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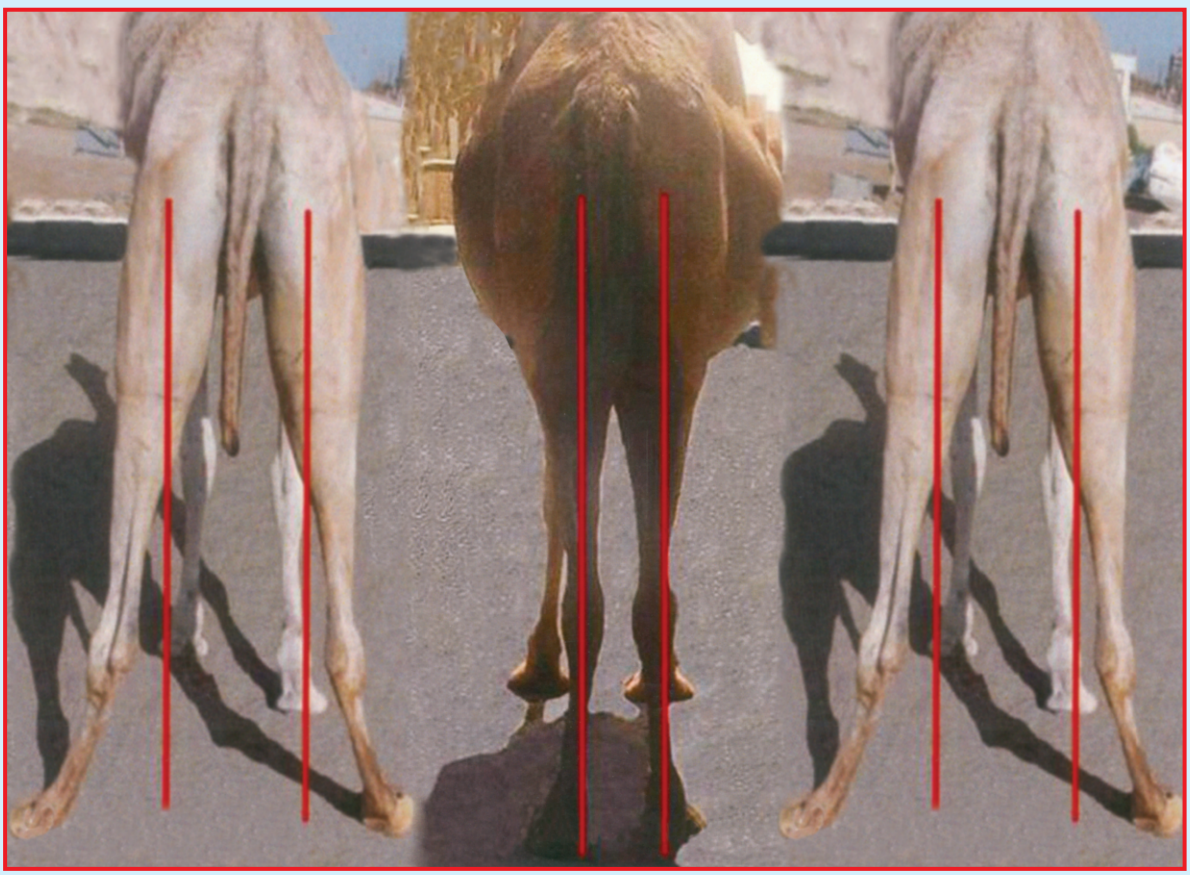

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Manuscripts and other related correspondence may be made to :

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

Phone : 0091-151-2527029 (R)
: 0091-151-2521282 (O)
Mobile : 0091-9414137029

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

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Volume 25 **August 2018** **Number 2**

CAMELS MARCHING AHEAD THROUGH LITERATURE, RESEARCH AND CONFERENCES

The new book Camel Clinical Biochemistry and Haematology authored by Bernard Faye and Mohammed Bengoumi has fulfilled a long felt void on this important topic from clinical health and nutrition point of view. The book will prove a milestone to the researchers and clinicians as normal or reference values would quickly enable comparison with values of samples being analysed in the laboratory. Many kudos to the authors. The Central Veterinary Research Laboratory (CVRL) in Dubai has played an important role in research looking at whether antibodies produced by camels could be used to combat HIV. The aim is to achieve a high-level of expression of HIV neutralising nanobodies in the vagina for passive immunisation at the primary sites of sexual transmission of HIV-1. Establishment of stem cells lines from camels would tremendously facilitate regenerative medicine for genetically superior camels, permit the gene targeting of the camel genome and the generation of genetically modified animal and be a mean for genome conservation for the elite breeds. Researchers have discovered that camels, llamas, sharks, and a few other animals produce unusually small antibodies. Small antibodies and nanobodies are also being studied as potential treatments for diseases such as cancer, as imaging agents, and for diagnostic testing. The first nanobody-based drug, which targets a clotting disorder, could be approved this year. Scientists have carried out molecular study on *T. vivax* and mixed-infection with *T. vivax* and *T. evansi* in Sudanese camels and concluded that *T. evansi* is no longer the single cause of camel trypanosomosis in Sudan.

The 5th Conference of the International Society of Camelid Research and Development (ISOCARD 2018) with a theme "Recent advances in camelids biology, health and production" will be hosted at Institut Agronomique et Vétérinaire Hassan II, Rabat, Morocco from 12th-15th November 2018. I am sure that this conference would surface up the emerging topics of current research on camelids.

The current issue is full of latest trends of camelid research. The topics covered in this issue are type III receptor protein-tyrosine kinase (KIT) loci, flumethasone- pharmacokinetics and pharmacodynamics, limbs-Linear and angular biometric measurements, muscles-pesticide and antibiotic residues, effect of road transportation- blood and serum parameters, adenocarcinoma in the genital tract, tumours-prevalence, types and locations, IgG subclasses, anti-diarrhoea immune camel milk, milk efficiency in protecting rat testes against toxicity, histology and histomorphometry of first compartment, lead acetate toxicity, histomorphometry of heart, cystic echinococcosis and *Echinococcus canadensis* G6 strain.

It is noteworthy to point out that contributions of clinical and research manuscripts to the Journal of Camel Practice and Research has increased ever since it has become triannual because the wait period for publication is reduced. Thanks to all the authors and members of the editorial board.

(Dr. T.K. Gahlot)
Editor

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CORRELATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE AGOUTI SIGNALING PROTEIN (ASIP) AND A TYPE III RECEPTOR PROTEIN-TYROSINE KINASE (KIT) LOCI WITH COLOUR IN THE BACTRIAN CAMEL (*Camelus bactrianus*)

Liang Ming[1], Dalai Siren[2], Tuya Saren[1], Li Yi[1], Jing He[1], Le Hai[1], Fucheng Guo[1] and Rimutu Ji[1,2]

[1]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, 010018, China

[2]Camel Research Institute of Inner Mongolia, Alashan, Inner Mongolia, 737300, China

ABSTRACT

Coat colour is an important characteristic and economic trait in bactrian camels. The agouti signaling protein (ASIP) and a type III receptor protein-tyrosine kinase (KIT) that control melanogenesis are candidate coat colour genes in mammals. However, these genes are not well characterised in bactrian camels and their association with colour has not been established. Here, we sequenced the ASIP and KIT genes in a population of different coloured bactrian camels to gain an improved understanding of the effects of these genes on coat colour. Our analysis of the bactrian camel ASIP gene revealed one mutation that altered the amino acid sequence of the encoded protein. In the KIT gene, we detected nine mutations, of which six were silent mutations and three were single nucleotide polymorphisms (SNPs) that alter the amino acid sequence (V28A, H57R, T118A). Analysis of associations between phenotypic and genotypic characteristics revealed that none of the mutations in the ASIP and KIT loci correlated completely with coat colour in bactrian camels. Thus, our findings indicate that the coat colour phenotype of the bactrian camel is not related to the ASIP and KIT genes; however, further investigations using more advanced technology with larger numbers of animals are required to confirm this conclusion.

Key words: Agouti gene, bactrian camel, coat colour, KIT gene, polymorphism, SNP

The bactrian camel is an important livestock species in Asia and the surrounding areas with cooler climates. China has camel genetic resources consisting mainly of two humped camels (*Camelus bactrianus*), the domestic and wild bactrian camels. Five bactrian camel breeds (Alxa, Sunit, Qinghai, Tarim and Zhungeer) are derived from these resources (Ji *et al,* 2009). The bactrian camel provides milk, wool and meat and is an important mode of transportation in desert or semi-desert areas. Coat colour in animals is an obvious phenotypic trait that is relatively easy to assess and of broad public interest. During a long period of domestication and selective breeding in the bactrian population, a large number of different breeds have been established with different pigmentation phenotypes (Sponenberg, 1997).

The agouti gene encodes the agouti signaling protein (ASIP), which regulates pheomelanin (yellow) and eumelanin (black and brown) synthesis by the pigment-producing cells (melanocytes) within the hair follicle (Jackson, 1994). Melanocytes can switch between the production of these pigments during hair growth, resulting in hairs with different banding patterns. As an antagonistic ligand of the melanocortin-1 receptor (MC1R), the agouti protein contains a seven-transmembrane motif and is expressed on the surface of melanocytes (Bultman *et al,* 1992; Miller *et al,* 1993; Robbins *et al,* 1993). Functional mutation of the agouti gene can lead to variation in coat colours in some domestic animals, such as alpaca (Chandramohan *et al,* 2013), dog (Kerns *et al,* 2004), horse (Rieder *et al,* 2001; Ludwig *et al,* 2009), cat (Eizirik *et al,* 2003), sheep (Norris and Whan, 2008) and mouse (Bultman *et al,* 1994; Kuramoto *et al,* 2001; Miltenberger *et al,* 2002). KIT, which is another strong candidate gene for the control of coat colour variation in mammal, encodes is a type III receptor protein-tyrosine kinase. The KIT ligand (also called stem cell factor, SCF) binds to KIT via the second and third extracellular immunoglobulin

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domains (Haase *et al,* 2007). The KIT gene structure is complex, not only containing a coding region that plays an important role in the formation of melanin, but also a non-coding region that also has a great impact on hair traits. The KIT gene can determine the survival, differentiation, proliferation and migration of cells and is very important for survival and growth of melanocytes. KIT gene mutations have previously been shown to influence coat colour phenotypes in horses (Haase *et al,* 2007; Haase *et al,* 2009; Haase *et al,* 2013) and other mammals, including pigs, humans and mice (Giebel and Spritz, 1991; Guerif *et al,* 2002; Pielberg *et al,* 2002). To date, only a few studies have focused on genotype characterisation of candidate genes responsible for coat colour in Bactrian camels (Liang *et al,* 2016). In this study, we characterised the two coat colour candidate genes (agouti and KIT gene) in bactrian camels to investigate relationships between genotypes and the coat colour phenotypes.

Materials and Methods

Ethic Statement

No experiments with animals was performed for this study except the collection of blood from the jugular vein and the owner or researcher of the land gave permission to conduct the study on this site.

Animals and DNA extraction

Blood samples about 10mL were collected from 94 bactrian camels (55 Alxa bactrian camels, 14 Sunit bactrian camels, 13 Zhungeer bactrian camels, 12 Qinghai bactrian camels), with three coat colours: red (n = 14), brown (n = 60) and white (n = 20). Fibre colour charts for bactrian camels were unavailable; therefore, coat colour was determined according to the owner's assessment of the animal. Genomic DNA was extracted from 200 µl of EDTA anti-coagulated blood using the DNeasy tissue kit (Qiagen, Doncaster, Vic., Australia) according to the manufacturer's instructions. The quality and quantity of genomic DNA were determined with a NanoDrop spectrophotometer. Pre-tests was carried out on 9 animals (3 red, 3 white and 3 brown) and an additional 85 samples were subsequently analysed according to the results of the pre-test (exon1 mutations in the ASIP gene and exon2, exon4 and exon11 mutations in the KIT gene).

Amplification and sequencing ASIP and KIT genes

Sequences of the ASIP and KIT genes of bactrian camels were retrieved from the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) and conserved regions were identified for the design of primers to amplify the coding region. PCR amplification of complete coding sequences was performed with the primers Table 1 in 50 µl reactions containing 5 µl 10× Taq Buffer (100 mM Tris-HCl, pH 8.8, at 25°C; 500 mM KCl, 0.8% (v/v) Nonidet), 1 µl dNTP (10 mM) (Sangon Biotech., Shanghai, China), 25 mM MgCl2 (Sangon Biotech.) 5 µl, 5 unit TaqDNA polymerase 0.5 µl, 1 µl each of forward and reverse primers and 1 µl genomic DNA. The cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles, each consisting of 94°C for 30 s, 55–60°C for 35 s and 72°C for 40–50s; with the final extension at 72°C for 5–8 min. Amplified DNA was electrophoresed in 1% (w/v) agarose gels in TAE buffer, stained with ethidium bromide and visualised by UV transillumination. The PCR products were purified using the Sangon PCR Cleanup Kit (Sangon). ASIP and KIT sequencing primers were designed for selected each exon (Table 1) using Big Dye Terminator Technology v3.1 (Applied Biosystems, Mulgrave, Victoria, Australia) and sequencing was performed on a 3730 DNA analyser (Applied Biosystems, USA).

Bioinformatics analyses

Sequences were analysed using Chromas, EditSeq and Seqman software to determine the genotypes of two randomly selected camels to verify the identities of the ASIP and KIT genes. Genotype and allele frequencies were counted directly and

Table 1. Primer pairs designed for amplification of ASIP and KIT gene exons from genomic DNA.

Gene	Primer	Sequence (5'-3')	Product size (bp)
ASIP	Ex1F Ex1R	TTGCTTCAGTCTCCCTCCC TTTCTCACAGCCTCTAACATGC	641 bp
KIT-2	Ex2F Ex2R	ACATCTTGGCCTGCATACC CTCATTAGGAAGAGTCGCACA	739 bp
KIT-4	Ex4F Ex4R	GGGGTAGAGTGCGTGCTTA AATCATTCAGAGAAACAGCATAAA	621 bp
KIT-11	Ex11F Ex11R	ACATAACAATGGCTTTAGGGAA GACCGATCACAAGAGCCAG	572 bp

the sequencing alignment was obtained using the DNAStar v5.2.2 program. The MegAlign program was used to align the ASIP and KIT amino acid sequences. The bactrian camel ASIP and KIT protein sequences were predicted from their respective exon sequences using the SpliceView program coupled with the known cattle coding sequence.

Results

The bactrian camel ASIP gene

The complete coding region of the bactrian camel ASIP gene encoded a protein of 132 amino acids with a predicted molecular mass of 14,543 Da. Amino acid alignment showed that there was high identity between the bactrian camel ASIP sequence and that of the counterparts in dromedary (98.1%), wild bactrian camel (98.1) and alpaca (96.2%). Furthermore, the bactrian camel protein exhibited the same identity with the pig, sheep, wild yak and cattle ASIP proteins (86.8%) (Fig 1).

The bactrian camel KIT gene

The complete coding region of the bactrian camel KIT gene encoded a protein of 982 amino acids with a predicted molecular mass of 109,662 Da. Amino acid alignment showed that there was high identity between the bactrian camel KIT sequence and that of the counterparts in dromedary (99.5%), wild bactrian camel (98.8%), alpaca (98.4%), pig (94.8%), wild yak (93.2). Furthermore, the bactrian camel protein exhibited the same identity with the sheep, goat and cattle KIT proteins (92.8%) (Fig 2).

Mutations in the bactrian camel ASIP gene

Sequencing of the ASIP gene coding region in 94 bactrian camels revealed three polymorphism in exon 1; while in the KIT gene, we identified 5 polymorphisms in exon 2, three in exon 4 and one in exon 11 (Table 2).

In exon 1 of the ASIP gene, we identified 2 synonymous single nucleotide polymorphisms (SNPs) and one nonsynonymous SNPs, c.A56C, which was predicted to cause an asparagine-to-threonine substitution at codon 19 (N19T). In the KIT gene, we found three nonsynonymous SNPs and six synonymous SNPs. In exon 2, two nonsynonymous SNPs were found: c.T82C was predicted to cause a valine-to-alanine substitution at codon 28 (V28A) and c.A169G predicted to cause a histidine-to-argnine substitution at codon 57 (H57R). Three synonymous mutations were also found: c.T26A (p.P9), c.A110C (p.T37) and c.T164A (p.N55). In exon 4, we identified one nonsynonymous mutation, c.A9G, which predicted to cause a threonine-to-alanine substitution at codon 118 (T118A). We also detected two nonsynonymous mutations, c.G7A (p.R187) and c.A90G (p.D215). In exon 11, one nonsynonymous mutation was found, c.G68T (p.S509) (Table 2 and Fig 3).

We hypothesised that SNP mutations associated with a change in the amino acid sequence may correlate with different coat colour in bactrian camels. Based on this hypothesis, we compared each genotype with coat colour to determine the relationship between SNP mutations and coat colour.

In exon 1 of the ASIP gene, 12 animals were heterozygous AC at N19T and anothor 12 were homozygous AA; the remaining 36 animals were homozygous for threonine. Among these animals, the majority were brown (n = 20), while nine were white and seven were red. In exon 2 of the KIT gene,

Table 2. Polymorphisms identified in the bactrian camel ASIP and KIT genes.

Polymorphism	Location	Amino acid change	Effect of amino acid change on protein
c.T23A	ASIP, exon 1	Synonymous	N/A
c.G25A	ASIP, exon1	Synonymous	N/A
c.A56C	ASIP, exon 1	N19T	Polar to polar
c.T26A	KIT, exon 2	Synonymous	N/A
c.T82C	KIT, exon 2	V28A	Nonpolar to nonpolar
c.A110C	KIT, exon 2	Synonymous	N/A
c.T164A	KIT, exon 2	Synonymous	N/A
c.A169G	KIT, exon 2	H57R	Polar to polar
c.A9G	KIT, exon 4	T188A	Polar to nonpolar
c.G7A	KIT, exon 4	Synonymous	N/A
c.A90G	KIT, exon 4	Synonymous	N/A
c.G68T	KIT, exon 11	Synonymous	N/A

Percent identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1		98.1	98.1	96.2	86.8	86.8	86.8	86.8	81.1	80.8	80.8	79.2	79.2	79.2	**1**	bactrian camel
2	1.9		100.0	94.3	84.9	86.8	84.9	84.9	79.2	78.8	78.8	77.4	77.4	77.4	**2**	dromedary camel
3	1.9	0.0		94.3	84.9	86.8	84.9	84.9	79.2	78.8	78.8	77.4	77.4	77.4	**3**	wild bactrian camel
4	3.9	5.9	5.9		84.9	83.0	83.0	83.0	79.2	76.9	76.9	75.5	75.5	75.5	**4**	alpaca
5	14.6	16.9	16.9	16.9		81.1	84.9	84.9	84.9	80.8	80.8	77.4	77.4	75.5	**5**	pig
6	14.6	14.6	14.6	19.3	21.8		96.2	96.2	73.6	75.0	75.0	79.2	79.2	69.8	**6**	sheep
7	14.6	16.9	16.9	19.3	16.9	3.9		100.0	77.4	78.8	78.8	79.2	79.2	73.6	**7**	wild yak
8	14.6	16.9	16.9	19.3	16.9	3.9	0.0		77.4	78.8	78.8	79.2	79.2	73.6	**8**	cattle
9	21.8	24.4	24.4	24.4	16.9	32.6	27.0	27.0		80.8	80.8	75.5	75.5	75.5	**9**	human
10	19.7	22.3	22.3	24.9	19.7	27.6	22.3	22.3	22.3		100.0	80.8	80.8	78.8	**10**	goat
11	19.7	22.3	22.3	24.9	19.7	27.6	22.3	22.3	22.3	0.0		80.8	80.8	78.8	**11**	dog
12	24.4	27.0	27.0	29.7	27.0	24.4	24.4	24.4	29.7	22.3	22.3		100.0	69.8	**12**	donkey
13	24.4	27.0	27.0	29.7	27.0	24.4	24.4	24.4	29.7	22.3	22.3	0.0		69.8	**13**	horse
14	24.4	27.0	27.0	29.7	29.7	38.6	32.6	32.6	29.7	22.3	22.3	38.6	38.6		**14**	rabbit
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**		

Divergence

Fig 1. Amino acid sequence alignments of ASIP proteins from different species. 1. Bactrian camel (*Camelus bactrianus,* NW_011515153); 2. Dromedary camel (*Camelus dromedarius*, NW_011591043); 3. Wild bactrian camel (*Camelus ferus,* NW_006211580); 4. Alpaca (*Vicugna pacos*, NW-005882736); 5. Pig (*Sus scrofa*, NC_010459); 6. Sheep (*Ovis aries*, NC_019470); 7. Wild yak (*Bos mutus*, NW_005397034); 8. Cattle (B*os taurus,* AC_000170); 9. Human (*Homo sapiens*, NC_00002); 10. Goat (*Capra hircus*, EF587236); 11. Dog (*Canis lupus*, NC_006606); 12. Donkey (*Equus asinus*, NW_014638605); 13. Horse (*Equus caballuss*, NC_009165); 14. Rabbit (*Oryctolagus cuniculus*, NM_001082077).

Percent identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1		99.5	98.5	98.4	94.8	93.2	92.8	92.8	92.8	91.6	91.1	89.9	88.5	80.0	**1**	bactrian camel
2	0.3		99.5	99.3	95.2	93.7	93.2	93.6	93.7	91.9	91.8	90.4	89.3	80.4	**2**	dromedary camel
3	0.7	0.4		99.0	94.6	93.2	92.8	93.2	93.4	91.7	91.5	90.0	89.0	80.0	**3**	wild bactrian camel
4	0.8	0.7	0.7		94.9	93.4	93.0	93.4	93.6	91.9	91.7	90.2	89.1	80.4	**4**	alpaca
5	5.1	5.0	5.2	5.1		94.6	94.1	94.5	94.6	93.1	92.9	91.5	89.9	81.5	**5**	pig
6	6.7	6.6	6.6	6.6	5.4		99.0	99.4	99.7	92.3	92.0	91.4	89.8	80.9	**6**	sheep
7	7.4	7.2	7.3	7.2	6.0	1.0		99.3	99.9	91.8	91.6	91.2	89.6	80.5	**7**	wild yak
8	6.9	6.8	6.9	6.9	5.7	0.5	0.7		99.3	92.2	92.1	91.4	89.9	80.7	**8**	cattle
9	6.7	6.6	6.5	6.5	5.4	0.1	1.1	0.7		92.3	92.1	91.3	89.9	80.9	**9**	human
10	8.7	8.6	8.5	8.5	7.1	8.2	8.8	8.4	8.2		99.7	90.9	89.3	80.8	**10**	goat
11	8.9	8.7	8.7	8.7	7.2	8.3	9.0	8.5	8.3	0.3		90.6	89.2	80.6	**11**	dog
12	10.7	10.3	10.3	10.3	9.0	9.2	9.6	9.3	9.3	10.1	10.4		88.6	80.0	**12**	donkey
13	11.6	11.5	11.6	11.5	10.5	10.6	11.1	10.8	10.7	11.5	11.6	12.4		80.1	**13**	horse
14	22.6	22.4	22.7	22.4	21.4	21.9	22.6	22.3	21.9	22.1	22.3	22.8	22.9		**14**	rabbit
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**		

Divergence

Fig 2. Amino acid sequence alignments of KIT proteins from different species. 1. Bactrian camel (*Camelus bactrianus,* NW_011515153); 2. Dromedary camel (*Camelus dromedarius*, NW_011591043); 3. Wild bactrian camel (*Camelus ferus*, NW_006211580); 4. Alpaca (*Vicugna pacos*, NW-005882736); 5. Pig (*Sus scrofa*, NC_010459); 6. Sheep (*Ovis aries,* NC_019470); 7. Wild yak (*Bos mutus*, NW_005397034); 8. Cattle (*Bos taurus*, AC_000170); 9. Human (*Homo sapiens*, NC_00002); 10. Goat (*Capra hircus,* EF587236); 11. Dog (*Canis lupus*, NC_006606); 12. Donkey (*Equus asinus*, NW_014638605); 13. Horse (*Equus caballuss*, NC_009165); 14. Rabbit (*Oryctolagus cuniculus*, NM_001082077).

three animals (2 brown and 1 red) were homozygous for the alanine allele at V28A. The heterozygous genotype was present in 21 animals, while the remaining 67 were homozygous for valine, with three varying phenotypes. At H57R of the KIT gene, four animals (3 brown and 1 red) were homozygous for the arginine allele and no white colour homozygotes were detected in our population. Thirty animals were heterozygous for the histidine allele and the remaining 68 were homozygous. Two red animals red and two brown animals were homozygous for the alanine allele at T118A. There were no white

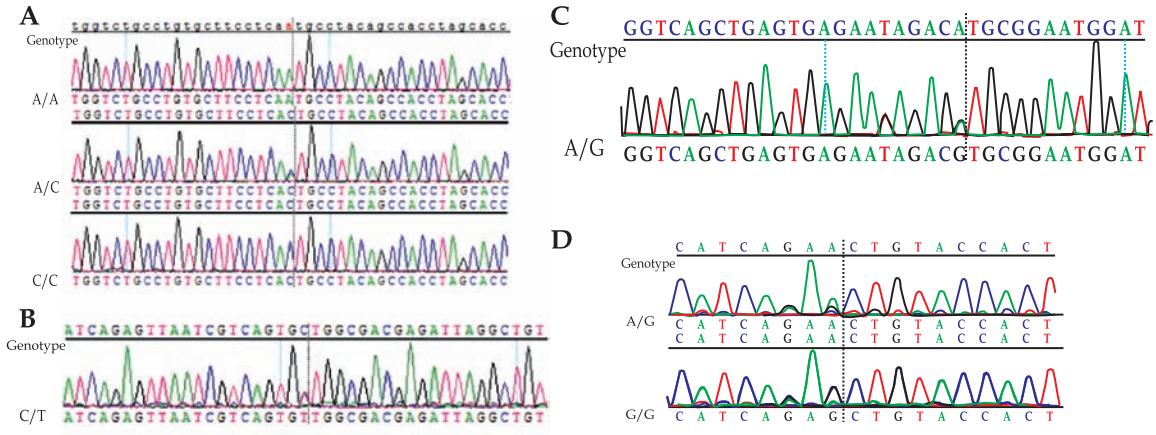

Fig 3. Mutations in the bactrian camel ASIP and KIT gene. (A) Exon 1 (ASIP), c.A56C; (B) Exon 2 (KIT), c.T82C; (C) Exon 2 (KIT), c.A169G; (D) Exon 4 (KIT), c.A9G.

animals with this genotype, 19 were heterozygous for the threonine allele and the remaining 48 were homozygous, with three varying phenotypes.

Discussion

In some domesticated species, coat colour is regulated by two main types of melanin: eumelanin and pheomelanin. The agouti protein is a paracrine-signaling molecule that is normally expressed in the skin and exhibits high homology among mammals. Loss-of-function mutations in the ASIP gene could perturb the ASIP signaling pathway and interfere with melanogenesis. Very few studies on functional genes in bactrian camels have been reported compared with those of other mammals, such as cattle and sheep. In particular, there are few studies on the funtional genes related to coat colour (Girardot *et al,* 2005; Royo *et al,* 2005; Li *et al,* 2014; Zhang *et al,* 2017), probably because of the genetic complexity of this characteristic. Assuming that colour is a complex phenotype, we investigated the ASIP and KIT genes, which have been identified as candidate genes. In this study, we detected a possible association between genotype (c.A56C, c.T82C, c.A169G, c.A9G) and coat colour phynotype in bactrian camels. All the SNPs in the ASIP and KIT gene can not bright out colour variation. Fiber colour charts for bactrian camels were unavailable; therefore, fibre colour was classified according to the owner's assessment, which may lead to in our analysis of the effects of SNPs on bactrian

Table 3. Genotype vs. phenotype.

Exon	Polymorphism	White individual	Red individual	Brown individual	Total
ASIP (exon1)	N19T				
	AA	4	3	5	12
	AC	1	1	10	12
	CC	9	7	20	36
KIT (exon2)	V28A				
	TT	14	8	45	67
	CT	6	4	11	21
	CC	0	1	2	3
KIT (exon2)	H57R				
	AA	14	8	46	68
	AG	6	3	21	30
	GG	0	1	3	4
KIT (exon4)	T118A				
	AA	9	3	36	48
	AG	6	4	9	19
	GG	0	2	2	4

camel fleece colour. Moreover, due to the complexity of the interactions among genes, studies of multiple candidate genes, including key regulatory regions and intronic regions of the ASIP and KIT genes and with larger sample numbers are required to fully elucidate the these association of these genes with coat colour in bactrian camels. In addition, the MC1R gene is a plausible choice for investigation because of its highly conserved nature among eutherian mammals.

Conclusion

Exon 1 of the ASIP gene and the exons 2, 4 and 11 of the KIT gene were sequenced to determine the existence of any correlation between ASIP and KIT gene polymorphisms and different coat colours in Bactrian camels. In total, 12 polymorphisms were identified in 94 bactrian camels. Three polymorphisms were detected in exon 1 of the ASIP gene, while the KIT gene contained five polymorphisms in exon 2, three polymorphisms in exon 4 and one polymorphism in exon 11. However, no association was identified between the genotypes of the polymorphisms in the ASIP and KIT genes and coat colour trait, possibly due to the complexity of coat colour gene interactions. Further investigations using more advanced technology with larger numbers of animals are required to confirm this conclusion.

Conflict of interest

The authors declare that they have no conflicts of interest.

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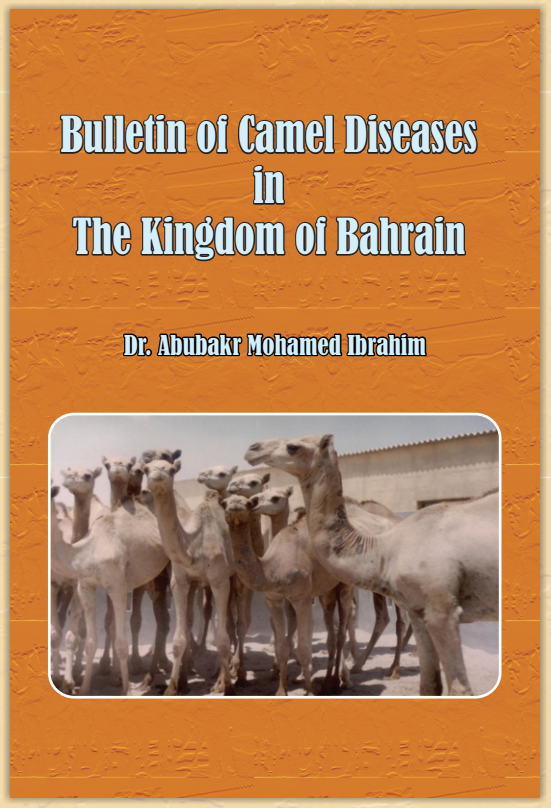
Bulletin of Camel Diseases in The Kingdom of Bahrain

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

About the Author

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



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THE PHARMACOKINETICS AND PHARMACODYNAMICS OF FLUMETHASONE IN CAMELS

I.A. Wasfi, Nasreen A Al Biriki, S.A. Wajid and B. Agha
Forensic Evidence Department, P.O. Box 253, Abu Dhabi, United Arab Emirates

ABSTRACT

The pharmacokinetics (PK) and pharmacodynamics (PD) of flumethasone was evaluated in 6 healthy camels after a single intravenous bolus doses of 5 µg/kg body weight. The PD was performed by applying PK/PD modeling using cortisol, circulating lymphocytes, neutrophils and plasma glucose as biomarkers. Plasma flumethasone and cortisol concentrations were measured by validated liquid chromatography/mass spectrometry methods (LC-MS/MS). Plasma flumethasone *versus* time concentration were fitted by nonlinear regression and were best described by a two compartment model. The PK parameters (mean ± SD) were; terminal elimination half-life was 10.45 ± 0.65 h, total body clearance was 115.8 ± 7.99 ml/h/kg and volume of distribution at steady state was 1631.6 ± 116.03 ml/kg. The PD parameters showed that flumethasone is a very potent steroidal anti-inflammatory drug as reflected by the estimated low IC_{50} of flumethasone for cortisol and lymphocytes.

Key words: Camel, flumethasone, pharmacodynamics, pharmacokinetics

Flumethasone is a synthetic corticosteroid structurally similar to dexamethasone but with an additional fluorine atom at position 6. Corticosteroids are widely used in veterinary medicine to treat various lameness conditions (Ferguson and Hoenig, 1995). Despite the wide use of injectable flumethasone in race camels in The United Arab Emirates, yet there are no pharmacokinetics (PK) or pharmacodynamics (PD) reports in camels or in large animals. The aim of the present study was to develop a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the detection of flumethasone and cortisol in camel plasma and to apply it for the evaluation of the PK and PD of flumethasone in camels after intravenous (i.v.) administration. Another goal of the study was to advise on a withdrawal period before camel racing following a therapeutic dose of flumethasone.

Materials and Methods

Animals and drug administration

Six clinically healthy male race camels 4-6 years old and body weight ranging in from 300-400 kg were used and kept in open pens. Good-quality hay and lucerne were fed once daily and water was provided *ad libitum*. This study was approved by the ethical committee in the Veterinary Department, Ministry of Agriculture. Flumethasone (Fluvet, 0.5 mg/mL; Zoetis, Mexico) was administered as a bolus intravenous (i.v.) injection (jugular vein) at a dose of 5.0 µg/Kg body weight (manufacturer recommended dose for bovine). Two blood samples were collected for analytical purposes in heparinised vacuum tubes from the opposite jugular vein at time 0 (predose) and at 5, 10, 15, 30, 45, 60 min and at 1.5,2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24 h timed after drug administration. A 3 mL blood sample was collected for the estimation of glucose, WBC, lymphocytes and neutrophils as reported previously (Al Katheeri *et al,* 2004a). Seven mL blood samples were collected for the quantification of plasma flumethasone and cortisol concentrations. Plasma was separated by centrifugation (2000 g for 10 min) and was stored at -20°C. Plasma samples were assayed within 10 days.

Analysis of plasma flumethasone

Flumethasone was extracted from plasma (1.0 mL) by solid phase extraction (C18) as reported previously (Al Katheeri *et al,* 2004a; 2004b). Cortisol-D3 was used as internal standard. The concentration of flumethasone and cortisol in plasma were measured by a validated liquid chromatography/mass spectrometry (LC/MS/MS). The LC system used was Agilent 1200 series with autosampler and column compartment

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(Agilent, USA). The chromatographic separation was performed using an Agilent Zorbax ZDB C18 column (3.5 m × 2 mm × 50 mm, Santa Clara, CA) linked to a Phenomenex pre-column filter (4 × 2 mm, Torrance, CA) operating in gradient mode at 35°C. The mobile phases were 0.1% formic acid (solvent A) and methanol (solvent B). A linear gradient was run at 0.3 mL/min, with 40% solvent B at the start (t = 0 min), increasing to 90% solvent B at t = 4 min. The gradient was then returned to 40% solvent B at t = 4.20 min and stabilised until t = 7.3 min before starting the next injection. The temperature of the autosampler tray and of the column compartment was set at 10 and 35°C, respectively. Ten µl was used for injection. Mass spectrometric analysis was performed on a 5500 Q-Trap mass spectrometer (ABSciex, Foster City, CA, USA) equipped with a turbo ion spray interface for electrospray ionisation (ESI) operated in positive ion mode. The source-dependent parameters were optimised using flow injection analysis (FIA) of flumethasone (20 ng/ml) into the mass spectrometer at 10 µL/min. Ion spray voltage was set at 5000 V and source temperature was at 550°C. Curtain gas, gas 1, and gas 2 were medium, 50, and 60 psi, respectively. Declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) used for each analyte were established and optimised during the tune procedure. Protonated molecules were used as precursor ions with selected reaction monitoring of the following transitions for flumethasone, cortisol and cortisol-D_3, respectively: 411–253 m/z, 363–121 m/z and 366–121 m/z.

The analytical method was validated at the beginning of the experiment. Linear calibration curves ($r > 0.999$) were obtained for flumethasone (0.5-20 ng/mL) and cortisol (0.5-50 ng/mL). The inter- and intra-assay coefficient of variations of the quality control samples (1.5, 7.5 and 15 ng/mL; n=9) were less than 9% and the accuracy was less than 15%. The limit of quantification of both analytes was 0.5 and ng/ml. The limit of detection (LOD) was 0.15 ng/ml.

Pharmacokinetic and pharmacodynamics analysis

Pharmacokinetic analysis of plasma flumethasone concentrations for each animal was performed using least – square nonlinear regression analysis program (WinNonLin Standard edition, version 4.0.1, Pharsight, Sunnyvale, CA, USA). One-, two- and three-compartment models were tested for the best fit to the i.v. administration data.

Weighting was achieved according to the variance modes: $var(t) = 1 / Y_{observed\ 2}$ where var (t) is the variance of the residual error of drug concentration at time t and Y observed is the observed drug concentration at time t. The best fit was based on Akaike (1976) and Schwarz (1978) criteria, analysis of residual plots and correlation matrix. The PK-PD surrogates, the reduction of plasma cortisol concentration and the number of lymphocytes and the increase of plasma glucose concentration and neutrophils number were calculated. Calculations were performed on individual data using least-square nonlinear regression analysis (WinNonLin Standard edition, version 1.5, USA). An indirect response model describing the PD effect of the drug with the mechanism producing the effect was used (Dayneka *et al,* 1993; Al Katheeri *et al,* 2004a; Al Katheeri *et al,* 2004b).

Results and Discussion

The mean serum concentration–time curves following the i.v. flumethasone administration are shown in Fig 1. Estimated PK parameters are summarised in Table 1. There are no reports on the PK of flumethasone in camels or in large species to compare our results with. However, flumethasone PK in camels was characterised by a long terminal elimination half-life, large volume of distribution and slow systemic clearance. Fluorinated compounds are reported to have increased lipophilicity (Wakefield, 2000) which might have contributed to the large volume of distribution and decreased clearance.

Table 1. Pharmacokinetic parameters of flumethasone following intravenous administration to 6 healthy male camels at a dose of 5.0 µg/Kg body weight. Data are expressed as mean and standard deviation.

Variable	Mean	Standard deviation
AUC (µg h^{-1} ml^{-1})	44.50	7.50
$t_{½\alpha}$ (h)	0.20	0.12
$t_{½\beta}$ (h)	10.40	1.59
VC (ml kg^{-1})	482.67	117.89
ClT (ml kg^{-1} h^{-1})	115.08	19.54
Vss (ml kg^{-1})	1631.64	284.21
AUMC (µg h^{-2} ml^{-1})	645.11	185.62

t½a= half–life of distribution phase; t½b= half–life of elimination phase; AUC= area under the curve to infinity; Vss= volume of distribution at steady state; ClT= total body clearance; Vc= volume of central compartment; AUMC= area under first moment curve.

Dexamethasone, a fluorine containing drug was also reported to have large volume of distribution, decreased clearance and long terminal elimination half-life (Al Katheeri *et al,* 2004a; Al Katheeri *et al,*

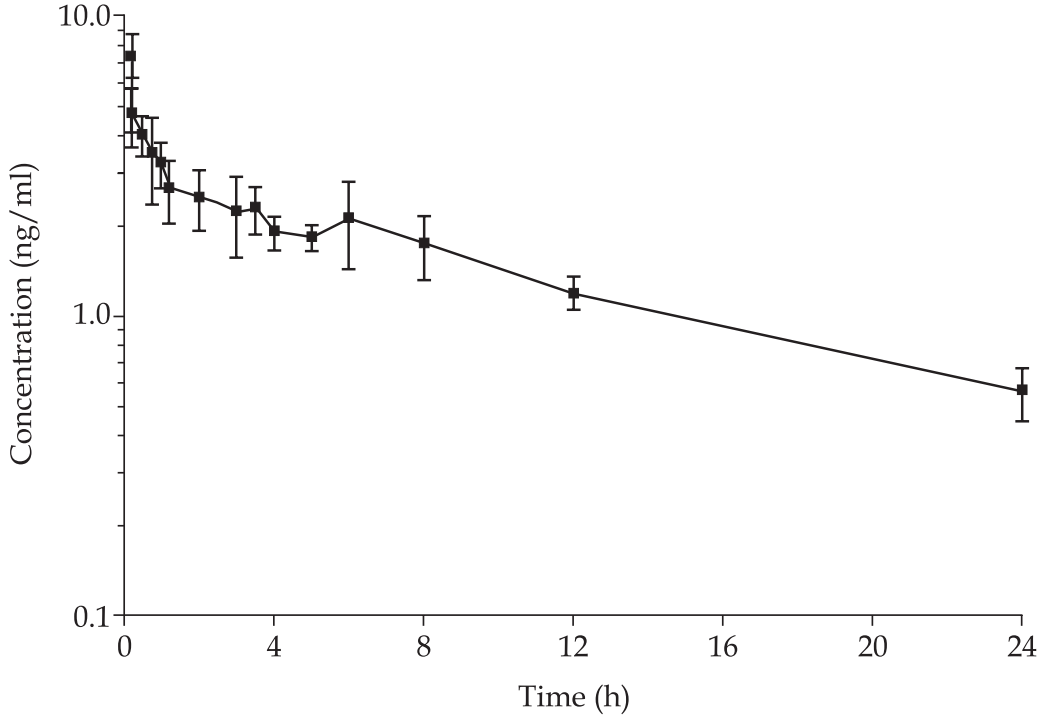

Fig 1. Flumethasone plasma concentrations-time profile of 6 male camels after an i.v. dose of 5.0 µg flumethasone/ Kg body weight. Values are presented as means ± standard deviation.

2004b). The estimated IC_{50} of flumethasone for cortisol and lymphocytes were 4.52 ± 1.33 and 3.57 ± 1.69 ng/ml, respectively. The EC_{50} for neutrophils and glucose were 33.5 ± 7.93 and 4.23 ± 0.82 ng/ml, respectively. The PD parameters showed that flumethasone is a very potent steroidal anti-inflammatory drug as reflected by the estimated low IC_{50} of flumethasone for cortisol and lymphocytes.

The plasma concentration of flumethasone at 24 h post administration was 0.56 ± 0.11 ng/ml. This means that in some animals, the plasma concentration of flumethasone would still be theoretically detectable 48 h post administration as reflected by the LOD of the method (0.1 ng/ml) and the long elimination terminal half-life (10.40 ± 1.59 h). Due to the large variation of pharmacokinetic parameters in animals, camel owners are therefore advised to withhold flumethasone use for a period of 2-3 days before racing.

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This research was supported by Brigadier Abdul Rahman Al Hammadi, director of the Forensic Science Laboratory. The assistance of M. ElGhazali and A. Al Juboori is highly appreciated.

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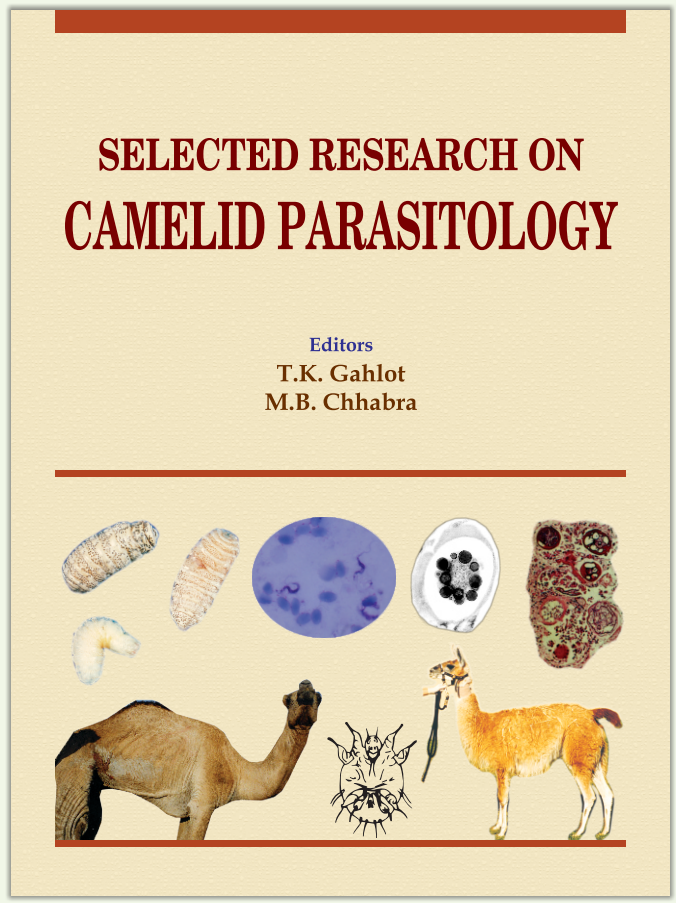

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LINEAR AND ANGULAR BIOMETRIC MEASUREMENTS OF LIMBS OF CAMEL (*Camelus dromedarius*)

M.B. Mostafa[1] and A.H. Khalil[2]
Department of Veterinary Surgery, Aanesthesiology and Radiology,
Faculty of Veterinary Medicine, Cairo University, Egypt[1] and Banha University[2]

ABSTRACT

Linear and angular limb conformations were carried on 208 camels of different breeds and sex using goniometer and software Auto CAD program 2013. Measurements of normal conformation in the fore and hind limbs showed the shoulder lengths in camels were nearly equal to the fore arm length. Thigh and gaskins lengths were found nearly equal in the hind limbs and the hind cannon were larger in length compared to the fore cannon. The recorded conformation traits were carpus valgus; calf knees; base wide, toe out and sloppy and upright pastern in the fore limbs. Cow, straight and sickle hocks, base wide, toe out sloppy and upright pastern were seen in the hind limbs. The mean normal angles for shoulder, elbow, carpal and pastern angles in the fore limbs and hip, stifle joints, hock and pastern joints in hind limbs were determined. The abnormal joint angles displayed carpal valgus and calf knees and these showed significant ($p<0.05$) decrease when compared with normal camels. Cow hocks and sickle hocks had significant ($p<0.05$) decrease value compare with the normal values. Sloppy pastern these showed significant ($p<0.05$) decrease while upright pastern had significant ($p<0.05$) increased value as compared with normal values. Objective conformation parameters in the present study established base line measurements for breeders and veterinarian for selecting camels with good conformations and performance.

Key words: Biometry camel, *Camelus dromedarius*, conformation, limbs, measurement, traits

Lameness in racing camels are considered to be a major welfare and economic issue encountered by camel owners in the terms of decreased milk production, decreased reproductive performance, growth retardation, culling of the camel from the competition or farm, decreased physiological vitality of the camel and additional cost in the care and treatment of the affected animal (Gahlot, 2007; Lira *et al,* 2011; Al-Juboori, 2013). There are many reason for not applying equine principles to camel because of the differences in anatomy of these animals and distinct different uses, biomechanics and geoclimatic adaptation (Gahlot, 2000).

Evaluation of conformation in equines was carried out through subjective methods (Stashak, 1987) and objective methods (McIlwraith *et al,* 2003; Anderson *et al,* 2004 and White *et al,* 2008). Digital photography has been demonstrated to provide a highly accurate method of conformation measurement in equines using linear and angular measurements (White *et al,* 2008 and Dyson *et al,* 2011).

Ideal conformation is that the body form which does not exert excess strain on any point of the body (Gahlot, 2000), Al- Ani (2004) and Anderson *et al* (2004). In general, there in lack of research done on camel conformation. Most of the research in camel was done in terms of body measurements (Osman *et al,* 2015 and Shag *et al,* 2013). Unfortunately, little attention has been given to know normal and abnormal camel limb conformations in camels. The objective of this study was to subjectively and objectively assess the normal and abnormal limb conformation parameters of one humped camel using linear and angular measurements.

Materials and Methods

This study was carried out on 208 camels of different breeds belonging to private camel farms. The camels were of both sex (159 males and 49 females) with a mean age 7.65 ± 3.88 years. Objective and subjective methods of evaluation were performed in squarely standing position on flat hard surface bearing its weight equally on all four limbs. Only one measurement was recorded for each part per day. Evaluation of camel conformations were carried out subjectively as methods (Fig 1) described for the horse (Magnusson and Thafvelin, 1985; Stashak, 1987).

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Fore limb evaluation: The conformation was taken by an imaginary line dropped from the point of the shoulder joint that should bisect the limb from a lateral view and another line dropped from the tuber spinae of the scapula that should bisect the limb down to the fetlock and end behind the foot pad (Fig 2 A and B). Deviation of the carpus medially, laterally, forward and backward; cold degree of extension of fetlock joint an deviation of the toes outward or downward were evaluated.

Hind limb evaluation: An imaginary line dropped from the point of buttock to the ground that normally was taken to touch the hock and end slightly behind the foot pad. From the rear, a line dropped from the point of the buttock to the ground that should essentially bisect the limb was also taken (fig 3 A and B).

Objective methods for conformation evaluation were applied in accordance to the Anderson *et al* (2004) in horses. The reference points and their anatomic locations were described in table 1. Lengths and angles (Table 2) were firstly measured by tape meter and goniometer, then the same measurements were confirmed by analysing the view images sing AutoCAD 2013 program (a commercial software application for 2D and 3D computer-aided design; Autodesk, Inc., California, USA). Lateral, frontal and rear views of the fore and hind limbs were taken by a digital camera (Samsung, PL80 28 mm 5X, 12 Megapixel) after labeling the reference points of the upper and the lower limb landmarks to enable easy identification during AutoCAD processing (Holmstrom, 2001). Different linear and angles measurements in apparently clinically normal and abnormal limb conformations were taken (Fig 1).

Photographic images: The examined camel should be centered within the photo frame and both the photographer and the camel should stand on the same level at a leveled ground surface, during lateral viewing the camera should be present just behind centre of gravity at midpoint of lateral thoracic wall. Lengths and angles were measured for each camel using measuring tape and goniometer used for calibration and scaling the measurements taken on the photos by AutoCAD 2013 program.

Statistical analysis: Descriptive statistical analysis for lengths and angles was done by IBM® SPSS® statistics V 20 program (IBM Corporation, 2009, New York, USA). Mean, variation coefficient % (C.V), minimum, maximum and percentile were determined for lengths according to Petrie and Watson (2006) and "t" test was used for comparing changes in joint angles. Differences were considered significant at a level of $P<0.05$.

Table 1. The reference points and their anatomic locations were described.

Reference points	Anatomic location
Withers	Highest point of camel wither
Point of shoulder (lateral)	Posterior part of the greater tubercle of humerus
Point of Elbow (lateral)	Caudal edge of lateral collateral ligament of elbow joint
Point of Carpus (lateral)	Just below the styloid process of ulna
Point of Fore-fetlock (lateral)	The central point of fetlock joint laterally
Point of Croup (lateral)	Highest point of croup (lumbosacral joint)
Point of Hip (lateral)	The groove between semitendinosus and biceps muscle just caudal to hip joint
Point of Stifle (lateral)	Distal end of the patella (over palpable tibial tuberosity)
Point of Tarsal (lateral)	Midpoint of lateral aspect of tarsal joint
Point of Hind pastern	Midpoint of lateral aspect of pastern joint

Results and Discussion

The mean shoulder lengths (Table 3) in fore limb measured lengths were 52.69 cm (SD ± 4.25 cm). Twenty five per cent of horses had shoulder length lower than 48.83 cm till 43.65 cm. Twenty five per cent had shoulder length higher than 55.86 cm 61.84 till cm. Amount of variation of shoulder length in the study was 20.63%. Mean arm lengths were 38.63±4.83 cm. Twenty five per cent had arm length lower than 35.07 cm till 32.08 cm. Twenty five per cent had higher arm length than 40.84 cm. till 50.46 cm. Amount of variation of arm length in present study was 23.37%.

The mean forearm lengths were 52.29±6.81 cm. Twenty five per cent had forearm length lower than 48.16 cm till 38.09 cm. Twenty five per cent had higher forearm length than 57.15 cm till 68.60 cm. Amount of variation of forarm length was 46.41%. The mean fore cannon lengths were 27.79±7.04 cm. Twenty five per cent had fore cannon length lower than 21.95 cm till 17.19 cm. Twenty five per cent had higher fore cannon length than 34.39 cm till 42.34 cm. Amount of variation of fore cannon length was 49.57%.

The mean fore digit lengths were 15.23±3.75 cm. Twenty five per cent had lower fore digit length

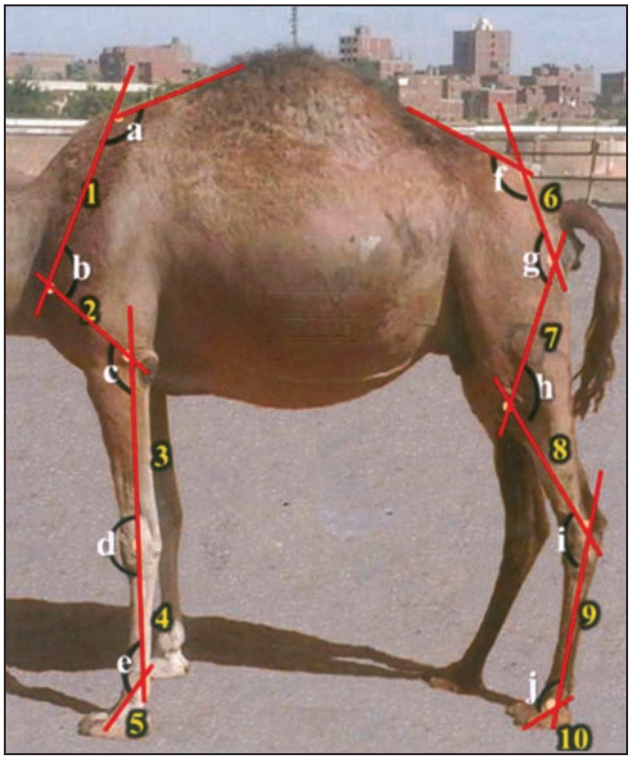

Fig 1. Reference points, legnths and angles in lateral view of camel fore limb (A) and hind limb (B). (**1.** shoulder length, **2.** Arm length, **3.** fore - arm length, **4.** fore cannon length, **5.** fore pastern length, **6.** pelvis length, **7.** thigh length, **8.** gaskin length, **9.** hind cannon length, **10.** hind pastern length. **a.** point (angle) of wither, **b.** point (angle) of shoulder, **c.** point (angle) of elbow, **d.** point (angle) of carpus, **e.** point of fore fetlock, **f.** point (angle) of croup, **g.** point (angle) of hip, **h.** point (angle) of stifle, **i.** point (angle) of hock, **j.** point (angle) of bind fetlock (after Anderson *et al,* 2004).

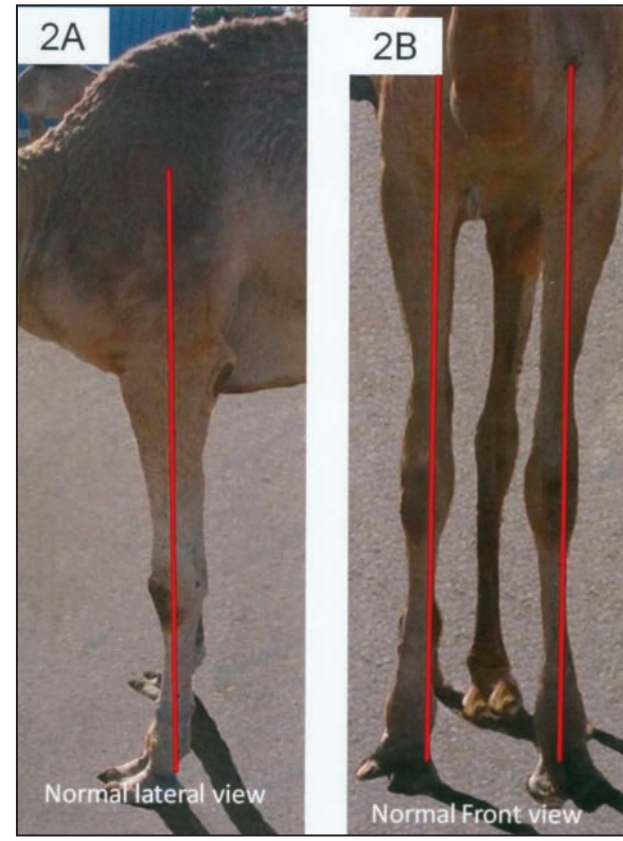

Fig 2. **A.** Normal forelimbs camel, an imaginary line dropped from the point of the shoulder joint that should bisect the limb from a lateral view **B.** Normal front forelimbs camel, an imaginary line dropped from the tuber spinae of the scapula should bisect the limb down to the fetlock and end behind the foot pad.

lower than 12.15 cm till 8.08 cm. Twenty five per cent had higher foredigit length than 17.55 cm till 23.34 cm. Amount of variation of foredigit length was 14.07%. The ratios of fore arm lengths to arm lengths were 1.35%. The ratios of fore cannon lengths to fore digit lengths represented 1.82%. The interesting findings in the present study was the shoulder lengths in camels which were nearly equal to the fore arm lengths.

The measurements of hind limb lengths are given in table 3. The mean pelvis lengths were 38.77±7.67 cm. Twenty five per cent had lower pelvis length than 34.28 cm till 24.32 cm. Twenty five per cent had higher pelvis length than 44.24 cm till 54.61 cm. Amount of variation of pelvis length was 58.76%. The mean thigh lengths were 45.34±4.81 cm. Twenty five per cent had lower thigh length than 41.35 cm till 37.45 cm. Twenty five per cent had higher thigh length than 49.39 cm till 54.71 cm. Amount of variation of thigh length was 23.17%.

The mean gaskin lengths was 46.83±5.06 cm. Twenty five per cent of gaskin lengths were lower than 42.26 cm till 38.06 cm. Twenty five per cent of gaskin lengths were higher than 50.85 cm till 55.36 cm. Amount of variation of gaskin length was 23.595%. The mean hind cannon lengths were 41.92±5.03 cm. Twenty five per cent had lower hind cannon length than 39.08 cm till 28.78 cm. Twenty five per cent had higher gaskin length than 46.42 cm till 50.44 cm. Amount of variation of gaskin length was 25.27%.

The mean hind digit lengths were 13.37±1.43 cm. Twenty five per cent had hind digit lower than 12.77 cm till 10.23 cm. Twenty five per cent had higher hind digit length than 14.32 cm till 15.82 cm. Amount of variation of hind digit was 2.05%. The ratios of pelvis lengths to thigh and gaskin lengths were 0.85% and 0.82%, respectively. The ratios of hind cannon to digit lengths were 3.14%. The interesting finding in camel hind limbs was that the lengths of the thigh and gaskin were nearly equal.

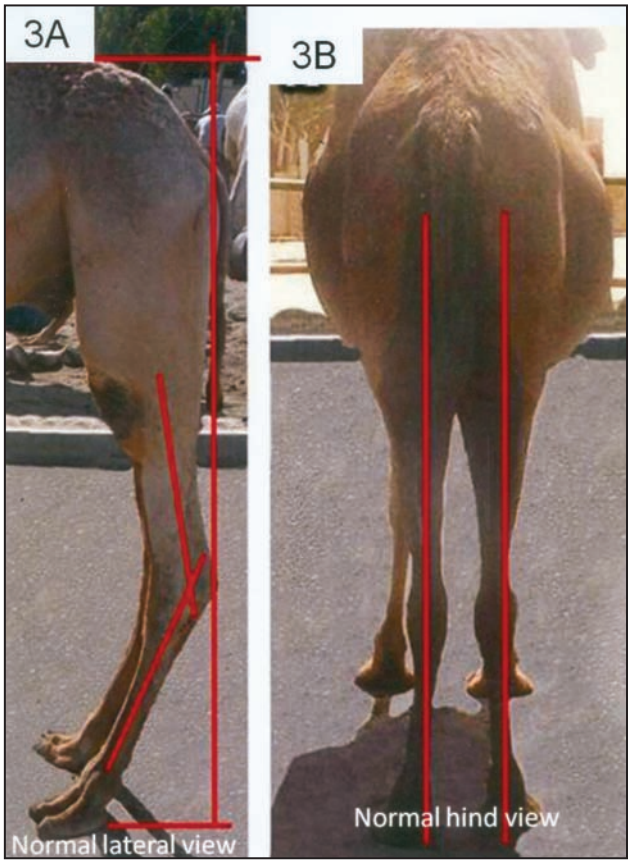

Fig 3. **A:** Normal lateral hind limbs camel, an imaginary line dropped from the point of buttock to the ground that normally was taken to touch the hock and end slightly behind the foot bad. **B:** Normal rear hind limbs, a line dropped from the point of the buttock to the ground should essentially bisect the limb.

The findings in the present study revealed that the shoulder and fore-arm were the longest regions of the limbs and the digits were the shortest. Thigh and gaskin lengths were nearly equal. Both shoulder and fore-arm lengths were nearly equal. The lengths of arm and pelvis were found nearly equal (39 cm). It is important to notice that pelvis was the highest variable length among camels. Both fore-arm and fore-cannon were second in the variability coefficient, whilst the least variabile were digits and the fore-digit.

Abnormal fore and hind limb conformations in 208 camels were represented in table 4. The carpus valgus and calf knee had the highest forelimbs conformations 32.2% and 31.7%, respectively. Base wide, toe out and sloppy pastern conformations were 26.9%, 25.4% and 18.2%, respectively. Fore limbs camped back, upright pastern, base narrow and steep shoulder were 15.3%, 13.9%, 10.1% and 5.2% consequently (Fig 4).

Cow hocks, base wide, toe out and sloppy pastern were the common prevelance hind limb conformations in camels and represented 29.3%, 28.3%, 25.9% and 19.7%, respectively. Upright pastern, sickle hocks, straight hocks and base narrow were represented 15.8%, 14.9%, 12.9% and 12.5%, respectively (Fig 5).

It is obvious in this study that carpus valgus, calf knee and cow hocks were the predominant abnormalities present in camels. They represented one third of the investigated population. Base wide

Table 2. Measuring pattern of body lengths and angles in one humped camel (McIlwraith *et al,* 2003).

Parameters	Items	Items	Exam. View	Description
Body Lengths	Fore limbs	Shoulder	Lateral	From point of withers to point of shoulder
		Arm	Lateral	From point of shoulder to point of elbow
		Fore-arm	Lateral	From point of elbow to point of carpus
		Fore cannon	Lateral	From point of carpus to point of fetlock joint
	Hind limbs	Pelvis		From point of croup to point of hip
		Thigh	Lateral	From point of hip to point of the stifle
		Gaskin	Lateral	From point of the stifle to point of tarsal joint
		Hind cannon	Lateral	From point of tarsal to point of fetlock joint
Body Angles	Fore limbs	Shoulder	Lateral	Between shoulder and arm
		Elbow	Lateral	Between arm and fore-arm
		Carpus	Lateral	Between fore-arm and fore cannon
		Fore pastern	Lateral	Between first and second phalanx
	Hind limbs	Hip	Lateral	Between pelvis and thigh
		Stifle	Lateral	Between thigh and gaskin
		Tarsal	Lateral	Between gaskin and hind cannon
		Hind pastern	Lateral	Between first and second phalanx

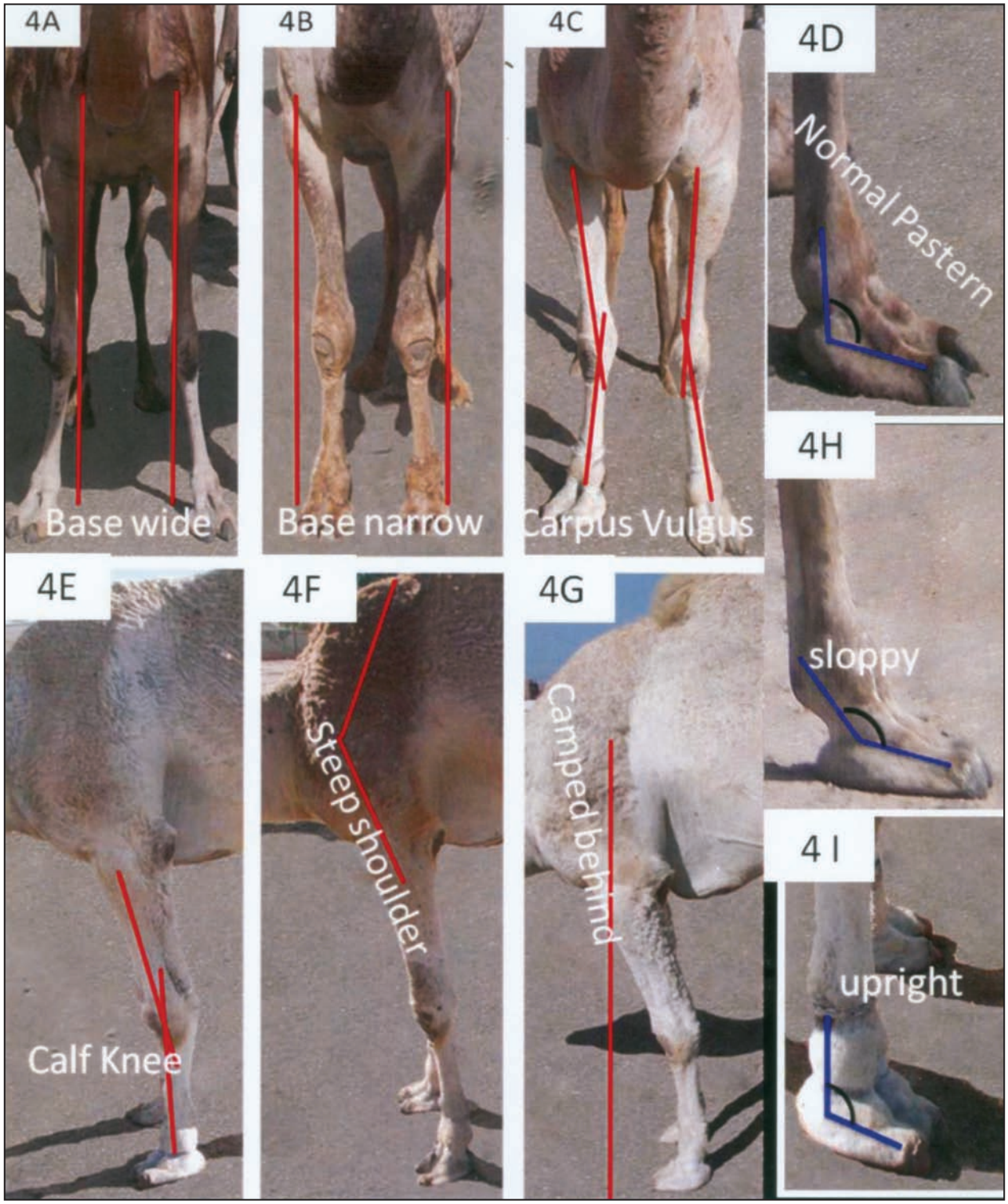

Fig 4. Abnormal limb conformation in camels: **A:** Base wide: the forelimbs are angled out from the perpendicular plumb line and the feet placed further apart than the top of the limb (Front view) **B:** Base narrow: The forelimbs are angled in from the perpendicular plumb line and the feet placed nearer together than the top of the limb (Front view). **C:** Carpus valgus: the carpal joint is directed medially from the front (Front view). **D:** Normal fore pastern angle: measured from the midpoint of the lateral pastern joint. **E:** Calf knee: the carpal joint is directed backward from lateral view. **F:** Steep shoulder: the scapular angle with the horizontal line and the scapula-humeral joint were wider. **G:** Standing under in front (camped behind): in which the entire forelimb is placed too far under the body when the camel is viewed from the lateral side. **H:** Sloppy pastern: low pastern angle when compared with normal angle. **I:** Upright pastern: high pastern angle when compared with normal, pastern more vertical.

Table 3. Mean lengths of fore and hind limbs in apparently normal camels.

Limbs	Lengths (cm)	Mean±SE (cm)	Var. Co. (%)	Min.	Max.	Percentiles		
						25	50	75
Forelimb	Arm	38.63 ± 4.83	23.37	32.08	50.46	35.07	37.21	40.84
	Forearm	52.29 ± 6.81	46.41	38.09	68.60	48.16	52.64	57.15
	Fore cannon	27.79 ± 7.04	49.57	17.19	42.34	21.95	24.89	34.39
	Fore Digit	15.23 ± 3.75	14.07	8.08	23.34	12.15	15.29	17.55
Hindlimb	Pelvis	38.77 ± 7.67	58.76	4.32	54.61	34.28	37.08	44.24
	Thigh	45.34 ± 4.81	23.17	37.45	54.71	41.35	45.42	49.39
	Gaskin	46.83 ± 5.06	25.59	38.06	55.36	42.26	47.51	50.85
	Hind cannon	41.92 ± 5.03	25.27	28.78	50.44	39.08	41.59	46.42
	Hind digit	13.37 ± 1.43	2.05	10.23	15.82	12.77	13.49	14.32

and toe out in both limbs were next in order in their percentage by one quarter of all animals under study. Sloppy pastern was nearer to them in its percentage by around one fifth. Lastly, steep shoulders were the least in their representation.

Table 4. Percentage (%) of abnormal fore and hindlimbs conformation in camels.

Limbs	Variables	Number	Percentage (%)
Forelimb	Base Wide	56	26.92 %
	Base Narrow	21	10.10 %
	Toe Out	53	25.48 %
	Upright Pastern	29	13.94 %
	Sloppy Pastern	38	18.27 %
	Carpal Valgus	67	32.21 %
	Calf Knee	66	31.73 %
	Open S-H angle	11	5.29 %
	Camped Back	34	16.35 %
Hindlimb	Base Wide	59	28.37 %
	Base Narrow	26	12.50 %
	Toe Out	54	25.96 %
	Upright Pastern	33	15.87 %
	Sloppy Pastern	41	19.71 %
	Cow Hocks	61	29.33 %
	Straight Hock	27	12.98 %
	Sickle Hock	31	14.9 %

The normal camels forelimb joint angles are given in table 5. Mean shoulder joint angles were 107 ± 5.38°. The mean elbow joint angles were 149.26± 6.09°. The mean carpal joints were 174.62±3.06°. The mean pastern joint angles were 123.17° ± 3.42°. The hind limb joints angle are given in table 5. The hip joint angles were measured between pelvis length and thigh length. Mean hip angles were 148.58° ± 25.06°. The mean stifle, hock and hind pastern joint angles were 160.32±5.93°; 153.58± 3.07° and 130.79± 4.53°, respectively.

Measurements of abnormal limb conformation angles in the fore limbs in the present study showed

Table 5. Mean joint angles in apparently clinically normal camels.

Normal angles		Abnormal angles	
Joints	Mean±SE Min.- Max.	Joints	Mean±SE Min.- Max.
Shoulder	107.43°±5.38 (96.10-117.41)	Steep shoulder	127.25*±1.55° (125.08-129.89)
Elbow	149.26°± 6.09 (140.03-165.51)	ND	ND
Carpal	174.62±3.06° (169.66-179.82)	Calf knee	157.32 **±1.65° (154.13-159.48)
		Carpus Valgus	159.67±4.47° (150.119-166.47)
Fore Pastern	123.75±3.42° (118.2-129.68)	Sloppy pastern	102.38 *±3.97° (98.42-109.59)
		Upright pastern	141.69±3.07 ** (136.82-148.29)
Hip	148.58±4.14° (140.13-155.13)	ND	ND
Stifle	160.32±5.93° (160.42-5.93)	ND	ND
Hock	153.58±3.07° (146.35-159.00)	Cow hocks	146.03 *±2.53° (142.53149.44)
		Sickle hock	128.83 **±3.15° (123.99- 133.32)
		Straight hock	165.42 **±2.31° 165.42.20-169.77
Hind Pastern	130.79±4.53° (121.29-138.55)	Sloppy pastern	97.0 **±4.38° (91.48-104.23)
		Upright pastern	157.0 *±4.69° (148.28-162.55)

*significant at (0.05) Min.-Max: minimum-maximum
ND: not determined

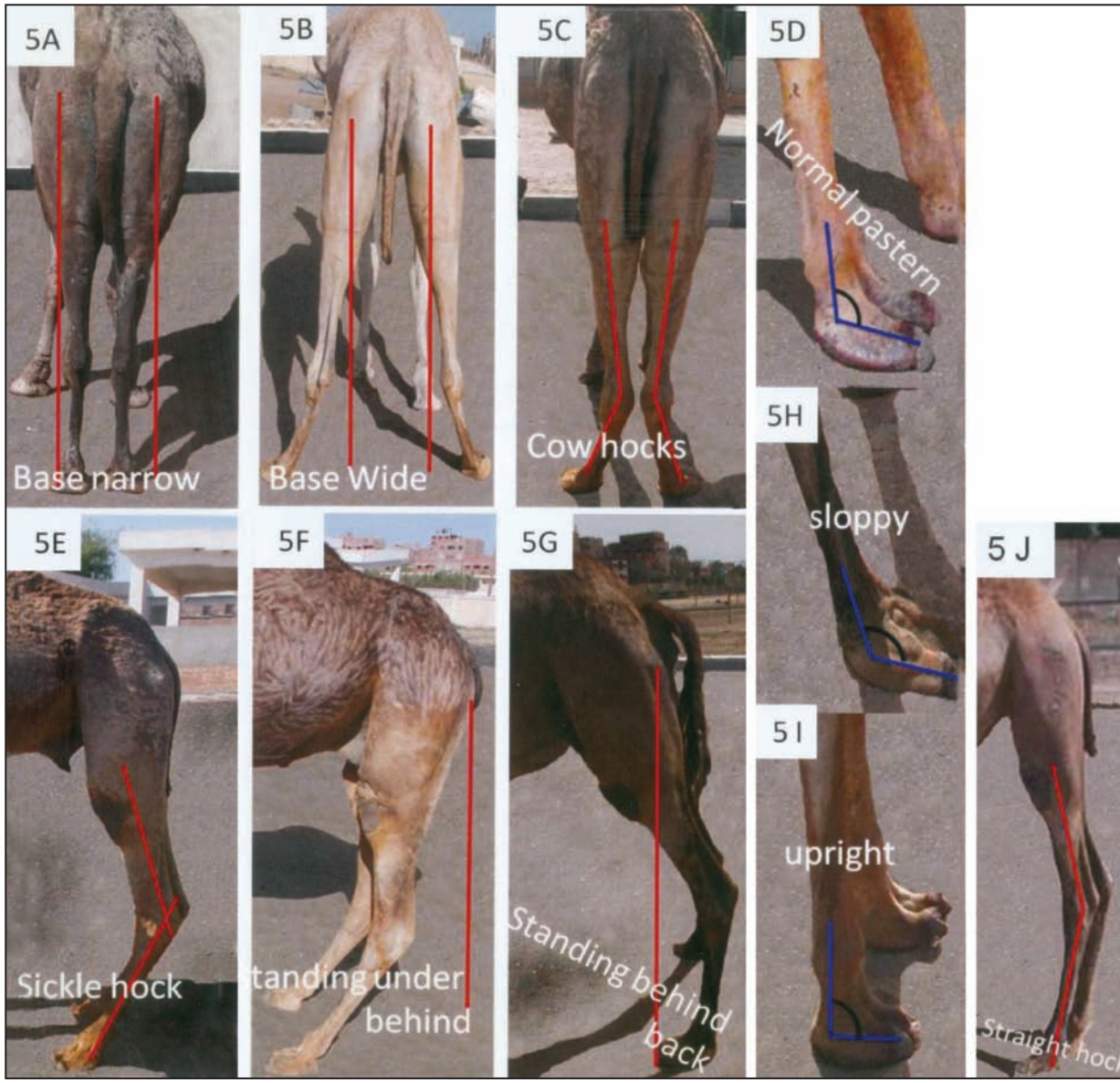

Fig 5. Abnormal hindlimb conformation: **A:** Base narrow: The hindlimbs are angled in from the perpendicular plumb line and the feet placed nearer together (rear view). **B:** Base wide: the hind limbs are angled out from the perpendicular plumb line and the feet placed further apart than the top of the limb (rear view). **C:** Cow hocks: The hocks are too close together and point toward one another and the feet are widely separated. **D:** Normal hind pastern angle : measured from the midpoint of the lateral pastern joint. **E:** Sickle hocks: small hock angle, the angle less than 150° to 153° are considered sickle. **J:** Straight hock: there is very little angle between the tibia and femur and the hock is excessively straight (large hock joint) when viewed from the lateral side. **F:** Standing under behind: the entire limb is placed too far forward when viewed from the side. The perpendicular plumb line drawn from the point of the buttock (*tuber ischii*) would strike the ground slightly far behind the limb. **G:** Standing behind back (Standing out behind): the entire limb is placed too far caudally when viewed from the lateral side. A perpendicular line dropped from the point of the buttock would be forward the foot pad. **H:** sloppy hind pastern: low pastern angle as compared with normal angle. **I:** Upright hind pastern: high pastern angle as compared with normal, pastern more vertical.

significant (P<0.05) increase in steep shoulder and upright pastern compared with normal shoulder angles. Camels with carpus valgus, calf knees and sloppy pastern conformations showed statistically significant (P<0.05) decrease in value as compared with the normal values (Table 5).

The abnormal hindlimbs joint angles were cow hock, straight hock and sickle hock. The mean values of cow and straight hocks and upright pastern had significant (P<0.05) increase as compared with normal values. The mean angles of sickle hocks and sloppy pastern had statistically significant (P<0.05)

decrease in the mean values. There were no significant differences between lengths and angles measured by AutoCAD program and that obtained by tape meter and goniometer.

In the current study subjective conformation evaluations of normal camels had straight limbs when viewed from the front and these were not too close together and hind limbs were far enough apart. Similar findings were reported in Alpaca and LIama (Fowler, 2011). Subjective evaluations of camels in the present study displayed abnormal fore and hind limbs conformation. The fore limbs had carpus valgus and calf knees, base wide, toe out and sloppy and upright pastern, base narrow and steep shoulder. The common hind limb conformations were cow, sickle, straight hocks base wide, toe out and sloppy and upright pastern, sickle hocks, straight hocks and base narrow. Similar findings were reported in fore and hind limbs in horses (McIlwraith *et al,* 2003) and Alpaca and Llama (Fowler, 2011).

Accordingly, the finding of present study showed that carpus valgus, calf knees and cow hocks were the dominant abnormality present in camels and represented one third of the investigated population. Base wide and toe out in both limbs were 2nd in their percentage by one quarter of all animals under study. Sloppy pastern was nearer to them in its percentage by around one fifth. Lastly, steep shoulders were the least in their representation.

The interesting findings in the present study the shoulder lengths in camels were nearly equal to the fore arm lengths. The ratios of hind cannon to digit lengths were 3.14%. Furthermore, the hind cannon were found larger in lengths compared to the fore cannon. Moreover, the lengths of the thigh and gaskin lengths were nearly equal. Contrary to our findings, Smuts and Bezuidenhout (1987) concluded that camel metacarpal and metatarsal were equal in lengths and tibia is slightly shorter than the femur. Therefore, the findings in the present study could be concluded that the shoulder and fore-arm were found the longest regions of the limbs and digits were the shortest. Similarly, thigh and gaskins were nearly equal in the hind limbs. In this respect, Robert *et al* (2013) reported that the ideal horse has a long gaskin, short hind cannon and low sets hocks. Similar findings were reported in thoroughbred horses (Elemmway, 2015).

The wide variations in pelvis measurements in the present study could be attributed to different types of camel breeds. Furthermore, the short pelvis length minimises the length of the muscles needed for powerful and rapid muscular contraction (Robert *et al,* 2013) in the horses.

The mean measurements of fore limbs angles related to the mean normal shoulder, elbow, carpal and pastern angles were 107.4° ± 5.3°; 149.2° ± 6.09°; 174.6° ± 3.06° and 123.7° ± 3.42°, respectively. The mean values of camel fore limb angles were found large in comparison with horses (Holmstrom *et al,* 2001; Anderson *et al,* 2004; Elemmway, 2015). This could be attributed to a unique anatomical structure and environmental adaptions of the camels (Janis *et al,* 2002). Steep shoulder was reported in 5.2% of the camels and the mean joint angles showed significant ($P<0.05$) increase. Marks (2000) and Elemmway (2015) reported that steep shoulder were more common in jumper horses and provides the vertical propulsive forces for the fore limbs during jumping.

Carpal valgus and calf knees were recorded 32.% and 31.7% of the examined camels and the mean joint angles showed significant ($p<0.05$) decrease as compared with normal camels. Lawrence (2001) considered the carpus 'normal' if it was straight and any deviation forward or backward were considered abnormal. Carpus valgus and calf knees were considered normal findings in thoroughbred jumping horses (Weller *et al,* 2006 and Kawcak *et al,* 2009).

The common observed abnormal conformation in the hind limbs were cow, straight and Sickle hocks in animals of present study. Similar findings have been reported in horses with less mean hock angles in the horses and found the tarsal joint less than 150° - 153° (Marks, 2000 and Baxter *et al,* 2011). Sickle hocks bears more stress on plantar ligaments thus producing curby hocks, worn joint out from fatigue, limits the straightening and backward extension of hocks, predispose the horse to bone spavin (Lawrence, 2001 and Thomas, 2005). In addition, horses with lameness and back problems usually had significantly smaller hock angles than sound horses (Holmstrom, 2001). Straight hocks predispose to upward fixation of patella, suspensory desmitis and fetlock osteoarthritis in horses (Ross and Dyson, 2011). Ross and Dyson (2011) found cow-hocked conformation in combination with or without base-wide or base-narrow deformities. Cow-hocked faults lead to lameness but have a substantial effect on gait.

The mean measurements of normal camels fore and hind pastern joint angles were 123.7° ± 3.42° and 130.7° ± 4.53°, respectively. There is no data concerning measurement of pastern joint angles in camels. The abnormal pastern conformation

measured was seen in upright and sloppy pasterns. The mean sloppy pastern angles had significant ($p<0.05$) decrease and upright pastern angles were significantly ($p<0.05$) increased as compared with normal values. Ross and Dyson (2011) reported that pastern angle in the horse are related to the pastern length, long pastern have more slope or lower pastern angle.

Upright pastern in horses predisposes to concussion and injuries to the fetlock, phalangeal joints and soft tissue structures behind the third metacarpus (Marks, 2000 and Stashak and Hill, 2002). Base wide, base narrow and toe out conformations were commonly observed in both fore and hind camel limbs in animal of present study. Thomas (2005) and Anderson *et al* (2004) mentioned that toe-out creates excess strain on the inner side of the hoof, pastern and fetlock predisposing the horse to DJD, ringbone and strain of deep digital flexor tendon and extensors branches of the suspensory ligaments.

Ross and Dyson (2011) reported that base-narrow conformation may occur alone or in combination with toed-in or toed-out conformation, resulting in overload of the medial aspect of the lower limb, predisposing to lameness and appeared with carpus valgus deformities. Lameness in racing camels occupied the 4th position among economically important problems in camel cows after mastitis, reproductive problems and metabolic diseases and represented 67.76% in forelimbs and 32.24% in the hind limbs (Aljuboori, 2013).

In conclusion, objectives of morphometry in the present study showed that fore and hind limbs in camels had some abnormal limb conformations. Moreover, our findings of normal conformation parameter would prove an important guideline in selecting camels for breeding and sport purposes.

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News

Government keen to promote camel racing in Saudi Arabia

The camel field in Taif is witnessing the influx of camel owners from various Gulf and Arab countries to participate in the Crown Prince Festival for Camels. The event will be organized by the Saudi Federation of Camels.

A total of 658 rounds have been allocated to camel races during the festival, starting with warm-up rounds, followed by two production and marathon rounds, and concluding with closing rounds as the festival ends.

The organizing committee of the Crown Prince Festival for Camels has prepared and equipped a 10-km race track, including seven paved tracks, three for camel owners and one for the media surrounded by an outer fence that prevents people from entering the field. The Saudi Arabian Camels Federation has announced the opening of online registration for those wishing to take part in the Crown Prince Camel Festival.

Participants are invited to submit their registration applications from Aug. 4 via www.cpcf.scrf.sa. The website includes various forms to fill out, and information about the festival and accompanying activities.

(Courtesy: Arab News)

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DETERMINATION OF PESTICIDE AND ANTIBIOTIC RESIDUES IN MUSCLES OF SUDANESE CAMEL (*Camelus dromedarius*)

A. Ibrahim Ghada[1], A. Nour Ikhlas[2], Al-Maqbali Rabea[3] and I.T. Kadim[4]

[1]Department of Meat Production, College of Animal Production, University of Bahri, PO Box 1660, Sudan
[2]Institute for Studies and Promotion of Animal Exports, University of Khartoum, Sudan
[3]Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman,
[4]Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa, PO Box 33, PC 616, Birkat Al-Mouz, Nizwa, Sultanate of Oman

ABSTRACT

An effective analytical procedure was used for determination of organochlorine pesticides (Endrin, Aldrin, DDT, Endosulfan sulfate and heptachlor) and antibiotics (Tetracycline, Sulfonamides, Gentamycin and Cephalexin) in 12 Sudanese dromedary camel muscles. Forty-eight muscle samples from two age groups: group 1 (3-4 year old) and group 2 (6-7 year old) were collected. Meat samples from four muscles, i.e. longissimus thoracis (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) were extracted with acetonitrile and purified with acetonitrile-saturated n-hexane for removing impurities. After evaporation to dryness, the residue was passed through a Sep-Pak C18 cartridge for sample cleanup prior to Gas Chromatography coupled with various detectors such as Mass Spectrometer or electron capture detector (GC-ECD). Liquid Chromatography Mass Spectrometry (LC-MS) was also used to quantify of chemical concentrations in camel muscles. Pesticides residues in all camel muscles were below the Maximum Residual Limit (MRL). A Thin Layer Chromatographic (TLC) method was used to determine the residual of veterinary drugs and the results were confirmed by Liquid Chromatography-Mass Spectrometry (LCMSMS). With the exception of tetracycline, no antibiotic residues were detected in camel muscles. Tetracycline residues in some muscles was significantly higher than the MRL. Meat sample from group 1 had significantly ($P<0.05$) lower tetracycline residues than group 2 in LT (32.13 vs. 36.75 ppm), BF (34.35 vs. 36.94), ST (29.07 vs. 35.83) and SM (28.42 vs. 35.92). This study confirmed that Sudanese camel meat is free from organochlorine residues but tetracycline residues were accumulated in both age groups. Following medication treatment, a withdrawal period of two weeks should be practiced to avoid any hazard for human health.

Key words: Antibiotics, muscles, organochlorine pesticides, residues, sudanese camel

Livestock may have access to different chemical contaminates through feed and water and it may be accumulated in their products (MacLachlan and Bhula, 2008; Hamamoto *et al,* 2017). Synthetic pesticides are fat-soluble and rapidly absorbed to accumulate in the tissue of animals continuously exposed to them through spraying of the environment or feed contamination (Hansen and Lambert, 1987). Pesticides including hexachlorocyclohexane (HCH), chlorocyclodienes (aldrin,dieldrin, endrin, heptachlor and heptachlor epoxide), DDTs and the fungicide hexachlorobenzene (HCB) are widespread used in developing countries and extensive leading to serious public health cancer, immune system disturbances and disruption of hormonal functions and environmental problems (Blair and Zahm, 1993; Hamamoto *et al,* 2017; Vincenzo *et al,* 2002; Garcia and Gotah, 2017). Residues of pesticides have been found in meat and meat products at different levels (Hamamoto *et al,* 2017; Hernandez *et al,* 1991; Herrera *et al,* 1994; Gallo *et al,* 1996). More than 90% of human exposure to harmful materials is due to consumption of contaminated meat products (Bantobal and Jodral, 1995). Antibiotics are also widely prescribed as antimicrobial drugs for human and animals. It is estimated that 100–200 thousand tons of antibiotic substances are annually produced in the world (Kümmerer, 2003; Wise, 2002). The improper use of veterinary drugs could lead to drug residue in animal products. The residues of these substances or its metabolites in animal products may cause adverse effects on consumers' health (Shankar *et al,*

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2010). Consumer demand for safe food has resulted in the introduction of numerous laws and regulations designed to control environmental distribution of these potential food contaminants. Therefore, many countries strictly regulated the use of chemical in agriculture. Despite the longer life span of camel compared to cattle and sheep, lower levels of some of chemical contaminates were found in camel meat and meat products compared to cattle and sheep products (Sallam and Morshedy, 2008). However, effect of environmental chemical contaminants in different muscles residues at different age have not been investigated. The present study was aimed to determine the residues of Endrin, Aldrin, DDT, Endosulfan sulfate, Tetracycline, Sulfonamides, Gentamycin, Cephalexin and heptachlor in musdes, i.e. longissimus thoraces (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) muscles of two age groups (3-4 and 6-7 year-old) of Sudanese camels.

Materials and Methods

Meat samples

Forty-eight muscle samples from two age group, i.e. group 1 (3-4-year old) and group 2 (6-7 year old) were collected from 12 Sudanese camels slaughtered at Tambol slaughterhouse (yard) at Al-Butana State, Sudan. Animals were slaughtered after having been held in a lairage for 12 hrs and dressed following routine commercial slaughterhouse procedures. The LT, ST, SM and BF muscles were dissected from the left side of each carcass within 60 min of postmortem. Each individual muscle was trimmed of external fat, kept in zipped plastic bags and transported in insulated ice box and kept at -18ºC for 7 days at the Meat Science Lab, Faculty of Animal production, University of Khartoum, then transported to Meat Lab at the Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University. The samples were kept in a freezer at -18ºC until analysis.

Reagents

Individual stock solutions of Organochlorine pesticides standards of Carbadox (CDX) 50 mg and penicillin, cephalexin, aminoglycoside, tetracycline and sulfanilamide (100 mg of each) purities of greater than 99% were prepared in acetonitrile. Whereas (CLP 100mg) was prepared in acetonitrile/ water (1/1, v/v) solution. Working standard solution for each antibiotic were diluted with acetonitrile (0.05 M sodium hydrogen phosphate (3/7, v/v)) to a series of concentrations ranging from 0.2 to 2.0 µg/mL.

Sample extraction and cleanup

The methods used for extraction of Multi-residue (pesticides and antibiotic) carried out according to (Minkao *et al,* 2001). Concentration of pesticides in camel meat samples were determined used Gas Chromatography/ Mass spectrometer GC/ MS (Shimadzu GC-MS system QP2010 Ultra with GC-2010 Plus Advanced flow Controller (AFC). Concentration of antibiotics were determined using a thin Layer Chromatography and conformed by a Liquid Chromatography-Mass Spectrometry (LCMS/ MS) following the procedure described by Tajick and Shohreh (2006).

Pesticides

Approximately 5 g meat sample from each muscle was homogenised with 20 ml acetonitrile in a 50 ml centrifugation tube using Ultra Turrax T25 homogeniser. Twenty ml of acetonitrile was added and vigorously shaken for 3 min. The mixture was filtered and the residues were mixed with another 50 ml of acetonitrile. The mixing and filtration steps were repeated more than once. The filtrated materials were combined and transferred into a separation funnel containing 30 ml of acetonitrile-saturated n-hexane and shaken vigorously for 5 min. The acetonitrile layer was collected into a concentration flask and evaporated to dryness at 40ºC using a rotary evaporator. The dry residue was reconstituted with 20 mL of (0.05 M sodium dihydrogen phosphate) and was introduced in a Sep-Pak C18 cartridge, which was pre-conditioned with 10 mL of methanol and 10 mL of 0.05 M sodium dihydrogen phosphate. The concentration flask was washed twice with 5 mL of sodium dihydrogen phosphate and then applied into the cartridge. The eluate was discarded and the flask was washed twice with 5 mL of methanol and the resulting solution was passed through the cartridge. The eluate was collected and evaporated to dryness at 40°C using a rotary evaporator.

The dry matter was reconstituted with 1 mL of acetonitrile /water (3/7, v/v) solution. After spiking 0.5 mL of acetonitrile-saturated n-hexane, the resulting solution was thoroughly mixed and then centrifuged at 3000 rpm for 5 min. The acetonitrile layer was collected and filtered through a membrane filter .45µl into vial and stored in a freezer at -20ºC until analysis.

Analytical determination

GC-MS analysis of pesticides was performed on a Shimadzu GC-MS system QP2010 Ultra with

GC-2010 Plus Advanced Flow Controller (AFC). The GC/MS temperature set at 275°C in spilt less mode with a 10.6 psi pressure constant flow. The flow of He through a GC column is set at 1m l/min. The oven programme at 100°C for one min, ramp at 20°C column was set at 140°C, then ramp at 5°C/min until reached 280°C, then held for 8 minutes. Interface temperature of the GC to the MS was set at 250°C and the MS ion source was set at 200°C. The MS was operated in electron –ionization (EI) mode scan range 60-500 m/z. The GC/MS was calibrated with each new sample batch. Three calibration standards were run. The calibration range for GC/MS is 200 to 1000g/L.

Antibiotics

Thin Layer Chromatography (TLC) was used to detect tetracycline, Sulfonamides, Gentamycin and Cephalexin residues following the procedure of Tajick and Shohreh (2006). Two g of meat from each muscle was homogenised with 5 ml phosphate buffer (pH 6.5). The protein was precipitated by adding 1 ml of trichloroacetic acid (30%). The solvent transferred to a 15 ml centrifuge tubes and centrifuged at 7000 rpm for 15 min. The supernatant was collected, then extracted by an equal volume of diethyl ether. The mixture was kept at room temperature for 10 min for separating layers; the mixture was separated from each other by using separating funnel. The upper oily layer was discarded and the bottom layer was collected. The steps were repeated from 5-8 times with diethyl ether and evaporated until dryness. The evaporated sample were reconstituted with 2 ml of mobile phase was done (methanol and acetone 1:1) and transferred into in a screw cap vial and kept in a refrigerator for antibiotic analysis.

Glass plates washed in acetone bath had 10 × 20 cm dimensions. For each plate 2 gm of Silica gel F256 (Merck, Germany) mixed in 5 ml DW and shacked thoroughly to produce fine paste. Clean glass plates coated with silica paste by TLC gel spreader system (Shandon, England) in 0.25 mm thickness. Plates activated in 120 C for two hours o (Boyer, 1993). Raw antibiotics (sulfaamidine, tetracycline, amoxicillin, ciprofloxacin and gentamicin) were prepared by dissolving of 0.1g of each material in 4ml methanol (Thangadu *et al,* 2002). Approximately, 50 µl of methanol dissolved antibiotic were applied at certain point on the line of the silica plates. The treated sample was transferred to TLC tank containing acetone-methanol (1:1) as mobile phase. After the solvent front retching to end of plates, chromatograms observed on UV light at 256 nm (Thangadu *et al,* 2002).

Statistical analysis

The data were analysed by Statistical Analysis System Software (SAS, 1993), using General Linear Model procedure. The procedure was used to evaluate the concentrations of pesticides and antibiotics at two age groups across four muscle types. Least Significant Difference (LSD) test was used for mean separation.

Results and Discussion

Pesticides

The organochlorine residues (α-HCH, β-HCH, Heptachlor, hexachlorobenzene (HCB), Endosulfan, Endosulfansulfate, 1,1,1-Trichloro-2,2-bis (chlorophenyl) ethane (DDT), Aldrin, Endrin Dieldrin in camel meat and the influence of muscle types (*longissimus thoracic, Semitendinosus, semimembranosus* and *biceps femoris*) and the age of camel (3-4 and 6-7 year-old) are presented in Table 1. With the exception of Endrin residues, the analysis procedure used did not detect any pesticides residues in camel meat samples. Although the level of Endrine residues was below MRL value established by FAO/WHO (2006), camel meat samples from group 2 (6-7 year-old) had 0.034 ppb in *semitendinosus* muscle. This may probably be due to that the camel has the ability to evacuate organochlorine compounds from their body tissues more efficiently than other livestock. Similarly, Khalid *et al* (2007) found that Endrin residues in meat samples from camel, cattle and sheep were below the MRL. The same authors reported the mean values of the residual concentrations (ng/g wet weight) of DDTs in camel, cattle and sheep muscles were 13.9, 17.9 and 20.3, respectively, were below MRL. However, residues of pesticides have been found in meat and meat products in different species and at different levels (Hamamoto *et al,* 2017; Hernandez *et al,* 1991; Herrera *et al,* 1994; Gallo *et al,* 1996). An LC-MS/MS multiresidue method coupled with modified QuEChERS ((Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method was used by Hamamoto *et al* (2017) for the investigation of eight pesticide residues: prallethrin (PR), resmethrin (RMT), imidacloprid (IMC), diflubenzuron (DFB), cyromazine (CYR), etofenprox (EFP), dinotefuran (DNT) and phthalthrin (PTLT). The mean concentration of the pesticides ranged between 74.7% and 113.5%, for bovine, swine and chicken muscle and liver tissue samples. Organochlorine pesticides

Table 1. Concentrations of pesticide residues (ppb) in s *longissimus thoraces* (LT), *biceps femoris* (BF), *semitendinosus* (ST) and *semimembranosus* (SM) muscles of Sudanese dromedary camels.

Parameters	Muscle								MRL[2] (mg/kg^{-1})
	LD		BF		ST		SM		
	Age (years)								
	3-4	6-7	3-4	6-7	3-4	6-7	3-4	6-7	
Organochlorine (ppb)									
α-HCH	ND	ND	ND	ND	ND	ND	ND	ND	0.01
β-HCH	ND	ND	ND	ND	ND	ND	ND	ND	0.20
Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND	0.01
HCB	ND	ND	ND	ND	ND	ND	ND	ND	0.10
Endosulfan	ND	ND	ND	ND	ND	ND	ND	ND	0.10
Endosulfan sulfate	ND	ND	ND	ND	ND	ND	ND	ND	0.10
DDT	ND	ND	ND	ND	ND	ND	ND	ND	0.05
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	0.01
Endrin	ND	ND	ND	ND	ND	0.034	ND	ND	0.10
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	0.01

MRL*s of EU regulation guidelines (CE 396/2005) = Maximum Reside Limits. HCB =hexachlorobenzene. ND: Not detected

and in particular Aldrin, DDT, Endrin and Dieldrin are widely used by farmers as insecticides that act against a wide range of agricultural pests. In addition to organochlorine pesticides, polychlorinated biphenyls (PCBs) are other type of persistent organic pollutants that may contaminate meat (Garcia and Gotah, 2017). Although, PCBs were banned many years ago, their residues were still present in meat products because of their stability and lipid-soluble properties; these compounds have harmful effects on consumer health including carcinogenicity, neurotoxicity and developmental disorders in children. Moreover, potential mechanisms of Endrin action on humans at a toxic dose may lead within few hours to signs and symptoms of intoxication excitability and convulsions. Death may follow within 2–12 hr after exposure if appropriate treatment is not applied immediately. Public concern about the adverse environmental and human health impacts of organochlorine contaminated led to strict regulations on their use in developed country. Nonetheless, DDT and several other organochlorine pesticides are still being illegally used for agriculture and animal production programs in many developing countries and led to the contamination food stuffs, especially those having a high fat content such as meat and meat products which contributed to the higher dietary intakes of most of the organochlorine (Kannan *et al,* 1994). In Nigeria, 96% of bovine meat and organs contaminated with organochlorine chemicals (Osibanjo and Adeyeye, 1997), while 88% of meat and meat products contaminated within organochlorine in Spain (Herrera *et al,* 1996). In Canada, DDT was detected in 21% of analysed fat samples from different animals (Frank *et al,* 1990). The higher detection frequencies in DDT in developing countries could be due to illegal use of DDT for agriculture purposes.

Antibiotic

With the exception of the tetracycline, there were no antibiotics residuals in the longissimus thoraces, semitendinosus, semimembranosus and biceps femoris muscle from the two age groups of Sudanese camel (Table 2). The results showed that tetracycline residues increased with increasing age of the camel from 3-4 to 6-7 year (Table 3). Although, meat samples from group 2 (6-7 year) had higher level of tetracycline residue than those from group 1 (3-4 year), the amount below the MRL values established by the EU law of drugs (European Commission 2004). The biceps femoris muscle had the highest tetracycline residues among all muscles (Fig 1). The acceptable maximum residues level (MRLs) for tetracycline as recommended by the joint FAO and WHO expert Committee on food Additives is 200,600 and 1200 mg/kg for muscles, liver and kidney, respectively. Antibiotics can act as growth promoters even though these substances can contribute to an increase in the human exposure to antibiotics, development of antibiotic-resistant pathogens and increased allergies due to its presence in foods (Reig and Toldrá, 2008; Mungroo and Neethirajan, 2014; Jalal *et al,* 2015; Garcia and Goitah, 2017).

Table 2. Levels of antibiotic residues (ppb) in longissimus thoraces (LT), semitendinosus (ST), semimembranosus (SM) and biceps femoris (BF) muscles of two different age groups of dromedary camel

Parameters	Muscle[1]								STM[1]	MRL
	LD		BF		ST		SM			
	Age (year)									
	3-4	6-7	3-4	6-7	3-4	6-7	3-4	6-7		
Tetracycline										
TLC	32.13^{b}	36.75^{c}	34.35^{bc}	36.94^{c}	29.07^{a}	35.83^{c}	28.42^{a}	35.92^{c}	4.78	100
LCMS/MS	8.34^{a}	46.33^{b}	15.42^{ac}	50.62^{b}	9.97^{a}	36.72^{bc}	9.67^{a}	32.18^{abc}	4.78	
Sulfonamides										
TLC	ND	ND	ND	ND	ND	ND	ND	ND		50
LCMS/MS	ND	ND	ND	ND	ND	ND	ND	ND		
Gentamycin										
TLC	ND	ND	ND	ND	ND	ND	ND	ND		50
LCMS/MS	ND	ND	ND	ND	ND	ND	ND	ND		
Cephalexin										
TLC	ND	ND	ND	ND	ND	ND	ND	ND		50
LCMS/MS	ND	ND	ND	ND	ND	ND	ND	ND		

[1]STM: standard error of means. MRL (maximum residues limit: Tetracycline (ppb) part per billion. (TLC): thin layer chromatography and tetracyscline by (LCMS/MS): liquid chromatography-mass spectrometry. ND: not detected

Table 3. Effect of Sudanese camel age on tetracycline residue (ppb) by Thin Layer Chromatography (TLC) and Liquid Chromatography-Mass Spectrometry (LCMS/MS).

Antibiotic (ppb)	Age (year)		SEM[1]
	3-4	6-7	
TLC	11.296a	41.466b	3.34
LCMS/MS	30.995b	36.365a	0.48

[1]SEM: Standard Error of Mean

Widespread use of antibiotics in livestock without withdrawal periods may probably led to accumulate drugs in animal products (Jalal *et al,* 2015; Garcia and Gotah, 2017). The present results indicated that antibiotics have been used at least once during the animal's lifetime for treatment of bacterial infections. However, the improper use of veterinary drugs may result in the presence of their residues in edible animal tissues, which can be toxic and dangerous for human health and potentially cause allergic reactions. Small residues of antibiotic in products consumed for long periods can lead to the spread of drug-resistant microorganisms (Shalaby *et al,* 2001; Masawat and Slater, 2007; Yu *et al,* 2011; Beyene, 2016). Health hazard concerns are raised on the antibacterial resistance in zoonotic enteropathogens (*Salmonella* spp., *Campylobacter* spp.), commensal bacteria (*Escherichia coli, Enterococci*) and bacterial pathogens of animals (*Pasteurella, Actinobacillus* spp.) (Di Stefano and Avellone, 2014). Monitoring of antibiotic residues is very important in controlling the safety of products for human consumption (Koesukwiwat *et al,* 2007). Antibiotic residues in animal products may be the cause of numerous health concerns in humans. These problems include toxic effects, transfer of antibiotic resistant bacteria to humans, immunopathological effects, carcinogenicity (sulphamethazine, oxytetracycline and furazolidone), mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol) and allergy (penicillin) (Paige *et al* 1997). Failure to observe the instructions for antibiotic use can lead to antibiotic residues entering animal-derived foods (Darwish *et al,* 2013). The significance of this contamination depends on the pharmacodynamics of the compound and the species (McEvoy, 2002). In Sudan, the most commonly used antibiotics by farmers are quinolones and tetracycline. The majority of farmers use antibiotics for prevention and control of disease; only 5% of farmers use antibiotics for livestock health maintenance (Eltayb *et al,* 2012). The risk of tetracycline residues in meat include toxic and allergic reaction and development of resistant strains of bacteria following the ingestion of sub-therapeutic doses of antimicrobials (Botsoglou and Fletuvris, 2001).

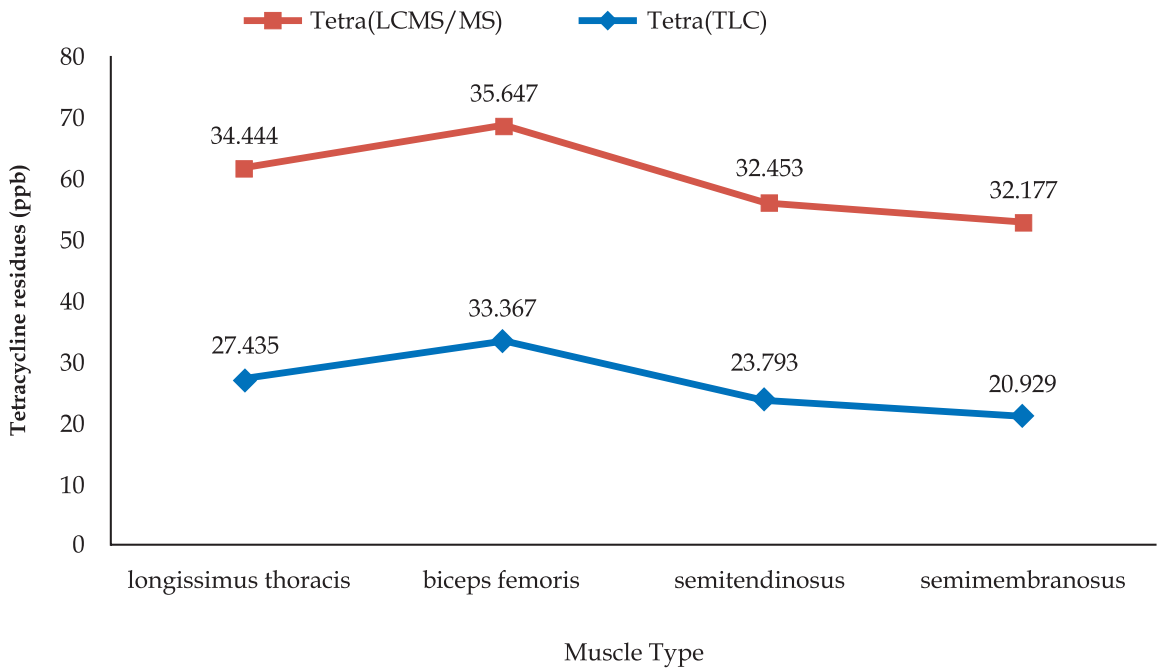

Fig 1. Tetracycline residues (ppb) in four muscles of Sudanese dromedary camel using (TLC) Thin Layer Chromatography (TLC) and Liquid Chromatography-Mass spectrometry (LCMS/MS).

Conclusion

The Organochlorine pesticides including DDT, Endosulfan sulfate and heptachlor were not detected in Sudanese camel muscle samples but trace residue of Endrin found in semitendinosus muscle from group 2. With the exception of tetracycline, Sudanese camel muscles were free from residue of sulfonamides, gentamycin and cephalexin. Tetracycline residue increased significantly with increase camel age. This study conclude that Sudanese camel meat is free from most pesticides and antibiotics, therefore it can marketed as a safe meat for human consumption.

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EFFECT OF ROAD TRANSPORTATION ON BLOOD AND SERUM PARAMETERS AND THYROID ACTIVITY IN SYMPTOMATICALLY HYPERGLYCAEMIC FEMALE DROMEDARY CAMELS

Tariq I. Almundarij

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

ABSTRACT

Ten animals were divided into two equal groups according to short (150–200 km) or long (250–400 km) transportation distance. Compared to 10 clinically healthy control animals, the glucose level, globulin concentration, neutrophil count, catalase and total antioxidant capacity activity were significantly higher in the camels after both the short- and long-distance lorry transportation, while the eosinophil count and super oxide dismutase activity were significantly lower. Long-distance transportation resulted in elevated creatinine, but lowered albumin in the camels. The long-distance group also exhibited a significant increase in total T3 and total T4 levels; however, their TSH levels were significantly lower than in the control camels. The short-distance lorry transportation of camels led to an elevation of the white blood cell count and haemoglobin concentration, but lower haematocrit % and lymphocyte count. Thus, the altered thyroid hormone levels and changes in the physiological metabolic profiles taken together may be effective biomarkers of transportation stress in this species. Finally, it was cleared that the obtained pathophysiological changes may be resulted from the transportation-induced stress in hyperglycaemic animals.

Key words: Blood, dromedary camel, hyperglycaemic lorry transportation, thyroid

In the Kingdom of Saudi Arabia (KSA), camels are commonly transported for a variety of purposes including clinical examination, slaughter and sale. Animal transportation has been linked to health disorders and economic loss (Padalino *et al,* 2015). Previous studies have reported that the long duration (Chacon *et al,* 2005) and density of animals in the lorry during transportation (Waas *et al,* 1997) lead to a stressful condition in the animals.

Ideally, blood glucose levels in camels range between 70 and 90 mg/dL (Poonia *et al,* 2016). Under stress conditions, the body shifts physiologically into fight-or-flight mode, hence elevating the blood glucose. It is well known that transportation involves many stressful factors for large animals, including manipulation, foreign environments, noise and fasting. These responses can change depending on the duration of the transportation (Padalino *et al,* 2015).

As an endocrine response to stress, thyroid hormones in particular are known as important modulators of energy metabolism (Kaneko *et al,* 2008; Eshratkhah *et al,* 2010). The alteration in thyroid hormone concentrations following different stresses has been reported in some domestic animals (Saeb *et al,* 2010). In addition, these hormones may have the capability to regulate the activities and metabolic pathways of the anti-oxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). In the serum of camels, these enzymes are changed during environmental stress (Kataria *et al,* 2010).

To the best of our knowledge, no study has been conducted to date describing the possible relationship between thyroid hormone status, antioxidant enzymes and serum and blood chemical profiles of symptomatically hyperglycaemic dromedary camels after short and long-distance lorry transportation. This study was therefore, undertaken to investigate the influence of lorry transportation on thyroid hormones, blood and serum biochemistry and antioxidant enzymes in the symptomatically hyperglycaemic camels admitted to Qassim University Veterinary Teaching Hospital.

SEND REPRINT REQUEST TO TARIQ I. ALMUNDARIJ email: dr.tariq3332@yahoo.com

Materials and Methods

This study was carried out at the Qassim University Veterinary Teaching Hospital and the Qassim University Farm in the Qassim region, KSA, during the November and December 2017. The experimental protocol was approved by the Animal Ethical Committee of Qassim University Deanship for Scientific Research.

Animals

Ten non-pregnant, non-lactating, clinically healthy female camels (*Camelus dromedarius*) aged 3–6 years were used as non-lorry-transported control camels in the present study. These camels were selected from the Qassim University farm based on the absence of any disease. Their body condition scores were considered normal, ranging between 3 and 3.5. Another ten female animals were admitted to the clinic after lorry transportation suffering from long- standing decrease in body weight and progressive weakness and had high glucose levels as measured by glucometer. These camels were divided into two equal groups according to the distance of transportation: short (150–200 km.) and long (250–400 km.).The body condition scores of these animals ranged between 2 and 3.5.

Blood samples

Five-mL blood samples were collected by sterile jugular venepuncture into a tube containing EDTA and used for the haematological parameters. Another 5-mL were collected and serum was separated by centrifugation at 3000 × g for 20 min and kept in a deep freezer at -20^{o}C till assayed.

Haematological, biochemical and hormonal analyses

Haemogram and leukogram evaluations were carried out using an automated analyser (Vet Scan HM5, Abaxis, California, USA). The haemogram included red blood cell (RBC counts, × 10^6/µL), haemoglobin (HGB) concentration, g/dL), haematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg) and mean corpuscular haemoglobin concentration (MCHC, g/dL). The leukogram included total leukocyte (WBC) count (× 10^3/µl), lymphocyte (LYM) count (× 10^3/µl), monocyte (MON) count (× 10^3/µl), neutrophil (NEU) count (× 10^3/µl) and eosinophil (EOS) count (× 10^3/µl). The serum samples were tested using an automated biochemical analyser (Vet Scan VS2, Abaxis, California, USA.) to determine the protein profile of total protein (TP, g/dL), albumin (ALB, g/dL), globulin (GLOB, g/dL), creatinine (CREA, g/dL), amylase (AMY, U/L), blood urea nitrogen (BUN, g/dL) and alkaline phosphatase (ALP, U/L). The electrolyte profiles included calcium (Ca, g/dL), phosphorus (P, g/dL), sodium (Na, mmol/L) and potassium (K, mmol/L).

Serum concentrations of total T_3, T_4 and thyroid stimulating hormone (TSH) were assayed by ELISA procedures using purchased kits (Immunotech Corporation, 90 Windom St., Boston, MA 02134, USA). For the antioxidant enzyme activity, Calorimetric measurement was performed using kits (Biodiagnostic) to determine total antioxidant capacity (TAC), super oxide dismutase (SOD) and catalase (CAT).

Statistical analyses

All values were expressed as mean and standard error (SE) with $P<0.05$ and $P<0.01$ seen as statistically significant. For comparison between the groups, the data were analysed by the Mann-Whitney U test and two-way analysis of variance (ANOVA).

Results and Discussion

Glucose level

The effects of short- and long-distance lorry transportation on the glucose level of the dromedary camels are shown in Table 1. Compared to the control group, the glucose levels were significantly higher in the camels after both short ($P<0.05$) and long ($P<0.01$) distance lorry transportation.

Protein profile

The effects of short- and long-haul lorry transportation on the protein profile of the symptomatically hyperglycaemic dromedary camels are shown in table 1. The ALB concentrations of the camels after long lorry transportation were significantly lower ($P<0.05$) than central group; however, non- significant difference was seen in the short-distance transportation group. In addition, there was no significant difference between the total protein in either the short- or long-distance transportation group compared to the control group of camels. Moreover, GLOB concentrations were significantly higher in both the short ($P<0.05$) and long ($P<0.01$) distance transport groups than in the control group.

ALP, CREA, BUN and Amylase

The effects of short- and long-haul lorry transportation on ALP, CREA, BUN and amylase

in the symptomatically hyperglycaemic dromedary camels were shown in table 1. The data revealed no significant difference in ALP and BUN levels of the camels in either group subjected to lorry transportation compared to the control group. Likewise, no significant difference in BUN was seen in either transported group compared to the control group. The CREA level of the short-haul transport group did not show any significant difference; however, the CREA levels of the camels in the long-haul transport group were significantly higher ($P<0.05$) than in the control group. Finally, the data showed that amylase activity in the camels under both short- and long-distance lorry transportation conditions were significantly lower ($P<0.05$) compared to that of the control group.

Leukogram

Table 2 shows the effects of short- and long-distance lorry transportation on the leukograms of the symptomatically hyperglycaemic dromedary camels. The WBC count of the camels after short-distance lorry transportation was significantly higher ($P<0.05$) compared to the control group, while after long lorry transportation, there was no significant difference in the WBC. There was a significantly greater decline ($P<0.05$) in the lymphocyte count of the camels after short lorry transportation than in the control group. On the other hand, compared to the control camels, there was no significant difference in the count after long-haul lorry transportation than lymphocyte group. In addition, no significant differences were observed in the monocytes count in any of the three camel groups. However, the netvoyage count was significantly higher in the camels in both the short ($P<0.01$) and long ($P<0.05$) distance transportation groups compared with the control group, while the eosinophil count was significantly lower in both the short ($P<0.01$) and long ($P<0.05$) distance transportation groups.

Haemogram

The effects of short- and long-distance lorry transportation on the haemograms of the symptomatically hyperglycaemic dromedary camels are shown in table 2. The data revealed no significant differences in the RBC count of camels in either the short-or long-distance lorry transportation groups compared to the control group. The hemoglobin concentration of camels after short-distance transportation was significantly higher ($P<0.05$) than that of the control group; similarly, their hematocrit % was also significantly higher ($P<0.05$). However, there was no significant difference in HGB concentration or the HCT %between the camels after long-distance transport and the control group. No significant differences were found in the MCV, MCH, or MCHC among all 3 groups.

Electrolyte profile

The data revealed no significant differences in calcium concentration in either the short- or long-distance lorry transport groups compared to the control camels. Similarly, neither lorry transport group showed any significant differences in the P levels, nor in Na and K, compared to the control group (Table 3).

Thyroid hormones

There was a significant increase ($P<0.01$) in the total T3 levels of the camels after long-distance lorry transportation compared to the control group. Similarly, total T4 was significantly higher ($P<0.05$) in camels after long-distance lorry transportation;

Table 1. Effect of short and long lorry transportation distance on biochemical profile of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	**Mean**	**SE (±)**	**Mean**	**SE (±)**	**Mean**	**SE (±)**
GLU (g/dL)	114.40	4.51	170.53[a]	15.81	224.82[b]	35.10
ALB (g/dL)	4.68	0.16	3.75	0.26	3.55[a]	0.51
GLOB (g/dL)	1.64	0.12	2.82[a]	0.41	3.03[b]	0.38
TP (g/dL)	6.18	0.16	6.57	0.29	6.58	0.35
ALP (U/L)	106.90	6.57	116.0	18.5	120.73	13.96
CREA (g/dL)	0.94	0.06	1.27	0.11	1.48[a]	0.29
BUN (g/dL)	26.31	0.84	20.23	2.74	26.97	2.73
AMY (U/L)	616.7	41.7	418[a]	69.0	412.2[a]	25.6

[a, b] Mean values of the short and long lorry transportation distance groups differed significantly from the value of the control group in the same row at $P<0.05$ and $P<0.01$, respectively.

however, their TSH levels were significantly lower (P<0.05) than those of the control camels (Table 4).

Antioxidant enzymes

A significant decrease was observed (P<0.05) and (P<0.01) in the SOD activity after short- and long-distance lorry transportation, respectively, compared to the control camels. Meanwhile, the CAT activity was significantly increased in the camels after short (P<0.05) and long (P<0.01) distance lorry transportation. However, the TAC activity in both transported camel groups was significantly higher (P<0.05) than in the control group (Table 5).

Animals exposed to unfavourable environments due to transportation experience very stressful conditions with a variety of physiological changes. These changes may have unfavourable effects on the camel's productivity and well-being.

Camels have developed resistance to harsh environments and found sustenance from sources unutilised by other species (Kadim *et al,* 2009). The complex physiological changes caused by lorry transportation stress can differ according to species and breeds within the same species (Ingram and Matthews, 2000). Different parameters, including metabolic, hormonal and behavioural changes, have been used to measure the physiological changes occurring during stress (Saeb *et al,* 2010; Nazifi *et al,* 2009).

Compared to the control group, glucose levels were significantly higher in the camels after short- and long-distance lorry transportation. This is in agreement with previous works conducted by Tadich *et al* (2005) and El Khasmi *et al* (2013) and with studies done on goats (Kannan *et al,* 2007; Minka and Ayo, 2010) and Holstein calves (Bernardini *et al,* 2012). However, results of other investigators did not support these findings, particularly those on cattle and goats (Earley *et al,* 2012). The elevated glucose level during lorry transportation may result from glycogenolysis in the muscle and liver due to an increased catecholamines from the sympathetic nervous system (Sanders and Straub, 2002; Almundarij *et al,* 2017) and glucocorticoids from the adrenal cortex (Tadich *et al,* 2005); however, these factors were not assessed in this study. In

Table 2. Effect of short and long lorry transportation distance on haematological profile of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	**Mean**	**SE (±)**	**Mean**	**SE (±)**	**Mean**	**SE (±)**
WBC ($\times10^3/\mu L$)	15.04	1.41	21.06[a]	3.79	16.37	4.22
LYM ($\times10^3/\mu L$)	3.21	0.54	1.53[a]	0.22	1.82	0.27
MON ($\times10^3/\mu L$)	0.100	0.01	0.14	0.02	0.13	0.04
NEU ($\times10^3/\mu L$)	8.81	1.04	18.80[b]	3.40	14.68[a]	3.73
EOS ($\times10^3/\mu L$)	2.92	0.53	0.58[b]	0.16	0.61[a]	0.33
RBC ($\times10^6/\mu L$)	8.69	0.32	10.53	0.76	8.77	0.92
HGB (g/dL)	13.60	0.49	17.00[a]	1.44	13.27	1.17
HCT (%)	24.14	0.66	28.81[a]	2.85	23.83	1.72
MCV (fl)	27.70	0.65	27.25	1.44	28.00	1.83
MCH (pg)	15.67	0.18	16.15	0.42	15.36	0.71
MCHC (g/dL)	56.31	1.18	59.45	2.04	55.42	1.09

[a, b] Mean values of the short- and long-distance lorry transportation groups differed significantly from the value of the control group in the same row at P<0.05 and P<0.01, respectively.

Table 3. Effect of short and long lorry transportation distance on electrolyte profile of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	**Mean**	**SE(±)**	**Mean**	**SE (±)**	**Mean**	**SE(±)**
CA (g/dL)	9.72	0.15	9.57	0.19	9.60	0.21
P (g/dL)	4.90	0.66	4.97	0.69	5.32	0.77
NA (mmol/L)	154.31	1.46	143.75	2.90	144.33	1.50
K (mmol/L)	5.57	0.09	4.92	0.22	4.56	0.30

contrast, a similar study in goats demonstrated that the elevation of blood glucose was due to the activation of the sympathetic nervous system rather than the plasma cortisol (Aoyama *et al,* 2008). In the present study, the elevated glucose levels resulting from the short-haul distance requiring a 2-3 h drive to the clinic could have been caused by a sympathetic outflow and increase in plasma cortisol either directly (O'Malley, 1971), or through ACTH (Maejima *et al,* 2006) mediated stress. On the other hand, both catecholamine administration and transportation have been shown to increase plasma glucose levels within 10-15 min in sheep (Parrott *et al,* 1994; Bassett, 1970). Additionally, a link was found between transportation stress, expression of the *c-fos* protein in the adrenal medulla and the increase in plasma glucose levels (Maejima *et al,* 2006). However, the blood glucose showed no significant change due to the length/duration of the transportation. Even when it increased from 170.5 g/dL to 224.8 g/dL, this might have been due to the variations of age among the animals, as previously mentioned by Eskandarzadeh *et al* (2014).

The increase in ALB levels during transportation may indicate the degree of stress in response to stressors (Križanović *et al,* 2008). The effects of short- and long-distance lorry transportation on the protein profiles of the symptomatically hyperglycaemic camels were recorded. Compared with the control group, the ALB concentration of the camels after long-haul lorry transportation was found to be significantly lower; however, no significant difference was seen in the short-haul transportation group. In addition, there was no significant difference between total proteins after either short- or long-distance lorry transportation compared to the control camels, whereas the GLOB concentrations were significantly higher in both groups of transported camels. As a result of lorry transportation, there was an elevation of GLOB and lowering of ALB without changes in the total protein. On the other hand, short lorry transportation had no effect on the ALB and total protein in the current study, suggesting that no dehydration occurred during the 2-3 h lorry transportation stress in the dromedary camels. The long-haul lorry transport of over 5-6 h had no significant effect on total protein concentration in the dromedary camels, as has been previously reported (Kataria and Kataria, 2004). It was concluded that no signs of dehydration had occurred in the dromedary camels during transportation, which may be a reflection of their ability to adapt to dehydration (Parker *et al,* 2003).

The data revealed that there was no significant difference in the ALP activity in either group of transported camels compared to the control group. The CREA and BUN levels of the camels after short-haul transportation were not significantly different; however, the CREA levels of the camels in the long-haul transportation group were significantly higher than those of the control group. This result is consistent with a previous study on different species (Hartung, 2003). Therefore, the elevated levels of CREA may have been due to the catabolism of protein by cortisol after food deprivation (Wensvoort *et al,* 2004). On the other hand, a different study showed no significant effect on CREA levels during drought stress (Kataria and Kataria, 2004). The present data indicated that neither short-nor long-haul transportation had extensive adverse effects on the protein catabolism of the transported camels.

The haemogram is one of the physiological stress indicators for transportation in camels (El Khasmi *et al,* 2013). The values of the control group were consistent with haematological values obtained in camels in Saudi Arabia (Al-Sultan, 2008). Camels have small, elliptical RBCs and a lower HCT % compared with other mammalian RBCs (Farooq *et al,* 2011). The data showed that the HCT % was significantly higher in the camels transported short-distance than in the control group. Moreover, the HCT % of the camels in the long-distance transport group was not significantly different from that of the control group. A previous study reported that the elevation of HCT % in animals might be attributed to haemoconcentration associated with splenic contraction induced by catecholamines (Tadich *et al,* 2005). It has been observed that HCT % may be increased during the handling and manipulation of animals (Tadich *et al,* 2005). The camel blood HGB concentration recorded in this study is in accordance with the values previously reported in Saudi Arabia byAl-Busadah (2007), in Iraq by Alsaad (2009) and in Iran by Ahmad *et al* (2004).

The blood index values (MCV, MCH and MCHC) and RBC count of the camels were similar to the reference values of Farooq *et al* (2011). Neither short-nor long-distance lorry transportation showed any significant difference from the control group. The HGB concentration of the camels after short-distance lorry transportation was significantly higher than that of the control group. However, there was no significant difference in HGB concentration between the camels after long transportation and the control

Table 4. Effect of short and long lorry transportation distance on total T3, T4 and TSH of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	**Mean**	**SE(±)**	**Mean**	**SE (±)**	**Mean**	**SE(±)**
Total T3 (ng/dL)	144.60	10.40	107.21[a]	33.91	214.2[b]	59.51
Total T4 (ug/dL)	10.46	0.76	9.98	2.46	18.52[a]	4.32
TSH (uIU/mL)	0.05	0.04	0.05	0.04	0.04[a]	0.02

[a, b] Mean values of the short and long lorry transportation distance groups differed significantly from the value of the control group in the same row at P <0.05 and P <0.01, respectively.

Table 5. Effect of short and long lorry transportation distance on serum antioxidant enzymes of symptomatically hyperglycaemic dromedary camels.

Groups	Control		Short-distance lorry transportation		Long-distance lorry transportation	
Parameters	**Mean**	**SE(±)**	**Mean**	**SE (±)**	**Mean**	**SE(±)**
SOD (U/mL)	305.51	5.89	232.72[a]	17.65	175.70[b]	31.50
CAT (u/L)	288.0	23.11	380.12[a]	36.11	602.32[b]	66.86
TAC (mM/L)	1.767	0.260	3.118[a]	0.528	2.701[a]	0.426

[a, b] Mean values of the short and long lorry transportation distance groups differed significantly from the value of the control group in the same row at P <0.05 and P <0.01, respectively.

group. The blood index values are in agreement with the previous works of Al-Sultan (2008) and Alsaad (2009) on camels. The variation in blood index values might be attributed to variable RBC size, age and physiological state.

The mean WBC count values recorded for the control animals in the present study were comparable to those reported by Ahmad *et al* (2004) and Alsaad (2009) and lower than those reported by Al-Busadah (2007) and Al-Sultan (2008). The WBC count of camels after short-distance lorry transportation was significantly higher than in the control group, which may be attributed to transportation stress, as reported in early studies (Tadich *et al,* 2005). However, there was no significant difference in WBC count after long-haul lorry transportation and in the control camels. Similar findings were presented by Minka and Ayo (2010). El Khasmi *et al* (2013) found no significant changes in WBC counts in camels transported under heat stress. Al-Wabel (2010) reported that the total WBC count in camels was not affected by the stressful conditions of transportation.

Differential leukocyte counts in the present study indicated that in the control group, lymphocyte were the most predominant leukocytes, followed by neutrophil, as has been previously reported (Al-Busadah, 2007) and contradictory to AL-Sultan (2008) and Al-Busadah and Osman (2000). Variation in the white cell values could be attributed to the differences in breeds or the stress of sample collection (Higgins and Kock, 1984). There was a significant decline in the lymphocyte count of the camels after short-haul lorry transportation when compared to the control group. On the other hand, no significant difference was seen between lymphocyte counts in the camels after long-haul lorry transportation and in the control camels. In addition, the monocyte count did not significantly differ among all three camel groups. However, the neutrophil count was significantly higher in the camels after both short- and long-distance lorry transportation compared with the control group. In contrast, the EOS count was significantly lower in both short- and long-distance lorry transported animals than in the control group. The observation of neutrophilia and lymphocytopeniain camels transported by lorry in the present study is supported by a previous study (Minka and Ayo, 2007). Al-Wabel (2010) found that transportation stress had no effect on the neutrophil counting camels, whereas it caused a slight reduction in the lymphocyte counts.

Endocrine responses constitute late response to stressors after nervous reflexes. Stress can affect the endocrine balancing of the essential parameters of production, metabolism, immunity and even growth. Hormonal signals play a pivotal role in homeostasis. Appropriate thyroid function and hormonal activity perform an essential task in the mechanisms that allow domestic animals to live and breed effectively (Todini, 2007). Many external factors are capable of

altering thyroid hormone concentrations by acting on the hypothalamus, pituitary and/or thyroid gland itself (Todini, 2007). A change in the thyroid blood concentrations has been used as an indicator of the stress response (Saeb *et al,* 2010). The serum concentrations of T4 and T3 in the control group parallel values reported earlier (Nazifi *et al,* 2009). The data obtained showed a significant increase of T3 and T4 levels in the serum of the symptomatically hyperglycaemic dromedary camels after long-distance lorry transportation compared to the control group. However, TSH levels in the camels after long-distance lorry transportation were significantly lower than in the control camels. Physiologically, as blood concentrations of T3 and T4 increase, both T3 and T4 inhibit TSH and as a result, the TSH level decreased during the long-distance transportation. These results are consistent with other studies which have shown that after long-distance lorry transport, T4 levels were elevated in Limousin calves (Fazio *et al,* 2001) and cattle (Fazio *et al,* 2005). In addition, Hartung (2003) stated that transportation stress might reduce animal fitness by inducing dysfunction of the pituitary and thyroid glands. The T3 and T4 variation during transportation may suggest the possible effect of stress on thyroid hormones (Saeb *et al,* 2010). Saeb *et al* (2010) found no significant change in serum T3 in camels; however, T4 increased after 1h of transportation, while T3 and T4 showed a significant increase after 5h of transport.

Antioxidant enzyme activities are major markers of oxidative stress. Results showed the effects of short- and long-haul lorry transportation on the activity of SOD, CAT and TAC in the symptomatically hyperglycaemic dromedary camels. A significant decrease was seen in SOD activity in camels after short- and long-distance lorry transportation compared to the control group. Meanwhile, CAT and TAC activity were significantly higher in the camels after short- and long-haul lorry transportation than in the control group. It has been suggested that the increase in both CAS and TAC acted as a compensation factor to balance the production of free radicals during the stress of transportation (Padalino *et al,* 2017). Both elevated and lowered antioxidant enzyme activities have been reported under different conditions as a result of enhanced reactive oxygen species (ROS) production, either by up-regulation of enzyme activity or depletion due to fighting the ROS during stress (El Khasmi *et al,* 2015). Pronounced deviations in the levels of ROS scavengers and free radical generation following or during stress have been noted in the serum of camels (Kataria *et al,* 2010). Transportation stress in camels leads to an increase in catalase activity (Nazifi *et al,* 2009). However, further studies should be focused on understanding the character of particular antioxidant enzymes in the tissues and under different stressful conditions.

In conclusion, this study has confirmed that the stress of long-distance lorry transport can be considered somewhat similar to severe stress. The altered thyroid hormone levels and changes in the physiological metabolic profiles taken together may be effective biomarkers of transportation stress in this species. Present study conclude that pathophysiological changes may result from the transportation-induced stress in hyperglycaemic animals.

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ADENOCARCINOMA IN THE GENITAL TRACT OF INFERTILE FEMALE DROMEDARY CAMELS

A Ali[1,2], R Derar[1,2], F Al Sobayil[1], M Tharwat[1,3], A Fathy[4] and M Khodeir[5]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia
[2]Department of Theriogenology, [4]Department of Surgery, Faculty of Veterinary Medicine, Assiut University, Egypt
[3]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt
[5]Department of Pathology, College of Medicine, Qassim University, Saudi Arabia

ABSTRACT

The aim of this study was to describe the clinical and ultrasonographic findings, gross and microscopic appearances and changes in the haemogram and blood chemistry of vaginal, cervical and uterine adenocarcinoma in dromedary camels. Tissue overgrowths were detected during clinical examination and ultrasonography in the vagina (*n*=9), cervix (*n*=2) and uterus (*n*=1). All females were multipara and aged between 9 and 13 years. A common history of post-mating vaginal bleeding of these females was noticed. Specimens were taken for histopathology and immunohistochemistry. Blood samples were obtained for haematology and biochemistry. The overgrown tissue masses bled easily upon palpation. By ultrasound, these tumuors were homogenous and echogenic, but sometimes with multiple hypo-echogenic cavities. Upon necropsy, metastasis was observed in the regional lymph nodes, mesentery as well as in the liver in one case. Diagnosis was confirmed by histopathological examination as vaginal, cervical and uterine adenocarcinoma. Immunohistochemically, all specimens with adenocarcinoma showed diffuse expression of epithelial membrane antigen and carcinoembryonic antigen. Compared to healthy controls (*n*=15), camels with adenocarcinoma showed significant decreases in lymphocytes, monocytes, erythrocytes, haemoglobin, haematocrit, total protein, albumin, calcium and phosphorus and increases of globulin, alkaline phosphatase and magnesium. In conclusion, this is the first report of adenocarcinoma in the genital tract of female dromedary. The vagina was the most frequent affected organ. Vaginal bleeding and anaemia were the common clinical and laboratory findings.

Key words: Adenocarcinoma, blood chemistry, female camel, genital tract

Tumuors of the female tubular genital tract in domestic animals are relatively rare, with the exclusion of bovine uterine carcinomas and vaginal fibropapillomas, bovine and canine leiomyomas and transmissible canine venereal tumuors (Bastianello, 1982; McEntee and Nielsen, 1976; Kumar *et al,* 2007; Stilwell and Peleteiro, 2010; Agnew and MacLachlan, 2016; Schlafer and Foster, 2016). Adenocarcinoma is a type of cancer that forms in mucous-secreting glands/ cells throughout the body (Agnew and MacLachlan, 2016; Schlafer and Foster, 2016).

Vaginal adenocarcinoma arises from the glandular/secretory cells in the lining of the vagina that produce some vaginal secretions (Kumar *et al,* 2007; Schlafer and Foster, 2016).

There were few reports describing invasive carcinomas including the cervix in domestic animals, but invasion from either a uterine or vaginal carcinoma could not be excluded (McEntee and Nielsen, 1976; Schlafer and Foster, 2016).

Uterine adenocarcinoma, commonly termed endometrial carcinoma, develops from cells in the endometrium. Carcinomas of the endometrium are rare neoplasms of domestic animals, however, they are more frequent in cattle, when compared to other species (Anderson and Sandison, 1969; Agnew and MacLachlan, 2016; Schlafer and Foster, 2016).

Adenocarcinoma of the female genital tract of camels has not been reported previously. Therefore, the objectives of this paper was to study the vaginal, cervical and uterine adenocarcinomas in female infertile dromedary camels.

Materials and Methods

Animals and gynaecological examination

During routine gynaecological examination of infertile female camels (n=1621) throughout two breeding seasons (September – March, 2015/2016 and 2016/2017) at the Veterinary Teaching Hospital of Qassim university of Saudi Arabia, tissue

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overgrowths were detected in the vagina, cervix and uterus of 12 animals. Age, parity and breeding history were obtained. Ultrasound examination of the genital tract was performed using Real-time, B-mode diagnostic ultrasonic equipment (Aloka SSD 500, Tokyo, Japan) attached to a 5 MHz trans-rectal transducer.

Necropsy findings

Due to poor prognosis, 5 females with vaginal masses were euthanised and postmortem examinations were performed. The reproductive tracts were immediately removed and examined morphologically. Necropsy results were corroborated with the ultrasonic images.

Histopathology and immunohistochemistry

Tissue specimens from the masses were obtained during necropsy (n=5) and from the living animals via biopsy (n=7) and were fixed in 10% buffered formalin and processed routinely and stained with hematoxylin and eosin. These were examined under light microscope. Immunolabelling was performed using the avidin–biotin– peroxidase complex (ABC) method. Sections 5 mm were cut from the paraffin blocks and mounted on positive charged adhesive glass slides ('Clipped Corner X-tra Slides', Leica Biosystems, Wetzlar, Germany). The slides were incubated at 37°C overnight for accurate adhesion of the section to the slide, deparaffinised by incubation in the oven at 56°C for 15 minutes and inserted in xylene for 30 minutes, rehydrated by transferring into graded ethanol, then washed in phosphate buffer saline (PBS) (pH 7.2) for 5 minutes. Slides were immersed in a solution of 90 ml methanol + 10 ml hydrogen peroxide (1.5%) for 30 minutes for blocking of endogenous peroxidase. Sections were then rinsed and incubated with primary antibodies EMA and CEA. The immunolabelling was performed on an automated immunostainer with appropriate positive and negative controls. DAB was used as a chromogen (3-3\ diaminobenzidine tetrahyrochloride) and Mayer's haematoxylin as a counter label.

Haematology and biochemistry

Blood samples were collected from the affected 12 camels and from 15 clinical health controls (7-12 years old), for haematology (Complete Blood Count) and biochemistry. The red blood cell counts, white blood cell counts, differential leukocytic counts including lymphocytes, monocytes and neutrophils, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration were determined by using Vet Scan HM5 (Abaxis, Union City, CA 94587, USA). Total protein, albumin, globulin, alkaline phosphatase, creatine kinase, aspartate aminotransferase, gamma glutamyl transferase, blood urea nitrogen, calcium, phosphorus and magnesium were determined in the harvested sera using Vet Scan 2 (Abaxis, California, USA).

Statistical analysis

A student t-test was used to compare between females with adenocarcinoma and the apparently healthy camels for the haematological and biochemical values. Significant values were set at $P < 0.05$. IBM-SPSS statistical program, version 24 (2016) was used for analysis.

Results and Discussion

All affected females were infertile but multipara and aged between 9 and 13 years. Difficult mating or vaginal bleeding after copulation were the attached history. On sonogram, these masses were homogenous and echogenic, sometimes with multiple hypo-echogenic cavities (Fig 1).

Vaginal exploration revealed multiple solidary circumscribed thickening (2 - 4 patches, 4 cm in diameter) palpated in the inner vaginal wall. In 2 animals, the external opening of the cervix was felt abnormally firm, enlarged, undilatable and partially or completely closed to allow the passage to the uterus. In 1 case, the uterine body, just anterior to the cervix had firm, thick and diffuse masses. The overgrown tissues of the vagina, cervix and uterus bled easily upon palpation.

On necropsy, 3 animals of vaginal tumuors showed circumferential pattern of growth, while in 2 animals these masses were multifocal (Fig 2). All tumuors were ulcerative and necrotic. On cutting, they reveal hard white nodules. In one case, metastasis was observed in the iliac lymph node, mesentery and liver. The lymph node was clearly enlarged (5 cm in diameter), the mesentery showed numerous rosary arranged nodules of about 1 cm in diameter.

Histopathologically, vaginal (n=9), cervical (n=2), and uterine (n=1) adenocarcinomas were found. Variable sized glands lined by malignant epithelial cells with mucin secretion were observed in all animals (Fig 4). Well differentiated adenocarcinomas were observed in 11 animals and a moderately differentiated in one cervical case. Immunohistochemically,

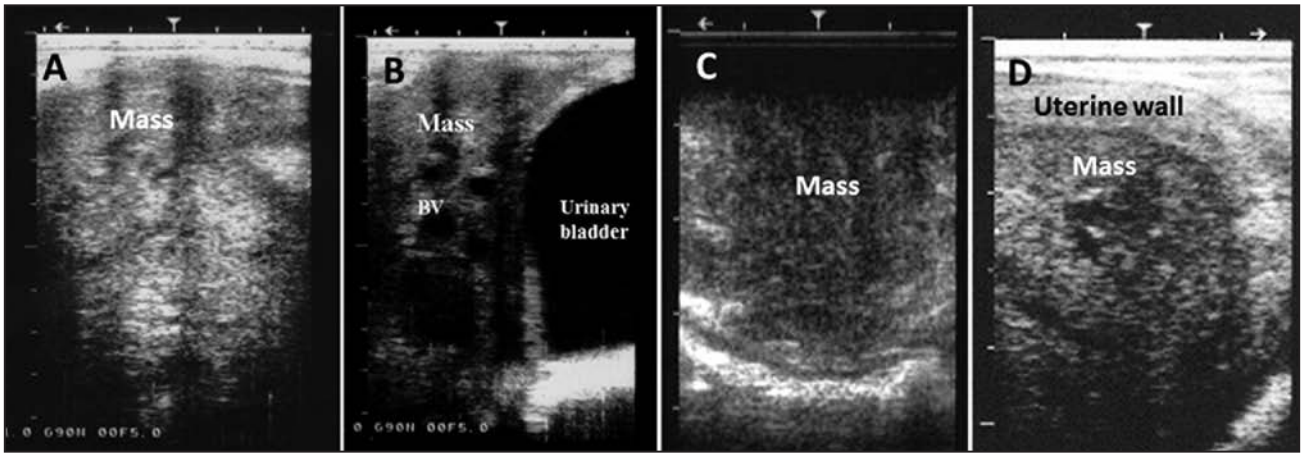

Fig 1. Sonogram of vaginal (A, B), cervical (C) and uterine (D) adenocarcinoma: the mass was echogenic, homogenous, mostly compact, but sometimes with enlarged blood vessels (BV).

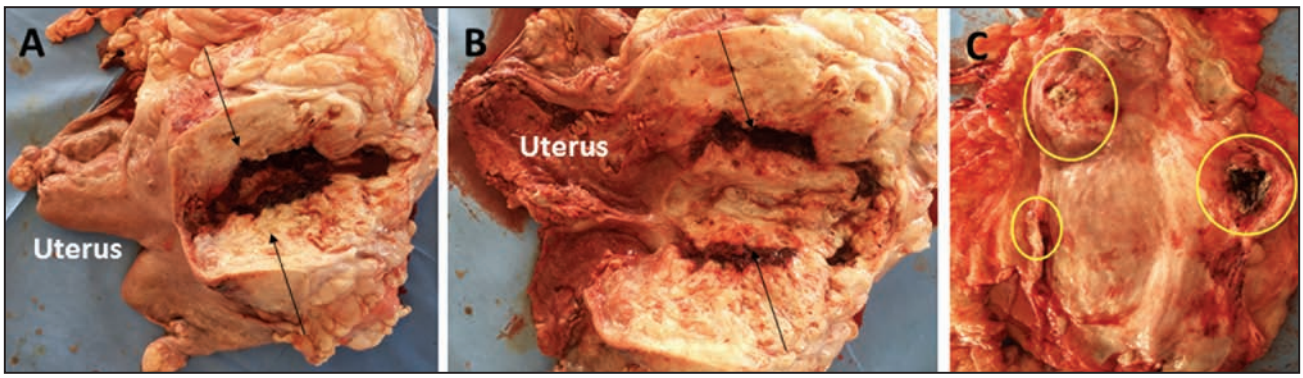

Fig 2. (A, B) Vaginal adenocarcinoma: the mass showed circumferential pattern of growth with marked narrowing of the vaginal lumen (arrows). (C) Vaginal adenocarcinoma: multifocal circumscribed ulcerated thickening in the vaginal wall.

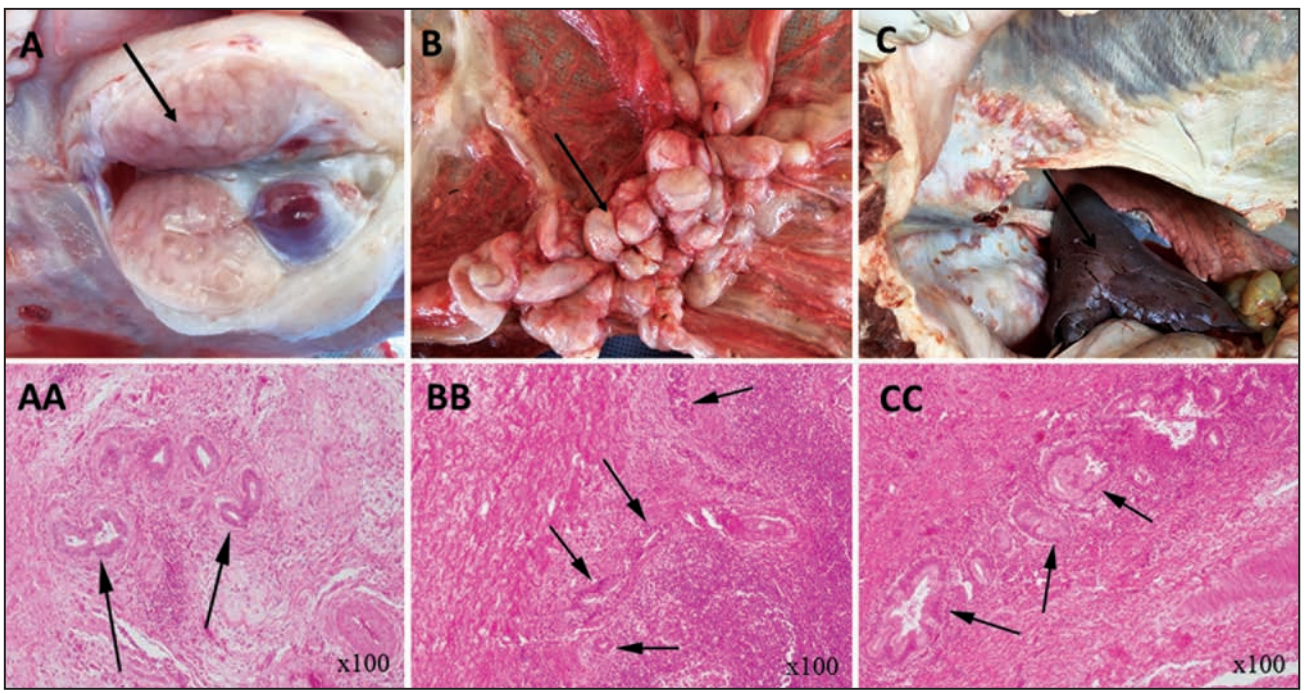

Fig 3. Metastatic vaginal adenocarcinoma. (A) The iliac lymph node was greatly enlarged (arrow). (B) Numerous small masses (1 cm in diameter) fixed to the mesentery (arrow). (C) The liver was large showed firm nodules (arrow). Multiple variable sized malignant glands (arrows) could be noticed in the mesentery (AA), lymph node (BB) and liver (CC), (H&E).

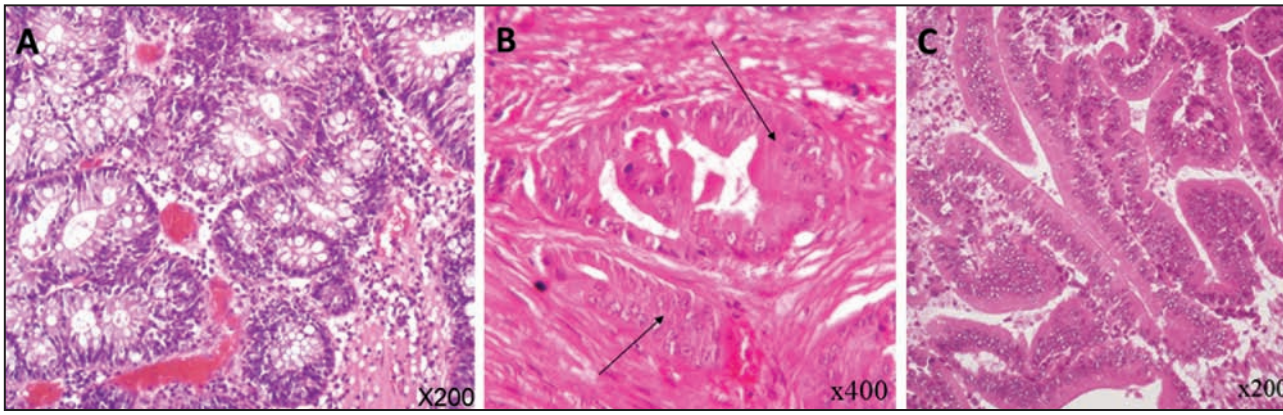

Fig 4. (A) Well differentiated invasive papillary adenocarcinoma, endometriod type, the malignant glands are lined by cuboidal tumour cells with open phase nuclei, prominent nucleoli, focal areas of haemorrhage were observed (H&E). (B) Moderately differentiated cervical adenocarcinoma (H&E). (C) Well differentiated, invasive uterine adenocarcinoma, myometrial tissue infiltrated by malignant infiltrate made up of irregularly shaped malignant glands (H&E).

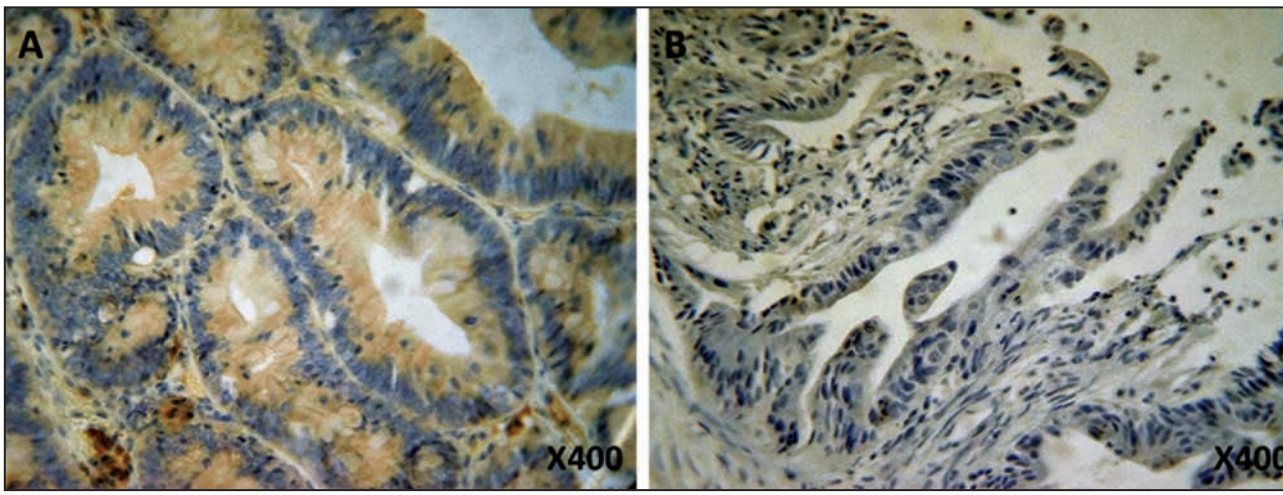

Fig 5. (A) Photomicrographs of animals with adenocarcinoma showed diffuse labelling of EMA (DAB Chromogen, H&E counter stain). (B) Photomicrographs of animals with adenocarcinoma showed labelling of CEA (DAB Chromogen, H&E counter stain).

all animals of adenocarcinoma showed diffuse cytoplasmic expression of EMA and CEA (Fig 5).

Camels with adenocarcinoma showed significant decrease in lymphocytes, monocytes, erythrocytes, haemoglobin, packed cell volume, total protein, albumin, calcium and phosphorus and increases of globulin, alkaline phosphatase and magnesium (Table 1).

The prevalence of adenocarcinoma of the genital tract of dromedary camel (0.007%) is slightly lower than that reported in cattle (0.03%) (Lucena *et al,* 2011). Clinically, affected camels showed difficult penile intromission during mating and post-mating bloody vaginal discharges were noticed. The symptoms of the tumuor differed from sero-sanguineous vaginal discharge in goat (Dockweiler *et al,* 2017), weight loss, in-appetence, mild respiratory signs, and reduced milk yield in cow (Stilwell and Peleteiro, 2010). Camels having adenocarcinoma were in middle age, however, uterine adenocarcinoma has been recorded in old cows (>6 y) (McEntee and Nielsen, 1976; Stilwell and Peleteiro, 2010).

The cause of vaginal cancer in camels is still unknown; however, papilloma virus should not be overlooked. About half of vaginal cancers in humans are associated with human papillomavirus (Viens *et al,* 2016). Papilloma viruses have been detected in camels. Non-licensed veterinary practitioners resort to place some odd materials (dates, margarine and other biological matter) inside the vagina/cervix in order to treat infertility, and these practices may additionally predispose the animal to vaginal cancers. Metastases of vaginal adenocarcinoma was detected in one case of this study. A form of contagious venereal vaginal

Table 1. Haematobiochmeical changes (mean ±SD) in camels with tumuors in compare with a control healthy group.

Blood parameter	Camels with Tumuors n=12	Control n=15	P value
White blood cell counts (10^9/L)	30.19±17.3[a]	18.85±3.58[a]	0.2
Lymphocytes (10^9/L)	1.88±0.75[a]	5.89±2.28[b]	0.003
Monocytes (10^9/L)	0.3±0.11[a]	0.89±0.55[b]	0.006
Neutrophils (10^9/L)	29.21±26.02[a]	10.79±2.89[a]	0.09
Red blood cell counts (10^{12}/L)	6.9±3.28[a]	11.38±1.31[b]	0.01
Haemoglobin concentration (g/dL)	11.48±3.34[a]	16.02±2.15[b]	0.003
Haematocrit (%)	20.5±9.27[a]	28.8±2.56[b]	0.03
Mean corpuscular volume (fl)	26±1.90[a]	25.7±1.39[a]	0.5
Mean corpuscular haemoglobin (pg)	13.40±1.07[a]	14.5±2.24[a]	0.08
mean corpuscular haemoglobin concentration (g/dL)	51.84±5[a]	57.58±8.6[a]	0.05
Albumin (G/L)	43.30±10.9[a]	60.88±2.8[b]	0.0005
Alkaline phosphatase (U/L)	55.61±23.57[a]	6.56±2.58[b]	0.001
Aspartate aminotransferase (U/L)	91.38±29.21[a]	79.5±15.5[a]	0.2
Calcium (MMOL/L)	2.1±0.16[a]	2.4±0.12[b]	0.0005
Gamma glutamyl transferase (U/L)	36.61±68.25[a]	12.18±4.98[a]	0.3
Total protein (G/L)	61.61±7.12[a]	67.25±4[b]	0.03
Globulin (G/L)	16.15±9.06[a]	6.9±3.53[b]	0.006
Blood urea nitrogen (MMOL/L)	6.36±2.37[a]	6.36±1.02[a]	0.9
Creatine kinase (U/L)	492.6±687.6[a]	138.8±20.24[a]	0.1
Phosphorus (MMOL/L)	1.55±0.32[a]	2.62±0.33[b]	0.001
Magnesium (MMOL/L)	0.82±0.16[a]	0.25±0.03[b]	0.006

[ab] Values with different superscripts in a row are significant at $P < 0.05$.

cancer has already been detected in animals (McEntee and Nielsen, 1976; Ganguly *et al,* 2016; Schlafer and Foster, 2016). This neoplasm is consisted of tumour cells creating diffuse masses or sheets underneath the mucosa. This tumour, first described 150 years ago in Europe, now has worldwide distribution (McEntee and Nielsen, 1976; Ganguly *et al,* 2016). The tumour is transmitted as intact cells by licking, by coitus, or by experimental injection. In female dogs, the neoplastic lesions are usually located at vestibule and less often at the vagina or invading the vulvar lips. Main lesions are almost always present at the junction of the vestibule and vagina. The tumour is cauliflower-like, pedunculated, nodular, papillary or multilobulated (Ganguly *et al,* 2016).

Cervical adenocarcinoma was recorded in 2 dromedary camels in this study. In fact, there were few reports describing invasive carcinomas including the cervix in domestic animals, but invasion from either a uterine or vaginal carcinoma could not be excluded (McEntee and Nielsen, 1976; Schlafer and Foster, 2016). Worldwide, cervical cancer is the fourth-most common cause of cancer and death from cancer in women (WHO, 2014). Human papillomavirus (HPV) infection appears to be involved in the development of more than 90% of cervical cancers (Kumar *et al,* 2007).

Uterine adenocarcinoma is rare in all species of domestic animal except cows and rabbits (Bastianello, 1982; Kufe, 2009; Stilwell and Peleteiro, 2010; Agnew and MacLachlan, 2016). In cows, it represents one of the 3 most common neoplasms encountered, following lymphoma and eye cancer (McEntee and Nielsen, 1976; Agnew and MacLachlan, 2016). Based on the histopathology, cancer glands invaded the myometrial layer. Similarly, the bovine form is a scirrhous adenocarcinoma that diffusely invades all layers of the wall (McEntee and Nielsen, 1976; Bastianello, 1982; Stilwell and Peleteiro, 2010). In contrast, in bitches and cats, the tumour is a non-sclerosing adenocarcinoma that typically produces a distinct mass with distortion of the mucosa (McEntee and Nielsen, 1976; Schlafer and Foster, 2016).

According to the present results, all animals of adenocarcinoma showed diffuse cytoplasmic expression of EMA and CEA. Epithelial membrane antigen (EMA) is an excellent marker of epithelial

differentiation, appears to be highly reliable for discriminating between poorly differentiated carcinomas and malignant lymphomas, and is especially helpful in characterising small cell anaplastic carcinomas (Pinkus and Kurtin, 1985). Antibodies to carcinoembryonic antigen (CEA) are commonly used in immunohistochemistry to identify cells expressing the glycoprotein in tissue samples. CEA is primarily expressed in cells of tumours but they are particularly associated with the adenocarcinomas, such as those arising in the colon, lung, breast, stomach, or pancreas. It can therefore be used to distinguish between these and other similar cancers (Ballesta *et al,* 1995).

A decrease in the number of lymphocytes and monocytes in the blood was observed in animals of present study in several diseases, but viral infections and undernutrition were the utmost common (Thrall *et al,* 2012; Roland *et al,* 2014; Vap and Bohn, 2015). The decreased erythrocytes, haemoglobin and packed cell volume may be attributed to the chronic blood loss from the haemorrhagic surface of the tumuors (Hawkey and Gulland, 1988; Stevens *et al,* 2012). The chronic long-standing nature of the tumours resulted in a significant increase in globulin but a decrease in total protein and albumin were seen (Hawkey and Gulland, 1988; Stockham and Scott, 2008; Thrall *et al,* 2012). The tumours also led to malnutrition that resulted in significant decrease of calcium and phosphorus (Garry *et al,* 1994; Tornquist, 2009; Thrall *et al,* 2012). Similar to the present study, an increase in serum alkaline phosphatase activity has been observed in human patients with ovarian cancer (Ben-Arie *et al,* 1999), metastatic breast and colon cancers (Walach and Gur, 1996; Usoro *et al,* 2010), osteosarcoma (Shimose *et al,* 2014) and skeletal metastatic cancer (Jin *et al,* 2015).

In conclusion, this is the first report pronouncing the occurrence of adenocarcinoma in the tubular genital tract of female dromedary camels. The vagina was the most frequent affected organ. Vaginal bleeding and anaemia were the commonly associated clinical and laboratory findings. Ultrasonography could be helpful in the primary diagnosis, especially in large sized masses. Most forms were diffuse and invasive. Further investigations should be focussed on associated metastasis and risk factors.

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BOOK REVIEW

Book: Camel Clinical Biochemistry and Haematology
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The new book Camel Clinical Biochemistry and Hematology authored by Bernard Faye and Mohammed Bengoumi has fulfilled a long felt void on this important topic from clinical health and nutrition point of view. The book is spread in more than 345 pages and 10 chapters. Every chapter is further divided into subtopics, conclusions and references. The book will prove a milestone to the researchers and clinicians as it contains normal or reference values of several haematobiochemical parameters, hormones, enzymes, macro and trace minerals etc. The book starts with a chapter on restraining technique which is required for blood sampling or biopsies. The instruments or technique used for estimating diverse haematobiochemical parameters are also named appropriately, i.e. Coulter Counter, cyanide method, haemocytometer of Neubauer, automatic blood cell counter etc.

Reading all the chapters arouse your interest as they unfold many mysteries specific to this species of animal, i.e. camels are able to withstand to haemoconcentration during water deprivation, they are able to lose 25% of its total body water without manifesting major symptoms of dehydration,etc. The chapter on Energetic metabolism is interesting and new because it explains that how camels have higher glycemia which is different from other ruminants. Chapter also explains that how camel is remarkable by its ability to well manage the storage or mobilisation of hump fat during various periods of nutrition. Camel being pseudoruminant, the protein metabolism is marked by the high level of nitrogen recycling, hence nitrogen parameters are important and these are discussed in detail. The relevant chapter gives good information on proteins like haptoglobin, fibrinogen, or pepsinogen whose references are believed to be scarce. Normal values in the main enzymes (ASAT, ALAT, ALP, and LDH) in camel are given which helps in diagnosis of camel in health and disease. The chapter concludes that blood enzymes in camel follow similar patterns than in other species.

The chapter on macro-minerals and electrolytes explains hydro-electrolytic balance and biological significance of values of various macro minerals. The adaptative advantage of the camel in desert areas during dehydration is explained nicely through the changes in electrolytic balance process and the role of the kidney. Another interesting chapter is on trace minerals and it explains the biological role of trace elements, the deficiency and excess (toxicity) of these minerals and their role in adaptation to desert life. The role of vitamins in camel health has been highlighted through an exclusive chapter on it. The level of vitamin D in blood and vitamin C in milk is quite higher in camels. Another interesting chapter is on hormones. Reproductive hormones (LH, FSH, testosterone, and estradiol) including prostaglandins, prolactin, and oxytocin, cortisol and the hormones involved in the regulation of the metabolism (thyroid hormones) and those for homeostasis (aldosterone and vasopressin) are appropriately discussed. The role of hormones during reproduction, stress and adaptation in extreme climates is discussed.

The last chapter on general conclusions highlights (among others) regarding the specificity of camel related to clinical and nutritional pathology, i.e. predominance of polynuclear neutrophils in its white cell formula, maintenance of hematocrit in case of physical effort, osmolality resistance, relative hyperglycemia, absence of ketone bodies, low plasma cholesterol in plasma concentration, susceptibility to hyperuremia, thermoresistance of alkaline phosphatises, maintenance of metalloenzyme activities in case of mineral deficiency, maintenance of electrolyte balance in dehydrated animals, low plasma zinc concentration in supplemented camel and higher sensitivity to selenium toxicosis.

The book is an excellent resource of literature on clinical biochemistry and haematology of camel and enlisted references occupy one third of volume of book which will prove as a ready reckoner to the clinicians and researchers. All the parameters and their values are depicted through several pertinent tables and graphs. Authors of the book deserve congratulations for designing most informative chapters containing specific and exhaustive information. I am sure that this book would find a space in shelf of library of camel vets, scientists and institutions.

Dr. T.K. Gahlot
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TUMOURS IN DROMEDARY CAMELS: PREVALENCE, TYPES AND LOCATIONS

F.A. Alsobayil[1], A. Ali[1,2], D.R. Derar[1,2], M. Tharwat[1], A.F. Ahmed[2] and M. Khodeir[3]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine,
[3]Department of Pathology, College of Medicine, Qassim University, Saudi Arabia
[2]Department of Theriogenology and Department of Surgery, Radiology and Anesthesiology,
Faculty of Veterinary Medicine, Assiut University, Egypt

ABSTRACT

The incidence, types and locations of tumours in 9576 dromedary camels were determined at the Veterinary Teaching Hospital of Qassim University. Grossly, tumour like growth was seen in 75 cases but histopathological examination confirmed the tumour in 59 cases (incidence 59/9576, 0.006%). Types of the tumours diagnosed were squamous cell carcinoma (*n*=22), fibroma (*n*=13), adenocarcinoma (*n*=13), fibromyxosarcoma (*n*=2), leiomyoma (*n*=2), angiosarcoma (*n*=1), schwannoma (*n*=1), lipoma (*n*=1), microcystic adnexal carcinoma (*n*=1), renal cell carcinoma (*n*=1), sertoli-leydig cell tumour (*n*=1) and granulosa cell tumour (*n*=1). These tumours were located at the limbs (*n*=14), vagina (*n*=9), abdominal wall (*n*=8), head (*n*=7), sacrum (*n*=4), neck (*n*=4), intraoral (*n*=3), cervix (*n*=2), ovary (*n*=2), rectum (*n*=2), uterus (*n*=1), soft palate (*n*=1), kidney (*n*=1) and compartment 3 (*n*=1). Females were more affected than males (54 vs 5). Age of the affected animals ranged between 4 months to 18 years. In conclusion, squamous cell carcinoma, fibroma and adenocarcinoma are the common types of tumours occurring in camel.

Key words: Adenocarcinoma, dromedary camels, fibroma, squamous cell carcinoma, tumours

The incidence of tumours reported in slaughter houses surveys indicates that tumours are most common in cattle (0.23%) and are uncommon in sheep (0.002%), goats (0.009%) and pigs (0.004%) (Valentine, 2004). Several studies have been done to determine the common types of tumours in sheep, goats, cattle and horses in Saudi Arabia (Al-Sobayil *et al,* 1993; Al-Sobayil *et al,* 2004a,b; Al-Sobayil *et al,* 2005; Ahmed 2011; Ahmed and Hassanein, 2012).

Neoplasms of the skin and subcutaneous tissues are the most frequently recognised neoplastic disorders in domestic animals (Hargis *et al,* 1977; Valentine, 2004; Ginn *et al,* 2007). Case reports of squamous cell carcinomas (Ramadan and Elhassan, 1989; Tageldin and Omar, 1986) and basal cell carcinoma (Al-Hizab *et al,* 2007; Fowler, 2010) have been described in camels. On the other hand, cases of internal neoplasia such as renal cell carcinoma (Vitovec, 1982), bronchoalveolar adenocarcinoma (Gameel *et al,* 1998), salivary fibro-adenocarcino-sarcoma (Ramadan *et al,* 2001), rhabdomyosarcoma (Zakia-Mohammed *et al,* 2007), seminoma with cholangiocarcinoma (Birincioglu *et al,* 2008; Ali *et al,* 2013a) and granulosa cell tumour (Ali *et al,* 2013b) have also been reported in camels.

Immunohistochemistry adds more depth for diagnosis of tumours. In addition, it has advantage of detecting status of important genes which have pivotal role for neoplasm pathogenesis irrespective of mechanism of gene activation or inactivation. Therefore, it provides broad information than would be obtained by any single genetic analysis alone (Fricke *et al,* 2003; Khodeir, 2005). Fortunately, all these valuable data can be demonstrated by reliable, easily performed techniques on paraffin embedded tumour specimens (Khodeir, 2005). The final goal is to cure the disease or considerably prolong life while improving the patient's quality of life. Immunohistochemical diagnosis of camel tumours has also been reported (Weiss and Walz, 2009; Khodakaram and Khordadmehr , 2011).

The objective of the present study was to determine the incidence, their common types and locations of tumours in camels of Kingdom of Saudi Arabia.

Materials and Methods

A total of 9576 dromedary camels were examined at the Veterinary Teaching Hospital of Qassim University in the Kingdom of Saudi Arabia for the occurrence of external or internal tumours.

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Biopsy from the suspected tumours was taken for histopathological and immunohistochemical examination. Histopathological examination was performed by hematoxylin and eosin (H&E) stain and diagnosis was done.

Immunostaining was performed using the avidin–biotin– peroxidase complex (ABC) method. Sections were then rinsed and incubated with primary antibodies cytokeratins (CK5/6) and Pan cytokeratin for squamous cell carcinoma cases. Adenocarcinoma cases were incubated with primary antibodies EMA, CEA. The immunostaining was performed on an automated immunostainer with appropriate positive and negative controls. DAB was used as a chromogen (3-3\ diaminobenzidine tetrahydrochloride) and Mayer's hematoxylin as a counter stain.

Results

Grossly, tumours like growths were diagnosed in 75 cases. However, after histopathological microscopic examination, 59 cases were confirmed to be tumours (incidence 59/9576, 0.006%). Types of the tumours were listed in Table (1) and illustrated in Figs (1-5). Squamous cell carcinoma, adenocarcinoma and fibroma were the common types of tumours occuring in camel.

Table 1. Types of tumours in dromedary camels.

Type of tumour	Number of cases	Incidence (Per cent)
Squamous cell carcinoma	22	37.3
Adenocarcinoma	13	22
Fibroma	13	22
Fibromyxosarcoma	2	3.4
Leiomyoma	2	3.4
Angiosarcoma	1	1.7
Schwannoma	1	1.7
Lipoma	1	1.7
Microcystic adnexal carcinoma	1	1.7
Renal cell carcinoma	1	1.7
Sertoli-Leydig cell tumour	1	1.7
Granulosa cell tumour	1	1.7
Total	59	100

Cases of adenocarcinoma showed diffuse expression of EMA (Fig 6) and CEA (Fig 7), while cases of squamous cell carcinoma showing diffuse strong expression of pancytokeratin (Fig 8) and cytokeratin5/6 (Fig 9).

The camel tumours were located at different parts and organs of the camel body (Table 2, Figs

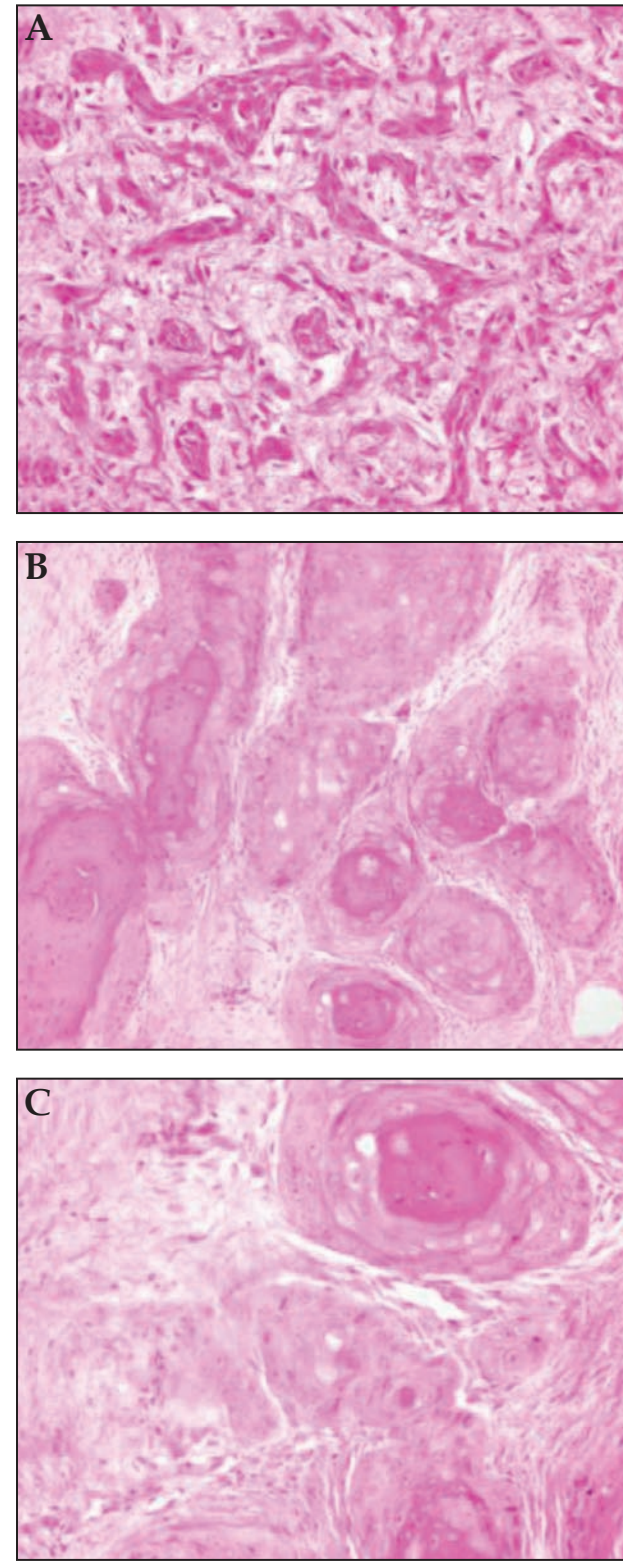

Fig 1. Histopathology of camel tumours: A: Sections in left side head tumour showing microcytic adnexal carcinoma with cords and nests of bland keratinocytes and ductal differentiation (H&E stain X 200), B and C: Abdominal tumour showing well differentiated keratinising squamous cell carcinoma multiple cell nests and keratin pearl formation (H&E X 200 and 400).

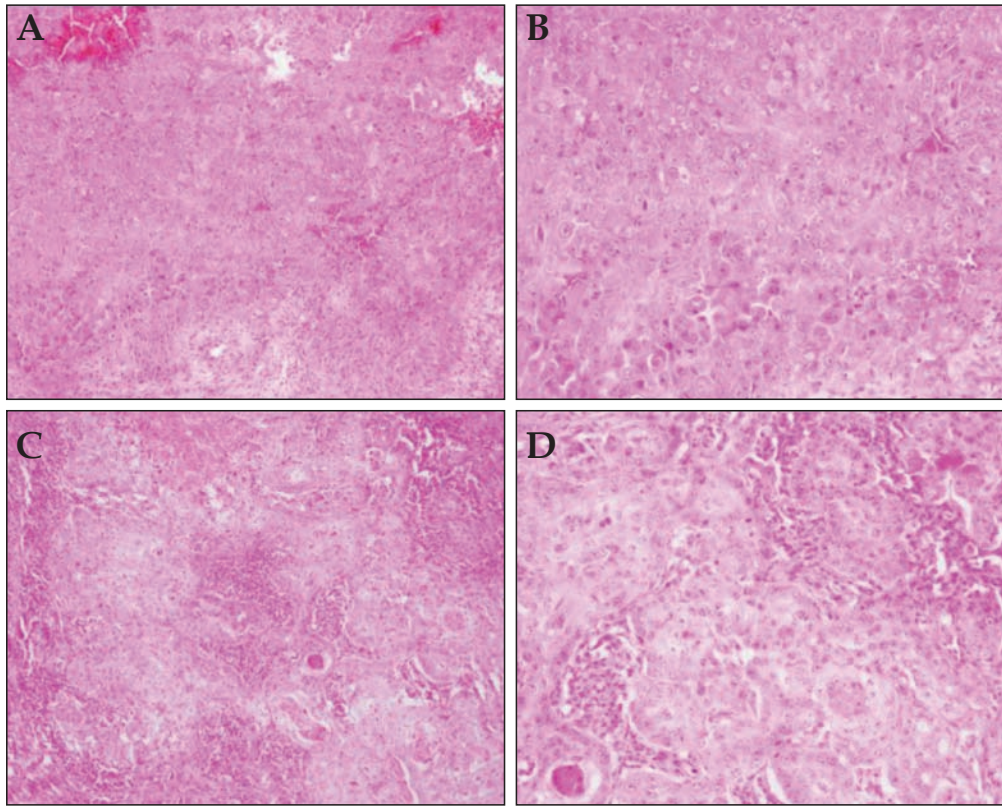

Fig 2. Histopathology of camel tumours: Different cases of moderately differentiated squamous cell carcinoma showing malignant epithelial cells with hyperchromasia and pleomorphism. A and B: non keratinising sheets of cells (H&E X 200 and 400), C and D: nests of malignant epithelial cells with focal keratinisation (H&E X 200 and 400).

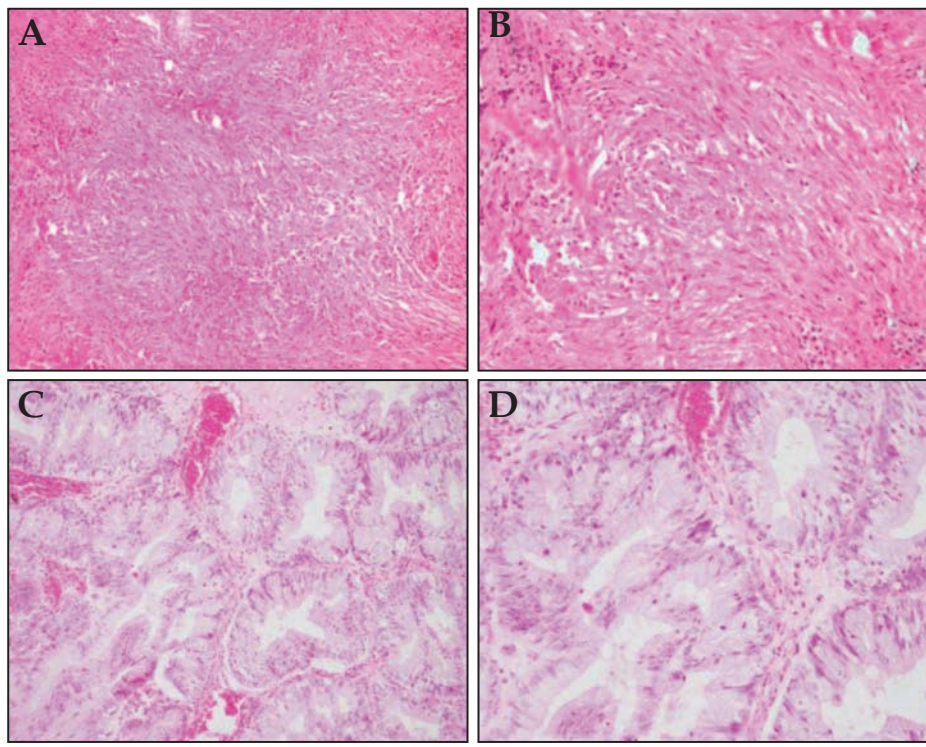

Fig 3. Histopathology of different cases of camel vaginal tumours: A and B: Leiomyoma showing interlacing bundles of bland looking smooth muscles with rod shaped nuclei (H&E X 200 and 400), C and D: well differentiated adenocarcinoma showing variable sized infiltrating mucin secreting malignant glands showing cellular and architectural atypia lined by columnar cells (H&E X 200 and 400).

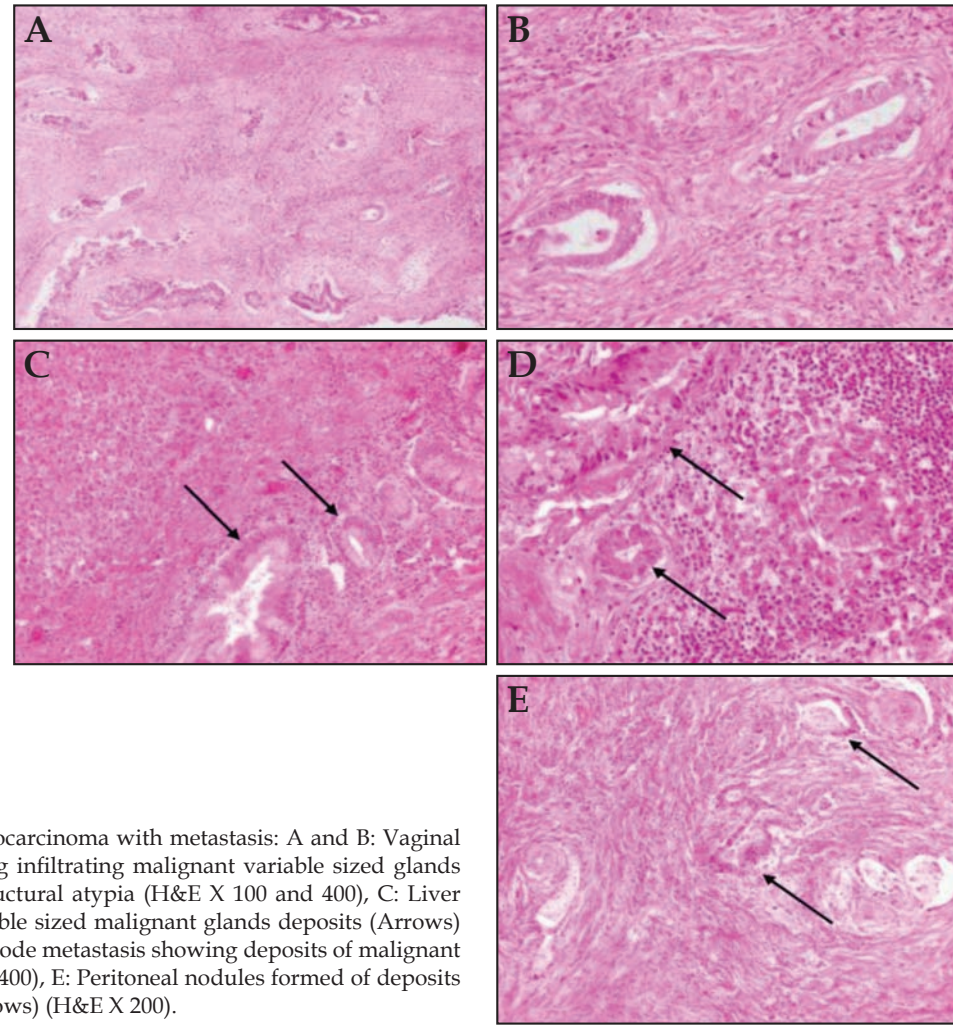

Fig 4. The case of vaginal adenocarcinoma with metastasis: A and B: Vaginal Adenocarcinoma showing infiltrating malignant variable sized glands showing cellular and structural atypia (H&E X 100 and 400), C: Liver metastasis showing variable sized malignant glands deposits (Arrows) (H&E X 400), D: Lymph node metastasis showing deposits of malignant glands (Arrows) (H&E X 400), E: Peritoneal nodules formed of deposits of malignant glands (Arrows) (H&E X 200).

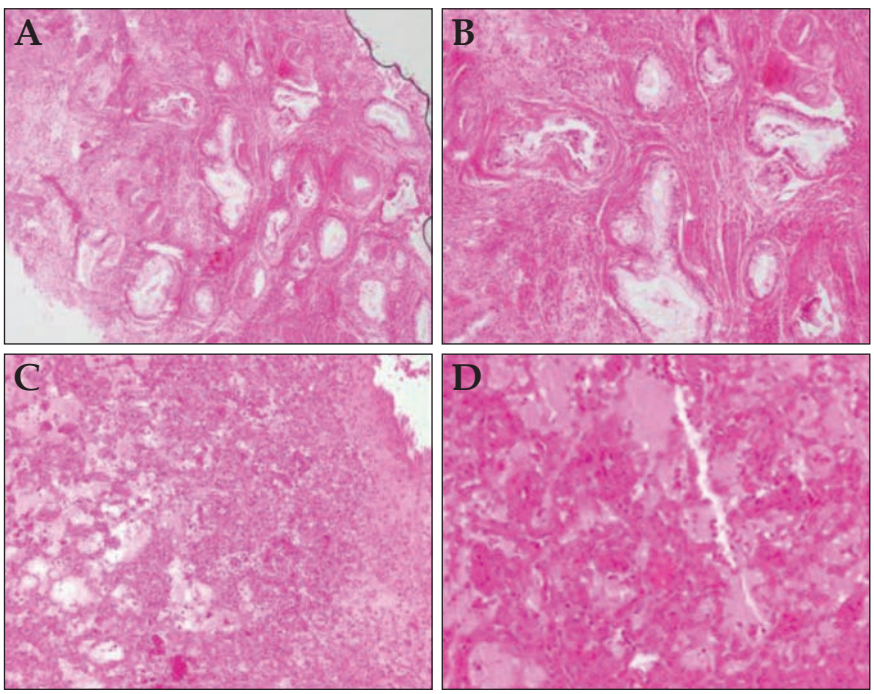

Fig 5. A and B: Well differentiated cervical adenocarcinoma showing infiltrating variable sized glands lined by malignant columnar epithelial cells with mucin secretion (H&E X 100 and 200), C and D: Renal cell carcinoma showing well differentiated tubules separated by cellular stroma (H&E X 100 and 400).

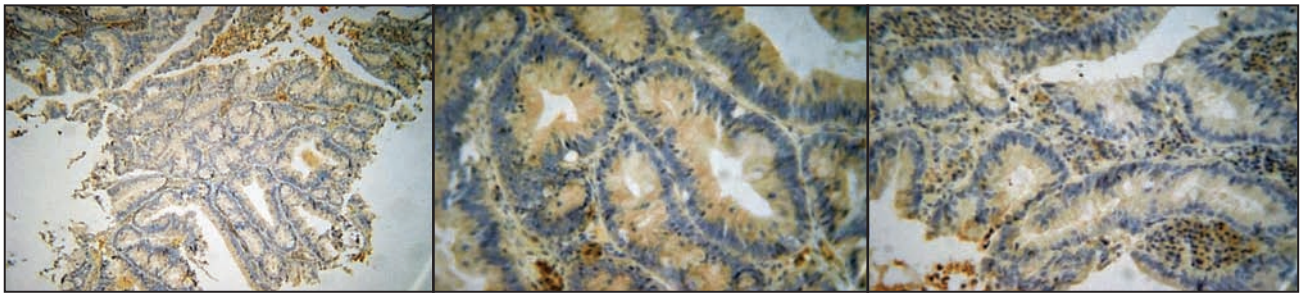

Fig 6. Photomicrographs of cases of adenocarcinoma showing diffuse expression of EMA (DAB Chromogen, Hx&E counter stain).

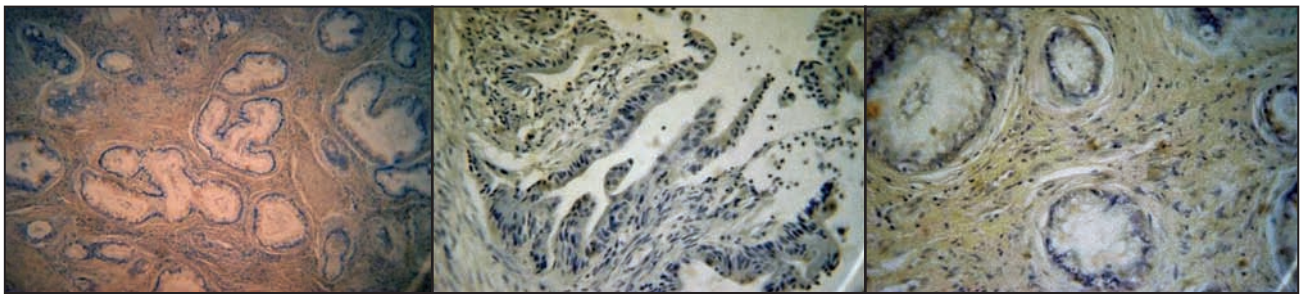

Fig 7. Photomicrographs of cases of adenocarcinoma showing expression of CEA (DAB Chromogen, Hx&E counter stain).

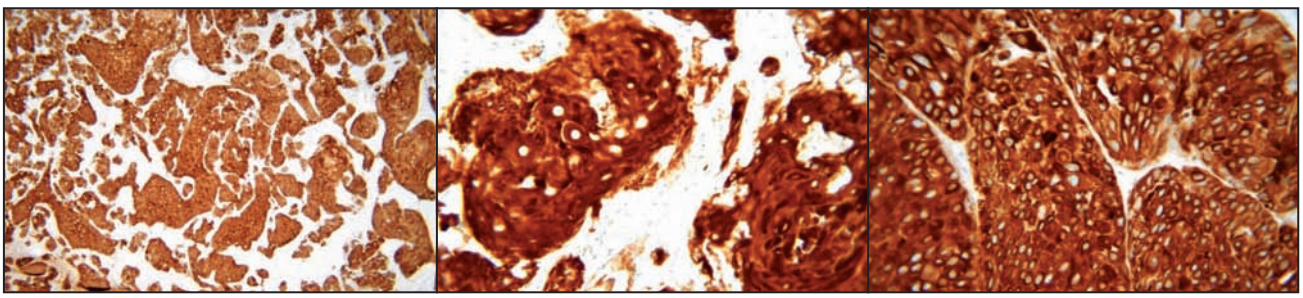

Fig 8. Photomicrographs of different cases of squamous cell carcinoma showing diffuse strong expression Pancytokeratin (DAB Chromogen, Hx&E counter stain x).

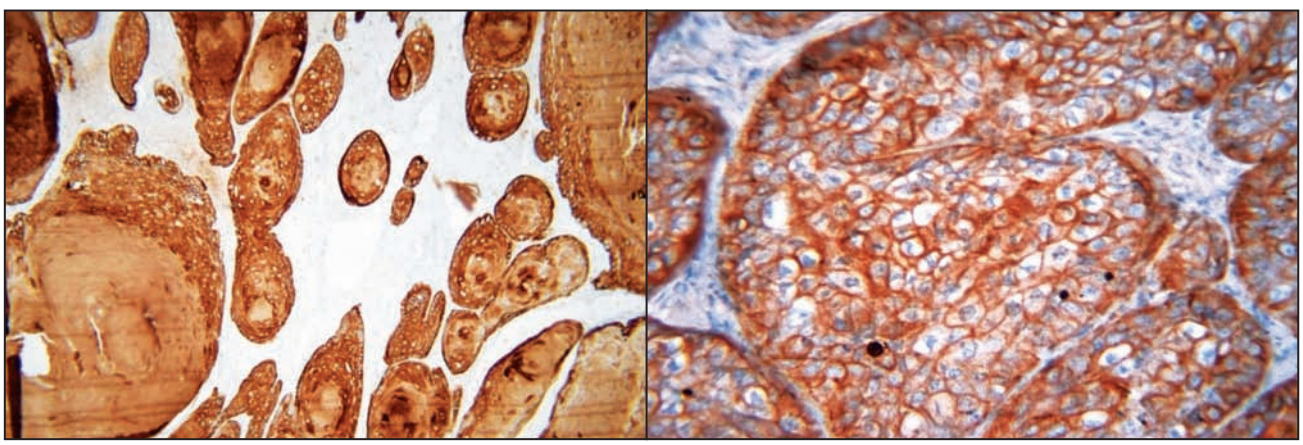

Fig 9. Photomicrographs of different cases of squamous cell carcinoma showing diffuse strong expression of cytokeratin 5/6 ((DAB Chromogen, Hx & E counter stain x).

10, 11). Only one case of metastasis was observed. Adenocarcinoma was observed firstly in the vagina. After postmortem examination, metastasis was observed in the regional and mesenteric lymph nodes (LN) and in the liver (Fig 11, H, I). Female camels showed tumours more frequently than males (54 vs. 5). Age of the affected animals ranged between 4 months to 18 years.

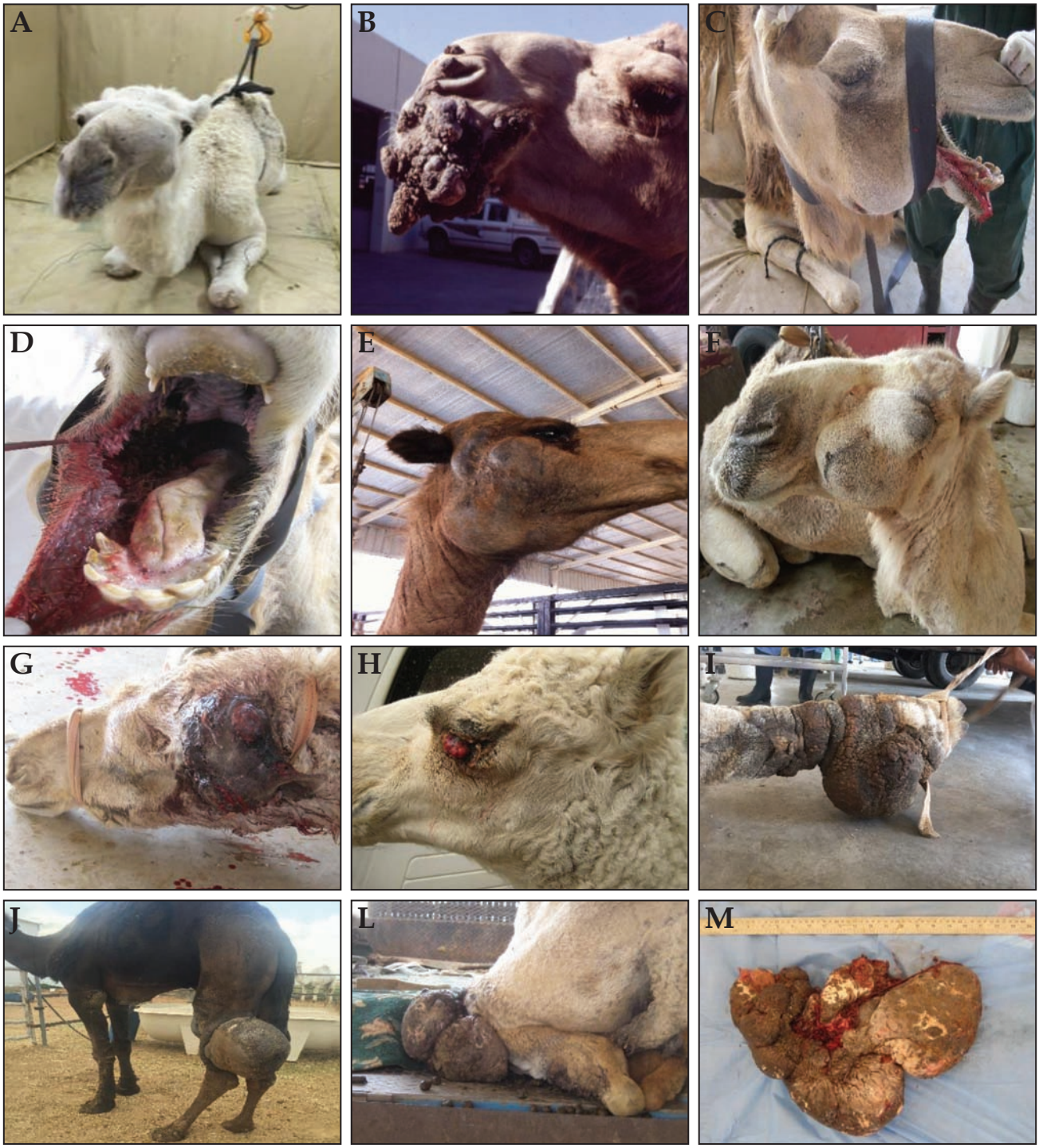

Fig 10. Location of camel tumours at the head (A-G), eye (H) and leg (I-M).

Discussion

The prevalence of camel tumours as reported in this study (0.006%) is extremely lower than that reported in cattle (0.23%), but nearly similar to that reported in sheep (0.002%), goats (0.009%) and pigs (0.004%) (Valentine, 2004). It was universally known that cattle are the most animal species affected with tumours.

Based on the present results, squamous cell carcinoma, fibroma and adenocarcinoma are the common types of camel tumours and the limbs, vagina, abdominal wall and head were the most affected organs. Neoplasms of the skin and subcutaneous tissues have been reported as the most frequently recognised neoplastic disorders

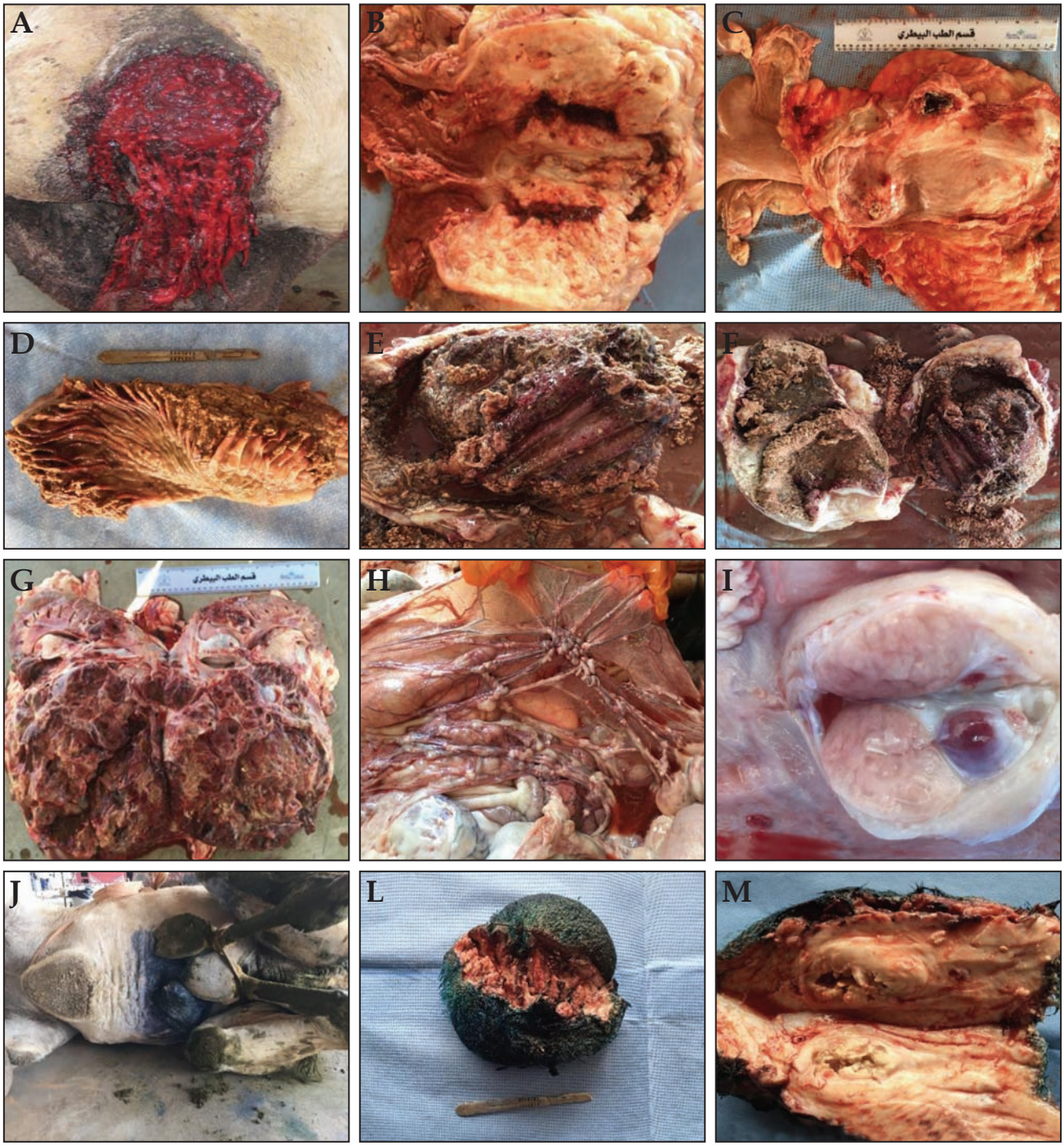

Fig 11. Location of camel tumours in the rectum (A), vagina and cervix (B, C), omasum and abomasum (D, E, F), kidney (G), mesenteric and regional LNs (H, I) and at the abdominal wall (L, M).

in domestic animals (Hargis *et al,* 1977; Valentine, 2004; Ginn *et al,* 2007). Case reports of squamous cell carcinomas (Ramadan and Elhassan, 1989; Tageldin and Omar, 1986) and basal cell carcinoma (Al-Hizab *et al,* 2007; Fowler, 2010) have been described in camels. On the other hand, cases of internal neoplasia such as renal cell carcinoma (Vitovec, 1982), bronchoalveolar adenocarcinoma (Gameel *et al,* 1998), salivary fibro-adenocarcino-sarcoma (Ramadan *et al,* 2001), rhabdomyosarcoma (Zakia-Mohammed *et al,* 2007), seminoma with cholangiocarcinoma (Birincioglu *et al,* 2008, Ali *et al,* 2013a) and granulosa cell tumour (Ali *et al,* 2013b) have also been reported in camels.

Table 2. Location of the tumours in dromedary camels.

Location	Number of camels	Incidence (Per cent)
Limbs	14	23.7
Vagina	9	15.2
Abdominal wall	8	13.6
Head	7	11.9
Sacrum	4	6.8
Neck	4	6.8
Intraoral	3	5.1
Cervix	2	3.4
Ovary	2	3.4
Rectum	2	3.4
Soft palate	1	1.7
Kidney	1	1.7
Uterus	1	1.7
Omasum and abomasum	1	1.7
Total	59	100

According to a recent report 4 different types of tumours were diagnosed in camels, which were SCC, fibroma, lipoma and fibromyxosarcoma. The most common type of tumour in Maghateer (white coloured coat) and Majaheem (dark brown to black coat) breeds were SCC and fibroma, respectively. In our study, cancer was common in white camels (n=33). A case of lipoma in the left ischiorectal fossa has been reported in camel (Kaswan *et al,* 2013).

Spontaneously or naturally occurring tumours in domestic animals are of particular interest for comparative studies. Prolonged and continuous exposure to sunlight is the best known etiologic factor and a sunlight-induced skin cancer relationship has been established in several domestic species (Valentine, 2006). Ultraviolet radiation (UV) is the major etiologic agent in skin cancer development (Storm and Yamamura, 1997), especially squamous cell carcinoma in cows, goats, sheep, cats and dogs (Nicola *et al,* 1992). However, other causes of cancers have been reported such as sex hormones, environmental pollution, radiation and viruses like papilloma virus.

According to the present results, age of the affected animals ranged between 4 months to 18 but most occurred during adulthood or old age. The overall occurrence of tumours cannot be determined with certainty because food animals are slaughtered for meat production at an early age, before tumours have time to develop. In most reported series of neoplasms there is a peak age of tumour incidence for each species. The peak age of incidence has not been clearly established in the food animals (Goldschmidt and Hendrick, 2002).

Female camels showed tumours more frequently than males; this might be attributed to slaughtering of male camels at young age for meat production, if compared to the females that are usually reared till old ages. However, other factors such as sex hormonal might have an effect on the higher occurrence of cancer in females than males.

One case of metastasis was observed. Adenocarcinoma was observed firstly in the vagina, after postmortem examination metastasis was observed in the regional and mesenteric lymph nodes and in the liver. To our knowledge this is the first report of spreading of vaginal cancer to other organs in camels.

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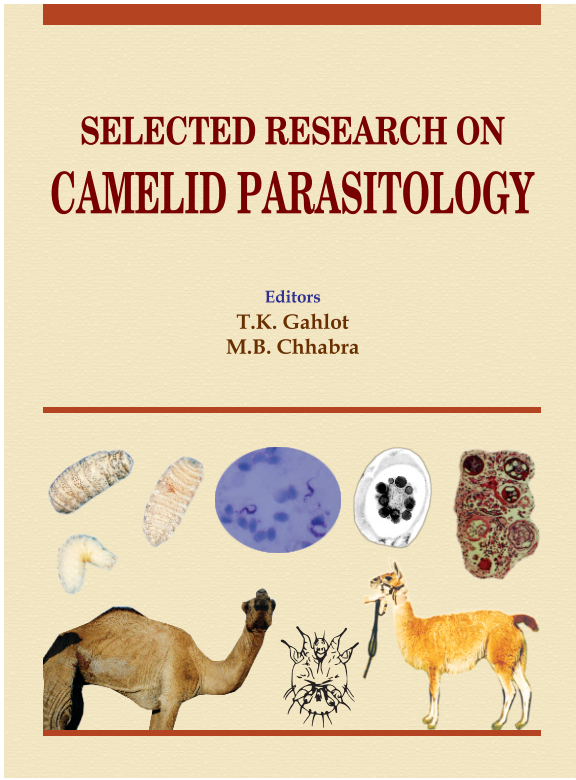

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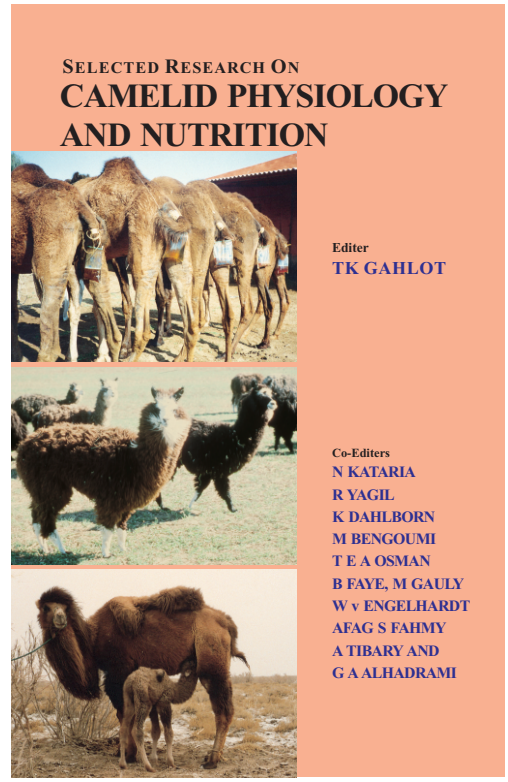

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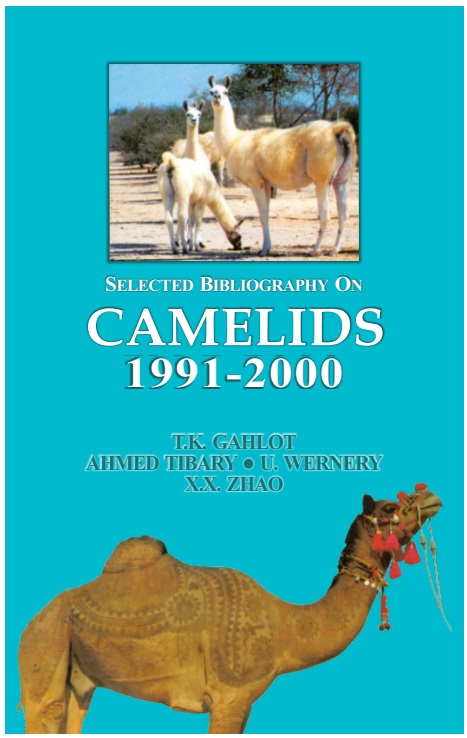

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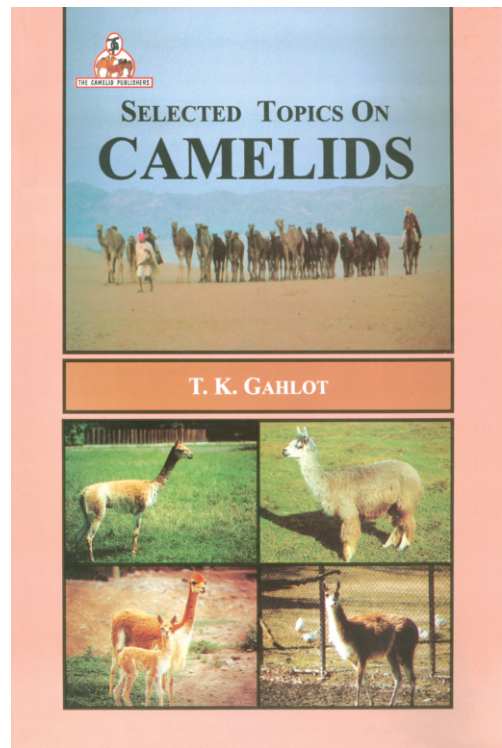

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PREPARATION OF ANTI-DIARRHOEA IMMUNE CAMEL MILK AND THE DETERMINATION OF THE ANTIGEN-BINDING ACTIVITY OF ITS SPECIFIC IgG SUBCLASSES

Li Yi[1,2], Liang Ming[1,2], Yisi Ai[3], Le Hai[1,2], Jing He[1,2], Xiang-yu Qiao[1,2] and Rimutu Ji[1,2]

[1]Key Laboratory of Dairy Biotechnology and Engineering Ministry of Education,
College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, China
[2]Inner Mongolia Institute of Camel Research, Alxa Right Banner, China
[3]College of Industrial Technology, Mongolian University of Science and Technology, Ulaanbaater, Mongolia

ABSTRACT

Infectious diarrhoea caused by a variety of pathogens such as bacteria, viruses and fungus, is a common and frequent-occurring disease that endangers human health. Immune milk containing specific immunoglobulins (IgG) obtained by using various pathogens to immunise pregnant animals has been widely used in the intestinal infectious diseases in recent decades. IgG in camel milk has found three subclasses are the same as those found in camel serum, of which IgG2 and IgG3 are heavy-chain antibodies (HCAbs). HCAbs are lacking light chains and constant domain 1 (CH1), but with complete antigen binding capacity and more stable than conventional IgG. It can pass the blood brain barrier and able to penetrate into tissues and cells that other animals' Igs were unable to do. In this study, we aimed to prepare immune camel milk from immunisation of lactating camels with a multivalent vaccine consisting of 3 strains of pathogenic diarrhoea bacteria. Then, we established indirect Enzyme-linked immunosorbent assay (ELISA) protocol to detect the antigen-binding activity of each IgG subclass in the serum and whey. The relative titres of immune serum (1:5000) and whey (1:1000) were significantly increased after the immunisation. The results of indirect ELISA showed that immunised camels produced both conventional and HCAbs specific to the antigen. The titres of each IgG subclass in the immune whey almost reached 1:64000. Our findings indicated that immunisation of lactating camels can produce immune milk containing specific IgGs, which may be exploited in therapies for prevention of pathogenic-induced diarrhoea.

Key words: Anti-diarrhoea, HCAbs, camel milk, immunoglobins, intestinal disease

Immunoglobulins (Igs) or antibodies are a family of globular proteins with antimicrobial and protective bioactivities (Gapper *et al,* 2007). They are found in blood serum, mucosal secretions and mammary secretions (colostrum and milk) with different concentrations. Based on their distinct structural and biological properties that are divided into five classes including IgA, IgD, IgE, IgG and IgM. The most predominant and abundant immunoglobulin in mammalian is the IgG class. It has the longest serum half-life of all immunoglobulin classes. It performs multifunctional properties, such as antimicrobial protection against pathogenic microorganisms, complement activation and especially, conferring passive immunity to the neonate until its own immune system is developed (Butler, 1983; Korhonen *et al,* 2000; Mix *et al,* 2006; Gapper *et al,* 2007; Schroeder and Cavacini, 2009). Immunisation of pregnant mammals with various pathogens can obtain a kind of milk enriched with target IgGs, called as hyperimmune milk. It is obtained by stimulating immune response of B lymphocytes, thereby leading to increase in serum specific IgG production and excreting into colostrum (Goldsby *et al,* 2000; Xu *et al,* 2006). In recent decades, hyperimmune milk has been investigated as a passive immunotherapeutic agent against various human gut pathogens, including *Escherichia coli* (Xu *et al,* 2006; Huang *et al,* 2008; Otto *et al,* 2011; Sears *et al,* 2017), *Helicobacter phlori* (Casswall *et al,* 1998), Shigellosis (Ashraf *et al,* 2001), Salmonellosis (Xu *et al,* 2006; Huang *et al,* 2008), Rotavirus (Mitra *et al,*1995), *Clostridium difficile* (Mattila *et al,* 2008; Wang *et al,* 2014) and Cryptosporidiosis (Tzipori *et al,* 1986).

Camel milk called as white gold of desert and has potential medicinal properties, such as

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antimicrobial, antiviral, anti-oxidative, antidiabetic, anticancer and antidiarrhoea (Yagil *et al,* 2013; Abrhaley and Leta, 2017). It is more similar with human milk than other mammals' milk because it contains low sugar and cholesterol, high minerals, high vitamin C and lots of protective proteins like as lysozyme, lactoperoxidase, lactoferrin and immunoglobulins (Kumar *et al,* 2015). Immunoglobulins, mainly IgG, in camelids differs from all other known antibodies, which consists of 3 subclasses, namely IgG_1, IgG_2 and IgG_3. The 2 latter subclasses are devoid of light chain and CH1 domain, called heavy-chain antibody (HCAbs) (Hamers-Casterman *et al,* 1993). The HCAbs have complete antigen binding capacity, enzyme and toxins neutralisation capacity. Compared with conventional antibodies, HCAbs were with smaller molecular weight, higher stability, stronger affinity and solubility and interfered with several biological processes (Riechmann and Muyldermans, 1999; Muyldermans *et al,* 2001; Jirimutu *et al,* 2012). El-Agamy *et al (*2009) has been reported that the level of IgG in camel milk was higher among goat, cow, sheep, buffalo and human milk. Additionally, camel milk lacks of β-lactoglobulin (β-LG) which caused allergenic properties (El-Hatmi *et al,* 2007). As shown above, camel milk has greater advantages for development of immune milk than bovine milk. However, to date, there was no relevant reports on the camel immune milk.

In this study, we attempted to immunise the lactating bactrian camels with highly concentrated multivalent vaccine of pathogenic bacteria causing diarrhoea to test the production of specific immunoglobulins in camel milk against the 3 strains of pathogens. Further, an indirect ELISA protocol was established to detect the antigen-binding activity of each IgG subclass in the serum and whey.

Materials and Methods

Vaccine preparation

Three different species of pathogenic bacteria including one strain of *Escherichia coli* (CMCC44104), one strain of *Salmonella typhi* (CMCC50115) and one strain of *Shigella dysenteriae* (CMCC51105) originated from human intestinal tract (China General Microbiological Culture Collection Centre, Beijing 100101, China) were prepared as vaccine by methods described previously (Xu *et al,* 2006; Zhang *et al,* 2004). Briefly, the bacteria were activated and cultured separately in nutrient broth (NB) for 24h at 37°C. Then added volume fraction of 0.4% formaldehyde inactivated the bacteria for 24h at 37°C. Inactivated bacteria were harvested by centrifugation at 4000 rpm for 15 min and washed 3 times with sterile saline and then adjusted the concentration as 1×10^9 CFU/ml. After that same aliquots of each inactivated suspension was separately plated on NB agar incubated for 72h at 37°C to confirm the absence of viable cells. The inactivated bacteria were mixed with each other in a ratio of 1:1:1 and then storage at 4°C until use.

Immunisation protocols

Eight healthy multiparous lactating bactrian camels living at Alxa left banner (Inner Mongolia, China) were chosen and divided randomly into control group (n=4) and immune group (n=4). After collection of pre-immune blood serum and milk, camels in immune group were injected intramuscularly with 2.5 ml vaccine mixed in 2.5 ml complete Freund's adjuvant (FCA, Sigma Chemical Co.). Two booster injections were given in 2 week intervals starting 2weeks after the initial injection with 2.5 ml vaccine in 2.5 ml of incomplete Freund's adjuvant (FIA, Sigma Chemical Co.). The camels in control group were injected with 0.85% sterile saline in the adjuvant at same dose and frequency (Table 1). All camels were in the same stage of lactation (1^{st}-2^{nd} month). None of them received any antibodies during the immunisation procedure in order to follow the specific anti-3 pathogenic bacteria antibody activity formed.

Sample collection

Blood and milk samples were collected from each camel prior to each immunisation and 2 weeks after final immunisation. The blood samples were collected from the jugular vein of each camel then placed at room temperature for 2-3h and centrifuged for 10 min at 4000rpm to collect the serum, labeled and stored at -20°C until further use. Milk samples were skimmed by centrifugation at 12000 rpm for 10 min to remove the fat components and collect the middle layer liquid. Then the whey samples were further passed through 0.22μm filter to get rid of particles and stored at -20°C.

Purification of total IgG and IgG subclasses by protein A/G chromatography

Based on the property of differential absorption of different IgG subclasses on protein G and protein A columns (GE Healthcare), a successive affinity chromatographic approach which applied on AKTA-FPLC system was used to purify and fractionate

immunoglobulins of serum and whey samples. Serum and whey samples were diluted 6-fold with 0.02 M PBS pH 7.4 prior to applying to the affinity columns. The diluted samples were injected into the column at a flow rate of 2ml/min and washed with equilibration buffer to remove the impurity until the absorbance at 280nm was <0.002. The purified procedure was as described previously (Hamers-Casterman *et al*,1993; Van *et al*, 2000; Salhi *et al*, 2015; Yao *et al*, 2017). In brief, total IgG was eluted from the protein A column with 0.02M glycine acid pH 2.7 (Sigma Chemical Co.). IgG3 and IgG1 were eluted from Protein G column with 0.2M acetic acid pH 3.6 and with 0.1M glycine pH 2.7, respectively. Subsequently, the protein G unbound fraction was adsorbed onto 10ml rProtein A agarose 4 fast flow (GE Healthcare) column. After washing at least 10-fold equilibration buffer to remove impurity and elution with 0.1M glycine-buffer pH 2.7, IgG2 was obtained. All collected IgG fractions were immediately neutralised by 1M Tris-HCl pH 9.0 then dialysed against 10 mM PBS pH 7.4 and stored at -20°C until analysis.

Detection and quantification of purified IgGs

Analysis of the purified IgG fractions was carried out sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) in reducing and non-reducing conditions by using Bio-Rad mini-Protean II system (Bio-Rad). Electrophoresis was performed at 10% acrylamide and 5% stacking gels and with heating for 5 min in a boiling water bath. Gels were stained with Coomassie blue R250 (Sigma Chemical Co.). After then, the protein concentrations of all collected fractions were determined with BCA Protein Assay kit (Sigma Chemical Co.), according to the manufacturer's instructions.

Preparation of polyclonal rabbit anti-camel antibody

In order to better identify the IgGs, we have prepared the corresponding secondary antibodies for each IgG subclass. The New Zealand White rabbits were divided into 3 groups of 2 rabbits per group. Three groups were first injected subcutaneously with 1ml 1mg/ml purified total IgG, IgG1 and IgG2 mixed in 1ml FCA, respectively. Two booster injection were given at 2 week intervals with same dose of antigen in FIA. The blood was collected 10 days post last injection from rabbit's heart puncture and antiserum were centrifuged at 10000 rpm for 30 min (4°C) and stored at -20°C. The purified camel whey total IgG, IgG1 and IgG2 were separately coupled with CNBr-activated Sepharose 4B (GE Healthcare) at a density of 2mg protein/ml resin to prepare affinity columns and equilibrated with 10-fold volumes of 0.02 PBS pH 7.4, respectively. After that, the antiserum was loaded into the appropriate column and eluted with 0.1M citric acid pH 3 to collect the polyclonal rabbit antibodies and dialysed against 10mM PBS pH 7.4 overnight. Then, we labelled the rabbit antibodies with horseradish peroxidase (HRP) according to the method proposed by Abdelrahman (2017) and

Table 1. The detailed information of camel-immunisation protocol.

Group	Camel No.	Immunisation protocol (Dose=5 ml)		
		1st	2nd	3rd
Immune group (n=4)	1	2.5 ml mixture inactivated bacteria suspension + 2.5 ml FCA	2.5 ml mixture inactivated bacteria suspension + 2.5 ml FIA	2.5 ml mixture inactivated bacteria suspension + 2.5 ml FIA
	2			
	3			
	4			
Control group (n=4)	5	2.5 ml 0.85% Sterile saline + 2.5 ml FCA	2.5 ml 0.85% Sterile saline+ 2.5 ml FIA	2.5 ml 0.85% Sterile saline + 2.5 ml FIA
	6			
	7			
	8			

Table 2. Concentration of each purified IgG subclass in serum and whey of the immune and control group. Values represent mean ± SD of serum and whey from four bactrian camels in the immune and control group, respectively.

Group	Serum (mg/ml)				Whey (mg/ml)			
	Total IgG	IgG1	IgG2	IgG3	Total IgG	IgG1	IgG2	IgG3
Immune group	14.58 ±1.01	4.58 ±0.33	3.19 ±0.64	2.19 ±0.15	0.70 ±0.08	0.13 ±0.04	0.13 ±0.02	0.12 ±0.02
Conrol group	10.73 ±0.84	2.99 ±0.33	2.21 ±0.28	1.45 ±0.12	0.60 ±0.03	0.12 ±0.02	0.12 ±0.01	0.11 ±0.02

removed the unbound HRP by salting out method (El-Agamy *et al,* 2009; Muro *et al,* 1997; Toaleb *et al,* 2013; Abdelrahman *et al,* 2017). For camel IgG3, we used monoclonal mouse anti-camel IgG3 antibody which was prepared previously in our lab as its secondary antibody in this work.

Relative avidity immunoassay of the serum and whey

The IgG response in serum and whey of each camel during the course of the immunisation was measured by means of an indirect ELISA as previously described (Otani *et al,* 2003; Xu *et al,* 2006; Cook *et al,* 2010; Gao *et al,* 2010) with some modification. Briefly, 96 wells microplates were coated with 100µl of inactivated mixture pathogen suspension in carbonate buffer (CBS) pH9.6 (1×10^8CFU/ml) and incubated at 4°C overnight. After coating, the wells were washed 5 times with 10mM pH7.4 phosphate buffered saline (PBS). The coated wells were blocked with 100µl 5% bovine serum albumin (BSA) in CBS and incubated in room temperature for 2h and washed as above to remove the non-bound material. The test serum and whey were both diluted as 1:1000 and 1:5000 in 5% fat-dried milk-PBS, respectively and then added to each well incubated for 2h at 37°C. After washing, the wells were incubated with 100µl horseradish peroxidase-conjugated rabbit anti-camel total IgG at 1:10000 for 30 min at 37°C. The wells were washed as before, TMB was used as the substrate added 100µl to each well. After 10 min at 37°C, the reaction was stopped with 50µl 2M H_2SO_4 and the absorbance (A) was measured at 450 nm by a BioTek Synergy H1 Microplate Reader (BioTek).

Determination of the specific activity of purified IgG subclasses

In indirect ELISA assay, the concentration of coating antigen, primary antibodies and secondary antibodies and the blocking conditions may affect the accuracy of the testing results to a certain extent. Thus, we optimised the indirect ELISA described in Section 2.7 by chessboard titration to determine the optimal concentration of coating antigen, secondary antibodies and the blocking conditions (blocking buffer, blocking temperature and blocking time). Then, we determined the pathogenic-binding activity of specific total IgG and IgG subclasses which were purified from immunised and non-immunised serum and whey by the optimised indirect ELISA method. The microplates were coated with 100µl of mixture pathogen suspension incubated for 24h at 4°C (optimal dilution: 1×10^8CFU/ml in CBS pH9.6), respectively. After the coating, the wells were washed as above and blocked with 100µl 5% BSA in CBS and incubated in room temperature for 1h and washed again. The test antibodies (total IgG, IgG1, IgG2 and IgG3 were diluted at 1:1000 ~ 1:64000 in 5% fat-dried milk in PBS, respectively) were added to each well (100µl) and incubated for 2h at 37°C. After washing, the wells were incubated with 100µl HRP-conjugated rabbit anti-camel IgG of the optimal dilution was added to each well and incubated for 30min at 37°C. After incubation, the wells were washed as before, TMB was used as the substrate added 100µl to each well. After 10min at 37°C, the reaction was stopped with 50µl 2M H_2SO_4 and the absorbance (A) was measured at 450nm using BioTek Synergy H1 Microplate Reader (BioTek). The specific activity for each purified IgG subclass was expressed as the dilution at which absorbance matched that of the negative control (PBS) > 2.1 standard deviations.

Statistical analysis

All analyses were performed in triplicate measurement and values were given as mean±SD. All reported data were analysed by ANOVA and level of significance was considered when $p < 0.05$.

Results and Discussion

Relative titre of immunised camel serum and whey

During the whole immunisation procedure, camels tolerated well and none of them showed side effect. Three doses of mixture pathogen suspension were given to camels in immune group at 2 week interval frequency. During the course of the immunisation, the serum and whey samples were diluted at 1:5000 and 1:1000, respectively, to assess the relative titres by indirect ELISA assay. Due to individual differences, we measured samples from each immunised camel separately instead of mixing to avoid the samples with poor immune response affected the result. As a result of multiple immunisation (Fig 1), specific antibodies were appeared in variable titres in serum and whey of camels in immune group and showed a significant difference of the specific activity between immune and control group ($p<0.005$). Moreover, the relative titre of serum was significantly higher than that of whey ($p<0.005$). In previous studies, most of researchers used various human gut bacteria to immunise pregnant cow multiple times to obtain hyperimmune colostrum which with high levels of immunoglobulins against the target pathogens (Korhonen *et al,* 1995; Freedman *et al,* 1998; Ashraf

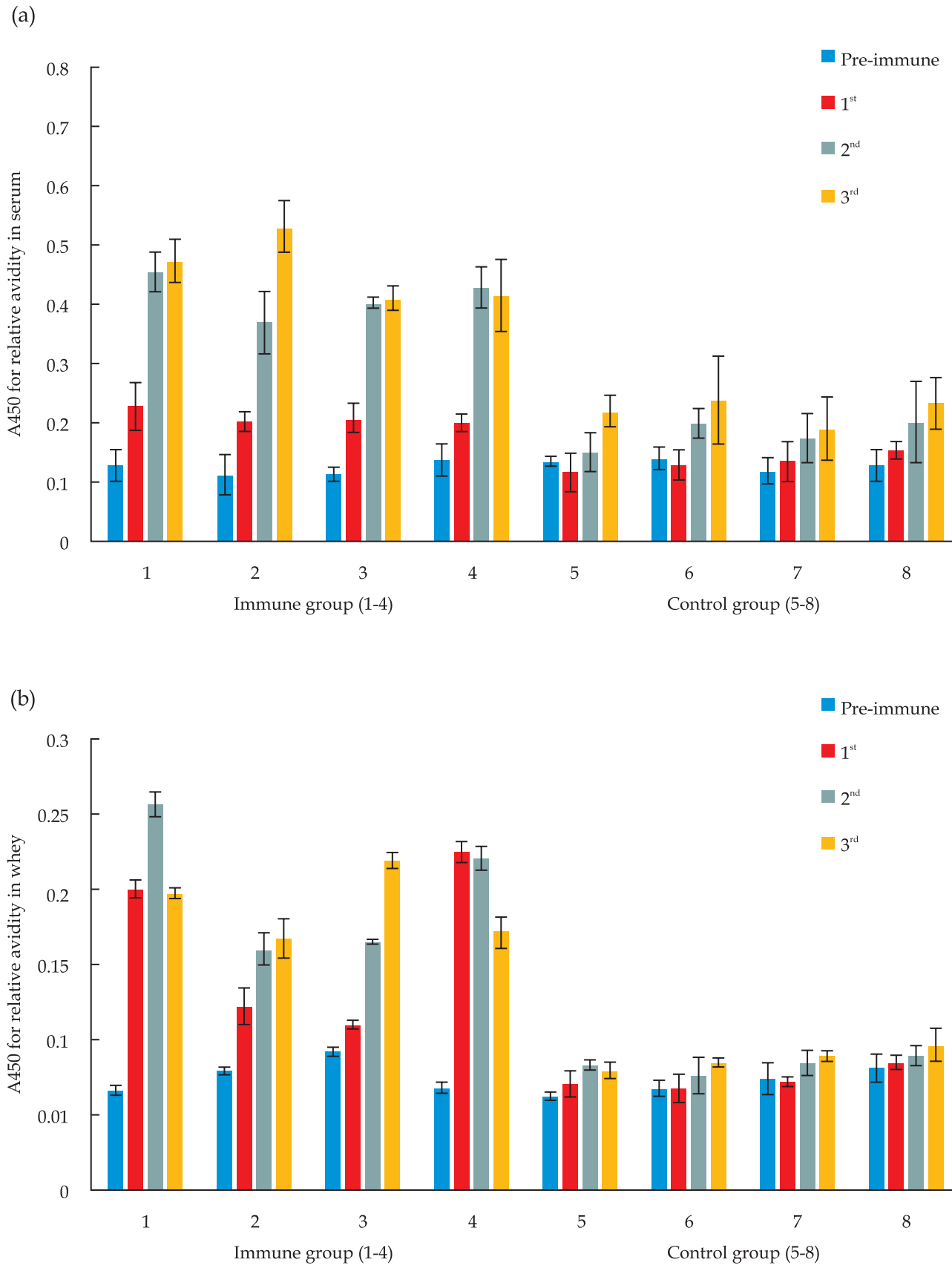

Fig 1. Relative titre against mixture pathogenic suspension in serum (a, dilution 1:5000) and whey (b, dilution 1:1000) from immune and control camels at different immunisation times.

et al, 2001; Xu *et al*, 2006; Huang *et al*, 2008; Gao *et al*, 2010; Otto *et al*, 2011; Wang *et al*, 2014; Sears *et al*, 2017). However, we had successfully obtained the immune milk with specific IgG activities by immunising the postpartum camels.

Purification and identification of total IgG and IgG subclasses from camel serum and whey

The serum and whey samples from the immunised and control camels were fractionated by sequentially adsorption on protein A and protein G columns which showed considerably different elution profiles (Fig 2). Such findings were correlated to differences in composition and structure between IgG subclasses of camels. The total IgG in serum and whey were both eluted with 0.02M glycine acid pH 2.7 through rProtein A agarose 4 FF column. Three major peaks were obtained by Protein G affinity chromatography of serum and whey, respectively and the first peak was their respective fluid of non-bound. The second peak was the first eluted fraction at pH3.6 corresponds the heavy-chain IgG subclasses called IgG3. The conventional IgG (IgG1) was found in 2nd eluted fraction (pH 2.7). The other heavy chain

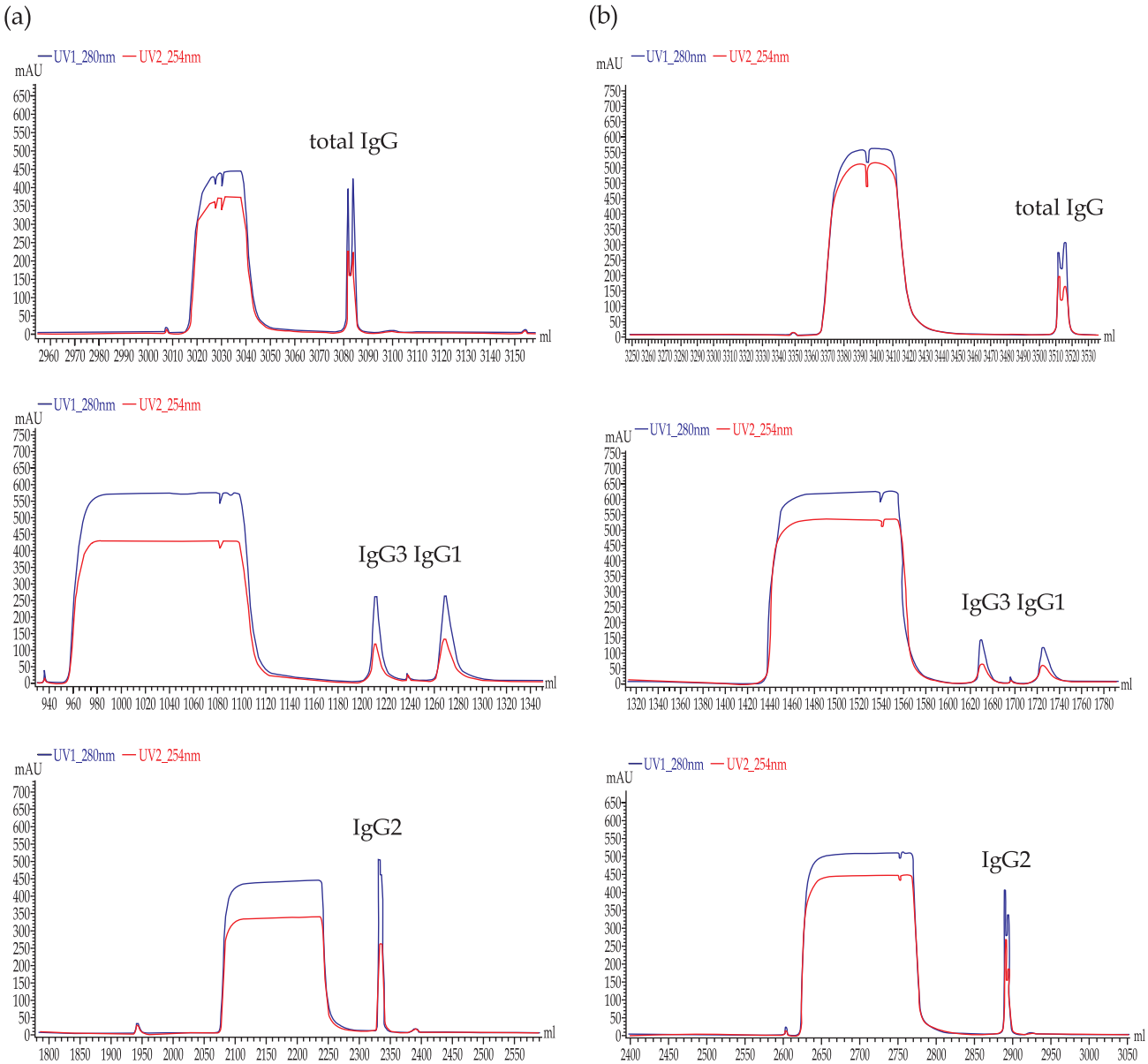

Fig 2. Affinity chromatography of IgGs from camel serum (a) and whey (b). (a) Column: Protein G agarose 4 fast flow; The first peak was the fluid of camel whey through Protein G column; The second peak was the first eluted fraction IgG3 at pH3.6; The third peak was the second eluted fraction IgG1 at pH2.7. (b) Column: Protein G agarose 4 fast flow and Protein A agarose 4 fast flow; The first peak was the fluid of camel whey through the protein G column then protein A column; The second peak was the eluted fraction IgG2.

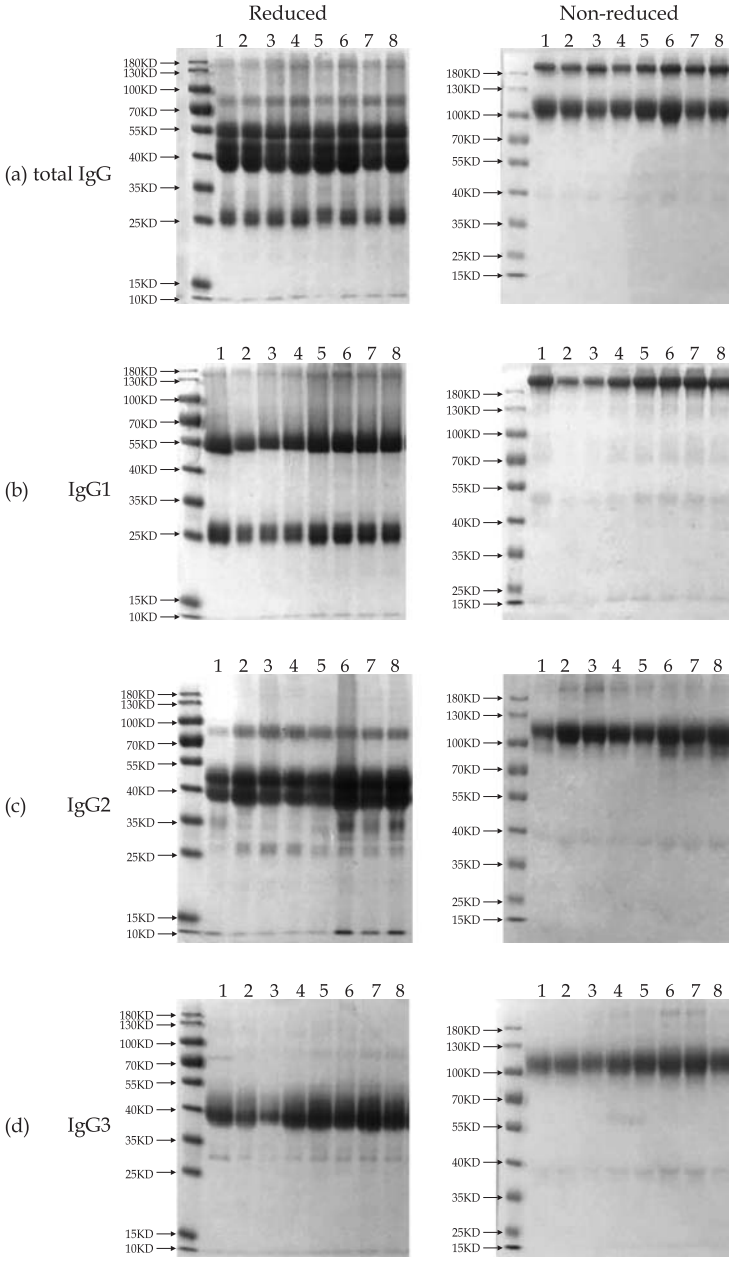

Fig 3. Coomassie blue-stained SDS-PAGE gel of purified IgG subclasses in serum and whey runder reducing and non-reducing conditions. (a) total IgG; (b) IgG1 (eluted from protein G); (c) IgG2 (eluted from protein A); (d) IgG3 (eluted from protein G). Marker proteins with known molecular masses. The lines in each electrophoresis represented: Marker proteins with known molecular masses, line 1 and 2 represented the correspond IgG from immunised serum, line 3 and 4 represented the correspond IgG from the control serum, line 5 and 6 represented the correspond IgG from the immunised whey, line 7 and 8 represented the correspond IgG from the control whey.

IgG subclasses IgG2 was purified from protein G non-absorbed fraction through elution at pH 2.7 on rProtein A agarose 4 fast flow column. Further, we identified the molecular size of each purified IgG subclass by reducing and non-reducing SDS-PAGE (Fig 3). The reducing SDS-PAGE results revealed that the purified total IgG was composed of 3 heavy-chains with molecular weight as 40kDa, 45kDa and 55kDa and one light-chain with molecular weight of 25kDa, which upon non-reduction yielded 2 molecules with 190kDa and 100kDa, respectively. The purified IgG1 has a molecular weight of ~190kDa (non-reduced) and dissociated upon reduction into one heavy chains of 55kDa and one light chains of 25kDa. Two band of heavy chains with molecular weight of around 40-45kDa were detected from purified IgG2 under reduced condition and with molecular weight of 110kDa under non-reduced condition. Our results were consistent with those of previous studies showing that there were different subtypes of IgG2 in camel serum (Hamers-Casterman *et al,* 1993; Muyldermans and Lauwereys, 1999; Daley *et al,* 2005; Tillib *et al,* 2014). The IgG3 fractions were only composed of one heavy-chain with a molecular weight as 40kDa, under non-reducing condition, yielded 100kDa. The results we got here were similar with those reported previously (Hamers-Casterman *et al,* 1993; Maass *et al,* 2007; Yao *et al,* 2017). It was indicated that the high purity of camel IgGs were obtained and could be used in further analysis.

Quantification of purified IgG subclasses

Based on the above results, we determined the relative amounts of each purified IgG subclass in serum and milk samples by BCA assay. The average content of each IgG in serum and whey of immune and control group from pre-immune and final immunisation was shown in table 2. The content of purified total IgG, IgG1, IgG2 and IgG3 in immunised serum was 14.58±1.01mg/ml, 4.58±0.33mg/ml, 3.19±0.64mg/ml and 2.19±0.15mg/ml, respectively, which was significantly higher than that of control group (10.73±0.84mg/ml, 2.99±0.33mg/ml, 2.21±0.28mg/ml and 1.45±0.12mg/ml, respectively) ($p<0.05$). The content of each IgG in the immunised whey was increased slightly, but there was no significant difference with the control group (total IgG was 0.70±0.08mg/ml *vs.* 0.60±0.03mg/ml, IgG1 was 0.13±0.04mg/ml *vs.* 0.12±0.02mg/ml, IgG2 was 0.13±0.02mg/

ml *vs.* 0.12±0.01mg/ml, IgG3 was 0.12±0.02mg/ml *vs.* 0.110.12±0.02mg/ml, respectively) (p>0.05). These results were in accordance with those reported by Sheldrake and Husband (1985), Zhang (2000) and You (2006) who indicated that systematic immunisation of ruminants could increase the content of IgG in the serum but did not necessarily increase the content of IgG in the colostrum and milk.

The content of each IgG in whey was about 20 times lower than that in serum. It could be explained by the decrease of the concentration of IgG in milk. Due to the placenta of camels with a thick layered epitheliochorial that prevents transplacental transfer of IgG, so that the IgG in camel milk are derived mainly from serum and accumulate in the mammary gland few days before prepartum (Wernery. 2001; Hurley and Theil, 2003; Gapper, 2007). The content of IgG in milk was gradually decreased with the lactation period. Fan (2006) showed that the IgG concentration decreased from 170.5mg/ml in colostrum to 2.1mg/ml in the milk at 90 days postpartum of the Alxa Bactrian camel. We purified about 0.60~0.70mg/ml of total IgG from camel whey and the proportion of HCAbs in total IgG of serum and whey were 55% and 62%, respectively. These results were similar to data previously reported in the literature but also with some differences (Hamers-Casterman *et al,* 1993; El-Agamy, 2009; Van *et al,* 2000; Wernery, 2001; Fan, 2006; El-Hatmi *et al,* 2006; Levieux *et al,* 2006; Konuspayeva *et al,* 2007; Konuspayeva

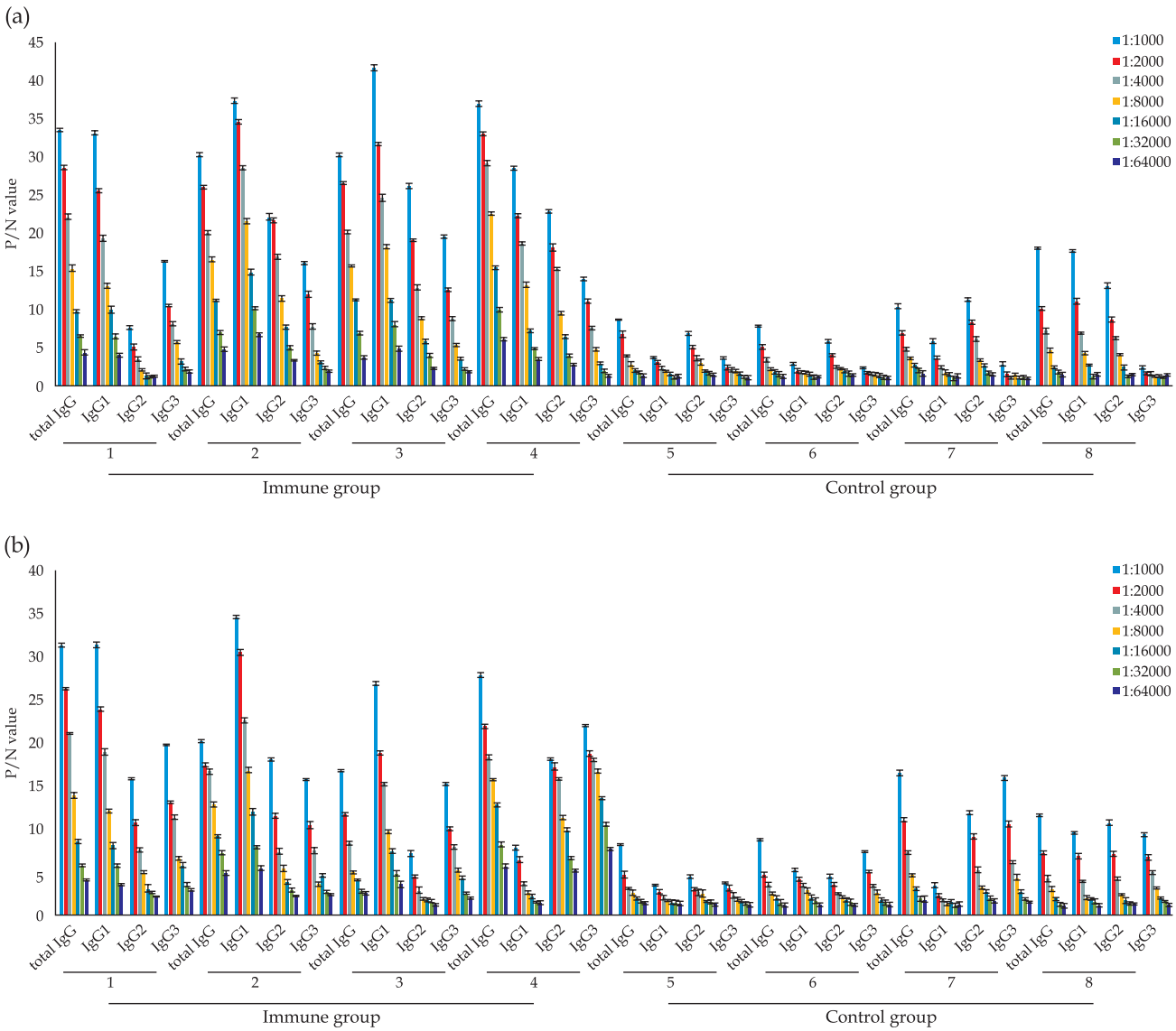

Fig 4. The specific anti-pathogenic activity of each purified IgG subclass in serum and whey from immune and control group. Each IgG was diluted from 1:1000 to 1:64000 then added in the microplate to detect the titre by optimal indirect ELISA. Each measurement was repeated five times and data were presented as mean ± SD.

Table 3. The specific activity of each IgG against 3 strains of pathogenic bacteria was determined by optimal indirect ELISA assay. All data shown in here was the dilution of P/N > 2.1.

Group	Camel No.	Serum				Whey			
		Total IgG	IgG1	IgG2	IgG3	Total IgG	IgG1	IgG2	IgG3
Immune group	1	1:64000	1:64000	1:4000	1:16000	1:64000	1:64000	1:64000	1:64000
	2	1:64000	1:64000	1:64000	1:32000	1:64000	1:64000	1:64000	1:64000
	3	1:64000	1:64000	1:64000	1:32000	1:64000	1:64000	1:4000	1:32000
	4	1:64000	1:64000	1:64000	1:16000	1:64000	1:16000	1:64000	1:64000
Control group	5	1:8000	1:4000	1:8000	1:2000	1:8000	1:2000	1:8000	1:4000
	6	1:8000	1:1000	1:8000	1:1000	1:8000	1:8000	1:4000	1:8000
	7	1:8000	1:4000	1:8000	1:1000	1:16000	1:2000	1:16000	1:16000
		1:16000	1:16000	1:16000	1:1000	1:8000	1:4000	1:8000	1:8000

et al, 2008; Xia *et al,* 2010; Muyldermans, 2013; Yao *et al,* 2017). These differences in the content of IgG and the proportion of HCAbs in camel milk could be attributed to several factors including geographical locations, feeding conditions, different breeds, stage of lactation, age and measurement methods (Haj and Kanhal, 2010). In addition, the sum of the purified IgG subclasses was much less than the amount of the purified total IgG. This might be caused by the loss during the purification process. Therefore, in the future research, we should optimise the purification steps to reduce the loss.

Determination of antigenic specific activity for each purified IgG subclass

ELISA is a highly specific, sensitive and simple biochemical assay, which has been widely used in qualitative or quantitative determinations of either antigen or antibody. The testing results of ELISA were affected by the various working conditions such as concentrations of coating antigen, secondary antibodies, blocking conditions. Therefore, we optimised several working conditions in the indirect ELISA method that was initially established. By the dot matrix method, we determined the optimal concentration of coating antigen (mixture of 3 strains pathogenic bacteria) was 1×10^8CFU/ml. The optimal dilutions of each secondary antibody was 1:16000 (HRP-conjugated rabbit anti-camel total IgG), 1:4000 (HRP-conjugated rabbit anti-camel IgG1), 1:16000 (HRP-conjugated rabbit anti-camel IgG2) and 1:16000 (HRP-conjugated mouse anti-camel IgG3 monoclonal antibody), respectively. Then, we used three factors combinations with different blocking buffers (5% BSA-CBS and 5% fat-dried milk-CBS), blocking temperature (Room temperature and 37°C) and blocking time (1h and 2h) to select the combinations that with the maximum positive / negative (P/N) values for the best blocking conditions. The results showed that the best blocking condition was that the plates blocked with 100µl 5% BSA in CBS buffer and incubated in room temperature for 1h.

All purified IgG subclasses were tested individually in the optimal indirect ELISA for their capacity to recognise 3 strains of pathogenic bacteria. As shown in Table 2 and Fig 4, these data suggested strongly that immunised camels produced both conventional and HCAbs specific to the antigen. The titres of total IgG in the immune group were same (1:64000), but the titres of each IgG subclass were with slight variation between individual camels. The result indicated that there was no single IgG subclass dominates the titre of the IgG response to immunisation with mixture pathogenic bacteria. It means that IgG taken from immunised camel could be processed into antidiarrhoeal agent without requiring the chromatographic fractions to select or exclude any specific IgG subclass. Moreover, the antibody activity was increased despite no increase in the amount of antibody in milk after immunisation. The results also showed that more than 60% of the antibodies in the camel milk were HCAbs. Compared with conventional IgG, the HCAbs are more stable, smaller and they can pass the blood brain barrier and easily absorbed by the gut into the general circulation (Gader and Alhaider, 2016). In addition, there was lack of β-LG in camel milk which could cause allergenic properties, so that could consume by lactose- and/or immune-deficient population (EI-Hatmi *et al,* 2006; EI-Agamy *et al,* 2009). All of the above results together with the characteristics of the composition of camel milk showed that immune camel milk has prodigious potential for development.

In conclusion, we demonstrated for the first time that conventional and heavy chain specific

immunoglobulins to *Escherichia coli, Salmonella typhi* and *Shigella dysenteriae* can be produced in high titres in camel mature milk by immunising lactating camels. We also established the indirect ELISA protocol to estimate the titre of each IgG subclass. This work in our opinion, is very important for developing camel immune milk. On the basis of this study, we need to further validate the efficacy of the immune camel milk by *in vitro* and *in vivo* experiments.

Acknowledgements

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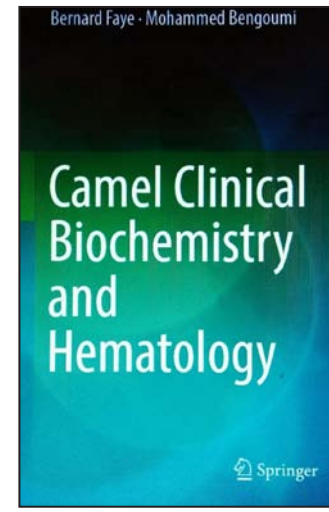
News

A new book- Camel Clinical Biochemistry and Hematology

The new book Camel Clinical Biochemistry and Hematology authored by Bernard Faye and Mohammed Bengoumi has fulfilled a long felt void on this important topic from clinical health and nutrition point of view. The book is spread in more than 345 pages and 10 chapters. Every chapter is further divided into subtopics, conclusions and references. The book will prove a milestone to the researchers and clinicians as normal or reference values are given now which can be quickly browsed to compare with values of samples being analysed in the laboratory. The book is excellent resource of literature and references occupy one third of volume of book which will prove as a ready reckoner to the clinicians and researchers. All the parameters and their values are given through several pertinent tables and graphs.



Reading all the chapters arouse your interest as they unfold many mysteries specific to this species of animal, i.e. camels are able to withstand to hemoconcentration during water deprivation, they are able to lose 25% of its total body water without manifesting major symptoms of dehydration, etc.

The last chapter on general conclusions highlights (among others) regarding the specificity of camel clinical and nutritional pathology, i.e. predominance of polynuclear neutrophils in its white cell formula, maintenance of hematocrit in case of physical effort, osmolality resistance, relative hyperglycemia, absence of ketone bodies, low plasma cholesterol in plasma concentration, susceptibility to hyperuremia, thermoresistance of alkaline phosphatises, maintenance of metalloenzyme activities in case of mineral deficiency, maintenance of electrolyte balance in dehydrated animals, low plasma zinc concentration in supplemented camel and higher sensitivity to selenium toxicosis.

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CAMEL MILK EFFICIENCY IN PROTECTING RAT TESTES AGAINST LEAD ACETATE TOXICITY

A.D. Zakaria[1,2], Sh. M. Abdel-Raheem[3,4] and Kh. A. Al-Busadah[1]

[1]Department of Physiology, Biochemistry and Pharmacology, [3]Department of Veterinary Public Health and Animal Husbandry, College of Veterinary Medicine, King Faisal University, Al-Asha 31982, Saudi Arabia

[2]Physiology Department, Faculty of Veterinary Medicine, Alexandria University, Rosetta Line, Behera Province 22758, Egypt

[4]Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, 71526, Egypt

ABSTRACT

Two experiments were conduted to evaluate the role of camel milk in preventing the detrimental effect of lead on rat. In the first, 6 groups of adult male rats were administered daily for 60 days the following : group 1 saline; group 2 camel milk; group 3 cow milk, group 4 lead acetate; group 5 camel milk plus lead and group 6 cow milk plus lead. In the second, pregnant female rats were divided and treated following the first experiment. The female were allowed to deliver pups, the treatment continued until weaning of the pups then, the male pups were left without treatment until puberty. Lead caused significant reduction in the body and reproductive organ weights; plasma and testicular testosterone, testicular zinc; antioxidant enzymes, luteinising and follicle stimulating hormones and semen characteristics, while it caused significant increase in malondialdehyde; testicular cholesterol and testicular and plasma lead. Camel milk treatment improved the estimated parameters in adult male rat. However, it could not alleviate these parameters in male rats born for mothers exposed to lead during pregnancy and lactation periods. Camel milk treatment improved the evaluated parameters in adult male rats exposed to lead intoxication albeit not all were identical to the control levels, however, it could not improve these parameters in adult male rats born for mothers exposed to lead during pregnancy and lactation.

Key words: Camel milk, lead acetate, oxidative stress, rat, testes, toxicity

Many metal ions drastically affect the reproductive process in both male and female. Lead can cross blood-testis barrier, produces its toxic effect on the primary and the secondary reproductive organs (Elgawish and Abdelrazek, 2014). Lead passes through placenta and mammary gland of mice and produces physiological and pathological adverse effects in the testes of both neonate and adult males (Sharma and Garu, 2011). Lead induced its toxicity in most biological systems via oxidative stress (Flora *et al,* 2012). Many researchers attempted to use nutrients and medicinal plants with antioxidant activity to protect against this toxicity. In addition, some chelating agents and certain antioxidants such as vitamin C, E, methionine, N-acetylcysteine, homocysteine and α-lipoic acid were used to reduce lead toxicity (Jackie *et al,* 2011). However, most of these chelating agents elicit many side effects and are ineffective to reduce lead exposure (Flora and Pachauri, 2010).

Camel milk contains high minerals, vitamins and insulin contents but have low protein, cholesterol and sugar (Alavi *et al,* 2017). Thus imparts medicinal properties, which are exceedingly exploited for human health, as in many developing countries and ex-Soviet Union (Konuspsyeva *et al,* 2009). Many potential therapeutic advantages for camel milk were reported for few diseases e.g. diabetes and wound healing in diabetic patient (Badr *et al,* 2012), hepatitis C infection (El-Fakharany *et al,* 2017), autism (Adams, 2013) and hypertension (Ayyash *et al,* 2018). Camel milk diminished oxidative stress status and free radicals production in aluminum chloride (Al-Hashem, 2009) and cadmium (Al-Hashem *et al,* 2009) treated rats.

Effect of camel milk against lead-induced testicular toxicity are not traceable in available literature. Hence, present study was done to assess the conservative efficiency of camel milk in the induced lead toxicity in the testes of rats.

Materials and Methods

Camel and cow milk samples were collected daily early in the morning, by hand milking from a healthy 5 and 7 years old she camel and Holstein cow, respectively kept at Experimental Veterinary and

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Agriculture Station, King Faisal University, Al-Ahsa, Saudi Arabia. The milk was collected in sterile screw capped bottles and kept in cool boxes until transported to the laboratory. The unpasteurised camel milk (pH 6.3 and specific gravity 1.029) and cow milk (pH 6.5 and specific gravity 1.034) were intubated daily to rats at dose of 2ml/rat at a fixed time.

Experimental animals and protocol

Experiment 1

Thirty six 36 adult male Wister rats, weighed 215-240 g obtained from the Experimental Veterinary and Agriculture Station, King Faisal University, at Al-Ahsa, Saudi Arabia, were housed in plastic cages at Physiology Laboratory. The rats were acclimatised in controlled environment (20-22°C and 12hours light/12 hours dark schedule). The rats were fed with standard food pellets (15% crude protein, 3.8 crude fat, 6% crude fiber, 1.1% calcium, 0.8% phosphorus) and water *ad libitum*. The maintenance and handling of the animals was done according to King Faisal University guidance from the Ethical Committee for Research on Laboratory Animals (KFU-REC/2017-04-04). Following one-week acclimatisation, the animals were assigned into 6 groups with 6 rats each. Group 1 was intubated with 2ml saline (S), group 2 was intubated with 2ml camel milk (CM); (Al-Hashem, 2009) group 3 was intubated with 2ml cow milk (W), group 4 was intubated with saline containing 20 mg lead acetate (Pb) (Sigma Chemical Co. St Louis, MO, USA)/ kg body weight (Abdel Moneim, 2016). Group 5 was intubated with camel milk 2 hours before intubation of lead acetate (CM + Pb). Group 6 was intubated with cow milk 2 hours before intubation of lead acetate (W+Pb). The treatment was performed daily for 60 days which represent complete spermatogenic cycle. Rats were weighed by the end of treatment.

Experiment 2

Female and male Wister rats (4-5 months) in the ratio of 2:1 were kept in plastic cages and the day at which sperm was detected in the vaginal smear was designed as day 1 of pregnancy. Pregnant rats were allocated into 6 groups (6 rats each) and were treated as in the experiment 1. All the animals allowed deliver pups. The treatments further continued throughout the lactation period (up to 22 days). At the time of weaning, the mothers were isolated and the pups grew alone without treatment until sexual maturity (60 days) in this experiment. Six male rat pups from each group were randomly selected and their body weight were recorded.

Reproductive organ weights

Twenty four hours after the end of the experimental period, all rats in both experiments, were anaesthetised intramuscularly with (85 mg and 15 mg of ketamine and xylazine/kg body weight respectively). Individual blood samples were collected by heart puncture. Then, all male rats were sacrificed by decapitation at the end of the 2 experiments. Blood samples were collected into heparinised test tubes. The samples were divided into 2 sets, one for lead determination and the other set was used for testosterone, Luteinising hormone (LH) and Follicle stimulating hormone (FSH) assay. Immediately after blood collection, the testes, prostate, seminal vesicles and epididymis were removed, blot dry; grossly examined and weighed. One testis was used for determination of antioxidant and oxidative stress biomarkers; the other one cut into 2 halves, one-half for lead determination and the other half for histopathological examination.

Semen analysis

Epididymal spermatozoa were counted by a modified method of Yokoi *et al* (2003). The epididymis was cut into head; body and tail, then they minced in 5 ml phosphate buffer (pH 7.4), after that it was shaken vigorously for homogeneity and dispersal of sperm cells. An aliquot (10µl) of epididymal sperm suspension was placed in the counting chamber of the haemocytometer and allowed to stand for 5 min for count under a microscope (×200). The heads of sperm were counted and expressed as million/ml. A drop of epididymal content of each rat was mixed with an equal drop of eosin–nigrosine stain and a thin film was made on a clean slide. The average viability % was determined from 200 sperms examined per slide. The progressive motility was evaluated. The content of cauda epididymis was obtained with a pipette and diluted to 2ml with tris buffer solution. The motility was evaluated at × 400 magnification and the average final motility score was estimated from the 3 different fields in each sample. The morphologically abnormal spermatozoa percentages were recorded, i.e. 40 µl of sperm suspension mixed with 10 µl of 1% eosin and nigrosine, 200 sperm examined on each slide using a microscope (×400) and the average taken.

Hormonal assay

Testicular and plasma testosterone levels were estimated using radioimmunoassay (RIA) kits from Diagnostic Products Cooperation (Los Angeles, California). The sensitivity was 0.2 ng/ml and intra-assay coefficient of variation was 12.8%.

LH and FSH levels were determined by generated electrochemiluminscence using kits supplied by Roche Diagnostics and using automat (Elecsys 2010, Roche Diagnostics, Mannheim). The hormones were assayed according to manufacture guide of the kits.

Antioxidant enzyme activities and oxidative stress assays

One of the 2 testes was kept at −70°C, then in cold potassium phosphate buffer (pH 7.4). This testis was homogenised, then centrifuged at 4°C for 10 minutes at 5000 rpm. The supernatant used for determination of glutathione (GSH), (SOD) superoxide dismutase, Catalase (CAT), malondialdehyde (MDA) using commercial available colorimetric assay kits (Cayman Chemical, Ann Arbor, Michigan, USA) according to the manufacturer guides.

Blood and testicular lead determination

In polystyrene test tube 100 µl, blood was placed then 150 µl deionised water and 150 µl of 2 M nitric acid was added, vigorously mixed for 30 seconds then centrifuged at 4000x g for 15 minutes. Ten µl of the supernatant was combusted in graphite furnace at 500°C for 24 hrs. The obtained ash was diluted with 5ml of 0.1 M nitric acid (Parsons and Slavin, 1993). The testicular tissue was thoroughly washed in distilled water and then dried for 48 hours at 60°C and finally combusted. The combusted tissue was digested with 10 ml nitric acid, after that few drops of perchloric acid were added then distilled water added to be 50ml (US EPA, 1986). Lead concentration was determined by AAS (Perkin Elmer Analyst, model 2180).

Histopathological examination

After the fixation of half of the testicle in 10% formalin solution, it was processed and sections were stained with hematoxylin and eosin (H & E) and examined under light microscopy (Lillie, 1965).

Statistical analysis

The obtained data expressed as means ± standard errors. The significance of differences calculated by one-way analysis of variance (SAS, 2001) followed by Duncan's multiple range test (Duncan, 1955). The difference between means was considered significant when $P<0.05$.

Results and Discussion

The final body weight and the index weight of the testis, accessory sex glands and the epididymis decreased significantly ($P<0.05$) in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control group. However, this reduction was less detected in the group treated with lead acetate plus camel milk (Table 1).

Epididymal sperm count, alive sperm and motility per cent decreased significantly ($p<0.05$) in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control group, this reduction was less conspicuous in the group treated with lead acetate plus camel milk (Table 2). Furthermore, there was a significant increase ($p<0.05$) in sperm abnormalities percentage in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control one. This increase was less marked in lead acetate plus camel milk treated group (Table 2).

Table 3 showed that MDA increased significantly ($p<0.05$) in lead acetate and lead acetate plus camel or cow milk treated groups compared to control one. The greatest increase was in lead acetate and lead acetate plus cow milk treated groups. Moreover, there was a significant decrease in GSH, SOD and CAT in lead acetate and lead acetate plus

Table 1. Effect of lead acetate, camel milk and cow milk on the body and reproductive organs index weight of adult male rats.

Groups	Parameters*					
	Initial body weight (gm)	Final bod weight (gm)	Feed intake	Testes (I.W)	Epididymis (I.W)	Accessory glands (I.W)
Group 1 (S)	226.17±3.22^{a}	285.33±3.70^{a}	20.92±0.48^{a}	1.64±0.02^{a}	0.70±0.02^{a}	0.82±0.02^{a}
Group 2 (CM)	226.67±3.11^{a}	285.83±5.07^{a}	15.87±0.26^{b}	1.69±0.03^{a}	0.73±0.02^{a}	0.83±0.02^{a}
Group 3 (W)	228.00±3.24^{a}	287.17±2.09^{a}	15.55±0.54^{b}	1.64±0.02^{a}	0.70±0.02^{a}	0.82±0.02^{a}
Group 4 (Pb)	225.83±3.75^{a}	235.00±3.42^{b}	12.87±0.21^{c}	1.04±0.02^{b}	0.53±0.01^{b}	0.63±0.01^{b}
Group 5 (CM+Pb)	226.50±0.43^{a}	251.67±4.01^{c}	13.22±0.15^{c}	1.37±0.03^{c}	0.62±0.01^{c}	0.72±0.01^{c}
Group 6 (W+Pb)	227.00±1.00^{a}	238.17±1.56^{b}	12.86±0.32^{c}	1.05±0.02^{b}	0.54±0.01^{b}	0.64±0.01^{b}

N= 6 * Means ± SE I.W = index weight
Means having different superscript letters in the same column are significantly different ($P<0.05$).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

camel or cow milk treated groups compared to control one. The greatest reduction was in lead acetate and lead plus cow milk treated groups.

Plasma testosterone, FSH and LH levels decreased significantly ($p<0.05$) in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control group, this reduction was less conspicuous in the groups treated with lead acetate plus Camel's milk (table 4). Furthermore, there was a significant increase ($p<0.05$) in blood lead concentration in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control one. This increase was less marked in lead acetate plus camel milk treated group (Table 4).

Table (5) showed that both testicular lead and cholesterol content increased significantly ($p<0.05$) in lead acetate and lead acetate plus camel or cow milk treated groups compared to control one. The greatest

Table 2. Effect of lead acetate, camel milk and cow milk on the epididymal sperm characteristics in adult male rats.

Groups	Parameters*			
	Sperm count ($x10^6$)	Sperm motility (%)	Alive sperm (%)	Sperm abnormalities (%)
Group 1 (S)	315.50±3.27[a]	91.00±0.58[a]	89.50±0.76[a]	7.33±0.71[a]
Group 2 (CM)	317.67±0.49[a]	91.67±0.56[a]	90.67±0.33[a]	6.83±0.48[a]
Group 3 (W)	318.00±1.39[a]	91.00±1.26[a]	90.50±1.43[a]	7.50±0.76[a]
Group 4 (Pb)	218.17±3.24[b]	69.00±0.97[b]	68.17±1.72[b]	17.33±0.67[b]
Group 5 (CM+Pb)	282.50±4.01[c]	80.00±1.71[c]	79.50±1.23[c]	12.00±0.73[c]
Group 6 (W+Pb)	221.00±3.51[b]	67.83±1.17[b]	69.17±1.17[b]	17.67±0.76[b]

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P<0.05).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 3. Effect of lead acetate, camel milk and cow milk on the oxidative stress markers and antioxidant in adult male rats.

Group	Parameters*			
	MDA (µmol/g)	GSH (mg/g)	SOD (µ/g)	CAT (µ/g)
Group 1 (S)	6.19±0.25[a]	16.47±0.45[a]	1.58±0.13[a]	18.36±0.35[a]
Group 2 (CM)	6.14±0.25[a]	17.33±0.72[a]	1.64±0.05[a]	16.76±1.61[a]
Group 3 (W)	6.22±0.30[a]	16..61±0.23[a]	1.67±0.03[a]	17.97±0.25[a]
Group 4 (Pb)	22.01±0.69[b]	6.87±0.37[b]	0.70±0.03[b]	8.61±0.24[b]
Group 5 (CM+Pb)	10.63±0.57[c]	10.96±0.48[c]	0.98±0.02[c]	11.16±0.20[c]
Group 6 (W+Pb)	22.52±0.72[b]	7.01±0.23[b]	0.71±0.04[b]	8.55±0.17[b]

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P<0.05).
MDA= malondialdehyde GSH= glutathione SOD= superoxidedimutase CAT= Catalase.
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 4. Effect of lead acetate, camel milk and cow milk on plasma testosterone; FSH & LH and blood lead content in adult male rats.

Groups	Parameters*			
	Plasma testosterone (ng/ml)	Plasma FSH (ng/ml)	Plasma LH (ng/ml)	Blood lead (µg/ml)
Group 1 (S)	5.53±0.22[a]	2.83±0.02[a]	6.33±0.35[a]	1.90±0.10[a]
Group 2 (CM)	5.57±0.37[a]	2.87±0.03[a]	6.45±0.39[a]	1.83±0.19[a]
Group 3 (W)	5 .55±0.11[a]	2.84±0.02[a]	6.60±0.08[a]	1.89±0.03[a]
Group 4 (Pb)	3.46±0.10[b]	1.52±0.10[b]	3.31±0.06[b]	17.34±1.69[b]
Group 5 (CM+Pb)	3.74±0.60[b]	1.63±0.08[b]	4.51±0.11[c]	14.72±0.48[c]
Group 6 (W+Pb)	3.56±0.71[b]	1.59±0.02[b]	3.23±0.19[b]	17.95±0.60[b]

N= 6 * Means ± SE
Means having different superscript letters in the same column are significantly different (P<0.05).
S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

increase was in lead acetate and lead acetate plus cow milk treated groups. Moreover, there was a significant ($p<0.05$) decrease in both of testicular testosterone and zinc content in lead acetate and lead acetate plus camel or cow milk treated groups compared to control one. The greatest reduction was in lead acetate and lead plus cow milk treated group.

The body weight and the index weight of the testes, accessory sex glands and the epididymis decreased significantly ($P<0.05$) in male rats born for mothers treated with lead acetate and lead acetate plus camel or cow milk during pregnancy and lactation periods compared to control group. There was significant ($p<0.05$) increase in body and index weight in the epididymis of lead acetate plus camel milk group compared to lead acetate and lead plus cow milk groups (Table 6).

Epididymal sperm count, alive sperm and motility percentage decreased significantly ($p<0.05$) in male rats born for mothers treated with lead acetate and lead acetate plus camel or cow milk during pregnancy and lactation periods compared to control group (Table 7). Furthermore, there was a significant increase ($p<0.05$) in sperm abnormalities percentage in groups treated with lead acetate and lead acetate plus camel or cow milk compared to control one. There was no significant ($p<0.05$) difference between lead acetate and acetate plus camel or cow milk treated groups in all parameters estimated (Table 7).

Plasma testosterone, FSH and LH levels decreased significantly ($p<0.05$) in male rat born for mothers treated with lead acetate and lead acetate plus camel or cow milk during pregnancy and lactation periods compared to control. Furthermore, there was a significant increase ($p<0.05$) in blood lead concentration in male rat born for mothers treated with lead acetate and lead acetate plus camel or cow milk during the pregnancy and lactation period compared to control one (Table 8). There was no significant ($p<0.05$) difference between lead acetate and lead plus camel or cow milk treated groups in plasma testosterone, FSH and LH levels and blood lead concentration (Table 8).

Table 5. Effect of lead acetate, camel milk and cow milk on testicular lead; testosterone cholesterol and zinc content in adult male rats.

Groups	Parameters*			
	Testicular lead (ng/g)	Testicular testosterone (ng/g)	Testicular cholesterol (mg/g)	Testicular zinc (µg/g)
Group 1 (S)	63.67 ± 1.45^{a}	8985.08±85.64^{a}	15.98±0.24^{a}	17.82±0.28^{a}
Group 2 (CM)	63.50 ± 0.50^{a}	8962.85±100.43^{a}	15.65±0.20^{a}	17.98±0.25^{a}
Group 3 (W)	63.44±0.35^{a}	8924.38±23.25^{a}	15.54±0.60^{a}	18.11±0.14^{a}
Group 4 (Pb)	128.91±0.51^{b}	5406.98±113.73^{b}	35.69±0.34^{b}	8.06±0.18^{b}
Group 5 (CM+Pb)	102.17 ±3.61^{c}	6400.53±79.25^{c}	20.95±0.28^{c}	12.47±0.17^{c}
Group 6 (W+Pb)	127.50±1.34^{b}	5391.96±41.61^{b}	35.49±0.0.53^{b}	8.16±0.22^{b}

N= 6 * Means ± SE

Means having different superscript letters in the same column are significantly different ($P<0.05$).

S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 6. Effect of lead acetate, camel milk and cow milk on the body and reproductive organs weight of male rats, which their mother exposed to lead acetate at pregnancy and lactation periods.

Groups	Parameters*				
	Body weight (g)	Testes (I.W)	Epididymis (I.W)	Prostate (I.W)	Seminal vesical (I.W)
Group 1 (S)	193.00±2.08^{a}	1.17±0.03^{a}	1.85±0.02^{a}	0.13±0.01^{a}	0.47±0.01^{a}
Group 2 (CM)	208.00±2.08^{b}	1.25±0.02^{b}	1.92±0.02^{b}	0.14±0.01^{a}	0.52±0.02^{a}
Group 3 (W)	208.17±0.79^{b}	1.14±0.03^{a}	1.83±0.03^{a}	0.15±0.01^{a}	0.54±0.02
Group 4 (Pb)	149.00±1.53^{c}	0.88±0.03^{c}	1.24±0.04^{c}	0.10±0.00^{b}	0.28±0.02^{b}
Group 5 (CM+Pb)	165.00±2.89^{d}	0.90±0.03^{c}	1.47±0.03^{d}	0.11±0.00^{b}	0.32±0.02^{b}
Group 6 (W+Pb)	157.17±2.76^{e}	0.88±0.01^{c}	1.25±0.0.1^{c}	0.11±0.01^{b}	0.28±0.1^{b}

N= 6 * Means ± SE I.W = index weight

Means having different superscript letters in the same column are significantly different ($P<0.05$).

S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

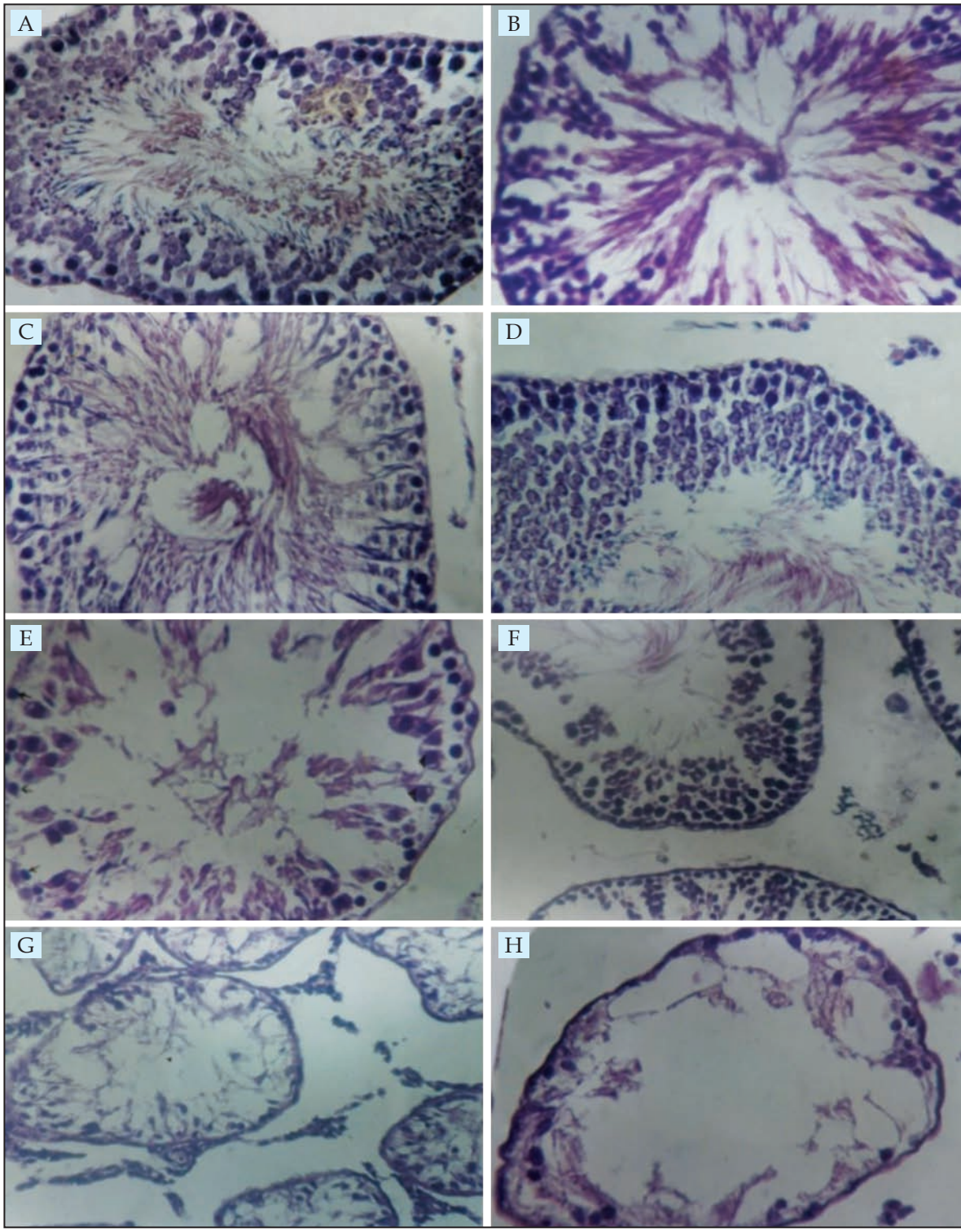

Fig 1. (A-H): Photomicrographs of rats testes (H&E): **A.** mature male (control) showing active seminiferous tubules containing all layers of spermatic cells (X100). **B.** mature rats treated with camel milk showing active seminiferous tubules containing all layers of spermatogenic cells (X400). **C.** male (control) born to mothers treated with saline during pregnancy and lactation periods (X400). **D.** male born to mothers treated with camel's milk during pregnancy and lactation period showing active spermatogenesis (X400). **E.** adult male treated with lead acetate showing degenerated spermatogonia cells and spermatocytes (X400). **F.** adult male treated with lead acetate plus camel milk showing poor spermatogenesis and few spermatozoa inside seminiferous tubules (X400). **G.** adult male born for mother exposed to lead acetate during pregnancy and lactation periods showing absence of spermatids and spermatocytes (X400). **H.** male treated with lead acetate plus cow milk showing degenerated seminiferous tubules and absence of 1ry & 2ndry spermatocytes and spermatids(X400).

Table 9 showed that both testicular lead and cholesterol content increased significantly ($p<0.05$) in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one and there were significant ($p<0.05$) decrease in testicular testosterone and zinc level in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one. There was no significant ($p<0.05$) difference between lead acetate and lead acetate plus camel or cow milk treated groups in all parameters estimated (Table 9).

Table 10 showed that MDA increased significantly ($p<0.05$) in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one. Moreover, there was a significant decrease in GSH, SOD and CAT in male rats born for mothers treated with both lead acetate and lead acetate plus camel or cow milk compared to control one. There was no significant ($p<0.05$) difference between lead acetate and camel or milk plus lead acetate treated groups in all parameters estimated (Table 10).

Histopathological examination

Testes of mature rats control (treated saline); treated with camel milk, male born to mother treated with saline during pregnancy and lactation and male born to mother treated with camel milk during pregnancy and lactation) showed normal structure of seminiferous tubules containing all layers of spermatogenic cells with huge amount of sperms (Figs 1A, B, C and D). Photomicrograph of the testes of male rats treated with lead acetate showed degenerative spermatogenic cells without sperm inside the seminiferous tubules (Fig 1E). Testes of mature rats treated with camel milk plus lead acetate show some activity of seminiferous tubules with few sperm inside seminiferous tubules (Fig 1F). Testes of mature rats born for mothers treated during pregnancy and lactation periods with camel or cow milk plus lead acetate showed degeneration of seminiferous tubules characterised by degeneration and necrosis of germ cells with the reduction of sperm in center of the seminiferous tubules with reduction of the interstitial cells (Figs 1 G and H).

The present study indicated that exposure to lead produced decrease in the final body weight. Aprioku and Siminialayi (2013) reported that the growth rate decreased in rat intoxicated with lead. The decrease in the body weight might be due to imbalance metabolism as result of disturbing zinc status in zinc dependent enzymes, which are essential for several metabolic processes. Testes, seminal vesicles, epididymis and prostate weights decreased in adult rats administered lead acetate for 60 days or in adult rat born for dam exposed to lead during pregnancy and lactation periods. Similar results were obtained in lead acetate exposed male rats (El Sayed and El-Neweshy, 2010). The decrease in the weights of the reproductive organs referred to the decrease in testosterone level or to the loss of the body weight. The decrease in the testosterone production and accessory sex gland weights may be a result of the decrease in the body weight (Rehm *et al,* 2008). Lead treated group (adult or born for mother exposed to lead at pregnancy or lactation period) in our study showed significant decrease in serum testosterone levels. El Sayed and El-Neweshy (2010) found that serum testosterone level fall in animals treated with lead. The decrease in plasma testosterone level might be due to reduction of testosterone production by Leydig cells which confirmed by its reduction in testicular tissues and increase in cholesterol testicular content. The reduction in testosterone level in the lead acetate treated rats may be due to reduction of utilisation of cholesterol by the Leydig cells. The stimulated Leydig cells function are impaired by the high cholesterol level (Tong *et al,* 2004). In the present study, lead acetate reduced LH and FSH levels in the lead treated group. Similar results were obtained by El Sayed and El-Neweshy (2010). FSH stimulates synthesis of LH receptors in Leydig cells the latter produce testosterone in response to LH stimulation (Ramaswamy and Weinbauer, 2014). Therefore, the reduction of testosterone production attributed to alteration in testicular steroidogenic enzyme activities because of decrease of FSH and LH secretion from pituitary. In the present study, sperm concentration, motility and viability reduced in lead acetate exposed rats (adult or born for mothers exposed to lead during pregnancy and lactation). These results are in accordance with the results of El-Sayed and El-Neweshy (2010). The decrease in the semen characteristics may be due to decrease of testosterone, FSH and LH or might be due to oxidative stress. The suppression of sperm motility is due to alteration of antioxidant system of spermatozoa (Ramah *et al,* 2015). In the present study, exposure to lead acetate for 60 days or at pregnancy and lactation periods resulted in significant decrease in SOD, GSH and CAT and increase in MDA. Exposure to lead in the present study produced testicular damage, which led to spermatogenic arrest. Similar results were noted by El-Sayed and El-Neweshy (2010). The

Table 7. Effect of lead acetate, camel's milk on the epididymal sperm characteristics in male rats, which their mother exposed to lead acetate at pregnancy and lactation periods.

Groups	Parameters*			
	Sperm count ($x10^6$)	Motility %	Live sperm %	Abnormal sperm %
Group 1 (S)	62.14±0.53[a]	88.50±0.43[a]	89.67±0.61[a]	12.00±0.97[a]
Group 2 (CM)	64.25±0.54[b]	90.00±0.58[a]	91.83±0.48[a]	12.83±0.83[a]
Group 3 (W)	62.39±0.49[a]	89.16±0.23[a]	90.33±1.09[a]	12.50±0.76[a]
Group 4 (Pb)	0.57±0.17[c]	20.14±1.03[c]	49.5±1.82[c]	29.50±0.99[b]
Group 5 (CM+Pb)	0.60±0.13[c]	20.20±2.01[c]	51.17±1.66[c]	28..17±2.15[b]
Group 6 (W+Pb)	0.58±0.01[c]	20.13±1.14[c]	50.50±1.61[c]	28.83±0.79[b]

N= 6 * Means ± SE

Means having different superscript letters in the same column are significantly different (P<0.05).

S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

Table 8. Effect of lead acetate, camel milk and cow milk on plasma testosterone; FSH & LH and blood lead content in adult male rats, which their mother exposed to lead acetate at pregnancy and lactation periods.

Groups	Parameters*			
	Plasma testosterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)	Lead (µg/dl)
Group 1 (S)	2.03±0.05[a]	6.05±0.09[a]	2.71±0.04[a]	0.93±0.04[a]
Group 2 (CM)	2.04±0.09[a]	6.34±0.18[a]	2.75±0.04[a]	0.97±0.09[a]
Group 3 (W)	2.07±0.05[a]	6.13±0.09[a]	2.72±0.03[a]	0.95±0.03[a]
Group 4 (Pb)	0.86±0.01[c]	3.41±0.09[b]	1.53±0.02[b]	7.62±0.40[b]
Group 5 (CM+Pb)	0.87±0.01[c]	3.50±0.08[b]	1.58±0.01[b]	7.36±0.25[b]
Group 6 (W+Pb)	0.89±0.03[c]	3.45±011[b]	1.55±0.02[b]	7.37±0.24[b]

N= 6 * Means ± SE

Means having different superscript letters in the same column are significantly different (P<0.05).

S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

LH =luteinizing hormone FSH =Follicle stimulating hormone

Table 9. Effect of lead acetate, camel milk and cow milk on testicular lead; testosterone cholesterol and zinc and testosterone content in adult male rats, which their mother exposed to lead acetate at pregnancy at lactation period.

Groups	Parameters*			
	Lead (ng/g)	Testosterone (ng/gm)	Cholesterol (mg/g)	Zinc (µg/g)
Group 1 (S)	36.78±1.01[a]	7105.24±176.84[a]	14.67±0.44[a]	15.24±0.61[a]
Group 2 (CM)	36.08±1.08[a]	7209.01±258.86[a]	14.71±0.35[a]	15.49±0.36[a]
Group 3 (W)	36.53±0.40[a]	7185.55±80.13[a]	14.80±0.23[a]	15.42±0.29[a]
Group 4 (Pb)	100.80±4.59[b]	4973.08±150.35[b]	36.78±0.035[b]	5.71±0.57[b]
Group 5 (CM+Pb)	98.86±2.31[b]	4990.92±133.92[b]	36.78±0.47[b]	5.64±0.45[b]
Group 6 (W+Pb)	100.01±3.49[b]	4979.14±3..26[b]	36.64±0.18[b]	5.53±0.32[b]

N= 6 * Means ± SE

Means having different superscript letters in the same column are significantly different (P <0.05).

S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

potential toxicity of lead caused decrease in sperm count, motility and viability as well as biochemical (decrease both SOD, GSH, CAT and zinc, while increase in MDA, lead and cholesterol) and hormones diruptions (FSH; LH and testosterone). The damage to the testicular cells induced by lead attributed to oxidative stress because it contains high quantity of unsaturated fatty acid. Lead exposure increased the level of ROS in male rat reproductive organs (Ramah *et al,* 2015). The lipid peroxidation oxidises the cell constituents resulting in their inactivation and finally damage of its membrane impartiality (Ramah *et al,* 2015). From our results, it is clear that administration of camel but not cow milk before exposure to lead significantly amended the estimated parameters although, not all were similar to control

Table 10. Effect of lead acetate, camel milk and cow milk on plasma oxidative stress marker and antioxidant content in adult male rats, which their mother exposed to lead acetate at pregnancy and lactation period.

Groups	Parameters*			
	MDA (µmol/g)	GSH (mg/g)	SOD (µ/g)	CAT (µ/g)
Group 1 (S)	5.69±0.21[a]	14.66±0.54[a]	1.51±0.07[a]	15.98±0.36[a]
Group 2 (CM)	5.61±0.32[a]	14.72±0.60[a]	1.52±0.95[a]	16.03±0.39[a]
Group 3 (W)	5.72±0.11[a]	14.42±0.37[a]	1.49±0.08[a]	16.21±0.31[a]
Group 4 (Pb)	20.47±0.36[b]	5.87±0.13[b]	0.62±0.04[b]	7.24±0.24[b]
Group 5 (CM+Pb)	20.17±0.62[b]	5.83±0.09[b]	0.61±0.04[b]	7.32±0.30[b]
Group 6 (W+Pb)	20.29±0.45[b]	5.79±0.19[b]	0.61±0.10[b]	7.33±0.11[b]

N= 6 * Means ± SE

Means having different superscript letters in the same column are significantly different (P <0.05).

S= saline; CM= camel milk; Pb= lead acetate and W= cow milk

MDA= malondialdehyde GSH= glutathione SOD= superoxidedimutase CAT= Catalase

levels. Vitamin C content in camel milk is higher than cow milk (EL-Hatmi *et al,* 2015). Moreover, the whey protein fraction and zinc in camel milk are higher than those of cow. The protective effect of camel milk against oxidative stress might be due to its high content of Ca++ and casein (Al-Ayadhi and Elamin, 2013), zinc and high content of vitamin C which act as antioxidants (Alavi *et al,* 2017). Interactions between lead and zinc inspected at absorptive and enzymatic sites, zinc occupy the same binding sites of lead on gastrointestinal transporting protein, metallothionin hence; it reduces the availability and absorption of lead and hence reducing its toxicity. Al-Hashem (2009) reported that camel milk could attenuate changes in oxidative stress parameters in testes intoxicated with aluminum chloride. Ebaid *et al* (2015) suggested that camel whey protein modulates oxidative stress and antioxidant defense system. El Bahr (2014) reported that camel milk improves liver damage induced by CCl_4 *via* activation of genes expression; upregulation of antioxidant enzyme gene expression and increasing GSH availability. Moreover, Ebaid *et al* (2015) found that camel milk peptides, digested by trypsin decreases nitric oxide, ROS and MDA. There were non-significant changes in parameters of the rat exposed to lead acetate during intrauterine life and lactation period when compared to the rats treated with lead acetate and camel's milk. It suggested that the amount of antioxidant present in the camel milk may pass through the placenta or mammary gland with low concentration that could not able to alleviate the deleterious effect of lead on these rats during pregnancy and lactation periods.

In conclusions, camel milk can alleviate the effect of lead intoxication in adult rats through its antioxidant properties, however, it could not able to do the same impact in rats born to mothers exposed to lead during pregnancy and lactation periods.

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Conflict of interests

The authors declare that there is no conflict of interest

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A HISTOLOGIC AND HISTOMORPHOMETRIC STUDY OF THE FIRST COMPARTMENT OF STOMACH IN THE DROMEDARY (*Camelus dromedarius*)

Ahmad Al Aiyan[1], Kenneth Richardson[2], Turke Shawaf[3], Saqib Abdullah[1], Robert Barigye[1], Al Aiyan A.[1], Richardson K.[2], Shawaf T.[3], Abdullah S.[1] and Barigye R.[1]

[1]Department of Veterinary Medicine, College of Food and Agriculture, United Arab Emirates University, Al Ain, UAE
[2]College of Veterinary Medicine, School of Veterinary and Life Sciences, Murdoch University, Perth, Australia
[3]Department of Clinical studies, College of Veterinary Medicine, King Faisal University, Al-Hasa, Saudi Arabia

ABSTRACT

In this study, tissue samples from 2 glandular and 2 non-glandular regions of the stomach's first compartment (C1) were collected from 48 healthy dromedaries of 4 age groups; 1 - 4 years, 5 - 7 years, 8 - 11 years and 12 - 16 years. After fixing in 10% buffered formalin, the specimens were processed routinely, stained with H&E and their histology examined and the thickness of the different layers measured. The histological data were similar to those previously reported whilst the histomorphometric data revealed significant intergroup variation ($p=0.001$) in the thickness of all layers in the caudodorsal glandular sac and the cranioventral non-glandular sac of C1 compartments. However, in the cranioventral glandular sac, the mucosal thickness was insignificant ($p>0.05$) and in the caudodorsal non-glandular sac the thicknesses of the mucosa, circular layer of the muscular layer and serosa were insignificant ($p>0.05$) in the different age groups. This study showed that the histological layers in the different regions of the first compartment of the dromedary stomach gradually increase in thickness with the animal's age.

Key words: Camel, dromedary, forestomach, glandular sac histology, histomorphometry

The true ruminants have a four-chambered stomach but camels have a three compartmented stomach consisting of a 'rumen', 'reticulum' and the 'abomasum' (Allouch, 2016; Eerdunchaolu *et al,* 1999; Langer, 1988; Singh *et al,* 1996; Vallenas *et al,* 1971; Wang *et al,* 2000). Whilst camelids do ruminate, their different stomach form has resulted in the camel being classified as a pseudoruminant (Eerdunchaolu *et al,* 1999; Langer, 1988; Singh *et al,* 1996; Vallenas *et al,* 1971; Wang *et al,* 2000). Confounding the different nomenclatures in use for the pregastric chambers of the camelid's stomach complex, several authors have reported a 4th chamber the 'omasum' (Czerkawski, 1985; Hansen and Schmidt-Nielsen, 1957; Hegazi, 1950; Smuts and Bezuidenhout, 1987). Reflecting the nomenclature that still exist in 2 recent studies of the bactrian camel stomach, Eerdunchaolu *et al (*1999) reported three compartments C1, C2 and C3, while Wang *et al (*2000) reported 2 ventricles and an abomasum. In their studies of the dromedary stomach, Abuagla *et al (*2014) and Osman (1999) reported 4 compartments namely C1, C2, C3 and C4. More recently, Pérez *et al (*2016) reported 3 compartments C1, C2 and C3 in the dromedary stomach.

In ruminants; the rumen, reticulum and omasum are nonglandular and lined by a keratinised stratified squamous epithelium while the abomasum is glandular (Banks, 1993; Eurell and Dellman, 1998). However, while most of the camel's first pregastric compartment is lined primarily by a nonglandular mucosa of keratinised stratified squamous epithelium, there are 2 large separate, distinct, glandular areas. The nomenclature used for these structures is varied ranging from; craniodorsal sac area and ventral sac area (Hansen and Schmidt-Nielsen, 1957), to glandular sac of cranioventral sac and glandular sac of caudodorsal sac (Smuts and Bezuidenhout, 1987), anterior and posterior glandular sac areas (Eerdunchaolu *et al,* 1999), cranial and caudal glandular sac areas (Wang *et al,* 2000) as well as cranioventral and caudodorsal glandular sacs (Abuagla *et al,* 2014). Other studies report 3 glandular areas (water sacs) in the camel rumen,

SEND REPRINT REQUEST TO AHMAD AL AIYAN email: a.alaiyan@uaeu.ac.ae

that also have a variety of designations ranging from; 1st, 2nd and 3rd water sacs (Hegazi, 1950) to cranioventral, caudodorsal and caudoventral water sacs (Allouch, 2016). Historically, the glandular areas were considered to play a role in the storage of water (Hegazi, 1950) but this was later disproven (Hansen and Schmidt-Nielsen, 1957). Earlier studies reported that the non-glandular region of the camel rumen, C1 of this study, was lined by keratinised stratified squamous epithelium and that the glandular regions were characterised by a simple columnar epithelium (Abdel-Magied and Taha, 2003; Amasaki *et al,* 1988; Hansen and Schmidt-Nielsen, 1957).

Many histomorphometric studies of the stomach complex have been reported in different ruminant species including cattle (Banks, 1993; Eurell and Dellman, 1998; Vivo *et al,* 1990), buffaloes (Sengar and Singh, 1970; Taluja and Saigal, 1988; Tiwari and Jamdar, 1970), goats (Chungath *et al,* 1985), sheep (Franco *et al,* 1992; Poonia *et al,* 2011) and reindeer (Mathiesen *et al,* 2000). Likewise, there have been numerous histomorphometric studies of the camel stomach complex (Abdel-Magied and Taha, 2003; Abuagla *et al,* 2014; Hansen and Schmidt-Nielsen, 1957; Naghani and Akradi, 2011). However, a comprehensive evaluation of histomorphometric changes in the histological layers of adult camel forestomach has not been reported. The aim of this study is to describe the histological characteristics of the mucosa, submucosa, muscularis and serosa of the dromedary's first stomach compartment. In addition, age-related histomorphometric changes were evaluated in animals aged from 1 to 16 years.

Materials and Methods

Study animals

The tissue samples for this study were taken from 48 healthy dromedaries of both sexes aged between 1 to 16 years old. In accordance with the research ethics code of the United Arab Emirates University Animal Ethics Committee, tissue samples were collected from camels slaughtered for food at the municipal slaughterhouse, Al Ain, Emirate of Abu Dhabi, UAE. Prior to slaughter, the age of the study animals was estimated by examining their dentition (Rabagliati, 1924). Based on age, 12 camels were assigned to each group where; Group 1 (1 - 4 years), Group 2 (5 - 7 years), Group 3 (8 - 11 years) and Group 4 (12 - 16 years). Immediately after slaughter, a 5×5 cm tissue sample was collected from the cranioventral non-glandular sac (CVNGS), cranioventral glandular sac (CVGS), caudodorsal non-glandular sac (CDNGS) and caudodorsal glandular sac (CDGS) of the first stomach compartment (Fig 1). The specimens were placed immediately in 10% buffered formalin and held at room temperature for at least 2 weeks prior to histological processing.

Tissue processing and staining

Following fixation, samples were trimmed to approximately 1.5×2.5 cm, placed into prelabelled tissue cassettes and loaded onto a spin tissue processor STP 250-VR (Pantigliate, Milan, Italy). The samples were processed routinely and 5 µm transverse sections were made using a YD-355AT microtome (Jinhua City, Zhejiang Province, China). The tissue ribbons were mounted onto microscopic slides, stained routinely with haematoxylin and eosin (H&E).

Light microscopy, histomorphometric and statistical analysis

A detailed histological evaluation of the H&E stained tissue sections was done using a light microscope (Olympus light microscope BX53, Japan). Each section was examined using 10x, 40x and 60x objectives, starting from the lumen and then scanning across the; tunica mucosa, tela submucosa, tunica muscularis, to the tunica adventitia. All histological features including the type of epithelium, connective tissue, blood and lymphatic vessels, autonomic nerve ganglia, smooth muscle and adipose tissue were examined. The thickness of the different histological layers were measured with the aid of an Olympus light microscope (BX53, Japan). An analysis of variance using Statistical Packages for Social Sciences software (SPSS Inc. Version 20, Chicago IL, USA) was conducted on all thickness data. The mean of the intergroup variation in the thickness of the different histological layers were calculated using Duncan's multiple range test and the differences in the dimensions of the different histological layers were considered significant at $p<0.05$.

Results and Discussion

Macroscopic assessment of the camel stomach

In the present study, a comprehensive gross and microscopic assessment of the camel stomach demonstrated that the stomach of the camel is divided into 3 compartments namely C1, C2 and C3 (Fig 1A and B). Compartment 1 is the largest and occupies most of left side of the abdominal cavity. Its parietal surface abuts the diaphragm and left abdominal wall while its visceral surface is oriented to the right and is related to C2, C3, liver

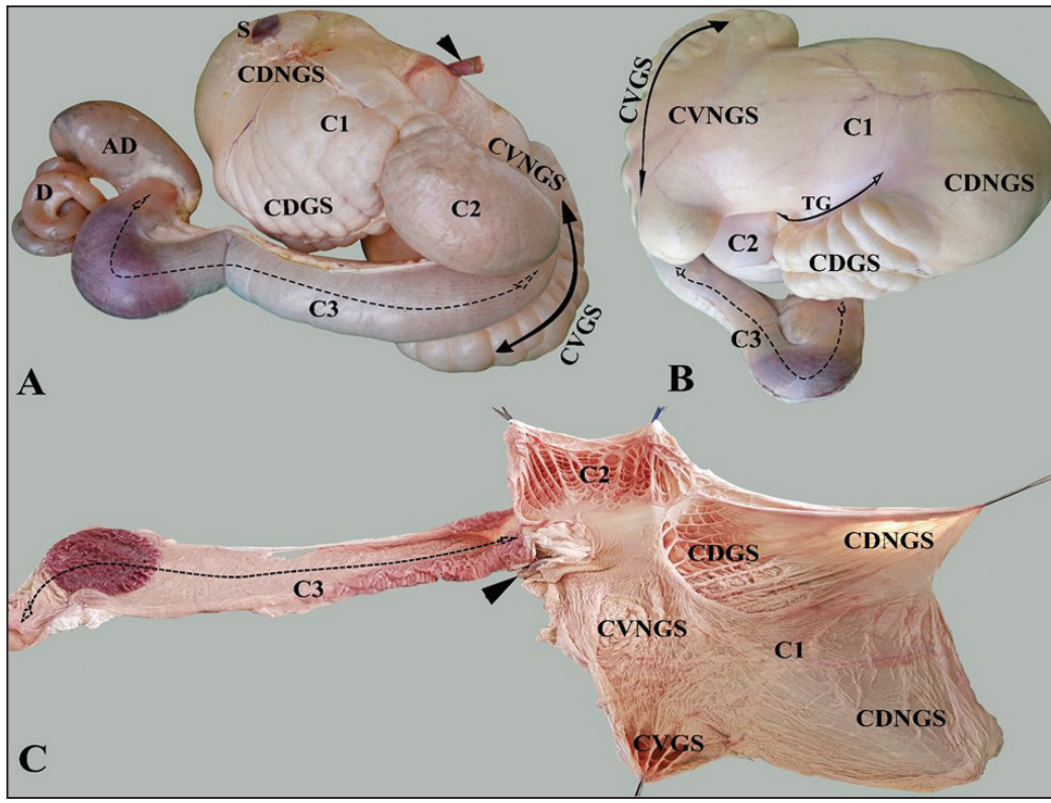

Fig 1. Composite photograph of the gross anatomy of the dromedary stomach complex where (A) right visceral aspect placed in its correct topographical position in the abdominal cavity, (B) ventral view and (C) luminal view of a dissected stomach complex. Here (C1) is the first compartment, (C2) second compartment, (C3) (interrupted black arrows) third compartment, (CDGS) caudodorsal glandular sac, (CDNGS) caudodorsal nonglandular sac, (CVGS) cranioventral glandular sac, (CVNGS) cranioventral nonglandular sac, (TG) transverse groove, (AD) ampulla duodeni, (D) duodenum, (S) spleen, (black arrowheads) oesophagus.

and intestines. The dorsal border of C1 lies against the diaphragm and the roof of the abdominal cavity, where the oesophagus opens craniodorsally into C1 (Fig 1A). The ventral border of C1 follows the contour of the abdominal floor. Topographically, C1 is divided by a strong oblique transverse groove into cranioventral and the caudodorsal sacs, in each of which a glandular sacculated area, namely cranioventral and caudodorsal glandular sacs, are situated (Fig 1A and B). Internally the nonglandular regions are characterised by laminae-like mucosal folds lined by stratified squamous epithelium whilst the glandular sacs are characterised by a retiform appearance, where robust radiating pillars (some being up to 1.5 cm in height) extend across the sac (Fig 1C). These pillars are connected by numerous cross struts forming a series of deep, mostly rectangular subcompartments (cells) that are lined by glandular mucosa characterised by rugae that are lined by a simple columnar epithelial cells.

The 2nd compartment (C2) is ovoid in shape, situated to the right and cranioventral to the cardia, directly cranial to the caudodorsal glandular sac of C1 (Fig 1A). Compartment 2 is separated externally from C1 by a small deep groove. Internally, the orifice between the C1 and the C2 is located cranial to the caudodorsal glandular sac of C1 and appears large compared to the small constricted orifice that connects C2 with C3 (Fig 1A and C). The elongated tubular 3rd compartment (C3) originates from C2 and lies cranially on the dorsal surface of the cranioventral sac and traverses caudally across the right side of C1 to merge with a small 'true' gastric gland region, immediately cranial to the duodenum (Fig 1A and B).

Histology of the non-glandular caudodorsal and cranioventral sacs

In all age groups, the non-glandular regions of (C1) of the dromedary stomach were characterised by a tunica mucosa, tela submucosa, tunica muscularis and tunica adventitia (Figs 2 and 3). The tunica mucosa has a luminal stratified squamous keratinised epithelium overlying a series of mucosal folds of varying height. Its epithelium is many cells thick and has a basal layer that is organised into

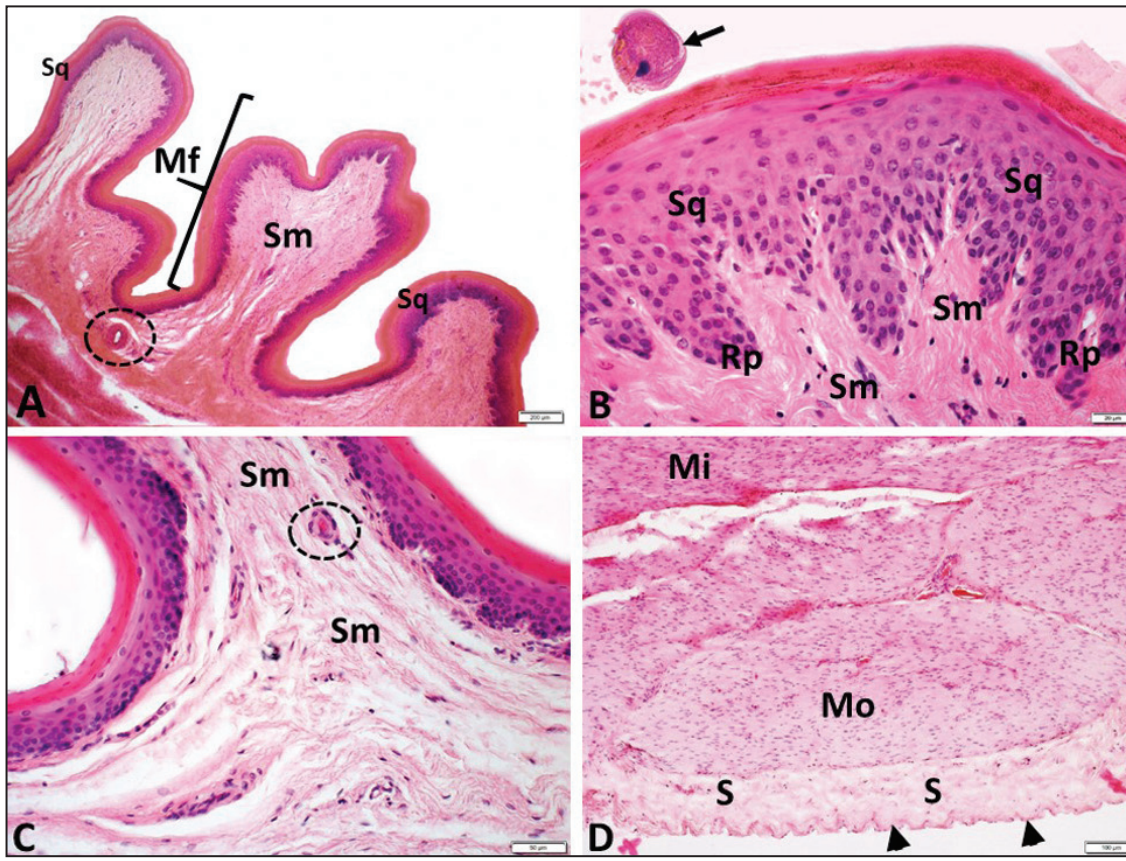

Fig 2. Light microscopy of the caudodorsal nonglandular sac where (A) mucosal folds, (B) mucosa, (C) base of a mucosal fold and (D) tunica muscularis and adventitia. Note the thick complex stratified squamous epithelium (Sq) covering the mucosal folds (Mf), the basal layer of the stratified squamous epithelium organised into short and stout downward projecting rete peg-like structures (Rp), (black arrow) *Balantidium* spp. symbiont, (Sm) tela submucosa with small blood vessels (interrupted black circles) in abundant connective tissue, (Mi) tunica muscularis circular and (Mo) tunica muscularis longitudinal, (S) adventitia with abundant connective tissue and lined by a simple squamous epithelium (black arrowheads). (H&E stain).

short, stout, peg-like structures extending into the adjacent submucosa. Occasional mitotic states are found within the basal layer. In addition, some keratinocytes located within the prekeratin layer have small amounts of intracytoplasmic keratohyalin granules (Fig 2B). The prekeratin layer gradually transitions into a parakeratotic keratin layer that is approximately one third of the entire thickness of the stratified squamous epithelium (Fig 2B and 3). Located within the lumen and attached to the epithelial surface were *Balantidium coli* like round to ovoid shaped protozoans (Fig 2B).

The submucosa occupying the core of the mucosal folds (Fig 2A, B and C) is characterised by large amounts of loose to moderately dense connective tissue that is rich in collagen fibres, numerous fibroblasts, a few small nerves, many blood vessels (Fig 3C), as well as several extensive adipose tissue deposits (Fig 3C). The tunica muscularis is composed of a broad inner circular and thinner outer longitudinal smooth muscle layer (Fig 2D and 3D). In the intervening region between the 2 smooth muscle layers there are; large amounts of connective tissue, moderate numbers of variably sized blood and lymphatic vessels, occasional post-ganglionic nerve fibres and clusters of ganglionic neuronal cells of the myenteric plexus, as well as adipose tissue deposits. The tunica adventitia has small accumulations of loose connective tissue interspersed with variably sized blood and lymphatic vessels all surmounted by a simple squamous epithelium.

Histology of the glandular caudodorsal and cranioventral sacs

In all age groups, the glandular sacs of C1 were characterised by a tunica mucosa, tela submucosa, tunica muscularis and tunica adventitia (Figs 4 and 5). The tunica mucosa bordering the lumen had a simple columnar epithelium, overlying the lamina propria and a distinct lamina muscularis mucosa. The mucosal surface was thrown into rugae that were lined by a glandular epithelium composed of

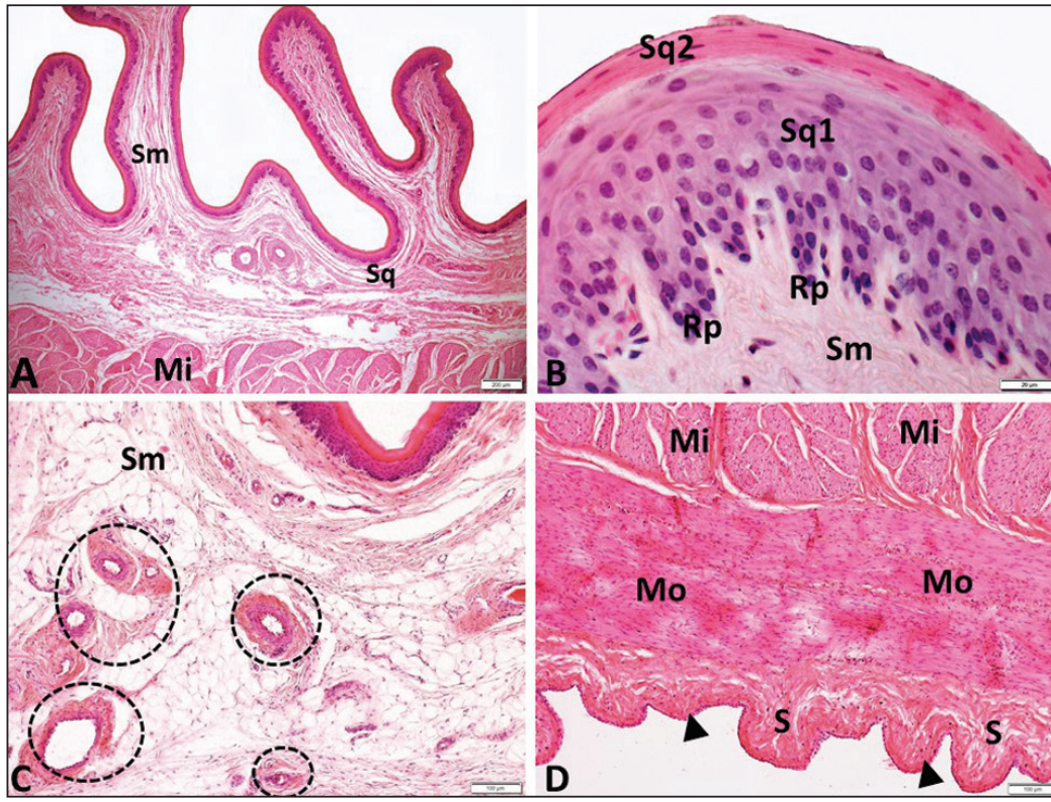

Fig 3. Light microscopy of the cranioventral nonglandular sac where (A) mucosal folds, (B) mucosa, (C) base of a mucosal fold and (D) tunica muscularis and adventitia. Where (Sq1) thick stratified squamous epithelium that gradually transitions into (Sq2) keratin layer, (Rp) rete peg-like structures of stratified squamous epithelium, (Sm) tunica propria submucosa with numerous blood vessels (interrupted black circles) and abundant adipose and loose connective tissue. (Mi) tunica muscularis circular and (Mo) tunica muscularis longitudinal. (S) adventitia characterised by abundant connective tissue and lined by a simple squamous epithelium (black arrowheads). (H&E stain).

numerous mucous neck cells supported by simple columnar epithelial cells seated on a barely visible basement membrane. The stomach glands were simple tubular and occasionally double branched sitting upon crypts that lied close to the lamina muscularis mucosae. The lamina propria had large numbers of lymphocytes, a few plasma cells together with scattered oeosinophils.

The tela submucosa (Figs 4A, C; Fig 5A, C) was an extensive area of loose connective tissue having many blood and lymphatic vessels, obvious neural plexuses, well defined lymphatic aggregations as well as extensive local adipose deposits. The tunica muscularis had a broad inner circular layer and a narrower outer longitudinal layer. The interstitial areas between the circular and longitudinal muscle layers was characterised by small amounts of loose connective tissue in which blood vessels, clumps of parasympathetic ganglion cells and postganglionic fibres as well as small deposits of adipose tissue occur. The tunica adventitia (Figs 4D and 5D) had small amounts of collagen-rich connective tissue in which occasional fibroblasts, large numbers of large lymphatic vessels and small numbers of variably sized blood vessels were present. The outer layer, the tunica serosa, is a simple squamous epithelium (Figs 4D and 5D).

Histomorphometric and statistical analyses

Caudodorsal non-glandular sac

There was statistically significant intergroup variation in the thickness of the tela submucosa and the longitudinal smooth muscle layer ($p=0.0001$). Thickness were least in the 1-4-year-olds and maximal 12-16-year-olds (Table 1). However, the thickness of the mucosa ($p=0.080$), muscularis circular layer ($p=0.958$) and adventitia ($p=0.060$) did not show significant intergroup variation (Table 1).

Caudodorsal glandular sac

The thickness of the mucosa, submucosa, tunica muscularis and adventitia in the CDGS showed significant intergroup variation among the 4 groups (Table 2). The thickness of all the histological layers increased progressively with the animal's age (Table 2).

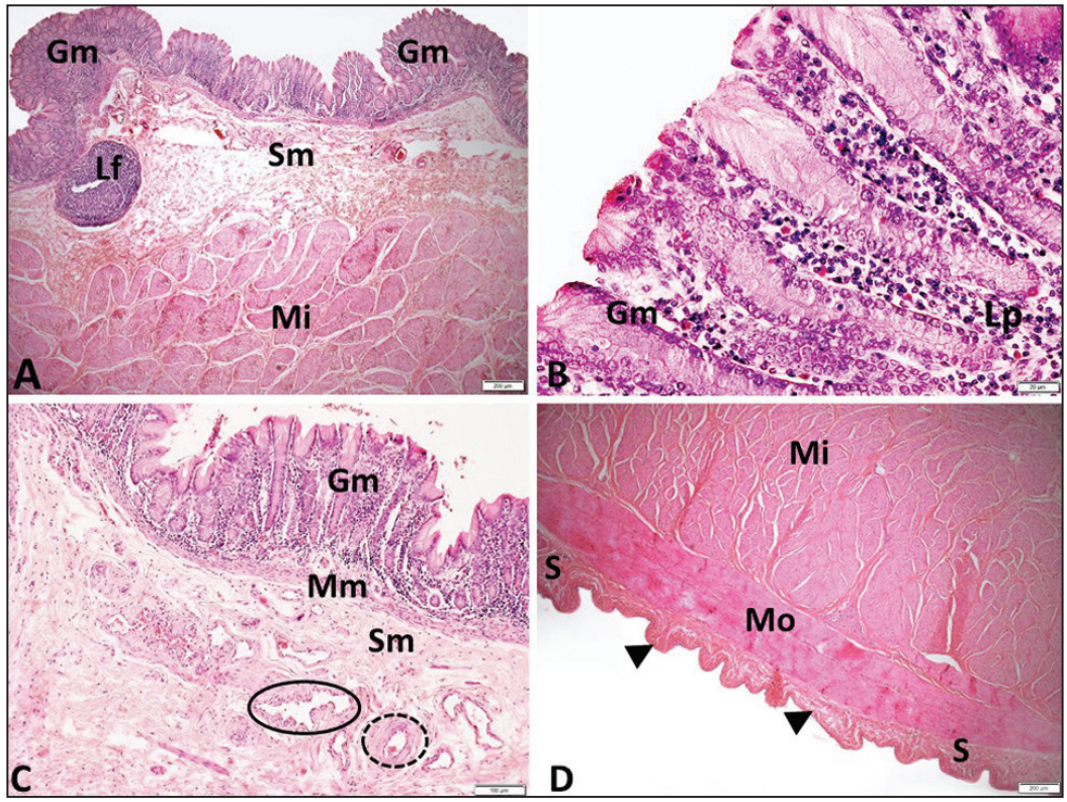

Fig 4. Light microscopy of the caudodorsal glandular sac where (A) over view, (B) mucosa, (C) mucosa and submucosa (D) tunica muscularis and adventitia. Note the glandular mucosa is organised into folds lined by a simple columnar epithelium that is rich in mucous producing cells (Gm). Lamina propria (Lp) is infiltrated by numerous lymphocytes, a few plasma cells along with occasional oeosinophils; muscularis mucosae (Mm). The tunica submucosa (Sm) has numerous blood (interrupted black circles) and lymphatic vessels (solid circles) within loose connective tissue. The tunica muscularis has circular (Mi) and longitudinal (Mo) layers. The adventitia (S) is lined by a simple squamous epithelium (black arrowheads). A single lymphoid follicle (Lf) is present in the submucosa. (H&E stain).

Cranioventral non-glandular sac

The thicknesses of the mucosa, submucosa, circular smooth muscle layer and the tunica adventitia of the CVNGS showed significant intergroup variation with thickness gradually increasing from the youngest to the oldest animals (Table 3). The muscularis longitudinal layer was significantly thicker in group 4 with decreasing thickness in groups 2, 1 and 3, respectively.

Cranioventral glandular sac

The mucosal thickness of the CVGS was similar in all groups (Table 4). However, the submucosal thickness varied significantly with age where maximal thickness occurred in the older animals and least in the youngest animals. The thickness of the muscularis circular layer had significant intergroup variation with the maximum thickness observed in group 2, followed by groups 4, 3 and 1, respectively. The thickness of the muscularis longitudinal layer also showed significant intergroup variation with maximum values observed in group 4 followed by groups 2, 3 and 1, respectively. The thickness of the adventitia increased significantly with age (Table 4).

Many studies have described the gross morphological features (Abdel-Magied and Taha, 2003; Abuagla *et al,* 2014; Osman, 1999; Smuts and Bezuidenhout, 1987; Wang *et al,* 2000) and others have reported the histological differences in the mucosal layers of the individual anatomical compartments of the camel stomach (Abdel-Magied and Taha, 2003; Dougbag and Berg, 1980; Eerdunchaolu *et al,* 1999; Hansen and Schmidt-Nielsen, 1957; Hegazi, 1950).

Conflicting interpretations and varying nomenclatures have arisen in the attempts to make a direct comparison between the stomach complex of the camel and that of ruminants. To highlight these inconsistencies, some researchers classified the camel as a pseudoruminant on the basis of a 3 compartment stomach (Abdel-Magied and Taha, 2003; Allouch, 2016; Dougbag and Berg, 1980; Eerdunchaolu *et al,* 1999; Pérez *et al,* 2016; Vallenas *et al,* 1971; Wang *et al,* 2000). On contrary,

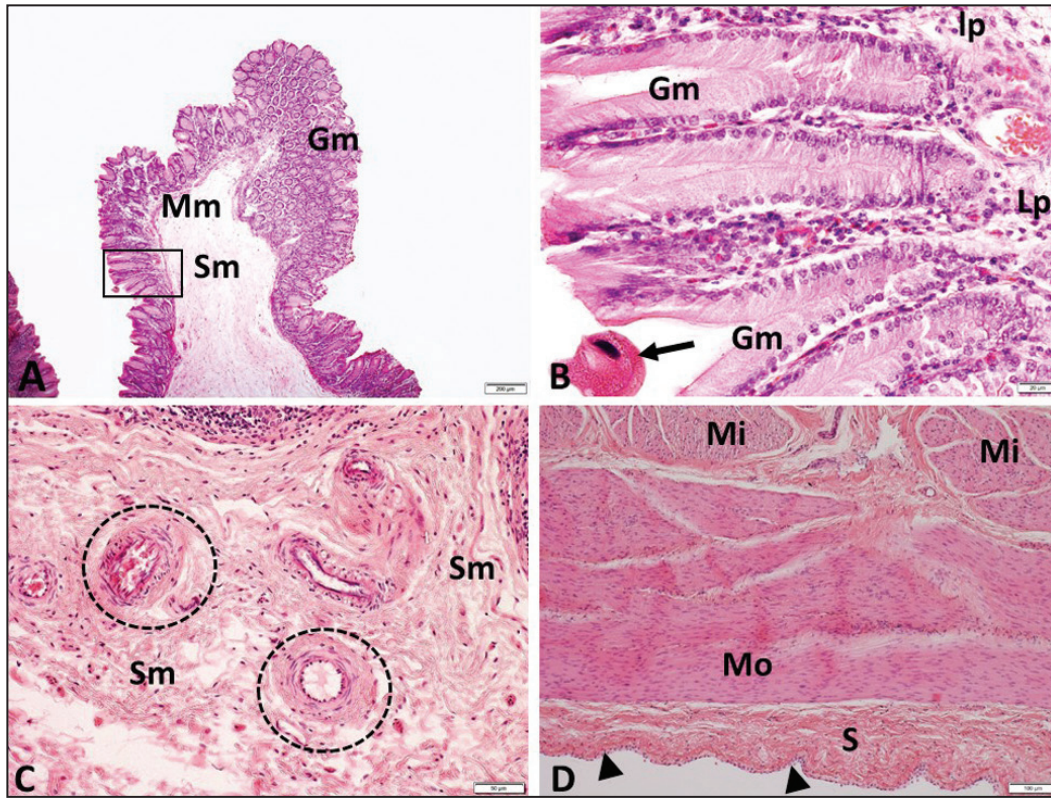

Fig 5. Light microscopy of cranioventral glandular sac where (A) mucosal fold, (B) mucosa, (C) base of a mucosal fold, (D) tunica muscularis and adventitia. Note the glandular mucosa is organised into folds lined by simple columnar epithelium (Gm) with many mucous neck cells. The lamina propria (Lp) has numerous lymphocytes, few plasma cells and occasional oeosinophils. (Mm) muscularis mucosae, (Sm) tunica submucosa with numerous blood vessels (interrupted black circles) and loose connective tissue. The tunica muscularis has circular (Mi) and longitudinal (Mo) layers. The adventitia (S) is lined by a simple squamous epithelium (black arrowheads). Black arrow, a *Balantidium* sp. protozoan.

others have claimed that the camel stomach has 4 compartments (Abuagla *et al,* 2014; Hansen and Schmidt-Nielsen, 1957; Hegazi, 1950; Osman, 1999; Smuts and Bezuidenhout, 1987). However, none of the compartments of the dromedary stomach has a similar suite of histological features as is found in the rumen, reticulum, omasum and abomasum of cattle or sheep (Banks, 1993; Eurell and Dellman, 1998). Consequently, the dromedary's stomach compartments cannot be considered homologous with any of those of the ruminants. The present study has revisited the gross anatomical structure of the dromedary stomach and on the basis of our data, we have adopted the terms; 1st compartment C1, 2nd compartment C2 and the 3rd compartment C3. Similarly, according to the topographical position of the 2 divisions of C1, we have adopted the terms, caudodorsal and cranioventral sacs of C1.

In present study of the dromedary stomach, the mucosal surface of the nonglandular regions of C1 share microscopic characteristics with the ruminal mucosa of the Ruminantia. However, we found that the first compartment of the dromedary stomach is characterised by mucosal folds that are morphologically dissimilar to the ruminal papillae of the Ruminantia. This has been reported by other authors (Eurell and Dellman, 1998; Hansen and Schmidt-Nielsen, 1957; Tamate *et al,* 1971). This study confirms the presence of the mucosal folds of the dromedary's first forestomach compartment reported by Hansen and Schmidt-Nielsen (1957) and in the bactrian camel reported by Amasaki *et al (*1988). Previously, Tamate *et al (*1971) and Singh *et al (*1983) have reported that ruminal papillae in cattle serve to increase the surface area for the absorption of volatile fatty acids and electrolytes. It is logical to conclude that the laminae-like mucosal folds seen in the nonglandular areas of C1 of the dromedary may also serve similar physiological functions.

In the present study, the muscularis mucosae is absent from the nonglandular areas of C1, as has been reported for the forestomachs in a number of domestic ruminant species including cattle (Eurell and Dellman, 1998; Vivo *et al,* 1990), sheep (Franco

Table 1. Thickness of the layers of the non-glandular part of the caudodorsal sac of the rumen in different age groups.

Group	1	2	3	4	P-value
	(1-4 year)	(5-7 year)	(8-11 years)	(12-16 year)	
Mucosa	138.02 ± 12.40[a]	145.73 ± 12.46[ab]	163.48 ± 21.26[ab]	188.50 ± 8.45[b]	0.08
Submucosa	560.52 ± 26.85[a]	813.57 ± 38.62[b]	956.33 ± 48.39[c]	987.79 ± 41.02[c]	0.00
Inner circular	1068.91 ± 24.76[a]	1183.83 ± 35.10[b]	1152.32 ± 35.59[ab]	1151.65 ± 20.80[ab]	0.958
Outer longitudinal	666.16 ± 21.82[a]	680.93 ± 27.82[a]	760.41 ± 38.52[a]	920.48 ± 37.58[b]	0.00
Serosa	193.35 ± 13.85[a]	188.76 ± 18.71[a]	230.36 ± 23.31[ab]	254.09 ± 19.33[b]	0.060

Values with different superscripts in a row differ significantly ($p<0.05$).

Table 2. Thickness of the layers of the caudodorsal glandular sac of the rumen in different age groups.

Group	1	2	3	4	P-value
	(1-4 year)	(5-7 year)	(8-11 years)	(12-16 year)	
Mucosa	129.04 ± 6.08[a]	135.38 ± 9.566[a]	175.18 ± 7.23[b]	181.85 ± 8.80[b]	0.000
Submucosa	609.04 ± 46.47[a]	742.53 ± 36.38[b]	781.88 ± 40.43[b]	1012.02 ± 18.60[c]	0.000
Inner circular	9994.62 ± 36.26[a]	1089.06 ± 27.26[a]	1341.79 ± 44.80[b]	1661.63 ± 48.13[c]	0.000
Outer longitudinal	780.93 ± 23.32[a]	969.03 ± 37.34[b]	916.53 ± 36.23[b]	983.85 ±31.29[b]	0.000
Serosa	133.53 ± 8.01[a]	208.21 ± 15.18[b]	216.07 ± 10.04[b]	227.72 ± 14.57[b]	0.000

Values with different superscripts in a row differ significantly ($p<0.05$).

et al, 1992; Poonia *et al,* 2011) and buffaloes (Taluja and Saigal, 1988). However, the muscularis mucosae was reported to be present as scattered bundles of smooth muscle cells near the glandular sac areas of the dromedary (Hansen and Schmidt-Nielsen, 1957). The muscularis mucosae may be involved in the mixing and digestion of the food as well as acting as sphincters of the subcompartments (cells) found in the glandular regions of C1 to retain their contents, while contraction of the muscularis mucosae in the wall and floor of these cells causes evacuation of their contents. The structure of the tunica muscularis in the nonglandular sacs of the dromedary reported in this study is similar to that reported in dromedaries (Hansen and Schmidt-Nielsen, 1957) and all Ruminantia studied to date (Banks, 1993; Chungath *et al,* 1985; Eurell and Dellman, 1998; Franco *et al,* 1992; Poonia *et al,* 2011; Vivo *et al,* 1990). The present study found that the tunica serosa of the glandular sacs has an outer layer of simple squamous epithelial cells overlying an inner layer characterised by abundant loose irregular connective tissue sparsely interspersed with occasional fibroblasts, large numbers of large lymphatic vessels and low numbers of variably sized blood vessels. Similar findings have been reported in buffaloes and sheep (Poonia *et al,* 2011; Taluja and Saigal, 1988).

Glandular areas like those found in the forestomach of the dromedary are absent in domestic ruminant species including cattle (Banks, 1993; Eurell and Dellman, 1998; Vivo *et al,* 1990), buffaloes (Sengar and Singh, 1970; Taluja and Saigal, 1988; Tiwari and Jamdar, 1970), goats (Chungath *et al,* 1985) and sheep (Franco *et al,* 1992, Poonia *et al,* 2011). The mucosa in the CDGS and CVGS of the dromedary's first stomach compartment is a simple columnar epithelium rich in mucous neck cells. The mucosa is continuous with extensive simple straight tubular glands together with a few branched tubular glands. Well-developed muscularis mucosae is present in the glandular sacs of C1.

Similar to other livestock species, numerous variably sized blood vessels, myenteric plexuses and abundant adipose tissue are present in the intervening interstitial areas between the inner circular and outer longitudinal muscle layers. The tunica serosa showed histological features similar to those present in the non-glandular sacs including the presence of small blood capillaries, adipose tissue and abundant collagen fibres in the subserosa lined by simple squamous epithelial cells.

In the present study, the histomorphometric analysis of the non-glandular sacs of C1 of the dromedary stomach revealed significant intergroup variation as the mucosa, submucosa, inner circular muscular and the serosal layers were thicker in the CVNGS, while the longitudinal smooth muscle layer was thicker in the CDNGS in all groups. In the present study the maximum mucosal thickness was 226.01 µm in the CVNGS in animals aged 12-16 years

Table 3. Thickness of the layers of the non-glandular part of the cranioventral sac of the rumen in different age groups.

Group	1	2	3	4	P-value
	(1-4 year)	(5-7 year)	(8-11 years)	(12-16 year)	
Mucosa	122.82 ± 9.46[a]	141.65 ± 15.68[a]	186.46 ± 15.40[b]	226.01 ± 12.86[c]	0.000
Submucosa	942.97 ± 20.49[a]	974.72 ± 23.78[a]	1226.61 ± 22.74[b]	1466.05 ± 25.52[c]	0.000
Inner circular	1309.20 ± 47.75[a]	1280.19 ± 32.57[a]	1352.60 ± 43.74[a]	1623.80 ± 29.63[b]	0.000
Outer longitudinal	669.53 ± 23.60[a]	743.20 ± 20.08[b]	641.78 ± 16.58[a]	749.41 ± 21.46[b]	0.000
Serosa	205.94 ± 14.66[a]	257.50 ± 17.10[b]	268.38 ± 16.04[b]	273.95 ± 6.12[b]	0.005

Values with different superscripts in a row differ significantly ($p<0.05$).

Table 4. Thickness of the layers of the cranioventral glandular sac of the rumen in different age groups.

Group	1	2	3	4	P-value
	(1-4 year)	(5-7 year)	(8-11 years)	(12-16 year)	
Mucosa	157.43 ± 17.05[a]	193.73 ± 16.16[ab]	194.09 ± 12.49[ab]	200.43 ± 5.04[b]	0.115
Submucosa	673.50 ± 19.16[a]	717.54 ± 21.87[a]	867.08 ± 23.19[b]	967.45 ± 12.46[c]	0.000
Inner circular	1040.93 ± 31.84[a]	1295.09 ± 28.35[c]	1111.70 ± 43.70[ab]	1170.15 ± 20.67[b]	0.000
Outer longitudinal	670.89 ± 25.49[a]	842.97 ± 23.71[c]	772.06 ± 24.62[b]	871.13 ± 17.38[c]	0.000
Serosa	200.54 ± 8.25[a]	207.66 ± 16.50[a]	210.82 ± 14.98[a]	295.86 ± 14.28[b]	0.000

Values with different superscripts in a row differ significantly ($p<0.05$).

old and the minimal mucosal thickness was 122.82 µm in animals aged 1-4 years old. Previously, Abdel-Magied and Taha (2003) reported the average mucosal thickness of 200 µm in the non-glandular areas of the first compartment of the dromedary stomach.

In both nonglandular regions of C1, maximum thickness of the submucosa, inner circular and outer longitudinal smooth muscle layers and serosa were found in 12-16 years old animals and minimum thickness were found in 1-4 years old animals. The average thickness of the submucosa, muscularis and serosa in the first compartment of the dromedary stomach reported by Abuagla *et al* (2014) were 1124.16±521.41 µm, 1826.02±349.91 µm and 263.60±142.75 µm in the cranioventral glandular sac and 982.75±490.20 µm, 1788.90±817.44 µm and 143.90±67.9 µm in the caudodorsal glandular sac, respectively. Abdel-Magied and Taha (2003) reported mucosal thickness of the glandular sacs of the 1st compartment of the dromedary to be 250 µm.

In the present study, the mucosal layer was thicker in the cranioventral glandular sac in all groups, while the thickness of the other histological layers in the CVGS and CDGS varied among the studied groups. With the exception of the mucosal thickness, these results are consistent with those published by Abuagla *et al* (2014) who reported average mucosal thicknesses of 235.60±28.10 µm and 285.70±42.72 µm in the cranioventral and caudodorsal glandular sacs, respectively.

Acknowledgements

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HISTOMORPHOMETRIC EVALUATION OF DROMEDARIAN (*Camelus dromedarius*) HEART

Muhammad Usman[1], Anas Sarwar Qureshi[2], Sarmad Rehan[3], Adeel Sarfraz[4] and Khizar Hayat[5]

[1,2,3,5]Department of Anatomy, University of Agriculture, Faisalabad 38040, Pakistan

[4]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur 63000, Pakistan

ABSTRACT

The primary objective of present study was comprehensive exploration of age related cardiac anatomy of camel which might be helpful for appropriate understanding of CVDs. Seven hearts of each, young (1-2 years) and adult (>5 years), group were collected and dissected for macroscopic and microscopic evaluation. Tissue sections were prepared by paraffin tissue technique and morphometry was done using Image J ®. Means of all parameters were compared by student T-Test. In macroscopic evaluation relative weight of heart was found significantly ($P<0.05$) higher in young dromedaries while absolute weight, circumference, thickness of left ventricle wall, width of moderator band and thickness of aortic wall along with lumen were found significantly ($P<0.05$) higher in adult dromedaries. Microscopic parameters of heart like thickness of endocardium, sub-endocardium and the purkinje fibres diameter were found significantly ($P<0.01$) thicker in adults than young dromedaries; however, it was otherwise for sub-endothelium. Parenchymal percentage in myocardium was found significantly ($P<0.01$) lower in young than adult dromedaries, however, the stromal content followed the inverse pattern. These cardiac anatomical results can be implicated for legitimate understanding of CVDs.

Key words: Camels, Heart, Histomorphometry

Cardiovascular diseases (CVDs) are the class of complications in which normal anatomy and physiology of heart along with arteries and veins are badly affected. Cardiac arrest is most common complication of faulty coronary circulation. Constraints in the coronary circulation lead to local ischaemia, acidosis (intra and extracellular), anaerobic glucolysis along with faulty membrane permeability of cardiac muscles (Custodis *et al*, 2013; Lelovas *et al*, 2014). Macroscopic and microscopic data in quantitative form on cardiovascular system organs i.e., heart, arteries, veins and blood is considered critical for the legitimate understanding of etiology and pathogenesis of heart related diseases (Qureshi *et al*, 2017).

The demand for a biomedical model that precisely approximates the human cardiovascular anatomy and physiology is indispensable. In recent era of biomedical research large animals are being focused to understand the CVDs like dogs and porcine (Lelovas *et al*, 2014).

Dromedaries are famous for their unique physiological adaptation in extreme conditions in which other animals cannot withstand. The normal physiological circulatory vitals of camelids are blood pressure of 76-115 mmHg with cardiac rate 50 beats/min. Camel contains more blood volume (93 ml/kg) than those of other livestock animals. About 15000 ml blood can be collect from 400 kg heavy camel after bleeding (Ouajd & Kamel, 2009).

The primary objective of present study was comprehensive exploration of age related cardiac anatomy of camel which might be helpful for appropriate understanding of CVDs in camels and other species.

Materials and Methods

This study included 14 clinically healthy dromedaries of either sex of two age groups (n=7) *i.e.*, young (1 to 2 years) and adult (more than 5 years). Live weight of these animals was calculated by using formula as follows: Live weight (kg) = S(m) x T(m) x A(m) x 52 ± 25kg given by Abebe *et al* (2002).

Where S: shoulder height, T: thoracic girth and A: abdominal girth m: meter.

Before slaughtering, dentition was used to estimate the age of the animals according to the dentition formula as described by Rabagliati (1924). Different physiological parameters like rectal temperature, respiration rate and pulse rate were determined in each animal before slaughter to ascertain health status of animals (Table 1). The

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heart specimens were collected from local abattoir immediately after slaughtering from October to December, 2016. Following the collection, these were transferred to the gross anatomy laboratory of Anatomy Department at University of Agriculture, Faisalabad. The weight of the heart was determined using digital weighing balance. Relative weight was worked out by using total body weight and heart weight. Different cardiac morphometric parameters like shape, colour of coronary fat, length (from base to apex) and circumference were measured by using measuring tape. The cardiac samples were dissected from right and left sides by using surgical scalpel for atrial and ventricular dimensions. Thickness of wall of atria and ventricles of both sides were measured by using Vernier's caliper. Along with these, thickness of moderator band, thickness of aortic wall and aortic lumen were also recorded. For microscopic evaluation, 1-2 cm^3 large tissue samples were dissected from left and right atria and ventricles immediately after slaughtering and fixed in buffered formalin after washing with normal saline. Tissues were cut into thin slices and processed by paraffin preparation technique. Sections were cut at 5 µm thickness and subjected to Hematoxylin and Eosin (H&E) staining procedure as described by Bancroft *et al* (2013). Prepared slides were subjected to Image J® analysis (version 1.46) software for measurement of histological parameters like thickness (µm) of endocardium, sub-endothelium and sub-endocardium, diameter (µm) of purkinje fibres, percentage stromal and parenchymal content of myocardium.

Statistical analysis:

Means, standard error of means (SEM) and ranges for each parameter were computed using Microsoft Excel®. Group means of young and adult animals were compared by Student's T-test done with the statistical software Minitab®. Group means were compared at 5 per cent level of confidence.

Results and Discussion

Macroscopic parameters:

The heart of dromedaries was reddish brown in colour, conical shaped with white coloured coronary fat regardless of their age (Fig 1). The mean ± SEM values of all macroscopic parameters are given in Table 2. Statistical findings showed that the mean values of absolute weight of heart were found significantly ($P<0.05$) higher in adult dromedaries as compared to young dromedaries while this situation was found reverse in case of relative weight of heart. The length of heart was found non-significantly ($P>0.05$) different in both groups and adult dromedaries had significantly ($P<0.05$) larger circumference as compared with young dromedaries. The left ventricle wall thickness was found much more than that of right's within the same heart (Fig 1). The thickness of right ventricle wall was found statistically similar ($P>0.05$) in both groups, however, left ventricle wall thickness was significantly ($P<0.05$) thicker in adult camelids. A similar trend was observed in case of moderator band width while its thickness remained non-significant ($P>0.05$) in both groups. The descending aortic lumen and wall thickness measurements reflected significantly ($P<0.05$) higher values in adult dromedaries.

Microscopic parameters:

Histomorphometric parameters of dromedary's heart are shown in Table 3, Fig 2-3. Endocardium was seen significantly ($P<0.01$) thicker in adult than young dromedaries but the result ($P<0.01$) was otherwise for sub-endothelium thickness. Sub-endocardium thickness and purkinje fibres diameter was significantly ($P<0.01$) more in adult than young dromedaries. The statistical analysis revealed that percentage of parenchymal content in myocardium was significantly higher ($P<0.01$) in adult than young ones. However, the stromal content was significantly higher ($P<0.01$) in young than adult dromedaries.

The heart of the dromedaries' appeared reddish brown in colour having conical shape, markedly broad at the base that narrows in the middle and pointed at the apex. It was lodged with white coronary fat. These findings were in line with those described by Rehan and Qureshi (2007) in camel calves. The absolute weight was found more in adult (1.93 ± 0.09 kg) as compared to young (1.28 ± 0.10 kg) dromedaries. These findings are supported by

Table 1. Physiological status of young and adult camels (n=7).

Dromedaries Group	Body Scoring	Age (year)	Body Weight (kg)	Rectal Temp. (°F)	Respiration Rate (per min.)	Pulse Rate (per min.)
Young	2.5 to 3	1 to 2	180-250	101.4-102.2	13-22	48-63
Adult	2 to 3	>5	350-400	99.8-102.6	11-16	39-54

Nawal *et al* (2002) and Babiker (2004) who reported that weight of heart ranges from 2 to 5 and 1.1 to 1.2 kg in adult and young dromedaries, respectively. Similarly, Rehan and Qureshi (2007) determined absolute heart weight (1.14±0.05 kg) of camel calves which supported the current study finding that might be due to the fact that with advancing age and body weight, the absolute weight of heart increases in all species. The values of absolute heart weight in case of buffalo and cattle are 2.56 and 2.23 kg, respectively reported by Panhwar *et al* (2004) cited by Qureshi *et al* (2017). Relative weight of heart was 6.68±0.19 g/kg in young dromedaries that significantly (P<0.05) higher than adult weight 5.71±0.13 g/kg. No previous data was available to describe the relative heart weight of dromedaries. These statistical figures showed that relative weight of heart decreases with advancing age. No previous literature was found to compare this finding in camels. However, this phenomenon is supported by Hussain *et al* (2006) for buffalo's heart. Heart to body weight ratio in different animals reported by Lelovas *et al* (2014) as 5g/kg in humans, 7g/kg in adult dog and 3g/kg in adult pig and sheep.

The mean length (cm) of heart in young and adult dromedaries was 19.43±1.3 and 20.83±0.40 respectively with no significant (P>0.05) difference. Rehan and Qureshi (2007) estimated camel calf heart length of 19.54±0.44 cm which was in line with the findings of current study. However, no previous literature is available to describe the influence of age on heart length in dromedaries. The heart length was directly proportional to age in buffalo as described by Hussain *et al* (2006). Similar proportion of heart and age was documented by Shah *et al* (2010) in kids (7.38cm) and adult (7.75cm) of goats. Hence, the current findings of age influence on heart length do not support the previous findings in other species. However, circumference was measured significantly (P<0.05) more in adult dromedaries as compared to young ones which are in agreement with the observations of Hussain *et al* (2006) in buffalo and Shah *et al* (2010) in goats. It may be suggested from this outcome that a non-significant increase in heart

Table 2. Mean ± SEM of morphometric parameters of heart in young (1-2 year) and adult (>5 years) dromedaries.

Morphometric parameters		**Young**	**Adult**	**P-value**
Shape		Conical	Conical	-------
Colour of Coronary Fat		White	White	-------
Absolute Weight (kg)		1.28±0.10	1.93±0.09*	0.0330
Relative Weight (g/kg)		6.68±0.19**	5.71±0.13	0.0055
Length (cm)		19.43±1.3	20.83±0.40	0.2587
Circumference (cm)		33.4±0.58	35±0.46*	0.0103
Atrial Wall Thickness (cm)	Right	0.366±0.04	0.5±0.05	0.2873
	Left	0.41±0.04	0.63±0.08	0.2044
Ventricle Wall Thickness (cm)	Right	1.03±0.20	1.1±0.22	0.8399
	Left	1.66±0.24	2.76±0.05*	0.0486
Moderator Band	Width (cm)	0.93±0.06	2.06±0.21*	0.0305
	Thickness (cm)	0.6±0.23	0.56±0.03	0.9098
Aorta	Wall thickness (cm)	0.5±0.05	0.88±0.05*	0.0142
	Lumen Diameter (cm)	2.6±0.25	3.53±0.18*	0.0339

*= Significant at P<0.05; **= Highly significant at p<0.01

Table 3. Mean ± SEM of microscopic parameters of heart in young (1-2 year) and adult (>5 years) dromedaries.

Microscopic parameters		Young	Adult	P-value
Endocardium (µm)		172.2±4.2	218.4±5.6**	0.002
Sub Endothelium (µm)		96.1±8.1**	58.8±2.9	0.003
Sub Endocardium (µm)		78.3±2.8	111.1± 5.6**	0.001
Purkinje Fiber Diameter (µm)		40.6±2.2	52.7±1.9**	0.003
Myocardium	Parenchyma (%)	76.6±0.8	87.5±0.7**	0.006
	Stroma (%)	23.4±0.8**	12.3±0.6	0.004

*= Significant at P<0.05; **= Highly significant at P<0.01

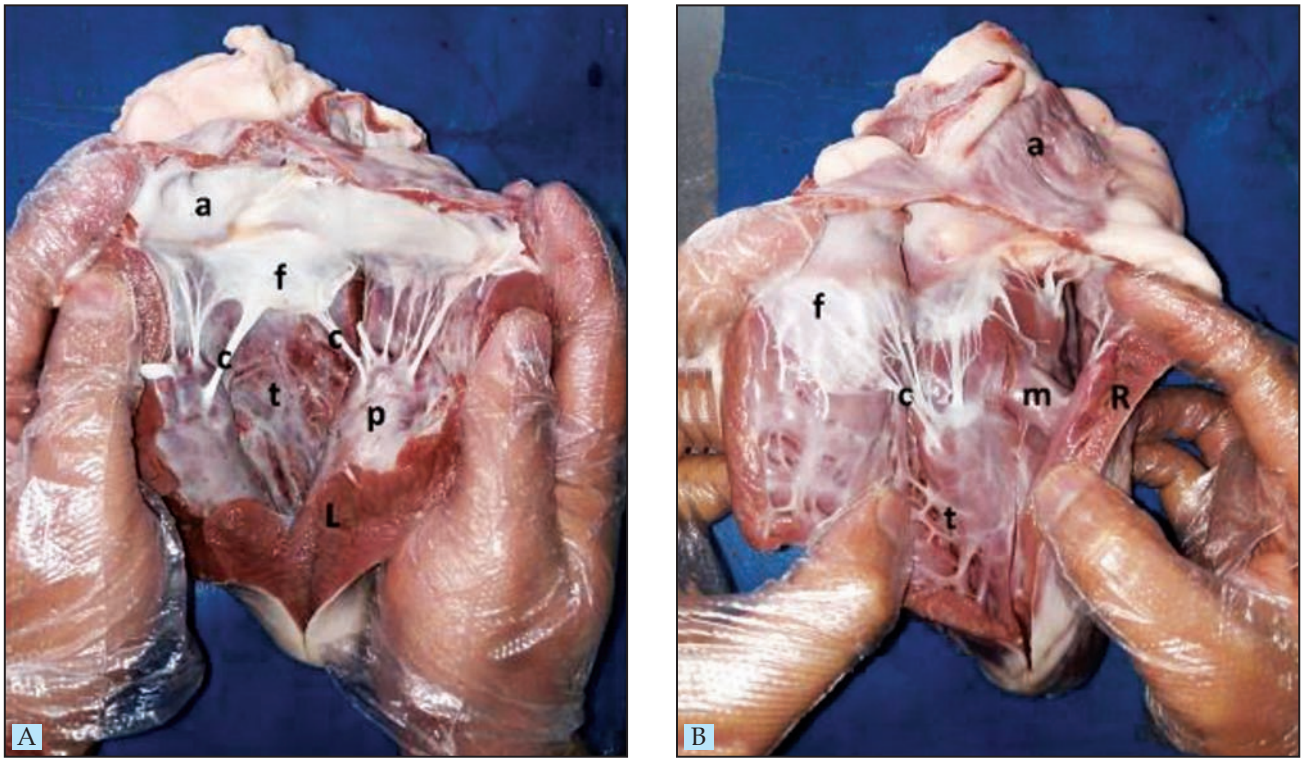

Fig 1. Pictorial view of left (A) and right side of heart: a; atrium, f; flap of semilunar cusp, c; chordae tendinae, p; papillary muscles, t; trabeculae carneae, L; left ventricular wall, R; right ventricular wall, m; moderator band.

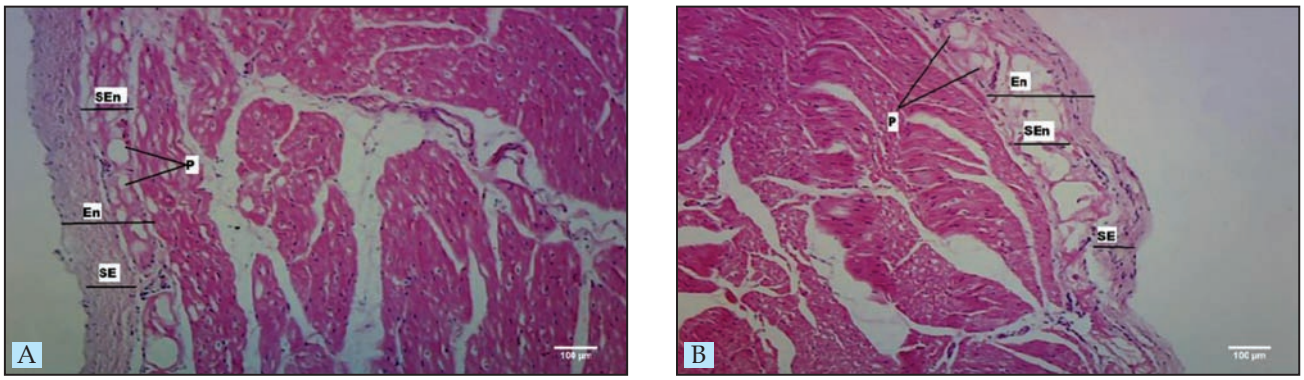

Fig 2. Cardiac histomicrograph of Young (A) and Adult (B) dromedaries (H&E 100X): Histological parameters i.e thickness of endocardium (En), Sub-endocardium (SEn) and diameter of purkinje fibre (P) is significantly (P<0.05) higher in adult dromedaries while sub-endothelium (SE) of young dromedaries is significantly (P<0.05) thicker.

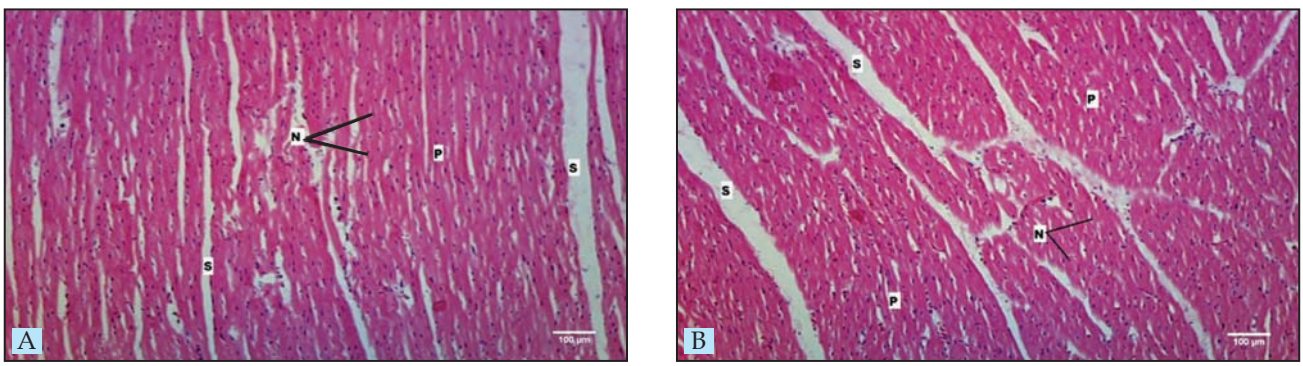

Fig 3. Cardiac histomicrograph of Young (A) and Adult (B) dromedaries (H&E 100X): Percentage of stromal content (S) is significantly (P<0.05) higher in young dromedaries while Parenchymal (P) percentage is found significantly (P<0.05) higher in adult dromedaries.

length may be accommodated for its significant increase in circumference. Age influence was witnessed non-significant (P>0.05) in case of right and left atrium, and right ventricle wall thickness while the left ventricle wall thickness was significantly (P<0.05) higher in adult camels as left ventricle requires more pressure for adequate blood circulation in the larger body. These results are in accordance to Tharwat *et al* (2012) results; they estimated the wall thickness (cm) of right and left ventricle as 1.5±0.2 and 2.8±0.7, respectively using echocardiography in adult dromedaries. The mean values of moderator band width and thickness depicted that width had significantly (P<0.05) increasing trend with increasing age while the later had remained unchanged with increasing age. This may be due to the fact that in adult dromedaries heart has to pump with more stroke volume, hence, a stronger moderator band is required to protect the overexpansion of left ventricle muscular wall. No anatomical statistics are available to compare these findings in animal species but mean thickness in adult human heart is reported 4.5±1.8 mm (Loukas *et al*, 2010). The luminal diameter and wall thickness of aorta at the base of heart were observed significantly (P<0.05) more in adult dromedaries. The age dependent literature is unavailable on these parameters of camel but Tharwat *et al* (2012) estimated the diameter of both atria and ventricles along with aortic diameter using echocardiography of adult dromedaries' heart that supported current findings of adult aortic parameters. This may be due to the fact that with advancing age and body weight thickening of these organs occur.

Microanatomical data revealed that thickness of endocardium and subendocardium layers were significantly (P<0.01) more in adult as compared to young dromedaries while subendothelium thickness followed the reverse trend. This can be linked with the higher absolute heart weight in adult dromedaries. The diameter of purkinje fibres was also seen significantly (P<0.01) increased in adult dromedaries as they require expanded conduction system for cardiac action. The parenchymal content (%) in myocardium of adult dromedaries was significantly (P<0.01) higher than young ones', while this pattern was observed inversed in case of stromal content (%). Rehan and Qureshi (2006) described the connective tissue percentage of right and left ventricle 7.26±0.28 and 6.8±0.32, respectively, in camel calves. In buffaloes, the connective tissues percentage in right and left ventricles was reported as 2.86 and 2.2, respectively (Panhwar *et al*, 2004). Age dependent intramural connective tissue in teddy goats reported by Qureshi *et al* (2013) showed a positive trend from young to adult followed by a negative trend from adult to old goats which is not in agreement to camel heart. The comprehensive age dependent data of microanatomy of camel heart is yet to be known to compare other microscopic structural findings.

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CYSTIC ECHINOCOCCOSIS IN DROMEDARY CAMEL: BIOCHEMICAL, HISTOPATHOLOGICAL AND PARASITOLOGICAL STUDIES

F.A. Al-Hizab[1], M.A. Hamouda[1], O.H. Amer[2], A.M. Edris[3], W.R. El-Ghareeb[3,4], S.M. Abdel-Raheem[3,5], Najoua Hawas[2], A.M. Elmoslemany[6] and A.M Ibrahim[1,7]

[1]Department of Pathology, [3]Department of Veterinary Public Health and Animal Husbandry (Meat Hygiene), College of Veterinary Medicine, King Faisal University, Box 400 hofof 31982 Saudi Arabia

[2]Department of Clinical Laboratory Science, College of Applied Medical Sciences, University of Hail, Saudi Arabia

[4]Food Control Department, Faculty of Veterinary Medicine (Meat Hygiene), Zagazig University, Egypt

[5]Department of Animal Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, Egypt

[6]Hygiene and Preventive Medicine Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 35516, Egypt

[7]Department of Pathology, College of Veterinary Medicine, Suez Canal University, Egypt

ABSTRACT

This study was conducted on 600 dromedary camels from March 2017 to December 2017 at Al Omran (n=330) and Al Ahsa (n=270) abattoirs (Al Ahsa Province, Saudi Arabia). The study was designed to determine the prevalence, cyst fertility and viability, biochemical and electrolyte analysis of fertile and infertile hydatid cyst as well as histopathological findings. In these 600/2600 (23.07%) camels were randomly selected for antemortem examintation. Total 171 (28.5%) were found infected with hydatid cyst, i.e. 91 (27.6%) from Omran and 80 (29.6%) from Al-Ahsa abattoirs, respectively. Furthermore, 171 hydatid cysts were examined for fertility and viability. In Al Omran, 4/91 (4.4%) were found to be fertile and viable, 1/91 (1.1%) was fertile non viable, 23/91 (25.3%) were sterile and 63/91 (69.2%) in Al Ahsa, 2/80 (2.5%) were found to be fertile and viable, whereas, 33/80 (41.2%) were sterile and 45/80 (56.2%) were calcified. Biochemical and electrolyte analysis of hydatid cysts showed significant increase in iron, total protein and alkaline phosphatase in fertile cyst. Whereas, sterile cysts showed significant increase in triglyceride, chloride and sodium. In conclusion, infertile cysts were either sterile or calcified in the dromedary camel and were predominant. The biochemical and electrolyte parameters of cyst fluid could help us to recognise different kind of cysts.

Key words: Biochemical, cystic echinococcosis, dromedary camel, fertility

Cystic echinococcosis (CE), formerly known as hydatid disease (HD) or hydatidosis, is considered an important worldwide disease that has economical and zoonotic impact. Several studies have documented the endemic and zoonotic properties of cystic echinococcosis in various provinces of Saudi Arabia (Al Mofleh *et al,* 2000; Fadaladdin *et al,* 2013; Toulah *et al,* 2017). However, this disease has public health importance as it is one of the neglected diseases (Ahmed *et al,* 2011; Ahmadic and Meshkehkar, 2012). Cystic echinococcosis remains to be a considerable cause of morbidity and mortality in many countries. It required strict control measure that may take around 20 years of sustained efforts to eliminate such disease (Craig *et al,* 2007). Liver is the main site of hydatid cysts, followed by the lungs. Other organs, like kidneys, spleen, abdominal and pelvic cavity, heart, brain and spinal cord (Geramizadeh, 2013) can be infected. Diagnosis of hydatid disease in living animals is very difficult with no routine reliable test. Detection of cysts during meat inspection or at post-mortem examination is considered the most reliable diagnostic tool (Collins and Huey, 2015). Many attempts were made to apply serological tests for diagnosis of the disease using crude Hydatid cyst fluid (HCF) (Lightowlers, 1990; Craig *et al,* 1996). Due to the great difference in antibody titre in natural CE infections, these tests show reduced sensitivity as well as cross-reactions with *T. hydatigena* (Yong and Heath, 1984; Soliman *et al,* 2014). Other techniques like a western blot showed higher sensitivity and specificity for the ovine disease (Gatti *et al,* 2007). Proteomic characterisation was used to highlight the complexity and heterogeneity of a wide range of

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proteins originated from the host and the parasites, found in HCF collected from sheep, cattle and humans (Aziz *et al,* 2011). The present study was designed to estimate the prevalence, cyst fertility and viability rates, histopathological findings and electrolyte profile and biochemical parameters in fertile and sterile cysts of cystic echinococcosis.

Materials and Methods

Study animals

The present study was conducted on local breed one humped camel of different ages and both sexes. The samples were collected from Al- Omran and Al-Ahsa abattoirs. The period of this survey was from March 2017 to December 2017.

Study design and sample size

This cross-sectional study was proposed to estimate the prevalence of CE. A representative random sample size was calculated using the formula: $N= (Z^2xP)(1-P)/e^2$ as described by Dohoo *et al* (2010), where the N = Total number of sample size, Z=1.96 for 95%confidence interval. Based on the criteria, a total of 197 samples per locality (abattoir) was required to get an accurate estimation of the prevalence. Hence, 330 and 270 were examined from Al Omran and Al Ahsa abattoirs, respectively. The samples were collected with the intention of maximising the sample size to increase precision. The sampling interval was computed based on to study period, the whole number of animals slaughtered and the required sample size. Therefore, the sampling interval was 5 (1600/330) in Al-Omran abattoir and 4 (1000/270) in Al Ahsa abattoir. The first animal was selected randomly (Thrusfield, 2005).

Abattoir survey

Visceral organs were subjected to a thorough visual inspection, palpation and systematic incision. The total number of cysts was counted and recorded. Cyst was cautiously removed with circular incisions around it and was separately collected in clean containers for further cyst characterisation. The cysts were subsequently subjected to a systematic size measurement using Vernier caliper and classified as small (<4cm), medium (4-8 cm) and a large cyst (>8cm) (Schantz, 1990).

Biochemical and electrolyte analysis

The cystic fluids were aspirated aseptically, centrifuged at 1500 rpm at 4°C for 30 min and the supernatants were analysed for various biochemical parameters including glucose, total protein, triglycerides, cholesterol, alkaline phosphatase (ALP) and electrolyte profiles using commercial kits.

Parasitological examination

Following sample collection, the individual cysts were carefully incised and its contents evacuated into a sterile test tube for microscopic examination of protoscolices.

A. Examination of cysts fertility

The cysts were classified into fertile and infertile based on the presence of either free protoscolices, appeared as white sand like material on the germinal layer. Further, the infertile cysts were subsequently categorised as sterile (fluid filled cysts), or calcified cysts which has a gritty sound sensation upon incision (McPherson, 1985; Assefa *et al,* 2015).

B. Examination of cyst viability

Viability test was applied to figure out whether they are alive or dead. Briefly, one drop of the residue containing the protoscolices added to a microscope slide then covered with a cover slip and examined microscopically to detect ameboid-like peristaltic movements of protoscolices. Identification of flame cells in the anterior and the posterior portion covered with a knob-like projection were characteristic for the invaginated protoscolices. To confirm the viability of cyst, one drop of hydatid fluid was mixed with one drop of aqueous solution 0.2% eosin (W/V) and examined microscopically (40×) (Dalimi *et al,* 2002). If the protoscolex is unable to gain the stain it considered live, whereas the dead one is stained uniformly. Several other vital stains like fast green were also used.

Histopathological examination

For histopathological examination, one cubic cm of the infected organ (liver or lung), including a part of the cyst wall and a part of the surrounded tissue, was collected from specimens, then fixed at 10% neutral formalin. The fixed tissue was prepared through paraffin section technique and 5 µ paraffin sections were stained with either haematoxylin and eosin (H&E) (Kim *et al,* 2013).

Statistical Analysis

The collected data from camels were recorded and analysed via SPSS 16.0. A statistically significant association among variables considered to exist if P value ≤ 0.05.

Results and Discussion

Distribution, Size, Shape and Nature of cystic echinococcosis

Gross examination revealed presence of cystic echinococcosis in the liver and lung. Liver possessed the vast majority of the cysts. The number of cysts ranged from only one detectable cyst to numerous cysts. Cyst's size was measured as 1-2 cm in diameter and reached up to 3 cm (<4cm). Some cysts were thin walled and contained clear, watery fluid (Fig 1A), others had a thick whitish wall with viscous fluid containing small sand like material (Fig 1B). Calcified cysts were also seen as firm whitish calcified nodules (Fig 1C). Cysts that were detected in the lung were large, with opaque wall and contained turbid sand like materials (Fig 1D)

Biochemical and electrolyte profile

Biochemical analysis revealed a significant increase in glucose, total protein, cholesterol, alkaline phosphatase, potassium, phosphorus, calcium and iron in fertile cysts, compared to sterile ones. Whereas, there was a significant increase of sodium, chloride and triglyceride in sterile cysts as compared to fertile ones (Table 1).

Cyst fertility and viability

The fertile cysts were examined directly without stains (wet amount) (Figs 2A, B, C). To confirm

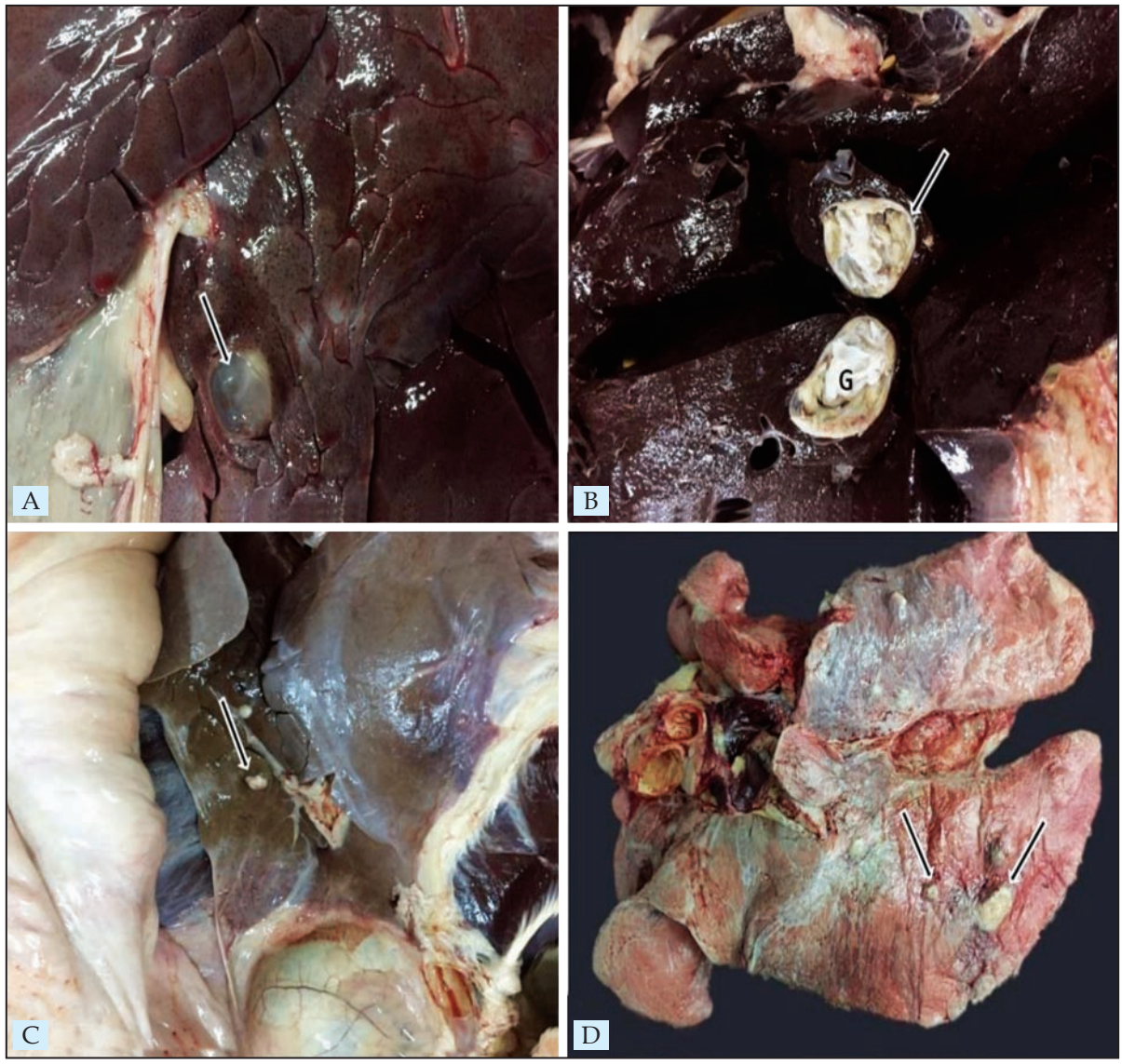

Fig 1. **A**, liver of a camel contains Cystic Echinococcosis with a thin walled and clear fluid (arrow). **B**, Large cyst (arrow) is seen embedded in the hepatic tissue containing germinal layer and sandy like materials (G). **C**, 1-2 cm diameter whitish, calcified cyst is located on the visceral surface of the liver (arrow). **D**, Multiple Cystic Echinococcosis were seen within the lung tissue (arrows).

Table 1. Comparison between various electrolyte and biochemical profiles based on fertility and sterility of cyst.

Item	Unit	Fertile (n=10) Mean ± SE	Sterile (n=10) Mean ± SE	P
Glucose, mmol/L	mmol/L	0.84±0.1**	0.12±0.00	<0.01
Total Protein	g/L	38.56±0.71***	3.04±0.39	<0.001
Cholesterol	mmol/L	1.11±0.02***	0.93±0.01	<0.001
Triglycerides	mmol/L	0.18±0.004***	0.25±0.01	<0.001
Alkaline phosphatase	U/L	2.37±0.23***	0.07±0.02	<0.001
Sodium	mmol/L	161.1±0.82*	165.2±1.49	0.02
Chloride	mmol/L	141.2±2.65***	159.75±1.19	<0.001
Potassium	mmol/L	6.12±0.16**	5.51±0.07	<0.01
Calcium	mmol/L	2.18±0.08***	1.68±0.02	<0.001
Phosphorus	mmol/L	0.7±0.24*	0.1±0.01	0.03
Iron	µmol/L	28.63±1.47***	0.85±0.37	<0.001

* = significant at p <0.05; ** = significant at p<0.01; *** = significant at p<0.001.

fertility, the slides stained with 0.1% eosin, Live cyst refuse eosin staining (Fig 3A) and stained with special stain (Fast green) (Fig 3B). Dead cysts take eosin stain (Fig 3C). Out of 171/600 (28.5%) cysts, the fertile cysts were examined for viability. In Al Omran, 4/91 (4.4%) were found to be fertile and viable, 1/91 (1.1%) was fertile non viable, 23/91 (25.3%) were sterile and 63/91 (69.2%) were calcified. In Al-Ahsa, 2/80 (2.5%) were found to be fertile and viable, 33/80 (41.2%) were sterile and 45/80 (56.2%) were calcified (Table 2).

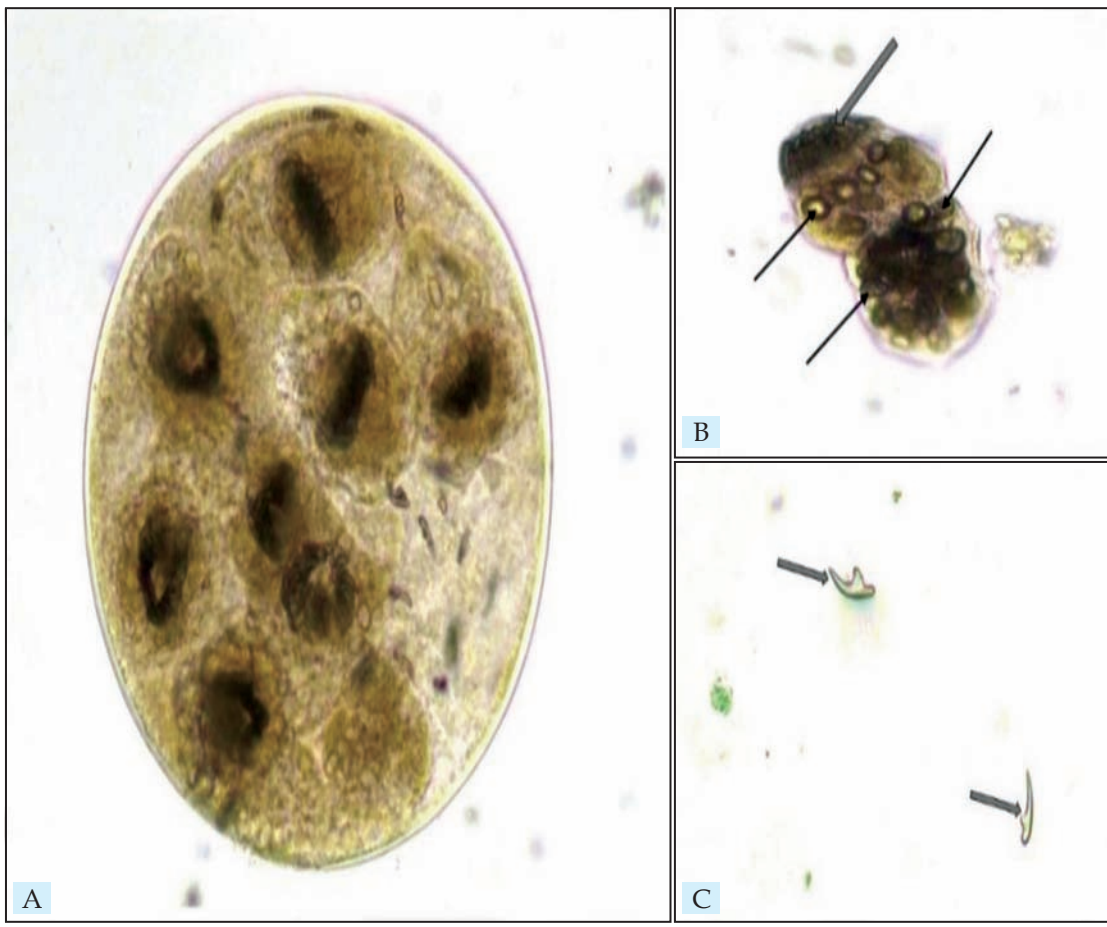

Fig 2. Wet mount - unstained fertile Cystic Echinococcosis: A, Invaginated scolices. B, Evaginated scolices with double row rostellar hooklets (Thick arrow) and calcareous corpuscles (Thin arrows). C, Note the hooks, Large hooks: 25.0 (µm), Small hooks: 17.5 (µm).

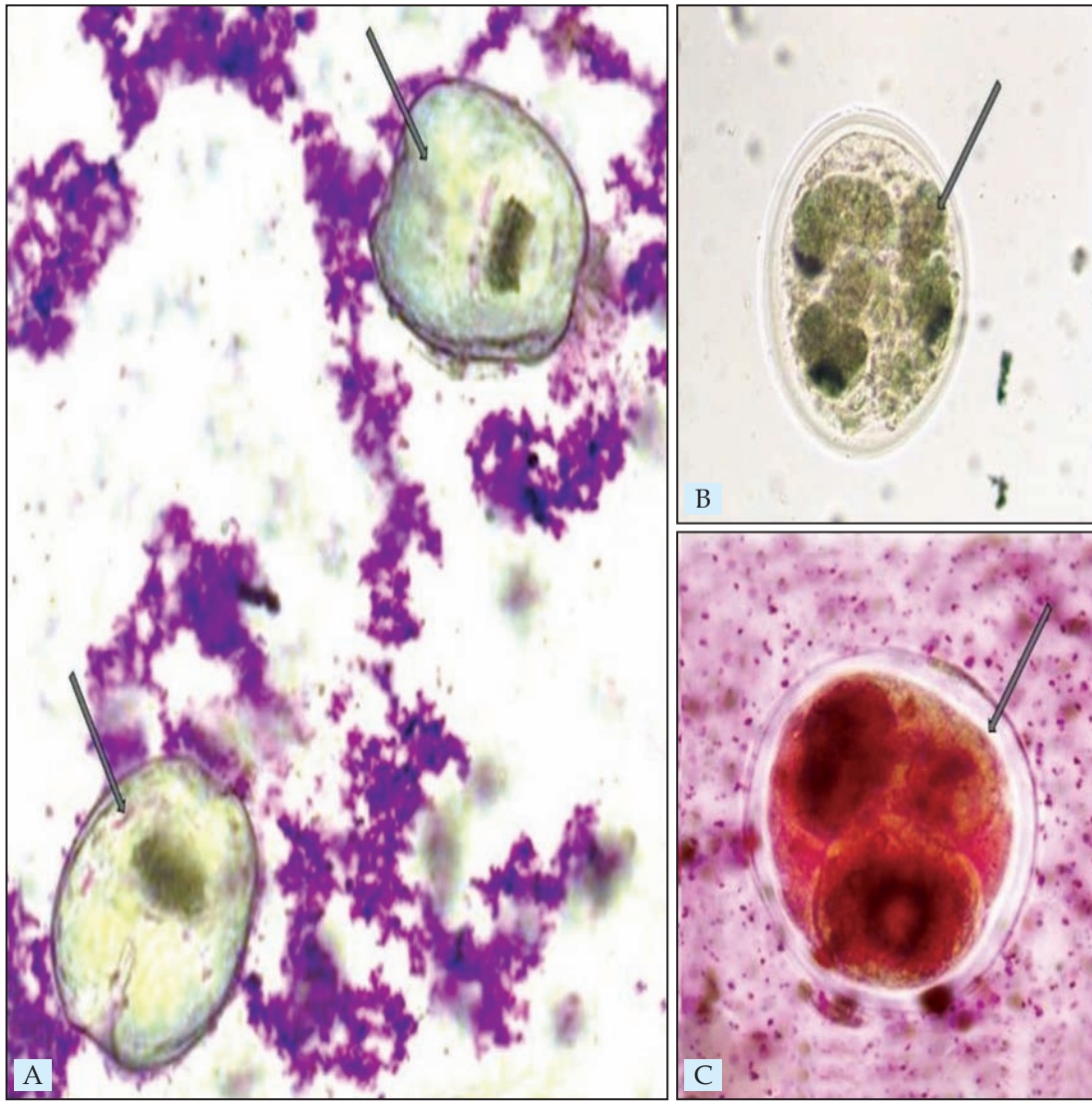

Fig 3. Wet mount - stained Cystic Echinococcosis: A, live invaginated protoscolices refuse staining with 0.1% eosin (arrows). B, Fast green stained viable cyst (arrow). C, Dead invaginated protoscolices staining with 0.1% eosin (arrow).

Table 2. Characterisation of cystic echinococcosis in two abattoirs.

Province	Abattoir	Total	Fertile (%)		Sterile (%)	Calcified (%)
			Viable (%)	Dead (%)		
Al Ahsa	Al Omran	91	4 (4.4%)	1 (1.1%)	23 (25.3%)	63 (69.2%)
	AL Ahsa	80	2 (2.5%)	0	33 (41.2%)	45 (56.2%)
Total		**171**	**6 (3.51%)**	**1 (0.58%)**	**56 (32.7%)**	**108 (63.16%)**

Microscopic findings

Cystic Echinococcus was effacing and replacing approximately 20-50% of the organ parenchyma. The majority of cysts had one chamber (unilocular). Some cysts were empty and others were filled with a cellular homogenous eosinophilic material. The cyst consisted of 3 layers, germinal, laminated, pericyst layer (P), (Fig 4 A,B). The fertile cyst was evident with the presence of multiple protoscolices, 200-250µm in diameter either free or attached to the germinal layer (protoscolices are absent in sterile cyst). Each protoscolex had thick tegument and contain suckers calcareous corpuscles and rostellum with birefringent hooks (Fig 4C). In calcified cysts, there was evidence of a large necrotic area with basophilic calcium mineralisation.The cyst was surrounded with a thick layer of fibrous connective tissue infiltrated with lymphocytes, plasma cells, macrophages, eosinophils and few multinucleated giant cells. The surrounding

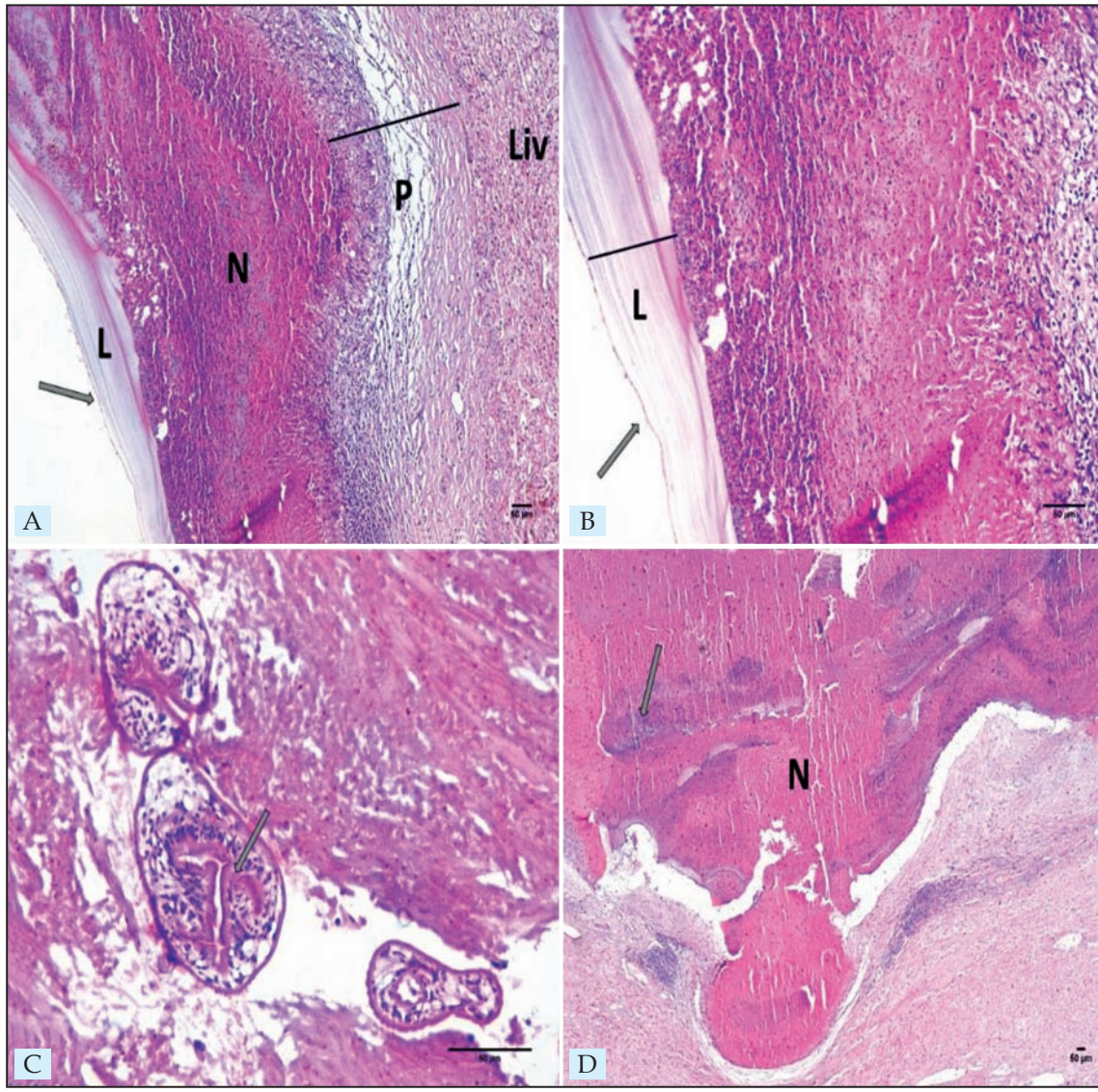

Fig 4. H&E- stained sections of camel's liver infested with cystic echinococcosis (Bar= 50µm): A, Hepatic tissue (Liver) is effaced by a parasitic cyst formed of 3 layers; germinal layer (arrow), laminated layer (L), pericyst layer (P), in addition to area of necrosis (N), B, Higher magnification of the previous image illustrates the germinal layer lined with single layer of cells (arrow) and laminated layer (L). C, Three protoscolices are seen inside the cyst containing rostrellum with birefringent hooks (arrow). D, A calcified cyst contains large necrotic area (N) with mineralisation (arrow).

hepatocytes showed necrotic cells associated with massive inflammatory cells.

Cystic echinococcosis is among the most neglected public health problems in humans and animals and causes serious socioeconomic effects throughout the world. The present study provides assessment of the magnitude of the disease in dromedary camels slaughtered in Al-Ahsa Province. The overall prevalence of cystic echinococcosis was 28.5%. Our findings were comparable with previous results in different localities in KSA, 34.64%, 16%, 32.85% and 6.86% in El-Madinah, Meka, Al-Baha and Jeddah cities, respectively (Fadaladdin *et al,* 2013; Haroun *et al,* 2008; Ibrahim, 2010; Toulah *et al,* 2017). This variation could be related to pastoralism practice, high dog population, inadequate medication and veterinary services, low hygiene and education standards, importing of animal from infected endemic areas are a continuous risk of re-introducing a disease and maintain its zoonotic life cycle (Daryani *et al,* 2007).

The fertility of cysts is an important factor that can influence the life cycle of a disease. The study showed that 3.5% of the cysts were fertile and viable, 32.7% was sterile and 63.2% was calcified. The fertility rate was lower than those observed in camels (51.57%) and sheep (18.18%) (Moghaddas *et al,* 2014; Hasona *et al,* 2017). This variation could be attributed

to the geographical situation, the nature of infected hosts, the sites of infection and genotype dependant (McManus, 2006). The high proportion of sterile cysts in camel may generally imply that most of the cysts in camel are infertile and this underscores the role of camel in maintaining the life cycle of a disease. Also, a high number of calcified cysts may be due to end stage to hydatid cysts or in the liver may be due to abundant fibrous tissue and reticuloendothelial cells (Haftu and Kebede, 2014). Histology of hydatid cyst in camel and its tissue damage were quite similar to those observed by Singh *et al* (2016) who reported that hydatid cyst results in a considerable damage to the affected organ. Hydatid cyst fluid is a mixture of chemical components arise from the parasite and host (Vuitton and Gottstein, 2010). There were variations in the biochemical contents of hydatid fluids inside intermediate hosts (Radfar and Iranyar, 2004; Osman *et al,* 2014). The variations of components play an important role in all biochemical reaction inside a hydatid cyst (Li *et al,* 2013; Osman *et al,* 2014). In the present study, biochemical analysis showed a significant increase in alkaline phosphatase in fertile cysts compared to sterile cysts. Alkaline phosphatase (ALP) is an enzyme which plays a critical role in biochemical reactions within hydatid cyst (Shaafie *et al,* 1999). Karibozorg *et al* (2014) reported that hydatid infection stimulates biliary cells to excrete ALP. So, an increase in ALP activity could be considered as a pathological biomarker in hydatid disease. Also the present results revealed a significant increase in calcium, phosphorus and iron in fertile cysts compared to sterile cysts. This finding was consistent with Radfar and Iranyar (2004) and Shaldoum *et al* (2017). There was a significant difference between the level of sodium, potassium and chloride in fertile and sterile one. The inflow of electolytes in the cyst has depended on parasite requirement and selective permeability (Rahdar *et al,* 2008).

In conclusion, infertile cysts in the dromedary camel, either sterile or calcified, were predominant and biochemical and electrolyte parameters of cyst fluid can help us to recognise the different kinds of cysts and also to promote distribution of the drug to the cyst.

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FIRST REPORT ON INCIDENCE OF *Echinococcus canadensis* G6 STRAIN FROM A DROMEDARY CAMEL OF INDIA

Shirish D. Narnaware[1] and Shyam S. Dahiya[2]

[1]ICAR- National Research Centre on Camel, Post Bag No. 07, Jorbeer, Bikaner 334001, Rajasthan, India

[2]ICAR- Directorate on Foot and Mouth Disease, IVRI Campus, Mukteswar, District Nainital Uttarakhand, India 263138

ABSTRACT

In the present study, a hydatid cyst was reported in the lung of an Indian dromedary camel during postmortem examination and it was investigated for genotype analysis. Total DNA was extracted from protoscolices and associated germinal layers of hydatid cyst and used in PCR amplification of mitochondrial *cytochrome C oxidase subunit 1* (*cox1*) gene. The PCR product was purified, sequenced and analysed using bioinformatics tools. The phylogenetic analysis showed that the isolate clustered with *Echinococcus canadensis* genotype 6 (G6). The circulation of the camel genotype (G6) in the Indian one-humped camel is reported for the first time in this study, which indicates that dromedary camel has an important role in the continuation of the *E. canadensis* life cycle.

Key words: Camel, cox 1 gene, *Echinococcus canadensis*, G6 strain, genotype, India

Hydatidosis is a zoonotic disease caused by larval stages of cestodes belonging to the genus *Echinococcus*. It is characterised by long term growth of the metacestode (hydatid cysts) in the intermediate host (McManus *et al,* 2003). It is a wide spread infection throughout the world including India and is found to occur in all domestic livestock including camels and cattle (Ibrahem *et al,* 2002; Sharma *et al,* 2013). Thus camels infected with cystic echinococcosis (CE) may represent an important source of transmission to dogs and hence indirectly to man (Lahmar *et al,* 2004). Because of the involvement of the vital organs, CE in humans is considered as a critical public health problem. Moreover, CE represents one of the neglected tropical diseases (Wahlers *et al,* 2012). Although cystic echinococcosis is highly endemic in human and animal population in India, there is still scarce information about species and/ or genotypes of the *Echinococcus granulosus* complex that infect humans and animals in India (Sharma *et al,* 2013).

Currently, ten distinct genotypes of *Echinococcus granulosus* (EG) designated as G1-G10 have been described worldwide on the basis of genetic diversity related to nucleotide sequences of the mitochondrial *cytochrome C oxidase subunit 1* (*cox1*) and NADH dehydrogenase subunit 1 (NADH 1) genes (Ahmed *et al,* 2013). These different genotypes are associated with distinct intermediate hosts including sheep, pigs, cattle, horses, camels, goats and cervids. Different genotypes would probably exhibit different antigenicity, transmission profiles and sensitivity to chemotherapeutic agents as well as different pathological consequences (Thompson and McManus, 2002). Therefore, due to epidemiological implementation and control strategies, it is essential that circulating EG genotypes in a given area of endemicity should be clearly defined (McManus *et al,* 2003). In present study a genotypic analysis of a hydatid cyst recovered from lung of a dromedary camel is reported.

Materials and Methods

An adult female dromedary camel from an organised herd located at Bikaner district of Rajasthan, India was presented for routine post mortem examination. This camel was raised under semi-intensive system of management and was regularly sent for grazing in nearby field area inhabited by large number of stray dog population. On postmortem, the camel was found to have single hydatid cyst in lung which was collected intact for determination of cyst fertility, histopathology and genotype identification. For determination of cyst fertility, the surface of the cyst was sterilised with alcoholic iodine solution and the cyst wall was penetrated using a large size needle and the contents

SEND REPRINT REQUEST TO SHIRISH D. NARNAWARE email: sdnarnaware1@gmail.com

were transferred into a sterile 15 ml centrifuge tube using a syringe. To ensure the maximum collection of protoscolices, the cyst was flushed 3-4 times with sterile normal saline. The contents were centrifuged at 5000 rpm for 5 min. The supernatant was discarded and a drop of sediment was placed on slide and examined under microscope. The part of the cyst along with lung tissue was also collected in 10% formal saline for histopathology. The formalin fixed tissue samples were embedded in paraffin, cut into 4-5 micron sections and stained with hematoxylin and eosin stain.

For genotype analysis, genomic DNA was extracted from protoscolices and associated germinal layers of hydatid cysts using a commercial kit (PureLink™ genomic DNA Kit, Thermo Fisher Scientific). The DNA was subjected to PCR for amplification of *cox1* gene of *Echinococcus* by using the specific primer sequence and the reaction procedure described previously (Pednekar *et al*, 2009). The amplified PCR product was purified and sequenced. The sequence was submitted in genebank database (accession number KY436827) and was aligned with available GenBank sequences for the *E. granulosus* genotypes. The phylogenetic analysis of the nucleotide sequence of the cox1 gene of *Echinococcus* isolated from Bikaner, Rajasthan was done by the maximum likelihood statistical method using molecular evolutionary genetics analysis software (MEGA 6) (Tamura *et al*, 2013).

Results

Grossly, the cyst collected from lung was thick walled, unilocular, tennis ball sized, spherical in shape, off white in colour with presence of slightly turbid fluid. The protoscolices collected from cystic fluid contained four suckers and a rostellum that has about 25–50 hooks. The histopathology of cyst revealed that the cystic wall consisted of three layers which are innermost germinal layer, eosinophilic laminated membrane beneath the germinal layer and outer fibrous layer with dense fibrovascular tissue, respectively. The histopathological changes in lungs were thickening of alveolar and bronchiolar wall and fibrous tissue proliferation around cystic wall.

The PCR for cox1 gene yielded amplification product of 434 bp. Molecular characterisation revealed that the strain involved in the infection is most identical to the G6 camel strain. In fact, the analysis of the variable sites of the cox1 sequences obtained for the sample indicates 99.5% nucleotide identity to G6 strain. The sequence obtained from the PCR products was found to align with corresponding regions for cox1 genes in the GenBank confirming

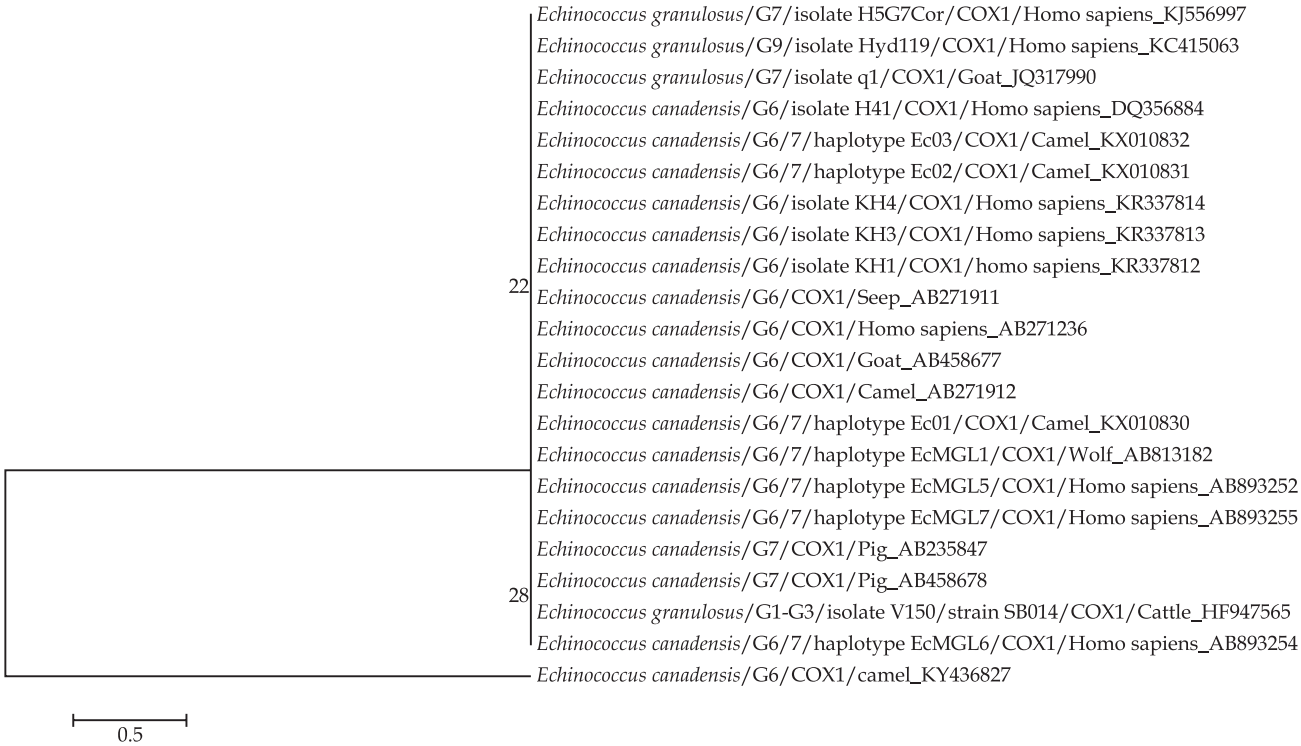

Fig 1. Phylogenetic analysis cox 1 gene of *E. canadensis* isolate from Bikaner. A total of 22 sequences were taken from GenBank. The phylogenetic tree was constructed by maximum likelihood method using Tamura-Nei model in Mega 6 program including 500 replica of bootstrap.

the cysts to contain the EG complex. Aligned with BioEdit, partial sequences for cox1 showed 100% homology in this study. To investigate for the relationship between this EG isolate and the other EG genotypes identified globally, phylogenetic tree was constructed which showed that the EG isolate (NRCC_EC_ KY436827) clustered with *E. canadensis* genotype G6 (Fig. 1).

Discussion

In Rajasthan, camels are owned by pastoralists as a source of milk, carrying loads and riding animals. Very scarce research has been conducted to evaluate the role played by camels in transmission of parasitic infections with special emphasis on cystic echinococcosis in India. The incidence of camel strain in Indian dromedary camel suggest that camel seem to play an important role in the transmission cycle of the parasite and the epidemiology of the disease particularly in camel rearing areas of Rajasthan. In the neighbouring country of Pakistan the prevalence and fertility of hydatid cysts was found highest in camels compared to other livestock species (Latif *et al,* 2010). However, phylogenetic analysis of the cox1 gene revealed that the common sheep strain (G1) and buffalo strain (G3) are cycling among camels of Pakistan and these strains are highly adapted to goats, camels and cattle (Latif *et al,* 2010).

In the present study, the phylogenetic analysis revealed the first report of *Echinococcus canadensis* camel genotype (G6) in the camels of India. This indicated that G6 genotype should equally be considered as an infectious form of EG-complex in the one humped camels in India. This finding provides an alarming evidence for the circulation of the camel genotype in the one humped camels. The close relationship between stray dogs and camels in the grazing area seem to play an important role in transmission and continuation of life cycle of *E. canadensis* in the present study.

Of the ten genotypes of EG, the strains to date reported from livestock population of India are the sheep (G1) (widely distributed), pig (G2), buffalo (G3) (widely distributed) and cattle (G5) strains (Bhattacharya *et al,* 2007; Gudewar *et al,* 2009; Pednekar *et al,* 2009; Singh *et al,* 2012). Whereas, sheep (G1), buffalo (G3), cattle (G5) and camel (G6) strains were reported from human population in India (Sharma *et al,* 2013). There is only a single case of incidence of camel strain from a human case from Rajasthan region of India (Sharma *et al,* 2013) which indicates potential of this strain to infect human population of camel rearing regions. In order to understand the transmission cycle, zoonotic potential of camel strain and epidemiology of the disease in India, a vigilant approach is required involving large number of human and camel population with full genome sequencing of some of the representative samples. This will be helpful to design and implement the control strategy for the disease.

Conclusion

The G6 genotype confirms a geographical distribution in India and demonstrates that camel can act as intermediate hosts. Therefore the importance of genotyping the isolates of *E. granulosus* complex has to be stressed in order to assess the contribution of G6 strain to the epidemiology of human hydatidosis.

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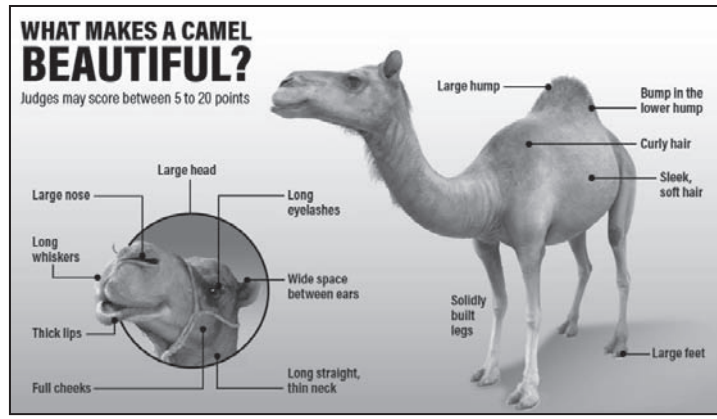
News

Genetics project focussed on camel beauty



Dr Jaime Gongora, a scieintist of wildlife and animal genetics and genomics at The University of Sydney, and his PhD student Mahmood Al Amri are developing an exhaustive scorecard for camel beauty contests. The scorecard will look at morphometric measurements, which are those that relate to the shape and dimensions of the parts of a camel's body assessed during judging. Genetic studies of the camels will be linked to the development of the scorecard which would allow the researchers to carry out an "association analysis" between the physical, or phenotypic, traits of individuals, and their genetic makeup. Two main types of markers, i.e. microsatellites, which are sections of repeated DNA sequences that vary from one animal to the next and Random Amplified Polymorphic DNA (RAPD) markers, in which random sequences of DNA are used for comparison.

(Source: The National, UAE)