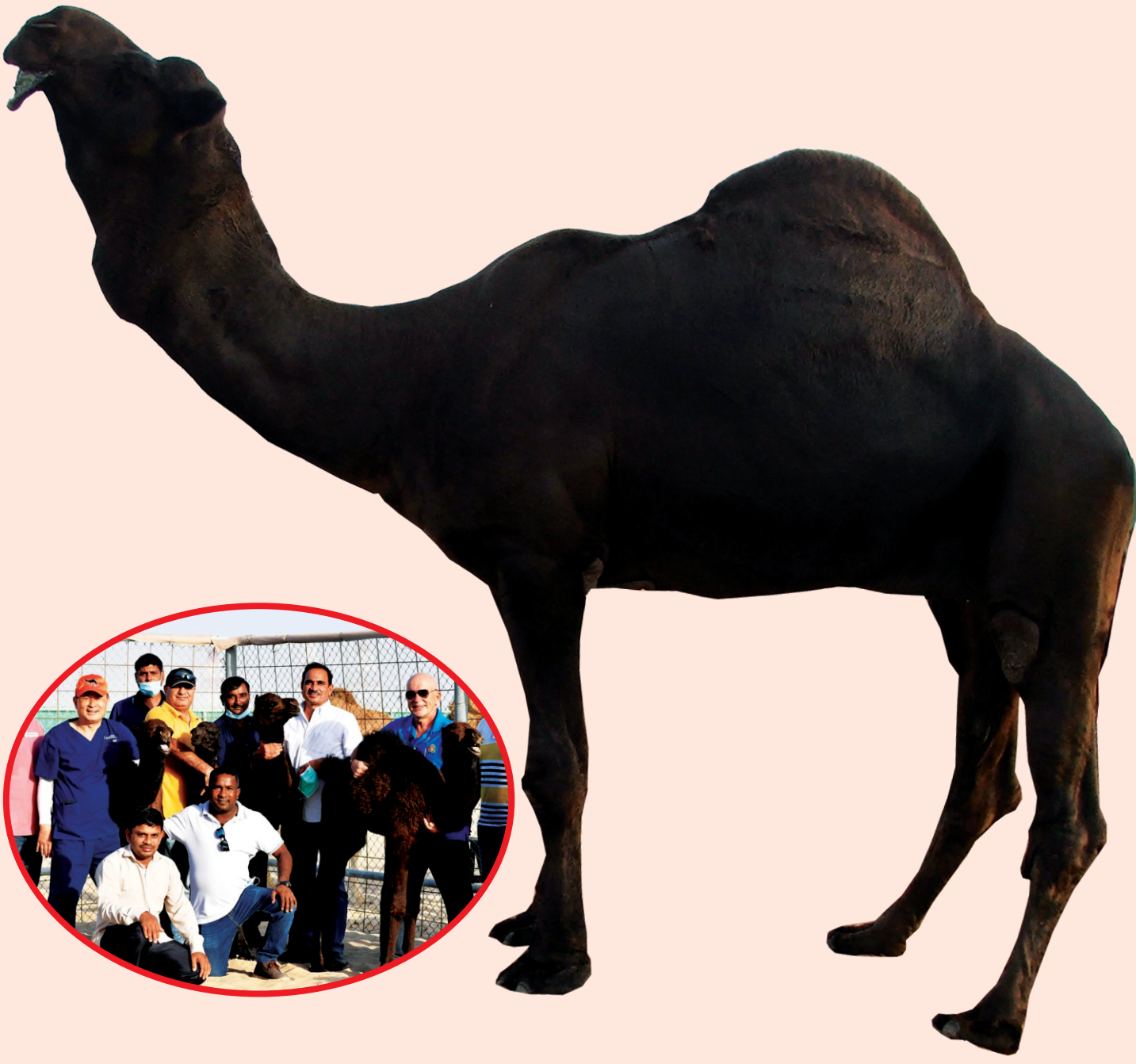
ISSN 0971-6777 (Print)
ISSN 2277-8934 (Online)

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com • www.indianjournals.com

Volume 28 December 2021 Number 3

MABROKAN BORN AGAIN BY SOMATIC CELL NUCLEAR TRANSFER



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

Mobile : 0091-9414137029
Email : tkcamelvet@yahoo.com
Website : www.camelsandcamelids.com • www.tkgahlotcamelvet.com • www.indianjournals.com

Scope of Journal of Camel Practice and Research

Journal of Camel Practice and Research (JCPR) publishes only research and clinical manuscripts related to the Camelids (Old and New World camelids), hence published contents are consistent with the title and scope of the journal. Review articles on emerging research are invited and published. JCPR also publishes the news related to the New or Old World camelids, specially those related to new products, conferences, books, trainings or workshops etc.

SUBSCRIPTION RATE - 2022

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. email: tkcamelvet@yahoo.com

Cover Design: T.K. Gahlot

Cover Photo: Mabrokan camel, UAE

Courtesy: Alex Tinson, UAE

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

CONTENTS

Volume 28 **December 2021** **Number 3**

CONTENTS

Volume 28 **December 2021** **Number 3**

FIRST REPORTED LARGE SCALE CLONING OF CAMELS

A biotechnology miracle of cloning, through somatic cell nuclear transfer (SCNT) was done by a team of scientists from UAE, Japan, UK and China. Multiple cloned camels from racing, show and dairy exemplars were produced in a recent research. Several parameters were compared including oocyte source, donor cell and breed differences, transfer methods, embryo formation and pregnancy rates and maintenance following SCNT. Researchers successfully achieved 47 pregnancies, 28 births and 19 cloned offspring who are at present healthy and have developed normally. It is first report on cloned camels from surgical embryo transfer and correlated blastocyst formation rates with the ability to achieve pregnancies. There was no difference in the parameters affecting production of clones by camel breed, and showed clear differences on oocyte source in cloning outcomes. Taken together researchers demonstrated that large scale cloning of camels is possible and that further improvements can be achieved. Congratulations to the team P. O. Olsson, Y. B. Son, Y. Jeong, Y. W. Jeong, L. Cai, S. Kim, E. J. Choi, X. Yu, W. S. Hwang, A. H. Tinson, N. Al Shamsi, K. S. Kuhad & R. Singh, K. Sakaguchi.

(Source: Olsson, P.O., Tinson, A.H., Al Shamsi, N. *et al,* Blastocyst formation, embryo transfer and breed comparison in the first reported large scale cloning of camels. Sci Rep 11, 14288; 2021).

This JCPR issue completes 28 years of age and is composed of twenty one manuscripts on diverse speciality of camels. Unique papers include those from CVRL, Dubai, i.e. *Rhodococcus equi* isolated from raw camel milk and 'Alpaca Fever' in dromedary camel calves. The manuscripts on infertility on female camels and DNA barcoding of spermatozoa make good contribution towards reproduction section. Histochemical and immunohistochemical study on the thyroid gland, biomarkers of stress, ultrasonography of the thorax, mold contamination and total aflatoxins in chilled muscle and edible offals, milk somatic cell count, sarcoptic mange, testosterone and growth hormone levels in female dromedary and camel culture with wrestling union in Turkey mark important section of dromedaries. This issue has a distinct segment of six manuscripts based on Bactrian camels. These manuscripts point to the advancement of research on this species. Important manuscripts of this section include transcriptome analysis of renal tubular epithelial cells, evaluation of recombinant antigen rCPI for iELISA detection of camel parabronemiasis, neuroglobin in the Bactrain camel brain, milk-derived exosomes, *In vitro* activity of CYP2J recombinant protease and antioxidant effect of milk on acute alcoholic liver. This issue includes book review and recent news also.

I wish all my authors and members of editorial team a Merry Christmas and Happy New Year 2022. I assure you that the year 2022 will bring a new flavour and colour to the Journal of Camel Practice and Research.

(Dr. T.K. Gahlot)
Editor

SUBSCRIPTION - 2022

FOR

JOURNAL OF CAMEL PRACTICE AND RESEARCH

(Triannual In English Language, April, August and December Issue Every Year)

SUBSCRIPTION RATE - 2022

ANNUAL Rs. 4500/- or US $ 450

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

Subscription Form

I want to become annual subscriber of the **Journal of Camel Practice and Research**, for/from the year 2022 ..... For this purpose I am enclosing herewith a cheque / demand draft number ..... dated ..... for Rs./US $..... in favour of "**Camel Publishing House**". The cheque or D.D. should be payable at State Bank of India, Code No. 7260, Bikaner. Payment may be made through payment portal of website www.camelsandcamelids.com or money transfer to bank account.

Name : .....

Permanent Address : .....

: .....

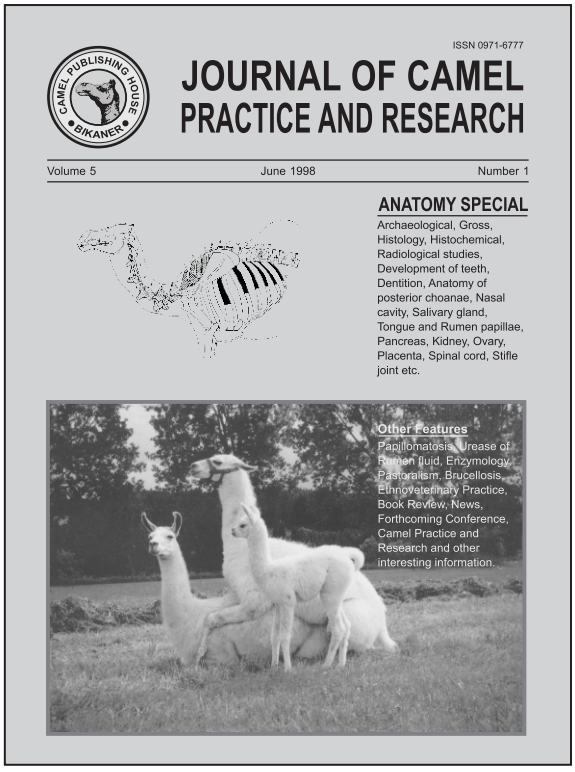
Country : .....

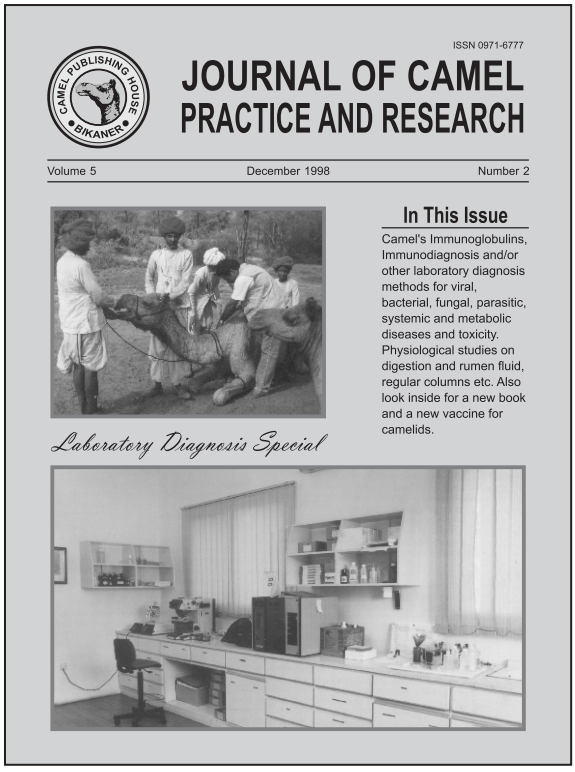
Signature : .....

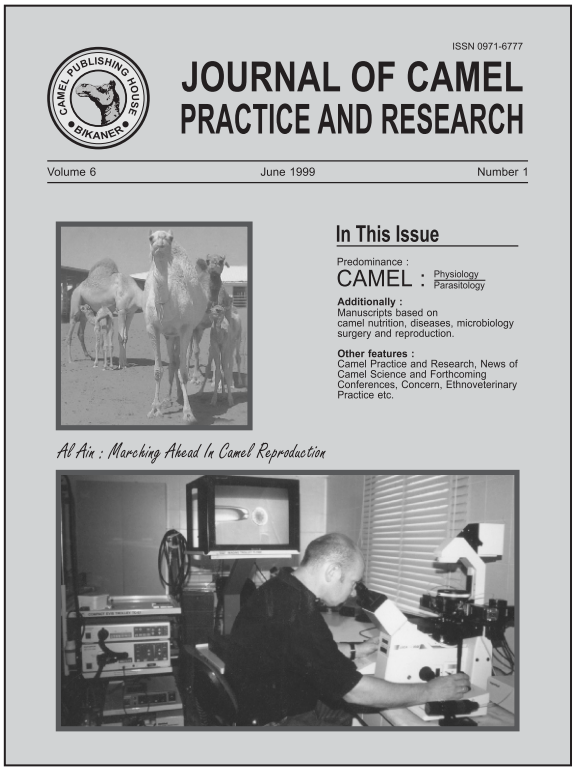
Mail to :

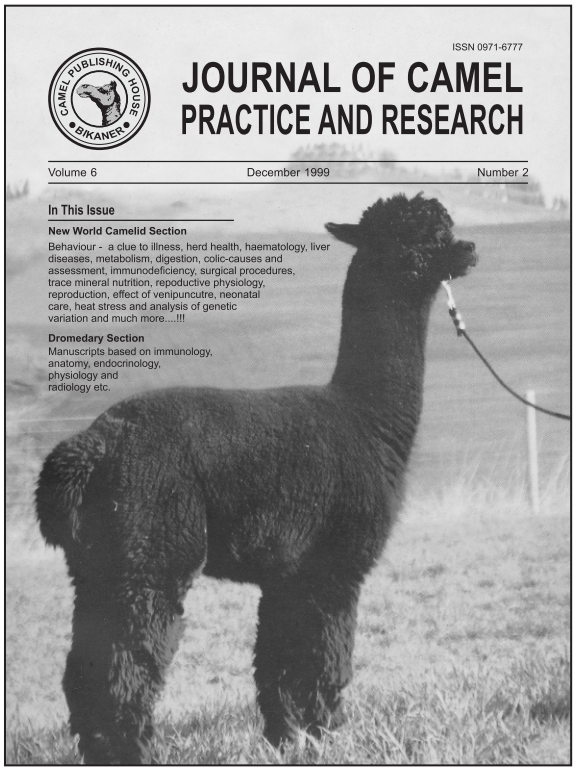
Camel Publishing House
67, Gandhi Nagar West
Near Lalgarh Palace
Bikaner - 334001, INDIA
Phone : 0091-151-2527029
email : tkcamelvet@yahoo.com
website : www.camelsandcamelids.com

BACK ISSUES OF JCPR AVAILABLE





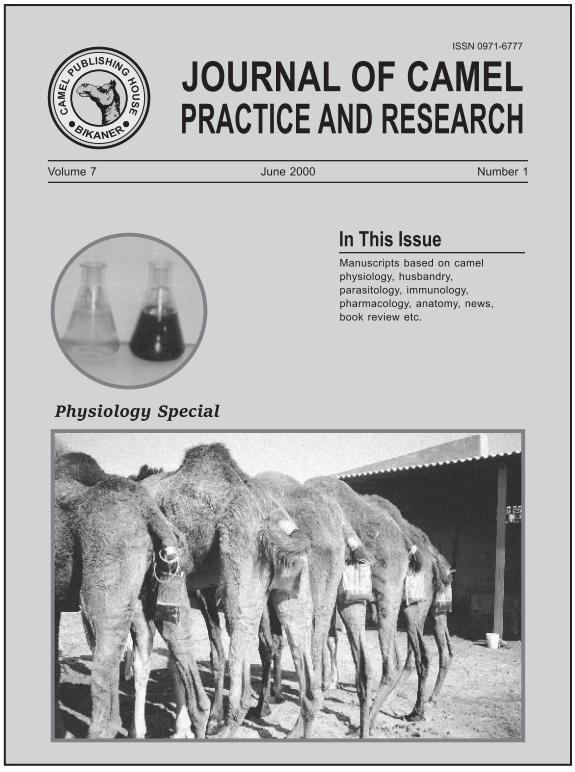


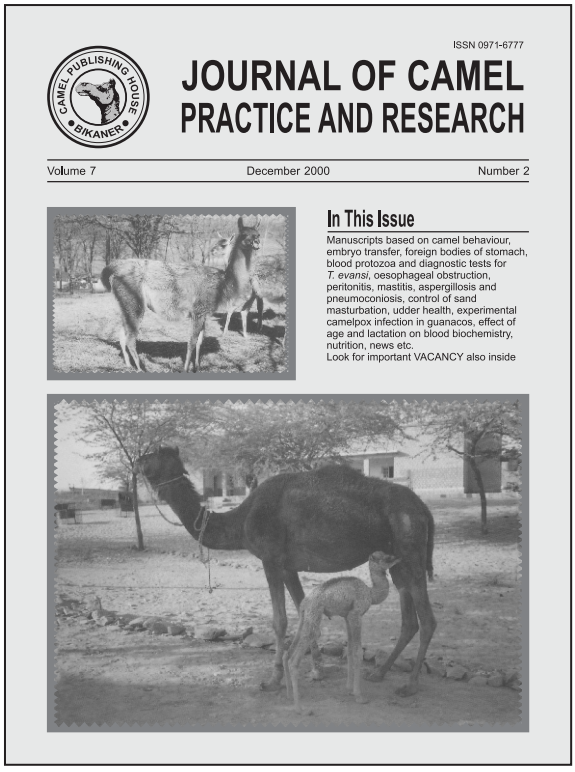


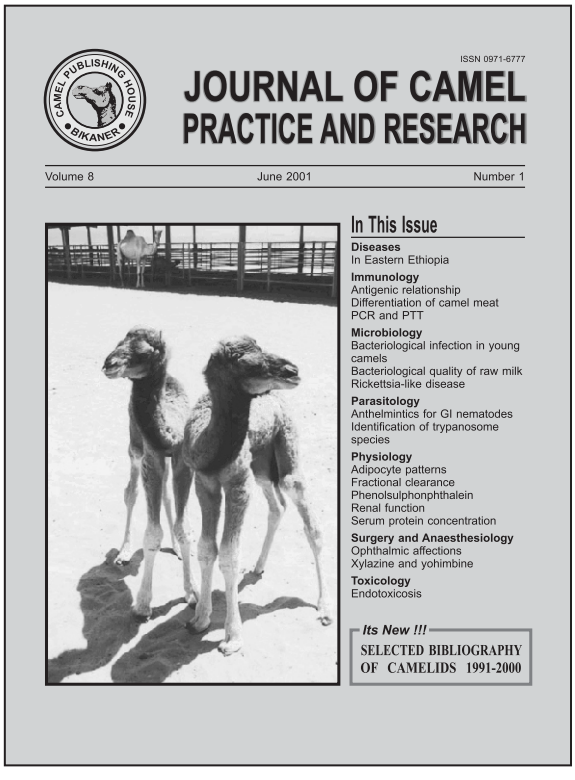
See the details of list of contents of all previous issues of JCPR on our website
www.camelsandcamelids.com

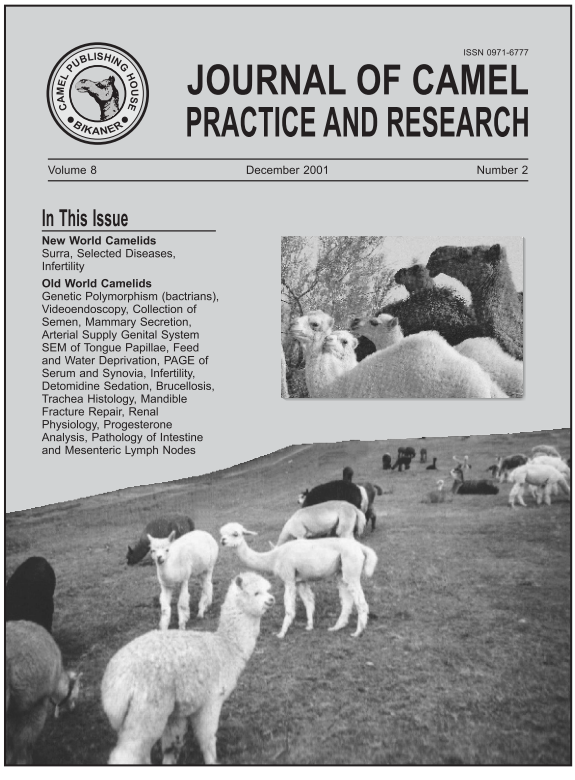
Kindly refer querries to: Dr. T.K. GAHLOT, Editor, JCPR
email: tkcamelvet@yahoo.com

BACK ISSUES OF JCPR AVAILABLE









See the details of list of contents of all previous issues of JCPR on our website
www.camelsandcamelids.com

Kindly refer querries to: Dr. T.K. GAHLOT, Editor, JCPR
email: tkcamelvet@yahoo.com

DOI : 10.5958/2277-8934.2021.00039.4 Vol 28 No 3, p 245-248

TRANSCRIPTOME ANALYSIS OF RENAL TUBULAR EPITHELIAL CELLS OF BACTRIAN CAMEL IN RESPONSE TO HYPEROSMOTIC STRESS

Bin Yang[1], Lihui Chen[1], Dong Ai[2], Lu Chu[3], Changmei Wang[4], Zhuwei Guo[1], Zhiyong Li[5], Tana Han[6] 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, Ministry of Agriculture and Rural Affairs of P. R. China, Hohhot, Inner Mongolia Autonomous Region, 010011, China
[2]Agriculture and Animal Husbandry Bureau of Alxa League,Bayanhot, 750306, China
[3]Brigade of Alxa Left Banner agriculture and animal husbandry comprehensive administrative law enforcement, Bayanhot, 750300, China
[4]Supply and Marketing Cooperative Union, Ejina Banner, Alxa League, Dalaihub, 735400, China
[5]Detachment of Alxa League Agriculture and Animal Husbandry Comprehensive Administrative Law Enforcement, Bayanhot, 750306, China
[6]Comprehensive Security and Technology Promotion Centre of DalaihubTown, Ejina Banner, Alxa League, Inner Mongolia Autonomous Region, 735400, China

ABSTRACT

The transcriptome alterations in renal tubular cells of Bactrian camel treated with NaCl hyperosmotic treatment were investigated. Primary tubular epithelial cells, obtained from kidney of Bactrian camel were treated with medium supplemented with NaCl to a total final osmolality of 600 mOsm. The transcriptome gene expression in primary tubular epithelial cells of Bactrian camel was examined using RNA sequencing technology. 5,114 genes from NaCl supplementation (600 mOsm) and control (300 mOsm) were significantly altered. The up-regulated genes in the 600 mOsm group include solute carrier family, ATP-binding cassette family, sodium channel, potassium channel, calcium channel, Na^+/K^+ ATPase, aquaporin, cytochrome P450 and heat shock protein. Some genes are associated with Bactrian camel's salt tolerance. This study will provide scientific basis to understand the mechanism of Bactrian camel's tolerance to high salt.

Key words: Bactrian camel; salt tolerance; transcriptome; tubular epithelial cells

Camel has evolved characteristics of salt- and drought-resistances to adapt to extreme desert environments (Cao *et al,* 2019). Early research on camel's thirst tolerance has reported that dehydration is associated with increased urine osmolarity, reduced urine production and increased Na excretion (Ben Goumi *et al,* 1993). Nowadays genomics and transcriptome techniques have been used to reveal the camel's resistance to stress. Comparative genomic analysis reveals camel's features related to desert adaptations. Transcriptomic analysis of Bactrian camels further reveals sodium reabsorption and water reservation of kidney in Bactrian camel (Wu *et al,* 2014). Some salt-resistance-related genes in renal cortex of Bactrian camel were identified by RNA-seq (Cao *et al,* 2019). This published paper focused on Non-coding RNAs *in vivo* experiment. However, transcriptional regulation in renal tubular epithelial cells of Bactrian camel in response to hyperosmotic stress is still not clear. In this study, we analysed gene transcription difference in renal tubular epithelial cells of Bactrian camel under hyperosmotic stress using RNA-seq to understand the mechanism of Bactrian camel's tolerance to high salt.

Materials and Methods

All the procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Inner Mongolia Agricultural University (12150000460029509N). Primary tubular epithelial cells (PTECs) were isolated from the kidney cortex segment of healthy Bactrian camel. The cortical fragments were incubated for 30 min at 37°C in a buffer containing 0.1% collagenase type I. The preparation was washed and filtered through a cell strainer. The obtained cells were incubated with cell

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

culture medium (300 mOsm) supplemented with NaCl to a total final osmolality of 600 mOsm in the medium. Control cells were cultured 24 h in cell culture medium (300 mOsmol).

After 24 h exposure to hyperosmotic medium, Total RNA was extracted from PTECs from isoosmotic and hyperosmotic groups using TRIzol reagent. The quality and quantity of the extracted RNA samples were determined with the NanoPhotometer spectrophotometer and the Bioanalyser 2100 system. Pooled RNA from samples was used for library preparation. The mRNA sequencing libraries were constructed by the IlluminaTruSeq RNA preparation kit. The samples were sequenced on the Illumina Hiseq 2500 platform (Novogene, Beijing, China) with125 bp/150 bp paired-end reads. Data quality was checked using the fastq software. The reads were compared with Bactrian camel genome and treated by Hisat2. Differential gene expression analysis was performed by the DESeq2 R package. For statistical analysis, two comparisons (hyperosmotic vs. isoosmotic) were analysed by *p* value and false discovery rate (*q* value).

Results

Transcriptomic analysis of PTECs after 24 h treatment with NaCl supplemented medium (total medium osmolality: 600 mOsm) was investigated using RNA-sequencing. Using |log2 fold change|>1 and *p* values<0.05 as a threshold, a total of 5,114 differentially expressed genes (DEGs) were identified. 3627 genes were up-regulated and 1487 genes were down-regulated in NaCl treated cells.

73 solute carriers (SLC) were up-regulated and 26 SLC were down-regulated in NaCl treated cells compared to the control (300 mOsm). The 5 up-regulated ATP-binding cassette family (ABC) were ABCC2, ABCC11, ABCC12, ABCG8 and ABCG5. The 4 down-regulated ABC were ABCA6, ABCA10, ABCC9 and ABCA3 (Table 1). The 5 up-regulated sodium channel (SCN) were SCN4A, SCN3A, SCNN1D, SCN9A and SCN10A. 33 potassium channel (KCN) and 10 calcium channel (CACN) were up-regulated in NaCl treated cells. The 4 up-regulated Na^+/K^+ ATPase were ATP1A3, ATP1B2, ATP1B4 and ATP1B3. The 1 up-regulated Ca^{++} ATPase was Ca^{++} ATPase plasma membrane 2. The 6 up-regulated aquaporin (AQP) were AQP6, AQP10, AQP8, AQP2, AQP1 and AQP3 (Table 2). 15 cytochrome P450 (CYP) were up-regulated and 2 CYP were down-regulated in NaCl treated cells (Table 3). 4 heat shock protein (HSP) and 3 small heat shock protein (sHSP) were up-regulated in NaCl treated cells.

Table 1. Up-regulated and down-regulated ATP-binding cassette family (ABC) in hyperosmotic treated PTECs of Bactrian camel.

Gene symbol	Gene name	log_2 fold change	*p* value
ABCC2	ATP-binding cassette sub-family C member 2	4.045	1E-38
ABCC11	ATP-binding cassette sub-family C member 11	3.804	2E-08
ABCC12	ATP-binding cassette sub-family C member 12	3.470	0.012
ABCG8	ATP-binding cassette sub-family G member 8	2.302	8E-03
ABCG5	ATP-binding cassette sub-family G member 5	1.722	0.021
ABCA6	ATP-binding cassette sub-family A member 6	−1.409	9E-03
ABCA10	ATP-binding cassette sub-family A member 10	−1.274	9E-04
ABCC9	ATP-binding cassette sub-family C member 9	−1.139	8E-11
ABCA3	ATP-binding cassette sub-family A member 3	−1.105	3E-25

Table 2. Up-regulated AQP in hyperosmotic treated PTECs of Bactrian camel.

Gene symbol	log_2 fold change	*p* value
AQP6	4.128	8E-03
AQP10	3.519	8E-12
AQP8	3.361	0.023
AQP2	2.462	7E-03
AQP1	1.431	6E-05
AQP3	1.053	4E-13

Discussion

The up-regulated SLC in NaCl treated cells included SLC12 family of sodium/potassium/chloride transporter, SLC4 family of bicarbonate transporters, SLC26 family of anion transporters, SLC2 family of facilitated glucose transporters and SLC38 family of amino acid transporters.

The multidrug resistance-associated protein 2 (MRP2/ABCC2) is a transporter that belongs to the ATP-binding cassette (ABC) superfamily. The multidrug resistance-associated protein 2 (MRP2/ABCC2), MRP11/ABCC11, MRP12/ABCC12 and MRP9/ABCC9 belong to the ATP-binding cassette (ABC) superfamily. ABCC1 has been reported to play a significant role in the protection of kidney epithelial cells against the stress caused by high sodium environment (Fonseca *et al*, 2013). Multidrug resistance-associated proteins may play crucial roles in the protection of internal organs, particularly the

kidney and liver, from various toxic agents (Pazik, *et al,* 2009). In the present study, hyperosmolality up-regulated ABCC2, ABCC11 and ABCC12. These findings suggested that some ABC transporters are related to the protection of the kidney against hyperosmolality.

Table 3. Up-regulated and down-regulated CYP in hyperosmotic treated PTECs of Bactrian camel.

Gene symbol	Gene name	log_2 fold change	*p* value
LOC105081748	cytochrome P450 2C23-like	5.651	1E-06
LOC105062873	cytochrome P450 2G1	5.225	1E-04
LOC105081809	cytochrome P450 2C21-like	5.002	9E-06
LOC105077059	cytochrome P450 3A12-like	4.365	2E-04
LOC105077600	cytochrome P450 3A29-like	3.772	2E-03
LOC105069191	cytochrome P450 1A2	3.356	2E-08
LOC105081827	cytochrome P450 26A1	3.110	4E-08
LOC105062871	cytochrome P450 2F1	2.998	2E-03
LOC105064834	cytochrome P450 2J2-like	2.357	0.036
LOC105067942	cytochrome P450 2U1	2.219	3E-08
LOC105068581	cytochrome P450 27C1	2.213	1E-03
LOC105077048	cytochrome P450 3A29-like	2.193	0.017
LOC105074543	cytochrome P450 4F22	2.093	2E-14
LOC105082362	cytochrome P450 1A1-like	2.037	3E-05
LOC105078126	cytochrome P450 2W1	1.371	0.013
LOC105067667	cytochrome P450 4V2	−1.508	3E-19
LOC105062914	cytochrome P450 2S1	−1.125	2E-08

The epithelial sodium channel (ENaC) plays a crucial role in salt and water homeostasis and is primarily involved in sodium reabsorption in the kidney (Chen *et al*, 2015). KCN mediate the K+ flux into the tubular lumen and Na+ reabsorption in the kidney (Welling and Ho, 2009). The Na^+/K^+ ATPase is the driving power for renal Na+ reabsorption (Khalaf *et al,* 2018). Na^+/K^+ ATPase also serves as the motor for the generation of the corticopapillary osmotic gradient that drives water reabsorption (Feraille and Doucet, 2001). The up-regulated SCN, KCN and Na^+/K^+ ATPase in NaCl treated cells indicate that the kidney of Bactrian camel is able to reabsorb large amounts of water to maintain water homeostasis in a dehydrated environment.

Aquaporins (AQP) serve as channels in the transfer of water and small solutes across the membrane (Takata *et al,* 2004). AQPs 1, 2, 3, 4, 6, 7, 8, and 11 are expressed in the kidney; of these, AQPs 1, 2, 3, 4, and 7 are shown to be involved in fluid homeostasis (Nishimura and Yang, 2013). AQP3 is regulated by thirst, arginine vasopression (AVP) and aldosterone (He and Yang, 2019). In present study, AQP3 with high FPKM value is highly expressed in renal tubular epithelial cells of Bactrian camel. The up-regulated AQP 1, 2, 3 ensure that Bactrian camel can reabsorb water in a water-deficient environment.

Genomic analysis revealed that Bactrian camel's salt tolerance may be due to having more copies of the *CYP2J* gene than other animals (Jirimutu *et al,* 2012). In present study, *CYP2J2-like* gene was up-regulated in NaCl treated cells. Moreover, *CYP 2C23-like, CYP 1A2, CYP 1A1-like, CYP 3A12-like* and *CYP 3A29-like* were also up-regulated under high salt treated condition. It has been reported that Nicardipine is used for anti-hypertension drug through increasing the expression of hepatic CYP isoforms such as CYP 1A1, CYP 1A2 and CYP3A subfamily (Miyajima *et al,* 2007). Another study reported that decreased renal CYP 2C enzymes including CYP 2C23 are associated with angiotensin salt-sensitive hypertension (Zhao *et al,* 2003). These findings suggested that some CYP genes play an important role in Bactrian camel's tolerance to a high-salt diet.

Stress proteins play a key role in the development of stress tolerance. αB-crystallin and HSP70 can be significantly upregulated by hyperosmotic stress in the mouse myocardial microvascular cell line and in the murine inner medullary collecting duct-3 (mIMCD3) cell line (Golenhofen *et al,* 2002; Valkova and Kultz, 2006). In present study, βA2-crystallin, βB2-crystallin, α-crystallin-related small heat shock protein B9 (HSPB9) and several HSP70 isoforms were up-regulated in NaCl treated cells. These findings suggested that stress proteins may be associated with Bactrian camel's salt tolerance.

Acknowledgements

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

References

Ben Goumi M, Riad F, Giry J, de la Farge F, Safwate A, Davicco MJ and Barlet JP. Hormonal control of water and sodium in plasma and urine of camels during dehydration and rehydration. General and Comparative Endocrinology. 1993; 89(3):378-386.

Cao Y, Zhang D and Zhou H. Key genes differential expressions and pathway involved in salt and water-

deprivation stresses for renal cortex in camel. BMC Molecular Biology. 2019; 20(1):11.

Chen MX, Gatfield K, Ward E, Downie D, Sneddon HF, Walsh S, Powell AJ, Laine D, Carr M and Trezise D. Validation and optimisation of novel high-throughput assays for human epithelial sodium channels. Journal of Biomolecular Screening. 2015; 20(2):242-253.

Feraille E and Doucet A. Sodium-potassium-adenosinetriphosphatase-dependent sodium transport in the kidney: hormonal control. Physiological Reviews. 2001; 81(1):345-418.

Fonseca LM, Alvarez AB, Rodrigues RC, Santos DH, Lopes AG and Capella MA. ABCC1 is related to the protection of the distal nephron against hyperosmolality and high sodium environment: possible implications for cancer chemotherapy. PLoS One. 2013; 8(6):e68049.

Golenhofen N, Ness W, Wawrousek EF and Drenckhahn D. Expression and induction of the stress protein alpha-B-crystallin in vascular endothelial cells. Histochemistry and Cell Biology. 2002; 117(3):203-209.

He J and Yang B. Aquaporins in Renal Diseases. International Journal of Molecular Sciences. 2019; 20(2).

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 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, Zhao L, Zhao W, Liu B, Li C, Tang X, Guo C, Liu W, Ming L, Temuulen, Cui A, Li Y, Gao J, Wurentaodi, Niu S, Sun T, Zhai Z, Zhang M, Chen C, Baldan T, Bayaer T and Meng H. Genome sequences of wild and domestic Bactrian camels. Nature Communications. 2012; 3:1202.

Khalaf FK, Dube P, Mohamed A, Tian J, Malhotra D, Haller ST and Kennedy DJ. Cardiotonic Steroids and the Sodium Trade Balance: New Insights into Trade-Off Mechanisms Mediated by the Na(+)/K(+)-ATPase. International Journal of Molecular Sciences. 2018; 19(9).

Miyajima S, Nemoto K, Sekimoto M, Kinae Y, Kasahara T, Souma S and Degawa M. Induction of hepatic cytochrome P450 isoforms by nicardipine at therapeutic doses in spontaneously hypertensive rats. The Journal of Toxicological Sciences. 2007; 32(1):79-90.

Nishimura H and Yang Y. Aquaporins in avian kidneys: function and perspectives. The American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2013; 305(11):R1201-1214.

Pazik J, Oldak M, Sitarek E, Lewandowski Z, Maksym R, Slubowska K, Ploski R, Malejczyk J and Durlik M. Multidrug resistance-associated protein 2 gene (ABCC2) variant in kidney allograft recipients. Transplantation Proceedings. 2009; 41(8):3009-3010.

Takata K, Matsuzaki T and Tajika Y. Aquaporins: water channel proteins of the cell membrane. Prog Histochem Cytochem. 2004; 39(1):1-83.

Valkova N and Kultz D. Constitutive and inducible stress proteins dominate the proteome of the murine inner medullary collecting duct-3 (mIMCD3) cell line. Biochimica et Biophysica Acta. 2006; 1764(6):1007-1020.

Welling PA and Ho K. A comprehensive guide to the ROMK potassium channel: form and function in health and disease. American Journal of Physiology - Renal Physiology. 2009; 297(4): F849-863.

Wu H, Guang X, Al-Fageeh MB, Cao J, Pan S, Zhou H, Zhang L, Abutarboush MH, Xing Y, Xie Z, Alshanqeeti AS, Zhang Y, Yao Q, Al-Shomrani BM, Zhang D, Li J, Manee MM, Yang Z, Yang L, Liu Y, Zhang J, Altammami MA, Wang S, Yu L, Zhang W, Liu S, Ba L, Liu C, Yang X, Meng F, Li L, Li E, Li X, Wu K, Zhang S, Wang J, Yin Y, Yang H and Al-Swailem AM. Camelid genomes reveal evolution and adaptation to desert environments. Nature Communications. 2014; 5:5188.

Zhao X, Pollock DM, Inscho EW, Zeldin DC and Imig JD. Decreased renal cytochrome P450 2C enzymes and impaired vasodilation are associated with angiotensin salt-sensitive hypertension. Hypertension. 2003; 41(3 Pt 2):709-714.

DOI : 10.5958/2277-8934.2021.00040.0 *Vol 28 No 3, p 249-263*

FEEDS AND FEEDING OF DROMEDARY CAMELS: A REVIEW

Lokesh Gupta
Department of Animal Production Rajasthan College of Agriculture
Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan 313001, India

ABSTRACT

Camels are remarkable animals that have evolved with a ruminant like digestive system to enable them to survive on low quality feeds. Being browsers, camels are able to select high quality diets, which they can efficiently digest. Camels are pseudo-ruminants, with a simple chambered fore-stomach, and are unlike the four chambered stomach found in cattle and sheep. Nevertheless, camels can digest high fibre feeds via fermentation pathways similar to those in true ruminants. The camel can survive on all sorts of vegetation including shrubs, weeds, grasses, tree leaves etc. and maintains their body condition. But camel usually prefers to browse (feeding tree leaves and twigs) rather than to graze particularly when green grasses are available. The crude protein content of grasses ranges from 5.9 to 10.2% except blue panic (*Panicum antidotale*) which had 15.6% CP contents. The dromedary camels spend 6-12 hours grazing daily under natural range conditions and plant matter intake varies from 5 to 55 kg/d depending on the season and feed availability. The camels have lower energy requirements than ruminants, and have evolved an efficient mechanism for nutrient recycling. Vitamins are included in feed supplement of livestock and poultry but in most of camel farming systems, only vitamins present in the natural diet are available for the animals. The mineral deficiencies which are widely present in domestic species notably may occur in camel both for major or minor elements. In general, the camels under rangelands feeding systems are not fed sufficiently to meet their nutrient requirements for pregnancy, lactation and growth, therefore dromedary camels should be supplemented with concentrate mixture during physiological conditions.

Key words: Dromedary camels, feeds, feeding, nutrient requirements

Camels (*Camelus dromedarius*) are reared by nomadic pastoralists mostly in marginal eco-zones of semi-desert lands in India and world. However, these systems are undergoing rapid adaptive changes and transformations to cope with emerging demographic and economic factors (Hashi, 1991). The methods of camel keeping are now fast changing due to the shrinkage of natural grazing land as a result of the establishment of mechanised irrigated or rain-fed agricultural schemes in parts of the natural camel range lands as well as the very severe and historical drought that hit several camel producing countries. The long run impact of natural disasters had aggravated the situation and forced many camel herders to start settling even nearby cities (Abbas and Omer, 2005). With its unique bio-physiological characteristics, the camel has become an icon of adaptation to challenging ways of living in arid and semi-arid regions because of their unique features of adaptability, survivability and draught performance under adverse climatic conditions (Nagpal and Jabbar, 2005). Thus, camels have a vital role in the subsistence economy of large sectors of rural pastoral communities. Camels are good source of draught, milk and they constitute the most important source of meat in arid areas (Knoess, 1977 and Farah *et al,* 1992).

Moreover, to keep pace with the alarming nutritional crisis, to make the ration economic for sustainable camel production, lot of research has been carried out in India and abroad to formulate rations for camels using conventional and non-conventional feed resources. In the current review, an attempt has been made to present a comparative account of different aspects of camel nutrition for sustainable production.

Digestive Behaviour of Dromedary Camels

Digestive System: In spite of the fact that the camel ruminates, its ingested feeds are subject to microbial digestion and the final metabolic products are similar to those as in true ruminants, it is classified as pseudo-ruminant, but this classification is mainly due to the significant differences in the structure and function of the digestive system of camelids (Tylopods) and the true ruminants (Bhattacharya, 1986).

SEND REPRINT REQUEST TO LOKESH GUPTA email: lokgupta76@gmail.com

The rumen of the camel is characterised by its unique exterior glandular sacs which secret a mucus like substance that differs in composition from rumen liquid. The third compartment is absent from the honey comb like structure which is not distinctively separated from the fourth compartment. Te camel does not have a gall bladder. Camels digest dry matter and crude fibre of range plants (El-Shami, 1985), alfalfa (Bhattacharya *et al,* 1985), straw and trifolium (Gihas *et al,* 1988) better than ruminants. This high dry matter and crude fibre digestibility was attributed to the unique movement of the forestomach and the longer retention time of the large feed particles in the fore-stomach of the camel (Engelhardt *et al,* 1988). Digestibility of proteins of feedstuffs was found lower in camels than sheep. However, camels utilise proteins better than sheep or goats in case of poor feeds such as straw, mainly due to urea recycling. Camels retain higher amounts of nitrogen (19.87%) than sheep (15.14%) and goats (12.68%) from the same diet. The proportion of retained nitrogen to digestible protein was 42.17% in camels, 32.63% in sheep and 27.98% in goats (Gihad *et al,* 1988).

Gordon and McAllister (1970) found a 24 h-period rhythmicity for rumination in sheep, with higher values observed in the second half of a 12 h dark period. In camels, it has been reported that rumination starts after midnight and lasts until 8:00, reaching peak values between 1:00 and 6:00 (Kaske *et al,* 1989) or between 4:00 and 7:00 (Iqbal and Khan, 2001). The acrophase of lying down and rumination considered as inactivity as for Bushbuck (Wronski *et al,* 2006) occurred at night whereas the acrophase of activities (feeding, stereotypy and walking) occurred during the light phase of the day and more precisely, around midday.

Rumen Microbiology and Physiology: The camel is a pseudo-ruminant with a forestomach differentiated into three compartments referred to as C1-equivalent to the rumen, C2-reticulum and the gastric secreting compartment C3-abomasum (Engelhardt *et al,* 2007). Similar to other ruminants, the camel rumen is an enlarged anaerobic fermentation chamber which houses a complex microbial community consisting of bacteria, archaea, protozoa and fungi (Flint, 1997 and Samsudin *et al,* 2012). The complex microbial community inhabiting the rumen has an obligate symbiotic association with the host animal, since plant lignocellulosic compounds by themselves are indigestible for the host digestive system.

The pH of camel's rumen (6.7) is generally higher than that of sheep (6.5) and goats (6.4) and less affected by diurnal variations due to higher buffering capacity due to the presence of higher concentrations of phosphate (18.3 mEq/L) and bicarbonate (100.4 mEq/L) ions in saliva and its high alkaline nature having pH 8.06 to 8.23 (Bhatia, 1996). In cattle, buffaloes, sheep and goats, the feed is mixed thoroughly into uniform digesta in the rumen before moving into the small intestine. In camel, the small feed particles in the rumen fluid are continuously removed and moved into the intestine while larger feed particles are retained for fine grinding and digestion. Retention time of rumen digesta has been estimated to be 46-50 hours (Bhatia *et al,* 1988; Engelhardt *et al,* 1988 and Maloiy, 1972), which is longer than retention time in cattle, sheep and goats. Higher retention time of digesta is advantageous to camel for ensuring increased microbial digestion and efficient utilisation of energy. Comparative studies between camel and sheep by Farid *et al* (1979) revealed that camel needed less water per unit dry matter intake on per unit body mass and thus are superior in water conservation during water deprivation and they also reduced water loss in faeces. The camel could digest dry matter and crude state of nitrogen better than sheep when maintained on similar diets. Nitrogen retention is further improved in camel as compared to sheep under water restriction in camel.

Watering Frequency

Harshi and Kamoun (1995) reported that environmental factors (thermal environment, level of dehydration, etc.) affect the intake capacity. The camel obtains most of its water requirements, for extended periods of time, from food selecting more succulent vegetation (Wilson, 1989). For this reason, together with its particular physiological characteristics, the camel is able to maintain appetite under conditions of dehydration. When the basal feed resources are mostly straw and stovers and other low quality roughages, there is little opportunity for those capacities of selective browsing of succulent vegetation. Camel may not tolerate restricted access to drinking water as may be the case under natural conditions. In an attempt to determine the effect of the frequency of watering on the voluntary intake of low quality roughages, adult female camels were offered wheat straw with-concentrate supplementation (2 kg per head per day) and subjected to infrequent watering. There was a gradual reduction in roughage intake as the distance from the last watering date increased. The depression in wheat straw consumption after 5 days without water was up to

25% even with the concentrate supplementation. However, even in this case, the camel economised water use, daily water intake decreased from 60 ml per kg lw0.82 when the non-lactating animals were watered daily to about 25 ml per kg lw0.82 after 5 days of water deprivation (Harshi and Kamoun, 1995). Nevertheless, the continued use of such poor quality roughages in camel feeding, calls for more frequent watering. Burgos *et al* (2001) reported that reducing water intake decreases saliva production and thus, the food intake decrease immediately after watering restriction.

Nutritive Value of Feeds and Fodders Fed to Dromedary Camels

Feeding of camel in his native land is the easiest and cheapest method of rearing compared with other domesticated animals. Nature has bestowed upon him the ability to digest any coarse bush or tree leaf, dry as well as green (Rathore, 1986). In general, camels at rest thrive solely on grazing and browsing, but when there are no facilities for sending the animals outside, or under lactation, or under hard work, stall feeding is required to provide the nutrients. In summer and rainy season, shrubs, bushes and trees provide the maximum nutrients but in winter, supplementary ration including feeding of grains becomes essential. The camel can survive on all sorts of vegetation including shrubs, weeds, grasses, tree leaves etc. and maintains their body condition (ICAR, 2013a). But camel usually prefers to browse (feeding tree leaves and twigs) rather than to graze particularly when green grasses are available. Camel usually require about 4 to 6 hours per day for consuming their feed and another 4 to 6 hours for ruminating the feed (Banerjee, 2016). The camel feeds can be divided into three groups viz., green fodders (green grasses, weeds, vines, leaves of shrubs, bushes and trees); dry roughages (straws of cultivated crops left after threshing) and concentrates (oil cakes, all types of grains, oils, fats etc.).

Green Fodders:

The camel graze or browse on green grasses like Siwan (*Lasiurus sindicus*), vines such as *Momordica dioica* (kakoda), *Tribulus terrestris* (gokhru), *Citrullus colocynthis* (indrayan) etc.; green leaves as shrubs such as *Solanum indicum* (oont kateri), *leptadenia spartium* (khimp), *Aerus tomentosa* (bui), *bushes like Calligonum polygonoides* (phog), *Capparis aphylla* (ker), *Salvadora oleoides* (khar), *Salvadora persica* (pilu), *Ziziphus rotundifolis* (pala or jharberi), *Ziziphus jujuba* (ber), *Prosopis spicigera* (khejra), *Prosopis juliflora* (pardesi khejri), *Acacia arabica* (babool), *Acacia catechu* (khair), *Acacia senegal* (kumta), *Albiza lebbeck* (siris), *Azardirachta indica* (neem). The green leaves of *Ficus religiosa* (peepal), *Ficus glonerata* (gular), *Mangifera indica* (mango), *Dalbergia sissoo* are also fed to camels in drought situation when there is nothing else to feed (Rathore, 1986 and ICAR, 2013a). When camels are in the fields they graze upon crops as moth (*Phaseolus acontifolius* Jacq.), guar (*Cyamopsis tetragonoloba*) and sorghum (*Sorghum bicolor*) but no one raises any crop especially for camels. In certain cases when a working animals gets weak and his condition becomes poor, the animals are also fed on green moth, green guar with grains, lucerne (*Medicaco sativa* L.) and white clover (*Trifolium repens*) so that the animal improves in health.

Grasses: ICAR (2013b) reported that grasses contained 29.3 to 46.6% DM with CP contents ranging from 5.9 to 10.2% except Blue Panic (*Panicum antidotale*) which had 15.6% CP contents. Among the grasses, Boor grass had low CP value of 7.3 while Grammna was highly promising with CP value of 15.6%. Sewan had low EE contents of 1.4 and Grammna had 4.8% EE. Bhurut grass had low CF contents of 20.25% while Sewan had high CF value of 33.25%. Boor grass contained low ash contents of 7.5% and Dachab grass had high ash contents of 20.5%. The NFE contents were minimum in Dhaman grass (39.89%) and maximum in Bhurut grass (51.5%). The nutritional value of grasses is affected by maturity of plants. With increase in the maturity, the DM contents of sewan grass increased from 30.6% to 78.3% with continuous fall in CP, EE and ash contents while NFE contents showed increase. The common grasses found in desert rangelands are available only for short periods of rainy season and disappear soon after the rainy season is over.

Creepers/Shrubs/Bushes: The common feeds in this category available for camels are phog leaves and khemp (ICAR, 2013a). The DM contents varied between 25.9% in Phog leaves to 30.3% in Kheemp at vegetative stage but the Pala leaves had highest DM contents of 53.2%. The crude protein contents were lowest with 10.5% in Kheemp and highest in Murali kakani (23.4%) indicating their rich nutritional worth (ICAR, 2013a). The EE contents varied between 1.6% in Bakeria/Phog and 3.8% in Pala leaves at flowering stage (ICAR, 2013a). Lowest crude fibre contents of 9.4% in Murali kakani and highest (21.75%) were observed in Phog. Crude fibre value in Kheemp could not estimate due to its tough stem texture. Phog contained low ash value of 9.5% and Bakeria leaves

contained high ash contents of 20.95%. Gokhru leaves had minimum NFE contents of 45.31% and Phog had maximum NFE contents of 56.56% (ICAR, 2013a).

The chemical composition of Tunisian browse fodder species is likely to have an impact on the consumption by the camels, as found by Osuga *et al* (2008) for Kenyan browse foliages by goats and sheep. *Limonium pruinosum, Retama raetam and Stipa tenacissima* browse species are generally considered of intermediate nutritional quality because they contain 6.2-14.7 g/kg DM of CP and less than 50 g/kg DM of NDF in *L. pruinosum, R. raetam* (Laudadio *et al,* 2009). The good CP content of the browse foliage justifies the use of browse foliages to supplement poor quality natural pastures and crop residues such as straw (Osuga *et al,* 2008). The low to moderate fibre content of browse foliages would positively influence the intake and digestibility of the foliages (Bakshi and Wadhwa, 2004). The fibre of the browse foliages has also been shown to be more digestible than that of grasses and crop residues (Diatta *et al,* 2008). Hence, when used as supplements, the foliages may improve the digestibility of low nutritional quality basal forages by providing easily fermentable non-structural carbohydrates and rumen degradable nitrogen (Osuga *et al,* 2008).

Top feeds: The chemical composition of tree leaves shows fairly constant nutritional values. The DM contents of tree leaves ranged between 30.5-41.5% with exception of Kikar leaves. The crude protein values varied between 15.8% in Jal to as high as 30.1% in Siras leaves. The EE contents ranged from 2.0% in Jal leaves to 7.0% in Siras. Crude fibre contents ranged from the lowest value (7.5%) in Siras leaves to as high as 20.7% in Khejri leaves. The ash contents were lowest in Siras leaves and high in Jal leaves (27.5%). The NFE contents were minimum in Khejri leaves (41.5%) and maximum in Kikar leaves (62.1%). In the semi arid and arid regions, it is the general practice to harvest Khejri (*Prosopis cineraria*) and Pala (*Zizyphus nummularia*) and other tree leaves during winter months, sun dried and stored for use as supplementation to the livestock during feed scarcity period. The tree leaves are extensively given to camels, sheep and goats during draught and scarcity periods which apart from providing maintenance ration can provide extra nutrients for production. Tree and shrub leaves in early stages of growth are fairly rich sources of proteins, contain very less fibre, though with the progressive plant growth and maturity, there is an decrease in the nutritional value and increase in fibre and tannic acid contents. The tannic acid renders the proteins of the leaves unavailable to the animals but camel has remarkable ability to digest tree leaves which needs to be explored (ICAR, 2013a).

In 2007, Towhidi and Zhandi studied the chemical composition of nine plant species fed to dromedary camels in Iran. The highest crude protein content was in *Alhagi camelorum,* and the lowest fibre content (NDF, ADF) were in *Haloxylon ammodendron.* The lowest crude protein was from *Hammada salicornica* and the highest fibre (NDF, ADF) were from *Halostachys* spp.

Dry Roughages:

In summer, camels are fed dry fodder and some concentrates. The dry fodder for camels consists of tree leaves such as khejri and pala leaves or grain crop straws left after threshing (Rathore, 1986). When straw of two leguminous crops such as moth (*Phaseolus aconifolius*) and gram (*Cicer arietinum*) or moth and green gram (*Vigna radiata* L.) is mixed it is called missa bhoosa. If camel owners have to purchase straw for feeding, they prefer the straw of moth. Besides straw, dry straw of grain crops left after harvesting is also fed to camels. These straws are chaffed into small pieces and fed to camels mixed with dried leaves or straw of legume crops in summer. The mixture of chaffed siwan grass (*Lasiurus indicus*) and straw is considered to be excellent feed for camels because the nutritive value of the mixture is greatly enhanced due to high protein content of siwan grass (*Lasiurus indicus*). Farmers also mix the straw with dried leaves of khejri (*Prosopis cineraria*) and pala (*Zizyphus nummuleria*) which are considered as very palatable and nutritive ration for the camels (Rathore, 1986). Khejri and Pala had 22.95 and 13.78% CP, 2.91 and 1.74% EE, 17.50 and 30.66% CF, 43.48 and 44.19% NFE and 8.06 and 9.63% TA, respectively on dry matter basis (Sharma and Dhuria, 2007).

The camels relish and prefer the straws in the order of groundnut haulms (*Arachis hypogea*), moth straw (*Phaseolus aconifolius*), gram straw (*Cicer arietinum*), green gram straw (*Vigna radiata* L.) and guar straw (*Cyamposis tetraganoloba*) (Rathore, 1986). Gupta *et al* (2008) conducted experiments on camels fed on moth straw (*Phaseolus aconitifolius*) and reported that moth straw contained 88.77% OM, 10.10% CP, 35.62% CF, 1.20% EE, 40.85% NFE and 11.23% TA on dry matter basis. However, Sharma and Dhuria (2007) noted 93.40% DM, 10.73% CP, 1.73% EE, 25.19% CF, 48.39% NFE and 14.11% TA in moth straw. The chemical composition of gram

straw was complied by Gupta *et al* (2011) and Sharma and Dhuria (2007) and noted that the CP, CF and EE content were varied from 5.99-6.51, 24.15-45.00 and 0.98-1.82%, respectively. Nagpal *et al* (2011) presented the chemical composition of guar phalgati (*Cymopsis tetragonoloba*) as 6.99% CP, 0.54% EE, 31.89% CF, 47.57% NFE and 13.01% TA on dry matter basis. In 2020, Gupta conducted experiments for evaluation of total mix ration with different proportions of roughages in draught camels and reported that crude protein (CP) content was 6.97 per cent in gram straw while, it was 6.61 per cent in soybean straw. Gram straw and soybean straw had ether extract (EE) of 1.21 and 1.52 per cent, respectively. Crude fibre (CF), nitrogen free extract (NFE) and organic matter (OM) were higher in soybean straw as compared to gram straw while, total ash (TA) was more in gram straw as compared to soybean straw.

Concentrates:

The concentrates given to camels consists of crushed grains of moth (*Phaseolus aconifolius*), gram (*Cicer arietinum*), guar (*Cyamposis tetraganoloba*), sorghum (*Sorghum bicolor*), bajra (*Pennisetum typhoides* L.) and black gram (*Vigna mungo* L.). Generally camel owners do not feed their animals with concentrates except when in a run down condition or during working period. During run down condition, farmers feed some bajra flour (*Pennisetum typhoides* L.) or barley (*Hordeum valgare* L.) flour along with molasses for some days till the animal regains his condition. In winter season, sometimes 200 to 250 gm of mustard (*Brassica juncea* L.) or sesame (*Sesamum indicum* L.) oil or 0.5 to 1 kg of oil cake is given to the animals (Rathore, 1986). When the camel is put to hard work, milk fat and milk are also given. Depending upon the work and season, camels are provided with special preparations made of Ajwain (*Trachyspermum ammi* L.), Kaliziri (local herb), Black salt, Fenugreek (*Trigonella foenumgraecum* L.), Alum and Molasses (Gupta *et al,* 2015).

Mineral Contents in Feeds and Fodders

The length of the camel neck and legs permitting to access handsomely to tree foliages, he didn't compete with other herbivorous. The camel consumes woody plants (wattle) rich in nitrogen and minerals. The camel is also known for its thirst, heat and protein deficiency resistance. It has developed in harsh conditions some remarkable physiological abilities (Faye, 1997) which contribute to its reputation. It is known also that this species is able to intake salty foodstuff and water without imbalance of the metabolic functions. Several applied and basic studies were performed on the mineral metabolism of the dromedary camel by several research teams, notably in Sudan (Abu Damir 1998); Morocco (Bengoumi *et al,* 1998), Djibouti (Faye, 1993), in India (Ghosal and Shekhawat, 1992) and in Arab Emirates (Wensvoort, 1992).

Mineral status of feed and fodders varies not only from area to area but within same locality. Intensive cultivation and change in agricultural practices has resulted in imbalance/deficiencies of certain soil minerals which in turn reflected in forage as well as in animals resulting in sub optimal production. Deficiency of mineral most frequently encountered in the livestock ration of tropical countries (Mc Dowell, 1923). The mineral requirement of camels for maintenance, growth or production has not been established accurately. Ayoub *et al* (1960) observed similar mineral composition of the body fluids of the dromedary camels to the other farm animals except for slightly higher chloride and phosphate levels. McDowell (1985) compared the mineral concentration with critical needs of ruminants. It should be in mind while interpreting the comparison between computed mineral concentration and their critical level that values less than predicated requirement may indicate deficiency but the value above doesn't necessarily indicate sufficiency because all elements present in feed are not fully utilised by microbes in the rumen or for absorption in the intestine of the animal (Norton, 1994).

The screening of minerals in locally available feeds and fodders fed to camels was carried out by Saini *et al* (2007) who reported that most of the straws offered to camels contained reasonable amount of calcium (1.03%) and phosphorus (0.67%), however, the average Ca and P content in shrubs and trees was (1.45 and 0.75%) comparatively higher than the straws. Zn is one of the elements, which is found to be deficient in most of the geographical zones of India (Garg *et al,* 2003). The Zn content in the feeds and fodders fed to camels varied from 8.40-14.09 ppm and was below the minimum requirement (40 ppm) and needed to be supplemented to overcome its deficiency (Saini *et al,* 2007). Among the dry roughages, sewan hay (10.22 ppm) had the highest Cu content followed by gram straw, groundnut haulms, moth straw and guar straw. The Cu content in top feeds varied from 9.5-10.4, it averaged around 9.9 ppm and also required supplementation as bioavailability play important role in absorption.

Nutrient Requirements and Utilisation in Dromedary Camels

Animal must be provided with a balanced diet which provides nutrients in proper ratio and amounts for its maintenance and production for 24 hours of the day. First of all, it is the dry matter consumption of the animal, which depends on the body weight, and the production status of the animal. Next is the formulation of diet from the fodders and feed ingredients for meeting the nutrient requirement of the animal. The camels generally eat about 1.0-2.5 kg dry matter per 100 kg of body weight depending on its physiological status (ICAR, 2013a). Dry matter intake of the animal depends on animal and its feed type. Feed factors include feed intake which in turn depends on feed quality of energy, protein, fibre, maturity stage, physical form and processing method. Animal factors are age, body weight, and physiological stage of pregnancy, growth, breeding, lactation and working etc. Once, dry matter intake of the animal is known, next step is the consideration of energy, protein and mineral requirements. The growing, pregnant, lactating and draft camels need extra dry matter and nutrients to meet the body needs for production. The development of feeding standards for the camel, is a very complex exercise given the wide ranging feeding conditions and the wide animal and feed component variations (Hashi and Kamoun, 1995).

The studies taken up so far regarding nutrient requirement illustrate apparently wide variation in nutrient intake by a relatively uniform population of camels observing similar exercise. The nutrient requirements (DM, DCP, TDN, Ca and P) of camels for growth and maintenance have been recommended by Indian Council of Agricultural Research (1985). However, Singh and Nagpal (2005) reported the nutrient requirements of camel calves growing @ 300g/day and adult camels.

Dry Matter Requirement:

The dromedary camels spend 6-12 hours grazing daily under natural range conditions. The plant matter intake varies from 5 to 55 kg/d depending on the season and feed availability (Gauthier-Pilters, 1979; Gauthier-Pilters and Dragg, 1981 and Wardeh, 1989). The amount of dry matter was estimated at 1.2-12 kg/d which represented 2.45 per cent of the body weight of a 500 kg camel or 104 g DM/$kg^{0.75}$ (Wardeh, 1989). When camels were offered fibrous ration at the maintenance level, the camels shows preference towards more concentrate components of the ration with a value of 8.37 MJ ME/kg DM as compared to the coarse ration (Wardeh, 2004). Farid *et al* (1985) reported that the dry matter intake was 46.8-52.8 g/$kg^{0.75}$ which was about 1.02 per cent of the average body weight of the camels but this average was lower than in the other ruminants.

Growth: Young growing camels (326.6 kg) consumed 1.33 per cent dry matter (3.35 kg/day/head) of their weight or 56.6 g/kg $W^{0.75}$. This amount consisted of 1.88 kg DM concentrate while the rest from wheat straw (Al-Motairy, 1991). In 2005, Nagpal *et al* conducted experiments on camel calves and reported that the dry matter intake (kg/100 kg body weight) ranged from 1.82 in gram straw based sole ration to 2.25 in complete ration containing gram straw, groundnut forage, molasses, guar churi, wheat bran, mineral mixture and salt. The dry matter intake of camel calves was studied by Nagpal *et al* (2007) and they reported that the DMI of camel calves varied from 1.77 to 2.53 over 9 fortnights of growth period. They further noted that the growth of animals and DMI has positive correlation indicating similar digestion and feed utilisation efficiency. Nagpal *et al* (2011) conducted experiments on male camel calves fed on isocaloric feed blocks containing 9.50, 12.00 and 14.50 per cent CP and reported the dry matter intake of 1.48±0.09, 1.55±0.02 and 1.38±0.05 kg/100 kg body weight, respectively which did not differ significantly among the groups. Jakhmola and Roy (1992) also did not observe significant variation in DMI among the groups of one year old camels, maintained on varying plane of nutrition. However, Saini *et al* (2007) reported significantly ($P<0.05$) higher DMI/100 kg body weight in growing camel calves fed on diets containing 2.35 to 5.69% DCP and 49.80 and 52.10% TDN. Safinaz *et al* (2010) fed camel calves on berseem hay and saltbush (*Atriples halimus*) forage and reported the DMI (kg/100 kg Body weight) of 1.84 to 2.07, respectively in saltbush forage and berseen hay. Osman *et al* (2014) conducted research to study the performance of Omani camels offered various levels of feed under stall-feeding and reported that the average daily feed intake (concentrate + hay) was 2.52, 4.23 and 5.15 kg for camels fed 1.5, 2.0 and 2.5% body weight, respectively. The feed intake expressed as a per cent of body weight was 1.2, 1.8 and 2.2% for camels fed 1.5, 2.0 and 2.5% body weight, respectively. There was a clear effect of feed intake on body growth with body weight gain decreasing with the decreasing feed intake. However, it is important to note that the camels on the 1.5% body weight did not lose weight throughout the trial. This indicates that this level

of intake from this diet combination is above their maintenance requirements.

Breeding and Lactation: Nagpal (2007) reported that the pregnant camels, weighing 565 kg in their 2-3 parity were fed on diets consisting of guar straw, groundnut haulms and concentrate mixture to provide 9.5% CP and 50% TDN (group 1), 10.5% CP and 55% TDN (group 2) and 12% CP and 60% TDN (group3), had DM intake kg/100 kg body weight of 1.53, 1.61 and 1.65, respectively. Moreover, individual feeding of dry camels decreased intake to 5.9 kg/day/head in Saudi Arabia (Basmail, 1989). Safinaz *et al* (2010) evaluated the performance of roughages in lactating camels and reported the DMI (kg/100 body weight) of 1.48 and 1.42 when fed on Egyptian clover and *Atriplex halimus*, respectively.

In 2009, Soliman supplemented different levels (T1-0%, T2-1.5% and T3-3%) of palm oil in the diets of she camels and reported that adding palm oil in the diets resulted in a significant decrease in feed consumption (8.79, 7.94 and 7.05 kg/d for T1, T2 and T3, respectively). The reduction in dry matter intake has been highly associated with the source and amount of fat supplement used (Coppock *et al,* 1987; Wu and Huber, 1994). In non-grazing conditions, total dry matter intake decreased linearly after protected unsaturated (2.6 kg DMI/d) or saturated (1.8 kg DMI/d) fat feeding. Another limitation to be considered when fat is added to the concentrate of grazing dairy cows is the potential reduction in the palatability of the concentrate supplement (Grummer *et al,* 1990). Camel's feed intake depends primarily on its selective feeding of a wide variety of vegetation and different parts of forage browse which differ in quality. However, feed intake studies, often based on uniform standard diets, do not take into account that ability. As a matter of fact, the few feed intake values reported for the camel in its natural conditions, are superior to those obtained under stall-fed conditions. The DM1 values for camels grazing natural pastures have been estimated to be kg per 1 kg lw (Richard, 1989). Dry matter intake (DMI) or energy intake, digestive capacity and feed utilisation have been studied in the dromedary camel using coarse roughages under staff fed conditions. DMI of meadow hay, wheat straw and oat hay was respectively 0.92, 0.65 and 0.66 kg Dm per 100 kg lw. With these types of feeds, camel's natural ability of selective browsing is reduces and there is reduced feed intake even with limited dehydration (Hashi *et al,* 1995).

Work: Nagpal *et al* (1996) found that the safari camels consumed 1.46 per cent DM (9.28 kg/d) when used for work for 6 hr/d and maintained their body weight. The DMI of draught camels ranged from 1.8 to 2.1 kg/100 kg body weight (Rai and Khanna, 1990). Rai *et al* (1995) recorded 1.33 per cent DMI of pack camels working for 2 hr during hot humid season. Chaudhary *et al* (2008) noted the DMI in the range of 1.98 to 2.03 in draught camels on feeding different levels of energy along with groundnut straw. Likewise, Gupta *et al* (2011) reported the DMI of 1.80, 2.07 and 2.27 per cent in draught camels on feeding gram straw with low, medium and high energy concentrates, respectively. Dry matter intake (DMI) of 1.70-1.85 (%BW) was reported by Gupta *et al* (2017) on feeding different levels of energy and protein along with groundnut haulms (*Arachis hypogaea* L.) in dromedary camels.

Protein and Energy Requirement:

Growth: Growth period of camel is longer than other livestock and it takes about 4 years to reach mature body of 500kg. Growth continues even after mature weight and adult camel weighs about 700-800 kg at the age 9-10 years (ICAR, 2013a). Under feeding of calves causes slow growth which results in a permanent and substantial loss of production by the camel and its progeny, hence a proper diet containing energy, protein, minerals and vitamins is recommended for optimum growth within genetic potential. Energy and protein requirements for growing calves increase with age, body weight and growth rate. The nutrient requirements for growth are higher than for maintenance (Nagpal *et al,* 1998a). More energy, protein, minerals are required during early growing period than the latter one.

When camels obtained 2.73 g DP/kg$W^{0.75}$ and 488.4 MJ ME/kg$W^{0.75}$ from natural rangelands east of the Mediterranean (Wardeh, 1989) and 2.60 g DP/kg$W^{0.75}$ in confined in Egypt (Farid *et al,* 1985) showed a very low growth rate and had a positive nitrogen balance. Kearl (1982) and Ranjhan (1980) reported higher values at 2.86 and 2.84 g DP/kg$W^{0.75}$, respectively for cattle in hot climates. Jakhmola and Roy (1992) conducted trials on one year old camel calves maintained on low (sole moth chara), medium (supplementation of 1.0 kg concentrate /day) and high plane of nutrition (1.5 kg concentrate/day). They reported the intakes of CP (gm) and ME (kcal /kg $W^{0.75}$) as 5.4 and 118.9 on low nutrition and 9.5 and 176.5 on medium plane of nutrition, respectively while these values on high plane of nutrition were in between the two groups.

The research conducted at National Research Centre on Camel, Bikaner (India) highlighted the

point that Indian Council for Agricultural Research (ICAR), New Delhi recommendations for nutrient requirements for camels need to be revised. Singh and Nagpal (2005) reported the nutrient requirements of camel calves growing @ 300g/day and adult camels. In 2018, Gupta *et al*. conducted research to study the influence of replacement of cotton seed cake with ambadi cake (*Hibiscus canabinus* L.) on performance of Mewari camels. They reported non-significant difference on nutrient digestibility, dry matter intake (8.36-8.40 kg/d), digestibility crude protein intake (553.69-561.61, g/day) and total dry matter intake (5.14-549, kg/d) on replacement of 50 and 100 per cent cotton seed cake with ambadi cake. Dereje *et al* (2016) evaluated feedlot performance of Ogaden intact dromedary camels of 24-30 months of age with 162.8±25.4 kg initial body weight. The experimental feed was urea (5%) treated maize stover basal diet fed *ad libitum* and a supplement consisting of wheat bran (66%), Noug (Gizotia abyssinica Cass), seed cake (13%), sorghum grain (20%) and mineral-vitamin premix (1%). The supplementary diet was offered to the camels in amount of 0.5 (low), 1.0 (medium) and 1.5 (high) % of body weight. Difference was observed in roughage, total DM and CP intakes, respectively for low, medium and high levels of supplements. Daily body weight gain was lower for the low supplement group as compared to the other treatments.

Breeding and Lactation: Pregnancy lasts from 12 to 13 months in the dromedary camels. The body weight gain of camels during last 4 months of pregnancy depend on plane of nutrition and it was 0.44 kg/d on sole moth chara (*Phaseolus aconifolius*) feeding which increased to 0.50 kg/d on daily supplementation of concentrate and to 0.82 kg/d on 2.0 kg/d concentrate supplementation (Jakhmola and Roy, 1996). Pregnant camels fed moth chara (*Phaseolus aconifolius*) with 2.0 kg/d concentrate ration consumed higher DM (23-29%), DCP (24-36%) and higher TDN (40-93%) as compared to dry camels because of higher nutrient demand (Nagpal *et al* 1998a). They further concluded that pregnant camels required 134-137% higher DCP and 66-72% higher ME as compared to dry camels. Nagpal (2007) fed pregnant camels on diets consisting of guar straw, groundnut haulms and concentrate mixture to provide 9.5% CP and 50% TDN (group 1); 10.5% CP and 55% TDN (group 2) and 12%CP and 60% TDN (group 3) and reported the daily intake of 0.95, 1.09 and 1.26 kg/d of CP and 4.95, 5.24 and 5.33 kg/d of TDN, respectively in group 1, 2 and 3. The gain in body weight of pregnant camels during last 4 months of pregnancy was significantly ($P<0.01$) different among the three groups and was 1.01, 1.22 and 1.44 kg/d in group 1, 2 and 3, respectively. The loss in body weight of pregnant camels on calving was to the tune of 14.79, 13.92 and 14.20% in group 1, 2 and 3, respectively. The respective average birth weight of camel calves was 43.25, 42.33 and 44.25 kg indicating non-significant influence of nutrition level during pregnancy.

Camels have been used mainly for draft since ages and have not been selected for higher milk production given high concentrate diet suitable for high milk production on scientific lines like high yielding cattle and buffaloes. The energy required for maintenance of camels depends on their activity. Energy requirement of camels of same size and breed may differ by 5-10%. For grazing, energy requirements for maintenance may increase by 10 to 20% depending on condition of pasture/rangeland (ICAR, 2013a).

The nutritional status of camels in late gestation and in lactation is a primary factor that influences milk production (Dereje and Uden, 2005). The camel milk contained 9-13% total solids, 2.0% fat, 3.0% protein and 4.0% lactose (Nagpal and Patil, 2011). The requirements for producing one kg milk (4.2% fat) will be 5.02 MJ ME, 55.0 g DP, 2.7 g calcium and 2.09 g phosphorus (Wardeh, 1989). The protein requirements for maintenance do not change during lactation but energy requirements should be increase by 12% in farm animals. However, 20% of the maintenance requirement was added to the growing lactating nouks during their first lactation and 10% during their second one (Wardeh, 1989). Maintenance requirement of 400 kg breeding female has been estimated to be 45 MJ ME and 260 g DCP and 5MJ ME and 50 g DCP is required for one litre milk production. An extra 20% and 10%, respectively ME and protein of the maintenance requirement should be given to growing lactating females during first and second lactation. Loss in body weight during early lactation because of milk production stress, particularly on feeding solely on roughages indicates the need of higher plane of nutrition. Lactating camels consume higher DM (58%), CP (50%) and ME (40%) than dry camels. An average milk yield of 1655 litre/10 months lactation with peak yield of 8.55 (litres/day) was recorded in Indian camels weighing 500 kg kept on sole moth fodder (MF) ration having an intake of 8.8 kg DM, 450 g DCP, 75.3 MJ ME and 5.0 kg TDN with body weight loss of 13.8% indicating nutrient stress on lactating camels (Nagpal *et al,* 1998b). Lactating

camels in 6th month of lactation given complete feed blocks (CFB) had significantly higher daily gain (1117 g/d) than those maintained solely on dry moth fodder (227 g/d) due to the improved voluntary DM intake (Nagpal and Jabbar, 2005). Milk yield was observed to be 10.4 kg/d in CFB group and it was economical and significantly higher than 7.2 litres/d in MF group. A significant ($P<0.05$) increase in milk total solids, protein and lactose was also seen in complete feed block fed camels(Nagpal and Jabbar, 2005).

The daily intakes of digestible protein (DP) and metabolizable energy (ME) were computed by Safinaz *et al* (2010) for the different physiological states of camels and reported that the DP intakes for late gestation, suckling and post weaning periods were 708.69, 859.16 and 859.77 g, respectively for group fed on *atriples*, as compared to 634, 636.63 and 659.25 g, respectively for the group fed on berseem hay. The calculated daily intakes of ME for late gestation, suckling and post weaning periods were 19.69, 19.33 and 19.93 Mcal, respectively for group fed on berseem hay as compared to 18.76, 18.90 and 18.98 Mcal, respectively for the group fed on *atriplex*. Rafat (2017) revealed that lactating camel does require 5.95 MJ of ME and 101g crude protein per kg milk production with 5% fat in milk. He also reported that 261g of DCP is required for maintenance of 550kg body weight of camel.

Likewise, Sagala *et al* (2020) conducted studies on 20 multiparous lactating Somali camels for assessing the quality of selected forage species browsed by the camels. They reported that the crude protein content of preferred forages during the short rains and dry season ranged from 9.64 to 17.71 % dry matter and 6.87 to 16.81 % dry matter for tree species and 9.73 to 12.19% dry matter and 8.92 to 10.66 % dry matter for shrubs, respectively. The neutral detergent fibre content during the short rains an dry season was in the range of 24.5 to 48.2% and 21.7 to 42.5% for tree species and, 40.4 to 41.4% and 35.4 to 39.8% for shrub respectively. The *in-vitro* dry matter digestibility during the short rains and dry season was in the range of 35.3 to 74.4% and 33.5 to 66.4% for tree species and, 51.1 to 59.2% and 45.5 to 58.3% for shrubs, respectively. It was concluded that there were species and seasonal differences in preferences and that the selected species were high in crude protein content and *in-vitro* dry matter digestibility.

Work: Wilson (1984) gave the maintenance requirement for 500 kg male as 54 MJ ME and 300 g DCP. For each additional one hour work camel required approximately 8.2 MJ ME and for 10 hr work 136 MJ ME and 300 g DCP with no additional protein. The ICAR (1985) has recommended 500 g DCP and 5.50 kg TDN as maintenance requirements for 500 kg camel with the provision of 25% more nutrients for work. Nagpal *et al* (1996) conducted trials on Bikaners camels carried 100 kg pack load and covered 40 km distance daily and reported the daily nutrient intake of 3.41 g DCP and 0.59 MJ ME/ $kgw^{0.75}$. Khanna and Rai (2000) calculated that the daily requirements of camels for maintenance and 10 hr work was 136 MJ ME and 300 g DCP. They reported that a 500 kg camel expend 0.21 MJ gross energy per minute at 15 to18 km/hr speed assuming an average metabolic efficiency of 60%. Racing camel would require approximately 18.9 MJ ME/hr which means that camels working for one hour would require approximately 49.6 MJ ME daily. The racing female camels had daily intake of 0.69 kg digestible protein and 99.2 MJ ME in comparison to daily intake of 70.35 kg digestible protein and 63.8 MJ ME at rest (Nagpal and Patil, 2011). The adult female camels during exercise consumed 94.62% more digestible protein and 55.30% more metabolizable energy than at rest. Draught camels weighing 495-562 kg body weight fed guar straw along with concentrate (2 kg/ day) could pull 2 wheeled cart on 24.5 km sandy tract in 4-5 hours and consumed 1.04 CP (kg), 902 DCP (g) and 6.36 TDN (kg) as compared to the respective values of 0.69, 558 and 4.80 by the camels fed only guar straw indicating higher nutrient demands of working camels (Chaudhary *et al,* 2003).

Gupta *et al* (2008) studies the effect of feeding different levels of energy along with moth straw (*Phaseolus acontifolius* Lacq.) on performance of dromedary camels and reported the DCPI (g/d) and TDNI (kg/d) of 728.73 and 6.06; 816 and 6.45 and 927.30 and 7.18, respectively in low, medium and high energy diets. Likewise, Gupta *et al* (2011) noted the similar trend for DCP (g/d) and TDN (kg/d) intakes on feeding gram straw (*Cicer arietinum*) supplemented with low energy (542.55 and 6.48), medium energy (670.24 and 7.80) and high energy (765.80 and 8.91) diets, respectively in the ration of dromedary camels. Gupta *et al* (2012) conducted experiment on feeding different proportions of groundnut haulms (*Arachis hypogaea*) and cluster bean straw (*Cyamopsis tetragonoloba*) in one of three ratios, 75:25, 50:50 and 25:75 in treatments T1, T2 and T3, respectively. They reported that DCPI (g/d) and TDNI (kg/d) were statistically ($P<0.05$) higher in T1 (664.5 and 7.3) followed by T2 (499.2 and 6.7) and T3 (352.8 and 6.2). Gupta and Tiwari (2017)

conducted experiment using dromedary camels (545-640 kg BW) to study the response of supplemental concentrates on nutrient utilisation under sustained working conditions. The results revealed that feeding of concentrate with higher levels of energy improved the nutrient digestibility and dry matter intake when fed on gram straw (*Cicer arietinum*) based basal diet.

Vitamin and Mineral Nutrition in the Dromedary Camel:

The camel is adapted to the life in the dry areas and their vegetations are characterised by their high heterogeneity in term of nutritive value with an important seasonal variation (Chehma *et al,* 2008). Faye and Bengoumi (2018) mentioned that vitamins are organic nutrients that all organisms require in small amount from their diet. Those nutrients are essential for the health, and their deficiency could be dramatic for animals and humans. They play different roles (cofactor of diverse enzymes, regulator of mineral metabolism and antioxidant activity, etc.) involved in the general metabolism of the organism. Vitamins are included in feed supplement of livestock and poultry (mineral-vitamin powders and blocks), but in most of camel farming systems, only vitamins present in the natural diet are available for the animals. The most studied vitamins in camel are vitamin A, B1, C, D and E. Plasma vitamin A increased with the age. The vitamin A deficiency could provoke crepuscular cecity in young camel. Deficiency in vitamin B1 causing polioencephalomalacia is described in racing camels. Vitamin C is particularly abundant in camel milk. Vitamin D is also in high concentration in plasma and is linked to bone metabolism. Vitamin E is contributing to reproduction efficiency of the camel.

Mineral supplementation is an important management aspect for production and productivity of camels (Simpkin, 1998). Mineral deficiencies which are widely present in domestic species from Africa (Van Schillorn *et al,* 1990) notably may occur in camel both for major or minor elements (Faye and Bengoumi, 1994). It has been suggested to provide salt allowances under normal dryland conditions range between 30 and 60 g/day. A camel working hard in the hot season may need as much as 140 g of salt daily. However, research has shown that camels suffers from specific mineral deficiencies due to complete lack or inadequate levels in the natural sources, which suggests properly formulated and balanced minerals for camels. Minerals contribute to the bone structure, to the electrolytic balance, to the protein structure, nervous and muscle activities or to the enzyme activities as well as vitamins.

The ability of the camel to intake more quantities of halophyte plants are well known and those resources are abundant in desert areas. This appetite for salty forages is explained by the adaptability of the species to the phyto-ecological context. Moty *et al* (1986) reported similar mineral composition of the body liquids of the dromedary camels to other farm animals except for slightly higher chloride and phosphate levels (Ayoub *et al,* 1960 and Kaneko, 1989). However, it seems that the dromedary camel requires high quantities of sodium chloride which might be about 6-8 times than that of other farm animals (Wilson, 1984). Increasing plasma sodium and chloride concentration is also linked with the water shortage, as they play a role in the osmotic pressure regulation. Camel is able to intake diet with high level of salt and to regulate the plasma concentrations at a high level which allow a metabolic resistance when transitory dehydration occurs. Camels which get 30-60 g salt/d might show lameness, skin necrosis and bone fracture. Such symptoms disappear when affected camels were supplemented with 140 g salt/d (Peck, 1983). The salt requirements of camel are higher than in other species (around 20g/100kg body weight or 24 g/kg of dry matter), whereas, the requirements for milk production are estimated to be 2.5g/kg of milk. Potassium is also important and ten days dehydration leads to an increase of potassium in blood (+3%) and in urine (+14%) for the same reason than sodium. The anti-diuretic hormone (ADH) appears to play a major role in its metabolism (Yagil and Berlyne, 1976).

Elmi (1989) reported the major problem encountered in camel mineral is the extremely low calcium to phosphorus ratio which was 26:1 in dry and 15:1 in wet seasons but these ratios are far below than that generally recommended (2:1) for domestic animals (McDowell *et al,* 1983). Vitamin D3 plays a central role in phosphor-calcic metabolism, notably by stimulating the intestinal absorption of food calcium and phosphorus. Thus, the plasma concentration in vitamin D3 (and more precisely in 1, 25 dihydroxycholecalciferol which is its main metabolite) in camel is 10 to 15 times higher than in other ruminants, indicating a best assimilation of calcium and phosphorus (Riad *et al,* 1994). Camels usually chew bones and eat snail shells during the dry season (Elmi, 1989 and Wilson, 1984) in order to obtain part of their phosphorus requirements (Wardeh, 2004). The maintenance requirements

are estimated to 4 g/100 kg body weight or 3.3 g/kg dry matter for calcium and 2.5 g/100 kg body weight or 2.1 g/kg dry matter for phosphorus. For lactating camel, the needs are 1.1g phosphorus and 1.9g calcium per litre of milk. Blood magnesium is generally stable, but camel seems to have no difference with other species. The normal value in adult camel is between 2.6 and 3mg/100ml (Barri *et al,* 2005).

Serum or plasma copper is a good reflect of copper intake. Copper is one of the trace elements, i.e. a mineral in very low quantity in the animal organisms, but with fundamental necessity for some metabolic functions. In ruminants, normal copper concentrations are between 70 to 120 µg/100ml (i.e. 12 and 19µmol/l). Most of the reported values in camel were inside those thresholds (Faye and Bengoumi, 1994). Copper is a component of cytochrome oxidase, lysil-oxidase, tyrosinase and Cp, molecules which contribute to different enzymatic functions for oxygen using or protein metabolism (Faye and Bengoumi, 1994). The maintenance requirements for copper are estimated to 15 mg/100 kg body weight or 12.5 mg/kg dry matter. Apart from copper, zinc is an essential biological element which is included in several enzymatic systems having action in protein synthesis (notably keratogenesis) and immune functions. So, zinc deficiency provokes skin affections and immunity depression. The maintenance requirements for zinc are estimated to 50 mg/100 kg body weight or 60 mg/kg dry matter (Faye and Bengoumi, 1994).

The selenium requirements of herbivorous are very low (1 to 2 mg/day), but this element acts in numerous biological oxidative process. Notably, it contributes to the cells protection and has a positive effect for the cancer protection in human. For a long time, selenium deficiency has been suspected to occur in camels kept in zoological parks affected by cardiopathy or myopathy (Finlayson *et al,* 1971; Wisner and Schotke, 1975 and Decker and McDermid, 1977) but no clinical descriptions and laboratory analysis have been made in these reports to confirm the role of selenium. In China also, Liu *et al* (1994) suspected selenium deficiency in cases of sway-back in Bactrian camel. In UAE, soils and feedstuffs are generally considered deficient in selenium, and many cases of degenerative myocarditis are observed with histological lesions similar to those in cattle (El-Khouly *et al,* 2001 and Seboussi *et al,* 2004). Selenium deficiencies have been also described in Morocco (Hamliri *et al,* 1990a andb). It seems essential to limit Se supplementation in camel at 0.01-0.02 mg/kg live weight i.e. approximately 4-8 mg per day for adult animals or 0.5-1 ppm in the diet.

The fluorine requirements are low and usually the supply is widely sufficient in the diet. However, fluorosis has been described in camel in Egypt (Karram *et al,* 1989) essentially in areas with phosphates deposits and phosphate manufacturing plants. The fluorine plasma concentration is the expression of the fluorine intake and the normal value remains within the limit of 0.3 ppm (Kessabi *et al,* 1984). The requirements for iron, manganese, cobalt, molybdenum, sulphur are similar than for other ruminants. Deficiencies or toxicity were not or rarely described in camel, and analysis not commonly achieved except for iron (Shekhawat *et al,* 1987). Only anaemia (iron deficiency) linked to parasitic disease as trypanosomosis was described (Ibrahim *et al,* 1992). The molybdenum and the sulphur in excess in the diet could provoke a secondary copper deficiency (Faye and Bengoumi, 1994) as for other ruminants. The sulphur is also an essential element for animals used in wool production. Karram *et al* (1989) reported one case of sulphur intoxication in camel in Egypt (Faye *et al,* 2011).

Conclusion

Camels are remarkable animals that have evolved with a ruminant like digestive system to enable them to survive on low quality feeds. Being browsers, camels are able to select high quality diets, which they can efficiently digest. Camels are pseudo-ruminants, with a simple chambered forestomach, and are unlike the four chambered stomach found in cattle and sheep. Nevertheless, camels can digest high fibre feeds via fermentation pathways similar to those in true ruminants. The camel can survive on all sorts of vegetation including shrubs, weeds, grasses, tree leaves etc. and maintains their body condition. But camel usually prefers to browse (feeding tree leaves and twigs) rather than to graze particularly when green grasses are available. The camels under rangelands feeding systems are not fed sufficiently to meet their nutrient requirements for pregnancy, lactation and growth. The camels have lower energy requirements than ruminants, and have evolved an efficient mechanism for nutrient recycling. The mineral deficiencies which are widely present in domestic species notably may occur in camel both for major or minor elements. It has been concluded that dromedary camels should be supplementation with concentrate mixture during pregnancy, lactation and work.

References

Abbas B and Omer OH. Review of infectious diseases of the camel. Veterinary Bulletin. 2005; 75:1-16.

Abu Damir H. Mineral deficiencies, toxicities and imbalances in the camel (*Camelus dromedarius*): A review. Veterinary Bulletin. 1998; 68:1103-1119.

Al-Motairy S. Feed resources in Saudi Arabia and the possibility of feeding urea treated straws to growing camels. M. Sc. Thesis. Gulf University, Bahrain. 1991.

Ayoub MH, Awad YI and Bayazed IA. Chloride in serum of Egyptian farm animals. Indian Journal of Veterinary Science. 1960; 30:34-37.

Bakshi MPS and Wadhwa M. Evaluation of forest tree leaves of semi-hilly arid region as livestock feed. Asian Australasian Journal of Animal Science. 2004; 17:777-783.

Banerjee GC. A Text Book of Animal Husbandry. Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi. 2016.

Barri MES, Al-Busadah KA and Homeida AM. Comparative calcium and magnesium status in adult and young camel (*Camelus dromedarius*). Science Journal of King Faisal University. 2005; 6:151-158.

Basmail S. The nutrition of Arabian camels under controlled management. Proceedings: International Symposium on Ruminant Production in Dry Subtropics. Constraints and Potential held in Cairo on Nov. 5-11, 1988. EAAP Publication No. 38. Wageningen. 1989; pp 259-261.

Bengoumi M, Essamadi K, Tressol JC and Faye B. Comparative study of copper and zinc metabolism in cattle and camel. Biological Trace Element Research. 1998; 63:81-94.

Bhatia JS. Some physiological studies on rumen of camel. Camel Research. Directorate of Research. Veterinary and Animal Science College, Bikaner. 1996; pp 48-49.

Bhatia JS, Goshal A K and Gupta HK. Rate of passage of digesta through the digestive tract of camel. International Journal of Animal Science. 1988; 3:45-52.

Bhattacharya AN. Structural peculiarities in the digestive system of camel. Range and Animal Development Centre. Saudi Arabia. 1986.

Bhattacharya AN, Al-Motairy S, Hashimi A and Economides S. Studies on energy and protein utilisation of alfalfa hay and barley grain by yearling camel calves. The British Society of Animal Production. 1985; 74:481-485.

Burgos MS, Senn M, Sutter F, Kreuzer M and Langhans W. Effect of water restriction on feeding and metabolism in dairy cows. Comparative Physiology. 2001; 280:418-427.

Chaudhary JL, Tiwari GS and Gupta L. Effect of feeding different levels of dietary energy on nutrient utilisation, draught performance and physiological reactions of camels. Journal of Camel Practice and Research. 2008; 15(2):195-200.

Chaudhary JL, Tiwari GS, Aminudeen and Sahani MS. A comparative utilisation of guar straw (*Cymopsis tetragonoloba*) fed with or without concentrate supplement to draught camels. Indian Journal of Animal Nutrition. 2003; 20(2):227-231.

Chehma A, Faye B and Reda Djebar M. Productivité fourragère et capacité de charge des parcours camelins dans le Sahara septentrional. Sècheresse. 2008; 19:115-121.

Coppock CE, Lanham JK and Horner JL. A review of nutritive value and utilisation of whole cottonseed, cottonseed meal and associated byproducts by dairy cattle. Animal Feed Science and Technology. 1987; 18:89-97.

Decker RA and McDermid A. Nutritional myopathy in young camel. Journal of Zoo Animal Medicine. 1977; 8:20-21.

Dereje M and Uden P. The browsing dromedary camel. II. Effect of protein and energy supplementation on milk yield. Animal Feed Science and Technology. 2005; 121:309-317.

Dereje M, Urge M, Animut G, Kurtu MY. Feedlot performance of Ogaden dromedary camels fed urea-treated maize stover and concentrate mixture. Livestock Research for Rural Development. 2016; 28 (10), Article#189.

Diatta S, Salifou I, Kaboré-Zoungrana SM, Banoin M and Akpo LE. Assessment of germinative potentialities of sahelian forage tree: Maerua crassifolia Forssk., Capparaceae. Livestock Research for Rural Development. 2008; 20(6).

El Khouly AA, Abbas TA and Moustafa. T. Myocardial dystrophy in camel calves in the United Arab Emirates (field cases). Emirates Journal of Agricultural Sciences. 2001; 13:11-17

Elmi A. Management, foraging behaviour, Diet Composition and Forage Quality of Free Ranging Camels in Ceeldheer District, Central Somali. PhD. Dissertation. Utah State University, Logan. 1989; pp 221.

El-Shami EM. Comparative study of utilisation of browse plants by camels and goats. in: Annual Report. Camel Research Unit, Faculty of Veterinary Science, University of Khartoum. 1985; pp 173-182.

Engelhardt WV, Dyckar CA and Dole ML. Absorption of short chain fatty acids, sodium and water from the forestomach of camels. Journal of Comparative Physiology. 2007; 177:631-640.

Engelhardt WV, Lechner-Doll M, Heller R, Schwartz HJ, Rutagwenda T and Schultka W. Physiology of the forestomach in camelides with particular reference to adaptation to extreme conditions. A comparative approach. Seminaire sur la Digestion la Nutrition et l'Alimentation du Dromadaire. Feb. 8-29. 1988. Ouargla, Algerie. 1988.

Farah Z, Rettenmayer R and Atkins D. Vitamin content of camel milk. International Journal of Vitamin and Nutrition Research. 1992; 62:30-33.

Farid MF, Shawket SM and Abdel-Rehman. The nutrition of camels and sheep under stress. The Camelid: An All-Purpose Animal. Proceedings: Khartoum Workshop on Camel. SIAA, Uppsala. 1979; pp 293-322.

Farid MFA, Sooud AO and Hassan NI. Effects of types of diet and protein intake on feed utilisation in camels and sheep. Proc. Third AAAP Animal Science Congress, Seoul. Korea. 1985; pp 781-783.

Faye B. Mangrove, sécheresse et dromadaire. Sécheresse. 1993; 4:47-55

Faye B. Guide de l'élevage du dromadaire. Ed. SANOFI, Libourne, France. 1997; pp 120.

Faye B and Bengoumi M. Trace-element status in camel. A review. Biological Trace Element Research. 1994; 41:1-11.

Faye B and Bengoumi M. Camel Clinical Biochemistry and

Hematology. Springer International Publishing. 2018; 978-3-319-95560-5. DOI 10.1007/978-3-319-95562-9.

Faye B, Bengoumi M and Seboussi R. Camels and their mineral nutrition. In: Strategic Livestock Feeding for Sustainable Production edited by Lokesh Gupta and Taparia, A.L. and published by Narendra Publishing House, Delhi (India). 2011; pp 77-100. ISBN: 978-93-80428-29-1.

Finlayson R, Keymer IF and Manton JA. Calcific cardiomyopathy in young camels (*Camelus* spp.). Journal of Comparative Pathology. 1971; 81:71-77.

Flint HJ. The rumen microbial ecosystem-some relevant developments. Trends in Microbiology. 1997; 5:483-488.

Gahlot TK. Selected Research on Camelid Physiology and Nutrition. Editor: T.K. Gahlot. The Camelid Publishers. 2004; ISBN-81-901141-2-3.

Garg MR, Bhandari BM and Sherasia PL. Macro mineral status of feeds and fodder in Kota district of Rajasthan. Indian Journal of Animal Nutrition. 2003; 20:252-261.

Gauthier-Pilters H. Some ecological aspects of the camel in the Eastern Sahara. In Cockrill (ed). The Camelid: An All Purpose Animal Proc. Workshop on Camels. 1979.

Gauthier-Pilters H and Dagg AI. The camel, its evolution, ecology, behaviour and relationship to man. The University of Chicago Press, Chicago and London. 1981.

Ghosal AK and Shekhawat VS. Observations on serum trace elements levels (zinc, copper and iron) in camel (*Camelus dromedarius*) in the arid tracts of Thar Desert in India. Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux (Paris). 1992. 45:43-48

Gihad EA, El-Gallad TT, Sooud AI, Abou El-Nasr HM and Farid M. Feed and water intake, digestibility and nitrogen utilisation by camels compared to sheep and goats fed low protein desert by products. Seminaire sur la Digestion, la Nutrition et l'Alimentation du Dromadaire. Feb. 8-29, 1988 Ouargla, Algerie. 1988.

Gordon J and McAllister. The circadian rhythm of rumination. The Journal of Agricultural Science. 1970; 74:291–297 DOI 10.1017/S0021859600022905.

Grummer RR, Hatfield ML and Dentine MR. Acceptability of fat supplements in four dairy herds. Journal of Dairy Science. 1990; 73:852-857.

Gupta L. Evaluation of total mix ration with different proportions of roughages in draught camels. Journal of Camel Practice and Research. 2020; 27(3):317-321.

Gupta L and Tiwari GS. Effect of feeding supplemental concentrate on nutrient utilisation in dromedary camels under sustained working conditions. Animal Nutrition and Feed Technology. 2017; 17:361-365.

Gupta L, Chaudhary JL and Tiwari GS. Effect of feeding gram straw on performance of Bikaneri camles. The Indian Veterinary Journal 2011; 88(9):38-40.

Gupta L, Roy AK and Tiwari GS. Supplemental effect of different levels of energy and protein along with groundnut straw (*Arachis hypogaea* L.) based ration on nutrient utilisation in dromedary camels. Indian Journal of Animal Science. 2017; 87(7):896-899.

Gupta L, Tiwari GS and Chaudhary JL. Effect of feeding different levels of energy on draught performance and physiological responses in camels. Indian Veterinary Journal. 2008; 85(8):869-871.

Gupta L, Tiwari GS and Garg R. Documentation of Ethno-veterinary remedies of camel diseases in Rajasthan, India. Indian Journal of Traditional Knowledge. 2015; 14(3):447-453.

Gupta L, Tiwari GS and Garg R. Influence of replacement of cotton seed cake with ambadi cake (*Hibiscus canabinus* L.) in the diets of Indian camels (*Camelus dromedarius*). Indian Journal of Animal Nutrition 2018; 35(1):66-70.

Hamliri A, Khallaayoune K, Johnson DW and Kessabi M. The relationship between the concentration of selenium in the blood and the activity of glutathione peroxidase in the erythrocytes of the dromedary camel (*Camelus dromedarius*). Veterinary Research Communications. 1990a; 14:27-30.

Hamliri A, Olson WG, Johnson DW and Kessabi M. Evaluation of biochemical evidence of congenital nutritional myopathy in the two-week prepartum fetuses from selenium-deficient ewes. Journal of the American Veterinary Medical Association. 1990b; 51:1112-1115.

Hashi AM. Pastoral resource use systems of Somalia. In: FAO Report-Subregional Seminar on the dynamics f pastoral land and resource tenure in the horn of Africa. Mogadishu, 8-11 April 1990. FAO, Rome. 1991.

Hashi AM and Kamoun M. Feed requirements of the camel. In: Tisserand J.L. (ed.). Elevage *et al*imentation du dromadaire. Zaragoza: CIHEAM. Options Méditerranéennes: Série B. Etudes et Recherches. 1995; 13:71-80.

Ibrahim A, Abdel Gaffar AA, Gameel AA, Nayel NM and Le Gailani M. A note on the haemogram of the dromedary camel in Bahrain. Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux (Paris). 1992; 45:318-320.

ICAR. Nutrient Requirements of Livestock and Poultry. Indian Council of Agricultural Research, New Delhi. 1985.

ICAR. Nutrient requirements of camel. Indian Council of Agricultural Research, New Delhi. 2013a.

ICAR. Nutrient composition of Indian feeds and fodder. Indian Council of Agricultural Research, New Delhi. 2013b.

Iqbal A and Khan BB. Feeding behaviour of camel–review. Pakistan Journal of Agricultural Sciences (Pakistan). 2001; 38:58-63.

Jakhmola RC and Roy AK. Effect of supplementation of concentrate on body weight gain and serum constituents in camel. Indian Journal of Animal Science. 1992; 62(8):782-784.

Jakhmola RC and Roy AK. Effect of feeding moth chara (*Phaseolus aconitifolius*) supplemented with concentrate and stage of pregnancy on certain blood metabolites in camel. Indian Journal of Animal Science. 1996; 66(1):68-73.

Kaneko JJ (1989). Clinical biochemistry of domestic animals. Ed. IV, Academic Press, New-York.

Karram MH, Mottelib AA, Nafie THS and Sayed AS. Clinical and biochemical studies in chronic fluorosis and sulphurosis in camels. Assiut Vétérinaire Médical Journal. 1989; 21:41.

Kaske M, Osman T, Lechner-Doll M, Larsson M, Engelhardt W. Circadian changes of fore stomach motility and of rumination in camels. Asian-Australasian Journal of Animal Sciences. 1989; 2:301–302 DOI 10.5713/ajas.1989.301.

Kearl IC. Nutrient requirements of ruminants in developing countries. International Feedstuffs Institute, Utah, U.S.A. 1982.

Kessabi M, Assimi B and Braun JP. The effects of fluoride on animals and plants in the South Safi zone. The Science of total Environment. 1984; 38:63-68.

Khanna ND and Rai AK. Reviewed papers, investigations on work potential of Indian Camel. Camel News Letter. 2000; 17:15-22.

Knoess KH. The camel as a meat and milk animal. World Animal Review. 1977; 22:39-44.

Laudadio V, Tufarelli V, Dario M, Hammadi M, Seddik MM, Lacalandra GM and Dario C. A survey of chemical and nutritional characteristics of halophytes plants used by camels in Southern Tunisia. Tropical Animal Health and Production. 2009; 41:209-215.

Liu ZP, Ma Z and Zhang YJ. Studies on the relationship between sway disease of bactrian camels and copper status in Gansu Province. Veterinary Research Communications. 1994; 18:251-260

Maloiy GMO. Comparative studies on the digestion and fermentation rate in the forestomach of one humped camel and Zebu steer. Research in Veterinary Science. 1972; 13:476-481.

McDowell LR. Minerals in Animals and Human Nutrition, Academic Press, Inc San Diego, CA. 1923; pp 47-50.

McDowell LR. Nutrition of grazing ruminants in warm climates. Academic Press Inc, San Diego, CA. 1985; pp 168-169.

Moty IA, Mulla A and Zaafer SA. Copper, iron and zinc in the serum of egyptian farm animals. Sudan Agriculture Journal. 1986; 3:146-151.

Nagpal AK. Nutrient utilisation and performance of pregnant camels kept on different levels of energy and protein. Journal of Camel Practice and Research. 2007; 14(1):79-82.

Nagpal AK and Jabbar A. Productivity of lactating camels on complete feed blocks. Indian Journal of Animal Nutrition. 2005; 22(2):102-106.

Nagpal AK and Patil NV. Feeding of Camels. In: Animal Nutrition, Advancements in feeds and feeding of livestock edited by Lokesh Gupta and Singhal, K.K. and published by AGROBIOS (India), Jodhpur (India). 2011; ISBN: 978-81-7754-447-3.

Nagpal AK, Arora M and Singh GP. Nutrient utilisation of gram straw (*Cicer arietinum*) based complete feed blocks in camel calves. Indian Journal of Animal Science. 2005; 71(1):64-68.

Nagpal AK, Rai AK and Khanna ND. Nutrient utiliztion and serum electrolytes in pack safari camels. Indian Journal of Animal Science. 1996; 66:1166-1169.

Nagpal AK, Roy AK, Chirania BL and Patil NV. Growth, nutrient utilisation and serum profile in camel calves as affected by dietary protein levels. Indian Journal of Animal Nutrition. 2011; 28(2):166-171.

Nagpal AK, Sahani MS, Roy AK and Mal G. Voluntary feed intake and utilisation of macro and micro nutrients in dry and pregnant camels. Indian Journal of Animal Nutrition. 1998a; 15:158-162.

Nagpal AK, Sahani MS, Roy AK and Mal G. Voluntary feed intake and utilisation of macro-and micro-nutrients in dry and lactating Bikaneri camels. Indian Journal of Animal Science. 1998b; 13:19-24.

Nagpal AK, Singh GP, Saini N and Jayant P. Voluntary feed intake, serum profile, growth performance and economics of weaned camel calves. Proceedings of the International Camel Conference, Bikaner, Rajasthan (India) from 16-17 Feb. 2007; pp 153-155.

Norton BW. The nutritive value of tree legumes. RC Gutteridge and Shelton (Eds.). Forage Tree Legumes in Tropical Agriculture. CAB International, Wallingford, Oxon, OX 108DE, UK. 1994.

Osman M, Isam TK, Waleed AM, Sadeq A, Al-Lawatia and Abdulla S Al-Abri. Effects of feed intake of a complete concentrate diet on performance of Omani camels raised under stall-feeding. Journal of Camel Practice and Research. 2014; 21(1):21-26.

Osuga IM, Wambui CC, Abdulrazak SA, Ichinohe T and Fujihara T. Evaluation of nutritive value and palatability by goats and sheep of selected browse foliages from semi arid area of Kenya. Animal Science Journal. 2008; 79:582-589.

Peck EF. The relationship of salt starvation to contagious necrosis and lameness in camels. Veterinary Record. 1983; 50:409- 410.

Rafat AJ. Camel Nutrition and Feeding. Australian Camel Milk Industry Forum, Adelaide, South Australia. 2017; pp 25-26.

Rai AK and Khanna ND. Effect of load pulling on physiological responses of camels. Proceedings of the International conference on camel production and improvement, Tobruk, Libya, 10-13 December. 1990; pp 207-220.

Rai AK, Nagpal AK and Khanna ND. Effect of water deprivation on nutient utilisation in Indian camels during winter. Indian Journal of Animal Science. 1995; 65:565-570.

Ranjhan SK. Animal nutrition in tropics. Vikas Publication House, India. 1980.

Rathore GS. Camels and their management. Indian Council of Agricultural Research, New Delhi 1986.

Riad F, Bengoumi M, Safwate A, Giry J, Davicco MJ and Barlet JP. Influence of the hydroxycholecalciferol on calcium and phosphorus concentration in camel milk. Journal of Dairy Science. 1994; 61:567-571.

Richard D. lngestibilité et digestibilité des aliments par le dromadaire. Options Méditerranéennes (CIHEAM). 1989; Série A(2):55-59.

Safinaz MS, Kamal MY and Mohamed HA. Comparative evaluation of Egyptian clover and Atriplex halimus diets for growth and milk production in camel (*Camelus dromedarius*). Animal Science Reporter. 2010; 4(1):9-21.

Sagala JI, Gachuiri CK, Kura SG and Wanyoike MM. Nutritive value of selected preferred forage species by lactating camels in the peri-urban area of Marsabit town, Kenya. Indian Journal of Animal Nutrition. 2020; 37(3):218-226.

Saini N, Singh GP and Nagpal AK. Nutrient utilisation from Clusterbean straw, supplemented with urea and Prosopis cineraria leaves in growing camel. Indian Journal of Dairy Science. 2007; 60:342-344.

Samsudin AA, Wright AD and Jassim RA. Cellulolytic Bacteria in the Foregut of the Dromedary Camel (*Camelus dromedarius*). Applied Environmental Microbiology. 2012; 78:8836-8839.

Seboussi R, Faye B and Alhadrami G. Facteurs de variation de quelques éléments trace (sélénium, cuivre, zinc) et d'enzymes témoins de la souffrance musculaire (CPK, ALT et AST) dans le sérum du dromadaire (*Camelus dromedarius*) aux Emirats Arabes Unis, Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux (Paris). 2004; 57:87-94.

Sharma T and Dhuria RK. Status of camel nutrition in arid India. Proceedings of the International Camel Conference, Bikaner, Rajasthan (India) from 16-17 Feb. 2007; pp 134-140.

Shekhawat VS, Bathia JS and Ghosal AK. Serum iron and total iron capacity in camel. Indian Journal of Animal Science. 1987; 57:168-169.

Simpkin SP. The effects of breed and management on milk yield of camels in Kenya. Ph. D Thesis, University of Newcastle 1998.

Singh GP and Nagpal AK. Feed processing for camel. Book: Roughage Processing Technology. (Eds.) S.S. Kundu, S.K. Mahanta, Sultan Singh and P.S. Pathak. Satish Serial Publishing House, Delhi. 2005; pp 273-284.

Soliman N and Al-Dobaib. Effect of Palm Oil Supplementation on the Milk Yield and Composition of Dromedary She Camels. Pakistan Journal of Nutrition. 2009; 8(6):710-715.

Towhidi A and Zhandi M. Chemical composition, in vitro digestibility and palatability of nine plant species for dromedary camels in the province of Semnan, Iran. Egyptian Journal of Biology. 2007; 9:47-52.

Van Schillorn, Van Veen TW and Loeffler IK. Mineral deficiency in ruminants in subsaharian Africa: a review. Tropical Animal Health and Production. 1990; 22:197-205.

Wardeh MF. The Nutrient Requirements of the Dromedary Camels. The Arab Centre for the Studies of Arid Zones and Dry Lands. Damascus, Syria. 1997.

Wardeh MF. The nutrient requirements of the Dromedary camels. Journal of Camel Science. 2004; 1(1):37-45.

Wardeh MF. Arabian camels, origin, breeds and husbandry. Al-Mallah Pub., Damascus, Syria. 1989; pp 500.

Wensvoort J. Copper, iron, manganese and zinc concentrations in livers of race animals. Proceedings of 1st International Camel Symposium, Dubai, UAE, 2-7 Feb. 1992; 91.

Wilson RT. The Camel. Longman, London and New York. 1984.

Wilson RT. The nutritional requirements of camel. Options Méditerranéennes (CIHEAM). 1989; Série A(2):171-179.

Wisner H and Schotke B. White muscle disease at The Hellabrun Zoo in Munich. Vet. Erkr. Zoo wild Tunis, Berlin. 1975; 1:717-720.

Wronski T, Apio A and Plath M. Activity patterns of bushbuck (*Trangelaphus scriptus*) in Queens Elizabeth National park. Behavioural Processes. 2006; 73:333-341.

Wu Z and Huber JT. Relationship between dietary fat supplementation and milk protein concentration in lactating cows: A Review. Livestock Production Science. 1994; 39:141-155.

Yagil R and Berlyne GM. Sodium and potassium metabolism in the dehydrated and rehydrated bedouin camel. Journal of Applied Physiology. 1976; 41:457-461.

BACK ISSUES OF JCPR AVAILABLE

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

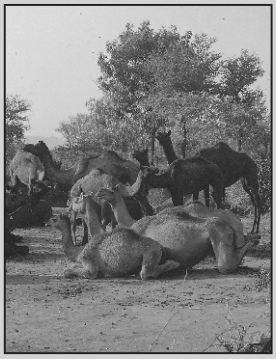
Volume 9 June 2002 Number 1

In This Issue

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

Volume 9 December 2002 Number 2



In This Issue

WORLD CAMEL POPULATION - 2002

19,321,812

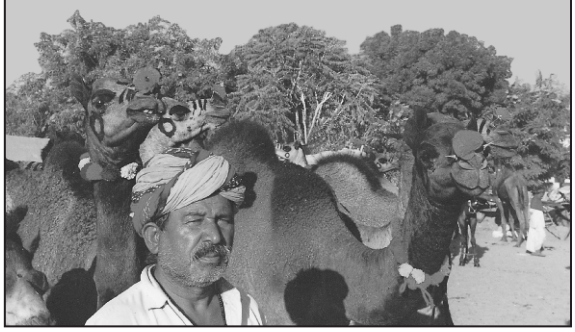
ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

Volume 10 June 2003 Number 1

10th Volume Contains.....

Experimental mange, Trypanosomiasis and efficacy of Trypan, internal parasites in llamas, Radiographic studies on the mandible and secondary ossification centres, anatomy of the major salivary glands, utilisation of some browse plants, efficacy of camel milk on glycemic control, biochemical parameters of Majaheem breed, colic, intramammary infections, burn, buccal infection and osteomyelitis associated with mandibular fractures and news.

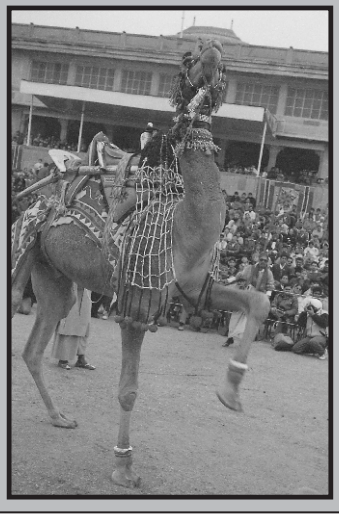


ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

Volume 10 December 2003 Number 2

Why is this camel so happy?
Because JCPR has completed 10 years of journey!!!



In This Issue

See the details of list of contents of all previous issues of JCPR on our website
www.camelsandcamelids.com

Kindly refer querries to: Dr. T.K. GAHLOT, Editor, JCPR
email: tkcamelvet@yahoo.com

DOI : 10.5958/2277-8934.2021.00041.2 Vol 28 No 3, p 265-266

Short Communication

Rhodococcus equi ISOLATED FROM RAW CAMEL MILK

Wernery U., B. Johnson, S. Raja and Sh. Jose
Central Veterinary Research Laboratory, Dubai, UAE

ABSTRACT

During a routine microbiological investigation of forty raw camel milk samples from one of Dubai's camel dairy farm for its suitability for human consumption, nine milk samples were contaminated with *Rhodococcus equi*. As this pathogen is a soil-borne bacteria, it was assumed that the contamination of the milk occurred via dust particles which are in the air during sand storms. Although the level of contamination was low and all other tested values of the milk were in the reference range, it was advised not to consume the milk, if *Rhodococcus equi* is present in the milk.

Key words: Dromedary camel, milk, *Rhodococcus equi*

Rhodococcus (R.) equi belongs to the Corynebacteriaceae family of the Actinomycetales order. It is the only significant veterinary pathogen in the genus. *R. equi* is an aerobe or facultative anaerobe which forms moist, mucoid colonies which are first white, but becomes pinkish when they are longer incubated. They do not produce haemolysis on blood agar and the mucoid colonies tend to merge. It is a Gram-positive coccus or rod which is catalase positive and possesses also an oxidative metabolism. It grows slowly on blood agar and cultures should be incubated from 48 hours to 72 hours at 37ºC. Trimethoprim sulfamethoxazole is effective and should be used as early as possible to eliminate the pathogen (Markey *et al*, 2013). *R. equi* has been found in several animal species, but it is most important as the cause of a purulent pneumonia in foals. It also infects other organs in the foal such as kidneys and the genital tract. The pathogen may also infect cattle, sheep, pigs and cats (Bisping and Amtsberg, 1988). Infections of human beings are very rare (Bürger and Müller, 1982).

We report here the isolation of *R. equi* from raw camel milk.

Materials and Methods

CVRL is testing pasteurised and raw milk samples from different camel dairy farms in Dubai on a daily basis for their safety for human consumption and mastitis screening. On 15-06-2021, forty raw camel milk samples were sent to CVRL on which the following tests were performed:

- California Mastitis Test (CMT) using Hauptner, Milchzelltest Farblos (Art. Nr: 96101000)
- Total Plate Count (TPC) following ISO 4833-1 test method
- Culture on Sheep Blood Agar (SBA) – (0.1ml spread plate culture)
- Coliform culture on Violet Red Bile Lactose Agar (VRBA) following ISO 4832 test method
- Culture on Brilliant Green Phenol Red Lactose Sucrose Agar (BPLS) to differentiate lactose fermenting and non- lactose fermenting bacteria (0.1ml spread plate culture)

Bacterial species grown from the raw milk samples were then identified after subcultures, by colony morphology on SBA, lactose metabolism on VRBA and BPLS agar, Gram-stain, oxidase reaction and identification using Vitek 2 identification system.

Results

Rhodococcus equi was isolated from 9 of the 40 raw camel milk samples (22.5%). The pathogen grew in small numbers between 20 to 90 colony forming units (cfu) per 1 ml milk especially well on blood agar. It exhibited the typical non-haemolytic mucoid appearance (Fig 1). All 40 milk samples were suitable for human consumption as all values found, were in the internationally acknowledged range of cow milk (Anonymous, 1992; Anonymous, 2000). International standards for camel milk are not yet available.

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

Fig 1. *Rhodococcus equi* isolated from raw dromedary camel milk on blood agar showing the typical mucoid appearance after 48 hours incubation at 37°C.

Discussion

The natural habitat of *R. equi* is soil and particularly soil which is contaminated with manure especially from horses, but also from other animals. Faecal contamination increases the rate of multiplication of this pathogen in the soil as volatile fatty acids in faecal material increases growth of *R. equi*. The main route of infection in equids is by inhalation. There is a clear correlation between the prevalence of *R. equi* foal pneumonia and airborne burden of the bacteria. *R. equi* possesses many virulence factors which play an important part in its pathogenesis. They include 7 types of capsular antigens, plasmid proteins and cell-wall mycolic acid (Markey *et al*, 2013). The pathogen produces suppurative broncho pneumonia in foals, and only very rarely in adult horses.

It has been reported in New World camels (NWCs) and recently also in Old World camels (OWCs) (Leite *et al*, 1975; Hong and Donahue, 1995; Cuteri *et al*, 2001; Kinne *et al*, 2011). In human beings it is an emerging opportunistic pathogen, especially in immunocompromised patients (Puthucheary *et al*, 2006).

Adult horses are typically resistant to *R. equi* infections (Giguère and Prescott, 1997) and only occasional cases have been reported in adult horses (Waldridge *et al*, 2008). Interestingly, in dromedary camels it is the opposite, no *R. equi* infections were reported in camel calves, only in adult dromedary camels (Kinne *et al*, 2011). It is not clear, how this pathogen had contaminated the camel milk, but it is presumed that it came with the dust as during the hot summer months, the United Arab Emirates experiences often severe sand-storms. The level of colony forming *R. equi* in the raw milk was low, but as a precautionary measure, this milk should not be consumed.

References

Anonymous. Council Directive 29/46 EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products. Official Journal of the European Communities No. L 368. 1992; pp 33-37.

Anonymous. Verordnung uber Hygiene – und Qualitätsanforderungen an Milch und Erzeugnisse auf Milchbasis (Milchverordnung). Bundesgesetzblatt Teil 1 No. 36. 2000; pp 1178-1207.

Bisping W and Amtsberg G. Colour Atlas for the Diagnosis of Bacterial Pathogens in Animals. Paul Parey Scientific Publishers. 1988; pp 51-56.

Bürger H and Müller HE. Rhodococcus isoliert aus Glutalabszeß. Infection. 1982; 10:343-346

Cuteri V, Takai S, Marenzoni ML, Morgante M and Valente C. Detection of antibodies against *Rhodococcus equi* in alpaca (*Lama pacos*) in Italy. European Journal of Epidemiology. 2001; D17:1043-1045.

Giguère S and Prescott JF. Clinical manifestations, diagnosis, treatment, and prevention of *Rhocococcus equi* infections in foals. Veterinary Microbiology. 1997; 56:313-334.

Hong CB and Donahue JM. *Rhodococcus equi* – associated necrotising lymphadenitis in a llama. Journal of Comparative Pathology. 1995; 113:85-88.

Kinne J, Madarame H, Takai S, Jose S and Wernery U. Disseminated *Rhodococcus equi* infection in dromedary camels (*Camelus dromedarius*). Veterinary Microbiology. 2011; 149:269-272.

Leite RC, Negrelli-Filho H and Langenegger CH. Infectiopor Corynebacterium equi en llama (*Lama glama*). Pesquisa Agropecuária Brasileria, Servicios Veterinarios. 1975; 10:57-59.

Markey B, Leonard F, Archambault M, Cullinane A and Maguire D. Clinical Vet. Microbiol., Mosby Elsevier 2nd ed. 2013; pp 135-160.

Puthucheary SD, Sangkar V, Hafeez A, Karunakaran R, Raja NS and Hassan HH. *Rhodococcus equi*: an emerging human pathogen in immunocompromised hosts: a report of four cases from Malaysia. Southeast Asian Journal of Tropical Medicine and Public Health. 2006; 37:157-161.

Waldridge BM, Morresey PR, Loynachan AT, Reimer J, Riddle WT, Williams NM and Bras R. *Rhodococcus equi* pneumonia in an adult horse. Equine Veterinary Education. 2008; 20:67-71.

DOI : 10.5958/2277-8934.2021.00042.4 *Vol 28 No 3, p 267-276*

INFERTILITY IN FEMALE DROMEDARY CAMELS

Ahmed Ali[1,2], Derar R. Derar[1,2] and Tariq I. Almundarij[1]

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

[2]Department of Theriogenology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

ABSTRACT

This review discusses the most common infertility-related reproductive disorders in female dromedaries. Four major categories are identified, namely, congenital, functional, pathological and management disorders. Congenital causes comprise ovarian agenesis, mesonephric duct segmental aplasia, endometrial agenesis, double cervix/vagina, imperforated hymen, vulvar atresia, and intersex. Functional causes cover ovarian inactivity, overgrown follicle, and ovulation failure. Pathological causes include ovarian hydrobursitis, hydrosalpinx and pyosalpinx, clinical and subclinical endometritis, hydrometra and pyometra, vaginal and cervical adhesions, and neoplasms. Management causes involve mating errors, use of traditional management systems, improper herder/camel ratio, and inadequate managerial experience. Pathological lesions and management errors are the main causes of female camel infertility. There are several effective protocols for the treatment of endometritis that offer a resilient prognosis. Ovarian hydrobursitis and vaginal adhesion are serious conditions and require appropriate preventive measures because treatment procedures are difficult and the prognosis poor. Enhanced management practices are essential in order to increase the reproductive efficiency of dromedary herds.

Key words: Dromedary female, endometritis, infertility, ovarian hydrobursitis, vaginal adhesion

Infertility in camels is quite different than in other farm animals. The reproductive organs of female dromedaries have certain characteristics that impact the development of unusual reproductive disorders. Camels are induced ovulators and therefore follicular growth occurs in waves of growing, mature, and regression phases (Musa and Abusineina, 1978; Skidmore *et al*, 1995; Manjunatha *et al*, 2012; Adams *et al*, 2016) rather than regular oestrus cycles. Camels do not have a regular luteal phase, as they only ovulate when mated; however, a mated non - pregnant camel has a short luteal phase of approximately eight days, which is much shorter than seen in other domestic species such as cows, mares, or ewes (Skidmore *et al*, 1995, 1996). The ovary is located entirely within the ovary bursa (Ali and Derar, 2017; 2020). It has been commonly reported that the fertility indices of dromedary camels are comparatively low when compared with other domestic animals. Fertility rates were assessed as between 34 and 57% in Kenya and Tunisia (Schwartz *et al*, 1983; Djellouli and Saint-Martin, 1992) and between 33 and 83% in Saudi Arabia (Arthur *et al*, 1985; Ali *et al*, 2018a). The low fertility rate in camels has been associated with non-developing follicles, failure of females to ovulate, and embryonic mortality, as well as genetic and acquired abnormalities of the genital tract (Novoa, 1970; Tibary and Anouassi, 1997; Al Eknah, 2000; Ali *et al*, 2010a; 2015; 2018a; Skidmore, 2011; Benaissa *et al*, 2017; Khalafalla *et al*, 2017). This paper discusses some possible aspects of reproductive disorders affecting female dromedaries, while at same time assigning these causes to specific categories.

Congenital causes of infertility

In many clinical studies and those conducted in abattoirs, dromedary genital tract abnormalities have been reported as 0.44 - 1.2% (Ali *et al*, 2010a; 2015; Tibary, 2015; Dawod and Elbaz, 2018; Gherissi *et al*, 2020). These anomalies include ovarian agenesis, mesonephric duct segmental aplasia, endometrial agenesis, double cervix/vagina, imperforated hymen, vulvar atresia, and intersex.

Ovarian agenesis (failure of ovaries to develop) was inadvertently observed in the abattoir (Fig 1 A); however, some studies have considered it to be the most prevalent ovarian anomaly in the dromedary (Tibary, 2015; Dawod and Elbaz, 2018). Ovarian agenesis has been pronounced in cows (Millward *et al*, 2019), mares (Basrur *et al*, 1969), and ewes (Regassa *et al*, 2009) and has been associated with numerous chromosomal aberrations, including monosomy X (Turner syndrome), trisomy XXX, and Klinefelter syndrome XXY.

SEND REPRINT REQUEST TO AHMED ALI email: drahmedali77@gmail.com, amaly@qu.edu.sa

Segmental aplasia of the paramesonephric ducts (lack of development of a portion of the Müllerian duct) results in anomalies in the uterine tubes, uterus, cervix, and anterior vagina (Yin and Ma, 2005). Segmental aplasia of the uterus may involve one horn (uterus unicornis), both horns, or only part of one horn, which may result in cystic dilatation of the uterine horn that is anterior to the obstruction site (Tibary, 2015). Accumulation of secretions occurring prior to the obstruction, secondarily, may lead to hydrosalpinx, hydrometra, or mucometra.

Endometrial gland agenesis associated with unilateral uterine aplasia and endometrial dysgenesis concomitant with metritis have been seen in female dromedaries from pastoral herds in Algeria (Gherissi *et al*, 2020).

Double vagina and double cervix have been detected in a dromedary (Fig 1 B1). The case was tentatively diagnosed by clinical examination and confirmed by ultrasonography (Fig 1 B2). The vaginal lumen was completely separated into two cavities, each leading to a separate cervix (Fig 1 B3). This rare Müllerian duct anomaly is in contrast with the conventional embryologic theory of caudal- to-cranial Müllerian development. However, it is consistent with another embryological hypothesis that fusion and absorption begin at the isthmus and continue simultaneously in both the cranial and the caudal directions (Varras *et al*, 2007; Shirota *et al*, 2009).

True hymen persistence or hymen imperforation is the most commonly reported paramesonephric duct abnormality in domestic animals, including the llama and dromedary (Egloff *et al*, 2013; Ali and Derar, 2017). Fluid accumulates in the vagina and uterus, which causes the hymen to protrude at the vulva when the animal lies down or strains. These cases are usually admitted to a clinic with a history of incomplete intromission (Ali *et al*, 2015).

Vulvar atresia has been predominantly described in the dromedary (Fig 1 C1, C2). Congenital vulvar atresia may interfere with the discharge of urine (Wilkins *et al*, 2006). The condition is believed to be caused by an autosomal recessive gene (Tibary, 2015).

Male-like behaviour has been reported in female dromedary camels which is associated with enlargement of the clitoris and narrowing of the vagina and vulva (Fig 1 D1, D2). All the cases recorded were nullipara females. Some cases had normal oestrus behavior prior to masculinity. No ovarian cysts or tumours were detected in any of these cases. Changes detected in the external genitalia may draw attention to the likelihood of hermaphroditism syndrome. True and false hermaphrodites have been described in many domestic animals (Batista *et al*, 2000; Takagi *et al*, 2005; Lear and McGee, 2012). Karyotyping, hormonal analysis, and postmortem examination are essential for the complete understanding of this disorder in dromedaries.

Apart from those affected by imperforated hymen or partial vulvar atresia (not accompanied by vaginal aplasia), females with congenital infertility should be rejected for breeding.

Functional causes of infertility

Ovarian inactivity is not seen as a major infertility problem in dromedary camels, with incidences ranging from 0.7 to 4.5% (Ali *et al*, 2010a,b; 2015; Elshazly *et al*, 2019). Due to the well-defined seasonality of dromedaries, the frequency is clearly lower in winter and spring than in autumn and summer seasons (Hegazy *et al*, 2004). The activity of the pituitary gland and plasma follicle-stimulating hormone (FSH) was lower in camels with inactive ovaries (Hegazy *et al*, 2004). Improper nutrition, adverse body conditions, and lactation may be predisposing to the condition (Tibary and Anouassi, 1997). Follicular growth could be induced two months before the natural breeding season by either eCG (2000 IU) or Ov-Synch (GnRH on day 0, prostaglandin F2α on day 7 and GnRH on day 9) (Quzy *et al*, 2013). Fertile oestrus induction was achieved when GnRH agonist was administered to ovarian follicles ranging from 0.9 to 1.9 cm in diameter between day 17 and day 31 postpartum (Derar *et al*, 2014). Female camels with inactive ovaries during the non-breeding season have been administered a single intramuscular injection of 250 mg of hexanoate hydroxyprogesterone, followed by 1000 IU eCG on days 2 and 3 of treatment. The camels were mated on day 5 after the last injection of the eCG. Ovulation was induced by intravenous administration of 3000 IU hCG or 40 mcg GnRH. Of the treated camels, 67% ovulated; however, the conception rate was only 16.6%, which was attributed to the uterine environment and/or the inadequacy of semen quality (Agarwal *et al*, 1997). Others have reported that dromedary camels did not ovulate persistently in response to either hCG or GnRH treatment (Cooper *et al*, 1990).

Infertility in female dromedaries may also be associated with the growth of a dominant follicle beyond the preovulatory diameter and the subsequent formation of a large anovulatory follicle (overgrown

follicle, OVGF) (Fig 2). Follicular fluid may remain clear/hypoechoic, but hyperechoic cellular debris may be seen moving within the follicle fluid. In some cases, the OVGF will contain blood inside the lumen. Escaping blood from the follicular capillaries may give the follicular fluid a disseminated, fine echo pattern as the blood cells float or form layers in the follicle lumen. This structure is referred to as a haemorrhagic anovulatory follicle. There is an interspersed fibrin network within the lumen of some haemorrhagic follicles. Changes in ovarian follicle vasculature could be an indicator of their vitality, activity and lutenisation (Fig 3). Blood flow increases with the growth of dominant follicles and lutenisation of the OVGF (Rawy *et al*, 2014). The mechanism for the formation of an OVGF is uncertain and may involve an untimely or attenuated release of luteinising hormone (LH), or may be due to a follicle defect that causes it to fail to respond to a normal LH surge, such as an atypical or reduced LH receptor (Hegazy *et al*, 2004). Abnormally high FSH concentrations have been found in camels with OVGF (Ali *et al*, 2019a, Table 1), assuming that this high concentration of FSH would stimulate the continued development of large follicles. Serum Zn was lower in female camels with OVGF compared to the control group (Ali *et al*, 2010b, Table 1). In support of this opinion, serum zinc levels in polycystic ovarian syndrome women were found to be lower than in healthy women (Abedini *et al*, 2019). Metabolic disorders and oxidative stress can contribute to the development of OVGF in dromedaries (El-Badry *et al*, 2020). Others have suggested that OVGF should regress spontaneously and rarely requires further management (Tibary and Anouassi, 1996; Skidmore, 2011; Manjunatha *et al*, 2012; Rawy *et al*, 2014). Cases of OVGF exhibited low concentrations of estradiol-17β (El-Bahr *et al*, 2015; Ghoneim *et al*, 2013) and did not respond to exogenous GnRH therapy (Skidmore, 2011). Ovulation failure after satisfactory mating is another cause of infertility in camels. Ovulation rates in well-managed camel herds range from 80 to 90% (Tibary and Anouassi, 1996). Ovulation failure may be caused by insufficient release of LH in response to copulation (Skidmore *et al*, 1995). This lack of LH or insufficient LH release could be due to a hypothalamo-pituitary function disturbance or the reduced stimulation effect of copulation (Zhao *et al*, 1992). Ovulation failure could also be caused by follicles growing too large to ovulate. Skidmore *et al* (1996) reported that when follicles increased to >2.0 cm in diameter, the ovulation rate dramatically decreased. Therefore, knowing the size of the mature follicle is important for successful ovulation. Exogenous GnRH or LH administration within 24 hours of mating may overcome this problem, which requires further research to confirm.

Table 1. Blood biochemistry in female camels affected with overgrown follicles.

Blood biochemistry	Control	Overgrown follicle	P value
FSH mIU	4.02±1.8	7.31±2.8	0.03
LH mIU	5.4±0.9	11.6±7.3	0.1
Estradiol 17 (pg ml^{-1})	12.4±34[a]	52.1±30[a]	0.08
Progesterone (ng ml^{-1})	1.7±2[a]	6.1±1.8[a]	0.0001
Thyroxin (g/dl)	14.7±1.3[a]	14.2±1.1[a]	0.1
Zinc (g/ml)	1.07±0.[1a]	0.48±0.1[b]	0.003
Copper (g/ml)	0.7±0.1[a]	0.7±0.1[a]	0.9
Calcium (mg/dl)	8.6±1.2[a]	9±0.8[a]	0.5
Phosphorus (mg/dl)	6.7±0.3[a]	6.9±0.3	0.5
Magnesium (mg/dl)	2.5±0.04[a]	2.5±0.03[a]	0.4
Cholesterol (mg/dl)	235±20[a]	245±17[a]	0.4
Triglyceride (mg/dl)	173±13[a]	173±11[a]	0.4
Glucose (mg/dl)	71±9[a]	86.7±7[a]	0.2
Total protein (g/dl)	7.9±0.4[a]	8.5±0.4[a]	0.07

Table 2. Types and frequencies of uterine bacterial isolates in relation to the characters of vaginal discharges in female camels.

Uterine bacterial isolates	Vaginal discharges			
	No discharge	Gelatinous	Mucopurulent	Purulent
Trueperella pyogenes	2x	1x	4x	7x
Staphylococcus aureus	3x	5x	1x	4x
Streptococcus pyogenes	2x	-	5x	2x
Corynebacterium diphtheroides	2x	2x	2x	3x
Bacillus anthracoides	2x	1x	2x	2x
Streptococcus zooepidemicus	-	3x	2x	2x
Staphylococcus epidermidis	1x	2x	1x	1x
Enterococci	2x	-	2x	1x
Streptococcus β-hemolytica	-	-	1x	1x
Coagulase –ve Staphylococcus	-	1x	1x	-
Escherichia coli	-	-	-	2x
Proteus	-	-	1x	1x
Pseudomonas aeruginosa	-	1x	-	-
Klebsiella pneumonia	-	-	-	1x
Total	14x	15x	22x	27x

Pathological causes of infertility

Ovarian hydrobursitis (OVHB) is a serious problem in dromedaries and is associated with

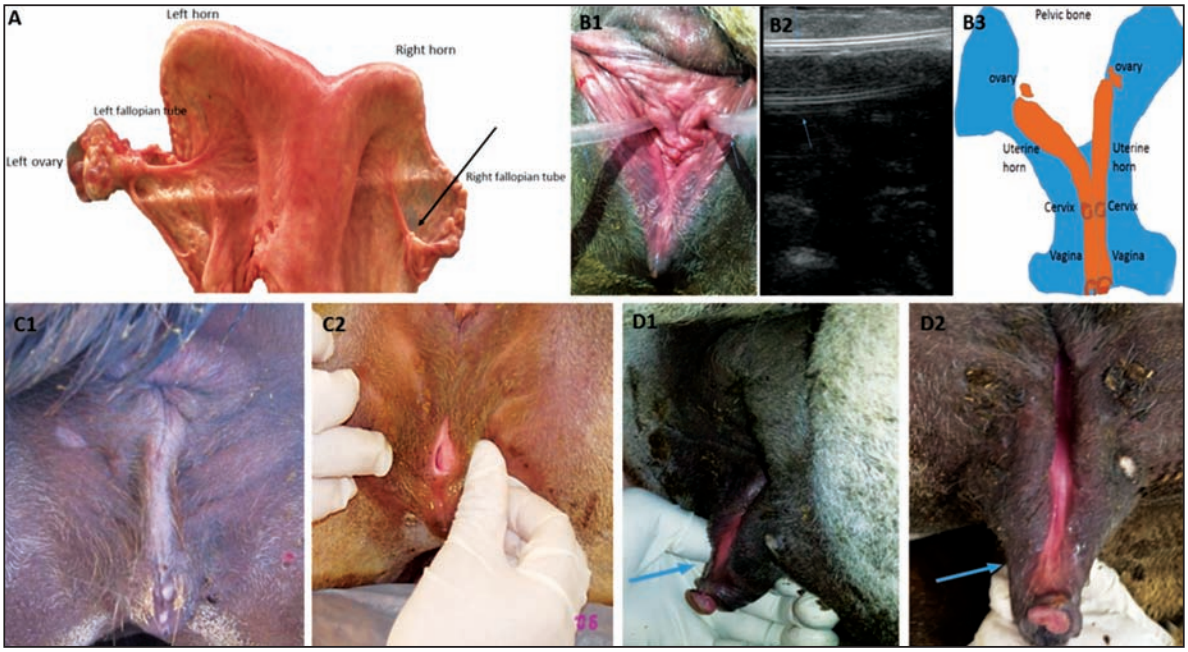


Fig 1. Congenital anomalies: A) unilateral ovarian aplasia in a female dromedary camel: Absence of the right ovary (arrow); B) Double cervix and vagina in a dromedary camel, the vaginal lumen is completely separated into two cavities, each leading to a separate cervix, two inseminating catheters are passing through the two vaginal cavities (arrows); C) vulvar atresia in virgin dromedary camels; D) hermaphroditism in infertile female dromedary camel, enlargement of the clitoris (arrow).

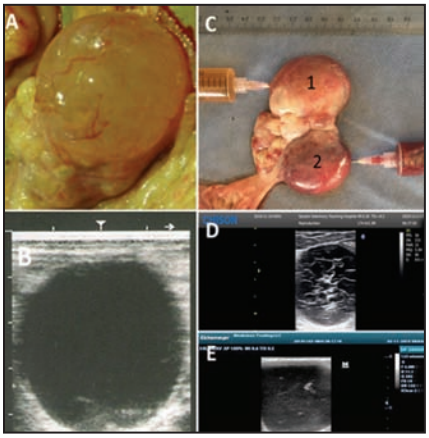


Fig 2. Morphology and sonogram of overgrown follicles (OVGF): (A, B) OVGF with a thin wall, clear content and transparent membrane; (C, D, E) OVGF with a thick wall and fibrous, opaque trabeculae with (1) straw-like and (2) bloody content.

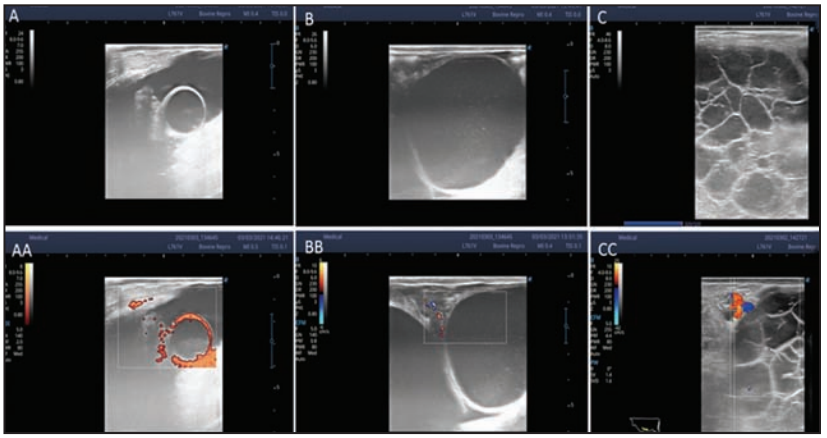


Fig 3. Blood flow of grown and overgrown follicles. Note that the blood flow increases with follicle growth (A, AA) and decreases in overgrown follicles (B, BB and C, CC).

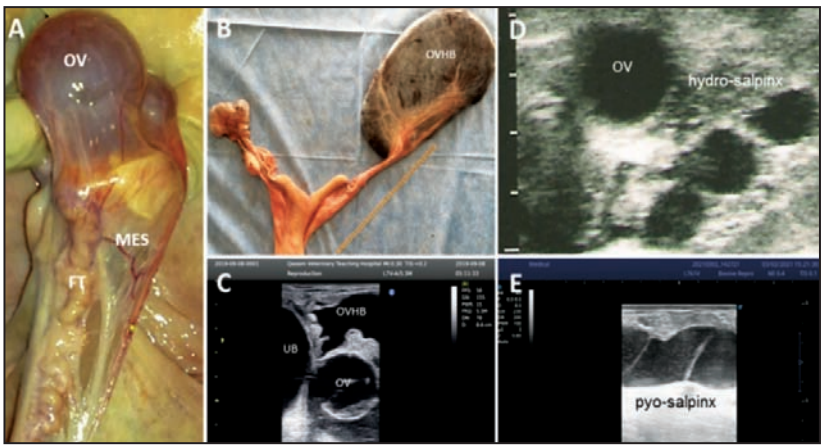


Fig 4. Ovarian hydrobursitis (OVHB) and hydro- and pyosalpinx in female dromedaries: (A) the ovary (OV) is normally completely covered by a thin fold of mesosalpinx (MES); (B, C) the OVHB appears as a large sac encapsulating the ovary; (D, E) the fallopian tubes appear beaded and filled with clear or turbid fluid, whereas the ovaries are located outside the distended organ.

long-term infertility, early embryonic death, and abortion (Tibary and Anouassi, 2001; Benaissa *et al*, 2014; Ali *et al*, 2015). The incidence range was 37.1% in nulliparous and 23.7% in multiparous barren dromedaries (Ali *et al*, 2015). It appears that the anatomical topography of the ovary bursa predisposes it to this serious condition in camels (Ali and Derar, 2020). The mesovarium, mesosalpinx, and proper ovarian ligament are joined together, completely encasing the ovary inside the bursa (Smuts *et al*, 1987; Srikandakumar *et al*, 2001; Salari *et al*, 2011) (Fig 4). As a result, accumulation of fluids inside the bursa may occur because of inflammatory reactions, rupture of large ovarian follicles, or obliteration of the infundibulum ostium abdominalis (Tibary and Anouassi, 2001; Ali *et al*, 2011a,b). *Chlamydia* is a commonly isolated pathogen in cases of salpingitis (inflammation of the oviduct) and cystic ovarian bursa in mice, koala, and camels (Obendorf and Handasyde, 1990; Khamesipour *et al*, 1994; Williams and Barker, 2001; Ali *et al*, 2012; Benaissa *et al*, 2020). *Chlamydia* can be transmitted through all methods of infection, including the venereal route (Obendorf and Handasyde 1990; Higgins *et al*, 2005; Faber *et al*, 2011). The disorder is assumed when retraction of the uterus and ovaries is extremely difficult or impossible upon palpation (Tibary and Anouassi, 2001; Ali *et al*, 2011b). However, ultrasonography is a reliable means of diagnosis for OVHB. The ovary is seen trapped and floating in a pool of fluids of varying volume and echogenicity, depending on the duration of the formation (Ali *et al*, 2017). Unilateral left, right, and bilateral OVHBs were detected at 45.8%, 12.5%, and 41.7%, respectively (Ali *et al*, 2011b). The condition has been associated with purulent endometritis, cervical and vaginal adhesions, pyometra, and enlargement of the fallopian tube, and also in cases where there were no apparent abnormalities in the genital tract other than the affected bursa (Ali *et al*, 2011b). Surgical removal of the affected bursa was recommended in unilaterally affected cases and the treated animals achieved a satisfactory conception rate (Tibary and Anouassi, 2001; Ali *et al*, 2011b). Small OVHBs can conceivably be treated with a combination of 20 mg/kg intramuscular oxytetracycline, 4% intrauterine lotagen, and 500 mg intramuscular cloprostenol (Ali *et al*, 2012). Because cases are usually detected at a late stage, making it difficult to deal with them, plans to vaccinate camels against *Chlamydia* infection are being considered.

Hydrosalpinx and pyosalpinx (distention of the fallopian tube with watery or purulent material) may be confused with cases of OVHB, particularly during rectal palpation. However, via ultrasonography, the ovary can be visualised outside the affected organ in the former cases, whereas the ovary is located inside the affected bursa in OVHB. In addition, the affected fallopian tubes often appear as beaded lobes, resembling peas in a pod (Fig 4). Hydrosalpinx and pyosalpinx have been estimated to affect 1.7% of barren female dromedaries (Ali *et al*, 2015). A relatively lower incidence (0.8%) was recorded in an abattoir survey (Benaissa *et al*, 2017). *Chlamydia* is associated with hydrosalpinx in mice and women (Shao *et al*, 2012; Harvie *et al*, 2019). These conditions occur as a result of the blockage of the fallopian tube or from the segmental aplasia of the paramesonephric ducts, which leads to the accumulation of secretions prior to the obstruction (Azawi *et al*, 2010; Tibary, 2015). Uterine inflammation may be extended to the utero-tubal junction, resulting in local inflammation, fibrosis, and tubal obstruction. Fertility is compromised in such cases, as there is no specific treatment (Tibary and Anouassi, 1997).

Clinical endometritis (presence of abnormal vaginal discharges) is the primary clinical finding of barren female dromedaries in most camel herds (Ali *et al*, 2015). Clinical endometritis (CE) may be mild (45%), moderate (32%), or severe (23%). Clinically, the majority of dromedaries with endometritis are repeat breeders, but others may refuse mating or accept mating after variable periods of anestrous (Ali *et al*, 2015). Unsanitary reproductive management, unhygienic procedures during parturition, and postpartum complications are causal factors for uterine contamination (Tibary *et al*, 2006). Infertility persists for the duration of CE infection, and subfertility may continue even after apparently successful treatment (Williams *et al*, 2008). In most females, infections are eradicated by the uterine defense mechanisms, but in some females, these defense mechanisms fail partially or completely, leaving the infection to develop (Tibary *et al*, 2006). *Trueperella pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus zooepidemicus,* and *b-hemolytic Streptococcus* are considered common causes of CE in dromedaries (Nawito, 1973; Hegazy *et al*, 1979; Wernery and Wernery, 1992; Tibary *et al*, 2006; Ali *et al*, 2015). No relationship has been observed between the characteristics of vaginal discharge and the prevalence of uterine bacterial isolation (Ali *et al*, 2009). The incidences of bacterial isolation were 85.7, 90.9, 81.8, and 87.5% in those with none, or gelatinous, mucopurulent, or purulent vaginal discharges, respectively (Table 2). Gram-positive isolates were

sensitive to chloramphenicol (91%), gentamicin (83.3%), and cotrimoxazole (82.1 %). By contrast, these isolates were less susceptible to neomycin (61.5%), penicillin G (55.1%), tetracycline (52.6%), and polymixin B (51.3%). At the same time, most Gram-negative isolates were sensitive to gentamicin (83.3%) and cefotaxime (83.3%). *Pseudomonas aeruginosa* was resistant to all antimicrobial drugs except gentamicin. Three regimens were compared for the treatment of CE in dromedaries (Ali *et al*, 2010c), consisting of intrauterine infusion that included 100-mL acriflavin 0.1%, 100-mL lotagen 4%, or 300 mg/100-mL gentamicin sulfate, complemented by administration of PGF25α at infusion and hCG at mating. These regimens were found to be effective in the treatment of mild and moderate levels of CE, but the intrauterine lotagen infusion was more effective in the treatment of severe CE.

Subclinical endometritis is a uterine inflammation with no clinical signs and is described as infiltration of polymorphicnuclear cells into the endometrium (Sheldon *et al*, 2006). Subclinical endometritis (SCE) was detected in 9.9% of infertile female dromedaries (Derar *et al*, 2020). Endometrial swabbing has been described as a more sensitive and specific procedure for SCE diagnostics compared to other techniques such as rectal palpation, ultrasonography, and Metricheck. As cytology is the gold standard test, a combination of cytological and bacteriological methods has been recommended to increase the sensitivity of diagnosis and to reduce misdiagnosis of SCE (Derar *et al*, 2020). The most commonly isolated microorganisms were *Bacillus* sp., *Staphylococcus* sp., and *Candida albicans*.

Hydrometra and pyometra (accumulation of aqueous or purulent material in the uterus) were observed in 7.1% of barren female camels (Ali *et al*, 2015). All cases were shown to have transluminal cervical and vaginal adhesions or persistent hymen. Transluminal cervical/vaginal adhesions in mares (Cozens, 2009) and persistent hymen in alpaca (Egloff *et al*, 2013) have been described as pyometra-related. *Trueperella pyogenes* has been isolated from camels with pyometra (Chauhan and Kaushik, 1992). Treatment involves opening the adhesion manually, emptying the uterine content, and washing several times with antiseptics. The administration of oxytocin and prostaglandin F2a (PGF2α) facilitates the emptying of the uterus.

Vaginal and cervical adhesions (occlusion of the vagina or cervix) and cervical stenosis (narrowing of the cervix) are problems that affect the reproductive life of female dromedaries. Incidences in barren female camels were estimated at 23.1% for vaginal adhesion, 10.3% for cervical adhesion, and 11.1% for cervical stenosis (Ali *et al*, 2015). Vaginal adhesions were seen in the caudal, mid, or cranial vagina at frequencies of 18%, 74%, and 8%, respectively. Injuries during parturition, foetotomy, chronic vaginitis, over-breeding, violent mating, and increasing parity have been suggested as the causes of these problems (Tibary and Anouassi, 1997; Derar *et al*, 2016; Benaissa *et al*, 2017). In addition, as part of ethno-veterinary practices, some farmers place unusual materials such as black seeds (*Nigella sativa*), dates, and salt in the vagina of camels with fertility problems, and these may irritate the mucous membrane of the vagina. Long-term refusal to mate, difficulty in mating, and bleeding after mating are characteristic clinical signs of vaginal adhesion. Most cases have shown an accumulation of fluid in the uterus (Derar *et al*, 2016). The rate of culling was higher in females with vaginal adhesion (54.4%) than in females with cervical adhesion (20%) and cervical stenosis (11.8%). Veterinarians should distinguish between cases of vaginal adhesion and cases of persistent hymen. Persistent hymen is a congenital condition that can only be found in nullipara females. Persistent hymen is usually located just cranial to the external opening of the urinary bladder, and the condition has a respectable prognosis. Vaginal adhesion, on the other hand, is an acquired condition that is typically detected in aged multiparous animals and has a poor fertility prognosis. Female camels with vaginal and cervical adhesions have been treated with manual rupture, local use of antibiotic anti-inflammatory suspension to prevent recurrence of adhesions, PGF2a for uterine evacuation, and intramuscular phenylbutazone (4.4 mg/kg) as a systemic anti-inflammatory agent. Cervical stenosis has been treated with manual dilation, administration of PGF2α and phenylbutazone. Pregnancy rates were 73.3%, 11.5%, and 0.0% for females with cervical stenosis, vaginal adhesion, and cervical adhesion, respectively (Derar *et al*, 2016).

Neoplasms of the reproductive tract in dromedaries have been assessed at 0.005% (Al-Sobayil *et al*, 2018; Ali *et al*, 2018b). The vagina was the most affected (63.6%), whereas adenocarcinoma was the most predominant type (81.8%). Other types included ovarian teratoma and dysgerminoma (Elwishy, 1990; El-Khouly *et al*, 1990), granulosa cell tumour (Ali *et al*, 2013), Sertoli-Leydig cell tumour (Ali *et al*, 2019b), and cervical and uterine adenocarcinoma (Ali *et al*, 2018b). The cause of vaginal cancer in camels is still unknown; however, papillomavirus should be

considered a causative factor. Approximately half of human vaginal cancers are associated with human papillomavirus (HPV), whereas all cervical cancers are primarily caused by HPV (McNamara *et al*, 2016; Viens *et al*, 2016). Papillomaviruses have been detected in camels (Ure *et al*, 2011). Risks associated with vaginal cancer include age, diethylstilbestrol exposure, history of cervical cancer, and vaginal irritation (Hellman *et al*, 2004; McNamara *et al*, 2016). Difficult mating, vaginal bleeding, and anemia were commonly associated with clinical and laboratory findings (Ali *et al*, 2018b). The prognosis of camels with vaginal adenocarcinoma is poor due to lack of a specific treatment.

Management causes of infertility

Infertility due to management errors is quite common and should be considered when dealing with camel herds. In one field study, 45% of dromedary females had no follicular structures or had follicles smaller than 9 mm (Tibary and Anouassi, 2000). Detection of oestrous in the dromedary is problematic and is not often related to follicular activity. In addition, oestrus behavior is extremely variable in length and intensity (Skidmore, 2011). Other management errors include breeding with a young male, overuse of males, and lack of verification of intromission during copulation (Tibary and Anouassi, 2000).

Tenacious use of traditional reproductive management systems in many breeding herds may be a key cause of decreased reproductive performance in dromedary camels (Ali *et al*, 2018a; Faye, 2018). In traditional management systems, camels usually calve once every two years (Matharu 1966; Merkt *et al*, 1990); however, with improved feed, they can calve every 18 months (Knoess, 1977). Under the semi-intensive system in North Kordofan, Sudan, about 78% of females became pregnant in the fifth to eighth month postpartum period, with a calving interval ranging from 17 to 20 months. Under the traditional system, only 44.5% of females became pregnant in the 11th to 16th months of postpartum, and the calving interval varied from 23 to 28 months (Bakheit *et al*, 2016). The calving interval may be reduced by mating during mid-lactation, but this will be accompanied by a decrease in the amount of milk (Nagy *et al*, 2013).

Herder/camel ratio and managerial experience are important factors affecting the reproductive performance of camel herds in Saudi Arabia (Ali *et al*, 2018a). Farms with a herder/camel ratio of 1:<25 had a higher pregnancy rate than farms with herder/camel ratios of 1:25–50 or 1:>50 (95% vs. 80% vs. 73%). Farms having managerial experience of >10 years had a higher pregnancy rate than farms having managerial experience of 5–10 years or <5 years (95% vs. 81% vs. 73%). Female/male ratio is also an issue in some herds, as there are frequently too many females in any one herd (Ali *et al*, 2018a). The refining of managerial performance and the maximum use of herders are therefore factors imperative for maintaining high reproductive efficiency in camel herds. Decreasing the age at first mating and the interval after calving can be achieved. In addition, it is recommended that the capabilities of veterinarians and camel herd managers be improved (Faye, 2018).

Conclusion

To our knowledge, this review is the first to identify and classify the causes of infertility in female dromedary camels. Applying appropriate preventive measures is important in order to prevent serious reproductive diseases such as ovarian hydrobursitis and vaginal adhesion. Enhanced management practices and rigorous selection are imperative for increasing the reproductive efficiency of dromedary herds. Meanwhile, further studies are needed to disclose the pathogenicity of overgrown follicles.

Acknowledgement

The authors extend their appreciation to the Saudi Arabian Deputyship of the Ministry of Education for Universities, Research and Innovation for funding this research work through the project numbered (QU - IF - 1-1-2). The authors also thank Qassim University for the technical support.

References

Abedini M, Ghaedi E, Hadi A, Mohammadi H and Amani R. Zinc status and polycystic ovarian syndrome: A systematic review and meta-analysis. Journal of Trace Elements in Medicine and Biology. 2019; 52:216-221.

Adams GP, Ratto MH, Silva ME and Carrasco RA. Ovulation inducing factor (OIF/NGF) in seminal plasma: a review and update. Reproduction in Domestic Animals 51 Suppl. 2016; 2:4-17.

Agarwal SP, Rai AK and Khanna ND. Induction of sexual activity in female camels during the nonbreeding season. Theriogenology 1997; 47:591-600.

Al Eknah MM. Reproduction in Old World camels. Animal Reproduction Science 2000; 60-61:583-592.

Ali A and Derar DR. Ovary and ovarian bursa in dromedary camels: Clinical relevance of the topographical features. Anatomia, Histologia, Embryologia. 2020; 49:325-332.

Ali A and Derar R. Atlas of Reproduction in Dromedary Camels. Qassim University-Saudi Arabia. 2017; ISBN-978-603-8176-66-5.

Ali A, Al-Sobayil FA and Al-Hawas A. Evaluating the effectiveness of different treatments of uterine infections

in female camels (*Camelus dromedarius*). Theriogenology 2010c; 74:40-44.

Ali A, Al-Sobayil FA, Hassanein KM and Al-Hawas A. Ovarian hydrobursitis in female camels (*Camelus dromedarius*): the role of *Chlamydophila abortus* and a trial for medical treatment. Theriogenology. 2012; 77:1754-1758.

Ali A, Al-sobayil FA, Tharwat M, Al-Hawas A and Ahmed AF. Causes of Infertility in Female Camels (*Camelus dromedarius*) in Middle of Saudi Arabia. Journal of Agriculture and Veterinary Sciences. 2010a; 2:59-66.

Ali A, Al-Sobayil FA, Tharwat M, Mehana EE and Al-Hawas A. Granulosa cell tumour in a female dromedary camel. Comparative Clinical Pathology. 2013; 22:1251-1254.

Ali A, Derar D, Alsamri A and Al Sobayil F. Echography of clinically relevant disorders in the genital tract of female dromedary camels. Animal Reproduction Science. 2017; 182:123-133.

Ali A, Derar D, Alsharari A, Khalil R, Almundarij TI, Alboti Y and Al-Sobayil F. Factors affecting reproductive performance in dromedary camel herds in Saudi Arabia. Tropical Animal Health and Production. 2018a; 50:1155-1160.

Ali A, Derar D, Khaled MA, Al-Howas A, Sadan M, El-Shafaey E and Al-Sobyil FA. Sertoli-Leydig cell tumour in a female dromedary camel. Journal of Camel Practice and Research. 2019b; 26:287-290.

Ali A, Derar DR, Zeitoun MM and Al-Sobayil. The interrelationship between the occurrence of oversized follicles and the peripheral and intra-follicular concentrations of E2, P4, FSH, and LH in female dromedary camels. Journal of Camel Health. 2019a; 1:11-19.

Ali A, Derar R, Al-Sobayil F, Al-Hawas A, Hassanein K. A retrospective study on clinical findings of 7300 cases (2007-2014) of barren female dromedaries. Theriogenology. 2015; 84:452-456.

Ali A, Derar R, Al-Sobayil F, Tharwat M, Fathy A and Khodeir M. Adenocarcinoma in the genital tract of infertile female dromedary camels. Journal of Camel Practice and Research. 2018b; 25:181-187.

Ali A, Hassanein KM, Al-Sobayil FA, Tharwat M, Al-Hawas A and Ahmed AF. Relationship between Characters of Vaginal Discharges and Uterine Bacterial Isolates Recovered from Repeat Breeding Female Camels (*Camelus dromedarius*). Journal of Agriculture and Veterinary Sciences. 2009; 2:89-98.

Ali A, Mehana EE, Ahmed AF, El-Tookhy O, Al-Sobayil A and Al-Hawas A. Ovarian hydrobursitis in female camels (*Camelus dromedarius*): clinical findings, histopathology and fertility after unilateral surgical ablation. Theriogenology. 2011b; 76:492-499.

Ali A, Tharwat M and Al-Sobayil FA. Hormonal, biochemical, and hematological Profiles in female camels (*Camelus dromedarius*) affected with reproductive disorders. Animal Reproduction Science. 2010b; 118:372-376.

Ali A, Al-Sobayil FA, Tharwat M, Hassanein KM. Ovarian hydrobursitis in female camels (*Camelus dromedarius*): biochemical, bacterial and protozoal evaluation. Theriogenology. 2011a; 75:734-741.

Al-Sobayil FA, Ali A, Derar DR, Tharwat M, Ahmed AF and Khodeir M. Tumours in dromedary camels: Prevalence, types and locations. Journal of Camel Practice and Research. 2018; 25:189-197.

Arthur GH, Rahim ATA and Al Hindi AT. Reproduction and genital diseases of the camel. British Veterinary Journal. 1985; 141:650-659.

Azawi O, Al-Abidy H and Ali AJ. Pathological and bacteriological studies of hydrosalpinx in buffaloes. Reprod. Domest. Anim. 2010; 45:416-420.

Bakheit SA, Faye B, Ahmed AI and Elshafei IM. Effect of Farming System on Camels Calving Interval in Western Sudan. Turkish Journal of Agriculture - Food Science and Technology. 2016; 4:418-423.

Basrur PK, Kanagawa H and Gilman JP. An equine intersex with unilateral gonadal agenesis. Canadian Journal of Comparative Medicine. 1969; 33:297-306.

Batista M, González F, Cabrera F, Palomino E, Castellano E, Calero P and Gracia A. True hermaphroditism in a horned goat with 60XX/60XY chimerism. Canadian Veterinary Journal. 2000; 41:562-564.

Benaissa MH, Faye B and Rachid K. Ovarian hydrobursitis in slaughtered female camels (*Camelus dromedarius*) in Southeast Algeria. Emirates Journal of Food and Agriculture. 2014; 26:915-920.

Benaissa MH, Mimoune N and Kaidi R. Prevalence and anatomohistopathologic studies of uterine anomalies in she-camels in southeast Algeria. HVM Bioflux. 2017; 9:137-145.

Benaissa MH, Mimoune N, Youngs CR, Kaidi R and Faye B. First report of *Chlamydophila abortus* infection in the dromedary camel (*Camelus dromedarius*) population in eastern Algeria. Comparative Immunology, Microbiology and Infectious Diseases. 2020; 73:101557.

Chauhan RS and Kaushik RK (1992). Pyometra in camels: case report. British Veterinary Journal 148:84-85.

Cooper MJ, Skidmore J, Ali M, Bilfah T, Wensvoort S, Rillah A, Allen WR. An attempt to induce and synchronise ovulation and superovulation in dromedary camels for embryo transfer. Proc. Workshop: IS it possible to improve the reproductive performance of the camel? Paris. 1990; 313-326.

Cozens ER. Pyometra and complete vaginal adhesion in a miniature horse. Canadian Veterinary Journal. 2009; 50:971-972.

Dawod A and Elbaz TH. Ovarian Reproductive Affections in She-Camels. Journal of Veterinary Science and Technology. 2018; 9:5.

Derar DR, Ali A and Al-Sobayil FA. The postpartum period in dromedary camels: uterine involution, ovarian activity, hormonal changes, and response to GnRH treatment. Animal Reproduction Science. 2014; 151:186-193.

Derar DR, Ali A, Al-Sobayil F, Al-Hawas A. Clinical findings and reproductive performance of female dromedary affected with vaginal and cervical adhesions and stenosis. Journal of Camel Practice and Research. 2016; 23:179-184.

Derar DR, Ali A, Saeed EM, Al-Sobayil F, Al-Samri A and Elbehiry A. Diagnostic evaluation of subclinical endometritis in dromedary camels. Animal Reproduction Science. 2020; 215:106327.

Djellouli M and Saint-Martin G. Productivity and economy of camel breeding in Tunisia. In: Allen WR, Higgins AJ, Mayhew IG, Snow DH, Wade JF. (Ed.). Proceedings of the 1st International Camel Conference. New market, UK: R&W Publications. 1992; pp 209-212.

Egloff C, Gerspach C, Rütten M, Dettwiler M, Reichler I and Bleul U. Pyometra and persistent hymen in an alpaca. Tierarztliche Praxis Ausgabe G: Grosstiere - Nutztiere. 2013; 41:185-189.

El-Badry DA, Ibrahimb MA and Leilc AZ. Hormonal and biochemical studies on female dromedary camels affected with multiple ovarian cysts. Small Ruminant Research. 2020; 188:106138.

El-Bahr SM, Ghoneim IM and Waheed MM. Biochemical and hormonal analysis of follicular fluid and serum of female dromedary camels (*Camelus dromedarius*) with different sized ovarian follicles. Animal Reproduction Science. 2015; 159:98-103.

El-Khouly ABA, El-Nasr A and Ontabli A. Some pathologic affections of camel ovaries in U.A.E. Zagazig Veterinary Journal. 1990; 18:210-217.

Elshazly MO, Abd El-Rahman SS, Hamza DA and Ali ME. Prevalence of non-neoplastic ovarian disorders in non-pregnant she-camels (*Camelus dromedarius*) and their correlation to bacteriological isolation. International Journal of Veterinary Science. 2019; 8:20-27.

Elwishy AB. Genital abnormalities in camels (*Camelus dromedarius*). In: Proceedings of the workshop "Is it possible to improve the reproductive performance of the Camel?", Paris. 1990; 163-174.

Faber MT, Nielsen A, Nygård M, Sparén P, Tryggvadottir L, Hansen BT, Liaw KL and Kjaer SK. Genital *Chlamydia*, genital herpes, Trichomonas vaginalis and gonorrhea prevalence, and risk factors among nearly 70,000 randomly selected women in 4 Nordic countries. Sexually Transmitted Diseases. 2011; 38:727-34.

Faye B. The improvement of camel reproduction performances: just a technical question? Revue Marocaine des Sciences Agronomiques et Vétérinaires. 2018; 6:265-269.

Gherissi DE, Lamraoui R, Chacha F, Bouzebda Z, Bouzebda FA and Hanzen C. Genital abnormalities associated to lack of uterine adenogenesis or endometrial gland dysgenesis of female dromedary camels. Open Veterinary Journal. 2020; 10:44-52.

Ghoneim IM, Waheed MM, El-Bahr SM, Alhaider AK and Al-Eknah MM. Comparison of some biochemical and hormonal constituents of oversized follicles and preovulatory follicles in camels (*Camelus dromedarius*). Theriogenology. 2013; 79:647-652.

Harvie MC, Carey AJ, Armitage CW, O'Meara CP, Peet J, Phillips ZN, Timms P and Beagley KW. *Chlamydia*-infected macrophages are resistant to azithromycin treatment and are associated with chronic oviduct inflammation and hydrosalpinx development. Immunology and Cell Biology. 2019; 97:865-876.

Hegazy A, Ali A, El-Eknah M and Ismail S. Studies on pituitary-ovarian axis in the female camel with special reference to cystic and inactive ovaries. Journal of Camelid Science. 2004; 1:16-24.

Hegazy A, Youseff HI and Selim SA. Bacteriological and histopathological studies on endometritis of the camel. Journal of the Egyptian Veterinary Medical Association. 1979; 39:81-97.

Hellman K, Silfversward C and Nilsson B. Primary carcinoma of the vagina: factors influencing the age at diagnosis. The Radiumhemmet series 1956-96. International Journal of Gynecological Cancer. 2004; 14:491-501.

Higgins DP, Hemsley S and Canfield PJ. Association of uterine and salpingeal fibrosis with *Chlamydia*l hsp60 and hsp10 antigenspecific antibodies in *Chlamydia*-infected koalas. Clinical and Diagnostic Laboratory Immunology. 2005; 12:632-639.

Khalafalla AI, Eknah MM, Abdelaziz M and Ghoneim IM. A study on some reproductive disorders in dromedary camel herds in Saudi Arabia with special references to uterine infections and abortion. Tropical Animal Health and Production. 2017; 49:967-974.

Khamesipour A, Pal S, Peterson EM and de la Maza LM. Induction of infertility by the *Chlamydia* trachomatis mouse pneumonitis biovar in strains of mice that differ in their response to the 60 kDa heat shock protein. Journal of Reproduction and Fertility. 1994; 101:287-294.

Knoess KH. The camel as a meat and milk animal. World Animal Review. 1977; 22:39-44.

Lear TL and McGee RB. Disorders of sexual development in the domestic horse, *Chlamydia*. Sexual Development. 2012; 6:61-71.

Manjunatha BM, Pratap N, Al-Bulushi NS and Hago BE. Characterisation of ovarian follicular dynamics in dromedary camels (*Camelus dromedarius*). Theriogenology. 2012; 78:965-73.

Matharu BS. Camel care. Indian Farming. 1966; 16:19-22.

McNamara M, Batur P, Walsh JM and Johnson KM. HPV update: vaccination, screening, and associated disease. Journal of General Internal Medicine. 2016; 31:1360-1366.

Merkt H, Rath D, Musa B and El-Nagger MA. Reproduction in camels. FAO, Rome, Italy, 1990. Review. 1990.

Millward S, Mueller K, Smith R and Higgins HM. A Post-mortem Survey of Bovine Female Reproductive Tracts in the UK. Frontiers in Veterinary Science. 2019; 6:451.

Musa BE and Abusineina ME. The oestrous cycle of the camel (*Camelus dromedarius*). Veterinary Record. 1978; 103:556-557.

Nagy P, Skidmore JA and Juhasz J. Use of assisted reproduction for the improvement of milk production in dairy camels (*Camelus dromedarius*). Animal Reproduction Science. 2013; 136:205-210.

Nawito M. Uterine infections in the camel. Egyptian Journal of Veterinary Sciences. 1973; 10:17-22.

Novoa C. Reproduction in Camelidae. Journal of Reproduction and Fertility. 1970; 22:3-20.

Obendorf DL and Handasyde KA. Pathology of Chlamydial infection in the reproductive tract of the female koala. In: Lee AK, Handasyde KA, Sanson GD, editors. Biology of the koala. Sydney, Australia: Surrey Beatty and Sons. 1990.

Quzy I, Anwar SS and Purohit GN. Hormonal management of ovarian activity in breeding camels two months ahead of the natural breeding season. Camel. 2013; 1:37-49.

Rawy MS, Derar RI, El-Sherry TM and Megahed GA. Plasma steroid hormone concentrations and blood flow of the ovarian structures of the female dromedary (*Camelus dromedarius*) during growth, dominance, spontaneous ovulation, luteinization and regression of the follicular wave. Animal Reproduction Science. 2014; 148:137-144.

Regassa F, Mengesha D, Dargie M and Tolosa T. Abattoir evidence on association between uterine and ovarian abnormalities in Ethiopian highland ewes. Animal Reproduction Science. 2009; 111:384-390.

Salari E, Raji AR and Farzaneh N. Comparative study of anatomy and histology on the ovary and oviduct in camel (*Camelus dromedarius*) and cow. Journal of Camel Practice and Research. 2011; 18:115-118.

Schwartz HJ, Dolan R and Wilson AJ. Camel production in Kenya and its constraints. I. Productivity. Tropical Animal Health and Production. 1983; 15:169-178.

Shao R, Wang X, Wang W, Stener-Victorin E, Mallard C, Brännström M and Billig H. From mice to women and back again: causalities and clues for *Chlamydia*-induced tubal ectopic pregnancy. Fertility and Sterility. 2012; 98:1175-1185.

Sheldon IM, Lewis GS, LeBlanc S and Gilbert RO. Defining postpartum uterine disease in cattle. Theriogenology. 2006; 65:1516-1530.

Shirota K, Fukuoka M, Tsujioka H, Inoue Y and Kawarabayashi T. A normal uterus communicating with a double cervix and the vagina: a müllerian anomaly without any present classification. Fertility and Sterility. 2009; 91:935.

Skidmore JA. Reproductive physiology in female Old World Camelids. Animal Reproduction Science. 2011; 124:148-154.

Skidmore JA, Billah M and Allen WR. The ovarian follicular wave pattern in the mated and non-mated dromedary camel (*Camelus dromedarius*). Journal of Reproduction and Fertility Suppl. 1995; 49:545-548.

Skidmore JA, Billah M and Allen WR. The follicular wave pattern and induction of ovulation in the mated and non-mated one-humped camel (*Camelus dromedarius*). Journal of Reproduction and Fertility. 1996; 106:185-192.

Smuts MMS, Bezuidenhout RJ and Bezuidenhout AJ. Anatomy of the dromedary. Oxford, UK: Univ Press. 1987.

Srikandakumar A, Johnson EH, Mahgoub O, Kadim IT and Al-Ajmi DS. Anatomy and histology of the female reproductive tract of the Arabian camel. Emirates Journal of Food and Agriculture. 2001; 13:23-26.

Takagi M, Yamagishi N, Oboshi K, Kageyama S, Hirayama H, Minamihashi A, Sasaki M and Wijayagunawardane MP. A female pseudohermaphrodite Holstein heifer with gonadal mosaicism. Theriogenology. 2005; 63:60-71.

Tibary A and Anouassi A. Ultrasonographic changes of the reproductive-tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy. Journal of Camel Practice and Research. 1996; 3:71-90.

Tibary A and Anouassi A. Theriogenology in Camelidae: anatomy, physiology, pathology and artificial breeding. Rebat, Morocco: Acetes Editions. 1997; pp 349-410, 501-410.

Tibary A and Anouassi A. Reproductive disorders in the female camelid. In: Skidmore JA, Adams GP, editors. Recent advances in camelid reproduction. International Veterinary Information Service. http://www.ivis.org/, access 24/4/2021. 2000.

Tibary A and Anouassi A. Retrospective study on an unusual form of ovario-bursal pathology in the camel (*Camelus dromedarius*). Theriogenology. 2001; 56:415-24.

Tibary A, Fite C, Anouassi A and Sghiri A. Infectious causes of reproductive loss in camelids. Theriogenology. 2006; 66:633-47.

Tibary A. Female Genital Abnormalities of Animals. https://www.msdvetmanual.com/reproductive-system/congenital-and-inherited-nomalies-of-the-reproductive-system/female-genital-abnormalities. Last access 24/4/2021. 2015.

Ure AE, Elfadl AK, Khalafalla AI, Gameel AA, Dillner J and Forslund O. Characterisation of the complete genomes of *Camelus dromedarius* papillomavirus types 1 and 2. Journal of General Virology. 2011; 92:1769-1777.

Varras M, Akrivis C, Demou A, Kitsiou E and Antoniou N. Double vagina and cervix communicating bilaterally with a single uterine cavity: report of a case with an unusual congenital uterine malformation. The Journal of Reproductive Medicine. 2007; 52:238-240.

Viens LJ, Henley SJ, Watson M, Markowitz L, Thomas CC, Thompson TD, Razzaghi H and Saraiya M. Human papillomavirus-associated cancers -United States, 2008-2012. MMWR Morbidity and Mortality Weekly Report 65:661-666. 2016.

Wernery U and Wernery R. Uterine infections in the dromedary camel "a review". In: Proceedings of the First International Camel Conference, February 2nd–6th, Dubai. 1992; pp 155-158.

Wilkins PA, Southwood LL and Bedenice D. Congenital vulvar deformity in 6 alpacas. Journal of the American Veterinary Medical Association. 2006; 229:263-265.

Williams EJ, Herath S, England GC, Dobson H, Bryant CE and Sheldon IM. Effect of *Escherichia coli* infection of the bovine uterus from the whole animal to the cell. Animal. 2008; 2:1153-1157.

Williams ES and Barker IK. Infectious Diseases of Wild Mammals (3rd ed.). Ames, Iowa, USA: Iowa State University. 2001.

Yin Y and Ma L. Development of the mammalian female reproductive tract. Journal of Biochemistry. 2005; 137:677-683.

Zhao XX, Huang YM and Chen BX. Biological activity of Gonadotrophin-releasing Hormone-like factors in the seminal plasma of the Bactrian camel. In: Proceedings of the 1st International Camel Conference Dubai, UAE. 1992; pp 163-168.

DOI : 10.5958/2277-8934.2021.00043.6 Vol 28 No 3, p 277-282

EFFECTIVENESS EVALUATION OF RECOMBINANT ANTIGEN rCPI FOR iELISA DETECTION OF CAMEL PARABRONEMIASIS

Yu Wang[1*], Chenchen Feng[1*], Chunxia Liu[2], Jianyun LI[3] and Wenlong Wang[1#]

[1]College of Veterinary Medicine, [2]College of Life Sciences, Inner Mongolia Agricultural University, Hohhot 010018, China Key Laboratory of Clinical Diagnosis and Treatment Technology in Animal Disease, Ministry of Agriculture, P.R. China, Hohhot 010018, China

[3]Inner Mongolia Comprehensive Centre for Disease Control and Prevention, Hohhot 010031, China

ABSTRACT

Parabronemiasis is a serious parasitic disease in ruminants and a major parasitic nematode in camels. There is still no ideal method for diagnosing the disease when the hosts are alive. In order to screen the antigen of *P. skrjabini* for establishing a serological diagnostic method for camel parabronemiasis, in this study, we amplified and cloned the gene encoding cysteine protease inhibitor (*cpi*) of *P. skrjabini* by RT-PCR and constructed the expression plasmid pET-*cpi*, which was then transferred into *Escherichia coli* BL21 (DE3) to obtain the recombinant protein rCPI. The purified recombinant protein was used as the coating antigen to establish an indirect ELISA diagnostic method for parabronemiasis. A total of 140 sera collected from camels in Inner Mongolia were tested. A recombinant *cpi* protein rCPI with a size of 20.2 kDa was obtained, which can specifically bind to IgG in the serum of camels infected with *P. skrjabini*. An iELISA method for the detection of parabronemiasis was established with good specificity. The positive rate of 140 camel sera was 84.3% (118/140), indicating that rCPI iELISA can be used as a serological diagnostic method for camel parabronemiasis.

Key words: Camels, cysteine protease inhibitor, *Parabronema skrjabini*, Parabronemiasis, serological diagnosis

Parabronemiasis is a parasitic disease caused by an infection with several species of trematodes belonging to the genus *Paragonimus*, including *Parabronema skrjabini*, which is usually found in the abomasum of ruminants such as camels, cattle, and sheep (Abd-Rabo *et al,* 1993; Hasheminasab *et al,* 2016a; Khalafalla *et al,* 2011). *P. skrjabini* has a wide distribution in Africa, Asia and some Mediterranean countries. It has been reported in a number of studies from Mongolia (Sharkhuu *et al,* 2001) ; Kazakhstan (Morgan *et al,* 2006), Saudi Arabia (Magzoub *et al,* 2000), Namibia (Krecek *et al,* 1990), Turkey (Umur and Yukari, 2005), Iran (Hasheminasab, 2015; Hasheminasab *et al,* 2016a) and China (Huang and Li, 2002; Yang *et al,* 2004). Parasitism of many worms can lead to inflammation, bleeding and ulcers in affected animals' abomasum. In severe cases, it can cause death of camel, thus causing severe damage to the camel industry, especially in China (Liu *et al,* 2020). In addition, it brings huge economic losses to breeders. Therefore, the diagnosis and control of parabronemiasis have been the focus of camel parasitic disease control.

Currently, there are relatively few researches on camel parabronemiasis. Over recent years, the studies mainly focused on classification and transmission media (Chen *et al,* 2016; Deng *et al,* 2017; Hasheminasab, 2016b; Zhao *et al,* 2012). At present, there is an important technical problem in the prevention and treatment of camel paragonimiasis, i.e., the lack of effective living diagnosis methods. Faecal egg examination methods suitable for many parasitic nematodes, such as the saturated sodium chloride solution egg flotation method, can be used to diagnose the disease. Still, the detection rate is low, and the detection effect is not ideal. The most accurate diagnosis mainly involves finding worms by autopsy (to find worms in the abomasum). Such diagnostic methods cannot provide a reference for early treatment (Jacobs *et al,* 1997; Liu *et al,* 2020; Wang *et al,* 2018). When raising camels, drugs are directly administered without diagnostic basis, which may cause economic losses, bringing drawbacks such as toxicity and side effects, drug-resistant insect strains, and veterinary drug residues in livestock products exceeding the standard, etc.

*These authors contributed equally to this work. Correspondence: WENLONG WANG
SEND REPRINT REQUEST TO email: wwl.imau@163.com

In this study, we used transcriptome sequencing analysis to screen the secreted protein gene cysteine protease inhibitor (cpi) of *P. skrjabini*: the gene was then cloned, expressed in prokaryotic cells, and its antigenic properties were verified. The gene's recombinant protein was used as the diagnostic antigen to establish a serological diagnostic method for the disease. The research results are of great significance for the prevention and treatment of camel parabronemiasis.

Materials and Methods

Specimen collection

P. skrjabini was collected from a Bactrian camel's abomasum in a slaughterhouse in northwestern Inner Mongolia, China. After morphological identification under a microscope, it was stored in liquid nitrogen. A total of 81 positive sera from camels infected by *P. skrjabini* were collected from northwestern Inner Mongolia, China. Additional 9 samples of negative sera of camels not infected with *P. skrjabini* were collected from Wulanchabu City, Inner Mongolia, China. Horseradish peroxidase-conjugated rabbit anti-camel IgG was prepared by Sangon Biotech.

Cloning and prokaryotic expression of cpi gene

Primers were designed according to the sequence of cpi gene (GenBank accession no. MW358934), and EcoR I and Xho I digestion sites were added to the upstream primers and downstream primers, respectively. The upstream primer sequence was 5'- GCC GAA TTC ATG TTG TGC TTT GCT GTG TTA T -3', and the downstream sequence was 5'- TTG CTC GAG TTA AGA CTT CAG CTG GAC TTG -3'. According to the RNAiso Plus specification, the total RNA of *P. skrjabini* was extracted, and the *cpi* gene was amplified by reverse transcription PCR. PCR reaction conditions were 94°C pre-denaturation for 5min; then, 94°C for 30s, 57°C for 30s, 72°C for 90s, 35 cycles; 72°C for 10min. The cDNA of *cpi* gene was cloned into a pMD19-T (TaKaRa, Dalian) simple vector. After sequencing, the cpi gene fragment was digested by *Eco*R I and *Xho* I, and then subcloned into *Eco*R I site of pET30a (+) (Novagen) plasmid to construct prokaryotic expression vector of cpi gene (pET-cpi) and transformed into competent cells of *Escherichia coli* BL21 (DE3)(TransGen Biotech, Beijing). The recombinant expression strain BL21 (pET-cpi) was induced by 1.0 mol/L IPTG. Samples were collected by centrifugation, lysed by lysozyme and ultrasonic wave, centrifuged, dissolved by 8mol/L urea, and filtered by 0.45 μm pore diameter filter. The recombinant protein (rCPI) was purified according to the instructions of the Ni-NTA Sefinose™ Resin Kit.

rCPI SDS-PAGE and Western blot detection

The recombinant protein was separated by SDS-PAGE and transferred to nitrocellulose membrane. The 5% skimmed milk powder was used for sealing at room temperature for 3 h; the positive serum (1:1000) of camel parabronemiasis was used as the primary antibody, and the negative serum control was set up at the same time. Rabbit anti-camel IgG labeled with horseradish peroxidase was used as the second antibody (1: 5000) for Western blot detection.

rCPI iELISA

According to the checkerboard titration, the purified rCPI was diluted with 50 mmol/L carbonate buffer solution (pH9.6) and microplates were coated overnight at 4°C with 1 μg, 2 μg, 4 μg, 8 μg, and 16 μg per well, respectively. After washing, 100μL blocking solution (PBS containing 3% bovine serum albumin) was added to the wells, and incubated for 3 h at 37°C. The positive serum and negative serum were diluted with 0.5% Tween-20 phosphate buffer (PBST) at 5 gradients of 1:25, 1:50, 1:100, 1:200, and 1:400, and rabbit anti-camel IgG-HRP was diluted at 1: 5000. The experiment was carried out according to the iELISA procedure indicated in the reference (Wang *et al,* 2014). The P/N value of each group was calculated according to the OD_{450nm} value. P/N= positive serum OD_{450nm}/negative serum OD_{450nm}. When the P/N value was the largest, the corresponding antigen dilution and serum dilution were optimal.

Determination of cut-off value

According to the optimal reaction conditions determined above, 9 negative sera were detected by rCPI iELISA, and the mean value ($\overline{X}$) and standard deviation (SD) of OD_{450nm} values of negative sera were calculated. According to the following formula, the recombinant antigen iELISA cut-off value is determined: Cut-off value = mean value of negative serum ($\overline{X}$) + 3 standard deviations (SD).

Specificity of iELISA

The specificity of iELISA was evaluated by using rCPI to detect 24 positive sera infected by *Moniezia* spp., *Haemonchus contortus, Dictyocaulus filaria, Chabertia ovina , Nematodirus* spp., *Trichuris ovis, Trichostrongylus* spp., *Oesophagostomum* spp. and *Oestrus ovis*.

Field sera testing

The established iELISA method was employed to detect 140 camel sera collected from Inner Mongolia, China.

Specimen collection (*P. skrjabini* was collected from Bactrian camel's abomasum)

The total RNA of *P. skrjabini* wa extracted

The *cpi* gene wa amplified by reverse transcription PCR

The cDNA of *cpi* gene was cloned into a pMD19–T simple vector

Construct prokaryotic expression vector of *cpi* gene (pET–*cpi*)

Prokaryotic expression of recombinant antigen *cpi* and Western–Blot analysis

Purification of recombinant proteins (rCPI)

Establishment of iELISA diagnostic method

Field sera testing: The established rCPI iELISA was used to detect 140 camel sera collected from Inner Mongolia.

Schematic representation of methodology of present study ranging from specimen collection of *P. skrjabini*. to the field sera testing.

Results

Clone and prokaryotic expression of cpi gene

The cpi gene was amplified by specific primers using the total RNA reverse transcription product of *P. skrjabini* as a template. The cpi gene was 399 bp by agarose gel electrophoresis detection (Fig 1). SDS-PAGE electrophoresis showed a specific expression band of the target gene fusion protein, which was consistent with the expected results compared with

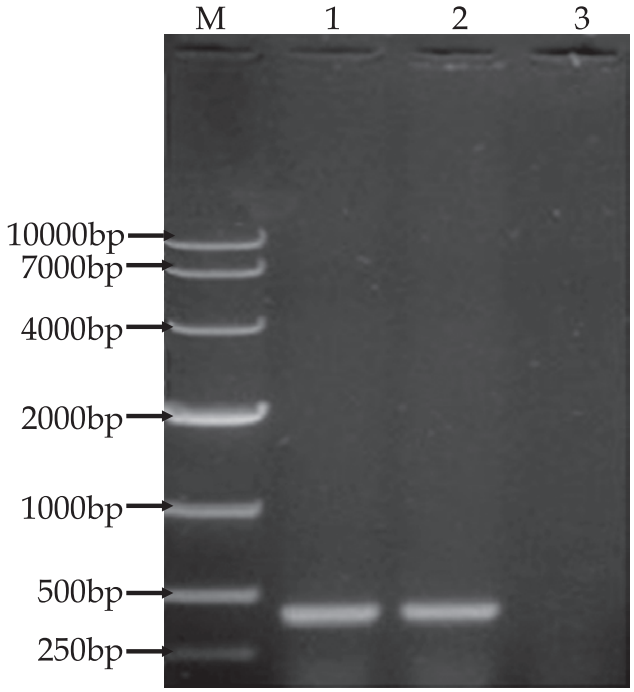


Fig 1. Electrophoresis of cpi Gene RT-PCR Product. M. DNA standard DL2000; 1-2. cpi gene; 3. Negative control.

the empty strain BL21 (DE3) and the empty vector transformed strain BL21 (pET30) (Fig 2).

rCPI Western blot

rCPI reacted with positive serum, and an aspecific band was observed at 20kDa, which was not the case for a negative serum control. This indicated that rCPI could specifically bind to IgG in camel serum infected by *P. skrjabini* (Fig 3).

Determination of reaction conditions and cut-off value of rCPI iELISA

According to the P/N value, when the coating amount of rCPI was 4 µg/well, and the serum dilution was 1:50, the P/N value was the maximum (7.47), determined as the best coating concentration and serum dilution for iELISA.

According to the OD_{450nm} values of 9 negative sera, the mean value ($\overline{X}$) = 0.210 and standard deviation (SD) = 0.023 of rCPI recombinant antigen detection, negative sera were calculated. Therefore, it was determined that the cut-off value was 0.279.

iELISA specificity

The iELISA established by rCPI was used to detect 24 positive sera infected by *Moniezia* spp., *Haemonchus contortus, Dictyocaulus filaria, Chabertia ovina, Nematodirus* spp., *Trichuris ovis, Trichosstrongylus* spp., *Oesophagostomum* spp. and *Oestrus ovis*. All 24 sera were negative, and there was

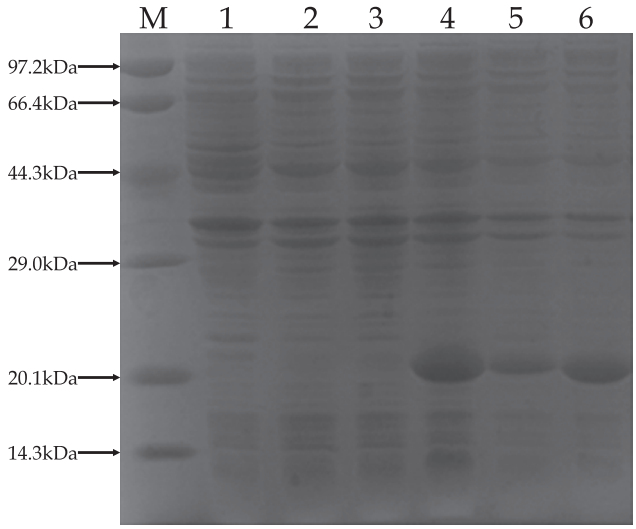


Fig 2. Electrophoresis results of induced expression of recombinant bacteria. M. Protein standard; 1. BL21 (DE3) empty bacteria; 2. Before induction of BL21 (pET30); 3. After induction of BL21 (pET30); 4-6. After induction of BL21 (pET-cpi).

no cross-reaction with the positive sera infected with the above 9 parasites, indicating that the rCPI iELISA method had good specificity.

Field serum test results

The established rCPI iELISA was used to detect 140 camel sera collected from Inner Mongolia. Among the 140 sera detected, 118 sera were positive (positive rate was 84.3%), 15 sera were negative, and the OD_{450nm} value of 7 sera was close to the cut-off value, which determined that they were suspected to be infected by *P. skrjabini*. The results showed that the infection of *P. skrjabini* was more serious in camels in Inner Mongolia.

Discussion

Some secretory protein homologues of parasites, such as aminopeptidases, proteases, and toxic secretions are considered as antigen proteins related to parasite and host immunity, which have immunogenicity and have an important role in parasite immune regulation, immune escape, and parasite-host interaction (Djafsia *et al,* 2015; Eberle *et al,* 2015; McSorley *et al,* 2013; Tritten *et al,* 2016). These functional antigens can provide the basis for screening vaccine antigens, exploring drug targets, and determining diagnostic antigens (Cao *et al,* 2016).

At present, there was no research report on immunological diagnosis and immune prevention of parabronemiasis. The cysteine protease inhibitor (*cpi*) gene selected in this study was an immune candidate gene screened out based on transcriptome and

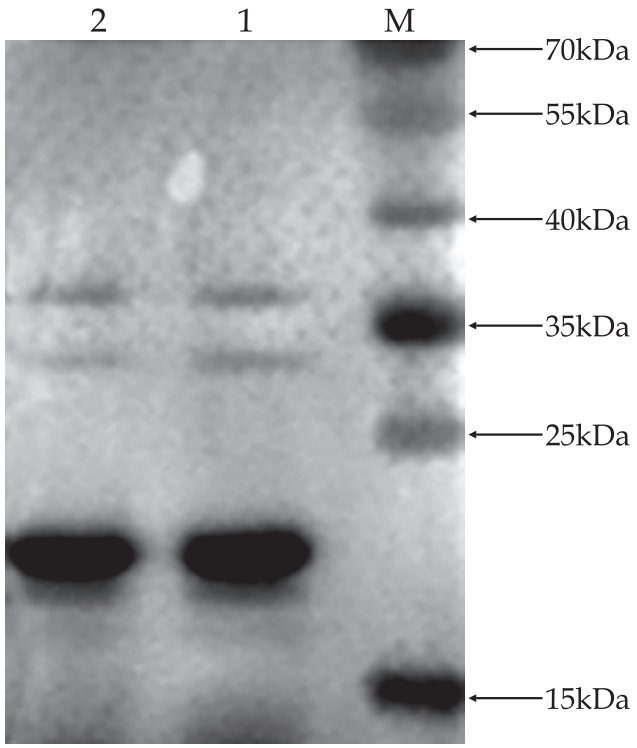


Fig 3. rCPI Western Blot Detection Results. M: Protein standard; 1-2: Positive serum test.

proteomic sequencing analysis of *P. skrjabini* and in combination with the characteristics of immunological candidate genes of other related nematodes (Feng *et al,* 2017). Bioinformatics analysis showed that the annotation function of the cpi gene coding product of *P. skrjabini* was a cysteine protease inhibitor, a secreted protein with a signal peptide sequence. Prediction of protein transmembrane region and subcellular localisation analysis of protein showed that the protein belongs to transmembrane protein. Antigenic epitopes predicted that the protein had 6 antigenic epitopes, indicating sound antigenicity. Through bioinformatics analysis, the *cpi* gene of *P. skrjabini* conformed to the characteristics of parasite immunological diagnosis and immune prevention candidate genes.

In this study, *cpi* gene was cloned from *P. skrjabini*, a prokaryotic expression vector of the gene was constructed, and recombinant expression was carried out. The results of SDS-PAGE showed that the molecular weight of rCPI protein was about 20.2 kDa. Western blot further indicated that rCPI protein could specifically bind to the antibody in the serum of camels infected with *P. skrjabini*, but did not react with the serum of camels not infected with *P. skrjabini*, thus indicating that CPI protein of *P. skrjabini* can stimulate the host to produce specific antibody during the natural sensory process. In this study, the purified rCPI

was used as the detection antigen to establish an iELISA method to detect camel parabronemiasis. According to the checkerboard experiment, the optimal reaction conditions were determined when the coating amount of rCPI was 4 μg/well, and the serum dilution was 1:50. The iELISA established by rCPI was used to detect 24 positive sera infected by *Moniezia* spp., *Haemonchus contortus, Dictyocaulus filarial, Chabertia ovina, Nematodirus* spp., *Trichuris ovis, Trichosstrongylus* spp., *Oesophagostomum* spp. and *Oestrus ovis*. No cross-reaction was found against the above parasites, which indicated that the established iELISA diagnostic method had good specificity and could be used for the diagnosis of parabronemiasis, and could provide basis for the monitoring and early treatment of camel parabronemiasis.

The established rCPI-iELISA was used to detect 140 field sera collected from Inner Mongolia, and 118 sera were positive, indicating that the epidemic of camel parabronemiasis in this area continues to be serious.

Conclusion

The present study, for the first time, evaluated the recombinant cysteine protease inhibitor (rCPI) as potential diagnostic markers for *P. skrjabini* infection in camels by iELISA, showing that the rCPI is highly sensitive and specific, and that rCPI is a potential serodiagnostic marker for the detection of *P. skrjabini* infection in camels.

Acknowledgements

We would like to thank all organisations which funded this work and all the teachers who cooperated in technical assistance.

Funding information

This work was supported by the National Natural Science Foundation of China (Grant No. 31260603 and 31760731), the Inner Mongolia Natural Science Foundation Project (Grant No. 2016MS0341).

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics statement

Our research was conducted strictly in accordance with the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China, and the protocol was reviewed and approved by the Research Ethics Committee of Inner Mongolia Agricultural University, Hohhot Inner Mongolia, China. However, the relevant document number is not available at Inner Mongolia Agricultural University. Permission was obtained from the local official slaughterhouse before collection of the specimens.

References

Abd-Rabo TMA, Amer OH and El-Sawak AA. Gastrointestinal nematodes in sheep in Kafr El-Sheikh Governorate. Egypt J Comp Path Clin Pathology. 1993; 6(2):261-271.

Cao XD, Fu ZQ, Zhang M, Han YH, Han HX, Han Q, Lu K, Hong Y and Lin JJ. iTRAQ-based comparative proteomic analysis of excretory–secretory proteins of schistosomula and adult worms of *Schistosoma japonicum*. Journal of Proteomics. 2016; 138:30-39.

Chen LJ, Shi HL, Yang LR, Yang XY, Deng Q, Li B and Li YY. Molecular Identification of *Parabronema skrjabini* Larvae in Different Instars Larvae of Horn Flies. Chinses Journal of Veterinary Medicine. 2016; 52(2):15-21(in Chinese).

Deng Q, Yang LR, Yang XY, Wang R, Li B, Zhang W and Yang B. Morphological study on camel parabronemiasis vectors of horn flies *Haematobia irritans* and *H.titillans* (Diptera:Muscidae). Chinese Journal of Veterinary Science. 2017; 37(6):1077-1083 (in Chinese).

Djafsia B, Ndjonka D, Dikti JV, van Hoorn S, Manchang K, Brattig N and Liebau E. Immune recognition of excretory and secretory products of the filarial nematode *Onchocerca ochengi* in cattle and human sera. Journal of Helminthology. 2015; 11:1-9.

Eberle R, Brattig NW, Trusch M, Schlüter H, Achukwi MD, Eisenbarth A, Renz A, Liebau E, Perbandt M and Betzel C. Isolation, identification and functional profile of excretory–secretory peptides from *Onchocerca ochengi*. Acta Tropica 2015; 142:156-166.

Feng CC, Wang WL and Hu HBTE. Sequencing and analysis of the transcriptome of *Parabronema skrjabini* in camle. Chinese Journal of Veterinary Science. 2017; 37(4):671-675 (in Chinese).

Hasheminasab SS. Molecular characterisation of the first internal transcribed spacer of rDNA of *Parabronema skrjabini* for the first time in sheep. Annals of Parasitology. 2015; 61(4):241-246.

Hasheminasab SS, Jalousian F, Meshgi B. Molecular and morphological characterisation of *Parabronema skrjabini* of sheep and goats at three different geographical zones in Iran. Annals of Parasitology. 2016a; 62:55-61.

Hasheminasab SS. 5.8S rRNA Sequence and Secondary Structure in *Parabronema skrjabini* and Related Habronematidae Species. Iran Journal of Parasitology. 2016b; 111(2):253-258.

Huang DS and Li SZ (2002). On the parasitic helminthum from an elephas maximus in Yunnan Province. Yunnan Journal of Animal Science and Veterinary Medicine. 2016b; 1:14-16 (in Chinese).

Jacobs DE, Zhu X, Gasser R and Chilton NB. PCR-based methods for identification of potentially zoonotic ascaridoid parasites of the dog, fox and cat. Acta Tropica. 1997; 68:191-200.

Khalafalla RE, Elseify MA and Elbahy NM. Seasonal prevalence of gastrointestinal nematode parasites of sheep in Northern region of Nile Delta, Egypt. Parasitology Research. 2011; 108(2):337-340.

Krecek RC, Boomker J, Penzhorn BL and Scheepers L. Internal parasites of giraffes (*Giraffa camelopardalis angolensis*) from Etosha National Park, Namibia. Journal of Wildlife Diseases. 1990; 26:395-397.

Liu Y, Zhao ZG, Yang XY, Yang LR, Yang B, Zheng WQ, Li WS, Luo XP, Wang R, Gu W and Wang PL. Haematobium irritans and Haematobium titillans as potential vectors of *Parabronema skrjabini* in camels (*Camelus bactrianus*) in Inner Mongolia, China. Parasitology. 2020; 147(13):1509-1514.

Magzoub M, Omer O, Haroun E and Mahmoud O. Effect of season on gastrointestinal nematode infection in Saudi Arabian camels (*Camelus dromedarius*). Journal of Camel Practice and Research. 2000; 7(1):107-108.

McSorley HJ, Hewitson JP and Maizels RM. Immunomodulation by helminth parasites: defining mechanisms and mediators. International Journal for Parasitology. 2013; 43:301-310.

Morgan ER, Torgerson PR, Shaikenov BS, Usenbayev AE, Moore ABM, Medley GF and Milner-Gulland EJ. Agricultural restructuring and gastrointestinal parasitism in domestic ruminants on the rangelands of Kazakhstan. Veterinary Parasitology. 2006; 139:180-191.

Sharkhuu T. Helminths of goats in Mongolia. Veterinary Parasitology. 2001; 101:161-169.

Tritten L, Clarke D, Timmins S, McTier T and Geary TG. *Dirofilaria immitis* exhibits sex- and stage-specific differences in excretory/secretory miRNA and protein profiles. Veterinary Parasitology. 2016; 232:1-7.

Umur Ş and Yukari BA. Seasonal activity of gastro-intestinal nematodes in goats in Burdur region, Turkey. Turkish Journal of Veterinary and Animal Sciences. 2005; 29:441-448.

Wang WL, Feng CC, Wang JL, Hu HBTR and Liu CX.Cloning and prokaryotic expression of Pj-CPR gene of *Parabronema skrjabini*. Progress in Veterinary Medicine. 2018; 39(3):30-34 (in Chinese).

Wang ZD, Ge W, Huang SY, Li JP, Zhu XQ and Liu Q. Evaluation of recombinant granule antigens GRA1 and GRA7 for serodiagnosis of *Toxoplasma gondii* infection in dogs. BMC Veterinary Research. 2014; 10:1-6.

Yang LR, Yang XY, Liu ZL, Zhang LS and Zhang WB. Investigation of *Parabronema skrjabini* disease of camels in Inner Mongolia region. Journal of Inner Mongolia Agricultural University. 2004; 25:43-45 (in Chinese).

Zhao ZG, Wang HY, Guo TZ, Yang LR, Wang R, Zhao LL, Ao WH, Liu H and Yang XY. Infection Situation of *Parabronema skrjabini* and pathological lesions in Alashan Bactrian Camels. Progress in Veterinary Medicine. 2012; 33:56-58 (in Chinese).

DOI : 10.5958/2277-8934.2021.00044.8 Vol 28 No 3, p 283-290

DNA BARCODING OF MAMMALIAN SPERMATOZOA

Hussein Y.A.[1,2], Youssef M.M.[3,4], Hereba A.M.[5,6], Al-Shokair S.S.[1] and Waheed M.M.[1,7]

[1]Department of Clinical Sciences, [5]Department of Microbiology and Parasitology, College of Veterinary Medicine,
[3]Department of Chemistry, College of Science, King Faisal University, P.O. Box: 400 Al-Ahsa 31982, Saudi Arabia
[2]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Alexandria University, Egypt
[4]Department of Chemistry, College of Science, Mansoura University, Egypt
[6]Medical Research Institute, Egypt
[7]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

ABSTRACT

One of the impressive tasks of recent natural science is to improve perfect and consistent knowledge for a quick selection of semen DNA variants. This subject of investigation is of major significance for the recognition and documentation of kinds in several fields of exploration. Diversity of DNA grounded attitudes have been established for the documentation of entities in a numerous of taxonomic clusters. The genomic DNA was isolated from semen samples collected from different eukaryotic kinds and matched the outcomes of the results acquired in expressions of magnitude (concentration of DNA isolated and DNA gotten per ml of semen used) and superiority (260/280 relation of the gotten results). A sequences of random oligonucleotide primers were planned and utilised it with the genome DNA of entities' kind of different eukaryotic kinds identify and estimate these kinds by using polymerase chain reaction (PCR) established assays. The DNA purified from semen was amplified and the migration outline of the amplified precise long and short amplified DNA features was measured by the benefit of agarose gel electrophoresis. The kinds specificity of the PCR amplification was confirmed by the aptitude of the analyses to precisely identify and recognise kinds definite DNA from varied sources. A critical assessment of all procedures is accessible concentrating on their biased authority, reproducibility and user kindliness. The present tendency was utilised to improve trivial measure devices with a high amount capability. Results of morphological parameters of sperms heads were tabulated, there were significant difference between human and animal groups (camel, ram and buck) ($P<0.05$). It is concluded that image J system and DNA barcode of individual spermatozoa provide perfect calculation of different semen parameters.

Key words: DNA barcodes, scanning electron microscope, sperm evaluation, sperm morphometry

The objective of DNA barcoding is to improve a kind's convinced categorisation library for eukaryotes. A 650 bp DNA fragment of the cytochrome c oxidase 1 gene has been utilised effectively for kind's level documentation in numerous animal collections (Meusnier *et al*, 2008). Kitano *et al* (2007) recognised a simple process utilising general primers for kinds documentation based on direct PCR sequencing. In humans, the estimated sizes of PCR yields of the 12S and 16S rRNAs were 215 and 244 bp, respectively. Both primer sets effectively augmented the predictable PCR products from different classes of vertebrates comprising mammals, birds, reptiles, fish, amphibians, and the sequenced sections contained adequate nucleotide variances to categorise each animal kind (An *et al*, 2007).

The problems faced in the separation and purification of DNA particularly from eukaryotic kinds comprise degradation of DNA owing to endonucleases, exceptionally sticky polysaccharides and other secondary metabolites which straight or indirectly inhibit with the enzymatic reactions. Different techniques for DNA extraction have been effectively useful to many eukaryotic kinds (Doyle and Doyle, 1987; Ziegenhagen *et al*, 1993), which were added improved to deliver DNA appropriate for numerous types of analyses (Wang and Taylor, 1993; Ziegenhagen and Scholz, 1998).

The integrated primate biomaterials and material source affords vital inquiry elements to the technical public by launching, approving, preserving, and allotting RNA and DNA consequent from primate cell cultures (Lorenz *et al*, 2005). Common primers were utilised for the magnification of the mitochondrial 12S rRNA gene from genomic DNA different kinds (Gupta *et al*, 2008).

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

The mensuration of sperm of different animals, including head area, head length and width was performed (Mortimer, 2018). The sperm flagellum distributed into four pieces the neck, midpiece, principal piece and the end piece (Cummins and Woodall, 1985). There are large variations of the head and length flagellum of different mammals' species (Fawcett, 1970; Serres *et al*, 1983). Scanning electron microscope (SEM) provides accurate dimensions, resolution and interpretation of various head part (Fujita *et al*, 1970; Liakatas *et al*, 1982; Conradie *et al*, 1988; Bonet, 1990; Van der Horst *et al*, 1991; Soley, 1992).

The public, Scanning Electron Microscopy (SEM) is used for the classification and identification of morphological features of spermatozoa (Sathananthan, 1996 and Sathananthan, 2013). SEM can be easily viewed the area of a sperm at good resolution of nanometer and in a vast range of magnification (Goodhew *et al*, 2000). Image of three-dimensional (3-D) can be gain through lenses with a field of high depth (Watson *et al*, 1980). Samples for SEM preparation depend on basic requirements (Nowell and Pawley, 1980).

The morphological differences of normal spermatozoon are due to the methods of staining (Soler *et al*, 2003; Henkel *et al*, 2008). Maree *et al* (2010) stated that these differences were due to fixatives and stains also, the technician play a role of these differences (Kruger *et al*, 1986).

The aim of this study was the analysis of sperms from human, camel, ram and buck by Scanning Electron Microscope (SEM) combined with DNA barcode to confirm the schedule required for its use in forensic practice.

Materials and Methods

2.1. Samples collection

Ten semen samples were collected from each fertile camels, rams and bucks using artificial vaginas while the samples of fertile humans were collected by masturbation.

2.2. Fixation of samples for SEM analysis

The fixation of semen was carried out with solution containing 2% glutaraldehyde, 1 % formaldehyde, 1.5 mM $CaCl_2$, 0.2 M sucrose and 0.1% picric acid 1% in 0.1 M cacodylate buffer at pH 7.0-7.2. Moreover, to this solution, 25 ml of 8 % aqueous glutaraldehyde to 25 ml of aqueous 4 % formaldehyde (yield an aqueous mixture of 4 % glutaraldehyde and 2 % formaldehyde) were added.

Fixation of all samples was done in fixative solution for one hour at 4°C. Clumped semen was removed from fixative, and the remaining fluid was centrifuged at 500-700 rpm for 10 minutes. The fixatives were removed and samples were suspended in 0.1 M cacodylate buffer and centrifuged then buffers were removed. The samples were re-suspended in new buffer and repeated centrifugation until most of the yellow color was gone. The samples were dehydrated by ethanol from 50 - 100 %. Ethanol was removed and replaced with hexamethyldisibenzene at room temperature in a petri dish under fume hood for overnight for complete dryness. The samples were mounted on SEM stuba, sputter coat and examined with SEM.

Ram, buck and camel spermatozoa head were calculated according to ImageJ system and confirmed by van Duijn (1957) and van Duijn (1960) for human, using the mathematical equation "**A** = {(*1.05* - 0.225 *Bbasis/Bmax*) X (0.36 *Bbasis* + 0.69 *Bma)L}*" and "A = 0.73 x L X Bmax"

Where A = head area, Ba = width of maximal head, Bbasis = width of head at connection to midpiece, L = length of head (Table 1).

Table 1. Sperm head criteria.

Variable	Formula
Length of head (µm)	L
Width basis of head (µm)	Wb
Width max of head (µm)	Wm
Area of head (μm^2)	A
Perimeter of head (µm)	P
Ellipticity of head	L/W
Elongation of head	(L-W)/L+W)
Roughness of head	4π(A/P2)
Regularity of head	π(L*W/4*A)

2.3. Extraction and purity of DNA

The DNA was isolated from the semen, allowing to the process described by Sambrook *et al* (1989). The DNA yield per ml of semen was examined via Spectrophotometry at 260 nm. The DNA purity was identified by manipulative the absorbance ratio at 260/280 nm. Any protein impurity of the DNA samples resulted in excessive difficulties through the successive management. The concentration and purity of DNA was identified by successively the samples on agarose gel (1 %) electrophoresis. The DNA concentration was considered subsequent (Sambrook *et al*, 1989).

2.4. Optimisation of RAPD reaction

The RAPD-PCR was performed utilising four common oligonucleotide primers; the first was MansI primer (5`-CGACTGCTAGACGACCCTGGCG-`3), the second was MansII primer (5′-AACTGAGCAG CAGCGTATCCAG-3′), the third was MansIII primer (5′-ACGTTAGTGTCTGCAGTGCGAG-3′) and the fourth was MansIV primer (5′-TGCAATCACA GCTACGATACGG-3′) (metabion international AG, Lena-Christ-Strassa 44/I, D-82152 Martinsried/ Deutschland). The reactions were carried out in a DNA Thermocycler (9700 thermal cycler PCR, Eppendorf, Germany). Reactions without DNA were used as negative controls. The PCR reaction mixtures were organised with 1µl of semen genomic DNA, 5 µl of Pfu buffer 10x, 200 µmol of each deoxynucleoside triphosphate, 20 pmol each primer, and 2.5 U Pfu DNA polymerase (Promega, Germany) and sterile filtered mille water to a final volume of 50 µl. The PCR program was as follows: Primary denaturation at 95°C for 5min, 30 cycles was applied as follow: 95°C for one min., 52°C for one min., 72°C for 2 min., and in final extension step at 72°C for 10 min., and PCR reaction kept on 4°C until removing the PCR tubes.

2.5. Agarose gels

DNA was examined by utilising agarose gel horizontal electrophoresis according Youssef *et al* (2016).

2.6. Statistical analysis

Statistical analyses were completed by using package of statistics for science SPSS software program, version 24.0 (SPSS, 2016).

Results and Discussion

The current study represent the stereology values for ram, buck, camel and human sperm surface area not measured by linear dimensions. The accurate estimation of the surface area of sperm head from two-dimensional images was aided through stereology of sperm. The values of ram, buck, camel and human spermatozoa were recorded in Table 2. There was a significance difference ($P<0.05$) of head area, length and elongation of head between human and other species, between camels with ram and buck. Moreover, there were significant differences ($P<0.05$) of Wm between human and camel with buck and ram. There were differences ($P<0.05$) in L/W and roughness of head between human and other groups, and between camel with ram and buck. The values of regularity of head were significant ($P<0.05$) different between human and other animals. Results of head perimeter were highly significant ($P<0.05$) between all groups each other.

Morphology of spermatozoa in different animal species shows constant dimensions (Sancho *et al*, 1998). On the other hand, there are differences of sperm morphology in other species including Camelid spermatozoa (Rai *et al*, 1997; Von Baer and Hellemann, 1998). This reality has need to encourage the researchers spent more efforts toward these direction.

de Monserrat *et al* (1995) studied the standardised methods by using Sperm Class Analyser (SCA) in humans. The variation of substantially and low coefficients was observed with the accuracy of the system in other species (Gago *et al*, 1998; Buendia *et al*, 2002). Davis and Gravance (1993) and Boersma *et al* (2001) were stated that discipline and replicates of this system to animal species for the preparation of the samples and staining are required.

The present results were in disagreement with that mentioned by Curry *et al* (1996), and Duncan and Watson (1992), who observed that the surface area of human and ram were 20.50 ± 2.25 µm and 33.75 ±

Table 2. Morphometric variables of ram, buck, camel and human sperm head measured manually using micrometers (Mean ± SE).

Morphometric parameter	Ram	Buck	Camel	Human
Number of sperm cells	50	50	50	50
Area of head (μm^2)	19.218 ± 0.538^{a}	19.953 ± 0.691^{a}	16.486 ± 0.489^{b}	12.862 ± 0.119^{c}
Perimeter of head (µm)	18.511 ± 0.216^{a}	19.493 ± 0.437^{a}	16.492 ± 0.247^{b}	13.998 ± 0.109^{c}
Length of head (µm)	7.056 ± 0.101^{a}	6.825 ± 0.109^{a}	6.357 ± 0.148^{b}	5.142 ± 0.105^{c}
Width basis of head (µm)	1.236 ± 0.530^{a}	1.153 ± 0.447^{ab}	1.063 ± 0.425^{b}	0.891 ± 0.600^{c}
Width max of head (µm)	3.96 ± 0.396^{a}	3.844 ± 0.996^{a}	3.112 ± 0.211^{b}	2.985 ± 0.362^{b}
Length/width max	1.817 ± 0.066^{a}	1.533 ± 0.153^{a}	2.161 ± 0.207^{b}	1.725 ± 0.041^{a}
Elongation of head (L-W) /L+W)	10.397 ± 0.183^{a}	10.089 ± 0.171^{a}	8.982 ± 0.314^{b}	7.544 ± 0.121^{c}
4 π (A/P2)	0.704 ± 0.014^{a}	0.663 ± 0.019^{a}	0.971 ± 0.064^{b}	0.828 ± 0.013^{c}
π (L*W/4*A)	1.001 ± 0.098^{a}	0.954 ± 0.028^{a}	1.040 ± 0.069^{a}	3.142 ± 0.000^{b}

Means with dissimilar superscripts in the same row are significantly different at $P < 0.05$.

1.06 µm, respectively. Therefore, the current results disagreed with that recorded by WHO (1996) and Kristina (2017) who reported that Length, width, and length/width ratio of human normal sperm were 4–5, and 2.5-3.5 µm, and 1.5-1.75 ratio, respectively. The head length of ram was about 8-10 µ length and 4 µ width (Setchell, 1978; Evans and Maxwell, 1987).

Despite the interest of speculate morphology of sperm, this dimensions are highly debatable in its use for the identification of forensic origin due to the variety of methods and criteria of classification used including the staining techniques (Cooper *et al,* 2004). The inefficiency of morphology techniques of sperm has well attests by Maree *et al* (2010), who showed that the parameters of sperm heads concerned on the fixatives and stains used. Kondracki *et al* (2014) concluded that the ejaculate volume exerted an effect on dimension of sperm morphology, and the narrow and short sperm head had a higher fertility (Hirai *et al*, 2001).

Morphology of spermatozoa of ram and human are similar (van Duijn, 1960; Curry *et al,* 1995). Duncan and Watson (1992) estimated head morphology of bull, their results were in close agreement with that obtained earlier by van Duijn (1960) and with that obtained by image analyser.

The problem of linear measurements to determine sperm surface area did not solve by image analysis. The grade model was used experimentally to acquire a formula of head area of human spermatozoa (van Duijn, 1957). It was recorded that the values of (4.4 and 3.2 µm) and (5.3 and 3.4 µm) for L and B max tabulated by van Duijn (1957) and Katz *et al* (1986) were somewhat smaller than the present results (6.46 and 3.85) but parallel to 6.7 and 4.2 µm (Curry *et al*, 1996). Concerning the head area of human spermatozoa, the current results were in agreement with that previously obtained 10.3 µm and 13.2 µm (van Duijn, 1957; Katz *et al*, 1986), but less than 20.5 µm (Curry *et al*, 1995).

In this study, the sperm mensuration of rams and bucks were parallel with that recorded by Curry *et al*, (1996) and Cummins and Woodall (1985), who observed that the length and width of the head of buck were 8.27 and 4.25 µm, respectively. Results of camel were partially similar to L 6.10, 5.62, and 6.18 µm, and W 3.62, 2.92, and 4.13 µm, obtained by Cummins and Woodall (1985), Buendia *et al* (2002), and Alhaider (2002), respectively.

Genomic DNA is an important constituent so as to attain molecular requests including genomic research. The plan of RAPD PCR is ongoing with

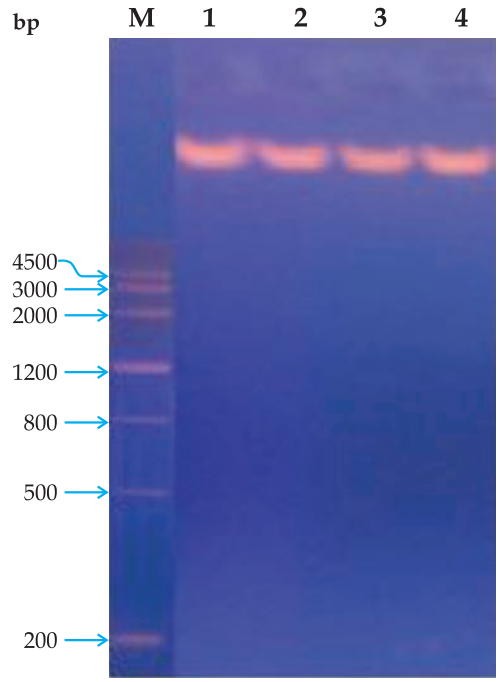


Fig 1. 1% agarose gel electrophoresis viewing the semen DNA isolated from camel, buck, ram of sheep and human as eukaryotic kinds.
Lane M: Gelpiolt DNA molecular weight marker (QIAGEN) Wide Range Ladder (100) Lane 1: DNA of camel Lane 2: DNA of buck Lane 3: DNA of ram of sheep. Lane 4: DNA of human.

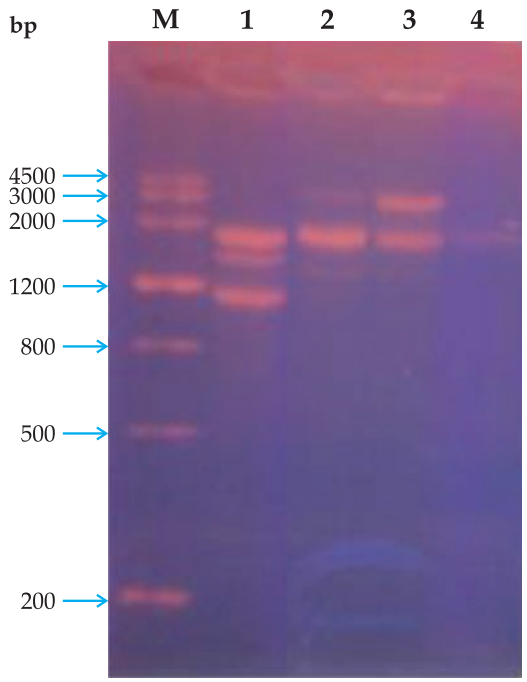


Fig 2. Showing 2% agarose gel electrophoresis containing the RAPD PCR product of the MansI nucleotide primer with the semen DNA of variant eukaryotic species.
Lane M: Gelpiolt DNA molecular weight marker (QIAGEN) Wide Range Ladder (100). Lane 1: DNA of camel Lane 2: DNA of buck Lane 3: DNA of ram of sheep. Lane 4: DNA of human.

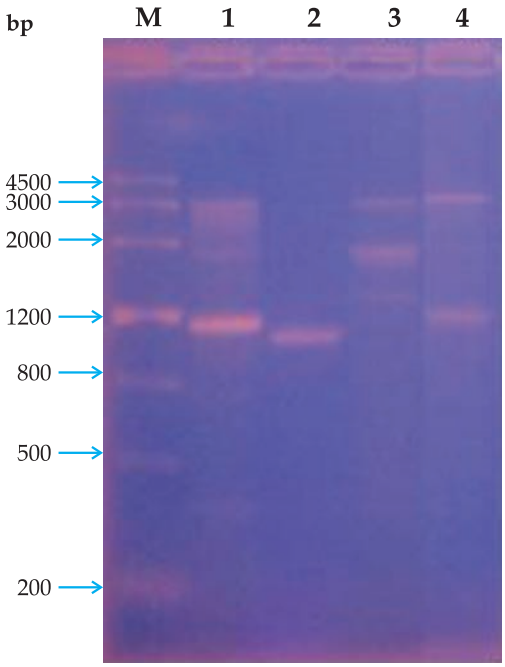


Fig 3. Showing 2% agarose gel electrophoresis containing the RAPD PCR product of the MansII nucleotide primere with the genomic DNA of variant eukaryotic species. Lane M: Gelpiolt DNA molecular weight marker (QIAGEN) Wide Range Ladder (100) Lane 1: DNA of camel, Lane 2: DNA of buck, Lane 3: DNA of ram of sheep, Lane 4: DNA of human.

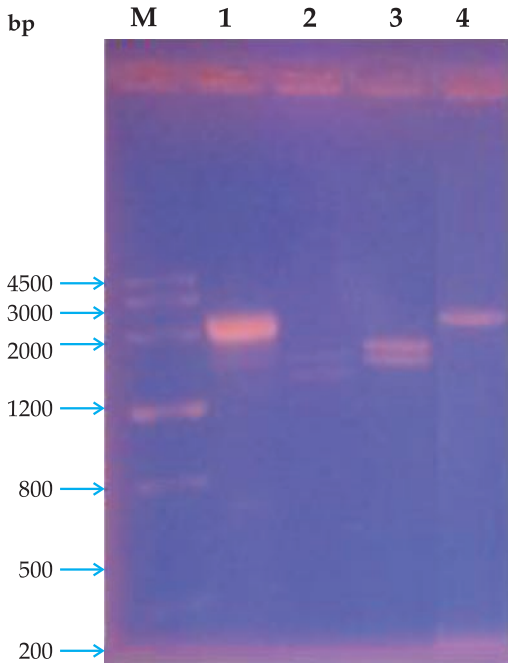


Fig 4. Showing 2% agarose gel electrophoresis containing the RAPD PCR product of the MansIII nucleotide primere with the genomic DNA of variant eukaryotic species. Lane 1: Gelpiolt DNA molecular weight marker (QIAGEN) Wide Range Ladder (100). Lane 2: DNA of camel. Lane 3: DNA of buck. Lane 4: DNA of ram of sheep. Lane 5: DNA of human.

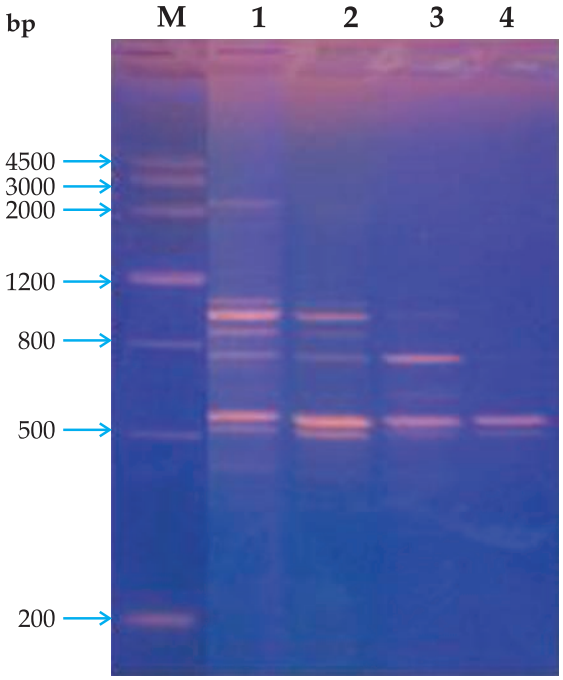


Fig 5. Showing 2% agarose gel electrophoresis containing the RAPD PCR product of the MansIV nucleotide primere with the genomic DNA of variant eukaryotic species. Lane 1: Gelpiolt DNA molecular weight marker (QIAGEN) Wide Range Ladder (100). Lane 2: DNA of camel. Lane 3: DNA of buck. Lane 4: DNA of ram of sheep. Lane 5: DNA of human.

separation the genomic DNA from the different eukaryotic kinds. Consequently, the seminal DNA of the male eukaryotic species were extracted and purified. It is difficult to isolate extraordinary superiority DNA from eukaryotic kinds as camel, buck, ram of sheep and human because of the extraordinary materials of polysaccharides (Mayes *et al*, 1992). Furthermore, it has been stated that the reproducibility of RAPD band profile in eukaryotic kinds was affected by the technique of DNA isolation (Mejjad *et al*, 1994). The superiority of the extracted chromosomal DNA of variant eukaryotic kinds was estimated by migrating the DNA in 1% agarose gel electrophoresis as shown in Fig 1. The technique used in the DNA isolation (Sambrook *et al*, 1989) offered a good DNA yield ranging from 7.6 to 8.3 µg ml^{-1}

Table 3. DNA yield and purity, isolated from different male eukaryotic species using protocol of Sambrook *et al*, 1989.

Eukaryotic Species	A260	A280	A260/ A280	DNA concentration (µg/ml^{-1}) blood
Camel	0.162	0.092	1.761	7.6
Buck	0.129	0.071	1.817	8.1
Ram	0.157	0.086	1.825	8.3
Human (F)	0.153	0.084	1.821	8.3

Table 4. DNA sequences of the primers used for random amplified polymorphic DNA (RAPD) analysis.

Primer	DNA sequence (5′ → 3′)	G+C content %	No. bands Products
MansI	(5`-GACTGCTAGACGACACTGACG-`3),	57.14	12
MansII	(5′-AACTGAGCAGCAGCGTATCCAG-3′)	54.55	12
MansIII	(5′-ACGTTAGTGTCTGCAGTGCGAG-3′)	54.55	11
MansIV	(5′-CGCAATCACAGCGACGATACGA-3′)	54.54	24

semen (Table 3) across camel, buck, ram of sheep and human as eukaryotic kinds. This DNA yield was reasonably high to that of Pereira *et al* (2011) where the yield of DNA extended from 2.3 to 5.0 µg ml^{-1}. The process used in the extraction of DNA shown A260/280 ratio ranging from 1.7 to 1.8 (Table 3) indicating the absence of polyphenol and proteins impurities and demonstrated that the extracted DNA has a good superiority and pure adequate to be used with RAPD PCR procedure. The DNA purity was additional confirmed by restriction digestion examination by the restriction endonucleases Pst I, Hind III and Eco RI (data not shown).

Of 12 primers verified in the existing effort, only 4 were lastly nominated for study; these produced strong and reproducible bands with all planned eukaryotic kinds. The whole numbers of 59 bands, extending from 200 to 2500 base pair (bp). The illustrative RAPD PCR of the camel, buck, ram of sheep and human seminal DNA electrophoresis profile with MansI, MansII, MansIII and MansIV primers are shown in Figs 2, 3 4, and 5, respectively. The sequences and G+C contents of the primers, together with the integer of bands they formed, are planned in Table 4. The semen DNA of all eukaryotic kinds in the current study was endangered to RAPD PCR utilising four common primers, MansI, MansII, MansIII and MansIV.

Conclusions

The present study suggests image J system and DNA barcode analyses of individual spermatozoa could be used to identify, to assess and to distinguish between camel, buck, ram of sheep and human eukaryotic species.

Acknowledgements

This research article was supported by grants from the Deanship of Scientific Research, King Faisal University, Kingdom of Saudi Arabia (Project # 140242).

References

Alhaider AK. Collection and evaluation of camel (*Camelus dromedarius*) semen. Master Degree.; King Faisal University, Saudi Arabia. 2002.

An J, Lee MY, Min MS, Lee MH and Lee H. A molecular genetic approach for species identification of mammals and sex determination of birds in a forensic case of poaching from South Korea. Forensic Science International. 2007; 167(1):59-61.

Boersma R, Rasshofer R and Stolla R. Influence of sample preparation, staining procedure and analysis conditions on bull sperm head morphometry using the morphology analyser integrated visual optical system Reproduction in Domestic Animals. 2001; 36:222–229.

Buendía P, Soler C, Paolicchi F, Gago G, Urquieta B, Pérez-Sánchez F and Bustos-Obregón E. Morphometric characterisation and classification of alpaca sperm heads using the sperm-class analyser computer-assisted system. Theriogenology. 2002; 57:1207-1218.

Bonet S. Immature and aberrant spermatozoa in the ejaculate of *Sus domesticus*. Animal Reproduction Science 1990; 22(1):67-80. https://doi.org/10.1016/0378-4320(90)90039-I

Conradie E, Selby PJ, Coetzee K and Menkveld R. The comparison of techniques for the preparation of spermatozoa for scanning electron microscopy. Med Tech South Africa. 1988; 2(2):152-154.

Cooper TG, Yeung CH, Fetic S, Sobhani A and Nieschlag E. Cytoplasmic droplets are normal structures of human sperm but are not well preserved by routine procedures for assessing sperm morphology. Human Reproduction. 2004; 19:2283-2288. doi:10.1093/HUMREP/DEH410

Cummins JM and Woodall PF. On mammalian sperm dimensions. Journal of Reproduction and Fertility. 1985; 75(1):153-175.

Curry MR, Millar JD and Watson PE. The presence of water channel proteins in ram and human sperm membranes. Journal of Reproduction and Fertility. 1995; 104:297-303.

Curry MR, Millar JD, Tamuli SM and Watson PF. Surface area and volume measurements for ram and human spermatozoa. Biology of Reproduction. 1996; 55:1325-1332.

Davis RO and Gravance CG. Standardisation of specimen preparation, staining, and sampling methods improves automated sperm-head morphometry analysis. Fertility and Sterility. 1993; 59:412-417.

de Monserrat JJ, Pe´rez-Sa´nchez F, Tablado L and Soler C. The Sperm-Class Analyser: a new automated system for human sperm morphometry and classification. Contraception Fertilite Sexualite. 1995; 23(Suppl. 9): S126, Abstract. pp 238.

Doyle JJ and Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 1987; 19:11-15.

Duncan AE and Watson PE. Predictive water loss curves for ram spermatozoa during cryopreservation: comparison with experimental results. Cryobiology. 1992; 29:95-105.

Evans G and Maxwell WMC. Salamon's Artificial Insemination of Sheep and Goats. Sydney, Butterworths. 1987.

Fawcett DWA. Comparative view of sperm ultrastructure. Biology of Reproduction. 1970; 2:90-127.

Fujita T, Miyoshi M and Tokunaga J. Scanning and transmission electron microscopy of human ejaculate spermatozoa with special reference to their abnormal forms. Zeitschrift für Zellforschung und Mikroskopische Anatomie. 1970; 105:483-497. https://doi.org/10.1007/BF00335423

Gago C, Perez-Sanchez F, Yeung CH, Tablado L, Cooper TG and Soler C. Standardisation of sampling and staining methods for the morphometric evaluation of sperm heads in the Cynomolgus monkey (*Macaca fascicularis*) using computer-assisted image analysis. International Journal of Andrology. 1998; 21:169–176. PMID: 9669201.

Goodhew PJ, Humphreys J and Beanland R. Electron Microscopy and Analysis. 3rd ed. New York: Taylor & Francis. 2000.

Gupta AR, Patra RC, Das DK, Gupta PK, Swarup D and Saini M. Sequence characterisation and polymerase chain reaction restriction fragment length polymorphism of the mitochondrial DNA 12S rRNA gene provides a method for species identification of Indian deer. Mitochondrial DNA. 2008; 4:394-400.

Henkel R, Schreiber G, Sturmhoefel A, Hipler UC, Zermann DH and Menkveld R. Comparison of three staining methods for the morphological evaluation of human spermatozoa. Fertility and Sterility. 2008; 89:449-455. DOI: 10.1016/j.fertnstert.2007.03.027

Hirai M, Boersma A, Hoeflich A, Wolf E, Föll J, Aumüller TR and Braun J. Objectively measured sperm motility and sperm head morphometry in boars (Sus scrofa): Relation to fertility and seminal plasma growth factors. Journal of Andrology. 2001; 22(1):104-110.

Katz DF, Overstreet JW, Samuels S, Niswander PW, Bloom TD and Lewis EL. Morphometric analysis of spermatozoa in the assessment of human male fertility. Journal of Andrology. 1986; 7:203-210.

Kitano T, Umetsu K, Tian W and Osawa M. Two universal primer sets for species identification among vertebrates. International Journal of Legal Medicine. 2007; 121(5):423-427.

Kondracki S, Górski K, Wysokińska A and Jóźwik I. Correlation of ejaculate parameters and sperm morphology with the ejaculate volume of pietrain boars. Bulgarian Journal of Agricultural Science. 2014; 20(3):703-709.

Kristina L. Assessment of Human Sperm Cells Morphological Parameters. 2017; http://dx.doi.org/10.5772/intechopen.71413

Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, Smith K. Sperm morphologic features as a prognostic factor in *in vitro* fertilisation. Fertility and Sterility. 1986; 46:1118-1123.

Liakatas MD, Williams AE and Hargreave TB. Scoring sperm morphology using scanning electron microscope. Fertility and Sterility. 1982; 38(2):227-231.

Lorenz JG, Jackson WE, Beck JC and Hanner R. The problems and promise of DNA barcodes for species diagnosis of primate biomaterials. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2005; 360(1462):1869-1877.

Maree L, du Plessis SS, Menkveld R and van der Horst G. Morphometric dimensions of the human sperm head depend on the staining method used. Human Reproduction. 2010; 25:1369-1382. DOI: 10.1093/humrep/deq075.

Mayes C, Saunders GW, Tan IH and Druehl LD. DNA extraction methods for kelp (Laminariales) tissue. Journal of Phycology. 1992; 28:712-716.

Mejjad M, Vedel F and Ducreux G (1994). Improvement of DNA preparation and of PCR cycling in RAPD analysis of marine macroalgae. Plant Molecular Biology Reporter 12:101-105.

Meusnier I, Singer GA, Landry JF, Hickey DA, Hebert PD and Hajibabaei M. A universal DNA mini-barcode for biodiversity analysis. BMC Genomics. 2008; 9:214-217. DOI: https://doi.org/10.1186/1471-2164-9-214

Mortimer D. The functional anatomy of the human spermatozoon: relating ultrastructure and function. Mol Molecular Human Reproduction. 2018; 24(12):567-592. DOI: 10.1093/molehr/gay040.

Nowell JA and Pawley JB. Preparation of experimental animal tissue for SEM. In: Murphy JA, Romans GM, eds. Preparation of Biological Specimens for Scanning Electron Microscopy. O'Hare: Scanning Electron Microscopy. 1980; pp 1-19.

Pereira JC, Chaves R, Bastos E, Leitão A and Guedes-Pinto H. An Efficient Method for Genomic DNA Extraction from Different Molluscs Species. International Journal of Molecular Sciences. 2011; 12(11):8086-8095.

Rai AK, Sharma N, Manivannan B and Khanna ND. Camel semen during breeding and non-breeding seasons. Indian Journal of Animal Sciences. 1997; 67:397-399.

Sambrook J, Fritschi EF and Maniatis T. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York. 1989.

Sancho M, Pe´rez-Sa´nchez F, Tablado L, de Monserrat JJ, Soler C. Computer assisted morphometric analysis of ram sperm heads: evaluation of different fixative techniques. Theriogenology 1998; 50:27-37. PMID: 10734471.

Sathananthan AH. Visual Atlas of Human Sperm Structure and Function for Assisted Reproductive Technology. Melbourne: La Trobe and Monash Universities. 1996.

Sathananthan AH. Ultrastructure of human gametes, fertilization and embryos in assisted reproduction: a personal survey. Micron. 2013; 44:1-20. https://doi.org/10.1016/j.micron.2012.05.002.

Serres C, Escalier D and David G. Ultrastructure morphometry of the human sperm flagellum with a stereological analysis of the lengths of the dense fibres. Biology

of the Cell. 1983; 49(2):153-161. DOI: 10.1111/j.1768-322x.1984.tb00233.x

Setchell BP. Development of the Testis. In: Setchell BP, ed. The Mammalian Testis, Cornell University Press, Ithaca, New York. 1978; pp 30-43. ISBN: 9780801411403.

Soler C, De Monserrat JJ, Gutierrez R, Nunez J, Nunez M, Sancho M, Perez-Sanchez F and Cooper TG. Use of the sperm-class analyser for objective assessment of human sperm morphology. International Journal of Andrology. 2003; 26:2620270.

Soley JT. A histological study of spermatogenesis in the ostrich (*Struthio camelus*). Ph.D. thesis, University of Pretoria, Pretoria. 1992.

SPSS. Statistical Package for Social Sciences, SPSS Inc., Armonk, NewYork: IBM Corp, IL, USA. Copyright © for Windows; version 24.0. 2016.

Van der Horst G, Curry PT, Kitchin RM, Burgess W, Thorne ET, Kwiatkowski D, Parker M and Atherton RW. Quantitative light and scanning electron microscopy of ferret sperm. Gamete Biology. 1991; 30(3):232-240. https://doi.org/10.1002/mrd.1080300311

van Duijn C. II. Biometry of human spermatozoa. Journal of the Royal Microscopical Society. 1957; 77:12-27.

van Duijn C. Mensuration of the heads of boar spermatozoa. Mikroscopie. 1960; 15:142-156.

Von Baer L and Hellemann C. Semen variables in llama (Lama glama). Archivos de Medicina Veterinaria. 1998; 30:171-176.

Wang Y and Taylor DE. A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. Biotechniques. 1993; 14:748-750.

Watson LP, McKee AE, Merrell BR. Preparation of biological specimens for scanning electron microscopy. In: Murphy JA, Romans GM, eds. Preparation of Biological Specimens for Scanning Electron Microscopy. O'Hare: Scanning Electron Microscopy. 1980; 45-56.

World Health Organisation. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. 4th ed. Cambridge University Press. 1996.

Youssef MM, Arafa RK, Ismail MA. Synthesis, antimicrobial, and antiproliferative activities of substituted phenylfuranylnicotinamidines. Drug Design, Development and Therapy. 2016; 10:1133–1146.

Ziegenhagen B, Guillemaut P and Scholz F (1993). A procedure for mini-preparation of genomic DNA from needles of silver fir (Abies Alba mill). Plant Molecular Biology Reporter 11:117-121.

Ziegenhagen B and Scholz F. Methods for Difficult Plant Species. In: Karp A, Issac PG, Ingram DS, eds. Molecular Tools for Screening Biodiversity. Kluwer Academic Publishers, Belgium. 1998; pp 32-35.

DOI : 10.5958/2277-8934.2021.00045.X Vol 28 No 3, p 291-295

'ALPACA FEVER' IN DROMEDARY CAMEL CALVES–A CASE REPORT

Wernery U.[1], J. Kinne[1], S. Jose[1], A. Das Gupta[2], A.A. Taha[2], A. A. Ismail[2], M. Joseph[1], P. Nagy[2] and J. Juhasz[2]

[1]Central Veterinary Research Laboratory, Dubai, UAE

[2]Emirates Industry for Camel Milk & Products, Dubai, UAE

ABSTRACT

'Alpaca Fever' in 4 young female dromedary camel calves is reported here. Alpaca Fever is caused by *Streptococcus equi* subsp. *zooepidemicus* in *Camelidae*, but infects mainly alpacas in Peru. In three of the four dromedary calves, *Streptococcus equi* subsp. *zooepidemicus* induced a fibrinopurulent to haemorrhagic pneumonia, which was fatal.

Key words: Alpaca Fever, dromedary calf, *Streptococcus equi* subsp. *zooepidemicus*

Streptococcus equi subsp. *zooepidemicus* is one of the β-haemolytic commensal organism with a large host range especially in horses, but also in camelids (Markey *et al,* 2013). *Streptococcus equi* subsp. *zooepidemicus* is the aetiological agent of 'Alpaca Fever' in Peru, but also has been isolated from dromedaries and their milk in Kenya and Somalia (Younan *et al,* 2005; Jones *et al,* 2009; Fowler, 2010). Alpaca fever occurs in acute, subacute and chronic forms and is an important disease in the Altiplano of South American camelids (Fowler, 2010). Transmission occurs via ingestion or direct contact with infected animals. Polyserositis, meningitis and internal and external abscesses have been described in association with the disease. In adult dromedaries, the infection could result in sporadic, but severe, live threatening mastitis with udder necrosis, and occasionally internal abscess formation. On the other hand, in youngstock, clinical signs are observed on larger number of animals at the same time, usually showing mild upper respiratory tract infections characterised by serous or mucopurulent nasal discharge. Some calves, but not all, also develop fever. However, severe manifestation of *Streptococcus equi* subsp. *zooepidemicus* infection occurs rarely in young dromedaries. In these severe cases, mainly meningitis was diagnosed, but pleuropneumonia has not been detected earlier (Juhasz, Nagy, Ismail personal communication).

In this manuscript, we describe four per-acute and acute cases caused by *Streptococcus equi* subsp. *zooepidemicus*. in dromedary camel calves.

Materials and Methods

Clinical evaluation of the animals

The four dromedary calves were kept at the premises of the Emirates Industry for Camel Milk and Products (EICMP), located in Dubai, United Arab Emirates (N25°, E55°). EICMP owns the largest dairy camel herd in the world, with a total dromedary population of over 8000 heads. At the farm, a herd health management program is implemented and is under strict veterinary control. The program includes, among many other elements, the clinical evaluation of the health status of all animals twice a day. The details of the calves and their clinical history are presented in Table 1.

Post-mortem examination of the animals

Three female camel calves (weighing 63, 74 and 87kg) and one male calf (weighing 125kg) were sent to the Central Veterinary Research Laboratory (CVRL) in Dubai for necropsy. During necropsy, samples were taken from all organs for bacteriology and histological investigations using routine known methods.

The affected lung samples were streaked onto sheep blood agar (Neogen), brilliant-green phenol-red lactose sucrose agar (BPLS-Merck) and nutrient agar (Oxoid). Duplicate sheep blood agar plates were used for culture to incubate at aerobic and anaerobic conditions, whereas the other two plates were incubated aerobically only, at 37°C. Pure cultures were obtained by subculturing and strains

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

Table 1. History, clinical, pathological findings and treatment of dromedary calves affected by severe *Streptococcus equi* subsp. *zooepidemicus* infection.

Calf ID	Gender	Age (Days)	Weight (KG)	DIED	Previous History	Clinical signs	Blood test result (CBC)	Treatment	Post mortem findings
4836	Female	180	87	24-Aug-2018	Under developed calf. No other previous history of illness.	Sudden death	Not Available	Not Applicable	Congested lung and liver. Yellowish intestinal fluid. Swollen mesenterial lymph nodes. *Strep. zooepidemicus* in Liver, lung and lung lymph nodes.
1926	Male	138	125	7-Jun-2021	Mother died next day after birth due to pelvic fracture. Well-developed calf. No previous history of illness.	Had fever and greenish diarrhea two days before.	WBC - 3.2 (10^9/L) [Low]; LYM - 63.4% [High]; NEU - 32.8% [Low]	Fluid Theraphy, Supportive vitamins. Wondercef (Ceftiofur 1.06 mg/kg BW), Baytril (Enrofloxacin 5 mg/kg BW), Sulprim Paste (Sulfadimidine 2 mg/kg BW & trimethoprim 0.4 mg/kg BW)	Congested lungs with brownish consolidated right cranial parts. Greenish C-1 content. Swollen intestinal lymph nodes. Severe diffuse suppurative bronchopneumonia. Strep. zooepidemicus in lung.
5596	Female	111	63	29-Jun-2021	Under developed calf. No other previous history of illness.	Had fever and diarrhea one day before.	Not Available	Supportive treatment. Vitamins. Marbox (Marbofloxacin 2 mg/kg BW), Benacillin(procaine Penicillin 7.5 mg/kg BW, Benzathine Penicillin 7.5 mg/kg BW), Scourban (Sulfadimidine 42.6 mg/kg BW, Sulfadiazine 42.6 mg/kg BW, Streptomycin (as sulfate) 7.28 mg/kg BW, Neomycin sulfate 2.16 mg/kg BW)	Frontal lobe of left lung and part of caudal left lobe solid, dark reddish blue. Right caudal lobe with subpleural petechiae. Severe fibrinupurulent to hemorrhagic pleuropneumonia. C-3 filled with green liquid content. Strep. zooepidemicus from lung.
5204	Female	447	74	18-Jun-2021	Retarded calf. Treated twice before. Once for colic and fever. Other time only fever for a long time.	Sudden death. No other sign of illness.	Not Available	Not Applicable	Hard mass blocking the tracheal bifurcation to the bronchi. Large hard, brown mass in the left cranial lobe. Cranial area of right lobe of lung is hemorrhagic and with small dots. Lung- necrotic material. C-1 content greenish. Swollen intestinal lymph nodes. *Strep. zooepidemicus* from lung.

were identified by API 20 Strept (bioMérieux) identification system and by Gram-stain.

Results

Clinical findings: One case occurred in the summer of 2018, while the other three cases happened within a period of three weeks at the beginning of the summer 2021. Animals were kept in separate locations, far from each other and other calves in the same groups showed no signs of diseases. Therefore, a direct link between the cases was not established. In general, the disease occurred in per-acute and acute forms. In the per-acute cases, the calves were found dead in the paddock, while in acute cases, animals developed high fever and diarrhoea, but respiratory signs were not detected. Blood sample was taken from one of the animals (C 1926) that showed severe lymphocytopenia and neutrocytopenia.

Post-mortem examinations: Four cases of 'Alpaca Fever' in dromedary calves were caused by *Streptococcus equi* subsp. *zooepidemicus*, which died suddenly. In the first per-acute case (C4836) no pneumonia was diagnosed. However, in the other three cases a severe fibrinopurulent to haemorrhagic pneumonia was detected. *Streptococcus equi* subsp. *zooepidemicus* was isolated on blood agar from the affected lung in pure culture showing strong β-haemolysis. The bacteria were also isolated

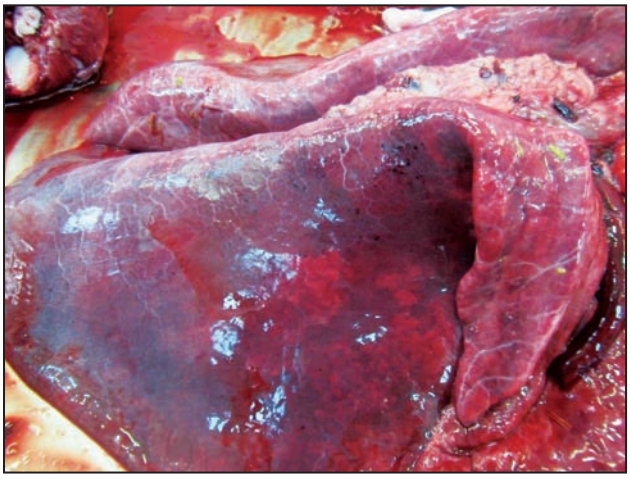


Fig 1. Acute haemorrhagic pneumonia caused by *Streptococcus equi* subsp. *zooepidemicus*.

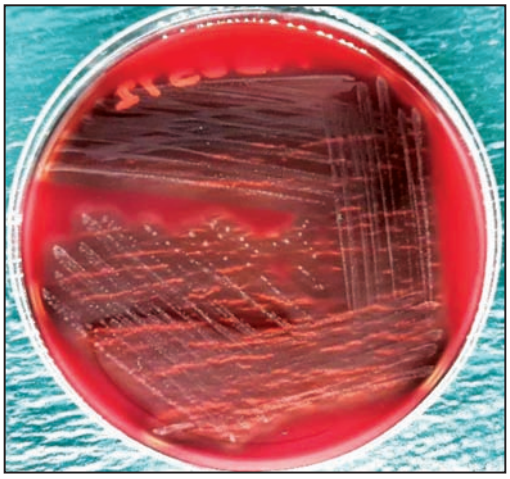


Fig 3. Colonies of *Streptococcus equi* subsp. *zooepidemicus* on Sheep blood agar incubated anaerobically for 48hours. Note the small diameter (1-2mm) of the colonies and beta haemolysis.

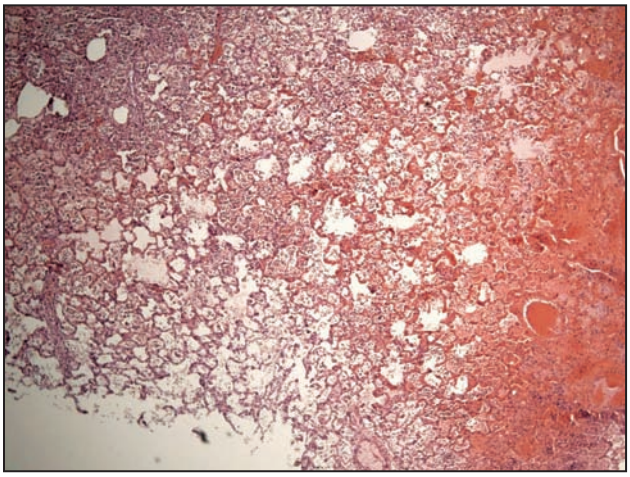


Fig 2. Histology of the lung shown in Fig 1 demonstrating fibrinopurulent haemorrhagic pneumonia, HE staining, 40x.

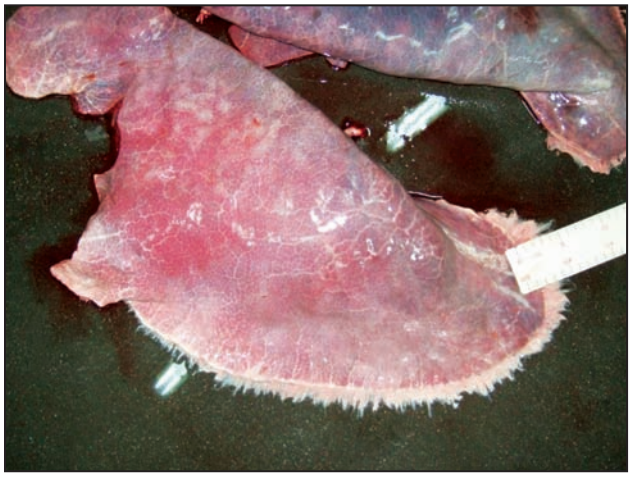


Fig 4. Pleural curtain in an adult camel: Note the length of 15 to 20mm.

from the lung and other organs in large numbers from the first per-acute case. During necropsy it was observed that one or both front lobes of the lungs were consolidated and dark reddish-blue (Fig 1) with several petechiae, from which the pathogen was also isolated. All other organs were without any pathological alterations. The compartment 1 (C1) contained green liquid and proper fodder.

In histopathology, the lungs exhibited an acute fibrinopurulent to haemorrhagic pneumonia with a great amount of coccoid bacteria, but without fungal elements (Fig 2).

After 24 hours of incubation, colonies, typical for *Streptococcus equi* subsp. *zooepidemicus* (small, about 1-2mm in diameter, beta haemolytic, mucoid with colony-size variation) were observed. Scanty growth was seen on BPLS agar and tiny colonies on Nutrient agar. Colonies were best seen on sheep blood agar which was incubated anaerobically than aerobically (Fig 3).

Discussion

In general, respiratory diseases in camelids are rare. When they occur, pneumonia is usually initiated by predisposing factors as well as underlying debilitating conditions in young camels (Wernery *et al,* 2014). This phenomenon may be explained by the unique immune system of camelids and also by the existence of the so-called pleural "curtain" that is found along the basal margins of the lung, cleaning, like a windscreen wiper, the pleural fluid and supporting phagocyting cells (Buzzel *et al,* 2010). The pleural curtain is a feature of camelids and has

also been seen in giraffes which are also apparently resistant to respiratory disease (Letzner, 1987). However, in camel calves this curtain is just visible (1 to 2mm long) in contrast to adult camels where it can reach up to 25mm (Fig 4). Hence, most likely this curtain in not fully functioning in young camel calves yet.

In alpacas, lesions of acute cases of coccal infection produce significant quantities of fibrinopurulent exudation of the thoracic cavity along with pleuritis and ecchymotic haemorrhages of serosal surfaces (Fowler, 2010). In our case in the dromedary calves, pathological changes observed might be similar, but the most significant changes were seen in the lungs, which are not described to occur in alpacas. Cebra *et al* (2000) infected llamas intratracheally with *Streptococcus equi* subsp. *zooepidemicus* which produced fever, anorexia and signs of depression. Corpa *et al* (2018) described three spontaneous cases of alpaca fever in alpacas with disseminated fibrinosuppurative polyserositis, vascular thrombosis and intralesional Gram-positive cocci. Two of the animals developed also severe fibrinosuppurative pneumonia, endocarditis, and myocardial necrosis.

Stoughton and Gold (2015) cultured *Streptococcus equi* subsp. *zooepidemicus* from venous blood, peritoneal fluid, and pleural fluid samples of a 12-week-old female dromedary camel calf showing severe bicavitary effusion, and peripheral lung consolidation. The animal was treated for 11 days with IV broad-spectrum antimicrobials, an NSAID, and pleural drainage was initiated. Antimicrobial treatment continued for 2 weeks after discharge and 6 weeks after the initial examination, there were no clinical signs suggestive of relapse or any reported complications.

Younan and Bornstein (2007) isolated 13 strains of *Streptococcus equi* subsp. *zooepidemicus* from camels (*Camelus dromedarius*) in Kenya and Somalia. The five mucoid strains of *Streptococcus equi zooepidemicus* were all isolated from the respiratory tract, similar to our four cases. However, strains of *Streptococcus equi* subsp. *zooepidemicus* isolated from cases of respiratory disease in Somalia differed in their ability to ferment lactose and were sensitive to penicillin G and oxytetracycline from Kenya-strains. This difference in biochemical activity between those and our strains may also reflect difference in virulence and pathogenicity.

In our case, *Streptococcus equi* subsp. *zooepidemicus* infection was endemic on the farm. Time to time, we observe mild upper respiratory tract infections with serous or mucopurulent nasal discharge in large number of calves and yearlings affecting entire groups of animals. Some calves might also develop fever. However, severe manifestation of the infection is rare in young dromedaries and such mild infections respond quickly and favorable to long-acting beta-lactam antibiotic treatment. The reason why in these four cases the *Streptococcus equi* subsp. *zooepidemicus* infection caused such a severe pathological changes and death is likely to be related to the impaired immunological status of those particular animals. One of the calves was retarded and two females were underdeveloped (lower body weight compared to their age group). Even the well-developed, male calf had a predisposing factor as its dam died shortly after delivery. We can anticipate that this animal might not had sufficient colostrum intake and might had disturbed maternal immunity. The history of these four cases further supports our assumption that compromised host immunity plays a vital role in the development of 'Alpaca Fever' in dromedaries and explain why an endemic, commensal bacteria cause such severe lesions and death sporadically in individual animals.

In conclusion, in this case report we described four severe manifestation of *Streptococcus equi* subsp. *zooepidemicus* infection. It seems that all Camelidae species are susceptible to an infection with this bacteria. However, the severity of clinical signs is determined by predisposing factors such as the immune status of the individual animal.

Acknowledgement

The authors would like to thank all the hard work and commitment of the veterinary assistant team at EICMP and are also grateful to the management of the company for supporting scientific research and development.

References

Buzzel GR, Kinne J, Tariq S and Wernery U. The pleural curtain of the camel (*Camelus dromedarius*). Anatomical Record. 2010; 293(10):1776-1786.

Cebra CK, Heidel JR, Cebra ML, Tornquist SJ and Smith BB. Pathogenesis of Streptococcus *zooepidemicus* infection after intratracheal inoculation in llamas. American Journal of Veterinary Research. 2000; 61(12):1525-1529.

Corpa JM, Carvallo F, Anderson ML, Nyaoke AC, Moore JD, Uzal FA. *Streptococcus equi* subspecies *zooepidemicus* septicemia in alpacas: three cases and review of the literature. Journal of Veterinary Diagnostic Investigation. 2018; 30(4):598-602.

Fowler ME. Medicine and Surgery of Camelids. 3rd Ed. Wiley Blackwell, Oxford, UK. 2010; pp 212.

Jones M, Miesner M and Grondin T. Outbreak of *Streptococcus equi* spp. *zooepidemicus* polyserositis in an alpaca herd. Journal of Veterinary Internal Medicine. 2009; 23:220-223.

Letzner G. Ein Beitrag zur Immobilisation, Physiologie und Pathologie der Giraffen. Dissertation, Tierärztliche Hochschule Hannover, Germany. 1987.

Markey B, Leonard F, Archambault M, Cullinane A and Maguire D. Clinical Veterinary Microbiology. 2nd ed. Mosby Elsevier. 2013; pp 121-134.

Stoughton WB and Gold J (2015). *Streptococcus equi* subsp *zooepidemicus* pleuropneumonia and peritonitis in a dromedary camel (*Camelus dromedarius*) calf in North America. Journal of the American Veterinary Medical Association 247(3):300-303.

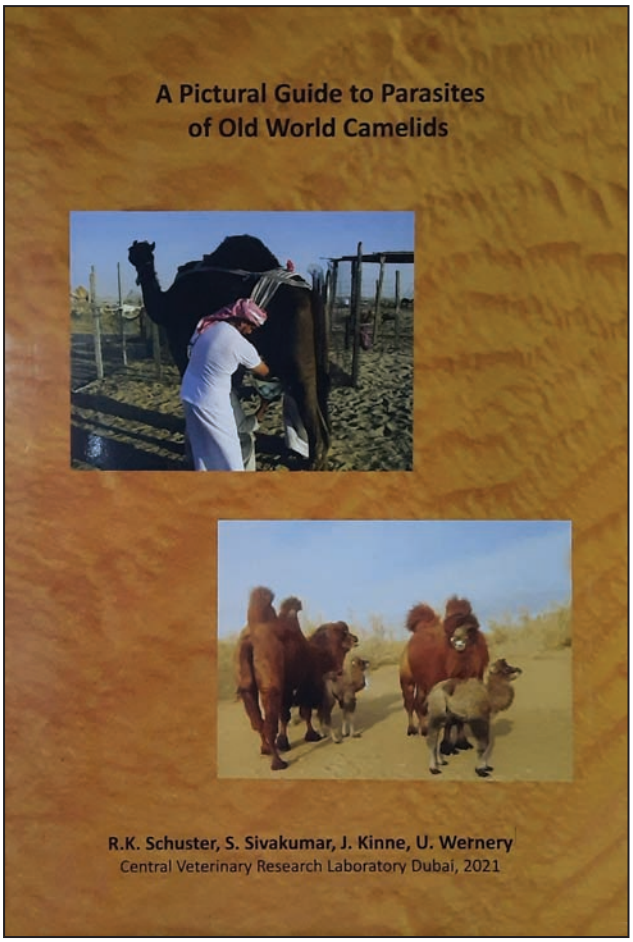
Wernery U, Kinne J and Schuster RK. Camelid Infectious Disorders. OIE Book. 2014; pp 113.

Younan M, Estoepangestics, Cengiz M, Alber J, El-Sayed and Lämmler C. Identification and molecular characterisation of *Streptococcus equi* subsp. *zooepidemicus* isolated from camels (*Camelus dromedarius*) and camel milk in Kenya and Somalia. Journal of Veterinary Medicine. B. Infectious Disease and Veterinary Public Health. 2005; 52:142-146.

Younan M and S Bornstein. Lancefield group B and C streptococci in East African camels (*Camelus dromedarius*). Veterinary Record. 2007; 160:330-335.

BOOK REVIEW

Title: A Pictural Guide to Parasites of Old World Camelids
Authors: R.K. Schuster, S. Shivakumar, J. Kinne and U. Wernery
Published by: Central Veterinary Research Laboratory, Dubai, UAE
Year: 2021



The new book- A Pictural Guide to Parasites of Old World Camelids is a handbook which will be highly useful to all laboratories engaged in the study of camel parasites. Book contains a very nice classified description of all the parasites. The illustrations (185 Figures) are in high definition and have come out to be remarkable in printing. Clinical, laboratory and microscopic pictures are highly illustrious. Those of *T. evansi*, Giardi, *Infundibulorium cameli,* Eimeria spp, Cryptosporidium, *Cystoisospora orlovi*, Fasciola, Dicrocoeliidae spp, Schistosomes, Anoplocephalidae I, II, Hydatids, Intestinal Trichostrogylids, Trichuris spp, Filarids, Ticks, *Sarcoptes scabiei*, Myiasis, Flesh flies, *Wohlfahrti magnifica*, Chrysomya, *Cephalopina titillato*r, blood sucking flies are best representative pictures, most suitable for clinicians and students to understand the diverse camel parasites. The last section of the book describes parasitological laboratory and its procedures. It includes description of equipments and other subheadings such as sending material, faecal samples, floatation method, direct smear and iodine smear, negative staining for Cryotosporidium oocyst, modified Ziehl-Neelsen staining, sedimentation method, larvae isolation, blood examination, haematocrit method, Knott's test and microfiltration, and skin scrapings. I really appreciate the cover pictures specially authors working in the field, laboratory and postmortem room...speaks of dedication of team committed to the diagnosis of parasitic diseases of camels.

My congratulations to the team of Central Veterinary Research Laboratory, Dubai for compiling, editing and authoring this useful publication.

(Dr. T.K. Gahlot)
Reviewer

DOI : 10.5958/2277-8934.2021.00046.1 *Vol 28 No 3, p 297-302*

BIOMARKERS OF STRESS IN HEALTHY AND DISEASED DROMEDARY CAMELS: A MINI REVIEW

Mohamed Tharwat[1,2] and Wael El-Deeb[3,4]

[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

[3]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

4Department of Internal Medicine, Infectious Diseases and Fish Diseases, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

ABSTRACT

This review was designed to describe the commonly used stress and inflammatory biomarkers in healthy and diseased dromedary camel medicine through providing insights by ongoing research on these biomarkers. The review especially focuses on the stress and inflammatory biomarkers in some of the very important disorders affecting camels such as paratuberculosis, trypanosomiasis, mange, pneumonia, urinary tract infection, liver abscessation and stress during camel transport. It is believed that these biomarkers will be increasingly used in the future in the diagnosis and prognosis of different disorders of camels.

Key words: Camels, glutathione, malondialdehyde, stress biomarkers, superoxide dismutase

In dromedary camels, biomarkers are used as an aid in the early detection, diagnosis and prognosis in various conditions such as bone affections, cardiac problems, infection and inflammation (El-Deeb *et al,* 2019; Tharwat, 2020 a,b,c,).

Oxidative stress (OS) occurs when the oxidant/antioxidant imbalance results in excess production of ROS and leads to cellular and tissue damage. Oxidative stress may occur during and after stressful events such as transport, exercise and intensive management in both humans and animals (Kirschvink *et al,* 2002). Therefore, is an imbalance between radical-generating and radical-scavenging activity processes necessary to detoxify these toxic molecules resulting in damage of all components of the cell, including proteins, lipids, and DNA (Kowaltowski and Vercesi, 1999; Niki, 2009). OS can occur because of either heightened reactive oxygen species (ROS) generation, impaired antioxidant system, or a combination of both. In the presence of oxidative stress, uncontained ROS attack, modify, and denature functional and structural molecules leading to tissue injury and dysfunction (Vaziri, 2008). Thus, OS can cause disruptions in normal mechanisms of cellular ability to detoxify the reactive intermediates or to repair the resulting damage (Lands *et al,* 2000).

The antioxidative status consists of two mechanisms: nonenzymatic and enzymatic mechanisms. Nonenzymatic mechanisms are composed of antioxidants, scavengers of free radicals, transition metal ions, sequester transition metal ions, albumins, ceruloplasmin, and metallothioneins. On the other hand, enzymatic mechanisms are composed of superoxide dismutase (SOD), peroxidase, catalase and reductase (Kleczhowski *et al,* 2003).

A complex association exists between OS and inflammation. The OS is believed to be an integrated part and major component in the pathogenesis of skin diseases expressed as erythema, oedema, wrinkling, hypersensitivity, keratinisation abnormalities and cancer (Bickers and Athar, 2006; Portugal *et al,* 2007). This review was written to shed light on the commonly used stress biomarkers in dromedary camel medicine which is believed that providing insights by ongoing research on these biomarkers, it will be increasingly used in the future in the diagnosis and prognosis of different disorders affecting this species.

Biomarkers of stress

A byproduct of normal aerobic metabolism is the generation of dangerously reactive intermediates of the reduction of oxygen. These

SEND REPRINT REQUEST TO WAEL EL-DEEB email: weldeeb@kfu.edu.sa, drwaeleldeeb@yahoo.com

ROS, and others that they can engender, threaten all cellular macromolecules, and defenses are needed. These defenses are aided by enzymes that repair or recycle oxidatively damaged nucleic acids and proteins. A role for such oxidative damage in aging and neurodegenerative diseases is well supported (Freidovich, 1999).

The reactive oxygen intermediates are formed in many parts of liver cells. A balance between free radical reactions and antioxidant activities is very important for normal liver functioning. This balance is altered in pathological processes (Blazovics *et al,* 1992). The antioxidant system consists of antioxidant enzymes, including SOD, catalase (CAT) and glutathione peroxidase (GSH-Px), glutathione (GSH), ancillary enzymes such as glutathione reductase (GSHR), glutathione S-transferase and glucose 6-phosphate dehydrogenase (G6PD), metal-binding proteins such as transferrin, ceruloplasmin (Cr) and albumin, vitamins such as alpha-tocopherol, ascorbate and beta-carotene, flavonoids and urate (Halliwell, 1994; Abd Ellah *et al,* 2007).

In a study carried out by Corbera *et al* (2001), the mean GSH-Px level in the total of 442 females and 267 males dromedary camels was 288.5+/-157.2 IU x g(-1) haemoglobin (Hb). Reference ranges were estimated and enzymatic activities below 51 IU x g(-1) Hb were considered inadequate. GSH-Px activities obtained in females (298.1+/-155.7 IU x g(-1) Hb) were significantly higher than in males (272.6+/-157.2 IU x g(-1) Hb).

Lipid peroxidaion is a general mechanism whereby free radicals induce tissue damages, and implicated under several diverse pathological conditions (Halliwell and Chirico, 1993). Malondialdehyde (MDA) has been widely applied as the most common biomarker for the assessment of lipoperoxidation in biological and medical sciences (Bird and Draper, 1984; Suttnar *et al,* 2001; Salar-Amoli *et al,* 2009). Therefore, MDA is one of the end-products of lipid peroxidation, and the extent of lipid peroxidation is most frequently measured by estimating MDA levels (Lata *et al,* 2004). In a research study, investigating the oxidative stress markers in healthy camels (Salar-Amoli *et al,* 2009), the authors measured MDA levels in serum and plasma of 24-48 month male camels in Iran. The MDA was detected as 0.43±0.009 (nmol/ml) in serum and 0.47±0.009 (nmol/ml) in plasma. Moreover, they estimated the total thiol groups which was detected as 27.86±0.44 and 29.44±0.47 (nmol/mg protein) in camel-calves and adult camels respectively. Furthermore, the total thiols content in serum and plasma of 24-48 month Iranian camels were about 0.09±0.03 in serum and 0.10±0.02 (mmol/l) in plasma.

Application of stress biomarkers in camel medicine

Important infectious diseases in farm animals, such as pneumonia and enteritis, are thought to be associated with the so-called oxidative stress, i.e. a chemical phenomenon involving an imbalance in the redox status of the individual animal. The specifics of oxidative stress and how it may result in disease or be prevented are complex questions with no simple answers. A particularly intriguing aspect is that, at least theoretically, oxidative stress should be easily prevented with antioxidants yet the use of antioxidants as therapy remains controversial (Lykkesfeldt and Svendsen, 2007). Following, we will discuss stress biomarkers in some diseases affecting the dromedary camels.

Stress and inflammatory biomarkers in paratuberculosis

In camels with paratuberculosis, the activities of SOD and CAT and reduced glutathione (RGSH) level are reduced significantly in infected camels compared to the control. On the contrary, lipid peroxidation is increased significantly as reflected on higher MDA value in the serum of infected camels compare to control. In addition, the values of all pro-inflammatory cytokines, IL-1α, IL-1β, IL-6, IL-10, TNF-α and IFN-γ were also increased significantly ($P \leq 0.05$) in paratuberculosis infected camels compared to control (El-Deeb *et al,* 2014).

Stress and inflammatory biomarkers in trypanosomiasis

In camels with trypanosomosis, the activities of SOD, catalase and RGSH levels are reduced significantly in camels with trypanosomosis compared with the control whereas, lipid peroxidation is increased significantly as reflected on higher MDA values in the serum of camels with trypanosomiasis compared to the healthy controls (El-Deeb and El-Moslemany, 2015; El-Bahr and El-Deeb, 2016). In addition, the values of all pro-inflammatory cytokines namely, interleukins-1α, 1β, 6, 10, tumour necrosis factor-α and interferon gamma are significantly higher in *T. evansi* camels compared with the control (El-Bahr and El-Deeb, 2016). In another study carried out by Eljalii *et al* (2015), it was concluded that infection by *T. evansi* in camels was

associated with lipids peroxidation and oxidative stress.

Stress biomarkers and mange

Scabies is a major global health problem in human and animal populations (McClain *et al*, 2009). Sarcoptic mange caused by *Sarcoptes scabiei* var *cameli* is a widespread, contagious and debilitating skin disease, ranking among the most serious and economically important diseases of the camel (Pegram and Higgins, 1992). The disease affects mainly camels reared under poor nutrition, management and hygienic conditions (Kumar *et al,* 1992). Symptoms of the disease include intense pruritis, exudative dermatitis, parakeratotic scaly crust formation, alopecia and dark thickened skin. Fissures develop in the crust and underlying epidermis resulting in haemorrhages. Emaciation, debilitation, anaemia and subcutaneous oedema are common signs in mangy camels (Kumar *et al,* 1992; Amer *et al,* 2006).

In skin diseases, the body possesses an array of a potent antioxidant protection such as SOD, CAT, GSH, GSH-Px, and the antioxidant vitamins A, E and C (Bickers and Athar, 2006). Synergistic and co-operative interactions of these antioxidants rely on the sequential degradation of peroxides and free radicals (Portugal *et al,* 2007). In camels with sarcoptic mange, concentrations of MDA did not differ in mild, but elevated in moderate and severe cases compared to the values in the control group. In comparison with the healthy camels, the values of SOD and catalase are significantly higher in mild and significantly lower in moderate and severe cases. The same trend is noticed for GSH concentration, where it is higher in mild and lower in moderate and severe cases compared to the healthy animal values (Saleh *et al,* 2011).

Stress and inflammatory biomarkers in pneumonia in camels

In a study conducted on 10 camels that suffered from bilateral nasal discharge, inappetence, dyspnoea, cough with harsh lung sounds, there was a significant increase in the levels of MDA in diseased camels when compared to its levels in the healthy ones. Moreover, a significant decrease in the levels of total antioxidant capacity (TAC) and catalase compared to the healthy group. In addition, there were static variations in the values of MDA and catalase between young aged pneumonic camels (2-4 years) and pneumonic camels aged from 5-8 years (Kamr *et al,* 2020). The decreased antioxidants (TAC and CAT), as identified in the later study, was attributed to the cell protection consumption of those enzymes by preventing the initiation of peroxidisation and production of products, that are capable of leading to serious cell damage (Halliwell, 1994). The significant elevation of the MDA level in the study of Kamr *et al* (2020) may be also associated with the risk effect of cellular damage and inflammation, which are linked to bronchopneumonia and bronchointerstitial pneumonia, in addition to the destruction of epithelial cells and fibrinous reaction resulting from vascular damage (Jarikre *et al,* 2017).

In another study conducted on 20 diseased she-camels manifested by moist painful harsh cough, rhinitis, congested mucous membranes and serous or mucoid nasal discharges, the mean values of serum concentrations of MDA, nitric oxide (NO) and plasma hydrogen peroxide (H_2O_2) were significantly increased in the diseased group compared to the controls (n=20). On the other hand, serum enzymatic activities of catalase, glutathione reductase (GR) and GSH-Px showed a significant decrease in the diseased group. Compared to controls, diseased she-camels had significantly higher serum concentrations of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6 and TNF-α), while the serum concentrations of anti-inflammatory cytokine (IL-10) were significantly decreased (Allam *et al,* 2017). In a third study investigating the oxidative stress markers in pneumonic camel calves, there was a significant increase in the levels of MDA and significant decrease in the levels of GSH, SOD and CAT in the pneumonic camel calves versus healthy controls (El-Deeb, 2015).

Stress biomarkers and urinary tract infection in camels

In a study with 74 camels with urinary tract infection, the concentrations of erythrocytic MDA (eMDA) (180.8 mmol/g Hb versus 109.4 mmol/g Hb), serum MDA (sMDA) (19.01 mmol/g Hb versus 10.85 mmol/g Hb) were significantly higher ($P<0.0001$) in diseased camels compared to healthy ones. However, catalase (11.63 U/mg Hb versus 15.67 U/mg Hb), super oxide dismutase (SOD) (3.73 U/mg Hb versus 4.98 U/mg Hb) and GSH levels (4.1 mmol/g Hb versus 6.78 mmol/g Hb) were significantly lower ($P<0.0001$) in diseased camels when matched with the same levels in control group. Interleukin-6 was also higher in camels with urinary tract infection compared to controls (15.08 pg/mL versus 12.35 pg/mL, $P=0.003$) (El-Deeb and Buczinski, 2015).

Stress biomarkers and liver abscessation in camels

In addition to the significant elevations in the serum activity of liver enzymes including AST, ALT,

GGT, GLDH, and ALP, bilirubin was also increased significantly in camels with liver abscesses compared to healthy ones. Increased levels of MDA and G6PD with significant reduction in the levels of SOD, CAT and GSH are also evident when compared to their levels in healthy camels (El-Deeb and Fouda, 2013).

Stress biomarkers and transport

In a study of camels subjected to road transportation stressor for 2 h by truck during the hot-dry season (El Khasmi *et al,* 2013). The authors revealed that road transportation allied to heat may be measured as a strong stressor which is able to persuade numerous cellular changes in camels. In another study investigating transport distance on stress biomarkers, El Khasmi *et al* (2015) concluded that road transport is very stressful for the camel, and the effects of this stress on the relevant indicators increase much with distance which was categorised as short (72-80km), medium (160-170km) and long (350-360km) distance. In the later study, the serum cortisol has increased dramatically with distance where it measured 88.32±19.4 ng/mL, 152.4±25.18, 231.7±23.75 ng/mL at the short, medium and long distance, respectively. The serum concentration of MDA was estimated to be 1.58±0.38 ng/mL, 3.88±0.2, 6.44±0.52 nmol/mL at the short, medium and long distance, respectively. Parallel, the serum concentration of catalase was found to be 60.08±3.18 ng/mL, 79.13±3.84, 93.95±3.62 KU/L at the short, medium and long distance, respectively (El Khasmi *et al,* 2015).

In another study of 300 km, 5-hour journey in truck by road conducted during hot summer, Nazifi *et al* (2009) found that the mean concentration of MDA (1.87±0.26 nmol/mL) and GSH-Px activity (297.86±25.68 U/g Hb) in basal pre-transport conditions show significant increase 24 h after arrival. However, the mean concentration of SOD activity (1742.5±74.36 U/g Hb) in basal pre-transport conditions had no significant change during and after transportation. It was concluded from this study that transport stress causes an oxidative challenge in dromedary camels and represent novel biomarkers for stress-associated disease susceptibility and welfare assessment (Nazifi *et al,* 2009).

In dromedaries, transport by trucks induces more stress than long-distance walk travel. The manipulation of camels during loading and unloading has a more stressful impact compared to the travel by walk for very long transport distance (Mohamed *et al,* 2021). In another trials, road transport and loading density were also found to be very stressful to the camel, and the effects of this stress on the relevant indicators raising much with the distance and density, respectively (Mohammed *et al,* 2015; Abdelilah *et al,* 2018). The effect of transport distance on so stress biomarkers in the dromedary camel meat was investigated. As conclusion, the results showed a significant gradual increase of meat MDA levels and a significant decrease of meat CAT activity with road transport distance, and could be explained by an antemortem OS activity during travel raising much with distance (Kaoutar *et al,* 2016).

References

Abd Ellah MR, Okada K and Yasuda J. Oxidative stress and bovine liver diseases: role of glutathione peroxidase and glucose 6-phosphate dehydrogenase. Japanese Journal of Veterinary Research. 2007; 54:163-173.

Abdelilah L, Mohamed F, Fouad R, Najia E, Elhassane T, Abderrahmane B, Bernard F and El Mohammed K. Evaluation of stress responses induced by the loading density in dromedary camel (*Camelus dromedarius*). Emirates Journal of Food and Agriculture. 2018; 30:803-808.

Allam TS, Saleh NS, Abo-Elnaga TR and Darwish AA. Cytokine Response and Immunological Studies in Camels (*Camelus dromedarius*) with Respiratory Diseases at Matrouh Province. Alexandria Journal of Veterinary Science. 2017; 53:116-124.

Amer AA, Abou El-Ela A and Ratib HZ. Some Hematobiochemical studies on sarcoptic mange infested camels before and after treatment by doramectin at Assiut governorate. Proceedings of the International Scientific Conference on Camels, 9-11 May 2006, KSA. 2006; pp 686-691.

Bickers DR and Athar M. Oxidative stress in the pathogenesis of skin disease. Journal Investigative Dermatology. 2006; 126:2565-2575.

Bird RP and Draper HH. Comparative studies on different methods of malonaldehyde determination. Methods in Enzymology. 1984; 105:299-305.

Blazovics A, Fehér E and Fehér J. Role of free radical reactions in experimental hyperlipedemia in the pathomechanism of fatty liver. In: Csomos G, Feher J (Eds) Free Radicals and Liver. Berlin Springer Verlag. 1992.

Corbera JA, Gutierrez C, Morales M, Montel A and Ontoya JA. Assessment of blood glutathione peroxidase activity in the dromedary camel. Veterinary Research. 2001; 32:185-191.

Eljalii IM, EL-Deeb WM, Fouda TA, Almujalli AM and El-Bahr SM. Blood Picture and Selected Oxidative Stress Biomarkers in Dromedary Camels Naturally Infected With *Trypanosoma evansi*. International Journal of Veterinary Sciences Research. 2015; 1:46-53.

El Khasmi M, Chakir Y, Riad F, Safwate A, Tahri EH, Farh M, El Abbadi E, Abouhafs R and Faye B. Effects of

Transportation Stress during the Hot-Dry Season on Some Haematological and Physiological Parameters in Moroccan Dromedary Camels (*Camelus dromedarius*). Journal of Life Sciences. 2013; 7:13-25.

El Khasmi1M, ChakirY, Bargaâ R, Barka K, Lektib I, El Abbadi N, Belhouari A and Faye B. Impact of transport distance on stress biomarkers levels in dromedary camel (*Camelus dromedarius*). Emirates Journal of Food and Agriculture. 2015; 27:507-512.

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

El-Deeb WM, Fouda TA and El-Bahr SM. Clinico-biochemical investigation of paratuberculosis of dromedary camels in Saudi Arabia: Proinflammatory cytokines, Acute phase proteins and oxidative stress biomarkers. Pakistan Veterinary Journal. 2014; 34:484-488.

El-Deeb WM and El-Moslemany AM. Cardiac and oxidative stress biomarkers in *Trypanosoma evansi* infected camels: diagnostic and prognostic prominence. Parasitology, Cambridge University. 2015; 142(6):767-772.

El-Deeb WM and Fouda TA. Liver Abscesses in Dromedary camels (*Camelus dromedarius*): oxidative stress biomarkers and proinflammatory cytokines. Journal of Veterinary Science and Technology. 2013; 4:4

El-Deeb WM. Acute phase response and oxidative stress parameters in pneumonic camel calves (*Camelus dromedarius*). Bulgarian Journal of Veterinary Medicine. 2015; 18:258-269.

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

El-Deeb WM, Ghoneim I, Fayez M, Elsohaby I, Alhaider A and El Gioushy M. Acute phase proteins, proinflammatory cytokines and oxidative stress biomarkers in sheep, goats and she-camels with *Coxiella burnetii* infection induced abortion. Comp. Immunol. Microbiol. Infect. Dis. 2019; 67:101352.

Freidovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? Annals of the New York Academy of Sciences. 1999; 893:13-20.

Halliwell B and Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. American Journal of Clinical Nutrition (Suppl. 1). 1993; 57:715S-725S.

Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet. 1994; 344:721-724.

Jarikre TA, Ohore GO, Oyagbemi AA and Emikpe BO. Evaluation of oxidative stress in caprine bronchoalveolar lavage fluid of pneumonic and normal lungs. International Journal of Veterinary Science and Medicine. 2017; 5:143-147.

Kamr A, Gadallah S, Arbaga A and Hassan HY. Oxidant and antioxidant biomarkers and the risk factor of age on their concentrations in pneumonic Arabian camels (*Camelus dromedarius*). Journal of Camelid Science. 2020; 13:40-48.

Kaoutar B, Mohamed F, Fouad R, El Hassane T, Abdarrahmane B and Mohammed E. Impact of transport distance on some stress biomarkers levels in camel meat. MOJ Food Processing Technology. 2016; 2:130-134.

Kirschvink N, Moffart BD, Smith N, Marlin D, oberts C and Lekeux P. Relationship between markers of blood oxidant status and physiological variables in healthy and heaves-affected horses after exercise. Equine Veterinary Journal. 2002; 34:159-164.

Kleczhowski M, Klucinski W, Sikora J, Jdanocviez M and Dziekan P. Role of antioxidants in the protection against oxidative stress in cattle: non-enzymatic mechanisms (part 2). Polish Journal of Veterinary Science 2003; 6:301-308.

Kowaltowski AJ and Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. Free Radical Biology and Medicine. 1999; 26:463-471.

Kumar D, Raisinghani PM and Manohar GS. Sarcoptic mange in camel: a review. Proceeding of the first international camel conference, Dubai, UAE, 2-6 February 1992. pp 79-82. 1992.

Lands LC, Grey V, Smountas AA, Kramer VG and McKenna D. Lymphocytes glutathione levels in children with cystic fibrosis. Chest. 2000; 116:201-205.

Lata H, Ahuja GK, Narang APS and Walia L. Effect of immobilisation stress on lipid peroxidation and lipid profile in rabbits. Journal of Clinical Biochemistry. 2004; 19:1-4.

Lykkesfeldt J and Svendsen O. Oxidants and antioxidants in disease: oxidative stress in farm animals. Veterinary Journal. 2007; 173:502-511.

McClain D, Dana AN and Goldenberg G. Mite infestations. Dermatologic Therapy. 2009; 22:327-346.

Mohamed RH, Abo El-Maaty AM, Abd El Hameed AR and Ali AH. Impact of travel by walk and road on testicular hormones, oxidants, traces minerals, and acute phase response biomarkers of dromedary camels. Heliyon. 2021; 7:e06879.

Mohammed E, Youssef C, Rita B, Kaoutar B, Islah L, Najia E, Abdarrahmane B and Bernard F. Impact of transport distance on stress biomarkers levels in dromedary camel (*Camelus dromedarius*)". Emirates Journal of Food and Agriculture. 2015; 27:507-512

Nazifi SS, Mahdi B, Hasan and Saeedeh S. Influence of road transportation during hot summer conditions on oxidative status biomarkers in Iranian dromedary camels (*Camelus dromedarius*). African Journal of Biochemistry Research. 2009; 3:282-287.

Niki E. Lipid peroxidation: physiological levels and dual biological effects. Free Radical Biology and Medicine. 2009; 47:469-484.

Pegram RJ and Higgins AJ. Camel ectoparasites: a review. Proceedings of the First International Camel Conference, Dubai, UAE, 2-6 February 199, pp 69-78. 1992

Portugal M, Barak V, Ginsburg I and Kohen R. Interplay among oxidants, antioxidants, and cytokines in skin disorders: present status and future considerations. Biomedicine and Pharmacotherapy. 2007; 61:412-422.

Salar-Amoli J, Hejazy M and Ali Esfahani T. Comparison between some oxidative Stress Biomarkers values in serum and plasma of clinically healthy adult camels (*Camelus dromedarius*) in Iran. Veterinary Research Communications. 2009; 33, 849.

Saleh MA, Mahran OM and Al-Salahy MB. Circulating oxidative stress status in dromedary camels infested with sarcoptic mange. Veterinary Research Communications. 2011; 35:35-45.

Suttnar J, Masova L and Dyr E. Influence of citrate and EDTA anticoagulants on plasma malondialdehyde concentrations estimated by high-performance liquid chromatography. Journal of Chromatography B. 2001; 751:193-197.

Tharwat M. Biomarkers of infection and inflammation in camels (*Camelus dromedarius*). Journal of Camel Practice and Research, 2020a; 27:159-163.

Tharwat M. The cardiac biomarkers troponin I and creatine kinase myocardial band in camels (*Camelus dromedarius*) - review. Journal of Camel Practice and Research. 2020b; 27:121-128.

Tharwat M and Al-Sobayil F. A review on biomarkers of bone metabolism in camels (*Camelus dromedarius*). Journal of Camel Practice and Research. 2020c; 27:23-29.

Vaziri ND. Causal link between oxidative stress, inflammation, and hypertension. Iranian Journal of Kidney Diseases. 2008; 2:1-10.

DOI : 10.5958/2277-8934.2021.00047.3 Vol 28 No 3, p 303-311

A HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY ON THE THYROID GLAND OF DROMEDARY CAMEL (*Camelus dromedarius*)

Devendra Singh[1], Sanjeev Joshi[1], Pankaj Kumar Thanvi[1], Aruna Panwar[1] and Om Prakash Choudhary[2]

[1]Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001, Rajasthan, India

[2]Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University (I), Selesih, Aizawl-796015, Mizoram, India

ABSTRACT

The present study was designed to provide the histochemical and immunohistochemical features of the thyroid gland in dromedary camel. The thyroid glands were collected from naturally dead camels (n=16) of both sexes. Different thyroid gland regions showed positive reactions in different intensities with different histochemical stains for sulphated mucopolysaccharides, polysaccharides, calcium, iron, glycogen and nucleic acid. The immunohistochemical staining with the anti-calcitonin antibody demonstrated moderate activity in the epithelial lining of large or inactive follicles. The epithelial lining of small and distended follicles showed a stronger affinity to calcitonin antibodies. The highest affinity was observed in the area of SCNs and parafollicular cells. It can be concluded that the present study has significance in identifying the various metabolic diseases related to the thyroid gland of camel by histochemical analysis of sulphated mucopolysaccharides, polysaccharides, calcium, iron, glycogen and nucleic acid. The present immunohistochemical study might be good candidates for diagnosing medullary thyroid carcinoma in camels and its treatment.

Key words: Dromedary camel, histochemical, immunohistochemical, metabolic diseases, thyroid gland

Adaptation of the dromedary to its natural habitat has great relevance with the activity of its endocrine glands, such as the thyroid gland and others. Anatomical particularities of thyroid gland of camel has been studied previously (Rejeb *et al,* 2011). The thyroid gland is one of the endocrine glands present in all mammals. It regulates the physical and chemical processes that occur at the cellular level through the hormones, i.e. thyroxin (T4), triiodothyronine (T3) (Kausar and Shahid, 2006; Banks, 1993) and calcitonin (Machado-Santos *et al,* 2013). Among others, these hormones play an important role in metabolism (Kausar and Shahid, 2006), regulation of nutrient absorption, energy regulation and calorigenesis (McNabb and Wilson, 1997) and normal reproductive function (Boswell *et al,* 1994; Schwartz *et al,* 1996). Thyroid hormones have several effects on carbohydrate and lipid metabolism, although their role in these instances is not nearly as important as that of other metabolic hormones (Prosser, 1973).

The scanning and transmission electron microscopy of the thyroid gland of the dromedary camel has already been studied in detail (Singh *et al,* 2021a, b). However, scarce work on the histochemical, immunohistochemical aspect of the thyroid gland of camel evoked interest in undertaking the present study.

Materials and Methods

Collection of the samples

The thyroid gland samples were collected from naturally dead camels (n=16) of both sexes from the Veterinary Clinical Complex, College of Veterinary and Animal Sciences, RAJUVAS, Bikaner, Rajasthan. The dead animals were free from any pathological condition of the thyroid gland. All the procedures involving thyroid gland sample collection from camel were conducted as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

SEND REPRINT REQUEST TO PANKAJ KUMAR THANVI ● OM PRAKASH CHOUDHARY email: drpankajthanvi@gmail.com ● dr.om.choudhary@gmail.com

Processing of the samples for histochemistry

Representative samples of thyroid glands were collected from identical sites and fixed in 10% formalin for 72 hours and Bouin's fluid for 24 hours, respectively followed by a recommended process for preparation of paraffin blocks (Luna, 1968). These were numbered and stored at 4°C in the refrigerator. Thick sections (5 to 6 micron (µm)) were prepared by using a semi-automatic rotary microtome. These were mounted on albuminised slides and kept overnight on the hot plate at 36°C. Finally, these slides were stained (Table 1) for histochemical observations.

Processing of the samples for immunohistochemistry

The immunostaining technique was performed on 5 µm thick sections using an LSAB-plus kit [Rabbit specific HRP/DAB (ABC) Detection IHC kit, ABCAM, Cambridge, US] as used earlier by Narendra *et al* (2015). The sections were deparaffinised and the endogenous peroxidase activity of the sections was blocked using a 3% (v/v) hydrogen peroxide (H_2O_2) solution in water for 5 minutes. Slides were later rinsed in a 0.01 M phosphate-buffered saline solution (PBS, pH 7.4) for 5 minutes. Then, the sections were microwaved at 750 W for three cycles of 7 minutes each. The processed sections were allowed to cool for 20 minutes, followed by rinsing with PBS. The sections were then incubated for 1 hour at room temperature with monoclonal rabbit anti-human calcitonin antibodies with a dilution of 1:50, which was the primary antibody. After incubation with primary antibodies, the slides were rinsed with PBS and then incubated for 15 minutes with a secondary antibody with a dilution of 1:200 i.e. biotinylated goat anti-rabbit IgG antibody (LSAB-plus kit). After that, the slides were rinsed in PBS and subsequently incubated for 15 minutes with the streptavidin peroxidase component of the LSAB-plus staining kit. Then the slides were rinsed in PBS and bound antibodies were visualised after adding a 3, 3′-diaminobenzidine tetrachloride solution (LSAB-plus kit). The sections were counterstained with Mayer's hematoxylin before being dehydrated in graded concentrations of ethanol. The thyroid tissues of the same species without primary antibody were used as negative control.

Sections were viewed under the microscope and the labeling intensity of cells was scored on a subjective scale as negative (-), weak (+), moderate, (++) strong (+++) and very strong (++++).

Results and Discussion

Histochemical study

The histochemical study was performed to detect the various chemical constitutions (sulphated mucopolysaccharides, polysaccharides, calcium, iron, glycogen and nucleic acid) in the thyroid gland of the camel, which may be used to diagnose various metabolic disorders related to the thyroid gland in the dromedary camel.

Capsule

In the present histochemical study, the thyroid gland capsule showed a positive PAS Alcian blue reaction for pH 1.0 for sulphated mucopolysaccharides and pH 2.5 for polysaccharides (Fig 1A and 1B). The capsule reacted positive for calcium in both Dahl's and Kossa's stain (Fig 1C and 1D), however, the same reaction was negative for calcium in the thyroid capsule of cattle (Sanap *et al,* 1998). The capsule also showed a positive reaction for iron in Gomori's and Pearl's stain (Fig 2A and 2B) as reported earlier in male goats (Joshi, 2016). A moderately positive PAS reaction was observed in the capsule for glycogen as described earlier in male Assam goats (Sarma *et al,* 2013), sheep of South Iraq (Ali *et al,* 2015), male goats (Joshi, 2016) and Pati ducks (Sinha *et al,* 2016), whereas capsule of the thyroid gland showed weakly PAS positive in

Table 1. Various histochemical stains used for the thyroid gland of the camel.

Sl. No.	Stain used	Purpose of staining	Reference
1.	McManus method	Glycogen	Singh and Sulochana (1997)
2.	PAS-Alcian blue method (pH 1.0)	Sulphated mucosaccharides	Luna (1968)
3.	PAS-Alcian blue method (pH 2.5)	Polysaccharides	Luna (1968)
4.	Gomori's method	Iron	Luna (1968)
5.	Pearl's method for iron	Iron	Luna (1968)
6.	Dahl's method	Calcium	Luna (1968)
7.	Kossa's method	Calcium	Singh and Sulochana (1997)
8.	Demonstration of Nucleic acid Feulgen reaction for DNA)	Nucleic acid	Singh and Sulochana (1997)

sheep (Rajalakshmi *et al,* 2019). Feulgen's reaction for nucleic acid was noticed positive in the capsule but the nucleus was not present. On the other hand, very weak Feulgen's reaction in the connective tissue of the capsule was observed in Assam goats (Sarma *et al,* 2013). A moderately positive PAS reaction was observed in the capsule for glycogen (Fig 2C). Feulgen's reaction for nucleic acid was noticed positive in the capsule, but the nucleus was not present in the present study (Fig 2D).

Trabeculae

The trabeculae showed a positive reaction for PAS Alcian blue stain for both pH 1.0 for sulphated mucopolysaccharides and pH 2.5 for polysaccharides (Fig 3A and 3B). The trabeculae also showed a positive reaction for calcium in Dahl's and Kossa's stains (Fig 3C and 3D) with different intensities. In other studies, a negative reaction for calcium was observed in thyroid trabeculae in cattle (Sanap *et al,* 1998) and male goats (Joshi, 2016). A positive reaction was seen for iron in trabeculae during Gomori's and Pearl's staining (Fig 4A and 4B) procedure as mentioned in male goats (Joshi, 2016). The trabeculae showed a moderate positive reaction for glycogen in PAS stain (Fig 4C), whereas a complete positive reaction for glycogen in PAS was noticed in male goats (Joshi, 2016). The trabeculae were noticed positive in Feulgen's reaction for nucleic acid (Fig 4D), whereas a very weak Feulgen's reaction in septae was noticed in Assam goats (Sarma *et al,* 2013).

Follicles

The follicles were observed positive for PAS Alcian blue stain for both pH 1.0 for sulphated mucopolysaccharides and pH 2.5 for polysaccharides (Fig 5A and 5B) as reported earlier in humans (Harach, 1985) and carabao (Maala and Reynoso, 1987). Follicles were revealed a positive reaction for calcium in both Dahl's and Kossa's stains (Fig 5C and 5D) as also described in male goats (Joshi, 2016). The follicles gave a positive result for iron in Gomori's and Pearls stain (Fig 6A and 6B) as elucidated in male goats (Joshi, 2016). The interfollicular tissue showed moderate reaction for glycogen in PAS stain (Fig 6C). In another study, the interfollicular area showed a weak PAS positive reaction in Pati ducks (Sinha *et al,* 2016). The thyroid follicles were also given positive for Feulgen's reaction to the nucleic acid (Fig 6D) as reported in Assam goats (Sarma *et al,* 2013).

Colloid

It was revealed that the colloid of follicles gave positive results for the PAS Alcian blue in pH 1.0 for sulphated mucopolysaccharides and pH 2.5 for polysaccharides with different intensities (Fig 7A and 7B). The colloid showed a positive reaction for calcium in Kossa's stain; however, few colloids were positive for calcium in Dahl's stain (Fig 7D and 7C) as reported earlier in male goats (Joshi, 2016). The colloid reacted positive for iron in Gomori's stain (Fig 8A), whereas active thyroid follicles showed a stronger positive reaction than the inactive follicles in Pearl's stain (Fig 8B). A strongly positive reaction was observed in colloid of follicles for glycogen in PAS stain (Fig 8C) as mentioned earlier in Indian buffalo (Roy and Yadava, 1975), bovines (Dellmann and Brown, 1981), mithun and yak (Baishya *et al,* 1991), Black Bengal goat (Adhikary *et al,* 2003), White Fulani cattle (Igbokwe and Ezeasor, 2015b), male goat (Joshi, 2016) and sheep (Rajalakshmi *et al,* 2019). On the other hand, the colloid in the follicles was negative to PAS reaction in one-humped camel (Abubakar, 2015). The Feulgen's reaction (Fig 8D) was not observed in the colloid due to the lack of a nucleus in the present study.

Isthmus

The thyroid isthmus showed a moderate positive reaction for PAS Alcian blue stain in both the pH 1.0 for sulphated mucopolysaccharides and pH 2.5 for polysaccharides (Fig 9A and 9B). The isthmus showed positive results for calcium in Kossa's method, whereas negative result was observed in Dahl's stain for the isthmus (Fig 9C and 9D). A positive reaction (Fig 10A and 10B) was observed for iron in the isthmus during Gomori's and Pearl's staining procedure. A moderate positive reaction was noticed for glycogen in PAS stain (Fig 10C). The isthmus showed positive Feulgen's reaction in the present study (Fig 10D).

Immunohistochemical study

Thyroid cancer is the most common malignancy of endocrine tumors (Mitchell *et al,* 2007). The medullary thyroid carcinoma (MTC) is a thyroid malignancy originating from C cells. The present immunohistochemical study might be good candidates for the diagnosis of thyroid cancer in camels.

In the present study, the immunohistochemical staining with anti-calcitonin revealed that the epithelial lining of small and distended follicles showed stronger affinity (+++) to calcitonin antibodies as reported in camel (Al-Ramadan, 2013). It was also reported in the present study that the anti-calcitonin showed stronger (+++) affinity to the

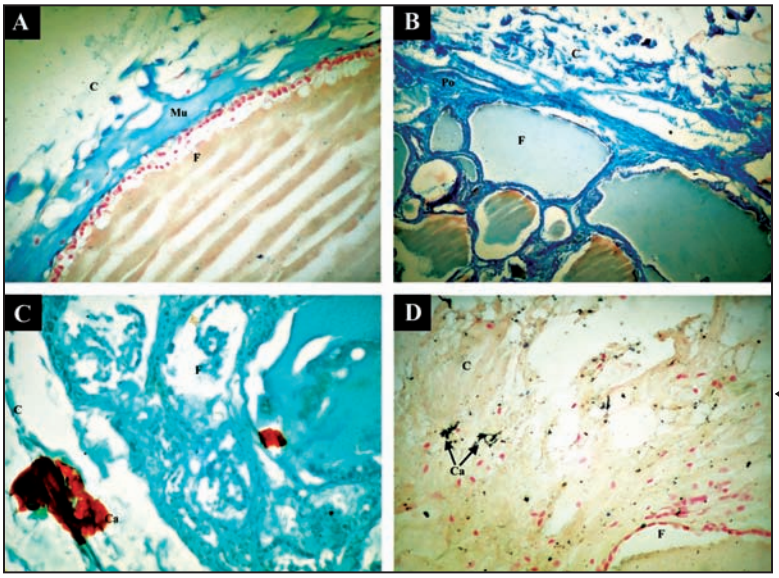


Fig 1. **A.** Photomicrograph of thyroid gland showing capsule (C), follicle (F) and mucopolysaccharides (Mu) in PAS Alcian Blue Stain pH 1.0 400x. **B.** Photomicrograph of thyroid gland showing, capsule (C), follicle (F) and polysaccharides (Po) in PAS Alcian Blue Stain pH 2.5 100x. **C.** Photomicrograph of thyroid gland showing, capsule (C), follicle (F) and calcium (Ca) in Dahls Stain 400x. **D.** Photomicrograph of thyroid gland showing, capsule (C), follicle (F) and calcium (Ca) in Kossa's Stain 400x.

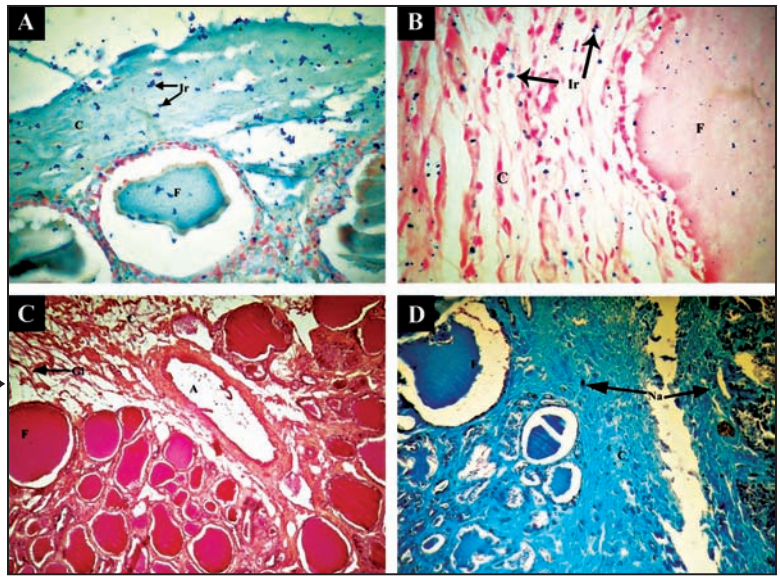


Fig 2. **A.** Photomicrograph of thyroid gland showing capsule (C), follicle (F) and iron (Ir) in Gomori's Stain 400x. **B.** Photomicrograph of thyroid gland showing capsule (C), follicle (F) and iron (Ir) in Pearls Stain 400x. **C.** Photomicrograph of thyroid gland showing, capsule (C), follicle (F), artery and glycogen (Gl) in McManus method for PAS Stain 100x. **D.** Photomicrograph of thyroid gland showing, capsule (C), follicle (F) and nucleic acid (Na) in demonstration of nucleic acid (Feulgen's reaction for DNA) 100x.

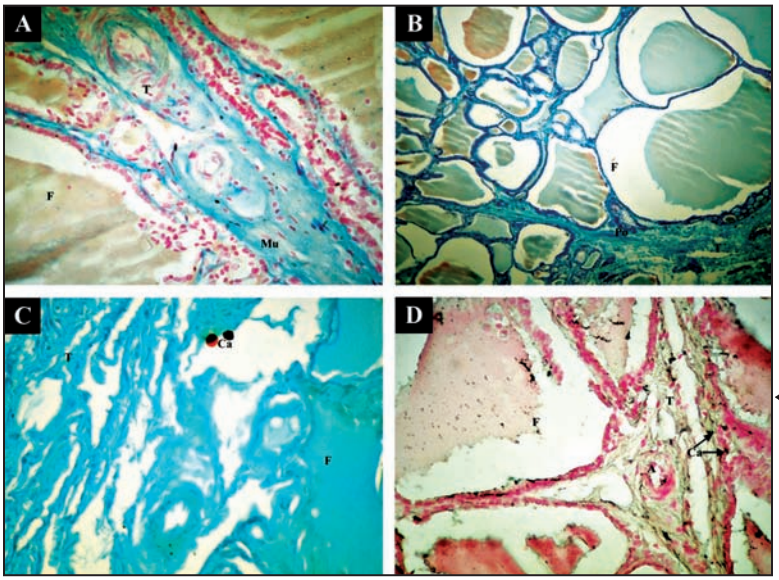


Fig 3. **A.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F) and mucopolysaccharides (Mu) in PAS Alcian blue pH 1.0 Stain 400x. **B.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F) and polysaccharides (Po) in PAS Alcian blue pH 2.5 stain 100x. **C.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F) and calcium (Ca) in Dahl's Stain 400x. **D.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F), artery (A) and calcium (Ca) in Kossa's Stain 400x.

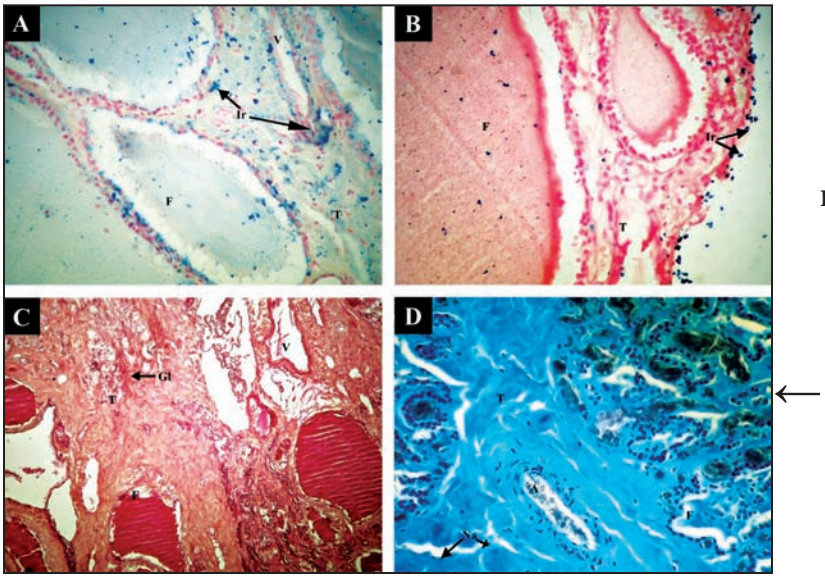


Fig 4. **A.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F), vein (V) and iron (Ir) in Gomori's stain 400x. **B.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F), and iron (Ir) in Pearl's stain 400x. **C.** Photomicrograph of thyroid gland showing trabeculae (T), follicle (F), vein (V) and glycogen (Gl) in McManus method for PAS stain 100x. **D.** Photomicrograph of thyroid gland showing, trabeculae (T), follicle (F), artery (A) and nucleic acid (Na) in demonstration of nucleic acid (Feulgen's reaction for DNA) 400x.

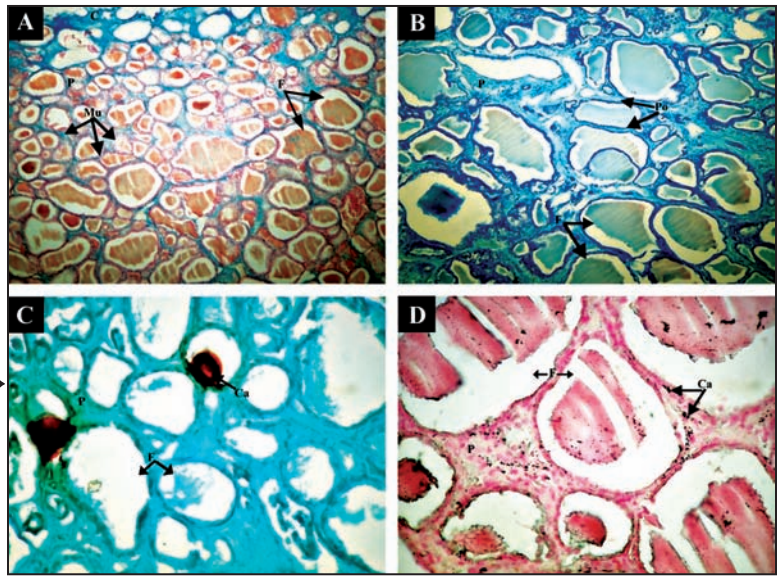


Fig 5. **A.** Photomicrograph of thyroid gland showing capsule (C), parenchyma (P), Follicle (F) and mucopolysaccharides (Mu) PAS Alcian blue pH 1.0 Stain 100x. **B.** Photomicrograph of thyroid gland showing, parenchyma (P), follicle (F) and polysaccharides (Po) PAS Alcian blue pH 2.5 stain 100x. **C.** Photomicrograph of thyroid gland showing, parenchyma (P), follicle (F), and calcium (Ca) in Dahl's stain 400x. **D.** Photomicrograph of thyroid gland showing parenchyma (P), follicle (F) and calcium (Ca) in Kossa's stain 400x.

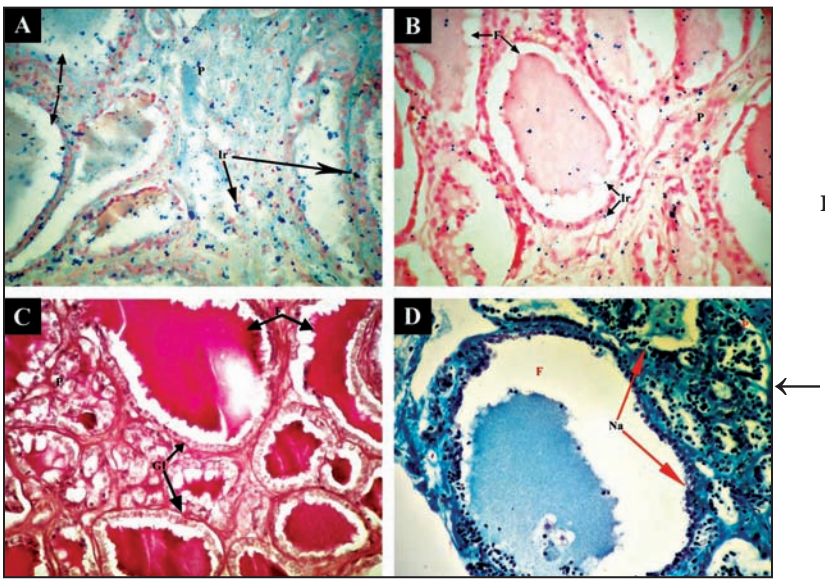


Fig 6. **A.** Photomicrograph of thyroid gland showing parenchyma (P), follicle (F), and iron (Ir) in Gomori's stain 400x. **B.** Photomicrograph of thyroid gland showing parenchyma (P), follicle (F), and iron (Ir) in Pearl's stain 400x. **C.** Photomicrograph of thyroid gland showing parenchyma (P), follicle (F) and glycogen (Gl) in Mcmanus method for PAS Stain 400x. **D.** Photomicrograph of thyroid gland showing, parenchyma (P), follicle (F) and nucleic acid (Na) in demonstration of nucleic acid (Feulgen's reaction for DNA) 400x.

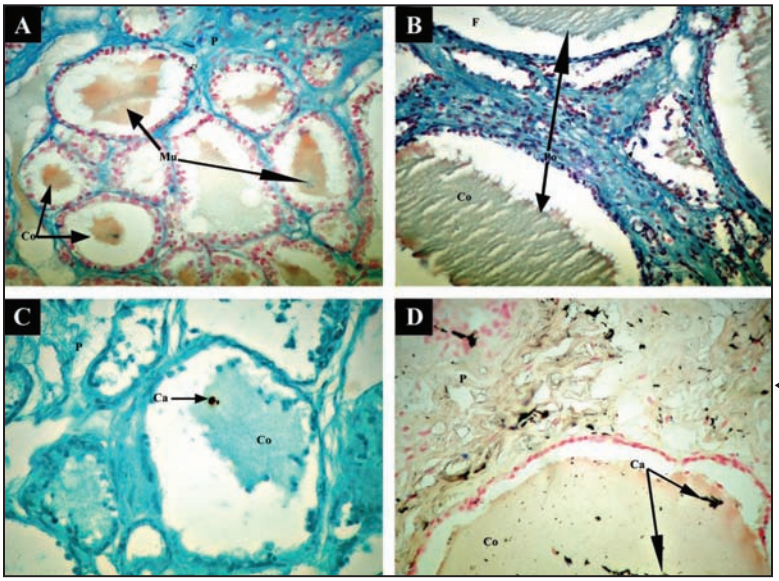


Fig 7. **A.** Photomicrograph of thyroid gland showing parenchyma (P), colloid (Co) and mucopolysaccharides (Mu) PAS Alcian blue pH 1.0 Stain 400x. **B.** Photomicrograph of thyroid gland showing, colloid (Co), follicle (F), and polysaccharides (Po) PAS Alcian blue pH 2.5 stain 400x. **C.** Photomicrograph of thyroid gland showing parenchyma (P), colloid (Co) and calcium (Ca) in Dahl's stain 400x. **D.** Photomicrograph of thyroid gland showing parenchyma (P), colloid (Co) and calcium (Ca) in Kossa's stain 400x.

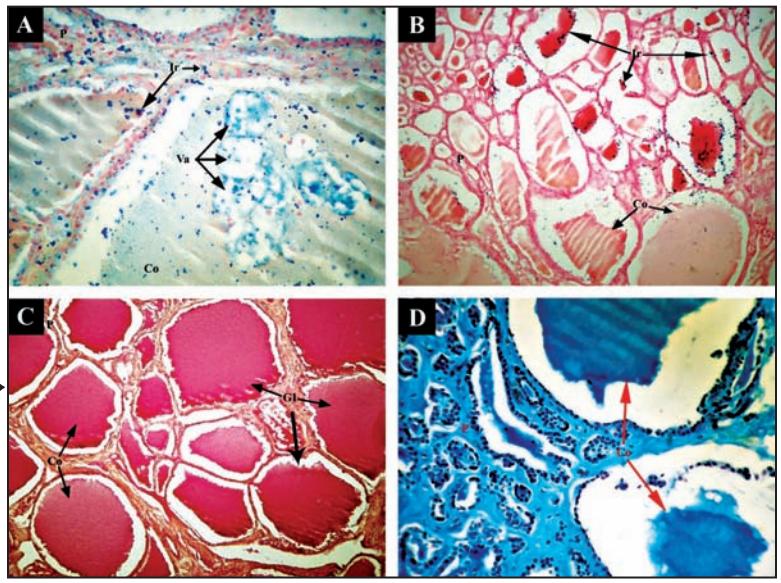


Fig 8. **A.** Photomicrograph of thyroid gland showing parenchyma (P), colloid (Co), vacuoles (Va) and iron (Ir) in Gomori's stain 400x. **B.** Photomicrograph of thyroid gland showing parenchyma (P), colloid (Co) and iron (Ir) in Pearl's stain 100x. **C.** Photomicrograph of thyroid gland showing parenchyma (P), colloid (Co) and glycogen (Gl) in McManus method for PAS Stain 100x. **D.** Photomicrograph of thyroid gland showing parenchyma (P) and colloid (Co) in demonstration of nucleic acid (Feulgen's reaction for DNA) 400x.

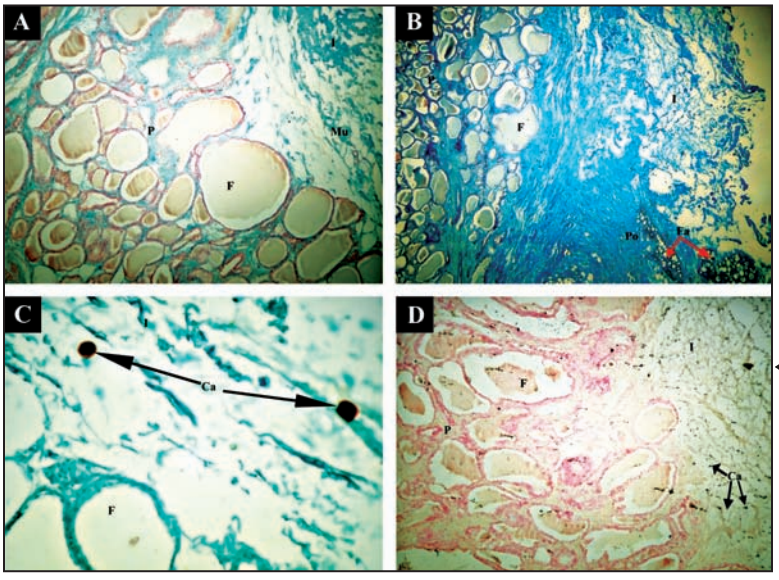


Fig 9. **A.** Photomicrograph of thyroid gland showing isthmus (I), parenchyma (P), follicle (F) and mucopolysaccharides (Mu) PAS Alcian blue pH 1.0 stain 100x. **B.** Photomicrograph of thyroid gland showing isthmus (I), parenchyma (P) follicle (F), fat cells (Fa) and polysaccharides (Po) PAS Alcian blue pH 2.5 Stain 40x. **C.** Photomicrograph of thyroid gland showing isthmus (I), follicle (F) and calcium (Ca) in Dahl's stain 400x. **D.** Photomicrograph of thyroid gland showing isthmus (I), follicle (F), parenchyma (P) and calcium (Ca) in Kossa's stain 100x.

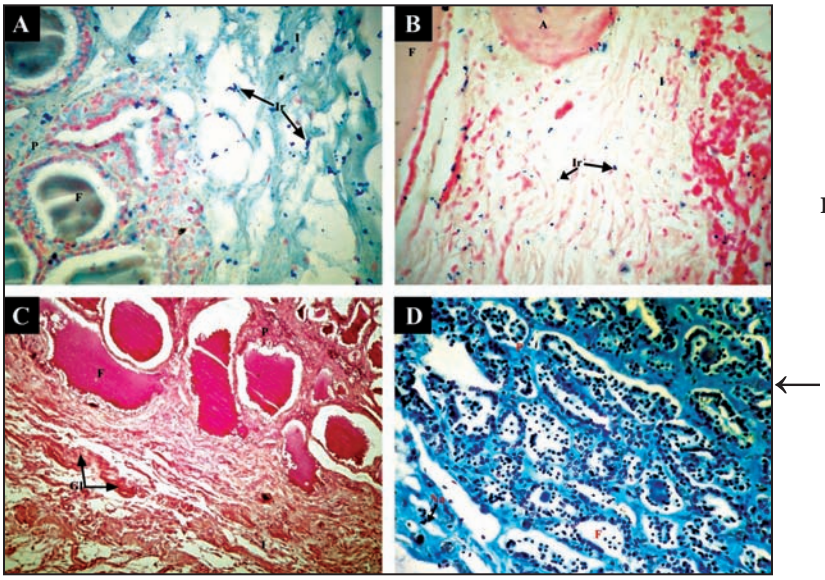


Fig 10. **A.** Photomicrograph of thyroid gland showing isthmus (I), parenchyma (P), follicle (F) and iron (Ir) in Gomori's stain 400x. **B.** Photomicrograph of thyroid gland showing isthmus (I), follicle (F) and iron (Ir) in Pearls's stain 400x. **C.** Photomicrograph of thyroid gland showing parenchyma (P), isthmus (I) and glycogen (Gl) in McManus method for PAS stain 100x. **D.** Photomicrograph of thyroid gland showing parenchyma (P), follicle (F), isthmus (I) and nucleic acid (Na) in demonstration of nucleic acid (Feulgen's reaction for DNA) 400x.

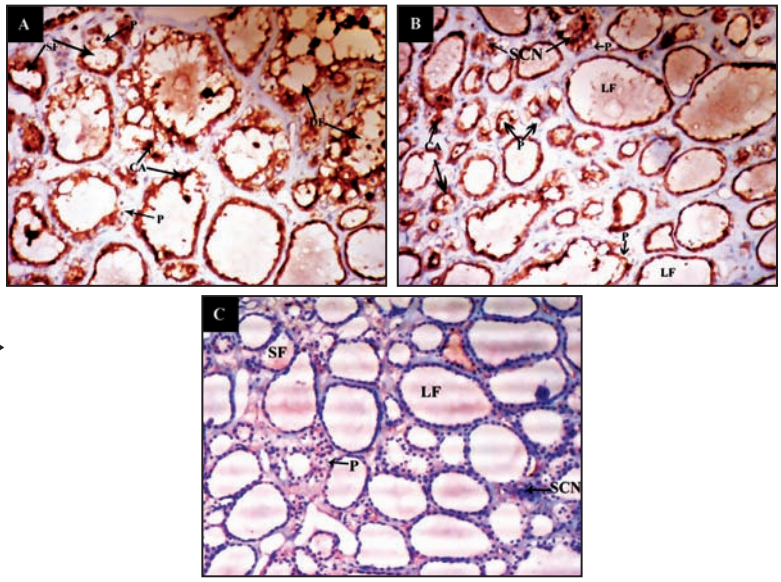


Fig 11. **A.** Photomicrograph of the thyroid gland in immunohistochemical reaction with anti-calcitonin showing small follicles (SF), distended follicles (DF), parafollicular cells (P) and calcitonin antibodies brick red (CA) 400x. **B.** Photomicrograph of the thyroid gland in immunohistochemical reaction with anti-calcitonin showing, large follicles (LF), solid cell nests (SCNs), parafollicular cells (P) and calcitonin antibodies brick red (CA) 400x. **C.** Photomicrograph of thyroid gland showing negative immunohistochemical reaction without anti-calcitonin in small follicle (SF), large follicle (LF), parafollicular cells (P), solid cell nests (SCNs) 400x.

epithelial lining of the distended follicles; whereas the calcitonin antiserum did not cross-react with normal follicular cells in canines (Moore *et al,* 1984). The highest affinity (++++) to calcitonin antibodies was observed in the area of SCNs (Fig 11A and 11B) as mentioned earlier in camel (Al-Ramadan, 2013), whereas few calcitonin-positive cells were present and distributed in the periphery of the solid cell nests in human (Mizukami *et al,* 1994). The moderate immunohistochemical activity (++) was found in the epithelial lining of large or inactive follicles as noticed earlier in camel (Al-Ramadan, 2013).

The thyroid gland also reacted very strong positive (++++) for calcitonin within parafollicular cells (Fig 11A and 11B) as elaborated earlier in one-humped camels (Nakamura *et al,* 2018). However, moderate immunopositive reactivity to calcitonin antibody in the foetal pig thyroid gland was observed (Igbokwe and Ezeasor, 2015a). The thyroid sections were used as negative control and gave no positive result in the present study.

Conclusion

It can be concluded for the present study that different thyroid gland regions showed positive reactions in different intensities with different histochemical stains for sulphated mucopolysaccharides, polysaccharides, calcium, iron, glycogen and nucleic acid. The immunohistochemical staining with anti-calcitonin antibody revealed that moderate activity was noticed in large or inactive thyroid gland follicles' epithelial lining. The epithelial

lining of small and distended follicles showed a stronger affinity to calcitonin antibodies. The highest affinity was observed in the area of SCNs and parafollicular cells.

References

Abubakar UM. Anatomical studies on thyroid, parathyroid and adrenal glands of indigenous one-humped camel. M.Sc. thesis submitted to the Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria. 2015.

Adhikary GN, Quasem MA, Das SK and Khalil M. A prospective study on histochemical observation of thyroid gland at prepubertal black Bengal goat. Mymensingh Medical Journal. 2003; 12(2):108-111.

Ahren BO. Regulatory peptides in the thyroid gland-a review on their localisation and function. European Journal of Endocrinology. 1991; 124(3):225-232.

Ali MA, Sadoon AH, Samera A and Sawsan AA. Anatomical and histological study of thyroid gland in local Iraqian sheep. Journal of International Academic Research for Multidisciplinary. 2015; 3(3):195-200.

Al-Ramadan S. Immunohistochemical study of the ultimobranchial remnants in the camel. Assiut Veterinary Medical Journal. 2013; 59(137):124-130.

Baishya G, Bhattacharya M, Talukdar SR and Kalita SN. Morphology of the thyroid gland and oxidoreductases in the liver of mithuns (*Bos frontalis*) and yaks (*Bos grunniens*). Indian Journal of Animal Sciences. 1991; 68(2):111-114.

Banks WJ. Applied Veterinary Histology. 3rd Edition. Mosby-Year Book, Inc. USA: 1993; 414-415.

Boswell T, Woods SC and Kenagy GJ. Seasonal changes in body mass, insulin, and glucocorticoids of free living Golden-Mantled Ground Squirrels. General and Comparative Endocrinology. 1994; 96(3):339-346.

Dellmann HD and Brown EM (1981). Textbook of Veterinary Histology. 2nd Edition. Lea and Febiger, Philadelphia, USA.

Harach HR. Thyroid follicles with acid mucins in man: a second kind of follicles? Cell Tissue Research. 1985; 242:211-215.

Igbokwe CO and Ezeasor DN. Histological and immuno-histochemical changes of the thyroid gland during the foetal and postnatal period of development in indigenous large white crossbred pigs. Bulgarian Journal of Veterinary Medicine. 2015a; 18(4):313-324.

Igbokwe CO and Ezeasor DN. Histologic and ultrastructural observations on the thyroid gland of the White Fulani (zebu) cattle in Northern Nigeria. African Journal of Biotechnology. 2015b; 14(2):156-166.

Joshi S. Gross and histological studies on the thyroid gland of goat (*Capra hircus*). Ph.D. Thesis submitted to the Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India. 2016.

Kausar R and Shahid RU. Gross and microscopic anatomy of thyroid gland of one-humped camel (*Camelus dromedarius*). Pakistan Veterinary Journal. 2006; 26(2):88-90.

Luna LG. Manual of Histological Staining Methods. Armed Forces Institute of Pathology, 3rd Edition. McGraw Hill Book Co. New York, USA. 1968.

Maala CP and Reynoso LV. Some histological and histochemical features of the thyroid gland and its isthmus in the Philippine carabao (*Bubalus Bubalis*). Philippine Journal of Veterinary and Animal Science. 1987; 23(1-2):45-60.

Machado-Santos C, Teixeira MJ, Sales A and Abidu-Figueiredo M. Histological and immunohistochemical study of the thyroid gland of the broad-snouted caiman (*Caiman latirostris*). Acta Scientiarum Biological Sciences. 2013; 35(4):585-589.

Mc-Nabb AFM and Wilson MC. Thyroid hormone deposition in avian eggs and effects on embryonic development. American Zoology. 1997; 37(6):553-560.

Mitchell I, Livingston EH, Chang AY, Holt S, Snyder WH, Lingvay I, Nwariaku FE. Trends in thyroid cancer demographics and surgical therapy in the United States. Surgery. 2007; 142:823-828.

Mizukami Y, Nonomura A, Michigishi T, Masakuni, Noguchi M, Hashimoto T and Ishizaki T. Solid cell nests of the thyroid: a histologic and immunohistochemical study. American Journal of Clinical Pathology. 1994; 101(2):186-191.

Moore FM, Kledzikh S, Wolfe J and Delellis RA. Thyroglobulin and calcitonin immunoreactivity in canine thyroid carcinomas. Veterinary Pathology. 1984; 21(2):168-173.

Nakamura T, Elewa YHA, Ichii O, Hosotani M, Ghonimi WAM, Tatsumi O, Nagasaki KI and Kon Y. Restricted localisation of ultimobranchial body remnants and parafollicular cells in the one-humped camel (*Camelus dromedarius*). Journal of Veterinary Medical Science. 2018; 80(9):1368-1372.

Narendra SC, Chalise JP, Magnusson M and Uppugunduri S. Local but not systemic administration of uridine prevents development of antigen-induced arthritis. PLOS ONE. 2015; 10(10):e0141863.

Prosser CL. Endocrine Mechanisms. In: Comparative Animal Physiology. 3rd Edition, Saunders, Philadelphia, USA. 1973.

Rajalakshmi K, Ramesh G, Kumari U, Kannan U, Kumar MS, Sridevi P and Lakkawar AW. Microanatomy of the thyroid gland in sheep (*Ovis aries*). International Journal of Chemical Studies. 2019; 7(6):404-415.

Rejeb A, Amara A, Matoussi A, Rezeigui H and Sautet J. Anatomical particularity of the thyroid gland of the dromedary (*Camelus dromedarius*). Revue de Medecine Veterinaire. 2011; 162(4):177-185.

Roy KS and Yadava CP. Histological and certain histochemical observations on the light cells of the thyroid gland in the Indian buffalo. Indian Journal of Animal Sciences. 1975; 45:201-208.

Sanap SM, Mugale RR, Bhosale NS and Mamde CS. Histochemistry of thyroid glands in prepubertal, pubertal and castrated cattle. Indian Veterinary Journal. 1998; 75(7):655-657.

Sarma K, Kalita SN and Devi J. Age related histochemical studies on the thyroid gland in male Assam goats

(*Capra hircus*) from birth to ten months of age. Indian Journal of Animal Research. 2013; 47(3):254-256.

Sawicki B. Evaluation of the role of mammalian thyroid parafollicular cells. Acta Histochemica. 1995; 97(4):389-399.

Schwartz MW, Seeley RJ, Campfield LA, Burn P and Baskin DG. Identification of targets of leptin action in rat hypothalamus. Journal of Clinical Investigation. 1996; 98(5):1101-1106.

Singh D, Joshi S, Thanvi PK and Choudhary OP. Ultrastructural studies on the thyroid gland of dromedary camel (*Camelus dromedarius*). Indian Journal of Animal Research. 2021a; DOI:10.18805/IJAR.B-4363.

Singh D, Joshi S, Thanvi PK, Saini MK and Choudhary OP. Scanning electron microscopic studies on the thyroid gland of dromedary camel (*Camelus dromedarius*). Journal of Camel Practice and Research. 2021b; 28(1):107-109.

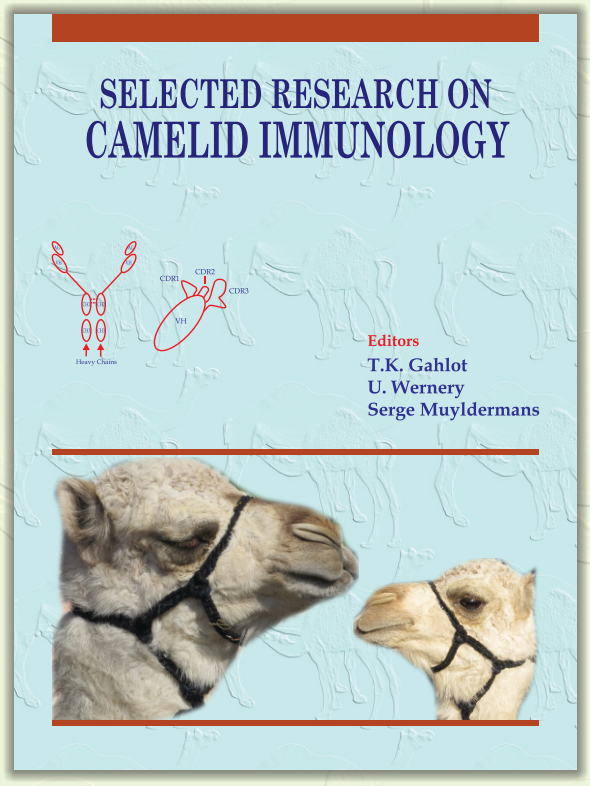
Singh UB and Sulochana S. Handbook of Histological and Histochemical Techniques. 2nd Edition, Premier Publishing House, Hyderabad, India. 1997.

Sinha S, Sarma M, Goswami S and Devchoudhury KB. Histochemical and ultrastructural studies on the thyroid gland of Pati ducks (*Anas platyrhynchos domesticus*) of Assam. Journal of Animal Research. 2016; 6(2):101-104.

SELECTED RESEARCH ON CAMELID IMMUNOLOGY

(Hard Bound, 392 pages, few figs coloured, Edition 2016)

In 1989 a group of biologists led by Raymond Hamers at the Free University Brussels investigated the immune system of dromedaries. This discovery was published in Nature in 1993. Based on their structure, these peculiar camelid antibodies have been named Heavy Chain Antibodies (HCAb), as they are composed of heavy chains only and are devoid of light chains. Sera of camelids contain both conventional heterotetrameric antibodies and unique functional heavy (H)-chain antibodies (HCAbs). The smaller size and monomeric single domain nature make these antibodies easier to transform into bacterial cells for bulk production, making them ideal for research purposes. Camelid scientists world over were greatly fascinated by a new field of research called "Camelid Immunology". Significant research has been done on camelid immunology in recent decade. In order to benefit future camelid immunology researchers, this book was planned in the series of "Selected Topics" by Camel Publishing House with a title- "Selected Research on Camelid Immunology" edited by T.K. Gahlot, U. Wernery and Serge Muyldermans. This book is a unique compilation of research papers based on "Camelid Immunology" and published in Journal of Camel Practice and Research between 1994-2015. Research on this subject was done in 93 laboratories or institutions of 30 countries involving about 248 scientists. In terms of number of published papers in JCPR on the immunology the following countries remain in order of merit (in parenthesis), i.e. Iran (1), India and UAE (2), China and Saudi Arabia (3), Sudan (4), Kenya and Belgium (5), USA (6), Germany (7) and so on. The book contains 11 sections and is spread in 384 pages. The diverse sections are named as overview of camel immune system; determinates of innate immunity, cells, organs and tissues of immune system; antibodies; immunomodulation; histocompatibility; seroprevalence, diagnosis and immunity against bacteria, viruses, parasites and combination of other infections; application of camel immunoglobulins and applications of immune mechanisms in physiological processes. The camelid immunology has to go a long way in its future research, therefore, this reference book may prove quite useful for those interested in this subject. Book can be seen on www.camelsandcamelids.com.



Editor:
T.K. Gahlot
U. Wernery
Serge Muyldermans

Edition: 2016

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 $ 475
INR 12500

ISBN 81-903140-4-1

DOI : 10.5958/2277-8934.2021.00048.5 *Vol 28 No 3, p 313-318*

ULTRASONOGRAPHY OF THE THORAX IN HEALTHY AND DISEASED CAMELS (*Camelus dromedarius*) – AN OVERVIEW

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, 44519, Zagazig, Egypt

ABSTRACT

In camels (*Camelus dromedarius*), pulmonary diseases are common. However, heart diseases are mostly diagnosed at slaughterhouses or incidentally discovered at postmortem examination. Of the most important pulmonary disorders are atelectasis, bronchiectasis, pneumoconiosis, pneumonia, hydatidosis, pleuritis, emphysema, pneumothorax, hydrothorax, haemothorax, empyema and pulmonary tumours. In camels, heart diseases include pericarditis, vegetative valvular endocarditis, hypertrophic cardiomyopathy, necrotic myocarditis and congenital defects including septal defects, patent ductus arteriosus, transposition of the aorta and pulmonary artery, persistent aortic trunk, and persistent right aortic arch and sarcocystosis. Thoracic ultrasonography is applicable in camel medicine and provides valuable diagnostic information on various cardiopulmonary affections. Echocardiography has also been carried out showing the normal cardiac chamber appearance and quantitative dimensions in adult dromedary camels. The procedure supplements the clinical and laboratory examinations by providing additional information on thoracic affections for diagnosis antemortem. By thoracic ultrasonography, the veterinarian can scan different layers of the thoracic wall, pulmonary parenchyma, pleura, and obtain the measurements for the dorsal and the ventral lung borders and the resulting dorsoventral dimensions of the lungs. It is also possible to obtain good-quality echocardiograms in camels as well as normal cardiac dimensions. Of the thoracic disorders evaluated by ultrasonography are cardiac muscle dystrophy, pneumonia, pulmonary abscessation and emphysema, pleural effusion, pleuritis and pleuropneumonia. This review article describes the results of thoracic ultrasonography in healthy camels as well as in camels with some thoracic disorders.

Key words: Camels, dromedary, echocardiogram, imaging, thorax, ultrasonography

Ultrasonography of the thorax enables the pleural surface of the lung and superficial lung parenchyma, heart and mediastinal region to be visualised and evaluated (Mohamed and Buczinski, 2011; Tharwat and Oikawa, 2011; Buczinski *et al,* 2013). Pulmonary ultrasonography is a diagnostic tool for various lung troubles and assessment the grade and severity of pulmonary diseases, as well as it can be used as a follow-up tool for evaluating the prognosis of respiratory troubles and monitoring the efficacy of therapies (Hussein *et al,* 2018). The procedure may be interpreted as an indirect 'estimator' of lung porosity (Soldati *et al,* 2014).

In small animals, ultrasound of the non-cardiac thorax is an important supplemental imaging modality in the diagnosis of pulmonary, mediastinal, pleural, and chest wall disease (Larson, 2009). Ultrasonographically, it is possible to detect bronchopneumonia, consolidation, pleural effusion, pulmonary emphysema and pleuritis in cattle (Flock, 2004). By thoracic ultrasonography, lung consolidation can also be detected in animals that did not show signs of respiratory disease. These findings emphasise the importance of using thoracic ultrasonography for an early and accurate detection of clinical and sub-clinical bovine respiratory disease (Cuevas-Gomez *et al,* 2021).

Ultrasonography can also be of tremendous help in the management of cardiac diseases. The procedure permits an antemortem diagnosis that can be especially useful in cases with a poor prognosis to avoid ineffective treatment. This early diagnosis can also be helpful in highly valuable animals by allowing an earlier therapeutic attempt and for monitoring the healing process (Buczinski, 2009; Mohamed and Buczinski, 2011).

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

Thoracic ultrasonography is applicable in camel medicine and provides valuable diagnostic information on various cardiopulmonary affections (Tharwat *et al,* 2012; Tharwat, 2013; Tharwat and Al-Sobayil, 2016). Echocardiography has also been carried out recently showing the normal cardiac chamber appearance and quantitative dimensions in adult dromedary camels (Tharwat *et al,* 2012). The procedure supplements the clinical and laboratory examinations by providing additional information on thoracic affections for diagnosis of such affections antemortem. Current review article was written to describe the results of thoracic ultrasonography in healthy camels as well as in camels with some thoracic disorders.

Thoracic ultrasonography

Pulmonary diseases are common in dromedary camels. Of the most important pulmonary disorders are atelectasis, bronchiectasis, pneumoconiosis, pneumonia, hydatidosis, pleuritis, emphysema, pneumothorax, hydrothorax, hemothorax, empyema and pulmonary tumours (Köhler-Rollefson *et al,* 2001; Al-Ani, 2004; Fowler, 2010). In a study conducted in Addis Ababa, Ethiopia, it was found that one or more gross lesions were encountered on 300 lungs (77.5%). The gross and histopathological examination of these lesions had revealed 60.2% emphysema, 21.2% hydatidosis, 18.6% pneumonia, 10.6% atelectasis, 4.9% aspiration of blood, 3.9% pneumoconiosis, 2.6% pulmonary oedema and congestion, 1.6% abscess, 1% pleurisy, and 0.8% granulomatous pneumonia (Jenberie *et al,* 2012).

Technique of pulmonary ultrasonography

The camel should be firstly slightly sedated, if necessary, using an intravenous xylazine injection (0.02 mg/kg) (Tharwat, 2013). Both sides of the thorax are then clipped and the skin shaved. After the application of transmission gel to the transducer, the lungs are examined transcutaneously on the right and then the left sides beginning at the 11th through the 4th intercostal space (ICS). Ultrasonographic examination is carried out using a 3.5 MHz sector or linear transducer. Each ICS is examined dorsal to ventral with the transducer held parallel to the ribs. Visualisation of the pleura and lungs can be assessed. The pleural space is examined for possible fluid accumulation (Tharwat, 2013).

Normal appearance of pulmonary parenchyma

The different layers of the thoracic wall appear as narrow bands of variable echogenicity. Medial to the thoracic wall, there is an echogenic line that represented the costal and pulmonary pleurae. Lung borders can easily be differentiated from the parietal pleura during inspiration/expiration in real time. Reverberation artefacts appear as lines of variable echogenicity that ran parallel to the pulmonary surface medial to the pleura. They results from reflection of the ultrasound waves by air in the lungs. The reverberation artefacts become progressively weaker as distance from the body surface increases; they are no longer seen at depth of 7 to 8 cm. The pulmonary parenchyma is not visualised because of its air content (Tharwat, 2013).

On the right side, back musculature, thoracic wall with pleura and pulmonary surface and parts of the liver and the omasum are seen. The ventral lung border has a caudodorsal course, so that the distance between the dorsal midline and the ventral lung border progressively decreases from cranial to caudal. The ventral lung border is largest at the 4th ICS and smallest at the 11th ICS. The dorsoventral dimension of the lung is largest at the 8th ICS and progressively decreases posterior to the 11th ICS and anterior to the 4th ICS (Table 1). It should be noted that the lung size in these ICSs is not the actual dimension of the lung; therefore it is only the ultrasonographically examinable part of the lungs. The echogenic line on the surface of the lung, consisting of the costal and the parietal pleurae, is 1 to 4 mm thick. On the left side, from dorsal to ventral, back musculature, thoracic wall with pleura and pulmonary surface, and depending on the ICS, rumen is seen. The pulmonary surface and the pleurae are imaged with approximately the same frequencies on the right side. The measurements for the dorsal and the ventral lung borders and the resulting dorsoventral dimensions of the lungs were similar to those on the right side (Table 1).

Echocardiography

Camels are affected by various cardiac diseases such as pericarditis, vegetative valvular endocarditis, hypertrophic cardiomyopathy, necrotic myocarditis and congenital defects including septal defects, patent ductus arteriosus, transposition of the aorta and pulmonary artery, persistent aortic trunk, and persistent right aortic arch and sarcocystosis (Fatani *et al,* 1996; Bekele, 1999; Woldemeskl and Gumi, 2001; Al-Ani, 2004; Valinezhad *et al,* 2008; Abdel-Ghaffar *et al,* 2009; Fowler, 2010). These heart diseases are mostly diagnosed at slaughterhouses or incidentally discovered at postmortem examination (Fowler, 2010), showing that the diagnosis of camel heart disease is a

Table 1. Dimensions (means ± SD) of the right and left lungs obtained at the 4th through the 11th intercostal spaces in healthy camels as estimated by ultrasound*

	Intercostal space							
	11th	10th	9th	8th	7th	6th	5th	4th
Right lung								
Dorsal margin*	34±2	33±3	29±2	29±2	53±6	55±4	62±5	62±2
Ventral margin*	43±9	46±7	51±7	60±5	64±3	69±4	75±6	76±6
Size (cm)	9±4[a]	13±3[a]	22±4[b]	31±2[b]	11±4[a]	14±4[a]	13±7[a]	14±7[a]
Left lung								
Dorsal margin*	35±4	34±2	28±2	28±2	51±5	52±4	60±6	64±4
Ventral margin*	46±4	43±8	48±5	57±7	63±5	65±5	72±7	76±7
Size (cm)	11±1[a]	9±5[a]	20±6[b]	29±4[b]	12±3[a]	13±2[a]	12±5[a]	12±5[a]

*Centimetres ventral to the dorsal midline. * Tharwat (2013)

challenging task especially when typical clinical signs of heart failure are absent.

Procedures of echocardiographic protocol

All echocardiographic examinations are usually performed in the recumbent camel which is sedated using xylazine (0.02mg/kg IV). In preparation for the echocardiography, the intercostal spaces (3rd to 6th) on both sides of the thorax are clipped, shaved and swabbed with alcohol to remove excess oil, and coupling gel was applied. The third, fourth and fifth ICS in the cardiac region can be examined ultrasonographically on both sides of the thorax. The thoracic limbs are moved cranially to facilitate better contact between the probe and the intercostal space. In the cardiac area, the heart, valves and major blood vessels are imaged (Tharwat *et al,* 2012). On the right side, the images are obtained in the following order: a caudal long-axis view of the right and left ventricles, a caudal long-axis view of the left ventricular outflow tract (LVOT), a caudal short-axis view of the ventricles and a cranial long-axis view of the right ventricular outflow tract (RVOT). On the left side, a caudal long-axis view of the heart (four-chamber view), a caudal long-axis view of the LVOT and a cranial long axis-view of the RVOT are obtained (Tharwat *et al,* 2012).

Eighteen measurements can be recorded from the 2-D images. Right ventricular diameter in systole (RVs) and diastole (RVd), right atrial diameter in systole (RAs) and diastole (RAd), right ventricular wall thickness in systole (RVWs) and diastole (RVWd), interventricular septal thickness in systole (IVSs) and diastole (IVSd) and tricuspid valve diameter in systole (TVDs) can be measured from the right parasternal caudal long-axis four-chamber view with the probe placed in the 5th ICS with a slight clockwise rotation or perpendicular in the 4th ICS. Left ventricular diameter in systole (LVs) diastole (LVd), left atrium diameter in systole (LAs) and diastole (LAd), left ventricular wall thickness in systole (LVWs) and diastole (LVWd) and mitral valve diameter in systole (MVDs) can also be measured from the left parasternal view with the transducer positioned in the 5th or 4th ICS and directed slightly caudodorsally. Aortic diameter in diastole (AOd) can be obtained from the left parasternal view with the transducer placed in the 4th ICS turned slightly more cranially and rotated slightly counter clockwise. Pulmonary artery diameter in diastole (PAd) can be measured from the left parasternal view with the transducer placed obliquely in the 3rd ICS. Systolic measurements are best measured during closure of the atrioventricular valves and opening of the semilunar valves, whilst diastolic measurements are measured during opening of the atrioventricular valves and closure of the semilunar valves. Ventricular measurements are measured at the level of the papillary muscles close to the chordae tendinae, whilst atrial measurements are measured at the widest part of the atria (Tharwat *et al,* 2012).

Echocardiographic findings in healthy camels

Right parasternal ultrasonograms

When the probe is placed longitudinally in the 5th ICS with a slight clockwise rotation or perpendicular in the 4th ICS, the caudal long-axis four-chamber view of the ventricles, atria, and the interventricular septum can be imaged. In this position, the right and left ventricles and the tricuspid and mitral valves can be imaged. From this position, the right and left ventricles and interventricular septum (IVS), the right and left atria, the tricuspid valve can be imaged. In some camels, a hybrid view

between a "four-chamber" and "LVOT view" can be imaged from the same position (Tharwat *et al,* 2012). Placement of the transducer in the 3rd ICS allows the visualisation of the RVOT in which the right ventricle, the pulmonary valve, pulmonary artery, aorta and aortic valve can be imaged (Tharwat *et al,* 2012).

Left parasternal ultrasonograms

When the probe is placed longitudinally in the 5th or 4th ICS and directed slightly caudodorsally, a view of the ventricles, atria, and the atrioventricular valves can be obtained. The LVOT can be imaged in the 4th ICS and the probe is turned slightly more cranially and rotated slightly counter clockwise. The RVOT can be seen from the 3rd ICS. In this position, the right ventricle, the tricuspid valve, the right atrium, an oblique section of the aorta, pulmonary artery can be imaged. The minimum, maximum, mean values, standard deviations and coefficient of variation for the measured variables are summarized in Table 2 (Tharwat *et al,* 2012).

Ultrasonography and detecting cardio-pulmonary disorders

Myocardial degeneration

Careful searching the veterinary literature found no published data concerning diagnosis of cardiac diseases in dromedary camels by ultrasonography. In our clinic, myocardial degeneration was detected by ultrasonography in a group of camel calves admitted with symptoms of Vitamin E/selenium deficiency (unpublished data). Ultrasonographic examination of the cardiac muscles in these calves revealed an overall increased echogenicity of myocardial tissue. Postmortem examination of the calves showed pale discolouration of the cardiac muscle with white streaks in muscle bundles. The lesions extended to involve the interventricular septum and papillary muscles and had a gritty character consistent with mineralisation.

Pneumonia

Inflammation of the pulmonary tissue or pneumonia is considered the most common pathological respiratory condition affecting camels (Wernery and Kaaden, 2002; Kane *et al,* 2005; Abubakar *et al,* 2010). In domestic animals including camels, pneumonia is usually caused by viruses, bacteria, fungi or parasites. However, non-infectious causes of pneumonia include aspiration, as well as toxins arriving haematogenously or by inhalation. Most infectious causes result from opportunistic bacteria (Elghazali *et al,* 2011; Ahmed *et al,* 2015; Ismail, 2017). Ultrasonography of the pulmonary parenchyma shows lung consolidation accompanied sometimes with pleural effusions. The non-ventilated lung tissue had a liver parenchyma-like echotexture. Sporadically, fluid bronchograms and anechoic vascular structures are discernible in the hepatised lung area and the ventilated lung deep to the consolidation can be identified by the weak, defined, and blurry reverberation artifacts. In cases with drenching pneumonia, ultrasonographic examination of the lung tissue reveals hypoechoic zones on the surface of the lung which represents ''superficial fluid alveolograms'' with comet-tail artifact (Tharwat and Al-Sobayil, 2016).

Table 2. Internal echocardiographic measurements in healthy adult camels*

Variable	Min	Max	Mean	SD	CV
RVd (cm)	4.3	8.0	5.3	1.2	22%
RVs (cm)	4.2	4.6	4.1	0.4	10%
LVd (cm)	9.9	15.5	11.8	1.6	14%
LVs (cm)	7.8	9.4	8.2	0.6	10%
RAd (cm)	5.0	6.5	5.9	0.5	10%
RAs (cm)	3.0	4.5	3.9	0.4	10%
LAd (cm)	6.8	8.8	7.6	0.6	10%
LAs (cm)	4.7	6.4	5.6	0.5	10%
RVWd (cm)	1.5	2.4	1.8	0.2	10%
RVWs (cm)	1.2	2.0	1.5	0.2	13%
IVSd (cm)	1.7	3.0	2.1	0.3	14%
IVSs (cm)	1.2	3.9	2.8	0.7	30%
LVWd (cm)	1.8	3.4	2.8	0.4	14%
LVWs (cm)	1.1	2.4	1.9	0.4	21%
AOd(cm)	6.0	8.2	7.0	0.8	11%
PAd (cm)	6.0	9.7	8.1	1.2	15%
TVDs (cm)	2.6	6.8	4.1	1.1	27%
MVDs (cm)	4.3	7.8	6.2	1.0	16%

Min, minimum value; Max, maximum value; SD, standard deviation; CV, coefficient of variation; RV, right ventricular diameter; LV, left ventricular diameter; RA right atrium diameter; LA, left atrium diameter; RVW, right ventricular wall thickness; IVS, interventricular septal thickness; LVW, left ventricular wall thickness; AO, aortic diameter; PA, pulmonary artery diameter; TVD, tricuspid valve diameter; MVD, mitral valve diameter; d, diastole; s, systole. * Tharwat *et al,* 2012.

Pulmonary abscessation

Chronic pneumonia in camels is often suppurative, and many organisms can be involved. Typical signs of pulmonary abscessation include dullness, loss of condition, an extended tongue, head

and neck, and severe dyspnea, leading to froth on the lips (Fowler, 2010). Ultrasonographic examination of the affected lung reveals hyperechogenic pleura, medium echogenicity of the pulmonary parenchyma and a heterogeneous appearance resembling liver parenchyma. In the compressed lung parenchyma, relatively well-defined abscesses exist in the form of round to ovoid anechoic areas. The abscess capsule is recognised as a slender reflective band and acoustic enhancement is imaged just below the lesion (Tharwat and Al-Sobayil, 2016).

Pulmonary emphysema

Hypersensitivity or allergic respiratory disease syndrome leads to an enlargement of the air spaces distal to the terminal bronchioles, with destruction of their walls but without fibrosis. Its major etiologies include chronic obstructive pulmonary diseases, acute interstitial pneumonia, large lung abscesses, strenuous exercise, and idiopathic unknown etiology. Pulmonary emphysema causes pulmonary oedema, congestion, interstitial emphysema, and alveolar changes. It is characterised by severe respiratory distress, frothing of saliva, and open-mouth breathing (Köhler-Rollefson *et al,* 2001; Fowler, 2010). Pulmonary emphysema is diagnosed based on the physical examination including increased resonance, enlarged lung field upon percussion and reduced vesicular breath sounds. Ultrasonographic examination in camels with pulmonary emphysema shows numerous comet-tail artifacts in the form of bright, closely situated echo bands starting at the lung surface and running perpendicular to the pleura in the lung tissue (Tharwat and Al-Sobayil, 2016).

Pleural effusion, pleurisy and pleuropneumonia

Pleural effusion is usually secondary to pleuritis, pericarditis or right heart insufficiency. In cases with clinical symptoms of pleuritis and pleural effusion, fluid located in the pleural cavity appears hypoechoic to anechoic fluid between the parietal pleural surface, diaphragm and lung on ultrasonographic examination. Echogenic bands or fibrin may be detected in the hypoechoic fluid. The fluid lead to compression atelectasis in the cranial lobes, which is imaged seen as hypoechoic, and where air is trapped in larger bronchi, they were hyperechoic with comet-tail artifacts. Fibrin appears as filmy and filamentous strands floating in the effusion with loose attachments to the pleural surfaces. Pockets of fluid separated by fibrin are commonly imaged (Tharwat and Al-Sobayil, 2016).

Inflammation of the pleura is known as pleuritis or pleurisy. It is usually associated with inflammation of the lung; the condition is called pleuropneumonia (Al-Ani, 2004). Inspiratory dyspnea is the most prominent sign. It is imperative that the nature of the pleural fluid be determined, because it may be a modified transudate or exudate. Ultrasonography of the thoracic cavity can monitor the pulmonary and pleural changes. It also can detect pleural effusion and aid in determining the prognosis (Tharwat, 2013; Tharwat and Al-Sobayil, 2016). Thoracic ultrasonography revealed anechoic fluid with fibrin net of the and right and left pleurae. In advanced cases, thoracic ultrasonography revealed bilateral heterogeneous pleural effusions with fibrin threads. In another severe case, thoracic ultrasonography revealed a considerable amount of heterogeneous fluid with large fibrin net at both the left and right pleural sacs. Results of postmortem examination included massive adhesions between the pleurae and costal arch, large amount of serosanguineous fluid in both pleural sacs and consolidation of anterior part of the left lung (Tharwat and Al-Sobayil, 2016).

In conclusion, thoracic ultrasonography is an extremely valuable imaging modality for diseases and disorders of the heart, lungs and pleura in healthy camels as well as in those with cardio-pulmonary affections. Of the common chest disorders that can be imaged and evaluated by ultrasonography are cardiac muscle dystrophy, inflammation of the lung parenchyma, pulmonary abscessation, pulmonary emphysema, pleural effusion, pleuritis and pleuropneumonia.

References

Abdel-Ghaffar F, Mehlhorn H, Bashtar AR, Al-Rasheid K, Sakran T and El-Fayoumi H. Life cycle of Sarcocystis camelicanis infecting the camel (*Camelus dromedarius*) and the dog (*Canis familiaris*), light and electron microscopic study. Parasitology Research. 2009; 106:189-195.

Abubakar MS, Fatihu MY, Ibrahim NDJ, Oladele SB and Abubakar MB. Camel pneumonia in Nigeria: Epidemiology and bacterial flora in normal and diseased lung. African Journal of Microbiology Research. 2010; 4:2479-2483.

Ahmed MA, Musa MT and Mohammed AE. Bacteria associated with pneumonia in camels (*Camelus dromedarius*) in the Sudan and sensitivity of some isolates to antibiotics using Vitek 2 Compact. Global Journal of Science Frontier Research: Biological Science 15 Version 1. 2015.

Al-Ani FK. In Al-Ani, F.K. (Ed.), Camel Management and Diseases. Al-Sharq printing press, Jordan. 2004.

Bekele ST. Gross and microscopic pulmonary lesions of camels from eastern Ethiopia. Tropical Animal Health and Production. 2008; 40:25-28.

Bekele T. Studies on the respiratory disease 'sonbobe' in camels in the eastern lowlands of Ethiopia. Tropical Animal Health and Production. 1999; 31:333-345.

Buczinski S. Cardiovascular ultrasonography in cattle. Veterinary Clinics of North America: Food Animal Practice. 2009; 25:611-632.

Buczinski S, Tolouei M, Rezakhani A and Tharwat M. Echocardiographic measurement of cardiac valvular thickness in healthy cows, cows with bacterial endocarditis, and cows with cardiorespiratory diseases. Journal of Veterinary Cardiology. 2013; 15:253-261.

Cuevas-Gomez I, McGee M, Sánchez JM, O'Riordan E, Byrne N, McDaneld T and Earley B. Association between clinical respiratory signs, lung lesions detected by thoracic ultrasonography and growth performance in pre-weaned dairy calves. Irish Veterinary Journal. 2021; 74:7.

Elghazali F, Hamid FM, Ali AMM and Shallali AO. Isolation of Mycoplasmas and Sero-surveillance for *Mycoplasma mycoides* subsp. *mycoides* in the Dromedary Camel in Some Areas of the Sudan. Sudan Journal of Veterinary Research. 2011; 26:13-22.

Fatani A, Hilali M, Al-Atiya S and Al-Shami S. Prevalence of Sarcocystis in camels (*Camelus dromedarius*) from Al-Ahsa, Saudi Arabia. Veterinary Parasitology. 1996; 62:241-245.

Flock M. Diagnostic ultrasonography in cattle with thoracic disease. Veterinary Journal. 2004; 167:272-280.

Fowler ME. Pleural effusion. In: Medicine and Surgery of Camelids. 3rd ed., Blackwell Publishing, Iowa. 2010; pp 346.

Ismail ZB. Pneumonia in dromedary camels (*Camelus dromedarius*): a review of clinico-pathological and etiological characteristics. Journal of Camel Practice and Research. 2017; 24:49-54.

Jenberie S, Awol N, Ayelet G, Gelaye E, Negussie H and Abie G. Gross and histopathological studies on pulmonary lesions of camel (*Camelus dromedarius*) slaughtered at Addis Ababa abattoir, Ethiopia. Tropical Animal Health and Production. 2012; 44:849-854.

Kane Y, Diop A, Isselmon E, Kaboret Y, Ould MM, Diallo BC, Kane Y, Kadja MC, Bada-Alambedji R, Bezeid OE, Akakpo JA and Kaboret Y. Lung lesions and bacteria of the one-humped camel (*Camelus dromedarius*) at Nouakchott slaughterhouse in Mauritania. Revue d'élevage et de Médecine Vétérinaire des Pays Tropicaux. 2005; 58:145-150.

Köhler-Rollefson I, Mundy P and Mathias E. Managing and treating camels. In: A Field Manual of Camel Diseases: Traditional and Modern Healthcare for the Dromedary. ITDG publishing, London. 2001; pp 1-67.

Larson MM. Ultrasound of the thorax (noncardiac). Veterinary Clinics of North America: Small Animal Practice. 2009; 39:733-745.

Mohamed T and Buczinski S. Clinicopathological findings and echocardiographic prediction of the localisation of bovine endocarditis. Veterinary Record. 2011; 169:180.

Soldati G, Smargiassi A, Inchingolo R, Sher S, Nenna R, Valente S, Inchingolo CD and Corbo GM. Lung Ultrasonography May Provide an Indirect Estimation of Lung Porosity and Airspace Geometry. Respiration. 2014; 88:458-468.

Tharwat M. Ultrasonography of the lungs and pleura in healthy camels (*Camelus dromedarius*). Acta Veterinaria Hungarica. 2013; 61:309–318.

Tharwat M and Al-Sobayil F. Ultrasonographic findings in camel calves (*Camelus dromedarius*) with thoracic affections. Journal of Camel Practice and Research. 2016; 23:287-290.

Tharwat M, Al-Sobayil F, Ali A and Buczinski S. Echocardiography of the normal camel (*Camelus dromedarius*) heart: technique and cardiac dimensions. BMC Veterinary Research. 2012; 8:130.

Tharwat M and Oikawa S. Ultrasonographic evaluation of cattle and buffaloes with respiratory disorders. Tropical Animal Health and Production. 2011; 43:803-810.

Valinezhad A, Oryan A and Ahmadi N. Sarcocystis and its complications in camels (*Camelus dromedarius*) of eastern provinces of Iran. Korean Journal of Parasitology. 2008; 46:229-234.

Wernery U and Kaaden OR. Infectious Diseases in Camelids. 2nd Edition, Blackwell Germany. 2002; pp 97-109.

Woldemeskel M and Gumi B. Prevalence of sarcocysts in one-humped camel (*Camelus dromedarius*) from southern Ethiopia. Journal of Veterinary Medicine B. 2001; 48:223-226.

DOI : 10.5958/2277-8934.2021.00049.7 Vol 28 No 3, p 319-325

PHYSIOLOGICAL PERSPECTIVE OF MILK SOMATIC CELL COUNT IN LACTATING CAMELS

Kaskous S[1], Ahmad Q Al-Momani[2], Azzam N Al-Yacoub[3] and Khaled A Al-Najjar[2]

[1]Department of Research and Development, Siliconform, Schelmengriesstrasse 1, 86842 Türkheim, Germany
[2]Animal production and Protection Department, Faculty of Agriculture, Jerash University, Jordan
[3]Biology Department, Faculty of Applied Science, Umm Al-Qura University, Makkah, Saudi Arabia

ABSTRACT

The present study sheds light on some physiological aspects of milk SCC in lactating camels. The somatic cells in camel milk contain the following cells: macrophages, polymorphonuclear neutrophils (PMN), lymphocytes, and a large number of cell fragments. Lymphocytes are the predominant cell type in camel milk in the healthy udder. So far, there is no established physiological level for SCC in healthy camel milk. It is suggested that 150 x 10^3 SCC cells/ml in milk is a limit value for healthy camel milk. If the SCC exceeds this limit, subclinical or clinical mastitis of the udder may occur and the milk may be contaminated with microbes. In order to maintain camel milk hygiene, proper machine milking such as StimuLactor for camels mainly be used in the intensive housing systems. An increase in the SCC above the physiological level not only indicates a problem with the health of the udder but also reduces milk production, changes the milk composition, affects milk processing and changes the bioactive ingredients of camel milk.

Key words: Camel, camel milk, somatic cell count, StimuLactor

Camel milk is one of the foods available in arid and Semi-arid regions. It covers a significant part of the qualitative and quantitative nutritional needs. Camel milk is traditionally consumed raw in many parts of the world. Therefore, the safety of raw camel milk plays a crucial role (Kaskous, 2019b). Somatic cell count (SCC) in camel milk could be the main indicator of milk hygiene, milk quality and udder health (Hadef *et al,* 2016). So far there is no official SCC limit for healthy udders in camels. However, preservation of camel milk samples must be used to study the correct concentrations of milk SCC. In addition, it has been observed that freezing camel milk at -20 °C and thawing at room temperature is not recommended due to the significant decrease in SCC (Najafabadi *et al,* 2013).

It is noteworthy that SCC in camel milk contains the following cells: macrophages, polymorphonuclear neutrophils (PMN), lymphocytes, and a large number of cell fragments (Abdurahman, 1998; Hamed *et al,* 2010; Nagy *et al,* 2013a; Hamed *et al,* 2016). These cell fragments can have an impact on the diagnosis of udder health by determining the SCC since they could be counted with somatic cells of the immune system. Therefore, most of the methods used have not yet been validated. The mode of milk secretion in this species is apocrine secretion. That is, camel milk contains a large number of non-nucleated cell fragments (Abdurahman *et al,* 1992; Abdurahman, 2006). The size and shape of these cytoplasmic particles resemble leukocytes and contain cytoplasmic organelles such as endoplasmic reticulum and mitochondria. On the other hand, Hamed *et al* (2010) found that lymphocytes in camel milk and macrophages in cow milk are the predominant cell types in healthy udders. However, both milk samples contain an SCC of less than 100 × 10^3 cells/ml. Abdurahman (2006) and Hamed *et al* (2010) found that an increase in the SCC, particularly PMN in camel milk, is a good indicator of inflammation (Fig 1). Moreover, Hamed *et al* (2017) found that during the rainy season in Tunisia, macrophages were the predominant cell type in camel milk and PMN in cow milk. However, macrophages in camel milk tended to maintain high levels as the season changed. Consequently, compared to cows, camels are more resistant to seasonal fluctuations. In the following sections, the physiological aspects of SCC in camel milk are presented and discussed.

Physiological level of somatic cells in dairy camels:

The physiological levels of SCC are not yet established in camel milk. There has been a problem establishing the basal levels of cells and their physiological variations in camel milk (Abdurahman

SEND REPRINT REQUEST TO KASKOUS S email: skaskous@siliconform.com

et al, 1992). However, the use of SCC is not very common but was applied as an indirect diagnostic tool for detecting uninfected and infected quarters (Abdurahman, 1995; Abdurahman *et al,* 1995; Saleh and Faye, 2011; Saleh *et al,* 2013). An earlier study by Kospakov (1978), reported a mean cell count of 1300 x 10^3 cells/ml of milk during normal lactation, 3300 x 10^3 cells/ml during pregnancy and 7900 x 10^3 cells/ml at the start of the dry period in camel milk bacteria samples.

The investigation performed by Hamed *et al* (2012) found that the arithmetic means of SCC in camel milk were 100 x 10^3 cells/ml. Another study found that the mean number of SCC in raw camel milk was 126.43 x 10^3±7.21 cells/ml from healthy udders under German conditions (Kaskous, 2019a). However, this concentration of SCC in the raw camel milk could be considered as a normal physiological level. Hence, no mastitis could be detected either in clinical or subclinical analysis in the camels. A similar concentration of SCC has been obtained by Saleh and Faye (2011) and the mean value of SCC was 125 x 10^3 cells/ml in Saudi Arabia. The results from Golestan province in Iran have shown that out of 243 camel milk samples from individual quarters (95 milking camels), 18.1% were subclinical mastitis and SCC values beyond 306 x 10^3 cells/ml could be considered as subclinical mastitis in the camel (Niasari-Naslaji *et al,* 2016). Abbood (2016) suggested that SCC level 250 x 10^3 is indicated for a healthy camel.

Nagy *et al* (2013a) found higher milk SCC in the bulk tank with geometric means of 394 × 10^3 cells/mL in a large-scale farm in the United Arab Emirate and the authors emphasised that SCC concentration in bulk milk of healthy camels can be higher than the threshold of acceptance for dairy cows, but more studies are required to determine the normal physiological range of SCC in camel milk (Nagy *et al,* 2013b). Due to the observation from Al-Majali *et al* (2007), camel milk samples with isolated bacteria as subclinical mastitis, the SCC was lower than 200 x 10^3 cells/ml. The following table 1 gives us the SCC levels in camel milk from some countries in the world. In general, SCC in healthy dairy camels is determined by physiological factors such as stage of lactation, parity and breed or type of dairy camels (Obied *et al,* 1996). However, the authors did not find any such physiological significant difference in SCC during the lactation or between lactation numbers in healthy camels. The differences between levels within parity were not also significant by Saleh and Faye (2011) and Saleh *et al* (2013) due to the high variability. Furthermore, Saleh *et al* (2013) also found that the SCC decreased all along stage of lactation, but the trends were not significant. On the contrary, various studies have reported that SCC levels increased with parity (Aljumaah *et al,* 2011). Moreover, a negative relationship between stage of lactation and SCC levels was found in dromedary camel milk (Hamed *et al,* 2012). Another study indicated that the risk of subclinical mastitis increased significantly with parity and with the early stage of lactation (Aljumaah *et al,* 2011). It is noteworthy that, PMN numbers in camels' milk were higher immediately after parturition and declined gradually with advanced lactation, while the macrophages number increased through lactation (Hamed *et al,* 2010).

Table 1. Somatic cell count of camel milk in some countries.

Country	Source of milk sample	SCC level x 10^3 cells /ml	Author
Algeria	Animal	240	Hadef *et al*, 2016
Ethiopia	Animal	300 -15000	Woubit *et al,* 2001
Germany	Animal	126	Kaskous, 2019a
Iran	Animal	306 *	Niasari-Naslaji *et al,* 2016
Iraq	Animal	560	Abbood, 2016
Kenya	Animal	162	Tinggren, 2019
Saudi Arabia	Animal	125	Saleh and Faye, 2011
Saudi Arabia	Animal	473	Aljumaah *et al,* 2020
Saudi Arabia	Animal	11 - 298 ** 14 - 643 ***	Saleh *et al*, 2013
Tunisia	Animal	100	Hamed *et al,* 2012
UAE	Bulk milk	340	Wernery *et al*, 2008
UAE	Bulk milk	394	Nagy *et al*, 2013a

*: limit to subclinical mastitis
: machine milking, *: hand milking

Increase of somatic cells over physiological level:

There are many factors affecting milk SCC over physiological level in lactating camels. The most important factor is infection status (Eberlein, 2007). If the mammary gland is infected, higher mean values of milk SCC can be found (Abdurahman *et al,* 1995; Woubit *et al,* 2001; Abdel Gadir Atif *et al*. 2006; Saleh and Faye, 2011; Wanjohi *et al,* 2013). Abdurahman, (2006) found that quarters infected with bacteria had higher mean values for SCC than non-infected quarters (P<0.0001). However, in inflammed quarters PMN dominated the sample and in non-inflamed quarters the dominant cells were epithelial cells and cell fragments (Abdurahman, 2006). Moreover, it was found that SCC in camel milk ranged between 300

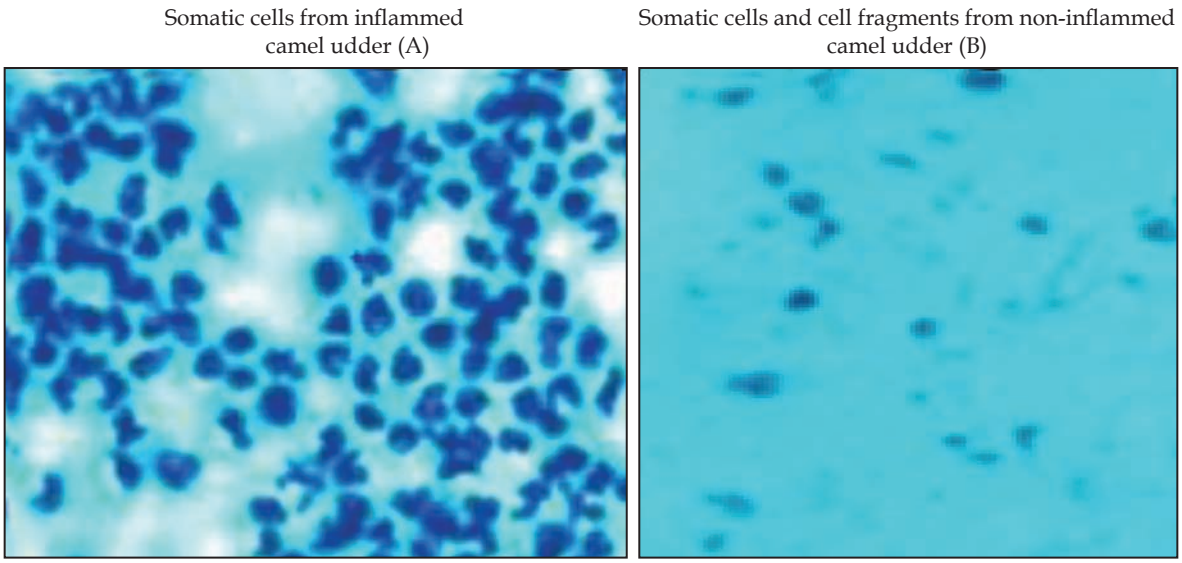


Fig 1. SCC from inflammed (A) and uninflammed (B) camel udder according to Abdurahman (2006).

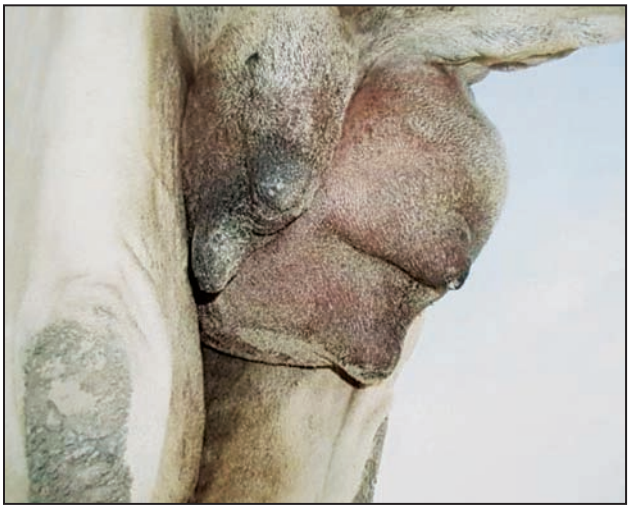


Fig 2. Healthy and inflammed quarters (same camel) according to Eberlein (2007).

x 10^3 and 15000 x 10^3 cells/ml in Lowland pastoral, Southwestern Ethiopia (Woubit *et al,* 2001). These high values could clearly prove that the udder had subclinical or clinical mastitis (Fig 2). However, a high percentage of subclinical mastitis in camels is reported by several authors (Obied *et al,* 1996; Almaw and Molla, 2000; Wanjohi *et al*, 2013; Niasari-Naslaji *et al,* 2016) and the values varied between 15 and 70% (Bhatt *et al,* 2004; Abera *et al,* 2010; Seifu and Tafesse, 2010; Alamin *et al*, 2013; Kashongwe *et al,* 2017). According to the previous studies, Chaffer *et al* (2000) and Kashongwe *et al* (2017) come to the conclusion that the SCC can be used as an effective indicator for udder infections in camels. On the other hand, Kashongwe *et al* (2017) have shown that herd-level prevalence of mastitis was lower in smallholders than in pastoral herds.

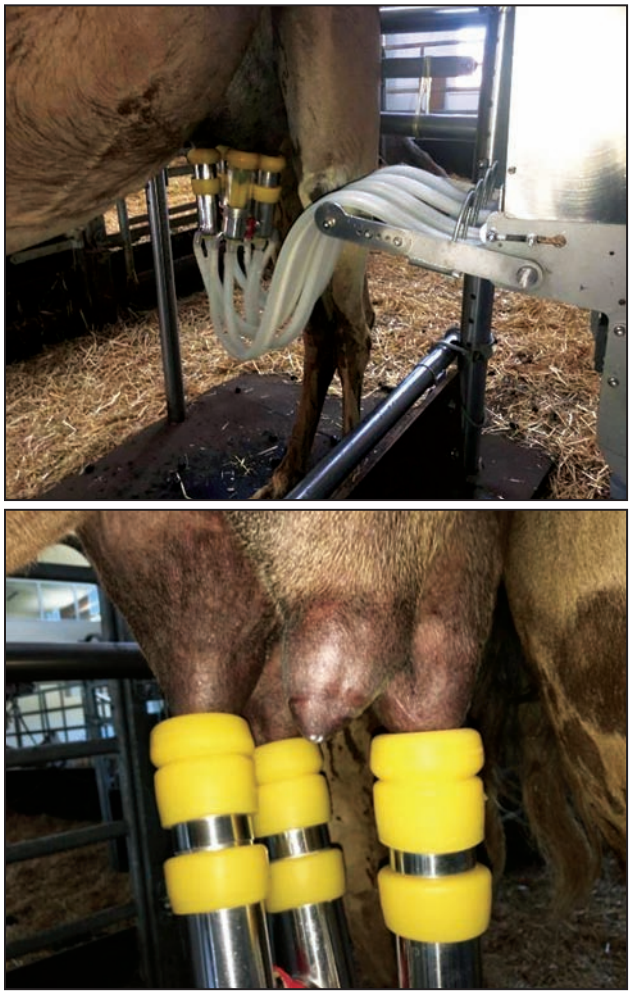


Fig 3. StimuLactor (ST-C) for camels during milking.

Moreover, an increasing number in the mean values of somatic cell counts was obtained for increasing scores of California Mastitis Test (CMT)

and 4 (1.9%) of the lactating camels examined were detected as clinical cases of mastitis (Table 2).

Table 2. Log SCC (Mean ± Standard Deviation, SD) with California mastitis test (CMT) scores according to Woubit *et al* (2001).

CMT Scores	Log SCC Mean±SD	Number of observations
0	2.78±2.57	577
Trace	4.02±2.31	182
1+	3.47±2.74	33
2+	5.27±2.04	9
3+	6.05±0.86	2

Thus, an increase in the SCC in camel milk with infected quarters has also been reported by many researchers (Abdurahman *et al,* 1995; Aljumaah *et al,* 2011; Saleh and Faye, 2011). Abdel Gadir Atif *et al* (2006) have found a positive correlation between SCC, CMT scores and bacteriological classes (P<0.001) in Ethiopia. Similarly, Saleh and Faye (2011) have found in Saudi Arabia that infected quarters in camels had higher values for SCC and CMT scores. In addition, it was observed that infected udders were significantly affected by increased age and parity of the animals (Abera *et al,* 2010). Wanjohi *et al* (2013) concluded that CMT is a useful screening test in the detection of subclinical mastitis in camels since a positive correlation of CMT with the presence of mastitis pathogens in camel milk was observed.

Influence of the milking procedures on the SCC level:

It is known that SCC levels and milk quality depend mainly on milking management and routine in camels. Therefore, hygiene around milking and milking equipment is essential. However, camels are milked by hand in most countries of the world in traditional farming systems. Many researchers emphasised that poor management and unhygienic milking practices prevalent in the traditional husbandry systems are responsible for contamination of udders (Obied *et al,* 1996; El-Ziney and Al-Turki, 2007; Almaw and Molla, 2000; Wanjohi *et al,* 2013; Asfour and Anwer, 2015; Kashongwe *et al*, 2017). It is also noted that the isolation of environmental *Streptococcus* species at high rates in traditional farming systems revealed the cross-contamination of raw camels' milk either from the animal itself or from the milker (Asfour and Anwer, 2015). Toroitich *et al* (2017) demonstrated an example of traditional hand milking. The results showed that many camel owners milked their camels thrice a day and washed their hands before milking, whereas most of them do not wash the udder. Furthermore, most of the treatment of camel mastitis was done by the owners, whereas only 26.9% requested assistance from the community-based animal health workers. During mastitis infection, most camel owners do not follow the milking order (Table 3).

Table 3. Characterisation of camel milking in the practices according to Toroitich *et al* (2017).

Household practices	Results	Frequency	Percentage (%)
Milking Rates	Thrice a day Twice a day and below	47 5	90.4 9.6
Hand washing before milking	Yes No	43 9	82.7 17.3
Udder washing before milking	Yes No No response	4 40 8	7.7 76.9 15.4
Who milks the camels?	Owners Wives Herders	14 16 22	26.9 30.8 42.3
Who treats camels with mastitis?	Self CBAHWs*	39 14	75 25
Follow milking order during mastitis	Yes No	13 39	25 75

*: community-based animal health workers

The results further showed that the most affected quarters were the right quarters with a high prevalence of 45.3% in the right forequarter and 48.4% in the right hindquarter. This suggests that in most cases during milking, the calf was left to suckle the left quarters and the right quarters were milked by the owners and due to poor hygienic milking procedures, these quarters were at a higher risk of getting infected. This study and others clearly showed that hand milking hygiene practices were not suitable for maintaining udder health and low SCC in the milk produced (Abera *et al,* 2016). Johnson *et al* (2015) reported that the camel's udder is protected by a variety of defense mechanisms like innate or specific immunity as well as physiological particularities. Therefore, the udder is free of microorganisms and so is the milk. Consequently, machine milking in camels must be spread in order to maintain the health of the udder and to avoid contamination of camel raw milk. Saleh *et al* (2013) found that machine milking reduced SCC in camel milk in comparison with hand milking and the microbiological contamination was higher in farms with hand milking than in farms with machine milking. Likewise, the results obtained by Kashongwe *et al* (2017) demonstrated that hand milking may have contributed to maintaining mastitis pathogens in herds. This has led to substantial pre-and post-

Table 4. Effect of isolated bacteria on some biochemical constituents of camel's milk whey (Mean±SE) according to Asfour and Anwer (2015).

Type of Bacteria	Total protein g/dl	Total globulin g/dl	Total albumin g/dl	Nitric oxide m/ml	Lysozyme g/ml	Lacto-ferrin mg/ml
S. aureus mixed(1)	2.8±0.3^{a}	1.2±0.2^{a}	1.6±0.1^{a}	46.3±11.3^{ab}	164.2±7.1^{a}	2.3±0.1^{b}
CNS(2)	3.5±0.8^{a}	1.6±0.7^{a}	1.9±0.4^{a}	73.4±7.6^{a}	166.9±6.3^{a}	3.4±0.6^{a}
CNS mixed(3)	2.7±0.1^{a}	1.1±0.3^{a}	1.6±0.1^{a}	42.2±8.5^{ab}	147.8±6.0^{ab}	3.2±0.6^{a}
E. coli mixed (4)	3.3±0.2^{a}	1.5±0.3^{a}	1.8±0.1^{a}	73.9±11.6^{a}	160.3±4.5^{a}	2.0±0.1^{b}
Y. enterocolotica(5)	2.8±0.1^{a}	1.3±0.1^{a}	1.5±0.0^{a}	10.21±2.0^{b}	122.7±11.8^{ab}	4.1±0.0^{a}
Control	2.9±0.4^{a}	1.0±0.1^{a}	1.8±0.3^{a}	9.5±1.0^{b}	95.2±5.5^{b}	4.1±0.2^{a}

Different superscript letters within a column indicate significant differences ($p<0.05$).
(1): *Staphylococcus aureus* with other bacteria; (2): Coagulase negative *Staphylococci*; (3): Coagulase negative *Staphylococci* with other bacteria; (4): *Escherichia coli* with other bacteria; (5): *Yersinia enterocolotica*.

harvest milk losses in smallholder and pastoral herds. Furthermore, the daily milk yield was 38% higher in machine milking compared with hand milking of camels (Hammadi *et al,* 2010). Currently, machine milking makes only slow progress and is limited to intensive dairy camel farms in a few countries (Nagy and Juhasz, 2016; Ayadi *et al,* 2018; Kaskous, 2018, 2019a). However, some studies clearly showed that completeness of milking by machine with the available equipment is not satisfactory. The amount of residual milk after machine milking is high (up to 30% or even more) (Ayadi *et al,* 2014; 2018; Kaskous, 2018). The remaining milk after milking may serve as a substrate for pathogens and increase the risk of mastitis (Bruckmaier and Wellnitz, 2008). Therefore, a special milking machine for camels is necessary, to allow fast and complete milk removal and to maintain good udder health. Only then is machine milking efficient for the farmer and guarantees milk production at high-quality standards (Kaskous, 2018). Siliconform Company in Germany has been developing a new milking machine "StimuLactor for Camels" (Kaskous, 2019a). The development was based on two main points to achieve the right milking technology for camels: first, on the technical basis of the milking machine which must be adapted to the requirements of a camel's udder and teats. Second, on the basis of the suckling behaviour of the calf. Now, the right milking machine for camels is ready to be used in the field (Fig 2).

Influence of high somatic cell counts on milk quality and processing

The effect of SCC on milk components, especially on fat and protein content, is known. The studies by Hamed *et al* (2012) have shown that a positive correlation between total protein and SCC in dromedary camel milk was found. However, a significant decrease in β-casein associated with increased SCC was observed. This demonstrates that a concomitant increase in SCC plays a key role in casein breakdown, apparently due to its impact on indigenous protease activity in milk. Furthermore, the results obtained by Hamed *et al* (2016) demonstrated that macrophages play a role in the degradation of dromedary milk fat. This means that lipolysis level increased as macrophage level in camel's milk increased. This suggests that the macrophages secreted lipolytic enzymes into the gradient while fractions containing PMN leukocytes and lymphocytes did not possess lipolytic activity. It is also noted that significant decreases in protein, lactose, and solid non-fat, Ca and K concentrations and an increase in Na concentrations were associated with subclinical mastitis (Aljumaah *et al,* 2011). Furthermore, subclinical mastitis in dairy camels reduces milk production, alters milk composition and affects milk processing (Tibary and Anouassi, 2000; Atasever and Koc, 2016). Consequently, milk production losses can be reduced by lowering SCC levels (Atasever and Koc, 2016). It is noteworthy that mineral composition in camel milk was significantly affected by the SCC level (Hamed *et al,* 2016). In the same way, the results obtained by Asfour and Anwer (2015) demonstrated that inflamed udders with bacteria in resulted changes in the bioactive ingredients of camel's milk (Table 3). Thus, the therapeutic and immunogenic benefits of camel's milk may deteriorate. Hence, there is a great need for strict hygienic measures during milking and husbandry.

References

Abbood AS. compare between somatic cell count (SCC) in she-camel and cow milk and genetic study: Indian Journal Research. 2016; 5(7):145-146.

Abdel Gadir Atif E, Hildebrandt G, Kleer JN, Molla B, Kyule MN and Baumann MP. Comparison of California

Mastitis Test (CMT), somatic cell counts (SCC) and bacteriological examinations for detection of camel (*Camelus dromedarius*) mastitis in Ethiopia. Berl. Munch. Tierärztl. Wochenschr. 2006; 119(1-2):45-49.

Abdurahman OA SH. The detection of subclinical mastitis in the camel (*Camelus bactrianus*) using somatic cell count and California mastitis tests. Veterinary Research Communication. 1995; 20:9-14.

Abdurahman OA SH. Udder health and milk quality among camels in the Errer valley of eastern Ethiopia. Livestock Research for Rural Development. 2006; 18:1-9.

Abdurahman OA SH, Agab H, Abbas B and Aström G. Relations between udder infection and somatic cells in camel (*Camelus dromedarius*) milk. Acta Veterinaria Scandinavica. 1995; 36(4):423-432.

Abdurahman OA SH. Detection of subclinical mastitis in camels: Relationship between udder infection and inflammatory indicators in milk, Dromadaires et chameaux, animaux laitiers/ Dromedaries and camels, milking animal. Actes du colloque 24-26 octobre 1994, Nouakchott, Mauritanie. Montepellier, France, Cirad. 1998; pp 31-34.

Abdurahman OSH, Coo Ray R and Born Stein S. The Ultrastructure of Cells and Cell Fragments in Mammary S*ecretions of Camelus bactrianus*. Journal of Veterinary Medicine Series A 1992; 39:648-655.

Abera M, Abdi O, Abunna F and Megersa B. Udder health problems and major bacterial causes of camel mastitis in Jijiga, eastern Ethiopia: implication for impacting food security. Tropical Animal Health and Production. 2010; 42:341-347.

Abera T, Legesse Y, Mummed B and Urga B. Bacteriological quality of raw camel milk along the market value chain in Fafen zone, Ethiopian Somali regional state. BMC Research Notes. 2016; 9:285-290.

Alamin MA, Alqurashi AM, Elsheikh AS and Yasin TE. Mastitis incidence and bacterial causative agents isolated from lactating she-camel (*Camelus dromedarius*). IOSR Journal of Agriculture Veterinary Science. 2013; 2:7-10.

Aljumaah RS, Almutairi FF, Ayadi M, Alshaikh MA, Aljumaah AM and Hussein MF. Factors influencing the prevalence of subclinical mastitis in lactating dromedary camels in Riyadh region, Saudi Arabia. Tropical Animal Health and Production. 2011; 43(8):1605-1610.

Aljumaah RS, Almutairi F, Ayadi M, Alshaikh MA, AlHaidary AA and Samara EM. Practicability of somatic cell count and electrical conductivity as subclinical mastitis diagnostic tests in camels (*Camelus dromedarius*). Sci. Agric. 2020; 77, n.4, e20180373:1-12.

Al-Majali AM, Ismail ZB, Al-Hami Y and Nour AY. Lactoferrin concentration in milk from camels (*Camelus dromedarius*) with and without subclinical mastitis. International Journal of Applied Research in Veterinary Medicine. 2007; 5(3):120-124.

Almaw G and Molla B. Prevalence and etiology of mastitis in camels (*Camelus dromedarius*) in Iraq. Journal of Camel Practice and Research. 2000; 7:97-100.

Asfour HAE and Anwer AM. Some bacteriological and immunological studies on camel's milk. Alexandria Journal of Veterinary Sciences. 2015; 47:38-46.

Atasever S and Koc A. Milk yield losses caused by high somatic cell count in dromedary camels (*Camelus dromedarius*). 1st international Selcuk-Ephesus Symposium on culture of camel-dealing and camel wrestling, Seluc Izmir-Turkey. 2016; November 2016.

Ayadi M, Aljumaah RS, Musaad A, Bengoumi M and Faye B. Effect of vacuum level and pulsation rate on machine milking efficiency in lactating dromedary camels. Seminaire international sur lelevage et la faune sauvage dans les zones arides et desertiques SIEFAD 2014, 16-18 decembre 2014, Djerba, Tunisie. 2014; 1-5.

Ayadi M, Musaad A, Aljumaah RS, Matar A, Konuspayeva G, Abdelrahman MM, Abid AI, Bengoumi M and Faye B. Machine milking parameters for an efficient and healthy milking in dairy camels (*Camelus dromedarius*). Journal of Camel Practice and Research. 2018; 25(1):81-87.

Bhatt L, Chahar A, Tuteja FC and Verma D. Prevalence etiology and antibiogram of subclinical mastitis isolates from camel. Veterinary Practitioner. 2004; 5:61-65.

Bruckmaier R and Wellnitz O. Induction of milk ejection and milk removal in different production systems. Journal of Animal Science, 2008; 86:15-20.

Chaffer M, Leitner G, Glickmann A, Van Creveld C, Winkler M, Saran A and Yagil R. Determination of udder health in camels. Journal of Camel Practice and Research. 2000; 7:171-174.

Eberlein V. Hygienic status of camel milk in Dubai (United Arab Emirates) under two different milking management systems. Doctoral thesis, Ludwig-Maximilians University, Munic, Germany. 2007; 1-101.

El-Ziney M and Al-Turki A. Microbiological quality and safety assessment of camls milk (*Camelus dromedarius*) in Saudi Arabia (Qassim Region). Applied Ecology and Environmental Research. 2007; 5(2):115-122.

Hadef L, Aggad H, Hamad B, Mahmoud MS and Adaika A. Subclinical mastitis in dairy camels in Algeria: Comparison of screening tests. Acta Argiculturae Slovenica. 2016; 108/2:85-92.

Hamed H, El Feki A and Gargouri A. Effect of total and differential somatic cell counts, lactation stage and lactation number on lipolysis and physicochemical composition in camel (*Camelus dromedarius*). Iranian Journal of Applied Animal Science. 2016; 6(4):777-782.

Hamed H, El Feki A and Gargouri A. Influence of season on total and differential Bulk cow milk somatic cell counts in Dromedary (*Camelus dromedarius*) and bovine milk. Research Journal of Animal Sciences. 2017; 11(5-6):7-11.

Hamed H, Gargouri A, Hachana Y and Elfeki A. Comparison between somatic cell and leukocyte variations throughout lactation in camel (*Camelus dromedarius*) and cows milk. Small Ruminant Research. 2010; 94(1):53-57.

Hamed H, Trujillo A-J, Juan B, Guamis B, Elfeki A and Gargouri A. Interrelationships between somatic cell counts, lactation stage and lactation number and their influence on plasmin activity and protein fraction distribution in dromedary (*Camelus dromedarius*) and cow milk. Small Ruminants Research. 2012; 105:300-307.

Hammadi M, Atigui M, Ayadi M, Barmat A, Belgacem A, Khaldi G and Khorchani T. Training and short time effects of machine milking on milk yield and milk composition in Tunisian Maghrebi Camels (*Camelus dromedarius*). Journal of Camel Practice and Research 2010; 17(1):1-7.

Johnson B, Joeph M, Jose Sh, Jose S, Kinne J and Wernery U. The microflora of teat canals and udder cisterns in non-lactating dromedaries. Journal of Camel Practice and Research. 2015; 22(1):55-59.

Kashongwe OB, Bebe BO, Matofari JW and Huelsebusch CG. Associations between milking practices, somatic cell counts and milk postharvest losses in smallholder dairy and pastoral camel herds in Kenya. International Journal of Veterinary Science and Medicine. 2017; 5(1):57-64.

Kaskous S. Physiology of lactation and machine milking in dromedary she-camel. Emirates Journal of Food and Agriculture. 2018; 30(4):295-303.

Kaskous S. Camel milk composition, udder health and effect of different storage times and temperatures on raw milk quality using camel milking machine "StimuLactor". Agriculture and Food Sciences Research. 2019a; 6(2):172-181.

Kaskous S. Prevalence of microbes in raw camel milk – an overview. IOSR Journal of Agriculture and Veterinary Science. 2019b; 12(2):51-60.

Kospakov ZK (1978). Cell content in the milk of Bactrian camels, depending on stage of lactation and condition of the udder. Vet. Bull. 48:6960.

Nagy P and Juhasz J. Review of present knowledge on machine milking and intensive milk production in dromedary camels and future challenges. Tropical Animal Health and Production. 2016; 48:915-926.

Nagy P, Faye B, Marko O, Thomas S, Wernery U and Juhasz J. Microbiological quality and somatic cell count in bulk milk of dromedary camels (*Camels dromedarius*): descriptive statistics, correlations, and factors of variation. Journal of Dairy Science. 2013a; 96(9):5625-5640.

Nagy P, Thomas S, Marko O and Juhasz J. Milk production, raw milk quality and fertility of dromedary camels (*Camelus dromedarius*) under intensive management. Acta Veterinaria Hungarica. 2013b; 61(1):71-84.

Najafabadi HA, Niasari-Naslaji A, Atakpour AB, Ghaffari S, Barin A, Samiei HR, Amjadi M, Moradlou AAH, Salimi A, Narimani I and Gerami A. Preservation of camel milk for somatic cell count and bacteriological investigation. Journal of Camel Practice and Research. 2013; 20(1):39-43.

Niasari-Naslaji A, Pezeshk H, Atakpour AB, Ghaffari S, Nickchi P, Safi S, Shirazi-Beheshtiha SH, Arabha H, Samiei R, Amjadi M, Haji Moradlou AA, Narimani I and Moosavi-Movahedi AA. Estimation of somatic cell count, as gold standard to detect subclinical mastitis, in dromedary camel. Journal of Camel Practice and Research. 2016; 23(1):175-178.

Obied AI, Bagadi HO and Mukhtar MM. Mastitis in *Camelus dromedarius* and the somatic cell content of camel's milk. Research in Veterinary Science. 1996; 61(1):55-58.

Saleh SK and Faye B. Detection of subclinical mastitis in dromedary camels (*Camelus dromedarius*) using somatic cell counts, California Mastitis Test and udder pathogen. Emirates Journal of Food and Agriculture. 2011; 23:48-58.

Saleh SK, Al-Ramadhan G and Faye B. Monitoring of monthly SCC in the she-camel in relation to milking practice, udder status and microbiological contamination of milk. Emirates Journal of Food and Agriculture. 2013; 25:403-408.

Seifu E and Tafesse B. Prevalence and etiology of mastitis in traditionally managed camels (*Camelus dromedarius*) in selected pastoral areas in eastern Ethiopia. Ethiopian Veterinary Journal. 2010; 14:103-113.

Tibary A and Anouassi A. Lactation and udder diseases. International Veterinary Information Service. Reproductive Disorders in the Female Camelid. 2000.

Tinggren S. Udder health inflammatory markers in camel milk (*Camelus dromedarius*) and milk yield. Master in Animal Science, SLU, Swedish University of Agricultural Sciences. 2019; 1-90.

Toroitich KC, Gitau GK, Kitala PM and Gitao GC. The prevalence and causes of mastitis in lactating traditionally managed one-humped camels (*Camelus dromedarius*) in West Pokot County, Kenya. Livestock Research for Rural Development. 2017; 29(4) (www.lrrd.org/lrrd29/4/gita29062.html)

Wanjohi M, Gitao CG and Bebora L. Subclinical mastitis affecting hygienic quality of marketed camel milk from North eastern Province, Kenya. Microbiology Research International. 2013; 1(1):6-15.

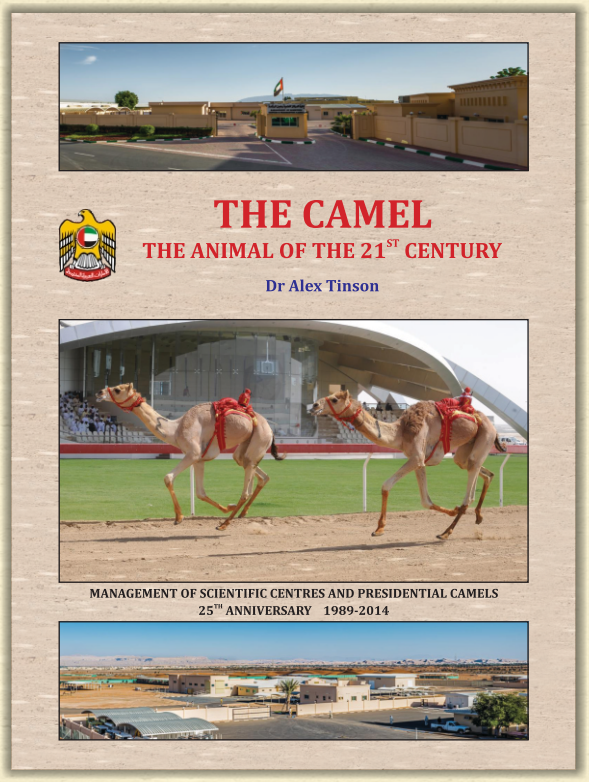
Werenry U, Fischbach ST, Kletzka S Johnson B and Jose SH. Evaluation of some camel milk parameters used in mammary health. Journal of Camel Practice and Research. 2008; 15:49-53.

Woubit S, Bayleyegn M, Bonnet P and Jean-Baptiste S. Camel (*Camelus dromedarius*) Mastitis in Borena Lowland pastoral area, southwestern Ethiopia. Revue D'élevage et de Médecine Vétérinaire des Pays Tropicaux. 2001; 54(3-4):207-212.

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 : 10.5958/2277-8934.2021.00050.3 Vol 28 No 3, p 327-336

MOLD CONTAMINATION AND TOTAL AFLATOXINS IN CHILLED MUSCLE AND EDIBLE OFFAL OF CAMEL (*Camelus dromedarius*): A STUDY OF THEIR HUMAN DIETARY INTAKE, AND HEALTH RISK ASSESSMENT

Waleed Rizk El-Ghareeb[1], Ahmed Aljazzar[2], Wageh Sobhy Darwish[3] and Sherief M. Abdel-Raheem[1]

[1]Department of Public Health, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Al-Ahsa, 31982, Saudi Arabia

[2]Department of Pathology, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Al-Ahsa, 31982, Saudi Arabia

[3]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt

ABSTRACT

Camel meat and edible offal are among the emerging sources of animal-derived protein worldwide, particularly in Saudi Arabia. The objectives of the present study were first to investigate the mold contamination (count and identification) of the camel meat (round muscle), and edible offal (liver, kidney, neck muscles, and masseter muscles). Second, to estimate the total aflatoxins levels (AFTs) by the use of VICAM AflaTest. Third, to calculate the dietary intakes and potential health risks of the total AFTs among the Saudi population. The public health significance of the isolated mold genera and AFTs was additionally discussed. Mycological examination of the examined samples revealed mold contamination of the examined samples at 30% (muscle), 40% (liver), 30% (kidney), 70% (neck muscles), and 70% (masseter muscles). Identification of the isolated molds revealed detection of *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Mucor* spp. and *Fusarium* spp. at variable rates. In addition, 25%, 25%, 45%, and 25% of the examined camel liver, kidney, neck muscles, and masseter muscles, respectively were found contaminated with AFTs. The liver had significantly the highest average residual contents of AFTs (5.80±0.48 µg/kg). Calculation of the daily intake and margin of exposure of AFTs due to consumption of camel's edible offal revealed a high potential cancer risk. Therefore, it is highly recommended to reduce the daily intake of the camel's edible offal among Saudi consumers. To the best of our knowledge, this is the first report to investigate the mold contamination and aflatoxin content in the camel edible offal.

Key words: Camel, edible offal, mold, risk assessment, total aflatoxin

Camel meat and edible offal are among the emerging sources of animal-derived protein, particularly in the Middle East, and China. The camel meat industry is increasing worldwide and represents about 0.7% of the total world meat production (Darwish *et al,* 2010; El-Ghareeb *et al,* 2019; Tang *et al,* 2020). Camel meat is gaining its popularity because of its high content of amino acids, polyunsaturated fatty acids, minerals and vitamins (Kadim *et al,* 2008; Tegegne *et al,* 2019). Edible offal (such as neck muscles, masseter muscle, liver, and kidney) is a popular food in the Middle East due to its specific aroma and flavour, low cost compared to meat, and high nutritive value (Tang *et al,* 2020). Therefore, ensuring the safety and wholesomeness of such meat sources is of high significance for the food safety and public health sectors.

Mold contamination of the camel meat and edible offal reflects the sanitary status and the hygienic measures adopted during slaughtering, dressing, evisceration, transportation, and chilling or freezing of camel carcasses. Mold spores are widely spread in the environment including animal excreta, air, water, and soil. Operators' hands, equipment, and utensils are also among the potential sources for mold contamination of the meat and meat products. The occurrence of fluctuation chilling and freezing temperatures is one factor that favours mold growth. Occurrence of mold contamination of a certain food matrix, particularly of the aflatoxigenic strains, affects both the quality and safety of such food (Aberle *et al,* 2001; Darwish *et al,* 2016). There is limited information about the mycological status of camel meat and offal in the Middle East, and particularly in Saudi Arabia.

SEND REPRINT REQUEST TO WALEED RIZK EL-GHAREEB email: welsaid@kfu.edu.sa

Aflatoxins (AFTs) are secondary metabolites produced by some fungal species, such as *Aspergillus flavus* and *Aspergillus parasiticus* (Alcaide-Molina *et al*, 2009). Contamination of the meat and edible offal with AFTs might start during the animal's life time via ingestion of contaminated feed and water or as metabolites because of the growth of certain fungi on the meat substrate. Then, AFTs find their way into the human body via contaminated foods, leading to several health adverse effects (Darwish *et al*, 2014). AFTs are known for their mutagenicity, carcinogenicity, particularly, hepatocellular carcinoma, and immunosuppressive effects (Abd-Elghany and Sallam, 2015). However, there is no information available about the contamination of the camel meat and edible offal with AFTs and their health risk assessment in Saudi Arabia.

Given the preceding facts, the current investigation's objectives were to first investigate the mold contamination status of chilled camel meat (round muscle) and edible offal (neck muscles, masseter muscles, liver, and kidney) retailed in Saudi meat markets by determining total mold counts and identifying the prevalent mold genera. Secondly, estimation of the total AFTs in the examined samples was followed. Thirdly, calculation of the dietary intakes and potential health risks associated with the detected levels of AFTs was performed. In addition, the public health significance of the isolated mold genera and AFTs was discussed.

Material and Methods

All experiments were conducted according to the guidelines of King Faisal University, Saudi Arabia. All used chemicals were of the highest available quality.

Collection of Samples

A hundred samples including round muscle, neck muscles, masseter muscles, liver, and kidney (n = 20 each, each sample weighs 100 g) were collected chilled from butchery shops and grocery stores at Al-Ahsa governorate, Saudi Arabia during the period of May to December 2020. The collected samples were transferred cooled directly with undue delay to the laboratory for mycological examination.

From May to December 2020, a hundred samples of round muscle, neck muscles, masseter muscles, liver, and kidney (n = 20 each, each sample weighing 100 g) were collected chilled from butchery shops and grocery stores in Al-Ahsa governorate, Saudi Arabia. The collected samples were immediately cooled and transported to the laboratory for mycological examination.

Organoleptic examinations

Organoleptic examination for the examined samples was conducted using the method of Varnam and Sutherland (1995). All selected samples showed fresh odour, firm in consistency and brick red colour for muscles, dark brownish for the liver, and brown for the kidney.

Preparation of samples

Samples were prepared according to Vanderzant and Splittstroesser (2001). In short, 25 g of each sample was collected under aseptic precautions, first homogenised in 225 ml of sterile buffered peptone water 0.1% (LAB104, LAB M, UK) for 2 min at 2000 rpm using sterile meat homogeniser (type M-p3-302, mechanic, Precyzina, Poland). Then, ten-fold decimal serial dilutions were prepared.

Determination of total mold count (TMC)

Total mold counts (TMC) were determined by the pour plate technique using both malt extract agar media for ordinary molds and Czapeck-Dox agar with 5% NaCl for xerophilic molds (Oxoid, Basingstoke, UK) followed by incubation in the dark at 25ºC for 5-7 days. During the incubation time, the plates were examined daily for mold growth. Estimation of TMC was obtained by direct counting of the cultured plates using the colony counter (Vanderzant and Splittstroesser, 2001).

TMC/g = average No. of colonies × reciprocal of the dilution counted colonies expressed as log 10 cfu/g.

Identiication of the isolated molds

Identification of molds was conducted according to the protocol of Pitt and Hocking (2009) using the macroscopical and microscopical characteristics of the mold colonies. The mold cultures were examined daily for the rate and pattern of growth during the incubation period. The consistency of the surface growth and folding, the colony margins, and the surface and reverse pigmentation were observed. Both the surface and backside of the colonies were examined. The prevalence rate and relative density (RD %) were calculated according to Pacin *et al* (2003).

RD % = number of samples with genus or species/ total number of isolates * 100

Molecular identification of the isolated molds

Molecular identification by sequencing the ITS regions of fungal DNA was performed

for some molds to avoid any uncertainty in mold identification. Fungal DNA was extracted by Cetyl trimethylammonium bromide (CTAB) DNA extraction protocol (Murray and Thompson, 1980). The fungus-specific universal primers ITS1 (5′-TCCGTAGGT-GAACCTGCGG-3′) and ITS4 (5′-GCATATCAATAAGCGGAGGA-3′) were used to amplify genes encoding the ITS region according to White *et al* (1990). Sequencing was performed using an AB1 Prism automated DNA sequencer (Applied Biosystems, Foster city, CA). Big Dye Terminator cycle sequencing kit (version 3.1; Applied Biosystems) was used following the manufacturer's manual on both strands with the same primers. Sequence comparisons were performed using the basic local alignment search tool (BLAST) in GenBank (www.ncbi.nlm.nih.gov/blast).

Estimation of total aflatoxins levels

Total AFTs was estimated using Series-4EX Fluorimeter (VICAM, Milford, USA) according to previous methods (El-Ghareeb *et al,* 2013; Abd-Elghany and Sallam, 2015). In brief, 25 g of each muscle and offal sample and 5 g NaCl were mixed and blended in 100 mL methanol: water (4:1 v/v) at a high speed for 3 min. The mixture was then diluted 4 times in DDW and followed by filtration. 4 mL of the filtrate was passed through AflaTest®-P affinity column at a rate of 2 drops/s. The elution of the AFTs from the affinity column was done using methanol at a rate of 1 drop/s. One ml of the sample elute was collected in a glass cuvette (VICAM part # 34000). AflaTest® Developer (1 mL) was then added and mixed well with the sample elute. Then, the cuvette was placed in the calibrated fluorimeter. The limit of detection (LOD) for meat and offal is 0.1 µg/kg.

Dietary intakes:

Calculation of the estimated daily intakes (EDI) for AFTs was done using the equation described by US Environmental Protection Agency) (USEPA, 2010):

EDI = C * FIR / BW

Where EDI is based on µg/kg/day for AFTs; C is the concentration of AFTs (µg/kg); FIR is the meat ingestion rate in Saudi Arabia. The meat ingestion rate in Saudi Arabia is 139 g/day for meat and offal (Adam *et al,* 2014). Body weight (BW) was set at 70 kg and 30 kg for Saudi adults and children, respectively (El-Ghareeb *et al,* 2019). The residual contents of AFTs were compared with the established MPL for AFTs in meat (4 µg/kg) (European Commission, 2006).

Health risk Assessment

The margin of exposure (MOE) approach (EFSA, 2005; FAO/WHO, 2006) was used for the assessment of the cancer risk among the Saudi population. MOE is the ratio of the benchmark dose that causes a 10% increase in cancer incidence in fisher rats (BMDL10) to the average level of total intake in humans. The BMLD10 for AFTs was estimated to be 250 ng/kg body weight/day. MOE was calculated according to Benford *et al* (2010) from the following equation:

MOE = BMDL10/EDI

MOE values lower than 10000 represent a major health concern (EFSA, 2020)

Statistical analysis

Mold counts were converted into log 10 colony forming units per g (log 10 cfu/g). All values are expressed as means ± SE. Statistical significance was evaluated using one way analysis of variance (ANOVA), followed by the Tukey–Kramer HSD post hock test. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

Results and Discussion

Prevalence of different mold genera in camel meat and edible offal

Organoleptic examination of the collected samples in the present study revealed that all samples had normal sensory parameters (Data are not shown). Mycological examination revealed that the prevalence rates of mold contamination among the examined samples were 30% (muscle), 40% (liver), 30% (kidney), 70% (neck muscles), and 70% (masseter muscles) (Fig 1A). Neck muscles had the highest TMC among the examined samples. The average TMC in the examined samples were 2.04 ± 0.12, 2.62 ± 0.11, 2.42 ± 0.12, 3.19 ± 0.16, and 2.80 ± 0.19 log 10 cfu/g in the examined camel round, liver, kidney, neck muscles, and masseter muscles, respectively (Fig 1B). Likely, Nasser (2015) reported that 70% of the examined canned meat products sold in Riyadh, Saudi Arabia were contaminated with molds. Besides, Darwish *et al* (2016) recorded high mold counts ranging between 2.69-3.60 log cfu/g in frozen chicken meat and giblets including liver and kidney. Mold contamination of camel meat was also reported by Tegegne *et al* (2019) who recorded a mold contamination rate of 8.57% in meat samples collected from Jigjiga municipal abattoir and retail houses in Ethiopia, with an average total mold and yeast count of 4.95 ± 0.07-log 10 cfu/g.

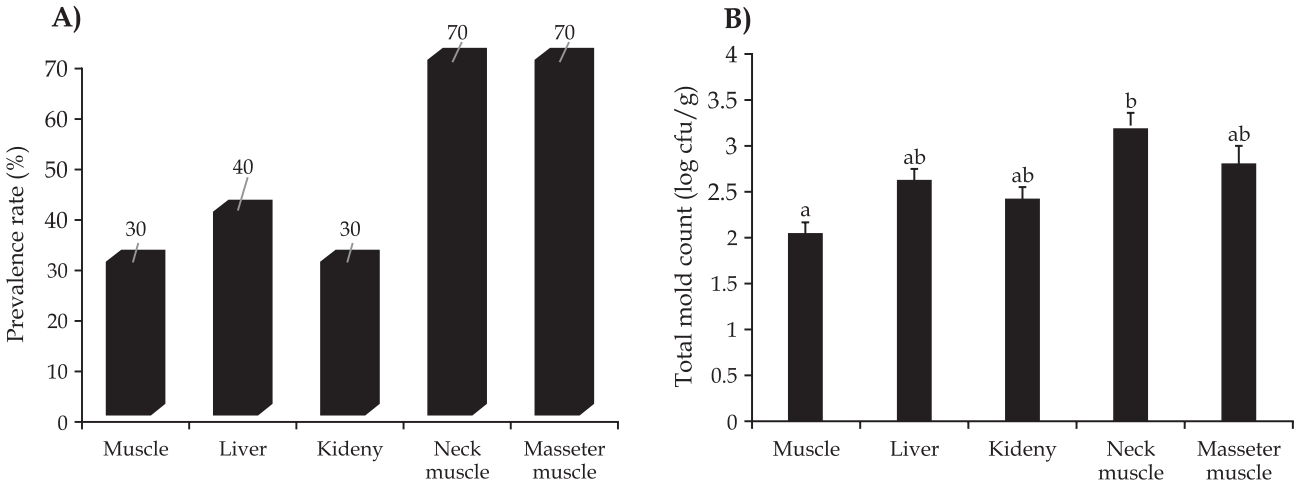


Fig 1. A) Prevalence (%) of mold contamination; B) Total mold count in camel meat and edible offal: Values represent means ± SE (Log 10 cfu/g). Columns with different letters differ significantly at $P < 0.05$.

The high mold contamination, particularly among the offal, reflects unsatisfactory hygienic measures adopted during slaughtering, skinning, evisceration or fluctuation of the chilling temperature during transportation and storage (Mizakova *et al,* 2002). Similar unsatisfactory hygienic measures associated with high mold contamination reaching 100% were recorded in luncheon samples retailed in Assiut city, Egypt (Ismail and Zaki, 1999).

Identification of the isolated molds revealed detection of *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Cladosporium* spp., *Mucor* spp., and *Fusarium* spp., at variable rates (Fig 2A). *Aspergillus* spp., and *Penicillium* spp. were the dominant isolated mold genera in all examined samples. *Aspergillus* spp. could be isolated from the examined round, liver, kidney, neck muscles, and masseter muscles at 20%, 30%, 25%, 55%, and 35%, respectively; while in case of *Penicillium* spp., such prevalence rates were 20%, 30%, 15%, 55%, and 45%, respectively. The calculated relative densities of the identified mold genera were 43.48%, 29.57%, 8.69%, 8.69%, 5.22%, and 4.35% for *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Mucor* spp., *Fusarium* spp., and *Cladosporium* spp., respectively (Fig 2B). Further identification of the isolated *Aspergillus* spp. revealed detection of *A. niger*, *A. flavus*, *A. fumigatus*, *A. ochraceous*, and *A. parasiticus* at variable rates among the examined samples. *A. niger* was the most dominant *Aspergillus* spp. (21.74%) followed by *A. flavus* (17.39%), *A. fumigatus* (1.74%), *A. parasiticus* (1.74%), and *A. ochraceous* (0.87%), respectively (Fig 2C). It notes worthy to confirm that neck muscles had the highest mold contamination rate which could be attributed to the presence of high residual blood from the slaughtering process, and it is known that blood is an ideal medium for growth and multiplication of microorganisms as it is rich in nutrients, high pH (~6.0), and high-water activity (~0.97), and difficult in preservation (Pereira *et al,* 2015). The obtained results in the current study are consistent with the findings of Pitt and Hocking (2009) which indicated that the common isolated mold genera from retailed meats were Aspergillus, Penicillium, Alternaria, Cladosporium, Mucor and Rhizopus. In addition, Nasser (2015) demonstrated that *Aspergillus* spp., and *Penicillium* spp. were the dominant mold genera isolated from canned meat products retailed in Saudi Arabia. Besides, *Aspergillus* spp., and *Penicillium* spp. were dominant molds isolated from frozen chicken giblets (Darwish *et al,* 2016), and chicken meat products (Habashy *et al,* 2019) retailed in Egypt. The high prevalence of *Aspergillus* spp., and *Penicillium* spp. is possibly because of their abilities to grow over a wide range of pH from 2 to 11, over a broad array of water activity (0.62 to 0.99), over a temperature range from 10 to around 60°C, and over a wide range of nutrient limitations (Pitt and Hocking, 2009).

Mold growth in meat and meat products has serious implications for the product safety, quality, marketability, and for public health. Such molds might introduce some secondary metabolites to the contaminated products. Table 1 summarises the potential health hazards and the possible secondary metabolites secreted by molds such as aflatoxins, ochratoxins, citrinin, kojic acid, asperotoxin, cyclopiazonic acid, sterigatocystin, and gliotoxin for *Aspergillus* spp., (Bennett, 1980; Denning *et al,* 2003; Darwish *et al,* 2014). *Penicillium* spp. produce toxic metabolites such as cyclopiazonic acid (organ damage in mammals), meleagrin (mutagenic), mycophenolic

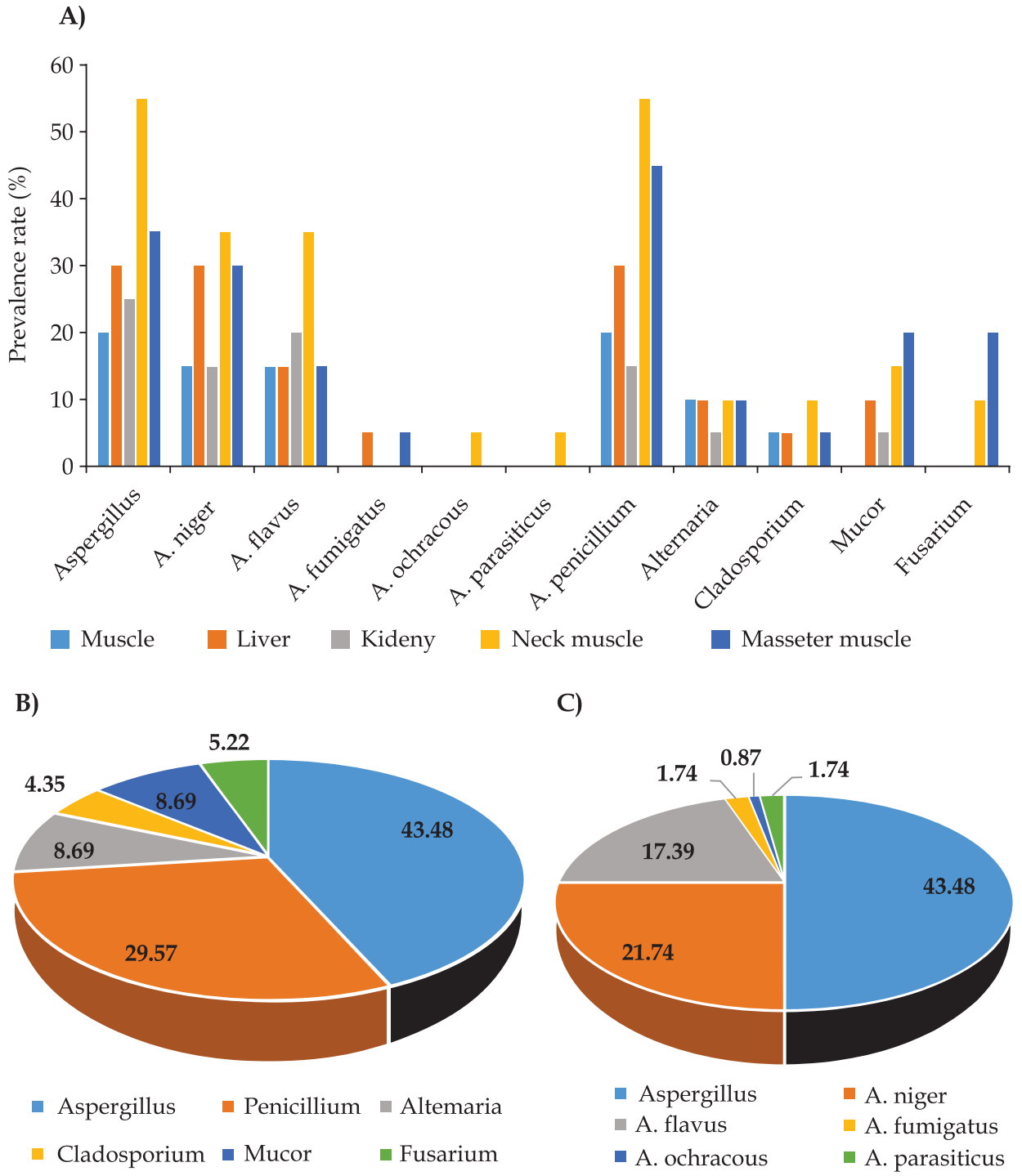


Fig 2. A) Prevalence rate (%); B) Relative density (%) of different mold genera; C) Relative density (%) of the identified *Aspergillus* spp. in the examined camel meat and edible offal.

acid (immunosuppressive), penitrem A (tremorgenic), roquefortine C (neurotoxic), rugulovasine A (Anti-hypotensive), terrestric acid (cardiotoxic) (Pitt and Hocking, 2009). While other identified molds might cause allergic reactions, pneumonia, and intoxications (Schoch *et al*, 2006; De Lucca, 2007; Patriarca *et al*, 2014; Tupaki-Sreepurna and Kindo, 2018).

Detection of aflatoxins in the camel offal, and their health risk assessment

Aflatoxins are classified as mutagenic and carcinogenic food-chemical contaminants that produce several fungal species and might contaminate meat and edible offal directly via ingestion of AFTs-contaminated feed and water or as secondary

Table 1. Public health hazards of the isolated molds.

Mold spp.	Public health hazards	Reference
A. niger	Allergic reactions, pulmonary aspergillosis Produce toxic metabolites such as oxalic acid, kojic acid, and malformins	Bennett, 1980
A. flavus	Aspergilloma, allergic bronchopulmonary aspergillosis, Craniocerebral aspergillosis Produce toxic metabolites like aflatoxins, aspergillic acid, kojic acid, asperotoxin, and sterigatocystin	Denning *et al,* 2003
A. fumigatus	Aspergillosis, aspergilloma and allergic reactions Produce toxic metabolite called gliotoxin	Chakrabarti *et al,* 2002
A. ochraceous	Produce toxic metabolites like ochratoxin A and citrinin	Darwish *et al,* 2014
A. parasiticus	Produce toxic metabolites like AFTs	Darwish *et al,* 2014
Penicillium spp.	Produce toxic metabolites such as cyclopiazonic acid (organ damage in mammals), meleagrin (mutagenic), mycophenolic acid (immunosuppressive), penitrem A (tremorgenic), roquefortine C (neurotoxic), rugulovasine A (Anti-hypotensive), terrestric acid (cardiotoxic)	Pitt and Hocking, 2009
Cladosporium spp.	Allergic reactions	Schoch *et al,* 2006
Fusarium spp.	Fusariosis, and peritoneal dialysis	Tupaki-Sreepurna and Kindo, 2018
Alternaria spp.	Alternaria toxins, and genotoxicity	Patriarca *et al,* 2014
Mucor spp.	Allergic reactions, pneumonia	De Lucca, 2007

metabolites for the growth of the aflatoxigenic molds (Darwish *et al,* 2014; Nishimwe *et al,* 2019; van der Fels-Klerx *et al,* 2018). The results in Fig 3A show that AFTs contaminated 25%, 25%, 45%, and 25% of the examined camel liver, kidney, neck muscles, and masseter muscles. The liver had the highest average residual content of AFTs (5.80±0.48 µg/kg), followed by the kidney (4.60±0.37 µg/kg), neck muscles (2.49±0.31 µg/kg), and masseter muscles (1.90±0.20 µg/kg), respectively (Fig 3B).

Drawing of scatter plots between the mold contamination and detection of AFTs revealed insignificant positive correlation only in the liver (r2 = 0.33, P = 0.15), with no clear correlations in the other examined samples (Fig 4). These results suggest a pre-slaughter exposure of the camel to AFTs-contaminated feed with a subsequent dissemination of AFTs to liver, kidney, neck muscles, and masseter muscles rather than a post-slaughter contamination of the examined samples with AFTs from the existing mold spores, possibly because of the short time of exposure of the samples to the mold contamination. Contamination of meat and meat products with AFTs is recorded worldwide, such as in retailed meat products (Abd-Elghany and Sallam, 2015), chicken meat and giblets in Egypt (Darwish *et al,* 2016), liver and intestine of ducks in central Thailand (Tulayakul *et al,* 2018), and in processed meat products in Saudi Arabia (Elzupir and Abdulkhair, 2020).

Ingestion of foods contaminated with AFTs represents a significant public health concern as the exposure to even repetitive small concentrations of AFTs is highly associated with liver damage, mutagenesis, and carcinogenesis (American Cancer Society, 2011). The recorded concentrations of the total AFTs in the present study revealed that 15%, 15%, and 5% of the examined liver, kidney and neck muscle samples exceeded the established MPL for AFTs (4 µg/kg) (European Commission, 2006). The EDI values of AFTs among Saudi adults and children via ingestion of AFTs-contaminated camel's offal were further calculated (Table 2). The obtained results indicated that EDI values (ng/day) for total AFTs were 11.52 (adults) and 26.87 (children) via consumption of camel's liver,

Table 2. Estimated daily intake and margin of exposure of AFTs among Saudi population.

	%	EDI		MOE	
		Adults	Children	Adults	Children
Muscle	0	NA	NA	NA	NA
Liver	15	11.52	26.87	21.71	9.30
Kidney	15	9.13	21.31	27.37	11.73
Neck muscles	5	4.95	11.56	50.46	21.63
Masseter muscles	0	3.77	8.80	66.26	28.40

%: refers to percentage of samples exceeding the maximum permissible limits of total AFTs (4 µg/kg)
EDI: Estimated daily intake MOE: Margin of exposure
NA: Not applicable

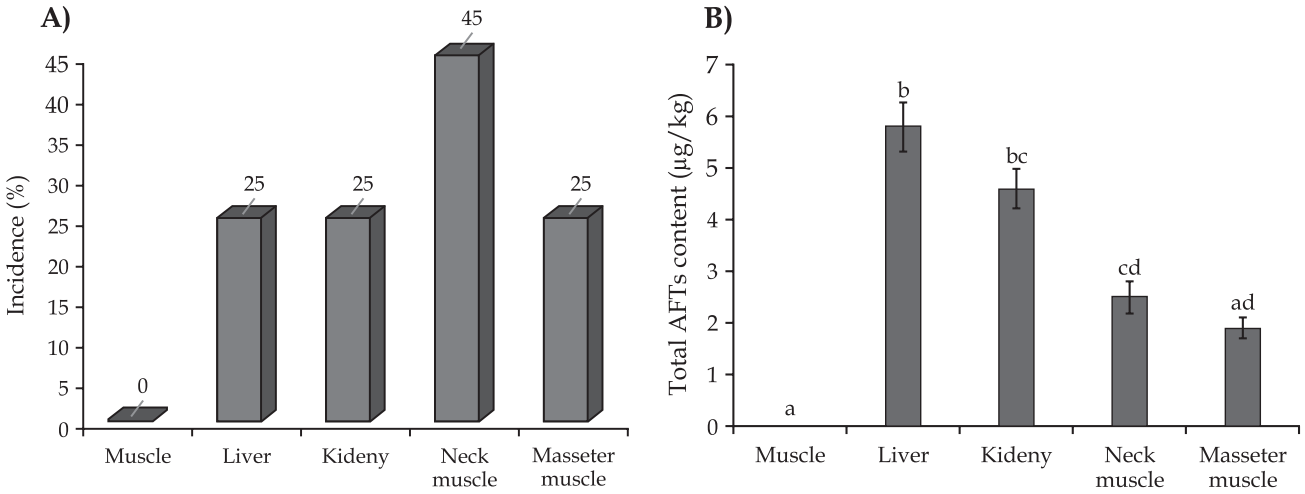


Fig 3. A) Prevalence (%); B) Total aflatoxin residues in the examined camel meat and edible offal: Total aflatoxin residues (µg/kg) in the examined samples. Values represent means ± SE. Columns with different letters differ significantly at $P < 0.05$.

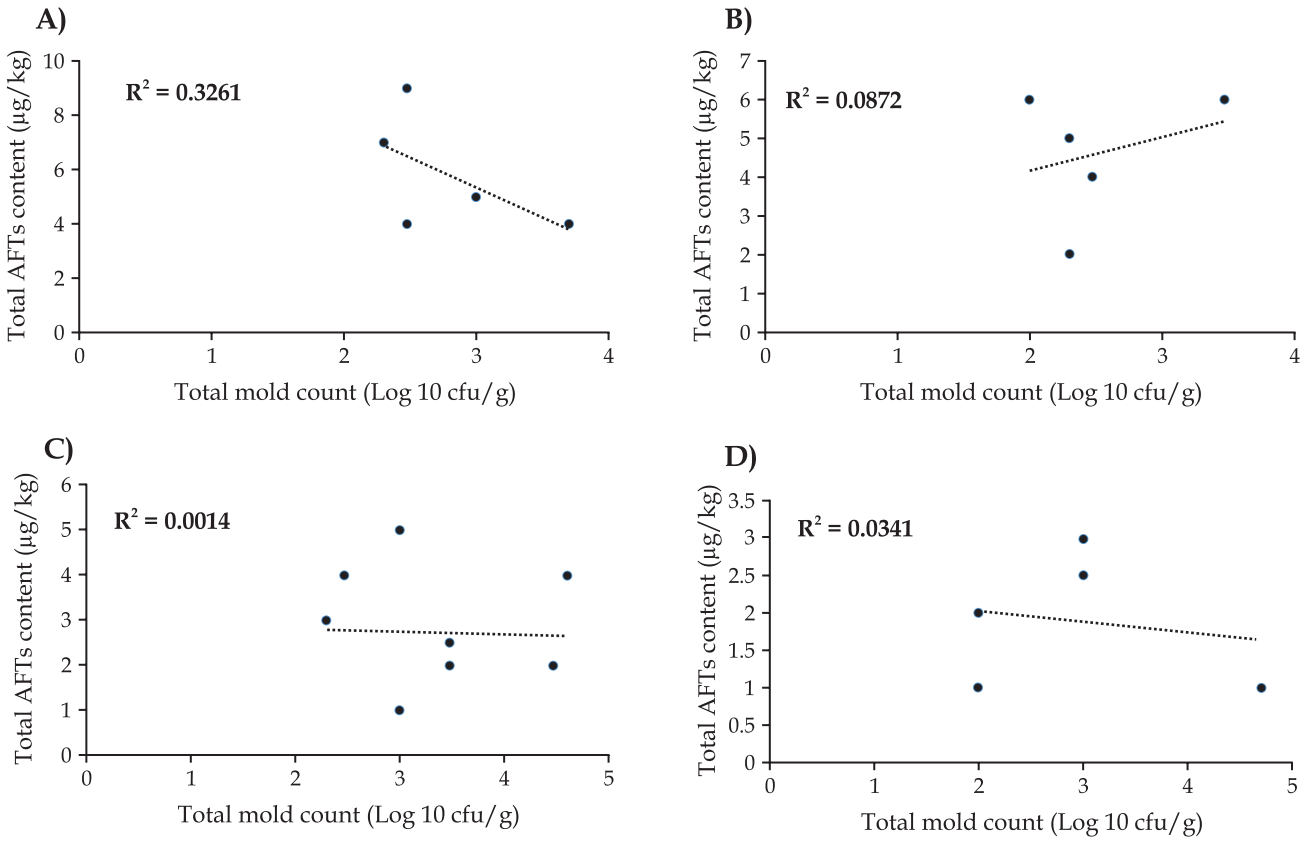
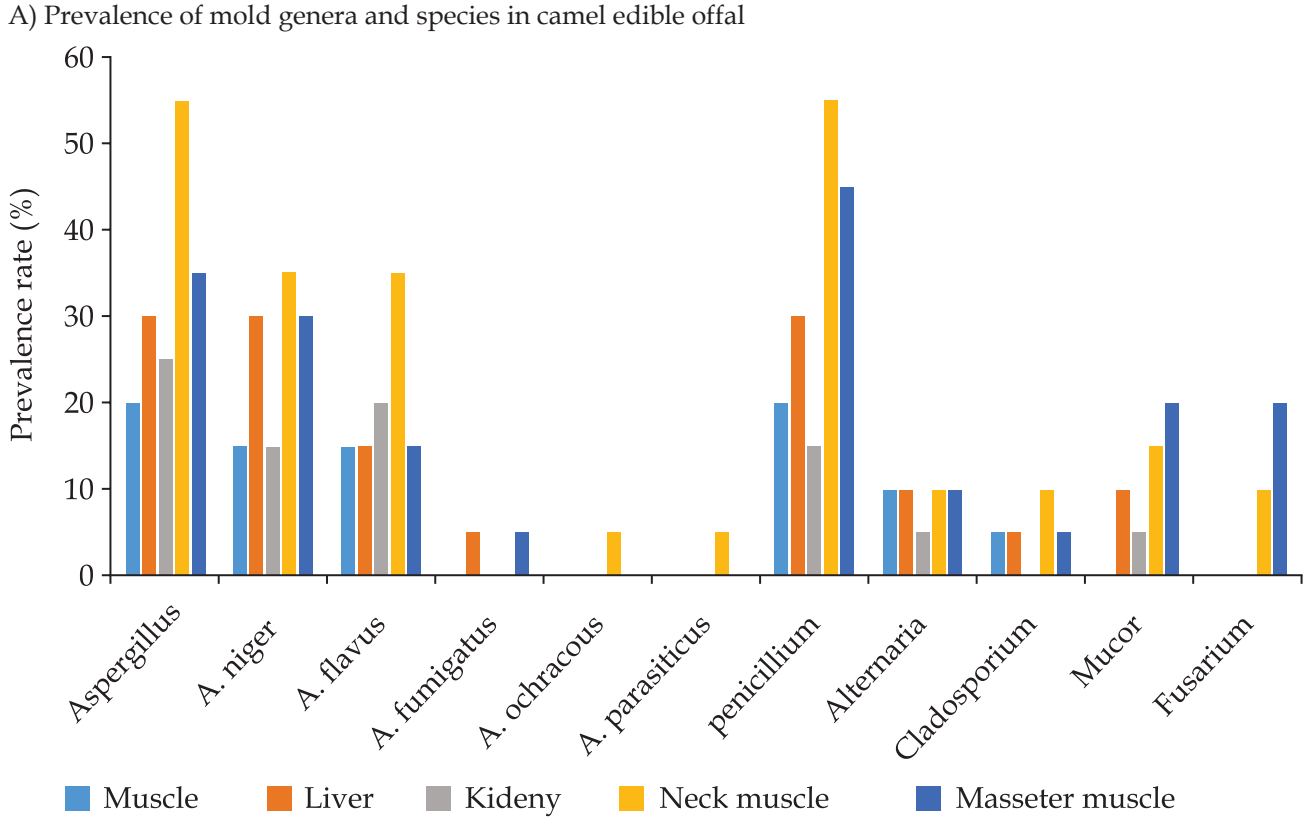


Fig 4. Pearson's correlations between total mold count and total aflatoxin content in the examined camel meat and edible offal: Scatter plots were drawn between TMC and total AFTs content in the examined camel's A) liver, B) kidney, C) neck muscles, D) masseter muscles.

9.13 (adults) and 21.31 (children) via consumption of camel's kidney, 4.95 (adults) and 11.56 (children) via consumption of camel's neck muscles, 3.77 (adults) and 8.80 (children) via consumption of camel's masseter muscles, respectively. To estimate the cancer risk for AFTs among the Saudi populations, the MOE approach was followed. MOE was considered as a reliable, accurate, and informative approach to assess the cancer risk for AFTs (EFSA, 2005; FAO/WHO, 2006). In the present study, MOE values of AFTs among adults were 21.71 (liver), 27.37 (kidney), 50.46 (neck muscles), and 66.26 (masseter muscles); while these values for children were 9.30 (liver), 11.73 (kidney), 21.63 (neck muscles), and 28.40 (masseter muscles). MOE values



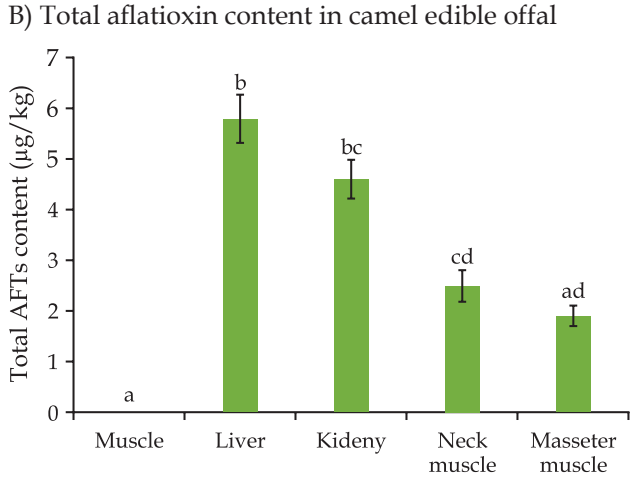


Fig 5. Graphical abstract

lower than 10000 represent a major health concern (EFSA, 2020). Therefore, consumption of camel offal in Saudi Arabia might possess a potential cancer risk. Similarly, Elzupir and Abdulkhair (2020) calculated MOE values for AFTs in the processed beef meat and poultry meat products. Their obtained values were 175, and 311 for beef meat and poultry meat products, respectively suggesting a public health concern among the Saudi population. To the best of our knowledge, this is the first report to investigate mold contamination and AFTs formation in the camel meat and edible offal in an Asian or African country.

Conclusions

The obtained results in the present study revealed contamination of the camel meat and edible offal including liver, kidney, neck muscles, and masseter muscles with mold and AFTs. *Aspergillus* spp., and *Penicillium* spp. were the most dominant mold genera identified in the present study. Calculation of the daily intakes and MOE values for total AFTs revealed a high risk for the exposure of the Saudi population to AFTs-induced cancer risks. Therefore, strict hygienic precautions should be taken during handling of camel meat and offal during handling, processing, and storage. Reduction of the daily intake of the edible camel offal is highly recommended.

Acknowledgements

The authors acknowledge the Deanship of Scientific Research at King Faisal University for the financial support under the research group support track (Grant No. 1811022).

Declarations

Compliance with ethical standards

Ethical Approval: This study was conducted according to the guidelines of King Faisal University, Saudi Arabia

Consent to Participate: All authors approved to participate in this research work and in the manuscript.

Consent to Publish: All authors approved this manuscript to be published.

Availability of data and materials: All data and materials will be available upon request.

Competing Interests: The authors declare that they have no conflict of interest.

Funding: This study was supported by the Deanship of Scientific Research at King Faisal University for the financial support under the research group support track (Grant No. 1811022).

Authors Contributions: All authors contributed to the study conception and design. Material preparation and sample collections were performed by Waleed Rizk El-Ghareeb and Sherief M. Abdel-Raheem. Chemical analysis and data management were conducted by Wageh Sobhy Darwish, Waleed Rizk El-Ghareeb, and Ahmed Aljazzar. The first draft of the manuscript was written by Wageh Sobhy Darwish, Ahmed Aljazzar and Waleed Rizk El-Ghareeb. All authors commented on the previous versions of the manuscript and approved the final version before submission.

References

Abd-Elghany SM and Sallam KI. Rapid determination of total aflatoxins and ochratoxins A in meat products by immuno-affinity fluorimetry. Food Chemistry, 2015; 179:253-256. doi:10.1016/j.foodchem.2015.01.140

Aberle ED, Forrest JC, Gerrard DE and Mills EW. Principles of Meat Science. 4th Ed., Kendall/ Hunt Publishing Co., Dubuque, IA. 2001.

Adam A, Osama S and Muhammad KI. Nutrition and food consumption patterns in the kingdom of Saudi Arabia. Pakistan Journal of Nutrition. 2014; 13:181-190.

Alcaide-Molina M, Ruiz-Jiménez J, Mata-Granados JM and Luque de Castro MD. High through-put aflatoxin determination in plant material by automated solid phase extraction on-line coupled to laser-induced fluorescence screening and determination by liquid chromatography–triple quadrupole mass spectrometry. Journal of Chromatography A. 2009; 1216:1115-1125.

American Cancer Society. Global Cancer Facts and Figs, 2nd ed. American Cancer Society, Atlanta. 2011.

Benford D, Bolger PM, Carthew P, Coulet M, DiNovi M, Leblanc J-C, Renwick AG, Setzer W, Schlatter J and Smith B. Application of the Margin of Exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. Food and Chemical Toxicology. 2010; 48:S2-S24.

Bennett JE. Aspergillosis, pp. 742-744. In K.J. Isselbacher, R.D. Adams, E. Braunwald, R.G. Petersdorff, and J.D. Wilson (eds.), Harrison's Principles of Internal Medicine, McGraw-Hill, New York. 1980.

Chakrabarti A, Sethi S, Raman DS and Behera D. Eight year study of allergic bronchopulmonary aspergillosis in an Indian teaching hospital. Mycoses. 2002; 45:295-299.

Darwish WS, Ikenaka Y, Nakayama SM and Ishizuka M. An overview on mycotoxin contamination of foods in Africa. Journal of Veterinary Medical Science 2014; 76(6):789-797. doi:10.1292/jvms.13-0563.

Darwish WS, Morshdy AE, Ikenaka Y, Ibrahim ZS, Fujita S and Ishizuka M. Expression and sequence of CYP1A1 in camel. Journal of Veterinary Medical Science. 2010; 72:221-224.

Darwish W, Bayomi RM, El-Moaty AM and Gad TM. Mould contamination and aflatoxin residues in frozen chicken meat-cuts and giblets. Japanese Journal of Veterinary Research. 2016; 64(Supplement 2):S167-S171.

De Lucca AJ. Harmful fungi in both agriculture and medicine. Revista Iberoamericana de Micología. 2007; 24(1):3-13.

Denning DW, Riniotis K, Dobrashian R and Sambatakou H. Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature and review. Clinical Infectious Diseases. 2003; 37:S265-S280.

El-Ghareeb WR, Darwish WS and Meligy AMA. Metal contents in the edible tissues of camel and sheep: human dietary intake and risk assessment in Saudi Arabia. Japanese Journal of Veterinary Research 2019; 67(1):5-14.

El-Ghareeb WR, Darwish WS, Tharwat AE, El-Desoky KI and Hussein MA. Aflatoxin and ochratoxin A residues in some meat additives. Life Science Journal. 2013; 10:3411-3417.

Elzupir AO and Abdulkhair BY. Health risk from aflatoxins in processed meat products in Riyadh, KSA. Toxicon. 2020; 181:1-5. doi:10.1016/j.toxicon.2020.04.092

European Commission (EC). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff. Official Journal of European Union. 2006.

European Food Safety Authority (EFSA). Scientific opinion on on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. European Food Safety Association Journal. 2005; 282:1e31.

European Food Safety Authority (EFSA). Opinion of the scientific committee on Risk assessment of aflatoxin in foods. European Food Safety Association Journal. 2020; 18(3):e06040.

FAO/WHO. Evaluation of Certain Food Contaminants. Sixty-fourth Report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organisation, Geneva (WHO Technical Report Series, No. 930. 2006; http://hqlibdoc.who. int/trs/WHO_TRS_930_eng.pdf.

Habashy AHA, Darwish WS, Hussein MA and El-Dien WMS. Prevalence of different mould genera in meat and meat products with some reduction trials using essential oils. Advances in Animal and Veterinary Sciences. 2019; 7(s2):79-85. http://dx.doi.org/10.17582/journal.aavs/2019/7.s2.79.85

Ismail MA and Zaky ZM. Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues. Mycopathologia. 1999; 146:147-154.

Kadim I, Mahgoub O and Purchas R. A review of the growth, and of the carcass and meat quality characteristics of the one-humped camel (*Camelus dromedarius*). Meat Science. 2008; 80(3):555-569.

Mizakova A, Pipova M and Turek P. The occurrence of moulds in fermented raw meat products. Czech Journal of Food Science. 2002; 3:89-94.

Murray MG and Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 1980; 8:4321-4325.

Nasser LA. Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia. Saudi Journal of Biological Sciences. 2015; 22(5):513-20. doi: 10.1016/j.sjbs.2014.08.003.

Nishimwe K, Bowers E, Ayabagabo JD, Habimana R, Mutiga S and Maier D. Assessment of Aflatoxin and fumonisin contamination and associated risk factors in feed and feed ingredients in Rwanda. Toxins (Basel). 2019; 11(5):270.

Pacin AM, Gonzalez HHL, Etcheverry M, Resnik SL, Vivas L and Espin S. Fungi associated with food and feed commodities from Ecuador. Mycopathologia. 2003; 156(2):87-92.

Patriarca A, Vaamonde G and Pinto VF. Alternaria. In Encyclopedia of food Microbiology. 2nd edition, Academic Press. 2014; pp 54-60.

Pereira JA, Dionísio L, Patarata L and Matos TJ. Effect of packaging technology on microbiological and sensory quality of a cooked blood sausage, Morcela de Arroz, from Monchique region of Portugal. Meat Sciences. 2015; 101: 33-41. doi: 10.1016/j.meatsci.2014.09.147.

Pitt JI and Hocking AD. Fungi and Food Spoilage, 3rd Ed. Published by Blackie Academic and Professional Academic Press New York, London. 2009.

United States Environmental Protection Agency (USEPA). Integrated Risk Information System (IRIS). Cadmium (CASRN-7440-43-9) 2010. 2010; http://www.epa.gov/iris/subst/0141.htm

Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, Spatafora JW and Crous PW. A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia. 2006; 98(6):1041-1052.

Tang H, Darwish WS, El-Ghareeb WR, Al-Humam NA, Chen L, Zhong RM, Xiao ZJ and Ma JK. Microbial quality and formation of biogenic amines in the meat and edible offal of *Camelus dromedarius* with a protection trial using gingerol and nisin. Food Science and Nutrition. 2020; 8(4):2094-2101. doi: 10.1002/fsn3.1503.

Tegegne HA, Berhanu A, Getachew Y, Serda B, Nölkes D, Tilahun S and Sibhat B. Microbiological safety and hygienic quality of camel meat at abattoir and retail houses in Jigjiga city, Ethiopia. Journal of Infection in Developing Countries. 2019; 13(3):188-194. doi: 10.3855/jidc.9686.

Tulayakul P, Mingkhwan R, Hananantachai H, Netvichian R, Khaodhiar S and Songserm T. Heavy metal (Cd and Pb) and aflatoxin contamination in tissues and eggs from free grazing ducks and their environment in Central Thailand. Biological Trace Element Research. 2018; 186(2):514-520. doi:10.1007/s12011-018-1321-2

Tupaki-Sreepurna A and Kindo AJ. Fusarium: The versatile pathogen. Indian Journal of Medical Microbiology. 2018; 36(1):8-17. doi: 10.4103/ijmm.IJMM_16_24.

van der Fels-Klerx HJI, Adamse P, Punt A and van Asselt ED. Data analyses and modelling for risk based monitoring of mycotoxins in animal feed. Toxins (Basel). 2018; 10(2):54.

Vanderzant C and Splittstroesser D. Compendium of Methods for the Microbiological Examination of Foods (4th Ed ed.). Washington, DC, USA: American Public Health Association. 2001.

Varnam AH and Sutherland JP. Meat and meat products: Technology, Chemistry and Microbial. 1st Ed. Champman and Hall, London, U.K. 1995.

White TJ, Burns T, Lee S and Taylor J. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Academic Press, New York. 1990.

DOI : 10.5958/2277-8934.2021.00051.5 Vol 28 No 3, p 337-340

CLINICAL MANAGEMENT OF SARCOPTIC MANGE IN DROMEDARY CAMELS REARED UNDER HIGH ALTITUDE COLD DESERT

Achin Arora[1], Vijay K. Bharti[1], Rakesh Ranjan[2] and SS Balaje[1]
[1]DRDO-Defence Institute of High Altitude Research (DIHAR), Leh, Ladakh UT-194101, India
[2]ICAR-National Research Centre on Camel, Bikaner, Rajasthan- 334001, India

ABSTRACT

A total of four adult dromedary camels (2 male and 2 female) inducted at DRDO-Defence Institute of High Altitude Research (DIHAR), Leh were reported with anamnesis of reduced appetite, frequent itching and development of skin lesions. The animals were kept in open and stall fed under high altitude cold desert conditions. Physical examination revealed development of diffused skin lesions like alopecia, numerous small vesicles, erythema and abrasions. Though lesions were diffused, they were more prominent in certain areas like head, neck, inguinal region and brisket. Skin scraping examination revealed presence of *Sarcoptes scabiei* var. *cameli* mite in abundance. The affected animals cured successfully using ivermectin and multivitamin injections and topical application of seabuckthorn (*Hippophae rhamnoides*) pulp, Himax and melathion 0.2% in petroleum jelly base.

Key words: Camel, High-altitude, Sarcoptic-mange, Scabies

Dromedary camel (*Camelus dromedarius*) is largely distributed in arid and semi-arid regions across the world. These are used as pack animals in harsh and hostile terrain of Ladakh, a high altitude region of India, where movement of mechanical transport is not easy, so local people and armed forces use them to carry their load and luggage (Lamo *et al,* 2020). These camels get gradually acclimatised to the prevailing climatic conditions under high altitude cold desert.

Highly prurutic and contagious dermatitis, caused by the mite *Sacroptic scabiei* var *cameli*, can lead to dramatic decline in health and productivity of diseased animal and may even result into death of the affected animal if no treatement is given (Driot *et al,* 2011). The present report describes successful management of sarcoptic mange in dromedary camel that developed one month after their induction to high-altitude cold desert conditions. The disease responded well to the treatment comprising subcutaneous administration of ivermectin in combination with topical application of Himax cream seabuckthorn pulp and melathion in petroleum jelly base.

Materials and Methods

Animal details and clinical observations

Four apparently healthy dromedary camels (2 male and female, each), aged 2-3 years were brought from plain low-altitude (Bikaner, Rajasthan, India) to high-altitude Ladakh (11500 msl), India. Ambient temperature and humidity of study period during June-August (summer months) were 8-26°C and 30-45% RH. Body condition of all four animals were good and after a month of arrival at Leh, all the camels gradually developed symptoms of pruritis, thickening of skin that were more evident in the head, groin, neck, inguinal and brisket region. There was no lesion on the hump region. The lesions were accompanied with alopecia, numerous small vesicles, arrhythmia, and white to grey heavy crusts (Fig 1). The affected camels were slightly anorectic, restless and showed itching and rubbed skin against the wall and iron pole of the shed.

Sample collection

Body weight of affected animals were recorded once at onset of symptoms and thereafter on monthly basis till recovery. Deep skin scrapings were taken from the periphery of skin lesions until bleeding started (Soulsby, 1982). Collected scrapings were transferred to 10% potassium hydroxide solution kept in sterile glass vials and fitted with screw cap. The samples collected were heated till the mixture became homogenised. After cooling the mixture was centrifuged at 1500 RPM/min for 5 minutes. The

SEND REPRINT REQUEST TO VIJAY K. BHARTI email: vijaykbharti@rediffmail.com

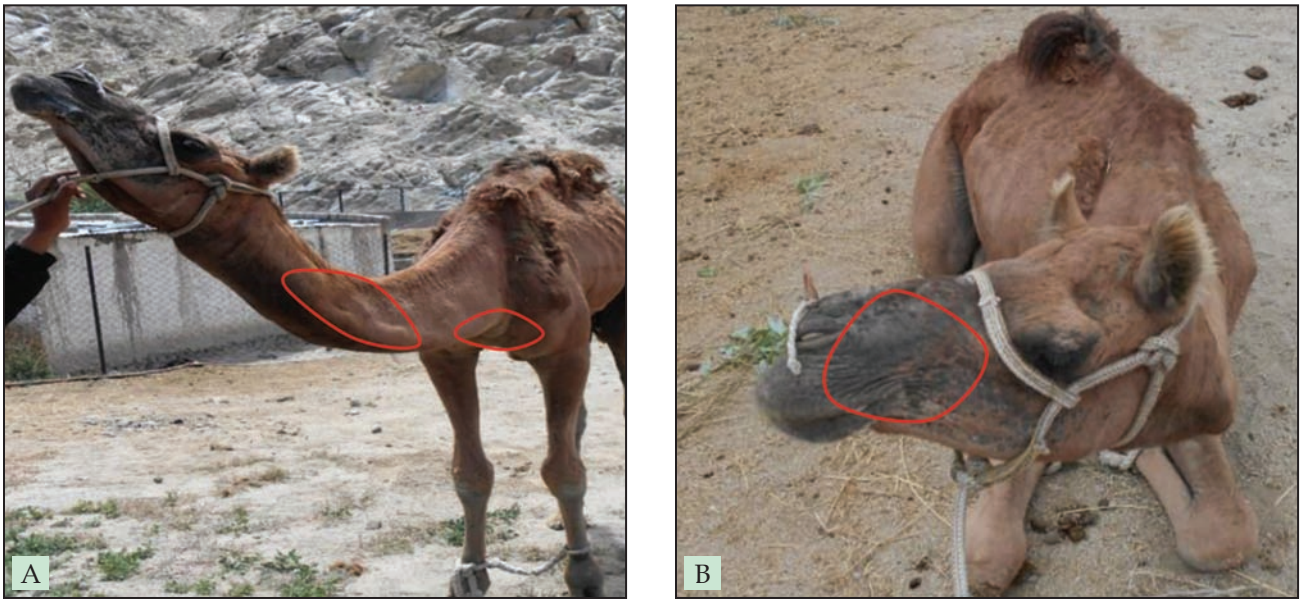


Fig 1. Male dromedary camel with sarcoptic mange infestation (Red colour round marked is affected neck, brisket region, frontal face).

supernant fluid was discarded and the sediment was put over a glass slide and examined under microscope. Blood samples were also collected using vacutainers containing EDTA and various haematological parameters were estimated using Auto-Blood Analyser.

Diagnosis and Treatment

Microscopic examinations of skin scrapings revealed that all the four camels were infested with *Sarcoptes scabiei* var *cameli* mites. The mites were identified on the basis of the characteristic morphological features viz. circular outline with four pairs of short and stumpy legs (Fig 2), the third and fourth pair of legs did not project beyond the body margins (Georgi, 1985; Nayel and Abu-Samra, 1986; Arora, 2003). The PCV, haemoglobin and RBC were within the normal range in all camels, but in one male camel, there was marked eosinophilia and neutropaenia, an indication of an allergic reaction due parasite infestation (Table 1).

All the infected camels were administered with the ivermectin (200µg/kg b. wt. s/c) at an interval of 15 days for about a 3 months period. Apart from this, as soon as the symptoms developed, topical application of a mixture containing Seabuckthorn pulp, Himax cream* Malathion (0.2%) in petroleum jelly once daily was continued for one month. The camels were also fed a routine diet along with Biovet –YC** @ 20 gm in meals once daily for 3 months and intramuscular multivitamin injections on alternate day for 15 days.

Table 1. Haematological parameters of camels affected with sarcoptic mange at high altitude.

Parameters	Animal and sex			
	J-78 (Male)	J-404 (Male)	J-79 (Female)	J-299 (Female)
Hb (gm %)	13.2	15.7	14.0	12.8
Erythrocytes count (million/ mm^3)	7.03	7.23	7.12	6.98
Leokocytes count (no./µl)	8000	15200	14000	11100
PCV (%)	30.20	30.60	31.30	32.40
Neutrophils (%)	28.0	67.0	70.0	68.0
Eosinophils (%)	17.0	4.0	7.0	9.0
Basophils (%)	0.0	0.0	0.0	0.0
Monocyte (%)	10.0	2.0	2.0	3.0
Lymphocytes (%)	45.0	27.0	21.0	20.0

Results and Discussion

All the treated animals showed clinical recovery after 3 months period (Fig 3). Dromedary camels are frequently infested with Sarcoptic mange mites. The clinical disease is characterised by alopecia, pruritis, thickening of skin, erythma and crust formation (Fowler, 1998). Dromedary camels included in the present report were inducted to high altitude cold desert conditions a month ago. The affected animals

* HIMAX Manufactured by Natural Remedies Pvt ltd, Ayurvedic ointment.

** Manufactured by Vetoquinol, Composition–Saccharomyces, Lactobacillus, Propionibacterium freudenreichii, Seaweed Powder.

Fig 2. Sarcoptic mange mite -*Sarcoptes scabiei* var *cameli*.

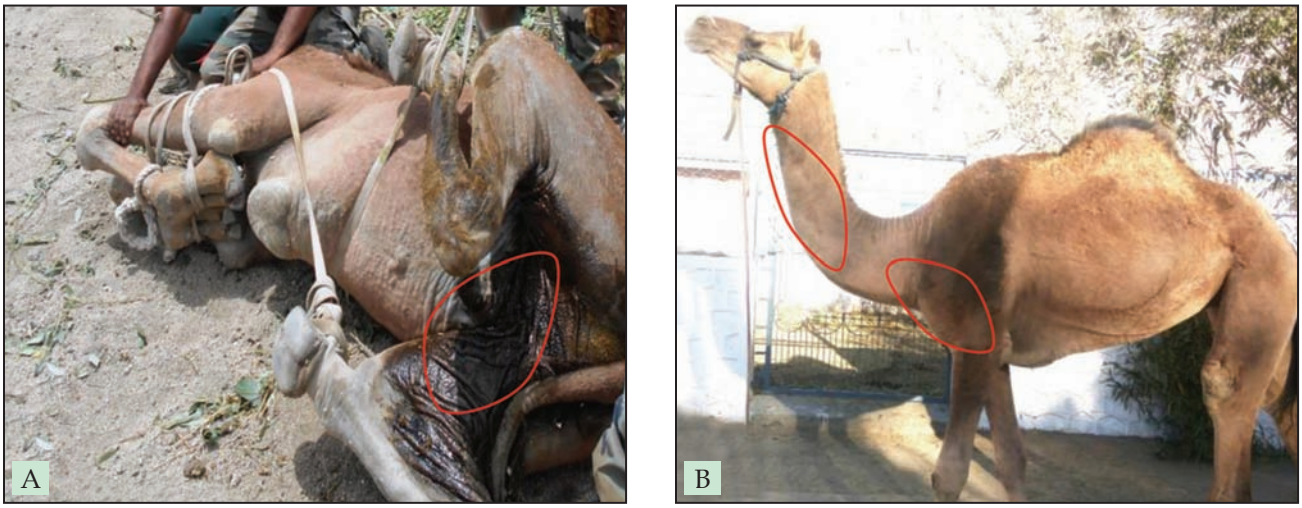


Fig 3. Recovery from Sarcoptic mange after the treatment, A. Red colour round marked showing topical application of medicine, B. Red color round marked is recovered neck, brisket region in male camel at high altitude.

also revealed alopecia, thickening of skin, pruritis alongwith with vesicle formation and erythematic reaction in the vicinity of the lesion which further caused loss of vitality of skin and crust formation. The lesions were found scattered throughout the body, especially in the head, neck, inguinal region, limbs, and the brisket region (Fowler, 2010; Ahmed *et al,* 2020). Treatment with ivermectin for sarcoptic mange (@ 200 µg per kg b.wt) against scabies in the camel was done based on the reports given by Singh *et al* (2001) and Fowler (2010). Multivitamin supplements were also administered as malnutrition and nutritional defienciey (particularly vitamin A deficiency) is reported to predispose the animal for sarcoptic mange infestation (Fassi-Fehri, 1987). As per Zielinska and Nowak (2017), the fruits of seabuckthorn contain flavonoids, catechins and procyanidins, cyclitols, phospholipids, tannins, sugars: galactose, fructose, xylose, and approximately 3.9% organic acids (maleic acid, oxalic acid, malic acid, tartaric acid), phenolic acids, e.g. ferulic acid as well as fatty oil that have a potential anti-allergic action. The leaf extract of seabuckthorn plant has been found reported to have anti-influenza and anti-cancer activities (Enkhtaivan *et al,* 2017). Thus topical application of its fruit pulp appeared to help early recovery from mange in dromedary camels. The therapeutic effect was supported by improvement in skin texture, subsided skin lesion, new hair follicular growth and improved body condition.

Conclusions

A treatment with parenteral administration of ivermectin of along with topical application of a mixture containing seabuckthorn pulp, Himax cream, 0.2% Malathion in petroleum jelly base was found effective and therefore can be recommended for the treatment of Sarcoptic mange in camel under high altitude conditions.

Acknowledgement

All authors acknowledge Director, DIHAR and Defence Research & Development Organization (DRDO) Headquarter, New Delhi, India, for providing financial support and research facility. The authors would like to thank all the technical staff and animal attendents for of Animal Science Division, DIHAR, Leh for their logistic support and assistance during the study.

References

Ahmed MA, Elmahallawy EK, Gareh A, Abdelbaset AE, El-Gohary FA, Elhawary NM, Dyab AK, Elbaz E and Abushahba MFN. Epidemiological and

histopathological investigation of sarcoptic mange in Camels in Egypt. Animals. 2020; 10(9):1485.

Arora BM. Indian Wildlife Diseases and Disorders. Association of Indian Zoo and Wildlife Veterinarians, Bareilly. 2003.

Driot C, Kamili A, Bengoumi M, Faye B, Delverdier M and Tligui N. Study on the epidemiology and histopathology of sarcoptic mange and ringworm in the one-humped camel in south of Moracco. Journal of Camel Practice and Research. 2011; 18:107-114.

Enkhtaivan G, John KMM, Pandurangan M, Hur JH, Leutou AS and Kim DH. Extreme effects of Seabuckthorn extracts on influenza viruses and human cancer cells and correlation between flavonol glycosides and biological activities of extracts. Saudi Journal of Bilogical Sciences. 2017; 24:1646-1656.

Fowler ME. Medicine and surgery of South American Camelids. In: Medicine and Surgery of Camelids, Eds., Fowler ME. 3rd Edn., Iowa State University Press, Blackwell Publishing, USA. 1998; pp 549.

Georgi JR. Parasitology to Veterinarians. W.R.Saunders. London. 1985.

Lamo D, Gahlawa G, Kumar S, Bharti VK, Ranjan P, Kumar D and Chaurasia OP. Morphometric, haematological and physio-biochemical characterisation of Bactrian (*Camelus bactrianus*) camel at high altitude. BMC Veterinary Research. 2020; 16:291.

Nayel NM and Abu-Samra MT. Experimental infection of the one-humped camel (*Camelus dromedarius*) and goats with *Sarcoptes scabiei var. Cameli* and *S. scabiei var. caprae*. British Veterinary Journal. 1986; 142:264-269.

Singh V, Momin RR and Parsani HR. Therapeutic efficacy of doramectin against sarcoptic mange in camel. Journal of Veterinary Parasitology. 2001; 15:75-76.

Soulsby EJL. Helminthes, Arthropods and Protozoa of Domestic Animals. 7th ed. London, UK: Clows limited. 1982; pp 523-530.

Zielińska A and Nowak I. Abundance of active ingredients in sea-buckthorn oil. Lipids in Health and Disease. 2017; 16:95.

DOI : 10.5958/2277-8934.2021.00052.7 *Vol 28 No 3, p 341-348*

DISTRIBUTION AND EXPRESSION PATTERN OF NEUROGLOBIN IN THE BACTRAIN CAMEL BRAIN

James Blackar Mawolo[1], Du Xiaohua[2], Liu Xia[3], Wang Haifang[3] and Haqi Astika Marela[3]

[1,3]College of Life Science and Technology, [2]College of Veterinary Medicine, [3]College of Life Science and Technology, Gansu Agricultural University, Lanzhou City, Gansu Province, People's Republic of China

ABSTRACT

Neuroglobin (Ngb) is a member of the vertebrate globin family involved in cellular oxygen homeostasis and reactive oxygen/nitrogen scavenging. It is an intracellular haemoprotein expressed in the central and peripheral nervous system, cerebrospinal fluid, retina and endocrine tissues. The study examines the distribution of Ngb in the brain of Bactrian Camel and compared the results with published results of yak, and cattle. The immunohistochemical staining method was used to observe the distribution of Ngb in the brain of healthy adult Bactrian camels. Ngb is significantly expressed in all tissues of the telencephalon except the hypothalamus. The cerebellar cortex, hippocampus, amygdala, cerebellum, and corpus callosum recorded the highest expression and each plays an important role in the Bactrian camel. In the Yak and cattle brain, Ngb were scattered in the cerebral cortex and were significantly higher than that in the cerebellar cortex, hippocampus, medulla oblongata, striatum, and olfactory bulb.

Key words: Bactrian camel, cattle, immunohistochemistry, neuroglobin, retina, yak

Neuroglobin (Ngb) is a kind of specific protein involved in the transport of oxygen (Burmester *et al*, 2000) and was found in mammalian brain. Further studies also observed Ngb expression in the retina, nerve system, testicles, and uterus (Geuens *et al*, 2003; Schmidt *et al*, 2003; Gao, 2015). Zhao *et al* (2012) reported that Ngb is highly expressed in the brains of mice with traumatic brain injury (Zhao *et al*, 2012) while in the hypothalamus, amygdala, and pontine tegmental nuclei of human, Ngb was significantly expressed (Hundahl *et al*, 2013). In the pig brain, Ngb levels in the hypothalamus were higher than the frontal cortex. The lowest difference was found in sheep, which showed Ngb expression in the hypothalamus and cerebrum (Fabrizius *et al*, 2016). The expression level of NGB in the brain retina was much higher than in the brain tissue (Wei *et al*, 2010; Anderson, 1968), suggesting that there exists a close relationship between NGB and retinal functions. Studies have shown that Ngb is involved in the elimination of reactive oxygen species (ROS) regulation which may play an important role in the oxygen homeostasis (Greenberg *et al*, 2008). Further research have demonstrated the important functions of Ngb in oxygen supply, anti-oxidative stress, apoptosis and signal transduction. At present, several researches have been conducted on the human, adult sheep, rabbits and rats about Ngb expression (Ran *et al*, 2005; Li *et al*, 2006; Ostoji *et al*, 2008; Ostoji *et al*, 2006; Yang *et al*, 2015a; Yang *et al*, 2015b). Ngb mRNA was detected in all brain especially the peripheral nervous system of rat (Ostoji *et al*, 2006), suggesting that Ngb could serve a neuroprotective role as scavengers of reactive oxygen species and developing therapeutic strategies for treatment of hypoxia-related ocular diseases. Despite all these results, there is no report of Ngb expression in the brain of the Bactrian camel. The current research examined Ngb expression in the brain of healthy adult Bactrian camels by employing Immunohistochemical staining procedures and IPP analysis. The researchers compared the existing results with those of the yak and cattle.

Materials and Methods

Animals and setting

The Animal Ethics and Welfare Committee of Gansu Agricultural University in October of 2019 (AEWC-GAU-2019-057) reviewed and approved all experimental procedures performed in this study. All animals were housed in a full facility at the Zhangye areas of Gansu Province, China. Three healthy adult Bactrian camel at the age of 3 years were purchased from the centre. The animals were housed and monitored by trained personnel and fed on grasses and sedges, such as Carex, Stipa, and Kobresia. In the Zhangye environment, the altitude

SEND REPRINT REQUEST TO JAMES BLACKAR MAWOLO email: donmawolo26@gmail.com

was 3000m. Experiments were carried out using adult Bactrian camel weighing 550-720 kg. The weight ranges from 10-15µm. The animals were maintained at a temperature between -7° C and -8° C and had free access to food and water.

Treatment and Specimen Techniques

Animals were retrieved one at a time from their living areas and minimally immobilised to facilitate sacrificing and then extraction of the brain by craniotomy. Subsequently, the cerebral cortex, frontal lobe, temporal lobe among others were extracted. Tissue samples prepared for immunohistochemistry were fixed in 4% paraformaldehyde (PH 7.4, w/v) and samples for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and western-blotting were stored at -80 °C.

Reagents and instruments

The description of instruments and reagent is given below with manufacture in parenthesis.

(1) KD-BM bio-tissue embedding machine, kd-h bio-tissue baking machine and KD-P bio-tissue spreading machine (Zhejiang Jinhua Cody Equipment Co. LTD). (2) DHP- 9082 thermostatic incubator (Shanghai Duofu Industrial Co., LTD). (3) Rm-2235 precision rotary semi-automatic helical slicer and 13395H2X optical microscope (LEICA, Germany). (4) PBS phosphate buffer (zli-9062), immunohistochemical staining kit (sp-9001) and DAB colour developing kit (zli-9018) (Beijing Zhongshan Jinqiao Biotechnology Co., LTD). (5) Trypsin (t8150-25) (Beijing Solabao Biotechnology Co., LTD). (6) Rabbit anti-mouse polyclonal antibody (bs-1859r) (Beijing Boorson Biotechnology Co., LTD). (7) Formaldehyde solution, glacial acetic acid, anhydrous ethanol, n-butanol and xylene, and other conventional chemical reagents (Tianjin Damao Chemical Industry co., LTD).

Immunohistochemical staining

Tissue samples from the brain of Bactrian camel were fixed (4% paraformaldehyde) and trimmed (2 cm×2 cm). Then, the tissue samples used conventional gradient alcohol dehydration, made tissue wax blocks with paraffin embedding, cutting tissues with serial sections (thickness 4 µm), exhibiting, patching, baking sheet processing, hematoxylin-eosin (HE) routine staining, microscopy. The paraffin-embedded tissue sections were deparaffinised in xylene and then rehydrated in graded alcohol. The PBS (0.01mol/L, pH =7.2) was rinsed 3 times, each time 5minutes. 0.125% trypsin antigen was repaired 30 minutes and rinsed in PBS for 2 times. The endogenous peroxidase activity was blocked by incubating the sections for 10 minutes in 30mL/L hydrogen peroxide blocking solution, followed by rinsing 3 times with PBS for 5 minutes each time to reduce non-specific binding of the first antibody. Normal sheep serum was added for blocking and incubated at room temperature for 15 minutes. The corresponding primary antibody was added in the sections, incubated at 37 °C for 2 hours, and rinsed in PBS 3 times. The appropriate secondary antibody was added after been removed from PBS and incubated at 37 °C for 15 minutes. The streptomyces avidin-peroxidase solution was added in the sections, incubated at 37 °C for 15 minutes, PBS was rinsed 3 times, 5 minutes each time. The immuno-peroxidase colour reaction was developed with the HRP-DAB substrate chromogen solution after removed PBS. Distilled water stopped the reaction and the sections were lightly counterstained with hematoxylin, dehydrated, in increasing concentrations of ethanol, cleared and covered with mounting medium and coverslips (at 4 °C). Then the sections were stored at 20 °C until used for taking photographs and microscopic analysis. To assess the specificity of the immunolabelling, the negative controls were performed by substituting the primary antibody with PBS. Other procedures remained constant.

Statistical analysis

SPSS 19.0 was utilised to analyse and compare the differences between MD values. The level of significant was cheked (P >.05).

Results

The trend of Ngb expression were widely distributed in the brain tissues of the adult Bactrian camel. Ngb expressions were significantly expressed in the cerebellar cortex, hippocampus, amygdala, cerebellum, and corpus callosum while other regions demonstrated less expression. However, it was recorded that the hypothalamus showed higher but without significant (Table 1).

The Ngb expression levels in different areas of the adult yak brain were significantly different (Liang *et al,* 2013). The relative expression of the Ngb gene in the cerebral cortex was significantly higher than that in the cerebellar cortex, medulla oblongata, striatum, and olfactory bulb. The expression level in the hippocampus was different from that in the other regions, with a high level of significance.

Ngb was mainly expressed in the following areas of the brain as compared to the study done

by the researchers (Tong *et al*, 2015). The quantities of expression and distribution pattern differed but localisation of distribution was almost similar.

Table 1. The comparison of the Ngb expression in the brain of Bactrian camel.

Regions	Mean ± SD	Significant
Cerebellar Cortex	12.179 ± 0.150	000***
Hippocampus	11.538 ± 0.118	002**
Amygdala	11.125 ± 0.470	0.003**
Olfactory lobe	10.690 ± 0.321	0.000***
Basal ganglia	11.022 ± 0.152	0.015**
Thalamus	10.884 ± 0.108	0.008**
Hypothalamus	11.134 ± 0.043	0.210
Cerebellum	11.805 ± 0.212	0.000***
Frontal lobe	10.707 ± 0.065	0.014**
Corpus Callosum	11.961 ± 0.008	0.000***

Discussion

According to Reuss *et al* (2002) Ngb was solely expressed in the cerebellar cortex of the rodent brain. Purkinje cells of the cerebellar cortex also showed a level of Ngb mRNA expression (Reuss *et al*, 2002). A study performed by Christian *et al* (2013) also confirmed the significant Ngb expression in the cerebellar cortex of the adult mouse brain but Fabrizius *et al* (2016) interestingly revealed a lower Ngb expression in the cerebellar cortex during foetal development of the mouse brain and had a tendency to increase as the mouse approaches adulthood. The current study displayed a significant level of Ngb expression in the cerebellar cortex of Bactrian camel. Ngb played a protective role in the control movement and influences many other functions in the cerebellar cortex (Hundahl *et al*, 2013). Its expression might play a role in protecting the cerebellar cortex and other nerve cells from permanent demage as suggested by the current findings. Ngb expression had neuroprotective and antiapoptotic functions also (Alekseeva *et al*, 2017). The expression also involved the metabolism of reactive oxygen and nitrogen species. Burmester *et al* (2000) reported that Ngb is expressed at 11% in the hippocampus of the human brain while finding reported by Reuss *et al* (2002) recorded positive expression of Ngb in the formation of the rodent's hippocampus. The present findings revealed a significant level of Ngb levels in the hippocampus of the Bactrian camel. In the Bactrian camel hippocampus, growth hormones such as age, sex, and stress require a significant level of oxygen for these changes to occur. Ngb played a role in oxygen supply and may detoxify reactive oxygen or nitric oxide (Burmester and Hankeln, 2009). Significant expression Ngb has a protective function as these changes take place in the hippocampus and influence Bactrian camel behaviour. In the hippocampus, it was observed that Ngb can decrease for long days after physiological changes but increase after a few days (Brayn *et al*, 2012). In the mouse brain, Ngb revealed its highest concentration in the amygdala and other regions (Hundahl *et al*, 2013) while a considerable level of Ngb mRNA expression was seen in the amygdala of rodent brain (Reuss *et al*, 2002). Similarly, the current result found a significant level of Ngb in the amygdala of the Bactrian camel. The behaviour changes and other functions in the Bactrian camel, such as protecting young adults from predators, long-distance movement at high-altitude, mitochondrial dysfunctions, and neurodegenerative disorders require strong protection of the neuron tissues from damage. Ngb mRNA was found distributed in the olfactory lobe and it was suggested that Ngb is a conserved gene in evolution and is very important in the nervous system (Chenggang *et al*, 2002). The present results showed a significant level of Ngb in the olfactory bulb of Bactrian camel and the expression contribute to essential neuronal senses. A significant level of oxygen was needed when the Bactrian camel was covering a long distance and the breath rate increase as the Bactrian camel moves. The Ngb expression in the olfactory bulb regulates the oxygen rate during breathing. The response to oxygen stimuli depended on the ability to successfully adapt to hypoxia. The pattern of Ngb expression facilitated oxygen movement between neural tissues and provided a level of neuronal protection during hypoxia.

In a 26 years old male, Ngb was found highly expressed in the basal nuclei while a female of 42 years showed low expression (Hu *et al*, 2017). Age factors may have an influence on the expression of Ngb in neuronal tissues especially the basal ganglia. The current findings reported a significant Ngb expression in the basal nuclei of the Bactrian camel. The expression pattern may participate in protecting neuron tissues during transportation or movement in the high altitude environment. During transportation, the breath rate of Bactrian camel often increased and oxygen was paramount in this process. The significant expression of Ngb was involved in protecting the movement and coordination of neuronal tissues in the basal ganglia.

The present findings recorded that Ngb expression in the Bactrian camel thalamus had shown

a significant rate of expression. During sensory signals in the Bactrian camel, Ngb regulated oxygen expression and played a neuroprotective function. Activities such as responding to predators can be stressful and require a significant level of oxygen. Ngb not only responded to the oxygen demand but protected neurons tissues from damage. It was recorded that Ngb expression in the yak, mouse, and murine thalamus read similarly (Della *et al*, 2010; Reuss *et al*, 2002). Although researches had a focus on Ngb expression in the thalamus, there exists limited references.

The current results showed a higher Ngb expression in the hypothalamus of Bactrian camel but were non significant. Ngb expression in the hypothalamus promoted neuronal survival (Eliana *et al*, 2016). Christian *et al (*2013) reported that Ngb was highly expressed in the hypothalamus of human while in the adult mouse brain the Ngb expression was non significant (Fabrizius *et al*, 2016). The high expression of Ngb in the Bactrian camel hypothalamus could help in supply of oxygen to the blood flow in the body and may act as endogenous protectants in the nerve cells while Hif-1α in the hypothalamus can have an oxygen-independent regulation such as oxidative stress and because the hypothalamus was located at the base of the forebrain and around the walls of the third ventricle which received signals from the periphery through the bloodstream (Cramer *et al*, 2003; Catrina, 2014). Ngb expression in the Bactrian camel hypothalamus may also be involved in preventing an imbalance in the blood flow and nutrients such as glucose and lactate, leading to biochemical and molecular changes that caused neuronal damage.

The distribution and expression of Ngb in various regions of the adult yak brain, were demonstrated by the immunohistochemical staining ISPs method and real-time fluorescence quantitative PCR (Tong-fang *et al*, 2015). The results indicated that Ngb was widely distributed in different regions of the adult yak brain (Tong-fang *et al*, 2015), while in the human brain (Hundahl *et al*, 2013), Ngb had a more limited levels of distribution, weaker expression, and fewer effects on neuronal morphology (Tong-fang *et al*, 2015). These differences could be the result of the varied methods and laboratory procedures used in each study. Ngb participation in the uptake and storage of oxygen by nerve cells can improve the rate of oxygen usage by nerve cells (Sun *et al*, 2001). Ngb upregulation can protect nerve cells, improving the tolerance of brain tissue to ischemia and hypoxia and reducing damage to the brain under these conditions (Greenberg *et al*, 2008). As yaks live in a high-altitude hypoxic environment for a long period, the levels of Ngb in different regions of the brain perform key functions in enhancing the oxygen utilisation rate. The nervous system maintained the normal physiological function of the brain (Zhang *et al*, 2008). Due to the high expression of Ngb in functional nuclei, the function of oxygen storage may be closely related (Dewilde *et al*, 2001); however, this expression may also reflect the difference in activity and oxygen consumption in different areas of the brain. In addition, the distribution of Ngb in other regions of the brain and in the cells of the yak may also be related to the oxygen-consuming activities of these regions and cells (Zivin *et al*, 2009). First, the expression of the Ngb gene in different regions of the yak brain was found by fluorescence quantitative PCR, and the results showed significant differences in the expression of Ngb in various areas of the yak brain. The level of Ngb quantity of expression in the cerebral cortex was the most significant (Tong-fang *et al*, 2015). Compared to its expression in humans, a large amount of Ngb was observed in the hypothalamus, but the difference was non significant (Hundahl *et al*, 2013). Both yaks and mice showed Ngb in the cerebral cortex, but the levels of expression differed (Tong-fang *et al*, 2015; Wei-De *et al*, 2013). The rat brain also showed higher expression in the cerebral cortex, but the difference was non significantly compared to the other brain regions (Guo *et al*, 2011). The positive expression of Ngb in the cerebral cortex of yaks was significantly higher than that in the cerebellar cortex, hippocampus, medulla oblongata, striatum and olfactory bulb (Wang *et al*, 2008).

The study of Liang *et al* (2013) used the immunohistochemistry to show the distribution of Ngb in the brain of the cattle and yak. All six layers of the cerebral cortex contained Ngb-positive cells that were distributed throughout the layers, and the level of expression was significantly higher than that in the cerebellar cortex, hippocampus, and striatum. Ngb-positive cells were also found in the medulla. The Ngb distribution and localisation were similar in the cerebral cortexes of the cattle and yaks (Tong-fang *et al*, 2015). The overall levels of Ngb expression in the brains of cattle were lower than those in the brain of yaks. In the cerebellar cortex of the yak, Ngb-positive cells showed high levels of expression in purkinje cell layers and lower levels in the granular layers. The distribution and localisation

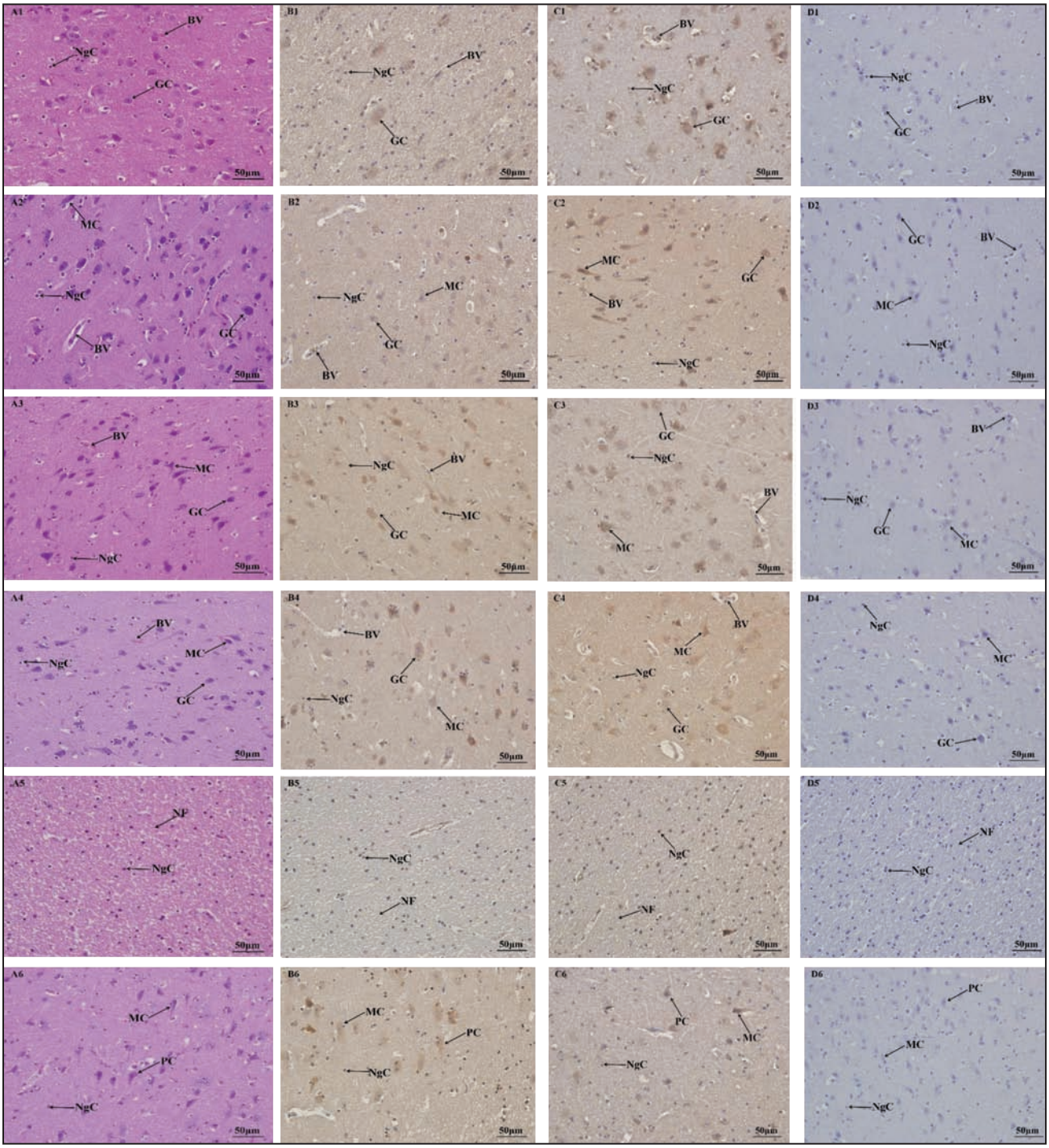


Fig 1. A1-10. Ngb expression in the brain tissues of Bactrian camel.

Plate A1-D1. The expression of Ngb in the cerebellar cortex of bactrain. Positive and negative controls are indicated by arrows.
Plate A2-D2. Ngb is found in the upper regions of hippocampus.
Plate A3-D3. Ngb is observed in the middle region of the amaydala.
Plate A4-D4. Ngb are revelaed in the middle region of cerebral.
Plate A5-D5. Ngb as seem are located in lower region of the White matter.
Plate A6-D6. Ngb are heavily found in the entire region of the Basal ganglia

of Ngb-positive cells in the cerebellar cortex of cattle was similar to that in the yak, but the intensity of the reaction was weaker overall. In various regions of the hippocampus in the yak, Ngb-positive cells were mostly found in pyramidal cells, with positive reaction sites but weak Ngb expression found in nerve

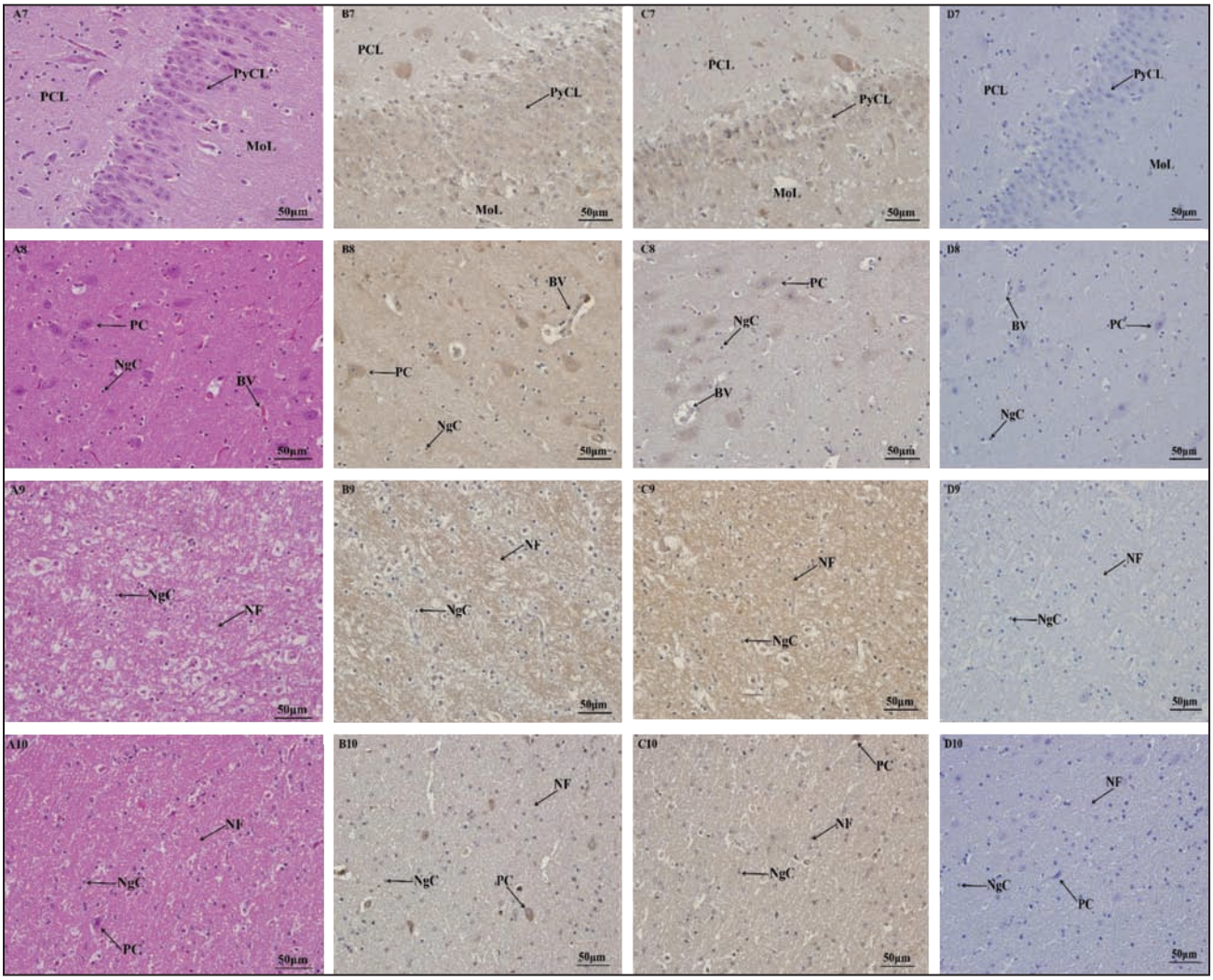


Plate A7-D7. Ngb is found in the middle region of the cerebrum.
Plate A8-D8. Ngb is observed in the middle region of the cerebellum.
Plate A9-D9. Ngb is distributed in the lower region of the frontal lobe.
Plate A10-D10. Ngb is found in the upper region of the corpus callosum.

processes (Liang *et al*, 2013). The similarities of these results might be due to the identical methods and laboratory procedures used. In separate areas of the cattle hippocampus, the distribution and localisation of Ngb-positive cells were similar to those of the yak, but the intensity of the reaction was weaker in the yak brain. Additionally, in the medulla oblongata of cattle and yaks, the distribution and localisation of Ngb-positive cells were weakly expressed, and the overall intensity was weaker in the cattle than in the yak. Ngb-positive cells in the striatum of the yak were widely distributed in the caudate nucleus and the Ngb-positive reactions were stronger than those in the hippocampus, medulla oblongata, and olfactory bulb, but Ngb was more weakly expressed than in the cerebral cortex and cerebellar cortex. In the medulla oblongata of the yak, Ngb-positive cells were mainly distributed in the gray matter and Ngb-positive cells were also scattered in the white matter. Ngb was also expressed in the mitral cell layer of the yak olfactory bulb, with notable staining and large cells; however, the staining intensity of the Ngb-positive cells was weaker than that of medulla oblongata and stronger than that of the hippocampus. The distribution and localisation of Ngb-positive cells in the mitral cell layer of the olfactory bulb of the cattle was similar to that of the yak; the staining intensity was higher than that of the hippocampus, weaker than that of the medulla oblongata and significantly weaker than that of yak (Tong-fang *et al*, 2015). Ngb-positive cells were distributed primarily in the peripheral nerve plexus and ganglia and mostly scattered in some of the nerve cells in the peripheral nervous system but at low quantities (Liang *et al*, 2013). In the peripheral nervous

system of the cattle, the distribution and localisation of the Ngb-positive cells were similar to those of the yak, but the intensity of the reactions was on average weaker than that of the yak (Tong-fang *et al*, 2015).

Conclusion

Camel

The study documented that Ngb may have a significant function in the maintenance of oxygen homeostasis and participation in the brain tissues. The study further provided explanations for Ngb physiological function and its relationship for the Bactrain camel to adapt to the extreme environmental conditions.

Yak and Cattle

The high expression of Ngb in different brain tissues of adult yak and cattle is suggested to play an important role in the utilisation of oxygen and physiological functions. Additionally, It enables the mammals to adapt to the extreme hypoxia conditions.

Acknowledgements

The researchers acknowledged the Gansu Agricultural University Library Team for their technical support and advice during the literature review.

Funding

The researchers extend their heartfelt thanks to the National Natural Science Foundation of China (Grant No. 31760305) and the Youth Tutor Foundation of Gansu Agricultural University (Grant No. GAU-QNDS-201501) for the financial and technical support. This work would not have been possible without assistance rendered from these groups.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

JBM, DX, LX WH and HAM contributed to the literature search. JBM, DX and HAM organised, investigated and interpreted the data. JBM, LX and WH wrote the first draft of the manuscript. DX and LX performed the study methodology and formal analysis. JBM designed the study concept. All authors contributed to this manuscript revision and read and approved the submitted version.

References

Alekseeva O, Grigor'ev, I and Korzhevskii D. Neuroglobin, an oxygen-binding protein in the mammalian nervous system (localisation and putative functions). Journal of Evolutionary Biochemistry and Physiology. 2017; 53:249-258. 10.1134/S0022093017040019.

Anderson B Jr. ocular effects of changes in oxygen and carbon dioxide tension [J]. Transactions of the American Ophthalmol society. 1968; 66(1):423-474.

Brayn H, Marco D, Xiao M, Lin X, Judith C, Kunlin J and David AG. Hypoxia-inducible factor-1 and neuroglobin expression. Neuroscience Lett. 2012; 514:137-140. doi: 10.1016/j.neulet.2012.01.080.

Burmester T, Weich B and Reinhardt S. A vertebrate globin expressed in the brain. Nature. 2000; 407(6803):520-523.

Burmester T and Hankeln T. What is the function of neuroglobin? J. The Journal of Experimental Biology. 2009; 212:1423-8. 10.1242/jeb.000729.

Catrina SB. Impaired hypoxia-inducible factor (HIF) regulation by hyperglycemia. Journal of Molecular Medicine. 2014; 92:1025-1034. doi: 10.1007/s00109-014-1166-x.

Chenggang Z, Chunli W, Meiyu D, Lin L and Hangyan W. Full-Length cDNA Cloning of Human Neuroglobin and Tissue Expression of Rat Neuroglobin. Biochemical and Biophysical Research Communications. 2002; 290:1411-1419. doi.org/10.1006/bbrc.2002.6360.

Christian A, Hundahl J, Kelsen B, and Hay-Schmidt A. Neuroglobin and Cytoglobin expression in the human brain. Brain Structure and Function. 2013; 218:603-609. doi: 10.1007/s00429-012-0480-8.

Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R and Mackman N. HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell. 2003; 112:645-657. doi: 10.1016/S0092-8674(03)00154-5.

Della-Valle B, Hempel C, Kurtzhals JA and Penkowa M. *In vivo* expression of neuroglobin in reactive astrocytes during neuropathology in murine models of traumatic brain injury, cerebral malaria, and autoimmune encephalitis. Glia. 2010; 60:1220-1227. doi: 10.1002/glia.21002.

Dewilde S, Kiger L, Burmester T, Hankeln T, Baudin-Creuza V, Aerts T, Marden MC, Caubergs R and Moens L. Biochemical characterisation and ligand binding properties of neuroglobin, a novel member of the globin family. Journal of Biological Chemistry. 2001; 276(42):38949-55 [PubMed] [Google Scholar]

Eliana B, Valentina E, Ricardo C, Marco ÁR and Luis MG. Protection by Neuroglobin Expression in Brain Pathologies. Frontiers in Neurology. 2016; https://doi.org/10.3389/fneur.2016.00146.

Fabrizius A, Andre D, Laufs T, Bicker T, Reuss S, Burmester T and Hankeln T. A critical re-evaluation of neuroglobin expression reveals conserved patterns among mammals. Neuroscience. 2016; S0306-4522 16, 30357-30358. doi. org/10.1016/j.neuroscience.2016.07.042.

Gao XJ. Research of distribution of neuroglobin in major organs and tissues of the Simmental [D]. Lanzhou: Gansu Agricultural University (in Chinese). 2015.

Geuens E, Brouns I and Flamez D. A globin in the nucleus[J]. Journal of Biological Chemistry. 2003; 278(33):30417-30420.

Greenberg DA, Jin K and Khan S. Neuroglobin: an endogenous neuroprotectant [J]. Current Opinion in Pharmacology. 2008; 8(1):20-24.

Guo ZD, Sun XC and Zhang JH. Mechanisms of early brain injury after SAH: matrix metalloproteinase 9. Acta Neurochir Supplement. 2011; 110(Pt 1):63-65. doi:10.1007/978-3-7091-0353-1_11.

Hundahl CA, Hay-Schmidt A and Kelsen J. Neuroglobin and Cytoglobin expression in the human brain. Brain Struct Funct. 2013; 218:603-609. doi:10.1007/s00429-012-0480-8.

Hu J, Xiyue C, Dejiang P, Qihui L, Yuanfeng Z, Bin F, Lixia, L, Zhengli C and Chao H. Tumour grade related expression of neuroglobin is negatively regulated by PPARγ and confers antioxidant activity in glioma progression. Redox Biology. 2017; 12:682-689. doi.org/10.1016/j.redox.2017.03.023.

Hundahl CA, Allen GC, Hannibal J, Kjaer K, Rehfeld JF, Dewilde S, Nyen-gaard JR, Kelsen J and Hay-Schmidt A. Anatomical characterisation of cytoglobin and neuroglobin mRNA and protein expression in the mouse brain. Brain Research. 2010; 1331:58-73. doi.org/10.1016/j.brainres.2010.03.056.

Liang C, Xia L, Ning-ning S and Xiaohua D. Distribution of neuroglobin in different tissues and cells of the adult yak and cattle. Acta Anatomica Sinica. 2013; 44(1):68-72. doi:10.13207/j.cnki.jnwafu.2015.06.012.

Li YY, Liu H and Tong Y. Normal distribution of neuroglobin in proteins in the eyeball of adult rats [J]. Chinese Ophthalmic Research. 2006; 24(5):461-464. (in Chinese).

Ostoji J, Grozdani SD and Syed NA. Patterns of distribution of oxygen-binding globins,neuroglobin and cytoglobin in the human retina [J]. Archives of Ophthalmology. 2008; 126(11):1530-1536.

Ostoji J, Sakaguchi DS and De Lathouder Y. Neuroglobin and Cytoglobin: oxygen-binding proteins in retinal neurons[J]. Investigative Ophthalmology and Visual Science. 2006; 47(3):1016-1023.

Ran J, Sun SQ and Bi X. Expression of neuroglobin mRNA in rats [J]. Chongqing Medicine. 2005; 34(7):1035-1036.

Reuss S, Saaler-Reinhardt S, Weich B, Wystub S, Reuss M, Burmester T and Hankeln T. Expression analysis of neuroglobin mRNA in rodent tissues. Neuroscience. 2002; 115:645-656. doi.org/10.1016/S0306-4522(02) 00536-5.

Schmidt M,Giessl A and Laufs T. how does the eye breathe? Evidence for neuroglobin-mediated oxygen supply in the mammalian retina [J]. Journal of Biological Chemistry. 2003; 278(3):1932-1935.

Sun Y, Jin K, Mao XO, Xie L, Peel A, Childs JT, Logvinova A, Wang X and Greenberg DA. Effect of aging on neuroglobin expression in rodent brain. Neurobiol Aging. 2001; 26(2):275-278.

Tong-fang L, Xia L, Xue-Jing S and Xiaohua D. Distribution of Neuroglobin in the brain of adult yak. Journal of Northwest A&F University. 2015; 05(11):15-03. doi:10.3969/j.issn.0529-1356.2013.01.01.013.

Wang X, Liu J, Zhu H, Tejima E, Tsuji K, Murata Y, Atochin DN, Huang PL, Zhang C, Lo EH. Effects of neuroglobin overexpression on acute brain injury and long-term outcomes after focal cerebral ischemia. Stroke. 2008; 39(6):1869-1874.

Wei X,Deng Y and Liu X. how can photoreceptor cells breathe in the retina? Neuroglobin may be the answer[J]. Neural Regeneration Research. 2010; 5(9):720-720.

Wei-De Li, Qing Sun, Xiang-Sheng Zhang, Chun-Xi Wang, Song Li, Wei Li and Chun-Hua Hang. Expression and Cell Distribution of Neuroglobin in the Brain Tissue After Experimental Subarachnoid Hemorrhage in Rats: A Pilot Study, Cell Mol Neurobiol. 2013; DOI 10.1007/s10571-013-0008-7.

Yang Y, Liu X and Zeng G. Distribution of neuroglobin in the retina of adult rabbits [J]. Chinese Journal of Histochemistry and Cytochemistry. 2015a; 24(2):139-144. (in Chinese)

Yang Y, Liu X and Gao X. Distribution of neuroglobin in the retina of adult sheep[J]. Chinese Journal of Anatomy. 2015b; (3):275-278. (in Chinese)

Zhang Lei, Aaron GL Fletcher, Vanessa Cheung, Fred Winston, and Laurie A Stargell. Spn1 regulates the recruitment of Spt6 and the Swi/Snf complex during transcriptional activation by RNA polymerase II. Molecular and Cellular Biology. 2008; 28(4):1393-1403.doi: 10.1128/MCB.01733-07.

Zhao S, Yu Z, Zhao G, Xing C and Hayakawa K. Protection by Neuroglobin Expression in Brain Pathologies. Neuroscience. 2012; 13:67-132. doi:10.1371/journal.pone.0076565.

Zivin JA, Albers GW, Bornstein N, Chippendale T, Dahlof B, Devlin T, Fisher M, Hacke W, Holt W, Ilic S, Kasner S, Lew R, Nash M, Perez J, Rymer M, Schellinger P, Schneider D, Schwab S, Veltkamp R, Walker M, Streeter J. Effectiveness and safety of transcranial laser therapy for acute ischemic stroke. Stroke; a journal of cerebral circulation. 2009; 40(4):1359-1364. https://doi.org/10.1161/STROKEAHA.109.547547.

DOI : 10.5958/2277-8934.2021.00053.9 Vol 28 No 3, p 349-353

ISOLATION AND CHARACTERISATION OF BACTRIAN CAMEL MILK-DERIVED EXOSOMES

Zhuwei Guo[1], Xiangjun Xu[2], Yongsheng Zhong[3], Zhorigtu[4], Chunhua Cao[3], Xinji Jiang[5], Demtu Er[1*] and Bin Yang[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 of P. R. China, Hohhot, 010018, China

[2]Alxa Left Banner Bayannorogon Comprehensive Administrative Law Enforcement Bureau, Bayannorogon, 750300, Inner Mongolia Autonomous Region, P.R. China

[3]Ejina Banner Centre of Animal Disease Prevention and Control, Dalaihub, 735400, Alxa League, Inner Mongolia Autonomous Region, P.R. China

[4]Alxa League Institute of Animal Husbandry, Bayanhot, 750306, Inner Mongolia Autonomous Region, P.R. China

[5]Alxa Left Banner Centre of Animal Disease Prevention and Control, Bayanhot, 750300, Alxa League, Inner Mongolia Autonomous Region, P.R. China

ABSTRACT

The morphological features were identified by TEM and total exosome protein concentration was determined by the BCA Protein assay. Under the electron microscope, it was observed that Bactrian camel milk exosomes were a typical vesicle enrichment with a large bilayer lipid membrane structure and the size of 30-200 nm round or oval membranous vesicles in the shape of a cup holder. The centre was evenly dyed black, the film was lightly dyed white and the edges were clear. The total protein concentration of the exosome extract measured by the BCA Protein assay was 18.6404 ± 1.7297 μg/μL. The study provided new ideas for exploring the biological function and principle of action of biological nano-vehicles in Bactrian camel milk.

Key words: Bactrian camel, exome, milk, TEM

Exosomes are extracellular vesicles (EVs) secreted by cells with a diameter of 30-150nm (Beuzelin and Kaeffer, 2018). It is a carrier for transmitting biological information such as nucleic acids, lipids and proteins and can transport various biologically active molecules. Recipient cells are widely involved in the exchange of information between cells and play an important role in various physiological and pathological processes, especially in immune responses (Zeng *et al,* 2021a) and tumours (Zeng *et al,* 2021b). Exosomes are derived from the intracellular membrane and are released into the extracellular environment when multivesicular bodies (MVB) fuse with the plasma membrane. Many cells can release exosomes, including reticulocytes (Johnstone *et al,* 1987), dendritic cells (Jung *et al,* 2020), B cells (Calvo and Izquierdo, 2020), T cells (Lopez *et al,* 2020, Wen *et al,* 2021), mast cells (Lecce *et al,* 2020), mesenchymal stem cells (Kim *et al,* 2021), epithelial cells (Du *et al,* 2021) and tumours cells (Srivastava *et al,* 2021, Zeng *et al,* 2021b). After the release of exosomes, these are widely present in blood, lymph (Saunderson *et al,* 2014), urine, saliva (Witwer *et al,* 2013), milk (Mirza *et al,* 2019), amniotic fluid, ascites (Jayaseelan, 2020) and other physiological or pathological fluids. Indeed, there are certain differences in the composition and function of exosomes from different sources.

Research has confirmed the physiological functions of milk-derived exosomes play an active role in infant intestinal development, innate immunity and prevention of inflammation (Martin *et al,* 2018). Camel milk and its exosomes successfully improved CTX-induced immunosuppression and oxidative stress in albino rats (Ibrahim *et al,* 2019). Exosomes isolated from milk can prevent experimentally induced necrotic intestinal damage by increasing goblet cell production and endoplasmic reticulum function. Milk exosomes provide a possible preventive strategy for human infants at risk of necrotising enterocolitis (Li *et al,* 2019). Through RNA sequencing and proteomics analysis of porcine milk exosomes, many mRNAs and proteins have been predicted to be involved in immunity, proliferation

SEND REPRINT REQUEST TO CORRESPONDING AUTHOR DEMTU ER* ● BIN YANG* email: eedmt@imau.edu.cn ● yangbin_nm@126.com

and cell signal transduction (Chen *et al,* 2017). The mRNA profiles of milk-derived exosomes were analysed from buffalo, dairy cow, swine, human and panda, in which several candidate genes that regulate disease resistance, immune response and metabolism were selected (Chen *et al,* 2020). In addition, the mRNAs of porcine milk exosomes have also been found to have the potential to protect intestinal epithelial cells from deoxynivalenol (DON) damage by regulating cell proliferation and tight junction proteins (TJs) (Xie *et al,* 2020). There has been no report on the research of Bactrian camel milk-derived exosomes. In this study, Bactrian camel milk was used as the research object to study the extraction and identification of exosomes and determination of total protein content by the BCA protein assay.

Materials and Methods

Milk sampling

The samples were taken from the mixed milk of 6 adult Bactrian camels transport on dry ice and stored at -80°C ultra-low temperature.

Preparation of exosomes from milk

The ultracentrifugation was used for separation, some changes were made (Badawy *et al,* 2018). Camel milk was centrifuged at 8,000 g at 4 °C for 30 minutes to remove fat, casein, cell debris and other crumbs. Skimmed milk supernatant was taken and centrifuged at 13,000 g at 4 °C for 30 minutes to remove the remaining fat and cell debris. The fat-free supernatant was ultracentrifuged at 100,000g at 4 °C for 120 minutes and then the supernatant was removed to obtain exosome pellets. The particles were suspended in PBS to obtain a uniform suspension. Bacteria were filtered with a 0.22 μm filter and either used immediately or stored in a freezer -80 °C.

Transmission electron microscope (TEM)

The isolated exosomes were identified by TEM (JEM2100, JEOL Ltd.) at a voltage of 80-120 kV. The exosome suspension were put in an ice bath, pipetted 5 L, fixed with a special fixative for exosomes, dropped on a copper mesh and allowed to dry, rinse with PBS 3 times and excess liquid was absorbed with filter paper. Observed by TEM after staining with uranyl acetate for 3 minutes, the 30-200 nm cup-holder-like vesicle structure in the field of view was typical exosome morphology.

BCA Protein assay

The standard protein was completely dissolved and diluted to 0.5 mg/mL. Depending on the number of standard wells, BCA reagent A and reagent B (V50: V1) were mixed and 0, 1, 2, 4, 8, 12, 16, 20 μL standards were added to the wells in order and PBS were topped up to 20 μL. The concentration was set to 0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 μg/μL, respectively. The fully mixed exosome solution (2 μL) was added to the sample well of the plate and up to 20 μL was repeated 6 times using PBS. 200 μL BCA working solution was added to each well and was put in a 37 °C thermostat for 30 min. The absorbance was measured at the wavelength of A562 with a microplate reader and calculated the protein concentration of exosomes after making a calibration curve.

Results and Discussion

Under the TEM, many vesicle-like concentrates with a two-layer membrane structure was seen. A circular or oval membranous vesicle sized 30-200 nm in the shape of a saucer and cup holder was typical (Fig 1B). The slightly larger translucent vesicles over 200 nm in diameter were EVs (Fig 1). Since the periphery of the exosome was coated with a bilayer lipid membrane, the centre was uniformly stained black, the membrane was stained lightly white, the periphery was stained darkly and the edges were clear. This was consistent with the size and shape of exosomes secreted by other cells reported so far (Fig 1). According to the BCA Protein assay, the fitting equation is $y = 20.693 x - 2.3014$, $R^2 = 0.9948$. The measured total protein concentration was 18.6404 ± 1.7297μg/μL, based on the results shown in Fig 2.

Morphological observations to identify exosomes rely primarily on TEM, which allowed direct observation of morphology and measurement of exosome size and this method was simple and practical. In this experiment, we directly observed elliptical vesicle-like nanoparticles with a horizontal diameter of 76 nm and a vertical diameter of 89 nm, with band-like fragments on the top. The background was darker, the spheres were uniformly stained white, the membrane boundaries were visible and stained black uniformly. Dense black bands that were darker than the background were visible around the exosomes. Due to the adhesion phenomenon of some exosomes, it was inferred that the attachments on the surface of the membrane body may be part of the membrane fragments that broke the exosomes, as shown in Fig 1.

The two exosomes were round membranous capsules with an average diameter of about 90 nm. The exosome membrane in the lower right foot of the visual field clearly showed that a darker, wider, uniformly stained ring banded surrounded the vesicle membrane (Fig 1).

Interestingly, in this study, a typical bilayer cup holder-like exosome structure was directly observed under the TEM (Fig 1). After software measurement and analysis, the observed exosomes were 156 nm in lateral diameter and 178 nm in vertical diameter, with oval and disc-shaped, complete membrane structure, clear outlines and hollow vesicle-like shapes. The background of the visual field was clear, the film was divided into two layers, an inner layer and an outer layer, there was a gap between the homogeneous white dyed film, a cavity was formed between the inner and outer sheet and the gap was uniform in texture and black and the colour was lighter than the background. In the centre of the membrane, a uniform black circular cavity can be seen. The capsule containing the material carried by the exosomes was highly consistent with the size and shape of the exosomes reported in previous literature (Wang *et al,* 2020).

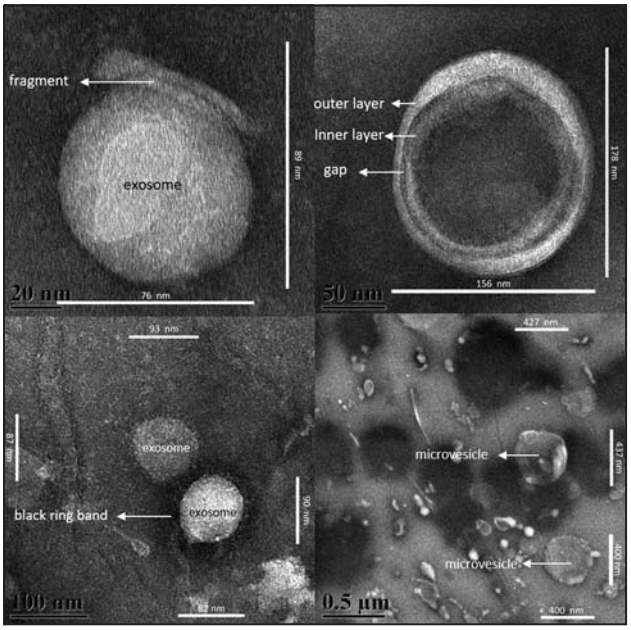
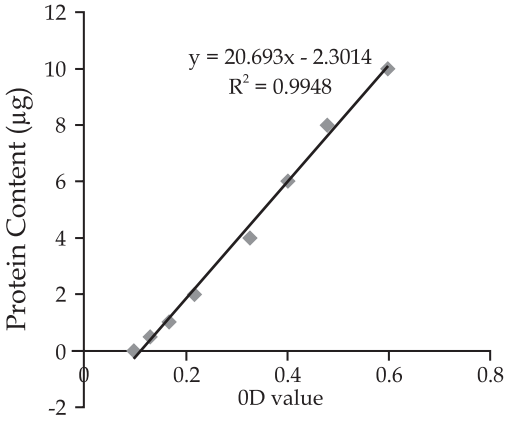


Fig 1. Observation of the exosomes by TEM. (A): Exosomes adhere to each other after disintegration and death (B): Typical double-layer cup holder-like exosomes (C): Dense staining (black ring band) around the exosome membrane (D): Extracellular vesicles larger than 200 nm.

EVs can be divided into 3 categories, depending on their size and source: exosome (30-200nm), microvesicle (100nm-1000nm) and apoptotic body (50-5000nm) (Galley and Besner, 2020). In this study, a transparent vesicle structure with a size of about 400 nm was successfully observed. The surface of the layers was rough and the overall shape was irregular (Fig 1). It is preliminarily inferred that this structure is typical microvesicle. The size and shape were consistent with previous studies (Ibrahim *et al,* 2019).

Exosomes from the same source were similar in shape and size and the diameter of exosomes from different sources may be different, but the diameter was between 30-100 nm. Serum-derived exosomes have the same diameter as other cell-derived exosomes, both of which were lipid bilayer-coated secretions. The exosomes observed in this study showed a typical model vesicle structure with



Number	0D value	Protein Content (μg)	Protein concentration μg/mL
1	0.098	0	0
2	0.13	0.5	25
3	0.166	1	50
4	0.216	2	100
5	0.326	4	200
6	0.4	6	300
7	0.478	8	400
8	0.598	10	500

Standard Curve Data Sheet

Number	1	2	3	4	5	6
0D value	1.975	1.597	2.106	1.805	2.019	1.975
Protein concentration (μg/μL)	19.2836	15.3727	20.6390	17.5247	19.7389	19.2836
Mean ± Sem (μg/μL)	18.6404 ± 1.7297					

Fig 2. Determination of the total protein concentration by BCA Protein assay.

round or oval morphology, darkly stained periphery and low-density regions within the cavity, with a diameter between 30-150 nm, with an average of about 50 nm in diameter, consistent with previous reports (Badawy *et al,* 2018).

Currently, people do not have a uniform standard for exosome extraction and various extraction methods have their strengths and weaknesses. The use of a series of ultracentrifugation methods to extract exosomes is the choice of most researchers. The physiological state of exosomes can be maintained as much as possible without the use of chemical reagents. Ultracentrifugation is the current "gold standard" for extracting exosomes, but it also has its flaw. After pretreatment, some large pollutants can be removed, but there are still some other small molecule proteins, which will affect the quantification and analysis eventually. The electron microscope can directly observe the structure, morphology and particle size of exosomes, which is a piece of direct evidence for the macroscopic identification of the presence of exosomes. Exosomes can also be comprehensively identified by nanoparticle tracking analysis, western blotting, flow cytometry, etc.

There is no doubt that camel milk has a significant positive effect in treating chronic diseases and improving body immunity. Studies show that camel milk can be used as a natural nutritional supplement to improve the body's immune system and help treat diabetes and its metabolic complications. Camel milk is a natural medicine for lowering blood glucose and suppressing lipids, which can significantly reduce the levels of total cholesterol, triglycerides and high-density lipoprotein in the blood of diabetic rats (Mansour *et al,* 2017). Camel milk exosomes have the properties of insulin-like protein and are not easily destroyed by gastric acid, which creates the possibility of oral insulin preparation (Shori, 2015). However, it remains to be seen whether camel milk neuropeptides and exosomes act in cells through specific signaling pathways or regulate physiological functions by binding to specific cell surface receptors. This also offers more possibilities for our next study. By increasing the molecular level of exosomes and studying their effects and signaling pathways, we can also better understand the internal mechanisms of the anti-diabetic properties of camel milk. Therefore, additional *in vivo* and *in vitro* experimental studies will provide new therapeutic ideas and theoretical support further for clinical T2D (Mansour *et al,* 2017), which can be employed.

As exosomes are new stars in the study of intercellular signal transduction, researchers have continuously discovered their important roles in the development of various diseases, physiological conditions and body immunity. The electron microscope observation of the shape and size of exosomes allows researchers to fully understand its own unique basic structural characteristics, thereby laying a certain foundation for better application in clinical disease treatment. certainly, the extraction and purification of exosomes from different species and origins have greatly enriched the breadth and innovation of exosomes research.

Acknowledgements

This work was supported by Inner Mongolia Agricultural University high-level talents research initiation fund project (Grant No. NDYB2018-27) and project (No. 201502069) of the Inner Mongolia Science and Technology Plan.

References

Badawy AA, El-Magd MA and AlSadrah SA. Therapeutic effect of camel milk and its exosomes on MCF7 cells *in vitro* and *in vivo*. Integrative Cancer Therapies. 2018; 17(4):1235-1246.

Beuzelin D and Kaeffer B. Exosomes and miRNA-loaded biomimetic nanovehicles, a focus on their potentials preventing Type-2 Diabetes linked to metabolic syndrome. Frontiers in Immunology. 2018; 9:2711.

Calvo V and Izquierdo M. Inducible polarized secretion of exosomes in T and B lymphocytes. International Journal of Molecular Sciences. 2020; 21(7):2631.

Chen T, Xi QY, Sun JJ, Ye RS, Cheng X, Sun RP, Wang SB, Shu G, Wang LN, Zhu XT, Jiang QY and Zhang YL. Revelation of mRNAs and proteins in porcine milk exosomes by transcriptomic and proteomic analysis. BMC Veterinary Research. 2017; 13(1):101.

Chen Z, Xie Y, Luo J, Chen T, Xi Q, Zhang Y and Sun J. Milk exosome-derived miRNAs from water buffalo are implicated in immune response and metabolism process. BMC Veterinary Research. 2020; 16(1):123.

Du J, Sun Q, Wang Z, Wang F, Chen F, Wang H, Shang G, Chen X, Ding S, Li C, Wu D, Zhang W, Zhong M and Li Y. Tubular epithelial cells derived-exosomes containing CD26 protects mice against renal ischaemia/reperfusion injury by maintaining proliferation and dissipating inflammation. Biochemical and Biophysical Research Communications. 2021; 553:134-140.

Galley JD and Besner GE. The Therapeutic Potential of Breast Milk-Derived Extracellular Vesicles. Nutrients. 2020; 12(3):745.

Ibrahim HM, Mohammed-Geba K, Tawfic AA and El-Magd MA. Camel milk exosomes modulate cyclophosphamide-induced oxidative stress and immuno-toxicity in rats. Food and Function. 2019; 10(11):7523-7532.

Jayaseelan VP. Emerging role of exosomes as promising diagnostic tool for cancer. Cancer Gene Therapy. 2020; 27(6):395-398.

Johnstone RM, Adam M, Hammond JR, Orr L and Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). Journal of Biological Chemistry. 1987; 262(19):9412-9420.

Jung BK, Kim ED, Song H, Chai JY and Seo KY. Immunogenicity of exosomes from dendritic cells stimulated with *Toxoplasma gondii* lysates in ocularly immunized mice. Korean Journal of Parasitology. 2020; 58(2):185-189.

Kim GB, Shon OJ, Seo MS, Choi Y, Park WT and Lee GW. Mesenchymal stem cell-derived exosomes and their therapeutic potential for osteoarthritis. Biology (Basel). 2021; 10(4):285.

Lecce M, Molfetta R, Milito ND, Santoni A and Paolini R. Fc epsilon RI signaling in the modulation of allergic response: role of mast cell-derived exosomes. International Journal of Molecular Sciences. 2020; 21(15):5464.

Li B, Hock A, Wu RY, Minich A, Botts SR, Lee C, Antounians L, Miyake H, Koike Y, Chen Y, Zani A, Sherman PM and Pierro A. Bovine milk-derived exosomes enhance goblet cell activity and prevent the development of experimental necrotising enterocolitis. PLoS One. 2019; 14(1):e0211431.

Lopez P, Rodriguez-Carrio J, Caminal-Montero L and Suarez A. Relationship between T-Cell exosomes and cellular subsets in SLE according to Type I IFN-signaling. Front Med (Lausanne). 2020; 7:604098.

Mansour AA, Nassan MA, Saleh OM and Soliman MM. Protective effect of camel milk as anti-diabetic supplement: biochemical, molecular and immunohistochemical study. African Journal of Traditional, Complementary and Alternative Medicines. 2017; 14(4):108-119.

Martin C, Patel M, Williams S, Arora H, Brawner K and Sims B. Human breast milk-derived exosomes attenuate cell death in intestinal epithelial cells. Innate Immunity. 2018; 24(5):278-284.

Mirza AH, Kaur S, Nielsen LB, Storling J, Yarani R, Roursgaard M, Mathiesen ER, Damm P, Svare J, Mortensen HB and Pociot F. Breast milk-derived extracellular vesicles enriched in exosomes from mothers with type 1 diabetes contain aberrant levels of microRNAs. Frontiers in Immunology. 2019; 10:2543.

Saunderson SC, Dunn AC, Crocker PR and McLellan AD. CD169 mediates the capture of exosomes in spleen and lymph node. Blood. 2014; 123(2):208-216.

Shori AB. Camel milk as a potential therapy for controlling diabetes and its complications: A review of in vivo studies. Journal of Food and Drug Analysis. 2015; 23(4):609-618.

Srivastava A, Rathore S, Munshi A and Ramesh R. Extracellular vesicles in oncology: from immune suppression to immunotherapy. The AAPS Journal. 2021; 23(2):30.

Wang K, Wei Y, Zhang P, Wang J, Hu J, Wang L and Li B. Progress in extracellular vesicle imaging method. Nan Fang Yi Ke Da Xue Xue Bao. 2020; 40(2):279-286.

Wen H, Peng L and Chen Y. The effect of immune cell-derived exosomes in the cardiac tissue repair after myocardial infarction: Molecular mechanisms and pre-clinical evidence. Journal of Cellular and Molecular Medicine. 2021; 25(14):6500-6510.

Witwer KW, Buzas EI, Bemis LT, Bora A, Lasser C, Lotvall J, Nolte-'t Hoen EN, Piper MG, Sivaraman S, Skog J, Thery C, Wauben MH and Hochberg F. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. Journal of Extracellular Vesicles. 2013; 2(1):

Xie MY, Chen T, Xi QY, Hou LJ, Luo JY, Zeng B, Li M, Sun JJ and Zhang YL. Porcine milk exosome miRNAs protect intestinal epithelial cells against deoxynivalenol-induced damage. Biochemical Pharmacology. 2020; 175:113898.

Zeng B, Wang H, Luo J, Xie M, Zhao Z, Chen X, Wang D, Sun J, Xi Q, Chen T and Zhang Y. Porcine milk-derived small extracellular vesicles promote intestinal immunoglobulin production through pIgR. Animals (Basel). 2021a; 11(6):

Zeng W, Yin X, Jiang Y, Jin L and Liang W (2021b). *In vitro* and ex vivo evaluation of tumour-derived exosome-induced dendritic cell dysfunction in mouse. STAR Protocols 2(1):100361.

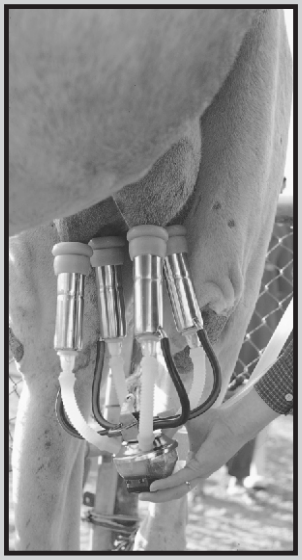
BACK ISSUES OF JCPR AVAILABLE

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 11 June 2004 Number 1



In This Issue

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 11 December 2004 Number 2

In This Issue

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 12 June 2005 Number 1

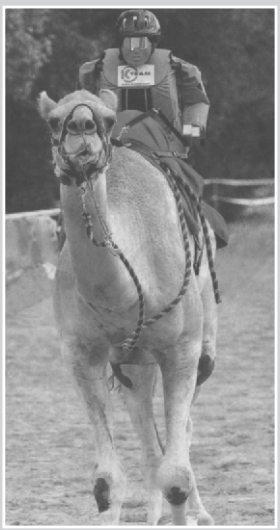
In This Issue

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 12 December 2005 Number 2



In This Issue

See the details of list of contents of all previous issues of JCPR on our website
www.camelsandcamelids.com

Kindly refer querries to: Dr. T.K. GAHLOT, Editor, JCPR
email: tkcamelvet@yahoo.com

DOI : 10.5958/2277-8934.2021.00054.0 Vol 28 No 3, p 355-359

INFRARED THERMOGRAPHY IN DROMEDARY CAMELS WITH INJECTED AND STRETCHED LIPS IN CAMEL BEAUTY PAGEANTS

Mohamed Tharwat[1,2], Abdulla Al-Hawas[1,3] and Yaser Alboti[3]

[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, 44519, Zagazig, Egypt

[3]University Veterinary Hospital, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia

ABSTRACT

This study was carried out during the events of the 5th King Abdulaziz Camel Festival (KACF), Saudi Arabia. It was designed to evaluate the infraed thermography (IRT) in 4627 dromedary camels with either injected lips with cosmetic medicine or stretched lips. Ninety camels with healthy lips were tested for IRT and were used as a control group. During the KACF, 14 camels were found to be injected by cosmetic fillers in the lips and 60 had stretched lips. When tested by IRT, the injection sites appeared darker than that the surrounding tissue. The stretched lips appeared longer, flabby and puffy. When tested by IRT, the mucosal surface in the stretched lips appeared lighter and heterogeneous compared to the darker and homogenous pattern in the non-stretched lips. In conclusion, IRT proved highly feasible for the diagnosis of injected or stretched lips in camel beauty pageants.

Key words: Beauty pageants, camel, cosmetic medicine, infrared thermography, plastic surgery

The camel beauty shows hosted in Saudi Arabia have high fame due to high prize money and camels are enrolled from various countries. Some camel owners resort to performing plastic surgery for their camels through injection of cosmetic fillers in the head region or stretching the lips to win the competitons (Tharwat and Al-Hawas, 2021).

Infrared thermography (IRT) can measure the surface body temperature from skin surface points to external inflammations or differences in blood circulation (Stelletta *et al,* 2012). The infrared camera measures the thermal energy discharged from the surface and converts it into an electrical signal proportional to the power of the infrared radiations (Usamentiaga *et al,* 2014). The camera lens has a capacity to read the temperature when taking a photo or immediately afterwards without using a computer (McCafferty, 2007; Cilulko *et al,* 2013).

In human medicine, the technology of IRT is used in several disorders such as studying the circulatory and lymphatic systems, rheumatic diseases, cosmetic surgery and in cancer diseases especially for the diagnosis of mammary gland cancer in women (Fauci *et al,* 2001; Vargas *et al,* 2009). In veterinary medicine, IRT is currently used in the diagnosis of inflammation of the sensitive structures of the hoof and the mammary tissue (Schaefer *et al,* 2004; Stelletta *et al,* 2007). Infrared thermography has also been used for scanning mammary stress induced by milking (Tangorra *et al,* 2019) and to measure stress and fear of humans in sheep (Cannas *et al,* 2018). In camels, the feasibility of utilising an infrared-thermographic technique for early diagnosis of mastitis in dairy camels has also been investigated (Samara *et al,* 2014).

This study was carried out to evaluate the feasibility of IRT in camel beauty pageants with either injected or stretched lips.

Materials and Methods

Camels

This study was carried out during the events of the 5th season of the KACF in the Kingdom of Saudi Arabia (November 29th - December 28th, 2020). A total number of 4627 camels (*Camelus dromedarius*) were thoroughly examined especially for injection of cosmetic fillers in the head region as well as stretching the lips. Of them 90 healthy camels (72 females and 18 males) aged 2 to 10 years were used as a control group based on a history of absence of diseases,

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

normal haematological and biochemical parameters and complete absence of skin lesions (tested by IRT). In addition, a total number of 74 camels with injected (n=14) or stretched lips (n=60) aged 5 to 10 years were also examined by IRT.

Thermography of the camel lips

The thermographic camera used was a FLUKE® TiX580 (USA) (thermal sensitivity of 0.05°C, temperature range of 20 negative degrees to 1000°C, automatic hot/cold detection). The infrared camera was also equipped with laser pointer, LaserSharp® Auto Focus for consistently in-focus images and laser distance meter that calculates distance to the target for precisely focused images and displays distance on screen. Thermographic measurements of the injected and stretched lips were taken at 7.00 to 10.00 AM. During the festival period, ambient temperature was minimum 6-17°C (11.5±4.7°C) and maximum 20-27°C (24.5±3.0°C), relative humidity 26-80% (50.7±18.7%) and wind speed 11-23 km/h (15.2±6.3 km/h).

Results

Camels evaluated by IRT were selected on the basis of absence of scars, injuries, or any skin diseases, which could interfere with the thermographic temperature. Camels enrolled for the study was of various colours including white, black, red, yellow, flame, and brindle that are represented in the beauty contests. The average age of the camels ranged from 5 to 10 years, and their weights ranged from 450 to 700 kg. Of the 4727 examined camels during the 5th KACF, 14 animals (0.30%) were found to be injected by cosmetic fillers in the upper lips and 60 camels (1.27%) had stretched lips. None of these animals had a history of recent illness.

Abnormalities of the injected lips included swelling, hardness of the lip tips and presence of multiple and hard nodules. When tested by IRT from the dermal surface, the injection sites appeared darker than that the surroundings. In another case, 5 injected sites were detected by IRT in the mucosal surface of the upper right lip that appeared darker than the surrounding tissue (Fig 1; a, b, c, d). The values of IRT of the upper right and left injected lips were 32.5±2.2°C and 32.7±3.4°C, compared to values of 30.3 ±1.1°C and 30.2 ±2.2°C, respectively in the control group. There were no significant differences between the values in injected lips compared to that in controls (P=0.07 and P=0.16, respectively).

Concerning the group of stretched lips, 3 points were tested by IRT that included upper right lip, upper left lip and lower lip. The IRT values for the 3 sites were 31.0 ±2.6°C; 31.0 ±2.6°C; 31.4 ±2.0°C, respectively compared to values of 30.3 ±1.1°C; 30.2 ±2.2°C; 31.4 ±1.8°C, respectively. There were no significant differences between the values in stretched lips compared to that in controls (P=0.50, 0.48 and 0.90, respectively). Of the 60 camels with stretched lips, 52 lips (88.3%) appeared longer than usual and flabby and in the remaining 7 camels (11.7%) they were longer, flabby and puffy. When tested by IRT the mucosal surface in the stretched lips appeared lighter and heterogeneous compared to the darker and homogenous pattern in the non-stretched lips (Fig 1; e, f, g, h). When the lips of this group were pressed, more saliva came out compared to the healthy lips in the control group.

Discussion

For the Arab peoples, especially in the Gulf area, camels constitute also cultural, literary, heritage and civilizational legacies. During the past decade, there has been increasing interest in camel racing competitions (Tharwat *et al,* 2013; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018; Tharwat, 2021). In addition, during the last 5 years camel beauty shows are being held regularly in Saudi Arabia where tremendous prizes are awarded. For this reason, plastic surgery in camels is currently rampant in the Gulf countries (Tharwat and Al-Hawas, 2021).

Thermography is a satisfactory technology for use in animals as it is safe and the infrared camera can be held at a distance from the subject (Stewart *et al,* 2005) and is a non invvoassive tool to study animal welfare (Stewart *et al,* 2005). Air temperature, convection and radiation and insulation influence the body surface temperature of animals which is determined also by the blood flow and metabolic rate of the underlying tissues. Thus, measurement of surface temperature using IRT may detect changes in local blood flow due to infection and/or inflammation (Eddy *et al,* 2001). Infrared thermography captures the spatial temperature profile of a target area and produces a visual map or thermogram of the surface temperature of this area by utilising false colour scales to represent pre-defined temperatures. Infrared thermographic devices contain an array of sensors and algorithms that measure incoming radiation and convert the values into temperatures (Harris-Bridge *et al,* 2018).

In veterinary medicine, the results of IRT use are controversial. In a study conducted on dogs,

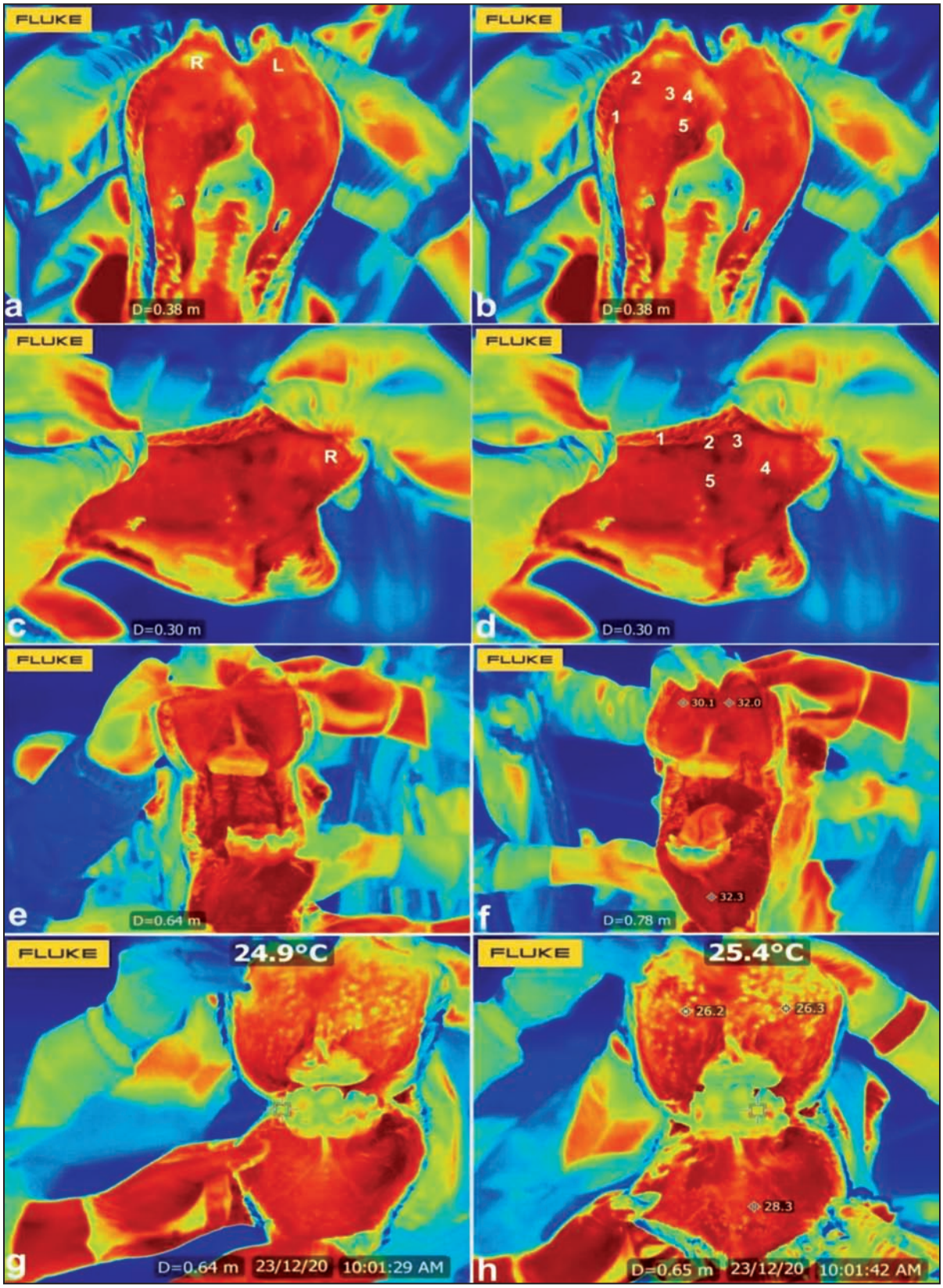


Fig 1. Infrared thermography of injected and stretched lips. Images a-d show injected upper lip in a camel where 5 injected sites (1-5) were detected and appeared darker than the surrounding tissue. Images e-h shows infrared thermography of stretched lips in a camel. The mucosal surface in the stretched lips appeared lighter and heterogeneous compared to the darker and homogenous pattern in the non-stretched lips. R=upper right lip; L=upper left lip.

Omobowale *et al* (2017) concluded that temperature measure obtained using non-contact infrared thermometry (forehead and nasal region of the head) was poor in consistency and agreement compared to rectal thermometry and usefulness of infrared thermometry in routine clinical practice depends on accurate calibration and therefore not recommended. On the contrary, in a study conducted on cattle by Stumpf *et al* (2021) concluded that by thermography of the udder lateral side, the clinician will be able to accurately predict an animal's rectal temperature.

Cosmetic medicine is a rapidly growing field in humans, and it includes minimally invasive treatments using resorbable dermal fillers, with hyaluronic acid fillers being the most commonly used products. The use of fillers has 5 major complications; it includes 1; injection site complications as erythema, edema, pain and ecchymosis 2; inappropriate injection technique related complications as formation of palpable nodules, visible implants, over- or under correction 3; allergy and hypersensitivity reaction 4; vascular adverse effects due to inadvertent intravascular injection of a filler and 5; wide varieties of bacterial, viral and fungal infections (Schutz *et al,* 2012; Shahrabi-Farahani *et al,* 2014; Kassir *et al,* 2020). Because of the high awards in camel beauty contests, some methods of cheating are carried out in some participated camels such as injection of the lip fillers or stretching the lips (Tharwat and Al-Hawas, 2021).

Injection of cosmetic lip fillers in this study led to the induration of the lips tip and formation of several hard nodules. By the infrared camera in the current investigation, the injected lip sites appeared darker than the surrounding tissue as its temperature was relatively higher when compared to healthy non-injected lip tissue. The darker appearance of the injected lip sites may be explained by the presence of inflammation near the injection site. Similarly, Stelletta *et al* (2012) stated that abnormal thermal images that can be monitored from skin point to superficial inflammations or alterations in blood flow. It should be clarified that in camels that have been injected since a long time, the image will be different, perhaps due to the disappearance of inflammation in the injected areas. Therefore, in these situations judgment should be accompanied by physical and ultrasound examinations. With regard to camels with stretched lips, there was no significant difference in temperature between stretched and healthy lips. In addition, stretched lips appeared by IRT lighter and heterogeneous compared to the darker and homogenous lips of healthy camels. The light colour of the image in this group could be explained by saliva retention within the salivary glands as found when examining such lips.

In conclusion, the obtained results of this study clearly showed that IRT, as an indirect non-invasive screening measure, is highly feasible and shows promise for detecting lesions such as injected or stretched lips in camel beauty pageants. However, thermographic monitoring alone is not enough for detecting these lip lesions especially in camels injected since long time. Therefore, a combination of IRT with clinical and ultrasonographic examinations would be a helpful for detecting such pathology.

Acknowledgements

The authors would like to express their deep gratitude to the Chairman of the Board of Directors of the Camel Club and the Chairman of the International Camel Organisation (ICO), Sheikh Fahd bin Falah bin Hithleen for his continuous support during the 5th season of the KACF (Nov. 29th – Dec. 28th, 2020).

References

Cannas S, Palestrini C, Canali E, Cozzi B, Ferri N, Heinzl E, Minero M, Chincarini M, Vignola G and Costa ED. Thermography as a non-invasive measure of stress and fear of humans in sheep. Animals. 2018; 8:146.

Cilulko J, Janiszewski P, Bogdaszewski M and Szczygielska E. Infrared thermal imaging in studies of wild animals. European Journal Wildlife Research. 2013; 59:17-23.

Eddy AL, Van Hoogmoed LM and Snyder JR. The role of thermography in the management of equine lameness. Veterinary Journal. 2001; 162:172-181.

Fauci MA, Breiter R, Cabanski Fick W, Koch R, Ziegler J and Gunapala SD. Medical infrared imaging - Differentiating facts from fiction, and the impact of high precision quantum well infrared photodetector camera systems, and other factors, in its reemergence. Infrared Physics and Technology. 2001; 42:337-344.

Harris-Bridge G, Young L, Handel I, Farish M, Mason C, Mitchell MA and Haskell MJ. The use of infrared thermography for detecting digital dermatitis in dairy cattle: What is the best measure of temperature and foot location to use? Veterinary Journal. 2018; 237:26-33.

Kassir M, Gupta M, Galadari H, Kroumpouzos G, Katsambas A, Lotti T, Vojvodic A, Grabbe S, Juchems E and Goldust M. Complications of botulinum toxin and fillers: A narrative review. Journal of Cosmetic Dermatology. 2020; 19:570-573.

McCafferty DJ. The value of infrared thermography for research on mammals: previous applications and future directions. Mammal Review. 2007; 37:207-223.

Omobowale TO, Ogunro BN, Odigie EA, Otuh PI and Olugasa BO. A Comparison of Surface Infrared with Rectal Thermometry in Dogs. Nigerian Journal of Physiological Sciences. 2017; 32:123-127.

Samara EM, Ayadi M and Aljumaah RS. Feasibility of utilising an infrared-thermographic technique for early detection of subclinical mastitis in dairy camels (*Camelus dromedarius*). Journal of Dairy Research. 2014; 81:38-45.

Schaefer AL, Cook N, Tessaro SV, Deregt D, Desroches G, Dubeski PL, Tong AKW and Godson DL. Early detection and prediction of infection using infrared thermography. Canadian Journal of Animal Science. 2004; 84:73-80.

Schutz P, Ibrahim HH, Hussain SS, Ali TS, El-Bassuoni K and Thomas J. Infected facial tissue fillers: Case series and review of the literature. Journal of Oral and Maxillofacial Surgery. 2012; 70:2403-2412.

Shahrabi-Farahani S, Lerman MA, Noonan V, Kabani S and Woo SB. Granulomatous foreign body reaction to dermal cosmetic fillers with intraoral migration. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology. 2014; 117:105-110.

Stelletta C, Fiore E, Vencato J, Morgante M and Gianesella M. Thermographic applications in veterinary medicine. In: Prakash RV, editor. Infrared hermography. In: INTECH Open Access Publisher; https://www. intechopen. com/books/infrared-thermography/thermographic-applicationsin-veterinary-medicine. 2012.

Stelletta C, Murgia L, Caria M, Gianesella M, Pazzona A and Morgante M. Thermographic study of the ovine mammary gland during different working vacuum levels. Italian Journal of Animal Science. 2007; 6:600.

Stewart M, Webster JR, Schaefer AL, Cook NJ and Scott SL. Infrared thermography as a non-invasive tool to study animal welfare. Animal Welfare. 2005; 14:319-325.

Stumpf M, McManus CM, Daltro DS, Alfonzo EPM, Dalcin V, Kolling GJ, Vieira RA, Louvandini H, Fischer V and da Silva MVGB. Different methods of assessing udder temperature through thermography and their relation with rectal temperature. Tropical Animal Health and Production. 2021; 53:44.

Tangorra FM, Redaelli V, Luzi F and Zaninelli M. The use of infrared thermography for the monitoring of udder teat stress caused by milking machines. Animals. 2019; 9:384.

Tharwat M. Influence of 8 km training on cardiac biomarkers alongside hematobiochemical profiles in race camels. Journal of Camel Practice and Research. 2021; 28:79-84.

Tharwat M and Al-Hawas A. Ultrasound detection of cosmic filler injection of lips in camel beauty pageants: first report in veterinary medicine. Tropical Animal Health and Production. 2021; pp 53:53.

Tharwat M and Al-Sobayil F. The impact of racing on the serum concentrations of acute phase proteins in racing dromedary camels. Comparative Clinical Pathology. 2015; 24:575-579.

Tharwat M and Al-Sobayil F. The impact of racing on serum concentrations of bone metabolism biomarkers in racing Arabian camels. Journal of Camel Practice and Research. 2018; 5:59-63.

Tharwat M, Al-Sobayil F and Buczinski S. Effect of racing on the serum concentrations of cardiac troponin I and CK-MB in racing camels (*Camelus dromedarius*). Veterinary Research Communications. 2013; 37:139-144.

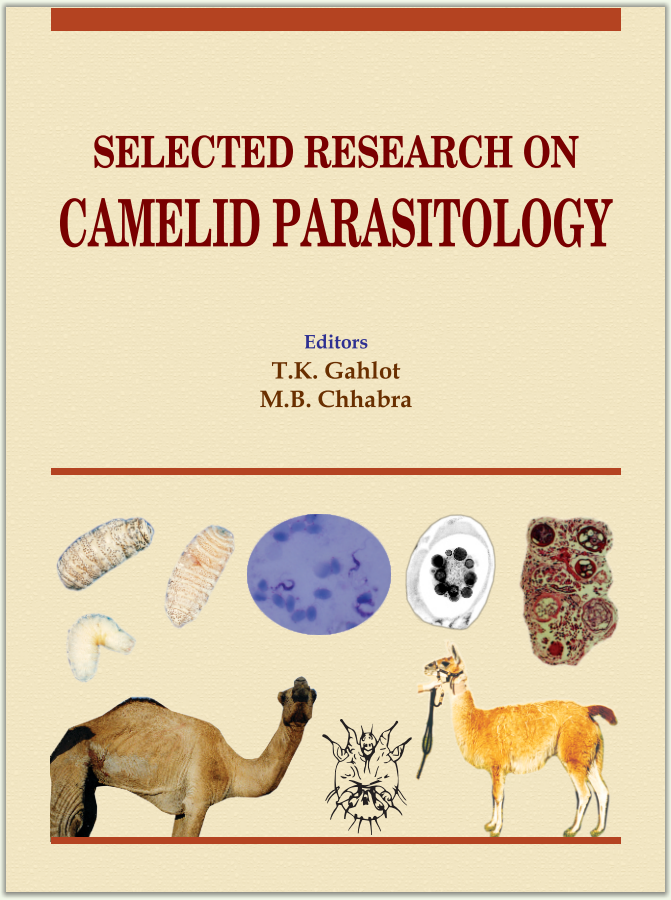
Usamentiaga R, Venegas P, Guerediaga J, Vega L, Molleda J and Bulnes F. Infrared thermography for temperature measurement and non-destructive testing. Sensors. 2014; 14:12305-12348.

Vargas JVC, Brioschi ML, Dias FG, Vargasa JVC, Brioschia ML, Diasb FG, Parolinc MB, Mulinari-Brennerd FA, Ordoneze JC and Colmanc D. Normalized methodology for medical infrared imaging. Infrared Physics and Technology. 2009; 52:42-47.

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 : 10.5958/2277-8934.2021.00055.2 *Vol 28 No 3, p 361-366*

ANALYSIS OF HAIR QUALITY ATTRIBUTES OF MEWARI AND JALORI CAMELS MANAGED UNDER NATURAL HABITAT

S.C. Mehta and S.S. Dahiya
ICAR-National Research Centre on Camel, Post Box-07, Bikaner-334001, Rajasthan, India

ABSTRACT

The hair quality attributes of two important Indian dromedary breeds viz. Mewari and Jalori, managed under natural habitat were analysed. Fifty and 49 samples were collected from the mid body side region of Mewari and Jalori camels, respectively. The average fibre diameter was 40.44±2.14 µ in Mewari breed as compared to 42.49±2.31 µ in Jalori breed. The fibre diameter was significantly (P<0.05) less among calves up to 1 year of age (31.55±2.72 µ) as compared to the higher age groups of 1-4 years' age (43.68±2.84 µ) and above 4 years' age (43.41±2.20 µ). The quality of fibre was assessed on the basis of medullation. The pure fibres were 4.87±0.73% in Mewari and 8.17±2.08% in Jalori with non-significant (P>0.05) effect of breed, sex and age groups. The hetero and hairy fibres were 25.46±2.21 % and 69.67±2.82 % in Mewari camels and 38.37±2.95 % and 53.32±3.62 % in Jalori camels, respectively with significant (P<0.01) effect of breed. The average staple length was 5.65±0.23 cm in Mewari (n=47) and 6.66±0.35 cm in Jalori (n=33) with significant (P<0.01) effect of breed. The study reveals that the quality of fibre produced by Jalori breed is better than that of the Mewari breed. In general, the calf hair can be optimally utilised in making outer wear and decorative handicraft items. The medium quality clippings can be used for making blankets, carpets, rugs and wall hangings whereas the clippings rich in hairy fibre can be utilised in making strings and lace for making designer garments. The study presents the fibre attributes of the two Indian dromedary breeds for the first time.

Key words: Camel hair, fibre characteristics, Jalori, medullation, Mewari

The population of dromedary camel in Rajasthan state has declined to 0.25 million (Livestock Census, 2019). The draught utility of camel is decreased due to mechanised farming. Nevertheless the therapeutic utility and development of various products of camel milk got significant attention (Mehta *et al,* 2009; 2014a; Govindasamy *et al,* 2019). However, in Indian context, the camel hair has been used extensively by the camel rearing families in making blankets, carpets, bags, ropes and other day to day use items for their own household use (Mehta *et al,* 2007). At several places in Rajasthan and Gujarat, it has taken the shape of rural cottage Industry (Sahani and Khanna, 1993). The finest natural fibre is produced by a camelid, i.e., Vicuna with the fibre diameter of 6-10 µ. Even the well-known producer of fine wool viz. Merino sheep and Angora rabbit has an average fibre diameter of 12-20 µ and 13 µ, respectively. Furthermore, the New World camelids viz. Guanaco, Alpaca and Llama too produce fibre with 10-30 µ diameter (Mehta *et al,* 2014b). Nevertheless, the dromedary hair is much coarser, hence utilised in different manner. Efforts were made to prepare the blends of camel hair with wool, silk waste and polyester with encouraging results (Gupta *et al,* 1987; 1989). Patni and Dhillon (1988) also evaluated the blends of camel hair with wool, silk waste and polyester and found it worthwhile. However, the use of camel hair in textile industry has several limitations due to the reduced number of camels in the country (Livestock Census, 2019) and limited production potential of the Indian dromedary breeds (Bhakat *et al,* 2002; 2003). There is tremendous scope of utilisation of camel fibre in the making handicraft items such as wall hangings, hair bands, purse, winter wears (Jackets), camel decoration items, ancient clothing, kurtas, cushion covers, embroidery items, patch work etc. The success of Ruma Devi (https://en.wikipedia.org/wiki/Ruma_Devi) model from the western Rajasthan gave wings to this thought. The use of camel hair under such model would certainly add to the income of the camel farmers and would support the conservation and propagation efforts of the scientists and stakeholders. In light of above facts, the hair quality and production of Mewari and Jalori camel reared under the natural habitat was evaluated.

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

Materials and Methods

Hair samples

The hair samples were collected from the breeding tract of Mewari and Jalori camel. The breeding tract of Mewari camel encompassed Mewar (Udaipur, Chittorgarh, Rajsamand districts), Vagad (Dungarpur, Banswara) and Hadoti (Kota, Bundi and Jhalawar) regions of Rajasthan state and the breeding tract of Jalori camel encompassed Jalore and Sirohi districts of state. For the assessment of hair quality; 50 and 49 hair samples from mid body side region were collected randomly from Mewari and Jalori camel, respectively while reording the age and sex of the animal. The hair production figures were collected from the camel householders in response to a questionnaire from Mewari and Jalori camel breeding tracts.

Hair quality

Fifty and 49 samples were analysed for assessment of hair diameter and type of fibre based on medullation, i.e., non-medullated (Pure) and medullated (hetero and hairy). However, 47 and 33 samples of Mewari and Jalori camels were assessed for staple length and the were measured before washing with petroleum ether. However, for rest of the parameters, the hair samples were cleaned and degreased in liquid petroleum ether thoroughly and dried overnight. The individual samples were mixed thoroughly to prepare a representative sample. The cleaned samples were loaded in the sample holder of microtome for the preparation of snippet. The snippet was put on a glass slide with the help of mounting media and covered with a glass cover slip. The images of short pieces of fibres in the glass slide were projected on screen and the diameters of fibres were measured by using a graduated scale on a projection microscope. The classification of type of fibre was also carried out at the same time. In each slide 300 observations were recorded to minimise the error.

Statistical analysis

Univariate analysis of variance by using general linear model was carried out by utilising SPSS version 26 (IBM Corp. Released 2019) for studying the effect of breed, sex, age and their interaction for each of the fibre characteristics under investigation.

Results and Discussion

The analysed fibre diameter, staple length and medullation of collected hair samples are presented in Table 1.

Fibre diameter

The average fibre diameter was 40.44±2.14 µ in Mewari camel as compared to 42.49±2.31 µ in Jalori camel with non-significant effect of breed (P>0.05). Pooled over breed, the average fibre diameter was 38.72±3.61 µ in males and 41.98±1.74 µ in females. The effect of sex was non-significant (P>0.05). However, it was noticed that the fibre diameter in Jalori males (26.98±1.28 µ) was quite less than the one observed for Mewari males (44.06±4.36 µ). The examination of data revealed that this difference was mainly due to the fact that all of the Jalori males (n=5) belonged to the less than one year age group. The fibre diameter in the females of the two breeds were quite comparable. The effect of age on fibre diameter was significant (P<0.05). The mean separation analysis showed that the fibre diameter of calves aging less than one year (31.55±2.72 µ) was significantly (P<0.05) less as compared to the animals of 1 to 4 years of age (43.68±2.84 µ) and adult camels (43.41±2.20 µ) of more than 4 years' age. However, relatively smaller fibre diameter in the three Indian dromedary breeds managed at the Institute have been reported by Bhakat *et al* (2001). The fibre diameter in Bikaneri, Jaisalmeri and Kachchhi breeds have been reported as 32.76±0.52, 35.52±0.59 and 38.19±1.07 µ, respectively. Accordingly, these differences were evident in the two sexes (34.05±0.59 µ in males and 36.93±0.55 µ in females) and the three age groups (26.05±0.72 µ in 1-year, 31.72±0.68 µ in 4-year and 48.70±0.66 µ in 8-year age group), respectively (Bhakat *et al,* 2001). These differences were obvious because in the present study the samples were collected only from mid body side region whereas in the study done by Bhakat *et al* (2001), the samples were collected from neck, shoulder, hump and mid body side. In spite of this, the differences in the two studies even for the mid body side were also large, which could be due to the breeds involved, selective breeding of farm animals for about 2-3 decades versus breeding by different camel owners as per their choice and limitations, difference in the management of an institutional herd (semi-intensive system) versus camel owners' herds (extensive system) belonging to different districts of the state, difference in the sample size and the differences in vegetation, rainfall, temperature and associated features of different breeding tracts and the Institute.

The fibre diameter is a single most important criteria in deciding its cost and use in the textile industry. The natural fibres having less than 25 µ diameter are used in apparel making and are one

among the costliest fibre. The second category of 25 to 35 µ diameter is also used in textile but for the preparation of outer wear. The next category consists of fibres having the diameter in the range of 35 to 45 µ is considered best for the preparation of carpets and other similar products (Teasdale, 1995; Singh and Mehta, 2002). The other closely link criteria of importance in the textile industry are the type of fibre, staple length and the production, i.e., the quantity that can be available for product making (Teasdale, 1995). The average fibre diameter of the calves (<1 yr.) was 31.55±2.72 µ with 88% of the animals having the fibre diameter in the range of 19.53-39.82 µ which shows that the calf fibre can optimally be utilised in making outer wear items like coat, jacket and day to day use items like cushion covers, embroidery and patch work items. However, since the production of hair from camels of this group has been reported to be 630±26 gm per annum (Bhakat *et al,* 2003), so the utilisation in textile industry would certainly require blending with other natural or artificial fibres. However, this can better be utilised in making handicraft items which can fetch better renumeration to the camel stakeholders.

The fibre diameter in the camels of 1-4 years' age group (43.68±2.84 µ) and that of the camels of greater than 4 years' age groups (43.41±2.20 µ) differed non-significantly (Table 1) and about 95 % of the values were covered in the range of 25.30-63.71 µ. Bhakat *et al* (2001) reported fibre diameter of

Table 1. Hair quality attributes with effect of breed, sex and age in the two Indian dromedary breeds.

Trait			Mewari	Jalori	Pooled
Fibre diameter (µ)	Breed		40.44±2.14 (50)	42.49±2.31 (49)	41.46±1.57 (99)
	Sex	Male	44.06±4.36 (11)	26.98±1.28 (5)	38.72±3.61(16)
		Female	39.42±2.45 (39)	44.25±2.43 (44)	41.98±1.74 (83)
	Age*	<1 yr.	27.93±3.15 (4)	32.67±3.42 (13)	31.55±2.72^{a} (17)
		1-4 yr.	44.53±2.95 (18)	42.41±5.72 (12)	43.68±2.84^{b} (30)
		>4 yr.	39.60±3.14 (28)	47.85±2.85 (24)	43.41±2.20^{b} (52)
Pure fibre (%)	Breed		4.87±0.73 (50)	8.17±2.08 (49)	6.50±1.10 (99)
	Sex	Male	1.46±0.59 (11)	16.94±7.75 (5)	6.29±2.93 (16)
		Female	5.83±0.87 (39)	7.18±2.13 (44)	6.54±1.20 (83)
	Age	<1 yr.	2.42±1.48 (4)	11.26±3.70 (13)	9.18±2.97 (17)
		1-4 yr.	3.50±0.99 (18)	5.89±2.75 (12)	4.46±1.24 (30)
		>4 yr.	6.09±1.09 (28)	7.64±3.53 (24)	6.81±1.71 (52)
Hetero fibre (%)	Breed**		25.46±2.21 (50)	38.37±2.95 (49)	31.85±1.94 (99)
	Sex	Male	17.48±3.94 (11)	56.13±7.99 (5)	29.56±5.81 (16)
		Female	27.71±2.51 (39)	36.35±3.04 (44)	32.29±2.04 (83)
	Age	<1 yr.	26.67±7.40 (4)	51.38±5.82 (13)	45.57±5.36^{b} (17)
		1-4 yr.	20.81±3.41 (18)	40.03±5.96 (12)	28.50±3.54^{a} (30)
		>4 yr.	28.27±3.07 (28)	30.48±3.52 (24)	29.29±2.30^{a} (52)
Hairy fibre (%)	Breed**		69.67±2.82 (50)	53.32±3.62 (49)	61.58±2.42 (99)
	Sex	Male	81.06±4.48 (11)	26.93±2.48 (5)	64.14±7.19 (16)
		Female	66.46±3.22 (39)	56.32±3.76 (44)	61.09±2.55 (83)
	Age	<1 yr.	70.92±8.78 (4)	37.36±5.76 (13)	45.25±5.92^{a} (17)
		1-4 yr.	75.69±4.27 (18)	53.86±7.52 (12)	66.96±4.35^{b} (30)
		>4 yr.	65.63±3.95 (28)	61.71±4.88 (24)	63.82±3.08^{b} (52)
Staple length (cm)	Breed**		5.65±0.23 (47)	6.66±0.35 (33)	6.07±0.21 (80)
	Sex	Male	5.20±0.38 (13)	9.15±2.75 (2)	5.72±0.55 (15)
		Female	5.83±0.29 (34)	6.50±0.33(31)	6.15±0.22 (65)
	Age	<1 yr.	3.88±0.48 (3)	7.43±0.95 (7)	6.36±0.86 (10)
		1-4 yr.	5.62±0.50 (11)	6.88±0.38 (4)	5.95±0.40 (15)
		>4 yr.	5.82±0.27 (33)	6.38±0.43 (22)	6.05±0.24 (55)

*Significant (P<0.05); **Significant (P<0.01); Figures with different superscripts differ significantly

31.72±0.68 μ in camels of 4 years age and 48.70±0.66 μ in camels of 8 years' age. Since, in present study these two age groups were clubbed together, so the reported values are slightly less to almost comparable because of factors discussed above. The values suggest that the quality carpet cannot be made out of this kind of fibre but the felts, carpets, rugs, blankets, bags, foot mats, wall hangings, decorative items, ropes etc. can be made out of this kind of hair.

Pure fibre

The utilisation of any natural fibres in the textile and associated industries also depend on the extent and type of medullation in the fibre and the per cent sharing of different fibres in the sample. The pure fibres were 4.87±0.73% in Mewari and 8.17±2.08% in Jalori. However, the effect of breed was non-significant (P>0.05). The percentage of pure fibres also did not differ significantly (P>0.05) among the two sexes. However, it was observed that the pure fibres were only 1.46% in Mewari males as compared 16.94% in Jalori males but this difference was observed mainly due to the fact that all Jalori males (n=5) belonged to youngest age group (<1 year) whereas the Mewari males (n=11) belonged to all the 3 age groups. Although, the effect of age group was non-significant but pure fibres in age group one (<1 year) were relatively higher than that in the higher age groups (Table 1). Significantly higher percentage of pure fibres in Indian dromedary breeds have been reported by Bhakat *et al* (2001), where the Bikaneri, Jaisalmeri and Kachchhi breeds have been shown to have 36.60±0.63, 36.31±0.71, and 40.68±1.28% pure fibres. These differences were obvious because in the present study the samples were collected only from mid-body side region whereas in the study done by Bhakat *et al* (2001), the samples were collected from neck, shoulder, hump and mid-body side. In spite of this, the differences in the two studies even for the mid body side were also large; which could be due to the breeds involved, management practices, breeding pattern, sample size, meteorological features of the breeding tract etc., as discussed above. The morphological structure of the natural fibres consists of the cuticle, the outer most layer of scales; the cortex, the intermediate portion consisting of ortho- and para-cortical cells; and the Medulla, hollow or partially filled central canal running continuously or in fragmented form along the length of the fibre (McGregor, 2012). The pure fibres do not have any medulla or hollow cavity in the inner core. The fibres having medulla has three different categories viz. hetero, hairy and kemp. The hetero-type fibres have interrupted or fragmented medulla and irregular scale pattern. The hairy type fibres have continuous medulla covering less than 50% width of the fibre, mosaic scale pattern and complete absence of crimps. The kemp fibres are chalky white in colour and tapering towards the tip, the medulla occupies ≥50% width and they are comparatively brittle, lack resiliency and has very thin walls that collapse to a flat ribbon (McGregor, 2012; Baxter, 2001; 2002). Hence, the clippings having higher proportion of pure fibres is considered better for apparel production.

Hetero fibre

The hetero fibres were significantly (P<0.01) less in Mewari (25.46±2.21%) as compared to Jalori breed (38.37±2.95%). These differences are clearly reflected in the animals of two sexes and three age groups (Table 1). However, pooled over breed, the hetero fibres differed non-significantly among males (29.56±5.81%) and females (32.29±2.04%). Further, the hetero fibres were 17.48% in Mewari males as compared to 56.13% in Jalori males for the reasons explained about their belongingness to only youngest age group in case of Jalori breed. The mean separation analysis indicated significant differences among the age groups with 45.57±5.36% hetero fibres in calves (<1 yr.) and 28.50±3.54 % and 29.29±2.30% hetero fibres, respectively in 1-4 yr. age group and adult animals (>4 yr.). Slightly higher values of hetero fibres have been reported by Bhatak *et al* (2001) where the Bikaneri, Jaisalmeri and Kachchhi breeds 39.68±0.53, 38.41±0.60, and 42.15±1.08 % hetero fibres. These differences can well be attribute to the reasons discussed above. The hetero fibres were significantly more in Jalori breed as compared to the Mewari breed making it relatively more suitable for carpet making. The hetero fibres are very important as the medullation in them imparts resiliency, which is one among the most important properties of the natural fibres which makes them suitable for carpet making. The pure and hetero fibres of the camel are thus suitable for the making of carpets and other types of floor covers.

Hairy fibre

The hairy fibres were significantly (P<0.01) higher in Mewari (69.67±2.82%) as compared to Jalori breed (53.32±3.62%). These differences were clearly reflected in the animals of two sexes and three age groups (Table 1). However, pooled over breed, the hairy fibres differed non-significantly among males (64.14±7.19%) and females (61.09±2.55%). Further, the hetero fibres were 81.06% in Mewari males as

compared to 26.93% in Jalori males for the reasons explained about their belongingness to only youngest age group in case of Jalori breed. The mean separation analysis indicated significant differences among the age groups with 45.25±5.92 % hairy fibres in calves (<1 yr.) and 66.96±4.35 % and 63.82±3.08% hairy fibres, respectively in 1-4 yr. age group and adult animals (>4 yr.). Significantly lower values of hairy fibres have been reported by Bhakat *et al* (2001) where the Bikaneri, Jaisalmeri and Kachchhi breeds have been shown to have 20.49±0.57 %, 23.85±0.64 % and 14.99±1.15 % hairy fibres, respectively. These differences could be due to the site of collection of samples, breed, selective breeding, difference in management, difference in breeding tract etc. as discussed above. Further, in present study the hairy and kemp fibres have been presented in this class of fibre. Also, it has been observed that the Mewari breed has significantly higher percentage of hairy fibres as compared to the Jalori breed, though the total medullation in the two breeds (95.13±0.73% in Mewari and 94.75 ±1.08% in Jalori) is quite comparable. Higher percentage of hairy and kemp fibres is not a desirable trait for the use of fibre in textile and other industries. However, products like ropes, strings, lace and other similar products can be made and used in the handicraft industry to make designer products.

Staple length

The Mewari breed (5.65±0.23 cm) has significantly (P<0.01) lower staple length as compared to the Jalori breed (6.66±0.35 cm). These differences were clearly reflected in the animals of two sexes and three age groups (Table 1). However, pooled over breed, the staple length differed non-significantly (P>0.05) among the two sexes and three age groups. Almost similar staple length was reported by Bhatak *et al* (2001) where the Bikaneri, Jaisalmeri and Kachchhi breeds had 6.68±0.21, 6.16±0.24 and 4.65±0.42 cm staple length, respectively. The staple length is also one among the important criteria in considering suitability of fibres for textile and other product making. Longer the fibre; stronger, smoother and perfect will be the yarn with better tenacity (Parsi *et al,* 2016). The present results suggest that the Jalori breed is relatively better in terms of staple length.

Hair production

Efforts were made to record the hair production of Mewari and Jalori camel in the breeding tract. It was observed that the farmers do clipping once in a year and some of them still use the camel hairs in making daily use house hold items but they do not weigh and record the production of hairs from each camel. Hence, it was not possible to give exact figures of the hair production in the two breeds. However, a rough etimate of 700 gm per adult animal per year was construed in both the breeds. Almost similar production figures have been reported in Bikaneri (933.85±17.99 gm and 976±31 gm), Jaisalmeri (733.43±17.84 gm and 746±15 gm) and Kachchhi (623.22±25.97 gm and 587±46 gm) breeds of Indian dromedary in the year 2002 and 2003 by Bhakat *et al* (2002) and Bhakat *et al* (2003), respectively.

The study reveals that the quality of fibre produced by Jalori breed is better than that of the Mewari breeds. In general, the calf hair can be optimally utilised in making outer wear and decorative handicraft items. The medium quality clippings can be used for making blankets, carpets, rugs and wall hangings whereas the clippings rich in hairy fibres can be utilised in making strings and lace for designer products.

Acknowledgements

This work was carried out under the Network Project on Animal Genetic Resources: Characterisation of Mewari and Jalori Camel. The project funding from the Indian Council of Agricultural Research through National Bureau of Animal Genetic Resources, Karnal is duly acknowledged.

References

Baxter BP. On-farm classing of animals and fleeces with the OFDA2000. Wool Technology and Sheep Breeding. 2001; 49:133-55.

Baxter BP. Raw-wool metrology: Recent developments and future directions. Wool Technology and Sheep Breeding. 2002; 50:766-79.

Bhakat C, Yadav B and Sahani MS. Effect of certain factors on hair quality attributes in Indian dromedary camel managed in an organised farm. The Indian Journal of Animal Sciences. 2001; 71(10):992-4.

Bhakat C, Mehta SC and Sahani MS. Studies on hair production attribute in Indian dromedary camel managed in an organised farm. The Indian Journal of Animal Sciences. 2002; 72(3):275-276.

Bhakat C, Mehta SC and Sahani MS. Annual hair yield attribute in indigenous camel breeds. The Indian Journal of Animal Sciences. 2003; 73(10):1189-91.

Govindasamy N, Swami SK, Mehta SC, Singh R, Meignanalakshmi S, Selvaraju S, Pourouchottamane R, Thirumaran SMK and Patil NV. Camel: A Medicinally Important Animal. Acta Scientific Veterinary Sciences. 2019; 1(4):12-22.

Gupta NP, Pokharna AK, Arora RK and Sugumar S. Mechanical processing of wool, specialty hair and

their blends. Annual Report, Central Sheep and Wool Research Institute, Avikanagar, India. 1987.

Gupta NP, Patni PC and Sugumar S. Properties and processing of camel hair in India. Indian Textile Journal. 1989; 99(4):180.

IBM Corp. Released. *IBM SPSS Statistics for Windows, Version 26.0.* Armonk, NY: IBM Corp. IBM Corp., New York. 2019.

Livestock Census. 20th Livestock census-2019, All India Report, Ministry of Agriculture and Farmers Welfare, Govt. of India. New Delhi. 2019.

McGregor BA. Properties, processing and performance of rare and natural fibres : a review and interpretation of existing research results. Rural Industries Research and Development Corporation. Publication No. 11/150. 2012.

Mehta SC, Bhardwaj B and Sahani MS. Status and conservation of Mewari and Jaisalmeri camels in India. Animal Genetic Resources Information. 2007; 40:87-101.

Mehta SC, Pathak KML, Bhardwaj B, Arora S and Bhatnagar CS. Camel Dairying: An Indian Perspective. The Indian Journal of Animal Sciences. 2009; 79(4):454-456.

Mehta SC. Genetic and demographic bottleneck analysis of Indian camel breeds by microsatellite markers. Tropical Animal Health and Production. 2014; 46(8):1397-406.

Mehta SC, Yadav SBS, Singh S and Bissa UK. Sire evaluation and selection of Indian dromedary for milk production: issues and strategies. Journal of Camel Practice and Research. 2014a; 21(1):93-98.

Mehta SC, Bissa UK, Singh S and Patil NV. Evolution, Status and Conservation of Camelids. In : Agro-Biodiversity and Sustainable Rural Development published by NIPA publisher. 2014b; pp 193-204.

Parsi RD, Madhuri VK, Pawar K and Patil RSP. Influence of fibre length on ring spun yarn quality. International Journal of Research and Scientific Innovation. 2016; III(VIII):154-156.

Patni PC and Dhillon RS. Areas and prospects of utilisation of camel hair and hide. National Seminar on Perceptions and Potentials of Camel Research in India, Bikaner (India). 1988; October 9-10.

Ruma Devi. https://en.wikipedia.org/wiki/Ruma_Devi date of access. 2021; June 22, 2021.

Sahani MS and Khanna ND. The camel fibre and its prospective utility. National Seminar on Production and Utilisation of Animal Fibres, Bikaner (India). 1993; December 13-14.

Singh VS and Mehta SC. A model of marketing and grading of raw wool. The Indian Textile Journal. 2002; 112(5):27-30.

Teasdale DC. The Wool Handbook – The A to Z of Fibre to Top. ISBN 0 646 24034. 1995; pp 49-53.

DOI : 10.5958/2277-8934.2021.00056.4 Vol 28 No 3, p 367-371

IN VITRO ACTIVITY OF CYP2J RECOMBINANT PROTEASE FROM BACTRIAN CAMEL

Xiaoxia Jing[1] and Surong Hasi[1,2]

[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, Hohhot, Inner Mongolia 010018, China
[2]Inner Mongolia Institute of Camel Research, Badain Jaran 737300, China

ABSTRACT

The purpose of this experiment was to study the *in vitro* activity of recombinant protease CYP2J of Bactrian camel. The activity of CYP2J enzyme was determined by fluorescence method according to the change of fluorescence peak after the conversion of ethoxycoumarin to hydroxycoumarin in NADPH generation system and ethoxycoumarin deethylase reaction system. Then, the affinity of the recombinant protease with its specific substrates arachidonic acid (AA) and astemizole was detected by localised surface plasmon resonance (LSPR) technique, and its activity was further determined. The results showed that the standard curve is y=17 533x+190.73 and R^2=0.998; The fluorescence detection result of the repeated experiments was 0.1350±0.0251 nM/min/mg, and the significant value between the repeated experiments was greater than 0.05, indicating that the difference was not statistically significant. The affinity of the recombinant protease for arachidonic acid was 3.53×10^{-5} M and that for astemizole was 4.07×10^{-5} M, respectively. Therefore, the Bactrian camel CYP2J recombinant protease has sufficient stable activities *in vitro* and can meet the basic requirements of further research.

Key words: Bactrian camel, CYP2J, *in vitro* activity, recombinant protease

CYP2J enzyme is the most abundant in cytochrome P450s, and its content accounts for 1/3 of the total enzyme content of CYP family. Mainly involved in the metabolism of most exogenous and endogenous substances including drugs and environmental compounds. CYP2J is widely distributed in different tissues of Bactrian camels, and mainly existed in digestive and metabolic organs such as small intestine, pancreas and liver (Maayah *et al*, 2019; Lu *et al*, 2020). Therefore, it is speculated that these unique biological characteristics of Bactrian camels are related to the specific CYP2J enzyme. The activity of the recombinant protease can be effectively detected by using the change of fluorescence peak after ethoxycoumarin is converted into hydroxycoumarin and ethoxycoumarin deethylase in NADPH generation system (Messina *et al*, 2010).

LSPR is a surface plasmon resonance technology that has been widely used in such fields as biomolecular interaction, proteomics, drug screening and real-time monitoring of related pharmacokinetics (Park *et al*, 2021). This method can obtain important information such as biomolecular interaction, interaction between different drugs or drug modified structures and biomolecules, the speed of molecular interaction and separation (Acimovic *et al*, 2014), when molecular interaction reaches equilibrium, and magnitude of interaction force in real time without labeling.

Materials and Methods

Recombinant protease

Bactrian camel CYP2J gene was expressed by *E. coli* prokaryotic expression system, purified and stored in the refrigerator at -80°C in laboratory (Jia, 2018).

Main reagents and instruments

In vitro fluorescence quantitative detection kit for cytochrome P450 sub-enzyme CYP2J (ECOD) activity: GENMED Scientifics Inc. USA; Tris-HCl buffer and PBS buffer: Solarbio company; Multifunctional microplate reader: Biotek Synergy H4 Hybrid Reader, USA; Open SPR biomolecular interaction analyser: Restar Communications Corporation, Japan; Arachidonic acid standard (SA9940-20mg): Solarbio company; Astemizole standard (YZ100301-50mg): National Institutes for Food and Drug Control.

SEND REPRINT REQUEST TO SURONG HASI email: baohaas@163.com

Determination of standard curve

The reaction conditions were set as follows: temperature 37°C, excitation wavelength of 370 nm and emission wavelength of 450 nm. According to table 1, prepared 20μL standard solutions with different concentrations, then 155μL buffer solution, 20μL reaction solution and 5μL substrate solution were sequentially added and mixed, incubated them at 37°C in the dark for 30 min, added stop solution and incubated for another 5 min for detection.

Determination of total activity of CYP2J recombinant protease

Added 20μL reaction solution, 5μL substrate solution and 20μL sample to 155μL buffer successively and mixed thoroughly. Incubated at 37°C for 30 min, and then added 75μL stop solution, and incubated for another 5 min in the dark. The relative fluorescence unit of the total activity of the sample was determined. The concentration of hydroxylcoumarin in the enzyme was obtained according to the standard curve.

Determination of nonspecific activity of CYP2J recombinant protease

20μL colourimetric solution, 20μL reaction solution, 5μL substrate solution and 20μL sample were added sequentially to the 135μL of buffer solution, and mixed thoroughly. Incubated at 37°C for 30 min, added 75μL of stop solution, then incubated again for 5 min. The relative fluorescence unit of nonspecific activity of the sample was detected. The concentration of hydroxylcoumarin in the enzyme was obtained according to the standard curve.

Detection of interaction between CYP2J recombinant protease and its specific substrates by LSPR

Sample pretreatment

The CYP2J recombinant protease was 0.6mg/mL and its molecular weight was 57.98 kDa. The specific substrates were arachidonic acid (AA) with molecular weight of 304.47Da and astemizole with molecular weight of 458.57Da. Standards were diluted with anhydrous ethanol to 10, 20, 40 and 100 μM.

Experimental process

The NTA chip was installed according to the standard operating procedure of OpenSPR instrument, and the PBS buffer (pH7.4) was run at the 150 μL/min. After reaching the signal baseline, 200μL 80% IPA(isopropanol) was added and the reaction temperature was 25°C. After running for 10s, the sample loop was flushed with buffer, then the flow rate of buffer was adjusted to 20 μL/min. The NTA chip was charged by Ni^{2+} ions by adding 200 μL of 40 mM $NiCl_2$ solution. 200 μL of CYP2J recombinant protein was injected and run for 4min. Then the samples were loaded at different concentrations of specific substrates to observe the binding and dissociation time of the enzyme and substrates.

Table 1. Preparation of standard tube concentration.

Centrifuge tube	buffer solution	Standard solution	Determination system standard hydroxycoumarin concentration
1	25 μL	25 μL	1 μmol/L
2	25 μL	25 μL Centrifugal tube No. 1	0.5 μmol/L
3	25 μL	25 μL Centrifugal tube No. 2	0.25 μmol/L
4	25 μL	25 μL Centrifugal tube No. 3	0.125 μmol/L
5	25 μL	Blank control	0

Result

Specific activity of CYP2J recombinant protease

According to the standard curve, the total activity and non-specific activity values of the recombinant protease were obtained by the above detection, and the specific activity of the recombinant protease was further obtained by calculation (Messina, 2010).

Sample activity = [corresponding hydroxycoumarin concentration × 0.2 × sample dilution times] ÷ [0.02 × reaction time] ÷ concentration of recombinant protein

Specific activity of sample = total activity of sample - nonspecific activity of sample

Table 2. Standard curve parameters.

Concentration of standard hydroxycoumarin	1	0.5	0.25	0.125	0
RFU (actual relative fluorescence unit)	17739	8683	5099	2307	0

Construct a standard curve: the vertical coordinate (y axis) is the actual relative fluorescence unit (RFU); The abscissa (x axis) is the standard hydroxycoumarin concentration (μmol/L) (Table 2). The standard curve is $y = 17\,533x+190.73$, $R^2 = 0.998$

(Fig 1). The correlation coefficient is close to 1, and the agreement between theory and practice is high.

Three repeated experiments were conducted to determine and compare the specific activities of protease. The results are shown in table 3. The specific activities of the three groups were as follows: 0.1333± 0.0175, 0.1450±0.0214 and 0.1267±0.0342, respectively. After the significance test, P=0.467, which was much higher than 0.05, indicated that there was no significant difference between groups. The total average value of the three groups was 0.1350±0.0251. Therefore, the Bactrian camel CYP2J recombinant protease obtained by *E. coli* prokaryotic expression system had certain *in vitro* activity.

Detection of interaction between CYP2J recombinant protease and its specific substrates by LSPR

It could be seen from the Fig 2 that the recombinant protease of bactrian camel CYP2J could be well combined with AA and astemizole, with smooth curve and high coincidence. Local module and one-to-one analysis model were used to determine the binding rate constant of AA and CYP2J was Ka=6.55×10^{-1} Lmol^{-1}s^{-1}, dissociation rate constant was Kd= 2.31×10^{-3}Ls^{-1}, and affinity constant

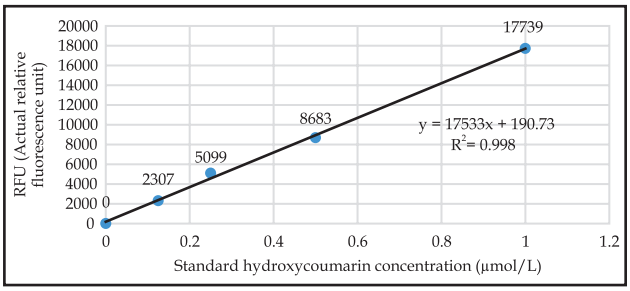


Fig 1. Standard curve.

was KD=3.53×10^{-5}M. After Trace Drawer analysis, the binding curve of AA and CYP2J recombinant protease had good waveform and the kinetic fitting accuracy was high. The results showed that AA had a good affinity with CYP2J recombinant protease, and the activity of CYP2J recombinant protease was stable.

Table 3. Comparison of specific activity and significance of repeated experiments in three groups.

Repeat group	1	2	3
Specific activity	0.1 333± 0.017 5	0.1 450± 0.021 4	0.1 267± 0.034 2
Significance test	0.467		
Total average	0.1 350±0.025 1		

Table 4. Interaction of CYP2J recombinant protease with arachidonic acid and astemizole.

Substrate	Ka(1/m*s)	Kd(1/s)	KD (M)
AA	6.55×10^{-1}	2.31×10^{-3}	3.53×10^{-5}
Astemizole	4.04×10^{-2}	1.64×10^{-2}	4.07×10^{-5}

Ka: Binding rate constant: Kd: Dissociation rate constant: KD: Dissociation equilibrium constant, also called affinity constant.v

As an exogenous specific substrate of CYP2J, astemizole could combine well with the recombinant protease of CYP2J in Bactrian camel. The binding rate constant was Ka=4.04×10^{-2} Lmol-1s^{-1}, the dissociation rate constant was Kd=1.64×10^{-2} Ls^{-1} and the affinity constant was KD=4.07×10^{-5} M.These parameters were obtained by fitting with the global module and one-to-one analysis model. Through the analysis of Trace Drawer, The smooth curve and good fitting directly indicate that astemizole had good affinity with recombinant protease. The activity characteristics of the recombinant protease *in vitro* of bactrian camel CYP2J are well represented.

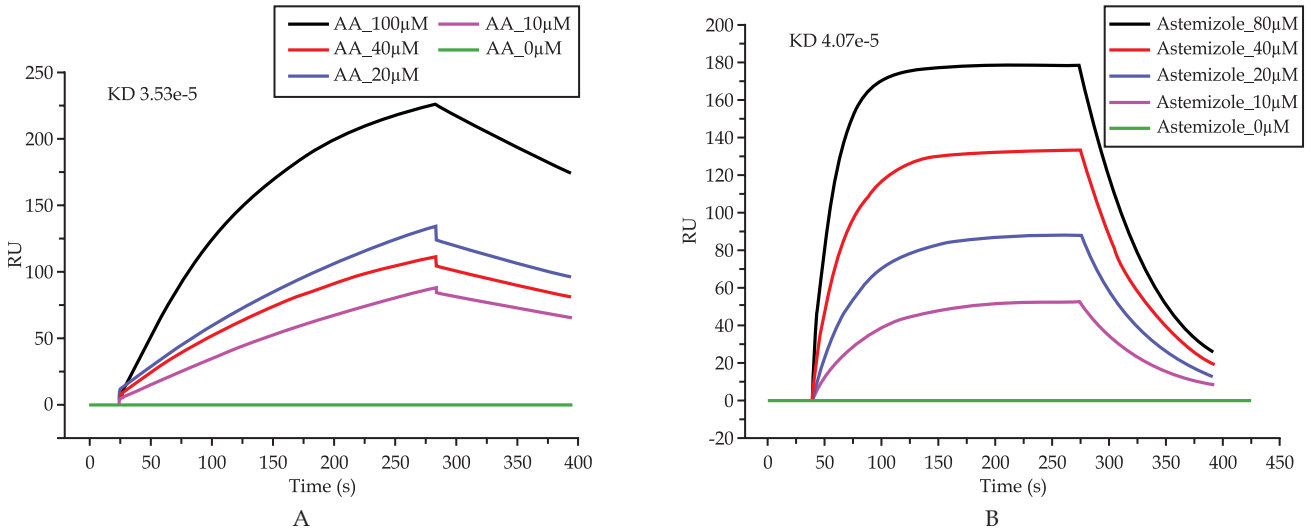


Fig 2. The interaction of CYP2J recombinant protease with AA (A) and astemizole (B) was detected by LSPR.

Discussion

Current studies had shown that the CYP2J subfamily genotypes were detected in different animals included, i.e. rabbit CYP2J1, rat CYP2J3/ 4, Mouse CYP2J8/ 11/ 12/ 13 (Graves *et al*, 2013), Human, ape, and cattle CYP2J2 (Lu *et al*, 2020). The substrates and reactions of CYP2J mainly included aniline hydroxylation, aminopyrine demethylation, ethoxycoumarin deethylation, testosterone hydroxylation, all-trans retinoic acid oxidation, astemizole-O-demethylation, diclofenac hydroxylation, arachidonic acid epoxidation and so on. The activity of ethoxycoumarin deethylase was a diagnostic marker of CYP2J (Hanif *et al*, 2017). The activity of cytochrome P450 subenzyme 2J was quantitatively determined by the change of fluorescence peak after the conversion of cytochrome P450 subenzyme to hydroxylcoumarin catalysed by ethoxycoumarin deethylase in the presence or absence of danazole, the sensitive inhibitor, and to screen inducers and inhibitors (Messina *et al*, 2010).

Bactrian camel CYP2J protein was mainly distributed in the small intestine, pancreas, liver, heart and other important organs, with anti-inflammatory, vasodilation, relaxation of smooth muscle, promote angiogenesis and other functions (Hasi *et al*, 2018; Peng, 2019). It mainly acts on aminopyrine, all trans retinoic acid, arachidonic acid and its derivatives prostaglandins, leukotrienes and epoxy eicosatrienes, among others. It could also oxidised and metabolised other exogenous compounds included astemizole, ebastine, terfenadine, albendazole, amiodarone, phentermine and diclofenac, etc. Thereby regulated hormone secretion, vascular tension, renal microtubule secretion, etc (Kang *et al*, 2011). CYP2J was also highly expressed in tumour tissues, which promoted tumour growth and reproduction. Abnormal CYP2J might lead to pathological conditions such as vascular diseases, tumour manifestations, inflammation, hormone secretion variation and renal microtubule filtration failure (Yu *et al*, 2000). Therefore, in this experiment, the activity of recombinant protease CYP2J of Bactrian camel was detected *in vitro*, so as to provide convenience for the later research of disease experiment or clinical medication.

In order to better confirm the activity of the recombinant protease, the specific activity of the recombinant protease was detected by conventional fluorescence method. According to the repeated tests, it was confirmed that the recombinant protease was relatively completely expressed and had good activity. Since CYP2J is widely distributed and abundantly expressed in the body, participating in endogenous and exogenous multiple metabolism, the specific substrates unique to CYP2J are selected to further use from both endogenous and exogenous substrates (Messina *et al*, 2010; Arnold *et al*, 2017), and the activity of recombinant protease can well be expressed by the sensitive reaction and intuitive effect of LSPR.

Arachidonic acid was one of the essential polyunsaturated fatty acids, which had many biological functions and participates in multiple metabolic reactions in the animal body, and was a specific substrate of CYP2J enzyme. Therefore, detecting the affinity of CYP2J recombinant protease and arachidonic acid could well show that the constructed recombinant protease could be used for later research and had a certain effect (Kamel *et al*, 2018; Olivares-Rubio and Espinosa-Aguirre, 2020). As a representative of exogenous CYP2J metabolism, astemizole affinity test not only could prove the activity of the recombinant protease, but also showed that the recombinant protease constructed could play a highly similar role with the protease actually existing *in vivo* (Lu *et al*, 2020; Matsumoto *et al*, 2002).

Localised surface plasmon resonance was generated by metal nanoparticles, typically gold and silver, as continuous thin films of gold. LSPR produced a strong resonance absorption peak in the visible light range, and the location of which was highly sensitive to the local refractive index around the particle (Chen *et al*, 2020). This technique only needs a small number of samples, which greatly saves the cost (Faridfar *et al*, 2020). The experiment could be easily repeated by reproducible sample injection and accurate results were obtained from reproducible measurements.

In conclusion, the recombinant protease was transformed into hydroxycoumarin under the catalysis of ethoxycoumarin deacetylase in NADPH production system. According to the change of fluorescence peak, the activity of recombinant protease was confirmed to be good, and the specific endogenous and exogenous active substances were further detected by LSPR technology. Therefore, the recombinant protein expressed in *E. coli* showed good activity, which could provide the basis for further research or clinical application.

Acknowledgements

This work was supported by National Natural Science Foundation of China (31560710).

Reference

Acimovic SS, Ortega MA, Sanz V, Berthelot J, Garcia-Cordero JL, Renger J, Maerkl SJ, Kreuzer MP and Quidant R. LSPR chip for parallel, rapid, and sensitive detection of cancer markers in serum. Nano Lett. 2014; 14:2636-2641.

Arnold WR, Baylon JL, Tajkhorshid E and Das A. Arachidonic acid metabolism by human cardiovascular CYP2J2 is modulated by doxorubicin. Biochemistry. 2017; 56:6700-6712.

Chen JS, Chen PF, Lina HT and Huang N-T. A Localised surface plasmon resonance (LSPR) sensor integrated automated microfluidic system for multiplex inflammatory biomarker detection. Analyst. 2020; 145:7654-7661.

Faridfar G, Zeinoddini M, Akbarzedehkolahi S, Faridfar S and Nemati AS. Immunodiagnostic of Vibrio cholerae O1 using localised surface plasmon resonance (LSPR) biosensor. International Microbiology. 2020; 24(1):115-122.

Graves JP, Edin ML, Bradbury JA, Gruzdev A, Cheng J, Lih FB, Masinde TA, Qu W, Clayton NP, Morrison JP, Tomer KB and Zeldin DC. Characterisation of four new mouse cytochrome P450 enzymes of the CYP2J subfamily. Drug Metabolism and Disposition. 2013; 41:763-773.

Hanif A, Edin ML, Zeldin DC, Morisseau C, Falck JR and Nayeem MA. Vascular endothelial overexpression of human CYP2J2 (Tie2-CYP2J2 Tr) modulates cardiac oxylipin profiles and enhances coronary reactive hyperemia in mice. Plos One. 2017; 12:e0174137.

Hasi S, Yao J, Yu S, and Tian Y. Diversity and distribution of CYP gene family in Bactrian camel. Functional and Integrative Genomics. 2018; 18:23-29.

Jia ZP. Cloning of Full-length c DNA, Bioinformatic Analysis and Prokaryotic Expression of CYP2J of Bactrian Camel. Master's thesis. Inner Mongolia Agricultural University. 2018.

Kamel S, Ibrahim M, Awad ET, El-Hindi HMA and Abdel-Aziz SA. Differential expression of CYP2J2 gene and protein in *Camelus dromedarius*. Journal of Biological Regulators and Homeostatic Agents. 2018; 32:1473-1477.

Kang W, Elitzer S, Noh K, Bednarek T and Weiss M. Myocardial pharmacokinetics of ebastine, a substrate for cytochrome P450 2J, in rat isolated heart. British Journal of Pharmacology. 2011; 163.

Lu J, Chen A, Ma X, Shang X, Zhang Y, Guo Y, Liu M and Wang X. Generation and Characterisation of Cytochrome P450 2J3/10 CRISPR/Cas9 Knockout Rat Model. Drug Metabolism and Disposition. 2020; 48:1129-1136.

Maayah ZH, McGinn E, Al BR, Gopal K, Ussher JR and El-Kadi AOS. Role of Cytochrome p450 and Soluble Epoxide Hydrolase Enzymes and Their Associated Metabolites in the Pathogenesis of Diabetic Cardiomyopathy. Journal of Cardiovascular Pharmacology. 2019; 74:235-245.

Matsumoto S, Hirama T, Matsubara T, Nagata K and Yamazoe Y. Involvement of CYP2J2 on the intestinal first-pass metabolism of antihistamine drug, astemizole. Drug Metabolism and Disposition. 2002; 30:1240-1245.

Messina A, Nencioni S, Gervasi PG, Gotlinger KH, Schwartzman ML and Longo V. Molecular cloning and enzymatic characterisation of sheep CYP2J. Xenobiotica. 2010; 40:109-118.

Olivares-Rubio HF and Espinosa-Aguirre JJ. Role of epoxyeicosatrienoic acids in the lung. Prostaglandins and Other Lipid Mediators. 2020; 149:106451.

Park H, Ma GJ, Yoon BK, Cho NJ and Jackmanv JA. Comparing Protein Adsorption onto Alumina and Silica Nanomaterial Surfaces: Clues for Vaccine Adjuvant Development. Langmuir. 2021.

Peng XB. Expression and Distribution of CYP2J Gene in visceral tissues of bactrian camel. Master's thesis. Inner Mongolia Agricultural University. 2019.

Yu Z, Huse LM, Adler P, Graham L, Ma J, Zeldin DC and Kroetz DL. Increased CYP2J expression and epoxyeicosatrienoic acid formation in spontaneously hypertensive rat kidney. Molecular Pharmacology. 2000; 57:1011-1020.

BIOTECHNOLOGY MIRACLE IN UAE: CHAMPION MABROKAN BORN AGAIN

Mabrokan – a male camel, weighing around a 1,000 kg, was a champion pure black "Hasmi" show camel who had been purchased as an adult animal by His Highness Sheikh Sultan bin Hamdan al Nahyan for the equivalent of $5 million US dollars. He won many beauty competitions over a number of years and was always the number one champion of males. Mabrokan was not a male to be "messed" with but when he died suddenly in 2010, a quick decision to preserve some testicular tissue and skin in liquid nitrogen at -196 deg C was made. After 10 years until they were examined "post thaw" from their frozen state by members of Prof Hwang's team to discover against the odds that there were viable cells in the skin tissue samples. These cells were then cultured into larger numbers and used in a process known as Somatic Cell Nuclear Transfer (SCNT), the same method which had given the team successful births the previous year from living donor camels. SCNT involves very accurate micromanipulation of the donor DNA cell and the surrogate oocyte. The DNA of the oocyte was removed and replaced by the "donor cell" DNA using micro needles and "robotic like" micromanipulation under a high magnification microscope. The DNA from Mabrokan's stored fibroblast skin cells was fused into a new egg, a micro pulse of electricity allowing the fusion to occur and a significant number of embryos were created. These embryos were cultured for 6 days in the laboratory under a "delicate" incubation process and transferred into synchronised surrogate female camels. This resulted in a total of 45 pregnancies with an identical DNA match to the deceased donor camel Mabrokan. Following the successful birth of the first Mabrokan calf a further 20 calves were born over a few months and these individuals are now almost a year old. Unique work was done at Hilli E.T. and Cloning Centre in Al Ain (Dr Alex Tinson, Dr Kuhad Kuldeep Singh and Dr Rajesh Sambyal) working with a team of experts from Abu Dhabi Biotech headed by Prof Hwang Woo-Suk. Prof Hwang and his team were well known for their previous work at Sooam Biotech Korea (now Abu Dhabi Biotech) on the Worlds' First Dog and Coyote Clones in 2005, as well as continuing work on trying to resurrect and clone the Woolly Mammoth.

Mabrokan – a male camel, weighing around a 1,000 kg, was a champion pure black "Hasmi" show camel

The camel has looked after generations of people on the Arabian Peninsula but now more recently the tables have turned and through camel racing, milking camels and camel showing competition the people of Arabia are returning a "debt of service" to this incredible animal. This is a world first achievement in the resurrection of a long dead champion camel and sets the scene for more amazing advances to come from U.A.E. Biotechnology Centre.

(Courtesy: Dr Alex Tinson)

UAE Biotech Research Centre in Abu Dhabi working with a well known team of experts headed by Professor Hwang: The DNA from Mabrokan's stored fibroblast skin cells was fused into a new egg and resultant embryo transplantation gave birth to the calves

DOI : 10.5958/2277-8934.2021.00057.6 Vol 28 No 3, p 373-376

TESTOSTERONE AND GROWTH HORMONE LEVELS IN FEMALE DROMEDARY CAMELS

Mohamed Tharwat[1,2], Abdulla Al-Hawas[1,3] and Ahmed Aldhubayi[3]

[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, 44519, Zagazig, Egypt

[3]Veterinary Teaching Hospital, Qassim University, P.O. Box 6622, Buraidah, 51452, Saudi Arabia

ABSTRACT

This study was designed to establish the normal level of testosterone and growth hormone (GH) in female camels (*Camelus dromedarius*) starting at 6 months until 10 years of age. Two groups each of 55 clinically healthy female camels were used in this study. The first group was aged from 6 till 15 months while the second group was aged over 15 months until 10 years of age. Both camel testosterone and GH were analysed using double-antibody sandwich enzyme-linked immunosorbent one-step processes. For testosterone, the detectable assay range was 0-320 pg/mL and the minimum detected level was typically less than 1.0 pg/mL. For GH, the detectable assay range was 0-16 ng/mL and the minimum detected level was typically less than 0.1 ng/mL. Compared to a value of 51±9 pg/mL in camels aged 6 to 15 months, the testosterone value was 52±7 pg/mL in camels aged over 15 month until 10 years of age. The minimum and maximum levels in the first group were 34 pg/mL and 75 pg/mL, while the minimum and maximum levels in the second group were 37 pg/mL and 66 pg/mL, respectively. There was no statistically significant difference when compared testosterone values in both groups. Compared to a value of 2.7±0.4 ng/mL in camels aged 6 to 15 months, the GH value was 2.2±0.3 ng/mL in camels aged over 15 month until 10 years of age. The minimum and maximum levels in the first group were 1.9 ng/mL and 4.3 ng/mL, while the minimum and maximum levels in the second group were 1.6 ng/mL and 2.9 ng/mL, respectively. There was a highly statistically significant difference when compared GH values in both groups.

Key words: Beauty pageants, camel, dromedary, growth hormone, testosterone

In order to enhance the growth and performance, many camel owners use growth and testosterone hormone in the young camels participating in King Abdulaziz Camel Festival (KACP) at Saudi Arabia. Medical committee of KACF Examines levels of these hormones and elevated levels disqualify the camel and it is a punishable offence (Tharwat and Al-Hawas, 2021).

Growth hormone (GH) is an important polypeptide of vertebrates commercially important in the areas of veterinary medicine, animal husbandry and animal production (DeNoto *et al,* 1981; Ayson *et al,* 2000). Biotechnology is used to enhance milk and meat production through Lactating animals' growth hormones and growth development of the animal are also controlled. GH also regulates complex physiological processes such as metabolism, reproduction and cell proliferation (Devlin *et al,* 1994).

Three key hormones are considered the "anabolic giants" in cellular growth and repair: testosterone, the growth hormone superfamily, and the insulin-like growth factor (IGF) superfamily (Kraemer *et al,* 2020).

Doping control in Horse racing and animal-related events poses different challenges, in comparison with other sports where humans are involved, because both performance-enhancing and performance impairing substances (or methods) can be used to manipulate and change the outcome of the competition while the controls are not standardised and rarely applied (Wong and Wan, 2014).

The androgenic-anabolic steroids (AAS) are synthetic derivatives of the male hormone testosterone. Testosterone is a precursor of other steroid hormones. The major functions of testosterone are pubertal development for spermatogenesis, regulation of the differentiation of the prostate, stimulation of erythropoietin production in the kidney and stem cells of the haematopoietic system, and the acceleration of growth during puberty in conjunction with growth hormone (GH) (Jeong *et al,* 2010). The GH belongs to a family of

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

somatolactogenic hormones that have classically included prolactin and placental lactogen and more recently have been expanded to include a number of the hematopoietic growth factors. The synthesis and secretion of GH are stimulated by GH-releasing hormone, a hypothalamic peptide hormone (Burton *et al,* 1994). The aim of the present study was therefore to evaluate the normal level of testosterone and GH in female camels (*Camelus dromedarius*) starting at 6 months until 10 years of age during competition in camel beauty pageants at KACF, Saudi Arabia.

Materials and Methods

The study area was KACF, Saudi Arabia and apparently healthy camels were included in the present investigation. These camels were aged between 10 months to 10 years and divided into two groups.

The first group had camels from 6 till 15 months of age while the second group had camels over 15 months until 10 years of age. Enrollment in the study was based on normal complete physical examination findings, normal cardiac auscultation, normal complete blood cell counts, and normal biochemistry profiles. Blood samples (7 mL) were collected in plain vacutainer tubes and sera were harvested.

Testosterone and growth hormones assays

Both testosterone and GH were analysed using double-antibody sandwich enzyme-linked immunosorbent one-step processes (Camel testosterone and Camel GH ELISA kits, CAT., NO: CKCA-0011 & CKCA-0013; LOT#: P20201127, Melsin Medical Co., Limited, China). For testosterone, the detectable assay range was 0-320 pg/mL and the minimum detected level of testosterone was typically less than 1.0 pg/mL. For GH, the detectable assay range was 0-16 ng/mL and the minimum detected level of GH was typically less than 0.1 ng/mL. Both assays had high sensitivity and excellent specificity for detection of camel testosterone and GH with intra-assay CV (%) < 10% and inter-assay CV (%) < 15%. The assays recognised recombinant and natural testosterone and GH and no significant cross-reactivity or interference between testosterone and GH and analogues was observed.

Statistical analysis

Data normality was examined using the Kolmogorov–Smirnov test. The data were presented as means ± SD and were analysed statistically using the SPSS statistical package (2009). A Student's t-test was used for comparisons testosterone and GH in both groups. Significance was set at $P \leq 0.05$.

Results

Table 1 summarises testosterone and GH levels mean ± SD) in camels, minimum and maximum values, alongside the 25th, 50th, 75th, 95th and 99th percentiles. Compared to a value of 51±9 pg/mL in camels aged 6 to 15 months, the testosterone value was 52±7 pg/mL in camels aged over 15 month until 10 years of age. The minimum and maximum levels in the first group were 34 pg/mL and 75 pg/mL, while the minimum and maximum levels in the second group were 37 pg/mL and 66 pg/mL, respectively. There was no statistically significant difference when compared testosterone values in both groups (P=0.19). Compared to a value of 2.7±0.4 ng/mL in camels aged 6 to 15 months, the GH value was 2.2±0.3 ng/mL in camels aged over 15 month until 10 years of age. The minimum and maximum levels in the first group were 1.9 ng/mL and 4.3 ng/mL, while the minimum and maximum levels in the second group were 1.6 ng/mL and 2.9 ng/mL, respectively. There was a highly statistically significant difference when compared GH values in both groups (P<0.0001).

Table 1. Testosterone and growth hormones levels in camels aged 6-15 months and in those over 15 months up to 10 years (n=55).

Parameter	Testosterone (pg/mL)		Growth hormone (ng/mL)	
Age category	06-15m	15m-10y	06-15 month	15m-10y
Mean ± SD	51±9^{a}	52±7^{a}	2.7±0.4^{a}	2.2±0.3^{b}
Minimum	34	37	1.9	1.6
Maximum	75	66	4.3	2.9
0.25% Percentile	45	46	2.4	2.0
0.50% Percentile	50	51	2.7	2.2
0.75% Percentile	55	57	2.9	2.3
0.95% Percentile	65	62	3.4	2.6
0.99% Percentile	71	64	3.9	2.9

Discussion

Camel beauty pageants are being held regularly in Saudi Arabia since last 5 years where tremendous prizes are awarded. Plastic surgery in camels is currently rampant in the Gulf region (Tharwat and Al-Hawas, 2021).

Injection of hormone such as testosterone and GH in widely used for enabling partiapating camels to have enhanced performance, stamina and looks (Tharwat and Al-Hawas, 2021). For investigation

of the last point, this study was carried out. To the best of the authors' knowledge, this is the first study measuring testosterone and GH values in female camels from 6 months until 10 years of age.

For many years, Androgenic Anabolic Steroid use (AAS) have been popular among athletes both for performance improvement and for aesthetic reasons. The first documented reports of misuse of AAS by athletes stem from the 1950s. Since the first results were less motivating, he concluded that androgens might exert particular psychological effects (Hartgens and Kuipers, 2004). However, since several AAS-using athletes won competitions and championships in that period, the abuse of these agents in sport began to spread (Celotti and Cesi, 1992; Yesalis *et al,* 2000).

Recently, it has become apparent that AAS may exert strong effects on psyche and behaviour (Medras *et al,* 2018). The potential for anabolic steroid abuse in equine sports has increased in recent years due to the growing availability of designer steroids (Waller and McLeod, 2017). Administration of recombinant ontogeny endocrine (rCGH) in animals is a useful approach to manipulate endocrine system and metabolic pathways towards faster growth rate, muscle deposition, milk yield and better feed efficiency (Khan *et al,* 2016).

Shah and Ashraf (2018) reported that liquid chromatography mass spectrometry (LC-MS) to detect four common doping drugs, i.e. cortisol, dexamethasone, methylprednisolone, and flumethasone in camel hair samples.

The GH is a substance produced by the body in order to help stimulate the production of new tissue. In younger animals, the hormone will be present in much higher levels as the animals are growing to their optimum adult size. The results of this study agree well with this scientific fact where the level of GH was significantly higher in female camels less than 15 months compared to second group up to 10 years of age. Once they reach adulthood, the levels of GH within their body will substantially decrease but will maintain a constant background level in order to help with functions such as recovery from injury and building extra muscle. Clinical and preclinical studies have suggested that anabolic hormones, such as GH, insulin-like growth factor I (IGF-I), and IGF binding protein 3, may reverse the catabolic state associated with cachexia in patients (Colao *et al,* 1999; Kotler, 2000; Wang *et al,* 2000). In conclusion, the testosterone and GH levels recorded in this study can be used as reference values when evaluating these hormone levels in camel beauty pageants.

References

Ayson FG, Jesus DE, Amemiya EGT, Moriyama Y, Hirano S and Kawauchi TH. Isolation, cDNA cloning, and growth promoting activity of rabbitfish (Siganus guttatus) growth hormone. General and Comparative Endocrinology. 2000; 117:251-259.

Burton JL, McBride BW, Block E, Gtimm DR and Kennelly JJ. A review of bovine growth hormone. Canadian Journal of Animal Science. 1994; 74:167-201.

Celotti F and Cesi PN. Anabolic steroids: a review of their effects on the muscles, of their possible mechanisms of action and of their use in athletics. Journal of Steroid Biochemistry and Molecular Biology. 1992; 43:469-477.

Colao A, Cuocolo A, Di Somma C, Cerbone G, Della Morte AM, Nicolai E, Lucci R, Salvatore M and Lombardi G. Impaired cardiac performance in elderly patients with growth hormone deficiency. Journal of Clinical Endocrinology and Metabolism. 1999; 84:3950-3955.

Denoto FM, Moore DD and Goodman HM. Human growth hormone DNA sequence and mRNA structure: possible alternative splicing. Nucleic Acids Research. 1981; 9:3719-3730.

Devlin RH, Byatt JC, Mclean E, Yesaki TY, Krivi GG, Jaworski EG, Clarke WC and Donaldson EM. Bovine placental lactogen is a potent stimulator of growth and displays strong binding to hepatic receptor sites of coho salmon. General and Comparative Endocrinology. 1994; 95:31-41.

Hartgens F and Kuipers H. Effects of androgenic-anabolic steroids in athletes. Sports Medicine. 2004; 34:513-554.

Jeong SH, Kang D, Lim MW, Kang CS and Sung HJ. Risk assessment of growth hormones and antimicrobial residues in meat. Toxicology Research. 2010; 26:301-313.

Khan R, Shahzad MI and Iqbal MN. Role of Camel in Pastoral Mode of Life and Future Use of rCGH as a Therapeutic Agent in Milk and Meat Production. PSM Veterinary Research. 2016; 01(1):32-37.

Kotler DP. Cachexia. Annals of Internal Medicine. 2000; 133:622-634.

Kraemer WJ, Ratamess NA, Hymer WC, Nindl BC, Fragala MS. Growth Hormone(s), testosterone, insulin-like growth factors, and cortisol: roles and integration for cellular development and growth with exercise. Front Endocrinol (Lausanne). 2020; 11:33. Published 2020 Feb 25. doi:10.3389/fendo.2020.00033.

Medras M, Brona A and Jóźków P. The Central Effects of Androgenic-anabolic Steroid Use. Journal of Addiction Medicine. 2018; 12:184-192.

Shah I and Ashraf SS. An undergraduate forensic biochemistry laboratory experiment to detect doping in animal hair using LCMS. International Journal for Innovation Education and Research. 2018; 6(1):133-148. https://doi.org/10.31686/ijier.vol6.iss1.927

SPSS. Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA Copyright© for Windows, version 18. 2009.

Tharwat M and Al-Hawas A. Ultrasound detection of cosmic fillers injection of lips in camel beauty pageants: first report in Veterinary Medicine. Tropical Animal Health and Production. 2021; 53:53.

Waller CC and McLeod MD. A review of designer anabolic steroids in equine sports. Drug Testing and Analysis. 2017; 9:1304-1319.

Wang W, Iresjo BM, Karlsson L and Svanberg E. Provision of rhIGF-I/IGFBP-3 complex attenuated development of cancer cachexia in an experimental tumour model. Clinical Nutrition. 2000; 19:127-132.

Wong JKY and Wan TSM. Doping control analyses in horseracing: A clinician's guide. Veterinary Journal. 2014, 200:8-16.

Yesalis CE, Courson SP and Wright JE. History of anabolic steroid use in sport and exercise. In: Yesalis C, editor. Anabolic steroids in sports and exercise. 2nd ed. Champaign (IL): Human Kinetics. 2000; pp 51-72.

DOI : 10.5958/2277-8934.2021.00058.8 Vol 28 No 3, p 377-382

ANTIOXIDANT EFFECT OF CAMEL MILK ON ACUTE ALCOHOLIC LIVER INJURY IN MICE

Bule QI[1], Dandan Wu[1], Xiaoyun Wu[1], Naqin[1], Shiqi Hao[1], Rimutu Ji[1,2] and Liang Ming[1,2]

[1]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, 010018, Hohhot, China

[2]Camel Research Institute of Inner Mongolia, 737300, Alashan, China

ABSTRACT

This study aims to explore the protective effect of camel milk (CM) on oxidative stress in mice and analyse its mechanism of action. The experimental animals were randomly divided into four groups: NC (normal diet), ET (normal diet, then ethanol), CM (normal diet and CM) and ET+CM (normal diet and CM, then ethanol). Then by measuring serum and liver tissue superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activity, glutathione (GSH), malondialdehyde (MDA) and other indicators to explore its antioxidant capacity. The results showed that CM attenuated ethanol-induced hepatotoxicity by reducing elevated liver enzyme, decreasing ethanol-induced reactive oxygen species (ROS) generation, inhibiting malonaldehyde (MDA) levels and reversing depletion of antioxidative defense system in mice liver. Furthermore, CM ameliorated ethanol-induced oxidative stress by down-regulating the expression of cytochrome P4502 E1 (CYP2E1) enzyme. These data suggest that CM, a health-promoting antioxidant food, exhibits hepatoprotective effects. The hepatoprotective mechanisms of CM were associated with alleviation of ethanol-induced oxidative stress through inhibiting CYP2E1 enzyme.

Key words: Alcoholic liver injury, Camel milk, CYP2E1, Oxidative damage

Liver is the main organ for alcohol metabolism, more than 80% of the ingested alcohol is metabolised in the liver (Stornetta *et al*, 2018). The course of Alcoholic liver disease (ALD) usually manifests as alcoholic fatty liver (AFL) at the beginning and turns into alcoholic steatohepatitis (ASH), alcoholic liver fibrosis and alcoholic cirrhosis. Excessive drinking can also induce extensive hepatocyte necrosis or even liver failure during severe hepatocellular cancer (HCC) (Banerjee *et al*, 2013; Liu *et al*, 2017). Alcohol metabolism will produce reactive oxygen species (ROS) and oxidative stress, which is the main mechanism implicated in the pathogenesis of ALD (Cai *et al*, 2018; Leung and Nieto, 2013).

Camel milk is considered one of the most valuable food sources for nomadic people in arid and semi-arid areas and has been consumed for centuries due to its nutritional values and medicinal properties (Dowelmadina *et al*, 2014; Yadav *et al*, 2015; Kaskous, 2016; Kula and Dechasa, 2016). It has high quality of composition and various bioactive ingredients, showing special properties that make it distinct and unique compared to other species milk (Wernery, 2007; Smits *et al*, 2011; Hamed *et al*, 2012; Yadav *et al*, 2015). The past decade, researchers have found a dramatic increase and growing interest in the use of camel milk for its medicinal values; while bioactive ingredients of camel milk have gained significant attention to scientists all over the world to study their potential health benefits (Rasheed *et al*, 2016; Gader and Alhaider, 2016; Mullaicharam, 2014). Scientifically, it has been proved that camel milk ingredients are excellent for nutritional view point as it contains high levels of lacto-peroxidase, immunoglobulin G (IgG), secretory immunoglobulin A (SIgA), copper, iron and vitamin C which giving it superior antioxidant and antimicrobial activities (Haj and Kanhal, 2010; Hailu *et al*, 2016). Camel milk can reduce liver damage caused by alcohol and protect liver tissue from alcoholism by improving liver tissue malondialdehyde, total antioxidant activity, tumour necrosis factor and caspase activity in alcoholic liver disease rats (Darwish *et al*, 2012).

In preent study, ICR mice were used to establish an animal model of acute alcohol liver injury to explore the protective effect of camel milk on oxidative stress in mice and to analyse its mechanism of action.

Materials and Methods

Preparation of vacuum freeze-dried skimmed milk powder

After the camel milk was collected, it was quickly cooled to 4°C and the upper fat was removed

SEND REPRINT REQUEST TO LIANG MING email: bmlimau@163.com

by centrifugation at 3500rpm/min for 40min. After heating in a 60°C water bath for 30min, it was vacuum freeze-dried. The obtained camel milk powder was stored in a refrigerator at -20°C and sealed.

Animals and experimental design

Male ICR mice (25±1g) were obtained from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd. (China; License Number SCXK 2016 -0006). The mice were housed in ventilated polypropylene cages (3 mice per cage) maintained at 22 ± 2°C with 50 ± 5% humidity and subjected to a 12h light/dark cycle. After 1 week of acclimation, all the mice were randomly separated into 4 groups as follows: (I) normal group (NC, n = 10); (II) camel milk group (CM, n = 10); (III) ethanol group (ET, n = 10); (IV) ethanol and camel milk group (ET+CM, n = 10). The initial body weights were controlled similar, and there were no significant differences among the groups. CM and ET+CM groups were subjected to oral camel milk treatments twice daily for 14 days via oral gavage, NC and ET groups only received 0.3mL of distilled water. The mice fasted for 6h after the last medical treatment for inducing hepatotoxicity by administering 50% alcohol (7.3 g/kg bw) to groups ET and ET+CM via oral gavage in 3 equal doses administered at 1h intervals. After 6h, the mice were anaesthetised with ethylether for sampling blood from eyeball extirpation. After incubating the blood samples at 37°C for 45min, serum was collected by centrifugation at 3000rpm for 20min at 4°C. The liver was excised and weighed after mouse were dissected and stored at -80°C.

Analysis of AST and ALT

The Blood samples were centrifuged at 3000rpm (20min, 4°C), and then detected alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum via the kits (C010-2-1 and C009-2-1). All operating steps were carried out in strict accordance with the instructions and then the absorbance was measured via using a microplate reader.

Determination of serum ROS production

According to the manufacturer's instructions (Shanghai Enzyme-linked Biotechnology Co, Shanghai, China; ml009876-1) and as previously described, ROS antibody was precoated on an ELISA plate. The serum/liver homogenate samples were added into ELISA plate wells and incubated for 60min at 37°C. Detecting HER labeling polyclonal antibody was then added into ELISA plate. Next, the avidin-peroxidase conjugates were added into ELISA plate and incubated for 15min. Finally, TMB substrates for coloring were added into ELISA plate for 15min. Absorbance of serum samples at 450nm was measured using a plate reader and serum ROS was obtained.

Determination of biochemistry

The levels of Malondialdehyde (MDA), Glutathione Peroxidase (GSH-Px), NADPH oxidase, Glutathione (GSH) and Superoxide Dismutase (SOD) were detected using kits (ml114150, ml058194, ml214840, ml063305 and ml001998) in accordance with the protocols from the manufacturer.

Quantitative RT-PCR analysis

Total liver RNA was extracted using RNAiso Plus (TaKaRa Bio, Otsu, Japan) according to the manufacturer's instructions and reverse-transcribed into cDNA with the PrimeScript RT reagent kit (TaKaRa Bio, Otsu, Japan). cDNA was amplified by real-time quantitative polymerase chain reaction (RT-qPCR) using the SYBR Premix Ex TaqII (TaKaRa Bio, Otsu, Japan) and the appropriate primers shown in Table 1. The thermocycler conditions used were 95°C for 30s, followed by 40 cycles of 95°C for 5s and 60°C for 30s. The relative mRNA expression of carnitine cytochrome P4502E1 (CYP2E1) was calculated by the $2-\Delta\Delta Ct$ method; β-actin genes were used as internal controls.

Data analysis

All data were presented as means ± standard error of the mean (SEM) and analysed by GraphPad prism 5.03 (GraphPad Software Inc., San Diego, CA, USA). Comparisons among all groups were performed with one-way analysis of variance (ANOVA) test.

Results and Discussion

Effect of camel milk on the activity of serum enzymes

ALT and AST are commonly used as indicators of liver function and health status, which can reflect

Table 1. Oligonucleotide sequence of primers for RT-qPCR.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
β-Actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
CYP2E1	AGGGGACATTCCTGTGTTCC	TTACCCTGTTTCCCCATTCC

the liver damage and health status of animals. To further confirmed the protective effects of camel milk, the degree of cellular injury caused by ethanol was estimated by the leakage of ALT and AST enzymes from the hepatocytes. Table 2 shows that the effects of CM on serum biochemical values in mice with acute alcoholic liver injury. As compared with the NC group, the serum ALT and AST activities of the ET group were significantly increased, indicating that the ALD mice were successfully modeled. The activities of the two enzymes in serum were decreased to various extents by intragastric administration of CM. Collectively, these results indicate that CM suppresses ethanol-induced liver damage in mice and has protective effect on alcoholic liver injury.

Effect of camel milk on liver index and oxidative stress in the liver

The liver index of mice can partly reflect the degree of liver injury. Ethanol causes an increased liver index in experimental animal models including rats and mice (Luo *et al*, 2013; Liu *et al*, 2015). Ethanol causes liver enlargement, hyperplasia, hyperemia, oedema and other diseases. After intragastric administration of alcohol, the liver index (liver weight/body weight) of the ET group increased significantly as compared with the NC group (Table 3). There was no significant difference in liver index between the mice in the ET+CM and NC group. Compared with the ET group, the liver index of the ET+CM group was significantly lower ($P<0.001$), indicating that camel milk can alleviate liver damage in mice.

Ethanol-induced liver injury is a common occurrence in liver disease, which has been considered to be a global health problem (Koch *et al*, 2004). Ethanol-induced oxidative stress is widely considered to play a major role in mechanisms of ethanol-induced liver injury (Cederbaum *et al*, 2009). Therefore, using appropriate antioxidant agents to inhibit oxidative stress is an attractive approach for prevention and treatment of ethanol-induced liver injury. SOD is an endogenous antioxidant enzyme that can catalyse the disproportionation of superoxide to generate H_2O_2, which is then decomposed into water under the action of catalase in the cytoplasm (Kalyanaraman, 2013; Wang *et al*, 2018). MDA is known as an end product of lipid peroxidation and is a significant marker of the increased oxidative stress. GSH is a major nonprotein thiol antioxidant, which plays a central role in coordinating antioxidant defense in the living organisms. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reactions (Hu *et al*, 2015).

Thus several indicators for hepatic oxidative stress including GSH, GSH-Px, MDA and SOD were assayed to examine whether camel milk could ameliorate liver oxidative stresses induced by alcohol-caused injuries. Excessive drinking could cause a significant increase in hepatic MDA level (Fig 1C) and sharp decline of hepatic GSH activities, GSH-Px activities and SOD contents (Fig 1A, B and D) in comparison with the NC groups. This indicated that the oxidative stress level in the liver of ALD mice was significantly increased. Compared with the ET group, the MDA level of the ET+CM group was significantly reduced, and the GSH, GSH-Px and SOD were significantly increased. Taken together, these data suggest that CM can alleviate ethanol-induced oxidative stress through inhibition of MDA levels and elevation of antioxidative defense system.

Recent studies have demonstrated that excess ethanol triggers the increase of ROS generation in liver tissues (Ye *et al*, 2015; Sid *et al*, 2013). As a vital marker of oxidative stress, ROS production plays an important role in the pathogenesis of various liver

Tabel 2. Effects of camel milk on serum ALT and AST in mice.

Parameters	NC	ET	CM	ET+CM
ALT(U/L)	15.91±1.01	30.65±3.625$^{\#\#}$	7.497±0.415$^{\#**}$	18.96±0.799**
AST(U/L)	22.18±0.667	40.61±4.311$^{\#\#}$	14.36±0.551**	26.17±1.104**

Data are expressed as mean ± SEM of 10 animals. Significantly different from the normal group at $^{\#}p< 0.01$, $^{\#\#}p< 0.001$. Significantly different from the ethanol-treated group at $*p< 0.01$, $**p< 0.001$. NC: normal group, ET: ethanol group, CM: camel milk group, ET+CM: ethanol and camel milk group.

Table 3. Effects of camel milk on liver index in ALD mice.

Group	NC	ET	CM	ET+CM
Liver index± SEM	3.77±0.051	4.0±0.038$^{\#\#}$	3.766±0.058**	3.782±0.046**

Liver index (%) = Liver weight/Body weight × 100%.Significantly different from the control group at $^{\#\#}P< 0.001$; significantly different from the model group at $**P< 0.001$. NC: control group, ET: ethanol group, CM: camel milk group, ET+CM: ethanol and camel milk group.

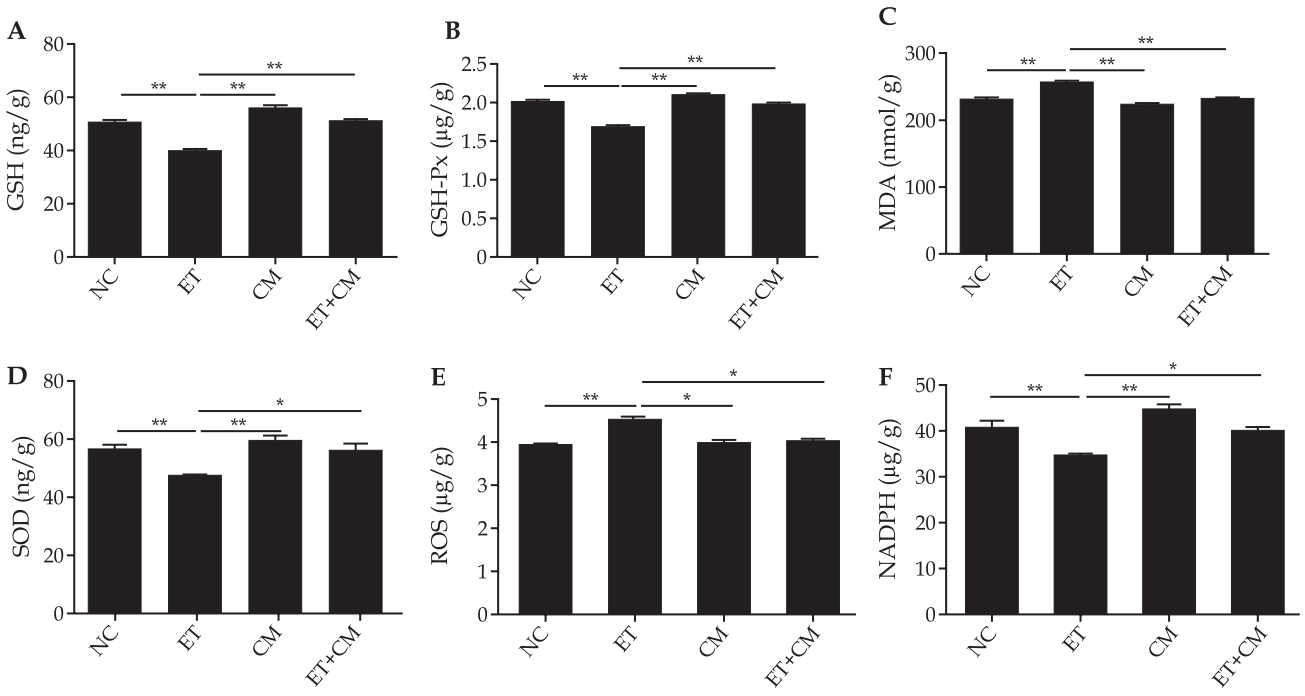


Fig 1. Effects of camel milk on the antioxidant capacity of the liver tissues in ALD mice. (A) GSH level, (B) MDA level, (C) GSH-Px level, (D) SOD level, (E) ROS level and (F) NADPH level were measured with microplate reader. Data are expressed as mean ± S.E.M. (n = 10) for each group. *P < 0.01, **P < 0.001. NC: normal group, ET: ethanol group, CM: camel milk group, ET+CM: ethanol and camel milk group.

disorders including alcoholic liver disease (Jiang *et al*, 2014). To test the effects of CM on oxidative stress induced by alcohol, the production of ROS was further measured. As shown in Figure 1E, ethanol treatment significantly increased ROS level compared with the control group, whereas CM pretreatment markedly reduced ROS generation in ethanol-treated mice. Accordingly, our findings indicate that CM exhibits hepatoprotective capability and suppresses ethanol-induced liver damage via inhibition of oxidative stress.

NADPH oxidase in hepatic Kupffer cells plays a predominant role in the pathogenesis of early alcohol-induced liver injury by stimulating the generation of ROS and proinflammatory cytokines (Mochel *et al*, 2010; Lee and Yang, 2012). To further elucidate the mechanism involved in the protective effect of CM on ethanol-induced liver injury, activation of NADPH oxidase was analysed. As shown in Figure 1F, ethanol treatment significantly decreased NADPH level compared with the NC group, whereas CM pretreatment markedly increased the production of NADPH in serum and liver homogenate of ethanol-treated mice. The results imply that CM improves the level of NADPH oxidase, which can alleviate ethanol-induced liver injury.

Effect of camel milk on oxidative stress in the serum

It can be seen from Fig 2 that at a dose of 7.3g/kgbw of 50% ethanol solution, the serum levels of GSH, GSH-Px, MDA, SOD, ROS and NADPH in the ET group were significantly different from those in the NC group ($P<0.01$, $P<0.001$), indicating that acute alcohol induction mice produce oxidative damage, which lead to a decrease or increase in the level of antioxidant substances in the mice serum. Compared with the ET group, the serum GSH ($P<0.001$), GSH-Px ($P<0.001$), MDA ($P<0.001$), SOD ($P<0.01$) ROS ($P<0.01$) and NADPH ($P<0.001$) level of mice in the ET+CM group was significantly reduced or increased. It shows that camel milk has the effect of regulating the level of antioxidants in acute alcohol mice.

Effects of camel milk on mRNA expression of CYP2E1 genes in the liver

CYP2E1, a central functional enzyme in alcohol metabolism, has been generally considered as a major contributor to the over-production of ROS during its catalytic circle (Leung and Nieto, 2013). Long-term and large amounts of alcohol consumption activate the expression of hepatic CYP2E1 and trigger oxidative stress, leading to tissue inflammation and injuries (Lu and Cederbaum, 2008).

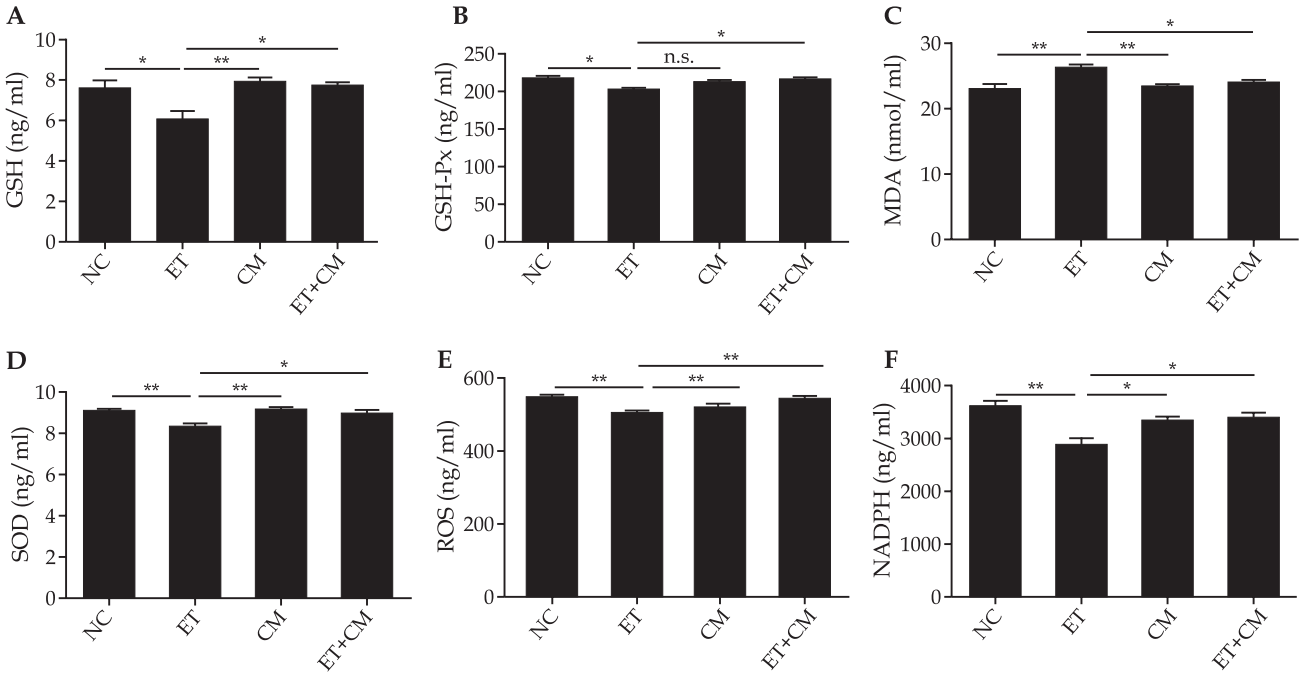


Fig 2. Effects of camel milk on the antioxidant capacity of the liver in ALD mice. (A) GSH level, (B) MDA level, (C) GSH-Px level, (D) SOD level, (E) ROS level and (F) NADPH level were measured with microplate reader. Data are expressed as mean ± S.E.M. (n = 10) for each group. *P < 0.01, **P < 0.001. NC: normal group, ET: ethanol group, CM: camel milk group, ET+CM: ethanol and camel milk group.

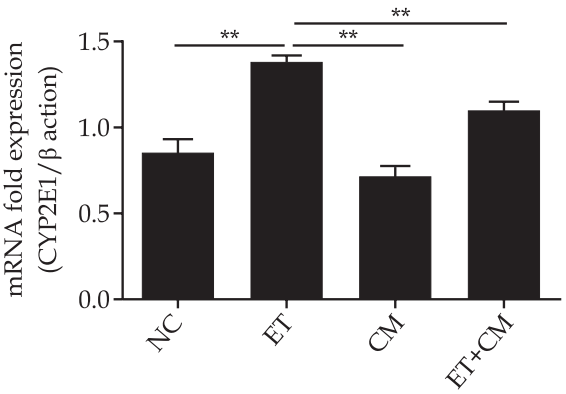


Fig 3. Effect of camel milk on the expression of CYP2E1 in ethanol-induced liver injury in mice. *P < 0.05, **P < 0.01. NC: normal group, ET: ethanol group, CM: camel milk group, ET+CM: ethanol and camel milk group.

To illuminate the mechanisms underlying the protective effects of CM on ethanol-induced oxidative stress, CYP2E1 was analysed by RT-qPCR. As shown in Fig 3, after ethanol exposure, the expression level of CYP2E1 in livers was significantly increased compared with the NC group. However, CM pretreatment markedly suppressed the increase of ethanol-induced CYP2E1 expression. Taken together, the results demonstrated that CM attenuates ethanol-induced oxidative stress by inhibiting CYP2E1 expression.

Conclusions

In conclusion, the present study demonstrated that feeding CM to alcohol-treated mice can prevent ethanol-induced liver damage. The protective effects of CM were associated with alleviation of ethanol-induced oxidative stress via inhibiting CYP2E1 and NADPH oxidase activities. Our findings provide new insights into hepatoprotective mechanisms of CM and a promising antioxidant-rich food for prevention of alcoholic liver injury.

Acknowledgements

This work was supported by grants from the Science and Technology Project of the College of Food Science and Engineering (SPKJ201901), and the Inner Mongolia Natural Science Foundation Project (2018BS03017).

Conflicts of Interest

The authors declare no conflict of interest.

References

Banerjee P, Jana S, Chakraborty S and Swarnakar S. Inflammation and MMPs in alcohol-induced liver diseases and protective action of antioxidants. Indian Journal of Biochemistry and Biophysics. 2013; 50(5):377-386.

Cai Z, Song L, Qian B, Xu W and Oey I. Understanding the effect of anthocyanins extracted from purple sweet

potatoes on alcohol-induced liver injury in mice. Food Chemistry. 2018; 245:463-470.

Cederbaum AI, Lu Y and Wu D. Role of oxidative stress in alcohol-induced liver injury. Archives of Toxicology. 2009; 83:519-548.

Darwish HA, Raboh NRA and Mahdy A. Camel's milk alleviates alcohol-induced liver injury in rats. Food and Chemical Toxicology, 2012; 50(5):1377-1383.

Dowelmadina IMM, El Zubeir IEM, Salim ADA and Arabi OHMH. Influence of some factors on composition of dromedary camel milk in Sudan. Global Journal of Animal Scientific Research. 2014; 2(2):120-129.

Gader AG and Alhaider AA. The unique medicinal properties of camel products: A review of the scientific evidence. Journal of Taibah University Medical Sciences. 2016; 11:98-103.

Hailu Y, Hansen EB, Seifu E, Eshetu M, Ipsen R and Kappeler S. Functional and technological properties of camel milk proteins: a review. Journal of Dairy Research. 2016; 83:422-9.

Haj OAA and Kanhal HAA. Compositional, technological and nutritional aspects of dromedary camel milk. International Dairy Journal. 2010; 20(12):811-821.

Hamed H, Trujillo A-J, Juan B, ElFeki A and Gargouri A. Interrelationships between somatic cell counts, lactation stage and lactation number and their influence on plasmin activity and protein fraction distribution in dromedary (*Camelus dromedarius*) and cow milks. Small Ruminant Research. 2012; 105(1-3):300-307.

Hu Y, Zhao Y, Yuan L and Yang X. Protective effects of tartary buckwheat flavonoids on high TMAO diet-induced vascular dysfunction and liver injury in mice. Food Function. 2015; 6:3359-3372.

Jiang J, Yu S, Jiang Z, Liang C, Yu W, Li J, Du X, Wang H, Gao X and Wang X. Nacetyl-serotonin protects hepg2 cells from oxidative stress injury induced by hydrogen peroxide. Oxid Med Cell Longev, 310504. 2014.

Kalyanaraman B. Teaching the basics of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. Redox Biology. 2013; 1(1):244-257.

Kaskous S. Importance of camel milk for human health. Emirates Journal of Food and Agriculture. 2016; 28(3):158-163

Koch OR, Pani G, Borrello S. Oxidative stress and antioxidant defenses in ethanol-induced cell injury. Molecular Aspects of Medicine. 2004; 25(1-2):191-198.

Kula J and Dechasa T. Chemical composition and medicinal values of camel milk. International Journal of Research Studies in Biosciences. 2016; 4(4):13-25.

Lee IT and Yang CM. Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases. Biochemical Pharmacology. 2012; 84(5):581-590.

Leung TM and Nieto N. CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease. Journal of Hepatology. 2013; 58:395-398.

Liu J, Wang X, Peng Z, Zhang T, Wu H, Yu W, Kong D, Liu Y, Bai H, Liu R and Zhang X. The effects of insulin preadministration in mice exposed to ethanol: alleviating hepatic oxidative injury through anti-oxidative, antiapoptotic activities and deteriorating hepatic steatosis through srbep-1c activation. International Journal of Biological Sciences. 2015; 11:569-586.

Liu Y, Wang J, Li L, Hu W, Qu Y, Ding Y, Meng L, Teng L and Wang D. Hepatoprotective Effects of *Antrodia cinnamomea:* The Modulation of Oxidative Stress Signaling in a Mouse Model of Alcohol-Induced Acute Liver Injury. Oxidative Medicine and Cellular Longevity. 2017; 2017:7841823.

Lu Y and Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. Free Radical Biology and Medicine. 2008; 44:723-738.

Luo Z, Liu H and Sun X. RNA Interference against discoidin domain receptor 2 ameliorates alcoholic liver disease in rats. Plos One, 8. 2013.

Mochel NSRD, Seronello S, Wang SH, Ito C, Zheng JX, Liang TJ, Lambeth JD and Choi J. Hepatocyte NAD(P)H oxidases as an endogenous source of reactive oxygen species during hepatitis C virus infection. Hepatology. 2010; 52(1):47-59.

Mullaicharam AR. A review on medicinal properties of camel milk. World Journal of Pharmaceutical Sciences. 2014; 2:237-42.

Rasheed N, Alghasham A and Rasheed Z. Lactoferrin from Camelus dromedarius inhibits nuclear transcription Factorkappa B activation, cyclooxygenase-2 expression and prostaglandin E2 production in stimulated human chondrocytes. Pharmacognosy Research. 2016; 8(2):135-141.

Sid B, Verrax J and Calderon PB. Role of oxidative stress in the pathogenesis of alcohol-induced liver disease. Free Radical Research. 2013; 47(11):894-904.

Smits MG, Huppertz AC and Alting AC. Composition, constituents and properties of dutch camel milk. Journal of Camel Practice Research. 2011; 18(1):1-6.

Stornetta A, Guidolin V and Balbo S. Alcohol-derived acetaldehyde exposure in the oral cavity. Cancers. 2018; 10(1):20.

Wang Y, Branicky R and Hekimi S. Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling. Journal of Cell Biology. 2018; 217(6):1915-1928.

Wernery U. Camel milk new observations. Proceedings of the International Camel Conference "Recent trends in camelids research and future strategies for saving camels", Rajasthan, India, 16-17 Febraury, 2007; pp 200-204.

Yadav AK, Kumar R and Priyadarshini L. Composition and medicinal properties of camel milk: A Review. Journal of Dairying, Foods and Home Science. 2015; 34(2):83.

Ye H, Qi X, Jiang NH, Xin YH, Wei L and Li CZ. Maltol, a food flavoring agent, attenuates acute alcohol-induced oxidative damage in mice. Nutrients. 2015; 7(1):682-696.

DOI : 10.5958/2277-8934.2021.00059.X Vol 28 No 3, p 383-393

CAMEL CULTURE IN TURKEY AND THE LEGAL AND SOCIO-ECONOMIC STRUCTURE OF THE CAMEL WRESTLING UNION

Aysun KOÇ[1] and Devrim ERTÜRK[2]

[1]Assistant Professor, Adnan Menderes University, Faculty of Veterinary Science, Efeler, Aydın, Türkiye
[2]Associate Professor, Dokuz Eylül University, Efes Vocational School, Selçuk, İzmir, Türkiye

ABSTRACT

Camels have been used in transportation in Turkey as in all geographies. The struggle of male camels with each other has been turned into wrestling matches over time and has gained an institutional dimension for people who own camels. As a result of the matches, camel culture has been able to reproduce itself today. Camel wrestling has become widespread and increased its popularity in the western coast of Turkey and in the provinces close to the coast. With the spread of camel wrestling especially after 1980, a demand and search for institutionalisation and organisation emerged. In this process, coffeehouses where camel owners came together had an important function. These coffeehouses, which can be regarded as the first step of the organisation, have first transformed into associations and then into a union with the merging of the associations. The organisation steps of camel wrestling, which has been maintaining its traditional structure for centuries in Turkey, were taken in 2012 and 2013. As a result of the corporate identity and organisation requirement of camel wrestling, which continues to exist in the coastal provinces of the Aegean, Marmara and Mediterranean Region, the "Camel Culture and Camel Wrestling Union (CCCWU)" was formed in 2012, with the merging of eight camel wrestling associations in Aydın. The Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture has been established by five camel culture associations in İzmir with the decision of the Governorship of İzmir dated 31.10.2013 and numbered 96462 and the union started to work with the approved statute. The CCCWU gained its legal structure after it was approved by the Aydın Governorship Animal Protection Board in 2014.

This study examined the content and principles of the regulations and by-laws of both unions and evaluated their structure in terms of animal rights and animal welfare. The study also aimed to reveal the contributions of the legal qualities of camel wrestling, whose sustainability is important due to its traditional structure, to camel breeding and organisations. Furthermore, the study aimed to analyse the representation capability of the union by involving the members of the Board of Directors and the Supervisory Board, who are on duty since the establishment of the union, in the study. Our study aimed to reveal the economic structure of the unions and its contributions to the economy of camel culture by including the budget information of the unions (income-expense table and donations, membership fees, income from wrestling, etc.).

Key words: Camel associations, camel owner coffee houses, camel ownership, camel wrestling, organisation, union

Camel ownership and camel wrestling have emerged in time in Turkey and have gained a traditional structure and become a cultural element. Camel wrestling has primarily become an organisation which has ensured the continuation of camel existence by maintaining camel culture in Turkey.

The functioning of the traditional structure has continued with its own traditional rules. With the spread of settled life and entertainment culture, the camel, which has primarily been used in transportation, has started to be a part of camel wrestling, which involves the leadership struggle among male animals in a controlled way. With the impact of the solidarity observed in the Yörük (nomads in Anatolia) culture, camel wrestling has been organised for charity (Kılıçkıran, 1987; Ertürk, 2019; Yılmaz *et al*, 2014).

Camel wrestling organisations which has a history of about two hundred years in Turkey (Kılıçkıran, 1987; Ertürk, 2017; Çalışkan, 2010) started to increase their popularity in the 1980s. This period, which corresponds to a period in which traditional elements started to gain importance on a world scale, revealed the demand and necessity of organisation in the camel and camel wrestling culture (Ertürk, 2018).

SEND REPRINT REQUEST TO AYSUN KOÇ email: aysunkoc@hotmail.com

It has been observed that the coffeehouses, where camel owners came together, were the basis of the organisation demand for camel breeding and camel wrestling. Prior to organisation period, camel owner coffeehouses emerged as areas where camel owners came together, talked about their common problems, and socialised (Ertürk, 2018). In parallel with the increase in interest in camel wrestling after the 1980s, the number of people engaged in camel breeding has also increased. This increase has led to being organised under an association (Ertürk, 2018; Çalışkan, 2016). The need for organisation that emerged over time facilitated the transition from coffeehouse to association. The social sphere formed around the camel culture showed itself on the way to become an association both with its internal dynamics and as a result of the need to gain a legal identity.

As a result of the necessity of forming an organisation of camel wrestling, which continues its existence in the provinces of the Aegean, Marmara and Mediterranean Regions of Turkey and preserves its traditional structure for centuries, two unions were established in 2012 and 2013. With the merging of eight camel associations in Aydın, "Camel Culture and Camel Wrestling Union (CCCWU)" was established in 2012 and gained its legal entity as a result of the approval and enforcement of the Aydın Governorship Animal Protection Board in 2014 (Anonymous, 2020a). In Izmir, five camel culture associations merged and established the "Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture" with the decision of the Governorship of İzmir, dated 31.10.2013 and numbered 96462, and the union started to act with the approved statute structure (Anonymous 2020b). The study aims to examine the legal and socio-economic structure of the unions established in 2012 and 2013 and to evaluate the steps taken towards institutionalisation, their contribution to the camel breeding and camel wrestling culture.

Materials and Methods

The fundamental material of the study is the regulations and sub-regulations of the Camel Culture and Camel Wrestling Union and the association charter of the Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture, which was established in Izmir in 2013 and started its activities[1]. In order to add detail to the work, the bylaws of the accessible camel culture associations, which are affiliated to the Union, were also included in the work. The structure and content of the regulations were evaluated in terms of the Animal Rights Law. The study also includes information on the administration and supervisory board since the establishment of the CCCWU and the budget of the union between 2012-2020.

Findings

For the first time in the world, the foundations of modern animal breeding were laid in England in 1760, and the organisations for those breeding animals were established by the state in Turkey with the "Islah-ı Hayvanat Kanunu (Animal Breeding Law)" in 1926 (Pekel and Ünalan, 1997). In the years following the establishment of the breeders' union in Turkey in 1926, many animal associations were established and they carried out their activities.

The Camel Culture and Camel Wrestling Union in Aydın, the foundations of which were laid with the establishment of an association, was approved by the Aydın Governorship Animal Protection Board and started its activities. The purpose, scope, basis and definitions section of this regulation includes a definition in Article 1- (1): "Protecting camels within the framework of Animal Protection Law and arranging camel wrestling organisations within the framework of this regulation." The Board of the Union determined the principles under eight titles as "Observer Regulation, Mouth Strap Controller Regulation, Mouth Treadmill Regulation, Chief Tether Regulation, Discipline Instruction, License Regulation, Referee Regulation, Presenter (Cazgır) Regulation." In addition, the principles and objectives of the Regulations of the CCCWU also constitute the legal structure of the union. Today, the union in Aydın continues its activities actively with its 135 delegates, and based on the regulation, three people from each association participate in the union elections (Anonymous, 2020a).

With the decision of the Governorship of İzmir, dated 31.10.2013 and numbered 96462, the Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture was established as the second union. In Article 2 of the Regulations, the aim of the union was stated as follows: "The Union was established to gather everyone who is interested in camel breeding and wrestling, which is the most deep-rooted cultural tradition in our country, under one roof and thus to ensure unity and solidarity, and to support people and organisations working on this culture and other organisations and volunteers who are interested in this culture." The Regulations

1. Aydın Governorship Provincial Animal Protection Board Decision, Date: 12/02/2012, Decision no: 21.

Fig 1. Camel Wrestling: A view of traditional camel wrestling in Turkey (Devrim Ertürk's archives).

DEVECİLİK KÜLTÜRÜ ve DEVE GÜREŞLERİ FEDERASYONU
DEGÜF
•2012•

Fig 2. Camel Culture and Camel Wrestling Union's Logo

include the organs of the Union, the membership conditions, the duties and powers of the board of the union, and the procedures and principles. In the Regulations, it is stated that three members representing each association participate in the elections of the union, formed by the five camel culture associations, and the first member needs to be the president of the association and the other two should be the delegates of the union.

The Regulation of the Camel Culture and Camel Wrestling Union in Aydın

With the approval of the Union Regulation by the Aydın Governorship Animal Protection Board in 2014, the infrastructure and principles of the camel breeding and camel wrestling culture gained a legal entity. The regulation of the union includes fifteen main sections.

The purpose, scope, basis and definitions section of this regulation is stated in Article 1-

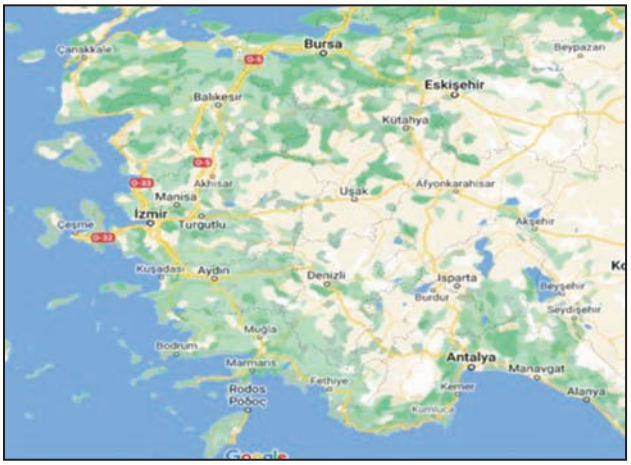


Fig 3. Cities where Camel Wrestling activities are organised.

(1) as follows: "Protecting camels within the framework of Animal Protection Law and ensuring the organisation of camel wrestling activities within the framework of this regulation." The legal basis of the regulation is the Law on Associations[2] dated 4.11.2004 and numbered 5253, the relevant provisions of the Turkish Civil Law[3] dated 22.11.2001 and numbered 4721, the Animal Rights Law[4] No.5199 and dated 2.6.2004, the relevant provisions of the Law on Charity[5] dated 23.6.1983 and numbered 2860, the relevant provisions of the Veterinary Services, Plant Health, Food and Feed Law[6] dated 11.6.2010 and numbered 5996, and the relevant provisions of the Regulation on the Transport of Animals and Animal Products in the Country[7]. In Article 3 of the Regulation, the Union determined some provisions regarding the rules and operation of camel wrestling with this regulation text. In the second part of the regulation, the concepts of "Camels, Camel Breeding and Camel Breeding in History" have been explained in detail. In the third chapter, detailed information was given on how camels are grouped, classified, and rated. Section 4 states that the Union consists of ten different bodies: General Assembly, Board of Directors,

2. Official Gazette, Date: 4.11.2004, Issue: 25649, Law No: 5253. Law on Associations.
3. Official Gazette, Date: 22.11.2001, Issue: 24607, Law No: 4721. Turkish Civil Law.
4. Official Gazette, Date: 01.07.2004, No: 5199, Issue: 25509, Animal Rights Law.
5. Official Gazette, Date: 01.07.2004, No: 5199, Issue: 25509, Animal Rights Law.
6. Official Gazette, Date: 11.06.2010, No: 5996, Issue: 27610, Veterinary Services, Plant Health, Food and Feed Law.
7. Official Gazette, Date: 17.12.2011, Issue: 28145. Regulation on the Transport of Animals and Animal Products within the Country.

Supervisory Board, Disciplinary Board, Arbitration Board, Inspection Board, Health Board, Board of Referees, Matching and Classification Board, and Education Board. In the fifth section, a definition was made for the "Organisational Structure and Duties and Authorities of the Disciplinary Board". Section 6 includes the Organisational Structure, Duties and Powers of the Arbitration Board. In the seventh section, the organisational structure of the Inspection Board, its Duties and Authorities, and the Procedures for Assignment are explained in detail. "The Organisational Structure, Duties and Powers of the Health Board and Appointment Procedures" and the Organisational Structure, Duties and Powers of the Board of Referees and the Procedures for Appointment were explained in Section 8 and 9, respectively. In Section 10, the organisational structure, duties and authorities of the Matching Board and the Appointment Procedures have been explained. Section 11 includes information on the "Organisational Structure, Duties and Powers of the Board of Education". The organisational structure, duties and powers of the Investigation and Observation Board are shared in Section 12, while section 13 gives information about the "Definition, Duties and Powers of Chief Referee, Midfield Referee, Scorekeeper, Field Commissioner, Mouth Fastener, Mouth Fastening Controller, Tether/ Separator, and Presenter." In Section 14, the "Form and Conditions of Arrangement of Camel Wrestling Organisations" are explained and in Section 15, a legal definition has been made under the headings and subheadings of "Various Provisions, Enforcement, and Execution."

Apart from the main regulation of the Union, there are also regulations and disciplinary instructions that set the principles under eight headings, namely "Observer Regulation, Mouth Fastener Controller Regulation, Mouth Treadmill Regulation, Chief Tether Regulation, Discipline Instruction, License Regulation, Referee Regulation, and Presenter Regulation", which were prepared in accordance with the Animal Protection Law No. 5199. According to the laws, the Union determined "The procedures and principles regarding the registration and visa procedures of the institutions and organisations organizing the protection of animals, observing animal rights and wrestling." According to the main regulation of the Union and Article 11 of the Animal Protection Law; "Animals cannot be trained in ways that exceed their natural capacity or strength, or cause them to injure, cause unnecessary pain, or encourage them to bad habits. It is forbidden to make animals fight with another living animal. Traditional demonstrations for folkloric purposes, which do not include violence, can be organised by obtaining the approval of the Ministry and obtaining permission from the Provincial Animal Protection Boards." These regulations and instructions are concluded with the statement that they are executed by the CCCWU and the Provincial Representatives of the Union (Anonymous, 2020a).

The Statutory Rules of the Camel Culture and Camel Wrestling Union in Aydın

In the statutory rules, the foundation purpose and administration of the Union and the steps to be taken for the establishment of the Camel Culture Research Institute, which aims to spread the camel culture, are explained[8]. This has been detailed under the heading of the studies to be carried out by the Union. Founding associations of the Union (Aydın Camel Breeders' Association; Incirliova Camel and Camel Wrestling Lovers Association; Çanakkale Çan District Camel Owners, Organisers and Audience Association; Çanakkale Ezine Camel and Camel Wrestling Lovers Association; İzmir Bergama Camel Culture and Camel Wrestling Association; Kuşadası Camel Culture and Camel Wrestling Association; Balıkesir Burhaniye Camel Culture and Camel Wrestling Association; Association for the Protection, Research and Promotion of Domestic Breeds-Manisa). In the statutory rules, the union's bodies, membership conditions, duties and powers of the general assembly, and procedures and principles are specified (Anonymous, 2020a).

Board of Directors and Supervisory Board Members of the Camel Culture and Camel Wrestling Union in Aydın

Members of the Union Administration and Supervisory Board, which continue their activities in their third term (2012-2021), are shared in the tables below.[9,10,11]

The Union has member associations in provincial and district centres where camel wrestling takes place. Considering the geographical distribution of the forty-five associations that are members of

8. Official Gazette; Date: January 8, 2018, Issue: 30295
9. Table 1 and 2 Source, CCCWU Board of Directors; Date 26.05.2013, Decision no: 8
10. Table 3 and 4 Source, CCCWU, Board of Directors; Date 29.05.2016, Decision no: 24
11. Table 5 and 6 Source, CCCWU, Board of Directors; Date 29.12.2016, Decision no: 48

the Union, it is seen that there are 14 associations in Aydın, 8 in İzmir, 3 in Manisa, 1 in Denizli, 7 in Balıkesir, 6 in Çanakkale, four in Muğla, and 2 in Antalya. The associations within the union are those in the provincial and district centres where wrestling takes place. It can be said that depending on the places where wrestling takes place, camel owners formed associations, which were then affiliated with the Union for representation.

Our data also revealed that the members who can be present in the Union's administrative and supervisory boards are also from the geographical regions where wrestling takes place. It can be said that those who are capable of representation in the Union are mostly camel owners, and those who are not camel owners are closely related to the camel breeding and camel wrestling culture.

Table 1. Board Members in the First Term (2013-2016).

Name/Last name	Connection with Camel Culture	Hometown
Metin ÇETİN	Camel Owner	Efeler/AYDIN
Hüsamettin BAŞOĞLU	Not a Camel Owner	Kemalpaşa/İZMİR
Murat AYDINLIGİL	Camel Owner	Burhaniye/ BALIKESİR
Aytekin KAYA	Camel Owner	İncirliova/AYDIN
Recep PULAT	Camel Owner	Çan/ÇANAKKALE

Table 2. Supervisory Board Members in the First Term (2013-2016).

Name/Last Name	Connection with Camel Culture	Hometown
Necati SARICA	Camel Owner	Demre/ANTALYA
Ercan SEVİNÇ	Not a Camel Owner	Bergama/İZMİR
İbrahim GÖR	Camel Owner	Gömeç/BALIKESİR

Table 3. Board Members in the Second Term (2016-2019).

Name/Last Name	Connection with Camel Culture	Hometown
Aytekin KAYA	Camel Owner	İncirliova/AYDIN
Halil AĞLAR	Camel Owner	Çan/ÇANAKKALE
Çağrı UZUN	Not a Camel Owner	Milas/MUĞLA
Musa SEZEK	Not a Camel Owner	Köşk/AYDIN
İbrahim ARSLANTAŞ	Camel Owner	Kumluca ANTALYA
Metin KAHVECİOĞLU	Camel Owner	Efeler/AYDIN
Ali ÇAKIR	Not a Camel Owner	Edremit/ BALIKESİR

Table 4. Supervisory Board Members in the Second Term (2016-2019).

Name/Last Name	Connection with Camel Culture	Hometown
Vedat ÖZTÜRK	Not a Camel Owner	Kuşadası/AYDIN
Şeref CANBOLAT	Camel Owner	Bergama/İZMİR
İbrahim GÖR	Camel Owner	Gömeç/ BALIKESİR

Table 5. Board Members in the Third Term (2019- ongoing).

Name/Last Name	Connection with Camel Culture	Hometown
Özgür DÜLGER	Camel Owner	Germencik/ AYDIN
Fidel AKGÜN	Camel Owner	Bodrum/Muğla
Mehmet YAVAŞ	Camel Owner	Torbalı/İZMİR
Feridun BALKIŞ	Camel Owner	Kepez/ ÇANAKKALE
Ali Enisi ISPARTA	Camel Owner	Edremit/ BALIKESİR
Mehmet YİĞİT	Camel Owner	Sarayköy/ DENİZLİ
Üzeyir KOTAN	Camel Owner	Biga/ ÇANAKKALE
İsmail DURAL	Camel Owner	Ayvalık/ BALIKESİR
Sadettin BALCI	Camel Owner	Bergama/İZMİR

Table 6. Supervisory Board Members in the Third Term (2019-2021).

Name/Last Name	Connection with Camel Culture	Hometown
Mehmet YAVAŞ	Camel Owner	Selçuk/İZMİR
İlker ŞAHİN	Not a Camel Owner	Sarıgöl/MANİSA
Hakan ÇELEBİ	Not a Camel Owner	Sultanhisar/AYDIN

In the light of all these data, it should be stated that the CCCWU has reached an important representation power in terms of camel breeding and camel wrestling culture. The presence of actors in the union administration and supervisory boards from provincial and district centres where wrestling is performed confirms this. In addition, the geographical distribution of the union's member associations shows that the camel culture in Turkey has started to reach an important level of representation.

The Economic Structure of the Camel Culture and Camel Wrestling Union (Income Sources-Expenses)

The CCCWU's entire income and expenses have been recorded since 2012, the date of establishment,

and have been declared open to inspection by the "Aydın Governorship Associations Department." Between the years 2012-2020 (2013-2014-2015-2016-2017-2018-2019-2020), in the eight-year period, the total official registered revenue was 46.950 TL and the revenue comes from donation, membership fee, and the participation fee received from wrestling. The annual membership fees are 250 TL, the entrance fees to be taken from the associations to be registered are 250 TL, the donations from individuals or institutions and the participation money received for each camel wrestling constitute the sources of income.

The total official registered expenditure of the union was 21.700 TL[12] (aid to wrestling, rent, personnel, equipment purchase, etc.) in the eight-year period between 2012 and 2020.

The income of the CCCWU within the framework of its legal structure is composed of elements such as the fees received from the member associations. The expenses include the Union's rent and the activities carried out regarding camel culture.

It is also noteworthy that the total budget of the Union is not at a very large level. With the increase in institutionalisation and representation ability, it can be said that the budget and income sources of the Union as a supreme control mechanism will increase, consequently the expenses will increase, and the budget will be higher as the camel breeding and wrestling culture develops and becomes widespread.

The Statutory Rules of the Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture

As a result of the application made by the Union to the Provincial Associations Directorate of the Governorship of İzmir on 07.10.2013, a registration number (35.061.030) was given and the Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture was established. Upon the examination of the necessary documents regarding the establishment and the statutory rules, the Union was approved on the basis of Associations Law[13] No. 5253 dated 4.11.2004, Turkish Civil Code[14] No. 4721 dated 22.11.2001 and Associations Regulation[15] No. 25772 dated 31.03.2005. The aim of the Union was stated as "to create a common solidarity among the camel breeders in the Mediterranean, Aegean and Marmara Regions and to serve this deep-rooted cultural heritage with all the stakeholders". It has been put under legal judgment that the centre of the Union is Izmir and that it cannot have a branch. The Union was formed by 5 associations, namely "Harmandalı Camel and Camel Wrestling Lovers Association, Kemalpaşa Town Camel Owners Association, Menemen Türkelli Camel Owners Association, Pınarbaşı Camel Breeding and Camel Wrestling Lovers Association, and Torbalı Camel Breeding and Camel Wrestling Culture Lovers Association." The Statute has established the legal structure of the bodies of the board of directors, giving details about their operations and procedures, and the elections to be held, and the Union started to work in 2013. It is understood that the Union specifically aims to organise and promote camel wrestling by being in contact with central and local governments in Anatolia and Thrace provinces, districts and towns. When the Statutory Rules are evaluated in general, it is seen that the Union aims to contribute to the development of the camel culture and to the region both culturally and economically (Anonymous, 2020b). The list of the members of the board of directors and supervisory board and the data on budget sources could not be reached through the negotiations with the Union.

Discussion and Conclusion

Throughout history, issues such as the human-animal relationship, the legal status of animals, the responsibilities of humans towards animals, crimes against animals and the penalties for these crimes have been included in the oldest legal texts. The oldest known laws were uncovered in Mesopotamian clay tablets. These are Ur-Nammu Laws (2100 BC), Lipit-Ishtar Law (900 BC), Eshnunna Laws (1920 BC) and Hammurabi Laws (1728 BC) (Gürler & Osmanağaoğlu, 2009). In Ottoman history, in the 15th and 16th centuries, establishments under the name of "Zoos" were established for the purpose of animal breeding and production, and it is seen that steps were taken to ensure that animal husbandry was supported and controlled by the state, and it was encouraged to raise high-quality animals (Edhem, 1918). Again, it is reported that the first association related to the racing competitions of animals in the Ottoman Empire was established in the time of Sultan Abdulaziz, according to the newspaper "Ceride-i Havadis" dated 1280 (1864 in the Gregorian

12. CCCWU Board of Directors, Date 21.04.2021, Decision no: 66.
13. Official Gazette, Date: 4.11.2004, Issue: 25649, Law No: 5253. Associations Law.
14. Official Gazette, Date: 4.11.2004, Issue: 25649, Law No: 5253. Associations Law.
15. Official Gazette, Date: 4.11.2004, Issue: 25649, Law No: 5253. Associations Law.

calendar) and numbered 829. On this date, the horse racing association was established (Erk, 1962). In the Ottoman Empire, "Specialisation Laws", which can be defined as municipal laws containing regulations regarding the goods and services produced and sold in the city, came into force. These laws, which were prepared on the basis of customs and traditions and religious laws, also include issues regarding the protection of animals. For example, in Bursa Specialisation Law (1502), which is one of these laws, it is stated that; *"And porters will not use horses without horseshoes and will not bring more than two burdens of the vineyard load. The mule wood should be three feet wide and camel wood should be six feet wide. And it should come to the city just as it was loaded in Uludağ. The load should be divided and shortened. This was determined by law"*. In the "Kanunname-i Osmanî" prepared before 1630, the standards of the wood load were determined as follows: *"And the woodsmen should make the mule wood four feet long, donkey load three feet long and camel load six feet long"*. In addition, it was stated in a law code of 1680 that "The mule wood load should be four feet long, the camel load six feet long, and the donkey load two and a half feet long" (Gürler and Osmanagaoğlu, 2009). The first association for the protection of animals in Turkey, "Istanbul Himâye-i Hayvânât Society" (Istanbul Animal Protection Society), was established in 1912 with the initiatives of military and civilian bureaucrats who played important roles in Ottoman history (Melikoğlu, 2009). With the first laws passed, it can be concluded that legislators took initiatives for the protection of animals, and activities including animals were carried out. Based on this information, it can be said that at that time, it was aimed to bring animal rights to a legal status and to develop them.

Apart from the state organisation, it can be said that non-governmental organisations also carry out a number of activities centered on animals. The essence of non-governmental organisation includes factors such as friendship, sense of achieving something together, collectiveism, and people coming together voluntarily and trying to do something. "Civil society", which includes a sense of synergy and unity of power, represents unity, volunteering and solidarity, meaning that people do things together that they cannot be done individually (Talas, 2011). Non-governmental organisations undertake important roles in the formation and raising of public awareness and in carrying the discussions to the political field (Ertürk, 2005). Countries that are members of the European Union have very effective non-governmental organisations on issues such as animal welfare, agricultural production, marketing, trade, food hygiene, environmental protection, and rural development in the agricultural sectors (Arabacı, 2005). It is seen that there are organisations related to animal breeding among non-governmental organisations that have emerged as a new concept in time.

Camel wrestling, which continues its existence in a traditional structure, has been organised since the 1980s, when the effects of modernity were rapidly felt. The association process started and unions were established in 2012. The Camel Culture and Camel Wrestling Unions have been legalized by taking steps towards institutionalisation (Ertürk, 2018). Similar to camel wrestling in Turkey, races with animals operate under the "Turkish Traditional Sports Union[16]." These races are Rahvan Horse Riding, Equestrian Javelin, Kökbörü, Horse Sleigh, and Equestrian Archery, which are included in the "Traditional Sports Union." The Camel Culture and Camel Wrestling Union, on the other hand, has been institutionalised as an independent Union.

With the adoption of the regulation in accordance with the 6th article of the Animal Protection Law numbered 5199 and the decision of the Animal Protection Board of Aydın Governorship dated 12.02.2014 and numbered 12, *"Weak animals cannot be used for commercial and demonstration purposes or for riding and transportation in any way"*. It can be concluded that the regulation approved by the Board is prepared by taking into account the appropriate age, quality of life and health conditions of the animals for demonstration-commercial purposes and will only be subject to permission on the basis of the protection of all kinds of rights of animals.

In Article 1 of the Union Regulation (Anonymous, 2020a), it is seen that the statement "ensuring the regulation of camel wrestling organisations by protecting camels" was created on the basis of the Law on the Protection of Animals. As stated in Article 1 of the Law, the aim is *"ensuring comfortable lives of animals and good and appropriate treatment of animals, protecting animals against suffering in the best way possible and preventing all kinds of victimization."* It is seen that importance is given to the prevention of suffering and to the continuation of sustainable lives, and the union administration aims to organise these competitions within the framework

16. https://www.gsdf.gov.tr/tr/spor-dallari; Retrieved 25.11.2020.

of animal rights and constitutes legal sanctions. The statutes and regulations of both Unions in our study confirm these statements.

As stated in Section 3 of the regulation, camels to wrestle should be classified according to measurements (height and weight) and the principle of equality should be taken as basis during the competitions. The structure, duties and powers of the Union board of directors and supervisory boards are defined in Sections 4 and 5 of the Regulation. It has been observed that the Union has been established in accordance with the Associations Law[17] and has a legal structure.

In Section 6, the duties and powers of the Arbitration Board are clarified and it is stated that the Board includes seven lawyers with at least three years of professional experience. In Section 7, the details regarding the Inspection Board are shared and it is stated that it is composed of seven individuals who are inspectors with at least three years of professional experience. Section 8 gives details about the duties and powers of the Health Board, and it is stipulated that it should include seven veterinarians with at least three years of professional experience. Section 11 gives details about the duties and authorities of the Board of Education, which includes five educators with at least three years of professional experience. Under the relevant heading in each of the four sections, it is stated that experts and authorized persons are appointed and qualification rules are followed.

In the Organisation Structure of the Arbitration Board and the Organisation Structure of the Matching Board, which are the 9th and 10th sections of the Regulation, it is seen that the boards consist of five members, and the principles of the selection of the referees in the competitions and how the competition of animals will be conducted have been clarified by legal provisions and trying to prevent favorable situations.

Section 12, on the other hand, states that the Examination and Observation Committee consists of four persons and includes their duties and powers. It also includes provisions regarding the care and feeding conditions of camels on certain dates of the year. It can be said that this provision coincides with the principles of animal welfare rules that should be applied during camel wrestling and similar animal races, according to Koç (2018).

In Section 13, the definition, duties and authorities of the Chief Referee, Midfield Referee, Scorekeeper, Field Commissioner, Mouth Fastener, Mouth Fastening Controller, Tether/Separator, and Presenter are given and the limits of the authority of each official are drawn; the rules of the competitions are shared, and unlawful acts are tried to be prevented.

In Section 14, the legal duty definition of the delegation, which is assigned to ensure that camel wrestling activities are carried out under legal responsibilities and according to the Terms and Conditions of the Regulation. In order to prevent any negative effects that may occur in the organisations, the rules have been written in detail and it has been observed that they are bound by the provisions of the regulation.

Section 15 includes Various Provisions, Enforcement, and Execution. It has been observed that the Union is trying to achieve a legal structure and an institutional vision regarding the competitions.

It has been determined that the Camel Culture and Camel Wrestling Union has been created by taking all regulations and instructions from the Animal Protection Law[18] No. 5199 and adhering to its practices. It is seen that the legal content of the regulations is based on animal rights and the use of animals in traditional non-violent demonstrations for folkloric purposes. In this context, it can be said that camel wrestling activities, which are a part of traditional culture, set an example for animal races that are not legally defined and that do not violate the laws by adhering to the Animal Rights Law.

However, it is noteworthy that how the camels should be moved to wrestling areas or what animal welfare norms should be followed/applied during wrestling were not mentioned at any point in the regulation. Although this situation is not directly stated in the bylaws and regulations of the Union, it can be said that the statutes and regulations take animal welfare into consideration. Even if the bylaws and regulations of the union seem to be incomplete in this respect, specific indicators for assessing camel welfare in the world have not yet been developed and camels have been clearly neglected by international legislation. The "World Animal Health Organisation" included camels in its land transport recommendations document, but the welfare aspects of camel breeding systems were not addressed in

17. Official Gazette, Date: 4.11.2004, Issue: 25649, Law No: 5253. Associations Law.

18. Official Gazette, Date: 01.07.2004, No: 5199, Issue: 25509, "Animal Rights Law, Law."

the special parts of the "Terrestrial Code" and the first European project called the "Welfare Quality" project, although they focused on other species, It is stated that it does not appear in the Animal Welfare Indicators Project (AWIN), the second largest project in Turkey (Padalino and Menchetti, 2020). Again, in the studies of Preveti *et al* (2016), although camels have needs to be taken into account in order to protect their welfare, there appears to be a lack of interest in the Directive 98/58 / EC (Council of Europe 1998), which has a wide scope and implemented in Italy by Legislative Decree 146/01 (Italian Law 2001). In this context, it can be concluded that the absence of animal welfare within the scope of the union regulation is in line with the animal welfare legislation and literature in the world.

The Regulation of Camel Culture and Camel Wrestling Union is composed of twenty-three articles and a temporary article. It is seen that the Union's statutory rules, which were formed by the eight member associations that established the union, includes the objectives of the Union and the activities to be carried out, aiming to ensure development of the camel culture and to gain a national identity. Especially placing the word "Turkey" at the title of the Union and targeting the establishment of Camel Culture Research Institute are the most important indicators of this. In addition, it can be said that it aims to develop relations in the international arena and to bring its prevalence to the world scale as understood from the statement "Producing projects related to the culture of camel wrestling and camel wrestling, providing support from public and private institutions and organisations, local governments and international organisations". Under the heading of the "projects to be continued" is "Creating a common platform with the Unions, foundations and other non-governmental organisations having the same purpose." The statement of "sharing opinions on" Animal Protection Laws "with relevant official institutions and non-governmental organisations" is remarkable. It can be said that trying to achieve partnerships with institutions and non-governmental organisations with a similar structure, i.e., stakeholders and uniting on the basis of animal rights are the goals. In the continuation of the statutory rules, the conditions for membership, the principles of general assembly election and the duty of the general assembly are regulated within the framework of the Associations Law[19].

19. Official Gazette, Date: 4.11.2004, Issue: 25649, Law No: 5253. Associations Law.

It has been observed that since the establishment of Camel Culture and Camel Wrestling Union in 2012, the 3rd Term Board of Directors and Supervisory Board has been established[20,21,22]. In Article 72 of the Turkish Civil Code, the board of directors is defined as a mandatory body that performs administration of internal affairs such as the implementation of association decisions, the fulfillment of daily current affairs, and the representation duty outside. It also fulfills the duties and powers of execution and representation in accordance with the laws and regulations of the association. Another mandatory body included in the Turkish Civil Code is the supervisory board. Supervisory boards serve as the association's internal audit bodies (Sabuncu, 2020). It has been observed that the Union Board of Directors and the Supervisory Board have continued without interruption from its establishment to the present day with its activities in accordance with their legal status in terms of duty-scope and internal relations.

When the profiles of both the board of directors and the members of the supervisory board are examined, it has been observed that it was formed by people who are closely related to camel culture and its geographical distribution is composed of people living in the coastal strip of the Aegean, Mediterranean and Marmara Regions and regions where camel wrestling is performed. It has been determined that 20 of these people are camel owners, while 10 of them are not camel owners, but they are raised within camel culture; they are interested in camel breeding, and they are involved in the Union activities. It can be said that the service provided on a voluntary basis for the continuation of camel wrestling, which is a cultural heritage, and to support its corporate structure, is meaningful and has made great contributions to the livestock sector in Turkey.

The income source of the Union is donations, membership fees, wrestling revenues and 500 TL participation money received for each camel wrestling. On the other hand, Union expenditure items are aid for wrestling, rent, personnel expenses, and equipment purchase. When the eight-year budget of the Union's economic structure is evaluated, it was concluded that it could balance its income and expenditure, especially by spending money in camel

20. CCCWU Board of Directors; Date:26.05.2013, Decision no: 8

21. CCCWU Board of Directors, Date: 29.05.2016, Decision no: 24

22. CCCWU Board of Directors Date:29.12.2016, Decision no: 48

wrestling organisations and completing the services with this budget.

The Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture was established in 2013 in accordance with the Law on Associations, the Turkish Civil Code and the Associations Regulation. It is noteworthy that although it is an association established on the basis of the development of animal husbandry and to support of breeders, the Animal Rights Law No. 5199[23] has not been included in the legal structure. However, while determining the relevant principles in the Camel Culture and Camel Wrestling Union in Aydın, animal rights provisions have been included and compliance with the rights of animals is legally guaranteed.

When the regulation is examined in general, it is seen that it consists of twenty articles and is structured according to the law of associations. It has been drawn up within the framework of the establishment, operation, procedures and principles of the associations. The Camel Culture and Camel Wrestling Federation in Aydın, with all its legislative structure, can be said to be in an important organisation not only for the establishment of a union but also for the development and encouragement of sustainable camel breeding.

As a result, it can be said that the existence of the Camel Culture and Camel Wrestling Union in Aydın and the Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture, which were established in 2012 and 2013, is very important for both camel breeding and camel wrestling to have a legal and institutional structure. The Union in Aydın has been established in a manner that serves the purpose with all legal structures and in accordance with all relevant laws.

The Camel Culture and Camel Wrestling Union in Aydın continues its activities with the participation of forty-five associations established in the regions where wrestling takes place as of 2021. On the other hand, the Union of Anatolian Camel Owner Nomads and Camel Wrestling Culture has completed the establishment phase but has not reached the effective representation power yet.

It has been observed that the main purpose of both organisations is to ensure the continuity of activities of camel wrestling on a legal basis. Based on all these developments, it can be judged that it is promising in terms of carrying the camel culture, which is a traditional element in Turkey, to the future, gaining the ability to represent, promoting it on national and international platforms, and keeping the camel breeding and wrestling culture alive. Finally, as an institution that will ensure that camel wrestling is carried out within the framework of animal welfare and animal rights issues in the future, the activities of the camel unions are extremely important.

23. Official Gazette, Date: 01.07.2004, No: 5199, Issue: 25509, Animal Rights Law.

References

Arabacı A. Avrupa Birliği üyelik sürecinde tarım sektöründeki sivil toplum kuruluşlarının önemi. II. Ulusal Sivil Toplum Kuruluşları Kongresi, Çanakkale, 15-16 Ekim, s. 209-214. 2005.

Anonymous. Devecilik Kültürü ve Deve Güreşleri Federasyonu, Yönetmelik Dosyası, Aydın, 2020. 2020a.

Anonymous. Anadolu Yörükleri Deveciler ve Deve Güreşleri Kültürü Federasyonu, Yönetmelik Dosyası, İzmir, 2020. 2020b.

Çalışkan V. Bir Dünya Kültür Mirası: Anadolu Devecilik Kültürü ve Deve Güreşleri, 1. Baskı, İncirliova Belediyesi Kültür Yayınları. 2016.

Çalışkan V. Kültürel Bir Mirasın Coğrafyası: Türkiye'de Deve Güreşleri, 1. Baskıı Selçuk Belediyesi Yayınları, No: 3, Anka Matbaacılık, İstanbul. 2010.

Edhem S (1334=1918): Nevsal-i Baytari, İstanbul: Agop Matasyon Matbaası.

Erk N. Memleketimizde At Yarışları İle İlgili İlk Derneğin Kuruluşu, Ankara Üniversitesi Veteriner Fakültesi Dergisi, Cilt: 9, no: 3-4, Ayrı Basım, Ankara: Ankara Üniversitesi Basımevi. 1962.

Ertürk D. Bireyden cemaate sivil toplum. Yayınlanmamış Yüksek Lisans Tezi, Dumlupınar Üniversitesi Sosyal Bilimler Enstitüsü, Kütahya, Türkiye. 2005.

Ertürk D. Deveci kahvesinden federasyona: Devecilik ve deve güreşlerinde örgütlenme modeli, II. Uluslararası Selçuk-Efes Devecilik Kültürü ve Deve Güreşleri Sempozyumu, I.Cilt Sosyal Bilimler, Selçuk-İzmir, 18-20 Ocak, s. 51-53. 2018.

Ertürk D ve Şanlı S. Türkiye'de Deve Güreşleri, (Edt.) Devrim Ertürk and Süleyman Şanlı, 1. Baskı, Gece Kitaplığı, Ankara. 2017.

Ertürk D ve Şanlı S. Kültür, Gelenek ve Eğlence: Deve Güreşleri, (Edt.) Devrim Ertürk&Süleyman Şanlı, 1. Baskı, Çizgi Kitabevi, Konya. 2019.

Gürler MA ve Osmanağaoğlu Ş. Türkiye'de hayvanları koruma kanununun tarihsel gelişimi. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2009; 15(3):325-330. DOI:10.9775/kvfd.2008.62-A.

Kılıçkıran MN. Ege'de kış turizminin kurtarıcısı "Deve Güreşleri", III. Milletlerarası Türk Folklor Kongresi Bildirileri, Ankara, Başbakanlık Basımevi, s. 125-146. 1987.

Koç A. Türkiye'de deve yetiştiriciliği ve hayvan refahı, II. Uluslararsı Selçuk-Efes Devecilik Kültürü ve Deve Güreşleri Sempozyumu, II. Cilt, Fen ve Sağlık Bilimleri, Selçuk-İzmir, 18-20 Ocak, s. 129-137. 2018.

Melikoğlu B. Türkiye'de Kurulan İlk Hayvanlar Koruma

Derneğinin Tarihsel Gelişimi. Veteriner Hekimler Derneği Dergisi. 2009; 80(1):37-44.

Padalino B and Menchetti L. The first protocol for assessing welfare of camels. Front. Vet. Sci. 7. 1230, doi. org/10.3389/fvets.2020.631876. 2020.

Pekel E ve Ünalan A. Türkiye süt sığırcılığının geliştirilmesinde damızlık hayvan yetiştiricileri birliklerinin rolü, Hayvancılıkta Örgütlenme Sorunları Sempozyumu, İzmir, 27-28 Kasım, s. 126-133. 1997.

Previti A, Guercio B and Passantino A. Protection of farmed camels (*Camelus dromedarius*): Welfare problems and legislative perspective. Animal Science Journal. 2016; 87:183-189 doi.org/10.1111/asj.12446

Sabuncu B. Dernek organlarının iç ilişkide birbirlerine karşı hukuki durumları. Ankara Barosu Dergisi 2020/1: 155-190. 2020.

Talas M. Sivil toplum kuruluşları ve Türkiye perspektifi. Türk Bilim Araştırmaları. 2011; (29):387-401.

Yılmaz O, Ertürk YE ve Ertuğrul M. Türklerde deve güreşlerinin Orta Asya'dan Anadolu'ya 4.000 yıllık geçmişi. ÇÖMÜ Ziraat Fakültesi Dergisi (COMU Journal of Agriculture Faculty). 2014; 2(1):37-44.

BACK ISSUES OF JCPR AVAILABLE

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 13 June 2006 Number 1

In This Issue

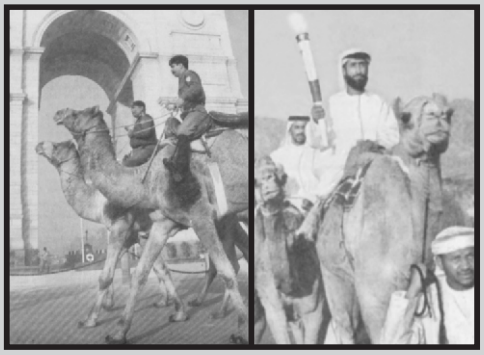
ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 13 December 2006 Number 2

In This Issue

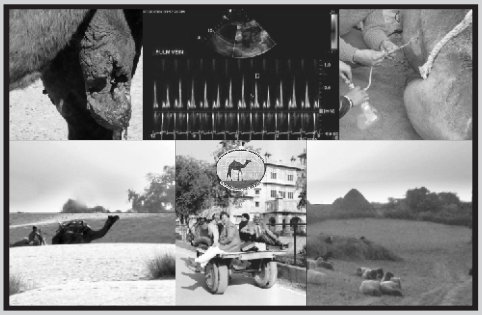


ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 14 June 2007 Number 1



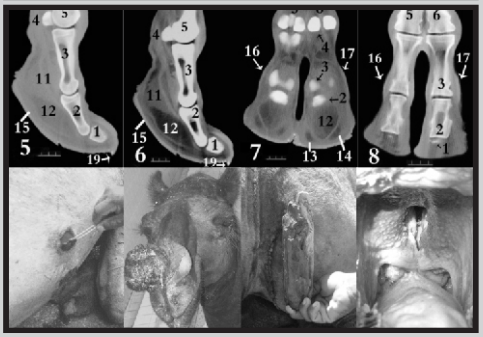
In This Issue

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 14 December 2007 Number 2



In This Issue

See the details of list of contents of all previous issues of JCPR on our website
www.camelsandcamelids.com

Kindly refer querries to: Dr. T.K. GAHLOT, Editor, JCPR
email: tkcamelver@yahoo.com

AUTHOR INDEX

SUBJECT INDEX

(Journal of Camel Practice and Research - triannual -April, August and December issues every year)

INSTRUCTIONS TO CONTRIBUTORS

The Journal of Camel Practice and Research is a triannual journal (April, August and December issues) published by 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 three 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. The Journal of Camel Practice and Research will occasionally contain an **invited editorial** commenting on the current research and papers in the issue.

Submission of manuscript: Manuscripts should be submitted in word files to **Dr. T.K. Gahlot,** Editor, Journal of Camel Practice and Research at tkcamelvet@yahoo.com. The figures can be submitted preferably as a high pixel JPEG or other format. The manuscript should be accompanied by a covering note and author consent 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.

Preparation of the manuscript: Manuscript should be typed in Book Antiqua font size 12 using British English, spellings and generic names of drugs. 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. Kindly remain restricted to the relevant subject matter of given title of manuscript only. The review of literature should be up to the submission year of manuscript. Kindly check every reference for its accuracy from the relevant book, journal or through google.

Each manuscript should have the following sections:

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:

PROTEOMIC CHARACTERISATION OF SERUM DURING THE BREEDING CYCLE IN MALE BACTRIAN CAMELS

Le Hai[1], Rendalai Si[2], Fu-Cheng Guo[1], Jing He[1], Li Yi[1], Liang Ming[1], Jun-Wen Zhou[3], La Ba[3], Rigetu Zhao[3] and Rimutu Ji[1,2]

[1]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China

[2]Inner Mongolia Institute of Camel Research, Badanjiran, Inner Mongolia, China

[3]Alxa League Institute of Animal Husbandry, Alxa, Inner Mongolia, China

SEND REPRINT REQUEST TO RIMUTU JI email: yeluotuo1999@vip.163.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 commence after key words. 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 or software, 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 about twice the size desired for reproduction that is 17 cm for double column or 8.3 cm for single column. Photographs should be of good quality with adequate contrast and high pixels. 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.

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: Shawaf T, El Nahas A, Melegi A, Al Bulushi S, Aiyan AA and Eljalli I (2020). Investigation on biochemical parameters of cerebrospinal fluid in camels with neurological disorders. Journal of Camel Practice and Research 27(2):165-171.

Wilson R Trevor (2020). The one-humped camel in Eritrea and Ethiopia: a critical review of the literature and a bibliography. Journal of Camel Practice and Research 27 (3): 229-262.

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): Faye B and Bengoumi M (2018). Camel Clinical Biochemistry and Haematology: Springer International Publishing. pp 275-286.

Chapter from multiauthored books: Wernery U, Kinne J and Schuster RK (2014). Unusual arboviruses and other minor viral infections. In: Camelid Infectious Disorders. OIE Book. pp 319-322.

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: Christina Adams (2014). Camel milk: a miracle cure for children with autism?. Gulf News, Published: April 09.

Personal communication: Hall LW (1995). Reader in Comparative Anaesthesia, Department of Clinical Veterinary Medicine, Madingley Road, University of Cambridge, Cambridge, CB3 0ES, England.

Reprints or Publication charges: 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 to receive a Final PDF or for 10 reprints for the year 2021. Charges for colour and black and white pictures: Author(s) has to pay for production of colour plates in his/her manuscript as per the invoice provided to them. Publication charges would be double if a manuscript is more than 10 printed pages in length. The publication charges should be paid into the bank account of Camel Publishing House. Author(s) will be sent final prepublished PDF for verification and corrections, if any alongwith invoice and bank account details for making advance payment, before publication of manuscript in JCPR.

Author Consent Letter

{Note: Kindly take print out of the Author Consent letter form, fill, scan and send along with your manuscript. Without Author Consent letter your manuscript will not be considered for publication}

To
The Chief Editor
Journal of Camel Practice and Research
Camel Publishing House
Bikaner

Sub: Submission of manuscript for publication in Journal of Camel Practice and Research reg.

Dear Sir

I hereby submit the manuscript for publication in 'Journal of Camel Practice and Research". I assure that this manuscript has neither been published in any other journal nor submitted for publication in any other journal. I also undertake along with the other authors that present study (on animals, human or laboratory animals, if any) was undertaken after the prior approval of relevant country/institutional ethical committee. I and on behalf of other co-author(s), I declare "No conflict of interest". I assure that this article do not have any plagiarism. Kindly consider the manuscript for publication in your journal. I abide all rules and regulations of the journal. In future if any litigation arises in this article I will cooperate with the editor to resolve the issue. I shall accept the decision of the editor and that would be final.

Thanking You

Kindly enter the details in the following

Manuscript

TITLE:

SUBJECT:

LABORATORY/RESEARCH CENTRE/INSTITUTE:

Corresponding Author

NAME

ADDRESS

SIGNATURE