathways and energy metabolism. The identified proteome profile will help us understand the function of the hump of Bactrian camel.

Key words: Bactrian camel, hump, proteome, shotgun

The camel can survive long periods without feed as it stores energy reserve in a the form of fat in the hump (Kadim *et al,* 2002). The physicochemical properties and the fatty acid composition of fat from the hump of camels has been measured (Kadim *et al,* 2002; Sbihi *et al,* 2013). Proteomic technology has been used for finding differences between different organs in the camel and the rat (Warda *et al,* 2014). There is limited knowledge about detailed proteome of camel hump. To understand functions of the hump of Bactrian camel, it is important to define the molecular constituents of the hump. The aim of the present study was to identify whole proteome profile of the hump of Bactrian camel using shotgun proteomic approach.

Materials and Methods

Transcriptome databases construction

One adult 10-years-old Bactrian camel was used to harvest hump tissue which was collected and frozen in liquid nitrogen, and stored at −80°C. Total RNA from hump tissue sample was extracted according to the manufacturer's protocol. The total RNA quantity and purity were analysed. Sequencing library was constructed and sequenced on an Illumina Hiseq platform. After reads mapping to the reference genome, one-frame translation of our own hump database was constructed.

Shotgun

Sample pretreatment

The sample was added SDT lysis buffer and homogenated. The sample was sonicated and incubated at 100°C for 10 min, then centrifuged at 12000g for 30 min. The supernatant was collected. Protein concentration was determined with BCA method.

FASP

The proteins were added with DTT and incubated at 100°C for 5 min. The sample was added with UA buffer and centrifuged at 14000g for 15 min. Then, IAA buffer was added and incubated for 30 min at room temperature in the dark. After centrifugation, UA buffer was added and centrifuged at 14000g for 15 min. NH_4HCO_3 was added and centrifuged at 14000g for 15 min. Trypsin buffer was added and incubated at 37°C for 16-18 h.

LC-MS/MS

The LC-MS/MS analysis was performed on an Easy nLC system and Q-Exactive mass spectrometer

SEND REPRINT REQUEST TO CORRESPONDING AUTHER DEMTU ER* email: eedmt@imau.edu.cn

system (Thermo Scientific). The sample was loaded onto a trap column (2cm×100µm, 5µm-C18, Thermo Scientific), and then separated onto an analytical column (75µm×100mm, 3µm-C18, Thermo Scientific) at a flow rate of 300 nL/min. Mass spectra were acquired with an Exactive mass spectrometer (Thermo Scientific). MaxQuant software was employed for protein quantitation. Only the protein identifications with false discovery rate (FDR) 1% or less were accepted in the final dataset.

Results and Discussion

A total of 1077 proteins in hump tissue were identified using shotgun proteomic approach. The 27, 12 and 19 catalogs of biological process, molecular function and cellular component were clustered, respectively. In the category of biological process, most annotated proteins were involved in cellular and metabolic processes. In the category of molecular function, most annotated proteins were associated with binding and catalytic activity. In the category of cellular component, most annotated proteins were associated with cell, cell part and organelle (Fig 1).

In present study 301 different pathways were enriched. Signal transduction pathways are the major pathways. Most of the signal transduction pathways involved in lipid metabolism, for example PI3K-Akt signalling pathway (Huang *et al,* 2018), MAPK signalling pathway (Carmen and Victor, 2006), Hippo signalling pathway (Ardestani *et al,* 2018), cAMP signalling pathway (Rogne and Tasken, 2014), Apelin signalling pathway (Bertrand *et al,* 2015), AMPK signalling pathway (Ceddia, 2013), PPAR signalling pathway (Wahli and Michalik, 2012), Sphingolipid signalling pathway (Lambert *et al,* 2018), Insulin signalling pathway (Tencerova *et al,* 2019), TGF-beta signalling pathway (Margoni *et al,* 2012), Wnt signalling pathway (Christodoulides *et al,* 2009) (Fig 2) and Adipocytokine signalling pathway (Lee and Shao, 2014).

Many pathways were associated with metabolic pathways, such as amino acid metabolism, carbohydrate metabolism, lipid metabolism and nucleotide metabolism (Fig 3).

Several pathways involved in energy homeostasis, such as glycolysis/gluconeogenesis, fatty acid degradation, citrate cycle (TCA cycle), fatty acid elongation and biosynthesis of unsaturated fatty acids (Fig 4). The results may indicate that part of the energy required by Bactrian camels is derived from the ATP produced by the hump through

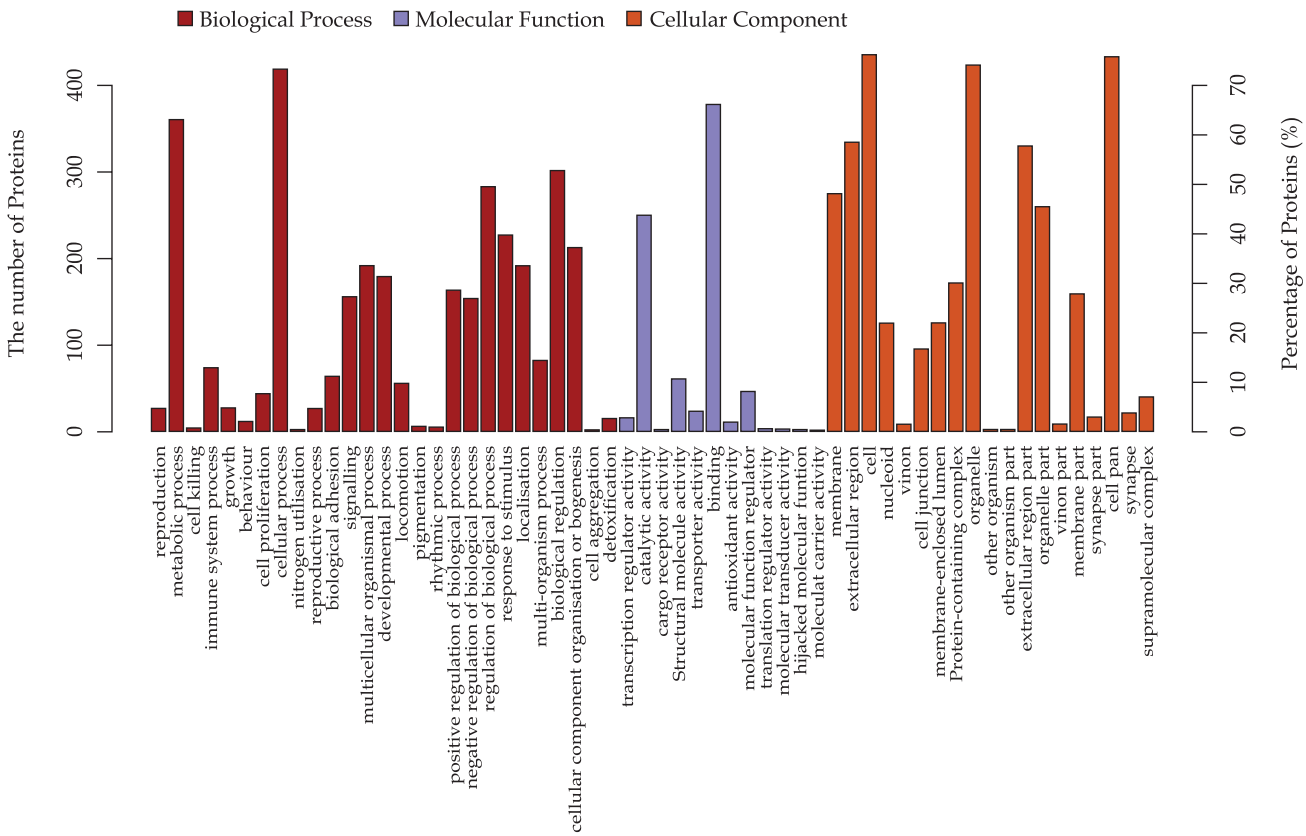


Fig 1. Gene Ontology (GO) categories of proteins identified in the hump of Bactrian camel.

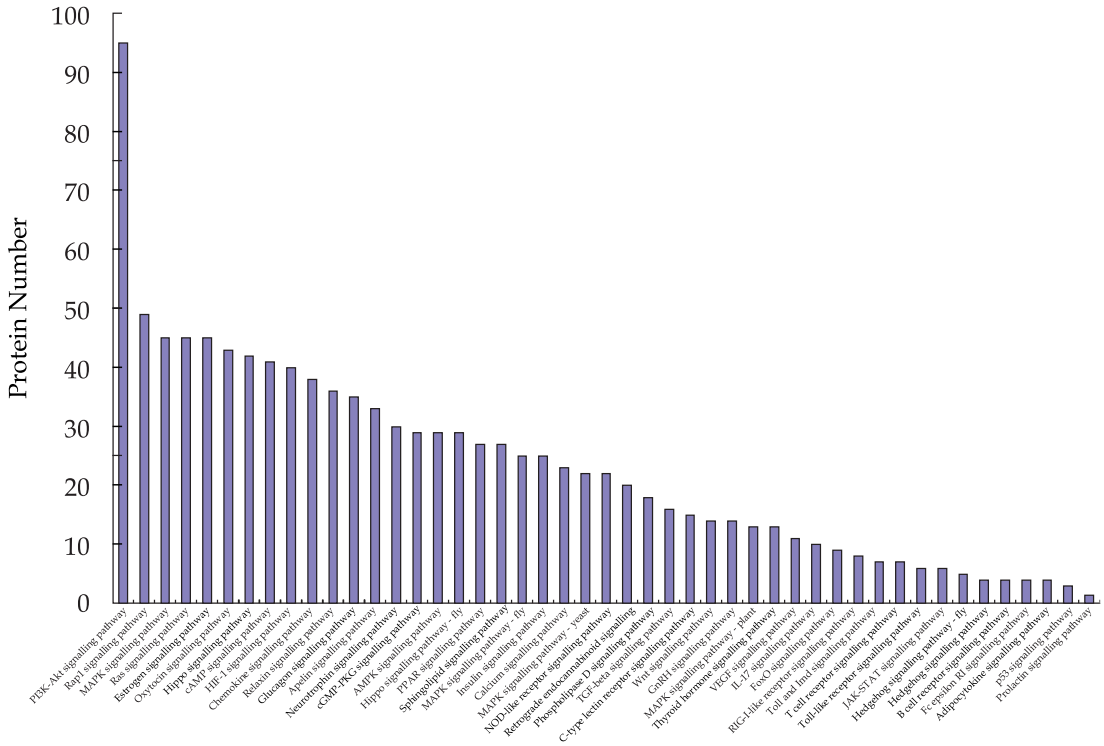


Fig 2. The pathways involved in signal transduction.

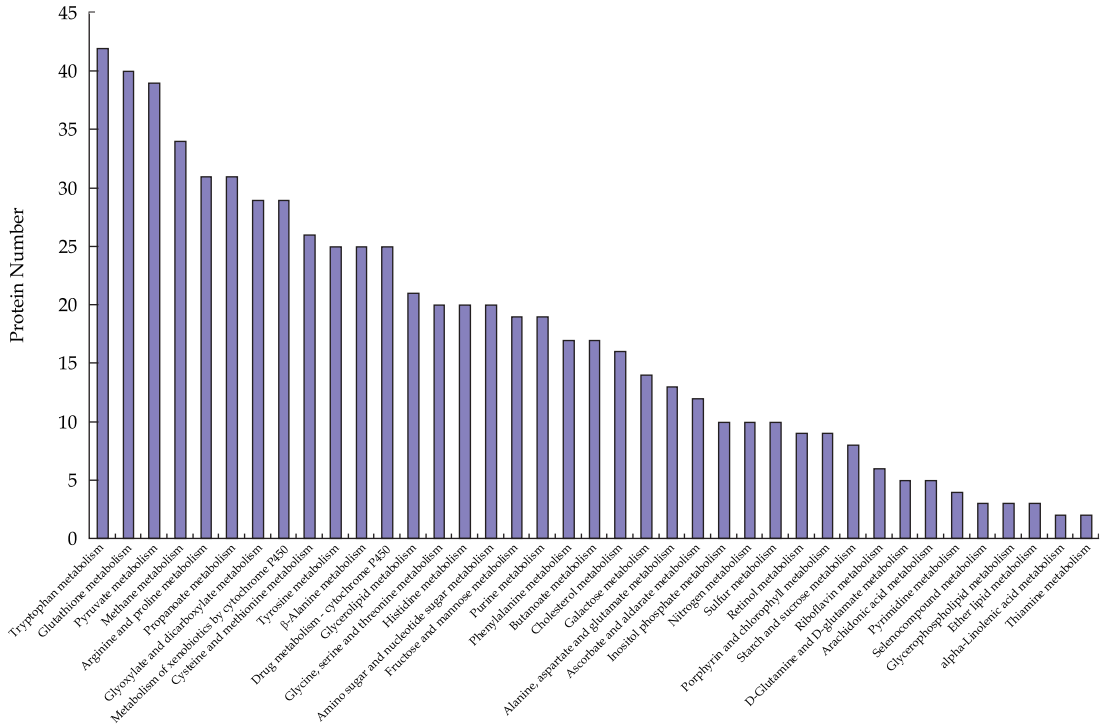


Fig 3. The pathways involved in metabolism.

glycolysis and TCA cycle. The maintenance of blood glucose in Bactrian camel may be due in part to hump gluconeogenesis.

Several pathways were associated with transport and catabolism, such as Endocytosis, Phagosome, Peroxisome, Proteasome and Autophagy (Fig 5). Furthermore, the pathways associated with signalling molecules and interaction, such as ECM-receptor interaction, SNARE interactions in vesicular transport and neuroactive ligand-receptor interaction

were represented. The extracellular matrix (ECM) is essential for tissue architecture and has an important role in adipogenesis (Mariman and Wang, 2010). The results indicate that the accumulation of hump fat is associated with ECM-receptor interaction. The precent study can help us to understand the function of the hump of Bactrian camel.

Acknowledgements

This work was supported by Inner Mongolia Agricultural University high-level talents research initiation fund project (Grant No. NDYB2018-27)

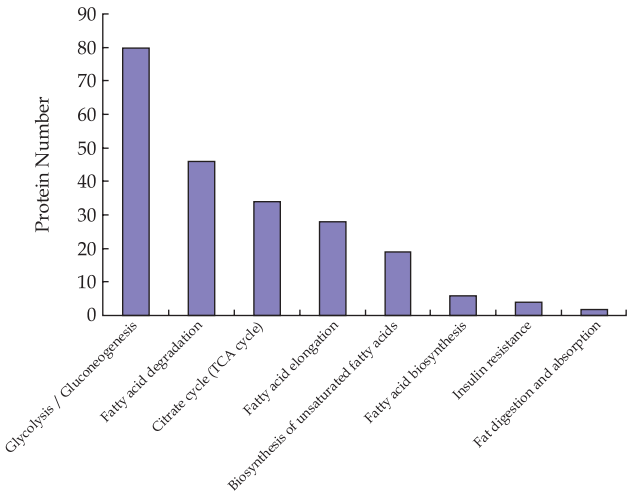


Fig 4. The pathways involved in energy homeostasis.

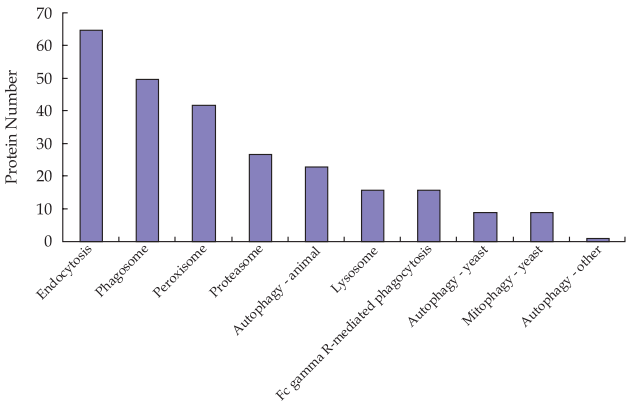


Fig 5. The pathways involved in transport and catabolism.

References

Ardestani A, Lupse B and Maedler K (2018). Hippo Signalling: key emerging pathway in cellular and whole-body metabolism. Trends in Endocrinology and Metabolism 29(7):492-509.

Bertrand C, Valet P and Castan-Laurell I (2015). Apelin and energy metabolism. Frontiers in Physiology 6:115.

Carmen GY and Victor SM (2006). Signalling mechanisms regulating lipolysis. Cellular Signalling 18(4):401-408.

Ceddia RB (2013). The role of AMP-activated protein kinase in regulating white adipose tissue metabolism. Molecular and Cellular Endocrinology 366(2):194-203.

Christodoulides C, Lagathu C, Sethi JK and Vidal-Puig A (2009). Adipogenesis and WNT signalling. Trends in Endocrinology and Metabolism 20(1):16-24.

Huang X, Liu G, Guo J and Su Z (2018). The PI3K/AKT pathway in obesity and type 2 diabetes. International Journal of Biological Sciences 14(11):1483-1496.

Kadim IT, Mahgoub O, Al-Maqbaly RS, Annamalai K and Al-Ajmi DS (2002). Effects of age on fatty acid composition of the hump and abdomen depot fats of the Arabian camel (*Camelus dromedarius*). Meat Science 62(2):245-251.

Lambert JM, Anderson AK and Cowart LA (2018). Sphingolipids in adipose tissue: What's tipping the scale? Advances in Biological Regulation 70:19-30.

Lee B and Shao J (2014). Adiponectin and energy homeostasis. Reviews in Endocrine and Metabolic Disorders 15(2):149-156.

Margoni A, Fotis L and Papavassiliou AG (2012). The transforming growth factor-beta/bone morphogenetic protein signalling pathway in adipogenesis. International Journal of Biochemistry and Cell Biology 44(3):475-479.

Mariman EC and Wang P (2010). Adipocyte extracellular matrix composition, dynamics and role in obesity. Cellular and Molecular Life Sciences 67(8):1277-1292.

Rogne M and Tasken K (2014). Compartmentalisation of cAMP signalling in adipogenesis, lipogenesis, and lipolysis. Hormone and Metabolic Research 46(12):833-840.

Sbihi HM, Nehdi IA and Al-Resayes SI (2013). Characterisation of Hachi (*Camelus dromedarius*) fat extracted from the hump. Food Chemistry 139(1-4):649-654.

Tencerova M, Okla M and Kassem M (2019). Insulin Signalling in Bone Marrow Adipocytes. Current Osteoporosis Reports 17(6):446-454.

Wahli W and Michalik L (2012). PPARs at the crossroads of lipid signalling and inflammation. Trends Endocrinol Metab 23(7):351-363.

Warda M, Prince A, Kim HK, Khafaga N, Scholkamy T, Linhardt RJ and Jin H (2014). Proteomics of old world camelid (*Camelus dromedarius*): Better understanding the interplay between homeostasis and desert environment. Journal of Advanced Research 5(2):219-242.

DOI : 10.5958/2277-8934.2020.00022.3 Vol 27 No 2, p 145-158

BIOINFORMATICS OF PYRIMIDINE METABOLISM IN CAMELS AND *Trypanosoma evansi*: URIDINE 5′-DIPHOSPHATE (UDP) METABOLIC PATHWAYS AND TARGETING ATP DIPHOSPHATASE

Mahmoud Kandeel[1,3,*], Abdulhafez Dalab[2], Abdulkarem Al-Shabebi[2] and Abdulla Al-Taher[1]

[1]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Alahsa, 31982, Saudi Arabia
[2]Department of Anatomy, College of Veterinary Medicine, King Faisal University, Alahsa 31982, Saudi Arabia
[3]Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, 35255, Egypt

ABSTRACT

Comparison of host and parasite genomes can lead to discovery of metabolic or structural differences that can be used in finding new drug targets. In this study, the pyrimidine metabolic pathway uridine 5′-diphosphate (UDP) and deoxyuridine 5-diphosphate (dUDP) paths were researched. In this perspective, several UDP/dUDP metabolising enzymes were investigated by bioinformatics tools. UMP/CMP kinase was not detected in *Trypanosoma evansi* (*T. evansi*) allowing ATP-diphosphatase as an important target for inhibition studies as the interconversion between UMP and UDP are affected. While 100% similarity rate was evident in camel species, there was about 30% differences between the camel and human ATP-diphosphatase. The great sequence differences between the camel and *T. evansi* ATP-diphosphatases (73% differences) suggests for vulnerability of using this target for future studies. However, further investigations are required to establish the biological aspects of the enzyme.

Key words: Camel, CTP synthase, genome, nucelotide, *Trypanosoma evansi*, UDP

The sequence of camel genome was recently published (Jirimutu *et al,* 2012). Resolving the sequence of the genes in various metabolic pathways gives comprehensive insights into the metabolic pathways and the requirements for camel adaptation to its harsh environment. In our group, bioinformatics tools were used for characterisation of new drug target (Alfuwaires *et al,* 2017; Alnazawi *et al,* 2017; Kandeel *et al,* 2018; Mahmoud *et al,* 2019). Recently, we provided some interesting differences in the metabolic pathways of pyrimidines in camel and *T. evansi* (Kandeel and Al-Taher 2020a; 2020b; Kandeel *et al,* 2020).

In this exploration, the pyrimidine metabolic pathways was investigated in both camels and the blood protozoan, *T. evansi*. During this work, the enzymes metabolising UDP/dUDP were reviewed. UDP/dUDP metabolism was traced by the KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016; Ogata *et al,* 1998).

The ultimate goal of this work was to explore the UDP/dUDP metabolic enzymes and compare its sequence in camel, *T. evansi*, human and other prokaryotes and eukaryotes. The enzymes involved in this pathway were listed and searched for its coding genes in camel and *T. evansi* genomes. The expected map of UDP/dUDP metabolism was drawn and compared to find potential new drug targets against the blood protozoa.

Materials and Methods

Retrieval of genomic data

Collection of genomic data was carried out by extracting the information from the gene database (http://www.genedb.org) (Hertz-Fowler *et al,* 2004), Kinetoplastom genome resources (http://tritrypdb.org/tritrypdb/), protein and genome databases (http://www.ncbi.nlm.nih.gov) and the Arabian camel genome project (http://www.camel.kacst.edu.sa). Information obtained from these sources included protein and nucleotide sequence information, gene annotation, paralogs and orthologs information, metabolic functions, signal peptides, transmembrane domains, predicted protein export domains, protein expression profiles, isoelectric point (pI), the number of predicted transmembrane helices,

SEND REPRINT REQUEST TO MAHMOUD KANDEEL email: mkandeel@kfu.edu.sa

any predicted sequence motifs and the E value of the closest PDB sequence homolog.

Protein sequence homologues were searched using the NCBI, BLAST (Basic Local Alignment Search Tool) (Madden, 2013) or PSI-BLAST (Position-Specific Iterated-BLAST) servers (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searching against the non redundant (nr) database with filtering of low complexity regions.

Multiple sequence alignment programme for proteins and construction of phylogenetic tree was carried out using the tools available (*https://www.ebi.ac.uk/Tools/msa/clustalo/*) (Sievers and Higgins, 2014). Multiple sequence alignment programme (ClustalW2) was used to calculate the best match of the selected sequences. The resultant alignment was used to generate a phylogenic tree, which is visualised by Dendroscope phylogenic tree viewer (Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains was searched by the domain prediction program available (*http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml*) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (*http://www.genome.jp/kegg/*).

Proteomic and genomic tools ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) were also used for analysis of nucleotide and gene sequences. The protein parameters including the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity was searched (http://us.expasy.org/tools/protparam.html). Protein sequences of target genes was analysed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam. PROSITE (http://www.expasy.org/proteomics/families__patterns_and_profiles) was used to search for patterns and profiles in the protein sequences of the target genes. The protein domains, families and functional sites was searched (http://prosite.expasy.org/).

Results and Discussion

Uridine 5′- diphosphate (UDP) production process is proposed by 3 production routes, derived from the metabolic pathways of the KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa *et al,* 2016). In the synthetic direction, uridine-5′-monophosphate (UMP) is phosphorylated by the kinase activity of UMP/CMP kinase and/or UMP kinases. The phosphorylation of uridine 5′-triphosphate (UTP) by nucleoside diphosphate phosphatase, thymidine triphosphatase and/or ATP-diphosphatase can yield uridine 5′-diphosphate (UDP) as shown in (Fig 1). In the catabolic pathway, UDP is phosphorylated by nucleoside diphosphatase or ATP diphosphatase to generate uridine 5′-monophosphate (UMP) (Fig 1 and Table 1). After bioinformatics tools application, the revised metabolic pathways of camels and *T. evansi* was provided in Fig 2 and 3, respectively.

The generation of UDP in camels was brought by the action of NDK, ATP diphosphatase and UMP/CMP kinase (Fig 2 and Table 2). In contrast, *T. evansi* was similar to camel profile, but devoid of UMP/CMP kinase (Fig 3, Table 3). Therefore, inhibition of ATP diphosphatase might be deleterious for the parasite life as it is the only predicted enzyme for catabolising UTP and UDP to UMP.

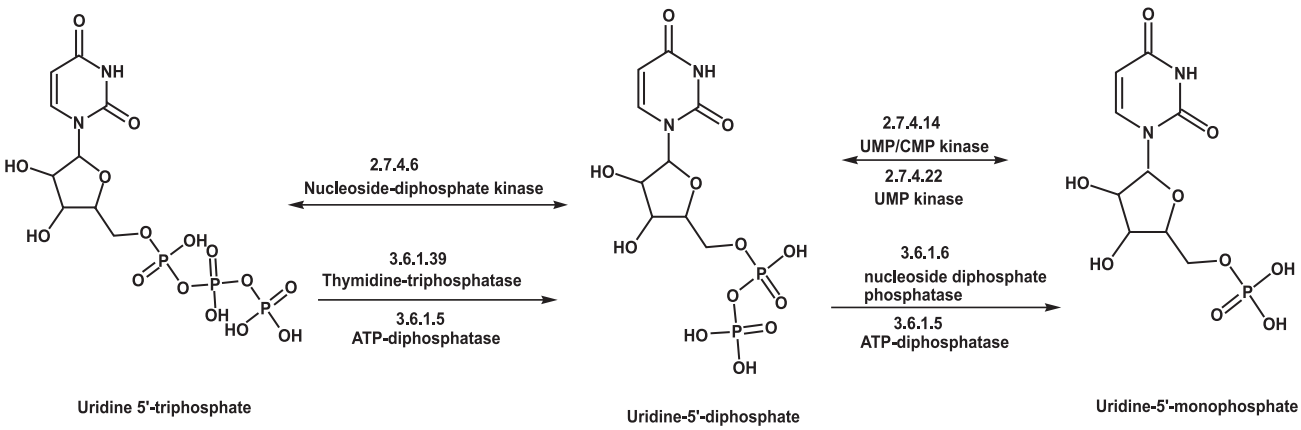


Fig 1. The proposed metabolic pathways of uridine 5′-diphosphate (UDP).

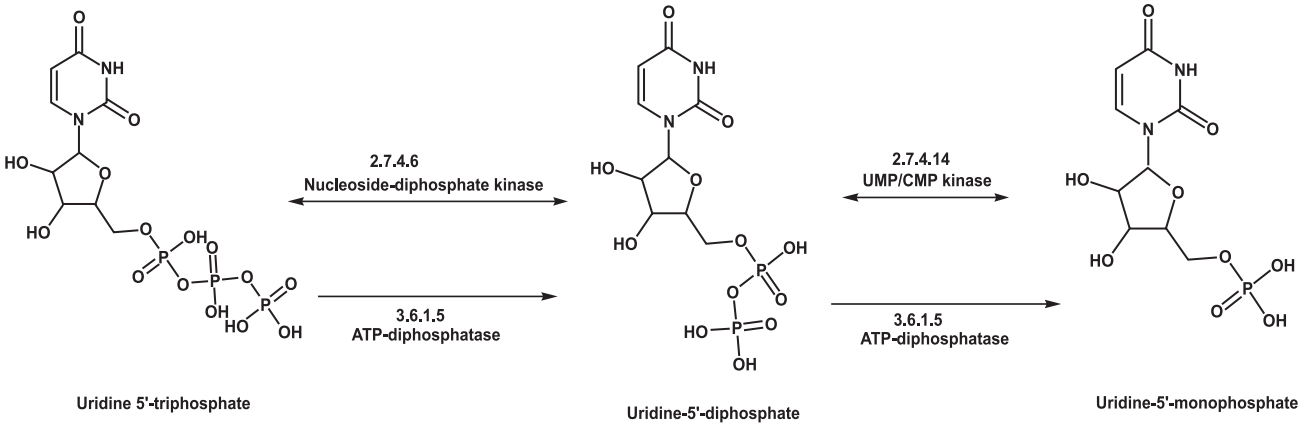


Fig 2. The proposed metabolic pathways of uridine 5′-diphosphate (UDP) in camels.

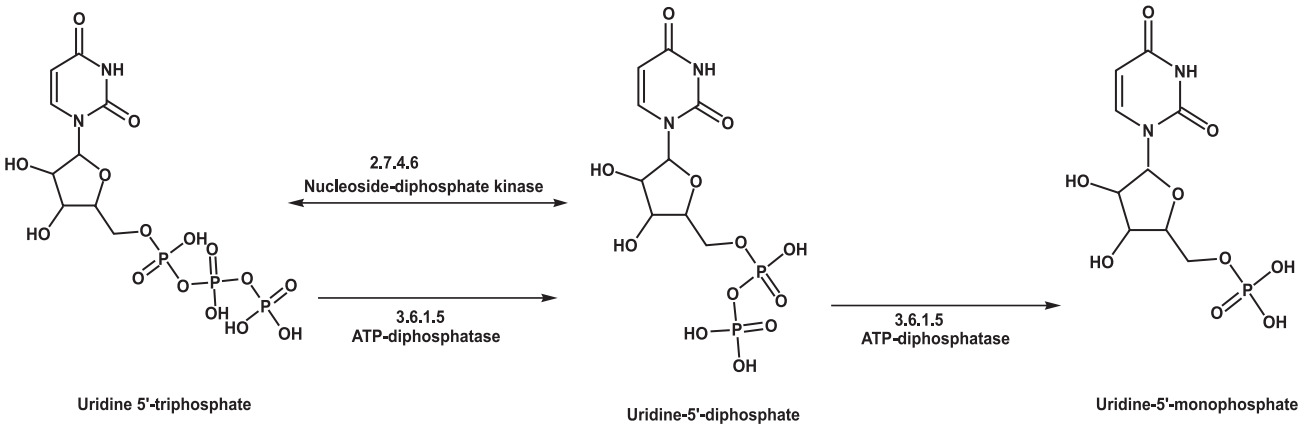


Fig 3. The proposed metabolic pathways of uridine 5′-diphosphate (UDP) in *Trypanosoma evansi*.

Table 1. Enzymes involved in metabolic pathways of UDP.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	nucleoside-diphosphate kinase
3.6.1.39	thymidine-triphosphatase, thymidine triphosphate nucleotidohydrolase
3.6.1.5	nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP-diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
2.7.4.14	UMP-CMP kinase, cytidylate kinase; deoxycytidylate kinase; CTP: CMP phosphotransferase
2.7.4.22	UMP kinase; uridylate kinase; UMPK
3.1.3.6	3′-nucleotidase; 3′-mononucleotidase

Table 2. The expected enzymes involved in metabolic pathways of UDP in camels.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	nucleoside-diphosphate kinase
3.6.1.5	nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP-diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
2.7.4.14	UMP-CMP kinase, cytidylate kinase; deoxycytidylate kinase; CTP: CMP phosphotransferase

Table 3. The expected enzymes involved in metabolic pathways of UDP in *Trypanosoma evansi*.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	nucleoside-diphosphate kinase
3.6.1.5	nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP-diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase

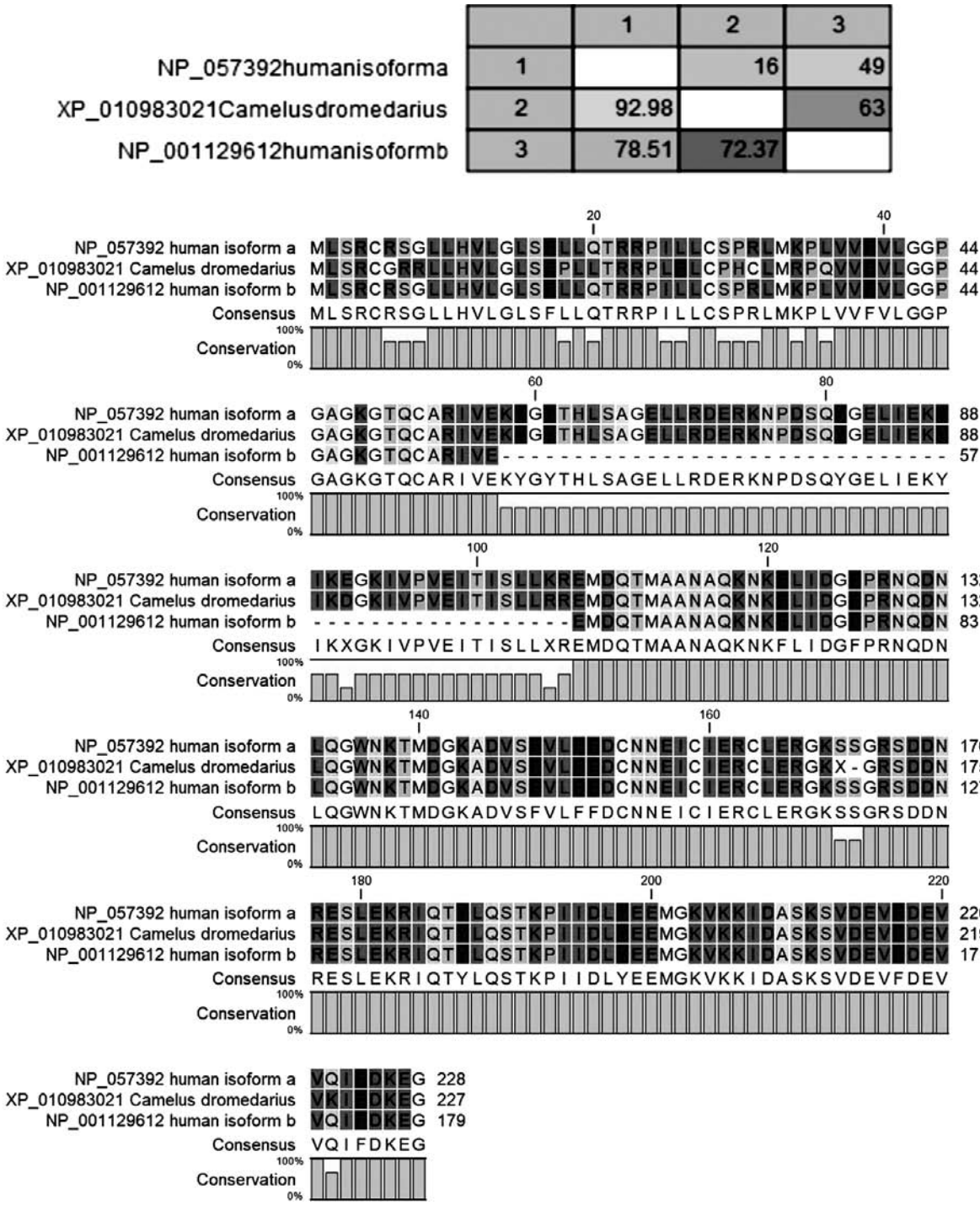


Fig 4. Multiple sequence alignment of dromedary camel and human bifunctional UMP/CMP kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region explain the number of differences between 2 sequences, while the lower left diagonal region explain the per cent of identity between 2 sequences.

Thymidine-triphosphatase

Thymidine-triphosphatase (dTTP; 3.6.1.39) an enzyme also named thymidine triphosphate monophosphohydrolase (dTTPase); thymidine triphosphate nucleotidohydrolase; dTTPase; deoxythymidine-5′-triphosphatase. This is a hydrolase enzyme, induces the dephosphorylation of deoxythymidine-triphosphate (dTTP) to the equivalent deoxythymidine-diphosphate (dTDP). Likewise, it acts on deoxyuridine-triphosphate (dUTP) and uridine-triphosphate (UTP) but very slowly. Therefore, its molecular weight is 48 500.

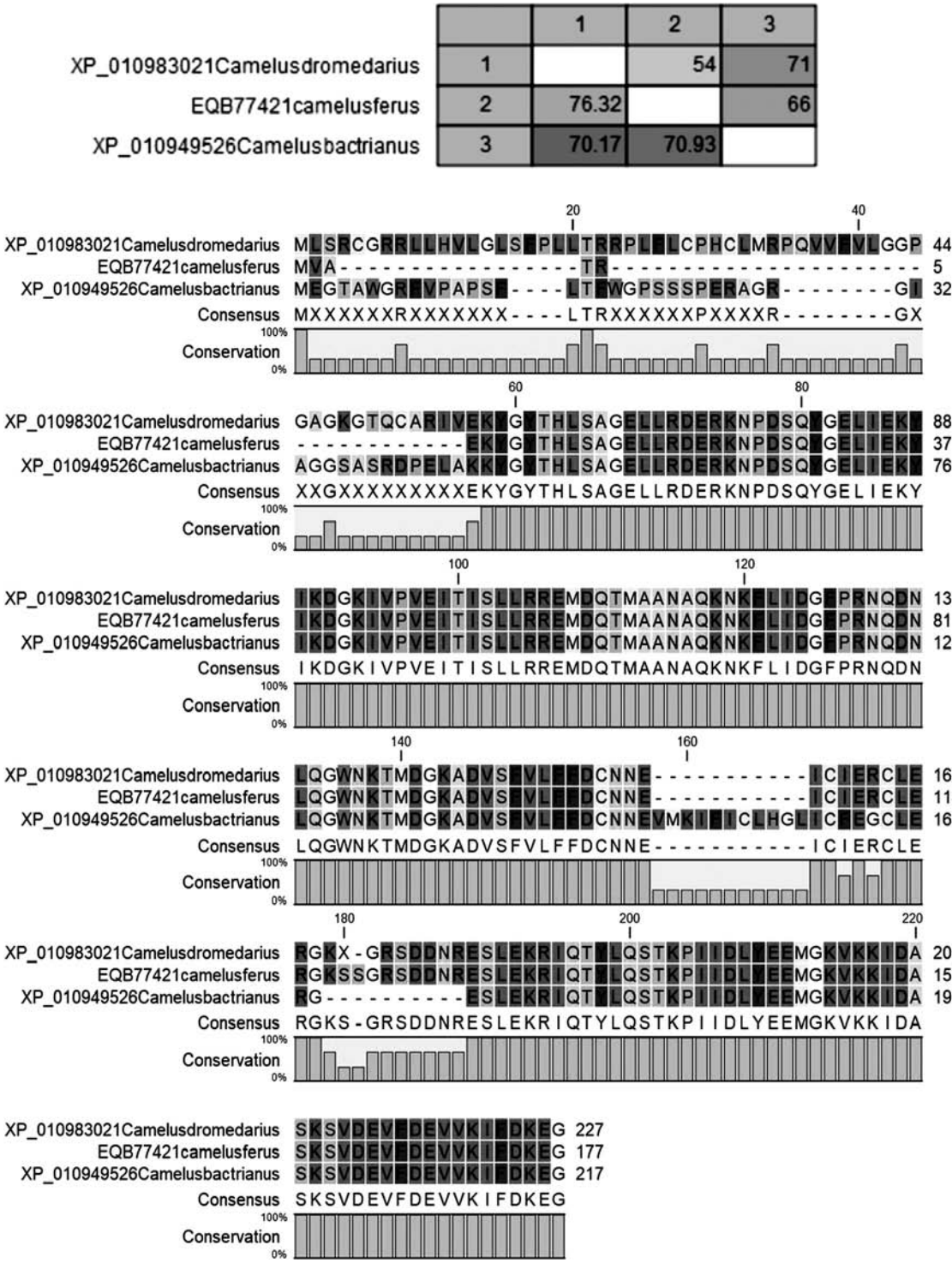


Fig 5. Multiple sequence alignment of dromedary, bactrian and feral camels bifunctional UMP/CMP kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

The dTTPase is formed of 2 equivalent subunits. Moreover, dTTPase displays optimal activity at pH 7 - 9 and the activation energy is valued to be 7.1 kcal/mol at pH 7.8. Meanwhile, the existence or absence of divalent cations does not affect dTTPase activity and the ethylenediaminetetracetic acid (EDTA) but not phenanthroline which can inhibit the enzyme activity. The Mn^{2+} reverses the inhibition

		1	2	3	4	5	6	7	8	9	10	11	12
NP_001301182Caprahircus	1		4	7	9	13	17	16	23	21	35	55	70
NP_001039509Bostaurus	2	98.25		8	10	14	18	15	23	21	35	53	70
XP_010983021Camelusdromedarius	3	96.93	96.49		10	12	16	16	23	21	34	56	68
XP_023114431Feliscatus	4	96.05	95.61	95.61		11	15	14	21	19	37	55	72
XP_023486088Equuscaballus	5	94.30	93.86	94.74	95.18		15	17	23	21	36	52	73
NP_057392human	6	92.54	92.11	92.98	93.42	93.42		14	23	21	37	51	75
XP_002715160Oryctolaguscuniculus	7	92.98	93.42	92.98	93.86	92.54	93.86		21	18	34	50	72
EDL90325Rattusnorvegicus	8	89.91	89.91	89.91	90.79	89.91	89.91	90.79		9	40	53	76
NP_079923Musmusculus	9	90.79	90.79	90.79	91.67	90.79	90.79	92.11	96.04		37	52	75
BAA06130swine	10	84.65	84.65	85.09	83.77	84.21	83.77	84.89	82.38	83.70		23	72
NP_001026735Gallusgallus	11	75.88	76.75	75.44	75.88	77.19	77.63	77.78	76.65	77.09	88.27		94
XP_010949526Camelusbactrianus	12	70.71	70.71	71.43	69.87	69.46	68.62	69.87	68.20	68.62	69.36	60.00	

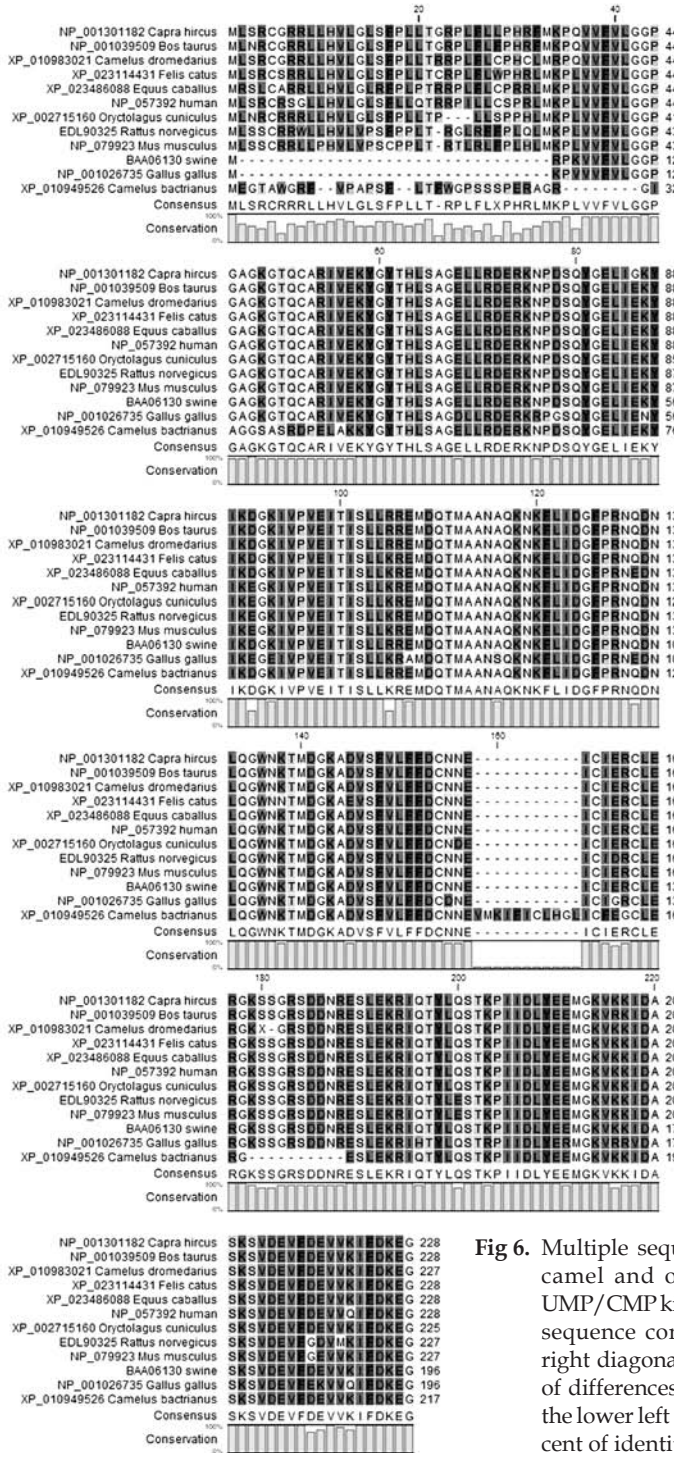


Fig 6. Multiple sequence alignment of dromedary camel and other eukaryotes bifunctional UMP/CMP kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

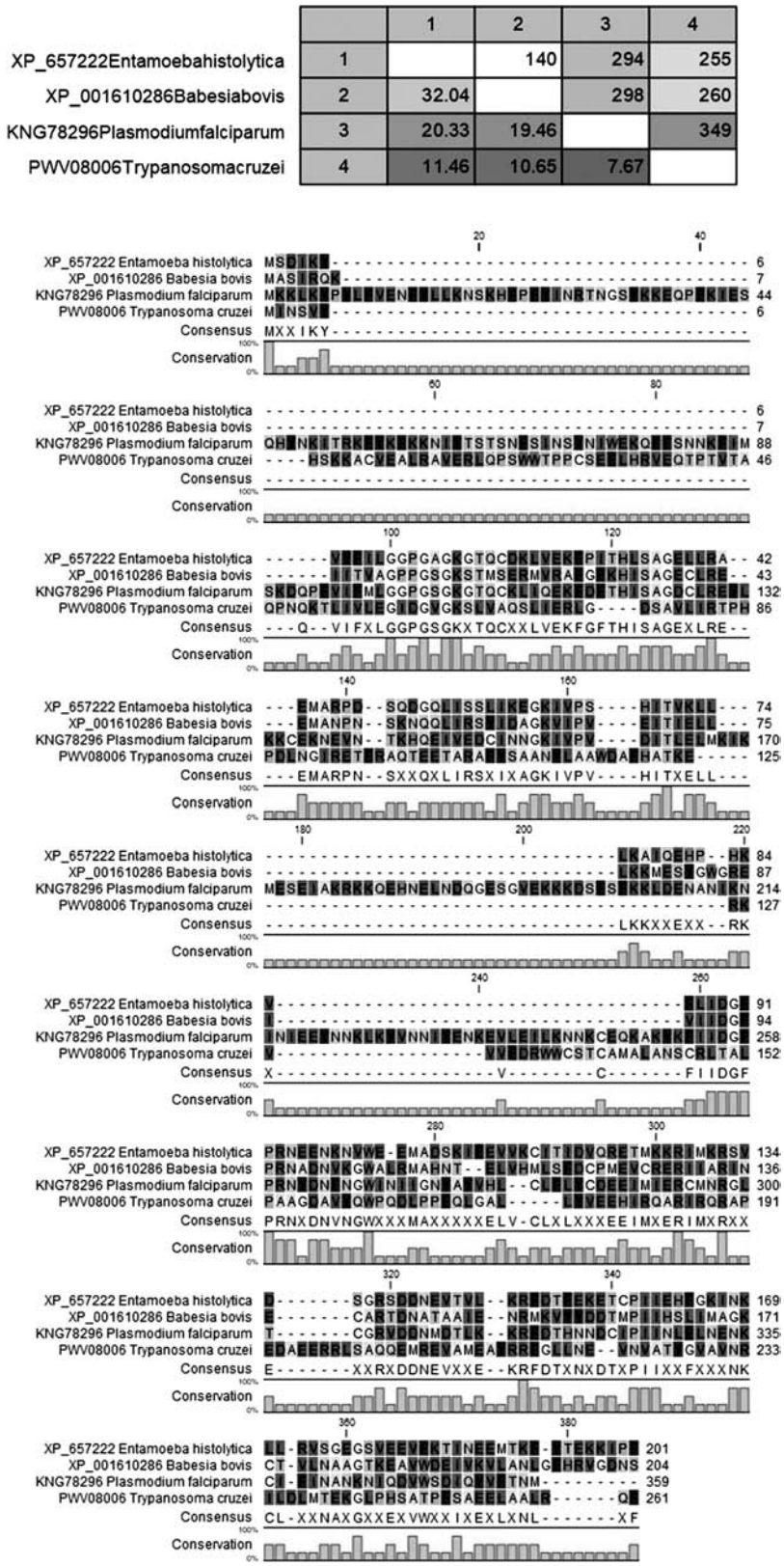


Fig 7. Multiple sequence alignment of *Trypanosoma cruzei* and some protozoal bifunctional UMP/CMP kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

caused by EDTA and Zn^{2+} triggers the absolute inhibition of dTTPase activity. The breakdown of dUTP and UTP are by about 50 and 20% of the rate of dTTP hydrolysis, respectively. Other deoxyribonucleosides or ribonucleoside triphosphates do not act as substrates for the dTTPase. This enzyme could play a significant role in the regulation of the cellular dTTP levels (Dahlmann, 1982). Thymidine-triphosphatase (thymidine triphosphate nucleotidohydrolase, dTTPase, EC no. 3.6.1.39) has been previously purified from human serum (Dahlmann, 1982). However, genetic and sequence data for other organisms were not available and the sequence of human gene was not present.

UMP-CMP kinase is a bifunctional enzyme present in eukaryotes that catalyses the phosphorylation of CMP and UMP. Prokaryotes has 2 different monofunctional enzymes EC 2.7.4.25, which act as CMP kinase and EC 2.7.4.22, which act as UMP kinase.

UMP/CMP kinase

UMP kinase (uridylate kinase; UMPK, 2.7.4.22) has restricted kinase activity for UMP only and specific for prokaryotes. It is replaced by the bifunctional UMP-CMP kinase.

Pyrimidine nucleoside monophosphate kinase (UMP/CMP kinase; 2.7.4.14); cytidylate kinase; deoxycytidylate kinase; CTP: CMP phosphotransferase; dCMP kinase; deoxycytidine monophosphokinase; UMP-CMP kinase; ATP:UMP-CMP phosphotransferase; pyrimidine nucleoside monophosphate kinase; uridine monophosphate-cytidine monophosphate phosphotransferase. This is eukaryotic bifunctional transferase enzyme (UMP/CMP kinase) with dual-specificity, transferring phosphorus-containing groups catalyses the phosphorylation of both substrates CMP and UMP

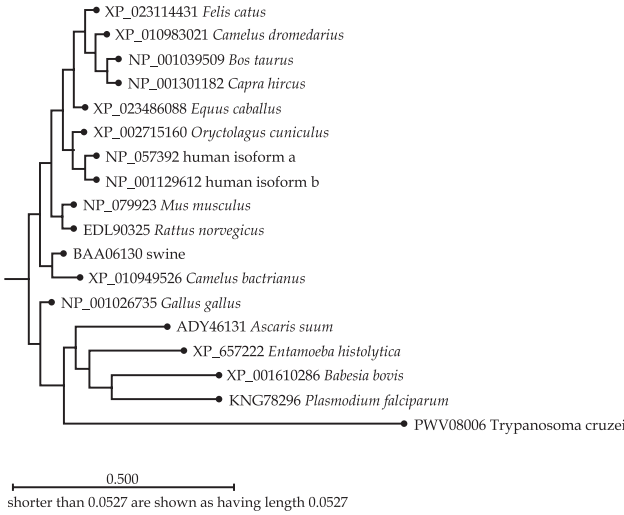


Fig 8. Phylogram of camel and *Trypanosoma cruzei* bifunctional UMP/CMP kinase in relation to a set of eukaryotic organisms.

with analogous efficacy forming UDP, CDP and dCDP, which are required for cellular nucleic acid synthesis. Also, dCMP may possibly act as acceptor. This eukaryotic enzyme dissimilar from the monofunctional prokaryotic enzymes EC 2.7.4.25, CMP kinase and EC 2.7.4.22, UMP kinase. Moreover, several cytidine and deoxycytidine analogues are important anticancer and antiviral drugs. These drugs exert their therapeutic effects *via* phosphorylation of their triphosphate structures. All of the nucleoside triphosphates used by UMP/CMPK as phosphate donors, the best donors are ATP and dATP while CTP being the poorest. Likewise, UMP/CMPK also able to phosphorylate all of the deoxycytidine analogue monophosphates. Strongly, DTT, 2-mercaptoethanol and thioredoxin, are reducing agents which could activate this enzyme, suggesting that its activity might be regulated by redox potential *in vivo*. The localisation of UMP/CMPK chiefly in the cytoplasm (Liou *et al,* 2002; Pasti *et al,* 2003).

Uridine monophosphate kinase (UMP kinase; 2.7.1.48); uridylate kinase; UMPK; PyrH; UMP-kinase; SmbA. UMP kinase is an enzyme transferring phosphate groups, precisely specific for UMP in prokaryotes and used for the *de novo* synthesis of pyrimidines nucleotides. The feedback control of this process *via* repression of carbamoxyl phosphate synthetase gene. Moreover, UMPK activated by GTP and inhibited by UTP. Conversely, UMP/CMP kinase is bifunctional for the same purpose in eukaryotes. The structure of UMPK differ from animals nucleoside monophosphate kinases, comprising the UMP/CMP kinase therefore, UMPK

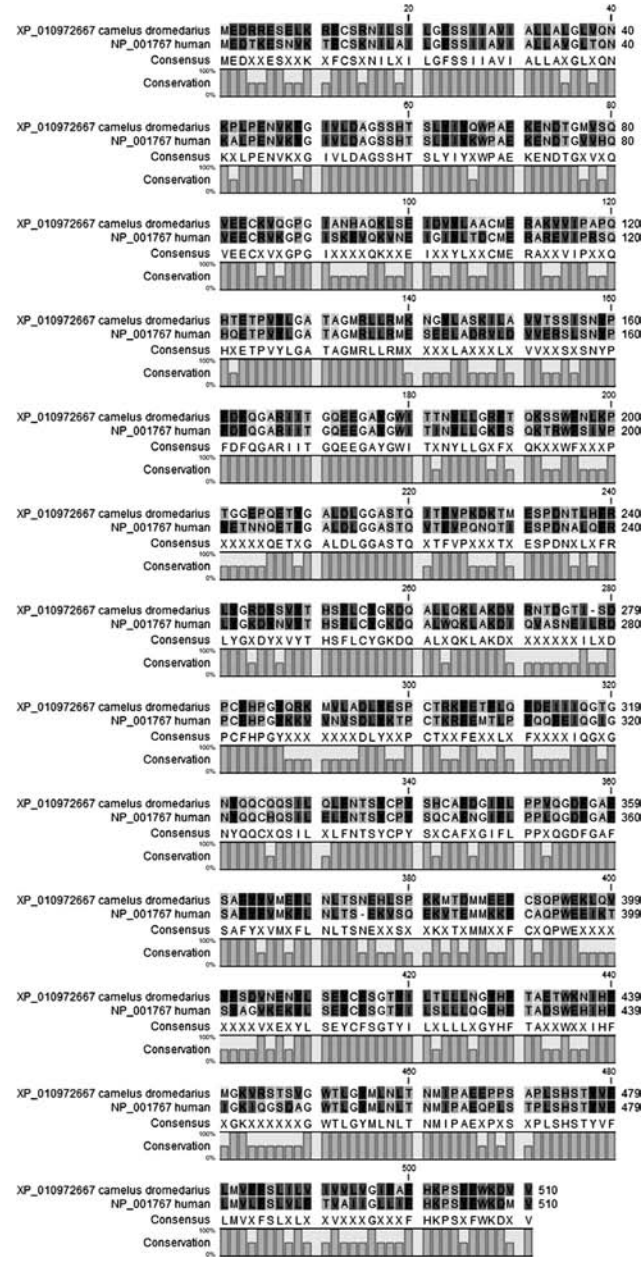


Fig 9. Multiple sequence alignment of dromedary camel and human ectonucleoside triphosphate diphosphohydrolase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

may act as antimicrobial target. This enzyme has a homohexameric assembly centred around a hollow nucleus and is organised as a trimer of dimers. The polypeptide of UMPK displays the amino acid kinase family fold, which described in carbamate kinase and acetylglutamate kinase. The substrates of acetylglutamate kinase bind within each subunit at identical, sufficiently adjusted sites. While, the

		1	2	3
XP_010972667camelusdromedarius	1		0	0
XP_014421250Camelusferus	2	100.00		0
XP_010969434bactrianus	3	100.00	100.00	

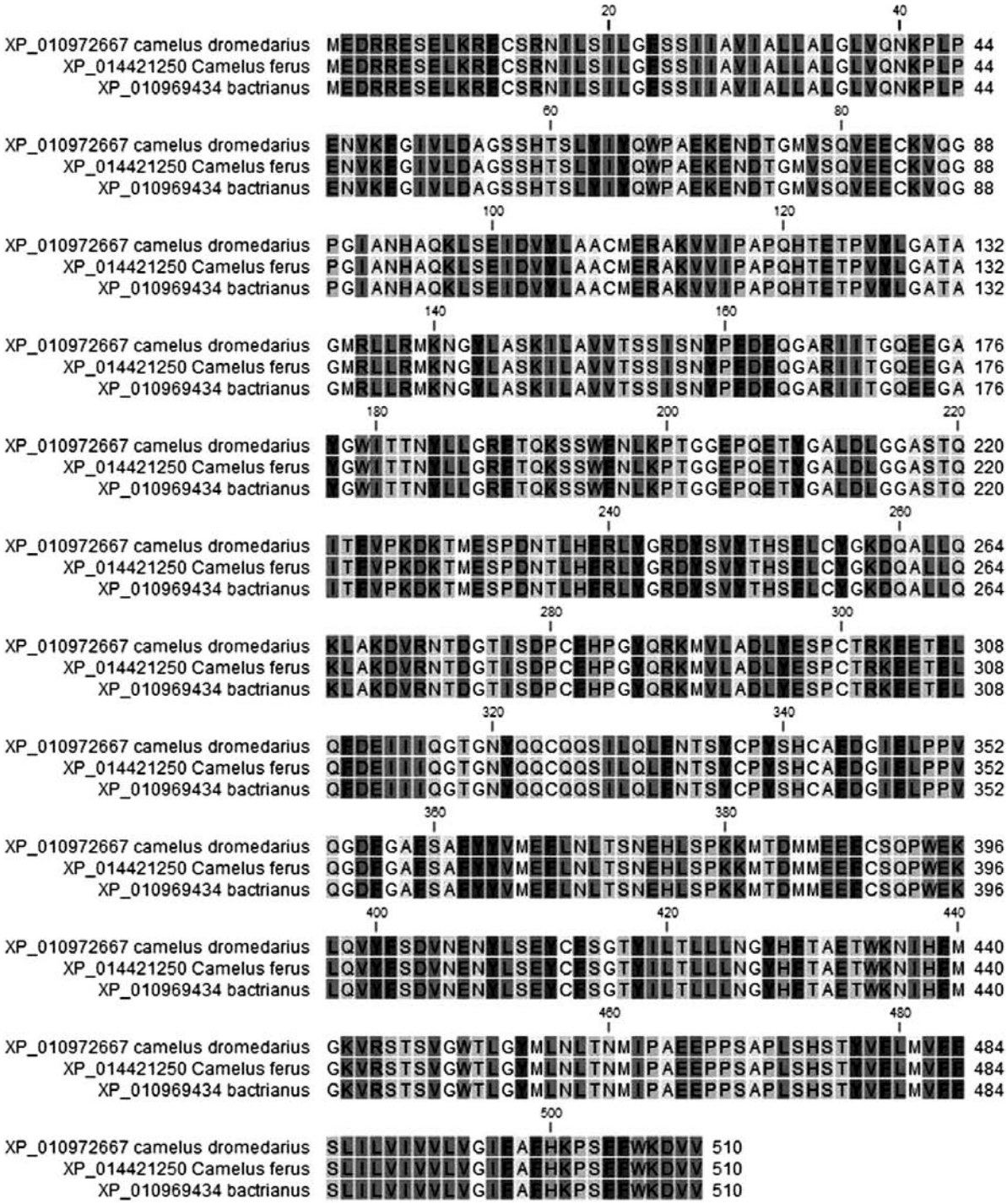


Fig 10. Multiple sequence alignment of dromedary, Bactrian and feral camels ectonucleoside triphosphate diphosphohydrolase. The upper panel represents sequence comparison statistics. The upper right diagonal region explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

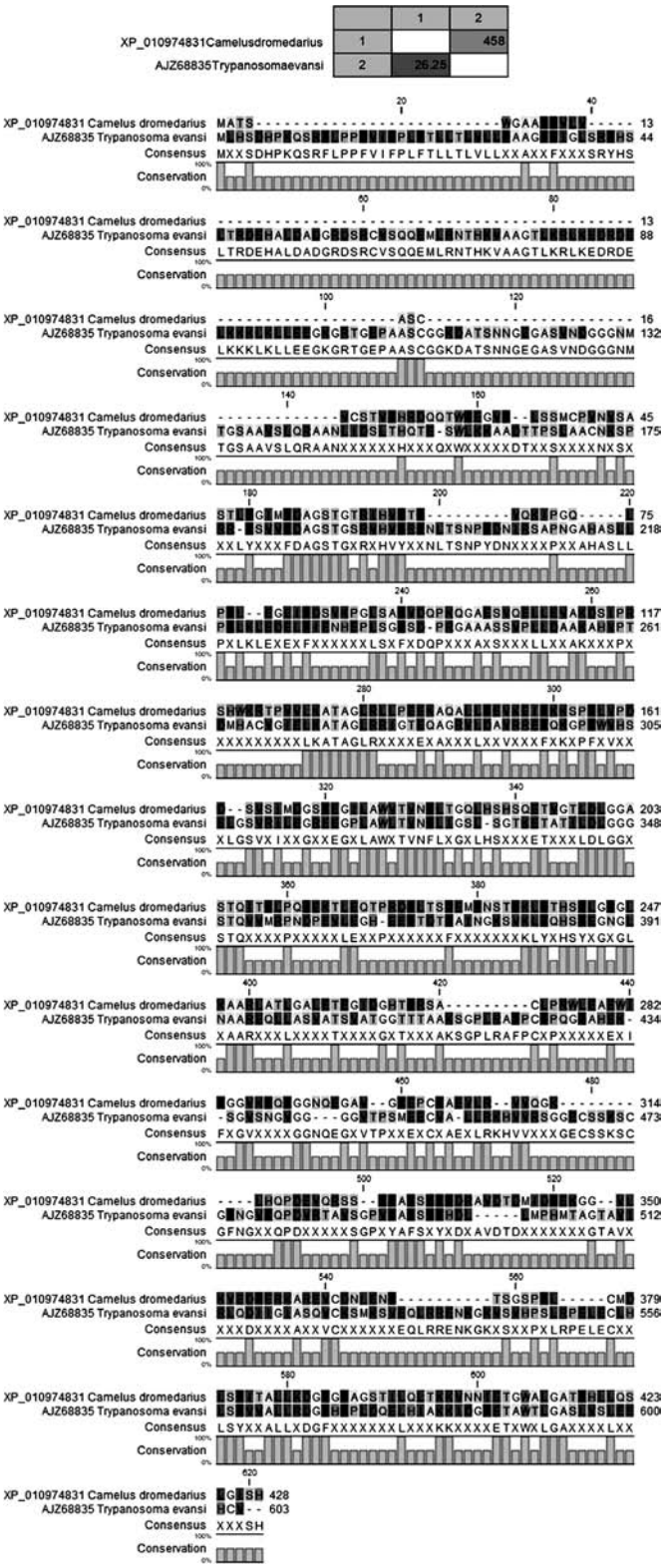


Fig 11. Pairwise sequence alignment of *T. evansi* and dromedary camel (isoform 5) ectonucleoside triphosphate diphosphohydrolase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

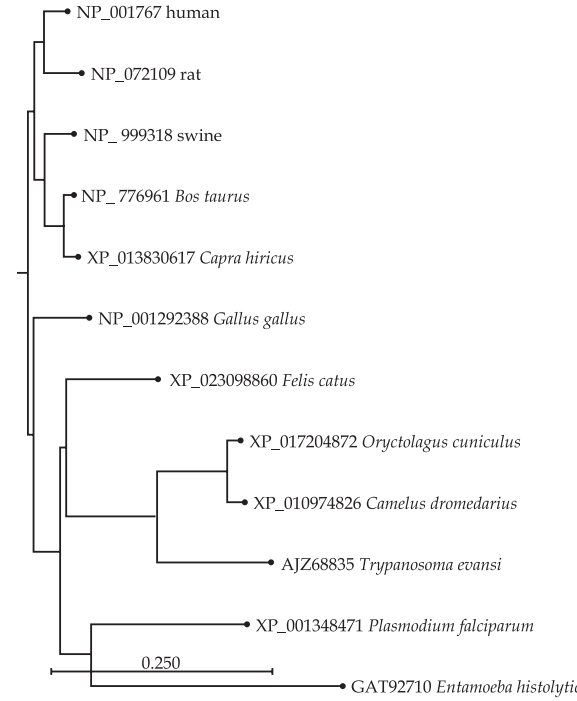


Fig 12. Phylogram of camel ectonucleoside triphosphate diphosphohydrolase in relation to a set of eukaryotic organisms.

structure of UMPK comprises 2 bound Mg^{2+} ions, one ion assists the maintenance of the transition status, hence having the same catalytic role as one lysine residue found in acetylglutamate kinase. Corresponding to carbamate kinase and acetylglutamate kinase, UMPK presents a radically dissimilar dimer architecture, lacking the characteristic 16-stranded beta-sheet backbone (Marco-Marin *et al,* 2005; Serina *et al,* 1995).

The sequence of human UMP/CMP kinase isoform A was aligned to its counterpart in dromedary camels (Fig 4). Among 228 AA, there were only 15 mismatches (6.5%) between human and dromedary camel UMP/CMP kinase. In addition to a missing residue in dromedary camel and insertion in human, it is worth noting that 12 mismatches (80%) are in the first 40 residues of the protein sequence. However, it is yet to determine whether variations at the beginning of the sequence have a considerable effect on the protein function.

Protein sequences of UMP/CMP kinase of the 3 camel species were aligned and shown in figure 5. The alignment showed the same phenomenon observed in figure 4 where the variations were concentrated in the first 60 AA. Only UMP/CMP kinase of *Camelus bactrianus* has

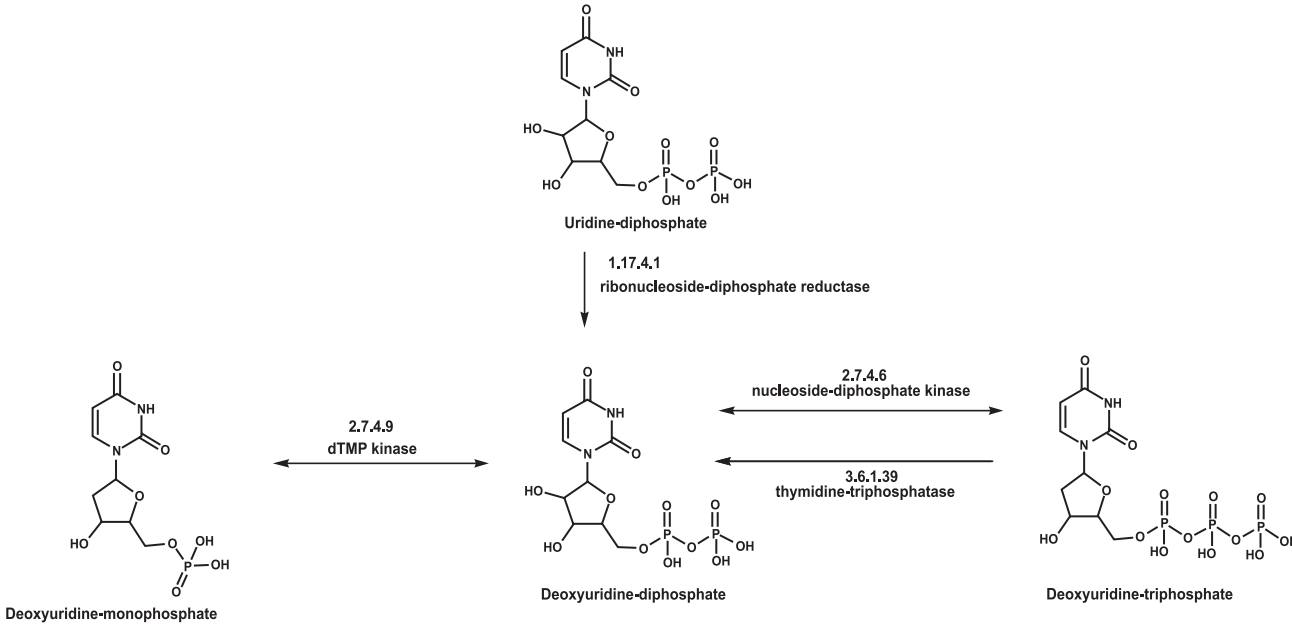


Fig 13. The proposed metabolic pathways of deoxyuridine-diphosphate (dUDP).

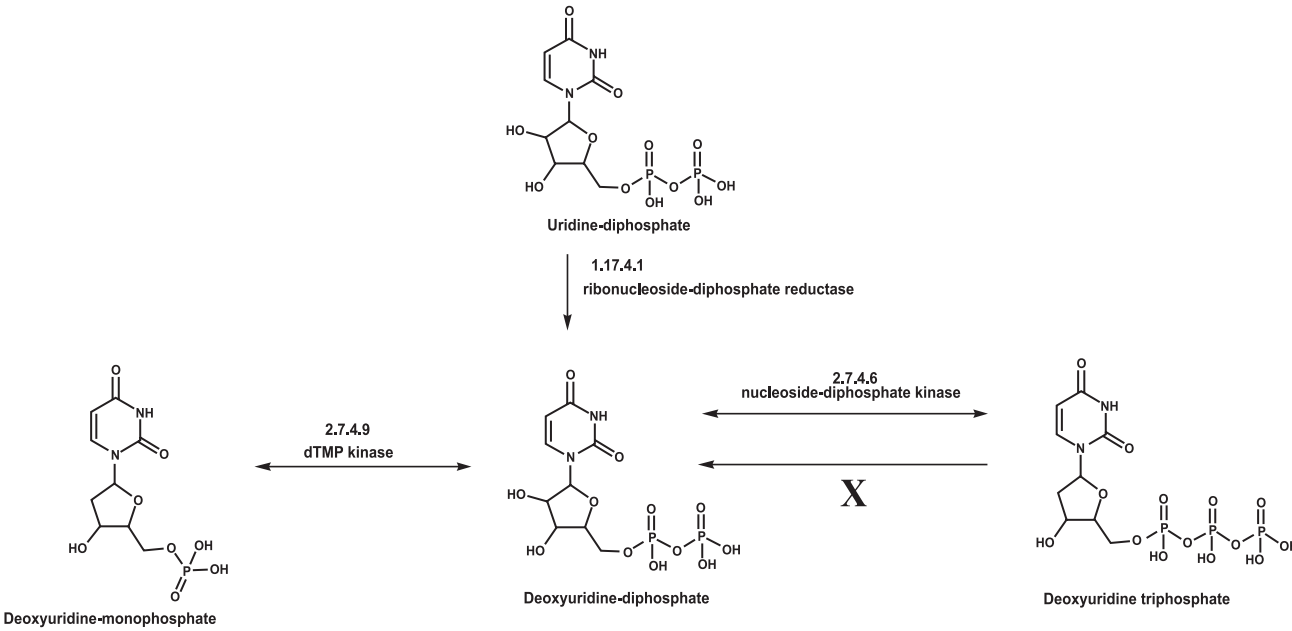


Fig 14. The proposed metabolic pathways of deoxyuridine-diphosphate (dUDP) in camels and *Trypanosoma evansi*.

insertion and deletion of 11 and 8 AA, respectively. This observation suggests that the positions of insertion/deletion may be not crucial for the protein function.

The sequence of UMP/CMP kinase of dromedary camel and other 11 species were aligned to get a larger view of variations across different species (Fig 6). The variations concentrated within the first 40 AA while, the rest showed a relative conservativeness. Only UMP/CMP kinase of *Camelus bactrianus* has insertion and deletion as described previously. In contrast to sequence of UMP/CMP kinase of the studied eukaryotic species, sequence UMP/CMP kinase of *Trypanosoma cruzei* and some protozoal is highly variable (Fig 7). We tried several BLAST searches of *T. brucei* and *T. evansi* genome using several UMP/CMP kinases, however, there outcome was zero hits.

Phylogenetic tree were reconstructed to visualise the evolutionary distance between camel

and *T. cruzei* bifunctional UMP/CMP kinase in relation to UMP/CMP kinase of a set of other eukaryotic organism (Fig 8). The protozoal enzyme was highly related to its protozoal orthologs and highly distant form the camel enzyme.

ATP - diphosphatase/ectonucleoside triphosphate diphosphohydrolase

ATP- diphosphatase (apyrases (ATP diphosphohydrolases; EC 3.6.1.5; the nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming); ATP- diphosphatase) act correspondingly on di- and triphosphate nucleotides (NDPs and NTPs) and dephosphorylate NTPs to nucleotide monophosphates (NMPs) by 2 separate succeeding phosphate-releasing steps, in which NDPs are intermediates. Unlike, apyrases differ from ATPases that distinctively hydrolyse ATP, by dephosphorylating both ATP and ADP. The eukaryotic enzymes necessitates Ca^{2+} and Mg^{2+} can alternative. The bulk of the ecto-ATPases found on be the cell surface and hydrolyse extracellular nucleotides that are target substrates for this enzyme family (Komoszynski and Wojtczak, 1996). The cells of *Trypanosoma brucei* can hydrolyse extracellular ATP. The absence of any divalent metal reduced the hydrolysis level of ATP in this protozoon parasite. Moreover, $MgCl_2$ promote ATP hydrolysis. Similarly, when $MnCl_2$ replacing $MgCl_2$, the acceleration of activity was also detected. Correspondingly, $CaCl_2$ and $ZnCl_2$ could speed up the ATPase activity, while less than $MgCl_2$. This ecto-ATPase activity was not sensitive to inhibitors than other ATPase and phosphatase activities. Living cells successively hydrolysed the ATP molecule creating ADP, AMP and adenosine and administration of ATP and adenosine was able to support the multiplication of *T. brucei*. Furthermore, the availability of purines controlled the activity of *T. brucei* ATP- diphosphatase (de Souza Leite *et al,* 2007).

Alignment of dromedary camel and human ectonucleoside triphosphate diphosphohydrolase showed variations among the length of the two protein sequences (Fig 9). The longest consecutive conserved sequence did not exceed 25 AA showing 149 differences and homology about 70.84%. However, sequence of ectonucleoside triphosphate diphosphohydrolase of the 3 camel species is identical and have 100% similarity (Fig 10). BLAST search of the obtained *T. evansi* protein against camel database revealed close similarity to ectonucleoside triphosphate diphosphohydrolase 5 isoform X2 (*Camelus dromedarius*). Fig 11 shows comparison of camel and *T. evansi* enzymes. There was low similarity rate of 26.25%.

The evolutionary distance between camel ectonucleoside triphosphate diphosphohydrolase and a set of eukaryotic organisms were shown by constructing a phylogenetic tree (Fig 12). The camel enzyme was distant from other vertebrates and highly related to the protozoal enzymes.

Motif and domain search by using prosite, NCBI domain or motif finder revealed conservative one domain content of nucleoside phophatease family in both of camel and *T. evansi* ATP-diphosphatase.

Nucleoside triphosphate phosphohydrolase

Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming, ATP-diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase, EC no. 3.6.1.5) present in vertebrates including camel and some protozoa. The enzyme was previously found by experimental procedures (Weiss *et al,* 2015), yet it was not found in the gene sequence databases after searching by accession number or name of enzyme.

Deoxyuridine 5-diphosphate (dUDP)

The production of dUDP was proposed by three production routes. In the synthetic direction, deoxyuridine-5′-monophosphate (dUMP) is phosphorylated by the kinase activity of dTMP kinase. The degeneration of deoxyuridine 5′-triphosphate (dUTP) by nucleoside diphosphate phosphatase, thymidine triphosphatase can yield dUDP as shown in (Fig 13). The reduction of UDP will give dUDP by the action of ribonucleoside diphosphate reductase. After bioinformatics tools application, the revised metabolic pathways of camels and *T. evansi* is provided in Fig 14. Camel and *T. evansi* shareed was common features of dUDP pathways comprising the lack of thymidine triphosphatase.

Acknowledgements

The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programs, National Transformation Program, under Research and Development Grants Program for National Research Institutions and Centers (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

References

Alfuwaires M, Altaher A and Kandeel M (2017). Molecular dynamic studies of interferon and innate immunity resistance in MERS CoV non-structural protein 3. Biological and Pharmaceutical Bulletin 40:345-351.

Alnazawi M, Altaher A and Kandeel M (2017). Comparative genomic analysis MERS CoV isolated from humans and camels with special reference to virus encoded helicase. Biological and Pharmaceutical Bulletin 40:1289-1298.

Dahlmann N (1982). Human serum thymidine triphosphate nucleotidohydrolase: purification and properties of a new enzyme. Biochemistry 21:6634-6639.

de Souza Leite M, Thomaz R, Fonseca FV, Panizzutti R, Vercesi AE and Meyer-Fernandes JR (2007). *Trypanosoma brucei*: biochemical characterisation of ecto-nucleoside triphosphate diphosphohydrolase activities. Experimental Parasitology 115:315-323.

Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD and Bairoch A (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Research 31:3784-3788.

Hertz-Fowler C, Peacock CS, Wood V, Aslett M, Kerhornou A, Mooney P, Tivey A, Berriman M, Hall N and Rutherford K (2004). GeneDB: a resource for prokaryotic and eukaryotic organisms. Nucleic Acids Research 32:D339-D343.

Huson DH, Richter DC, Rausch C, Dezulian T, Franz M and Rupp R (2007). Dendroscope: An interactive viewer for large phylogenetic trees. BMC Bioinformatics 8:460.

Jirimutu, Wang Z, Ding G, Chen G, Sun Y, Sun Z, Zhang H, Wang L, Hasi S, Zhang Y, Li J, Shi Y, Xu Z, He C, Yu S, Li S, Zhang W, Batmunkh M, Ts B, Narenbatu, Unierhu, Bat-Ireedui S, Gao H, Baysgalan B, Li Q, Jia Z, Turigenbayila, Subudenggerile, Narenmanduhu, Wang Z, Wang J, Pan L, Chen Y, Ganerdene Y, Dabxilt, Erdemt, Altansha, Altansukh, Liu T, Cao M, Aruuntsever, Bayart, Hosblig, He F, Zha-ti A, Zheng G, Qiu F, Sun Z, Zhao L, Zhao W, Liu B, Li C, Chen Y, Tang X, Guo C, Liu W, Ming L, Temuulen, Cui A, Li Y, Gao J, Li J, Wurentaodi, Niu S, Sun T, Zhai Z, Zhang M, Chen C, Baldan T, Bayaer T, Li Y and Meng H (2012). Genome sequences of wild and domestic bactrian camels. Nature Communications 3:1202.

Kandeel M and Al-Taher A (2020a). Bioinformatics of uridine/deoxyuridine paths in *Trypanosoma evansi* revealed targeting uridine phosphorylase and cytidine deaminase. Journal of Camel Practice and Research 27:111-119.

Kandeel M and Al-Taher A (2020b). Uridine 5′-monophosphate (UMP) metabolising enzymes uracil phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase/ump synthase in camels and *Trypanosoma evansi*. Journal of Camel Practice and Research 27:81-88.

Kandeel M, Al-Taher A, Li H, Schwingenschlogl U and Al-Nazawi M (2018). Molecular dynamics of Middle East Respiratory Syndrome Coronavirus (MERS CoV) fusion heptad repeat trimers. Computational Biology and Chemistry 75:205-212.

Kandeel M, Dalab A, Al-Shabebi A, Al-Taher A, Altaher Y and Abozahra M (2020). Uridine 5-triphosphate (UTP) metabolising enzymes nucleoside diphosphate kinase and cytidine triphosphate (CTP) synthase in camels and *Trypanosoma evansi*. Journal of Camel Practice and Research 27:61-68.

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S and Tokimatsu T (2007). KEGG for linking genomes to life and the environment. Nucleic Acids Research 36:D480-D484.

Kanehisa M, Furumichi M, Tanabe M, Sato Y and Morishima K (2016). KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Research 45:D353-D361.

Komoszynski M and Wojtczak A (1996). Apyrases (ATP diphosphohydrolases, EC 3.6.1.5): function and relationship to ATPases. Biochimica et Biophysica Acta 1310:233-241.

Labarga A, Valentin F anderson M and Lopez R (2007). Web services at the European Bioinformatics Institute. Nucleic Acids Research 35:W6-W11.

Liou JY, Dutschman GE, Lam W, Jiang Z and Cheng YC (2002). Characterisation of human UMP/CMP kinase and its phosphorylation of D- and L-form deoxycytidine analogue monophosphates. Cancer Research 62:1624-1631.

Madden T (2013). The BLAST sequence analysis tool. In: The NCBI Handbook (Internet) 2nd ed. National Centre for Biotechnology Information (US).

Mahmoud K, Kamal E, Wael E, Mahmoud F and Ibrahim G (2019). Species specificity and host affinity rather than tissue tropism controls codon usage pattern in respiratory mycoplasmosis. Journal of Camel Practice and Research 26:1-12.

Marchler-Bauer A anderson JB, Cherukuri PF, DeWeese-Scott C, Geer LY, Gwadz M, He S, Hurwitz DI, Jackson JD and Ke Z (2005). CDD: a Conserved Domain Database for protein classification. Nucleic Acids Research 33:D192-D196.

Marco-Marin C, Gil-Ortiz F and Rubio V (2005). The crystal structure of *Pyrococcus furiosus* UMP kinase provides insight into catalysis and regulation in microbial pyrimidine nucleotide biosynthesis. Journal of Molecular Biology 352:438-454.

Ogata H, Goto S, Fujibuchi W and Kanehisa M (1998). Computation with the KEGG pathway database. Biosystems 47:119-128.

Pasti C, Gallois-Montbrun S, Munier-Lehmann H, Veron M, Gilles AM and Deville-Bonne D (2003). Reaction of human UMP-CMP kinase with natural and analog substrates. European Journal of Biochemistry 270:1784-1790.

Sequencing H (2011). CLC Genomics Workbench. Workbench.

Serina L, Blondin C, Krin E, Sismeiro O, Danchin A, Sakamoto H, Gilles AM and Barzu O (1995). *Escherichia coli* UMP-kinase, a member of the aspartokinase family, is a hexamer regulated by guanine nucleotides and UTP. Biochemistry 34:5066-5074.

Sievers F and Higgins DG (2014). Clustal Omega, accurate alignment of very large numbers of sequences. In: Multiple Sequence Alignment Methods. Springer. pp 105-116.

Weiss PH, Batista F, Wagner G, Magalhaes Mde L and Miletti LC (2015). Kinetic and biochemical characterisation of *Trypanosoma evansi* nucleoside triphosphate diphosphohydrolase. Experimental Parasitology 153:98-104.

News

LOOK CAMEL FOR MILK AND BEYOND MILK

The astonishing adaptations of the camel aren't restricted to its milk. Here are a few more that have their roots in the animal's genes.

1. The urea cycle conserves nitrogen, used to make protein in the face of limited food.
2. The heart makes a lot of the protein α-actinin, enabling it to beat steadily through fluctuating drought and wetness.
3. Hiked enzyme levels in the brain and liver keep up energy supplies.
4. Vimentin protein in cells in the hump mobilizes fat, releasing energy.
5. A camel's streamlined nanobodies arose from a mutation that removed the hinges that connect the Y-shaped arms of more conventional antibodies. Sometimes a mutation is a good thing!
6. Further infection protection comes from the milk protein lactoferrin, which fights hepatitis C.
7. Tolerating High Blood Sugar by camel-herding people in India, the Raika, drink camel milk and don't get diabetes.

(Camel Milk and Autism: Connecting the Genetic Dots, November 21, 2019 Ricki Lewis, PLOS BLOGS DNA Science)

DOI : 10.5958/2277-8934.2020.00023.5 Vol 27 No 2, p 159-163

BIOMARKERS OF INFECTION AND INFLAMMATION IN CAMELS (*Camelus dromedarius*)

Mohamed Tharwat[1,2]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

Inflammation and infection biomarkers or acute phase proteins (APPs) can be used in diagnosis, prognosis and in monitoring response to therapy, as well as in general health screening. It has also been suggested that APPs may be useful in the assessment of animal welfare. Acute phase reaction or response (APR) is a positive (increase) or negative (decrease) response of APPs to infection, inflammation, trauma or other causes and it might be a physiological protective mechanism during inflammatory events. The APPs have received attention as biomarkers for APR due to its low physiological levels, a fast incline, marked rise in concentration during APR that eases detection and a fast decline after cessation of a stimulus. In ruminants, the major APPs are haptoglobin (Hp) and serum amyloid A (SAA), and have been proposed as sensitive and rapid indicators of inflammatory disturbances. Hp is a major APP in numerous species of livestock and domesticated animals as well as in camels. Elevated Hp concentrations occur not only with inflammation, but also with some conditions not generally associated with inflammation or tissue damage. SAA is considered also as one of the major acute-phase reactants in vertebrates. Elevated serum SAA levels are found following inflammation and also under conditions unrelated to inflammation such as physical stress and parturition. In camels, it was found that chronic infections as well as non-inflammatory stressors following parasitic infections, at parturition, following stimulation by electroejaculation and following race stimulate APPs production. This review describes the commonly used biomarkers in camel medicine as indicators of infection and inflammation.

Key words: Acute phase proteins, biomarkers, camels, haptoglobin, serum amyloid A

Biomarkers of infection and inflammation or acute-phase proteins (APPs) are a class of proteins whose blood concentrations increase (positive APPs) or decrease (negative APPs) in response to infection, inflammation or trauma (Murata *et al,* 2004; Petersen *et al,* 2004; Ceron *et al,* 2005; Tharwat *et al,* 2014; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2015, 2018a,b). This response is called the acute-phase reaction or acute-phase response (APR). The APR is a rapid, nonspecific, systemic response occurring secondary to many types of tissue injury and might be a physiological protective mechanism during inflammatory events (Yazwinski *et al,* 2013). The origin of APR can be attributed to infection, inflammation, surgical trauma, or other causes (Petersen *et al,* 2004; Ceron *et al,* 2005), and the purpose of the response is to restore homeostasis and to remove its disturbance (Ebersole and Cappelli, 2000; Ceron *et al,* 2005). The APR is induced by the pro-inflammatory cytokines IL-1, TNF-α and especially IL-6 (Tizard, 2009). These cytokines activate receptors on various target cells and promote hormonal and metabolic changes leading to local and systemic effects, including APP synthesis in the liver (Petersen *et al,* 2004; Tizard, 2009).

In response to injury, local inflammatory cells (neutrophil granulocytes and macrophages) secrete a number of cytokines into the bloodstream. The liver responds by producing a large number of APPs (Petersen *et al,* 2004). The negative APPs include albumin, the most abundant constitutive plasma protein, and transferrin. The positive APPs include Haptoglobin (Hp), C-reactive protein, serum amyloid A (SAA), ceruloplasmin, fibrinogen and alpha 1-acid glycoprotein (Eckersall and Bell, 2010).

In domestic animals, a critical mass of knowledge on the use of APPs as biomarkers of inflammatory conditions has accumulated over recent years, so there is now sufficient understanding of the pathophysiology of the response to support the use of these compounds as diagnostic tools in clinical settings (Eckersall and Bell, 2010; Tharwat *et al,* 2014; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018a,b). In ruminants, the APPs have

SEND REPRINT REQUEST TO MOHAMED THARWAT email: mohamedtharwat129@gmail.com

been proposed as sensitive and rapid indicators of inflammatory disturbances (Eckersall, 2000; Eckersall and Bell, 2010; Schneider *et al,* 2013).

In veterinary medicine, the APPs can be used in diagnosis, prognosis and in monitoring response to therapy, as well as in general health screening (Eckersall and Bell, 2010). In ruminants, the major APPs are Hp and SAA (Murata *et al,* 2004). In cattle, Hp and SAA are effective in the diagnosis and prognosis of mastitis, enteritis, peritonitis, pneumonia, endocarditis, and endometritis (Gronlund *et al,* 2003; Murata *et al,* 2004; Petersen *et al,* 2004). Elevations in this protein have also been reported in cows with fatty liver syndrome, at parturition, and during periods of starvation and transport stress (Uchida *et al,* 1993; Nakagawa *et al,* 1997; Katoh and Nakagawa, 1999). It has also been suggested that APPs may be useful in the assessment of animal welfare (Eckersall, 2000; Murata *et al,* 2004; Murata, 2007; Baghshani *et al,* 2010). Six-fold increases in Hp concentration were found in dairy cows with infectious and metabolic disease at slaughter compared to animals with minor lesions (Hirvonen *et al,* 1997), and 40-fold and 7-fold increases in HP and SAA, respectively were found in culled dairy cattle with acute lesions, relative to healthy beef-type animals (Tourlomousis *et al,* 2004).

Although not studied to the same extent, the APR in camels appear similar to that in cattle and chronic infections continue to stimulate APPs production (Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018a,b). The changes in APPs due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species (Murata *et al,* 2004; Murata, 2007). The APPs have received attention as biomarkers for APR due to its low physiological levels, a fast incline, marked rise in concentration during APR that eases detection and a fast decline after cessation of a stimulus (Ceron *et al,* 2005). This review describes the commonly used biomarkers in camel medicine as indicators of infection and inflammation; and to the best of the author's knowledge, this review is the first to discuss such title.

Application of inflammation biomarkers in camel medicine

Hp and SAA are the common used APPs in camel medicine. Haptoglobin is an α2-globulin synthesised in the liver (Eckersall and Bell, 2010) and is a major APP in numerous species of livestock and domesticated animals as well as in camels (Baghshani *et al,* 2010; Nazifi *et al,* 2012). Elevated Hp concentrations occur not only with inflammation, but also with some conditions not generally associated with inflammation or tissue damage (Murata *et al,* 2004; Baghshani *et al,* 2010). Another APP that is considered one of the major acute-phase reactants in vertebrates is SAA. Elevated serum SAA levels are found following inflammation and also under conditions unrelated to inflammation such as physical stress and parturition (Murata *et al,* 2004; Baghshani *et al,* 2010).

APR manifested by significant increases in Hp, SAA, fibrinogen and interleukins were reported in camels naturally infected with *Trypanosoma evansi* (El-Bahr and El-Deeb, 2016). From another study, it was also concluded that APPs could be used as diagnostic and prognostic biomarkers in camels with urinary tract infection (El-Deeb and Buczinski, 2015). Similarly, Hp and SAA were markedly elevated in camels naturally infected with *Toxoplasma gondii* (Azma *et al,* 2015). APR occurred in female

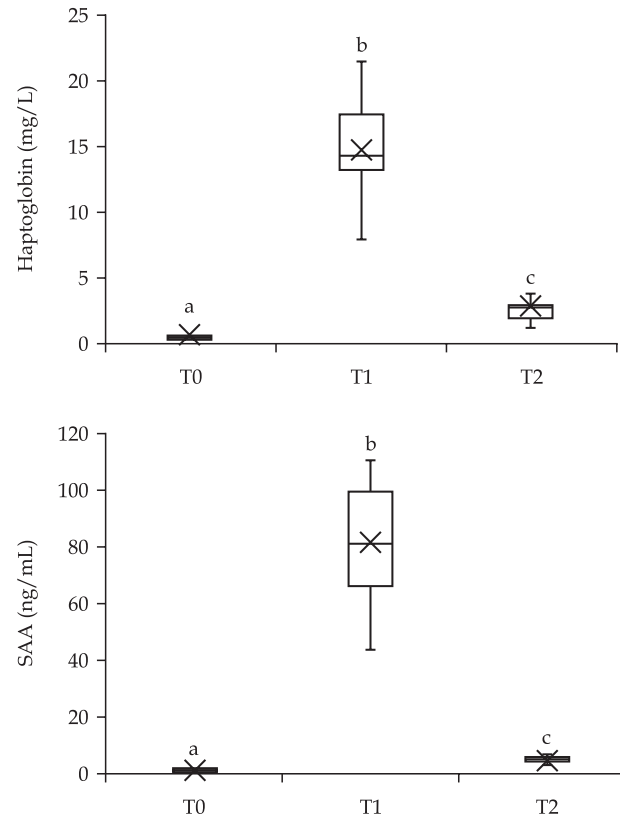


Fig 1. Box and whiskers plots of serum haptoglobin and serum amyloid A (SAA) in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. T0, 3 wk before expected parturition; T1, within 12h of parturition; T2, 3 wk after parturition. Values with different letters differ significantly (P<0.5) (Tharwat and Al-Sobayil, 2015).

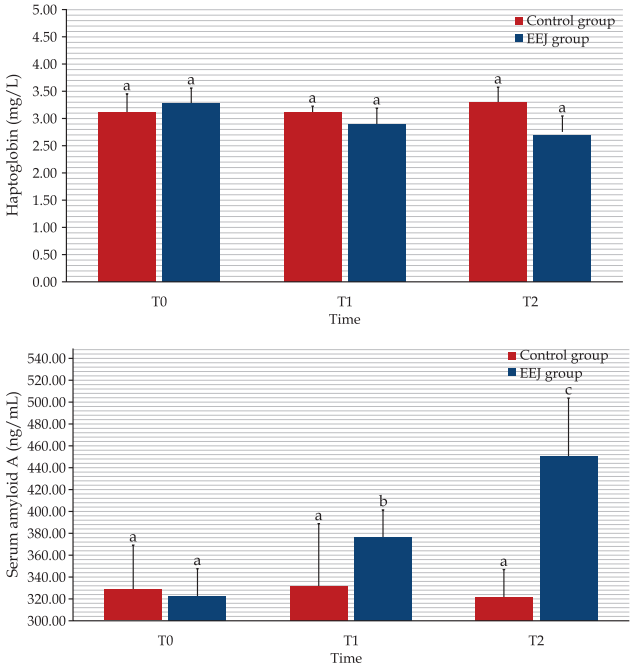


Fig 2. Effect of stimulation by electroejaculation (EEJ) on concentrations of haptoglobin and serum amyloid A in male dromedary camels (mean ± SD, n=20) compared to control group (n=10). T0: just before EEJ; T1: directly after EEJ; T2: 24h after EEJ. [a,b,c] Values differ significantly. (Tharwat and Al-Sobayil, 2018a).

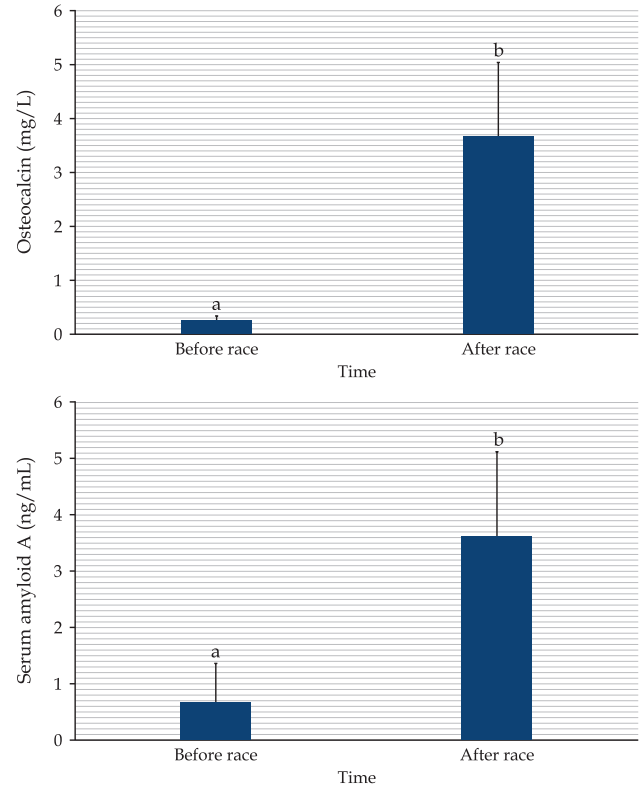


Fig 3. Mean serum concentrations of serum amyloid A in racing camels before and after a 5 km race. [a,b] Differ significantly at P< 0.05. (Tharwat and Al-Sobayil, 2018b).

dromedary camels at parturition that was manifested by significant increases in Hp and SAA as compared to values before or after parturition (Fig 1) (Tharwat and Al-Sobayil, 2015).

Generally, the APPs are secreted during the inflammatory response (Eckersall and Bell, 2010). However, in our recent study (Tharwat and Al-Sobayil, 2015), significant increases of Hp and SAA at parturition was found. These elevation detected at parturition were not attributed to pathological conditions, as the WBCs did not change significantly at that time, thus confirming the absence of pathological conditions. These elevations could be due to cortisol and hormone release and to stress resulting in numerous changes (Huzzey *et al,* 2011). Parturition, often considered as a physical stress, represents a variety of physical and psychological stimuli that alter homeostasis and metabolism (Trevisi *et al,* 2012). The mechanism behind the stress-induced APPs release at parturition is not known, but a hypothesis based on a neuroendocrine-immune network concept has recently been put forward, indicating that non-inflammatory and psychophysical stressors activate the combined action of the sympatho-adrenal axis and the hypothalamic–pituitary–adrenal axis. This would affect both the immunity-related cells and the release of glucocorticoids, and would ultimately lead to the production and release of APPs. Therefore the elevations in APPs or inflammation biomarkers clearly differentiate the physiological response to parturition versus the inflammation related to pathologies during the periparturient period.

APR manifested by significant increases of SAA has also been reported in camels subjected to electroejaculation (EEJ) (Fig 2) (Tharwat and Al-Sobayil, 2018a). It was not attributed to pathological conditions, as the WBCs did not change significantly, thus confirming the absence of pathological conditions. Therefore, these elevations, together with the cortisol increases, could be due to a physical "stress" resulting in the numerous changes (Huzzey *et al,* 2011; Bauer *et al,* 2012). EEJ, often considered as a physical stress, represents a variety of physical and psychological stimuli that alter homeostasis and metabolism (Tharwat and Al-Sobayil, 2018a).

As a result of stress, APR manifested by significant increases of Hp and SAA was also reported in camels after race (Fig 3) (Tharwat and Al-Sobayil, 2018b). In this study, the significant increases of Hp and SAA after racing cannot be associated with

pathological conditions as WBCs did not change significantly after racing, thus confirming the absence of pathological conditions. Therefore, these elevations, together with the cortisol increase, could be due to a physical "stress" resulting in the numerous changes that occur during racing (Bauer *et al,* 2012). Exercise, often considered as a physical stress, represents a variety of physical and psychological stimuli that alter the homeostasis and metabolism of animal organisms (Mastorakos *et al,* 2005).

The mechanism behind the stress-induced SAA release after EEJ and race is not known, but a hypothesis based on a neuroendocrine-immune network concept has recently been reported (Murata, 2007). This indicates that the non-inflammatory and psychophysical stressors activate the combined action of the sympatho-adrenal axis and the hypothalamic–pituitary–adrenal axis. This would affect both the immunity-related cells and the release of glucocorticoids, and would ultimately lead to the production and release of APPs (Murata, 2007). In addition, glucocorticoids have been shown to induce or facilitate hepatic synthesis of APPs *in vitro* (Alsemgeest *et al,* 1995).

Conclusions

This mini review sheds light on the commonly used inflammation biomarkers or APPs (Hp and SAA) in camel medicine. These biomarkers are elevated in camels following non-inflammatory stressors as parasitic infections, at parturition, following stimulation by electroejaculation and following race stimulate APPs prsoduction. It is expected that APPs might be used in facilitating the diagnosis and prognosis of camel diseases.

References

Alsemgeest SPM, Lambooy IE, Wierenga HK, Dieleman SJ, Meerkerk B, van Ederen AM and Niewold, TA (1995). Influence of physical stress on the plasma concentrations of serum amyloid A (SAA) and haptoglobin (Hp) in calves. Veterinary Quaternary 17:9–12.

Azma F, Razavi SM, Nazifi S, Rakhshandehroo E and Sanati AR (2015). A study on the status of inflammatory systems in camels naturally infected with *Toxoplasma gondii*. Tropical Animal Health and Production 47:909-914.

Baghshani H, Nazifi S, Saeb M and Saeb S (2010). Influence of road transportation on plasma concentrations of acute phase proteins, including fibrinogen, haptoglobin, serum amyloid A, and ceruloplasmin, in dromedary camels (*Camelus dromedarius*). Comparative Clinical Pathology 19:193–198.

Bauer NB, Er E and Moritz A (2012). Effect of submaximal aerobic exercise on platelet function, platelet activation, and secondary and tertiary hemostasis in dogs. American Journal of Veterinary Research 73:125-133.

Ceron JJ, Eckersall PD and Martinez-Subiela S (2005). Acute phase proteins in dogs and cats; current knowledge and future perspectives. Veterinary Clinical Pathology 34:85-99.

Ebersole JL and Cappelli D (2000). Acute-phase reactants in infections and inflammatory diseases. Periodontology 23:19-49.

Eckersall PD and Bell R (2010). Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Veterinary Journal 185:23-27.

Eckersall PD (2000). Acute phase proteins as markers of infection and inflammation: monitoring animal health, animal welfare and food safety. Irish Veterinary Journal 53:307-311.

El-Bahr SM and El-Deeb WM (2016). *Trypanosoma evansi* in naturally infected dromedary camels: lipid profile, oxidative stress parameters, acute phase proteins and proinflammatory cytokines. Parasitology 143:518-522.

El-Deeb WM and Buczinski S (2015). The diagnostic and prognostic importance of oxidative stress biomarkers and acute phase proteins in Urinary Tract Infection (UTI) in camels. PeerJ. 3:e1363.

Gronlund U, Hulten C, Eckersall PD, Hogarth C and Waller KP (2003). Haptoglobin and serum amyloid A in milk and serum during acute and chronic experimentally induced Staphylococcus aureus mastitis. Journal of Dairy Research 70:379-386.

Hirvonen J, Hietarkopi S, Saloniemi H (1997). Acute phase response in emergency slaughtered cows. Meat Science 3:249-257.

Huzzey JM, Nydam DV, Grant RJ and Overton TR (2011). Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. Journal of Dairy Science 94:5878-5889.

Katoh N and Nakagawa H (1999). Detection of haptoglobin in the high-density lipoprotein and the very high-density lipoprotein fractions from sera of calves with experimental pneumonia and cows with naturally occurring fatty liver. Journal of Veterinary Medical Science 61:119-124.

Mastorakos G, Pavlatou M, Diamanti-Kandarakis E and Chrousos GP (2005). Exercise and the stress system. Hormones (Athens) 4:73-89.

Murata H, Shimada N, Yoshioka M (2004). Current research on acute phase proteins in veterinary diagnosis: an overview. Veterinary Journal 168:28-40.

Murata H (2007). Stress and acute phase protein response: an inconspicuous but essential linkage. Veterinary Journal 173:473-474.

Nakagawa H, Yamamoto O, Oikawa S, Higuchi H, Watanabe A and Katoh N (1997). Detection of serum haptoglobin by enzyme-linked immunosorbent assay in cows with fatty liver. Research in Veterinary Science 62:137-141.

Nazifi S, Oryan AM, Ansari-Lari M, Tabandeh MR, Mohammadalipour A and Gowharnia M (2012). Evaluation of sialic acids and their correlation with acute-phase proteins (haptoglobin and serum amyloid A) in clinically healthy Iranian camels (*Camelus dromedarius*). Comparative Clinical Pathology 21:383-387.

Petersen HH, Nielsen JP, Heegaard PMH (2004). Application of acute phase protein measurement in veterinary clinical chemistry. Veterinary Research. 35:163-187.

Schneider A, Corrêa MN, Butler WR (2013). Acute phase proteins in Holstein cows diagnosed with uterine infection. Research in Veterinary Science 95:269-271.

Tharwat M and Al-Sobayil F (2018a). Influence of electro-ejaculator on serum concentrations of acute phase proteins and bone metabolism biomarkers in male dromedary camels (*Camelus dromedarius*). Journal of Applied Animal Research 46:1226-1232.

Tharwat M and Al-Sobayil F (2018b). The impact of racing on serum concentrations of bone metabolism biomarkers in racing Arabian camels. Journal of Camel Practice and Research 25:59-63.

Tharwat M, Al-Sobayil F and Buczinski S (2014). Influence of racing on the serum concentrations of acute phase proteins and bone metabolism biomarkers in racing greyhounds. Veterinary Journal 202:372-377.

Tharwat M, and Al-Sobayil F (2015). Serum concentrations of acute phase proteins and bone biomarkers in female dromedary camels during the periparturient period. Journal of Camel Practice and Research 22:271-278.

Tizard IR (2009). Veterinary Immunology. An Introduction, 8th ed. Saunders Elsevier, St. Louis, Missouri.

Tourlomousis P, Eckersall PD, Waterston M and Buncic S (2004). A comparison of acute phase protein measurements and meat inspection findings in cattle. Foodborne Pathogens and Disease 1:281-290.

Trevisi E, Amadori M, Cogrossi S, Razzuoli E and Bertoni G (2012). Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Research in Veterinary Science 93:695-704.

Uchida E, Katoh N and Takahashi K (1993). Appearance of haptoglobin in serum from cows at parturition. Journal of Veterinary Medical Science 55:893-894.

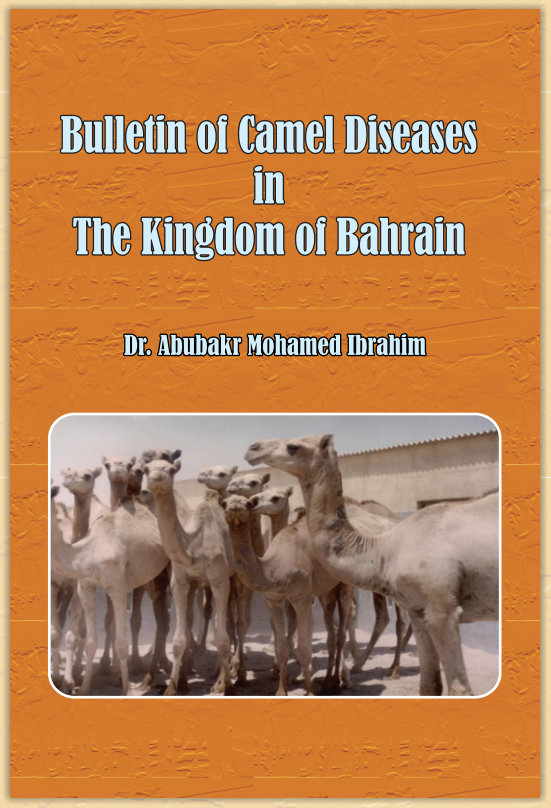
Yazwinsk M, Milizio JG and Wakshlag JJ (2013). Assessment of serum myokines and markers of inflammation associated with exercise in endurance racing sled dogs. Journal of Veterinary Internal Medicine 27:371-376.

Bulletin of Camel Diseases in The Kingdom of Bahrain

This is a unique book which contains chapters on infectious and non-infectious diseases. The chapter on infectious diseases contains six sections. The section of bacterial diseases is subclassified as corynebacterium abscesses, paratuberculosis, hepatic necrobacillosis, mastitis, *Streptococcus zooepidemicus,* bacterial Infection in young camels, uterine Infection, infection of the vagina and vulva and other disorders. The section of protozoal diseases has narrations on trypanosomiasis, anaplasmosis and babesiosis. The section on parasitic infections is composed of gastrointestinal parasites in young camels, echinococcosis and mange. The section of mycotic diseases contains phycomycosis and ringworm. The section of viral diseases contains subsections on camel pox and contagious ecthyma. Edema Disease is described in miscellaneous section. The chapter on noninfectious diseases has three sections. Other section on poisoning describes pyrethroid, nitrate and toxic jaundice. The section describes zinc deficiency. The miscellaneous section describes foreign bodies, sand colic, bloat, caecal impaction, hydrocephalus, corneal opacity and osteochondroma.

About the Author

Dr. Abubakr Mohamed Ibrahim is a Veterinary Pathologist and worked for a long period as head of Royal Court Veterinary Laboratory. Kingdom of Bahrain which led to genesis of this publication out of his rich experience in diagnosing camel diseases in the Kingdom of Bahrain. This would be counted as his significant contribution and future researchers will find it easy to understand the pattern of camel diseases in this part of the world. Dr. Abubakr had majority of his publications based on camel diseases of Bahrain. Thus publication of this book would prove an important reference book for the camel practitioners and researchers.



Editor:
Dr. T.K. Gahlot

Edition: 2014

Publisher:
Camel Publishing House
67, Gandhi Nagar West,
Near Lalgarh Palace,
Bikaner-334001 Rajasthan, India
email: tkcamelvet@yahoo.com

Website:
www.camelsandcamelids.com
www.tkgahlotcamelvet.com

Price: US $ 90
INR 1000

ISBN 81-903140-2-5

DOI : 10.5958/2277-8934.2020.00024.7 Vol 27 No 2, p 165-171

INVESTIGATION ON BIOCHEMICAL PARAMETERS OF CEREBROSPINAL FLUID IN CAMELS WITH NEUROLOGICAL DISORDERS

Shawaf T[1], El Nahas A[1], Ahmed Melegi[1], Al Bulushi S[2], Ahmad Al Aiyan[3] and Isam Eljalli[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Hasa, Saudi Arabia,
[2]Animal Health Department, Animal Wealth General Directorate, Ministry of Agriculture and Fisheries, Sultanate of Oman
[3]Department of Veterinary Medicine, College of Food and Agriculture, United Arab Emirates University, Al Ain, UAE

ABSTRACT

This study was aimed to determine and compare CSF biochemical parameters in 8 healthy camels and 15 camels with neurological signs. The important neurological signs observed were shivering, tremour, staggering, rotation of the head, slight vision impairment and progressive worsening of general condition. Serum and CSF were collected for the biochemical analysis of the parameters. The results revealed that there was an increase in ALB, AST, TP, MG, NA, and TBIL values in the CSF obtained from camels showing neurological sings as compared to healthy camels.

Key words: Biochemical, camel, CSF, neurological signs, serum

Cerebrospinal fluid CSF) normally surrounds the brain and spinal cord and protects these from injury and nourishes and support central nervous system (Scott, 2010; Simon and Iliff, 2016). However, CSF is considered as a source of nutrition for the parenchyma of the brain and spinal cord (Achard *et al,* 2017). The normal chemical values of CSF in different animal species has been documented, i.e. horses, sheep, cattle, cats, dogs and various laboratory animals (Abate *et al,* 1998; Ahmed *et al,* 2009a; Ameri and Mousavian, 2007; Di Terlizzi and Platt, 2006; Nazifi and Maleki, 1998b; Stocker *et al,* 2002; Stokol *et al,* 2009; Welles *et al,* 1992b). Shawaf *et al* (2018) recently reported values for CSF constituents from healthy camels in Saudi Arabia.

The comparison of CSF analysis to serum has been used for diagnostic investigation of systemic abnormalities (Benedicenti *et al,* 2018; Stokol *et al,* 2009). However, the composition of CSF is strongly dependent on blood plasma constituents. Alterations in cerebrospinal fluid could be due to physiological and environmental conditions and diverse neurological diseases (Achard *et al,* 2017; Stokol *et al,* 2009).

Cerebrospinal fluid examination is done for inflammatory, neoplastic, traumatic, infectious, or degenerative disorders of the nervous system (NS) (Windsor *et al,* 2008), however, CSF analysis can rarely deliver a definitive diagnosis (Di Terlizzi and Platt, 2009).

Chemical analysis of CSF can provide evidence and information about the metabolism of the brain. It can also aid in the evaluation of CNS disruption, and it aids in biomarkers identification for the diagnosis of CNS diseases (Johanson *et al,* 2008). Cellular and chemical analysis of cerebrospinal fluid in different animal species have been used to monitor neurological disorders (Kumar and Kumar, 2012; Lampe *et al,* 2020; Simon and Iliff, 2016; Stokol *et al,* 2009). The cellular and biochemical parameters of cerebrospinal fluid helps in the evaluation of nervous system health status of living animals (AI-Sagair *et al,* 2005; Bellino *et al,* 2015; Frosini *et al,* 2000; Welles *et al,* 1992a).

Neurological signs observed in camels can be classified as infectious or noninfectious (Babelhadj *et al,* 2018; Shoeib *et al,* 2019). The infectious causes include viral, bacterial and prions diseases while the non-infectious causes are nutritional disorders. The neurological signs in camels manifest behavioural and neurological changes, i.e. meningitis, encephalitis, rhomboencephlistis, meningoencephalitis, stillbirth and abortion El Dobab *et al* (2008). Babelhadj *et al* (2018) studied neurological signs in camels and found severe spongiform degenerative changes in the brain tissues along with disease-specific prion protein.

SEND REPRINT REQUEST TO TURKE SHAWAF email: tshawaf@kfu.edu.sa

The main objective of this study was to determine and compare CSF biochemical parameters in healthy camels, and in camels with neurological signs.

Materials and Methods

Animals and sampling

Fifteen dromedary camels (age 2-21 years) with a history of neurological symptoms presented to the Veterinary Teaching Hospital, King Faisal University were investigated in this study. The main neurological symptoms observed in these animals were shivering, tremour, staggering, rotation of the head, slight vision impairment and progressive worsening general condition. Eight apparently healthy camels were used for comparison. Clinical examination was performed on all the subjected animals, venous blood samples were collected from the jugular vein. Animals were sedated and cerebrospinal fluid samples were collected aseptically from the Atlanto-occipital articulation as described by Shawaf *et al* (2018).

Biochemical samples analysis

Cerebrospinal fluid and serum samples were processed using Vet scan vs 2 analyser (ABAXIS, USA) to determine the concentration of albumin (ALB), gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase, total serum protein (TP), creatine kinase (CK), blood urea nitrogen (BUN), inorganic phosphorus (PHOS), potassium (K), magnesium (Mg), chloride (Cl), Sodium (Na), calcium (Ca), amylase, alanine aminotransferase, total bilirubin (TBIL), glucose (GLU) and creatinine (CRE).

Statistical analysis

Data was recorded in Excel spreadsheets and imported into Graph Pad Prism 7 software for further analyses. The data was analysed to determine the range, mean and standard error of the mean. To determine the significance, the student's t-test was used with a significance factor of $P < 0.05$.

Results

Table 1 shows the mean±SEM values of the chemical parameter's in the serum of clinically healthy dromedary camels (n=8) and camels with neurological disorders (n=15). The obtained results showed a significant increase in the serum parameters, i.e. ALB, AST, CK, Na, ALT, and TBIL in the affected camels, while a significant decrease in ALP, PHOS, Mg, K, and AMY were observed. No significant difference was observed in the following serum parameters of the affected and healthy camels, i.e. GGT, TP, BUN, Ca, Cl, CRE and Glucose.

Table 2 showed the mean±SEM values of the chemical parameters in the serum and CSF in camels with neurological disorders (n=15). Most parameters showed significant decrease in the CSF in comparison to values in serum. Only the chloride value was increased significantly in CSF compared to its value in serum of affected animals. No significant changes were observed in Mg, Na and K values among serum and CSF in affected animals.

The mean±SEM of CSF chemical parameters (Table 3) revealed that there was a significant increase in ALB, AST, TP, Mg, NA, and TBIL values in the CSF of affected animals. There was a significant decrease in CSF-Glucose in affected camels compared to healthy camels. However, there were no significant differences in ALP, GGT, CK, ALT, PHOS, Ca, Cl, K, ALT, BUN, AMY and CRE values.

Discussion

CSF chemical parameters compared to serum parameters are frequently used for diagnostic purposes, and to explain the nature of the disease process in CNS diseases and its severity (Kulkarni *et al,* 2009; Shawaf *et al,* 2018). Clinically, it was difficult to determine the main cause of neurological disorders in camels because these symptoms could be manifested by various other diseases (El Dobab *et al,* 2008).

In the present study, the concentrations of most biochemical parameters of the CSF for the camels with neurological symptoms were decreased compared to the levels in the serum. The same findings were reported in healthy camels (Nazifi and Maleki, 1998a; Saladin, 2012; Shawaf *et al,* 2018). This result may conclude that the values of biochemical parameters for camels are generally lesser in CSF vis-a-vis serum in both healthy and diseased animals, hence must be evaluated carefully for diagnostic purposes.

The results of this study revealed that AST levels were lesser in the CSF than serum which were in agreement with Ahmed *et al* (2009b) and Shawaf *et al* (2018) who also found similar results in camels. On the other hand, increased values of CSF-AST of affected camels as compared with healthy camels was in agreement with Tapiola *et al* (1998) in the case of Alzheimer's disease in humans. Increased AST in CSF for patients with meningitis was reported possibly due to outflow of enzymes from the destroyed cells

of CNS (Kulkarni *et al,* 2006). However, Thrall (2004) stated that the bigger molecules of AST, which can't pass through the blood brain barrier, leads to increased activities of this enzyme in the CSF, which could indicate damage of CNS cells. Moreover, Feldman (1997) and Indrieri *et al* (1980) stated a poor prognosis in affected animals with high AST levels in CSF. Normally, all the proteins which are found in the CSF are derived from plasma (Reiber *et al,* 2013), the levels are inversely related to its molecular weight (William Vernau *et al,* 2008). CSF protein levels are considered as one of the most sensitive indicators of a pathological process within the CNS (Kumar and Kumar, 2012). Similar results for the total protein in CSF of healthy camels were reported in healthy sheep (George, 1996). The total protein in CSF was in contrast with observations from a previous study in cattle (Welles *et al,* 1992a) and dogs (Hoerlein, 1978). Kumar and Kumar (2012) reported increased total protein in CSF in animals with viralor bacterial disease with was in agreement with the results of present study. According to Polizopoulou (2014) the increased concentrations of CSF total protein especially albumin can be attributed to the increased permeability of the blood-brain barrier. In contrast to our results, Bellino *et al* (2015) and Kumar (2012) reported less total protein concentration in CSF for affected cattle. However, Kumar and Kumar (2012) also reported decreased CSF protein in animals suffering from degenerative changes in CSN.

The main CSF protein is albumin which is produced only in the liver (William Vernau *et al,* 2008). Albumin synthesis only occurs extrathecally, while its increased level in CSF indicates damage to the CSF blood-brain barriers or CNS trauma. Barrier dysfunction can also be indicated by the ratio between CSF and serum albumin (William Vernau *et al,* 2008). A significant increase in CSF albumin was detected in the neurologically-manifested camels. Accordingly, this could indicate brain or meningeal diseases in the examined camels.

Creatinekinase (CK) consists of M and B subunits and may be traced in the brain, muscle, and heart (Ferreira *et al,* 2016). The results obtained indicated a highly significant elevation in the serum CK value for affected camels, compared to the CSF-CK value. This could be attributed to muscle damage during irregular movements, ataxia, or recumbency as a result of neurological disorders (Teodoro *et al,* 2019). The increased CK levels in CSF for affected animals compared to healthy animals was in agreement with Indrieri *et al* (1980); Jackson *et al* (1996); Kjekshus *et al* (1980) who observed a close correlation between the amount of brain injury and CSF-CK activity.

Table 1. Mean, SEM and range of biochemical value of blood serum in clinically normal dromedary camels (n=8) and camels with neurological disorders (n=15).

Parameter	Serum in healthy camels		Serum in affected camels		P value
	Mean ± SEM	Range	Mean±SEM	Range	
ALB (g/dl)	3.21 ± 0.24	2.4-4.0	5.28 ± 0.36**	4.1-6.7	0.009
ALP (IU/l)	183.8 ± 11.32	166-199	112.4 ± 13.96**	48-156	0.007
AST (IU/l)	143 ± 11.33	121-174	235.9 ± 93.01**	93-754	0.0095
GGT (IU/l)	14.45 ± 1.76	12.5-16.4	17.86 ± 5.02	10-47	0.25
TP (g/dl)	6.96 ± 0.33	6.43-7.23	7.17 ± 0.25	6.5-8.3	0.28
BUN (mg/dl)	14.2 ± 0.82	12.43-16.2	20.59 ± 2.37	12-47	0.14
CK (IU/l)	111.2 ± 25.7	95-145	277.1± 73.2***	168-714	0.008
PHOS (mmol/l)	5.98 ± 1.32	3.3-7.7	3.09 ± 0.28**	2.2-4.2	0.01
Mg (mmol/l)	2.83 ± 0.22	2.3-3.1	2.13 ± 0.15**	1.9-2.2	0.008
Ca (mmol/l)	9.68 ± 0.33	9.2-10.5	10.1 ± 0.34	8.9-11.2	0.35
Na (mmol/l)	151.1 ± 1.60	148-154	158.9 ± 2.37*	148-166	0.03
Cl (mmol/l)	113.5 ± 1.68	111-120	118 ± 1.31	114-122	0.1
K (mmol/l)	5.8 ± 0.56	4.8-6.2	4.66 ± 0.18*	418-5.5	0.02
ALT (IU/l)	14.11 ± 1.29	12-15	18.14 ± 1.33*	14-25	0.017
AMY (IU/l)	488.3 ± 21.8	461-533	270.9 ± 42.2**	105-450	0.003
TBIL (µmol/l)	0.2 ± 0.03	0.1-0.3	0.46 ± 0.14*	0.1-1.3	0.05
CRE (mg/dl)	1.39 ± 0.18	1-1.75	1.27 ± 0.12	0.8-1.8	0.28
GLU (mg/dl)	101.8 ± 15.61	71-143	97.7 ± 19.19	64-119.4	0. 1

Table 2. Mean, SEM and range of biochemical values of serum and CSF in affected dromedary camels with neurological disorders (n=15).

Parameter	Serum in affected camels		CSF affected camels		P Value
	Mean ± SEM	Range	Mean ± SEM	Range	
ALB (g/dl)	5.28 ± 0.36	4.1-6.7	0.77 ± 0.09***	0.41-1.02	0.0009
ALP (IU/l)	112.4 ± 13.96	48-156	53.43 ± 1.72**	48.2-59.3	0.008
AST (IU/l)	235.9 ± 93.01	93-754	33.14 ± 2.24***	25.8-41.35	0.0008
GGT (IU/l)	17.86 ± 5.02	10-47	4.54 ± 0.53***	4.12-9.98	0.00075
TP (g/dl)	7.17 ± 0.25	6.5-8.3	1.06 ± 0.18***	0.82-1.52	0.00033
BUN (mg/dl)	20.59 ± 2.37	12-47	9.33 ± 1.32**	9.90-10.90	0.006
CK (IU/l)	277.1± 73.2	168-714	30.14 ± 3.01***	21.3-39.8	0.0008
PHOS (mmol/l)	3.09 ± 0.28	2.2-4.2	0.51 ± 0.08***	0.20-0.89	0.0009
Mg (mmol/l)	2.13 ± 0.15	1.9-2.2	2.63 ± 0.11	2.1-2.92	0.38
Ca (mmol/l)	10.1 ± 0.34	8.9-11.2	5.14 ± 0.31**	3.48-6.01	0.008
Na (mmol/l)	158.9 ± 2.37	148-166	161 ± 3.18	144-171	0.19
Cl (mmol/l)	118 ± 1.31	114-122	136 ± 1.18*	127-140	0.045
K+ (mmol/l)	4.66 ± 0.18	418-5.5	4.52 ± 0.14	3.92-4.88	0.21
ALT (IU/l)	18.14 ± 1.33	14-25	10.57 ± 0.53**	8.2-12.12	0.007
AMY (IU/l)	270.9 ± 42.2	105-450	11.43 ± 0.78***	10.65-14.47	0.0006
TBIL (µmol/l)	0.46 ± 0.14	0.1-1.3	0.24 ± 0.04*	0.11-0.42	0.018
CRE (mg/dl)	1.27 ± 0.12	0.8-1.8	0.57 ± 0.06**	0.38-0.83	0.0035
GLU (mg/dl)	97.7 ± 19.19	64-119.4	72.3 ± 10.4*	59-83.6	0.031

Table 3. Mean, SEM and range of biochemical values of CSF in healthy (n=8) and affected dromedary camels with neurological disorders (n=15).

Parameter	CSF in healthy camels		CSF in affected camels		P Value
	Mean± SEM	Range	Mean± SEM	Range	
ALB (g/dl)	0.15 ± 0.03	0.1-0.19	0.77 ± 0.09**	0.41-1.02	0.0014
ALP (IU/l)	51.07 ± 1.4	48-55	53.43 ± 1.72	48.2-59.3	0.36
AST (IU/l)	25.24 ± 2.82	22-30	33.14 ± 2.24*	25.8-41.35	0.026
GGT (IU/l)	5.3 ± 2.35	3-8.2	4.54 ± 0.53	4.12-9.98	0.21
TP (g/dl)	0.71 ± 0.04	0.62-0.86	1.06 ± 0.18*	0.82-1.52	0.018
BUN (mg/dl)	8.98 ± 0.75	8.8-12.9	9.33 ± 1.32	9.90-10.90	0.26
CK (IU/l)	24.85 ± 3.39	17-30	30.14 ± 3.01	21.3-39.8	0.08
PHOS (mmol/l)	0.64 ± 0.08	0.5-0.91	0.51 ± 0.08	0.20-0.89	0.095
Mg (mmol/l)	2.11 ± 0.07	2-2.3	2.63 ± 0.11*	2.1-2.92	0.018
Ca (mmol/l)	4.77 ± 0.08	4.6-5.0	5.14 ± 0.31	3.48-6.01	0.17
Na (mmol/l)	153.5 ± 0.82	151-156	161 ± 3.18*	144-171	0.019
Cl (mmol/l)	128.3 ± 2.2	123-133	136 ± 1.18	127-140	0.095
K+ (mmol/l)	4.22 ± 0.04	4.2-4.33	4.52 ± 0.14	3.92-4.88	0.11
ALT (IU/l)	10.54 ± 0.43	10.1-11.8	10.57 ± 0.53	8.2-12.12	0.47
AMY (IU/l)	9.71 ± 1.23	8-15	11.43 ± 0.78	10.65-14.47	0.075
TBIL (µmol/l)	0.14 ± 0.02	0.1-0.2	0.24 ± 0.04*	0.11-0.42	0.028
CRE (mg/dl)	0.6 ± 0.06	0.5-0.7	0.57 ± 0.06	0.38-0.83	0.35
GLU (mg/dl)	90.8 ± 9.69	100.3-80.2	72.3 ± 10.4*	59-83.6	0.037

In the current study an increase in the concentrations of sodium values was found in both the serum and the CSF from affected camels. Kulkarni *et al* (2009) reported the similar results for sodium in CSF in poisoned cases. Our results agreed with Kumar and Kumar (2012), who reported that Na concentrations are similar in both CSF and serum.

The obtained results for Cl and Ca levels were in agreement with Kumar and Kumar (2012). Chloride levels were higher in the CSF than in the blood, while the calcium concentration was less in CSF for healthy and affected groups. Contrary to our results Tan *et al* (2014) reported decreased values of Cl in CSF patients with bacterial meningitis. According to Kumar and Kumar (2012), the increased concentrations of CSF-Ca indicates the presence of damage in the blood brain barrier.

Magnesium has an important role in the nervous system for ideal nerve transmission, calcium channel antagonism and neuromuscular coordination. It also helps to prevent excitotoxicity (Grober *et al,* 2015). The results for Mg in serum and CSF in affected camels were interesting. The Mg concentration decreased in serum from affected animals in comparison to its levels in serum from healthy animals, while there was an increase in CSF-Mg for affected animals compared to its levels in CSF of healthy animals. Our results for decreased magnesium in serum from affected camels were in agreement with earlier observations in camels affected with Dubduba Syndrome (Al-Mujalli *et al,* 2011). However, it is well known that insufficiency of Mg results in muscle cramps, increased irritability, weakness, tremors and jerking (Al-Mujalli *et al,* 2011). Similar to our results Reynolds *et al* (1984) reported increase in CSF-Mg and decrease in serum of calves affected with hypomagnesaemia. Similarly, results of increased CSF-Mg in people with epilepsy was reported (Alvarez-Domínguez *et al,* 1978; Ileana Benga *et al,* 1985). In contradiction to our results, Bayir *et al* (2009) reported lower CSF-Mg in people with brain injuries. The difference in Mg concentration among serum and CSF in affected animals could be attributed to the fact that there is no direct relation between Mg in serum and CSF, which confirms the theory that the infusion of Mg compounds into blood had no influence on CSF-Mg concentrations (Mercieri *et al,* 2012).

CSF glucose is transported through facilitated diffusion from the plasma, in which its levels in CSF are relying on glucose levels in the blood-CNS metabolic rate, and glucose amount delivered into the CSF (William Vernau *et al,* 2008). The ratio of CSF/serum glucose are ranged between 40-60% in healthy camels (Ahmed *et al,* 2009b; Shawaf *et al,* 2018). Decreased CSF-glucose in affected camels compared to healthy camels in the present study was in agreement with previous studies in humans with several disorders of the nervous system like meningitis (Roberts *et al,* 2014; Troendle and Pettigrew, 2019) and severe encephalomyelitis (Troy *et al,* 2008); and also with dogs with nervous distemper (Ettinger and Feldman, 2005). Additionally, a decreased glucose levels in CSF (hypoglycorrhachia) in animals is related with bacterial meningitis or systemic hypoglycemia (Bailey and Vernau, 1997; George, 1996), which agreed with the results of this study in camels that are affected by neurological disorders. The serum glucose of affected camels was decreased in the present study compared to the levels in serum of healthy camels, which could explain its lower levels in CSF in affected camels. However, Deisenhammer *et al* (2011) also reported that the elevated levels of CSF glucose had no significant diagnostic importance, as it proportionately increased with blood glucose in case of diabetes.

Acknowledgements

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number: IFT20025 with reference to the research grant number.

References

Abate O, Bollo E, Lotti D and Bo S (1998). Cytological, immunocytochemical and biochemical cerebrospinal fluid investigations in selected central nervous system disorders of dogs. Zentralbl Veterinarmed B 45(2):73-85 doi:10.1111/j.1439-0450.1998.tb00769.x

Achard D, Francoz D, Grimes C, Desrochers A, Nichols S, Babkine M and Fecteau G (2017). Cerebrospinal fluid analysis in recumbent adult dairy cows with or without spinal cord lesions. Journal of Veterinary Internal Medicine 31(3):940-945 doi:10.1111/jvim.14705

Ahmed SH, Omer SA and Gameel AA (2009a). Some normal constituents in serum and cerebrospinal fluid in sudanese camels (*Camelus dromedarius*). Assiut Veterinary Medical Journal 55(123):163-170.

Ahmed SH, Omer SAA and Gameel AA (2009b). Some normal constituents in serum and cerebrospinal fluid in Sudanese camels (*Camelus dromedarius*). Assiut Veterinary Medical Journal 55(123):163-170

AI-Sagair OA, Fathalla S and Abdel-Rahman H (2005). Reference values and age related changes in cerebrospinal fluid and blood components in the clinically normal male dromedary camel. Journal of Animal and Veterinary Advances 4:467-469.

Al-Mujalli AM, Al-Naeem AA, Al-Ghamdi Ghanem, Al-Swailem Abdulaziz, Alyamani Essam, Shehata AM, Al-Dubaib Musaad, Hashad M, El-Lithy DA, Mahmoud Osama and Alfayez M (2011). Cellular and biochemical blood profile in camels suffering from dubduba syndrome. Scientific Journal of King Faisal University 12(2).

Alvarez-Domínguez L, Prats-Quinzanños J, Calvet-Micas E, Alsina-Kirtchner MJ and Ramón-Bauza F (1978). Study of calcium and magnesium in cerebrospinal fluid and Its' relation to different neurological diseases. Anales Españoles de Pediatría 11(11):753-62.

Ameri M and Mousavian R (2007). Analysis of cerebrospinal fluid from clinically healthy Iranian fat-tailed sheep. Veterinary Research Communications 31(1):77-81. doi:10.1007/s11259-006-3374-5

Babelhadj B, Di Bari MA, Pirisinu L, Chiappini B, Gaouar SBS, Riccardi G, Marcon S, Agrimi U, Nonno R and Vaccari G (2018). Prion disease in dromedary camels, Algeria. Emerging Infectious Diseases 24(6):1029-1036. doi:10.3201/eid2406.172007

Bailey CS and Vernau W (1997). Cerebrospinal Fluid, 5th edn. Academic Press, London.

Bayir A, Ak A, Kara H and Sahin T (2009). Serum and cerebrospinal fluid magnesium levels, Glasgow Coma Scores, and in-hospital mortality in patients with acute stroke. Biological Trace Element Research 130:7-12.

Bellino C, Miniscalco B, Bertone I, Cagnasso A, Occhiena E, Gianella P and D'Angelo A (2015). Analysis of cerebrospinal fluid from cattle with central nervous system disorders after storage for 24 hours with autologous serum. BMC Veterinary Research 11:201-201 doi:10.1186/s12917-015-0502-x

Benedicenti L, Gianotti G and Galban EM (2018). Comparison between cerebrospinal fluid and serum lactate concentrations in neurologic dogs with and without structural intracranial disease. Canadian Journal of Veterinary Research 82(2):97-101.

Deisenhammer F, Bartos A, Egg R, Gilhus NE, Giovannoni G, Rauer S, Sellebjerg F, Tumani H (2011). Routine Cerebrospinal Fluid (CSF) Analysis. European Handbook of Neurological Management. Vol 1, 2nd edn. Blackwell Publishing Ltd, pp 5-17.

Di Terlizzi R and Platt S (2006). The function, composition and analysis of cerebrospinal fluid in companion animals: part I - function and composition. Veterinary Journal 172(3):422-431 doi:10.1016/j.tvjl.2005.07.021

Di Terlizzi R and Platt SR (2009). The function, composition and analysis of cerebrospinal fluid in companion animals: Part II – Analysis. The Veterinary Journal 180(1):15-32 doi:https://doi.org/10.1016/j.tvjl.2007.11.024

El Dobab MA, Aboelhassan DG and Hashad M (2008). Dubduba Syndrome: An emerging neurological disease of camels with a possible viral etiologic agent. Journal of Camel Practice and Research 15(2):147-152.

Ettinger SJ and Feldman EC (2005). Textbook of Veterinary Internal Medicine, 6th edn. Elsevier Saunders, St Louis, Missouri.

Feldman BF (1997). Cerebrospinal fluid. In: Clinical Biochemistry of Domestic Animals KANEKO. JJ, 5.ed. edn. Academic Press, San Diego. pp 786-822.

Ferreira Christina R, Yannell Karen E, Mollenhauer Brit, Espy Ryan D, Cordeiro Fernanda B, Ouyang Z and Cooks RG (2016). Chemical profiling of cerebrospinal fluid by multiple reaction monitoring mass spectrometry. Analyst 141(18):5252-5255.

Frosini M, Sesti C, Palmi Mitri, Valoti M, Fusi F, Mantovani PL, Carlos Dantas Bianchi L, Della Corte L and Pietro Sgaragli G (2000). Heat-stress-induced hyperthermia alters CSF osmolality and composition in conscious rabbits. American Journal of Physiology Regulatory, Integrative and Comparative Physiology 279:2095-2103

George L (1996). Diseases of the Nervous System, 2nd ed. edn, Mosby, St Louis.

Grober U, Schmidt J and Kisters K (2015). Magnesium in prevention and therapy. Nutrients 7:8199–8226.

Hoerlein BF (1978). Canine Neurology, 3rd edn. W B Sounders Company, Philadelphia.

Ileana Benga, Valeria Baltescu, Rozalia Tilinca, Viorel Ghiran, Muschevici D and Benga G (1985). Plasma and cerebrospinal fluid concentrations of magnesium in epileptic children. Journal of the Neurological Sciences 67(1):29-34

Indrieri RJ, Holliday TA and Keen CL (1980). Critical evaluation of creatine phosphokinase in cerebrospinal fluid of dogs with neurologic disease. American Journal of Veterinary Research 41(8):1299-1303.

Jackson C, de Lahunta A, Divers T and Ainsworth D (1996). The diagnostic utility of cerebrospinal fluid creatine kinase activity in the horse. Journal of Veterinary Internal Medicine 10(4):246-251

Johanson CE, Duncan JA, Klinge PM, Brinker T, Stopa EG and Silverberg GD (2008). Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. Cerebrospinal Fluid Research 5(1):10 doi:10.1186/1743-8454-5-10

Kjekshus JK, Vaagenes P and Hetland O (1980). Assessment of cerebral injury with spinal fluid creatine kinase (CSF-CK) in patients after cardiac resuscitation. Scandinavian Journal of Clinical and Laboratory Investigation 40:437-444.

Kulkarni MD, Samant SR, Yadav GB, Khanvilkar AV and Khasn is MW (2009). Diagnostic importance of cerebrospinal fluid in pathognomic condition. Veterinary World 2(11):441-443.

Kulkarni SP, Mallikarjuna CR and Murthy DS (2006). Cerebrospinal fluid free sialic acid and aspartate transaminase levels in meningitis. Indian Journal of Clinical Biochemistry 21:185-188.

Kumar V and Kumar N (2012). Diagnostic value of cerebrospinal fluid evaluation in veterinary practice: an overview. Journal of Advanced Veterinary Research 2(3):213-217.

Lampe R, Foss KD, Vitale S, Hague DW and Barger AM (2020). Comparison of cerebellomedullary and lumbar cerebrospinal fluid analysis in dogs with neurological

disease. Journal of Veterinary Internal Medicine doi:10.1111/jvim.15700

Lecollinet S, Pronost S, Coulpier M, *et al* (2019). Viral Equine Encephalitis, a Growing Threat to the Horse Population in Europe? Viruses 2020, 12(1)23 (doi:10.3390/v12010023)

Mercieri M, De Blasi RA, Palmisani S, Forte S, Cardelli P, Romano R, Pinto G and Arcioni R (2012). Changes in cerebrospinal fluid magnesium levels in patients undergoing spinal anaesthesia for hip arthroplasty: Does intravenous infusion of magnesium sulphate make any difference? A prospective, randomised, controlled study. British Journal of Anaesthesia 109(2):208-215.

Nazifi S and Maleki K (1998a). Biochemical analysis of serum and cerebrospinal fluid in clinically normal adult camels (*Camelus dromedarius*). Research in Veterinary Science 65:83-84.

Nazifi S and Maleki K (1998b). Biochemical analysis of serum and cerebrospinal fluid in clinically normal adult camels (*Camelus dromedarius*). Research in Veterinary Science 65(1):83-84.

Polizopoulou Z (2014). Cerebrospinal fluid analysis. Journal of the Hellenic Veterinary Medical Society 65(3):215-224 doi:https://doi.org/10.12681/jhvms.15537

Reiber H, Ressel CB and Spreer A (2013). Diagnosis of neuroborreliosis-Improved knowledge base for qualified antibody analysis and cerebrospinal fluid data pattern related interpretations. Neurol Psychiatry Brain Res 19(4):159-169.

Reynolds C, Bell M and Sims M (1984). Changes in plasma, red blood cell and cerebrospinal fluid mineral concentrations in calves during magnesium depletion followed by repletion with rectally infused magnesium chloride. The Journal of Nutrition 114:1334-1341.

Roberts J, Custalow C, Thomsen T and Hedges J (2014). Roberts and Hedges' Clinical Procedures in Emergency Medicine, 6th edn. Elsevier/Saunders, Philadelphia.

Saladin KS (2012). Anatomy and Physiology: The Unity of Form and Function, by 6th Edn edn. McGraw.

Scott PR (2010). Cerebrospinal fluid collection and analysis in suspected sheep neurological disease. Small Ruminant Research 92:96-110.

Shawaf T, Ramadan RO, Al Aiyan A, Hussen J, Al Salman MF, Eljalii I and El-Nahas A (2018). Cerebrospinal fluid collection and its analysis in clinically healthy dromedary camels (*Camelus dromedarius*). Journal of Camel Practice and Research 25(1):75-79.

Shoeib S, Sayed-Ahmed M and El-khodery S (2019). Hypomagnesemic tetany in camel calves (*Camelus dromedarius*): Clinical consequences and treatment outcomes. Slovenian Veterinary Research 56(22):589-94.

Simon MJ and Iliff JJ (2016). Regulation of cerebrospinal fluid (CSF) flow in neurodegenerative, neurovascular and neuroinflammatory disease. Biochim Biophys Acta 1862(3):442-51 doi:10.1016/j.bbadis.2015.10.014

Stocker H, Sicher D, Rusch P and Lutz H (2002). Reference values in the cerebrospinal fluid of calves between four and eight weeks of age. Schweiz Arch Tierheilkd 144(6):283-8 doi:10.1024/0036-7281.144.6.283

Stokol T, Divers TJ, Arrigan JW and McDonough SP (2009). Cerebrospinal fluid findings in cattle with central nervous system disorders: a retrospective study of 102 cases (1990-2008). Vet Clin Pathol 38(1):103-12 doi:10.1111/j.1939-165X.2008.00094.x

Tan Q-C , Zhang J, Xing X-W, Tian C-L, Huang X-S and Yu S (2014). Significance of chloride contents in cerebrospinal fluid and plasma and their ratio in early diagnosis and differential diagnosis of central nervous system infections. Medical Journal of Chinese People's Liberation Army 39(5):401-405.

Tapiola T, Lehtovirta M, Ramberg J, Helisalmi S, Linnaranta K, Riekkinen Sr P and Soininen H (1998). CSF tau is related to apolipoprotein E genotype in early Alzheimer's disease. Neurology 50:169-174.

Teodoro J, Silva M, Zimerman L, Turke K, Silva L, Feder D and Carvalho A (2019). Symptomatology and prevalence of Pompe disease in patients with proximal muscle weakness and high CK levels. Neuromuscular Disorders 29:S60 doi:10.1016/j.nmd.2019.06.095

Thrall MAea (2004). Laboratory Detection of Muscle Injury. Lippincot, Philadelphia.

Troendle and Pettigrew A (2019). A systematic review of cases of meningitisin the absence of cerebrospinal fluid pleocytosis on lumbar puncture. BMC Infectious Diseases 19:692.

Troy S, Blackburn B, Yeom K, Caulfield A, Bhangoo M and Montoya J (2008). Severe encephalomyelitis in an immunocompetent adult with chromosomally integrated human herpesvirus 6 and clinical response to treatment with foscarnet plus ganciclovir. Clinical Infectious Diseases 47(12):93-96.

Welles EG, Tyler JW, Sorjonen DC and Whatley EM (1992a). Composition and analysis of cerebrospinal fluid in clinically normal adult cattle. American Journal of Veterinary Research 53:2050-2057.

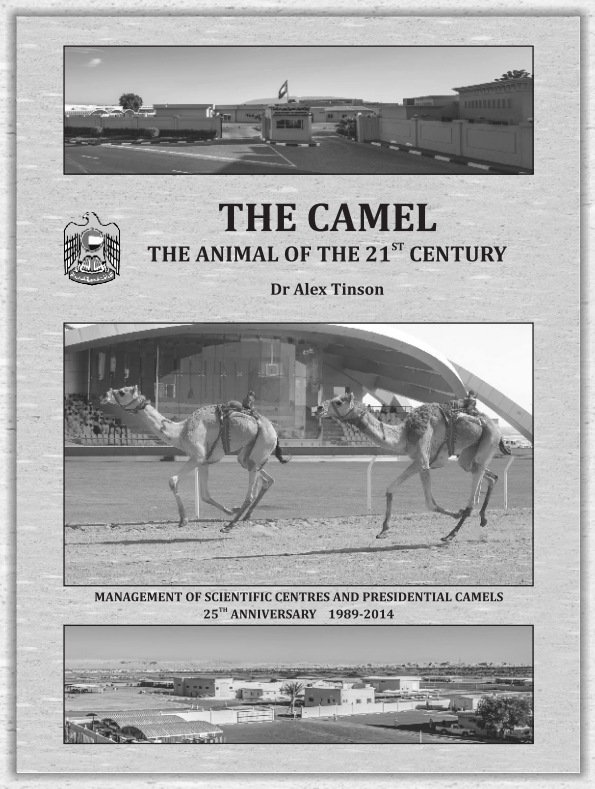
Welles EG, Tyler JW, Sorjonen DC, Whatley EM (1992b). Composition and analysis of cerebrospinal fluid in clinically normal adult cattle. American Journal of Veterinary Research 53(11):2050-2057.

William Vernau, A. K, Vernau and Cleta Sue Bailey (2008). Clinical Biochemistry of Domestic Animals, Sixth Edition. Academic Press.

THE CAMEL

THE ANIMAL OF THE 21ST CENTURY

This book authored by Dr Alex Tinson is an acknowledgement to the support and inspiration that His Highness Sheikh Khalifa Bin Zayed Al Nahyan has provided to the centre and to research in general. The last 25 years has been an incredible adventure for us, the noble camel and the people of the U.A.E. Dr Tinson has been involved with many world first's since moving to Abu Dhabi 25 yrs ago. First there was the establishment of pioneering centres in exercise physiology and assisted reproduction. The establishment of the Hilli Embryo Transfer Centre led to five world firsts in reproduction. The world's first successful embryo transfer calf birth in 1990, followed by frozen embryo transfer births in 1994, twin split calves in 1999, pre-sexed embryo births in 2001 and world's first calf born from A.I. of frozen semen in 2013. The hard bound book is spread in 288 pages with 5 chapters. The first chapter involves early history of the centre, world's firsts, world press releases, history of domestication and distribution, evolution of camel racing in the U.A.E. and historical photos the early days. Second chapter comprises camel in health and disease and it involves cardiovascular, haemopoetic, digestive, musculoskeletal, reproductive, respiratory, urinary and nervous systems in addition to the description of special senses. This chapter describes infectious, parasitic and skin diseases in addition to the nutrition. The third chapter is based on Examination and Differential Diagnosis. The fourth chapter is based on special technologies bearing description of anaesthesia and pain management in camels, diagnostic ultrasound and X-Ray, assisted reproduction in camels, drug and DNA testing and surgery. The last chapter entailed future scope of current research.



Author
Dr Alex Tinson

First Edition : 2017

Publisher:
Camel Publishing House
67, Gandhi Nagar West, Near Lalgarh Palace
Bikaner-334001, india
Email : tkcamelvet@yahoo.com

Website:
www.camelsandcamelids.com
www.tkgahlotcamelvet.com

ISBN : 81-903140-5-X
Printed in India

DOI : 10.5958/2277-8934.2020.00025.9 Vol 27 No 2, p 173-176

Klebsiella oxytoca ISOLATED FROM NOSTRILS OF DROMEDARY CAMELS: RESISTANCE PATTERN AND *pehX* GENE BASED GENOTYPING

P.S. Rathore, S.K. Sharma, N. Arora[1] and P. Nathiya
Department of Veterinary Microbiology and Biotechnology,
Post Graduate Institute of Veterinary Education and Research (PGIVER), Rajasthan University of Veterinary and Animal Sciences (RAJUVAS) Jaipur, Rajasthan, India
[1]Veterinary Officer, Department of Animal Husbandry, Government of Rajasthan

ABSTRACT

The present study was aimed to characterise *Klebsiella oxytoca* obtained from nostrils of camels (*Camelus dromedarius*) with special reference to antibiotic resistance pattern. Total 68 samples of infected camels were examined and out of these, 33 (48.53%) were found to be positive for *K. oxytoca*, which were further confirmed phenotypically and genotyped by species specific primer based on *pehX* gene of 344bp amplicon size. The isolates were characterised for biochemical properties and analysed to evaluate the susceptibility against 26 antimicrobials. It was found that 100% isolates were resistant to eight antibiotics namely amoxycillin, ampicillin, bacitracin, clindamycin, oxacillin, rifampicin, sulfadiazine and vancomycin, while all isolates were completely sensitive to cefepime and imipenem. Variable resistance patterns were recorded for other studied antibiotics.

Key words: Camels, *Klebsiella oxytoca*, *pehX* gene, respiratory tract infections, superbug

K. oxytoca is ubiquitous in the environment and is an opportunistic pathogen that can be cultured from the skin, mucous membranes, oropharynx and intestines as well as a variety of tissues from β-clinically affected humans and animals (Stojowska and Krawczyk, 2016). In animals, Klebsiella are mostly associated with sepsis, urinary tract infections, meningitis and mastitis (Chander *et al,* 2011). Antibiotic resistance and virulence of *K. oxytoca* is poorly understood (Herzog *et al,* 2014; Darby *et al,* 2014).

K. oxytoca exhibits both natural resistance to penicillins and has acquired various chromosomal and plasmid associated genetic mechanisms of antibiotic resistance, thus hampering disease management and it also has the enzymes such as extended spectrum β-lactamases (ESBL) and carbapenemases that have a role in resistance to β-lactam antibiotics and other antibiotics (Fenosa *et al,* 2009). Thus, investigation undertaken in present study becomes important not only in terms of biochemical profile but also to analyse the antibiotic resistance pattern of *K. oxytoca*.

Materials and Methods

Sample collection

A total of 68 deep nasal swab samples were collected from nostrils of camels with nasal discharge, high temperature, loss of appetite and pneumonic clinical signs. Samples for bacteriology were placed in polythene bags and kept in flask containing ice and taken to the laboratory as early as possible (Cheesebrough, 2000).

Isolation and identification

All samples were subjected to cultivation on Simmon's citrate agar with 1% inositol (SCIA), MacConkey agar (MCA) and Eosin methylene blue (EMB) agar followed by various other biochemical tests (Van Kregten *et al,* 1984). The phenotypically identified isolates were further confirmed genotypically on the basis of *pehX* gene amplification with amplicon size of 344bp including forward primer (5′ GAT ACGGAG TAT GCC TTT ACG GTG -3′) and reverse primer (5′- TAG CCT TTA TCA AGC GGA TAC TGG -3′). The PCR reactions were carried out as described earlier by (Kovtunovych *et al,* 2003) using Promega (USA) gene amplification kit and thermocycler (Mastercycler® nexus gradient) conditions were: Initial denaturation step of 2 min at 95°C, followed by 30 amplification cycles (94°C, 20s; 59°C, 20s; 72°C, 30 s) and a final elongation step of 10 min at 72°C. The PCR products were separated in an 8% native Polyacrylamide gel electrophoresis PAGE

SEND REPRINT REQUEST TO S.K. SHARMA email: drsharmask01@hotmail.com

(Sambrook and Russel, 2001). The gel was analysed under UV light (Azure 300 Chemi docu system).

Investigation of antibiotic susceptibility pattern

Total 26 antibiotics (Hi-Media) of various groups were examined for antibiotic susceptibility pattern of confirmed *Klebsiella oxytoca* isolates by disc diffusion method as per technique described by Kirby-Bauer method (Bauer *et al,* 1966) by using Mueller-Hinton agar. The log phase cultures were spread evenly on Meuller Hinton agar plates and antibiotic discs were aseptically placed on the agar surface. Samples were incubated aerobically for 18–24 hours at 37°C and the zones of inhibition were measured to the nearest millimetre. After inhibition zone measurement, organisms were classified as Sensitive (S), Intermediate sensitive (I) or Resistant (R) as per defined by CLSI (2017).

Results and Discussion

Out of 68 samples, 33 isolates were obtained after growth on Simmon's citrate agar (SCAI) with 1% inositol, where they showed yellow mucoid dome shaped colonies, all isolates were further confirmed by growth on Eosin Methylene Blue (EMB) agar where 100% isolates showed dark mucoid colonies without metallic sheen, expressed pink mucoid lactose fermenting colonies on Mac-Conkey agar and exhibited yellow mucoid colonies on Bromo Cresol Purple (BCP) agar. The phenotypically identified isolates were confirmed on the basis of *peh*X species specific gene primers with amplicon size of 344bp, which confirmed all isolates as *Klebsiella oxytoca* (Fig.1). In the present study, 100% isolates were observed with typical IMViC pattern of *K. oxytoca* viz. +-++. All the isolates were able to reduce the nitrates and utilise the malonate including hydrolysation of the aesculin and decarboxylation of the lysine. To study the phenotypic expression of antibiotic resistance, antibiogram of the *K. oxytoca* isolates were carried out, against the selected 26 commonly used antibiotics in veterinary therapeutics. Out of the total antibiotics, cefepime and imipenem were most effective with 100% efficacy while amoxycillin, ampicillin, bacitracin, clindamycin, oxacillin, rifampicin, sulfadiazine and vancomycin were found to be least effective. In the studied isolates, more than 90.0% sensitivity was observed for norfloxacin and gentamicin while for other sensitivity ranged from 36.3% to 87.8%.

Being ubiquitous, *Klebsiella oxytoca* has shown their presence in water, soil and on plants (Podschun & Ullmann, 1998). This bacteria has also been detected on the mucosal membranes of both healthy and diseased mammals including human (Darby *et al,* 2014), animals, poultry (chicken) and has also been reported in various reptiles such as green anoles, garter snakes reptile, wild tuatara and green turtles by different scientists (Goldstein *et al,* 1981; Santoro *et al,* 2006; Gartrell *et al,* 2007; Nthenge *et al,* 2008; Jackson, 2016).

Similar to present study, Santoro *et al* (2006) observed that 31.0% of green turtles were positive for *K. oxytoca*. Similarly, 20% & 17.0% occurrence was reported in garter snakes and green anoles by Goldstein *et al* (1981), Jackson (2016), respectively. However, lower prevalence (3.0%) was reported by Gartrell *et al* (2007) in wild tuatara. Present study as well as past studies such as haemorrhagic colitis (diarrhoea) in hospitalised patients (Cheng *et al,* 2012; Rath and Padhy, 2014), pneumonia, urinary tract and skin infections (Herzog *et al*, 2014) suggests certain adaptations on part of opportunistic *K. oxytoca* strains that are not only infecting animals but also humans as well.

Biochemical profile of Klebsiella isolates in present study is similar to profiles observed by Davis *et al* (1987), Rath and Padhy (2014) and Trivedi *et al* (2015) in various studies. However, few variations were also reported by Monnet and Freney (1994) who pointed out that 97.0% isolates were positive for indole production. Further, looking into the variability pattern of other biochemical tests such as positive gelatin liquefaction (Power and Calder,

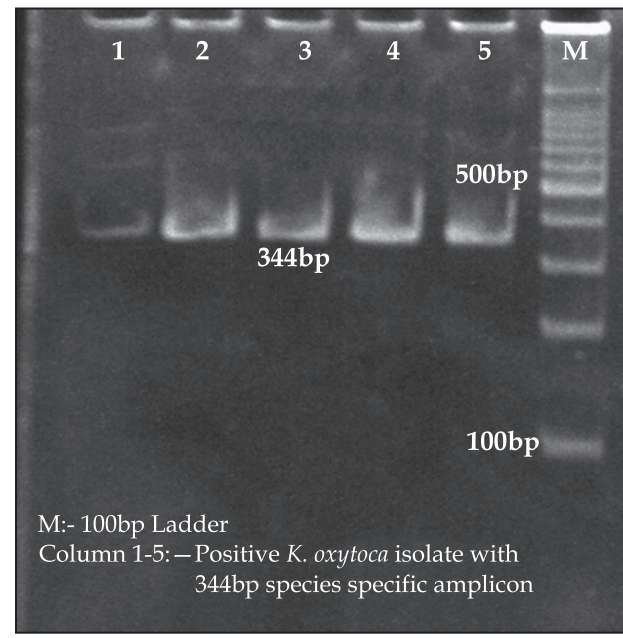


Fig 1. *peh*X species specific gene based genotyping of *Klebsiella oxytoca* (344bp) isolates (8% native PAGE)

1983), growth on 10°C (Kovtunovych *et al,* 2003) and negative citrate utilisation (Trivedi *et al,* 2015), the genotypic characterisation becomes significant. In the present study all isolates were characterised as *K. oxytoca* with species specific *peh*X gene. Similar to present study, Chander *et al* (2011) and Kovtunovych *et al* (2003) have also found that this genotypic method has good repeatability, sensitivity and specificity.

Similar to present study, other researchers have also reported good efficacy of third generation cephalosporins, β-lactamase inhibitor combinations, fluoroquinolones, trimethoprim-sulfomamides, gentamicin and carbapenem antibiotics (Brisse and Duijkeren, 2005; Rath & Padhy, 2014). Similarly, complete resistance to ampicillin and other beta lactams was reported by Trivedi *et al* (2016) in human clinical samples (Darby *et al,* 2014) in lab animals and non-human primates isolates and (Nthenge *et al,* 2008) also detected similar resistance pattern among *K. oxytoca* isolates obtained from chicken samples. In agreement to present study, ciprofloxacin and colistin efficacy was reported by Labrador & Araque (2014) and Singh *et al* (2016) for *K. oxytoca* isolates of human origin. However, indifferent to present study Singh *et al* (2016) observed some resistance for imipenem, meropenem, gentamicin and ciprofloxacin among human isolates. Labrador and Araque (2014) have also detected similar resistance pattern and further explained that the strains of *K. oxytoca* are resistant to amino-penicillins and carboxy-penicillins due to the production of β-lactamases and other similar enzymes. In present study and also reported in earlier studies (Darby *et al,* 2014; Sato *et al,* 2015) *K. oxytoca* has been observed with resistance to many antibiotics such as beta lactams, fluoroquinolone, cephalosporins, aminoglycosides etc.

Conclusion

Klebsiella oxytoca exists with variable phenotypic characteristics and genotypic confirmation with species specific primers based on *peh*X gene may be an important tool for molecular epidemiology as well as field diagnostics. Looking into the scarcity of literature of *K. oxytoca* with special reference to camel, this study suggests further genotypic characterisation of *K. oxytoca* isolates including investigation of virulence genes as well as study of antibiotics resistance dynamics of animal origin isolates.

Acknowledgements

We acknowledge the support and facilities provide by Head, Department of Veterinary Microbiology and Biotechnology and Dean, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jaipur for this study.

References

Bauer AW, Kirly WMM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by a standardised single disk method. Americal Journal of Clinical Pathology 45:493-496.

Brisse S and Duijkeren E (2005). Identification and antimicrobial susceptibility of 100 Klebsiella animal clinical isolates. Veterinary Microbiology 307-312. doi: 10.1016/j.vetmic.2004.11.010.

Chander Y, Ramakrishnan MA, Jindal N, Hanson K and Goyal SM (2011). Differentiation of Klebsiella pneumonia and *K. oxytoca* by multiplex polymerase chain reaction. International Journal of Applied Research in Veterinary Medicine 9(2):138-142.

Cheng VC, Yam WC, Tsang LL, Yau MC, Siu GK, Wong SC, Chan JF, To KK, Tse H, Hung IF, Tai JW, Ho PL and Yuen KY (2012). Epidemiology of *Klebsiella oxytoca*-associated diarrhoea detected by simmons citrate agar supplemented with inositol, tryptophan, and bile salts. Journal of Clinical Microbiology 50:1571-1579.

Cheesebrough M (2000). Distric Laboratory Practice in Tropical Countries. Chambridge University Press. pp 434.

CLSI (2017). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute 27:32-39.

Darby A, Lertpiriyapong K, Sarkar U, Seneviratne U, Park DS, Gamazon ER, Batchelder C, Cheung C, Buckley EM, Taylor NS, Shen Z, Tannenbaum SR, Wishnok JS and Fox JG (2014). Cytotoxic and pathogenic properties of *Klebsiella oxytoca* isolated from laboratory animals. PLoS One. doi:10.1371/journal.pone.0100542.

Davis JK, Gaertner DJ, Cox NR, Lindsey JR, Cassell GH, Davidson MK, Kervin KC and Rao GN (1987). The role of *Klebsiella oxytoca* in utero-ovarian infection of B6C3F1 mice. Laboratory Animal Science 37(2):159-166.

Fenosa A, Fuste E, Ruiz L, Veiga-Crespo P, Vinuesa T, Guallar V, Villa TG and Vinas M (2009). Role of TolC in *Klebsiella oxytoca* resistance to antibiotics. Journal of Antimicrobial Chemotherapy 63(4):668-674.

Gartrell BD, Youl JM, King CM, Bolotovski I, McDonald WL and Nelson NJ (2007). Failure to detect *Salmonella* species in a population of wild tuatara (*Sphenodon punctatus*). New Zealand Veterinary Journal 55:134-136.

Goldstein EJ, Agyare EO, Vagvolgyi AE and Halpen E (1981). Aerobic bacterial flora of garter snakes: Development of normal flora and pathogenic potential for snakes and humans. Journal of Clinical Microbiology 13:954-956.

Herzog AT, Schneditz G, Leitner E, Feierl G, Hoffmann KM, Schwetz IZ, Krause R, Gorkiewicz G, Zechner EL and Högenauer C (2014). Genotypes of *Klebsiella oxytoca* isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated haemorrhagic colitis. Journal of

Clinical Microbiology 52(5):1607-1616. doi: 10.1128/jcm.03373-13.

Jackson KA (2016). Prevalence of *Klebsiella oxytoca* in Anolis carolensis of Louisiana. Department of Biology, McNeese State University, Lake Charles, Louisiana. Vector-Borne And Zoonotic Diseases 16(12):800-801. doi: 10.1089/vbz.2016.2023

Kovtunovych G, Lytvynenko T, Negrutska V, Lar O, Brisse S and Kozyrovska N (2003). Identification of *Klebsiella oxytoca* using a specific pcr assay targeting the polygalacturonase *peh*X gene. Research in Microbiology 154:587-592. doi:10.1016/S0923-2508(03)00148-7

Labrador and Araque (2014). First description of KPC-2-producing *Klebsiella oxytoca* isolated from a pediatric patient with nosocomial pneumonia in Venezuela. Hindawi Publishing Corporation. Case Reports in Infectious Diseases. Volume 2014; Article ID 434987, 4 pages. doi: 10.1155/2014/434987

Monnet D and Freney J (1994). Method for differentiating *Klebsiella planticola* and *Klebsiella terrigena* from other *Klebsiella* species. Journal of Clinical Microbiology 32: 1121-1122.

Nthenge AK, Nahashon SN, Chen F and Adefope N (2008). Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and guinea fowl. Poultry Science 87: 1841-1848. doi:10.3382/ps.2007-00156

Podschun R and Ullmann U (1998). *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical Microbiology Reviews 11:589-603.

Power JT and Calder MA (1983). Pathogenic significance of *K. oxytoca* in acute respiratory tract infection. *Thorax* 38(3):205-208.

Rath and Padhy (2014). Prevalence of two multidrug-resistant Klebsiella species in an Indian teaching hospital and adjoining community. Journal of Infection and Public Health 7:496-507. doi: 10.1016/j.jiph.2014.05.002

Sambrook J and Russell DW (2001). Molecular Cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Santoro M, Hernandez G, Caballero M and Garcia F (2006). Aerobic bacterial flora of nesting green turtules (*Chelonia Mydas*) from tortuguero national park, Costa Rica. Journal of Zoo and Wildlife Medicine 37:549–552.

Sato T, Hara T, Horyama T, Kanazawa S, Yamaguchi T and Maki H (2015). Mechanism of resistance and bacterial susceptibility in extended spectrum beta lactamases phenotype *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated between 2000 and 2010 in Japan. Journal of Medical Microbiology 64:538-543. doi: 10.1099/jmm.0.000057

Singh L, Cariappa MP and Kaur M (2016). *Klebsiella oxytoca*: An emerging pathogen? Medical Journal Armed Forces India 1-3. doi: 10.1016/j.mjafi.2016.05.002

Stojowska K and Beata Krawczyk (2016). A new assay for the simultaneous identification and differentiation of *Klebsiella oxytoca* strains. Applied Microbiology and Biotechnology 100:10115-10123. doi: 10.1007/s00253-016-7881-1

Trivedi MK, Patil S, Shettigar H, Bairwa K and Jana S (2015). Phenotypic and biotypic characterisation of *Klebsiella oxytoca*: An impact of biofield treatment. Journal of Microbial and Biochemical Technology 7:(4)202-205. doi:10.4172/1948-5948.1000205

Trivedi MK, Branton A, Trivedi D, Nayak G, Shettigar H, Gangwar M amd Jana S (2016). Characterisation of antimicrobial susceptibility profile of biofield treated multidrug-resistant *Klebsiella oxytoca*. Applied Microbiology 1(1):1-6. doi:10.4172/2471-9315.1000101

Van Kregten E, Westerdaal NAC and Willers JMN (1984). New, simple medium for selective recovery of *Klebsiella pneumoniae* and *Klebsiella oxytoca* from human faeces. Journal of Clinical Microbiology 20:936-941.

DOI : 10.5958/2277-8934.2020.00026.0 Vol 27 No 2, p 177-183

CAMEL MILK ALLEVIATES ALCOHOL INDUCED LIVER INJURY IN MICE

Luping Bai, Liang Ming and Rimutu Ji[1]

Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China

[1]Camel Research Institute of Inner Mongolia, Alashan, Inner Mongolia, China

ABSTRACT

The present study aimed to investigate the effectiveness of camel milk (CM) in alleviating alcohol-induced hepatotoxicity as a model of clinical liver illness. Male mice were divided into 4 groups: pair-fed control (PE), ethanol (ET), camel milk treated group (CM) and cow milk treated group (NM). Mice from the PE ingested a Lieber-DeCarli controls liquid diet, while mice from the ET, CM and NM groups ingested a Lieber-DeCarli alcohol liquid diet of 8 weeks. Camel milk and cow milk were orally administered from the beginning of the 4th week until the end of the experiment. Mice from camel milk group was observed to significantly reduce the levels of serum ALT, AST and LPS compared with other groups. The hepatic steatosis was improved after camel milk administration and attenuated alcohol-induced oxidative stress by declining the level of hepatic oxidants. In addition, the production of apoptosis was inhibited by preventing the formation of TNF-α in the CM group. These results showed that camel milk may alleviate alcohol-associated hazards and protect hepatic tissue from alcohol-induced toxicity.

Key words: Alcohol liver injury, camel milk, hepatoprotective, lipopolysaccharide

Alcoholic liver disease (ALD) is one of the most prevalent liver diseases in Europe and the United States. The disease can be caused by the chronic consumption of alcohol exceeding a certain daily amount (>40g/day) (Seitz *et al*, 2018), which varies from different individuals (Akinyemiju *et al*, 2017). In the early stages of the disease, alcoholic fatty liver (an accumulation of triglycerides in hepatocytes) usually develop and progress to alcoholic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. According to the statistics, chronic heavy alcohol consumption (>40g of alcohol per day) of a sustained period (months or years) will result in 90-100% individuals developing alcoholic fatty liver. Among them, only 10-35% individuals who continue with chronic heavy alcohol consumption will develop alcoholic steatohepatitis, which is characterised by inflammation of the liver with concurrent fat accumulation in liver. While, only 8-20% chronic heavy drinkers develop alcoholic liver cirrhosis, of these patients, 2% develop hepatocellular cancer per year.

Comprehensive research shows that camel milk has inestimable value to human disease prevention, health and longevity (Hammam, 2019). In recent years, a great deal of research has been conducted on the therapeutic effects of camel milk on the improvement in liver disease symptoms and treatment side effects. The treatment of ethanol-induced hepatotoxicity in rats with camel milk had a tendency to alleviate liver damage by its antioxidant activity or to its chelate effects on toxicants (Elhag *et al*, 2017). A research on rats revealed that camel milk may improve hypercholesterolemia (Meena *et al*, 2018). Overall, camel milk contains factors that may be protective and therapeutic to the liver disease. Camel milk has a wide range of an antioxidant, antibacterial and immunomodulatory properties. However, scarce research has been done on the protective effect of camel milk on alcoholic liver injury. In this study, we investigated the therapeutic and repairing effects imparted by camel milk in a mouse model of ALD.

Materials and Methods

Chemicals

Ethanol (≥99.9%, absolute ethanol) was purchased from FUCHEN Chemical Reagents Company (Tianjin, China), while camel milk and cow milk were purchased from camel farm in Bayan Nur City, Inner Mongolia, China.

Alanine transaminase (ALT), aspartate transaminase (AST), malondialdehyde (MDA),

SEND REPRINT REQUEST TO RIMUTU JI email: yeluotuo1999@vip.163.com

superoxide dismutase (SOD), glutathione (GSH) and triglyceride (TG) kits were provided from Nanjing Jiancheng Bioengineering Institute, China.

Caspase-3, lipopolysaccharide (LPS), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor-a(TNF-α) were determined using enzyme-linked immunosorbent assay (ELISA) kits that were provided by Shanghai Enzyme-linked Biotechnology Co, Ltd, China.

Animal Treatment

Male C57BL/6N mice weighing 18-20 g were obtained from Charles River (Beijing Vital River Laboratory Animal Technology Co. Ltd, China). The animals were housed in standard plastic cages in an environmentally controlled room which was housed in a temperature (25±2°C) and relative humidity (65%-75%) with a 12 : 12 light dark cycle (Yu *et al*, 2016). The animals received humane care and all protocols were approved by Animal Care and Use Committee at Inner Mongolia Agricultural University. After one week of acclimatisation with chow diet, the animals were randomly divided into 4 groups of 6 animals in each group. Pair-fed with isocaloric Lieber-DeCarli liquid diets (TROPHIC Animal Feed High-Tech Co. Ltd, China) for 8 weeks as the following:

(1) Pair-fed control group (PF, n=6), receiving regular Lieber–DeCarli liquids diets;

(2) Ethanol group (ET, n=6), receiving ethanol containing Lieber–DeCarli liquid diets (28% of total calories as ethanol);

(3) Camel milk treated group (CM, n=6), receiving ethanol containing Lieber–DeCarli liquid diets (28% of total calories as ethanol) and administrated camel milk by the gavage (3g/kg body weight);

(4) Cow milk treated group (NM, n=6), receiving ethanol containing Lieber–DeCarli liquid diets (28% of total calories as ethanol) and administrated milk by the gavage (3g/kg body weight); The ethanol content of the diet gradually increased over a 7-day period (no ethanol for day 2, one fourth of the given amount for days 3-4, half of the given amount for days 5-6, two-thirds of the given amount for days 7-8 and full amount for the rest). At the ninth week, ethanol-fed and pair-fed mice were gavaged in the early morning with a single dose of ethanol (5 g kg- 1 body weight) or isocaloric maltose dextrin and euthanised 9 h later. Blood samples were collected by the retrobulbar vessels and allowed to clot for 45 min at room temperature. After standing for 3 h, the serum was separated by centrifugation (1500 rpm, 10 min and 4°C) and stored at - 80°C for biochemical analysis. In addition, body weight of the mice was weighed once a week, before and after the experiment. After the experiment, the mice in different groups were sacrificed promptly by cervical vertebra dislocation. Livers were dissected quickly, washed twice with phosphate buffer saline, blotted dry on a filter paper and wet weight were measured then stored at - 80°C (Li *et al*, 2015).

Biochemical Analysis

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum.

In order to evaluate the liver-protection capacity of camel milk, the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mice receiving camel milk were compared with the ET group. The levels of ALT and AST in mouse serum were determined using commercial assay kits according to the manufacturer's protocols.

Measurement of LPS level in serum

The LPS level in serum was measured by using enzyme-linked immunosorbent assay (ELISA) kit according to the procedure supplied by the manufacturer.

Determination of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) levels in the liver

After homogenisation and centrifugation, the supernatants of liver tissues were evaluated for the content of malondialdehyde (MDA), the activity glutathione (GSH) and superoxide dismutase (SOD) were measured according to the commercial assay kits.

Determination of triglyceride (TG), Caspase-3 activity and TNF-α, IL-1β and IL-6 levels in liver tissue

The level of TG in liver was measured according to the commercial assay kits.

Caspase-3 activity, TNF-α, IL-1β and IL-6 levels were measured by using enzyme-linked immunosorbent assay (ELISA) kit according to the procedure supplied by the manufacturer.

Statistical Analysis

The data were expressed as mean ± SD values. One-way analysis of variance (ANOVA) was used for

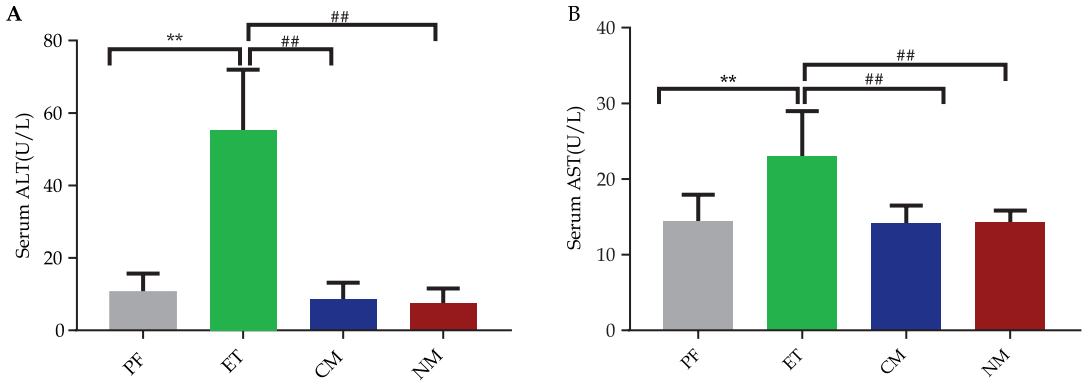


Fig 1. Effects of camel milk on serum ALT(A) and AST(B) activities. All data are expressed as mean ± SEM. ** $p < 0.01$ *versus* PF group, ## $p < 0.01$ *versus* ET group. PF= Pair-fed control group, ET= Ethanol group, CM= Camel milk treated group, NM=Cow milk treated group.

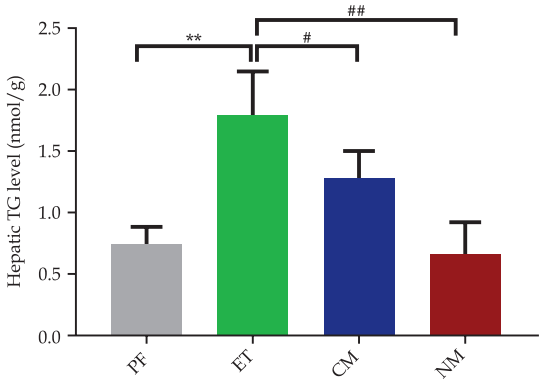


Fig 2. Effects of camel milk on TG level in liver tissue. All data are expressed as mean ± SEM. **$p < 0.01$ *versus* PF group, # $p < 0.05$, ##$p < 0.01$ *versus* ET group. PF= Pair-fed control group, ET= Ethanol group, CM= Camel milk treated group, NM= Cow milk treated group.

the comparison of more than 2 mean values. Results were considered to be statistically significant when $p < 0.05$ and $p < 0.01$.

Results and Discussion

The body weight of the mice from ET group did not change in comparison with PF group but in CM group it showed a decreasing trend ($p>0.05$) (Table 1).

Organ index of the liver was evaluated in mice. Liver index was increased in mice from ET group compared with the PF group. While the liver index from CM group had the decreasing trend ($p>0.05$) (Table 1).

The above results showed that consumption of the ethanol diet had a significant effect on mice weights, resulting in decreased body weight compared with PF group. There are no significant changes in final body weight, liver weight, or liver weight as a percentage of body weight of mice in CM group.

Table 1. Effects of camel milk on body weight and organ index in mice.

Group	Initial Wts (g)	Final Wts (g)	Liver Index (×100, mg·g^{-1})
PF	23.03±1.53	34.79±4.85	4.33±0.81
ET	24.91±0.63	29.78±2.33	4.16±0.23
CM	24.12±1.02	29.18±2.40	4.09±0.23
NM	22.7±2.04	26.32±3.11	4.49±0.50

All data are expressed as mean ± SEM. * $p < 0.05$ PF group, # $p < 0.05$ ET group. PF= Pair-fed control group, ET= Ethanol group, CM= Camel milk treated group, NM=Cow milk treated group.

The increase of AST and ALT in the blood indirectly reflects liver failure caused by ethanol induced hepatotoxicity. The levels of ALT and AST in serum were significantly elevated at 9 h following gavages of alcohol to mice ($p< 0.01$) (Fig 1A). This indicated liver cell damage in the mice and the model of alcoholic liver injury had been established successfully. The levels of ALT and AST were significantly decreased in the CM group and NM group of mice compared to the ET group ($p< 0.01$) (Fig 1B), indicating that the gavage of camel milk and cow milk can protect the liver of mice from the damage to ethanol and reduced the content of ALT and AST in serum.

Camel milk is widely used in various populations for the treatment and prevention of diseases (Dubey *et al*, 2016). Consumption of camel milk is known to have beneficial antioxidative properties in the treatment of many diseases and also that it inhibits lipid peroxidation (MDA) in mice (Lbrahim *et al*, 2017). Our results revealed that compared with the PF group, SOD and GSH levels in the liver of the ET group of mice showed a downward trend and the MDA level significantly increased

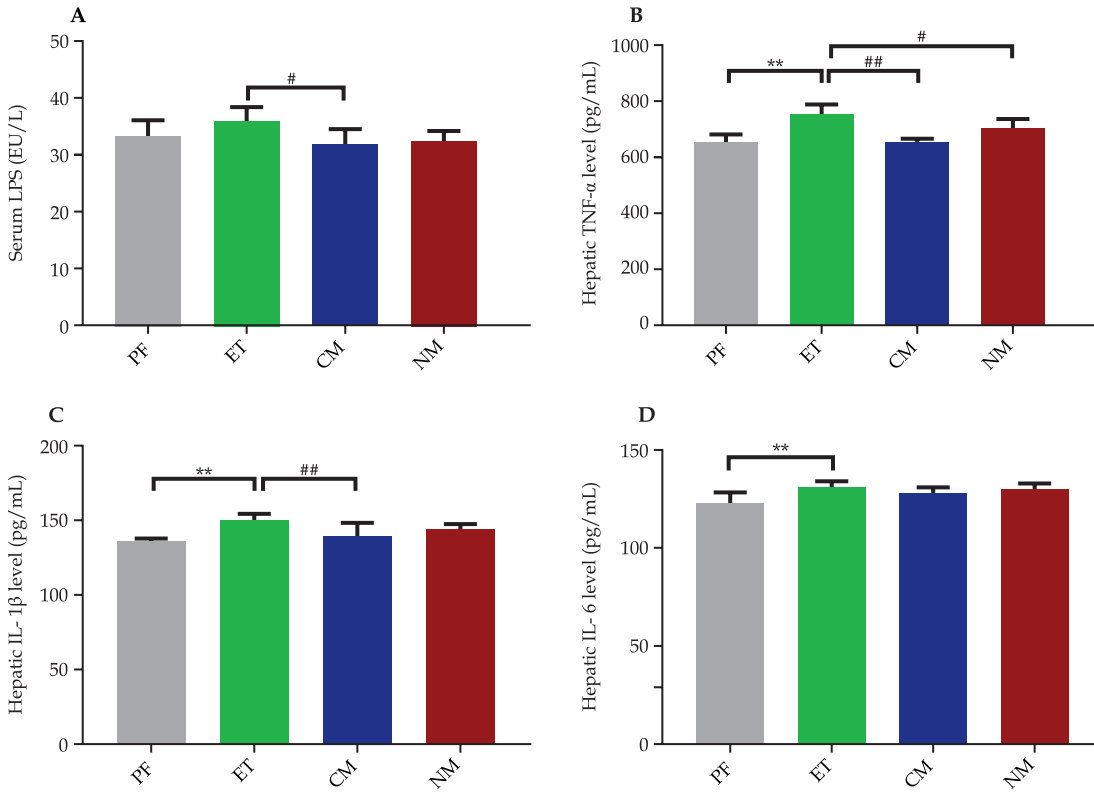


Fig 3. Effect of camel milk on alcohol-induced inflammation and on serum LPS level. All data are expressed as mean ± SEM. ** p < 0.01 *versus* PF group, # p < 0.05, ## p < 0.01 *versus* ET group. PF= Pair-fed control group, ET= Ethanol group, CM= Camel milk treated group, NM= Cow milk treated group.

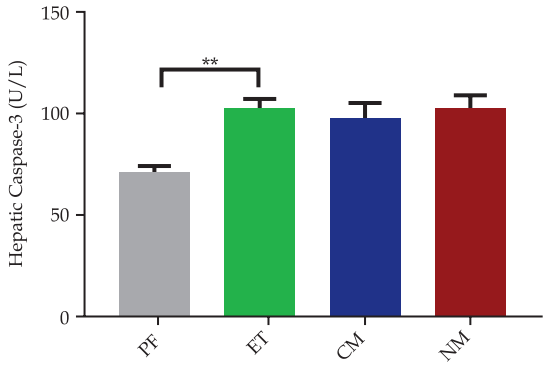


Fig 4. Effects of camel milk on Caspase-3 levels in liver tissue. All data are expressed as mean ± SEM. **p < 0.01 *versus* PF group. PF= Pair-fed control group, ET= Ethanol group, CM= Camel milk treated group, NM= Cow milk treated group.

(p<0.05) (Table 2). The results of this experiment indicated that a more severe lipid peroxidation occurred in the mice of the ET group. Compared with the ET group, the levels of SOD and GSH in the liver of the CM and NM groups of mice showed an upward trend. There was no difference in MDA levels in liver of NM group mice, while MDA levels in liver of CM group decreased significantly (p<0.05). We found that our results are consistent with previous studies (Mihic *et al*, 2016). Camel milk could protect the liver by decreasing the levels of MDA and increasing the activities of SOD and GSH in mice. An increase in the level of MDA in the liver enhances peroxidation and can lead to tissue damage and failure of the antioxidant defense mechanisms.

Chronic exposure to ethanol induces glutathione depletion, which makes hepatocytes more sensitive to oxidative stress (Wheeler *et al*, 2001) as reducing (not oxidised) glutathione protects cells against ROS (Louvet and Mathurin, 2015). Furthermore, SOD can also reduce oxidative stress and is an effective defense enzyme that converts the dismutation of superoxide anions into hydrogen peroxide (Li *et al*, 2012). Several studies have found that antioxidant enzymes such as SOD and GSH protect against oxidative tissue-damage (Diaz Castro *et al*, 2013) and suggest that these two enzymes contribute to the hepatoprotective effects of the mice CM group.

When alcohol is ingested excessively, fat metabolism in the body is impaired. The decomposition of fatty acids was inhibited and synthesis was strengthened (Ni *et al*, 2013), which would lead to fat accumulation in liver cells and

clinically manifested as liver triglyceride, high cholesterol levels (Wang *et al*, 2016). The level of TG was significantly increased in the liver of the mice from ET group compared with the PF group ($p<0.01$) (Fig 2). The level of TG was significantly decreased in the livers of the mice from CM group ($p<0.05$) and NM group ($p<0.01$) compared with the ET group. The above results effectively illustrated the inhibitory effect of camel milk and cow milk on liver lipid accumulation caused by alcohol.

Table 2. Effects of camel milk on MDA, SOD and GSH levels in mice liver.

Group	MDA (nmol/mg prot)	SOD (U/mg prot)	GSH (μmol/g prot)
PF	1.47±0.22	1137.61±74.43	14.16±6.35
ET	2.10±0.44*	981.82±143.65	9.69±2.23
CM	1.56±0.20#	1018.62±44.87	27.30±3.67##
NM	1.63±0.27	956.33±49.19	21.23±6.86##

All data are expressed as mean ± SEM. * $p < 0.05$ versus PF group, # $p < 0.05$, ## $p < 0.01$ *versus* ET group. PF= Pair-fed control group, ET= Ethanol group, CM= Camel milk treated group, NM= Cow milk treated group.

Increased serum level of LPS was commonly found in patients with ALD. Alcohol consumption not only caused enteric dysbiosis and bacterial overgrowth, but also increased gut permeability and the translocation of bacteria-derived LPS from the gut to the liver (Yan *et al*, 2011). LPS can stimulate Kupffer cells to produce reactive oxygen species and cytokines (including TNF-α) that causes hepatocellular damage and activate TLR4 signaling in liver sinusoidal endothelial cells, resulting in the regulation of angiogenesis and subsequent promotion of fibrogenesis (Tamai *et al*, 2002).

Collectively, the role of bacterial translocation in the pathogenesis of ALD had been clearly established (Crabb, 1999). Chronic alcohol models increased circulating concentrations of lipopolysaccharide (LPS) compared with the PF group and the severity of hepatic injury was correlated to serum level of LPS ($p>0.05$) (Fig 3A). The level of LPS was significantly decreased from the CM group of mice compared with the ET group ($p<0.05$). The above results indicated that gavage of camel milk can protect the liver of mice from the damage to ethanol and reduced the content of LPS in serum.

The production of TNF-α is one of the earliest events in many types of liver injury, triggering the production of hepatocyte, other inflammation and apoptosis (Takahashi *et al*, 2012; Marks *et al*, 1990). Chronic alcohol models increased liver tissue TNF-α (Fig 3B) and several TNF-α inducible cytokines (Parkin *et al*, 2019), such as interleukin-1β (Fig 3C) and interleukin-6 (Fig 3D) are also increased compared with the PF group ($p<0.01$). Compared with the ET group, we found that IL-6 in the liver of CM group mice showed a downward trend, while TNF-α and IL-1β levels were significantly decreased ($p<0.01$). In the NM group, liver TNF-α level was significantly decreased ($p<0.05$) and IL-6 and IL-1β levels were not significantly different. Through the above results, camel milk by inhibiting the formation of TNF-α to reduce the effect of inflammatory response is more obvious than cow milk.

Hepatocyte apoptosis is an important pathologic feature of human ALD. Apoptosis results from multiple mechanisms, including ethanol mediated hepatotoxicity, induction of oxidative stress, inhibition of survival genes (c-Met) and induction of proapoptotic signaling molecules (TNF-a and Fas ligand) (Takahashi *et al*, 2012). It could be postulated that the inflammation of liver cells, produced upon ethanol intoxication, might lead to apoptosis of hepatocytes *via* caspase-3 activation. Osawa *et al* (2001) had demonstrated the involvement of caspase cascade activation in TNF-α induced hepatocytes apoptosis (Osawa *et al*, 2001). The activity of Caspase-3 was significantly increased to the livers of the mice from ET group compared with the PF group ($p<0.01$) (Fig 4) and the level of Caspase-3 was decreased to the livers of the mice from CM group compared with the ET group ($p>0.05$) (Fig 4). We found that our results were consistent with previous studies. Gavage of camel milk can protect the liver of mice from the damage to ethanol, again explains hepatoprotective action of camel milk.

These results revealed that chronic ethanol feeding plus a single binge (the NIAAA model) can cause chronic liver damage (Stice *et al*, 2015), which was manifested by significantly increased serum ALT, AST and LPS levels in mice (Bertola *et al*, 2013). Camel milk treatment could protect the liver by decreasing the levels of ALT, AST, LPS, MDA and TG, increasing SOD and GSH activity as well as decrease levels of TNF-α, IL-1β and IL-6 content and Caspase-3 activity. Thus, regular consumption of camel milk could increase the antioxidant capacity of the liver and regulate inflammation and apoptosis, in this way camel milk can prevent the liver from being damaged. Camel milk is a potential liver-protective food without any side effects.

This study indicated the protective effect of camel milk in mice. It could be postulated that

camel milk exerted its effect of ethanol-induced hepatotoxicity via modulating the extent of lipid peroxidation, augmenting the antioxidant defense system decrease LPS. Inhibition of ROS and LPS generation might account for the observed decrease in TNF-α, IL-1β and IL-6 levels and caspase-3 activity following camel milk treat. Thus, it is likely that oxidative stress and LPS are a common signal transducer for a diverse cell death-inducing stimulate. Camel milk through enhancing the antioxidant ability and decreasing the LPS level of decreased various toxic substance-induced oxidative stresses in the liver. The protective effect of cow milk on mice liver is much worse than camel milk. Therefore, it is of great research value to further study the individual active compounds present in camel milk. This will enable us to understand the exact mechanisms responsible for liver protection.

Acknowledgements

We gratefully acknowledge support and funding received from the Science and Technology Major Project of Inner Mongolia Autonomous Region, Inner Mongolia Natural Science Foundation Project (2018BS03017) and the project of high-level talents introduce scientific research (NDYB2017-28). We thank, the researchers at the Animal Husbandry Workstation of West Sunid, Shiliingol Aimag, Inner Mongolia, for providing the samples and facilitating their identification. We would like to thank the native English speaking scientists of Elixigen Company (Huntington Beach, CA, USA) for editing our manuscript.

References

Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu M A, *et al* (2017). The Burden of Primary Liver Cancer and Underlying Etiologies From 1990 to 2015 at the Global, Regional and National Level: Results From the Global Burden of Disease Study 2015. JAMA Oncology 3(12):1683-1691.

Bertola A, Mathews S, Ki SH, Wang H and Gao B (2013). Mouse model of chronic and binge ethanol feeding (the NIAAA model). Nature Protocols 8(3):627-37.

Crabb DW (1999). Pathogenesis of alcoholic liver disease: newer mechanisms of injury. The Keio Journal of Medicine 48(4):184-8.

Diaz Castro J, Alferez MJ, Lopez Aliaga I, Nestares T, Sanchez Alcover A and Campos MS (2013). Bile composition, plasma lipids and oxidative hepatic damage induced by calcium supplementation; effects of goat or cow milk consumption. Journal of Dairy Research 80(2):246-54.

Dubey US, Lai M, Mittal A and Kapur S (2016). Therapeutic potential of camel milk. Emirates Journal of Food and Agriculture 28(3):164 176.

Elhag AE, Bernard F and ElBadwi SMA (2017). Protective activity of camel milk and urine mixture (*Camelus dromedarius*) against ethanol-induced hepatotoxicity in rats. Advances in Bioscience and Biotechnology 8:378-387.

Hammam ARA (2019). Compositional and therapeutic properties of camel milk: a review. Emirates Journal of Food and Agriculture 31(3):148-152.

Lbrahim MA, Shaik R and Wani F (2017). Hepatoprotective effect of olive oil and camel milk on acetaminophen induced liver toxicity in mice. International Journal of Medical Science and Public Health 6(1):186-194.

Li G, Ye Y, Kang J, Yao X, Zhang Y, Jiang W, Gao M, Dai Y, Xin Y, Wang Q, Yin Z and Luo L (2012). l-Theanine prevents alcoholic liver injury through enhancing the antioxidant capability of hepatocytes. Food and Chemical Toxicology 50(2):363-72.

Li W, Qu XN, Han Y, Zheng SW, Wang J and Wang YP (2015). Ameliorative effects of 5-hydroxymethyl-2-furfural (5-HMF) from *Schisandra chinensis* on alcoholic liver oxidative injury in mice. International Journal of Molecular Sciences 16(2):2446-57.

Louvet A and Mathurin P (2015). Alcoholic liver disease: mechanisms of injury and targeted treatment. Nature Reviews Gastroenterology Hepatology 12(4):231-42.

Marks JD, Marks CB, Ljuce JM, Montgomery AB, Turner J, Metz CA and Murray JF (1990). Plasma tumour necrosis factor in patients with septic shock. Mortality rate, incidence of adult respiratory distress syndrome and effects of methylprednisolone administration. American Review of Respiratory Disease 141(1):94-7.

Meena S, Rajput YS, Sharma R and Singh R (2018). Effect of goat and camel milk *vis a vis* cow milk on cholesterol homeostasis in hypercholesterolemic rats. Small Ruminant Research 171:8-12.

Mihic T, Rainkie D, Wilby HJ and Pawluk SA (2016). The therapeutic effects of camel milk: a systematic review of animal and human trials. Journal of Evidence-Based Complementary and Alternative Medicine 21(4):110-126.

Ni HM, Du K, You M and Ding WX (2013). Critical role of $FoxO_3a$ in alcohol-induced autophagy and hepatotoxicity. The American Journal of Pathology 183(6):1815-1825.

Osawa Y, Banno Y, Nagaki M, Nozawa Y, Moriwaki H and Nakashima S (2001). Caspase activation during hepatocyte apoptosis induced by tumour necrosis factor-alpha in galactosamine-sensitised mice. Liver 21(5):309-19.

Parkin GM, Clarke C, Takagi M, Hearps S, Babl FE, Davis GA anderson V and Ignjatovic V (2019). Plasma tumour necrosis factor alpha is a predictor of persisting symptoms post-concussion in children. Journal of Neurotrauma 36(11):1768-1775.

Seitz HK, Bataller R, Cortez Pinto H, Gao B, Gual A, Lackner C, Mathurin P, Mueller S, Szabo G and Tsukamoto H (2018). Alcoholic liver disease. Nature Reviews Disease Primers 4:16-37.

Stice CP, Liu C, Aizawa K, Greenberg AS, Ausman LM, Wang XD (2015). Dietary tomato powder inhibits alcohol-induced hepatic injury by suppressing cytochrome P_{450} 2E1 induction in rodent models. Archives of Biochemistry and Biophysics 572:81-88.

Takahashi N, Duprez L, Grootjans S, Cauwels A, Nerinckx W, Du Hadaway JB, Goossens V, Roelandt R, Hauwermeiren FV, Libert C, Declercq W, Callewaert N, Prendergast GC, Degterev A,Yuan J and Vandenabeele P (2012). Necrostatin-1 analogues: critical issues on the specificity, activity and *in vivo* use in experimental disease models. Cell Death Disease 3:e437.

Tamai H, Horie Y, Kato S, Yokoyama H and Ishii H (2002). Long-term ethanol feeding enhances susceptibility of the liver to orally administered lipopolysaccharides in rats. Alcoholism-Clinical and Experimental Research 26(8 Suppl):75S-80S.

Wang S, Ni H M, Dorko K, Kumer SC, Schmitt TM, Nawabi A, Komatsu M, Huang H and Ding WX (2016). Increased hepatic receptor interacting protein kinase 3 expression due to impaired proteasomal functions contributes to alcohol-induced steatosis and liver injury. Oncotarget 7(14):17681-98.

Wheeler MD, Nakagami M, Bradford BU, Uesugi T, Masonll RP, Connorll HD, Dikalovall A, Kadiiskall M and Thurman RG (2001). Overexpression of manganese superoxide dismutase prevents alcohol-induced liver injury in the rat. Journal of Biological Chemistry 276(39):36664-72.

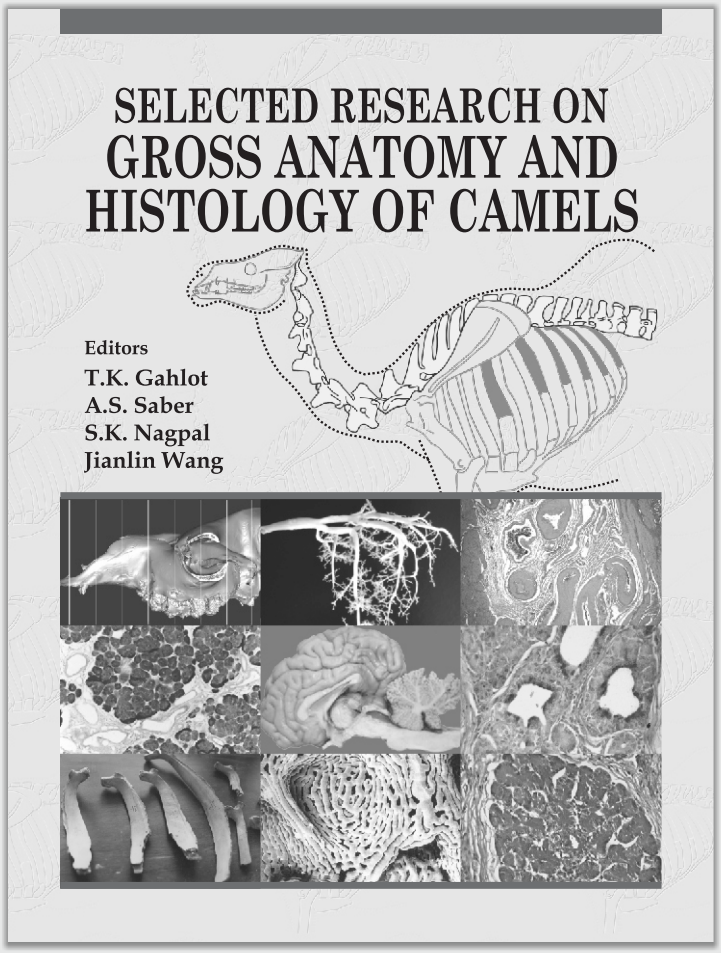
Yan AW, Fouts DE, Brandl J, Starkel P, Torralba M, Schott E, Tsukamoto H, Nelson KE, Brenner DA and Schnabl B (2011). Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatology 53(1):96-105.

Yu X, Xu Y, Zhang S, Sun J, Liu P, Xiao L, Tang Y, Liu L and Yao P (2016). Quercetin attenuates chronic ethanol-induced hepatic mitochondrial damage through enhanced mitophagy. Nutrients 8(1):27.

SELECTED RESEARCH ON
GROSS ANATOMY AND HISTOLOGY OF CAMELS

Hard bound, 452 pages, few figures coloured

Selected Research on Gross Anatomy and Histology of Camels is a unique reference book on anatomy of dromedary and bactrian camels. This book contains a first ever wide spectrum of histological description of various organs of camels which is depicted by special stains and scanning electronmicroscopy in addition to the gross anatomy, histochemical and immunohistochemical studies. The book has 92 manuscripts in 9 sections, e.g. radiographic anatomy, anatomy of various systems (skeletal, digestive, respiratory, circulatory, urogenital and nervous), common integument and miscellaneous. These manuscripts were published by 158 authors working in 37 laboratories or colleges or institutions from 14 countries in the Journal of Camel Practice and Research between June 1994 to June 2010. Bactrian camel anatomy research was exclusively contributed by the researchers of China. The countries involved in camel anatomy research were China, Egypt, India, Iran, Saudi Arabia, Iraq, Jordan, Japan, Pakistan, Sweden, United Arab Emirates, United States of America, France and Germany. Camel Publishing House has taken a step forward to compile this knowledge in form of a book and this herculian task was accomplished by its dedicated editors, viz. T.K. Gahlot (India), S.K. Nagpal (India), A.S. Saber (Egypt) and Jianlin Wang (China). This classic reference book will serve as a one stop resource for scientific information on gross anatomy and histology of camels.



Editors:
T.K. Gahlot, A.S. Saber, S.K. Nagpal and Jianlin Wang

Edition: 2011

Publisher: **Camel Publishing House**
67, Gandhi Nagar West,
Near Lalgarh Palace
Bikaner 334001 Rajasthan,
India
email: tkcamelvet@yahoo.com
website: www.camelsandcamelids.com

Price: US $ 375 (Abroad)
INR 7500 (India)

ISBN: 81-903140-1-7

DOI : 10.5958/2277-8934.2020.00027.2 Vol 27 No 2, p 185-192

DEOXYURIDINE 5-MONOPHOSPHATE (dUMP) METABOLISING ENZYME AND THE BIFUNCTIONAL DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE IN CAMELS AND *Trypanosoma evansi*

Mahmoud Kandeel[1,2,*] and Abdulla Al-Taher[1]

[1]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Alahsa, 31982, Saudi Arabia
[2]Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, 35255, Egypt

ABSTRACT

Bifunctional protein can be used as a drug target as it proves a classical difference between the host and parasite structures that can be used as a drug target. In this study, the metabolic pathways of deoxyuridine 5-monophosphate (dUMP) were compared in camel and the blood parasite *Trypanosoma evansi*. *T. evansi* shares similar profile with camel pathways but with predicted inability to degrade dUMP to uridine and devoid of dCTP deamination to yield dUMP. In the enzyme set of dUMP metabolism, thymidylate synthase was raised as a bifunctional enzyme in *T. evansi* with dihydrofolate reductase-thymidylate synthase (DHFR-TS) domains content, compared to a single TS domain in camels enzyme. Specific targeting of DHFR-TS in *T. evansi* is expected to yield specific anti-trypanosomal drugs.

Key words: Camel, DHFR-TS, dUTP, thymidylate synthase, *Trypanosoma evansi*

Computational techniques have largely enhanced drug discovery process by target identification and validation. It had been used to provide valuable knowledge about molecular targets in pathogens (Alnazawi *et al,* 2017; Kandeel *et al,* 2019a; Kandeel *et al,* 2019 in press; Kandeel *et al,* 2019b; Mahmoud *et al,* 2019). This process depends on comparison of genome sequences of the host and its pathogens. The sequence of camel genome was recently published (Jirimutu *et al,* 2012). This will give a new era in camel related research by defining the host spots in genome and proteome of camel and its pathogens against which drugs can be designed. Recently, we provided some interesting differences in the metabolic pathways of pyrimidines in camel and *T. evansi* (Kandeel and Al-Taher, 2020a; Kandeel and Al-Taher, 2020b; Kandeel *et al,* 2020).

In this study, enzymes of dUMP metabolism were compared in camels and the blood protozoan, *T. evansi*. In this respect, the KEGG maps were used to set up the enzymes set (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016; Ogata *et al,* 1998). The bioinformatics tools were used to assess the standard enzymes in camel and *T. evansi*. Finally, differences in metabolic enzymes were highlighted and evaluated for vulnerability to be a drug target.

Materials and Methods

Retrieval of genomic data

Collection of genomic data was carried out by extracting the information from the gene database (http://www.genedb.org) (Hertz-Fowler *et al,* 2004), Kinetoplastom genome resources (http://tritrypdb.org/tritrypdb/), protein and genome databases at (http://www.ncbi.nlm.nih.gov) and the Arabian camel genome project (http://www.camel.kacst.edu.sa).

Searching homologues Protein sequence homologues were searched using the NCBI BLAST (Basic Local Alignment Search Tool) (Madden, 2013) or PSI-BLAST (Position- Specific Iterated-BLAST) servers (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment programme for proteins and construction of phylogenetic tree was carried out using the tools available at (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins, 2014). Multiple sequence alignment program (ClustalW2) was used to calculate the best match of the selected sequences. The resultant alignment was used to generate a phylogenic tree, which is visualised by Dendroscope phylogenic tree viewer (Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

SEND REPRINT REQUEST TO MAHMOUD KANDEEL email: mkandeel@kfu.edu.sa

Putative domains were searched by the domain prediction programme available at (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Proteomic and genomic tools ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) was also used for analysis of nucleotide and gene sequences. The protein parameters including the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity was searched at (http://us.expasy.org/tools/protparam.html). Protein sequences of target genes was analysed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam. PROSITE (http://www.expasy.org/proteomics/families__patterns_and_profiles) was used to search for patterns and profiles in the protein sequences of the target genes. The protein domains, families and functional sites was searched at (http://prosite.expasy.org/).

Results and Discussion

Deoxyuridine-5′-monophosphate (dUMP) production process was proposed by 4 routes 1) dephosphorylation of deoxyuridine 5′-triphosphate by dUTP pyrophosphatase, 2) the dephosphorylation of deoxyuridine-5′-diphosphate by the actions of dTMP kinase 3) from deoxyuridine by the kinase activity of thymidine kinase 4) from dCTP by dCTP deaminase as shown in (Fig 1). In the catabolic direction, deoxyuridine was produced from dUMP by 5′-nucleotidase or 5′-deoxynucleotidase (Fig 1). In camel, the previous profile applied except for the absence of dCTP deamination to yield dUMP (Fig 2). *T. evansi* was found to be devoid of dCTP deamination and 5′-nucleotidase (Fig 3). Thus, dUMP was not converted to deoxyuridine. The summary of all enzymes in deoxyuridine pathways and the predicted enzymes in camels and *T. evansi* are provided in Tables 1-3.

Thymidylate synthase

Thymidylate synthase (EC 2.1.1.45; dTMP synthase), is a methyltransferase enzyme also called thymidylate synthetase; methylenetetrahydrofolate: dUMP C-methyltransferase; TMP synthetase. It acts on one-carbon moieties, as it catalyses methylation of dUMP with 5, 10-methylenetetrahydrofolate (methyl donor), creating dTMP and dihydrofolate (Slavik and Slavikova, 1980). Parasites, *Crithidia fasciculata, Crithidia oncopelti*, and a number of trypanosomatids (*Trypanosoma brucei, Trypanosoma congolense, Trypanosoma lewisi* and *Trypanosoma cruzi*) were able to synthesise several thymidylate synthases (TS). The trypanosotamid enzyme was inactivated by Mg^{2+}, was extensively more sensitive to mercaptoethanol, had elevated noticeable Km values for substrate (dUMP) and cofactor (tetrahydrofolate), had a greater obvious molecular weight and was significantly more sensitive to inhibition by suramin. Therefore, it is a promising target for chemotherapeutic agents, either on its own or in combination with a dihydrofolate reductase inhibitor (Chalabi and Gutteridge, 1977). Additionally, potency of inhibitory effects of antifolate drugs on targeted dual functionally dihydrofolate reductase-thymidylate synthase (DHFR-TS) that isolated from African trypanosomes, protozoan parasite causing both sleeping sickness in humans and nagana in cattle has been estimated. Thus, the most effective inhibitors for DHFR were methotrexate and trimetrexate, and for

Table 1. Enzymes involved in metabolic pathways of dUMP.

ID (E.C. number)	Definition (Enzyme name)
2.7.1.21	thymidine kinase
3.1.3.89	5′-deoxynucleotidase
3.1.3.5	5′-nucleotidase
3.5.4.30	dCTP deaminase (dUMP-forming)
3.6.1.23	dUTP diphosphatase; deoxyuridine-triphosphatase
2.7.4.9	dTMP kinase; thymidine monophosphate kinase
2.1.1.45	thymidylate synthase; dTMP synthase

Table 2. The expected enzymes involved in metabolic pathways of dUMP in camels.

ID (E.C. number)	Definition (Enzyme name)
2.7.1.21	thymidine kinase
3.1.3.5	5′-nucleotidase
3.6.1.23	dUTP diphosphatase; deoxyuridine-triphosphatase
2.1.1.45	thymidylate synthase; dTMP synthase

Table 3. The expected enzymes involved in metabolic pathways of dUMP in *Trypanosoma brucei.*

ID (E.C. number)	Definition (Enzyme name)
2.7.1.21	thymidine kinase
3.6.1.23	dUTP diphosphatase; deoxyuridine-triphosphatase
2.1.1.45	thymidylate synthase; dTMP synthase
2.7.4.9	dTMP kinase; thymidine monophosphate kinase

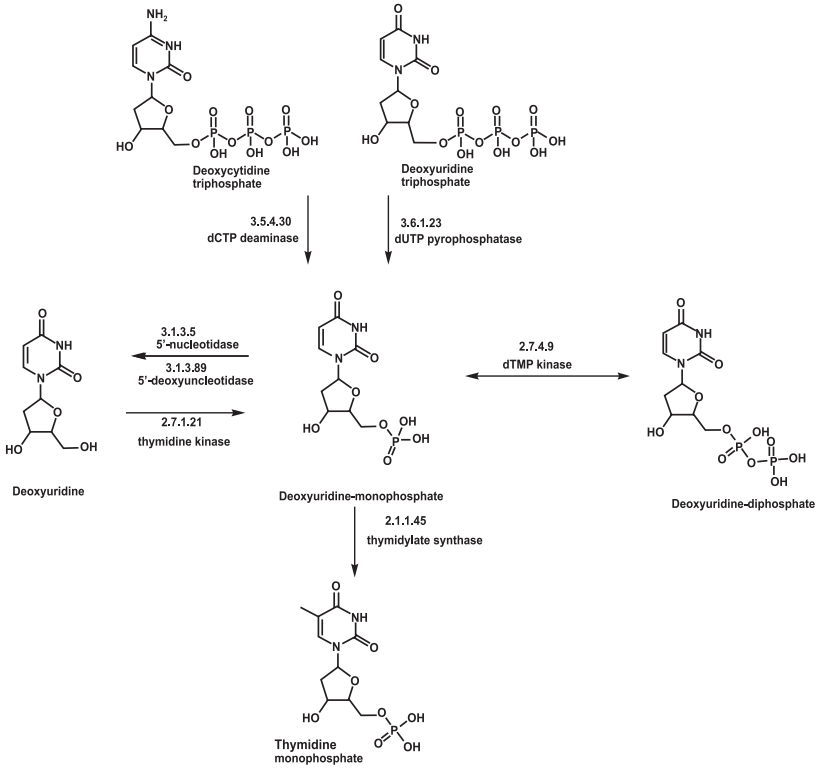


Fig 1. The proposed metabolic pathways of deoxyuridine monophosphate (dUMP).

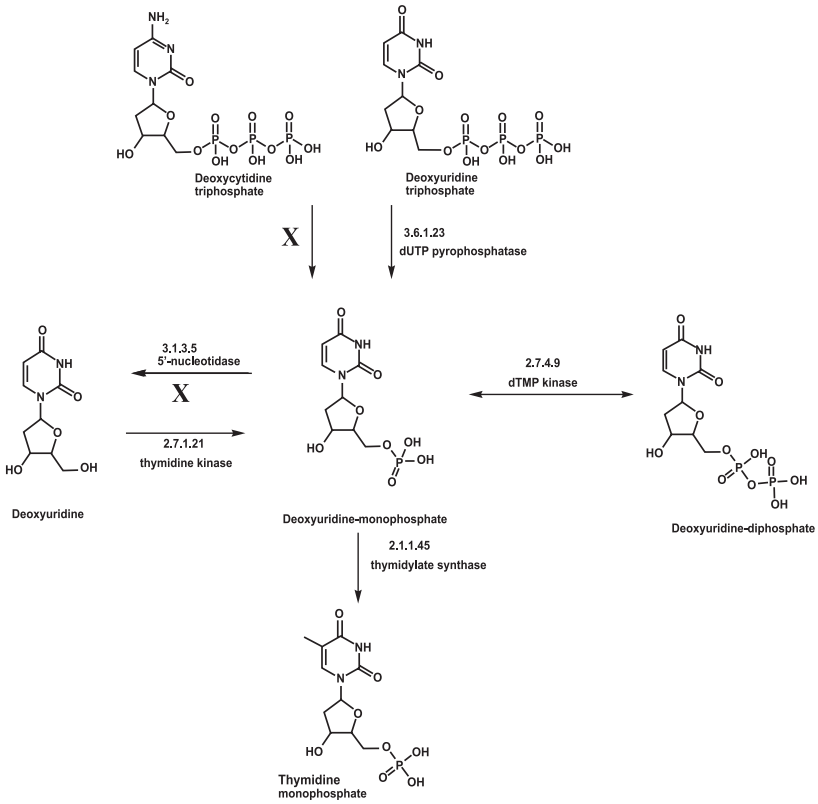


Fig 2. The proposed metabolic pathways of deoxyuridine monophosphate (dUMP) in camels.

thymidylate synthase were FdUMP and nolatrexed (Gibson *et al,* 2016).

Human and camel TS showed 77% similarity and 72 differences (Fig 4). This difference was attributed to the lack of 60 amino acids at the N-terminal of camel TS. In comparing the camel species, the dromedary and bactrian camels were 99.21% similar, while the similarity rate drops to 74.05 and 73.42% in comparing dromedary and bactrian camel with the feral camel. This was due to the lack of 58 amino acids at the N-terminal of dromedary and bactrian camels (Fig 5). Comparison of camel and *T. evansi* TS is provided in Fig 6. The camel protein was 253 amino acids, while the *T. evansi* TS is 527 amino acids in length forming 29.98% similarity and 369 differences. Motif and domain search using MotifFinder revealed the bifunctional dihydrofolate reductase-thymidylate synthase (DHFR-TS) was encoded by the *T. evansi* protein, while the camel enzyme encodes one thymidylate kinase domain (Fig 7).

The heat map of multiple comparison of TS sequences in several species showed 68.9-94.27% similarities among the selected vertebrates TS. Comparisons with protozoal and prokaryotes TS revealed low similarity rate in the range of 12-33.69% (Fig 8). The phylogenetics of camel and *T. evansi* TS is shown in (Fig 9). The *T. evansi* TS was related to the protozoal enzyme and highly related to the prokaryotic TS. The camel TS was related to the monofunctional eukaryotic thymidylate synthases.

The bifunctional DHFR-TS had been regarded as a promising antiprotozoal and anti-trypanosomal target (Gibson *et al,* 2016; Panecka-Hofman *et al,* 2017; Schormann *et al,* 2010; Senkovich *et al,* 2009; Valente *et al,* 2019). The present finding which highlights the differences between camel and *T. evansi* TS suggests using it as a drug target.

Molecular models of camel and human TS were requested from

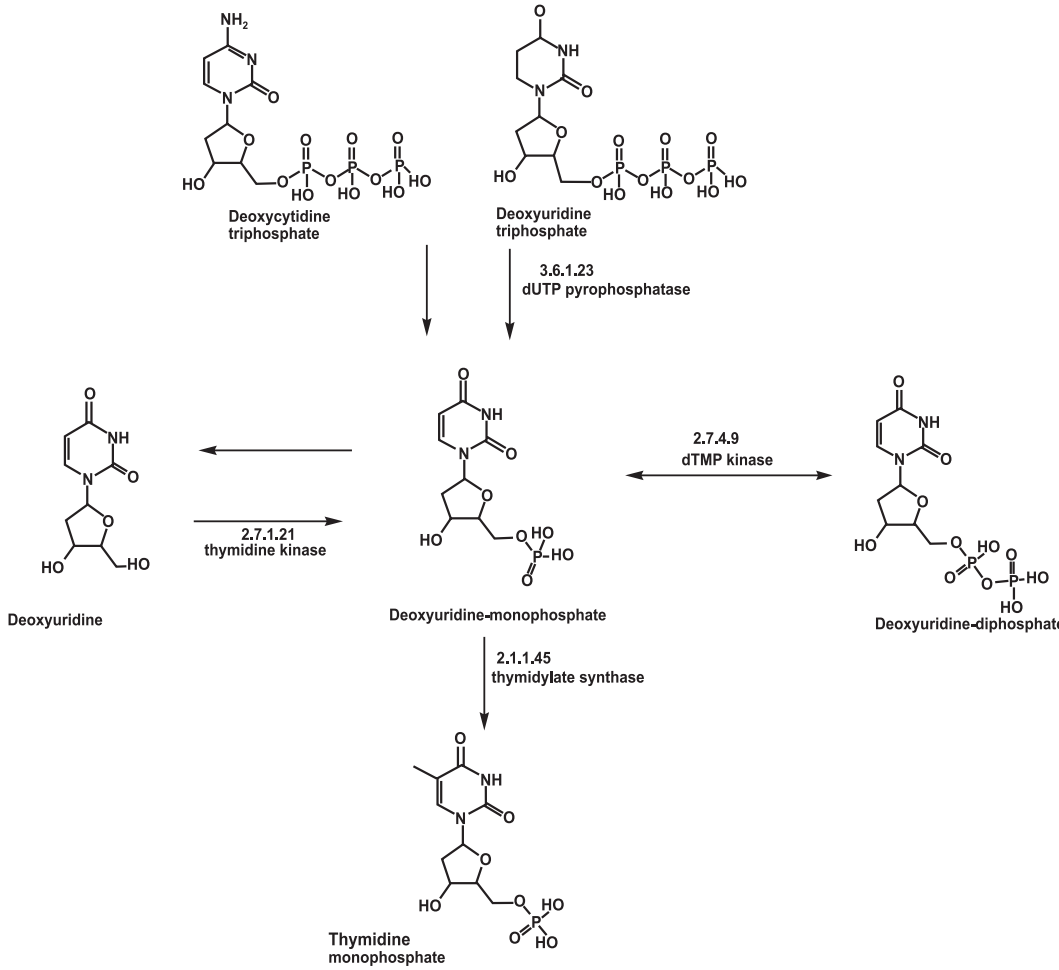


Fig 3. The proposed metabolic pathways of deoxyuridine monophosphate (dUMP) in *Trypanosoma brucei.*

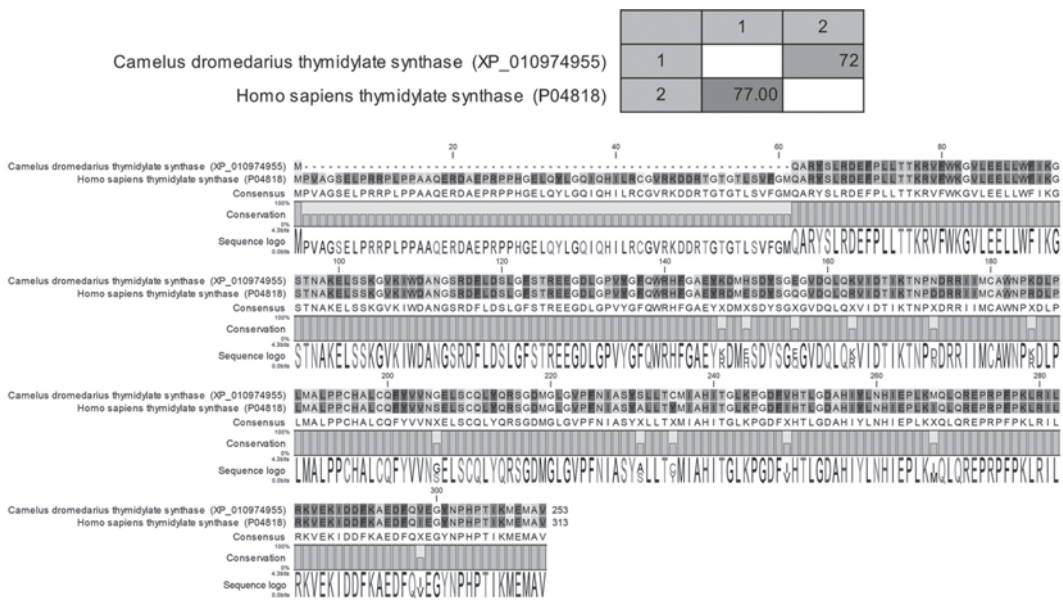


Fig 4. Multiple sequence alignment of dromedary camel and human thymidylate synthase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

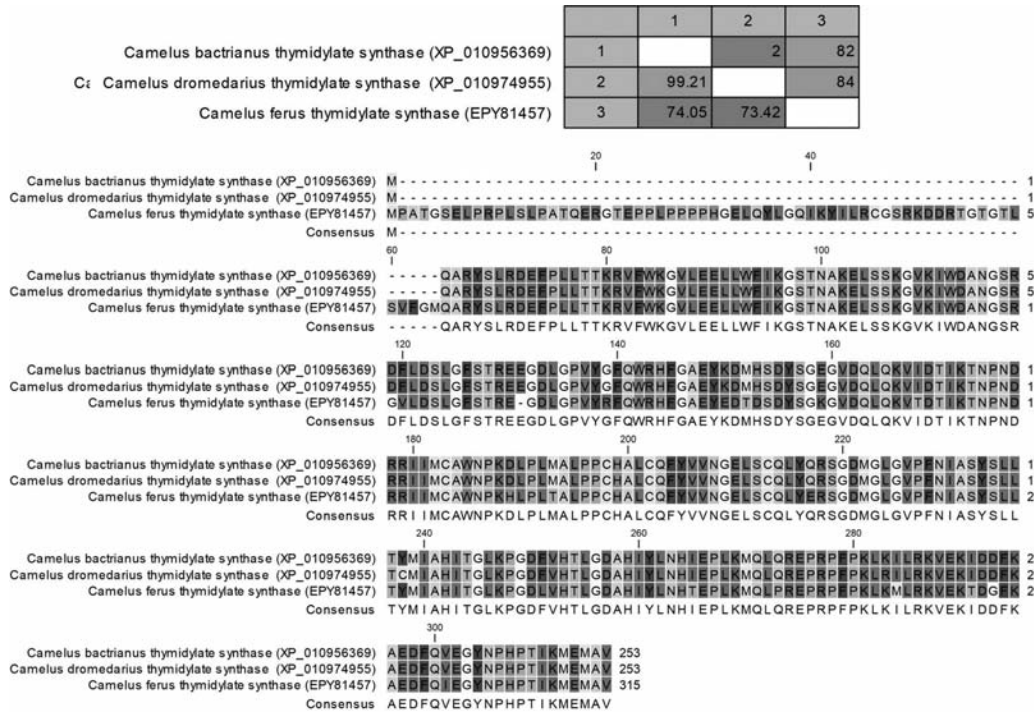


Fig 5. Multiple sequence alignment of dromedary, bactrian and feral camels thymidylate synthase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

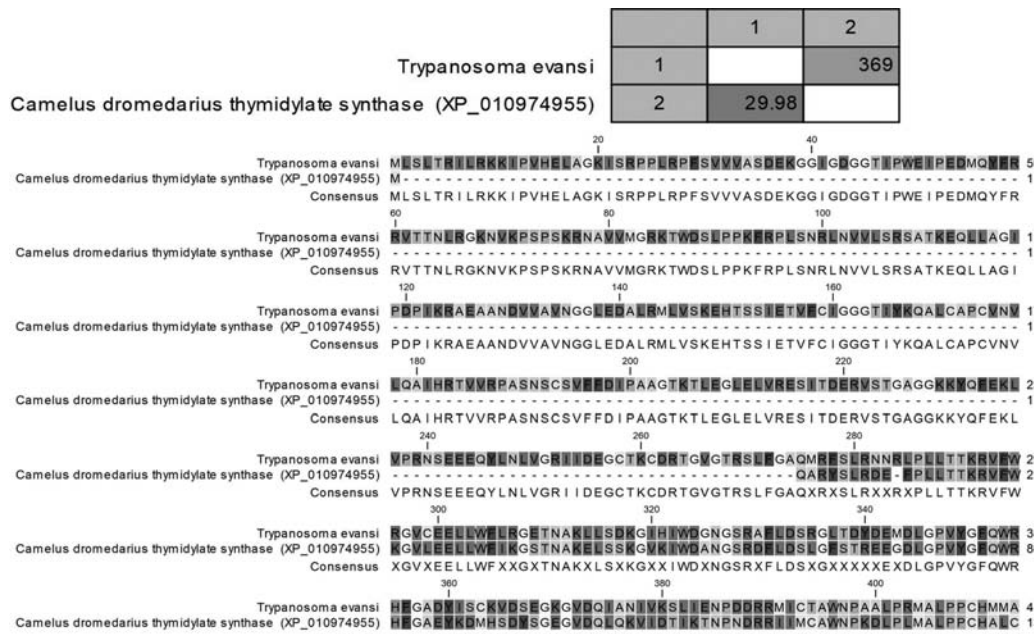


Fig 6. Multiple sequence alignment of dromedary camel and *Trypanosoma evansi* thymidylate synthase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

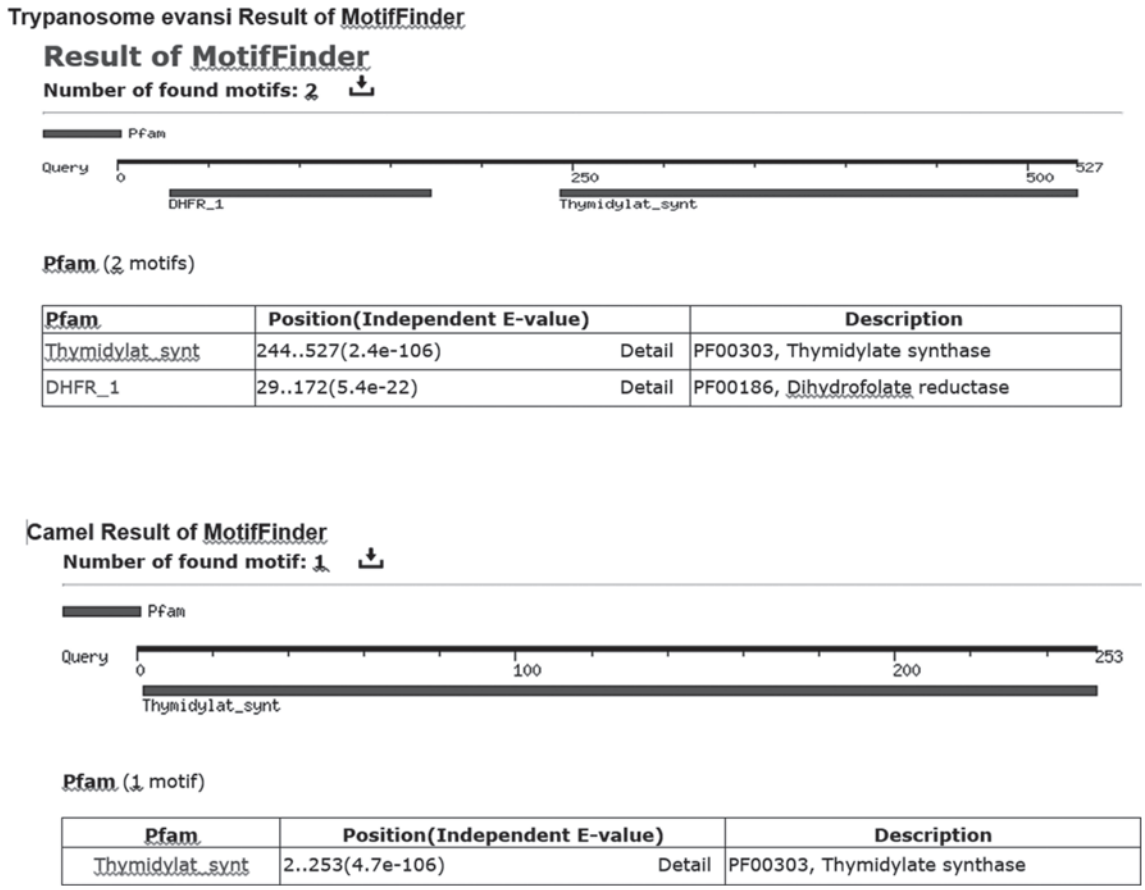


Fig 7. Motif and domain content of dromedary camel and *Trypanosoma evansi* thymidylate synthase.

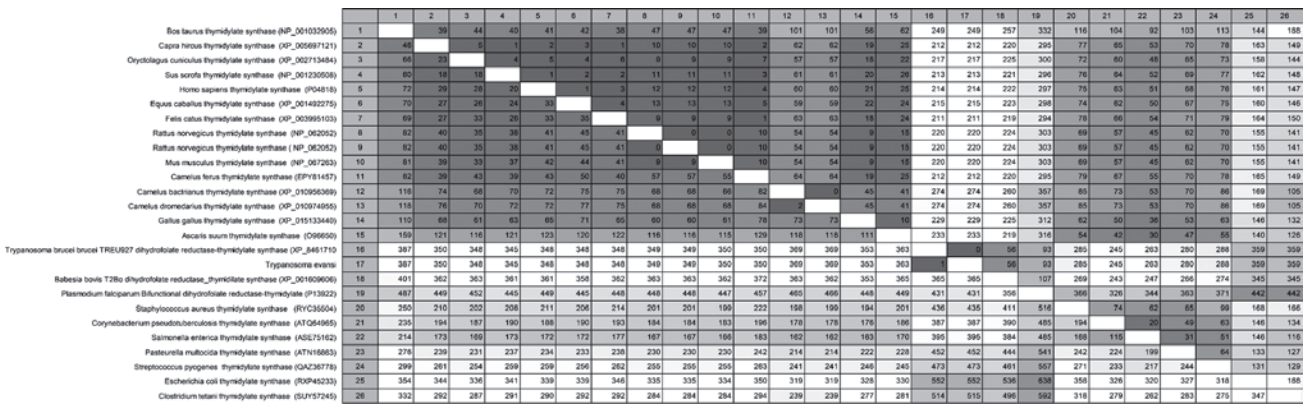


Fig 8. Multiple sequence alignment of camel, *Trypanosoma evansi*, prokaryotes and eukaryotes thymidylate synthase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

the Swiss-Model website (Waterhouse *et al,* 2018). The camel TS sequence was submitted to Swiss Model to predict its structure by automated model building tool. The website predicted the 3D camel structure based on the 3h9k pdf file which contains a homodimer of human TS. The best model was based on human TS with 100% sequence coverage and 61% similarity (Fig 10). The model of *T. evansi* TS showed the highest similarity with the bifunctional DHFR-TS. The highest similarity was with the structure of *T. cruzei* DHFR-TS (PDB ID 3irm) with coverage of 98% and similarity rate 0.52.

The bifunctional DHFR-TS in *T. evansi* is distinct from the camel enzyme. The structure and functional differences could put the enzyme as a hopeful target to develop specific drugs.

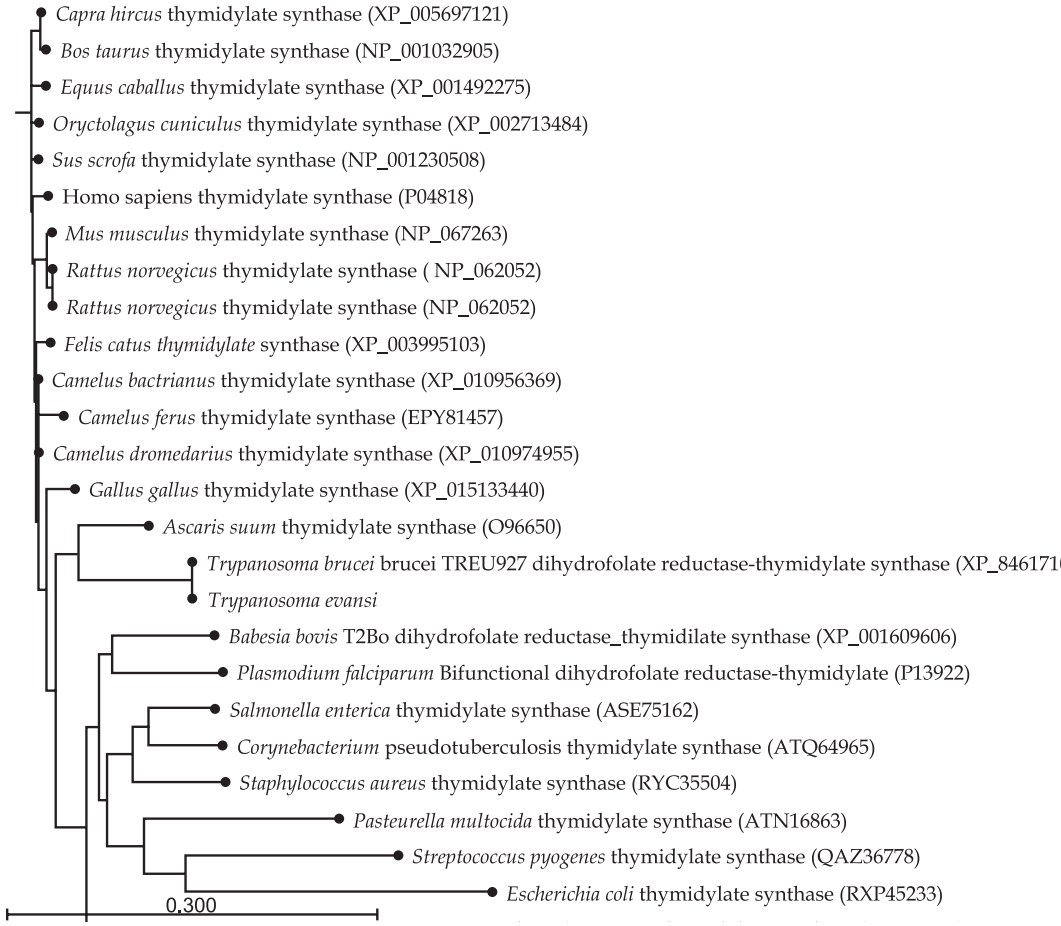


Fig 9. Phylogram of camel and *Trypanosoma evansi* bifunctional thymidylate synthase in relation to a set of prokaryotic and eukaryotic organisms.

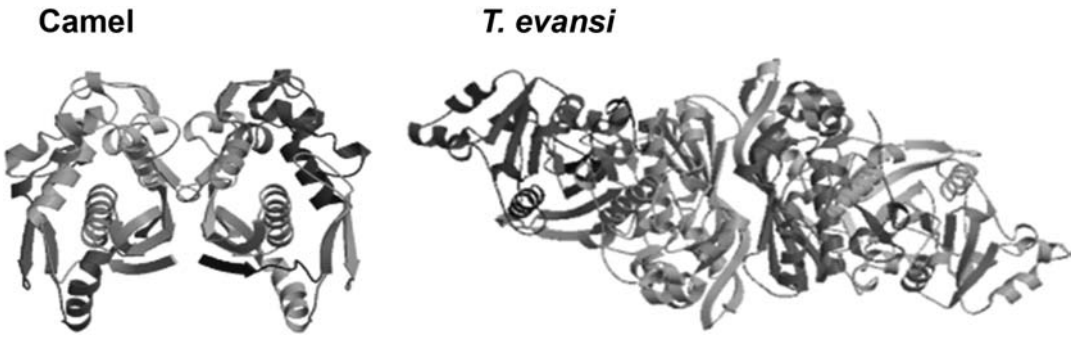


Fig 10. Molecular models of camel and *T. evansi* TS. The models were built by Swiss-Model server.

Acknowledgements

The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programs, National Transformation Program, under Research and Development Grants Program for National Research Institutions and Centres (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

References

Alnazawi M, Altaher A and Kandeel M (2017). Comparative genomic analysis *MERS Cov* isolated from humans and camels with special reference to virus encoded helicase. Biological and Pharmaceutical Bulletin 40:1289-1298.

Chalabi KA and Gutteridge WE (1977). Presence and properties of thymidylate synthase in trypanosomatids. Biochim Biophys Acta 481:71-79.

Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD and Bairoch A (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Research 31:3784-3788.

Gibson MW, Dewar S, Ong HB, Sienkiewicz N and Fairlamb AH (2016). *Trypanosoma brucei* DHFR-TS Revisited: Characterisation of a bifunctional and highly unstable

recombinant dihydrofolate reductase-thymidylate synthase. PLoS Negl Trop Dis 10:e0004714.

Hertz-Fowler C, Peacock CS, Wood V, Aslett M, Kerhornou A, Mooney P, Tivey A, Berriman M, Hall N and Rutherford K (2004). GeneDB: a resource for prokaryotic and eukaryotic organisms. Nucleic Acids Research 32:D339-D343.

Huson DH, Richter DC, Rausch C, Dezulian T, Franz M and Rupp R (2007). Dendroscope: An interactive viewer for large phylogenetic trees. BMC bioinformatics 8:460.

Jirimutu, Wang Z, Ding G, Chen G, Sun Y, Sun Z, Zhang H, Wang L, Hasi S, Zhang Y, Li J, Shi Y, Xu Z, He C, Yu S, Li S, Zhang W, Batmunkh M, Ts B, Narenbatu, Unierhu, Bat-Ireedui S, Gao H, Baysgalan B, Li Q, Jia Z, Turigenbayila, Subudenggerile, Narenmanduhu, Wang Z, Wang J, Pan L, Chen Y, Ganerdene Y, Dabxilt, Erdemt, Altansha, Altansukh, Liu T, Cao M, Aruuntsever, Bayart, Hosblig, He F, Zha-ti A, Zheng G, Qiu F, Sun Z, Zhao L, Zhao W, Liu B, Li C, Chen Y, Tang X, Guo C, Liu W, Ming L, Temuulen, Cui A, Li Y, Gao J, Li J, Wurentaodi, Niu S, Sun T, Zhai Z, Zhang M, Chen C, Baldan T, Bayaer T, Li Y and Meng H (2012). Genome sequences of wild and domestic bactrian camels. Nat Commun 3:1202.

Kandeel M and Al-Taher A (2020a). Bioinformatics of uridine/deoxyuridine paths in *Trypanosoma evansi* revealed targeting uridine phosphorylase and cytidine deaminase. Journal of Camel Practice and Research 27:111-119.

Kandeel M and Al-Taher A (2020b). Uridine 5′-monophosphate (UMP) metabolising enzymes uracil phosphoribosyltransferase and orotidine-5′-phosphate decarboxylase/ump synthase in camels and *Trypanosoma evansi*. Journal of Camel Practice and Research 27:81-88.

Kandeel M, Altaher A and Alnazawi M (2019a). Molecular dynamics and inhibition of MERS CoV papain-like protease by small molecule imidazole and aminopurine derivatives. Letters in Drug Design and Discovery 16:584-591.

Kandeel M, Altaher A and Alnazawi M (2019). Molecular dynamics and inhibition of MERS CoV papain-like protease by small molecule imidazole and aminopurine derivatives. Letters in Drug Design and Discovery 16:1-8 (in press).

Kandeel M, Dalab A, Al-Shabebi A, Al-Taher A, Altaher Y and Abozahra M (2020). Uridine 5-triphosphate (UTP) metabolising enzymes nucleoside diphosphate kinase and cytidine triphosphate (CTP) synthase in camels and *Trypanosoma evansi*. Journal of Camel Practice and Research 27:61-68.

Kandeel M, Kitade Y, Al-Taher A and Al-Nazawi M (2019b). The structural basis of unique substrate recognition by *Plasmodium* thymidylate kinase: Molecular dynamics simulation and inhibitory studies. PLoS One 14:e0212065.

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S and Tokimatsu T (2007). KEGG for linking genomes to life and the environment. Nucleic Acids Research 36:D480-D484.

Kanehisa M, Furumichi M, Tanabe M, Sato Y and Morishima K (2016). KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Research 45:D353-D361.

Labarga A, Valentin F, Anderson M and Lopez R (2007). Web services at the European bioinformatics institute. Nucleic Acids Research 35:W6-W11.

Madden T (2013). The BLAST sequence analysis tool. In: The NCBI Handbook (Internet) 2nd edition. National Centre for Biotechnology Information (US).

Mahmoud K, Kamal E, Wael E, Mahmoud F and Ibrahim G (2019). Species specificity and host affinity rather than tissue tropism controls codon usage pattern in respiratory mycoplasmosis. Journal of Camel Practice and Research 26:1-12.

Marchler-Bauer A, Anderson JB, Cherukuri PF, DeWeese-Scott C, Geer LY, Gwadz M, He S, Hurwitz DI, Jackson JD and Ke Z (2005). CDD: a conserved domain database for protein classification. Nucleic Acids Research 33:D192-D196.

Ogata H, Goto S, Fujibuchi W and Kanehisa M (1998). Computation with the KEGG pathway database. Biosystems 47:119-128.

Panecka-Hofman J, Pohner I, Spyrakis F, Zeppelin T, Di Pisa F, Dello Iacono L, Bonucci A, Quotadamo A, Venturelli A, Mangani S, Costi MP and Wade RC (2017). Comparative mapping of on-targets and off-targets for the discovery of anti-trypanosomatid folate pathway inhibitors. Biochim Biophys Acta Gen Subj 1861:3215-3230.

Schormann N, Velu SE, Murugesan S, Senkovich O, Walker K, Chenna BC, Shinkre B, Desai A and Chattopadhyay D (2010). Synthesis and characterisation of potent inhibitors of *Trypanosoma cruzi* dihydrofolate reductase. Bioorg Med Chem 18:4056-4066.

Senkovich O, Schormann N and Chattopadhyay D (2009). Structures of dihydrofolate reductase-thymidylate synthase of *Trypanosoma cruzi* in the folate-free state and in complex with two antifolate drugs, trimetrexate and methotrexate. Acta Crystallogr D Biol Crystallogr 65:704-716.

Sequencing H (2011). CLC Genomics Workbench. Workbench.

Sievers F and Higgins DG (2014). Clustal Omega, accurate alignment of very large numbers of sequences. In: Multiple Sequence Alignment Methods. Springer. pp 105-116.

Slavik K and Slavikova V (1980). Purification of thymidylate synthetase from enzyme-poor sources by affinity chromatography. Methods Enzymol 66:709-723.

Valente M, Vidal AE and Gonzalez-Pacanowska D (2019). Targeting Kinetoplastid and apicomplexan thymidylate biosynthesis as an antiprotozoal strategy. Curr Med Chem 26:4262-4279.

Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R and Schwede T (2018). SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 46:W296-w303.

DOI : 10.5958/2277-8934.2020.00028.4 *Vol 27 No 2, p 193-200*

ENZYMATIC AND ANTIOXIDANT ACTIVITY OF CAMEL MILK FERMENTED WITH DIFFERENT STRAINS OF LACTIC ACID BACTERIA

Vinay Kumar Mehra, Raghvendar Singh, Devendra Kumar and Rakesh Poonia
ICAR-National Research Centre on Camel, Bikaner, Rajasthan, India

ABSTRACT

The aim of the present study was to compare antioxidant activity of different LAB *viz Lactobacillus delbrueckii* sub sp. *Bulgaricus*, *Lactobacillus casei, Lactobacillus plantarum* and *Streptococcus thermophilus*. The camel milk was fermented with *Lactobacillus delbrueckii* sub sp. *Bulgaricus, Lactobacillus casei, Lactobacillus plantarum* and *Streptococcus thermophilus* at 37°C upto 12 hr. The resulting fermented camel milk was evaluated with respect to the changes of pH, acidification) and antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azino-di-(3-ethylbenzthiazoline sulfonic acid (ABTS) inhibition assay and enzymatic assay including Catalase, Sod and GST during 0 h to 12 hr fermentation time at 37°C. *L. plantarum* and *S. thermophilus* increases the acidityof fermented milk more rapidly ($p < 0.05$). The *Lactobacillus delbrueckii* showed highest ABTS activity (61.84 ± 0.34) and *S. thermophilus* showed lowest ABTs activity (43.60 ± 0.40), while *Lactobacillus casei* fermented milk had highest DPPH activity (7.52 ± 0.13) at different fermentation time. The *Lactobacillus plantarum* showed highest value of SOD (4.54 ± 0.013), catalase (200.17 ± 0.95) and GST activity (9.13 ± 0.155) at different fermentation time. In conclusion, compared to *Lactobacillus delbrueckii* sub sp. *Bulgaricus*, *Lactobacillus casei* and *Streptococcus thermophilus*, *Lactobacillus plantarum* showed higher antioxidant activity and can be used as a potential probiotics. It also found that the variation in antioxidant activity of fermented camel milk with 4 different LAB could be due to the change in pH, change in fermentation time or formation and breakdown of bio-active peptides during fermentation process.

Key words: Antioxidant activity, camel milk, catalase, fermentation, *Lactobacillus,* lactic acid bacteria, SOD

Lactic acid bacteria (LAB) have long been ingested by people in lot of fermented foods such as dairy products because of their pro-biotic properties which have the ability to produce various antimicrobial compounds and also show anti-tumour activity, alleviation of lactose intolerance stabilisation of gut microflora (Khedid *et al,* 2009). Several studies have been reported that reactive oxygen species (ROS) and free radicals play a crucial role in many diseases like cancer, atherosclerosis and diabetes (Beckman and Ames, 1998). The body has enzymatic antioxidants such as catalase, super oxide dismutase, glutathione S transferase and non-enzymatic antioxidant compounds which defend against ROS but these defense systems are not so efficient to entirely prevent the damage, so therefore, food supplements having antioxidant properties may be used to reduce oxidative damage (Zommara *et al,* 1996; Oxman *et al*, 2000; Terahara *et al*, 2001; Kullisaar *et al*, 2003).

In milk various bioactive peptides are present and the activity of these bio-peptides are further enhanced by fermentation of milk by LAB (Korhonen and Pihlanto, 2006). Fermented camel milk possess antioxidant properties, thus making it a potential candidate for functional and novel foods to improve health through nutrition (Korhonen, 2009). These peptides have various health beneficial effects such as immunomodulatory activities, antioxidative activities, antimicrobial and ACE inhibitory activities (Soleymanzadeh Nazila *et al,* 2016). Therefore, the aim of this study was to use of different strains of LAB for the fermentation of camel milk and to assess their enzymatic and antioxidant activities during the fermentation of the milk at different time intervals.

Materials and Methods

The milk samples collected from National Research Centre on Camel were pooled together and placed into a sterile container.

Sources of cultures

Lyophilised pure culture strains of *Lactobacillus delbrueckii* sub sp. *Bulgaricus* (NCDC- 009), *Lactobacillus casei* (NCDC- 017), *Lactobacillus plantarum*

SEND REPRINT REQUEST TO VINAY KUMAR MEHRA email: vinay28mehra@yahoo.com

(NCDC- 020), *Streptococcus thermophilus* (NCDC- 074) used in this study were obtained from National Dairy Research Institute, Karnal, Haryana (India). Each strain was first activated in sterilised skim milk medium (12.5%), at 37°C for 24 h. These were then cultured and maintain in sterilised litmus milk medium. For fermentation, 1% skim milk culture was used. Each strain was fermented in duplicate for 12 h in skim camel milk and pH, TA, antioxidant (ABTS, DPPH) and enzymatic activity (Catalase, SOD, GST) was determined periodically at 2 h interval (i.e - 0, 2, 4, 6, 8, 10, 12 h) .

Measurement of pH and titratable acidity

The pH was determined by inserting a pH electrode (HANNA) directly fermented camel milk at every 2 h interval. The titratable acidity was determined by titrating 10 ml of homogenised fermented camel milk with 0.1 N NaOH to the phenolphthalein end point at every 2 h interval.

Biochemical analysis

Measurement of ABTS (2,2'-azino-di-(3-ethylbenzthiazoline sulfonic acid)) radical scavenging activity

ABTS radical scavenging activity was assayed spectrophotometricaly according to method described by Salami *et al* (2009). This method is based on the ability of antioxidant to blow out ABTS cation radicals ($ABTS^+$), a blue/green chromophore with absorbance at 734 nm, in comparison to standard antioxidants. ABTS assay was carried out by oxidising 7 mM ABTS solution in 2.45 mM potassium persulphate buffer with equal volume (1 : 1) for 12-16 h in the dark to provoke the formation of ABTS radical ($ABTS^+$). Before use, ABTS+ stock solution was diluted with distilled water to obtain absorbance 0.7 ± 0.2 at 734 nm at t_0 (t = 0 min), then 3ml ABTS+ working standard solution was mixed with 60 ul of sample and absorbance was measured after 20 min (t_{20}) at 734 nm in UV spectrophotometer (Biorad Hercules, California, United States).

Measurement of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams *et al* (1994) with slight modification. DPPH make stable free radical in aqueous or ethanol solution. However, fresh DPPH solution was prepared before every measurement. Three ml DPPH reagent (100µM) was mixed with 0.75 ml of 0.1 M Tris-HCL (pH-7.4) and 75ml of hydrolysate sample in test tubes and mixed well. The absorbency in time t = 0 min (t_0) was measured using UV spectrophotometer (Biorad Hercules, California, United States). The sample tubes were also incubated at room temperature under dark for measurement of absorbancy in time t = 20 min (t_{20}). Ethanol was used as a blank. The free radical scavenging activity was calculated as decrease in absorbance.

Enzyme assay

Catalase activity

The catalase activity was performed according to method described by Aebi (1984) and Cuellar-Cruz *et al* (2009) with slight modification. In the ultraviolet range, H_2O_2 shows a continual increase in absorption with decreasing wavelength. The decomposition of H_2O_2 can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance (ΔA_{240}) per unit time is a measure of the catalase activity. The assay was conducted by taking 1 ml Phosphate buffer 50 mM, pH 7.0 and 1 ml hydrogen peroxide 30 mM and 1 ml diluted sample in phosphate buffer (1:10) in quartz cuvette and absorbance was measured at 240 nm at 60 sec interval for 3 min using UV spectrophotometer (Biorad Hercules, California, United States). Phosphate buffer was used as a blank. The catalase activity was calculated as decrease in absorbance.

SOD (Superoxide dismutase) activity

The SOD activity was performed according to method described by Marklund and Marklund (1974) with slight modification. In a spectrophotometric cuvette, 2.7ml 50 mM Tris Cacodylate buffer (pH - 8.2) containing 1 mM EDTA was added to 300 µl of pyrogallol solution and absorbance was measured at 420 nm at 60 sec interval for 3 min using UV spectrophotometer (Biorad Hercules, California, United States). The resultant absorbance was considered as the experiment pyrogallol blank. The same procedure was carried out using of 100 µl sample (homogenate supernatant) and obtained the ΔA420 nm/minute using the maximum linear rate for both the test and experimental blank. Tris Cacodylate buffer was used as a blank. The SOD activity was calculated as increase in absorbance. The enzyme activity was expressed in terms of units/mg protein in which one unit corresponds to the amount of enzyme that inhibited the autoxidation reaction by 50%.

Table 1. pH of the camel mik milk samples fermented by *L. delbrueckii* sub sp. *Bulgaricus, L. casei, L. plantarum, S. thermophilus*.

S.No.	Strain	0 hr	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs
1	Control	6.55 ± 0.002^{e}	6.54± 0.003^{De}	6.54 ± 0.002^{De}	6.51 ± 0.002^{Ed}	6.05 ± 0.003^{Cc}	5.85 ± 0.003^{Cb}	5.69 ± 0.003^{Ca}
2	*Lactobacillus delbrueckii* sub sp. *Bulgaricus NCDC- 009*	6.53 ± 0.004^{g}	6.48 ± 0.003^{Cf}	6.38 ± 0.002^{Ce}	6.23 ± 0.003^{Dd}	6.21 ± 0.003^{Dc}	6.18 ± 0.004^{Eb}	6.12 ± 0.003^{Ea}
3	*Lactobacillus casei NCDC- 017*	6.53 ± 0.004^{g}	6.48 ± 0.002^{Cf}	6.39 ± 0.002^{Ce}	6.22 ± 0.003^{Cd}	6.21 ± 0.002^{Dc}	6.09 ± 0.003^{Db}	5.91 ± 0.003^{Da}
4	*Lactobacillus plantarum NCDC- 020*	6.53 ± 0.004^{g}	6.28 ± 0.002^{Bf}	5.87 ± 0.004^{Be}	5.36 ± 0.004^{Bd}	5.21 ± 0.003^{Bc}	4.76 ± 0.003^{Bb}	4.58 ± 0.004^{Ba}
5	*Streptococcus thermophilus NCDC- 074*	6.53 ± 0.004^{g}	6.23 ± 0.003^{Af}	5.71 ± 0.003^{Ae}	5.02 ± 0.003^{Ad}	4.67 ± 0.004^{Ac}	4.37 ± 0.004^{Ab}	4.28 ± 0.003^{Aa}

Table 2. Titratable acidity of the camel milk samples fermented by *L. delbrueckii* sub sp. *Bulgaricus, L. casei, L. plantarum, S. thermophilus*.

S.No.	Strain	0 hr	2 hrs	4 hrs	6 hrs	8 hrs	10 hrs	12 hrs
1	Control	0.18 ± 0.003^{a}	0.18 ±0.002^{Aa}	0.19 ± 0.002^{Aab}	0.20 ± 0.002^{Ab}	0.21 ± 0.002^{Ac}	0.25 ± 0.002^{Ad}	0.27 ± 0.002^{Ae}
2	*Lactobacillus delbrueckii* sub sp. *Bulgaricus NCDC- 009*	0.18 ± 0.003^{a}	0.20 ±0.002^{Bb}	0.22 ± 0.004^{Bc}	0.23 ± 0.003^{Bc}	0.24 ± 0.002^{Bd}	0.26 ± 0.003^{Be}	0.28 ± 0.003^{Af}
3	*Lactobacillus casei NCDC- 017*	0.18 ± 0.003a	0.20 ±0.002^{Bb}	0.23 ± 0.003^{Bc}	0.24 ± 0.002^{Cd}	0.26 ± 0.002^{Ce}	0.28 ± 0.003^{Cf}	0.30 ± 0.002^{Bg}
4	*Lactobacillus plantarum NCDC- 020*	0.18 ± 0.003^{a}	0.23 ± 0.003^{Cb}	0.34 ± 0.002Cc	0.45 ± 0.004^{Dd}	0.50 ± 0.003^{De}	0.57 ± 0.002^{Df}	0.64 ± 0.003^{Cg}
5	*Streptococcus thermophilus NCDC- 074*	0.18 ± 0.003^{a}	0.23 ± 0.003^{Cb}	0.34 ± 0.002^{Cc}	0.55 ± 0.003^{Ed}	0.60 ± 0.003^{Ee}	0.65 ± 0.003^{Ef}	0.69 ± 0.003^{Dg}

GST (Glutathione-S-Transferase) activity

The GST activity was performed according to the method described by Habig *et al* (1974), Simons and Vander Jagt (1977) with slight modification. The reaction was measured by observing the conjugation of 1-chloro, 2, 4-dinitrobenzene (CDNB) with reduced glutathione (GSH). This was done by watching an increase in absorbance at 340 nm. One unit of enzyme will conjugate 10.0 nmol of CDNB with reduced glutathione per minute at 25°C. In a spectrophotometric cuvette, 100 mM potassium phosphate buffer containing 1 mM EDTA and 75 mM glutathione, reduced solution (G-SH) and 30 mM 1-chloro-2,4-dinitrobenzene solution (CDNB) were added and absorbance was measured at 340 nm at 60 sec interval for 5 min using UV spectrophotometer (Biorad Hercules, California, United States). The resultant absorbance was considered as the experiment blank. The same procedure was carried out using of 100 µl sample (homogenate supernatant) and obtained the ΔA340nm/minute using the maximum linear rate for both the test and experimental blank. Potassium phosphate buffer was used as a blank. The GST activity was calculated as increase in absorbance. The values were expressed as units/mg protein.

Statistical analysis

The experiments were carried out in 3 times and repeated in duplicate (n = 6). Data were expressed as mean ± S.E.M (standard error). All data were subjected to two-way analysis of variance (ANOVA) using SPSS 20.0 software (SPSS INC., Chicago, IL, USA, 2002) and the individual samples were compared by using Duncan's multiple range test (DMRT), at 95 % confidence level.

Results and Discussion

pH and titratable acidity of camel milk fermented with different cultures

pH and titratable acidity play an important role to determine the quality of fermented milk product. In the present study, the pH of all treatments and control was decreased significantly during the fermentation time i.e 0 to 12 h at 37°C (Table 1). Among all 4 type of bacterial culture *Lactobacillus plantarum* and *Streptococcus thermophilus* reduced the pH of milk more rapidly compared to *Lactobacillus delbrueckii* and *Lactobacillus casei* as shown in Fig 1. Similarly the titratable acidity of fermented camel milk was significantly increased ($P<0.05$) in all the strains during fermentation time (0 h to 12 h) (Table 2). It was found that *L. plantarum* and *S. thermophilus*

increases the acidity more rapidly as compared to *L. delbrueckii* and *L. casei* (Fig 2). It was reported that *L. plantarum* grow better between pH 3 and 4 and more metabolically active and produced different bioactive peptides which may affect the antioxidant property of the fermented product (Abubakr *et al,* 2013). Similarly, decreasing the pH and increasing the titratable acidity more rapidly by *S. thermophilus* suggested that it also produces bioactive peptides during fermentation, which may enhance the antioxidant property of the fermented milk (Julijana *et al,* 2016).

Determination of antioxidant activity:

The ABTS activity deepens on the presence of different amino acids in protein especially Cys, Trp and Met possess the highest antioxidant activity compared to the other amino acids (Salami *et al,* 2009), so during fermentation process various bioactive peptides are formed and degraded, which alter the antioxidant property of the fermented product. The antioxidant activity of camel milk fermented with different bacterial strains was evaluated against 2 different ABTS and DPPH radicals. ABTS assays indicated that the whey fraction fermented with *Streptococcus thermophilus* showed higher antioxidant activity (78.58 ±0.43) at 2 h fermentation time but at the end of the fermentation time (12h) *Lactobacillus delbrueckii* showed highest antioxidant activity (61.84 ± 0.34) and *S. thermophilus* showed lowest activity (43.60 ± 0.40) as shown in Fig 3.

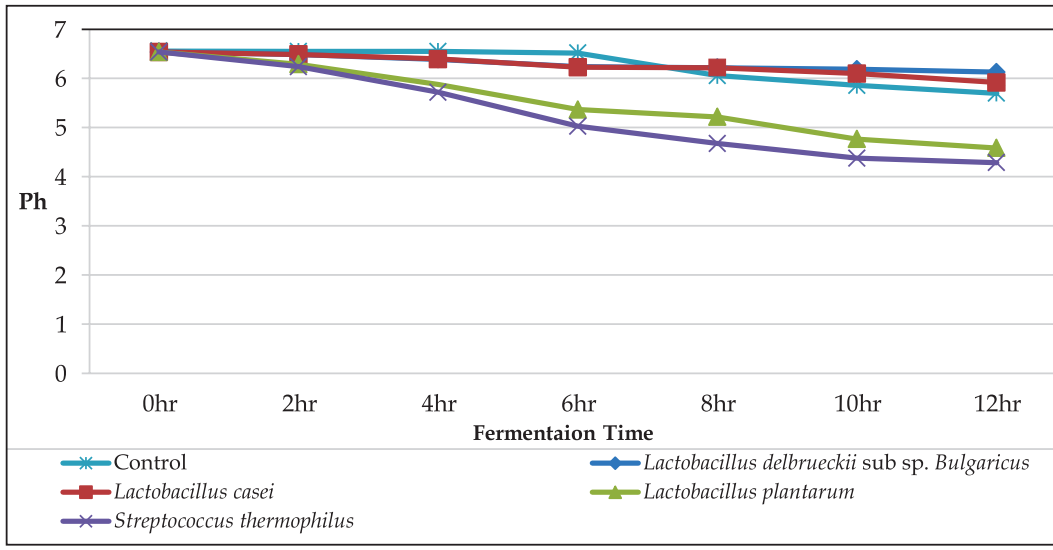


Fig 1. Graphical representation of change in pH of the camel milk samples fermented by 4 different LAB.

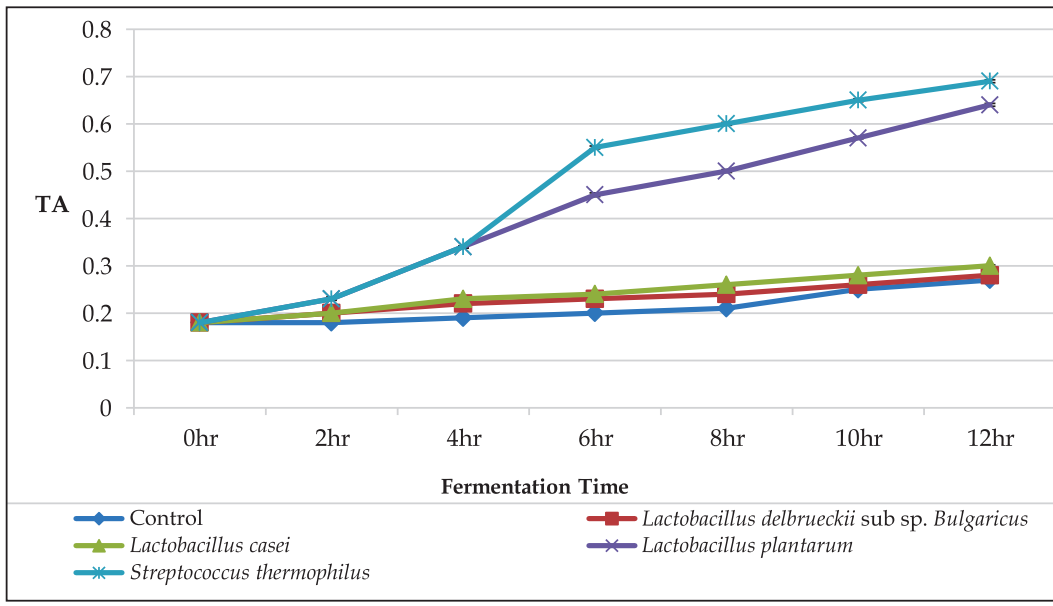


Fig 2. Graphical representation of change in titratable acidity of the camel milk samples fermented by 4 different LAB.

The DPPH activity varies in all the strains and result showed that all 4 strains fermented milk fraction have higher antioxidant activity compared to control at different fermentation time and *Lactobacillus casei* had highest antioxidant activity (7.52 ± 0.13) at 6 h fermentation time compared to all others as shown in Fig 4. The interaction of a potential antioxidant with DPPH depends on its structural conformation. Some compounds react very rapidly with the DPPH and reducing a number of DPPH molecules corresponding to the number of available hydroxyl groups (Brand-Williams *et al*, 1994). The variation in the DPPH activity revealed that all 4 types of bacterial strains produces different type of bioactive peptides according to their metabolic activity during different fermentation time, which may affect the antioxidant activity.

Determination of Enzymatic Antioxidants:

Antioxidant activity of milk and fermented products depends on many components such as sulfur containing amino acids, phosphate, vitamins A, E, carotenoids, zinc, selenium, enzyme like superoxide dismutase, catalase, glutathione

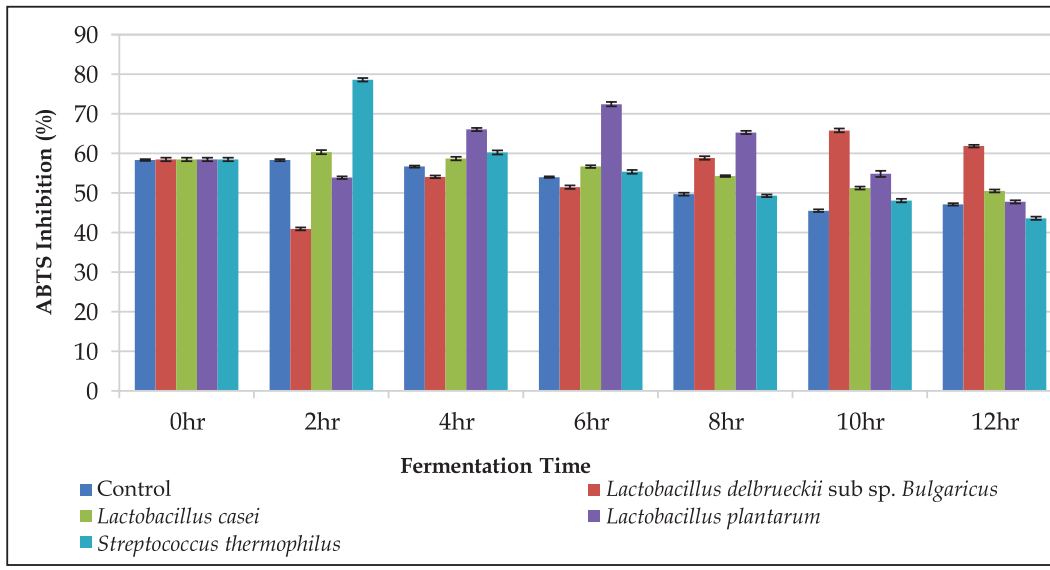


Fig 3. Changes in ABTS activity of fermented camel milk with four different LAB during 0 hr to 12 hr (incubation at 37°C). Values are presented as mean ± SEM (Error bars show standard error).

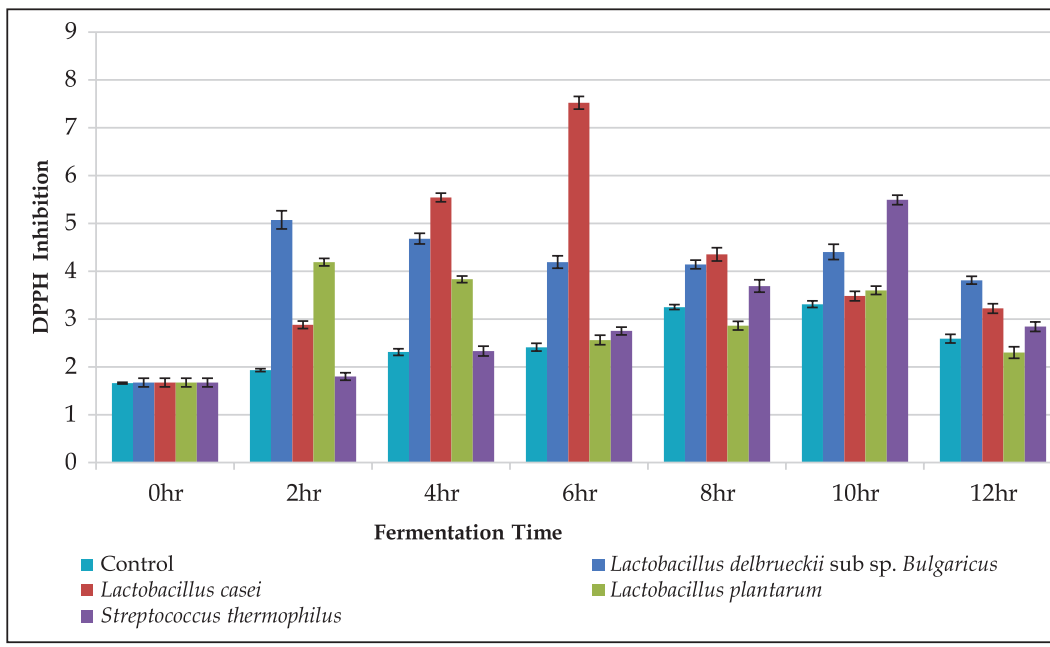


Fig 4. Changes in DPPH activity of fermented camel milk with four different LAB during 0 hr to 12 hr (incubation at 37°C). Values are presented as mean ± SEM (Error bars show standard error).

peroxidase and peptides which are produced during fermentation (Khan *et al,* 2019). In present study, it was found that the SOD activity in camel milk fermented with the strains *viz. L. delbrueckii* sub sp. *Bulgaricus*, *L. casei*, *L. plantarum*, *S. thermophilus* was increased significantly as compared to control during 0 h to 12 h fermentation time and *Lactobacillus plantarum* showed highest SOD activity (4.54 ± 0.013) at 12 h fermentation time (Fig 5).

Similarly, the Catalase and GST activity in all the strains were significantly increased as compared to control (Fig 6 and 7). The results showed that *Lactobacillus plantarum* had highest catalase (200.17 ± 0.95) and GST activity (9.13 ± 0.155) at 10 h and 12 h

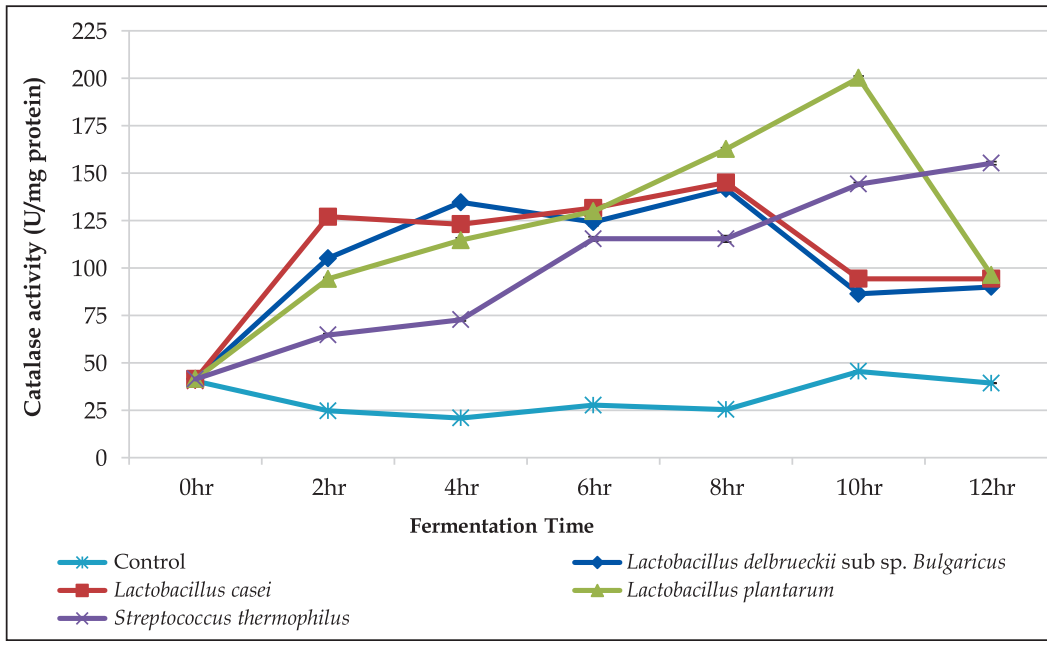


Fig 5. Catalase activity of the camel milk fermented by *L. delbrueckii* sub sp. *Bulgaricus, L. casei, L. plantarum, S. thermophilus* at different fermentation time (incubated at 37°C).

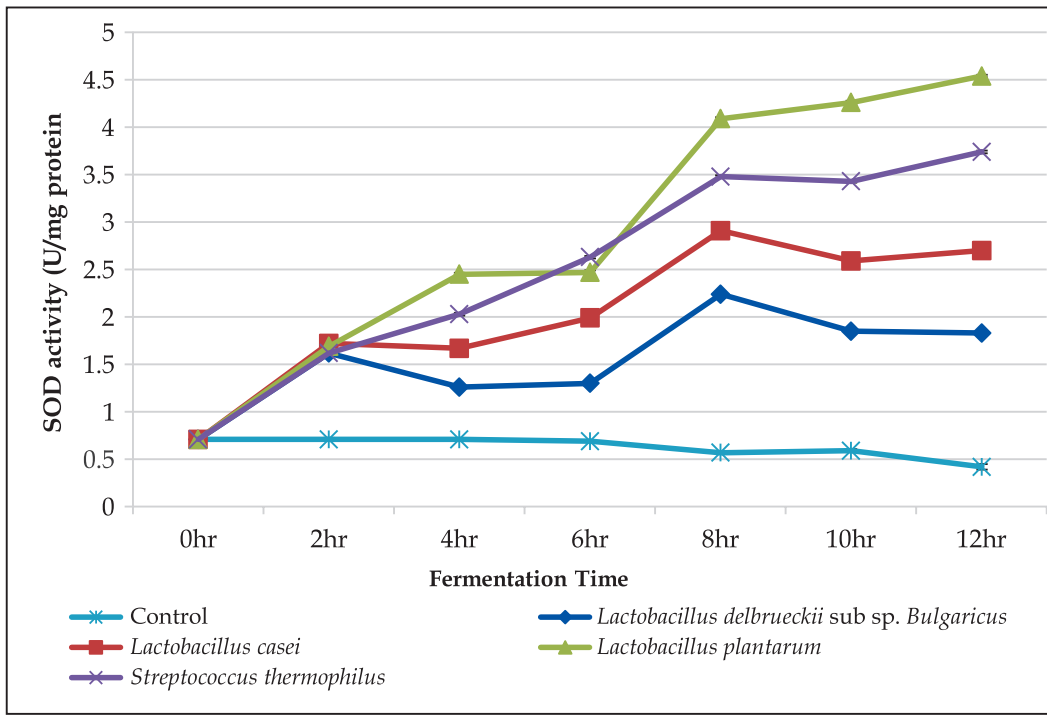


Fig 6. SOD activity of the camel milk fermented by *L. delbrueckii* sub sp. *Bulgaricus, L. casei, L. plantarum, S. thermophilus* at different fermentation time (incubated at 37°C).

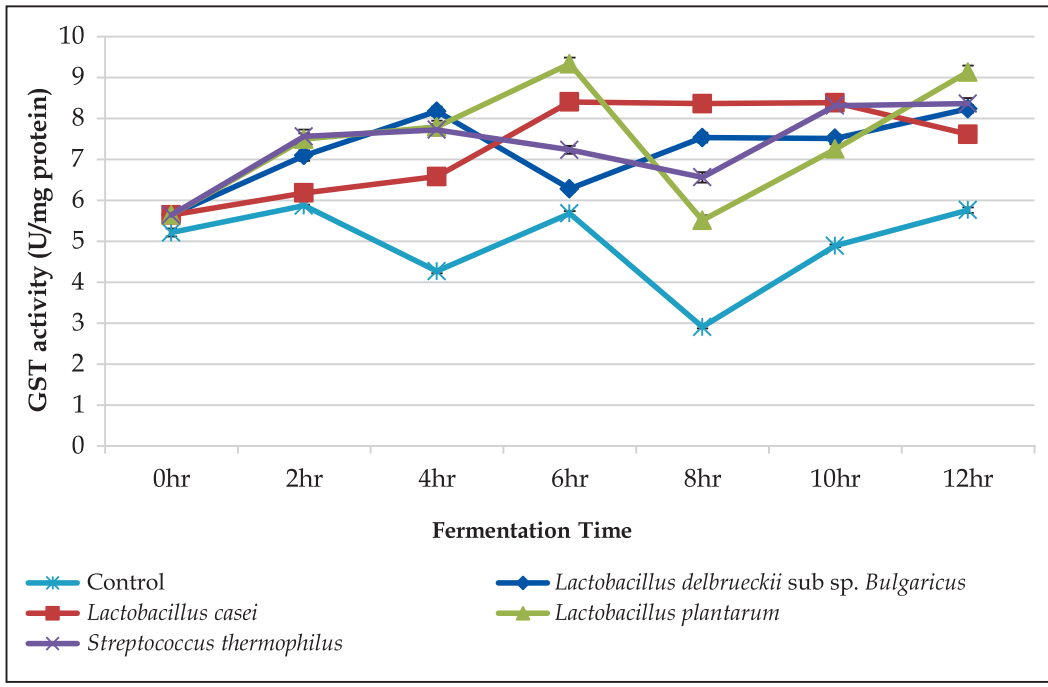


Fig 7. GST activity of the camel milk fermented by *L. delbrueckii* sub sp. *Bulgaricus, L. casei, L. plantarum, S. thermophilus* at different fermentation time (incubated at 37°C).

fermentation time, respectively. Tomusiak-Plebanek *et al* (2018) reported that *L. plantarum* showed highest catalase activity as compared to other *Lactobacillus* spp. These results revealed that the variation in enzymatic antioxidants activity may be due to the change in pH or formation of different bio-peptides or duration of fermentation time. Kleniewska *et al* (2016) noted that exopolysaccharides secreted by probiotic bacteria may play an important role in reducing the oxidative stress. These polysaccharides protect probiotics under starvation conditions or extreme temperature and pH.

Several reports suggested that Lactic acid bacteria (LAB) have antioxidant properties due to presence of enzyme such as catalase, superoxide dismutase and they are usually sensitive to oxidative stress caused by reactive oxygen species (ROS) and the ezymes superoxide dismutase (SOD) and catalase (CAT) can protect against these free radicals by eliminating superoxide and H_2O_2, respectively. In present study, the fermentation of camel milk with different strains of LAB increased the anti-oxidative property of the camel milk. The *Lactobacillus plantarum* showed the highest anti-oxidative activity which suggested that *L. plantarum* can be used as a potential probiotics. During the study we also found that the variation in DPPH, ABTS, Catalase, SOD and GST activity may be due to the change in pH or fermentation time or formation and breakdown of new bio-peptides during fermentation by different strains of LAB.

References

Abubakr, Maryam & Hassan, Zaiton & Salem, Galia. (2013). Antioxidant activity of milk fermented with lactobacillus plantarum 1 and leuconostoc mesenteroides isolated from non-dairy sources. Asian Journal of Pharmaceutical Research and Development. 1. 71-83.

Aebi H (1984). Catalase *in vitro*. Methods Enzymol 105:121-126.

Beckman KB and Ames BN (1998). The free radical theory of aging matures. Physiological Reviews 78:547-581.

Brand-Williams W, Cuvelier ME and Berset C (1994). Use of a Free Radical Method to Evaluate Antioxidant Activity. Lebensmittel-Wissenschaft und -Technologie 28:25-30 (1995).

Cuellar-Cruz M, Castano I, Arroyo-Helguera O and De Las Penas A (2009). Oxidative stress response to menadione and cumene hydroperoxide in the opportunistic fungal pathogen Candida glabrata. The Memórias do Instituto Oswaldo Cruz 104(4):649-654.

Habig WH, Pabst MJ and Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry 249(22):7130-7139.

Julijana Tomovska, Nikola Gjorgievski and Borche Makarijoski (2016). Examination of pH, titratable acidity and antioxidant activity in fermented milk. Journal of Materials Science and Engineering A 6 (11-12) (2016) 326-333 (doi: 10.17265/2161-6213/2016.11-12.006).

Khedid K, Faid M, Mokhtari A, Soulaymani A and Zinedine A

(2009). Characterisation of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. Microbiological Research 164(1):81-91.

Kleniewska Paulina, Hoffmann Arkadiusz, Pniewska Ewa and Pawliczak Rafa B (2016). The Influence of Probiotic Lactobacillus casei in Combination with Prebiotic Inulin on the Antioxidant Capacity of Human Plasma. Oxidative Medicine and Cellular Longevity Volume 2016, Article ID 1340903, 10 pages http://dx.doi.org/10.1155/2016/1340903.

Korhonen H and Pihlanto AD (2006). Bioactive peptides: production and functionality. International Dairy Journal 16 (2006) 945–960. http://dx.doi.org/10.1016/j.idairyj.2005.10.012.

Korhonen HJ (2009). Bioactive Components in Milk In: Young W. Park (ed) Bioactive Components in Milk and Dairy Products. 2009 edn. Wiley-Blackwell, Ames, Iowa, USA, pp 15-42

Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T and Zilmer M (2003). Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenity in human subjects. British Journal of Nutrition 90:449-456.

Marklund S and Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry 47:469-474.

Oxman T, Shapira M, Diver A, Klein R, Avazov N and Rabinowitz B (2000). A new method of long-term preventive cardioprotection using *Lactobacillus.* American Journal of Physiology - Heart and Circulatory Physiology 278:H1717–H1724.

Salami M, Yousefi R, Ehsani MR, Razavi SH, Chobert JM, Haertlé T, et al (2009). Enzymatic digestion and antioxidant activity of the native and molten globule states of camel α-lactalbumin: possible significance for use in infant formula. International Dairy Journal 19:518e523.

Simons PC and Vander Jagt DL (1977). Analytical Biochemistry 82:334-341.

Soleymanzadeh Nazila, Saeed Mirdamadi and Mehran Kianirad (2016). Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). Dairy Science and Technology 96:443-457 DOI 10.1007/s13594-016-0278-1.

Terahara M, Kurama S and Takemoto N (2001). Prevention by lactic acid bacteria of the oxidation of human LDL. Bioscience, Biotechnology and Biochemistry 65:1864-1868.

Tomusiak-Plebanek A, Heczko P, Skowron B, Baranowska A, Okoń K, Thor PJ and Strus M (2018). Lactobacilli with superoxide dismutase-like or catalase activity are more effective in alleviating inflammation in an inflammatory bowel disease mouse model. Drug Design, Development and Therapy 12:3221-3233. https://doi.org/10.2147/DDDT.S164559

Zommara M, Tachibana N, Sakomo M, Suzuki Y, Oda T, Hashida H and Imaizumi K (1996). Whey from cultured skim milk decreases serum cholesterol and increases antioxidant enzymes in liver and red blood cells in rats. Nutrition Research 16:293-302.

DOI : 10.5958/2277-8934.2020.00029.6 Vol 27 No 2, p 201-206

GENETIC POLYMORPHISM AT κ-CASEIN GENE IN INDIAN CAMEL BREEDS (*Camelus dromdarius*)

S.A. Jadhav, U.D. Umrikar, M.P. Sawane, V.D. Pawar, R.S. Deshmukh, S.S. Dahiya[1] and S.C. Mehta[1]

Bombay Veterinary College, Parel, Mumbai- 400012, Maharashtra

[1]ICAR-National Research Centre on Camel, Post Box-07, Bikaner-334001, Rajasthan

ABSTRACT

The present study was carried out in 112 camels belonging to Bikaneri, Jaisalmeri, Kachchhi and Mewari breeds of Indian dromedary to detect point mutation in κ- casein encoding gene. Amplification of 488 bp fragment of κ-Casein gene spanning from -137 (5′ flanking region) to +351 bp of κ-CN gene was carried out and genotyped for the g.1029T>C SNP using the restriction enzyme *Alu*I in PCR-RFLP analysis. Three restriction patterns were resolved on 3.5% agarose gels. The pattern, comprising of 203 bp, 158 bp and 127 bp bands, was resolved successfully for the TT samples. The g.1029T>C transition created an additional restriction site for the enzyme *Alu*I leading to the digestion of 158 bp band into two fragments of 120 bp and 38 bp resulting in the 5 band pattern of 203 bp, 158 bp, 127 bp, 120 bp and 38 bp for CT genotype and 4 band pattern of 203 bp, 127 bp, 120 bp and 38 bp for CC genotype. The genotype frequency, pooled over breed, was 0.045, 0.384 and 0.571 for the CC, CT and TT genotypes, respectively. The frequency of major allele T was observed to be 0.763 and that of C was observed to be 0.237. The existence of CT genotype in sizable number documents the dynamic nature of the locus g.1029T>C SNP, in Indian dromedary breeds. Almost comparable polymorphism was observed in both the sexes. The 3 genotypes, *viz*. CC, CT, TT, were almost equally distributed among the four Indian breeds (χ^2=3.4529; P = 0.750224). The frequency of C allele was lowest in Bikaneri and highest in the Mewari breed. Though the frequency of C allele (Cytosine) in Indian dromedary is relatively low (0.237), still a rapid directional selection might be attempted in favour of the C allele, which is responsible for the creation of an extra putative site for the Hepatocyte Nuclear Factor – 1 (HNF-1) transcription factor. The HNF-1 is reported to be involved in regulation of a number of genes associated with innate immunity, lipid and glucose transport, metabolism etc.

Key words: Camel, dromedary, kappa-casein, milk, polymorphism

The protein content in dromedary milk ranges from 2.3 to 4.9% in different camel rearing countries. The casein is the major protein (1.63 to 2.76%) in camel milk and constitutes about 52 to 87% of the total protein (Konuspayeva *et al,* 2009; Nikkah, 2011a; 2011b; Singh *et al,* 2017). In camel milk κ-CN is 3.5% (El Agamy, 2006) and is encoded by CSN3 gene (Kappeler *et al,* 1998). It is reported that κ-CN plays an essential role in stabilisation of casein micelle (Alexander *et al,* 1988). It is chiefly located on micellar surface and is the specific substrate of chymosin, responsible for the hydrolysation of the κ-CN into para-κ-CN and the caseino-macropeptide (CMP) (Kappeler *et al*, 2006; Moller *et al,* 2012). Existence of 16 alleles corresponding to 13 κ-CN variants have been reported in goat (Caroli *et al,* 2006) and 19 alleles corresponding to 14 κ-CN variants have been reported in cattle (Caroli *et al,* 2009). However, the information about the DNA sequence of dromedary κ-CN is coming at a slow pace. The cDNA sequence and comparison of the 5′ flanking regions of CNS3 gene in Somali camel has been reported by Kappeler *et al* (1998) and Kappeler *et al* (2003), respectively. Recently, Pauciullo *et al* (2013) reported full length sequence of CSN3 gene along with 1045 nucleotides of 5′ flanking region of κ-CN in Sudanese dromedary. They have also reported 17 polymorphic sites in Sudanese camels. The information regarding genetic variability in dromedary populations existing elsewhere in the world is largely lacking including the Indian subcontinent. Looking at this and the increased importance of camel milk for human therapeutics, this study was planned to investigate the genetic variability in κ-Casein gene spanning from -137 (5′ flanking region) to +351 bp of κ-CN in Indian dromedary breeds.

Materials and Methods

Experimental animals

The blood samples were collected from 112 camel belonging to Bikaneri, Jaisalmeri, Kachchhi

SEND REPRINT REQUEST TO S.C. MEHTA email: scmehta64@gmail.com

and Mewari breeds (Table 1) at the ICAR-National Research Centre on Camel, Bikaner, Rajasthan. Approximately 10 ml of venous blood was collected in EDTA and were transported to the laboratory in ice box and stored at 4°C until use.

PCR conditions

DNA was isolated using phenol-chloroform method (Sambrook *et al,* 1989) with minor modifications. The PCR primers; forward: CACAAAGATGACTCTGCTATCG and reverse GCCCTCCACATATGTCTG were utilised (Pauciullo *et al,* 2013). PCR amplifications were carried out in 12.5 µl reactions containing 50 ng DNA, 12.5 pmol each primer (Sigma-Aldrich), 1.0 U *Taq* DNA polymerase, 0.2 mM each dNTP, 1.25 µl 10X Taq DNA polymerase buffer containing 10 mM Tris – HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl and 0.01% gelatin. The PCR amplification programme, performed on Eppendorf Mastercycler Gradient, consisted of an initial denaturation temperature of 95°C for 5 min, then 34 cycles at 94°C for 30s, 56°C for 30 s and 72°C for 45s. Final extension was carried out at 72 °C for 5 min. The κ-Casein bands were visualised in 1% agarose gel containing ethidium bromide. The electrophoresis was carried out in 1XTBE at 80 volts and the results were recorded using UVP gel-documentation system.

Restriction digestion

Around 250-500 ng of amplified PCR products were digested in 10 µl reaction using 5 units of *Alu*I restriction enzyme (BioLabs) with CutSmart Buffer and incubating at 37°C for 15 minutes. The restriction bands were analysed on 3.5% Agarose gel electrophoresis with appropriate marker DNA.

Statistical analysis

The Chi-square test ($\chi 2$) was performed using IBM SPSS Statistics 20 software (2017) to test the statistical significance of the differences between observed and expected frequencies in genotypic classes.

Sequencing and sequence analysis

The PCR products were got sequenced on ABI3730 DNA Sequencer. The SNPs were visualised on chromatograms using Chromas 2.6.6 software. The sequences were analysed using BioEdit Sequence Alignment Editor (Hall, 1999). Sequence phylogeny was derived using Nucleotide BLAST programme of NCBI.

Results and Discussion

PCR amplification of κ-Casein gene promoter

Amplification of 488 bp fragment spanning from -137 of 5′ flanking region (promoter) to +351 bp of κ- Casein gene was successfully achieved by PCR in Bikaneri, Jaisalmeri, Kachchhi and Mewari Camels (Fig 1). The present results are in agreement with the findings of Pauciullo *et al* (2013) in Sudanese camel (*Camelus dromedarius*), Othman *et al* (2016) in Maghrabi camel of Egypt and Yamini *et al* (2019) in Bikaneri camel of India, where the same set of primers was utilised for the amplification of 488 bp fragment of CSN3 gene and it's 5′flanking region.

PCR-RFLP of κ-Casein gene, promoter fragment

The detection of point mutation at κ-Casein gene, promoter was attempted using PCR-RFLP. The restriction fragments were resolved in 3.5% Agarose gel. The RE digestion of PCR product (488 bp) of κ-Casein gene from the Indian camel (*Camelus dromedarius*) breeds using *Alu*I lead to 3 fragments of 203 bp, 158 bp and 127 bp for the TT genotype camels. The 158 bp long band was further restricted into 2 daughter fragments of 120 bp and 38 bp in the presence of cytosine. Thus, four bands of 203 bp, 127 bp, 120 bp and 38 bp (not resolved) were observed in animals having CC genotype. Accordingly, the

Table 1. Genotype frequency and allele frequency in Indian dromedary at κ-Casein gene, promoter.

Genotype	Bikaneri			Jaisalmeri			Kachchhi			Mewari		
	M	F	P	M	F	P	M	F	P	M	F	P
N	13	15	28	6	22	28	13	15	28	13	15	28
CC	0.00	0.00	0.00	0.00	0.04	0.03	0.00	0.13	0.07	0.08	0.07	0.07
CT	0.38	0.33	0.36	0.17	0.50	0.43	0.38	0.27	0.32	0.23	0.60	0.43
TT	0.62	0.67	0.64	0.83	0.46	0.54	0.62	0.60	0.61	0.69	0.33	0.50
Allele T	0.81	0.83	0.82	0.92	0.70	0.75	0.81	0.73	0.77	0.81	0.63	0.71
Allele C	0.19	0.17	0.18	0.08	0.30	0.25	0.19	0.30	0.23	0.19	0.37	0.29

M-Male; F-Female; P-Pooled Sex; N-Number of Animals

heterozygous animals with CT genotype showed 5 fragments of 203 bp, 158 bp, 127 bp, 120 bp and 38 bp (not resolved). The results are presented in table 1 and Fig 2.

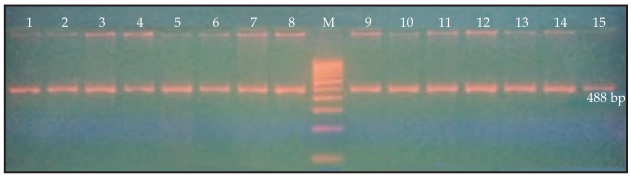


Fig 1. Amplification of 488 bp fragment of κ-Casein gene, promoter in Indian camel (Lane 1 – 15) ; M-100 bp marker.

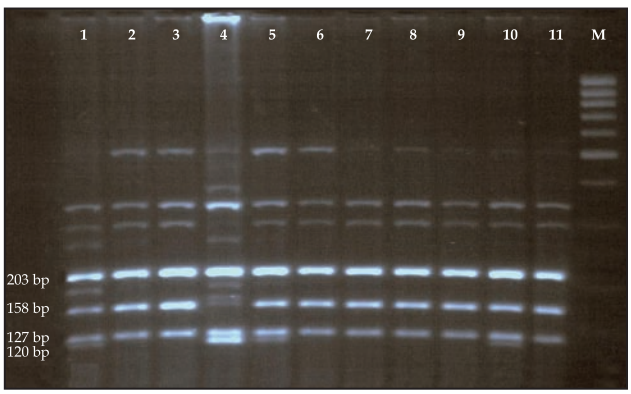


Fig 2. PCR-RFLP genotyping of camel κ- casein gene, promoter with *Alu*I. Lane 4 : CC genotype (203bp, 127 bp, 120bp and 38 bp) ; Lane 1, 5 and 10 : CT genotype (203 bp, 158 bp, 127 bp, 120 bp and 38 bp) ; Lane 2-3, 6-9 and 11 : TT genotype (203bp, 158 bp and 127 bp); M-50 bp DNA marker.

The results indicated that the TT genotype was the most numerous followed by CT, and the CC genotype was the least numerous. The nucleotide substitution at g.1029T>C SNP was observed in all the camel breeds studied but the CC genotype was not observed in Bikaneri camels analysed in the study. The frequency of CT genotype in Bikaneri, Jaisalmeri, Kachchhi and Mewari breeds were observed to be 0.357, 0.429, 0.322 and 0.429, respectively. The frequency of the CC, CT and TT genotypes, pooled over breeds, was 0.045, 0.384 and 0.571, respectively (Table 2). The existence of CT genotype in sizable number documents the dynamic nature of the locus g.1029T>C SNP, in Indian dromedary breeds. The frequency of major allele T was observed to be 0.763 and that of C was observed to be 0.237. Almost

Table 2. Genotype and allele frequency in Indian dromedary at κ-Casein gene, promoter.

Genotype	Male	Female	Pooled	Allele	Frequency
CC	0.02	0.06	0.045	C	0.237
CT	0.31	0.43	0.384	T	0.763
TT	0.67	0.51	0.571		

comparable polymorphism was observed in both the sexes (Table 2). The three genotypes, *viz.* CC, CT, TT, were almost equally distributed among the 4 Indian breeds (χ^2=3.4529; P = 0.750224; non-significant at 5% probability level of significance).

Othman *et al* (2016) studied the genetic polymorphism of κ-casein gene in Maghrabi camel reared in Egypt. The amplified fragments at 488-bp of κ-CN gene were digested with *Alu*I endonuclease. The results showed the presence of 3 genotypes; CC (12%), TT (48%) CT (40%). The finding of Othman *et al* (2016) are in agreement with the present findings in Indian camel breeds except the reporting of higher frequency of CC genotype in Maghrabi camel. Comparable results have also been reported by Pauciullo *et al* (2013) in Sudanese camel breeds where they also observed little higher frequency of CC genotype (0.18) as against (0.045) in present investigation indicating relatively higher replacement rate at the locus g.1029T>C SNP. Accordingly, the frequency of C allele in the sample of 188 Sudanese camels was 0.38, with a variation among the breeds ranging from 0.30 to 0.46 and that of T allele was 0.62 with the variation among the breeds ranging from 0.54 to 0.70. However, Yamini *et al* (2019) reported 3 fragments of 203 bp, 158 bp and 127 bp upon restriction digestion of 488 bp κ-Casein gene fragment with *Alu*I restriction enzyme in TT genotyped camels of Bikaneri breed, which is in agreement with the present findings but restriction digestion of 158 bp fragment with *Alu*I enzyme leading to 2 daughter bands of 146 bp and 12 bp was not observed in the present investigation involving Bikaneri, Jaisalmeri, Kachchhi and Mewari breeds of Indian dromedary; however, these were observed by Pauciullo *et al* (2013) in Shanbali, Kahli, Arabi and Lahaoi breeds of Sudanese camel; and by Othman *et al* (2016) in Maghrabi camel reared in Egypt. Thus, the CT genotype reported by Yamini *et al* (2019) was different from the CT genotype referred in above 3 studies. Though, the frequency of C allele (Cytosine) in Indian dromedary is relatively low (0.237), still a rapid directional selection might be attempted in favour of the C allele, which is responsible for the creation of an extra putative site for the Hepatocyte Nuclear Factor – 1 (HNF-1) transcription factor. The HNF-1 is reported to be involved in regulation of a number of genes associated with innate immunity, lipid and glucose transport, metabolism etc. Here it will be worth mentioning that the camelids are considered as evolutionary innovation because of their only heavy chain antibodies (Hamers-Casterman *et al*, 1993). Although, the three genotypes, *viz.* CC,

CT, TT, were almost equally distributed among the 4 Indian breeds (χ^2=3.4529; P = 0.750224), it was observed that the replacement to thymine with cytosine at g.1029 locus was lowest in Bikaneri breeds followed by Kachchhi and Jaisalmeri, and highest in Mewari breed of camel (Table 1). The Bikaneri, Jaisalmeri, Kachchhi and Mewari breeds of Indian dromedary are adapted in different geo-climatic

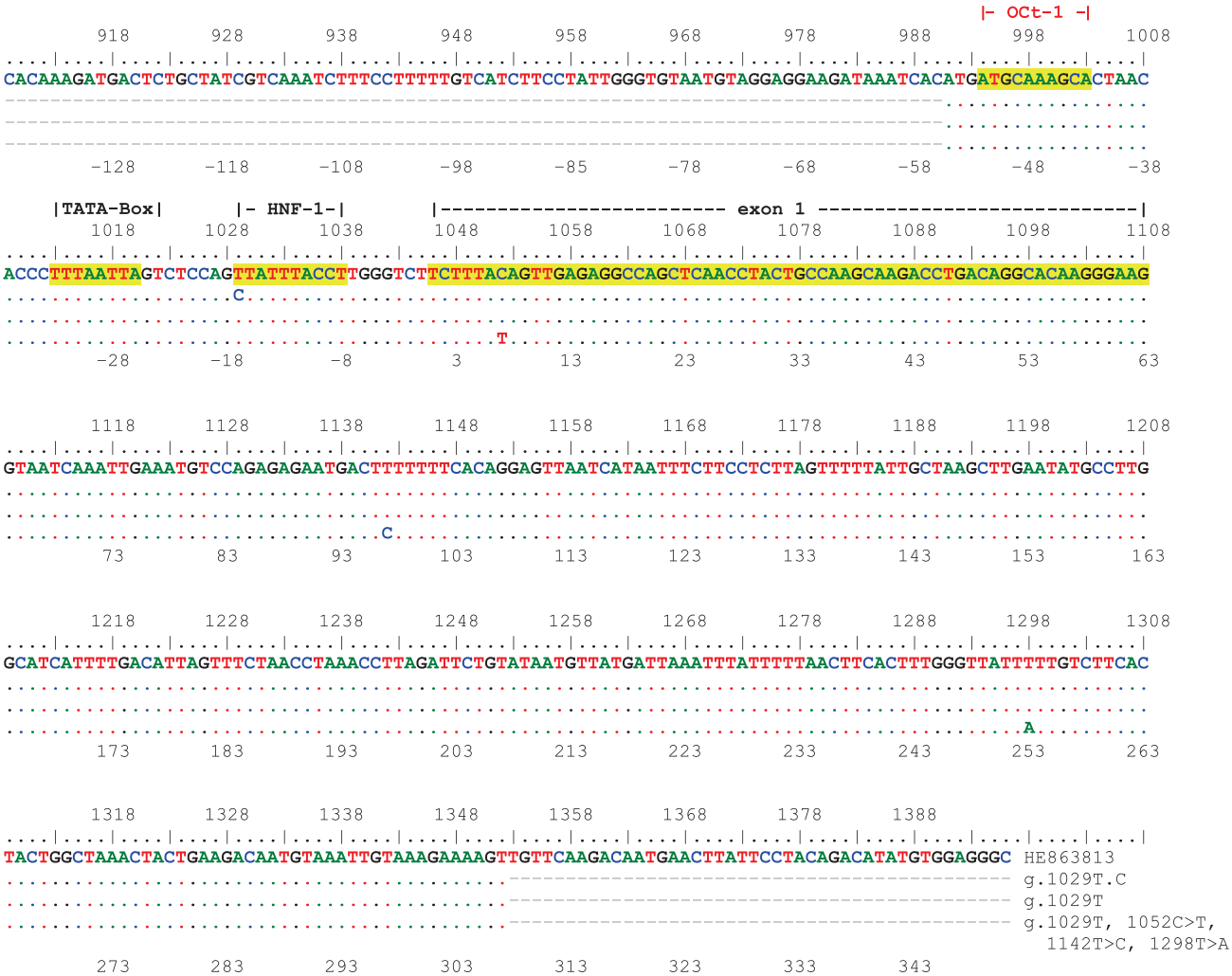


Fig 3. Alignment of nucleotide sequences of the promoter region, exon-1 and intron-1 of *C. dromedarius* CSN3 gene variants observed in the present study with the published sequence of the camel (NCBI GenBank ID HE863813). The first line numbering is as per the reference sequence HE863813 and the lower numbering is relative to the first nucleotide of first exon (+1).

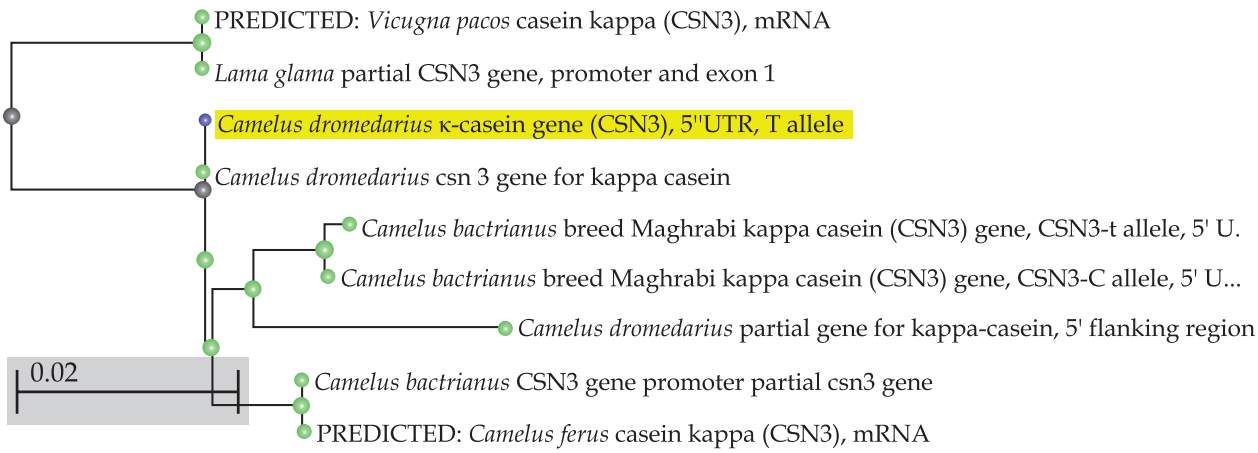


Fig 4. Phylogenetic analysis of sequence containing g.1029 T allele in κ-casein gene, promoter.

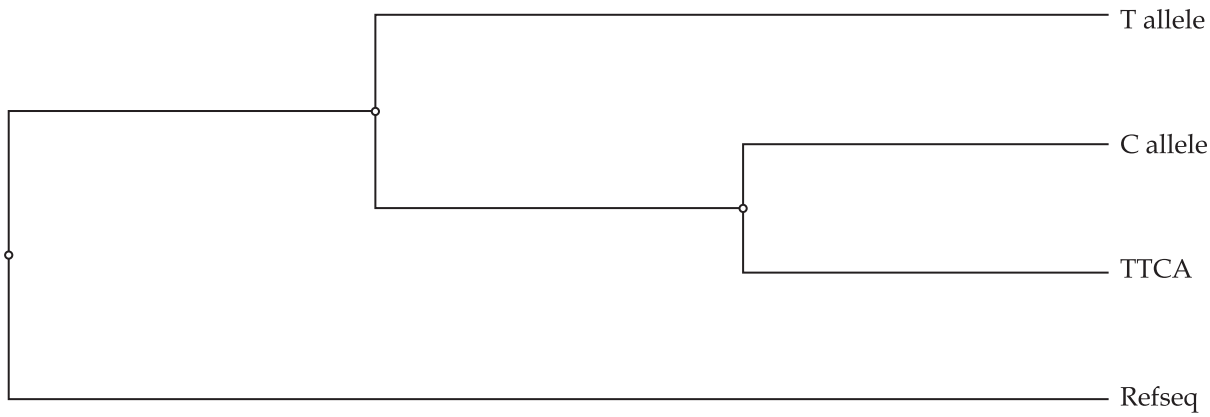


Fig 5. Phylogenetic analysis of sequence containing T allele (g.1029 T), C allele (g.1029T>C), TTCA alleles (g.1029T, 1052C>T, 1142T>C, 1298T>A) in κ-casein gene, promoter in Indian camel in relation to the published sequence (NCBI GenBank ID HE863813).

conditions and have recently been evaluated and/or selected for milk production (Mehta *et al,* 2011; 2014; 2015). Nevertheless, it will be too early to correlate this SNP with above classification and selection of Indian camel breeds because of relatively small sample size (28 animals per breed) to establish this relationship.

Nucleotide-substitution: SNP verification by sequencing

The samples identified as representing TT, CT and CC genotype with respect to the single g.1029T >C nucleotide substitution in CSN3 gene were sequenced. Fig 3 presents the alignment of nucleotide sequences of the promoter region, exon-1 and intron-1 of *C. dromedarius* CSN3 gene variants observed in the present study with the published sequence of the camel (NCBI GenBank ID HE863813). The analysis of sequences confirms the specificity of the sequences for the identified SNP genotypes. The TT homozygous animal was also observed to be heterozygous for g.1052C>T, g.1142 T>C and g.1298 T>A (Fig 3). The phylogenetic analysis reveals that the sequence containing g.1029T allele was placed at a distance of 0.017 from evolutionary point of reference in the node containing CSN3 gene and 5'UTR sequences of *Camelus dromedarius*, *Camelus bactrianus* and *Camelus ferus*. The other node of the phylogenetic tree had CSN3 gene and 5'UTR sequences of *Lama glama* and *Vicugna pacos* (Fig 4). The phylogenetic relationship between sequences containing g.1029T allele; g.1029T allele along with g.1052C>T transition; g.1142T>C transition and g.1298T>A trans-version; and g.1029T>C transition was studied in relation to the published sequence (NCBI GenBank ID HE863813) and presented in the Fig 5. The analysis suggested that the sequences containing C allele and other transition and transversion are of subsequent origin (Fig 4-5), which is also substantiated by the paucity of CC genotype animals and lower frequency of C allele in all the 4 breeds covered in the present investigation. The SNPs g.1052C>T transition; g.1142T>C transition and g.1298T>A trans-version needs to be further investigated by increasing the sample size and working out possible correlation with the traits of economic importance.

The study documents existence of genetic polymorphism in Indian dromedary breeds in the CSN3 gene promoter just upstream of the exon 1 creating extra putative site for the transcription factor HNF-1. The influence of HNF-1 allelic variants on CSN3 needs to be further substantiated. Nevertheless, the presence of C allele at g.1029 locus in the 5'UTR of CSN3 gene with the frequency 0.24 gives the opportunity for the rapid directional selection in favour of such allele. This DNA based PCR-RFLP test can be used for typing camel CSN3 variability independent of age, sex and stage of lactation of animals for selecting them in breeding and production programmes.

Acknowledgement

This work was carried in the Institutional Project of ICAR-NRC on Camel, Bikaner. The authors thank the Indian Council of Agricultural Research, New Delhi for providing the financial support and other facilities for the research.

References

Alexander LJ, Stewart AF, Mackinlay AG, Kapelinskaya TV, Tkach TM and Gorodetsky SI (1988). Isolation and characterisation of bovine kappa-casein gene. European Journal of Biochemistry 178:395-401.

Caroli A, Chiatti F, Chessa S, Rignanese D, Bolla P and Pagnacco G (2006). Focusing on the goat casein complex. Journal of Dairy Science 89:3178–87.

Caroli AM, Chessa S and Erhardt GJ (2009). Milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. Journal of Dairy Science 92: 5335–52.

El Agamy EI (2006). Camel milk. In: Handbook of Non-Bovine Mammals, eds., Park Y W and Haenlein F W, Blackwell Publisher Professional, Iowa, NJ, USA. pp 297-344.

Hall TA (1999). BioEdit : a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-8.

Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hammers C, Bajyana Songa E, Bendahman N and Hammers R (1993). Naturally occurring antibodies devoid of light chains. Nature 363:446-8.

IBM Corp. (2017). IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

Kappeler S, Farah Z and Puhan Z (1998). Sequence analysis of *Camelus dromedarius* milk caseins. Journal of Dairy Research 65:209-22.

Kappeler S, Farah Z and Puhan Z (2003). 5′ Flanking regions of camel milk genes are highly similar to homologue regions of other species and can be divided into two distinct groups. Journal of Dairy Science 86:498-508.

Kappeler SR, van den Brink HJ, Rahbek-Nielsen H, Farah Z, Puhan Z, Hansen EB and Johansen E (2006). Characterisation of recombinant camel chymosin reveals superior properties for the coagulation of bovine and camel milk. Biochemical and Biophysical Research Communications 342(2):647-54.

Konuspayeva G, Faye B and Loiseau G (2009). The composition of camel milk: a meta-analysis of the literature data. Journal of Food Composition and Analysis 22:95-101.

Mehta SC, Bissa UK, Patil NV and Pathak KML (2011). Importance of camel milk and production potential of dromedary breeds. Indian Journal of Animal Science 81(11):1173-7.

Mehta SC, Yadav SBS, Singh S and Bissa UK (2014). Sire evaluation and selection of Indian dromedary for milk production: issues and strategies. Journal of Camel Practice and Research 21(1):93-8.

Mehta SC, Sharma AK, Bissa UK and Singh S (2015). Lactation persistency, yield and prediction models in Indian dromedary. Indian Journal of Animal Science 85(8): 875-82.

Møller KK, Rattray FP, Sørensen JC and Ardö Y (2012). Comparison of the hydrolysis of bovine κ-casein by camel and bovine chymosin: a kinetic and specificity study. Journal of Agricultural and Food Chemistry 60(21):5454-60.

Nikkah A (2011a). Equidae, camel, and yak milks as functional foods: a review. Journal of Nutrition and Food Sciences 1(5):100-116.

Nikkah, A (2011b). Science of camel and yak milks: human nutrition and health perspectives. Food and Nutrition Sciences 2:667-73.

Othman EO, Nowier Am and El-Denary ME (2016). Genetic variations in two casein genes among Maghrabi camels reared in Egypt. Biosciences Biotechnology Research Asia 13(1):473-80.

Pauciullo A, Shuiep ES, Cosenza G, Ramunno L and Erhardt G (2013). Molecular characterisation and genetic variability at k-casein gene (CSN3) in camels. Gene 513:22-30.

Sambrook J, Fritsh E F and Manities T (1989). Molecular cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbour Laboratory Press, New York.

Singh R, Mal G, Kumar D, Patil NV and Pathak KML (2017). Camel Milk: An Important Natural Adjuvant. Agricultural Research 6(4):327-40.

Yamini, Gahlot GC, Pannu U, Ashraf M and Choudhary S (2019). Assessment of genetic variability in kappa Casein gene in Indian dromedary. Journal of Camel Practice and Research 26(3):255-8.

DOI : 10.5958/2277-8934.2020.00030.2 Vol 27 No 2, p 207-208

MIDDLE EAST RESPIRATORY SYNDROME (MERS) IN AN ADULT DROMEDARY CAMEL: SHORT COMMUNICATION

Wernery U[1], Joseph S[1] and Woo PCY[2]
[1]Central Veterinary Research Laboratory, Dubai, United Arab Emirates
[2]Department of Microbiology, University of Hong Kong, Hong Kong, China

ABSTRACT

MERS-CoV was isolated from nasal swabs for 10 days from an adult female camel which displayed clear nasal discharge from both nostrils. When MERS-CoV ELISA antibodies appeared in the camel's blood, the virus was no longer present in its nasal cavities.

Key words: Camel, MERS

Since the emergence of Middle East Respiratory Syndrome (MERS) in 2012 in Saudi Arabia, more than 2000 human cases have been reported worldwide with a case fatality of 30% (WHO, 2018). The causative agent of MERS has been confirmed to be a novel coronavirus (CoV) named MERS-CoV belonging to the lineage C of Betacoronavirus (Van Boheemen *et al,* 2012). Investigations have shown that the one-humped or dromedary camel (*Camelus dromedarius*) is so far the only reservoir of MERS-CoV (Alagaili *et al,* 2014; Wernery *et al,* 2015a, 2015b; Sabir *et al,* 2016), although we recently showed that the two-humped or Bactrian camel (*Camelus bactrianus*) in Dubai also possessed MERS-CoV antibodies (Lau *et al,* 2020). Adult dromedaries have almost 100% seropositivity against MERS-CoV while the virus is found mainly in dromedary calves (Wernery, 2014; Wernery *et al,* 2015a).

In this article a rare case of MERS-CoV infection in an adult female dromedary camel is reported.

Materials and Methods

One adult, more than 10-year-old breeding dromedary was presented with clear nasal discharge from both nostrils, but otherwise healthy, eating and drinking well. It was in a group of 2 other dromedaries which showed no clinical signs. Nasal swabs were taken from both nostrils of all 3 dromedaries as well as blood from the jugular veins. The nasal swabs were collected in viral transport medium containing minimal essential medium (MEM) with antibiotics. The blood was centrifuged and sera were stored at -20°C until tested. Virus isolation was attempted on vero cells with bacterial filtered samples. The sera of the 3 dromedaries were tested with the Euroimmun® MERS-CoV antibody ELISA. All test procedures are laid down in the upcoming MERS-CoV chapter of the OIE manual (2022).

Results

Results of this investigation is summarised in Table 1.

MERS-CoV was isolated from camel 341 which showed nasal discharge, but not from camels 342 and 513 which displayed no nasal discharge. Ten days later MERS-CoV was not any longer present in the nose of camel 341.

Table 1. MERS-CoV investigations of 3 adult dromedary camels.

Camel ID	Day 0		Day 10		Day 20	
	Virus isolation	Antibody ELISA	Virus isolation	Antibody ELISA	Virus isolation	Antibody ELISA
341	Positive	negative	negative	negative	negative	positive
342	Negative	positive	negative	positive	negative	positive
513	Negative	positive	negative	positive	negative	positive

SEND REPRINT REQUEST TO WERNERY U email: cvrl@cvrl.ae

ELISA antibodies were found in camels 342 and 513 from which no MERS-CoV was isolated. No antibodies against MERS-CoV were detected for 20 days in camel 341 from the day virus was isolated.

Discussion

It is rare to isolate MERS-CoV from adult dromedaries as most of them have neutralising antibodies (Wernery *et al*, 2017). In our case, dromedary 341 did not possess any ELISA antibodies and therefore shed MERS-CoV through its nose for 10 days. A similar result was found in camel calves. The virus was shed through the nose only for 8 days (Wernery, 2014). After MERS-CoV antibodies appeared, the virus disappeared as proven with camel 341 which displayed MERS-CoV ELISA antibodies 20 days after the virus was isolated for the first time.

In conclusion, dromedary adult camels which do not possess MERS-CoV antibody can get infected by the virus and may display nasal discharge. However, when antibodies appear, the virus is no longer isolated from the camel's nose.

References

Alagaili AN, Briese T, Mishra N, Kapoor V, Sameroff SC, Burbelo PD, de Wit E, Munster VJ, Hensley LE, Zalmout IS, Kapoor A, Epstein JH, Karesh WB, Daszak P, Mohammed OB and Lipkin WI (2014). Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. mBio 5(2):e00884-14.

Lau SKP, Li KSM, Luk HKH, He Z, Teng JLL, Yuen KY, Wernery U and Woo PCY (2020). Middle East Respiratory Syndrome coronavirus antibodies in Bactrian and hybrid camels from Dubai. mSphere 5:e000898-19.

OIE (2022 in press). MERS-CoV chapter in: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

Sabir JS, Lam TT, Ahmed MM, Li L, Shen Y, Abo-Aba SE, Qureshi MI, Abu-Zeid M, Zhang Y, Khiyami MA, Alharbi NS, Hajrah NH, Sabir MJ, Mutwakil MH, Kabli SA, Alsulaimany FA, Obaid AY, Zhou B, Smith DK, Holmes EC, Zhu H and Guan Y (2016). Co circulation of three camel coronavirus species and recombination of MERS-CoVs in Saudi Arabia. Science 351(6268):81-84.

Van Boheemen S, de Graaf M, Lauber C, Bestebroer TM, Raj VS, Zaki AM, Osterhaus AD, Haagmans BL, Gorbalenya AE, Snijder EJ and Fouchier RA (2012). Genomic characterisation of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. MBio 3(6):e00473-12.

Wernery U (2014). Some epidemiological studies on MERS coronavirus in dromedaries in the United Arab Emirates – a short communication. Journal of Camel Practice and Research 21(1)1-4.

Wernery U, Corman VM, Wong EY, Tsang AK, Muth D, Lau SK, Khazanehdari K, Zirkel F, Ali M, Nagy P, Juhasz J, Wernery R, Joseph S, Syriac G, Elizabeth SK, Patteril NA, Woo PC and Drosten C (2015b). Acute Middle East respiratory syndrome coronavirus infection in livestock dromedaries, Dubai, 2014. Emerging Infectious Disease 21(6):1019-1022. doi: 10.3201/eid2106.150038.

Wernery U, El Rasoul IH, Wong EY, Joseph M, Chen Y, Jose S, Tsang AK, Patteril NA, Chen H, Elizabeth SK, Yuen KY, Joseph S, Xia N, Wernery R, Lau SK and Woo PC3 (2015a). A phylogenetically distinc Middle East respiratory syndrome coronavirus detected in a dromedary calf from a closed dairy herd in Dubai with rising seroprevalence with age. Emerging Microbes and Infections 4(12):e74.

Wernery U, Lau SK and Woo PC (2017). Middle East respiratory syndrome (MERS) coronavirus and dromedaries. Veterinary Journal 220:75-79.

World Health Organisation (WHO) (2018). MERS situation update, July 2018. HWO, Geneva, Switzerland. pp 1.

DOI : 10.5958/2277-8934.2020.00031.4 Vol 27 No 2, p 209-211

A CASE OF ENDOCARDIAL FIBROELASTOSIS IN A LLAMA (*Llama cria*)

V. Ipek[1] and O. Kaplan[2]
Department of Pathology, [1]Institute of Health Sciences, [2]Faculty of Veterinary Medicine,
Mehmet Akif Ersoy University, Burdur, 15100, Turkey

ABSTRACT

In this communication, we aimed to describe a case of endocardial fibroelastosis in a *Llama cria*. Macroscopic examination revealed that the endocardial tissue of the heart had a gray-white appearance. However, cardiovascular malformations and myocardial necrosis/injury were not seen. Microscopic examination of the heart showed severe endocardial thickening due to the proliferation of elastic and collagen fibres.

Key words: Endocardium, fibroelastosis, heart, histopathology, llama

Endocardial fibroelastosis (EFE) is a rare heart disease characterised by thickening of the endocardium due to collagenous and elastic tissue (Rodriguez *et al,* 2018). Primary EFE is a congenital heart disease with an unknown cause that affects humans and animals. No other anatomical cardiac or vascular anomalies are encountered in this condition (Paasch and Zook, 1980; Robinson and Robinson, 2016; Zook *et al,* 1981). In the secondary form, there may also congenital cardiovascular malformations, viral myocarditis (Noren *et al,* 1974), cardiomyopathy (Liu, 1970), myocardiosis, localised endocardial thickening which in secondary to myocardial necrosis and endomyocardial injuries (Zook and Paasch, 1982), myocarditis observed with carnitine deficiency (Wolfson *et al,* 1990) and glycogen storage diseases (Zook *et al,* 1981), left hypoplastic heart syndrome (Lurie, 2010; McElhinney *et al,* 2010) and autoimmune reactions (Aoki *et al,* 2011) but distinction between primary and secondary forms may be difficult (Krahwinkel and Coogan, 1971). First EFE was reported in cats and dogs (Eliot *et al,* 1958). This condition was also reported in people, horse, cattle, chicken, tiger and pallas cat (Bentley, 1999; Carvalho *et al,* 2019; Cushing, 2013; Gudenschwager *et al,* 2019; Hananeh and Ismail, 2018; Lurie, 2010; Pass, 1983; Rodriguez *et al,* 2018). In this case report, we aim to present an EFE case in *Llama cria* with histopathological findings.

Case Report

A 7 months old *Llama cria* of Antalya Zoo, was necropsied after death, and internal organs were sent to the department. Tissue samples was fixed in 10% formalin and then following routine follow-up procedure, tissues embedded in paraffin, 5 µm cut sections were made and stained with Haematoxylin-Eosin and Verhoeff-van-Gieson. Stained sections were examined under light microscope. Macroscopically, there was no myocardial necrosis, endomyocardial injuries or cardiovascular malformations, but endocardium was grey-white colour. Liver was diffusely pale. Microscopically, there was diffuse mild degeneration in liver and necrosis in some hepatocytes. In the heart, left endocardium was markedly thick due to dense connective tissue proliferation (Fig 1). With Verhoeff-van-Gieson staining, dark stained, dense elastic fibrils within the area of endocardial thickening was observed (Fig 2).

The microbiological analysis of fresh tissue samples did not reveal any bacterial or viral agent.

Discussion

Macroscopically, fibroelastosis was mostly characterised by diffuse opaque and whitish endocardial thickening in the left ventricle (Lurie, 2010). Similarly, we observed diffuse grey to white thickening of the left ventricle. Microscopically, ventricular endocardial thickening due to accumulation of elastic and collagen fibres was demonstrated by Verhoeff-van-Gieson staining which has been reported previously (Eliot *et al,* 1958). EFE should be distinguished from a heart tumour fibroelastoma which affects heart valves and adjacent

SEND REPRINT REQUEST TO V. IPEK email: volkanipek@mehmetakif.edu.tr

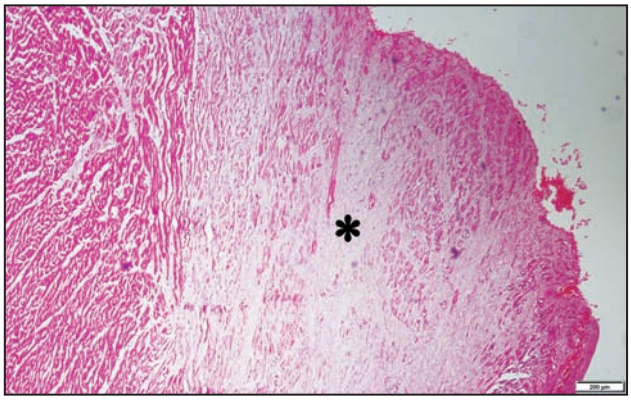


Fig 1. Wide fibroelastosis area in the endoardium extending into the myocardium. (asterisk). Haematoxylin-Eosin, Bar: 200 µm.

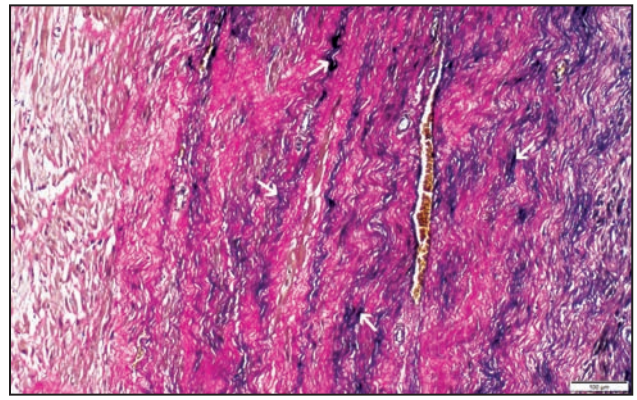


Fig 2. Elastic fibrils in the EFE area (arrows), Verhoeff-van-Gieson, Bar: 100 µm.

endocardium reported in humans (Lurie, 2010). We distinguished the case of fibroelastoma due to the diffuse appearance.

Primary EFE is a familial disease in humans (Paasch and Zook, 1980), feline species (Paasch and Zook, 1980; Rozengurt, 1994; Zook and Paasch, 1982). There have been suggested mechanisms of genetic transmission in some reports (Hanukoglu *et al,* 1986; Westwood *et al,* 1975). Most patients with primary EFE die due to congestive heart failure within 1 year after birth (Rozengurt, 1994). We believed that our case is primarily congenital EFE because of absence any other cardiac and vascular anomaly. However, genetic analysis could not be performed. There is a report of atrioventricular septal defect in llama (Cebra *et al,* 2015) but according to the authors' knowledge, there is no any report of EFE in llamas.

References

Aoki H, Inamura N, Kawazu Y, Nakayama M and Kayatani F (2011). Foetal echocardiographic assessment of endocardial fibroelastosis in maternal anti-SSA antibody-associated complete heart block. Circulation Journal 75:1215-1221.

Bentley DM (1999). Congenital endocardial fibroelastosis in a dog. Canadian Veterinary Journal 40:805-807.

Carvalho TP, Oliveira AR, Duarte MS, Rezende LA, Oliveira MS, Ribeiro BNT, Reis AMS, Serakides R and Ocarino NM (2019). Cardiac fibroelastosis associated with thromboembolism and paresis in a cat - Case report. Brazilian Journal of Veterinary Pathology 12:63-68.

Cebra ML, Cebra CK, Garry FB, Boon JA and Orton EC (2015). Atrioventricular septal defects in three Llamas (*Lama glama*). Journal of Zoo and Wildlife Medicine 29:225–227.

Cushing TL (2013). Endocardial fibroelastosis in a quarterhorse mare. Journal of Comparative Pathology 149:318-321.

Eliot TS, Eliot FP, Lushbaugh CC and Slager UT (1958). First report of the occurrence of neonatal endocardial fibroelastosis in cats and dogs. Journal of the American Veterinary Medical Association 133:271-274.

Gudenschwager EK, Abbott JA and LeRoith T (2019). Dilated cardiomyopathy with endocardial fibroelastosis in a juvenile Pallas cat. Journal of Veterinary Diagnostic Investigation 31:289-293.

Hananeh WM and Ismail ZB (2018). Concurrent occurrence of acute bovine pulmonary oedema and emphysema and endocardial fibroelastosis in cattle: A case history and literature review. Veterinary World 11:971-976.

Hanukoglu A, Fried D and Somekh E (1986). Inheritance of familial primary endocardial fibroelastosis. Clinical Pediatrics 25:272-275.

Krahwinkel DJ and Coogan PS (1971). Endocardial fibroelastosis in a Great Dane pup. Journal of the American Veterinary Medical Association 159:327-31.

Liu SK (1970). Acquired cardiac lesions leading to congestive heart failure in the cat. American Journal of Veterinary Research 31:2071-88.

Lurie PR (2010). Changing concepts of endocardial fibroelastosis. Cardiology in the Young 20:115-23.

McElhinney DB, Vogel M, Benson CB, Marshall AC, Wilkins-Haug LE, Silva V and Tworetzky W (2010). Assessment of left ventricular endocardial fibroelastosis in foetuses with aortic stenosis and evolving hypoplastic left heart syndrome. American Journal of Cardiology 106:1792-1797.

Noren CR, Staley NA, Kaplan EL and Jankus EF (1974). Cardiomyopathy. Comp. Pathol. Bull. 6:2-4.

Paasch LH and Zook BC (1980). The athogenesis of endocardial fibroelastosis in Burmese cats. Laboratory investigation; A Journal of Technical Methods and Pathology 42:197-204.

Pass DA (1983). A cardiomyopathy (Sudden death syndrome) of adult hens. Avian Pathology 12:363-369.

Robinson W and Robinson N (2016). Cardiovascular System. In: Pathology of Domestic Animals Vol. 3. Eds K. Jubb, P. Kennedy, *et al,* Elsevier. pp 572

Rodriguez KT, Cushing AC, Bernal C, Ramsay EC, Craig LE and Gompf RE (2018). Endocardial fibroelastosis in two related tiger cubs (*Panthera tigris*). Journal of Veterinary Cardiology 20:73-77.

Rozengurt N (1994). Endocardial fibroelastosis in common domestic cats in the UK. Journal of Comparative Pathology 110:295-301.

Westwood M, Harris R, Burn JL and Barson AJ (1975). Heredity in primary endocardial fibroelastosis. Heart 37:1077-1084.

Wolfson DJ, Pepkowitz SH, Velde R van de and Fishbein MC (1990). Primary endocardial fibroelastosis associated with hydrops foetalis in a premature infant. American Heart Journal 120:708-711.

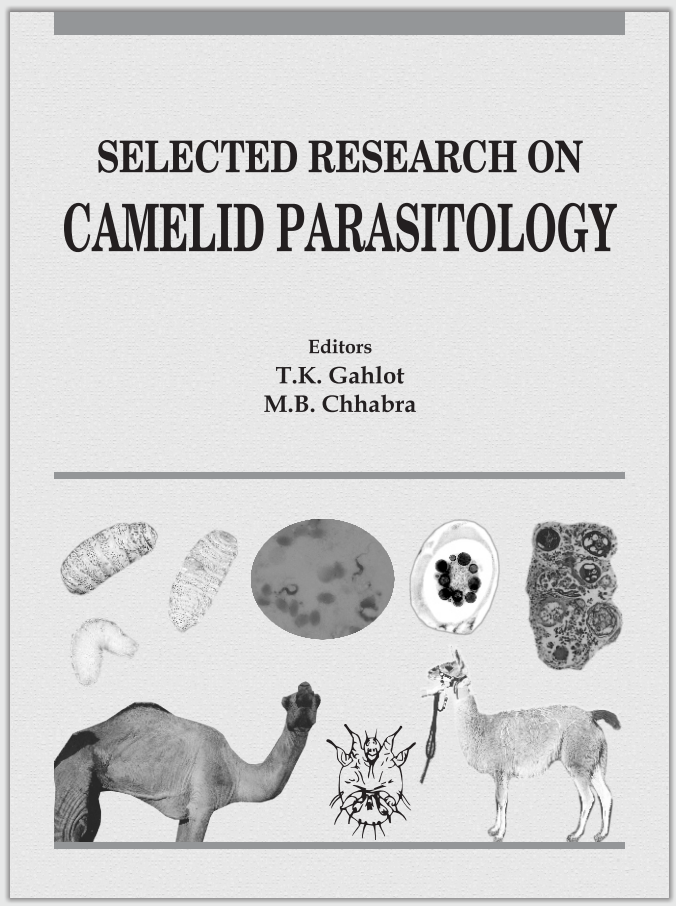
Zook BC and Paasch LH (1982). Endocardial fibroelastosis. Endocardial fibroelastosis in Burmese cats. American Journal of Pathology 106:435-438.

Zook BC, Paasch LH, Chandra RS and Casey HW (1981). The comparative pathology of primary endocardial fibroelastosis in burmese cats. Virchows Archiv A Pathological Anatomy and Histology 390:211-227.

SELECTED RESEARCH ON
CAMELID PARASITOLOGY

Hard bound, 291 pages, few figures coloured

New research and experience always broaden our knowledge, and help us adopting new diagnostic methods and treatments. Camel Publishing House has taken a step forward to compile this knowledge in form of a book and this Herculian task was accomplished with the help of dedicated editors. viz. Drs. T.K. Gahlot and M.B. Chhabra. ***Selected Research on Camelid Parasitology*** is most comprehensive guide to Camelid Parasitology. The classic reference book serves as a one stop resource for scientific information on major aspects of Camelid Parasitology. Featuring abundant photographs, illustrations, and data, the text covers camelid protozoa, helminths, and arthropods of dromedary and New World camelids. This hard bound book of 304 pages contains seroepidemiological studies, immunological and other diagnostic procedures, and new treatments of parasitic diseases. There are at least 17 countries involved in camelid parasitology research, viz. Ethiopia, France, India, Iran, Jordan, Kenya, Libya, Mauritania, Nigeria, Sultanate of Oman, Pakistan, Saudi Arabia, Sudan, Sweden, United Arab Emirates, Uganda and U.S.A. As per published papers in Journal of Camel Practice and Research (JCPR), 173 authors have contributed 72 manuscripts which are appropriately placed in 5 sections. The text of each manuscript published previously in JCPR remains the same except the pattern of numbering the references in the body of text. This book indicates a swing of camelid research during period 1994-2008 and will help identifying the missing links of research in this subject.



Editors:
T.K. Gahlot and M.B. Chhabra

Edition: 2009

Publisher: **Camel Publishing House**
67, Gandhi Nagar West,
Near Lalgarh Palace
Bikaner 334001 Rajasthan,
India
email: tkcamelvet@yahoo.com
website: www.camelsandcamelids.com

Price: US$ 200 (Abroad)
INR 3000 (India)

ISBN: 81-903140-0-9

DOI : 10.5958/2277-8934.2020.00032.6 *Vol 27 No 2, p 213-215*

HAEMATOLOGICAL STUDIES DURING LATE PREGNANT AND EARLY LACTATION STAGE IN JAISALMERI CAMELS (*Camelus dromedarius*)

Barkha Gupta, G.S. Gottam, P.K. Mittal, S.K. Jindal, D.R. Bilochi and Dinesh Maruti

Department of Veterinary Physiology and Biochemistry, Post Graduate Institute of Veterinary Education and Research (PGIVER), Jamdoli, NH-11, Agra Road, Jaipur, Rajasthan, 302031, India

ABSTRACT

In present study, haematological parameters were recorded during late pregnancy and early lactation in the Jaisalmeri breed of camel. Blood samples from 10 adult Jaisalmeri late pregnant and early lactating females were collected. The Complete Blood Count (CBC) was performed using automatic blood analyser. The Mean ± SE of Erythrocyte count (RBC), Total Leukocyte count (TLC), Haemoglobin (Hb) and Haematocrit value (PCV) were 6.18 ± 0.35 x 10^6/µL, 4.95 ± 0.91 x 10^3/µL, 10.20 ± 0.41 g/dl and 25.69 ± 1.16%, respectively in late pregnant females while 7.15 ± 0.41 x 10^6/µL, 7.69 ± 0.38 x 10^3/µL, 10.30± 0.50 g/dl and 23.38 ± 1.24%, respectively in early lactating females of camel. The Differential Leukocyte Count i.e. Mean ± SE values of Lymphocyte, Monocyte, Neutrophil, Eosinophil and Basophil percentage were 29.62 ± 5.82%, 10.36 ± 2.20%, 49.13 ± 5.85%, 10.59 ± 1.72% and 0.30 ± 0.04%, respectively in late pregnant females while 17.63 ± 1.79%, 5.58 ± 0.48%, 74.55 ± 1.96%, 1.91± 0.41% and 0.33 ± 0.03%, respectively in early lactating females of camel. Most of the findings of blood analysis were within the normal range except TLC and DLC. This may be due to the effect of physiological status of animal i.e. late pregnancy, early lactation and some diseased conditions. Total Leukocyte Count (WBC) was found to increase significantly (P ≤ 0.05) during early lactation as compared to late pregnancy.

Key words: Camel, haematology, Jaisalmeri breed, lactation, pregnancy

Pregnancy and lactation are physiological periods that result in increased metabolic demands. Although, homeostatic mechanisms keep substances in the blood at relatively constant levels, some changes in the concentrations of haematological parameters occur indicative of the metabolic stress during pregnancy and early lactation. Haematological values of camels have been reported (Tornquist *et al,* 2010; Faye and Bengoumi, 2018). The haematological variations in response to stress during pregnancy and early lactation has not been studied in Jaisalmeri camels of Rajasthan. Present study was planned to assess the effect of late pregnancy and early lactation on the haematological parameters in the Jaisalmeri breed of camels.

Materials and Methods

Ten pregnant and early lactating females of Jaisalmeri breed of dromedary camel from Phalodi Tehsil of Jodhpur District, Rajasthan were selected for study. Blood samples were obtained from the Jugular vein. Blood was collected in test tubes containing K_2-EDTA and gently mixed. To avoid alterations related to diurnal variations, blood samples were collected at the same time each day. The samples were stored in refrigerator till processing. The complete blood count (CBC) was performed using Automated Haematology Analyser i.e. (Spincell 5 compact Vet Mode. Spinreact, Ctra. Sta. Coloma, 717176 St. Esteve de Bas GIRONA – Spain). Data was analysed statistically using t-Test.

Results and Discussion

The effects of late pregnancy and early lactation on the haematological parameters in Jaisalmeri camel were expressed as mean ± standard error values (Table 1, Fig 1, 2 and 3). Total Leukocyte Count (WBC) was significantly increased (P ≤ 0.05) during early lactation compared to late pregnancy.

The results showed that the neutrophils increase was highly significant (P ≤ 0.001) during the early lactation compared to the late pregnancy. Postpartum neutrophilia observed in this study has also been reported previously (Ebissy *et al,* 2019), who attributed such finding to stress being associated with parturition and the beginning of lactation stage. Significant (P ≤ 0.05) decrease in lymphocytes percentage was observed during early lactation

SEND REPRINT REQUEST TO BARKHA GUPTA email: drbgvet@gmail.com

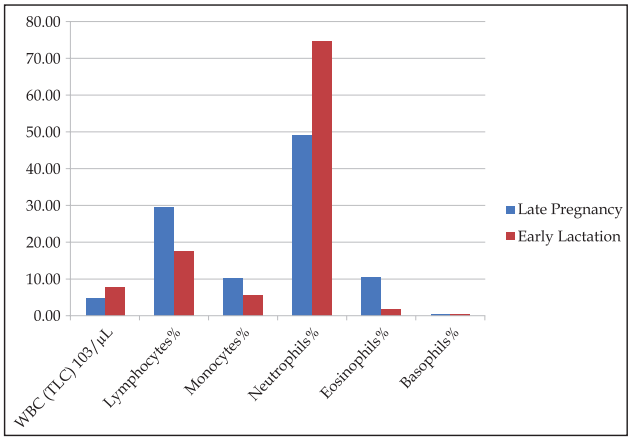


Fig 1. Leukocytes parameters of female camels during late pregnancy and early lactation (n = 10).

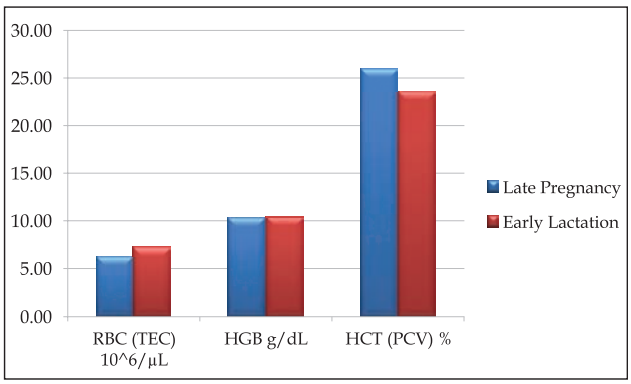


Fig 2. Erythrocytes parameters of female camels during late pregnancy and early lactation (n = 10).

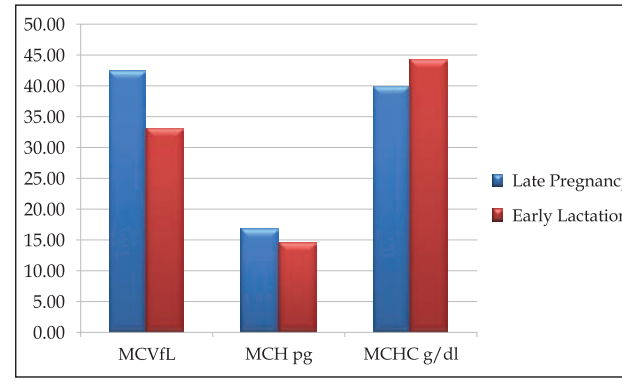


Fig 3. Erythrocytes parameters of female camels during late pregnancy and early lactation (n = 10).

compared to late pregnancy, whereas monocytes percentage increased during the late pregnancy and early lactation compared to the normal non-pregnant adult but monocytes percentage decreased significantly ($P \leq 0.05$) during early lactation compared to the late pregnancy.

Eosinophil percentage decreased significantly ($P \leq 0.001$) during the early lactation compared to the late pregnancy and increased in late pregnancy. This may be due to the effect of physiological status of animal i.e., late pregnancy.

The predominant white cells observed in the present study were neutrophils which in agreement with earlier results (Ayoub *et al,* 2003). The significant

Table 1. The effects of late pregnancy and early lactation on the haematological parameters in Jaisalmeri camel (n = 10).

S. N.	Parameter	Unit	Late Pregnancy Mean±SE	Early Lactation Mean±SE	Per cent Increase/ Decrease
1.	WBC (TLC)	$10^3/\mu L$	4.95 ± 0.91	7.69 ± 0.38*	+55.35
2.	LYM%	%	29.62 ± 5.82	17.63 ± 1.79*	-40.47
3.	MON%	%	10.36 ± 2.20	5.58 ± 0.48*	-46.14
4.	NEU%	%	49.13 ± 5.85	74.55 ± 1.96***	+51.74
5.	EOS%	%	10.59± 1.72	1.91± 0.41***	-81.96
6.	BASO%	%	0.30 ± 0.04	0.33 ± 0.03	-
7.	RBC (TEC)	$10^6/\mu L$	6.18 ± 0.35	7.15 ± 0.41	-
8.	HGB	g/dL	10.20 ± 0.41	10.30± 0.50	-
9.	HCT (PCV)	%	25.69 ± 1.16	23.38 ± 1.24	-
10.	MCV	fL	42.24 ± 1.74	32.87 ± 0.57***	-22.18
11.	MCH	pg	16.64± 0.60	14.39± 0.22**	-13.52
12.	MCHC	g/dL	39.70 ± 0.54	44.04± 0.27	-

NS = Non significant ($P > 0.05$) * = Significant ($P \leq 0.05$) ** = Significant ($P \leq 0.01$)
*** = Significant ($P \leq 0.001$)

changes in neutrophils and lymphocytes and monocytes percentage could be due to cortisol and ACTH release in response to pregnancy and lactation stress.

The values changes of TEC (RBC), haemoglobin (HGB) and haematocrit (PCV) did not attain statistical significance during late pregnancy and early lactation,. Such unchanged values of erythrocytes parameters during the transition period in camels can be taken as an indicator of the proper feeding and management regime. Although TEC, haemoglobin (HGB) and haematocrit (PCV) slightly increased during the late pregnancy and early lactation as compared to adult non-pregnant camels (Tornquist *et al,* 2010; Faye and Bengoumi, 2018) the MCV (($P \leq 0.001$) and MCH ($P \leq 0.05$) values decreased significantly, during the early lactation compared to the late pregnancy which may be attributed to the increased metabolic demand for oxygen consumption during late pregnancy as the animal has to fulfil the additional metabolic demands of the foetus.The effects of late pregnancy and early lactation on haematological parameters have not been studied in Jaisalmeri or any other breed of Rajasthani camels. However, a similar study in Jennies (Mariella *et al,* 2014) did not find significant differences in MCV, MCH and MCHC between late pregnancy and early lactation. It may be opined that camel females are under greater stress during late pregnancy in arid climates and hence the difference in response occurred. Most of the haematological values for Jaisalmeri camel (Mean ± SE) used in this study were within the range as reported earlier by Tornquist *et al* (2010) except for TLC and DLC which may be due to the difference in breeds and physiological stage of camels in this experiment.

Late pregnancy and early lactation have a negative influence on the haematological parameters in camels. The observed neutrophilia could be attributed to a stress factor that being associated with the initiation of the lactation period.

References

Ahmed MH (2017). Effects of Selenium and Vitamin E Injection during Transition period on Physiological Performance of Camels (*Camelus dromedarius*) and their Neonates Reared Under Semi-intensive System. MVSc Thesis, University of Khartoum, Sudan.

Ayoub MA, EL-Khouly AA and Mohamed TM (2003). Some haematological and biochemical parameters and steroid hormones levels in one- humped camels during different physiological condition. Emirates Journal of Agriculture Science 15(1):44-55.

Ebissy E, El-Sayed A and Mohamed R (2019). Haematological and biochemical profile in female camels (*Camelus dromedarius*) during the transition period. Slovenian Veterinary Research 56(22):571-577.

El-Zahar H, Zaher H, Alkablawy A, Al Sharifi S and Swelum A (2017). Monitoring the changes in certain haematological and biochemical parameters in camels (*Camelus dromedarius*) during postpartum period. Journal of Fertility Biomarkers 1(1):47-54.

Faye B and Bengoumi M (2018). Clinical Enzymology. Camel Clinical Biochemistry and Haematology. Springer, Cham. pp 123-172.

Jainudeen MR and Hafez ESE (1994). Gestation, prenatal physiology and parturition. In: Hafez ESE, eds. Reproduction in Farm Animals, Lippincott, Williams and Wilkins. pp 247-283.

Kelanemer R, Antoine-Moussiaux N, Moula N, Abu-Median AAK, Hanzen Ch and Kaidi R (2015). Effect of nutrition on reproductive performance during the peri-partum period of female camel (*Camelus dromedarius*) in Algeria. Journal of Animal Veterinary Advances 14(7):192-196.

Mariella J, Pirrone A, Gentilini F and Castagnetti C (2014). Haematological and biochemical profiles in Standardbred mares during peripartum. Theriogenology 81(4):526-34.

Tharwat M, Ali A, Al-Sobayil F, Selim L and Abbas H (2015). Haemato-biochemical profile in female camels (*Camelus dromedarius*) during the periparturient period. Journal of Camel Practice Research 22(1):101-106.

Tornquist SJ, Weiss DJ and Wardrop KJ (2010). Haematology of Camelids. Schalm's Veterinary Haematology. 6th edition. Ames (IA): Blackwell Publishing Ltd. pp 910-917.

3,000 CAMELS WERE EVACUATED FROM LIBYA'S CAPITAL

In an overnight evacuation 3000 camels have been walked out of Libya's capital Tripoli after the port where they arrived came under artillery fire in third week of February this year. The camels were herded along a highway some 45 km (30 miles) away leading west to the city of Zawiya, where they arrived next day morning. Camels looked for the food while passing through the roads. According to one report a local armed group had stolen 125 of the camels as they passed through the Tripoli suburb of Janzour. Security forces temporarily closed the road to let them pass.

(Source: The Indian Express, 24 August 2020)

ONLINE SOUTHWEST CAMEL CONFERENCE 2020

As a part of education programme, Doug Baum is launching Covid 19 affected year's only online Southwest Camel Conference on 28 October 2020. There will be many speakers in the conference. Few noted speakers are Dr T.K. Gahlot, India, Abdul Raziq Kakar, Al Ain, UAE, Ahmed Eisa, El Hag, Sudan, Gil Riegler, Oasis Camel Dairy, USA, Coralie Le Meur, Droma, Sud, France, Ivan French, Oklahoma Mini Mill and others. Second announcement will follow soon.

DOI : 10.5958/2277-8934.2020.00033.8 *Vol 27 No 2, p 217-224*

In-vitro CAPACITATION OF SPERMATOZOA AS ASSESSED BY CHLORTETRACYCLINE STAINING IN CAMELS (*Camelus dromedarius*)

M.M. Waheed[1,2], S.M. El-Bahr[3,4], A.K. Alhaider[1], I.M. Ghoneim[1,2] and A.M.A. Meligy[5,6]

[1]Department of Clinical Sciences, [3]Department of Physiology, Biochemistry and Pharmacology (Biochemistry), [5]Central Diagnostic Lab., College of Veterinary Medicine, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia

[2]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12515, Egypt

[4]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt

[6]Physiology Department, Plant Protection Research Institute (PPRI), Agricultural Research Centre (ARC), Giza, Egypt

ABSTRACT

Twelve ejaculates were collected from 6 adult healthy dromedary camels during the rutting season to study the effect of heparin, caffeine and calcium-ionophore on the induction of capacitation in dromedary spermatozoa. Each semen sample was evaluated (sperm progressive motility % and sperm concentration x 10^6/mL). Nine ejaculates out of twelve were diluted with Shotor buffer to obtain 15 aliquots of 5-10 x 10^6 motile spermatozoa/ 990µl. Five aliquots were mixed with 10 µl of heparin in concentrations of 0 control, 10 IU (2.5 µl/mL), 25 IU (5 µl/ mL), 50 IU (10 µl/mL) and 100 IU (20 µl/mL). Caffeine (10 µl) was added to another 5 aliquots in concentrations of 0 control, 2.5 mM (0.00485g/mL), 5 mM (0.0097g/mL), 10 mM (0.0194g/mL) and 20 mM (0.0388g/mL). The last 5 aliquots were mixed with 10 µl of calcium-ionophore A23187 in concentrations of 0 control, 0.05 mM (3.75 µl/mL), 0.1 mM (7.53 µl/mL), 0.2 mM (14.95 µl/mL) and 0.3 mM (20 µl/mL). All aliquots were incubated at 38°C in a 5% CO_2 atmosphere and 90% relative humidity for 60 min. Aliquots from replications were taken at 0, 5, 15, 30 and 60 min and evaluated for percentages of sperm motility, live sperm and spermatozoa with reacted acrosomes using eosin nigrosin and Chlortetracycline staining. Results revealed differences in viability indices (VI) between camel semen incubated with calcium-ionophore and both semen incubated with heparin and caffeine. Heparin 100 IU, caffeine 5 mM and calcium-ionophore 0.05 mM were the best capacitating factors. A marked increase existed in B (capacitated and acrosome intact) and AR (capacitated and acrosome reacted) patterns cells accompanied with a large decrease in F pattern (uncapacitated and acrosome intact) cells in aliquots with the capacitating factors than in control. In conclusion, heparin (100 IU), caffeine (5 mM) and calcium-ionophore A23187 (0.05 mM) are convenient capacitating factors for dromedary camels' semen. CTC fluorescent staining technique can be used for assessing capacitation status and acrosome reaction in dromedary camels.

Key words: Caffeine, camel, capacitation, chlortetracycline, heparin

Assisted reproductive technologies such as artificial insemination (AI), embryos transfer (ET) and *in-vitro* embryos production (Torner *et al,* 2003; Skidmore and Billah, 2006; Tibary *et al,* 2007; Wani, 2009) could improve the well-known poor reproductive efficiency of the camel. Several areas of *in-vitro* embryo production in dromedary, including sperm capacitation, need to be resolved before this technology could be used regularly. Capacitation is defined as a series of biochemical and biophysical changes prior to fertilisation (Wani, 2002). There is limited information on *in vitro* induction of capacitation in camels. When mammalian spermatozoa are first released from the male reproductive tract, they are unable to fertilise oocytes immediately, despite being morphologically mature and independently motile (Das Gupta *et al,* 1993). The spermatozoa must undergo a post-release maturation phase termed capacitation which is obligatory for mammalian spermatozoa to attain full fertilising potential (Sebkova *et al,* 2012; Tulsiani and Abou-Haila, 2012; Kwon *et al,* 2014). Chlortetracycline (CTC) fluorescence technique was first used to assess the functional status of mouse spermatozoa (Ward and Storey, 1984). CTC is a fluorescent antibiotic that binds to the surface of sperm cells in a Ca^{2+} dependent manner (Tsien, 1989). CTC staining is currently the assay of choice because it distinguishes 3 different stages of sperm activation; non-capacitated, capacitated acrosome-

SEND REPRINT REQUEST TO M.M. WAHEED email: mmwaheed@kfu.edu.sa

intact and capacitated acrosome-reacted spermatozoa (Rathi *et al,* 2001; Nakai *et al,* 2012). CTC staining has been used previously to assess the capacitation state of spermatozoa in mouse (Saling and Storey, 1979; Ward and Storey, 1984), stallion (Varner *et al,* 1987), bull (Fraser *et al,* 1995), dog (Guerin *et al,* 1999), ram (Paulenz *et al*, 2002) and camels (Crichton *et al*, 2015). However, unfortunately, a clear understanding of how CTC interacts with the sperm surface at the molecular level is lacking and the evaluation of CTC staining has been performed on fixed sperm cells (Rathi *et al,* 2001). Present investigation was done to study the effect of different concentrations and time of incubation of heparin, caffeine and calcium-ionophore A23187 on the *in vitro* capacitation of ejaculated dromedary spermatozoa and to assess this effect by aid of CTC stain.

Materials and Methods

Chemical reagents preparation

A Shotor buffer (Niasari-Naslaji *et al,* 2007) was made by dissolving 2.60 g Tris (Sigma, T8793), 1.35 g Citric acid (Sigma, C1857), 1.20 g Glucose (Sigma, G7528) and 0.90 g Fructose (Sigma, F2543) in 100 ml deionised water with an osmolality of 330 mOsm/kg and pH of 6.9. Heparin concentrations were made by dissolving heparin sodium salt (Sigma, H4784) in deionised water and preparing serial dilutions with final heparin concentrations of 0, 10, 25, 50 and 100 IU. Caffeine concentrations were made by dissolving caffeine powder (Sigma, C0750) in deionised water and preparing serial dilutions of caffeine as 0, 2.5, 5, 10 and 20 mM. Calcium-ionophore A23187 concentrations were prepared by dissolving calcium-ionophore A23187 (Sigma, C7522) in Dimethyl sulfoxide (DMSO; Sigma, D2650) and serial dilutions were done to obtain concentrations of 0, 0.05, 0.1, 0.2 and 0.3 mM. The CTC fluorescence stain was adopted from a previously stated method (Hewitt and England, 1998). A fixative buffer was prepared by dissolving 12.11 g Tris (Sigma, T8793) in 100 ml deionised water. The fixative was made by mixing glutaraldehyde 25% (Sigma, G5882) with the fixative buffer 1:1 (v/v) and the pH was adjusted to 7.4. A CTC buffer was prepared by dissolving 240 mg Tris (Sigma, T8793) and 760 mg sodium chloride (Sigma, S3014) in 100 ml deionised water. The buffer was filtered through Amicon® Ultra-15 Centrifugal Filter Device with 100,000 MWCO (UFC 910024, Merck Millipore Ltd., Tullagreen, Carrigtwohill, Co. Cork, Ireland) using a swinging bucket rotor at 4,000 x g for 30 min and stored at 4°C. A CTC solution was made by mixing 2 mg of CTC (Sigma, 26430) and 4.4 mg of DL-Cysteine (Sigma, 861677) with 5 ml of CTC buffer. The pH of the solution was adjusted to 7.8 and stored at 4°C.

Camels and semen collection

Six adult healthy camels with sound history of fertility in the herd and an average age of 7 years (range, 5–13 years) were used in this study during the rutting season (December to April; Arthur *et al,* 1985). These males were maintained under standard conditions of feeding and management at the Camel Research Centre, King Faisal University, Saudi Arabia and served as sires for breeding females. Twelve ejaculates were collected (one ejaculate/week) from these camels using electro-ejaculation method (Tingari *et al,* 1986). Quality of semen samples was established by evaluating sperm motility and concentration by the same trained individual using Sperm Vision® 3.5 (Minitube of America, Inc) and NucleoCounter® SP-100TM (Chemometec, Ser. no. 1110-020-03, Gydevang 43, DK-3450 Allerod, Denmark), respectively. Percentages of sperm abnormalities were determined using eosin nigrosin stain (Eosin G, 2% Ref. 15405/0025, miniTüb, GmbH, Germany; Nigrosin, 4% Ref. 15405/0029, miniTüb, GmbH, Germany).

Semen dilution and capacitation

Only semen samples that had sperm progressive motility equal to or more than 50% (9 out of 12) were used. Semen samples and all chemical reagents were put in a water bath at 34°C. Immediately after semen evaluation, semen samples were diluted with an appropriate volume of Shotor buffer to obtain 15 aliquots with a final concentration of 5-10 x 10^6 motile spermatozoa/aliquot (each aliquot was 990 µl).

One aliquot of Sperm-Shotor's suspension of 5-10 x 10^6 spermatozoa (990 µl) was mixed with 10 µl dose of 0 heparin (heparin control) and 4 aliquots were mixed with 10 µl of 1 of 4 concentrations of heparin (10 IU (2.5 µl/mL), 25 IU (5 µl/mL), 50 IU (10 µl/mL) and 100 IU (20 µl/mL). Another aliquot of Sperm-Shotor's suspension was mixed with 10 µl dose of 0 caffeine (caffeine control) and 4 aliquots were mixed with 10 µl of 1 of 4 concentrations of caffeine (2.5 mM (0.00485g/mL), 5 mM (0.0097g/mL), 10 mM (0.0194g/mL) and 20 mM (0.0388g/mL). Also, one aliquot of Sperm-Shotor's suspension was mixed with 10 µl dose of 0 calcium-ionophore A23187 (calcium-ionophore A23187 control) and 4 aliquots were mixed with 10 µl dose of 4 concentrations of

calcium-ionophore A23187 (0.05 mM (3.75 µl/mL), 0.1 mM (7.53 µl/mL), 0.2 mM (14.95 µl/mL) and 0.3 mM (20 µl/mL). All aliquots were incubated at 38°C in a 5% CO_2 atmosphere and 90% relative humidity for 60 min. Aliquots were taken at 0, 5, 15, 30 and 60 min and evaluated for percentage of sperm motility. The viability indices (VI, Change in sperm motility with time; Milovanov *et al,* 1964) were calculated from the following equation:

$$VI = \Sigma [M \times (T-R/2)]$$

where; VI is the viability index, Σ is a sign for the sum total, M is the percentage of sperm motility, T is the time of next determination of motility and R is the time of previous determination of motility.

Moreover, aliquots were taken at 0 and 60 min and percentages of live sperm were determined using eosin nigrosin exclusion technique (Björndahl *et al,* 2003; Cecere, 2014; Agarwal *et al,* 2016) and maturational state was evaluated by CTC staining assay (Wang *et al,* 1995). Aliquots from replications (20 µl) were mixed with an equal volume of eosin solution and 40 µl of nigrosin solution and smeared onto a pre-warm microscope slide (37°C). After drying of the slide, sperm were scored (at least 100 cell/slide) under light microscope (oil-immersion 100x). Live sperm showed no staining and dead cells showed pink colouration. The presence of spermatozoa with a partial colouration were considered as dead cell. In the CTC staining assay, the microscope slide of the CTC stain was prepared by mixing 45 µl of each of replications with 45 µl of the CTC solution and 8 µl of fixative. A droplet (10 µl) of the stained replication was placed on a prewarmed (37°C) microscope slide and a droplet of vectrashield (Vector Laboratories, Peterborough, UK) was added to retard fading of the fluorescence. These droplets were mixed on the slide using a pipette tip, a coverslip was applied and gently compressed using a tissue paper. The coverslip was sealed on the slide by colourless nail varnish (Hewitt and England, 1998). The slides were examined with an Olympus corporation microscope (TH4-200, Tokyo, Japan) equipped with epifluorescence optics (excitation at 405 nm BP filter and CTC fluorescence emission at 455 DM), Olympus optical high pressure mercury burner (BH2-RFL-T3, Ser no. 2206115, Japan) and Thermo plate (MATS-U55RH20, Ser.no. 120997, Tokai Hit Co., Ltd, Japan). The characters of the CTC stained sperms were determined, photographed and described. For each replication, 50 spermatozoa were evaluated.

Statistical analysis

Data are presented as means ± SEM for camel sperm parameters, motility, viability index, percentage of live sperm and sperm staining pattern with CTC. These parameters were compared by t-test using SPSS program, version 24.0 (SPSS, 2016).

Results and Discussion

The initial semen parameters (mean ± SEM) of camels' ejaculate volume, percentage of motile sperm, sperm concentration and percentage of sperm abnormalities were 3.90 ± 0.71 ml, 55.33 ± 2.72, 342.53 ± 86.82 x 10^6/ml and 25.33 ± 1.00, respectively. As shown in table 1, there are significant (P<0.05 - P<0.001) differences in viability indices among camel semen incubated with heparin. Heparin 100 IU resulted in the best result of viability indices of camel semen as a capacitating factor (Table 1). Significant (P<0.05 - P<0.001) differences in viability indices of camel semen incubated with caffeine are shown in table 2. Caffeine 5 mM is the best concentration of caffeine that exerted the highest viability index of the incubated camel semen (Table 2). Table 3 declares that calcium ionophore 0.05 mM is the best concentration resulted in good viability index of camel semen in comparison to the other calcium ionophore concentrations. Significant differences in live sperm percent between the 0 min and 60 min of incubation in all semen aliquots except the part incubated with calcium-ionophore 0.05 mM is shown in table 4. Three CTC fluorescent staining patterns are observed: F pattern with fluorescence is

Table 1. Sperm motility (%) and viability indices of incubated camel semen (n=9) with heparin (mean ± SEM).

Heparin concentrations	Motility %					Viability Indices
	0 min	5 min	15 min	30 min	60 min	
Heparin 100 IU	60.83^{a} ± 2.69	51.67^{a} ± 4.54	48.33^{a} ± 4.54	33.33^{a} ± 3.97	18.33 ± 4.54	2443.75^{a} ± 294.89
Heparin 50 IU	44.17^{ab} ± 6.79	38.33^{b} ± 6.91	35.83^{b} ± 6.89	26.67^{a} ± 5.69	16.67 ± 3.60	1945.83^{ab} ± 386.66
Heparin 25 IU	40.00^{b} ± 4.71	38.33^{b} ± 4.54	35.00^{b} ± 4.93	26.67^{ab} ± 3.70	14.17 ± 3.38	1850.00^{b} ± 258.36
Heparin 10 IU	41.67^{b} ± 4.84	38.33^{b} ± 4.22	30.00^{b} ± 4.41	28.33^{a} ± 3.88	15.00 ± 3.63	1854.17^{b} ± 278.34
Heparin 0 IU (Control)	40.00^{b} ± 7.07	40.00^{ab} ± 7.07	31.67^{b} ± 4.84	22.50^{b} ± 5.12	16.67 ± 3.60	1802.08^{b} ± 343.00

Means with dissimilar superscripts in the same column are significantly different from P<0.05 - P<0.001.

visible over the whole sperm head (uncapacitated and acrosome intact); B pattern with bright anterior head and faint fluorescence in the post-acrosomal region (capacitated and acrosome intact); AR pattern with dull fluorescence stain over the whole sperm head (capacitated and acrosome reacted). The capacitation and acrosome reaction of camel spermatozoa was recorded using CTC pattern at the start of incubation (0 min) and after 60 min in the presence or absence of the capacitating factors. As illustrated in Fig 1, just after the start of incubation (0 min), it was recorded that a marked increase in B and AR patterns cells accompanied with a large decrease in F pattern spermatozoa in aliquots with the capacitating factors than in control aliquots. The percentages of F, B and AR cells at 0 and 60 min were 32.75 and 25.75%, 40.12 and 45.50%, and 22.13 and 28.75%, respectively, in aliquots treated with heparin; 33.60 and 27.75%, 37.75 and 42.25%, and 28.65 and 30.00%, respectively, in aliquots treated with caffeine; 30.75 and 22.50%, 39.15 and 41.38%, and 30.10 and 36.12%, respectively, in aliquots treated with calcium-ionophore; 13.42 and 17.67%, 21.8 and 23.23%, and 12.78 and 17.10%, respectively, in control aliquots (Fig 1).

In the present study, semen parameters of the freshly collected ejaculates differed slightly from the previously reported parameters in dromedary camels. In 14 dromerary ejaculates (Waheed *et al,* 2018), the ejaculate volume, percentages of sperm motility, sperm cell concentration and percentages of sperm abnormalities were 4.72±0.72 ml, 66.11±2.32 %, 268.56±29.10 x 10^6/ml and 18.78±2.67 %, respectively. Higher values of the ejaculate volume and sperm cell concentration (5.4±4.7ml and 520.3±388.2 x 10^6/ml) have been found in 5 ejaculates of dromedaries (Monaco *et al,* 2013). In this study, by using eosin nigrosin stain, The percentages of live spermatozoa decreased significantly between the 0 min and 60 min of incubation period except in one replicate. However, by using Trypan blue and Giemsa stain,

n= number of ejaculates.

Table 2. Sperm motility (%) and viability indices of incubated camel semen (n=9) with caffeine (mean ± SEM).

Caffeine concentrations	Motility %					Viability Indices
	0 min	5 min	15 min	30 min	60 min	
Caffeine 20 mM	50.00^{a} ± 3.33	43.33^{a} ± 2.72	36.67^{aa} ± 2.72	26.67^{a} ± 3.97	11.67^{ab} ± 2.45	1858.33^{a} ± 185.90
Caffeine 10 mM	42.50^{b} ± 4.98	40.83^{ab} ± 4.60	36.67^{ab} ± 5.69	30.00^{a} ± 4.71	11.83^{ab} ± 4.10	1900.83^{a} ± 336.51
Caffeine 5 mM	50.83^{a} ± 4.89	45.83^{a} ± 4.60	36.67^{ab} ± 4.91	29.17^{a} ± 3.58	12.50^{ab} ± 3.31	1960.42^{a} ± 256.01
Caffeine 2.5 mM	45.00^{ab} ± 7.22	40.83^{ab} ± 6.40	34.17^{ab} ± 5.17	26.67^{a} ± 5.18	15.00^{a} ± 4.64	1895.83^{a} ± 331.18
Caffeine 0 mM (Control)	38.33^{b} ± 5.38	34.17^{b} ± 5.43	29.17^{b} ± 3.58	16.67^{b} ± 3.30	6.83^{b} ± 1.94	1293.67^{b} ± 200.61

Means with dissimilar superscripts in the same column are significantly different from P<0.05 - P<0.001.

n= number of ejaculates.

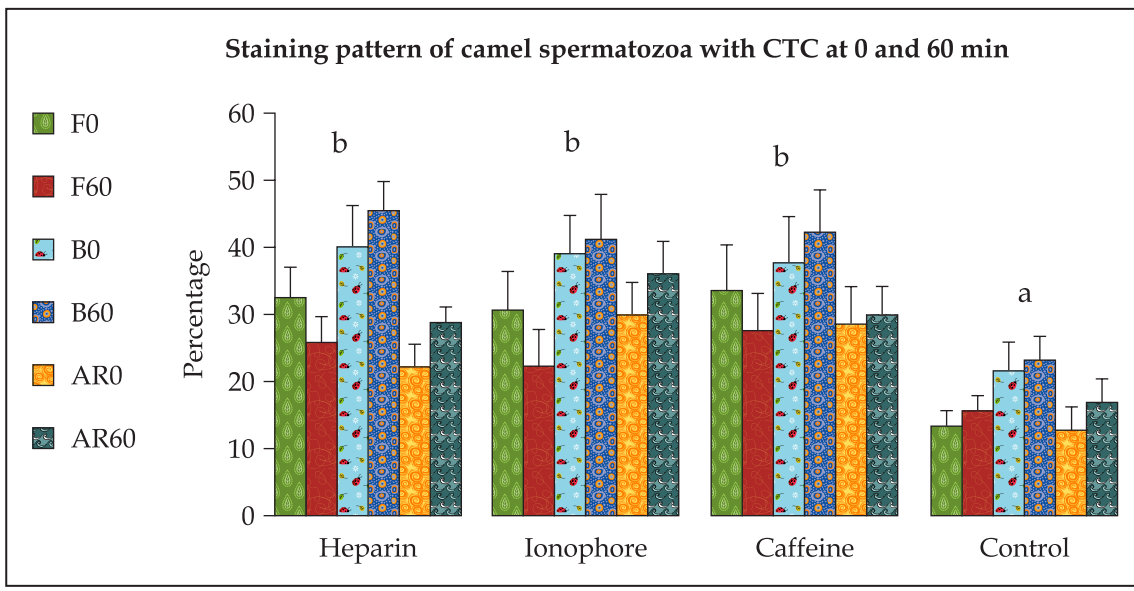


Fig 1. Staining pattern of camel spermatozoa with CTC at 0 and 60 min.

Table 3. Sperm motility (%) and viability indices of incubated camel semen (n=9) with Calcium ionophore (mean ± SEM).

Calcium ionophore concentrations	Motility %					Viability Indices
	0 min	5 min	15 min	30 min	60 min	
Calcium ionophore 0.3 mM	$9.17^{a} \pm 3.05$	$2.50^{a} \pm 1.10$	$0.17^{a} \pm 0.11$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$43.75^{a} \pm 15.42$
Calcium ionophore 0.2 mM	$13.33^{ab} \pm 3.08$	$6.00^{a} \pm 3.14$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$78.33^{a} \pm 29.39$
Calcium ionophore 0.1 mM	$17.50^{a} \pm 4.70$	$4.33^{a} \pm 2.09$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$76.25^{a} \pm 27.00$
Calcium ionophore 0.05 mM	$20.00^{b} \pm 4.71$	$11.67^{b} \pm 4.14$	$0.83^{a} \pm 0.54$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$147.92^{b} \pm 47.21$
Calcium ionophore 0 mM (Control)	$40.00^{c} \pm 4.41$	$33.33^{c} \pm 6.16$	$28.33^{b} \pm 6.10$	$24.17^{b} \pm 5.17$	$7.67^{b} \pm 2.56$	$1477.92^{c} \pm 302.97$

Means with dissimilar superscripts in the same column are significantly different from P<0.05 - P<0.001. n= number of ejaculates.

Table 4. Percentages of live sperm in incubated camel semen (n=9) with the capacitating factors using eosin nigrosin stain (mean ± SEM).

Capacitating factor	Live sperm % 0 min P< 0.05-0.001	Live sperm % 60 min P< 0.05
Heparin 100 IU	$68.78^{Aa} \pm 1.35$	$40.11^{Bac} \pm 4.38$
Heparin 50 IU	$54.11^{Abdgh} \pm 3.68$	$41.89^{Babc} \pm 5.46$
Heparin 25 IU	$60.67^{Adeh} \pm 2.74$	$41.00^{Babc} \pm 3.74$
Heparin 10 IU	$57.22^{Abegh} \pm 2.83$	$39.44^{Babc} \pm 3.96$
Heparin 0 IU	$56.11^{Abdgh} \pm 3.78$	$39.89^{Bac} \pm 4.23$
Calcium ionophore 0.3 mM	$41.11^{Ac} \pm 2.38$	$34.89^{Bab} \pm 2.23$
Calcium ionophore 0.2 mM	$51.11^{Abdgh} \pm 4.69$	$35.44^{Bab} \pm 4.40$
Calcium ionophore 0.1 mM	$49.78^{Acg} \pm 3.23$	$40.67^{Bac} \pm 3.93$
Calcium ionophore 0.05 mM	$49.00^{ch} \pm 3.96$	$36.33^{abc} \pm 6.86$
Calcium ionophore 0 mM	$62.00^{Abdg} \pm 3.23$	$28.33^{Bb} \pm 3.25$
Caffeine 20 mM	$59.67^{Aabdgh} \pm 3.70$	$35.44^{Babc} \pm 4.66$
Caffeine 10 mM	$57.89^{Abdgh} \pm 4.04$	$39.22^{Babc} \pm 4.88$
Caffeine 5 mM	$61.89^{Aabdgh} \pm 4.03$	$40.78^{Babc} \pm 4.90$
Caffeine 2.5 mM	$63.11^{Aabd} \pm 4.81$	$43.44^{Bc} \pm 2.58$
Caffeine 0 mM	$62.56^{Adf} \pm 1.48$	$39.56^{Bac} \pm 3.34$

Means with dissimilar superscript capital letters in the same row of each parameter and means with dissimilar superscript small letters in the same column are significantly different from P<0.05 - P<0.001.

n= number of ejaculates

the addition of calcium-ionophore to the bovine and caprine semen resulted in a significantly improved percentage of live spermatozoa with true acrosome reaction at all stages of incubation (Pereira *et al,* 2000). In the present study, heparin 100 IU and caffeine 5 mM exerted a significant beneficial effect on viability indices of camel semen. However, calcium-ionophore A23187 had very low values of viability indices resulted from the dramatic decrease in sperm motility as no spermatozoa were scored as motile after 15 min of incubation with all calcium-ionophore concentrations, even though approximately 35-40% of the cells were still live at 60 min of incubation. This is might be explained by ionophore A23187 might affect sperm metabolism and has less effect on sperm plasma membrane. Heparin, caffeine and calcium-ionophore have been used in initiation of acrosome reaction in several species including bovine and caprine (Pereira *et al,* 2000). In rams, heparin 250 IU, caffeine 5.15 mM and calcium-ionophore 1.55 mM have been used for *in-vitro* capacitation and acrosome reaction (El-Shahat *et al,* 2016). These concentrations of heparin and calcium-ionophore are much more than the concentrations used in the present study. However, in bovine and caprine, the effect of incubation with heparin, caffeine and calcium-ionophore on sperm motility is negligible (Pereira *et al,* 2000). Similar to the present study, calcium-ionophore has a negative effect on stallion sperm motility during incubation for 3.5 h (Rathi *et al,* 2001). Nevertheless, an alternative means of improving the *in-vitro* fertilising capacity of spermatozoa, in bovine (Byrd, 1981; Jiang *et al,* 1991; Pereira *et al,* 2000), caprine (Shorgan, 1984; Pereira *et al,* 2000), equine (Zhang *et al,* 1991; Rathi *et al,* 2001), dogs (Hewitt and England, 1998), sheep (El-Shahat *et al,* 2016), and mouse (Tateno *et al,* 2013; Navarrete *et al,* 2016) is the use of calcium-ionophore A23187. Concerning caffeine, It inhibits nucleotide-phosphodiesterase that is responsible for cAMP degradation. Hence caffeine treatment induces an increase in intracellular cAMP concentration (Niwa and Ohgoda, 1988). The

Talp Stock medium supplemented with 25.7 mM caffeine induced sperm capacitation and *in-vitro* fertilisation in swine (de Oliveira *et al,* 2011). The addition of caffeine 12.87 mM to the Whittingham's T6 medium containing human serum (T6 + 10% HS) promoted the sperm's motility and vitality and enhanced fertilisation in mouse (Nabavi *et al,* 2013). On using caffeine 5 mM with the universal IVF medium, there is hyperactivation efficacy of frozen bovine semen (Barakat *et al,* 2015). As a result of their hydrophobic characteristics, calcium-ionophores are able to transport ions across membranes. The increase in free calcium-ionophore within the cell directly induces the acrosome reaction and bypasses capacitation (First and Parrish, 1987). The CTC staining patterns (F, B and AR) have been found in human (DasGupta *et al,* 1993), bovine (Fraser *et al,* 1995) and dogs' spermatozoa (Hewitt and England, 1998). In the present study, 3 staining patterns were recorded in camels' spermatozoa and this method could be useful for identification of capacitation and acrosome reaction in this species. In this study, at 0 min of incubation, the percentages of F, B and AR cells in calcium-ionophore treated aliquots were 30.75, 39.15 and 30.10%, respectively and in control aliquots were 13.42, 21.8 and 12.78%, respectively. In stallions, the average percentages of spermatozoa showing F and AR patterns are 50.2±0.8% and 9.5±3.2%, respectively, in samples diluted with Tyrode medium + bicarbonate at 0 min of incubation (Rathi *et al,* 2001). In the present study, the percentages of F, B and AR cells after 60 min were 17.67, 23.23 and 17.10%, respectively in control aliquots and they were 22.50, 41.38 and 36.12%, respectively in calcium-ionophore treated aliquots. In dogs, mean percentages of F, B and AR cells after 1 h incubation were 58.75, 37.25 and 4.00%, respectively in control samples and they were 25, 52.5 and 22.5%, respectively in the ionophore (10 mM) treated samples (Hewitt and England, 1998).

Heparin (100 IU), caffeine (5 mM) and calcium-ionophore A23187 (0.05 mM) are convenient capacitating factors for dromedary camels' semen. CTC fluorescent staining technique can be used for assessing capacitation status and acrosome reaction in dromedary camels, and it may be useful in future studies of *in-vitro* culture and *in-vitro* fertilisation.

Acknowledgements

The authors would like to thank the Camel Research Centre, King Faisal University for support and for approving by the ethical committee.

Deanship of Scientific Research, King Faisal University, Kingdom of Saudi Arabia (Project # 140057) is thanked for providing necessary grants.

References

Agarwal A, Gupta S and Sharma R (2016). Eosin-Nigrosin Staining Procedure. In: Andrological Evaluation of Male Infertility (Agarwal A., Gupta S., Sharma R. eds). Springer. pp 73-77.

Arthur HG, Rahim AT and Hindi A (1985). Reproduction and genital diseases of the camel. British Veterinary Journal 141:650-659.

Barakat IAH, Danfour MA, Galewan FAM and Dkhil MA (2015). Effect of Various Concentrations of Caffeine, Pentoxifylline, and Kallikrein on Hyperactivation of frozen bovine semen. BioMed Research International 7 pages. http://dx.doi.ogr/10.1155/2015/948575.

Björndahl L, Söderlund I and Kvist U (2003). Evaluation of the one-step eosin-nigrosin staining technique for human sperm vitality assessment. Human Reproduction 18(4): 813-816.

Byrd W (1981). *In vitro* capacitation and the chemically induced acrosome reaction in bovine spermatozoa. Journal of Experimental Zoology 215:35-46.

Cecere JT (2014). Eosin-Nigrosin Staining in the Evaluation of Sperm. In: Dascanio JJ, McCue PM (eds). Equine Reproductive Procedures, John Wiley and Sons, Inc.

Crichton EG, Pukazhenthi BS, Billah M and Skidmore JA (2015). Cholesterol addition aids the cryopreservation of dromedary camel (*Camelus dromedarius*) spermatozoa. Theriogenology 83(2):168-174.

Das Gupta S, Mills CL and Fraser LR (1993). Ca^{2+} related changes in the capacitation state of human spermatozoa assessed by a chlortetracycline fluorescence assay. Journal of Reproduction and Fertility 99:135-143.

de Oliveira VP, Marques MG, Simöes R, Assumpção MEOD and Visintin JA (2011). Influence of caffeine and chondroitin sulfate on swine sperm capacitation and *in vitro* embryo production. Acta Scientiae Veterinariae 39(2):960-965.

El-Shahat KH, Taysser MI, Badr MR and Zaki KA (2016). Effect of heparin, caffeine and calcium ionophore A23187 on *In vitro* induction of the acrosome reaction of fresh ram spermatozoa. Asian Pacific Journal of Reproduction 5: 148-155.

First NL and Parrish JJ (1987). *In-vitro* fertilisation of ruminants. Journal of Reproduction and Fertility Supplement 34: 151-165.

Fraser LR, Abeydeera LR and Niwa K (1995). Ca 21-Regulating mechanisms that modulate bull sperm capacitation and acrosomal exocytosis as determined by chlortetracycline analysis. Molecular Reproduction and Development 40: 233-241.

Guerin P, Ferre M, Fontbonne A, Bénigni L, Jacquet M and MénézoIn Y (1999). *In vitro* capacitation of dog spermatozoa as assessed by chlortetracycline staining. Theriogenology 52:217-228.

Hewitt DA and England GCW (1998). An investigation

of capacitation and the acrosome reaction in dog spermatozoa using a dual fluorescent staining technique. Animal Reproduction Science 51:321-332.

Jiang S, Yang X, Chang S, Heuwieser W and Foote RH (1991). Effect of sperm capacitation and oocyte maturation procedures on fertilisation and development of bovine oocytes *in vitro*. Theriogenology 35:218.

Kwon W, Rahman MS, Lee J, Kim J, Yoon S, Park Y, Hwang YY and Pang M (2014). A comprehensive proteomic approach to identifying capacitation related proteins in boar spermatozoa. BMC Genomics 15(1):897. doi: 10.1186/1471-2164-15-897

Milovanov VK, Trubkin GD, Chubenko NS, Tsvetkov IV, Erzin ZK and Meschankin AB (1964). Artificial insemination of livestock in the U.S.S.R. Israel Program For Science and Translation, Jerusalem. pp 102-104.

Monaco D, Fatnassi M, Padalino B, Kchira B, El Bahrawy K, Rateb S, Khorchani T, Hammadi M and Lacalandra GM (2013). The experimental semen collection centers for dromedary camels in Egypt and Tunisia: current situation and future developments. In: 11th Congress of the Italian Society of Animal Reproduction, June 19-22. pp 132-136.

Nabavi N, Todehdehghan F and Shiravi A (2013). Effect of caffeine on motility and vitality of sperm and *in vitro* fertilisation of outbreed mouse in T6 and M16 media. Iranian Journal of Reproductive Medicine 11:741-746.

Nakai M, Nagai T, Tanihara F and Kikuchi K (2012). Image processing combined with chlortetracycline staining for assessment of boar sperm capacitation and fertility. Proceeding of Assisted Applied Animal Andrology Conference – Vancouver, Canada. pp 137-144.

Navarrete FA, Alvau A, Lee HC, Levin L, Buck J, Leon PM, Santi CM, Krapf D, Mager J, Fissore RA, Salicioni AM, Darszon A and Visconti PE (2016). Transient exposure to calcium ionophore enables *in vitro* fertilisation in sterile mouse models. 6, doi: https://doi.org/10.1038/srep33589.

Niasari-Naslaji A, Mosaferi S, Bahmani N, Gerami A, Gharahdaghi AA, Abarghani A and Ghanbari A (2007). Semen cryopreservation in Bactrian camel (*Camelus bactrianus*) using SHOTOR diluent: effects of cooling rates and glycerol concentrations. Theriogenology 68: 618-625.

Niwa K and Ohgoda O (1988). Synergistic effect of caffeine and heparin on *in vitro* fertilisation of cattle oocytes matured in culture. Theriogenology 30:733-741.

Paulenz H, Söderquist L, Pérez-Pé R and Berg K (2002). Effect of different extenders and storage temperatures on sperm viability of liquid ram semen. Theriogenology 57:823-836.

Pereira RJTA, Tuli RK, Wallenhorst S and Holtz W (2000). The effect of heparin, caffeine and calcium ionophore a 23187 on *in vitro* induction of the acrosome reaction in frozen-thawed bovine and caprine spermatozoa. Theriogenology 54:185-192.

Rathi R, Colenbrander B, Bevers MM and Gadella BM (2001). Evaluation of *in vitro* capacitation of stallion spermatozoa. Biology of Reproduction 65:462-470.

Saling PM and Storey BT (1979). Mouse gamete interactions during fertilisation *in vitro*: chlortetracycline as fluorescent probe for the mouse sperm acrosome reaction. Journal of Cell Biology 83:544-555.

Sebkova N, Cerna M, Ded L, Peknicova J and Dvorakova-Hortova K (2012). The slower the better: how sperm capacitation and acrosome reaction is modified in the presence of estrogens. Reproduction 143:297-307.

Shorgan B (1984). Fertilisation of goat and ovine *in vitro* by ejaculated spermatozoa after treatment with ionophore A23187. Bull Nippon Veterinary Zoology College 33: 219-221.

Skidmore JA and Billah M (2006). Comparison of pregnancy status in dromedary camels (*Camelus dromedarius*) after deep intra-uterine *versus* cervical insemination. Theriogenology 66:292-296.

SPSS. Statistical Package for Social Science (2016). SPSS Inc, Chic, IL, USA Copyright© for Windows; version 24.0.

Tateno H, Krapf D, Hino T, Sánchez-Cárdenas C, Darszon A, Yanagimachi R and Visconti PE (2013). Ca^{2+} ionophore A23187 can make mouse spermatozoa capable of fertilising *in vitro* without activation of cAMP-dependent phosphorylation pathways. Proceeding of National Academic Science USA 110(46):18543-18548. doi: 10.1073/pnas.1317113110

Tibary A and Anouassi A (1997). "Management of reproduction in camelidae". In: Tibary A, ed, Theriogenology in Camelidae: Anatomy, Physiology, BSE, pathology and artificial breeding: actes editions. Institut Agronomique et Veterinaire Hassan II. pp 459-476.

Tibary A, Anouassi A, Sqhiri A and Khatir H (2007). Current knowledge and future challenges in camelid reproduction. Society of Reproduction and Fertility Supplement 64:297-313.

Tingari MD, Manna MM, Rahim AT, Ahmed AK and Hamad MH (1986). Studies on camel semen. I. Electroejaculation and some aspects of semen characteristics. Animal Reproduction Science 12:213-222.

Torner H, Heleil B, Alm H, Ghoneim IM, Srsen V, Kanitz W, Tuchscherer A and Fattouh EM (2003). Changes in cumulus-oocyte complexes of pregnant and non pregnant camels (*Camelus dromedarius*) during maturation *in vitro*. Theriogenology 60:977-987.

Tsien RY (1989). Fluorescent indicators of ion concentrations. In: Taylor DL, Wang YL, eds, Fluorescence Microscopy of Living Cells in Culture. Part 6B. Quantitative Fluorescence Microscopy—Imaging and Spectroscopy. Methods Cell Biol., New York: Academic Press; Vol 30, chapter 5. pp 127-156.

Tulsiani DRP and Abou-Haila A (2012). Biological Processes that Prepare Mammalian Spermatozoa to Interact with an Egg and Fertilise It. Scientifica (Cairo) doi: 10.6064/2012/607427

Varner DD, Ward CR, Storey BT and Kenney RM (1987). Induction and characterisation of acrosome reaction in equine spermatozoa. American Journal of Veterinary Research 48:1983-1989.

Waheed MM, Meligy AMA and Dhalam SA (2018). Determination of some trace elements in seminal

plasma and serum of camels (*Camelus dromedarius*) and their correlation to fertility. Reproduction in Domestic Animals 53:1367-1374.

Wang WH, Abeydeera LR, Fraser LR and Niwa K (1995). Functional analysis using chlortetracycline fluorescence and *in vitro* fertilisation of frozen-thawed ejaculated boar spermatozoa incubated in a protein-free chemically defined medium. Journal of Reproduction and Fertility 104(2):305-313.

Wani NA (2002). *In vitro* maturation and *in vitro* fertilisation of sheep oocytes: Review. Small Ruminant Research 44:89-95.

Wani NA (2009). *In vitro* embryo production in camel (*Camelus dromedarius*) from *in vitro* matured oocytes fertilised with epididymal spermatozoa stored at 4 degree C. Animal Reproduction Science 111:69-79.

Ward CR and Storey BT (1984). Determination of the time course of capacitation in mouse spermatozoa using a chlortetracycline fluorescence assay. Developmental Biology 104:287-296.

Zhang JJ, Muzs LZ and Boyle MS (1991). Variations in structural and functional changes of stallion spermatozoa in response to calcium ionophore A23187. Journal of Reproduction and Fertility Supplement 44:199-205.

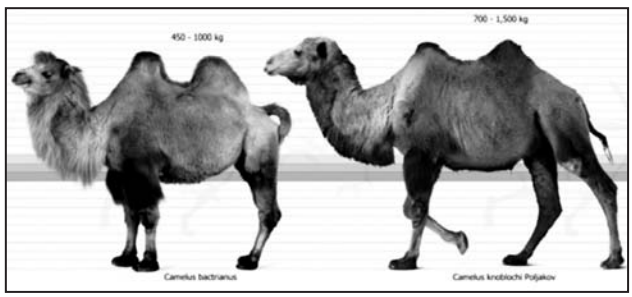
SWIMMING CAMELS, MANGROVE ISLANDS OF KUTCH FACE MOUNTING CHALLENGES

The Kharai camels of Kutch are dependent on mangroves for their food, and during monsoons, swim to mangrove islands in hordes. Pastoralists have complained about mangrove destruction to the state forest department in Gujarat. The sight of swimming camels, as one can imagine, is spectacular, but faced with challenges such as rapid industrialisation in the coastal areas, and mangrove destruction, their population is dwindling, making it a threatened breed.

The history may be hazy, but the fact remains that the Kharai camels are held with respect by the tribes and until a few years ago they did not even sell its milk or wool. For income, they depended on selling the male camels to traders who used them for transportation. With the advent of small commercial vehicles that can go deep into small towns and villages, camels are not used as much – one of the many reasons why these tribes are struggling socio-economically today. For eight months in a year, the Kharai camels are completely dependent on the mangrove islands, spending weeks together on these masses. They eat the saline plants and the mangrove species and drink rainwater accumulated in depressions on these islands. During winter, their handlers– Rabaris are typically the owners – graze them on the dry land.

LARGEST EURASIAN CAMEL—CAMELUS KNOBLOCHI

Camelus knoblochi (Camelus knoblochi Poliakov,1880) belongs to Order Artiodactyla and Family Camelidae. It has a gigantic Size, i.e. 3,5 m in length, 250 cm in height, 700 - 1,200 kg of weight. *C. knoblochi* was the largest Eurasian camel. Its stratigraphical range includes most of the Middle Pleistocene and Late Pleistocene. This species had its maximum distribution and highest abundance in the Late Middle Pleistocene, when its range extended from Eastern Europe to Transbaikalia. In the Late Pleistocene, *C. knoblochi* inhabited a considerable part of Asia from 391 to 541 N between the Urals and northeastern China. Palynological data and paleozoological contexts indicate steppe and forest–steppe environments of the species. Most likely, *C. knoblochi* fed mainly on grassy vegetation. However, its diet also included sprigs and leaves. Their extinction was caused by climatic aridization during the Late Pleistocene accompanied by the drastic change of plant communities. In this climatic situation, camels were displaced to relatively extreme conditions of dry steppe and semi-deserts because of high competition with more efficient phyllophagous and herbivorous animals such as large deer and ruminants. Large *C. knoblochi* became extinct, being replaced by *C. bactrianus*, which is better adapted to severe environments and to feeding on less nutritious vegetation.



(Source: Vadim V. Titov)

CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS ENDEMICITY IN UNITED ARAB EMIRATES, 2019

Camel scientists from the Austria and UAE conducted a cross-sectional survey of Crimean-Congo hemorrhagic fever virus (CCHFV) in dromedary camels and attached ticks at 3 locations in the United Arab Emirates. Scientists performed 2 conventional reverse transcription PCRs on the RNA-positive serum samples and on each tick from the 2 RNA-positive pools, 1 amplifying a 492-bp portion of the viral small (S) segment and 1 amplifying a 672-bp portion of the viral medium (M) segment (Appendix). These were then subjected to PCR products to Sanger sequencing (GenBank accession nos. MN516481–8). Results revealed a high prevalence of CCHFV-reactive antibodies in camels and viral RNA in ticks and camel serum, suggesting the virus is endemic in this country. Survey indicates that exposure to CCHFV is common among camels in the UAE, and transmission to camels might be occurring via native infected *H. dromedarii* ticks.

(Source: Camp JV *et al*, Crimean-Congo Hemorrhagic Fever Virus Endemicity in United Arab Emirates, 2019. Emerg Infect Dis. 2020; 26(5):1019-1021.)

WHOLE-GENOME SEQUENCING OF 128 CAMELS ACROSS ASIA REVEALS ORIGIN AND MIGRATION OF DOMESTIC BACTRIAN CAMELS

The domestic Bactrian camels were treated as one of the principal means of locomotion between the eastern and western cultures in history. However, whether they originated from East Asia or Central Asia remains elusive. To address this question, we perform whole-genome sequencing of 128 camels across Asia. The extant wild and domestic Bactrian camels show remarkable genetic divergence, as they were split from dromedaries. The wild Bactrian camels also contribute little to the ancestry of domestic ones, although they share close habitat in East Asia. Interestingly, among the domestic Bactrian camels, those from Iran exhibit the largest genetic distance and the earliest split from all others in the phylogeny, despite evident admixture between domestic Bactrian camels and dromedaries living in Central Asia. Taken together, our study support the Central Asian origin of domestic Bactrian camels, which were then immigrated eastward to Mongolia where native wild Bactrian camels inhabit.

(Source: Ming, L., Yuan, L., Yi, L. *et al,* Whole-genome sequencing of 128 camels across Asia reveals origin and migration of domestic Bactrian camels. Commun Biol 3, 1 (2020). https://doi.org/10.1038/s42003-019-0734-6)

BIOCHEMICAL BIODIVERSITY OF CAMEL MILK

Moroccan camel milk is characterised by slight hydronium potential (pH=6.5), low Dornic acidity (15°D), low density (1.029 kg/l), and high content of ashes (8.06 g/l). Likewise, samples had a high content of macronutrients (Fats: 34.09 g/l; Proteins: 32.4 g/l; Sugar: 49.8 g/l) and micronutrients (Vitamin C: 27.53 mg/l; Flavonoids: 29.05 mg EQ/l: total phenolic compounds: 35.45mg GAE/l). In this respect, working on multiple specimens from different Moroccan regions highlighted an analytical diversity from the south to the north. Comparative study of samples from numerous territories all over the world has confirmed this diversity. North African milk is characterized by high content of proteins, fats, and sugar. On the other hand, oriental milk is peculiarised by high vitamin C content.

(The Scientific World Journal Volume 2019, Article ID 2517293, 7 pages
https://doi.org/10.1155/2019/2517293)

INSTRUCTIONS TO CONTRIBUTORS

The Journal of Camel Practice and Research is published by half-yearly from the Camel Publishing House, 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner, 334 001 (India). It is in offset print size of 20.5x27.5 cm in two columns with a print area of 17x22 cm. It will be known as **Journal of Camel Practice and Research** with **Volume** number on yearly basis and **Number** on issues per volume basis (in exceptional cases there can be more than two issues in a volume).

Nature of coverage: This journal is dedicated to disseminate scientific information about new and old world camelids in form of: **Original research** articles in camel health, husbandry, pastoralism, sports, specific behaviour, history and socio-economics. **Reports** on unusual clinical case(s) or unreported management of clinical case(s). Review articles will be accepted on invitation only. **Book review** directly or indirectly related to camels will be reviewed by subject-matter specialists and included if sent to the journal for this purpose. **Masters or Doctorate thesis/dissertation abstracts** will be published only if sent by the candidate with due certification from advisor/supervisor and head of the department where the research was carried out. All thesis/ dissertation abstracts should be accompanied by attested or photocopy of their mandatory certificates only for official records. The Journal of Camel Practice and Research will occasionally contain an **invited editorial** commenting on the papers in the issue.

Each issue of the Journal of Camel Practice and Research will contain some titbits like My Camel Memories, Clinical Camelids, `**from the old literature', `cartoons' and interesting news** items'. Readers are welcome to contribute for these and due credit lines will suitably be included. However, all these are subject to scrutiny by members of the editorial board.

News of any International Association of Camel or Camelids will be included as and when necessary. `**Research in progress'**, is a special feature we intend to incorporate in the Journal of Camel Practice and Research. In this column researchers can report initial findings of their work in advance, so that others engaged in similar pursuit can exchange views about it. However, such short communications will be entertained on understanding that full article will also appear in this journal.

Submission of manuscript: Mail two hard copies of the manuscript and two complete sets of figures along with a CD or a soft copy in word files to **Dr.T.K. Gahlot**, Editor, Journal of Camel Practice and Research, Department of Surgery & Radiology, College of Veterinary & Animal Science, **Bikaner**, Rajasthan, 334 001 India. Send soft copy to Editor at tkcamelvet@yahoo.com.

The manuscript should be sent in a heavy paper envelope and photographs or illustrations should be enclosed in a cardboard to avoid damage during mail handling. The manuscript should be accompanied by a covering letter from the author responsible for correspondence. It should also contain a statement that manuscript has been seen and approved by all co-authors. Editor and members of the editorial board are not responsible for the opinions expressed by authors and reserves the right to reject any material or introduce editorial changes. Material will be accepted for publication on the understanding that it has not been published in any other form and is not being considered elsewhere. Any material once accepted for publication may not be republished in any form without prior permission of the author.

Manuscripts can also be accepted on 3.5" or 5.25" floppies, computers, PM5 or PM6 Microsoft-Word-5 or compatibles, Microsoft-Excel-4 or compatibles. It would be in the interest of authors to accompany a hard copy.

Preparation of the manuscript: Manuscript should be typed on white bond paper (A4 or 5 size) with a margin of 4 cm on right side, 3 cm on left side, top and bottom. British English, spellings and generic names of drugs should be used. International Code of Zoological Nomenclature, *Nomina Anatomica Veterinaria*, International Code of Nomenclature of Bacteria, International Code of Botanical Nomenclature and International Standards should be strictly followed. All terms should be identified by their scientific names and for easy comprehension common terms/names can be used. Population data and geographical distribution of camelids should invariably be avoided in introduction, unless it is warranted.

Each of the following sections should be types on separate pages:

Title page: This page should contain title of the article, name of the department/institution where work has been done, present postal address of each author and name of author with email to whom reprint request should be addressed. Following is the example:

Example: CLINICAL EVALUATION OF INTERDENTAL WIRING TECHNIQUE FOR MANDIBULAR FRACTURE REPAIR IN CAMELS

T.K. Gahlot[1], S.K. Chawla[2], R.J. Choudhary[3], D. Krishnamurthy[4] and D.S. Chouhan[5]

Department of Surgery & Radiology,[1,3 and 5] College of Veterinary and Animal Science,[2 and 4], College of Veterinary Sciences, CCS-Haryana Agricultural University, Hisar, 125004 INDIA.

SEND REPRINT REQUEST TO DR. T.K. GAHLOT email: tkcamelvet@yahoo.com.

Abstract and Key words: The abstract should begin with title of the article (in upper case), and have brief procedures, salient results and conclusions not more than 225 words, in one paragraph on second page. Proprietary names and abbreviations should be avoided. Provide four to six key words below the abstract for indexing services. Abstract is not necessary for short communications, case reports, news items etc.

Text: The proper text of the paper should start from third page and should again begin with title of the article (in upper case). The text should be divided into sections with headings, introduction, materials and methods, results, discussion, tables/illustrations and references.

Introduction: The logic of the introduction is to introduce the specificity and relevance of the topic to the readers. It should include the objective of the work in brief and most important related reference(s).

Materials and Methods: Should contain details regarding materials and brief account of procedures used.

However, sufficient details must be included to reproduce the results. For established or routine methods only related reference(s) can be cited. Any deviation from routine procedures should be specifically mentioned. Only generic names of the drugs and chemicals should be used in the running text. The trade names, source or other necessary related information should be mentioned in parenthesis there in.

In case reports, the case record sheet should also be included in materials and methods.

Statistical methods if used should be briefly described alongwith reference. If any analysis is done with the help of a computer programme, its complete name and source should be mentioned, however, it does not mean to exclude the mention of methods, level of significance and other relevant information.

Results and Discussion should be presented in logical sequence with implications of findings about other relevant studies. The data or information easily attainable from the tables or graphics need not be repeated in the results. Only important observations need to be summarised. Undue repetition of the text from results to discussion has to be avoided. To preclude it, depending on article, results and discussion can be combined. In discussion only significant results should be discussed. One should not always stick to the term `statistically significant' data rather biological importance or significance of any variation should be given due importance in discussion. Discussion should always end in conclusions linked with objectives of the study mentioned in the introduction and unqualified statements should be avoided.

Tables: Each tables should be typed on separate sheet. Large tables should be avoided and should not exceed one page. Each table should be numbered in Indo-Arabic numerals according to their sequence in the text that refers to it. In the text it should be referred as proper noun e.g., Table 1. The title of the table should be brief and self-explanatory. Footnotes can be included to enhance understanding ability of the contents of the table.

Illustrations and Legends: All illustrations should be submitted in duplicate and about twice the size desired for reproduction that is 17 cm for double column or 8.3 cm for single column. Photographs and photomicrographs should be printed on glossy paper with excellent details and contrast. Drawings and diagrams should be in India ink (Black) on smooth white paper. All illustrations should be referred as figures in the text and should also be numbered in Indo-Arabic numerals e.g., Fig 1. Legends of all these figures should be typed on a separate sheet. Each legend should be clear, concise and informative. A statement of magnifications or reductions should be given where it is applicable. Nothing should be written with pen or typed on the back of any illustration except it bears running title of the paper, figure number and arrow indicating top edge with light pencil. All graphs should be supported with data on a separate sheet to redo them (in certain special cases) according to format of the journal.

References: References to the work should be cited in the text with author's surname and year of publication in the parenthesis e.g., Gahlot (1995) or Gahlot *et al* (1995) or (Gahlot *et al*, 1995), depending upon construction of the sentence. In case there are two authors the conjunction `and' or its symbol `&' should be used according to construction of the sentence e.g., Gahlot and Chouhan (1995) or (Gahlot & Chouhan, 1995). When there are more than two authors the surname of first author will be followed by *et al*. When name of any author bears only first and second name, latter will be considered as surname for the text. However, in papers submitted to this journal both names should be used in the title page. Chronological order should be followed in citing several references together in the text.

References should be arranged in alphabetical order. Authors should not modify the title of the references. Mention full name of the journal. Examples of correct forms of references are given below:

Periodicals: Sharma SD, Gahlot TK, Purohit NR, Sharma CK, Chouhan DS and Choudhary RJ (1994). Haematological and biochemical alterations following epidural administration of xylazine in camels (*Camelus dromedarius*). Journal of Camel Practice and Research 1(1):26-29.

For edited symposium/congress/proceedings: Abdalla HS (1992). Camel trypanosomiasis in the Sudan. Proceedings First International Camel Conference, Dubai (UAE), February 2-6, p 401-403.

Books (Personal authors): Gahlot TK and Chouhan DS (1992). Camel Surgery, Ist Edn. Gyan Prakashan Mandir, Gauri Niwas, 2b5, Pawanpuri, Bikaner, India. pp 37-50.

Chapter from multiauthored books: Chawla SK, Panchbhai VS and Gahlot TK (1993). The special sense organs-Eye. In: Ruminant Surgery, Eds., Tyagi RPS and Singh J. Ist Edn., CBS Publishers and Distributors, Delhi, India. pp 392-407.

Thesis: Rathod Avni (2006).Therapeutic studies on sarcopticosis in camels (*Camelus dromedarius*). Unpublished Masters Thesis (MVSc), Rajasthan Agricultural University, Bikaner, Rajasthan, India.

Commercial booklets: Anonymous/Name (1967). Conray-Contrast Media. IIIrd Edn., 12-15, May and Baker Ltd., Dagenham, Essex, England.

Magazine articles: Taylor D (1985). The Constipated Camel. Reader's Digest. Indian Edn. RDI Print & Publishing (P) Ltd., Mehra House, 250-C, New Cross Road, Worli, Bombay, India. 126:60-64

News paper articles: Anonymous or name of correspondent (1985). Bright Sunlight causes Cataract. Times of India, New Delhi, City-1, India October-9 pp 3, Col 3-5.

Personal communication: Hall LW (1995). Reader in Comparative Anaesthesia, Department of Clinical Veterinary Medicine, Madingley Road, University of Cambridge, Cambridge, CB3 0ES, England.

Reprints: There is no provision for free reprints. Author or person in correspondence has to pay INR 4500/- (for Indian Citizens only) or US $ 450, in advance for 10 reprints for the year 2020. Additional reprints in multiples of 10 may be requested and will be charged at the same rate but with minimum order of 30 reprints and every request for extra reprints should be made, if required, before 30th March, July or November every year.

Charges for colour and black and white pictures: Author(s) has to pay for production of colour plates in his/her manuscript. More than 4 black and white picture will be charged from the author(s) towards printing charges.