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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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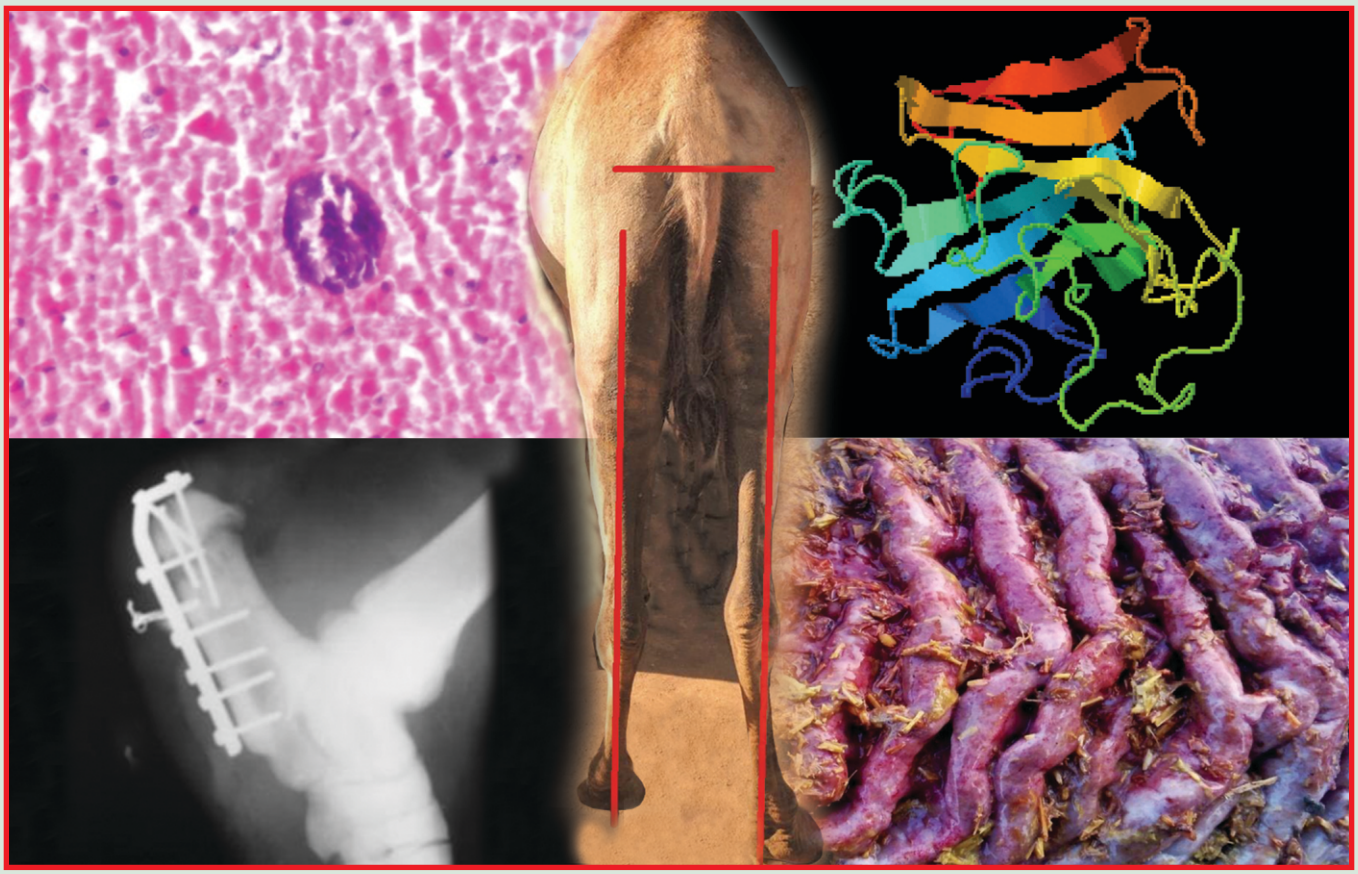
Volume 23 June 2016 Number 1

In This Issue



JOURNAL OF CAMEL PRACTICE AND RESEARCH

CAMEL PUBLISHING HOUSE
Bikaner - 334001, INDIA

Manuscripts and other related correspondence may be made to :

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SUBSCRIPTION RATE - 2016

ANNUAL Rs. 2500/- or US $ 250

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

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

Cover Design: Dr. T.K. Gahlot

Cover Photo: A sarcocyst in the cardiac muscle (arrow) **(**Top Left). Inverted L plate on plantar surface of tarsus for fracture fixation (Bottom Left). Predicted three dimensional structure of single hump camel c-Met protein depending on amino acids. (Top Right). Gross appearance of fundic region of Compartment 3 showing congestion and haemorrhages (Bottom Right). The rear side of an adult female of Somali breed showing three body areas that need to be assessed during a camel breed evaluation: a; rump width; b, rear legs vertical conformation; c, fetlocks (Centre).

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

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CAMELID IMMUNOLOGY AND OTHER RESEARCH ON CAMELIDS

The camelid functional immunoglobulin devoid of light chains is among the important discoveries made in the late 1980s by scientists at the ''Vrije Universiteit Brussel'' (Free University of Brussels). In 1989 a group of biologists led by Raymond Hamers at the Free University Brussels investigated the immune system of dromedaries. This discovery was published in Nature in 1993. Based on their structure, these peculiar camelid antibodies have been named Heavy Chain Antibodies (HCAb), as they are composed of heavy chains only and are devoid of light chains. HCAbs are not found in other mammals except in pathological cases. This peculiarity of camelid antibodies generated interest of camelid scientists world over and large number of papers on this topic were published in the Journal of Camel Practice and Research (JCPR). Camel Publishing House took a lead to compile all these published papers in form of a book entitled, "Selected Research on Camelid Immunology". This book is a unique compilation of research papers based on "Camelid Immunology" and published in JCPR between 1994-2015. Research on this subject was done in 30 countries involving about 248 scientists. In terms of number of published papers in JCPR on the immunology the following countries remain in order of merit (in parenthesis), i.e. Iran (1), India and UAE (2), China and Saudi Arabia (3), Sudan (4), Kenya and Belgium (5), USA (6), Germany (7) and so on. The book contains 11 sections and is spread in 384 pages. The diverse sections are named as overview of camel immune system; determinates of innate immunity, cells, organs and tissues of immune system; antibodies; immunomodulation; histocompatibility; seroprevalence, diagnosis and immunity against bacteria, viruses, parasites and combination of other infections; application of camel immunoglobulins and applications of immune mechanisms in physiological processes. The camelid immunology has to go a long way in its future research, therefore, this reference book may prove quite useful for those interested in this subject. Thanks to Drs U. Wernery and Serge Muyldermans for joining me in the team of Editors.

The current volume of JCPR covers many topics of interesting research on camelids. This includes an outstanding manuscript on camel breed judging by M.Dioli. Camel milk also remained subject of choice for researchers. It included papers on effect on drug metabolising cytochrome P450 enzymes, enzymatic hydrolysis of proteins and hepato-renal protection. Many diverse topics also found space in the current volume, i.e.muscle- effects of breed and type, mitochondrial genes, adjuvant role on safety and antibody modulation, SACASG and SACALG, GFP-labelled camel skin and lung fibroblast cell lines, *Staphylococcus aureus*- capsular typing isolates, udder stimulation-effect on milk, *Eimeria leuckarti*, Sarcocystosis, propofol-ketamine for total intravenous anaesthesia, histological and histochemical study- small intestine, tear fluid secretion rate, bulbourethral glands in bactrians, applied anatomy of the maxillofacial and mandibular regions, osteometric evaluation of the metapodial bones, ultrasonographic applications- review, bladder stones, inflammatory conditions- upper gastro-intestinal tract, subclinical mastitis, vaginal and cervical adhesions and stenosis, dystocia, fractures and luxations of the tarsus and book review. Additionally manuscripts describing tumours of camelids, i.e. Choroid plexus papilloma, multinodular thyroid gland hypertrophy and sweat-gland-tumour with osseous metaplasia ''chondroid syringoma" also marked the current issue which comprised 33 papers.

Camel Publishing House is thankful to its members of editorial board for screening the manuscripts submitted and to the authors for extending their support to this exclusive journal on camelids.

(Dr. T.K. Gahlot)
Editor

SUBSCRIPTION - 2016

FOR

JOURNAL OF CAMEL PRACTICE AND RESEARCH

(BIANNUAL IN ENGLISH LANGUAGE, JUNE AND DECEMBER ISSUE EVERY YEAR)

SUBSCRIPTION RATE - 2016

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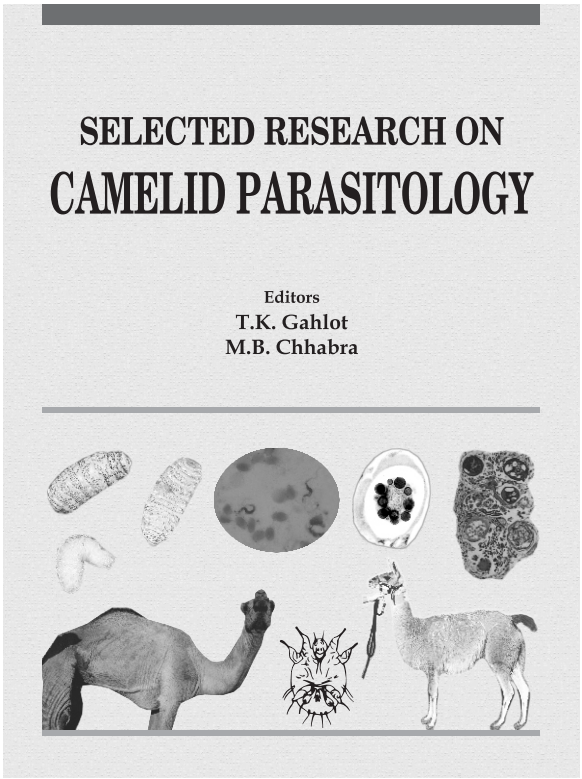
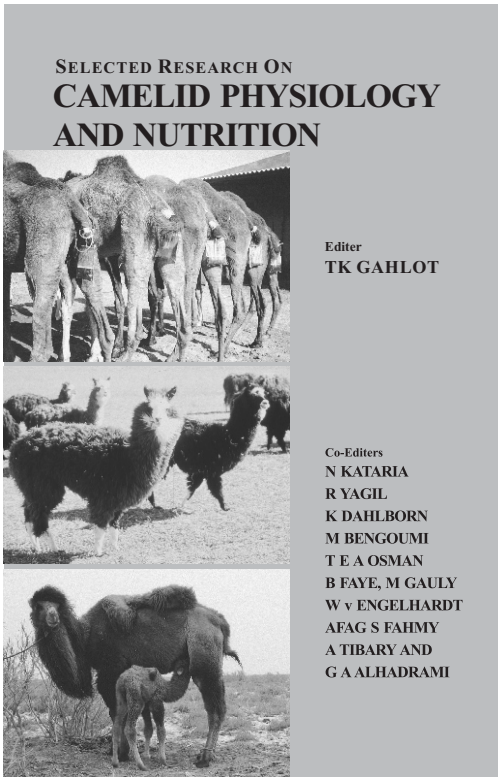


Selected Research on Camelid Parasitology (Edition 2009)
Edited by T.K. Gahlot and M.B. Chhabra and published by Camel Publishing House
website: www.camelsandcamelids.com
Price: US $ 200 (Abroad) INR (Rs) 3000 (India)
ISBN: 81-903140-0-9



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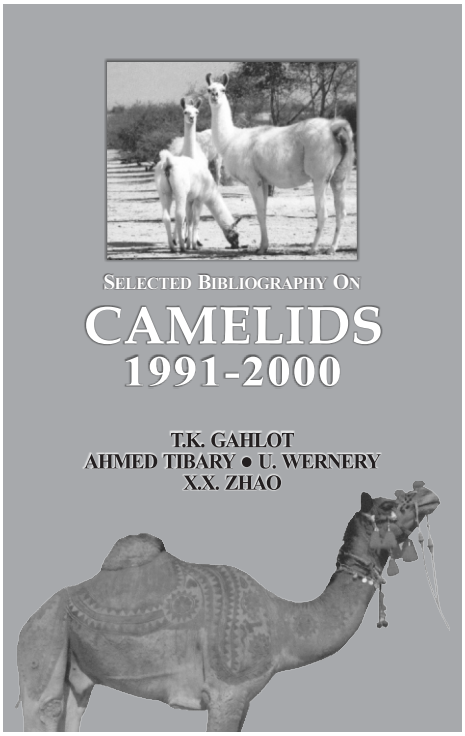
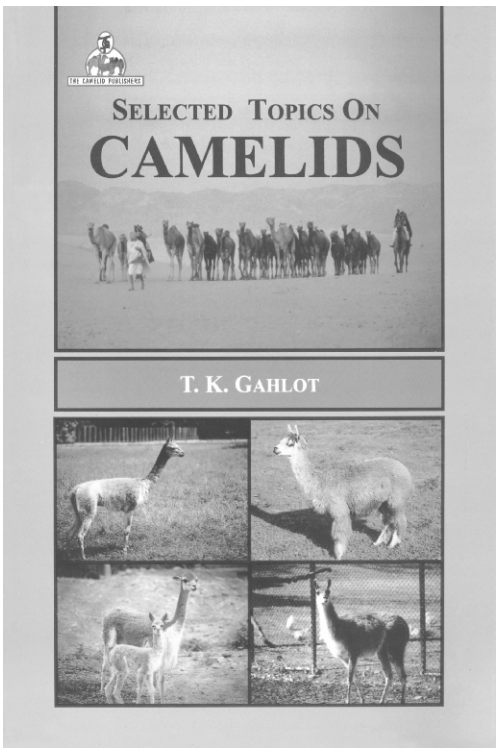
Edited by T.K. Gahlot and Marketed by Camel Publishing House.
website: www.camelsandcamelids.com
Price: US $ 180 (Abroad) INR (Rs) 1950 (India)
ISBN: 81-901141-2-3



Selected Bibliography on Camelids 1991-2000 (Edition 2002)
Edited by T.K. Gahlot, Ahmed Tibary, U. Wernery and X. X. Zhao
Marketed by Camel Publishing House
website: www.camelsandcamelids.com
Price: US $ 90 (Abroad) INR (Rs) 950 (India)
ISBN: 81-901141-1-5



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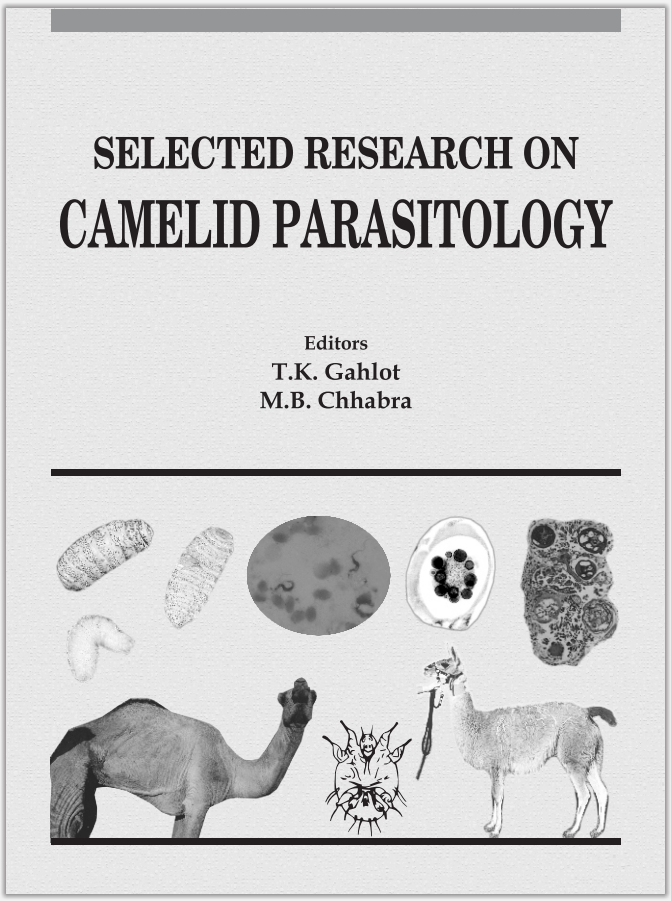
Edited by T.K. Gahlot and Marketed by Camel Publishing House.
website: www.camelsandcamelids.com
Price: US $ 180 (Abroad) INR (Rs) 1950 (India)
ISBN: 81-901141-0-7

SELECTED RESEARCH ON
CAMELID PARASITOLOGY

Hard bound, 291 pages, few figures coloured

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



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

Edition: 2009

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

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

ISBN: 81-903140-0-9

DOI : 10.5958/2277-8934.2016.00001.1 Vol 23 No 1, p 1-12

TOWARDS A RATIONAL CAMEL BREED JUDGING: A PROPOSED STANDARD OF A CAMEL (*Camelus dromedarius*) MILK BREED

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Riu Tajo 15 B, 03580 Alfaz del Pi, Spain

ABSTRACT

Each modern livestock breed has a clearly defined "breed standard" that assist in the selection to breed the animal with the most desirable traits and to cull the one with poor phenotype or faults in terms of poor conformation or blemishes or unsoundness. The camel (*Camelus dromedarius*) does not have yet a written rational breed standard. The article proposes a draft breed standard for an hypothetical lowland camel breed selected for milk production. The draft standard list various body areas and physiological parameters and suggest baseline values based on available studies and empiric observations of the author on East African and Arabian camel milk breeds. Anatomical and behavioural faults leading to possible disqualification of a camel from the standard of a camel milk breed are mentioned.

Key words: Breed standard, breeds, *Camelus dromedarius*, phenotype

Man and domesticated animals have thousands of years of shared history. Since the beginning of this shared relationship man has manipulated and controlled the breeding of domesticated animals in order to select specific desired qualities such as docility, strength, milk production and easiness of milking etc, or to increase the one already existing. These actions have created a multitude of breeds of domesticated animals able to perform various specialised tasks. For every livestock breed, the quest to achieve the perfect combination of physical and mental characters best suited to a specific purpose has been done by selecting and breeding only animals that possessed a set of the desired characteristics combined with the absence of undesirable traits. Such task has been greatly facilitated by the adherence of the breeders towards the accepted "breed standard" of that specific livestock species. A livestock species "breed standard" is basically a summarised list of specific morphologic and psychological characteristics, agreed by livestock owners and livestock associations of that species, that a livestock breed has to posses in order to belong to that specific breed. A livestock "breed standard" is therefore, an essential tool for a modern and sound livestock husbandry minimising individual opinions and unambiguously defining phenotypes and psychological characteristics of a certain livestock breed.

Today, virtually every livestock species has a specific written breed standard and the only exception to this rule is the one-humped camel (*Camelus dromedarius*) where up to now breed standards have not been developed. The various camel breeds in existence are still selected on a basis of personal parameters and opinions of the local camel breeders. An example of such approach is the set of parameters listed in the "criteria for show camels" (Anonymous, 2014) used to asses show camels in UAE and presented in table 1.

While various body measurements of well established camel breeds have been recorded from various countries (Droandi, 1936; 1932; Faye *et al,* 2011; Ishag *et al,* 2010; Oulad Belkhir *et al,* 2013; Raziq *et al,* 2011; Shah *et al,* 2015; Schulz *et al,* 2005; Yilmaz *et al,* 2011); none of these measurements have subsequently been assembled to produce a rational camel breed standard capable to guide camel breeders in the selection of the desired traits of a specific camel breed. It is obvious that the use of vague terminology to describe specific body areas, the absence of verifiable measurements of anatomically structures and the focusing on characteristics irrelevant for a productive livestock breed such as : "lips", "beard", "curve of the nose" instead of well recognised productive traits such as udder size and conformation, calving interval or lactation length and yield render the task of evaluating a camel inevitably

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Table 1. Body areas and terminology used to evaluate show camels breeds in UAE.

Body area	Desired Conformation
Head	Large
Lips	Big and with extra dropping on the bottom lip
Beard	long
Tail	Broad and long
Cheek	Broad and high
Hump	Broad, round and long
Leg	For Majaheem solid tibia, and for pure bred camels the reverse is desired
Neck	Long and extended forward
Chest	Wide, high up and extending forward
Arm	For Majaheem the arm bone is broad and dignified, while for pure bred camels the reverse is preferred
Ears	Long and sharp
Height	As tall as possible
Soles	Large and widely rounded, while for pure bred camels a small sole is preferred
Hocks	A wide distance between the hocks and they should be big while in pure bred camels the reverse is preferred
Back	Short distance between the end of the hump and the base of the tail and a substantial width between the hips
Curve of the nose	Preference is for a highly curved nose
Size	Large

confusing, prone to highly subjective individual interpretations and most importantly ineffective toward the task of judging and selecting the best productive camel among a group of animals.

The development of a rational and measurable and non subjective set of criteria to evaluate a camel breed is therefore much needed. This paper will attempt to do just that by suggesting a draft set of criteria for a generic lowland "camel milk breed" since, the camel is most importantly used as milk producing livestock.

Materials and Methods

The approach used to prepare a draft "camel milk breed standard" to establish a list of important camel phenotypic characters, or individual camel observable characteristics, and performance traits thought important for a camel breed utilised for milk production. The list was assembled utilising the authors, frequent and extensive discussions with camel keeping nomads of various geographical areas: Horn of Africa, Middle East, India (Dioli, 2013; Schwartz and Dioli, 1992) and phenotypic values reported in a number of articles by various authors on phenotypes of various camel breeds (Abdallah and Faye, 2012; Al-Hazmi *et al*, 1994; Chniter *et al,* 2013; Droandi, 1936; 1932; Faye *et al,* 2011; Ghiasuddin *et al,* 2014; Ishag *et al,* 2010; 2011; Oulad Belkhir *et al,* 2013; Shah *et al,* 2015; Tibary and Anouassi, 1997; Yılmaz *et al,* 2013; Yosef *et al,* 2014).

Results and Discussion

Phenotypic characters and performance traits important for a camel milk breed have been combined in two tables. One table is listing morphological characteristics (Table 2) and another table is listing camel production and behavioural traits (Table 3). Both tables are structured in a way that each phenotypic character and productive trait mentioned in the tables is associated with the most desirable condition, described with a clear numerical range of values, and with the poor conformation and blemishes again described with clear numerical values. To facilitate the evaluation process and the detection of the commonest phenotypic faults mentioned in table 2, a set of figs are included that illustrate the common phenotypic characters that should be assessed (Fig 1, 2, 3), poor conformation of body (Fig 4) and udder (Fig 5) encountered.

Since the main task of the hypothetical camel breed is milk production, the shape and size of the udder and teats have a direct impact on such task. This paper therefore introduce (Table 2) various udder measurements to better and more clearly define the characteristic of a correct udder of camel milk breed (Table 2).

Articles that have specifically dealt with camel udder morphology have been published (Atigui *et al,* 2015; Ayadi *et al,* 2013; 2015) however, not about calculation of camel udder volume. Camel udder volume could be estimated possibly adopting various measurements that have been specifically devised to calculate udder volume for cows (Alshaikh *et al,* 1995; Davis and Hughson, 1988) or small ruminants (Milerski *et al,* 2006; Makovický *et al,* 2013; Rovai *et al,* 2004). However, to use measurements specifically made up for cattle or small ruminants udders would probably be difficult and time consuming in camels because of the not very easy access of the camel udder, deep between the camel hind legs, and because of the well known reluctance of camels to allow manipulation of their udder by strangers. In this article, the udder measurements and values

Table 2. Suggested body areas that should be subjected to assessment during an evaluation of a lowland camel breed specialising in milk production in a semi-intensive production husbandry system. Females over 4 years, males over 7 years.

Body areas	Desired Characteristics	Undesired Characteristics (if excessive may warrant disqualification)	Points that may be assigned	Comments on evaluating
1	2	3	4	5
Coat colour	According to the prevalent colour existing for that breed	Large differences with the agreed breed colour	**up to 1**	➢ *If various colour varieties in the breed exists they **should not be considered as poor conformation.***
Hair distribution	Smooth, uniform and/or with localised hair growth in specific body areas	Large areas without hair and/or with thickened and wrinkled skin (indicating an active or past scabies infestation)	**up to 1**	➢ *Active or past episodes of mange can be responsible for large body areas to be without hair and with thickened and profusely wrinkled skin. Such animals **should be considered as poor conformation.*** ➢ *Females with clear and well demarcated hairless areas on the back/top of the hump **should be considered as poor conformation,** since this is a sign of poor fertility; the airless area is caused by mechanical pressure and friction of the pedestal of the male during mating. Overly frequent mating sessions cause these typical skin traumas.* ➢ *Camel kept in areas with cold seasons will grow longer coats even if their original breed type has short smooth coats.*
Withers height	females over 185 cm males over 210 cm Wither clearly higher than the lumbar area	females 175 cm or less males 200 cm or less Wither with same height of lumbar area	**up to 4**	➢ *Excessively tall females **should be considered as poor conformation** points since they may have reached such height only because of sterility or manmade prevention of breeding.*
Body weight *(females > 4 years males > 7 years)*	Females: 550-700 kg Males: 750-900 kg	450 kg or less for females 650 kg or less than for males	**up to 5**	➢ *Watering regime **should not be over 3 days** otherwise body weight may be significantly affected.* ➢ *Obviously obese males **should be considered as poor conformation** since they are probable not experiencing a long rut season.* ➢ *Obviously obese females **should be considered as poor conformation** and if with small udder must be **considered as poor conformation** since such animals are often sterile or with poor reproductive or milking capabilities.* ➢ *Males at the end of rut season may be considerably lighter in weight and should not **be considered as poor conformation**.*
Head size (*Measured from nose to occipital crest*) and conformation	Length approx 45-55 cm (higher values for males) Males with a well marked forehead. Lips may be pendulous or completely without hair	Small: 40 cm or less Males with flat forehead Mandible undershot or overshot Presence of "wry face" (lateral deviation of the face)	**up to 2**	➢ *The presence of any firing scar around the orbit without obvious eye lesions **should be considered as poor conformation** since it may be an indication of a tentative to correct idiopathic blindness with a traditional treatment.* ➢ *"Wry face" is a genetic abnormality so affected animals **should be considered as poor conformation**.*
Neck (*Measured from base of the jaw to base of the neck in front of the chest. Circumference measurement taken in the middle of the neck*)	Length 110-120 cm or more (higher values for males) Circumference 90-100 cm or more (higher values for males)	Length 90 cm or less in females 110 cm or less in males Circumference: 70 cm or less in females 80 cm or less in males	**up to 2**	➢ *The presence of any firing scar **should be considered as blemish** since could be an indication of past pathologies such as "wry neck" or "impacted dulaa".* ➢ *A thick neck in male camel is a sign of masculinity and therefore capacity to breed a high number of females. It is also probably related to fertility.*

1	2	3	4	5
Front legs front view	Vertically straight, parallel or slightly toe out	brushing knees	**up to 3**	➢ *The presence of any firing scar **should be considered as blemish** since it may be an indication of a tentative to correct a poor conformation with a traditional treatment: firing (thermocautery).*
Front legs side view	Vertically straight with well develop elbow callosity	undershot knees	**up to 3**	➢ *The presence of any firing scar **should be considered as blemish** which results following friring to correct poor conformation Blemishls on unsoumdoors scoral firing marls.*
Chest width (*Measure of distance between inside surfaces of top front legs*)	30-35 cm or more (higher values for males)	Narrow space with width less than 30 cm and evidence of brushing elbows and/or pedestal	**up to 4**	➢ *when lower chest width measures are recorded it is compulsory to verify that there are no areas of friction between front leg/elbow pad with pedestal/ chest wall*
Chest girth (*Measured from the front of the hump and behind the pedestal*)	Circumference over 210 cm for females and over 220 cm for males	200 cm or less for females 210 cm or less for males	**up to 4**	➢ *Animals that have been recently sick or convalescent may have very low chest girth values.*
Chest callosity (*pedestal*)	well developed without irregular growths, misshapen areas or obvious side friction areas with inner side of front legs	Callosity small, with obvious misshapen areas and/or obvious areas of friction on the sides with inner side front leg	**up to 2**	➢ *Moderately abnormal chest callosity may be tolerated in females, however males with deformed/small chest callosity **should be considered as poor conformation** since a healthy functional chest callosity is essential for breeding males.* ➢ *presence of friction areas **should be considered as poor conformation.***
Hump	Medium size (shape irrelevant), centrally positioned not extending over the lumbar area or on the lateral sides over the chest	Very large	**up to 1**	➢ *A very large hump should not be preferred since is often a sign of reproductive failure/mastitis in a female or of absence/short rut season in a male.* ➢ *males 7-8 years old may have a small hump and **should not be considered as poor conformation.*** ➢ ***C. dromedarius** breeds who have received genetic input from **C. bactrianus** have very large humps extending from the wither to the rump, such animals **should not be considered as poor conformation.*** ➢ *females with humps hanging on the sides **should not be considered as poor conformation** since these are often a sign of an abundant and sustained milk production.*
Lumbar area angle (*Measured as the angle made by the lumbar vertebrae over the horizontal line*) and height	Moderately inclined approximately 15 degrees over the horizontal line. Lumbar height lower than the wither height	Insufficiently inclined (horizontal) same height than the wither	**up to 3**	➢ *Mature females over 12 years with good calving history tend to exhibit a less inclined lumbar area angle trait and **should not be considered as poor conformation**.*
Rump area angle (*Measured as the angle of the rump structure, from iliac crest or hip bone to the ischial tuberosity or pin bone, over the horizontal*)	highly sloped: approximate 45 degrees over the horizontal line	with insufficient slope: 30 degrees or less or with excessively slope: 60 degree or more	**up to 2**	➢ *The presence of any firing scar **should be considered as poor conformation** since it may be an indication of a tentative to correct a conformation fault with a traditional treatment: firing (thermocautery).*
Rump width (*Measured as the distance between the most posterior points of the ischial tuberosities or pin bones*)	As wide as possible, 30 cm or more (higher values for males)	Less than 25 cm	**up to 3**	➢ *All females with low measures should have their vaginal area examined to detect eventual recto-vaginal lacerations possibly caused by dystocia episodes because of a very narrow birth canal. If lacerations are present the animal **should be considered as poor conformation**.* ➢ *Females with any degree of vaginal prolapse **should be considered as poor conformation**.*

1		2	3	4	5
Rear legs rear view		Parallel or slightly toed out	Excessively toed out with hocks touching each other	**up to 3**	➢ *The presence of any firing scar **should be considered as blemish** that results followings firing on area of chronic inflammation since it may be an indication of a tentative to correct a conformation fault with a traditional treatment: firing (thermocautery).* ➢ *swollen hocks should also be **considered as poor conformation** and the ability of the animal to smoothly couch and stand up tested.*
Rear legs side view		Almost vertically straight (stifle angle approx 150 degrees) with a large and very well defined hock joint and a large heel bone	sickle-hocks (stifle angle approx 120-130 degrees), hock joint not defined, swollen Achilles tendon and/ or hock joint	**up to 3**	➢ *The presence of any scar **should be considered as blemish** that results followings firing on area of chronic inflammation.*
Feet		Front feet larger than the rear feet with uniform digits	Irregular size of digits	**up to 2**	➢ *Presence of abscess or wounds should **not** be considered as poor conformation.* ➢ *Front feet should be larger than rear feet.*
Fetlocks (*the first digital bones at the distal end of the metacarpal and metatarsal bones*)		Short (approx 8-9 cm). In the front feet almost in vertical line with the metacarpal bone; in the rear feet more sloped	Long (over 10 cm) Externally deviated: splayed toes, or internally deviated: pigeon toes, too inclined making them almost horizontal (dropped fetlocks: horizontal or touching the ground) or too vertical (contracted tendon)	**up to 4**	➢ *The presence of any firing scar **should be considered as blemish** that results followings firing on area of chronic inflammation.* ➢ *Animals that have spent many hours forcibly couched because of transport on trucks may show swollen fetlocks and **should not be considered as poor conformation**.* ➢ *dropped fetlocks are particularly common in the front feet, such fault may have a genetic component, affected males, even if mildly affected, **should be considered as poor conformation**.*
Tail		Length 50 cm or over, with very large base: circumference of the tail base over 25 cm (higher values for males)	Length 40 cm or less tail base circumference of 20 cm or less	**up to 3**	➢ *Hairy tails should be preferred over hairless tails.*
Female	Udder	Large symmetrical quarters that should all be on the same level ✓ udder depth (or height) of over 25cm udder horizontal circumference of over 120 cm ✓ total udder size (volume) of over 3000 cm^3 ✓ milk vein diameter (3 measurements) of 3 cm or over	Large differences in dimension between front and rear quarters. Rear quarters much lower than the front quarters and/or at the same level of the knee joint. Front quarters lower than rear quarters and/ or at the same level of the knee joint. All udder measures less than suggested parameters Milk vein not visible or with diameter of 2 cm or less	**up to 25 ***	➢ *Udder tissue should be spongy without any presence of hard areas. Mammary lymphnodes **should not be large**: over 6-8 cm.* ➢ ***udders should preferably be assessed on milking females**. Udder measurements should be taken after the camel has been milked or well before the scheduled milking session.* ➢ ***consideration should be given on number of parities: in females with the same udder size preference should be given to the one with lesser number of parities.*** ➢ *past episodes of mastitis diminish the size of the affected quarters, obvious size difference among the quarters should be critically evaluated.* ➢ *attention should be given to the size of "milk wells" (the area where the milk veins enter chest cavity) that should be very large.*
	Teats distance between each other and placement	Teats from front quarters well spaced from teats of rear quarters. Teats vertical by placed at the center of the quarters	Teats of front quarters close or touching the teats of rear quarters. Teats diverging towards the side or converging towards each other	**up to 10 ****	➢ *Females with fused teats **should be considered as poor conformation**.* ➢ *Females with teat lesions caused by inappropriate application of suckling control devices should not be penalised but the issue should be noted and the owner advised to use other methods.*

1		2	3	4	5
Female	Teats size	all teats in the 4 quarters must be uniform in size: 5-7 cm in length and of moderate thickness: 10-12 cm in circumference	Teats of different size between quarters, too short: less than 4 cm too long: over 7 cm too thick: over 12 cm of circumference	**up to 10*** **	➢ *Teats that are too small can be easily traumatised at milking while too thick or long cannot be properly sucked by the newborn calf requiring assistance from the herdsman. Wrong sized teats pose difficulties if using automatic milking equipment. Females with such teats* ***should be considered as poor conformation.***
Male	Testis	Both testes visible with longitudinal length of 8-10 cm or more and width of 5-7 cm or more	testes not visible or small: of less than 7 cm in longitudinal length and of less than 4 cm in width	**same points as***	➢ ***testes should be palpated to detect abnormalities.*** ➢ ***cryptorchid animal should be considered as poor conformation or unsoundness.*** ➢ ***testis size is affected by rut stage of the male.*** ➢ ***For males that have the same testis size preference must be given to the youngest.*** ➢ *males with testis of marked different size should be* ***considered as poor conformation or unsoundness.***
	Prepuce	Loose with large (1-2 cm long) accessory teats	Presence of prepuce eversion or prolapse. Prepuce tight to the belly with small or non visible accessory teats	**same points as****	➢ *In sandy desert areas prepuce eversion/prolapse shorten the life of breeding bulls so males with such faults* ***should be considered as poor conformation or unsoundness.***
	Scrotum	Large with abundant skin	Tight skin keeping the testes close to the body	**same points as *** **	➢ *males with a large swollen scrotum should be* ***considered as poor conformation or unsoundness.***
This number will represent the judged camel adherence towards the agreed standard. NB: only in exceptional cases it will be 100!				**TOTAL 100**	

mentioned in table 2 have been kept to a minimum and are a combination of the author own values and some of the values used by Eisa *et al* (2010) and Eisa (2006), slightly modified by the author. Measures mentioned in table 2 include udder depth: the distance between the abdominal wall and the base of the teats (4 measures should be taken and then averaged) and udder horizontal circumference: the distance starting from the front middle of the udder (in proximity of the median suspensory ligament) a few centimeter over the right teat, then going around the right front quarter clockwise towards and around the rear quarters and continuing to and around the left front quarter reaching the starting point (Fig 6).

While such measures provide an immediate estimate of a camel udder size, a more complete estimate would be udder volume. In this regard, camel udder volume may be estimated adopting the method used by Eisa *et al* (2010) that was adopted from Maskovskaja (1967); multiplying the udder depth with the udder horizontal circumference. Alternatively, assuming that the udder shape is similar to a vertical cylinder, the udder volume can be calculated using the common formula $V=\pi. radius^2. height$ (where estimated udder radius could be calculated from udder circumference $2r=circumference/\pi$). Obviously, because of the irregular shape of the udder volume calculated through these method is approximated. More accurate methods to rapidly and precisely calculate udder volume in camels could be devised, such as, the adoption of an aluminum foil molded manually to the udder and then using polyurethane particles to measure the inner volume of the aluminum cast (Magana-Sevilla and Sandoval-Castro, 2003).

Another udder parameter mentioned in table 2 is the diameter of the milk vein: subcutaneosly abdominal vein (Fig 7). While in cows it has generally been accepted that the size of the milk vein is not correlated with the amount of milk production in camels; there seem to exist a correlation between a large diameter of milk vein and an abundant milk production (Eisa *et al,* 2010). Although, such finding go against the present wisdom, it should not be outrightly rejected since recent studies on milk vein of lactating cows using ultrasonography to calculate the milk vein internal surface area seem to indicate that there is indeed a relationship between milk vein

Table 3. Suggested productive traits that should be evaluated during an assessment of a lowland camel breed specializing in milk production in a semi-intensive production husbandry system.

Productive Traits	Desired Traits	Undesired Traits (if excessive may warrant disqualification)	Points that may be assigned	Comments on evaluating
Age and weight at 1st calving (Female) Age and weight at 1st successful unassisted mating (Male)	4 years at 500 kg bw or over 6 years at 650 kg bw or over, proven fertility with long rut season	5 years or over and/or bw of 400 kg or less successful unassisted mating at 8-9 years bw of 600 kg or less Unsatisfactory fertility and short rut duration	**up to 15**	➢ *It is compulsory to inspect the vaginal area of primiparous females to detect eventual recto-vaginal lacerations possibly caused by dystocia at delivery caused by an insufficient development of the area and or a narrow birth canal. If lacerations or vaginal prolapse are present the animal* ***should be considered as unsoundness****.* ➢ *Male Bactrian camels (Camelus bactrianus) are able to mate at an earlier age then dromedaries. Mating precociousness should therefore be possible to achieve even in dromedaries (Camelus dromedarius).* ***Fertility of stud bulls should be verified for soundness.*** ➢ *Males 8-12 years old with short rutting season* ***should be considered as unsoundness.***
Female mothering instinct	Gentle, allows anybody to approach the calf and touch the udder	Excessively protective behavior strongly rejecting close contact with herdsman or requiring her legs to be tied to be milked Insufficient protective or total rejection of the calf	**up to 5**	➢ *care should be taken not to penalise good milking primiparous females since they are often overprotective and not yet used to the milking routine*
Calving interval	2 years (possibly verified over several reproduction cycles)	3 years or more or 1.5 years or less	**up to 15**	➢ *A female with calving interval of less than 1.5 years means often that she has poor milk production (naturally or because udder pathologies) that has caused the death of her calf and a subsequent new mating session and pregnancy. It may also mean that the female has a poor motherhood instinct resulting in rejection of her calf with consequently calf death/short lactation and precocious new breeding. Such females* ***should be considered as unsoundness****.*
Lactation length	12 months	6-8 months or over 16 months	**up to 10**	➢ *While in nomadic pastoral areas lactations over 12 months are common (14-16 months). Under an improved husbandry system it is better to select for 12 months long lactations the allows higher daily yields.* ➢ *Females with short lactation* ***should be considered as unsoundness.***
Lactation daily yield at the 4-6th months of lactation	10 liters or more	4-6 liters or less	**up to 35**	➢ *Daily milk yields are influenced by number of parities.* ***For females that have the same milk yield preference must be given to the one with lower number of parities.*** ➢ *Milk yields are obviously influenced by nutrition and abundant access to water and the period from when the animal was milked: animal that have not been milked for the last 12 hours produce more milk! These factors should be considered in the overall assessment.* ***Purely range bred camels on watering interval over 3 days should not be considered as unsoundness.*** ➢ ***Milking is easily affected by external influences, so care should be taken not to declare good milking animals as unsound.*** ➢ *The calf body weight of the judged female should also be assessed and maximum points only given to females with the heavier and most developed calf.*
Milk let down reflex Milking duration	easy even in unfamiliar areas Over 1.5-2 minutes or longer	Difficult or impossible when handled by strangers or in new surroundings shorter than 1 minute	**up to 20**	➢ *Maximum points should only be given to a female who require a minimum presence of her calf to be milked or that she is able to be milked without her calf.* ➢ *A short milk let down reflex is responsible for a small amount of milk produce so for high producing milking sessions a long milk let down reflex is necessary.*
This number will represent the judged camel adherence toward the agreed standard. NB: only in exceptional cases it will be 100!			**TOTAL 100**	

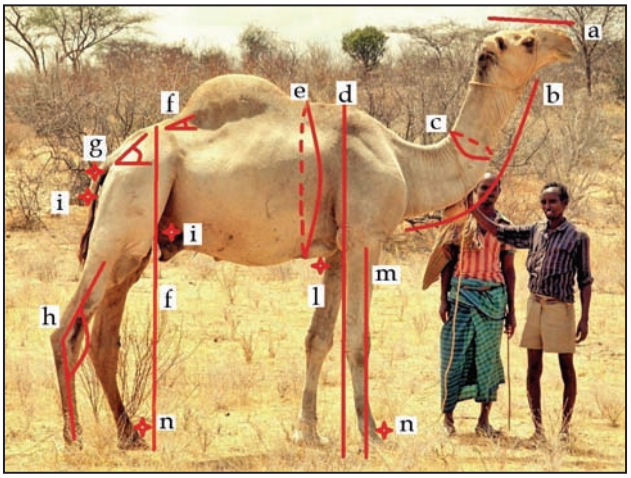

Fig 1. An adult male stud bull of Somali breed showing various body areas that need to be assessed during a camel breed evaluation: a, head length; b, neck length; c, neck circumference; d, wither height; e, chest circumference; f, lumbar area angle and height; g, rump area angle and base tail diameter; h, rear legs stifle angle; i, size and conformation prepuce/udder/testis; l, size and conformation pedestal callosity; m, front legs lateral vertical conformation; n, fetlocks.

size and milk production (Braun and Forster, 2012; Gracner *et al,* 2015).

To facilitate the tables understanding and interpretation, each table report a series of "comments on evaluating" that explain the rationale behind certain decisions, further explain issues, and warn about possible misinterpretations. Finally, to render the tables an effective tool to practically facilitate the evaluation task of assessing if a specific camel is belonging to a milk breed or not a score range on a 100-point scale is assigned to each phenotypic character and productive traits mentioned in the tables. The highest score allowed for each trait will be given when the phenotypic characters and productive traits of the assessed camel fully comply with the ones described in the tables, while a lower score or none at all will be given when phenotypic characters and productive traits do not conform to the one mentioned in the tables. In this regard, it is important to note that, as in other livestock species, if the detected faults are too severe the recommendation is that the affected camels should be disqualified.

Once the judgement process is finished, all the various phenotypic characters and productive traits mentioned in table 1 and 2 have been graded. The individual scores will be summed up and the overall number obtained will be the final assessment of that specific individual camel. The numerical data will make the work of judges tasked with assessing a specific camel easier, impartial and straightforward: a

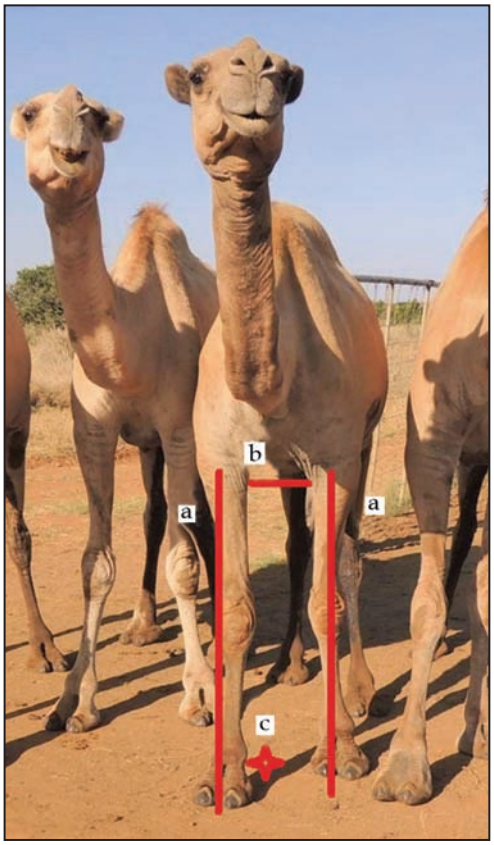

Fig 2. The front side of an adult female of Somali breed showing three body areas that need to be assessed during a camel breed evaluation; a, chest width; b, front legs vertical conformation; c, fetlocks.

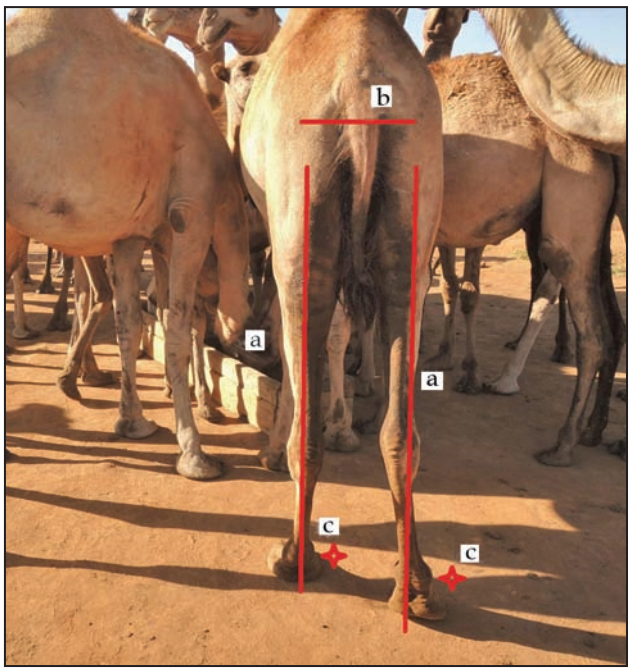

Fig 3. The rear side of an adult female of Somali breed showing three body areas that need to be assessed during a camel breed evaluation: a; rump width; b, rear legs vertical conformation; c, fetlocks.

Fig 4. Various examples of body conformation faults: from top left clockwise: a, narrow chest (right animal); b,c, brushing elbow; d, brushing pedestal; e,f,g, angular deformity of fetlocks; h, angular deformity of the right front leg; i,j, laxity of front leg flexor tendons; k,l, brushing knees; m,n,o, "sickle-hocks" or over-angulated hind legs; p, horizontal lumbar area; q,r, "wry face" a congenital malformation.

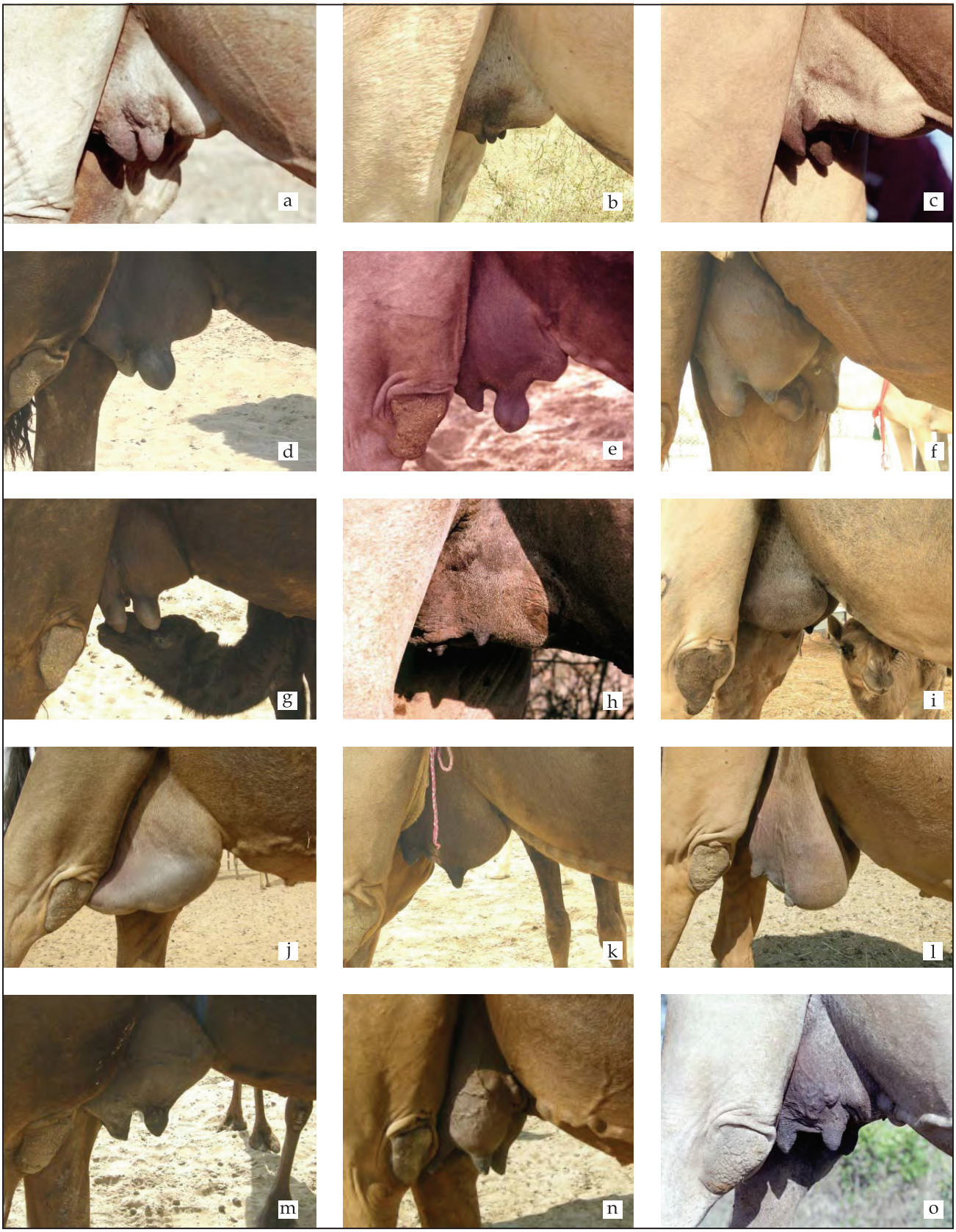

Fig 5. Various example of udder conformation faults: from top left clockwise: a,b,c, insufficiently spaced teats; d,e,f,g, teats too close, widely irregular in size and too large; h,i,j, teats too small; k,l, udders with poor "median suspensory legament"; m,n,o, acceptable to good udders.

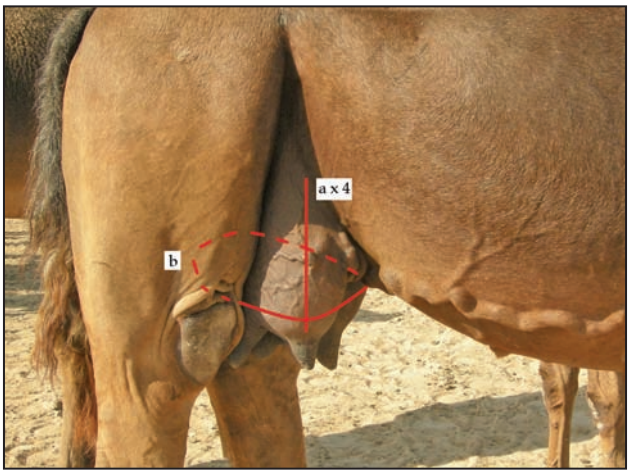

Fig 6. Lactating udder of an adult female of Majaheem breed showing udder measurements that need to be taken during a camel breed evaluation: a, distance between the abdominal wall and the base of the teats (4 measures); b, udder horizontal circumference.

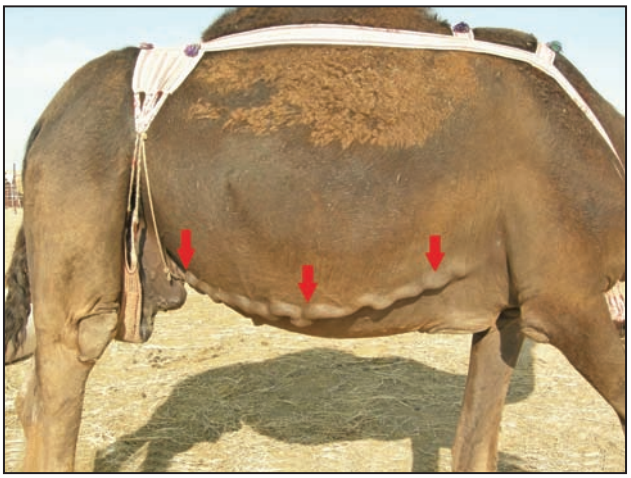

Fig 7. Lactating udder of an adult female of Majaheem breed showing the development of the milk vein (subcutaneously abdominal vein) and where measures of milk vein diameter should be taken (arrow).

camel that scored an overall total of 80 points will be considered a better camel than a camel that scored 60 points.

Conclusions

These drafts are obviously, the first step towards the development of a rational for "camel milk breed standard" and are open to improvement and suggestions. It is the hope of the author that this article stimulate further discussion among the camel breeding community and professional institutions involved in camel selection and development so that a consensus on the best "camel milk breed standard" may be officially devised and adopted. A rational, non subjective camel milk breed standard is an essential prerequisite to guide the efforts of camel research institutions and individuals camel farmers in the difficult and lengthy process of camel selection.

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DOI : 10.5958/2277-8934.2016.00002.3 Vol 23 No 1, p 13-17

EFFECTS OF BREED AND TYPE OF MUSCLE ON COMPOSITION, QUALITY AND TEXTURE TRAITS OF DROMEDARY CAMEL (*Camelus dromedarius*) MEAT

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ABSTRACT

This study was designed to evaluate the effects of breed and type of muscle on composition, quality characteristics and texture indices of one-humped camel meat. Two muscles; *Longissimus dorsi* (*LD*) and *Semimembranosus* (*SM*) were taken from 16 one-humped camels of 2 breeds; Najdi and Somali. The results showed that moisture and crude fat contents of muscles were significantly (P<0.05) different between the 2 breeds. Shearing force was also significantly (P<0.05) different between the treated camel groups. The *LD* muscle for both breeds was tenderer than their counterparts *SM* muscles. The *SM* muscle of the Somali breed showed the highest myofibril fragmentation index (MFI) value. The *LD* and *SM* muscles for Najdi breed showed the least MFI values. Cooking loss (CL) and water-holding capacity (WHC) were significantly (P<0.05) different between the muscles of the 2 breeds. Coinciding with its cooking loss value, the *LD* muscle of Najdi breed had the lowest value of WHC. Moreover, it was more red in colour than that of Somali, while the *SM* muscle of Najdi tended to be lighter than that of Somali. The breeds also showed significant (P<0.05) differences in texture profile parameters. It is concluded that meats from camels of both breeds Najdi and Somali differ in fat content and quality characteristics. Generally, meats of Najdi breed tended to be more tender and juicier than Somali breed which was leaner than Najdi breed.

Key words: Camel meat, longissimus dorsi, najdi, semimembranosus, somali

Meat represents the most preferable and dominant food all over the Arab countries. The situation in Saudi Arabia is not so different from that found in the region. The only disparity is the high demand for meat and meat products from the natives that led to increase in meat imports. The per capita consumption of meat in Saudi Arabia is estimated to be 54.5 kg (FAO, 2013). Although, thousands of tonnes of meats from different sources are produced, still the gap between demand and supply is expanding. During the last decade, this gap is shortened through importation of meat from different countries, including Australia, Brazil, New Zealand, Turkey, India, Pakistan, Sudan and Somalia. The majority of imported camels is obtained from the last 2 mentioned countries. The local herdsmen used to keep the imported camels under an intensive feeding regime for 2 weeks before slaughtering. This behaviour had raised the degree of competition with the locally produced meat to the maximum. A considerable recent studies were carried out to investigate the reasons behind differences in composition and quality of camel meat. Kadim (2014) studied the influence of feeding intake and type of muscle on the quality and histochemical characteristics of dromedary camel meat. Also, Kadim *et al* (2013) evaluated chemical composition, quality and histology characteristics of individual dromedary camel muscles. Additional studies had investigated the effects of breed and muscle on meat quality and composition of cattle (Peasonen *et al,* 2012; Xie *et al,* 2012; Muchakilla *et al,* 2014). Moreover, a recent review study had considered the influence of breed and feeding on meat and carcass quality of sheep (Ramírez-Retamal and Rodrigo, 2014). This study was conducted to study the effects of breed and muscle type on composition, quality and texture profile of meat from Najdi and Somail camel breeds.

Materials and Methods

Animals and muscle samples

A total of 32 *Longissimus dorsi* (*LD*) and *Semimembranosus* (*SM*) muscles were taken from 16 animals of one-humped camel breeds, i.e. local Saudi Najdi and imported Somali (8 intact males from each

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breed). The animals age and weight 18 months and 150 kg, respectively. The feeding background for both breeds was same.

Meat chemical composition

Muscle samples from both breeds were taken to estimate its chemical composition. Moisture, crude protein, ether extract and ash contents of the meat were determined according to AOAC (2006).

Meat physical, chemical and sensory properties

pH and colour were measured at 24 hrs postmortem, *pH* and colour on the raw muscles of the 2 breeds. A portable meat *pH*- meter (HI 99163, Hanna Ins., USA) was used to record *pH*, while the colour indices (L*, a* and b*) were measured using a Minolta Chroma Meter (CR-400, Konica, Japan) according to the colour system of CIELAB (1976).

Cooking loss, shear force and texture profile analysis were evaluated as described by Al-Owaimer *et al* (2014). Briefly, the muscle sample was cooked to an internal temperature of 70°C, where the temperature was observed by inserting a thermocouple thermometer probe (Ecoscan Temp JKT, Eutech Instruments) into the centre of the muscle. The cooking loss percentage (CL%) was calculated as the difference between the initial and final weights. Thereafter, the cooked sample was used to evaluate shear force (SF), according to the procedure described by Wheeler *et al* (2005) using Texture Analyser (TA-HD-Stable Micro Systems, England) fitted with a Warner-Bratzler annex. The texture profile analysis (TPA) was performed to determine hardness, cohesiveness, springiness, and chewiness using the same above mentioned device equipped with a compression-plate attachment.

Water-holding capacity (WHC) was determined as described by Wilhelm *et al* (2010), where 2 replicates of around 2 g muscle sample were placed between 2 filter paper (Whatmann No. 1) and 2 plexiglass plates. Then, a weight of 10 kg was placed over the plates for 5 minutes. The difference between the weights of the muscle sample before and after compression was WHC.

Myofibril fragmentation index (MFI) of the muscle sample was determined as described by Al-Owaimer *et al* (2014). A 4 g muscle sample was minced, then homogenised with a cold isolating MFI buffer. The absorbance of the solution was determined at 540 *NM* to quantify MFI value.

Subjective evaluation– The attributes tenderness, juiciness, flavour and overall acceptability were assessed by 8 semi-trained panelists, using a 6 point hedonic scale, where 1 was the least desirable and 6 was the most desirable.

Statistical analysis

The obtained data were statistically analysed using one-way ANOVA for a complete randomised block design with 2 x 2 research design method, using the general linear models procedure of SPSS software (SPSS Ver. 21). The mean separation was performed using least significant difference (LSD). The null and alternative hypotheses were as follows:

H_0: μNLD=μNSM=μSLD=μSSM (All the treatment means are equal)

Ha: at least one mean is different,

Where:

NLD = Najdi *Longissimus dorsi* muscle

NSM = Najdi *Semimembranosus* muscle

SLD = Somali *Longissimus dorsi* muscle

SSM = Somali *Semimembranosus* muscle

Results and discussion

Meat chemical composition

The chemical composition of meat from Najdi and Somali breeds is presented in table 1. The results showed no significant differences between the 2 breeds in protein and ash contents for both types of muscles. Contrary to protein and ash, moisture and fat were different (P<0.05) between the treatment groups. The Somali *semimebranosus* (*SM*) muscle attained the highest (77.27%) moisture content followed by Najdi *SM* (74.99%). It was justifiable for the former muscle to achieve the least fat content (0.77%), as known that fat and moisture contents of meat are inversely related. On the other hand, Najdi SM deviated from this rule. The fat content of the muscles was in the following order: Najdi *SM* > Somali *LD* > Najdi *LD* > Somali *SM*. The conclusion regarding differences in fat content between the 2 camel breeds obtained in this study was comparable to that stated by Suliman *et al* (2011) and Keane and Moloney (2008) who ascribed this variation to breed difference.

Meat physical, chemical and sensory properties

pH_{24} and colour components results are summarised in table 2. The muscle pH_{24} was different (P<0.05) between the treatment groups. The pH_{24} of Somali *SM* muscle was greater (P<0.05) than that attained by Najdi *LD* muscle, but not so with the

Najdi *SM* and Somali *LD* muscles. Kadim *et al* (2006) reported a pH_{24} of a *Longissimius thoracis* muscle that was comparable to the value obtained in this study. On the other hand, and contrary to the results obtained in this study, Suliman *et al* (2011) showed no significant differences between camel breeds in their pH_{24}. Nevertheless, the pH_{24} values of the all treatment muscles for both breeds were within the normal range. The lightness (L*) colour values did not differ between the treatments, while the redness (a*) and yellowness (b*) colour values were (P<0.05) different between the 2 breeds. The *LD* muscle of both breeds showed the highest (P>0.05) redness values compared to the *SM* muscles. The *SM* muscles of the 2 breeds also showed no significant differences between them. But the Somali *LD* was more (P<0.05) redder than the Najdi *SM* muscle. The Najdi *LD* and *SM* muscles were not significantly different in their yellowness values, while the Somali *LD* and *SM* muscles differed significantly (P<0.05). The Somali *LD* attained the highest (6.61) yellowness value compared to SM that attained the lowest value (3.83).

Cooking loss and water-holding capacity results are presented in table 2. Both parameters were different (P<0.05) between the treatments. The Somali *LD* muscle showed the highest (32.83%) percentage of cooking loss, followed by Najdi *SM* (32.67%), then Somali *LD* (32.83%), while Najdi *LD* came last (28.15%). The highest loss of cooking by Somali *LD* coincided with the highest ratio of WHC (5.41) attained by the same treatment group. The lowest ratio of WHC (3.73) was attained by Najdi *LD*, which is also corresponding to the lowest cooking loss value attained by the same group. Both muscles of the Najdi breed were different (P<0.05) from both muscles of the Somali breed in their WHC, whereas both muscles of the 2 breeds did not differ significantly between each other.

Shear force, myofibrillar fragmentation index and texture profile analysis

Results are displayed in table (3). The treatments were different (P<0.05) in shear force. The attained shear force values were as follows: Najdi *LD* < Somali *LD* < Najdi *SM* < Somali *SM*. Both muscles of Najdi breed were more tender than Somali muscles, but only Najdi *LD* was (P<0.05) tenderer than Somali *SM*. The *MFI* values for both muscles of Najdi were (P<0.05) different from those of Somali. But the muscles of the 2 breeds were not significantly different between each other. The components of the TPA were significantly different between the treatments, except for springiness. The obtained

Table 1. Proximate chemical composition of Longissimus dorsi (*LD*) and Semimembranosus (*SM*) muscles of Najdi and Somali camel breeds.

Parameter %	Najdi		Somali		S.E	L.S
	LD	*SM*	*LD*	*SM*		
Moisture	73.71[a]	74.99[ab]	74.31[a]	77.27[b]	0.46	0.02
Protein	21.50	20.99	21.19	18.85	0.46	0.15
Fat	0.90[a]	3.24[b]	2.51[b]	0.77[a]	0.28	0.001
Ash	1.63	1.64	1.65	1.55	0.03	0.53

[ab]Means in the row with different superscripts differ significantly

L.S. The level of significance (P < 0.05) S.E. standard error of the mean

Table 2. pH, Colour Components, Cooking loss, and Water-holding capacity of Longissimus dorsi (*LD*) and Semimembranosus (*SM*) muscles of Najdi and Somali camel breeds.

Parameter	Najdi		Somali		S.E	L.S
	LD	*SM*	*LD*	*SM*		
pH_{24}	5.76[a]	6.12[ab]	6.0ab	6.23b	0.06	0.04
Colour:						
L*	42.53	44.34	43.37	40.19	0.75	0.25
a*	15.82[b]	12.27[a]	15.73[b]	13.94[ab]	0.55	0.04
b*	5.99[ab]	5.70[ab]	6.61[b]	3.83[a]	0.40	0.01
Cooking Loss%	28.15[a]	32.67[b]	32.83[b]	30.88[ab]	0.67	0.04
WHC	3.73[a]	3.87[a]	5.41[b]	5.28[b]	0.26	0.02

[ab]Means in the row with different superscripts differ significantly

L.S. The level of significance (P < 0.05) S.E. standard error of the mean

Table 3. Shear force, MFI and Texture profile analysis (TPA) of *Longissimus dorsi* (*LD*) and *Semimembranosus* (*SM*) muscles of Najdi and Somali camel breeds.

Parameter	Najdi		Somali		S.E	L.S
	LD	*SM*	*LD*	*SM*		
Shear Force (kg)	3.21[a]	4.40[ab]	3.36[a]	5.16[b]	0.27	0.02
MFI	103.84[a]	100.43[a]	67.48[b]	56.91[b]	5.95	< 0.001
TPA:						
Hardness (kg)	1.90[a]	2.76c	2.77[bc]	1.94[ab]	0.18	0.03
Chewiness	0.82[bc]	0.43[ab]	1.09[c]	0.29[a]	0.09	< 0.01
Cohesiveness	0.49[ab]	0.44[ab]	0.54[b]	0.41[a]	0.02	0.03
Springiness	0.50	0.48	0.52	0.40	0.02	0.17

[abc]Means in the row with different superscripts differ significantly
L.S. The level of significance (P < 0.05) S.E. standard error of the mean

Table 4. Subjective evaluation of *Longissimus dorsi* (*LD*) and *Semimembranosus* (*SM*) muscles of Najdi and Somali camel breeds.

Parameter	Najdi		Somali		S.E	L.S
	LD	*SM*	*LD*	*SM*		
Tenderness	4.31[a]	3.50[b]	3.56[b]	3.44[b]	0.35	0.03
Juiciness	4.00	3.69	3.50	3.25	0.40	0.44
Flavour	4.75[a]	4.25[ab]	4.38[ab]	3.75[b]	0.37	0.01
Overall Acceptability	4.56[a]	3.81[ab]	4.00[ab]	3.56[b]	0.39	0.02

[ab]Means in the row with different superscripts differ significantly
L.S. The level of significance (P < 0.05) S.E. standard error of the mean

hardness value for *LD* muscle of Najdi breed (1.90 kg) was the least between the treatments, which was consistent with the results of SF and MFI for the same muscle. This result was (P<0.05) different to that of Najdi SM and Somali *LD*, but not so with Somali *SM*. The *LD* of Najdi was different (P<0.05) from the *SM* of Somali in chewiness. Likewise, the *LD* of Somali was (P<0.05) different from the *SM* of Najdi. On the other hand, only the *LD* and *SM* muscles of Somali breed were (P<0.05) different between each other in cohesiveness and not with those of Najdi breed which were also not significantly different.

Subjective evaluation Outcomes are presented in table 4. The treatments were different (P<0.05) in tenderness, flavour and overall acceptability. The juiciness showed no significant difference between the treated groups. The Najdi *LD* recorded the highest rate (4.31) (P<0.05) for tenderness between treatment groups. This rating matched that of shear force values, where Najdi *LD* > Somali *LD* > Najdi *SM* > Somali *SM*. The Najdi *LD* also revealed the most intense flavour (P<0.05) followed by Somali *LD*, then Najdi *SM* and lastly Somali *SM*. The Najdi *LD* achieved the most acceptable rating (P<0.05) compared to other treatment groups. The rating came in the following sequence: Najdi *LD* > Somali *LD* > Najdi *SM* > Somali *SM*. It was clear that the subjective evaluation consolidates most of the objective evaluation obtained in this study.

Conclusions

It is concluded that meats from camels of both breeds Najdi and Somali differ in fat content and quality characteristics. Generally, meats of the Najdi breed tended to be more tender and juicier than the Somali breed, which was leaner than Najdi breed.

Acknowledgements

The authors would like to express their appreciation to the Deanship of Scientific Research, King Saud University for funding this study through the Research Group Project No.: RGP-VPP-267.

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DOI : 10.5958/2277-8934.2016.00003.5 Vol 23 No 1, p 19-23

AMPLIFICATION AND SEQUENCING OF ENERGY RELATED MITOCHONDRIAL GENES FOR SOME DOMESTIC ANIMALS IN SAUDI ARABIA

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ABSTRACT

The sequences of mitochondrial genes encoding for cytochrome C oxidase subunits 1 and 2 (CO1 and CO2), ATPase synthase subunit 6 (ATP6) and cytochrome b (cytb) were collected from the Genbank database for some domestic animals and were aligned manually. The conserved regions were selected to design new primers for amplification and sequencing these genes. The designed primers produced positive amplification and sequencing results in spite of the high genetic variability shown among the studied animals. This method is expected to accelerate both amplification and sequencing domestic animals mitogenomes. This could aid to understand the domestic animals genetic framework necessary for conservation and breeding management.

Key words: Conserved primers, domestication, mitogenome, Saudi Arabia

Animal domestication is necessary for providing human with food, clothes, transportation and protection. Among the domestic animals, camel, sheep and goat are considered as farm animals accumulated domestication adaptations for thousands of years.

There are two surviving camel species nowadays which are the double-hump Bactrian (*Camelus bactrianus*) and the single-hump Dromedary (*Camelus dromedarius*). The single-hump camel is distributed in the region from Southwestern Asia to northern Africa and there is no wild dromedary is present (Grubb, 2005). However, the double-hump camel still survives in the wild of central Asia. Different ancestors and domestication events for both camel species have been suggested using mtDNA and microsatellite data (Jianlin *et al,* 2004). Domestication of the Bactrian camel took place 6000 years ago in the eastern part of Central Asia (FAO, 2007) whereas, dromedary has been domesticated 7000 years ago (Peters, 1997).

Sheep (*Ovis aries*) and goat (*Capra hircus*) were firstly domesticated approximately 10,000 to 12,000 years ago in the southwestern Asia, Turkey, Iran and Arabian Peninsula (Zeder *et al,* 2006; Naderi *et al,* 2008). MtDNA data were used to address the ancestral origin of sheep domestication (Hiendleder *et al,* 2002; Bruford *et al,* 2003) and the relationships among their different haplotypes origin (Meadows *et al,* 2007). A relatively little insight into the relationship between sheep breeds has been provided by SNPs information of Y-chromosome and microsatellites compared to the mtDNA data (Meadows *et al,* 2006) because of the unfortunate use of different microsatellite panels and the little phylogeographical structure obtained by these data (Groeneveld *et al,* 2010).

Goats have been domesticated in the same era and region of sheep domestication about 10,000 years ago in Southwestern Asia. Based on mtDNA data, Naderi *et al* (2008) have summarised haplogroup relationship of goats. Several Y-chromosome and microsatellite data were also used to infer the haplogroup origin of goats (Canon *et al,* 2006; Berthouly *et al,* 2009).

The molecular information between and within animal breeds are necessary to make informed management decisions (Simianer, 2005; Toro *et al,* 2009). One of the requirements for the effective management of farm animal genetic resources (FAnGR) is within- and between-breed genetic diversity and therefore, molecular data are essential for this purpose (Groeneveld *et al,* 2010). Data on the mitochondrial genes related to the functional environment may be informative regarding the animal adaptation to facilitate comparisons of their

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performance levels. It is therefore, necessary to find out conserved primers successful for amplification and sequence of the mitochondrial genes related to traits of energy production. These newly designed primers could accelerate collection of genetic information for domestic animals in order to aid in their management decisions and conservation.

Materials and Methods

The sequences of CO1, CO2, ATP6 and cytb genes were collected from the Genbank database for random samples of camel, sheep and goat that represent the common domestic animals in Saudi Arabia. The collected data were subjected to manual alignments and the conserved blocks have been selected for designing new primers. The individual genes were firstly prepared by DNASIS v. 3 and aligned by MacClade v. 4. The alignments were used to manually design new set of primers in the conserved regions (Table 1). The designed primers were checked for their reproducibility using specific computer programs (Olig4 and Umplify2) based on primer-design criteria. Freshly obtained animal samples from some individuals representing the studied domestic animals were collected. These samples involved 3 animals from each of Arabian camel (*Camelus dromedarius*), sheep (*Ovis aries*) and goat (*Capra hircus*). Blood samples were numbered, labeled immediately in the lab and preserved it -80°C for further molecular studies.

Table 1. Primers used in PCR and sequencing. Degenerate bases are as follows: R (A, G) and Y (C, T).

Gene	Product size	Annealing	Sequence (5` - 3`)
CO1	800 bp	54°C	TCAACCAACCACAAAGA AATATATGGTGGGCTCATAC
CO2	600 bp	50°C	TACCACACATTTGAAGAACC GCGTCTGTTTTYARTCCTAG
ATP6	450 bp	40°C	ACAAAATGAACGAAAAT ATTACTAGYATTGGGAT
cytb	850 bp	50°C	TCATCATGATGAAAYTT CGGAATATTATGCTTCGTTG

Qiagen spin-column kit was used for mitochondrial DNA extraction from 0.5ml blood sample according to the manufacturer's instruction (QIAamp DNA Micro Kit). The PCR was conducted in a 25 µl reaction volume as described by Amer and Kumazawa (2009) with some modifications. The PCR tube contained 2 µl DNA template, 0.2 µl of 10 µM from the forward and the reverse primers of the corresponding gene (Table 1), 12.5 µl PCR master mix (Promega Corporation, Madison, WI) and 10.3 µl autoclaved distilled water. PCR conditions were set as follows: 1 cycle for 4 minutes at 94°C; 35 cycles of 1 minute at 94°C, 1 minute at the corresponding annealing temperature (see Table 1 for each corresponding annealing temperature) and 1 minute at 72°C; one cycle for 5 minutes at 72°C. Because of the degenerate sites within the designed primers, we tried different annealing temperatures. The annealing temperatures which were listed in table 1 were the only one which produced positive amplifications. PCR products were purified with BioFlux spin column (BioFlux, Tokyo, Japan) according to the manufacturer instructions. The amplified products were visualised under UV light and photographed.

Purified products and PCR primers were submitted to Macrogen Inc. (Macrogen, Seoul, S. Korea) for conducting sequencing. Sequences were conducted in an ABI PRISM 3730xl sequencer (Applied BioSystems) and BigDyeTM Terminator Cycle Sequencing Kits with Ampli Taq-DNA polymerase (FS enzyme) (Applied Biosystems) following the protocols supplied by the manufacturer.

After reading the targeted genes, we deleted the first and the last 50 bp of the sequences because of the un-ambiguity always found in these flanking regions. The nucleotide sequences were treated with DNASIS, MacClade and PAUP (Swofford, 2003) for detecting success of the designed primers in sequencing the targeted genes. The genetic divergences among different studied domestic animals were estimated by calculating base composition and pairwise genetic distance (Tamuri-Nei distances).

Results and Discussion

The most common domestic animals in Saudi Arabia are camels, sheep and goat which belonged to two mammalian families (Camelidae and Bovidae). They are used for meat and milk production and therefore, studying their genetic framework is necessary for conservation and breeding management. In this study, 4 pairs of primers were designed for amplifying and sequencing 4 energy-related genes (CO1, CO2, ATP6 and cytb) from mitochondrial DNA of these animals (Table 1). The first pair of primers amplifies a 800-base-pair segment of CO1 gene. Likewise, the 2nd pair of primers amplifies a 600-base-pair segment of CO2 gene. The 3rd set amplifies a 450-base-pair segment of ATP6 gene and the 4th pair amplifies a 900-base-pair segment of cytb gene. The newly designed primers produced PCR products pure

enough to sequence directly (Fig 1). The primers contained 1 or 2 degenerate sites in the reverse primers of CO2 and AT6 genes and one site in the forward primer of cytb gene. All the forward and reverse primers consisted of 17 to 20 bases (Table 1) with notable low G/C contents of 24% to 40% and Tm of 40°C to 54°C. Although, the primers disobey the criteria of Miya *et al* (2015), successful amplifications of the hypervariable regions using extracted DNA from the 3 domestic animal groups have been confirmed. With these PCR products, their nucleotide sequences using the conventional Sanger sequencing method have been successfully determined. The sequence data are available with their accession numbers (KT750036-KT750059) in NCBI GenBank databases.

Percentages of base composition of the sequenced genes were shown in table 2. Adenine showed percentages ranged from 24.7 to 28.5 (CO1 gene), from 31.8 to 36.3 (CO2 gene), from 31.1 to 33.7 (ATP6 gene) and from 28.8 to 30.3 (cytb gene). Thymine showed a range of 29.7 to 31.1 in CO1 gene, from 26.7 to 29.9 in CO2 gene, from 26.4 to 28.1 in ATP6 gene and from 26.8 to 29.9 in cytb gene. For cytosine, percentages ranged from 25.0 to 25.5 (CO1 gene), from 22.8 to 24.1 (CO2 gene), from 29.4 to 30.1 (ATP6 gene) and from 26.1 to 28.6 (cytb gene). Guanine percentages were from 16.2 to 19.1 (CO1 gene), from 13.0 to 15.1 (CO2 gene), from 9.8 to 11.2 (ATP6 gene) and from 14.8 to 16.1 (cytb gene). The differences in base composition reflect the ambiguity in the sequences of these genes among the studied taxa with considerable genetic variability. The uncorrected pairwise genetic distances calculated by Paup (Swofford, 2003) among the studied animals were shown in table 3. The data indicated genetic differences among the taxa with a range of distance between 0.09 and 0.26.

There are great differences in mtDNA across animal species. Therefore, primers that amplify specific segments of mtDNA of a certain species would not be expected to amplify the corresponding segments of mtDNA from other species (Yang *et al*, 2014). Nonetheless, when we attempted to design conserved primers to obtain the mtDNA sequences of camel, goat and sheep, we found that some regions in the mitochondrial genomes of these taxa are conserved. We therefore, searched the 4 genes related to energy production and designed our primers. As we found a great success of amplifying these mitochondrial genes in Camelidae and Bovidae, we expect these primers will be useful to researchers

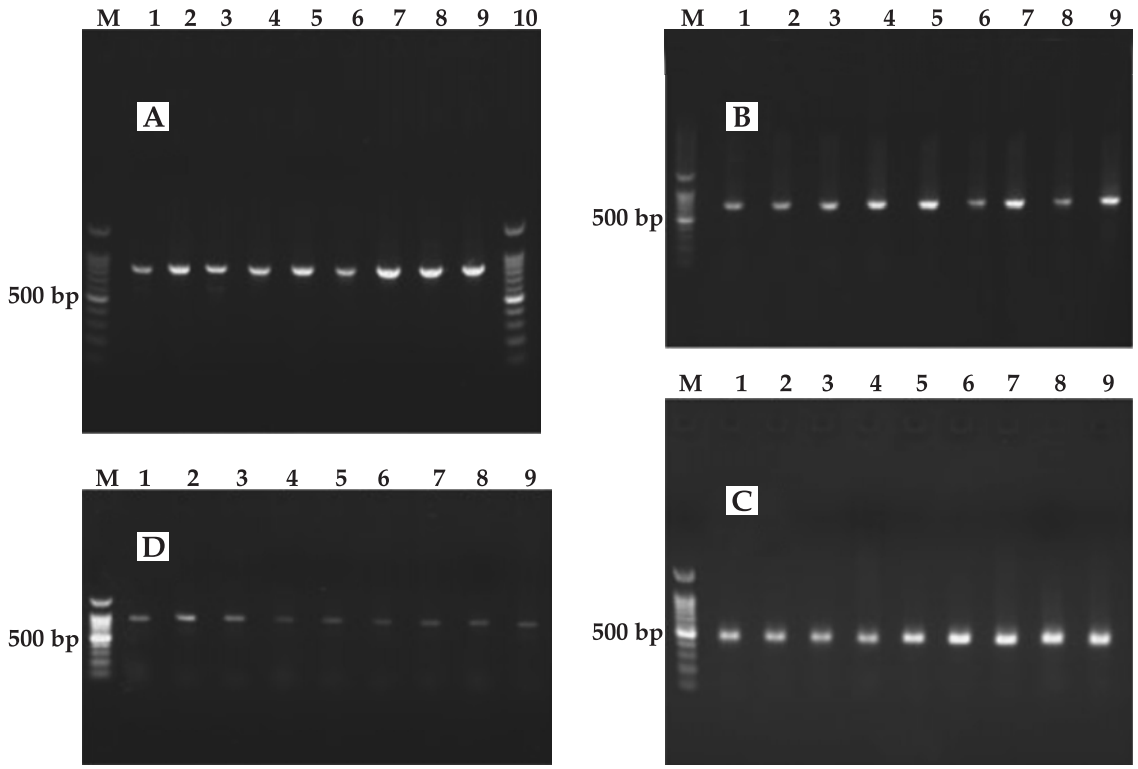

Fig 1. Agarose gel profiles of the PCR amplified products for CO1 gene (A), CO2 gene (B), ATP6 gene (C) and cytb gene (D). M refers to 100 bp DNA ladder and the amplified products are for camel (1-3), sheep (4-6) and goat (7-9).

Table 2. Percentages of base composition and sequence length of the studied genes.

Taxa	CO1					CO2					ATP6					Cytb				
	A	T	C	G	Tot.	A	T	C	G	Tot.	A	T	C	G	Tot.	A	T	C	G	Tot.
camel	24.7	31.1	25.1	19.1	760	36.3	27.7	22.8	13.0	569	31.1	28.1	29.4	11.2	356	27.8	29.9	28.6	16.1	574
sheep	27.6	30.4	25.0	16.9	760	36.2	26.7	24.1	13.0	569	32.3	26.4	30.1	11.2	356	29.8	26.8	26.1	14.8	574
goat	28.5	29.7	25.5	16.2	760	31.8	29.8	23.1	15.1	569	33.7	28.1	28.4	9.8	356	30.3	27.4	27.5	14.8	574

Table 3. Pairwise genetic distances among different domestic taxa calculated for the sequenced gene fragments.

Taxa	CO1		CO2		ATP6		Cytb	
	sheep	camel	sheep	camel	sheep	camel	sheep	camel
camel	–		–		–		–	
sheep	0.20	–	0.09	–	0.26	–	0.24	–
goat	0.21	0.09	0.19	0.21	0.24	0.11	0.23	0.24

looking at relationships above species and genus level. Similar finding were revealed by Concepcion *et al* (2006) and Sarri *et al* (2014), who showed that the universal primers amplified segments that could convey sufficient phylogenetic information to assign samples to species.

Avoiding degenerate sites is recommended in designing the primers (Miya *et al*, 2015). As we used protein-coding genes in which the 3rd codon positions almost always vary among taxa even if the amino acid sequence is completely conserved (Sorenson, 2003). We did not find a region conserved perfectly among the studied taxa and therefore, the degenerate sites were used in designing the primers. We applied the strategy of Sorenson (2003) and Amer *et al* (2013) in which degenerate sites were used to accommodate the variation likely to be present in the taxa we are working on. This resulted in a primer that has at least some molecules that match every taxon perfectly and then the primer with degenerate sites yielded products more consistently.

Acknowledgement

We are thankful to Taif University for funding this work under a project number 1-436-3794.

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DOI : 10.5958/2277-8934.2016.00004.7 Vol 23 No 1, p 25-32

EFFECTS OF CAMEL MILK ON DRUG METABOLISING CYTOCHROME P450 ENZYMES EXPRESSIONS IN RATS

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ABSTRACT

Cytochrome P450 (CYPs) constitutes the major enzyme family capable of catalysing the oxidative biotransformation of most drugs. These are affected by a large number of factors including environmental elements. Widely practiced drinking of camel milk as a nutrient, therapy for some disease or even adjuvant with some drugs make it mandatory to investigate the possible CYPs modulator effects of camel milk. Forty-eight male Wistar rats were divided into 6 groups of 8 rats each. Groups were allocated as control, Camel milk (CM) treated, Sudan III (S.III) treated, S.III +CM, Phenobarbital (PB) or PB+CM.

CYP3A2, CYP 2B1, CYP 1A1 and CYP 1A2 mRNA were measured by semi-quantitative RT-PCR. Results showed that camel milk supplementation reduced the mRNA expression of basal and PB induced CYP3A2 and CYP2B1. Camel milk also reduced the mRNA expression of the basal levels of CYP1A2 and the S.III or PB induced CYP1A2 and CYP1A1. These results indicates that camel milk supplementation down regulated hepatic CYP3A2, CYP2B1, CYP1A1 and CYP1A2 mRNA expressions either their basal control or induced levels with PB or S.III. This may operate mainly through camel anti-CAR effect. This may indicate camel milk potential anticancer effect through preventing the activation of procarcinogenes to carcinogens. These results also imply that CM affects the metabolism of drugs metabolised by these enzymes.

Key words: Camel milk, CYPs, PB, S.III

Abbreviations: (CYPs), Cytochrome P450; (PB), Phenobarbital; CAR, consistutive active androstane receptor; S.III, Sudan III; RT-PCR, reverse transcription-polymerase chain reaction; CM, camel milk; STAT3, signal transducer and activator of transcription 3; NF-κB, nuclear factor kappa beta; (AhR), Arylhydrocarbon receptor; CITCO, 6-(4-Chlorophenyl) imidazo [2,1-b] [1,3] thiazole-5-carbaldehyde O-3,4-dichlorobenzyl) oxime (A novel potent and selective CAR agonist; CCL3 (MIP-1α), Chemokine (C-C motif) ligand 3 (CCL3) also known as macrophage inflammatory protein 1-alpha; AMPK, 5′ adenosine monophosphate-activated protein kinase ; p38, Mitogen-activated protein kinase 14, also called p38-α; B[a]P, Benzo [a] pyrene; 7,12-DMBA, 7,12-Dimethylbenz [a] anthracene; 3-MC, 3-Methylcholanthrene; co-planar PCB, Coplanar polychlorinated biphenyls; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Cytochrome P450 enzymes (CYPs) play critical roles in the metabolism of a vast array of endogenous as well as exogenous substrates (Sangar *et al,* 2010). These enzymes constitute the major enzyme family capable of catalysing the oxidative biotransformation of most drugs and other lipophilic xenobiotics and are therefore of particular relevance for clinical pharmacology (Guengerich, 2008). Cytochromes P450 have been shown to be involved in metabolism of drugs and toxic chemicals (Schenkman and Grein, 1993; Gonzalez, 1990). As drug clearance depend on CYP enzymes activities, their inhibition can lead to overexposure and toxicity (Fowler and Zhang, 2008). On the other hand, enzyme induction can increase drug elimination and decreases its plasma concentration hence attenuate its pharmacological effect (Lazarou *et al,* 1998). A large number of factors including physiological factors (hormones, development and disease); and environmental elements can affect drug metabolising enzymes (Tang *et al,* 2005). It has been shown that hepatic CYP activities were affected by the component of feed as malnutrition in rats caused CYP reduction (Lee *et al,* 2004). Camel's milk exhibits a wide range of biological activities including antimicrobial, antioxidative, antithrombotic, antihypertensive,

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and immuno-modulatory effect (Fitz and Meisel, 2000; Kohonen and Pihlanto, 2003; Saltanat *et al,* 2009). It was therapeutically used to treat jaundice, splenic problems, asthma, anemia, piles, and diabetes (Knoess, 1979; Rao *et al,* 1970). Camel's milk is different from other ruminant milk, having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamins (C, B2, A, and E), low protein, and high concentrations of insulin (Korashy *et al,* 2012). Besides casein, camel milk contains high levels of lactoferrin, an iron-binding glycoprotein of the transferring family (Al-Majali *et al,* 2007). Camel milk anticarcinogenic, anti inflammatory and antioxidant activities was proposed to be caused mainly by Lactoferrin (Konuspayeva *et al,* 2004). A comparative survey of lactoferrin concentrations in different milks showed that camel's milk contain the greatest amount of lactoferrin (Konuspayeva *et al,* 2004). Due to the presence of peptides and protein in camel's milk it is considered an excellent source of well balanced nutrients and also exhibits a range of biological activities that influence digestion, metabolic responses to absorbed nutrients, growth and development of specific organs and resistance to diseases. (Yagil *et al,* 1984; Korhonen and Pihlanto, 2001). Camel's milk proved its potential effect in the treatment of food allergies, due to its inflammation-inhibiting proteins, and hypoallergenic properties, in addition to its smaller size nanobodies, which are different from those found in human (Al-Ayadhi and Elamin, 2013). Camel's milk nanobodies, as a single domain, show many promising and therapeutic potencies in infection and immunity (Zafra *et al,* 2011). Often, HCV-infected patients consume large amounts of camel's milk as an alternative and/or supportive medicine (Redwan and Tabll, 2007). A study by Mohamad *et al* (2009) suggested that as an adjunct to standard management, daily ingestion of camel's milk can aid metabolic control in young type 1 diabetes, at least in part by boosting endogenous insulin secretion. Widely practiced drinking of camel's milk as a nutrient, therapy for some disease or even adjuvant with some drugs in the Arab countries make it mandatory to investigate camel milk modulator effects on drug metabolism through affecting drug metabolising enzymes CYPs450 to enable physicians to predict the dosing and the kinetics of the prescribed drugs for patients who drink camel milk to avoid drug toxicity or subclinical dosing. In this study we are aiming to investigate the effect of raw camel's milk on the expression of cytochrome P450 enzymes in rats.

Materials and Methods

Sudan III and Phinobarbital were collected from Sigma Chemical Co., (St. Louise, Mo, USA) and camel's milk samples were collected daily early in the morning from camel farm in Taif Providence, Saudi Arabia. Milk with down by hand milking and in sterile screw bottles and kept in cool boxes until transported to the laboratory. The rats were given this fresh milk (120 mL/cage) as such without any further treatment at morning after deprivation of rats from water for 3 hours as to ensure drinking of milk within 2 hour to avoid souring of the milk.

Experimental animals: A total of 48 adult male Wistar rats weighing about 200 g each were used in the present study and kept under observation for about 4 days before the onset of the experiment. These were maintained in stainless steel cages at normal atmospheric temperature of 25±2°C and good ventilation.

Treatment

Group I: (8 Rats) received only water and served as control.

Group II: (8 Rats) received raw camel's milk by drinking 50ml/kg daily for 25 days

Group III: (8 Rats) received water daily for 21 days and 22nd, 23rd and 24th days Sudan III in a dose of 80mg/kg

Group IV: (8 Rats) received raw camel's milk in a dose of 50 ml/kg daily for 21 days then at the 22nd, 23rd and 24th days camel's milk 50 ml/kg plus S.III in a dose of 80mg/kg

Group V: (8 Rats) received water daily for 21 days then at the the 22nd and 24th days PB in a dose of 80mg/kg

Group VI: (8 Rats) received raw camel's milk in a dose of 50ml/kg daily for 21 days then at the 22nd and 24th days camel's milk plus Phenobarbital in a dose of 80mg/kg

Sampling

At the 25th day, rats were sacrificed through cervical dislocation under light ether anesthesia and tissues specimen of all groups from liver were collected, for RT-PCR studies.

RNA extraction:

Total RNA was extracted from the collected liver samples using Qiazol Reagent according to the manufacturer's instructions. Briefly, 100 mg of each tissue samples were homogenised in 1ml QIAzol

(QIAGEN Inc., Valencia, CA) then 0.3 ml chloroform were added to the homogenate. Then, the mixtures were shaken for 30s followed by centrifugation at 4°C and 12,500 rpm for 20 min. The supernatant layer were transferred into a new set of tubes, and an equal volume of isopropanol were added to the samples, and were shaken for 15 seconds and centrifuged at 4°C and 12500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, dried up and then were dissolved in DEPC water. The prepared RNA integrity were checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm.

cDNA synthesis: a mixture of 2 µg total RNA and 0.5 ng Oligo (dT)15 primers (Macrogen, Inc., Seoul, Korea) in a total volume of 11µl sterilised DEPC water was incubated in the Thermo Hybaid PXE 0.2 Thermal Cycler (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 70°C for 10 min for denaturing. Subsequently, 4 µl 5X RT-buffer, 2 µl 10 mM dNTPs and 100 units RevertAid Premium reverse transcriptase (Fermentas; Thermo Fisher Scientific, Inc., Pittsburgh, PA, USA) were added to the reaction mixture and DEPC water was used to bring the total volume to 20 µl. The mixture was then incubated in the thermal cycler at 30°C for 10 min, 42°C for 1 h and 90°C for 10 min. The resulting cDNA was then preserved at -20°C until use.

Semi-quantitative polymerase chain reaction (PCR): The mRNA expression levels of hepatic CYPs gene were analysed by semi-quantitative PCR using the corresponding gene-specific primers (Table 1). The genes analysed were CYP1A1, CYP1A2, CYP3A2 and CYP2B1. As a reference gene, the expression of β-actin mRNA was determined. The primers were designed using the Oligo Primer Analysis software, version 4 (www.oligo.net) and nucleotide sequences published in Genbank (www.ncbi.nlm.nih.gov/genbank; Table I). The primers were synthesised by Macrogen, Inc. PCR was conducted in a final reaction volume of 25 µl consisting of 1 µl cDNA, 1 µl of each 10 pM forward and reverse primers, 12.5 µl PCR master mix (Promega Corporation) and nuclease-free deionised water. PCR was conducted using the thermal cycler with a cycle sequence of denaturing at 94°C for 4 min for 1 cycle, followed by 25-28 cycles of denaturation at 94°C for 1 min, annealing at the specific temperature corresponding to each primer (see Table I) and extension at 72°C for 1 min, with an additional final extension step at 72°C for 5 min. The PCR products were electrophoresed on a 1.5% agarose A (Bio Basic Canada, Inc., Markham, ON, Canada) gel in Tris-EDTA buffer at 100 V for 30 min, and stained with ethidium bromide. The PCR products were visualised under UV light and images were captured using the GelDoc-It imaging system. The intensities of the bands were densitometrically quantified using NIH Image software (National Institutes of Health, Bethesda, MD, USA; rsb.info.nih.gov/nih-image).

Statistical analysis

Data were presented as means ± standard errors of means. Statistical analysis was done by SPSS software (SPSS version 13.0, IBM, Chicago, IL, USA.

Results

Effect of camel milk on CYP3A2 and CYP2B1mRNA expressions:

To examine the possible modulator effects of camel milk on the hepatic major metabolic enzymes CYP 2B1 and CYP3A2 expression, the mRNA expressions of these two enzymes in the liver of rats were measured by semi-quantitative RT-PCR. Camel milk drinking reduced the mRNA expression of CYP2B1 compared with control levels. Administration of Phenobarbital (PB) upregulated CYP 2B1 mRNA expression meanwhile when administrated with PB, camel milk prevented the PB-induction of CYP2B1 mRNA. Sudan III administration to rats showed suppressive effect on CYP2B1 mRNA expression either alone or with camel milk (Fig 1 A).

Camel milk drinking reduced the mRNA expression of hepatic CYP3A2 compared with control levels. Phenobarbital administrations upregulated CYP 3A2 mRNA expressions than control levels. Meanwhile when administrated with PB, camel milk

Table 1. Primers and polymerase chain reaction conditions used for tested genes amplification.

Name	sense 5´..... 3´	anti-sense 5´..... 3´	Ann. Tm.	BP
β-actin	ATGTACGTAGCCATCCAGGC	TCCACACAGAGTACTTGCGC	56°C	628
CYP3A2	TTGATCCGTTGTTCTTGTCA	GGCCAGGAAATACAAGCAA	52°C	342
CYP2B1	TCTCACTCAACACTACGTTC	CTGGGAAAGGATCCAAGCCTGGG	58°C	450
CYP1A1	CCATGACCAGGAACTATGGG	TCTGGTGAGCATCCAGGACA	56°C	341
CYP1A2	GCAGGTCAACCATGATGAGAA	CGGCCGATGTCTCGGCCATCT	56°C	334

decreased the PB-induction of CYP 3A2 mRNA. Sudan III administration to rats for 3 days showed suppressive effect on CYP 3A2 mRNA expression. Camel milk drinking before and in concomitant with S.III administration produced further suppressive effect on CYP3A2 mRNA expression (Fig 1 B).

Effect of camel milk on CYP1A1 and CYP1A2 mRNA expressions:

To examine the possible modulator effect of camel milk on hepatic CYP1A1/CYP1A2 mRNA expressions, the mRNA expressions of hepatic CYP1A1 and CYP1A2 mRNA expressions were measured by semi-quantitative RT-PCR. CYP1A1 mRNA expression which was not expressed in the normal state, was clearly induced with S.III administration for 3 days. PB administration to rats caused induction of CYP1A1 in rats' liver. Camel milk supplementation to rats before and in concomitant with S.III-induced did not affect CYP1A1 mRNA expression. However, Camel milk supplementation to rats before and in concomitant with Phenobarbital (PB) completely abolished the PB-induced CYP1A1 mRNA expression (Fig 2 A). Camel milk supplementation clearly inhibited CYP1A2 mRNA expression than the control levels. Sudan III or PB administration caused induction of CYP1A2 mRNA expression. Camel milk supplementation slightly suppressed the S.III-induced CYP1A2 mRNA expression meanwhile it strongly down regulated the PB-induced CYP1A2 mRNA expression (Fig 2 B).

Discussion

Drug clearance depend on CYP enzymes activities, their inhibition can lead to overexposure and toxicity (Fowler and Zhang, 2008), while their induction can increase drug elimination and decreases its plasma concentration and therefore attenuate its pharmacological effects (Lazarou *et al,* 1998). Induction or inhibition of CYP3A4 expression is considered a major clinical concern for drug-drug interactions in patients receiving multiple CYP3A4-metabolising drugs (Li *et al,* 2012). The inhibition of hepatic CYP3A2 mRNA expression by camel milk indicates its ability to affect the metabolism of drugs and chemicals that are substrates of CYP3A4 in human. Indeed, rat CYP3A2 exhibits a 73% homology of the amino acid sequences and some substrate preference to human CYP3A4 (Gibson *et al,* 2002). Rat CYP3A2 and human CYP3A4 are involved in the metabolism of erythromycin, nifedipine, lidocaine, testosterone, aflatoxin B1 and benzo (a) pyrene (Gibson *et al,* 2002; Nedelcheva and Gut, 1994). CYP3A4 is highly expressed in adult liver and small intestine (Lamba *et al,* 2002) and metabolises not only xenobiotics including majority of drugs and carcinogens (Nelson *et al,* 1996; Shayeganpour *et al,* 2006) but also many endogenous compounds such as cholesterol, bile acids, fatty acids, prostaglandins, leukotrienes, retinoids and biogenic amines (Nelson *et al,* 1996, Christians, 2004). Supplementation of diabetic mice with camel whey protein significantly restored the activation of STAT3 and NF-κB (Badr, 2012). CYP3A4 was reported to be down-regulates by JAK/Stat pathway (Jover *et al,* 2002) and NF-κB (Zangar *et al,* 2008). Therefore CYP3A2 inhibition in this study may be operated through camel milk-induced STAT3 and NF-κB. CYP3A4 catalyses more than 50% of clinically used drugs so any change of CYP3A4 activity will affect the pharmacokinetics of these drugs (Chen *et al,* 2014). This CYP3A down regulating effect of camel milk should be considered when camel milk is consumed with substrates of CYP3A4 to adjust the dose of those drugs. Of these drugs are the anti-cancer drug cyclophosphamide, Midazolam, Nifedipine and Testosterone (Zhang *et al,* 2005), cardiovascular drug nifedipine (Galetin *et al,* 2005), cyclosporine, Erythromycin, Estradiol, Hydrocortisone, Lidocaine, Tacrolimus, Tamoxifen, and Terfenidine (Waxman, 1999).

Prolonged treatment with phenobarbital results in the formation of altered hepatic foci and liver tumours through induction of CYP2B1/2 (Elcombe *et al,* 2010; Tien and Negishi, 2006). Treatment of diabetic mice with undenatured camel milk whey protein accelerates the wound healing process by enhancing the expression of MIP-1α (Badr *et al,* 2012). On the other hand, CCL3 (MIP-1α) was demonstrated to induce increase in cell migration through activation of the AMPK, p38, and NF-κB pathways (Hsu *et al,* 2013). Furthermore, Metformin was recently demonstrated to enhance CAR phosphorylation in human hepatocytes in part through an AMPK-dependent signaling pathway therefore suppress CYP2B6 induction (Yang *et al,* 2014). Taken together, camel milk suppressive effect on CYP2B1 mRNA expression revealed in this study may be mediated through AMPK-dependent enhancement of CAR phosphorylation. This suppression of CYP2B expression by camel milk and possible effects on the metabolism of drugs that are substrate of CYP2B is worth to be considered in application of drugs such as ketamine, artemisinin, nevirapine, efavirenz, bupropion, sibutramine, propofol, arachidonic acid, lauric acid, 17beta-estradiol, estrone, ethinylestradiol, and testosterone (Mo *et al,* 2009).

CYP1A1 is not expressed in the normal condition, while CYP1A2 is constitutively expressed in rat' liver. In this study camel milk consumption reduced CYP1A2 mRNA expression than control and reduced its induced levels either by S.III and PB. Camel milk consumption also suppressed the CYP1A1 mRNA induced with either S.III or PB. Arylhydrocarbon receptor (AhR) has been shown to play important roles in regulation and induction of CYP1A1 and CYP1A2 (Whitlock, 1999). It has been reported that Sudan dyes could potently induce CYP1A1 and 1A2 mRNA and protein expression in rats (Nahla *et al,* 2008). More than 90% of known chemical carcinogens, including aromatic amines and polycyclic aromatic hydrocarbons (PAH) s, are substrates of these cytochromes (Conney, 1982; Ioannides and Parke, 1993; Kawajiri and Fujii-Kuriyama, 1991) and their metabolism often results in the formation of active carcinogenic metabolites (Ioannides and Parke, 1990; Lewis *et al,* 1993). PAHs such as carcinogenic B[a]P, 7,12-DMBA, and 3-MC and co-planar PCB congeners, induce CYP1A1/1A2 enzymes through AhR-dependent mechanism (Nebert 1989, Whitlock 1999, Hankinson, 1995). AhR (–/–) mice, are rather resistant to teratogenicity by TCDD than AhR(+/+) mice (Mimura *et al,* 1997) and to carcinogenicity by B[a]P (Shimizu *et al,* 2000). CYP1A2 was shown to be induced also by PB through CAR activation (Shaban *et al,* 2013). In the current study the inhibition of rat hepatic CYP1A1 and CYP1A2 mRNA by Camel milk is consistent with a previous report of Cyp1a1 down-regulation in murine hepatoma Hepa 1c1c7 cells by camel milk (Korashy *et al,* 2012). This and the results of the current study indicate the ability of camel milk to suppress the AhR target genes activation and hence protect against AhR-dependent procarcinogen activations. Hepatic CYP1A2 participates in the metabolic activation of chemical mutagens in cooked food, such as 2-amino-3-methylimidazo [4,5-f] quinoline (IQ), 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (Boobis *et al,* 1995). Hepatic CYP1A2 is one of the key enzymes

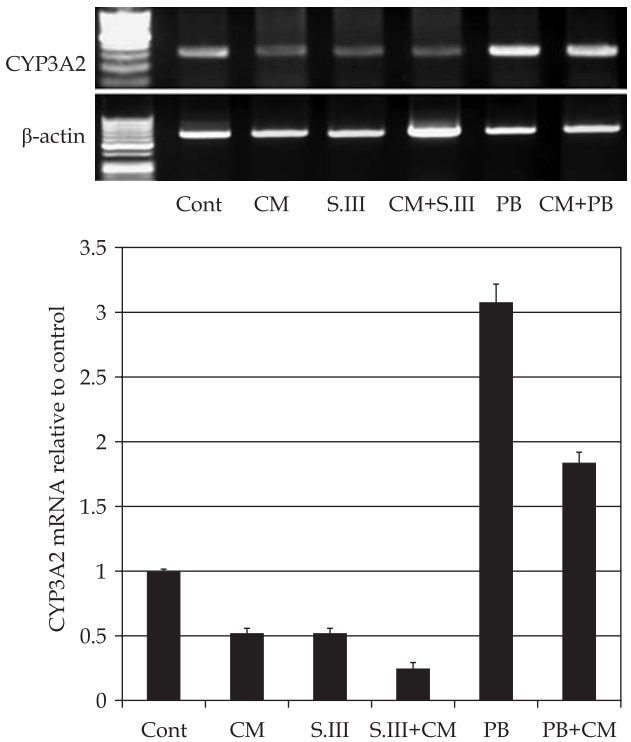

Fig 1. Effect of camel milk on hepatic CYP3A2 mRNA expressions. Total RNA was prepared from liver tissues and the expression levels of CYP3A2 mRNA were analysed by semi-quantitative RT-PCR. Values are presented as the mean ± standard error of 8 rats. *P<0.05 vs. cont group. #P<0.05 vs. PB-treated group. C, control; CM, Camel milk-supplement group; S.III, Sudan III treated group; S.III+CM, Sudan III+Camel milk treated group; PB, Phenobarbital treated group and PB+CM, PB + camel milk treated group.

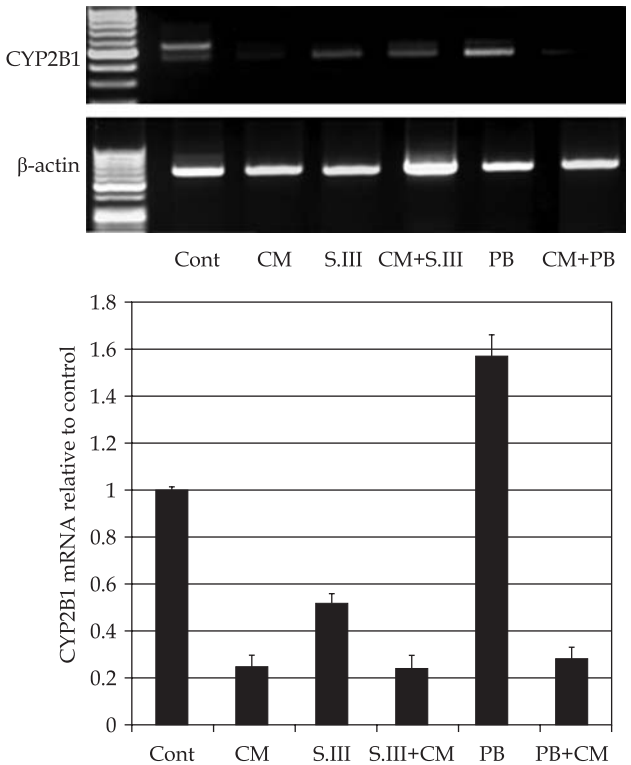

Fig 2. Effect of camel milk on hepatic CYP2B1 mRNA expressions. Total RNA was prepared from liver tissues and the expression levels of CYP2B1 mRNA were analysed by semi-quantitative RT-PCR. Values are presented as the mean ± standard error of 8 rats. *P<0.05 vs. cont group. #P<0.05 vs. PB-treated group. C, control; CM, Camel milk-supplement group; S.III, Sudan III treated group; S.III+CM, Sudan III+Camel milk treated group; PB, Phenobarbital treated group and PB+CM, PB + camel milk treated group.

having an important role in the metabolic clearance of 5% of currently marketed drugs (Faber *et al*, 2005). Of its substrates are drugs, such as theophylline, caffeine, phenacetin, and propranolol (Gonzalez, 1990). The activity of CYP1A2 is of the possible risk factors determining the carcinogenicity of heterocyclic amines in human beings (Zaher *et al*, 1998). It was demonstrated that CYP1A2 mRNA could be induced by phenobarbital in female C57BL/6Ncrj (C57BL/6) mice in vivo as well as in vitro experiments (Nemoto *et al*, 1995). A significant increase of theophylline clearance after chronic PB treatment was reported in humans (Saccar *et al*, 1985). AhR activation regulates the transcription of CYP1A1 and CYP1A2 and several other genes (Nebert and Dalton, 2006). However, CYP1A2 was reported to be induced by PB in mice in an AhR-indpenednt manner (Sakuma *et al*, 1999). The induction of CYP1A2 by PB in this study is in line with previous results showing CYP1A2 induction by PB through CAR activation (Shaban *et al*, 2013). Additionally the inhibition of CYP1A2 may indicate the suppressive effect of camel milk on the CAR activation. Moreover, in the current study the induction of CYP1A1 by PB is in line with a previous study where CAR was suggested to modify CYP1A1 transactivation by Sudan III (Ohno *et al*, 2012). The addition of CITCO, a selective CAR agonist, enhances transcriptional activity of AHR on the human CYP1A1 gene (Yoshinari *et al*, 2010). Moreover, down regulation of PB induced-CYP1A1 by camel milk may reflect its anti-CAR activation and indicates camel milk possible limitation of procarcinogens activation by CYP1A1 and hence protection against cancer development.

Conclusion Camel milk supplementation to Wistar rats down regulated hepatic CYP3A2, CYP2B1, CYP1A1 and CYP1A2 mRNA expressions either their basal or induced levels with PB or S.III. This may operate through camel anti-CAR effect. This effect may indicate CM potential anticancer effect through preventing procarcinogenes activation to carcinogens. It also implies that CM consumption may affect the metabolism of drugs that are substrates of these enzymes.

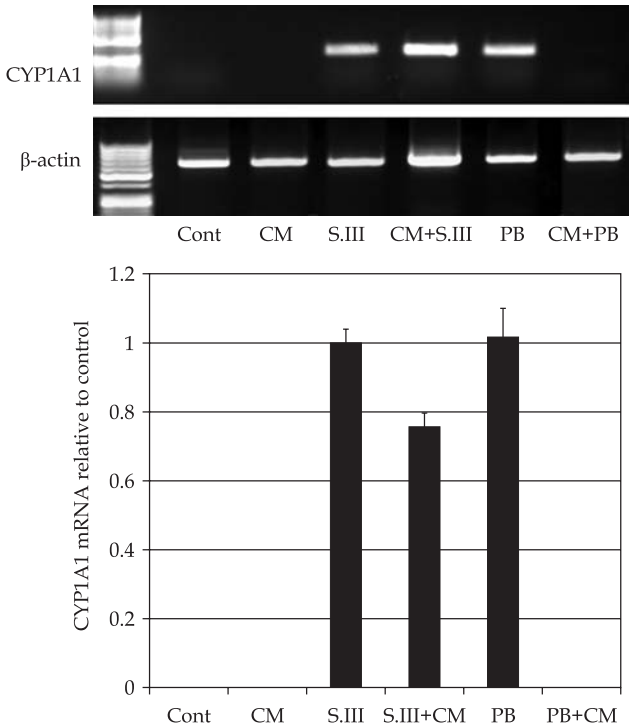

Fig 3. Effect of camel milk on hepatic CYP1A1 mRNA expressions. Total RNA was prepared from liver tissues and the expression levels of CYP1A1 mRNA were analysed by semi-quantitative RT-PCR. Values are presented as the mean ± standard error of 8 rats. *P<0.05 vs. cont group.*# P<0.05 vs. S.III treated group. #P<0.05 vs. PB-treated group. C, control; CM, Camel milk-supplement group; S.III, Sudan III treated group; S.III+CM, Sudan III+Camel milk treated group; PB, Phenobarbital treated group and PB+CM, PB + camel milk treated group.

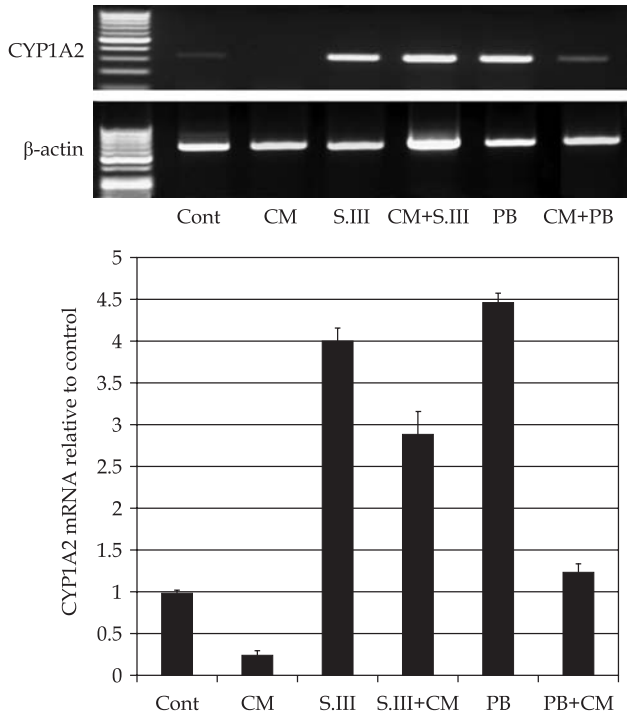

Fig 4. Effect of camel milk on hepatic CYP1A2 mRNA expressions. Total RNA was prepared from liver tissues and the expression levels of CYP1A2 mRNA were analysed by semi-quantitative RT-PCR. Values are presented as the mean ± standard error of 8 rats. *P<0.05 vs. cont group.*# P<0.05 vs. S.III treated group. #P<0.05 vs. PB-treated group. C, control; CM, Camel milk-supplement group; S.III, Sudan III treated group; S.III+CM, Sudan III+Camel milk treated group; PB, Phenobarbital treated group and PB+CM, PB + camel milk treated group.

Acknowledgement

The present work was supported in part by a research project grant (No. 1/435/3599) from Taif University, Saudi Arabia granted to the first author.

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DOI : 10.5958/2277-8934.2016.00005.9 Vol 23 No 1, p 33-40

ENZYMATIC HYDROLYSIS OF CAMEL MILK PROTEINS AND ITS ANTIOXIDANT PROPERTIES

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ABSTRACT

Camel milk proteins were hydrolysed with alcalase, α-chymotrypsin and papain and hydrolysates were assessed for antioxidant activity. Non-fat camel milk (NFCM) powder was reconstituted (5% TS) in phosphate buffer and enzymes were added at a ratio of 1:100 (enzyme: substrate). Hydrolysis was carried out at 55°C for Alcalase and Papain, and 37°C for α-Chymotrypsin for 6 hours and samples were drawn at 2h interval. The hydrolysates were analysed for change in pH, degree of hydrolysis (DH) and antioxidant activities *viz.* 2, 2′ azino bis (3 ethylbenzthiazoline 6 sulphonic acid) (ABTS), 2,2′ diphenyl 1 picrylhydrazyl (DPPH) and ferric reducing antioxidant power assay (FRAP). With the progress of hydrolysis time, pH of the hydrolysates were decreased and higher rate was observed for alcalase. The DH increased significantly ($p<0.05$) upto 6 h on hydrolysis with alcalase and papain, whereas upto 4h for chymotrypsin. In SDS-PAGE, the disappearance of major protein bands in hydrolysates samples confirm hydrolysis and production of low molecular weight peptides. The antioxidant activity was assessed by ABTS, DPPH and FRAP assay, increased significantly ($p<0.05$) with the increase in hydrolysis time and DH. The hydrolysis carried by chymotrypsin exhibited higher antioxidant activity as compared to alcalase and papain. The results suggested that camel milk proteins could be used as natural source of protein to produce hydrolysates with antioxidant activities and can be used for human consumption and as ingredient in nutraceutical and pharmaceuticals and also in health oriented food products.

Key words: Antioxidant activity, camel milk, enzymatic hydrolysis, protein hydrolysate

In recent years, extensive scientific evidence have been documented on beneficial effects of food derived bioactive peptides on human health (Korhonen and Pihlanto, 2006; Haque *et al,* 2009; Mao *et al,* 2011). For the first time, Marcuse (1960) reported that the peptides derived from dietary proteins have antioxidant activity. Since then, various protein sources *viz.* casein (Suetsuna *et al,* 2000), whey proteins, egg proteins (Sakanaka and Tachibana, 2006), fish proteins, muscle protein, plant proteins such as peanut proteins (Hwang *et al,* 2010), and larval proteins (Wang *et al,* 2013) have been explored to investigate the antioxidant properties.

The milk obtained from one-humped camels (*Camelus dromedarius*) differs from bovine milk in composition and structure of its protein components, which influences its functional and biological properties. The milk proteins constitute casein (CN) and whey proteins. Out of these proteins, casein proteins are the major proteins in camel milk and β-CN constitutes about 65% of total camel caseins (Kappeler *et al,* 2003), whereas, the whey proteins are present in smaller amount (20-25% of total protein) in which the β-lactglobulin is deficient. Many of the recent studies have suggested that camel milk could have significant therapeutic attributes such as anti-cancer and anti-diabetic properties (Agrawal *et al,* 2003; Magjeed, 2005), but search for milk based bioactive peptides has been focused until now mainly on bovine and to smaller extent on ovine and caprine milk proteins. Therefore, this study was undertaken to produce protein hydrolysates from camel milk using proteolytic enzymes and to investigate the antioxidant activity of the hydrolysates.

Materials and Methods

Chemicals and reagents

Enzyme alcalase (EC 3.4.21.62, activity ≥5 units/g protein) from Sigma–Aldrich Chemical Co., India and α-chymotrypsin (EC 3.4.21.1, activity 35 units/ mg protein) and papain (EC.3.4.22.2, activity ≥10 units/mg protein) (M P Biomedicals, India) were procured. Fine chemicals such as 2, 2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS),1,1-diphenyl-2-picrylhydrazyl (DPPH) obtain from Sigma–Aldrich Chemical Co. India and 2,4,6-tripyridyl-s-triazine (TPTZ) (MP Biomedicals,

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India). All solutions, prepared with double-distilled water, were kept at 4°C before further use.

Non-fat camel milk (NFCM) powder preparation

Fresh camel milk was collected from dromedary camels (*Camelus dromedarius*) maintained at Camel Dairy Farm, National Research Centre on Camel, Bikaner and immediately cooled to below 5°C for further use. The non-fat dry camel milk powder was prepared in Camel Milk Product Laboratory, NRC on Camel, Bikaner, Rajasthan. For production of non-fat dry camel milk powder, the whole milk was pre-heated to 35-40°C and subjected for skimming in the cream separator. After separation of fat to desired level (<0.5%), the skimmed milk was pasteurised in high-temperature short-time pasteuriser (72°C for 15 sec). The samples obtained were utilised for powder production by Spray dryer. The skimmed, pasteurised camel milk was dried using a mini spray dryer (Model: ADL311, Yamato, Japan) at an inlet temperature of 200-220°C and outlet temperature of 95-105°C for obtaining good quality milk powder. The powdered samples collected were packed in low-density polythene bags and stored for further use.

Enzymatic hydrolysis of NFCM powder

Camel milk powder was reconstituted (5% total solid) in phosphate buffer of different pH (8.0 for alcalase and α-chymotrypsin, and 6.5 for papain) for optimum enzymatic action. The reconstituted milk was heated in boiling water bath (Equitron, Model: 8414, Medica Instrument Mfg. Co., Mumbai, India) for 5 min. for complete solubility. The optimum pH and temperature for hydrolysis experiment was standardised by preliminary trials and by consulting manufacturer's instructions as well as available literature. The enzyme substrate ratio (E:S ratio) was kept constant (1:100) for all the proteases. The hydrolysis was carried out by incubating the samples in stirred water bath at 55±1°C for alcalase and papain, and 37±1 °C for α-chymotrypsin and samples were drawn at 0^{th}, 2^{nd}, 4^{th} and 6^{th} of incubation. Each hydrolysed samples were immediately heated to 85°C for 15 minutes in water bath to stop further enzymatic action. Then, the samples were cooled and centrifuged in a refrigerated centrifuge (Eltek, Model:MP 400R, Elektrocraft (India) at 10000 rpm for 25 min, supernatants were collected and stored at -20°C until further use.

pH measurement

The pH of hydrolysate samples was measured using combined glass electrode of Mettler Toledo pH meter (Model FiveEasy™ plus FEP 20, Switzerland).

Determination of degree of hydrolysis

The degree of hydrolysis (DH) of milk protein hydrolysates was determined by the percentage of solubilised protein in 10% (w/v) trichloroacetic acid (TCA), in relation to the total protein content of the sample according to Hoyle and Merritt (1994), with modifications. Aliquots of 500µL of the hydrolysed protein were mixed with 500µL of 20% (w/v) of TCA solution to obtain the soluble and insoluble fractions in 10% TCA. After 30 minutes of rest, the mixture was centrifuged (Cooling Microfuge Model CM 12, Remi Elektrotechnik Ltd, Vasai, India) at 3500 rpm for 15 minutes, and the soluble protein content of the supernatant was determined by the method of Lowry *et al* (1951), modified by Hartree (1972). Bovine serum albumin (BSA) was used as the standard. The total protein in the samples were estimate by Kjeldahl method. The DH was calculated according to the equation: DH (%) = [Solubilised protein content in 10% TCA (mg)/ Total protein content (mg)] x 100.

SDS-Polyacrylamide gel electrophoresis

SDS-PAGE was performed on a 4.0% (w/v) polyacrylamide in 0.125M Tris-HCl buffer, pH 6.8 stacking gel and a 16.5% (w/v) polyacrylamide in 0.38M Tris-HCl buffer, pH 8.8 containing 0.1% (w/v) SDS separation gel. Samples were mixed in equal proportion in sample buffer containing 2 mg/mL in 0.125M Tris-HCl buffer (pH 6.8), 0.1% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue. After heating the mixture at 100°C for 5 min, 20 µL of samples were loaded in the gel. The molecular mass standards used was of low range: 6500-66000 Da (M3913-1VL, Sigma™ chemical CO, Missouri, USA). After running the electrophoresis unit at 25mA, constant current for about 9-10h, the gel slabs were taken out carefully and proteins were fixed with 12% (w/v) trichloroacetic acid (TCA) for 30 min and then stained with 0.1% (w/v) R-250 Coomassie brilliant blue dissolved in a mixture of methanol: acetic acid: water (50:10:40, v/v/v), followed by destaining in a solution containing methanol, acetic acid and water in the ratio of 45:10:45.

Antioxidant activity

2-2 azinobis-3ethylbenthiazoline-6-sulphonic acid ($ABTS^+$)

The spectrophotometric analysis of ABTS+ radical scavenging activity was determined according to method described by Salami *et al* (2009). ABTS radical cation ($ABTS^+$) was produced by reacting $ABTS^+$ stock solution with equal volume of 2.45 mM

potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 h before use. Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.70 at t_0 (0 min) and equilibrated at 30°C exactly 6 min after initial mixing. About 1 ml of $ABTS^+$ working standard solution was mixed with 10µl of hydrolysate/standard and absorbance was measured after 20 min (t_{20}) at 734 nm in multimode reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, BioTek India, Mumbai). The $ABTS^+$ activity was calculated by using formula: ABTS activity (% inhibition) = $[(0.7\text{-}At_{20})/0.7] \times 100$.

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

The ability to scavenge 1,1diphenyl-2picrylhydrazyl (DPPH) radical by added antioxidants in samples was estimated following the method of Brand-Williams *et al* (1995) with slight modification. One ml of DPPH reagent (100µM) was mixed with 0.25 ml of 0.1M Tris-HCl buffer (pH 7.4) and 25µl of hydrolysate sample in test tubes. The content was gently mixed and the absorbancy in time t=0 min (t_0) was measured at 517 nm using multimode reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, Bio Tek India, Mumbai). The sample tubes were also incubated at room temperature under dark for measurement of absorbancy in time t=20 min (t_{20}). Ethanol was used as blank. The free radical scavenging activity was calculated as decrease in absorbance from the equation: Scavenging activity (% inhibition) = $100\text{-}[(At_{20}/At_0) \times 100]$.

Ferric reducing-antioxidant power (FRAP) assay

The FRAP was assessed according to Benzie and Strain (1999) using multimode reader. Briefly, 900µL of working FRAP reagent (300mM acetate buffer, pH 3.6: 20mM ferric chloride solution: 10mM TPTZ in 40mM HCl::10:1:1) prepared fresh was mixed with 100µL of hydrolysate sample; the absorbance at 593 nm was recorded using multimode reader (Synergy H1 Hybrid Multi-Mode Microplate Reader, Bio Tek India, Mumbai) after a 20 min. incubation at 37°C. FRAP values were obtained by comparing the absorption change in the test mixture with those obtained from increasing concentrations of Fe^{3+} and expressed as mmol of Fe^{2+} equivalents per mL of sample. Ferrous sulphate was used as standard for standard curve preparation.

Statistical analysis

Hydrolysis experiments were repeated 3 times and all the parameters were analysed in triplicate (n=9). Data were expressed as means with standard error. Two-way analysis of variance (ANOVA) was done by comparing the means by using Duncan's multiple range test (DMRT), at 95% confidence level using a SPSS package (SPSS 17.0 for Windows, SPSS Inc., USA).

Results and Discussion

Research efforts have been focused on the generation of bioactive peptides from camel milk proteins and subsequently the changes in pH, degree of hydrolysis, and different antioxidant assays were carried out and the data obtained were statistically analysed, presented in tables and figs, and are also discussed in detail in the following sections. In the current area of study, there are relatively few studies available in the literature, therefore, comparative inference was drawn on discussion with other milk and protein sources.

Change in pH during hydrolysis

Although, the enzymatic hydrolysis is affected by initial pH of the medium, it also depends upon several factors including the structure of the protein, temperature, enzyme: protein ratio, enzyme concentration, and change in pH of the buffer. Heat denaturation and pH adjustments are 2 common ways of making peptide bonds more susceptible to enzymatic action. Change in pH during hydrolysis may not only affect the enzyme structure, but also changes in structure or properties of the substrate takes place which in turn affect the enzyme-substrate binding and thereby hydrolysis. In the current study, phosphate buffers of specific pH for each enzymes were used to get optimum hydrolysis. However, a decrease in pH of the hydrolysates were observed with the advancement of hydrolysis time (Table 1). As compared to alcalase, the rate of pH decrease was higher for α-chymotrypsin and papain. This might be due to release of protons into the surrounding medium that results in reduction in the pH of the reaction mixture (Ovissipour *et al,* 2013). Daroit *et al* (2012) and Kumar *et al* (2016) also reported similar decrease in pH of ovine casein hydrolysates and camel casein hydrolysates, respectively with the progress in hydrolysis time.

Degree of hydrolysis (DH)

The progression in DH during the hydrolysis of camel milk proteins by different proteases are shown in table 1. The DH of food proteins is the measure of the soluble peptide released during hydrolysis and it affects the antioxidant activity of protein

hydrolysates. The DH increased significantly ($p<0.05$) with the increase in duration of hydrolysis, however, after 4h of hydrolysis, it increased slowly and after 6h of hydrolysis it became static. This might be due to decreased availability of cleavable peptide bonds within the substrate. Adler-Nissen (1986) attributed the reduction in hydrolysis rate due to the competition between unhydrolysed protein and the peptides being constantly formed during hydrolysis. The reduction of hydrolysis rate in latter hours might also be due to decrease in pH of the medium, which might cause denaturation of protein structure of the enzyme or the disturbances of the ionic character of the substrate, would in turn affect enzyme-substrate binding. In alcalase and papain treated proteins, the DH increased significantly ($p<0.05$) from 0 to 6h but in case of α-chymotrypsin, the DH increased significantly ($p<0.05$) upto 4h thereafter the increase was non-significant. The alcalase treated protein showed higher DH for first 2h as compared to other 2 enzymes. However, at 4 and 6h the DH values for α-Chymotrypsin treated protein had significantly ($p<0.05$) higher DH followed by those of alcalase and papain treated proteins. The highest levels of DH obtained with α-chymotrypsin suggested that this enzyme has more affinity for the substrate and thus more efficient than alcalase and papain for the production of protein hydrolysates of camel milk peroteins. Similar results were also reported by Graszkiewicz *et al* (2010) in egg-white protein precipitate hydrolysis in which chymotrypsin caused a higher DH in one-hour hydrolysates than trypsin and elastase. Lira *et al* (2010) also reported 28.17% DH of goat milk casein obtained with the use of papain. In the current study, the DH after 6 h of hydrolysis had not increased significantly (data not shown). This might be due to enzyme specificity which could not further hydrolyse the remaining bonds within the generated peptides. Carreira *et al* (2003) also recommended that the length of the hydrolytic reaction should not be more than 5h, because beyond this time can favour the microbial contamination of the protein preparations. Another important reason for keeping shorter hydrolysis time is that longer hydrolysis time often releases bitter-tasting mixture of peptide and amino acids, which may contribute bitter flavour to the hydrolysates, ultimately limits their application in food products (Belitz and Grosch, 1999). According to Tsou *et al* (2010), the limited hydrolysis was required to maintain the structure or sequence of active peptides and to ensure functionality.

Table 1. pH change and degree of hydrolysis of camel milk protein hydrolysates with different enzymes (Mean±SE).

Hydrolysis time (hr.)	Enzymes		
	Alcalase	α-Chymotrypsin	Papain
	pH		
0	7.98 ± 0.01^{Cb}	7.97 ± 0.01^{Cb}	6.49 ± 0.01^{Ba}
2	7.86 ± 0.02^{Bb}	7.80 ± 0.02^{Bb}	6.44 ± 0.01^{Ba}
4	7.81 ± 0.04^{ABb}	7.77 ± 0.02^{Bb}	6.41 ± 0.01^{Ba}
6	7.76 ± 0.02^{Ab}	7.69 ± 0.01^{Ab}	6.30 ± 0.03^{Aa}
	Degree of hydrolysis (%)		
0	0.98 ± 0.05^{A}	1.16 ± 0.16^{A}	1.01 ± 0.04^{A}
2	12.4 ± 0.19^{Bb}	10.91 ± 0.42^{Ba}	10.88 ± 0.64^{Ba}
4	18.74 ± 0.31^{Cb}	21.48 ± 0.24^{Cc}	12.99 ± 0.18^{Ca}
6	20.08 ± 0.28^{Db}	21.98 ± 0.30^{Cc}	16.49 ± 0.29^{Da}

Mean ± SE values bearing same superscripts row-wise (small alphabets) and column-wise (capital alphabets) do not differ significantly ($p<0.05$).

SDS-Polyacrylamide gel electrophoresis

The samples of reconstituted camel milk proteins, its different hydrolysate samples were compared with the standard molecular weight marker (Fig 1). In the hydrolystes samples (lane 3-10), the disappearance of bands of major proteins as in lane 2 and appearance of bands of lower molecular weight indicates hydrolysis of camel milk proteins to different extent which were also supported by the values observed for degree of hydrolysis. Similar findings were also reported by Saliha *et al* (2013) and Kumar *et al* (2016) in camel milk casein following enzymatic digestion with various proteases.

Antioxidant activity of camel milk protein hydrolysates

Antioxidant activities of camel milk protein hydrolysates were determined using ABTS, DPPH and FRAP assays.

The ABTS radical scavenging activity increased significantly ($p<0.05$) with the advancement hydrolysis time upto 6h for alcalase and papain treated samples (Figs 2 and 4) whereas, for α-chymotrypsin treated samples, it increased significantly ($p<0.05$) upto 4h thereafter, a non-significant increase was observed (Fig 3). As compared to other 2 enzymes, hydrolysates produced by α-chymotrypsin had significantly ($p<0.05$) higher antioxidant activity. However, among the 2 enzymes i.e, alcalase and papain, hydrolysates produced by alcalase showed significantly ($p<0.05$) higher ABTS activity. Similar findings were also reported by Salami *et al* (2011) and Kumar *et al* (2016) in camel milk casein hydrolysates. They

further reported that α-chymotrypsin could produce hydrolysates with higher antioxidant activity than trypsin and pepsin. Gomez-Ruiz *et al* (2008) also reported higher ABTS activity of ovine casein hydrolysates than intact casein.

The DPPH activity of camel milk protein hydrolysates increased significantly ($p<0.05$) with the progress in hydrolysis time (Figs 2, 3 and 4), and a positive relationship between hydrolysis time and DPPH activity could be established; however, the higher DPPH-scavenging activity was not exhibited after 6 h of hydrolysis (data not shown). Hydrolysates produced by all the 3 enzymes had significantly ($p<0.05$) increasing DPPH-scavenging activity upto 6h of hydrolysis time. As compared to other 2 enzymes, the α-chymotrypsin produced hydrolysates which had significantly ($p<0.05$) higher antioxidant activity at 2h of hydrolysis and it remained significantly higher upto 6th h of hydyolysis. However, after 2 hour, the protein hydrolysates produced by alcalase also showed significantly ($p<0.05$) higher DPPH-scavenging activity than hydrolysates produced by papain. From the observed pattern of DPPH-scavenging activity, it could be hypothesised that both hydrolysed and non-hydrolysed camel milk proteins contain some electron donating substances that could react with free radicals, making them more stable molecules and stopping the radical chain reaction. Mao *et al* (2011) also reported increase in DPPH-scavenging activity of yak milk protein hydrolysates obtained with alcalase with the progression of hydrolysis process for up to 7 h. The increase in DPPH radical scavenging activity of camel milk protein hydrolysates was also in agreement with Thiansilakul *et al* (2007) and Khantaphanta *et al* (2011) who reported the increases in DPPH radical scavenging activity upon increase in DH of the hydrolysate from round scad muscle protein prepared using flavourzyme and alcalase, and brownstripe red snapper muscle prepared using alcalase, respectively.

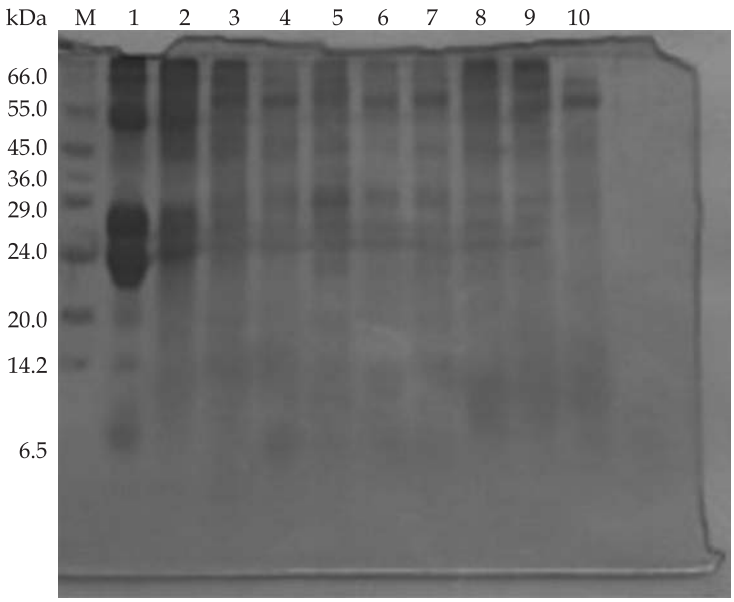

Fig 1. SDS-PAGE of camel milk proteins and its hydrolysates at different hydrolysis time (M: Molecular weight marker; 1: whole milk protein; 2-4: alcalase treated milk proteins at 2nd, 4th & 6th h; 5-7: α-chymotrypsin treated milk proteins at 2nd, 4th & 6th h; 8-10: papain treated milk proteins at 2nd, 4th & 6th h.).

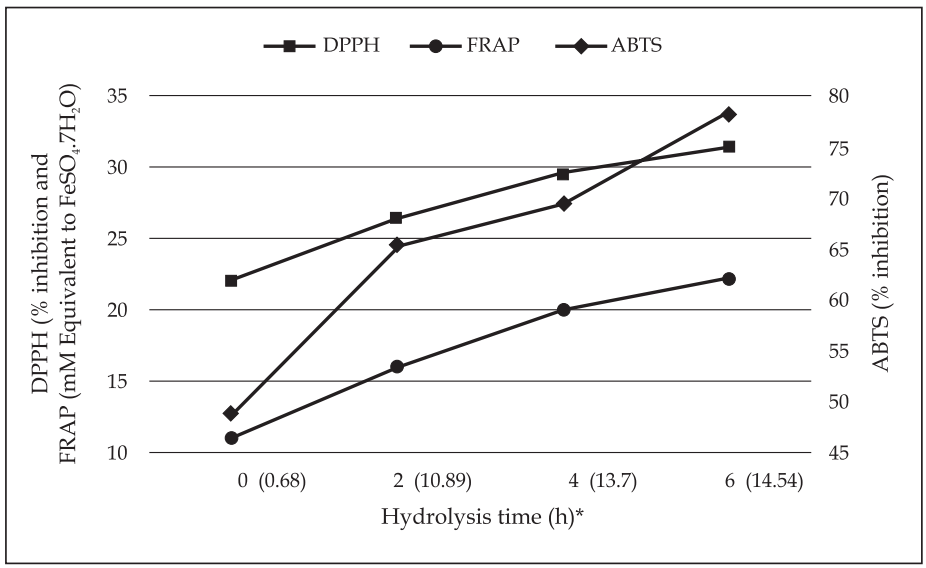

Fig 2. Antioxidant activities of camel milk protein hydrolysates with alcalase *: the value in parentheses indicates degree of hydrolysis at that time.

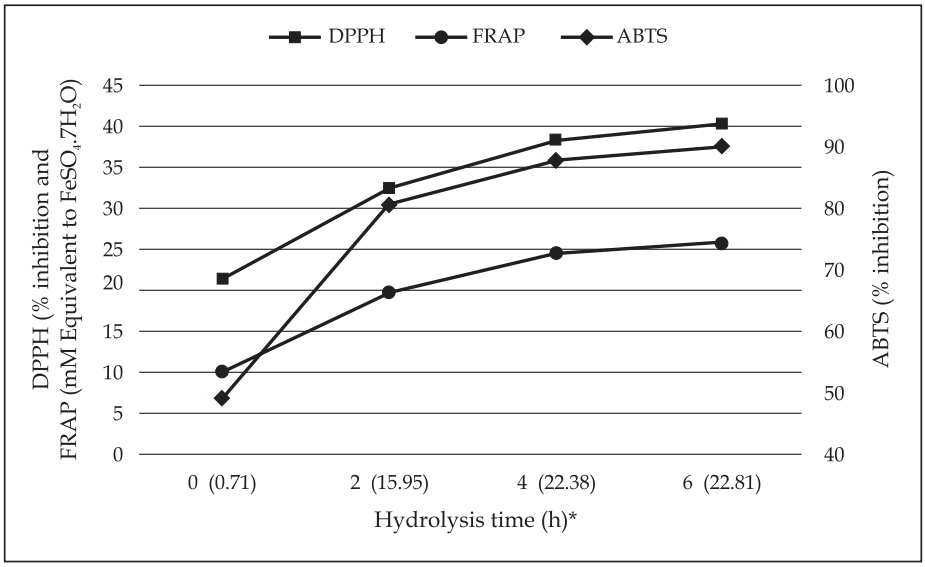

Fig 3. Antioxidant activities of camel milk protein hydrolysates with α-chymotrypsin *: the value in parentheses indicates degree of hydrolysis at that time.

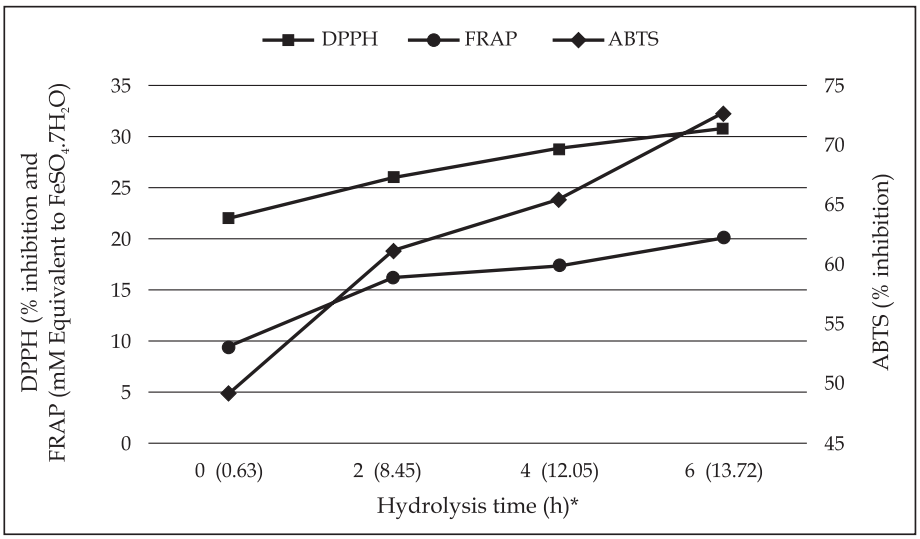

Fig 4. Antioxidant activities of camel milk casein hydrolysates with papain *: the value in parentheses indicates degree of hydrolysis at that time.

The Ferric Reducing Antioxidant Power (FRAP) method is based on the reduction of 2, 4, 6-tripyridyl-s-triazine (TPTZ) and ferric chloride complexes. The results of this experiment showed a positive relationship between hydrolysis time and FRAP value as these values increased significantly ($p<0.05$) with increase in hydrolysis time (Figs 2, 3 and 4). However, among all enzymes, a significant ($p<0.05$) higher activity was observed for hydrolysates with α-chymotrypsin. Previous works also suggested that smaller size peptides released by proteolytic enzymes exhibited better reducing power than high molecular weight fractions (Bougatef *et al,* 2009; Ajibola *et al,* 2011). Khantaphanta *et al* (2011) also reported the increase in FRAP activity of brownstripe red snapper muscle hydrolysate prepared using various proteases.

A positive correlation was also observed in degree of hydrolysis and antioxidant activity for all the enzymes. The increased antioxidant activity through hydrolysis suggested that this process contributed to antioxidant activity by releasing previously inactive peptides encrypted in the sequence of native proteins. Khantaphanta *et al* (2011) and Kumar *et al* (2016) also reported a positive relation between DH and antioxidant activity (DPPH, ABTS and FRAP assay) of the hydrolysate from brownstripe red snapper muscle and camel milk casein prepared using various proteases.

Conclusions: It was concluded that camel milk proteins could be hydrolysed with proteases such as alcalase, α-chymotrypsin and papain to increase its biological activity. The duration of hydrolysis varied with enzyme and 6h hydrolysis time was found optimum for alcalase and papain whereas, 4h for α-chymotrypsin to achieve maximum DH as well as antioxidant activities (ABTS, DPPH and FRAP assay). Among different enzymes, α-chymotrypsin produced hydrolysates with significantly higher DH and antioxidant activities. Results suggested that camel milk proteins could be used as natural source of protein to produce hydrolysates with antioxidant activities. It also encouraged the use of camel milk proteins and derived peptides as antioxidant agents for human consumption and as ingredient in nutraceutical and pharmaceuticals, as well as in different health oriented food products to enhance its functionalities and shelf life.

Acknowledgement

The authors sincerely acknowledge Dr. N. V. Patil, Director, National Research Centre on Camel, Bikaner, Rajasthan, India providing facilities for camel milk collection and sample preparation (spray drying) for this research work.

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DOI : 10.5958/2277-8934.2016.00006.0 Vol 23 No 1, p 41-46

PARTIAL CLONING OF c-MET cDNA AND ITS TISSUE DISTRIBUTION IN ARABIAN CAMEL (*Camelus dromedarius*)

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ABSTRACT

The present study was, aimed to clone Hepatic growth factors receptor (c-Met). Arabian camels RT-PCR was conducted using liver tissue RNA and primers designed based on data from different animal species published in gene bank. The PCR product was sequenced and analysed using different bioinformatics programs. The results confirmed that the obtained sequence is related to c-Met gene family. The nucleotides sequence was deposited in the DDJB with accession number KC794957. Furthermore, the data showed base frequencies of A = 27.5%, C = 23.9%, G = 20.6% and T = 28.00%. Of the 600 nucleotides used for tree analyses, 454 were constant and 146 were variables. The neighbour-joining tree showed clustering of the species of family Camelidae with each other with strong bootstrapping (100 BP for MP and NJ methods). The deduced amino acids showed two non-synonymous substitutions discriminating *C. dormedarius* from other camelids; aspartic acid (D94) into histidine (H94) in other camelids at G281→C281 and glutamine (Q144) into histidine (H144) at A430 →C430. The results exhibited ubiquitous expression of c-Met mRNA in the tested tissues; kidney, liver, skeletal muscle, spleen, testis and heart. The obtained results are expected to be important for addressing the genetic diversity of the Afro-Arabian camel and clarifying the relationships among its available breeds.

Key words: Arabian camel, cloning, c-Met, distribution

Growth Factors are protein molecules made by the body or can also be produced by genetic engineering in the laboratory and used in biological therapy. One of the most important growth factors is HGF and it's receptor c.Met. HGF is a heat-labile protein that was originally discovered as a mitogen of adult rat hepatocytes (Nakamura *et al,* 1987; Nakamura and Mizuno 2010). HGF is a multifunctional cytokine derived from stroma. It induces cell proliferation, differentiation, and motility in a variety of epithelial cells by binding to the product of the *c-Met* proto-oncogene (Trusolino and Comoglio, 2002; Birchmeier *et al,* 2003; Yamaji *et al,* 2006). Also HGF and c-Met have been involved in the embryonic and postnatal development of a variety of tissues including those of the mammary gland (Trusolino and Comoglio 2002; Birchmeier *et al,* 2003; Yamaji *et al,* 2006).

c-Met, a proto-oncogenic gene product, is receptor for HGF. While HGF was discovered in the sera of 70%-hepatectomised rats (Nakamura *et al,* 1984; Nakamura and Mizuno, 2010), c-Met was identified as a new member of the tyrosine kinase family, possibly as a new receptor of unknown growth factors. Researchers found that c-Met is a receptor for HGF (Bottaro *et al,* 1991; Nakamura and Mizuno 2010) and its is composed of a 50 kD α-chain and a 145 kD β-chain. (Bottaro *et al,* 1991; Nakamura and Mizuno, 2010). Binding of HGF to c-Met induces phosphorylation of C-terminally clustered tyrosine residues of tyrosine kinase. This results in biological activities in a wide variety of cells, including mitogenic, motogenic and morphogenic activities. A study have proven that HGF has essential regenerative roles for liver and other organs like lung, kidney and that it is produced by liver , kidney, spleen and exerts its action as an autocrine, paracrine and endocrine (Nakamura and Mizuno, 2010).

The local HGF-c-Met systems are involved not only in tissue repair but also metabolic homeostasis.

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HGF stimulates mitogenesis and insulin production in pancreatic β-cells (Nakamura And Mizuno, 2010).

The present study was aimed to clone hepatic growth factors receptor (*c.Met*) that plays an important role in cell growth and differentiation, in addition to study their tissue distribution in Arabian camels.

Materials and Methods

Sampling

Different tissue samples of liver, kidney, spleen, heart, skeletal muscle, testis and mammary gland were collected from the local slaughterhouse (Taif, KSA). These were exposed to sudden freezing in liquid Nitrogen, transferred to laboratory, and kept at - 80°C until used.

Primer Design

Two sets of degenerate primers were designed. The first (c-Met-1F and c-Met-1R) was from the highly conserved regions of *c-Met* gene available in the gene bank for different species including *C. bactrianus* XM_010947627, *C. ferus* XM_006177483, *Lama glama* KF042853), Equidae (*Equus caballus* NM_001114147) and Bovidae (*Bos taurus* BC146202 and *Capra hircus* XM_005679158). The second one (c-Met-2F and c-Met-2R) was internal primer and was designed according to the obtained sequence and used for studying tissue distribution. Another set of primer (GAPDH-F and GAPDH-R) was for GAPDH and designed from the sequence of *C. dromedarius* GAPDH XM_010975572 (Table 1).

RNA Extraction, cDNA Synthesis and Reverse Transcription PCR

Total RNA was extracted according to the method described by Ahmed *et al* (2015) using Qiazol lysis reagent following to the manufacturer's instructions. Briefly, 100 mg of each tissue sample was homogenized in 1ml QIAzol (QIAGEN Inc., Valencia, CA) then 0.3 ml chloroform was added to the homogenate. After that, the mixtures were shaken for 30 s followed by centrifugation at 4°C and 12,500 rpm for 20 min. The supernatant layer were transferred into a new set of tubes, and an equal volumes of isopropanol were added to the samples, shaken for 15 seconds and centrifuged at 4°C and 12500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, briefly dried up then, were dissolved in Diethylpyrocarbonate (DEPC) water. The prepared RNA integrity was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm.

For synthesis of cDNA, mixture of 2 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µl sterilized DEPC- water was incubated in the PeX 0.5 thermal Cycler (Thermo Electronic Corporation, Milford, Ma) at 70°C for 10 min for denaturing. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U RevetAid Premium reverse transcriptase (Fermentas Canada Inc. Harrington Court, Burlington Ontario) will be added and the total volume was completed up to 20 µl by DEPC water. The mixture was then will be re-incubated in the thermal Cycler at 30°C for 10 min, at 42°C for 1 h and at 90°C for 10 min then, will be preserved at -20°C until.

Polymerase Chain Reaction ((PCR)

For amplification of c-Met cDNA, polymerase chain reaction (PCR) and specific primers c-Met -1 for each genes (Table 1) were used. PCR was conducted in a final volume of 50 µl consisting of 1µl DNA, 1µl (10 picomolars) of each primer and 25 µl PCR master mix (Promega Corporation, Madison, WI, USA) the volume was brought up to 50 µl using sterilised deionized water. PCR was carried out using a PeX 0.5 thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the cycle sequence at 94°C for 5 min one cycle, followed by 25 cycles each of which consisted of denaturation at 94°C for 1 min, annealing at the specific temperature corresponding to each primer set (Table 1) and extension at 72 °C for 1min with an additional final extension at 72°C for 5 min. PCR products were electrophorized on 1.5% agarose (Bio Basic, Konrad Cres, Markham, ON, Canada), gel stained with ethidium bromide in TAE (Tris–acetate–

Table 1. Sequences, annealing temperatures and expected PCR products size of the used primers.

Primer Name	primer sequence 5´-3´	Annealing temp.	Expected size product
c-Met-1F	ARTGTGGCTGGTGCCA	51 °C	650 bp
c-Met-1R	GAGATCATYACTGGCTTTTC		
c-Met-2F	TCCTTCCACCTGACAATACT	53°C	420 bp
c-Met-2R	TTGTGTGAAAAGTCTGAGCA		
GAPDH-F	TGGGAAGCTAACTGGCATG	53°C	550 bp
GAPDH-R	AGGCAGGGCTCCCTAAGC		

EDTA) buffer (Sigma–Aldrich, St. Louis, MO, USA). PCR products were visualized under UV light and photographed using gel documentation system (UVP, Upland, CA, USA). Following that, PCR product were purified using FavorPrep PCR Clean-Up Mini Kit according to the manufacturer's instructions.

Sequence analysis of PCR product

Purified PCR products for c-Met were sequenced in an ABI PRISM 3730xl sequencer (Applied BioSystems) and BigDyeTM Terminator Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems) following the protocols supplied by the manufacturer. After reading the targeted genes, the nucleotide sequences have been treated with different software programs (DNASIS, MacClade, PAUP). Amino acid sequence was obtained by translating the sequenced DNA fragment using the DNAsis program and the deduced amino acid sequence was compared with sequences obtained from searches in the NCBI protein database using the BLASTP algorithm (ref).

Multiple Sequence Alignment and Phylogenetic Analysis

The phylogenetic analyses were conducted by using maximum-parsimony and neighbour-joining methods with PAUP* 4.0b10 (Swofford, 2002) by heuristic searches with the TBR branch swapping and 10 random taxon additions. Bootstrapping replicates were set to 5000 for both methods and the neighbour-joining method was adjusted by distance option of Tamura-Nei.

c-Met expression tissues distribution

The obtained sequence was used to design an internal primer (Table 1) for studding the tissue distribution of c-Met mRNA expression. RNA from kidney, spleen, muscle, testis and heart were reverse transcripted and the internal primers were used to perform PCR. The products were electrophoresed and photographed.

Secondary and 3D Structure Prediction of c-Met

1. The deduced amino acid sequence of c-Met was used to predict its secondary and 3D structure. The secondary structure was predicted using PSIPRED program while the 3D was predicted using Swiss-model server using homology structure modeling (Xu and Zhang, 2012).

Results and Discussion

In the Arabian Desert, the single-humped camel (*Camelus dromedarius*) is considered as one of the most important animals. Although the genome of double-hump camel has been identified, little is known about that of single-hump camel. Many attempts have been carried to identify some genes especially in the Arabic regions including Putative Stress-Induced Heat-Shock Protein (Elrobh *et al,* 2011), Putative Copper-Zinc SOD (Ataya *et al,* 2012) or even mitochondrial genes (Ahmed *et al,* 2013) and Kappa casein (Minoia *et al,* 198).

The analysis of camel genome might be helpful to give an insight on the mechanism of adaptation to climate change and disease resistance. For this reason an attempt was carried out to clone Hepatic Growth Factor receptor (c-Met) cDNA from a single hump camel liver tissue and also studying the expression in different tissues.

HGF is a pleiotropic cytokine with a receptor known as c-Met. They play an important role in the both embryonic and postnatal organ development (Yamaji *et al,* 2006). The c-Met had been identified as a new member of the tyrosine kinase family, possibly as a new receptor of unknown growth factor(s). Bottaro *et al* (1991) found that c-Met is a receptor for HGF (Bottaro *et al,* 1991; Nakamura and Mizuno, 2010). c-Met is composed of a 50 kD α-chain and a 145 kD β-chain. (Bottaro *et al,* 1991; Nakamura and Mizuno 2010). The α-chain is exposed extracellularly, while the β-chain is a transmembrane subunit containing an intracellular tyrosine kinase domain. Binding of HGF to c-Met induces phosphorylation of C-terminally clustered tyrosine residues of tyrosine kinase. This results in biological activities in a wide variety of cells including mitogenic, motogenic and morphogenic activities.

c-Met gene of Arabian camel was partially cell free cloned using PCR primers c-MET –1F and c-Met-1R with a product of 800 bp was obtained (Fig.1). This PCR product was sequenced and a clear 600 bp peaks were subjected to data analysis.

For data analysis the sequence nucleotides, six hundred nucleotides from c-Met gene of the Arabian *C. dromedarius* were aligned with their counterparts found in the Genbank database for the previously mentioned species. The aligned data were used for phylogenetic analyses and the same sequences for *Homo sapiens* NM_001127500 and *Mus masculus* (NM_008591) were used for tree rooting.

The data showed base frequencies of A = 27.5%, C = 23.9%, G = 20.6% and T = 28.00%. Of the 600 nucleotides used for tree analyses, 454 were constant and 146 were variables. About 85 of the variable

Table 2. The non-synonymous substitutions in the sequenced fragment of C-MET gene for the studied taxa. The letters refer to the corresponding amino acid and numbers refer to their exact positions. A= alanine, D = aspartic acid, E= glutamic acid, G= glycine, H= histidine, M= methionine, N= asparagine, Q= glutamine, R= arginine, S= serine, T = threonine, Y= tyrosine. Dashes refer to one amino acid deletion in rodents at the corresponding position.

Animal group	Species	Amino acids								
		94	**99**	**109**	**144**	**149**	**159**	**166**	**243**	**244**
Camelidae	*C. dromedarius*	D	E	D	Q	D	Y	T	Q	T
	C. dromedarius	H	E	D	H	D	Y	T	Q	T
	C. bactrianus	H	E	D	H	D	Y	T	Q	T
	C. ferus	H	E	D	H	D	Y	T	Q	T
	Lama glama	H	E	D	H	D	Y	T	Q	T
Bovidae	*Bos taurus*	D	Q	G	H	N	H	A	R	S
	Capra hircus	D	Q	G	H	N	H	E	Q	S
Equidae	*Equus caballus*	D	R	G	H	N	Y	Q	R	S
Human	*Homo sapiens*	D	Q	G	H	N	H	M	S	S
Rodents	*Mus musculus*	D	Q	G	H	D	H	--	Q	S

Table 3. Pairwise genetic distances among the different species of family Chamelidae as calculated from the sequenced C-Met gene fragment in this study.

	***C. dromedarius* (this study)**	***C. dromedarius* (Genbank)**	***C. bactrianus***	***C. ferus***
C. dromedarius (this study)	–			
C. dromedarius (Genbank)	0.0034	–		
C. bactrianus	0.0034	0.000	–	
C. ferus	0.0034	0.000	0.000	–
Lama glama	0.0067	0.0034	0.0034	0.0034

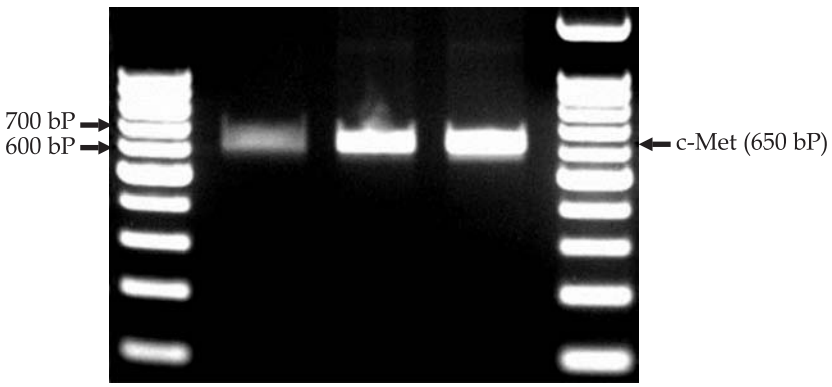

Fig 1. PCR product for c-Met using c-Met-1F and c-Met-1R designed according to the sequence published in Gene bank for Camelidae (*C. dromedarius* XM_010975572, *C. bactrianus* XM_010947627, *C. ferus* XM_006177483, *Lama glama* KF042853), Equidae (*Equus caballus* NM_001114147) and Bovidae (*Bos taurus* BC146202 and *Capra hircus* XM_005679158).

sites were parsimony uninformative and 61 were informative under parsimony criterion. The consensus parsimony tree constructed showed consistency index (CI = 0.87), homology index (HI = 0.25), retention index (RI = 0.801) and rescaled consistency index (RC = 0.67).

The neighbour-joining tree showed clustering of the species of family Camelidae with each other with a strong bootstrapping (100 BP for MP and NJ methods). The pairwise genetic distances among the studied camelid species are listed in Table 1. In concordance to the tree topology, the genetic distances showed the smallest values among all species except the present *C. dromedarius* which exhibited distance values of 0.0034 with other Camelus species and a distance value of 0.0067 with *Lama glama*. The distances among other camelid species are either zero or 0.0034.

Table 2 showed 9 positions in which two non-synonymous substitutions were found to differentiate camelids from other mammals. Among these substitutions, the translated amino acids showed two non-synonymous substitutions in positions 94 and 144 (Table 2) discriminating the present *C. dormedarius* from other camelids. These substations included changes of aspartic acid (D_{94}) in the current species into histidine (H_{94}) in other camelids at $G_{281} \rightarrow C_{281}$

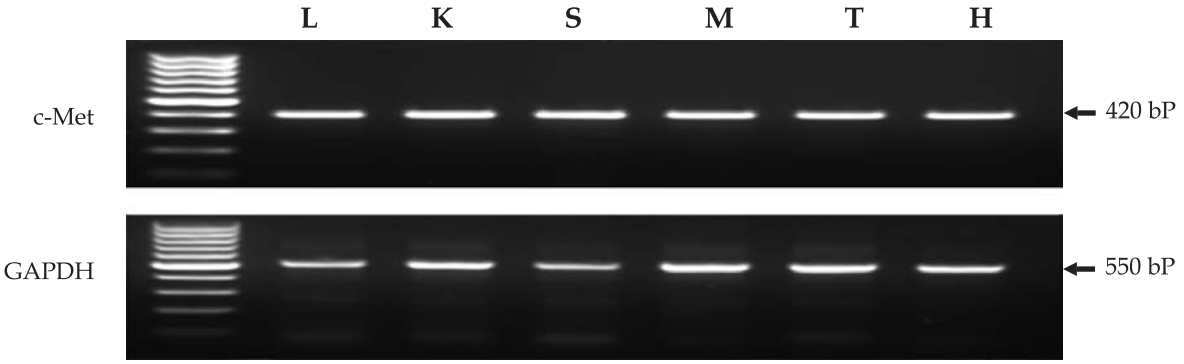

Fig 2. PCR showing tissue distribution of c-Met expression in different camel tissues, L means liver, K means kidne, H mean Heart, M means Muscle, T means testis and S means spleen. PCR carried out using c-Met-2F and c-Met-2R primers.

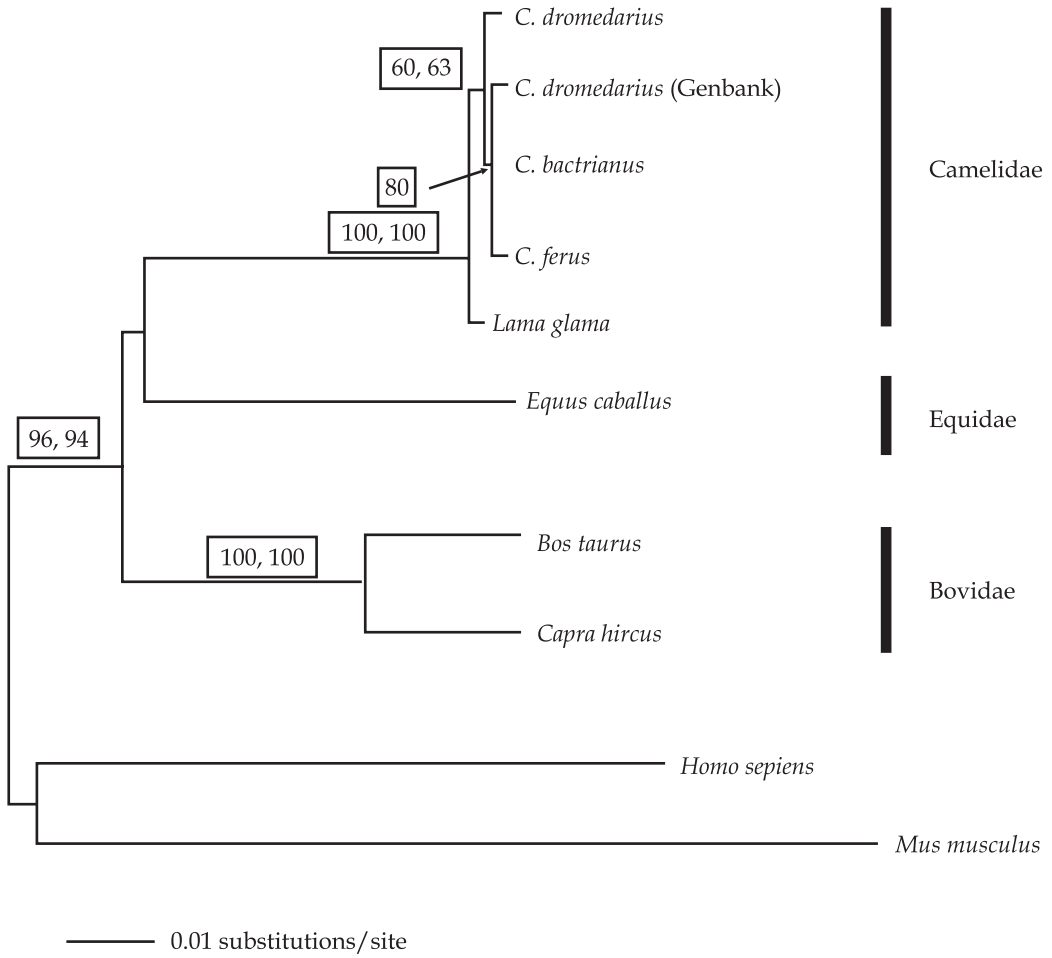

Fig 3. A neighbour-joining tree constructed from 600 bp sequenced fragments of MET proto-oncogene, receptor tyrosine kinase gene for the collected camelid and other mammalian taxa. Values at nodes refer to the bootstrapping of maximum-parsimony and neighbour-joining methods which were shown when they were over 50%.

and glutamine (Q_{144}) into histidine (H_{144}) at $A_{430} \rightarrow C_{430}$. The numbers below the letters referred to the corresponding positions of either the amino acid or the nucleotide inside the complete gene sequence.

To study the tissue distribution of c-Met mRNA expression, internal primers were used. The obtained results showed that c-Met is expressed in a variety of tissue including liver, kidney, spleen, heat, muscle and testis.

In the present stud we succeeded to clone a partial c-Met cDNA from dromedarius camel Live tissue and stud the expression in some different tissues. Further studies is needed to clone c-Met full length gen from dromedarius which excepted

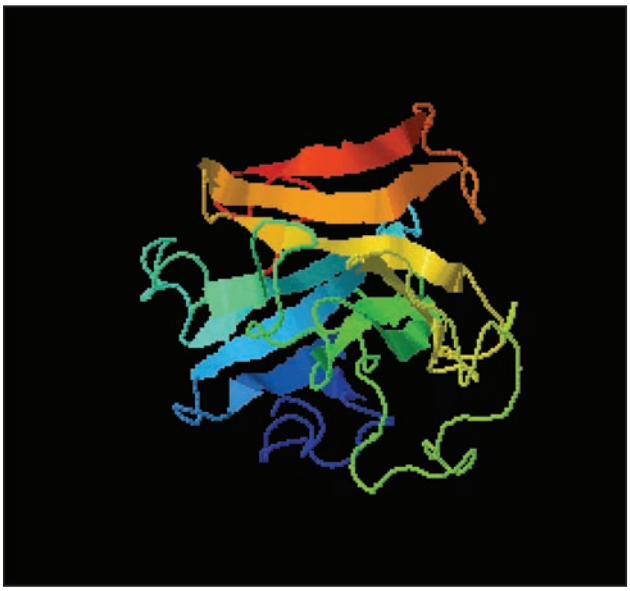

Fig 4. Predicted three dimensional structure of single hump camel c-Met protein depending on amino acids.

to be more informative for addressing the genetic diversity of the Afro-Arabian camel and clarifying the relationships among its available breeds as well as other species.

Acknowledgement

This work was supported by research Project grant (No. 1/435/3280) of Taif University, Kingdom of Saudi Arabia.

Conflict of interests

The authors declare that they have no conflict of interests.

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DOI : 10.5958/2277-8934.2016.00007.2 Vol 23 No 1, p 47-51

POLYMORPHISMS OF THE TYROSINASE (TYR) GENE IN BACTRIAN CAMEL (*Camelus bactrianus*) WITH DIFFERENT COAT COLOUR

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ABSTRACT

The aim of this study was to determine if any correlation exists between tyrosinase polymorphisms and different coat colour in bactrian camels. The coding region of the bactrian camel tyrosinase gene was sequenced. It was determined to encoded a protein that is 530 amino acids long. Six and three single nucleotide polymorphisms were identified within exon 1 and exon 5 of TYR gene, respectively. In exon 1, two were silent mutations and four were single nucleotide polymorphisms (SNPs) that alter the amino acid sequence (P38L, H211P, W238R, V258A); In exon 5, one was silent mutations and two were single nucleotide polymorphisms (SNPs) that alter the amino acid sequence (Q473R, K505E). No mutation correlated completely with coat colour in bactrian camels at the TYR genotypes. Further studies with larger numbers of animals were required to investigate or verify these association.

Key words: Bactrian camel, coat colour, polymorphism, SNP, *tyrosinase* gene

China is one of the main distribution area of bactrian camel in the world and is estimated at 0.29 million heads in 2014 year. Coat colour is an important form of camouflage and can be an integral part of social communication and recognition (Sponenberg, 1997). The mammalian's coat colour depends basically on the amount of two pigments, eumelanin (black or brown pigment) and pheomelanin (red or yellow pigment) (Klungland *et al,* 2000). The tyrosinase (TYR) is the rate-limiting enzyme in the metabolic pathway leading to coat colour pigmentation and hormone production (Shah *et al,* 2005). TYR is mainly involved in two reaction processes; on the one hand, TYR converts tyrosine to dopaquinone (DQ) and on the other hand, DQ undergoes a complex series of redox reactions leading to the production of melanin (Ito *et al,* 2000; Ito, 2006; Ray *et al,* 2007). Functional mutation of the tyrosinase gene are responsible for the albino phenotype and causing variation in coat colours have been described in domestic animals, such as rabbit (Aigner *et al,* 2000), cat (Schmidt-kuntzel *et al,* 2005), cattle (Sheila *et al,* 2003; Guibert *et al,* 2004), sheep (Hao, 2014), mink (Anistoroaei *et al,* 2008), *Mustela vison* (Siyuan, 2014), alpaca (Rhys and Kylie, 2011) and dromedary camel (Ishag *et al,* 2013). In contrast to other domesticated animals, the genetic variation of TYR gene and association with coat colour has not been investigated in bactrian camel. In per sent study, the TYR gene was characterised in bactrian camels and identied alleles were at the TYR locus in our population. These genotypes identified were compared to the phenotypes of each individual.

Materials and Methods

Animals and DNA extraction: Blood samples were collected from 77 bactrian camels of the three breeds: Gobi red camel, Alashan bactrian camel and Qing-hai bactrian camel from animals bred in the Bayannuur, Alashan and Qinghai regions China, (Table 1). Fibre colour was determined according to the owner's assessment of the animal. Genomic DNA was extracted from 200µl of EDTA anticoagulated blood using the DNeasy tissue kit (Qiagen) according to the manufacturer's instructions. The quality and quantity of genomic DNA were determined with nano-drop spectrophotometer. Initial sequence analysis was carried out on 9 animals (three black, three white and three brown). An additional 68 animals were subsequently analysed, but only for exon1 and exon5 mutations.

Amplification and sequencing of bactrian camel TYR gene: Polymerase chain reaction (PCR) primers

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Table 1. Colour phenotypes of bactrian camel used in the current study.

Fibre colour	Number of animals
White	17
Red	10
Brown	50
Total	77

were designed to amplify the five coding exons of the bactrian camel TYR gene (Table 2). These primers were based on the bactrian camel sequence assembly available on the NCBI database (http://www.ncbi.nlm.nih.gov/). All PCRs were carried out in an Eppendorf Mastercycler (Eppendorf, North Ryde, New South Wales, Australia), in 50µl reactions containing 5µl 10×Taq Buffer (100mM Tris-HCl, pH 8.8, at 25; 500 mM KCl, 0.8%(v/v) Nonidet), 1µl dNTP (10mM) (Sangon Biotech.Shanghai, China), 25mM $MgCl_2$ (Sangon Biotech.Shanghai, China) 5µl, 5 unit TaqDNA polymerase 0.5µl, 1µl each of forward and reverse primer and 1µl genomic DNA. Thermal cycles: initial denaturation at 95°C for 3 min, followed by 35 cycles, each consisting of 94°C for 30 s, 55-60°C for 35s and 72°C for 40-50s; with the final extension at 72°C for 5-8min. 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). TYR primers for each exon (Table 2) with Big Dye Terminator Technology v3.1 (Applied Biosystems, Mulgrave, Victoria, Australia) and analysed on a 3730 DNA analyser (Applied Biosystems, USA).

Genotyping TYR mutations: Once mutations were identified in bactrian camel TYR (Table 3) an additional sample group was genotyped for the exon 1 and exon 5 polymorphisms. The PCRs were carried out as above on an additional 68 animals (Table 1). Amplified DNA was electrophoresed in 1% (w/v) agarose gels in TAE buffer, stained with ethidium bromide and visualised by UV transillumination. PCR products were then sequenced using Big Dye v3.1 on a 3730 DNA analyser (Applied Biosystems, USA), using primers Ex1F, Ex1R and Ex5F, Ex5R.

Table 2. Primer pairs designed for amplification of TYR exons from genomic DNA.

Primer	Sequence (5′-3′)	Product size (bp)
Ex1F	GTGGGATTCATGCCAACTC	1260
Ex1R	TTAGTGAAGGAGGGTAGACAAATA	
Ex2F	GGTCATCAGGAATGCCCA	404
Ex2R	CCCAGGGTTTTGGATAAGAG	
Ex3F	CAGGCTTTCAATTGTAGTCGTA	346
Ex3R	TTTTTTACGAACCAGTTGGC	
Ex4F	CTGTCCAGGGCTTGAGATTT	422
Ex4R	GGATTGTAGAGCACTTTCCTAACTT	
Ex5F	TAAAGCATCCCAATAAGGTGA	686
Ex5R	TGGGCTTGAGGGAAACTG	

Bioinformatics analyses: Genotype and allele frequencies were counted directly and the sequencing alignment was obtained using the DNAStar v5.2.2 programme. A bactrian camel TYR protein sequence was predicted from the five exon sequences, using SpliceView program coupled with the known cattle coding Sequence.

Results

The bactrian camel TYR gene

The complete coding region of bactrian camel TYR encoded a protein of 530 amino acids with a predicted molecular mass of 60,567 Da (pI 8.97). The instability index (II) was 54.49 and this classifies the protein as unstable.

Amino acid alignment showed that there was high identity between the bactrian camel TYR sequence and that of dromedary (100%), alpaca (99.6%), pig (99.1%), cattle (99.1%), Hanwoo cattle (99.1%), River buffalo (99.1%), goat (98.9%), sheep (98.9%), dog (98.5%), human (98.1%), mouse (97.8%), rabbit (97.6%) and horse (96.3%) (Fig 1).

Mutations in bactrian camel TYR

Sequencing of the TYR coding region in 77 bactrian camels revealed six polymorphisms in

Table 3. Polymorphisms identified in the bactrian camel TYR gene.

Polymorphism	Location	Amino acid change	Effect on protein due to amino acid change
c.C113T	Exon 1	P38L	Nonpolar to nonpolar
c.A632C	Exon 1	H211P	Polar to nonpolar
c.T712C	Exon 1	W238R	Nonpolar to polar
c.T773C	Exon 1	V258A	Nonpolar to nonpolar
c.G309T	Exon 1	synonymous	N/A
c.T432C	Exon 1	synonymous	N/A
c.A1419G	Exon 5	Q473R	Polar to polar
c.A1514G	Exon 5	K505E	Polar to polar
c.A1507T	Exon 5	synonymous	N/A

exon 1 and two polymorphisms in exon 5. Four nonsynonymous single nucleotide polymorphisms (SNPs) were identified in exon 1: c.C113T, predicted to cause a proline-to-leucine substitution at codon 38 (P38L); c.A632C, predicted to cause a histidine-to-proline substitution at codon 211 (H211P); c.T712C, predicted to cause a Tryptophan-to-arginine substitution at codon 238 (W238R) and c.T773C, predicted to cause a valine-to-alanine substitution at codon 258 (V258A); two synonymous mutations: c.G309T (p.C103) and c.T432C (p.T144). In addition, two nonsynonymous single nucleotide polymorphisms (SNPs) were identified in exon 5: c.A1419G, predicted to cause a glutamine-to-arginine substitution at codon 473 (Q473R) and c.A1514G, predicted to cause a lysine-to-glutamic substitution at codon 505 (K505E); one synonymous mutations: c.A1507T (p.R502) (Table 3).

Each of the mutations resulting in a change in the amino acid sequence could possibly be causative in coat colour differentiation. We compared each individual genotype with the phenotype it induced in our population of camels to observe if there was a correlation between any one mutation and coat colour.

The exon 1 in TYR gene of camel, 14 animals were heterozygous AC at H211P; no proline homozygotes were detected in our population; 63 animals were homozygous for the histidine allele. Three animals, both red, one white were homozygous for the leucine allele at P38L; the heterozygous genotype was present in 6 animals; the remaining 68 animals were homozygous for proline with varying three phenotypes. Likewise, three animals, both red, one white were homozygous for the alanine allele at V258A; the heterozygous genotype was present in 9 animals; the remaining 65 animals were homozygous for valine with varing three phenotypes. At W238R, six animals, three white, three brown were homozygous for the arginine allele; 32 animals was present heterozygous genotype and remaining 39 animals were homozygous for tryptophan. In exon 5, four animals, both red, both white were homozygous for the arginine allele at Q473R; the heterozygous genotype was present in 11 animals; the remaining 62 animals were homozygous for glutamine with varying three phenotypes. Both brown animals were homozygous for the glutamic allele at K505E; no red and white animals in this genotype; 6 animals were heterozygous genotype; the remaining 69 animals were homozygous for lysine with varying three phenotypes (Table 4).

Discussion

In mammals, coat colour is relative to synthesis and distribution of melanin. Melanins are produced

	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1		100.0	99.6	99.3	99.1	99.1	99.1	98.9	98.9	98.5	98.1	97.8	97.6	96.3	**1**	Bactrian camel
2	0.0		99.6	99.3	99.1	99.1	99.1	98.9	98.9	98.5	98.1	97.8	97.6	96.3	**2**	Dromedary
3	0.4	0.4		98.9	98.7	98.7	98.7	98.5	98.5	98.5	97.8	97.4	97.2	95.9	**3**	Alpaca
4	0.7	0.7	1.1		98.7	98.7	99.1	98.9	98.9	98.5	98.1	97.8	97.6	96.3	**4**	Pig
5	0.9	0.9	1.3	1.3		100.0	99.6	99.4	99.4	98.0	97.6	97.2	97.4	95.7	**5**	Cattle
6	0.9	0.9	1.3	1.3	0.0		99.6	99.4	99.4	98.0	97.6	97.2	97.4	95.7	**6**	Hanwoo cattle
7	0.9	0.9	1.3	0.9	0.4	0.4		99.8	99.8	98.3	98.0	97.6	97.8	96.1	**7**	River buffalo
8	1.1	1.1	1.5	1.1	0.6	0.6	0.2		100.0	98.1	97.8	97.4	97.6	95.9	**8**	Goat
9	1.1	1.1	1.5	1.1	0.6	0.6	0.2	0.0		98.1	97.8	97.4	97.6	95.9	**9**	Sheep
10	1.5	1.5	1.5	1.5	2.1	2.1	1.7	1.9	1.9		97.4	97.0	96.9	95.6	**10**	Dog
11	1.9	1.9	2.3	1.9	2.5	2.5	2.1	2.3	2.3	2.6		97.8	97.6	95.9	**11**	Human
12	2.3	2.3	2.6	2.3	2.8	2.8	2.4	2.6	2.6	3.0	2.3		96.9	96.7	**12**	Mouse
13	2.4	2.4	2.8	2.4	2.6	2.6	2.3	2.4	2.4	3.2	2.5	3.2		95.2	**13**	Rabbit
14	3.8	3.8	4.2	3.8	4.4	4.4	4.0	4.2	4.2	4.6	4.2	3.4	5.0		**14**	Horse
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**		

Divergence

Fig 1. Amino acid sequence alignments of fourteen TYR. 1. Bactrian camel (*Camelus ferus*, XM_006192400) 2. Dromedary (*Camelus dromedarius*, XM_010979466) 3. Alpaca (*Vicugna pacos*, XM_006218369) 4. Pig (*Sus scrofa*, NP_001025212) 5. Cattle (*Bos taurus*, AF445639) 6. Hanwoo cattle (*Bos taurus* Hanwoo, JQ513975) 7. River buffalo (*Bubalus bubalis*, JN887462) 8. Goat (*Capra hircus*, NM_001287562) 9. Sheep (*Ovis aries*, NM_001130027) 10. Dog (*Canis lupus*, NM_001002941) 11. Human (*Homo sapiens*, NM_000372) 12. Mouse (*Mus musculus*, NM_011661) 13. Rabbit (*Oryctolagus cuniculus*, NM_001082077) 14. Horse (*Equus caballus*, XM_001492560).

Table 4. Genotype vs. phenotype.

exon	Polymorphism	White	Red	brown	Total
(exon1)	P38L				
	CC	15	4	49	68
	CT	1	4	1	6
	TT	1	2	0	3
(exon1)	H211R				
	AA	16	8	39	63
	AC	1	2	11	14
	CC	0	0	0	0
(exon1)	W238R				
	TT	10	4	25	39
	TC	4	6	22	32
	CC	3	0	3	6
(exon1)	V258A				
	TT	15	2	48	65
	CT	1	6	2	9
	CC	1	2	0	3
(exon5)	Q473R				
	AA	13	3	46	62
	AG	2	5	4	11
	GG	2	2	0	4
(exon5)	K505E				
	AA	17	10	42	69
	AG	0	0	6	6
	GG	0	0	2	2
Total		17	10	50	77

in pigment cells (mel- anocytes) in a specialised cytoplasmic organelle: the melanosome. Tyrosinase is widely existed in mammals, flora, microbe and is essential the process of melanin synthesis (Veronique and Friedrich, 1996). In some species, there are several alleles at the TYR locus, which varying effects on phenotype. In human, the Arg402Gln mutation of tyrosinase gene was significantly associated with skin colour (Nan *et al,* 2009); In addition, there are several mutation in the tyrosinase gene and are responsible for oculocutaneous albinism (OCA1); these mutations can catalysed regions of tyrosinase particularly in the histidine-rich regions that can complex copper (Veronique and Friedrich, 1996). In siamese coloured cat, a nonsynonymous transition G901A in TYR gene, which leaded to the substitution of a glycine (G) by an arginine (R) at codon position 301; In burmese-coloured cat, a nonsynonymous substitution G679T was found, which led to the substitution of a glycine (G) by a tryptophan (W) at codon position 227 (Schmidt *et al,* 2005). In albino cats, a cytosine deletion in TYR gene at 975 bp in exon 2 was found, and it caused a frame shift resulting in a premature stop codon nine residues downstream from the mutation (Imes *et al,* 2006). The expression of tyrosinase gene in lack plumage quails was higher than that in maroon plumage quails (Ying *et al,* 2013) and the expression quantity of tyrosinase in the skin of pigmented alpaca was higher than that of natural white alpaca (JunBing *et al,* 2010). In camel family species (bactrian camel, dromedary camel and alpaca), there are several research for tyrosinase with related to their coat colours. Ishag (2013) detected the allelic variant (G.200C>T) of tyrosinase gene (Exon 1) in six dromedary camel breeds and indicated that this allelic variant was insignificant association between camel coat colour; Rhys and Kylie (2011) selected the colour dilution candidate tyrosinase gene which has been associated with coat colour dilution in other species and attempted to find the relationship between colour dilution gene and alpaca fibre colour; the result showed that there was no polymorphism which were discovered in the tyrosinase gene coding region had an influence on dilution in alpaca fibre colour.

In our study, nine single nucleotide polymorphisms were found in the coding region of TYR gene, six of them are non-synonymous (P38L, H211P, W238R, V258A, Q473R, K505E) and three are synonymous (C103C, T144T, R502R). However, there is no significant correlation between coat colour and Tyrosinase genotypes in bactrian camels. Key regulatory regions and intronic regions of the TYR gene and larger numbers of animals will have to be examined to investigate or verify these association.

Acknowledgements

The work was supported by National International Scientific and Technological Cooperation Projects (2015DFR30680 and ky201401002) and Chinese National Natural Science Foundation project (31360397).

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"SELECTED RESEARCH ON CAMELID IMMUNOLOGY"- NEW BOOK

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

In 1989 a group of biologists led by Raymond Hamers at the Free University Brussels investigated the immune system of dromedaries. In addition to the expected four-chain antibodies, they identified simpler antibodies consisting only of two heavy chains. This discovery was published in Nature in 1993. Based on their structure, these peculiar camelid antibodies have been named Heavy Chain Antibodies (HCAb), as they are composed of heavy chains only and are devoid of light chains. HCAbs are not found in other mammals except in pathological cases. Sera of camelids contain both conventional heterotetrameric antibodies and unique functional heavy (H)-chain antibodies (HCAbs). The smaller size and monomeric single domain nature make these antibodies easier to transform into bacterial cells for bulk production, making them ideal for research purposes. Single-domain antibodies are being researched for multiple pharmaceutical applications and have potential for use in the treatment of acute coronary syndrome, cancer and myeloid (e.g. Alzheimer's) disease. Camelid scientists world over were greatly fascinated by a new field of research called "Camelid Immunology". Camel has low susceptibility to pathogens has led to a belief that camel's immune system is either more potent in combating the infections or is unique and different from other mammalian species. Significant research has been done on camelid immunology in recent decade. The term "nanoantibody" or "nanobody" was given to the recombinant single-domain antigen-binding variable fragments of special type of antibodies (i.e. HCAbs) that naturally exist (in addition to classical types of antibodies) in blood of Camelidae family animals. These nanobodies have a big potential for employment in immunobiotechnology and medicine. In order to benefit future camelid immunology researchers, this book was planned in the series of "Selected Topics" by Camel Publishing House with a title- "Selected Research on Camelid Immunology" edited by T.K. Gahlot, U. Wernery and Serge Muyldermans. This book is a unique compilation of research papers based on "Camelid Immunology" and published in Journal of Camel Practice and Research between 1994-2015. Research on this subject was done in 93 laboratories or institutions of 30 countries involving about 248 scientists. In terms of number of published papers in JCPR on the immunology the following countries remain in order of merit (in parenthesis), i.e. Iran (1), India and UAE (2), China and Saudi Arabia (3), Sudan (4), Kenya and Belgium (5), USA (6), Germany (7) and so on. The book contains 11 sections and is spread in 384 pages. The diverse sections are named as overview of camel immune system; determinates of innate immunity, cells, organs and tissues of immune system; antibodies; immunomodulation; histocompatibility; seroprevalence, diagnosis and immunity against bacteria, viruses, parasites and combination of other infections; application of camel immunoglobulins and applications of immune mechanisms in physiological processes. The camelid immunology has to go a long way in its future research, therefore, this reference book may prove quite useful for those interested in this subject. Book can be seen on www.camelsandcamelids.com.

(ISBN:81-903140-4-1)

Dr. T.K. Gahlot

DOI : 10.5958/2277-8934.2016.00008.4 Vol 23 No 1, p 53-63

MOLECULAR MECHANISM OF HEPATO-RENAL PROTECTION OF CAMEL MILK AGAINST OXIDATIVE STRESS-PERTURBATIONS

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ABSTRACT

Possible molecular mechanism of camel milk protection of liver and kidney against oxidative stress generated by CCl_4 injection was investigated. Rats injected with carbon tetrachloride (CCl_4) showed upregulation of the mRNA expression of hepatic IL-6 and renal IL-1β, TGF- β1, SREBP-1c and caspase-6 and down-regulation of anti-oxidative enzymes SOD, GST and CAT in addition to hepatocellular vacuolation, mononuclear cell infiltration and sinusoidal dilatation and renal glomerular atrophy, capsular space expansion, and adhesion between visceral and parietal layers of Bowman's capsule. Camel milk supplementation prior and with CCl_4 injection to rats attenuated CCl_4-induced hepatic and renal inflammatory cytokines (IL-6, IL-1β, TGF- β1 SREBP-1c and caspase-6), upregulated CCl_4-suppressed anti-oxidative markers (SOD, GST and CAT) and induced protective and regenerative mechanism (EPO and IL-10). Additionally camel milk protected the liver and kidney from CCl_4-induced histopathological changes. These results showed the mechanism of camel milk protection of liver and kidney against CCl_4-generated oxidative stress and injuries. These findings may support the beneficial use of camel milk as therapeutic adjuvant with drugs that always associated with production of oxidative stress that injured liver and kidneys as anti-tumor drugs as Cisplatin.

Key words: Camel, hepato-renal protection, milk, oxidative stress

List of abbreviations: (**SOD**) Superoxide dismutase, (**GST**) Glutathione S-transferases, (**EPO**) Erythropoietin, (**TG**) Triglycerol , (**SREBP-1**) sterol regulatory element-binding protein-1c, (**TGF-β1**) Transforming growth factor beta (**TGFβ1**), (HCV-infected) Hepatitis C virus infected, (**ROS**) Reactive Oxygen species, (**IL-6**) Interleukin-6, (**IL-10**) Interleukin-10, (**IL-1β**) Interleukin-1 beta, (**i.p**) Intraperitoneal injection, (**CAT**) Glutathione-S-transferase, (**RT PCR**) reverse transcription polymerase chain reaction.

Oxidative stress occurs in the body due to inability of the different body antioxidant mechanism to scavenge reactive oxygen species (ROS) due to overproduction and/or reduction of the body anti-oxidant defense mechanisms. This leads to many degenerative diseases, i.e. hepatopathies (Hensley *et al,* 2000), and nephropathies (Atessahin *et al,* 2003). Liver and kidney tissues are more prone to be affected by oxidative stress produced by infectious agent, alcohol consumption, drugs, toxic industrial chemicals, food additives, and pollutants in air and water. Free radicals and reactive oxygen species play a crucial role in the initiation and progression of liver diseases (Jemal *et al,* 2007).

Carbon tetrachloride (CCl_4)-induced oxidative stress is commonly used in rodent models to screen for protective effect of synthetic or natural product against drug-associated hepatotoxicity (Khan *et al,*2012) or nephrotoxicity (Haghi *et al,* 2014). After being biotransformed by the hepatic microsomal cytochrome P450 enzymes into trichloromethyl free radical (Khan *et al,* 2012), it produces chemical tissue toxicity by generating free radicals in liver, kidney, heart, lung, testes, brain, and blood (Shenoy *et al,* 2001; Khan *et al,* 2012). Reactive oxygen species (ROS) plays a main role in the development and progression of human diseases as liver disorders, lung and kidney damage, diabetes mellitus, atherosclerosis and aging (Singh *et al,* 2008), through free radicals-induced lipid peroxidation and cell membranes damage (Ogeturk *et al,* 2005). It causes inflammation, tissue damage, cancer and aging (Bhadauria and Nirala 2009). Studies showed that various natural products could protect organs against CCl_4 induced oxidative

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stress through enhancing the decreased activities of antioxidant enzymes, as catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD) (Rajesh and Latha, 2004; Saif *et al,* 2014).

HCV-infected patients consume large amounts of camel's milk as an alternative and/ or supportive medicine (Redwan and Tabll, 2007). Camel's milk exhibits a wide range of biological activities including antimicrobial, antioxidative, antithrombotic, antihypertensive, and immuno-modulatory effect (Fitz and Meisel, 2000; Kohonen and Pihlanto, 2003; Saltanat *et al,* 2009). It was therapeutically used to treat jaundice, splenic problems, asthma, anaemia, piles, and diabetes (Knoess, 1979; Rao *et al,* 1970). Besides casein, camel milk contains high levels of lactoferrin which is an iron-binding glycoprotein of the transferring family (Al-Majali *et al,* 2007). Camel milk anticarcinogenic, anti inflammatory and antioxidant activities was proposed to be mainly caused by Lactoferrin (Konuspayeva *et al,* 2004). Camel's milk was shown to be effective in food allergies treatment due to its inflammation-inhibiting proteins and hypoallergenic properties (Al-Ayadhi and Elamin 2013). A promising therapeutic potency of camel milk against several diseases was suggested also to be due its newly identified nanobodies content (Zafra *et al,* 2011).

Camel's milk was described to has antitoxic effect against alcohol (Darwish *et al*, 2012), CCl_4 (Althnaian *et al,* 2013), Cadmium chloride (Dallak, 2009), Cisplatin (Afifi, 2010). However, the molecular mechanism of the camel milk protective effects against these hepato-renal hazards is still not clear. This motivated us to explore the molecular mechanism of camel milk protection against oxidative stress using CCl_4-injected rats as a model.

Materials and Methods

Camel's milk samples were collected in sterile screw bottles daily early in the morning from camel farm in Taif Province, Saudi Arabia and kept in cool boxes until transported to the laboratory. Rats were given this fresh milk (120 mL/cage) without any further treatment at morning after deprivation of rats from water for 3 hours as to ensure drinking of milk within 2 hours to avoid souring of the milk. Carbon tetra-chloride was purchased from Sigma (Sigma. Aldrich Co, St. Louis, USA.).

Experimental animals

A total of 24 adult male Wistar rats weighing about 200-250g were used in the present study and were divided into equal 4 groups. Animals were kept under observation for about 4 days before the onset of the experiment. These were maintained in stainless steel cages at normal atmospheric temperature of 27 ± 5°C and good ventilation and 12/12 light dark automatic cycler.

Treatment: Group 1 served as control injected I/P with corn oil. Group 2 received water daily for 25 days and at the 18^{th} and 20^{th} days these were injected intraperitoneally with CCl_4 at day 18^{th} and 20^{th} CCl_4 in a dose of 1ml/kg in 50% corn oil. Group 3 received raw camel's milk orally 100 ml/kg daily for 25 days and injected with corn oil intraperitoneally at day 18^{th} and 20^{th}. Group 4 received raw camel's milk at a dose of 100 ml/kg daily for 25 days and injected with CCl_4 at day 18^{th} and 20^{th} CCl_4 in a dose of 1ml/kg 50 % in corn oil

Sampling: Rats were sacrificed at the day 25^{th} through cervical dislocation under light ether anaesthesia then tissues samples were taken from liver and kidneys for histopathological and RT-PCR studies.

Analysis of gene expression

RNA extraction and cDNA synthesis: Total RNA was extracted from 100 mg of each tissue sample using QIAzollysis reagent (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions and as detailed previously (Ahmed *et al*, 2014). Integrity of the prepared RNA was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm and 280 nm. The ratio of the OD260/280 of all RNA samples was 1.7-1.9. Two µg RNA were reverse transcribed with oligo-dT primer and Moloney murine leukaemia virus Virus (M-MuLV) reverse transcriptase (SibEnzyme Ltd. AK, Novosibirsk, Russia) as previous described (Ahmed *et al*, 2014). The resultant cDNA was preserved at -20°C until used

Semi-quantitative Ploymerase chain reaction (PCR): mRNA expression of some genes playing major roles in the oxidative stress, antioxidant defense mechanism, inflammation and regeneration were tested by semi-quantitative PCR using corresponding specific primers of these genes (Table 1). Of these tested genes, are Transforming growth factor Beta (TGF-β1), IL-1β, IL-6, IL10, TNFα, SOD, CAT, and GST. The used primers were designed using Oligo-4 computer program and nucleotide sequence published in Genebank (Table 3) and synthesised by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu. Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl

(10 picomoles) of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI). The final volume was brought to 25 µl using sterilised, nuclease-free deionised water. PCR was carried out using a PeX 0.5 thermal Cycler with the cycle sequence of denaturing at 94°C for 5 minute for one cycle, followed by 28-35 cycles each of which consisted of denaturation at 94°C for 1 minute, annealing at the specific temperature corresponding to each primer (Table 1) and extension at 72°C for 1 minute with an additional cycle as a final extension at 72°C for 5 minutes. As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was tested using specific primers (Table 1). PCR products were electrophoresed on 1.5% agarose A (Bio BAsic INC. Konrad Cres, Markham Ontario) gel in TE (Tris-EDTA) buffer at 100 volt for 30 minutes with ethidium bromide staining. PCR products were visualised under UV light and photographed. The intensities of the bands were quantified densitometerically using NIH image program (http://rsb.info.nih.gov/nih-image).

Histological examination:

Small specimens from the liver and kidney were fixed in 10% neutral buffered formalin (NBF) for 24 hours, then washed under running tap water and preserved in 70% ethanol. The samples were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in Paraplast Plus® (Sigma–Aldrich, St. Louis, MO, USA) and sectioned at 5µm thickness. Tissue sections were mounted on glass slides. Sections were stained with hematoxylin and eosin for studying the histopathological changes (Bancroft *et al*, 1996). Photomicrographs were taken with a Leica DM LB light microscope (Leica Microsystems, Wetzlar, Germany) and digital camera (Leica EC3, Leica Microsystems Ltd., Heerbrugg, Switzerland).

Statistical analysis

Data were presented as means ± standard errors of means. Statistical analysis for the obtained results was one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test for the multiple comparisons among the groups. The analysis was done by SPSS software (SPSS version 13.0, IBM, Chicago, IL, USA. Values for $P < 0.05$ were considered statistically significant.

Results

Effect of Camel milk on hepatic inflammatory and anti-inflammatory cytokines gene expressions

To examine the possible involvement of cytokines modulation by camel milk protective effect

Table 1. Primers and PCR conditions used for the tested genes.

Gene	Product size (bp)	Annealing	Direction	Sequence
GAPDH	309	52	Sense	AGATCCACAACGGATACATT
			Antisense	TCCCTCAAGATTGTCAGCAA
TGF-β1	456	58	Sense	TGAGTGGCTGTCTTTTGACG
			Antisense	TGGTTGTAGAGGGCAAGGAC
EPO	530	60	Sense	TACGTAGCCTCACTTCACTGCTT
			Antisense	GCAGAAAGTATCCGCTGTGAGTGTTC
SOD	410	55	Sense	AGGATTAACTGAAGGCGAGCAT
			Antisense	TCTACAGTTAGCAGGCCAGCAG
GST	575 bp	55	Sense	GCTGGAGTGGAGTTTGAAGAA
			Antisense	GTCCTGACCACGTCAACATAG
IL-6	485 bp	57	Sense	AGTTGCCTTCTTGGGACTGA
			Antisense	GAGCATTGGAAGTTGGGGTA
IL-1β	550 bp	57	Sense	TTCAAATCTCACAGCAGCATCT
			Antisense	TGTGCAGACTCAAACTCCACTT
IL-10	259 bp	57	Sense	ACCAGCTGGACAACATACTGC
			Antisense	TCATTCTTCACCTGCTCCACT
SREBP-1c	191	58	Sense	GGAGCCATGGATTGCACATT
			Antisense	AGGAAGGCTTCCAGAGAGGA
Caspase 6	289	56	Sense	AACCACATTTACGCATACGATG
			Antisense	CGGTGAGAGTAATACCCTTCTG

on the liver, the mRNA expressions of interleukin-6 (IL-6) and interleukin-10 (IL-10) were measured by semi-quantitative RT-PCR. IL-6 mRNA expression was up-regulated by CCl_4 injection. Meanwhile, when administrated with CCl_4, camel milk completely normalised IL-6 mRNA expression (Fig 1 A). Interleukin-10 the anti-inflammatory and regenerative cytokines mRNA expression was slightly induced in camel milk treated group and highly induced with camel milk when rat's livers were subjected to CCl_4 damaging effect, meanwhile, its expression was not induced in the rat group treated with CCl_4 alone (Fig 1 B).

Effect of camel milk on hepatic antioxidant enzymes expression

The mRNA expressions of superoxide dismutase (SOD) were clearly increased with Camel milk treatment, while glutathione-S-transferase (GST) mRNA expressions were not changed than control levels. CCl_4 injection suppressed the mRNA expression of (SOD) and (GST). When administrated pre or in concurrent with CCl_4, camel milk completely rescued the antioxidant enzymes mRNA expression from the CCl_4 suppressive effect and even upregulated their expression higher than control (Fig 2A & B).

Effect of camel milk on renal IL-1β, TGF-β1 and SREBP-1c gene expressions

Camel milk administration alone decreased the gene expression of TGF-β1 and SREBP-1c than control levels. Treatment with CCl_4 induced renal IL-1β, TGF-β1 and SREBP-1c gene expressions compared to control. When supplemented pre and in concurrent with CCl_4, camel milk normalised the CCl_4-induced gene expression of these cytokines (Fig 4 A, B & C).

Effect of Camel milk on renal Caspasae-6, EPO and SOD gene expressions

Caspase-6 mRNA expression was slightly induced with camel milk alone and highly induced with CCl_4 treatment compared to control group. However, when supplemented pre and in concurrent with CCl_4, camel milk completely normalised the CCl_4-induced caspase-6 mRNA expression (Fig 5 A). SOD mRNA expression was slightly induced with camel milk supplementation and suppressed with CCl_4 injection. When supplemented pre and in concurrent with CCl_4, camel milk not only reversed the CCl_4-suppressed but even up-regulated SOD mRNA expression than control group (Fig 5 B). Camel's milk supplementation up-regulated EPO mRNA expression. When supplemented pre and in concurrent with CCl_4, camel milk highly up-regulated EPO mRNA expression than control group (Fig 5 C).

Effect of camel milk on liver and kidney histopathological changes

The current histopathological findings in the hepatic sections from different groups (Fig 5) mostly support the molecular findings. Liver sections from the control group (Group1) displayed the classical hepatic lobule formed from hepatic cords radiating from a central vein towards the portal tracts at the periphery of the lobule (Fig 5A). Liver sections from CCl_4-treated rats (Group 2) showed typical centrilobular hepatocytic degenerative changes, steatosis (microvesicular and macrovesicular fatty degeneration), necrosis, congestion and sinusoidal dilatation (Fig 5B). Hepatic sections from rats received camel's milk (Group 3) showed a lobular pattern similar to those from the control (Fig 5C). Camel milk pre-and concurrent treatment of animals with CCl_4 (Group 4) reduced the CCl_4-hepatocellular vacuolation, mononuclear cell infiltration and sinusoidal dilatation, and achieved better preservation of the normal hepatic architecture (Fig 5D).

Kidney sections from the control and camel milk adminstered groups (Groups 1 and 3) displayed the normal histological structure (Fig 6A and 6C). Renal sections from CCl_4-treated rats displayed different forms of degenerative changes in the glomeruli. Some glomeruli showed atrophy and mild expansion of the capsular space, whereas a small number of others exhibited congestion in the capillary loops with an adhesion between visceral and parietal layers of Bowman's capsule (Fig 6B). Some of renal tubules were dilated and their epithelial cells tended to be vacuolated. Camel milk pre-and concurrent treatment of animals with CCl_4 (Group 4) reduced these CCl_4-histopathological changes (Fig 6D).

Discussion

In this study, the mechanism through which camel milk protects liver and kidney against damaging oxidative stress was elucidated at the molecular level. CCl_4 was demonstrated to cause hepato-toxicity (Lin *et al*, 2008) and nephro-toxicity (Javier *et al,* 1987), after being metabolised to generate trichloromethyl (CCl_3) and peroxy trichloromethyl ($OOCCl_3$) radicals that cause lipid peroxidation and protein deterioration (Recknagel *et al,* 1989). The results show that camel milk attenuates hepatic

and renal inflammatory cytokines, augments anti-oxidative markers and up-regulates protective and regenerative mechanism. In response to inflammatory mediators, IL-6 is produced in the liver by several cell types, including cholangiocytes (Park *et al,* 1999). Its expression was demonstrated to be markedly increased in the liver disease patients with non-alcoholic steatohepatitis as a marker of liver inflammation (Wieckowska *et al,* 2008). In this study, the up-regulation of liver IL-6 mRNA expression indicates CCl_4-induced inflammatory condition. Meanwhile, camel milk supplementation before and with CCl_4 was able to prevent liver IL-6 mRNA expression up-regulation which indicates its anti-inflammatory effect. Parallel with this result, camel milk was able to decrease IL-6 levels in the serum of diabetic wounded mice at the 4th day of

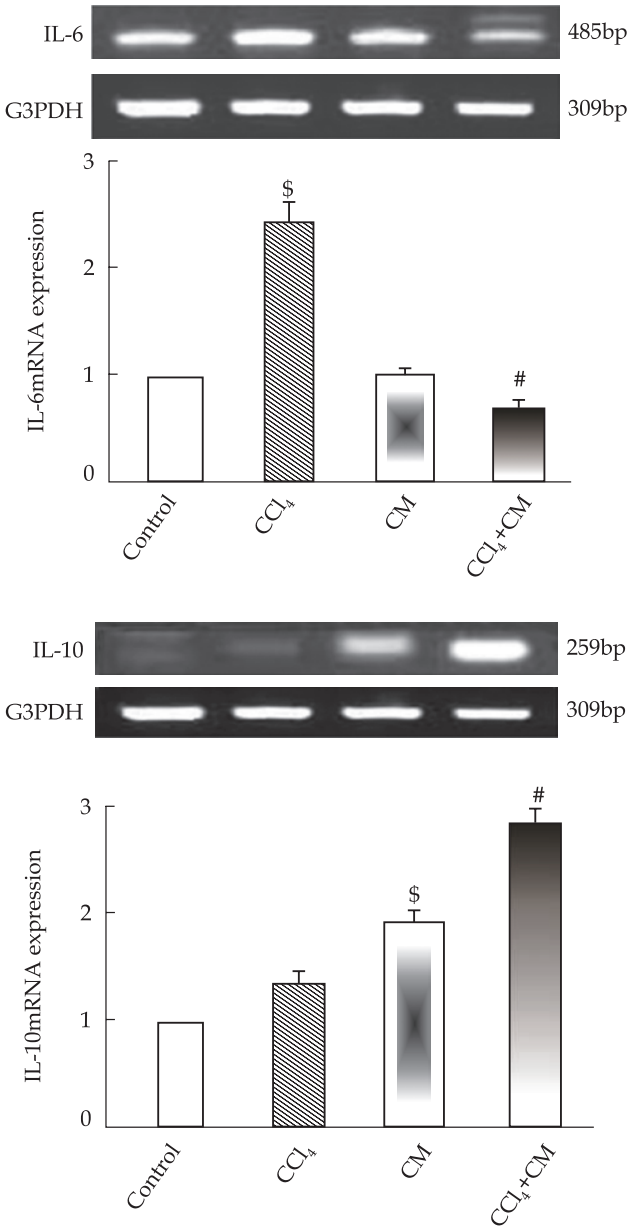

Fig 1. Effect of camel milk on hepatic inflammatory and anti-inflammatory cytokines gene expressions: Rats groups were treated with CCl_4, camel milk or camel milk before or with CCl_4: Total RNA was prepared from liver tissues and the expressions of IL-6 (A) and IL-10 (B) were analysed by semi-quantitative RT-PCR. Values are means ± SE of 6 rats. $P < 0.05 Vs control group, #P < 0.05 CCl_4 injected group.

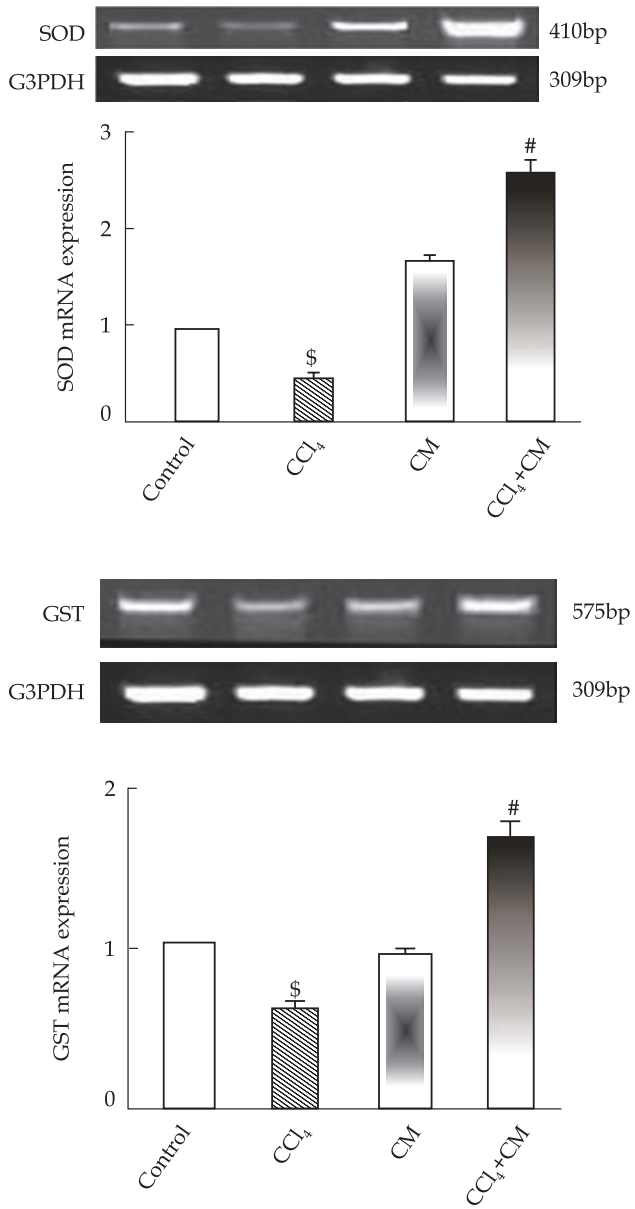

Fig 2. Effect of camel milk on hepatic antioxidant enzymes expression: Rats groups were treated with CCl_4, camel milk or camel milk before or with CCl_4: Total RNA was prepared from liver tissues and the expressions of SOD (A) and GST (B) were analysed by semi-quantitative RT-PCR. Values are means ± SE of 6 rats. $P < 0.05 Vs control group, #P < 0.05 CCl_4 injected group.

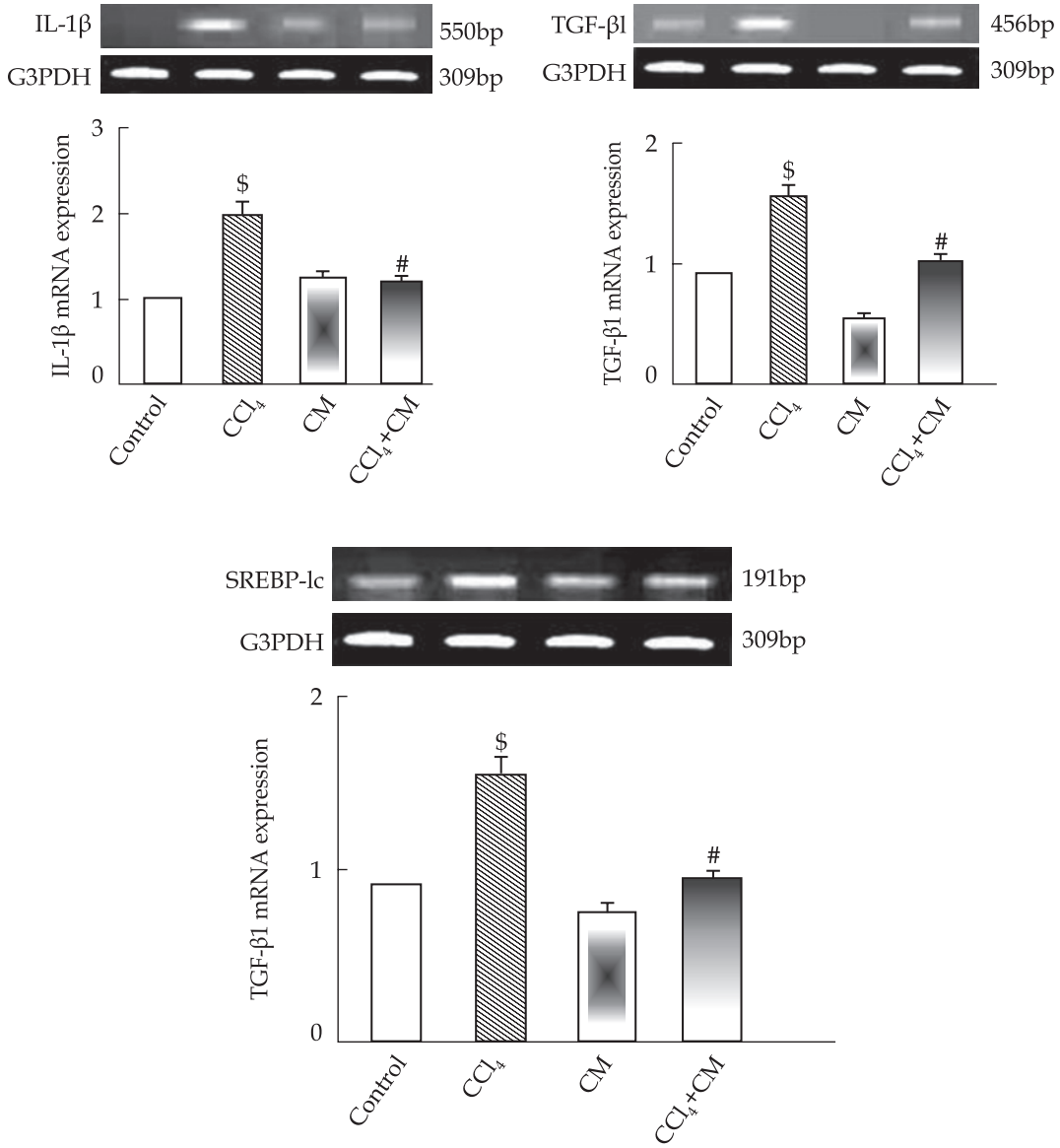

Fig 3. Effect of camel milk on renal IL-1β, TGF-β1 and SREBP-1c gene expressions: Rats groups were treated with CCl_4, camel milk or camel milk before or with CCl_4: Total RNA was prepared from liver tissues and the expressions of IL-1β (A) TGF- β1 (B) and SREBP-1c (C) were analysed by semi-quantitative RT-PCR. Values are means ± SE of 6 rats. $P < 0.05 Vs control group, #P < 0.05 CCl_4 injected group.

supplementation (Ebaid *et al*, 2013). An increase of renal IL-1β was demonstrated to be associated with Cisplatin-induced acute renal failure (Faubel *et al*, 2007). In our study, the increase of renal IL-1β by CCl_4 indicates its involvement in the CCl_4-induced renal injury. When administered before and with CCl_4, camel milk prevented the CCl_4-increased renal IL-1β mRNA expressions which imply that camel milk-renoprotective effect (Korish *et al*, 2015), may act through inhibiting IL-1β mediated inflammation. Indeed Taxilli Ramulus pretreatment prevented the cisplatin-increased IL-1β levels through which it inhibits cisplatin-nephrotoxicity (Lee *et al*, 2012).

The transforming growth factor beta (TGF-β1) is considered as one of the main cytokines that aggravates diabetic nephropathy (Basile, 2001). Our results show CCl_4-induced renal TGF-β1 mRNA expressions that imply its involvement in the process of CCl_4-nephro-toxicity (Javier *et al*, 1987). Intensive urine excretion of TGF-β1 occurred in chronic glomerulonephritis (CGN) patients with expression of this cytokine in renal interstitium. A correlation was found between urine levels of TGF-β1 and severity of tubulo-interstitial fibrosis (Bobkova *et al*, 2006). Cisplatin was reported to trigger renal nuclear factor-kappa B (NF-κB) activation and hence

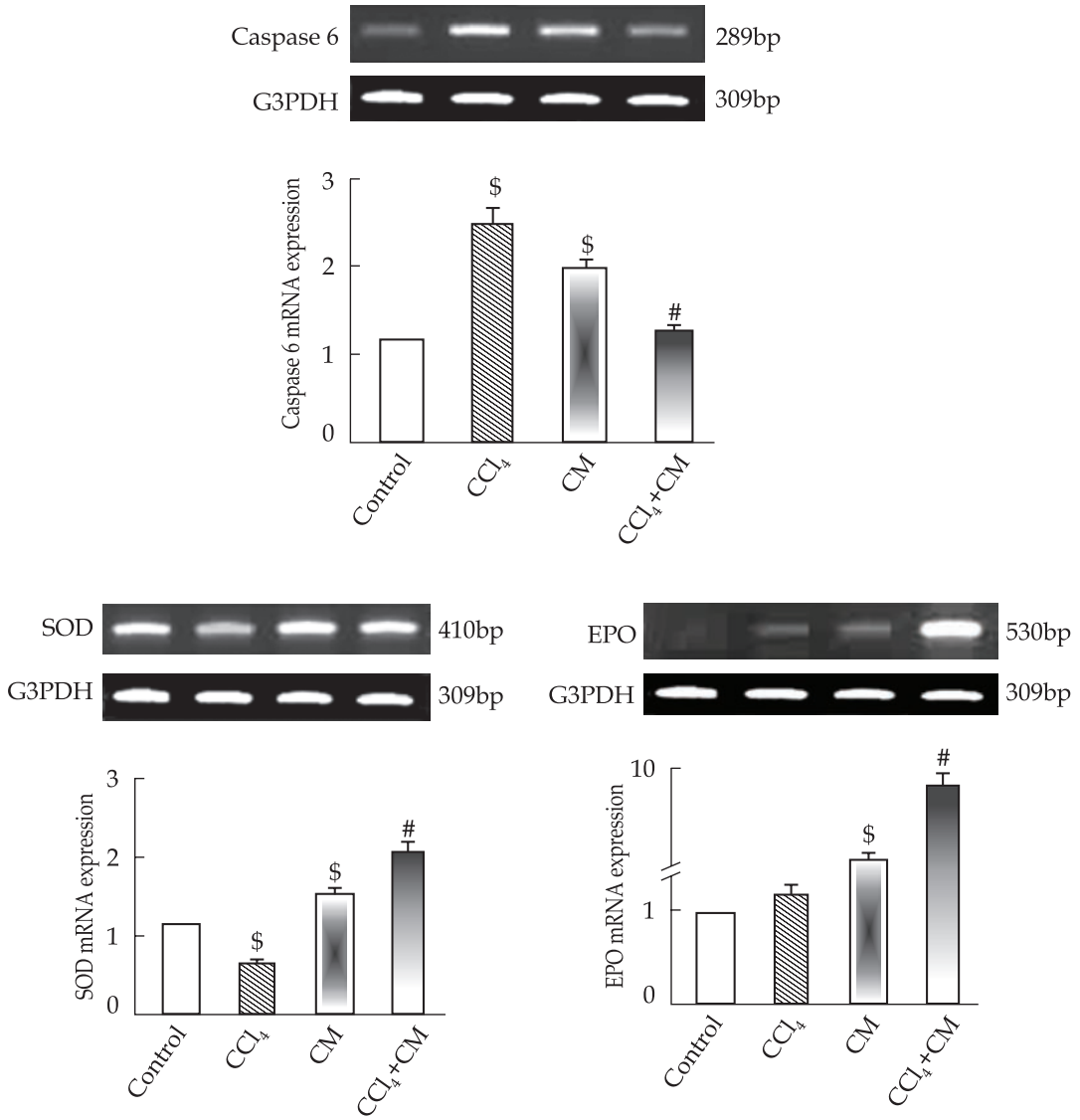

Fig 4. Effect of camel milk on renal caspasae-6, EPO and SOD gene expressions: Rats groups were treated with CCl_4, camel milk or camel milk before or with CCl_4: Total RNA was prepared from liver tissues and the expressions of Caspase 6 (A) SOD (B) and EPO (C) were analysed by semi-quantitative RT-PCR. Values are means ± SE of 6 rats. $P < 0.05 Vs control group, #P < 0.05 CCl_4 injected group.

inflammatory condition resulting in over-expression of set of factor including TGF-β (Ramesh and Reeves 2004). In the current study, the down-regulation of the CCl_4-induced renal TGF-β1mRNA expression due to camel milk supplementation may in part explain its renoprotective effect and possibility of its using as an adjuvant with the oxidative stress producing drugs as cisplatin. The CCl_4-upregulated renal sterol-regulatory element-binding protein (SREBP)-1 may be another mechanism through which CCl_4-generated oxidative stress-nephrotoxicity by enhancing (SREBP)-1 that in turn activate key lipogenic enzymes leading to triglyceride and cholesterol accumulation. Up-regulation of SREBP-1 was described to be one of the main factors causing renal TG accumulation and diabetic nephropathy (Ishigaki *et al,* 2007). The inhibition of CCl_4-upregulated SREBP-1c mRNA expression indicates the ability of camel milk to protect the kidney from oxidative stress derived renal TG and cholesterol accumulation and therefore its renoprotective effect.

Caspase-6 was reported to significantly contribute to cell death in cisplatin-induced renal injury (Yang *et al,* 2008). The CCl_4-upregulated renal caspase-6 mRNA expression indicates the acceleration of cell death by CCl_4-induced oxidative stress through caspases upregulation. The inhibition of the CCl_4-upregulated renal caspase-6 mRNA expression by

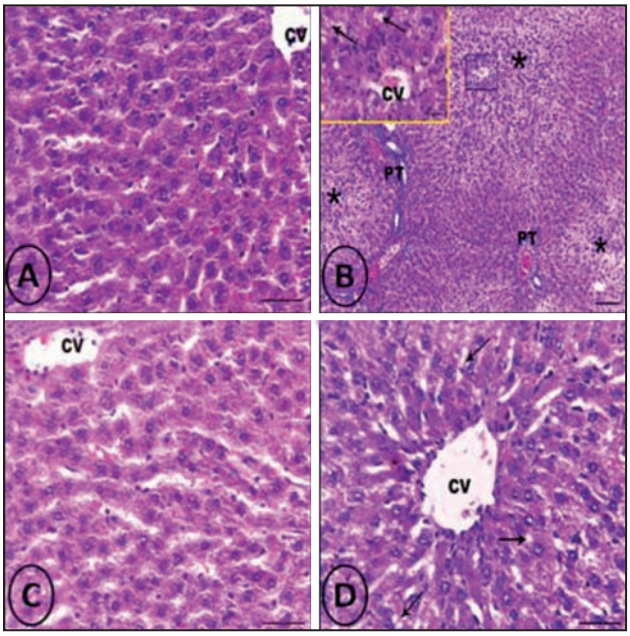

Fig 5. Effect of camel milk on liver histopathological changes: rats groups were treated with CCl_4, camel milk or camel milk before or with CCl_4: Liver sections from each group were histologically examined as detailed in the Material and methods. Control group show classical hepatic lobule (5A). CCl_4-treated rats showed typical centrilobular hepatocytic degenerative changes, steatosis necrosis, congestion (5B). Camel's milk showed a lobular pattern similar to those from the control (5C). Camel milk + CCl_4 group showed reduced the hepatocellular vacuolation, mononuclear cell infiltration and sinusoidal dilatation, (5D).

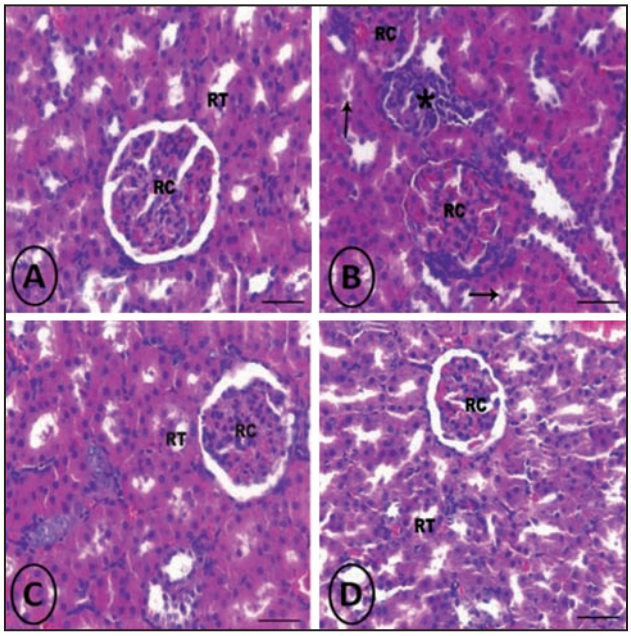

Fig 6. Effect of camel milk on kidneys histopathological changes: rats groups were treated with CCl_4, camel milk or camel milk before or with CCl_4: Kidney sections from each group were histologically examined as detailed in the Material and methods. Control (6A) and camel milk (6B) groups displayed the normal histological structure. CCl_4-treated rats displayed glomeruli with atrophy and mild expansion of the capsular space, other has congestion in the capillary loops with an adhesion between visceral and parietal layers of Bowman's capsule (6B). Camel milk + CCL_4 group showed greatly reduced glomerular histopathological changes (6D).

camel milk may indicate the caspases downregulation as a mechanism through which camel milk ameliorate oxidative stress-induced cell death. In consistence with this assumption, Inhibition of caspase-6 by its specific inhibitor VEID-CHO in caspase-3 (-/-) cells provided marked protection from cisplatin injury (Yang *et al,* 2008).

IL-10 configures the development of immune response and decrease pro-inflammatory cytokine expression (Moore *et al,* 2001). In our study the downregulation of IL-10 with CCl_4 is in line with the previously reported CCl_4-reduction of hepatic IL-10 level (Hou *et al,* 2014). Previous reports described IL-10 to be important in anti-fibrinogenesis during CCl_4-induced hepatic fibrogenesis (Huang *et al,* 2006; Zhang *et al,* 2007). Parallel with this, our results show IL-10 upregulation with camel milk indicates its anti-inflammatory effect through which camel milk protects liver from the CCl_4-inflammatory condition. Therefore camel milk through IL-10 upregulation could protect the liver from the CCl_4-oxidative stress-induced inflammation and fibrogenic effects by regulating the expression of IL-10 and hence its anti-inflammatory and regenerative action (Pestka *et al,* 2004).

Oxidative stresses disturb the balance between production and removal of ROS. Conversion of superoxide to less toxic H_2O_2 is catalysed by SOD, while conversion of the H_2O_2 into nontoxic H_2O is catalysed by CAT (Celi, 2010). GST is known to play a significant role in the detoxification of oxidative stress products (Fiander and Schneider, 1999). CCl_4-induced hepatic injury act through lipid peroxidation and protein deterioration that cause hepatocyte membrane damage followed by hepatic macrophages activation and release of a series of inflammatory mediators resulting in further generation of a variety of ROS (Sudo *et al,* 2005). In the current study, the CCl_4-down-regulated SOD and CAT mRNA expression indicates CCl_4-generated oxidative stress ability to weaken the tissue antioxidant defense mechanism production. In addition to this, the antioxidant defense, (SOD, GPX and CAT) were reported to decrease in the tissues due to their rapid consumption

after combatting free radical-induced oxidative stress (Dai *et al,* 2014). The camel milk-rescued hepatic and renal antioxidant defense enzyme SOD and CAT mRNA expression from the CCl_4-down-regulating effect seems to be crucial mechanism of the hepato-renal protective effect of camel milk against oxidative stress.

Erythropoietin (EPO) was reported to have protective effects in various tissues through modulation of metabolism and inhibition of apoptosis in non-haematopoietic tissue (Chatterjee, 2005). Correction of anemia by EPO administration was suggested to slow the progression of chronic kidney disease (Gouva *et al,* 2004). In the current study the up-regulation of renal EPO mRNA by camel milk administration indicates its ability to protect renal tissue from CCl_4-generated oxidative stress. Induction of endogenous EPO expression by a novel inducer (EH-201) in the heart, kidney and liver was postulated to be a potential therapeutic strategy for ischaemic diseases (Hsu *et al,* 2013). Moreover, recombinant human EPO injection was reported to enhance recovery from cisplatin-induced acute kidney injury through alleviating renal function impairment and preventing apoptosis (Kong *et al,* 2013). Our finding of camel milk protection against CCl_4-induced oxidative stress was confirmed also at histological levels as camel milk reduced CCl_4-induced hepatocellular vacuolation, mononuclear cell infiltration and sinusoidal dilatation, and preserved normal hepatic architecture which agrees with a previous reported (Khan and Alzohairy, 2011). Furthermore, the CCl_4-induced glomerular atrophy, capsular space expansion, and adhesion between visceral and parietal layers of Bowman's capsule were normalised with camel milk supplementation treatment. This is in agreement with milk-renoprotective effects against oxidative stress at tissue levels (Korish *et al,* 2015).

Conclusion: Camel milk attenuated CCl_4-induced hepatic and renal inflammatory cytokines (IL-6, IL-1β, TGF- β1 SREBP-1c and caspase-6), up-regulated CCl_4-suppressed anti-oxidative markers (SOD, GST and CAT) and augmented the protective and regenerative mechanism (EPO and IL-10). Hence camel milk showed hepato-renal protection from CCl_4-induced histopathological changes. These findings may support the beneficial use of camel milk as therapeutic adjuvant with drugs that always associated with production of oxidative stress that injures liver and kidneys as anti-tumour drugs especially Cisplatin.

Acknowledgements

This work was supported in part by research Project grant (No.1/435/3599) by Taif University of Kingdom of Saudi Arabia granted to the first Corresponding Author. The authors are thankful to Dr. Samir Ahmed El-shzely, Associate Prof., Department of Biotechnology, College of Science, for his help in the practical part of this study.

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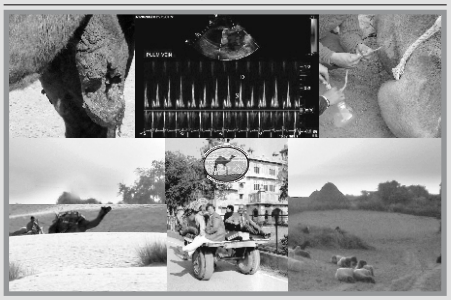

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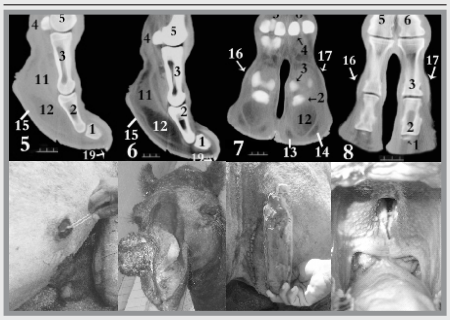

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DOI : 10.5958/2277-8934.2016.00009.6 Vol 23 No 1, p 65-72

THE ROLE OF ADJUVANT ON SAFETY AND ANTIBODY MODULATION OF DROMEDARY CAMEL

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ABSTRACT

Adjuvants selection is a subject of wide research in veterinary vaccines industry. In camels, adjuvant can modulate the production of either heavy chain (HCAbs) or conventional antibodies and both are of a valuable therapeutic interest. This study compared safety and immunogenicity of vaccine mixed with different adjuvants, Marcol™52, Stimune® and Alum in dromedary camels. Camels were injected with heat killed *Propionibacterium acnes (P. acnes)* bacteria mixed with different adjuvants. Reactogenecity (Inflammation and skinfold thickness), IgG response, isotypes modulation and complement fixation were measured. Stimune® vaccine showed severe reaction (87 mm) and induced marked percent increase in anti-*P. acnes* IgG1 and HCAbs (86.24 ± 3% and 58.76 ± 3.9%, respectively). However, Marcol™52 and Alum vaccines enhanced lower elevation in HCAbs (29.18 ± 9.3% and 8.4 ± 0.87 %, respectively). Both conventional and HCAbs induced by all vaccines against *P. acnes* were capable of activating the complement system. The highest complement fixing (CF) IgG2 was induced by Stimune® (28.46%), whereas, Marcol vaccine induced the highest CF IgG3 (9.94%). In conclusion, although Stimune® vaccine, was the most reactogenic adjuvant in camels it induced the highest HCAbs with complement fixing IgG2, whereas, Alum, the safest adjuvant induced the lowest antibody response but still capable of inducing complement fixing HCAbs.

Key words: Adjuvants, antibodies, camels, complement fixation test, heavy chain antibody. immunologic, inflammation

Camel antibodies have been raised against different bacterial, viral and toxin antigens (El Agamy *et al,* 1992, Harrison *et al,* 2006; El-Fakharany *et al,* 2012). In order to optimise antibody production; vaccines are used in a mix of specific antigens and adjuvant. Immunological adjuvant was originally described as substances used in combination with a specific antigen that elevate immune response (Humoral or cellular) (Mutiso *et al,* 2010). This broad definition encompasses a very wide range of materials.

Safety of the adjuvant is very important in vaccine development. Specifically, in camels, few studies have focused on developing safe and potent vaccine. In this study the aim was to compare the safety and immunogenicity of three different adjuvants mixed with bacterial antigen.

Materials and Methods

Animals

Six male camels (*Camelus dromedarius*) aged between 3 and 3.5 years old were stabled at company farm in Al-Ramtha city (Jordan). Camels were divided into three groups, each of 2 camels. All camels were designated codes based on numbers and adjuvant used. Group 1 received Marcol™52 vaccine and assigned as C1M52 and C2M52, Group 2 that received Stimune® vaccine assigned as C1St and C2St and Group 3, camels which were given Alum vaccine, assigned as C1Al and C2Al.

Vaccine Preparation

Bacteria and Outer Membrane Proteins (OMPs) Preparation:

Propionibacterium acnes strain (NCTC 737) was cultured on Mueller Hinton Agar (MHA) (BBL, Japan) under anaerobic conditions using Gas-Pak (Oxoid, UK) at 37°C for 72 hrs. *P. acnes* bacteria culture plates were scraped with autoclaved 0.15 M Phosphate Buffered Saline (PBS). Ten ml of the scraped culture were collected into a conical screw cap tube. Optical density reading was adjusted to 0.6 as measured at 600 nm (2.0 McFarland). For the preparation of the final concentration the bacteria was heat killed at 80°C for 30 minutes. One ml of the culture was streaked over a MHA plate (BBL, Japan) and incubated at 37°C for two weeks to ensure complete inactivation of *P. acnes*.

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Outer membrane proteins were prepared by sonication (Soniclean, Italy) of the inactivated bacteria (three pulses of 5 min) then cooling down in ice (Basal *et al,* 2004). The procedure of sonication and cooling was repeated three times then clarified by centrifugation (5000 xg/15 min/4°C). After that the supernatant was filtered using 0.2 µm syringe filter (Sartorius, UK) and concentrated on Vivaspin concentrator with molecular mass cut-off 10 KDa (Sartorius, UK). Protein quantification was determined using Bradford assay. Total inactivated bacteria was used for vaccine preparation whereas, bacteria OMPs were used for ELISA Assay.

Adjuvant Selection

Adjuvants were mixed with the antigens at ratio of 1:1. The selected adjuvants were: Marcol™52 (ESSO, USA)– which is a purified mixture of liquid saturated hydrocarbons; Stimune® (Prionics Lelysatd B.V, Netherlands)– water-in-oil adjuvant and Alum (Kool *et al,* 2012).

Vaccination Protocol

Vaccine formulas were made by mixing 1.5 ml of the heat killed *P. acnes* bacteria with 1.5 ml of each adjuvant. Three batches of the vaccine were formulated and injected. Each group of camels was injected with the designated vaccine. Group 1 was injected with vaccine containing Marcol™52, group 2 was injected with vaccine containing Stimune® and group 3 injected with vaccine containing Alum. Vaccines were injected on day 0 and 7.

Reactogenecity Measurements

The vaccine was injected at two different sites at one week interval on each group of animals. All groups were injected subcutaneously in the right sub-scapular site. After 7 days, a second injection of the vaccine was administered subcutaneously in the left side of the sub-scapular site. Reactogenicity which resembles the inflammation at site of injection was measured. Reactogenicity was interpreted by measuring the skinfold thickness (which resemble the area of inflammation) or skin reaction areas of sites of injection using a digital caliper (Eckersley *et al,* 2011). The measurements were recorded on day 0, 7, 14, 21 and 35.

2.5 Blood Collection and Processing

5 ml of blood was collected weekly from Jugular vein (jugular venipuncture) on day 0, 7, 14, 21 and 28 from each camel using sterile syringes. All samples were centrifuged at 3300 xg for 10 minutes at 4°C using benchtop centrifuge (Hermle, Germany). Serum collected before immunisation considered as negative controls for each camel samples. All serum samples were collected, labelled and stored at -20°C for further tests.

Measurement of Anti** P. acnes **Polyclonal Antibodies in Camel Sera

Camel polyclonal antibodies against *P. acnes* were measured using indirect Enzyme Linked Immunosorbent Assay (ELISA). Firstly, 5µg of bacteria OMPs were suspended in 1 ml carbonate/bicarbonate solution (pH 9.6). Subsequently, 0.1 ml of the suspension was added to each well of the 96-well flat-bottom ELISA plate (Greiner, Germany). The plate was covered and incubated overnight at 4°C. On the next day, wells were washed with 0.15 M PBS containing 0.05% Tween-20 (PBS-T). A blocking buffer was prepared by mixing 2% bovine serum albumin (BSA) in 0.15 M PBS. A 0.2 ml aliquot of this blocking buffer was added to each well. The plate was incubated overnight at 4°C. Wells were washed twice using PBS-T.

Antibodies in serum samples were measured at a dilution of 1/1000 in 1% BSA/PBS. Samples were processed in duplicates. The plate was incubated with 0.1 ml of diluted serum samples for 1 hour at room temperature. After washing, a 0.1 ml aliquot of the purified mouse anti-camel IgGs Abs (Previously prepared in-house) (Yousef *et al,* 2015) of a dilution of 1:2,000 in 1% BSA/PBS was added to each well and the plate was incubated for one hour at room temperature with shaking. After Washing with PBS-T, the peroxidase conjugate anti-mouse IgG produced in goat (Serotec, UK) at a dilution of 1/1000 (1% BSA/PBS) was added to each well. Subsequently, the plate was washed and a 0.1 ml of liquid substrate, O-phenylenediamine (OPD, Acros organics, USA) (1mg/ml in Citrate Buffer pH 4.5), was added to each well and the plate was incubated for 5 minutes at room temperature. Finally, 0.05 ml of 3M HCl was added to each well to stop the reaction. The plate was read at 492nm using Enzyme Linked Immunosorbent Assay reader (Thermo Scientific, Finland).

Measurement of IgG subclasses titre in camel serum

ELISA procedure was applied following previously described modification in some steps. Serum samples from camel groups injected with different adjuvant were analysed separately. Serum from pre-immunised camel was used as negative

control whereas serum collected on day 21 was used because of the high antibody titre according to ELISA results. Instead of adding the secondary mouse anti camel IgG (total), a specific monoclonal mouse anti camel IgG1 (diluted 1:2000) or mouse anti camel HCAb (IgG2 and IgG3) (diluted 1:500) (previously prepared in-house according to Monojo's standard operation procedures) were used. The plate was read at 450nm using Enzyme Linked Immunosorbent Assay reader (Thermo Scientific, Finland). Per cent increase in antibodies isotypes was calculated using the following formula:

$$\%\text{Increase in IgG Isotype}=\left\{\frac{\text{O.D reading of Post immune sample}-\text{O.D of preimmune sample}}{\text{O.D of Postimmune sample}}\right\}\times 100\%$$

Characterisation of Camel Antibodies

Characterisation of the camel dromedary antibodies was carried out using three different assays. Firstly, purification of the IgG subclasses using Protein G (PG) and Protein A (PA). Then the eluted peaks from both PG and PA were further analysed by 12.5% SDS-PAGE. Finally complement fixation ability of the purified isotypes was tested.

Purification of antibody subclasses from camel serum

Immunoglobulin subclasses were isolated from total serum using Protein G (PG) and Protein A (PA) chromatography as described by Hamers-Casterman *et al* (1993). Some minor modifications were introduced to the original procedure. Briefly, 0.5 ml from a pool of pre-immune or post-immune camel sera from each group was diluted 1:24 in 20mM sodium phosphate buffer (pH 7.0), and injected into a 5 ml Hitrap™ Protein G-Sepharose column (GE HealthCare, USA). Bound protein was eluted with 0.15M NaCl, 0.58% acetic acid, pH 3.5 (Peak A) and then with 0.1M glycine–HCl, pH 2.7 (Peak B). The flow through was subsequently applied to a 5 ml Hitrap™ Protein A-Sepharose Column (GE HealthCare, USA) and washed with 20mM sodium phosphate buffer (pH 7.0). The retained fraction was then eluted with 0.15M NaCl, 0.58% acetic acid, (pH 4.5) as (Peak C). Last elution was carried out using 0.1M glycine–HCl (pH 2.7) buffer and (Peak D) were collected. Neutralising buffer (1M Tris-base) of pH 9.0 was used to neutralise the pH of each fraction directly after the collection. All peaks were concentrated using Vivaspin® 10 kDa membrane cut-off column (Sartorius, Germany). Protein concentration was then determined for all fractions using Bradford assay.

Antibody Purity Assessment by SDS-PAGE.

Eluted peaks from PG and PA chromatography were analysed by 12.5% running gel SDS-PAGE. The electrophoresis was performed in a Bio-Rad Mini-PROTEAN system (Bio-Rad Laboratories, U.S.). The gel was stained with 0.2% Coomassie Brilliant Blue R-250 solution. The stained gel was then washed with a mixture of acetic acid: water (1:5) and bands were visualised.

Complement Fixation Tests (CFT) to Anti P. acnes Antibodies Isotypes.

Purified IgG subclasses (200µg/ml) from preimmune and post-immune sera were analysed for their ability to fix complement proteins. Subclasses were purified from serum of animals injected by different adjuvants. Guinea pig serum was used as a source of complement proteins (Sigma, USA). The haemolytic system consisted of a mixture (1:1) of 3% sheep red blood cells suspension and diluted rabbit anti-sheep RBC (dilution 1/100) (Sigma, USA). The reaction was performed in two steps. Firstly, 0.025 ml of the complement and 0.025 ml of the *P. acnes* were incubated at 37°C for 1 h with 0.05 ml of the pre- or post-immune IgG isotypes in a 96 U-shape plate. Secondly, the haemolytic system was added and the reaction was incubated for 1 hour at 37°C, then the plate was centrifuged 2000 xg (Hermle, Germany). Each sample was tested in duplicate. 0.15 ml Supernatants were withdrawn to flat-bottom ELSIA plate and plate was read at 415 nm using microplate reader (Thermo Scientific, Finland). Fixation per cent was calculated using following formulas:

$$\textit{Preimmune Lysis\%} = \left\{\frac{\textit{O.D reading of pre immune sample}}{\textit{O.D Full Lysis}}\right\}\times 100\%$$

$$\textit{Postimmune Lysis\%} = \left\{\frac{\textit{O.D reading of post immune sample}}{\textit{O.D Full Lysis}}\right\}\times 100\%$$

Lysis % = preimmune Lysis % – post immune Lysis %.

Fixation % = 100 – Lysis %.

System control of 100% fixation was determined using no complement but bacteria and antibodies. Zero % fixation was determined by using full lysis with distilled water.

Data Analysis

Statistical analyses were run using Excel 2013. Error bars were calculated for all samples of each adjuvant. Standard error was calculated based on standard deviation of the samples.

Results

Reactogenecity Results

A mild skin reaction (35 mm skinfold thickness) was noticed only at one out of four sites (C1M52 R) in camels injected with Marcol™52 adjuvant (Fig 1A). On the other hand, no reactions were observed in the other three injected sites (Fig 1). Stimune® was the most irritant adjuvant. Both right and left sites of injection showed a severe reaction (74 mm and 87 mm, respectively). On the contrary, in camel C2St right sub-scapular skinfold, Stimune® showed a very mild reaction which resolved after 14 days of injection. Severe reaction resulted after second injection with Stimune® in both camels, whereas no significant reaction was observed in Marcol™52 and Alum after second injection (Fig 1B). The skinfold thickness in camel C2St left site peaked after day 20 of injection. Reaction area was measured instead of skinfold thickness after day 30, this was because of difficulty of measuring skinfold thickness due to hardness of the inflamed skin. However, the only adjuvant not associated with significant reaction was Alum. No reaction at any site of injection was observed at any time during and after the injections.

Stimune® Induced the Highest Antibody Response to P. acnes.

Stimune® adjuvant showed the most significant increase in anti *P. acnes* antibodies titre (Fig 2). Moreover, vaccines made up of Alum or Marcol™52 induced significant increases in the antibodies titre that was prominent after second injection. Antibody response peaked on day 21 of vaccination time in all animals and started to show minor decline after day 28. The decline in the antibody titre highlighted the need for another boost to keep the titre significant in camel vaccination time line.

Antibody isotypes analysis using ELISA showed increase in conventional (IgG1) and HCAbs (IgG2 and IgG3) titres. The increase varies among camels (Fig 3). Stimune® vaccine induced the highest titre of both conventional and non-conventional IgGs. The average per cent increase in antigen-specific IgG1 and HCAbs from camels injected with Stimune® were 86.2 ± 3 % and 58.7 ± 3.9 %, respectively. Vaccines mixed with Marcol™52 and Alum also triggered an increase of IgG1 (76.9 ± 1.3 % and 54.2 ± 4.2 %, respectively). Moreover, average per cent increase of HCAbs from Marcol™52 and Alum vaccines were 29.18 ± 9.3 % and 8.3 ± 0.87, respectively.

Both Conventional and HCAbs Antibody were Capable of Complement Fixation

The purified IgG1, IgG2 and IgG3 were characterised and confirmed by 12.5% SDS (Fig 4). Results obtained from the CFT showed the ability of postimmune IgG1, IgG2 and IgG3 for all vaccines to fix and activate complement system. IgG1 was the highest complement fixing isotype in Marcol™52 and Stimune® immunised camels with about 33% fixing capability for both (Fig 5). However, the ability of HCAbs to fix and activate the complement system varies according to adjuvant used. IgG2 in Stimune®

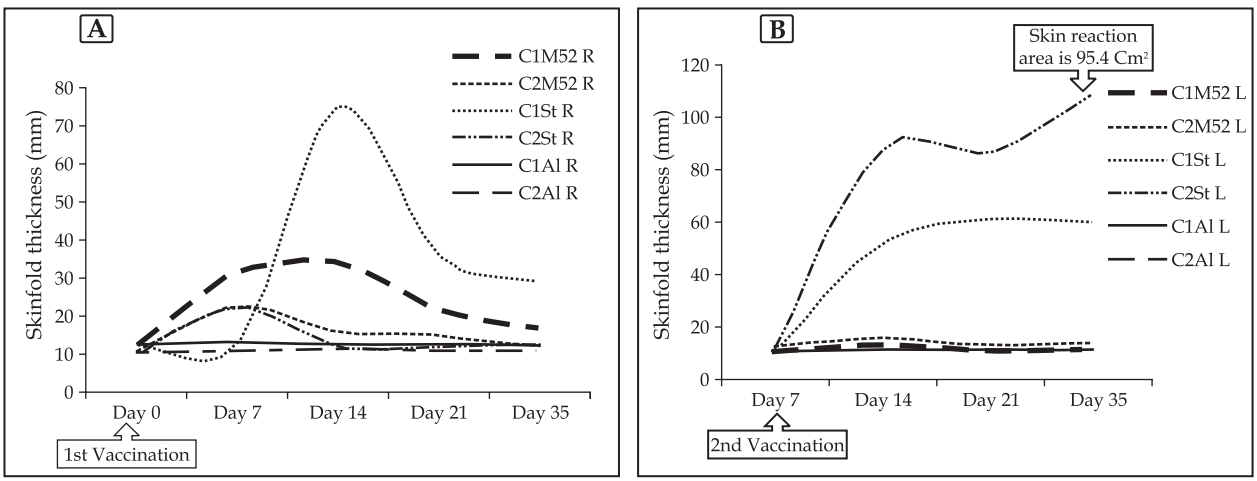

Fig 1. Skinfold thickness of camels injected with heat killed *P. acnes* mixed with different adjuvants. Two shots of each vaccine were injected. First was administered subcutaneously on the right sub-scapular skin area (A) and seven days later the second was administered in the subcutaneous area of left sub-scapular skin area (B).

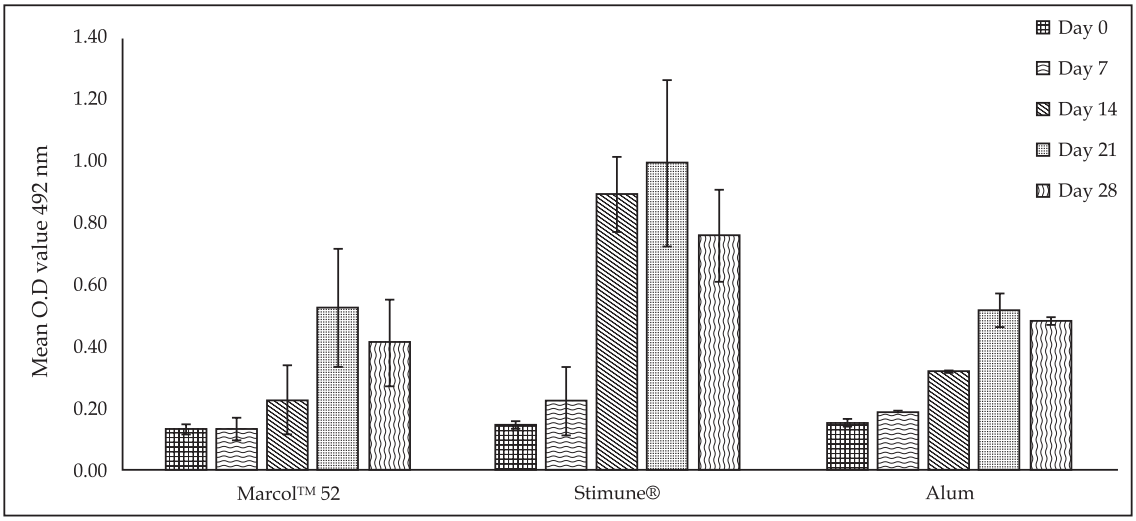

Fig2. Kinetic of total IgG response in serum (1:1000) of camel to *P. acnes* over 28 days of immunisation, as per ELISA. Camels were immunised with heat killed bacteria mixed with different adjuvant (Marcol™52, Stimune® or Alum). Each bar corresponds to the mean value of duplicate measure and the error bars represent the mean ±SEM.

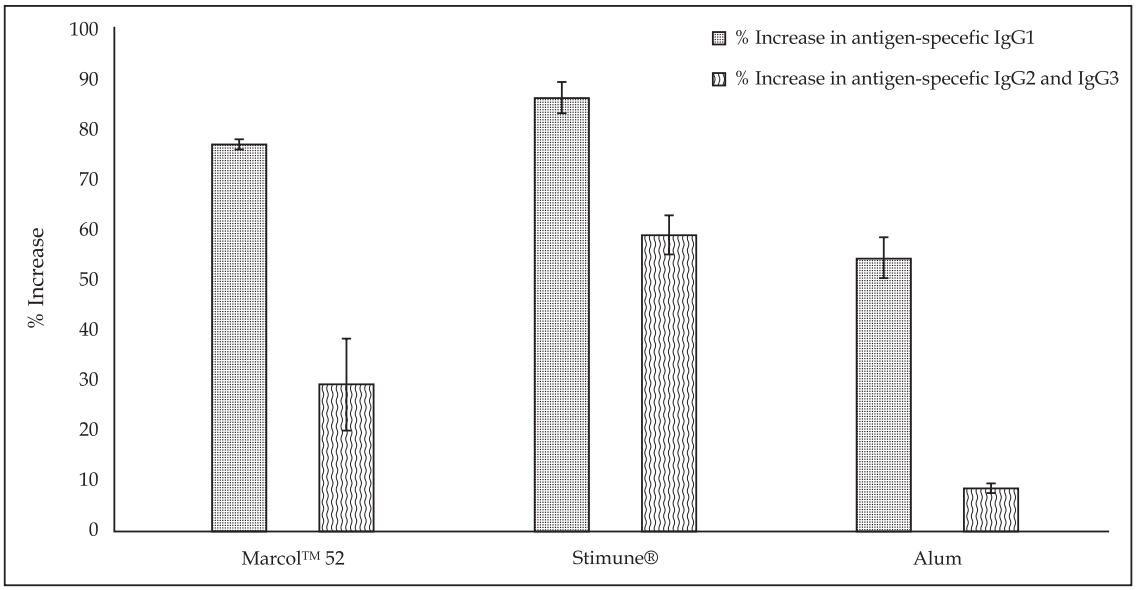

Fig 3. The % increase in antigen-specific camel IgG1 or IgG2 and IgG3 in serum samples after 21 days of immunisation with heat killed *P. acnes* in three different adjuvants. Antibody production was measured using ELISA after dilution of serum samples of 1:1000. Per cent increase in antibodies isotypes was calculated using the following formula:

$$\%\text{Increase in IgG Isotype} = \left\{ \frac{\text{O.D reading of Post immune sample} - \text{O.D of pre immune sample}}{\text{O.D of Postimmune sample}} \right\} \times 100\%$$

Each bar corresponds to the mean value of duplicate measure and the error bars represent the mean ±SEM.

and Alum immunised camels were able to cause 28.46% and 10.15% fixation, respectively, whereas in Marcol™52 IgG2 fixed 3% only. On the other hand, in Marcol™52, IgG3 was able to cause almost 10% fixation comparing to 1.5% and 6% in Stimune® and Alum, respectively (Fig 5).

Discussion and Conclusion

This study was conducted to compare the safety and immunogenicity of vaccine made of *P. acnes* antigens mixed in three different adjuvants to be used in camels. The effect of adjuvants on modulation of conventional and HCAbs, as well as, complement fixation ability were also studied.

Stimune® which is water-in-oil adjuvant induced the highest reaction at site of injection. In addition, Stimune® provoked the immune response with the highest increase in antibody titre. This could be explained by the nature of the adjuvant. Water-

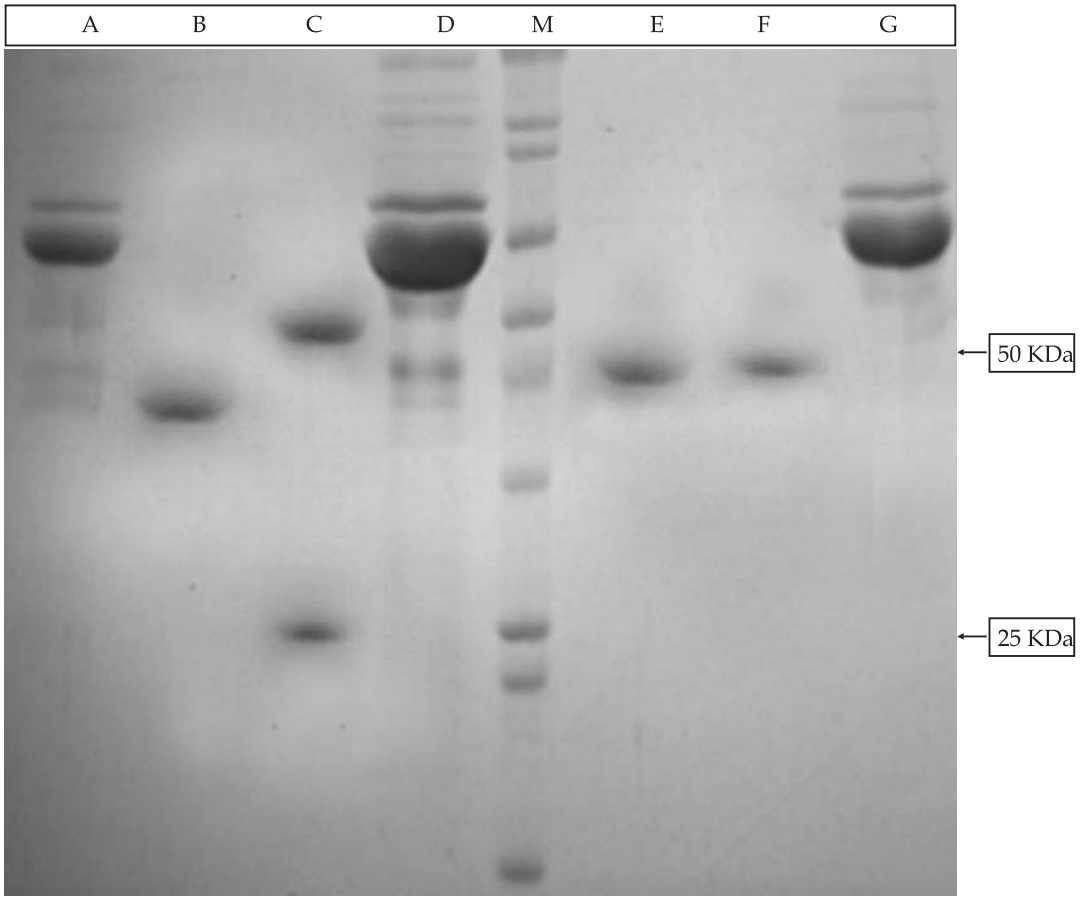

Fig 4. Reduced SDS-PAGE profile of Protein G and Protein A affinity of serum load and elution fractions. (A) denotes loaded serum, (B) denotes Peak A of PG (IgG3), (C) denotes Peak B of PG (IgG1), (D) denotes washout from PG, (E) denotes Peak C PA (IgG2), (F) denotes peak D of PA IgG3 and IgM, (G) denotes washout from PA and (M) denotes SDS protein marker.

in-oil adjuvants give antigens the long-lasting and slow release effect. Moreover, water-in-oil adjuvants give high opportunities of Ag epitopes detected by ELISA to be produced (Ten Hagen *et al,* 1993). Stimune® irritated the skin and tissues of site of injection which cause tissue damage and increase in the "damage –associated molecular pattern" DAMP (Danger hypothesis), this in turn will recruit more antigen presenting cells and activate more B cells and Th1 cells (Jounai *et al,* 2012). This might be caused by the proteins exposing reagents content in the formula of the adjuvant (As indicated by the manufacturer). Furthermore, Stimune® adjuvant leads to the highest increase in both the conventional and the HCAbs simultaneously.

Alum adjuvant was the safest adjuvant to be used in camels. But, the safest adjuvant induced the lowest modulation of the HCAbs (Fig 3). Although, the nature of Alum is thought to form the depots effect and cause antibody production (Cain *et al,* 2013). Different reasons could be behind the weak immune response of Alum, for example; Alum binds to antigens by electrostatic interaction and it's usually not as persistent as emulsions (Stils, 2005).

Marcol™52 increased the conventional IgG (76.9 ± 1.3 %) more than the HCAbs (29.18 ± 9.3 %). Marcol™52 has been reported as a potent adjuvant in animal vaccination trials (Singh and O'Hagan, 2003; Zhou *et al,* 2009). This might be due to the chemical composition of the saturated hydrocarbon and the oily nature of the adjuvant. Low viscosity of Marcol™52 makes administration of the vaccine easier and keeps local adverse effects at minimum. Oily adjuvant works as a vehicle carrying antigens and exposing them to the lymphocytes. This extends the period of antigen absorbent which leads to activation of B cell and increase the titre of antibodies.

HCAbs from all vaccines varies in their ability to fix complement system. These results could be explained by the molecular structure and stability of the binding site. HCAbs have the Fc region which is necessary as a C1q binding site of the classical pathway of complement system

(Dunkelberger and Song, 2010). Fixing and activation of the complement system plays a crucial role in the innate defence against pathogens and process of proteolysis (Dunkelberger and Song, 2010). This is favoured by antibody-dependent cell cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), opsonisation and phagocytosis for protection against intracellular pathogens, viruses and tumour (Saccodossi *et al,* 2012; Visciano *et al,* 2012; Mujic-Delic *et al,* 2014). Previous work focused on the ability of the HCAbs to fix the complement were published. A study carried out by De Simone and associates showed the anti-complementary activity (ability of antibody to fix the complement in the absence of antigens) of the heavy chain purified from llama (*Lama glama*) (De Simone *et al,* 2006). However, same group published a work later and proved the lack of complement fixation ability of HCAbs from llama after direct immunising with the sheep stromal antigens. Our results showed the ability of the HCAbs (Specifically IgG2) from camel serum to fix and activate the complement system, although the study proved that the C1q binding site is highly conserved in llamas HCAbs, it mentioned that the inability of the HCAbs in llamas to activate complement could be due to the hindrance of C1q binding site by the proximity of the variable domains (Saccodossi *et al,* 2012). This might not oppose with the previous works as molecular structure and hinge region of the dromedary HCAbs are different from llama.

Our results demonstrate the ability of HCAbs specifically; IgG2 to activate the complement system, but it also showed that IgG3 from Alum was a weak activator of the complement fixation (1.5%). Difference in the molecular structure of camel IgG2 and IgG3 could explain this difference. The long-hinge region in the IgG2 could be the reason for this variation (Nguyen *et al,* 1999). Still further research should be carried out to characterise the dromedary HCAb C1q binding site on a molecular base.

Overall, the data are compatible with the conclusion that different adjuvants caused variable

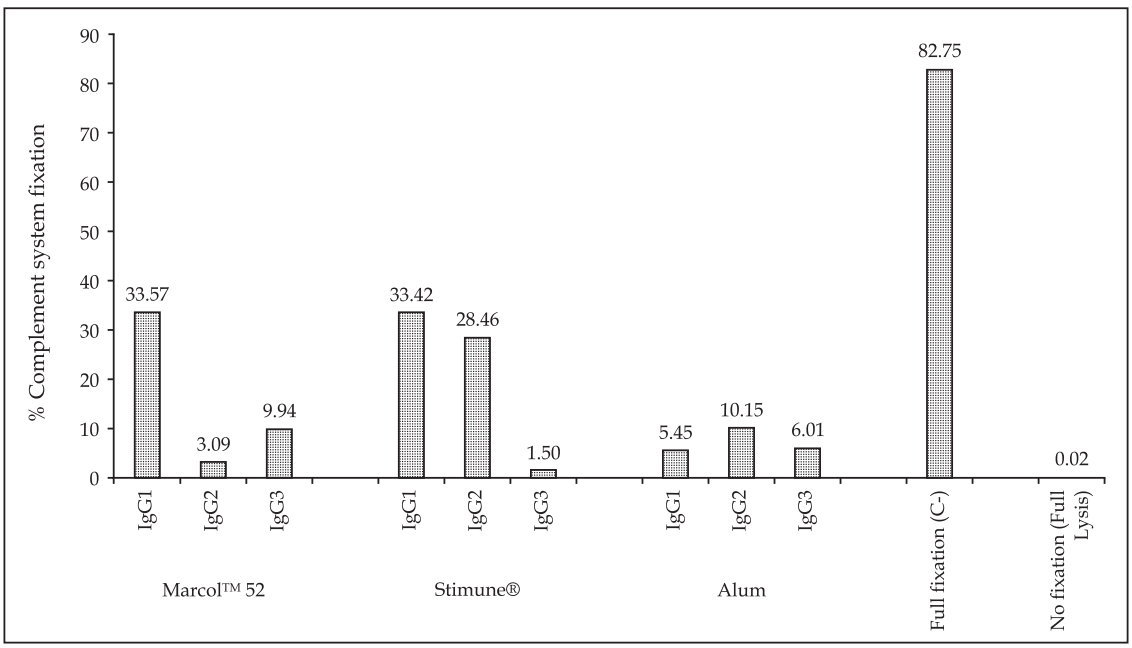

Fig 5. The complement fixation percentage of purified IgG fractions collected from camels injected with *P. acnes* mixed in three different batches each with different adjuvant. Fixation percentages were calculated using following formulas:

$$Preimmune\ Lysis\% = \left\{ \frac{O.D\ reading\ of\ pre\ immune\ sample}{O.D\ Full\ Lysis} \right\} \times 100\%$$

$$Postimmune\ Lysis\% = \left\{ \frac{O.D\ reading\ of\ post\ immune\ sample}{O.D\ Full\ Lysis} \right\} \times 100\%$$

Lysis % = preimmune Lysis % – post immune Lysis %.

Fixation % = 100 – Lysis %.

System control of 100% fixation was determined using no complement but bacteria and antibodies. Zero% fixation was determined by using full lysis with distilled water.

reactogenecity profile when used in camel. Also different adjuvants can modulate isotypes production and specificity. Alum is a preferable choice in camel vaccination as it has no reaction at site of injection and produce both neutralising and opsonising HCAbs. But still further improvement maybe taken to increase the response of Alum mixed vaccine. This might be through improving Alum efficacy as an adjuvant through combining Alum with a TLR agonist like Monophosphoryl lipid A (MPLA) or CpG motifs (Kool *et al,* 2012).

Acknowledgement

The study was totally funded by MONOJO.

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DOI : 10.5958/2277-8934.2016.00010.2 *Vol 23 No 1, p 73-80*

SACASG AND SACALG: NEW GFP-LABELLED CAMEL SKIN AND LUNG FIBROBLAST CELL LINES

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ABSTRACT

Camelids skin is claimed to respond differently under different physiological and pathological condition, however, there is no *in vitro* model with a reporter to investigate this claim. So generating camel fibroblast cell lines that expresses green fluorescent protein (GFP) would be important as a tool to monitor camel cell growth, migration and other processes. Hence, in this investigation, we created a GFP expressing fibroblast cell lines derived from a primary skin and lung fibroblast cell lines of the Arabian camel with a stable expression of GFP which was transfected using the pCAG-EGFP plasmid with CMV enhancer via Lipofectamine 2000 reagent. Stably transfected clones were selected by puromycin screening. The results revealed that camel fibroblasts can be efficiently transduced *in vitro* using pCAG-CMV-based vectors and that these vectors direct long-term transgene expression without evident toxicity, pathogenesis or alteration of native fibroblast morphology. Immunofluorescence staining showed no differences in the expression of fibroblast biomarkers between the transfected and non-transfected cell lines. The viability of thawed cells remained above 85% after cryopreservation in liquid nitrogen. The gene expression of the fibroblast markers was not different in the transfected cell lines. Taken together, we have established and fully characterised GFP expressing-fibroblast cell lines of Arabian camel. Based on our assays, we conclude that transfection of GFP into the Arabian camel skin and lung fibroblasts did not change their observed properties. The GFP-labelled cell lines may represent a new tool for convenient monitoring of live primary camel fibroblasts.

Key words: Arabian camel, fibroblast cell line, green fluorescent protein, tissue culture

Preserving the genetic traits of the Arabian camel by sequencing the complete DNA (Al-Swailem *et al*, 2010) and by establishing cell banks has been done previously (Ryder, 2002). Cell banks are extremely important for species preservation (Leon-Quinto *et al*, 2009) by preserving animals' semen, embryos, blood, tissue, cells, genomic libraries and cDNA libraries. Somatic cell lines can be produced from the cells at the bank and saved using cryopreservation (Li and Ma, 2012). Theses cell lines can be used in research and thus overcome the limitations of animal experiments by providing a practical, viable, and timely backup of genetic material. They also provide a versatile tool for virological, toxicological, epidemiological and animal cloning studies. Formerly, a camel skin fibroblast cell line named "DUBCA" was established and characterised (Kloprie*s et al*, 1995), however, that line was not labelled.

Labeling camel fibroblast cell lines would facilitate the study of chemotaxis, granulation tissue formation and other phenomena by tracing fibroblast migration and motility with high specificity and resolution (Grinnell and Petroll, 2010). The fibroblast cytological characteristics were assessed using fibroblast genetic markers; Vimentin (Goodpaster *et al*, 2008), fibroblast-specific protein 1 (FSP1) (Strutz *et al*, 1995), and prolyl 4-hydroxylase (Goodpaster *et al*, 2008). For more than 10 years, fluorescent proteins have offered a useful tool for live-cell imaging (Chalfie *et al*, 1994); the green fluorescent protein (GFP) gene isolated from the bioluminescent jellyfish *Aequorea victoria* (Prasher *et al*, 1992) was cloned to be used as a marker for gene activity. It has also been utilised for tagging subcellular compartments and proteins in living cells (Petrie and Yamada, 2012). Additionally, it is very indispensable for biochemical sensor applications and hence facilitates tracking of cells in tissues (Shaner *et al*, 2007) Therefore, steady GFP-expressing primary camel fibroblasts would be fundamental for studying camel cell growth and migration. Present study was therefore undertaken to establish a stable GFP-transfected Arabian camel

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skin and lung cell lines, SACASG and SACALG, respectively, as an *in vitro* model system for different cell processes. This model allowed cell visualising that did not require histological staining using fluorescent microscope.

Materials and Methods

Cell culture, skin and lung biopsies from healthy male *Camelus dromedarius* were collected from the slaughterhouse. The tissue samples were washed twice with phosphate buffered saline (PBS) containing ampicillin, gentamicin, kanamycin and ampicillin/streptomycin and necrotic, adipose and vascular tissues were discarded, then the samples were cut into small pieces. The fragments were then evenly placed in 6 well plate coated with 0.1% gelatin and cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (GIBCO) containing 10% foetal bovine serum (FBS), 2mM glutamine, 152µg/ml ampicillin, 76µg/ml kanamycin and 100U/ml ampicillin /streptomycin in 37°C and 5% CO_2 incubation condition until they reached 95% confluence and the skin and lung cell lines were considered the parental cell lines and named SACAS and SACAL. The confluent cells were then detached using 0.25% trypsin and subcultured at 1:3 ratio. Subculturing was repeated every 7-9 days.

GFP transfection of SACAS and SACAL– SACAL was transfected at P2 and SACAS at P3. A day prior to transfection, 1.5 x 10^5 cells were seeded into a new 6 well plate coated with 0.1% gelatin. SACAS and SACAL were transfected with 4 µg each of pCAG-EGFP plasmid using lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions, the transfected cells were cultured in serum-free DMEM/F12. The medium was changed 6 hours after transfection with DMEM-F12 containing 10% foetal bovine serum and 2µg/ml puromycin. The cells were examined 24, 48, and 72 h post-transfection using inverted fluorescent microscope to assess the transfection efficiency and GFP expression. The GFP-transfected SACAS and SACAL were named SACASG and SACALG.

Morphological Observation– SACAS, SACASG, SACAL and SACALG cells were cultured in culture chambers (Lab-Tek Chamber Slide, Nunc Naperville, Ill., USA) for 2 days and stained with Giemsa solution. Cell morphology was viewed under light microscope.

Measurement of cell numbers– Cells were detached with trypsin and then collected by centrifugation at 600 × g for 4 minutes and then resuspended in 500 µL PBS for counting after staining with trypan blue.

Immunofluorescence– SACAS, SACASG, SACAL, SACALG were allowed to grow overnight until they reached 60~80% confluence on sterile glass cover slips in 6-well plates. Then these were fixed with 4% paraformaldehyde for 10 min at room temperature. The primary rabbit anti-S100A4 (abcam) and rabbit anti-Vimentin (abcam) antibodies and the fluorescence-labelled secondary antibody FITC-donkey anti-rabbit IgG (abcam)) were used to identify fibroblast markers. The cover slips were rinsed with distilled water and sealed. To exclude non specific binding, staining with the primary antibody was skipped and the cells were incubated with secondary antibodies in some slides as a negative control. Nuclei were stained with Hoechst 33342 (Life technologies).

Assessment of cell viability and cell recovery after freezing– We evaluated SACAS, SACASG, SACAL and SACALG cell viability after cryopreservation using trypan blue (Gibco) staining method. The results were expressed as the percentage of living cells. The cryopreserved cells were resuspended in the culture medium and then were plated at 1 x 10^5 cells/well (48-well plate) after thawing and DMSO removal. After 5 days of culture, the cells were dissociated, re-suspended and counted.

Gene expression– To determine whether fibroblast markers genes were expressed in SACAS, SACASG, SACAL and SACALG, PCR amplification was employed. Total RNA was extracted from SACASG cells and SACAS cells by using TRIZOL reagent (Sigma) according to Chomczynski and Sacchi (2006) protocol. Cells were washed once with cold PBS, and then treated with 1 ml TRIZOL, followed by 0.2 ml of chloroform, and then cells were incubated at RT for 2 minutes. After incubation, the cells were centrifuged at 12,000 rpm for 10 minutes at 4°C. The aqueous phase was transferred to a new tube and 0.5 ml isopropyl alcohol was added to precipitate the RNA. Quantity and quality of the isolated RNA were measured using NanoDrop (ND-1000). One µg of the isolated RNA was used to synthesis cDNA with Reverse Transcription System (Promega). PCR was carried out in a 25µl solution. The Primers used (Table 1) were designed according to the data from the Arabian camel genome project. PCR Products were run in 1.2% agarose in sodium borate (SB) buffer with a 100-bp sized ladder.

Cell migration assay– The cell migration assay was utilised to assess cell migration (Rodriguez-

Table 1. List of primers used for PCR amplification.

No.	Primer	Sequence (5′-3′)
1.	FSP1	F – CGTACCCCCTGGAGAAGG R – ATTTCTTCCGGGGCTGTTTA
2.	Vimentin	F – GCTTCTCTGGCACGTCTTGA R – TTAGTCCCTTTGAGCGCATCC
3.	P4HA1	F – TCTAGCAAAACCGAGGCTGAG R – CAGTGGCTCCTCCTGCTAAC
4.	Beta-actin	F – TGACGATATTGCTGCGCTCG R – ACGTAACTAAGTCCGCCTAGAA

Menocal *et al*, 2012). In brief, SACAS, SACASG, SACAL, and SACALG were cultured in 10% FBS DMEM 6-well plates for 48 hours until they reached 70– 80% confluency. An 520 µm wide cell-free gap was created by scratching the monolayer using a sterile 200 µL pipette tip. Non-adherent cells were washed away by 2 washes with 1 mL DMEM. Serial digital images of the cell gap were captured and the number of cells emerged were counted at different time points.

Detection of microbial contamination– Antibiotic free media were used for testing for the presence of aerobic, anaerobic, facultative anaerobic bacteria and fungi. In short, prior to detection, SACAS, SACASG, SACAL and SACALG were cultured in antibiotic-free media until they reached 60-70% confluency and then the media were visually observed for turbidity.

Statistical analysis– Data were expressed as mean ± standard error of the mean. Unpaired numerical data were compared using the unpaired t test (2 groups) or ANOVA (more than 2 groups), with statistical significance at $p \leq 0.05$.

Results

Generation of GFP-labelled Arabian camel Fibroblast primary cell lines

A primary cell culture was established from the samples using primary explant technique. The primary culture system of SACAS and SACAL contained cells of mixed origins, including epithelial, spindle-shaped, and fibroblast cells (Figs 1A and 1B). A cell monolayer was formed on day 13-15 after explanation. Afterwards, the 1st subculture was conducted at a 1:3 split ratio, some plates were transfected with GFP and then cultured. All early passages (P2-P6) of the cell cultures shared very similar growth patterns, and reached 90% confluence within 7 d (Figs 1C, 1D, 1E and 1F). To assess whether GFP expression in the fibroblast would impact the population growth of SACAS and SACAL, fibroblasts were detached from the culture dishes at different time points and cell number was quantified. The cell number was expressed as a per cent of the day 0 count. The cell number gradually increased in both nontransfected SACAS, SACASG, SACAL, and SACALG during 4-day period, with no significant differences between the 4 cell lines (Figs 1G, H; $p \leq 0.10$). PCR was performed to differentiate the expression of fibroblast specific markers FSP1, Vimentin, and prolyl 4-hydroxylase in transfected and nontransfected cell lines. The result revealed similar expression of the 3 fibroblast specific genes in SACAS, SACASG, SACAL and SACALG (Fig 1i).

Stable GFP expression

Fig 2 shows the GFP expression in SACASG and SACALG under the fluorescent microscope. All transfected cells culture expressed GFP localised to the cytoplasm. GFP expression remains stable from early passage (Figs 2B and F) throughout subculturing until late passage (P26-P32) (Figs 2D and H). This visible GFP expression throughout the transfected cell populations hinted toward the efficiency of the transfection process.

Positive cell lines for fibroblast specific markers

To detect the Vimentin and S100A4 (Fibroblast specific markers) expression in SACAS, SACASG, SACAL, and SACALG cells, these were immunostained for Vimentin and S100A4 using indirect immunofluorescence. A fluorescence-labelled secondary antibody FITC-donkey anti-rabbit IgG (green, red fluorescence) was used. The nucleus was counterstained with Hoechst. As shown in Fig 3, red fluorescence was observed in SACAS and SACASG while green fluorescence was only observed in SACASG (Fig 3).

Effect of cryopreservation for GFP transfected and non-transfected cell lines

To detect the effect of cryopreservation, 2 different cell lines were utilised. SACAS and SACASG cells from early passages and SACAL and SACALG from late passages. The percentage of living cells in the whole cell population is shown in day 0 and day 5 (Figs 4A and B). For both cell lines no significant difference in cell viability was observed.

Similar cell migration ability with GFP transfected and non-transfected cell lines

We wanted to examine whether plasmid transfection and GFP expression would influence the fibroblast's ability to migrate in the culture dish. The scratch gap distance (in microns) between

nontransfected SACAS, SACASG, SACAL, and SACALG (Fig 5) was compared. There was no significant difference in gap distance crossed at 0, 3, or 9 hours between the nontransfected vs. SACASG and SACALG (n=3,p ≤ 0.05, ANOVA).

Discussion

The Arabian camel (dromedary) needs establishment of cell lines which would provide an optimal *in vitro* model for many applications in which the primary cell culture can be used as an alternative to whole animal experiments. Cell lines such as Dubca have been successfully established for the Arabian camel (Kloprie*s et al*, 1995). However, this cell line was lacking reporter labeling hence the green fluorescent protein gene, cloned from the bioluminescent jellyfish *A. Victoria* (Prasher *et al*, 1992) was selected. GFP is unique as it doesn't require any other Aequorea proteins, substrates, or cofactors to fluoresce (Yang, 1996; Moss *et al*, 1996). Its molecular weight is 27 kDa and its cDNA encodes a 283 amino acid monomeric polypeptide. It was initially used to identify metastatic cells in freshly collected tissue samples (Yang *et al*, 1998) and cell motility within primary tumours in the mammary fat pad was studied (Farina *et al*, 1998). Its fluorescence and expression was enhanced in mammalian cells and thus it became more useful for

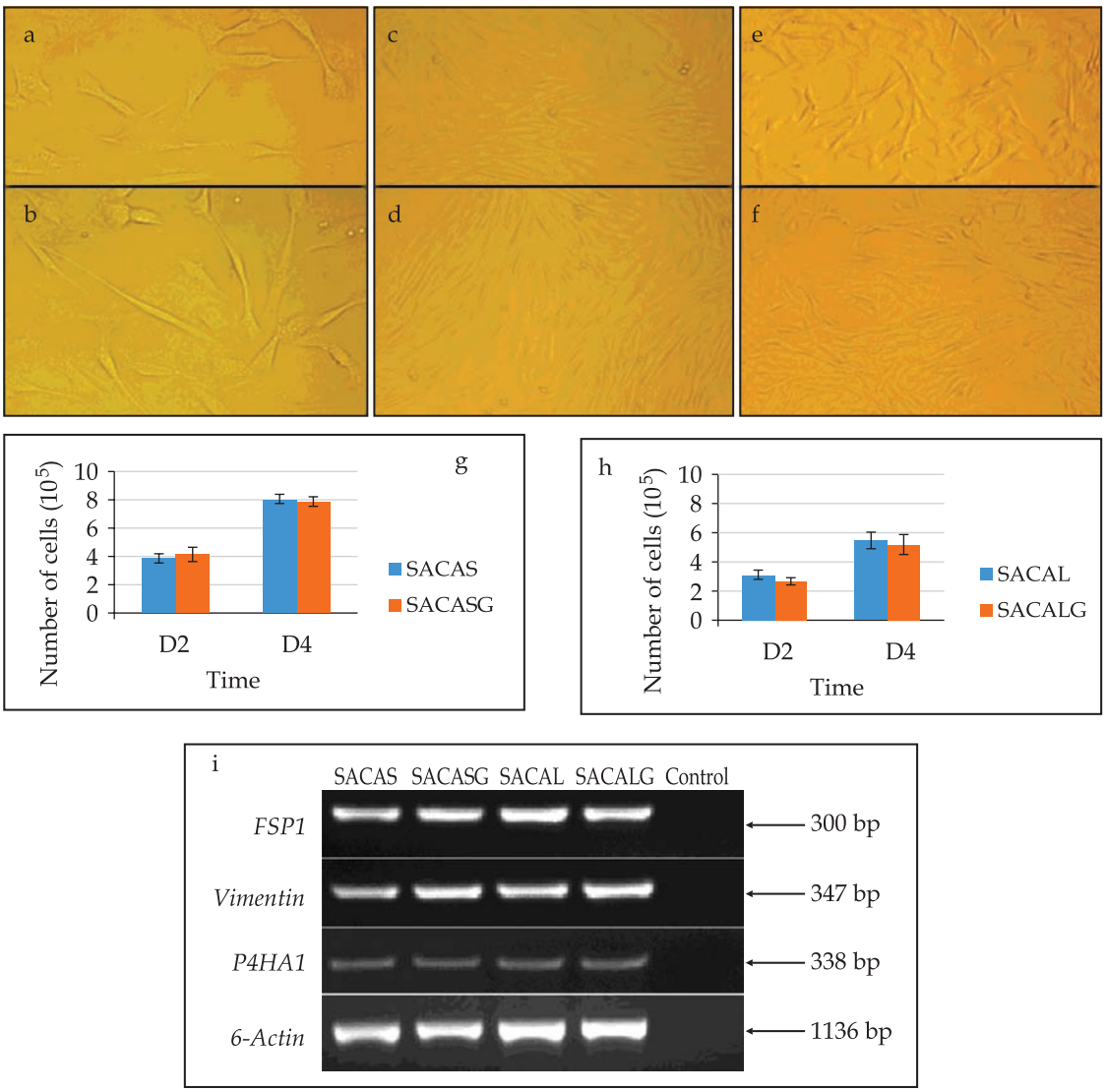

Fig 1. Characterisation of camel fibroblast cells (SACAS) and (SACAL). (a,b) SACAS and SACAL exhibited either epithelial, spindle-shaped or fibroblast morphology in (40X). (c,d) SACAS and SACAL prior to passage at 80-90% confluent (20X). (e,f) SACASG and SACALG prior to passage at 80-90% confluent (20X). (g,h) Cell number in non transfected SACAS and SACAL vs. SACASG and SACALG. (i) PCR analysis of the expression patterns of fibroblast specific genes; beta-actin was used as endogenous control (n=3, p ≤ 0.05).

in vivo imaging studies (Cubitt *et al*, 1995). To our knowledge, GFP-transfected SACAS and SACAL cells had never been developed before. Hence, in this study, we demonstrated for the first time, the feasibility of stable GFP expression in SACAS and SACAL transfected with a plasmid vector for use in tissue culture systems. Labelling SACAS and SACAL with GFP enables us to observe and monitor living cell morphology under fluorescence microscope and consequently facilitate *in vitro* testing. Moreover, it can advance real time tracking of cells while they engage in different processes (Wang *et al*, 2008).

Viral vectors offer high transfection efficiency thus these are widely used to deliver genetic material into cells. However, they haphazardly integrate into the host genome, which may disrupt vital genes, set off oncogenes or interrupt tumour suppressor genes and therefore, may lead to an inflammatory reaction and an insertional mutation (Woods *et al*, 2003). So, we chose to use the lipofectamine system to deliver GFP to both SACAS and SACAL. A potential shortcoming of using the Lipofectamine in large-scale operation is the poor transfection efficiency in prolonged high density culture (Codamo *et al*, 2011). However, it is credited with no mutagenesis, no restriction on the size of the packaged nucleic acid, low cytotoxicity and no extra-carrying DNA.

In the current study, there were no differences in cellular morphology, cellular migration, and cellular population growth between SACAS, SACAL and SACASG, SACALG. In addition, the cell survival rate after cryopreservation is regarded as an indicator, whether or not GFP transfection was safe. Our results showed that more than 85%

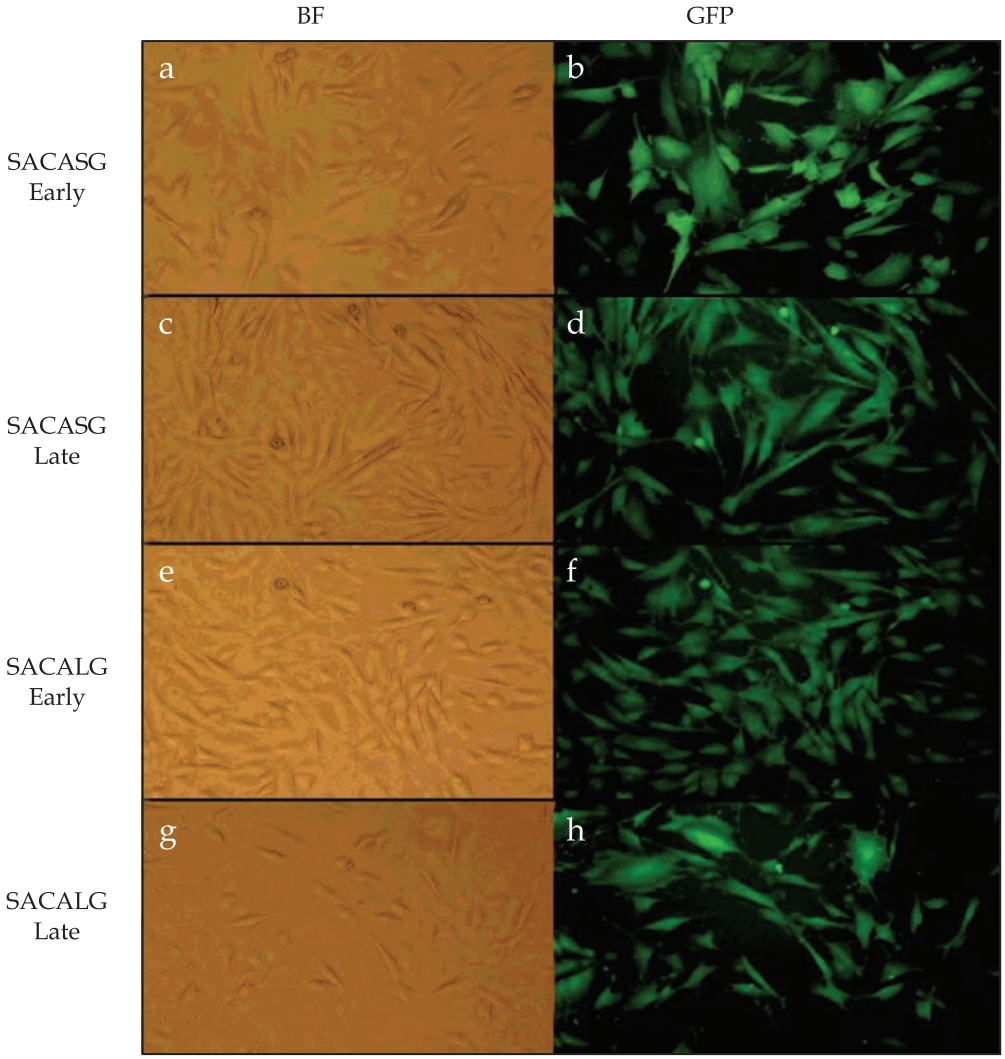

Fig 2. Expression of GFP in SACASG and SACALG under the fluorescent microscope (20X). (b,f) GFP expression in SACASG and SACALG at early passage. (d,h) GFP expression remains stable in late passage. (a,c,e,f) Phase contrast images for the transfected cell lines.

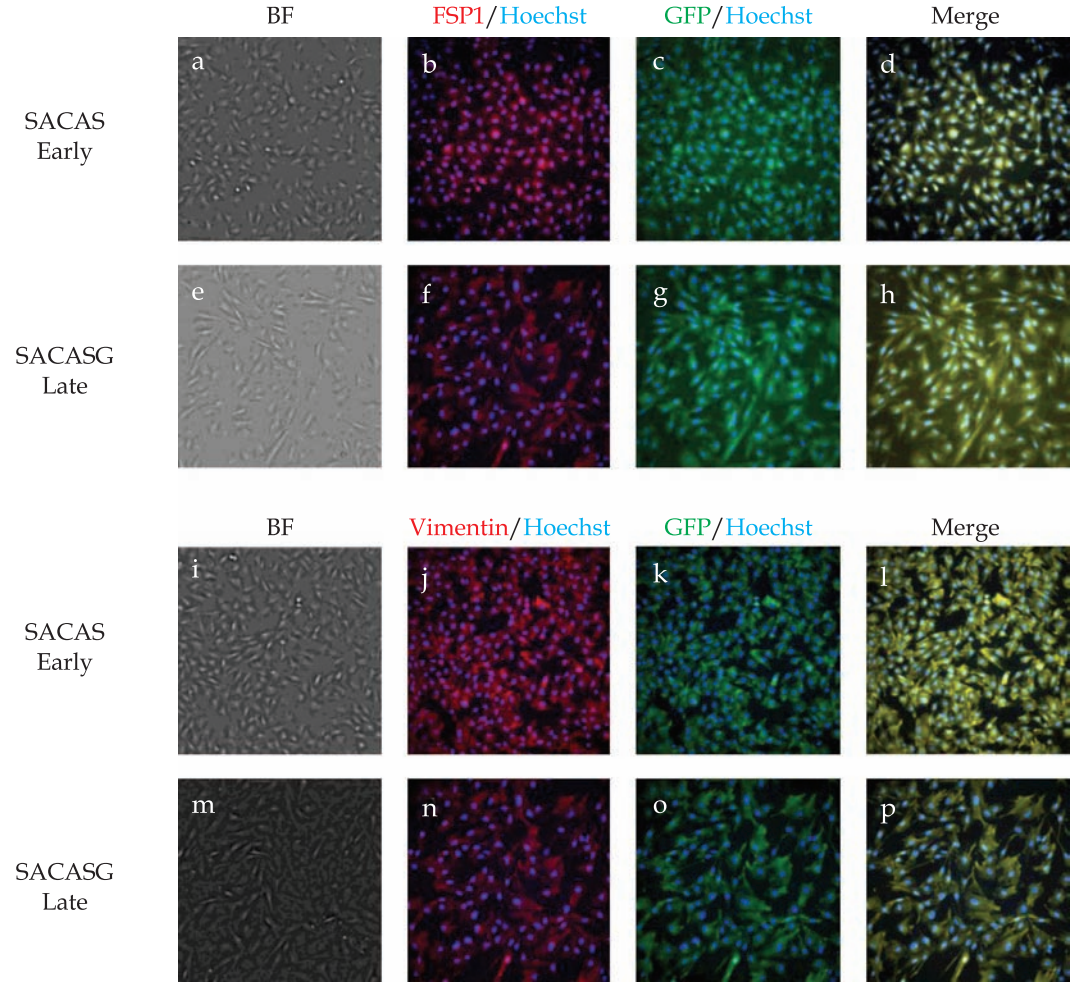

Fig 3. Immunofluorescence for FSP1 (red), Vimentin (red) and cell nuclei labelled with Hoechst (blue). Distribution of fibroblast markers and GFP expression in SACAS (a-d and i-l), SACASG (e-h and m-p) at early and late passages. (Scale bar. 30 µm).

of cells were alive immediately after thawing for both cell lines. Furthermore, the gene expression of 3 fibroblast markers was similar in nontransfected SACAS and SACAL and SACASG, and SACALG. Therefore, real time *in vitro* study of camel fibroblast physiology would be possible. The achievement of stable expression of GFP in SACAS and SACAL may set a role model for transferring several essential factors in cell reprogramming and generating iPS cells (induced pluripotent stem cells) that is central for Arabian camel stem cell research.

In spite of the apparent normality of the GFP-labelled SACAS and SACAL, we can't exclude the possibility that augmenting the degree of GFP expression within SACAS and SACAL will, at a certain point, interrupt the cellular physiology. Hence, using different assays or even boosting the sensitivity of assays used in our study might have identified a drawback of GFP expression. We could also use other surrogates for cell wellbeing such as cytokine release, gene transcription and protein synthesis. However, we chose to use cell functional proxies such as migration and cellular population growth because they require harmonisation of multiple cellular processes including cell wellbeing surrogates. Interestingly, these processes do not seem to be sufficiently disturbed to perturb the cell wellbeing surrogates. Therefore, we did not observe any impact of GFP transfection on camel fibroblast cell lines.

In conclusion, SACASG and SACALG were the 1st camel fibroblast cell lines that expressed GFP to our knowledge. Lipofectamine delivery of GFP to primary camel fibroblasts was an efficient method of generating steady expression of GFP in camel fibroblast cell lines. This GFP expression was exhibited by green fluorescence and did not show any deleterious effect on fibroblast phenotype. The availability of SACASG and SACALG may allow real time monitoring of cultured cells and consequently

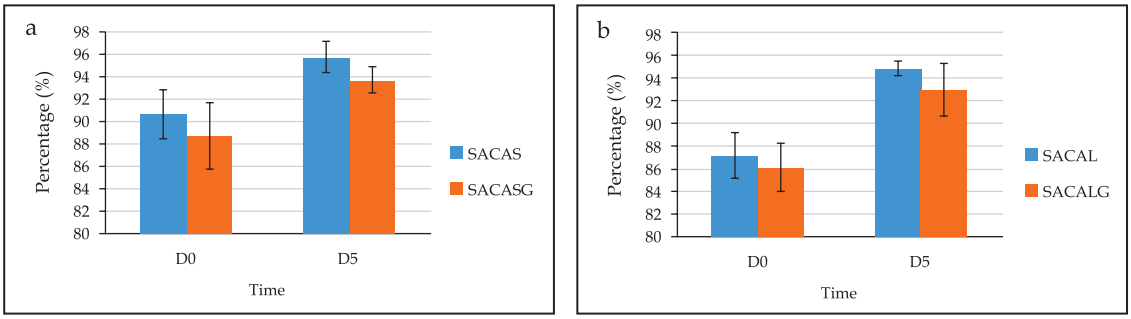

Fig 4. Percentage of cell viability after cryopreservation at day 0 and day 5. No significant difference was observed between GFP-transfected and non transfected camel (A) skin fibroblasts and (B) lung fibroblasts (n=3, $p \leq 0.05$).

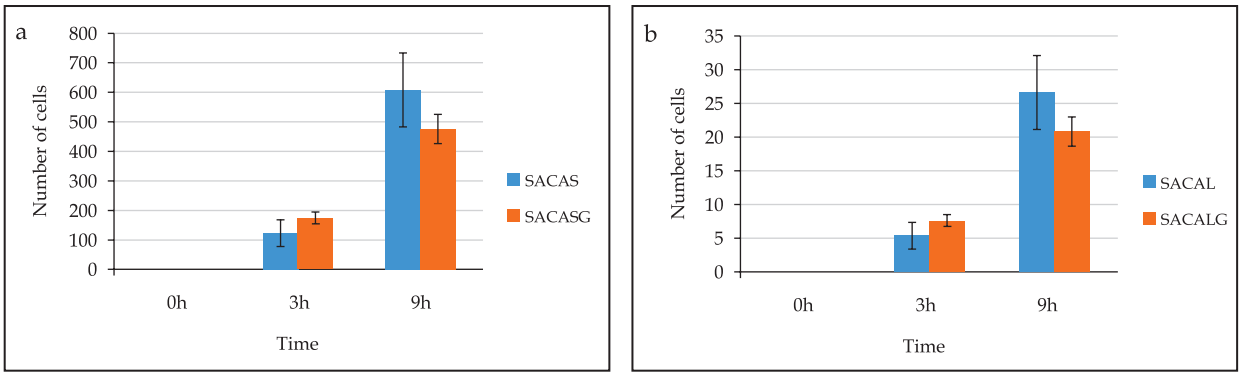

Fig 5. Migration of nontransfected vs green fluorescent protein-labelled camel (A) skin fibroblasts (SACAS) and (B) lung fibroblasts (SACAL). No significant difference was noticed after 3 and 9 hours in both cell lines (n=3, $p \leq 0.05$).

enhances the study of cellular and gene functions of camel fibroblast cell lines in different culture systems.

Acknowledgements

This study was supported by National Centre for Stem Cell Technology (NCSCT), King Abdulaziz City for Science and Technology (KACST), KACST 33-837. The authors also gratefully acknowledge the financial support from KACST under NSTIP grant (no. 32-685). Special thanks are due to SAAD Research and Development Centre at Saad Specialist Hospital for the help.

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DOI : 10.5958/2277-8934.2016.00011.4 Vol 23 No 1, p 81-84

CAPSULAR TYPING OF *Staphylococcus aureus* ISOLATES FROM CAMEL AND OTHER DOMESTIC ANIMALS USING DUPLEX POLYMERASE CHAIN REACTION

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ABSTRACT

The present study reports successful development of duplex PCR to detect *cap5K* and *cap8K* genes in single PCR reaction in 102 *S. aureus* isolates obtained in our study. The *cap5K* gene was detected in 54 (52.94%) isolates with single amplicon of 361bp, *cap8K* in 33 (32.3%) isolates with 173bp amplicon, both genes were detected in 12 (11.76%) isolates with both amplicons whereas 3 isolates (one each from dog, buffalo and cattle) were nontypeable. Among 8 camel isolates, 5 were found to be positive for *cap5K* gene, 2 isolates were positive for *cap8K* gene and one isolate with both genes.

Key words: Camel capsular gene (*cap5K* and *cap8K*), duplex PCR camel, *Staphylococcus aureus*

Phagocytosis is an important process of host defense system but *S. aureus* has developed many strategies to escape phagocytosis. Capsular polysaccharides (CP) possessed by *S. aureus* are considered to be important virulence factor as antiphagocytic and enables the organism to survive during course of infection. Of the 11 CP types, CP5 and CP8 are produced by majority of the human and animal staphylococcal infections. The cap genes are highly conserved and the capsular polysaccharides have got antigenic specificity thus, the capsular polysaccharides CP5 and CP8 also offer promise as target antigens for a vaccine to prevent staphylococcal infections (O'Riordan and Lee, 2004). It has been demonstrated that mice immunised with antibodies to CP5 or CP8 had significantly reduced tissue bacterial burden 4 days after intra-mammary challenge with encapsulated *S. aureus* strains. Similarly, other studies have also shown that antibodies to capsular polysaccharides have some protective efficacies for preventing infections in experimental animal (Fatton *et al,* 1996; Tuchscherr *et al,* 2008).

The variable occurrence of *cap5K* and *cap8K* genes has been reported from different geographical regions in *S. aureus* from different sources (Sordelli *et al,* 2000; Salasia *et al,* 2004; Upadhyay *et al,* 2010; Khichar and Kataria, 2014). The simplex PCR method to study capsular genes (*cap5K* and *cap8K*) was developed by Verdier *et al* (2007) and also used by many researchers (Upadhyay *et al,* 2010; Khichar and Kataria, 2014; Yadav *et al,* 2015). However, this is labour intensive, time consuming and expensive method. This study was designed to develop duplex PCR to detect both capsular genes (*cap5K* and *cap8K*) in single PCR reaction and to detect variability in capsular genes (*cap5K* and *cap8K*) among *S. aureus* isolates from camel and other animal species.

Materials and Methods

Bacterial isolates

A total of 102 *S. aureus* isolates were obtained from different sources and identified with conventional methods (Cowan and Steel, 1975) and later confirmed genotypically using species-specific primer-1 (5'-ACGGAGTTACAAAGGACGAC-3') and primer-2 (5'-AGCTCAGCCTTAACGAGTAC-3') as per the method of Straub *et al* (1999) described for ribotyping based on 23S rRNA gene. Out of 102 isolates, 3 were from clinical infections of horse, 2 from nasal swab of healthy pigs, 8 from camel wounds, 6 from dog skin infections and 6 from sheep with metritis and pneumonia (3 each), 21 from milk of mastitic buffalo and 28 mastitc milk samples each from goat and cattle.

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Detection of cap5K and cap8K genes

Genomic DNA extraction was carried out from overnight grown bacterial cultures, by the method of Nachimuttu *et al* (2001) and DNA quantification was carried out by spectrophotometric measurements (Sambrook *et al*, 1989).

Standardisation of PCR

The primer set of Verdier *et al* (2007) was used for amplification of *cap5K* and *cap8K* genes following the protocol as mentioned below for representative 10 samples. The sequences for primers used were 5′-GTCAAAGATTATGTGATGCTACTGAG-3′ (Primer-1) and ACTTCGAATATAAACTT GAATCAATGTTATACAG-3′ (Primer-2) for amplification of *cap5K* gene and it were 5′-GCCTTA TGTTAGGTGATAAACC-3′ (Primer-1) and 5′-GGAAAAACACTATCATAGCAGG-3′ (Primer-2) for amplification of *cap8K* gene. The reaction mixture for individual PCR was prepared by mixing12.5µl Go Taq® Green Master Mix (2X) containing PCR buffer, dNTPs, taq polymerase and MgCl2, 0.5µl Primer-1 (25 pM/µl), 0.5µl Primer-2 (25 pM/µl), 3µl DNA template (25 ng/µl) and Nuclease free water to make 25µl total reaction volume. Amplification was carried out in a thermal cycler (Applied Biosystems) as follows: One initial cycle of amplification at 94°C for 5min, 25 cycle (denaturation at 94°C for 60 sec., primer annealing at 55°C for 60 sec and primer extension at 72°C for 60 sec), and final extension at 72°C for 7 min (Fig 1).

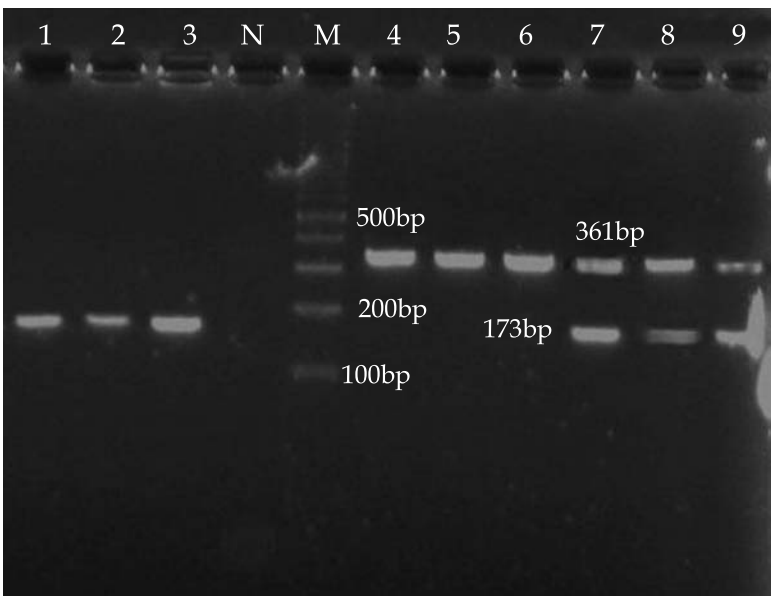

Fig 1. Development of duplex PCR for *cap5K* and *cap8K* genes detection. M-100bp Molecular Marker, N- Negative Control, N- Well No.1, 2, 3- isolates Positive for *cap8K* gene (Positive Control), Well No. 4, 5, 6- isolates Positive for *cap5K* gene (Positive Control), Well No. 7 and 8 Is mix of 1 & 4 and 2 & 5, respectively, Well No. 9-Isolate positive for both (*cap5K* and *cap8K*) genes.

Development of duplex PCR

A positive and a negative sample each for *cap5K* and *cap8K* gene in individual PCR was taken as positive and negative control, respectively. A positive sample for both genes was prepared by mixing 2 positive sample for each gene. The duplex PCR was developed by adding primers for both (*cap5K* and *cap8K* gene) genes together with following reaction mixture 15µl Go Taq® Green Master Mix, 2X, 1µl Primer-1 (25 pM/µl), 1µl, Primer-2 (25 pM/µl), 3µl DNA template (25 ng/µl) and Nuclease free water to make 25µl total reaction volume. Amplification was carried out as similar to simplex PCR with annealing 55°C for 60 sec (Fig 2) with positive and negative controls. Isolates which were found positive for both genes were further cross verified by simplex PCRs.

The PCR products were resolved in 1.2% agarose gels prepared in 0.5 x TBE buffer containing 0.5µg/ml of ethidium bromide and 100bp DNA ladder was used as molecular marker. The amplification products were electrophoresed at 80V. till complete resolving. The gel was then visualised and photographed under gel documentation system (ENDURO GDS).

Results and Discussion

Capsule production is an important virulence factor, which helps to survive the organism in the host by evading the host-immune system especially phagocytosis. Among 11 capsular serotypes, capsular types 5 and 8 have been found to be most common. However, there seems to be great variation in capsular type depending on source of isolation and geographical distribution of organism. In the present study duplex PCR was standardised and developed successfully (Fig 1) to detect both capsular gene in a single reaction.

Of the 102 *S. aureus* isolates, 3 isolates were detected not to possess *cap5K* nor *cap8K* genes and 99 isolates possessed either 1 of the 2 or both genes (Table 1). The *cap5K* gene was detected in 54 (52.94%) isolates, *cap8K* in 32 (32.35%) isolates and both in 12 (11.76%) isolates.

Out of the 8 isolates of the camel, 5 isolate were found to be positive for *cap5K* gene with single amplicon of 361bp, 2 isolates for *cap8K* gene with 173bp amplicon, one isolate with both

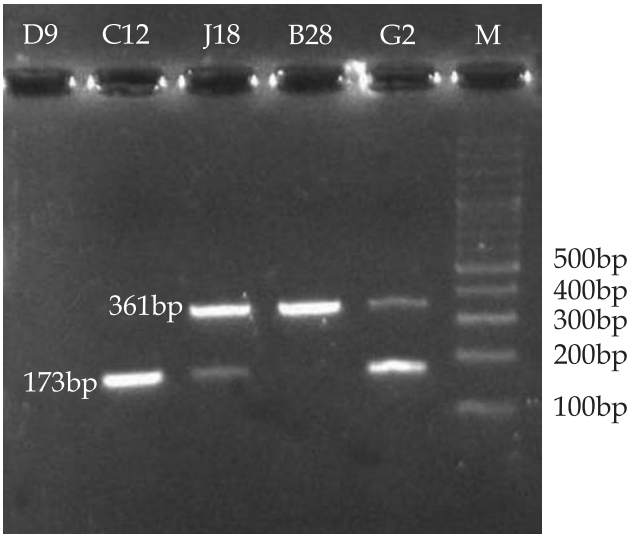

Fig 2. Detection of *cap5K* and *cap8K* genes by Duplex PCR in *S. aureus* isolates. D9- Non typeable dog Isolate, C12- Cattle Isolate positive for *cap8K* gene, J18 & G2- Camel and Goat Isolate positive for both *cap5K* and *cap8K* genes. B28- Buffalo Isolates positive for *cap5K* gene, M- 100bp Molecular Marker.

genes and none of the isolate was found to be non-typeable (Fig 2). None of the dog isolate carried *cap8K* gene.

A variable prevalence of *cap5K* and *cap8K* possessing *S. aureus* isolates from various sources have been reported by various workers from different geographical locations. Salasia *et al* (2011) reported that most of the isolates from bovine origin harboured *cap5* (74%), while most of the isolates from humans harboured *cap8* (91%) and isolates from food sources were positive for *cap5* (100%) and *cap8* (64%). They also found that both *cap5* and *cap8* genes were present in 7 of the 19 bovine isolates, 2 of the 11 human isolates and 7 of the 11 food origin isolates with overall 39% prevalence among 21 isolates.

Reinoso *et al* (2008) reported 21 (47%) isolates to be positive for *cap5* (11 human, nine bovine and 1 food sample strain), 7 (15%) for *cap8* gene (4 human and 3 food sample strains) while 17 (38%) isolates were non-*cap5* or *cap8* among 45 isolates from humans, bovine subclinical mastitis and food samples. Similar to our results, Khichar and Kataria (2014) also reported that *cap5K* gene was more prevalent in the isolates of bovine mastitic samples wherein they found 26 isolates (92.86%) with *cap5K* gene and 2 isolates (7.14%) with *cap8K* gene.

Our findings corroborated the earlier observations of Xu *et al* (2015) who reported *cap5* and *cap8* genes in 46.4% and 39.3% isolates, respectively among 28 isolates from mastitic milk samples of cow. Yadav *et al* (2015) found 68.75% isolates to carry *cap5K* gene and 21.87% isolates with *cap8K* gene whereas, 3 isolates (9.37%) were found nontypeable among 32 isolates from milk of cattle and buffalo with clinical mastitis. Similarly, Nathawat *et al* (2015) reported 68.38% *S. aureus* isolates with *cap5K* gene and 34.61% with *cap8K* while one isolate was detected nontypeable among 27 isolates from milk samples of goat with mastitis. The simplex PCR to detect *cap5K* or *cap8K* gene has been used by various workers over the periods in different laboratories. In the present study, the duplex PCR was developed to detect both the capsular genes in a single reaction which will reduce labour, cost and time in profiling of *S. aureus* for these 2 genes.

Acknowledgements

The authors are thankful to the Dean, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal sciences, Bikaner for the basic facilities provided to accomplish the work. The first author is highly thankful to Department of

Table 1. Detection of cap genes of *S. aureus* isolates from different sources.

S. No.	Source	No. of isolate	cap genes (%)			
			cap5K (361bp amplicon)	*cap8K* (173bp amplicon)	Both *cap5K* and *cap8K*	Non-typable
1.	Horse	3	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)
2.	Pig	2	1 (50.0)	1 (50.0)	0 (0.0)	0(0.0)
3.	Camel	8	5 (62.5)	2 (25.0)	1 (12.5)	0 (0.0)
4.	Dog	6	5 (83.3)	0 (0.0)	0 (0.0)	1 (16.7)
5.	Sheep	6	5 (83.3)	1 (16.7)	0 (0.0)	0 (0.0)
6.	Buffalo	21	8 (38.0)	9 (42.9)	3 (14.9)	1 (4.2)
7.	Goat	28	18 (64.2)	5 (17.9)	5 (17.9)	0 (0.0)
8.	Cattle	28	12 (42.9)	13 (46.4)	2 (7.1)	1 (3.5)
Total		102	54 (52.94)	33 (32.35)	12 (11.76)	3 (1.9)

Isolates D9, B55 and C34 were non-typable for both *cap5K* and *cap8K* genes.

Science and Technology, New Delhi for their financial assistance in the form of Inspire Fellowship (IF130331) under grant no. DST/INSPIRE Fellowship/2013/800, dated 28 May 2013.

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DOI : 10.5958/2277-8934.2016.00012.6 Vol 23 No 1, p 85-89

EFFECTS OF MANUAL UDDER STIMULATION ON MILK PARTITIONING AND FLOW TRAITS DURING THE MACHINE MILKING IN DAIRY CAMELS (*Camelus dromedarius*)

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ABSTRACT

A total of 9 multiparous dromedary camels in late stage of lactation (287±8 DIM; 3.8±0.8 kg/d) were used to study the effect of manual udder stimulation on machine milking efficiency of dairy camels under intensive management condition. Experimental design consisted of 3x3 Latin square with 9 animals allocated randomly and equally to 3 treatment (T_1=60 s, T_2=90 s and T_3=120 s). All camels were machine milked twice daily. Milk yield and milk flow parameters were recorded by Lactocorder® milk meters. Udder health was evaluated by California mastitis test (CMT) and somatic cell counts (SCC). Cisternal milk was determined 14 h after milking using Atosiban®. Volumes of machine milk (MM), machine stripping milk (MSM) and residual milk (RM) were recorded in duplicate. No subclinical mastitis was detected during the experimental period as indicated by the CMT (<1) and SCC (279×10^3±58 cells/mL). Camels were characterised by relatively small cistern (8.84±3.10%). There was a large variation in the proportion of RM (average= 25.1± 10.2%; max: 83.4%, min: 2.1%) between camels due to the duration of udder preparation. Consequently, camels were classified into easy milked camels (G_1: RM<25%) and hard milked camels (G_2=RM>25%). The increase of udder stimulation from T_1 to T_3 decreased ($p<0.05$) the lag time (LT) (3.83 to 2.24 sec) in camels of G_1 and the MSM (26.6 to 14.8%) as well as RM (46.1 to 31.4%) in camels of G_2. Bimodal curves tended (P=0.08) to decrease from 43.6% to 28.1% when duration of udder stimulation increased from T_1 to T_3 in camels of G_2. In conclusion, increase duration of manual udder stimulation to 90-120 s ameliorate the machine milking efficiency in harder milked camels at late stage of lactation.

Key words: Camels, cistern, machine milking, milk partitioning, udder stimulation

Milking routine in dairy camels differs significantly from that used for cows (Atigui *et al,* 2014a). Camels are known to be difficult to milk and many authors confirmed problems with disturbed milk ejection in this species (Yagil *et al,* 1999; Ayadi *et al,* 2009). Therefore, milk ejection can be induced by suckling or direct contact of the mother with calf. Nevertheless, in a large scale system, milking would be very difficult to manage if calves were present in the parlour (Juhasz and Nagy, 2008). For this reason, the pre-milking stimulation is of extreme importance for milk ejection removal in camels.

Milking routine and characteristics of milking cluster can affect udder health and the machine milking ability of dairy camels. Therefore, increasing the amount of machine milk (MM) and reducing the amount of residual milk (RM) is essential to ameliorate the machine milking efficiency in dairy camels. The RM represented 20% of total milk when milking clusters were attached immediately and 62% when milking were delayed for 4 min (Atigui *et al,* 2014b). Decrease of proportion of RM from 44.1 to 29.8% was observed when camels were milked with 50 kPa vacuum level and 60 pulsations/min (Ayadi *et al,* 2014). The consistency and duration of udder preparation are critical factors in milking efficiency in dairy ruminants (Labussiere, 1999). Twenty seconds of tactile stimulation is sufficient to elicit oxytocin secretion in dairy cows (Bruckmaier and Blum, 1998) and up to about 120 seconds necessary to open sphincter in buffalo (Borghese *et al,* 2007). Nevertheless, No data are available about the optimal duration of udder pre-stimulation in dairy camels.

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In dairy camels, approximately 85 to 90% of milk is stored in the alveolar compartment of the udder and milk ejection from the alveoli is required during machine milking (Caja *et al,* 2011; Ayadi *et al,* 2013; Atigui *et al,* 2014a). Therefore, a sufficient udder pre-stimulation ensures higher milk flow rate and faster milking in dairy cows (Bruckmaier and hilger, 2001; Dzidic *et al,* 2004) and buffaloes (Ambord *et al,* 2010). According to Atigui *et al* (2014a), bimodal curve occur in 42% of milk flow patterns in camels suggesting that udder pre-stimulation could be useful in this species.

The aim of this study was to investigate the effect of duration of manual udder stimulation on machine milking efficiency in lactating dromedary camels maintained under intensive management condition.

Materials and Methods

Animal and management

A total of 9 multiparous dromedary camels (8-12 years old) in late stage of lactation (287±8 DIM; 3.8±0.8 kg/d) from Conservation and Genetic Improvement Centre (Al-Kharj district, Riyadh, Kingdom of Saudi Arabia) were used. The daily feeding routine for lactating camels includes *ad libitum* alfalfa hay and 3 kg/day/head of commercial pellets (Wafi®, ARASCO, Riyadh, Saudi Arabia). Camel had free access to fresh water. Lactating dromedary camels suckled their calves freely during the 1st month of lactation. Thereafter, the dams were introduced progressively to machine milking. Calves usually weaned at 12 month of age, leaving the dams to continue their lactation for another 6 months. Camels were machine milked twice a day (06:00 and 16:00 h) in a single-tunnel milking parlour equipped with medium-pipeline (1.8 metres) milking stalls and electronic pulsator (BouMatic, Itak Company, Riyadh, Saudi Arabia). The weight of the milking cluster and the diameter of the mouthpiece liners were 1.9 kg and 25 mm, respectively. The milking machine was set at 50 kPa, 60 pulses/min, and 60:40 pulsation ratio. The milking routine included: milk let-down by calves (without suckling); udder preparation (teat and udder washing and drying), machine milking, and final stripping by the calf.

At the start of experiment, all camels were diagnosed free of mastitis by California mastitis test (CMT). Milk samples (100 mL) were collected weekly at each milking and analysed for udder health. The CMT was performed using Bovivet CMT kit (Bovi Vet, Kruuse, Germany) and the SCC was determined as cells/mL using Fossomatic Minor somatic cell counter (Fossomatic 90, FOSS Electric, Denmark).

Milk fraction and milk flow traits

Experimental design consisted of 3x3 Latin square with 9 animals allocated randomly and equally to 3 treatment (T_1=60 s, T_2=90 s and T_3=120 s) during 6 days. The 1st treatment (T_1) consists of milking following a pre-milking stimulation of 60 s prior to cluster attachment. The 2nd (T_2) and 3rd treatment (T_3) were similar to T_1 but with manual pre-milking stimulation of 90 s and 120 s prior to cluster attachment, respectively. Stimulation consisted of for-stripping and manual massage of the teats and floor of the udder.

Milk fractions at evening milking were recorded in 2 different days as follows: machine milk (MM; the quantity of milk that was taken after the setting of teat cups until milk flow dropped below 0.100 L/min), machine stripped milk (MSM; the amount of milk that was taken by udder stripping with hands without removing the teat cups) and residual milk (RM); the milk amount that was taken by machine milking after an i.m. injection of synthetic oxytocin (20 IU/camel; Biocytocine, Laboratoires Biove, Arques, France). Based on the above measures, total machine milk (TMM = MM + MSM) and udder volume (UV = TMM + RM) were calculated.

Milk flows rates were recorded in 2 different days by using 2 electronic mobile milk flow meters (Lactocorder®, WMB, Balgach, Switzerland) specially calibrated to low milk flow rate (<0.05 kg/min). The following milking traits were determined: milk yield (kg; total milk yield per head from the beginning to the end of the morning milking), total milking time (min; total milking time from attachment of the cluster till their removal), average milk flow rate (kg/min; average milk flow rate during milk ejection time), peak milk flow rate (kg/min; peak milk flow rate during milk ejection time). The measurement of milk flow traits was made by the associated software lactopro® (version, 6.0.28). The lag time (min; time from cup attachment to the 1st drops of milk being observed) was also visually determined during the experimental periods.

Cisternal milk fraction

To study the milk partitioning between cisternal and alveolar udder compartments, a total of 9 late lactating camels were used 14 h after milking. At first, and to prevent undesirable milk let-down during

udder manipulation, each camel was intravenously injected with 10µg/kg BW of an oxytocin receptor blocking agent (Atosiban®, Tractocile, Ferring Middle East, Jordan). At a.m. milking, cisternal milk was evacuated by machine milking and recorded. After the atosiban injection and the cisternal milk determination, camels were received i.m. injection of oxytocin (20IU/camel; Biocytocine, Laboratoires Biove, Arques, France) to induce milk let-down in order to determine alveolar milk. Alveolar milk was obtained by machine milking and recorded.

Statistical analysis

Data were statistically analysed for least square means procedures using the Proc GLM of Statistical Analysis System (SAS version 9.1, SAS Inst. Inc., Cary, NC). All percentage values were transformed to arcsine before the statistical analyses. Pearson correlation coefficients between traits were also calculated. The level for statistical significance was set at ($p<0.05$).

Results

The effects of the duration of udder stimulation on milk partitioning in the udder and flow traits in easy and hard milked camels were presented in Table 1 and 2. The change of the duration of udder stimulation from T_1 to T_3 decreased ($p<0.05$) the MSM (from 26.6 to 14.8%) and RM (from 46.1 to 31.4%) in camels of G_2. Also increase of duration of udder stimulation from T_1 to T_3 did not change milk flow traits except of LT, decreased ($p<0.05$) from 3.83 to 2.24 sec in camels of G_1 (Table 1) and tended to decrease ($P=0.10$) from 3.95 to 2.94 sec in camels of G_2, suggesting that 120 sec of udder stimulation open the teat sphincter and drain cisternal milk more rapidly. The percentage of cisternal milk obtained in our study at 14-h milking interval ranged between 2.1 to 17.2%, and on average is 8.84±3.10%.

There is a large variation in the proportion of RM (average= 25.1 ± 10.2%; max: 83.4%, min: 2.1%) between camels according to the duration of manual udder preparation. Therefore, camels were classified into easy milked camels (G_1: RM<25%) and hard milked camels (G_2=RM>25%). Milk partitioning and flow traits according to the proportion of residual milk are shown in Tables 1 and 2. The LT and TMT did not differ between easy or hard milked camels. Nevertheless, average and peak flow rate were higher ($p<0.05$) by 32.5% and 37.5% in G_1 compared to G_2, respectively.

No subclinical mastitis was detected in any of the udders quarters during the experimental period as indicated by the CMT (<1) and SCC (279×103 ± 58 cells/mL). On average, MM (kg), MSM (kg), RM (kg), LT(sec), TMT(min), PFR (kg/min) and AFR (kg/min) were 2.71 ± 0.65; 0.56± 0.12; 1.22± 0.65; 3.36 ± 1.15; 4.34±0.73; 2.12 ± 0.25 and 1.04 ± 0.15, respectively.

Positive correlations were found between MM and milk flow characteristics ($r=0.20$ to 0.49; $P<0.05$), although RM was never related to milk flow rate. The TMT was negatively correlated with AFR ($r= - 0.24$; $P=0.014$).

Discussion

Somatic cell counts (SCC) in milk are considered as good indicators for detecting subclinical mastitis in camels (Aljumaah *et al,* 2011). No subclinical mastitis was detected during the experimental period. The obtained results herein were in the same range than those reported previously in camel milk under intensive conditions with different milking routine (Hammadi *et al,* 2010; Atigui *et al,* 2014a).

The proportion of MSM (17.1%) and RM (25.1%) recorded in our work were higher with those previously reported in dairy camels machine milked (Atigui *et al,* 2014a). The lag time observed in our

Table 1. Effects of duration of manual udder stimulation on milk partitioning and milk emission kinetics of lactating camels with low residual milk (RM < 25%).

	Duration of udder stimulation (SEC)		
	60	**90**	**120**
Milk fraction[1]			
MM, kg	3.30 ± 0.40	3.54 ± 0.43	3.30 ± 0.44
MM , %	79.3 ± 8.2	67.8 ± 8.3	80.8 ± 8.3
MSM, Kg	0.65 ± 0.11	0.47 ± 0.12	0.53 ± 0.13
MSM, %	19.5 ± 3.3	12.9 ± 3.4	12.1 ± 3.5
TMM, kg	3.95 ± 0.45	4.01 ± 0.50	3.83 ± 0.50
RM, kg	0.84 ± 0.26	0.87 ± 0.27	0.65 ± 0.31
RM, %	17.5 ± 1.9	17.8 ± 1.9	14.5 ± 2.0
UV, kg	4.79 ± 0.46	4.88 ± 0.50	4.48 ± 0.52
Milk emission[2]			
LT, sec	3.83 ± 0.41[a]	3.38 ± 0.44[ab]	2.24 ± 0.42[b]
TMT, min	4.41 ± 0.40	4.01 ± 0.43	3.70 ± 0.46
AFR, kg/min	1.17 ± 0.14	1.12 ± 0.16	1.22 ± 0.16
PFR, kg/min	2.37 ± 0.30	2.35 ± 0.33	2. 53 ± 0.35
Bimodality, %	39.3	34.6	32.5

[1]MM, machine milk; MSM, machine striping milk; TMM (MM+MSM), total machine milk; RM, residual milk; UV (TMM+RM), udder volume.

[2]LT, lag time; TMT, total milking time; AFR, average flow rate; PFR, peak flow rate

[a-d]Means in the same line with different letters were significantly different ($p<0.05$).

Table 2. Effects of duration of manual udder stimulation on milk partitioning and milk emission kinetics of lactating camels with high residual milk (RM > 25%).

	Duration of udder stimulation (SEC)		
	60	**90**	**120**
Milk fraction[1]			
MM, kg	1.79 ± 0.46	2.22 ± 0.47	2.64 ± 0.50
MM , %	73.4 ± 8.2	78.7 ± 8.4	85.1 ± 8.3
MSM, Kg	0.65 ± 0.27	0.60 ± 0.20	0.46 ± 0.20
MSM, %	26.6 ± 5.4[a]	21.3 ± 6.5[ab]	14.8 ± 4.6[b]
TMM, kg	2.44 ± 0.58	2.82 ± 0.56	3.10 ± 0.52
RM, kg	2.09 ± 0.61	1.84 ± 0.59	1.49 ± 0.55
RM, %	46.1 ± 9.2[a]	39.5 ± 8.4[ab]	29.6 ± 7.3[b]
UV, kg	4.53 ± 1.04	4.66 ± 0.87	4.59 ± 0.88
Milk emission[2]			
LT, sec	3.95 ± 0.44	3.71 ± 0.45	2.94 ± 0.43
TMT, min	5.29 ± 0.78	5.13 ± 0.73	4.24 ± 0.72
AFR, kg/min	0.65 ± 0.20	0.83 ± 0.17	0. 89 ± 0.15
PFR, kg/min	1.69 ± 0.28	1.28 ± 0.19	1.48 ± 0.17
Bimodality, %	43.6	33.2	28.1

[1]MM, machine milk; MSM, machine stripping milk; TMM (MM+MSM), total machine milk; RM, residual milk; UV (TMM+RM), udder volume.

[2]LT, lag time; TMT, total milking time; AFR, average flow rate; PFR, peak flow rate

[a-d]Means in the same line with different letters were significantly different ($p<0.05$).

work was similar with those previously reported in Saudi dromedary camels by Ayadi *et al* (2013), but shorter than observed by Hammadi *et al* (2010) on Maghrebi dromedary camels. The discrepancy between the current and previous results may be attributed to a direct consequence of using calves (without suckling) for inducing milk let-down in our case.

The increase of duration of udder stimulation from T_1 to T_3 decreased ($p<0.05$) the LT in camels of G_1 and tended to decreased ($P=0.10$) in camels of G_2, suggesting that 120 sec of udder stimulation open the teat sphincter and drain cisternal milk more rapidly. Our finding agrees with those previously obtained in buffalos in which up to about 2 min of tactile stimulation are necessary to open the hard teat sphincter muscle (Borghese *et al,* 2007). In dairy cows, less than 10 seconds is inadequate stimulus for consistent milk letdown response in all cows, and 20 seconds of tactile stimulation is sufficient to elicit oxytocin secretion in high producing cows (Bruckmaier and Blum, 1998).

Camel udder differs slightly from that of the bovine. Approximately, 90% of the milk is stored in the secretory tissue after a milking interval of 9-10 hours of camel and it is only possible to extract this alveoli milk with an active milk ejection (Ayadi *et al,* 2013). Therefore, it is imperative that this milk is removed as completely as possible by complete milk ejection and an efficient milking technique. The cisternal milk fraction varied according to species, breed, lactation stage, parity and milking intervals (Dewhurst and Knight, 1994). The percentage of cisternal milk obtained in our study was higher (8.8%) than that reported by Atigui *et al* (2014a) using Atosiban (3.8%). The discrepancy between the current and previous results in camels could be explained by the difference between breeds or between lactation rank, some camels being accustomed to be milked by machine milking for several parities. Animals that store large amounts of milk in the gland cistern generally produce more milk, milked faster and tolerate extended milking intervals (Dewhurst and Knight, 1994; Ayadi *et al,* 2003). The relatively small cistern size of our camels may impair milk secretion and subsequently the total milk yield especially, for late lactation and extended milking intervals (Ayadi *et al,* 2009). Recently, Atigui *et al* (2014a) reported very small volume of milk available in the udder before milk ejection, especially at late stage of lactation, require special settings and practice during machine milking. Therefore, insufficient udder and teat stimulation of empty udder will lead to incomplete milking in dairy camels.

The increase of the duration of udder stimulation from T_1 to T_3 decreased ($p<0.05$) the MSM and RM in camels of G_2. These results agree with those previously reported by Ayadi *et al* (2009), in which increase of udder stimulation before milking is recommended in dromedary camels to maximise machine fraction and to minimise stripping fraction during machine milking. Wernery *et al* (2004) showed that milking time was short when udder stimulation time was long (123 s). Nevertheless, our results disagree with those recently reported in camels by Atigui *et al* (2014b) in which manual udder pre-stimulation for 30 s is sufficient to ameliorate milk ejection and reduce bimodality in dairy camels. Our results proved that the response of camel to the increase in duration of manual udder stimulation varied according to the aptitude of camel to the machine milking. Therefore, hard milked camels respond better to the udder stimulation compared to easy milked camels. Further research need to confirm these results without the presence of calves at proximity of camels in the milking parlour.

Positive correlations were found between MM and milk flow characteristics. Similar results were observed in dairy camels by Atigui *et al* (2014b). A total of 54 milk flow curves were recorded. Bimodal curves occurred in 36.2% of total recorded milk flow patterns. These results agree with those recently reported in dairy camels (Ayadi *et al,* 2014; Atigui *et al,* 2014a). In camel of G_1 the milk ejection bimodal curves did not change according to the manual udder stimulation. However, the increase of manual udder stimulation from T_1 to T_3 in camels of G_2 tended to decreased (P=0.08) the bimodal curves from 43.6% to 28.1%, respectively. These results are in accordance with those previously reported in dairy cows (Bruckmaier and Blum, 1998; Dzidic *et al,* 2004) and buffaloes (Ambord *et al,* 2010), in which increase of udder pre-stimulation reduced the occurrence of bimodal curves. Therefore, the reduction of bimodality in dairy camels is a good sign of efficient machine milking.

The increase of duration of udder stimulation to 90-120 s ameliorate the machine milking efficiency in harder milked camels (RM>25%) at late stage of lactation. Other types of stimulation could be assessed like the distribution of concentrates in the milking parlour or, the presence or not of the calves at proximity.

Acknowledgements

Study was supported by the FAO project of improvement of research for sustainable development of camel production in the KSA (UTF/SAU/021/SAU) and the Deanship of Scientific Research at King Saud University, Saudi Arabia through the Research Group Project No. RGP-042.

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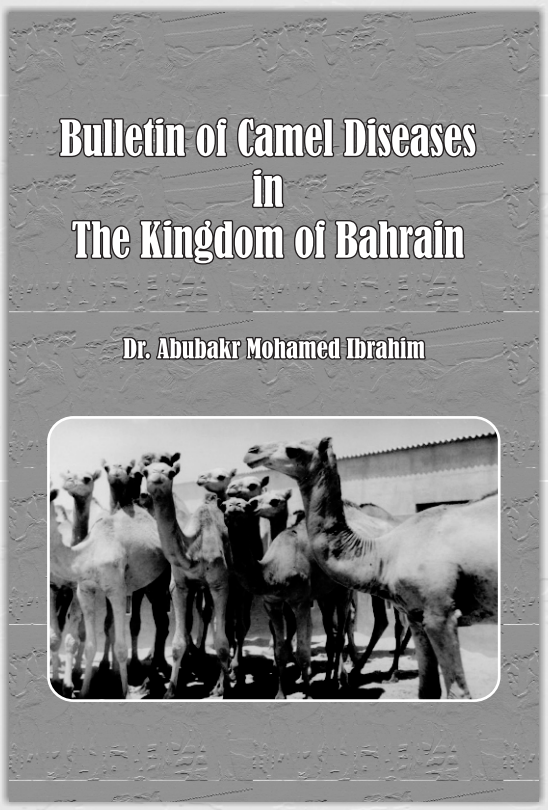
Bulletin of Camel Diseases in The Kingdom of Bahrain

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.



Editor:
Dr. T.K. Gahlot

Edition: 2014

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

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

Price: US $ 90
INR 1000

ISBN 81-903140-2-5

DOI : 10.5958/2277-8934.2016.00013.8 Vol 23 No 1, p 91-94

Eimeria leuckarti FROM DROMEDARIES CAMEL CALVES

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ABSTRACT

The current work reports *E. leukarti* from camel calves of a farm in Bikaner, India and makes some notes on the sporulation process, apart from the unsporulated oocyst morphology. Two out of the 34 faecal samples of camel calves under 2 years of age had unsporulated oocysts of *E. leuckarti*, which were recovered by sedimentation. Noteworthy, in the morphological study the identification of an enlarged region in the inner layer of the oocyst wall, always in the opposite end of the micropyle. The sporulation time recorded in the present study was 19 days at 37°C. Unexpectedly, some oocysts do not sporulate even after 30 days in appropriate conditions of sporulation at room temperature even when kept in petri dishes under aeration.

Key words: Bikaner, camel calves, coccidiosis, *Eimeria leuckarti*, oocyst, sporulation

Mainly 5 *Eimeria* species are believed to have capability of infecting the camel intestine (Soulsby, 1986; Kauffman, 1996), and several of these species are distributed widely with high prevalence rates among camels (Luckins, 1992). *Eimeria cameli* and *Eimeria dromedarii* are the most widely spread species of camelid *Eimeria*, and others (*Eimeria bactriani*, *Eimeria rajasthani* and *Eimeria pellerdyi*) are found in some particular geographical region. Several cases of coccidiosis causing enteritis and mortality rates of up to 10% in young camels have been reported in only a few reports such as Gruvel and Garber (1965). The study by Tafti *et al* (2000) indicated that the most important and frequent pathologic lesion in the digestive tract of camels are those resulting from *Eimeria* spp. infections (63% of 100 slaughtered camels). Young infected animals display haemorrhagic enteritis and diarrhoea. Animals with severe infections show signs of loss of appetite, dehydration and progressive weight loss. Dehydration and secondary infections can increase risk of mortality in camel calves (Kauffman, 1996). In severe *E. cameli* coccidiosis, camels died from general weakness were emaciated and most of them had passed bloody faecal drops. The parasites appear to be pathogenic to camel calves, causing destruction of the intestinal mucous membranes by their giant schizonts, while adult camels were found to be chronic shedders of oocysts without manifesting clinical signs (Mahmoud *et al,* 1998). Another research also concluded that older camels seem to be asymptomatic oocyst shedding carriers (Hussein *et al,* 1987).

Information on the occurrence *Eimeria leuckarti* in dromedary camel of India, is scarce. Present work documents the cases of intestinal coccidiosis caused by *Eimeria leuckarti* in camel calves.

Materials and Methods

Thirty four faecal samples were collected from camel calves under 2 years during the month of January and February, 2012 from NRCC farm. All the faecal samples were examined by McMaster egg counting slide and *Eimeria leuckarti* oocysts, were detected in 2 faecal samples. After 2 days, faecal samples were again collected directly from rectum of those 2 camel calves which were earlier found positive for *Eimeria leuckarti* oocysts and stored at 4°C.

Collected faecal samples from both the 2 camel calves were examined microscopically using sedimentation technique (Soulsby, 1982). The identification of *Eimeria leuckarti* oocysts was carried through following the key of Levine (1986) and Soulsby (1982).

After the faecal material was submitted to microscopic examination and *E. leuckarti* oocysts were confirmed, another 50 gm of faecal material were washed with distilled water, sieved and centrifuged at 2000 rpm for 10 minutes. The resulting sediment was placed on petri dishes containing thin layers (~5 mm) of 2.5% potassium dichromate solution, for

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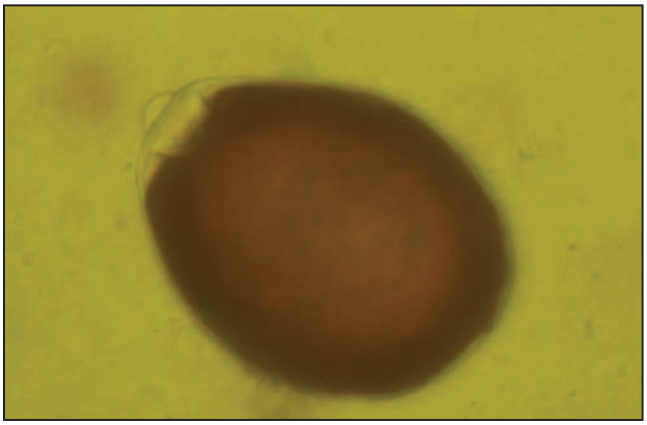

Fig 1. Unsporulated ocyst of *Eimeria leuckarti*.

sporulation of oocysts and further observation. The developmental analysis of *Eimeria leuckarti* oocysts 40X objective of trinocular microscope.

Results and Discussion

The identified unsporulated oocysts were ovoidal, thick, dark brown flattened with bilayered oocyst wall (Fig 1). The outer layer was dense, rough and large; while the inner layer was smooth and thin. The micropyle in the outer layer was prominent and easily distinguishable. The opposite end of the micropyle always showed a projection/enlargement in the inner layer, which was easily seen after the rupture of the outer layer (Figs 2a-d).

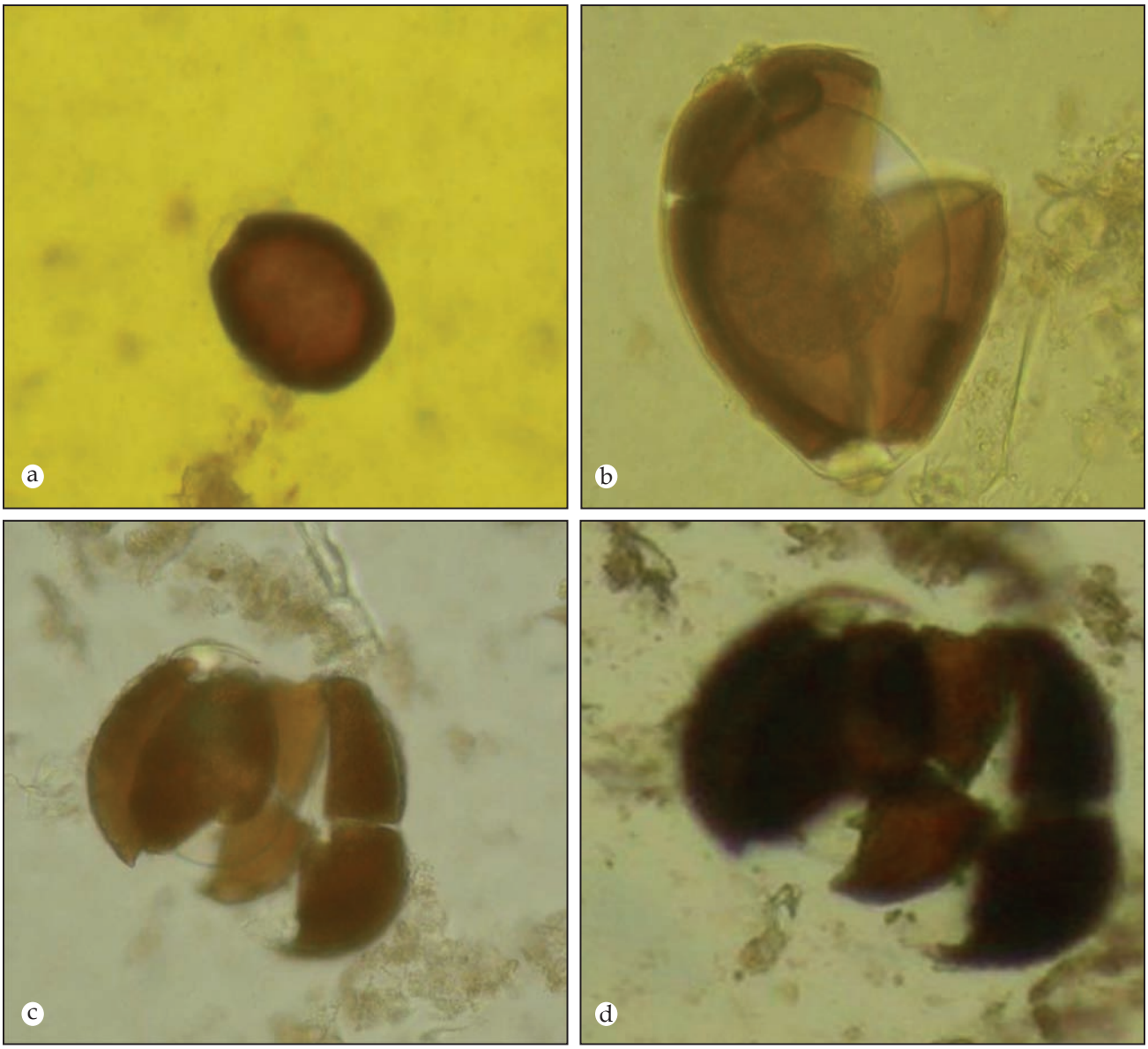

Fig 2. Sporulation of *Eimeria leuckarti* oocyst collected from camel calf faeces (a) Oocyst initiating sporulation containing sporozoites; (b) Sporulated oocyst showing start of fragmentation of external oocyst wall in four parts, exposing the inner oocyst wall; (c and d) Start of liberation of ellipsoid sporozoites.

The morphology of these identified unsporulated oocysts from camel calves were quite similar to those described by Sutoh *et al* (1975), Sheahan (1976), McQueary *et al* (1977), Bauer (1988), Lyons *et al* (1988), Reppas and Collins (1995), Hirayama (2002), Lyons and Tolliver (2004), Studzinska *et al* (2008), De Souza *et al* (2009), Kornas *et al (*2011) and Sudan *et al* (2013) in equine species. In the present work an enlarged region in the inner layer of the oocyst wall, always in the opposite end of the micropyle was observed which is similar with the findings of Barker and Remmler (1972). The sporulation time recorded in the present study was 19 days at 37°C. Unexpectedly, some oocysts do not sporulate even after 30 days in appropriate conditions of sporulation at room temperature. Sporulation time of *E. leuckarti* in horses is 20-22 days at 20°C (Soulsby, 1968; Pellerdy, 1965). By comparing the morphology of the oocysts reported by Dunlap (1970), Barker and Remmler (1972) and De Souza *et al* (2009), where the sporulation of the oocysts was observed, and sporoblast formed a subspherical compact mass within the oocysts, while in the present study the sporoblast was granulated and dispersed within the oocyst (Figs 1 and 2a).

Only 2 camels were found infected with *E. leuckarti* which was primarily an infection of Equidae. The present finding also reflected that there was no host restriction for this infection. The occurrence of natural infection of camel calves by *E. leuckarti* is probably influenced by the host individual susceptibility and access of pastures randomly contaminated by the Equine species.

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FORM IV

(See Rule 8)

1.	Place of Publication	:	Camel Publishing House, 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner 334001, India.
2.	Periodicity of its publication	:	Biannual
3.	Printer's Name	:	Smt. Leela Devi
	(Whether citizen of India)	:	Yes
	Address	:	Gahlot Kutir, Nagani Road, Bikaner (Raj.)
4.	Publisher's Name	:	Smt. Leela Devi
	(Whether citizen of India)	:	Yes
	Address	:	Gahlot Kutir, Nagani Road, Bikaner (Raj.)
5.	Editor's Name	:	Dr. T.K. Gahlot
	(Whether citizen of India)	:	Yes
	Address	:	Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Bikaner 334001 (India).
6.	Names and address of individual who own the newspaper and partners or share holders holding more than one per cent of total capital.	:	Dr. T.K. Gahlot 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner 334001 (India)

I, Smt. Leela Devi hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated : 01.06.2016

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DOI : 10.5958/2277-8934.2016.00014.X Vol 23 No 1, p 95-100

FIRST EVIDENCE OF NATURAL ANAPLASMOSIS IN *Camelus dromedarius* IN SAUDI ARABIA

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ABSTRACT

Ninety-six of 237 dromedary camels manifested fever, anorexia, diarrhoea, emaciation, pale mucous membranes, lacrimation, abortion and/or infertility. Parasitological examinations of blood and faecal samples were performed in all camels (n=237) using Giemsa-stained blood smears and standard flotation sedimentation techniques, respectively. Seventy-two of the clinically affected camels were diagnosed anaplasmosis with a 40.50% overall morbidity. The haematological analysis revealed significant reduction (P<0.01) in total RBC count, HGB concentration, HCT and MCV in the affected camels. Additionally, significant increases (P<0.01) in total WBC count, lymphocytes %, MCHC and platelets were observed. The biochemical analysis exposed significant reduction (P<0.001) in the iron level. Significant increases (P<0.01) in GGT, AST, ALT, total bilirubin, BUN and LDH blood levels were detected. The applied control measures succeeded in controlling anaplasmosis in affected herds. In conclusion, the successful in the control of camel anaplasmosis and its first diagnosis was achieved in Saudi Arabia dromedary camel.

Key words: Anaplasmosis, clinical, control, dromedary camel, haematobiochemical pictures

The Arabian camels (*Camelus dromedarius*) exhibit certain characteristic that enable them to survive famine, thirst, drought and produce on marginal resources in extreme climatic conditions (Bekele *et al,* 2011). Although, large numbers of ticks are often found on camels (Hamed *et al,* 2011; Nazifi *et al,* 2011), very few reports concerning tick-borne pathogens in camels have been published that describes the clinical and laboratory findings induced by natural theileriosis and babesiosis in *Camelus dromedarius* (Ismael *et al,* 2014; Swelum *et al,* 2014). In addition, *Theileria equi* and *Babesia caballi* in equine were identified by PCR in Jordanian dromedary (Qablan *et al,* 2012). Anaplasmosis is a tick-borne infectious disease associated with *Anaplasma* spp. that are obligate intra erythrocytic parasites belonging to the order Rickettsiales and infecting ruminants.The causative agent of anaplasmosis in cattle and wild ruminants is *Anaplasma marginale* while in sheep and goats is *A. ovis*. The disease is of great economic and veterinary interest worldwide (Radostits *et al,* 2007). The significant natural vectors for transmission are ticks in the family Ixodidae and flies in the family Tabanidae. Of the ticks, the one-host *Boophilus* spp. are of major importance in tropical and subtropical regions. Therefore, prevention of the disease by controlling ticks seems necessary and a prerequisite for improving camel meat and milk production (Radostits *et al,* 2007; Nazifi *et al,* 2011; Hekmatimoghaddam *et al,* 2012).

To the best of our knowledge, very little is known about anaplasmosis in camels and it has not been reported in camels in Saudi Arabia. Very few reports describe natural anaplasmosis in camels (Alsaad, 2009).

The aim of this investigation was to study the natural infection in an outbreak of anaplasmosis in dromedary camels.

Materials and Methods

Animals and clinical investigation

The present study was carried out on 237 dromedary camels (males and females), aged 3-15 years from the Riyadh and Makkah regions, Saudi Arabia between December 2012 and March 2014. Ninety-six camels had clinical signs which were indicative of anaplasmosis and were infested with ticks. Thirty-five apparently healthy non-pregnant camels from the same herds were selected as control

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animals. It was ensured that these animals were not medicated prophylactically and curatively for any disease since last 30 days. The selected animals had been reared under similar feeding systems, management and environmental conditions. The camels were subjected to careful clinical and laboratory investigations.

Samples

Faecal and blood (EDTA tube and plain tube) samples were collected from camels (n=237).

Parasitological examination

Giemsa stained blood films were examined microscopically for presence of *Anaplasma marginale* and other blood protozoa. Additionally, 10 ml of EDTA tube blood samples were centrifuged for 15 minutes in microhaematocrit tubes and analysed for *Trypanosoma evansi* in its buffy coat layer (Tejedor-Junco *et al,* 2011). Moreover, a standard floatation sedimentation technique was carried out on the faecal samples for the detection of gastrointestinal parasites and *Balantidium coli* (Coles, 1986).

Haematological analysis

A complete blood count was conducted using an automatic blood cell counter (BC-2800 Vet Analyzers – China) as previously described (Feldman *et al,* 2000).

Biochemical analysis

The biochemical analysis including liver, kidney and muscle function in addition to elements were performed in accordance with the automated biochemistry analyser (Bio-system A-15) Spain.

Treatment and control trails

Methods previously described by Radostits *et al* (2007) for the treatment and control of anaplasmosis were followed. These methods consist of (i) treatment of infected camels with a single injection of long-acting oxytetracycline at a dose of 20 mg/kg BW intramuscularly. (ii) placing the in-contact animals on a regimen of prolonged tetracycline protection. (iii) control of ticks and other vectors to prevent the transmission using the traditional methods because the chemotherapy as acaricide is not acceptable in the studied regions. The treated camels were observed daily for monitoring any clinical improvement. Additionally, blood samples were collected from treated (27 days post-treatment) and in-contact animals (at the end of the study) for presence of *Anaplasma marginale*.

Statistical analysis

The Statistical Products and Service Solutions programme (version 17, SPSS Inc., Chicago, IL, USA) were used for all analyses. Data were expressed as the mean±SE. Comparisons among groups were tested using an analysis of variance (ANOVA) and differences were considered to be significant at $P<0.05$.

Results

Clinical manifestation

Seventy-two out of 96 clinically affected camels were diagnosed anaplasmosis with 40.50% overall morbidity. The observed clinical signs included fever, anorexia, diarrhoea, emaciation, pale mucous membranes, lacrimation and anaemia that persisted up to 87 days in some cases. However, not all animals showed the typical clinical picture and the majority (n=57) showed mild manifestation. Mixed infections of *Anaplasma marginale* with other pathogens, including Theileria, Babesia, gastrointestinal nematodes and *Balantidium coli*, gave approximately the same clinical picture as infection with Anaplasma alone.

Parasitological results

Anaplasma marginale was detected in Giemsa-stained blood smears from 72 camels out of 96 clinically affected camels (Fig 1). The 72 camels with positive blood smears clinical signs were manifested while the other clinically affected camels (n=24) did not show *A. marginale* in their blood smears. *A. marginale* were observed at the periphery of infected RBCs and appeared as reddish-violet pleomorphic ordot-like forms (0.2-0.4 μm diameter) within erythrocytes (Fig 1). Thirty seven camels of 72 were infected with *A. marginale* only, and the other 35 camels were infected with *A. marginale* and other pathogens (2 cases showed mixed infection of *Anaplasma* and *Trypanosoma evansi*, 3 cases showed mixed infection of *Anaplasma* and *Babesia*, 5 cases showed mixed infection of *Anaplasma* and *Theileria*, 21 cases showed mixed infection of *Anaplasma* and gastrointestinal nematodes, and 4 cases showed mixed infection of *Anaplasma* and *Balantidium coli*). The degree of *Anaplasma* infection varied from mild, moderate to severe infection depending on the percentages of infected red cells of the various camels, which ranged between 2 and 19% (mild 2-7%, moderate 8-13%, severe 14-19%).

Haematological consequences

The mean values and standard error (SE) of the haematological parameters in clinically healthy

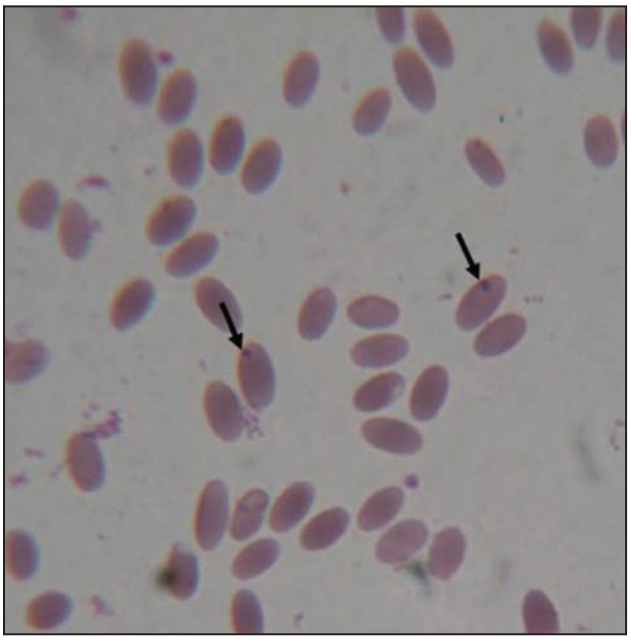

Fig 1. Blood smear from a naturally infected dromedary camel with *Anaplasma marginale* at the periphery of infected RBCs, Giemsa x100.

camels and *Anaplasma*-infected individuals are presented in table 1. Camels infected with *Anaplasma* alone showed a significant reduction (P<0.01) in the total RBC count, HGB concentration, HCT and MCV, indicating haemolytic anaemia. Significant increases (P<0.01) in total WBC count, lymphocytes %, MCHC and platelets were observed, whereas the other haematological parameters were close to normal values. However, significant differences in the levels of WBC count, lymphocytes % and RBC count were observed in the mixed infection group relative to *Anaplasma* alone group. Sex, age, lactation and seasonality slightly influenced the haematological parameters (results not shown).

Biochemical findings

The mean ± SE of biochemical parameters, including liver, kidney and muscle functions and elements, in clinically healthy and *Anaplasma*-infected camels are shown in table 2. A significant reduction (P<0.001) in the mean values of iron was determined in camels infected with *Anaplasma* alone when compared with the mean values of controls. While significant increases (P<0.01) in the mean values of GGT, AST, ALT, total bilirubin, BUN and LDH were found in affected camels when compared with the mean values of control camels. However, significant differences in the levels of GGT, AST, ALT, total bilirubin and LDH were observed in the mixed infection group relative to *Anaplasma* alone group. While the *Anaplasma* alone group showed significant differences in the levels of total protein, globulin, creatinine, BUN and iron relative to the mixed infection group.

Table 1. Haematological parameters (Mean+SE with minimum-maximum values) of clinically healthy and *Anaplasma*-infected camels.

Parameters	Clinically healthy camels (n = 35)	Camels infected with *Anaplasma* alone (n=37)	Camels infected with *Anaplasma* and others parasites (n=35)[a]
WBC count $\times 10^9$/L	12.08 ± 1.07 (10.01 -13.81)	15.03 ± 1.97** (12.71-17.14)	17.99 ± 2.1*** (11.82-19.65)
Lymphocytes %	43.03 ± 2.22 (39.64 -45.85)	49.19 ± 3.08*** (46.98 -53.78)	55.05 ± 4.45*** (47.32 -57.66)
RBC count $\times 10^{12}$/L	11.13 ± 0.22 (9.95 -13.77)	7.99 ± 0.51*** (7.24 -11.47)	6.07 ± 0.41*** (7.01 -9.72)
HGB g/dL	14.03 ± 0.52 (11.17 -16.91)	9.92 ± 0.67*** (8.01 -13.88)	9.21 ± 0.55*** (7.12 -11.69)
HCT %	33.82 ± 1.31 (29.44 -38.74)	29.21 ± 1.45** (25.63 -33.83)	27.01 ± 2.01*** (21.77 -33.17)
MCV fL	36.07 ± 1.61 (33.71 -41.82)	29.15 ± 0.69*** (23.06 -30.73)	28.89 ± 0.87*** (22.70 -31.78)
MCH pg	13.15 ± 0.23 (11.80 -14.00)	11.79 ± 0.48** (10.08 -13.60)	11.92 ± 0.55** (9.50 -13.80)
MCHC g/dL	40.11 ± 0.42 (38.39 -41.30)	42.79 ± 0.74** (39.91 -44.89)	42.97 ± 1.02** (38.99 -45.19)
RDW %	18.85 ± 0.41 (17.02 -21.05)	19.01 ± $0.38^{n.s}$ (16.91 -22.18)	19.63 ± 12.21* (18.20 -23)
PLT$\times 10^9$/L	127.17 ± 4.84 (115 -134)	139.25 ± 5.72** (121 -157)	140.12 ± 4.35** (128 -153)
MPV fL	5.79 ± 0.33 (4.72 -7.01)	6.13 ± $0.27^{n.s}$ (4.31 -7.19)	6.17 ± $0.29^{n.s}$ (5.12 -7.72)
PCT %	0.071 ± 0.004 (0.05 -0.09)	0.078 ± $0.003^{n.s}$ (0.057 -0.09)	0.08 ± $0.004^{n.s}$ (0.075 -0.09)
PDW %	14.05 ± 0.04 (13.71 -14.23)	13.98 ± $0.19^{n.s}$ (12.27 -14.51)	13.88 ± $0.17^{n.s}$ (13.16 -14.50)

WBCs: White blood cells, RBCs: Red blood cells, HGB: haemoglobin concentration, HCT: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red distribution width, PLT: Platelets, MPV: mean platelet volume, PCT: plateletcrit, PDW: platelet distribution widths.

[a]Other parasites: *Trypanosoma evansi, Babesia, Theileria*, gastrointestinal nematodes and *Balantidium coli*.

***=significant (P<0.001), **=significant (P<0.01), *=significant (P<0.05)

Table 2. Biochemical parameters (Mean+SE with minimum-maximum values) of clinically healthy and *Anaplasma*-infected camels.

Parameters	Clinically healthy camels (n = 35)	Camels infected with *Anaplasma* alone (n=37)	Camels infected with *Anaplasma* and others parasites (n=35)[a]
Liver function			
Total protein g/dL	5.81 ± 0.10 (5.17 -6.20)	6.80 ± 0.46 ** (4.90 -8.10)	5.24 ± 0.38n.s (3.79 -7.10)
Albumin g/dL	3.49 ± 0.16 (2.06 -3.90)	3.84 ± 0.41* (2.77 -4.90)	3.93 ± 0.24* (2.85 -4.98)
Globulin g/dL	2.76 ± 0.23 (2.00 -3.7)	4.60 ± 0.41** (2.90 -5.90)	2.97 ± 0.21* (2.21 -4.00)
GGT µ/L	9.12 ± 1.33 (5.51 -12.42)	17.77 ± 3.27* (10.97 -25)	20.01 ± 5.24** (13.41 -57)
AST µ/L	87.73 ± 3.81 (72.17 -93.67)	157.92 ± 10.5** (97.34 -182)	169.11 ± 23.19*** (105 -322)
ALT µ/L	10.45 ± 0.88 (7.56 -12.64)	17.98 ± 1.79*** (13.55 -22.19)	19.98 ± 2.49*** (14.76 -28.12)
Total bilirubin	0.29 ± 0.03 (0.23 -0.37)	0.67 ± 0.07** (0.39 -0.79)	0.75 ± 0.30*** (0.43 -1.80)
LDH µ/L	701.24 ± 21.03 (572.16-813)	1307.4 ± 123.5*** (990-1923)	1712.87 ± 234.5*** (937.27-2397)
Kidney function			
Creatinine mg/dL	1.46 ± 0.10 (1.00 -2.01)	1.68 ± 0.32* (0.75 -3.10)	1.21 ± 0.09n.s (0.78 -1.69)
BUN mg/dL	23.21 ± 1.25 (17.13 -31.19)	52.43 ± 2.76*** (37 -65)	49.74 ± 2.12*** (36.18 -63)
Elements			
Iron mg/dL	127.72 ± 2.97 (101.23 -145.65)	71.13 ± 5.43*** (53.19 -97.17)	67.88 ± 6.51*** (41.00 -95.73)

GGT: gamma-glutamyltransferase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, LDH: lactate dehydrogenase

[a]Other parasites: *Trypanosoma evansi, Babesia, Theileria*, gastrointestinal nematodes and *Balantidium coli*.

***=significant (P<0.001), **=significant (P<0.01), *=significant (P<0.05)

Table 3. Results of treatment and control trails of camel anaplasmosis.

Camels	Number	Clinical picture	Presence of *Anaplasma marginale* in blood smears[c]
Treated camels[a]	96	Treated camels were clinically improved	Not detected
In-contact camels[b]	141	Normal picture	Not detected
Total	237	–	–

[a]Treatment of infected camels with a single injection of long-acting oxytetracycline at a dose of 20 mg/kg BW intramuscularly.

[b]Placing the in-contact camels on a regimen of prolonged tetracycline protection.

[c]Blood samples were collected from treated (27 days post-treatment) and in-contact camels (at the end of the study) for presence of *Anaplasma marginale*.

Results of treatment and control trails

The treated camels were clinically improved with absence of *A. marginale* in their blood smears (Table 3). In addition, the in-contact animals that placed on a regimen of prolonged tetracycline protection did not show any *A. marginale* in their blood smears (Table 3). Ticks and other vectors were controlled by applied measures used in this study.

Discussion

This study presents the first description of anaplasmosis and its treatment and control in dromedary camel in Saudi Arabia. Most of the relevant previous studies described the clinical findings of anaplasmosis in cattle and other species, but very few studies have described the clinical picture of this disease in camels. In our study, 96 out of 237 (40.50%) camels were clinically infected with *Anaplasma marginale* and showed the clinical signs which were indicative of anaplasmosis. In Iraq, Alsaad (2009) examined 52 naturally infected camels with *A. marginale* and approximately described a similar clinical picture but he did not determine the infection rate because the source of camel herd and its total composition of camels were not recorded. In contrast to Wernery and Kaaden (2002) who mentioned that the infections with *Anaplasma* in dromedaries appear to be subclinical, the majority of infected dromedaries in our study showed clinical signs in variable degree. This could be explained by the first introduction of anaplasmosis in these regions which increase the susceptibility of dromedaries to infection or the expansion of the vector population into previously free areas or into the interface between endemic and non-endemic regions (Radostits *et al,* 2007). In addition, the presence of ticks on different parts of the body confirms the role of ticks in transmission of *Anaplasma* (Loftis *et al,* 2006).

A. marginale was detected in Giemsa-stained blood smears from 72 out of 96 clinically affected camels in the present study. This might indicate the high pathogenicity and the 1st introduction of anaplasmosis in these herds. Moreover, the percentages of parasitaemia ranged between 2-19%, which is indicative degree of infection. However, 24 of 96 clinically affected camels did not show *A. marginale* in their blood smears that might indicate the presence of mixed infection. A narrow range of parasitaemia (5-11%) was recorded in another study (Alsaad, 2009). The variation in parasitaemia could be attributed mainly to susceptibility of affected animals and the strain of *Anaplasma* (Radostits *et al,* 2007). In general, the pathogenesis of anaplasmosis is dependent on the infection of mature erythrocytes by an endocytic process and reproduction by binary fission to produce 2-8 infective initial bodies which leave by exocytosis to infect other erythrocytes. The number of infected erythrocytes doubles every 24-48 hours and about 10 to 90% of erythrocytes may be parasitised in the acute stage of the infection depending upon the strain and the susceptibility of the host (Radostits *et al,* 2007). The mixed infection with other parasites was observed in our study that affect both the blood parameters and the severity of the disease which was in accordance with other studies (Ismael *et al,* 2014; Rabana *et al,* 2011).

In the present study, the mean values of the haematological parameters of the clinically healthy camels were in the normal range (Mal *et al,* 2001). These normal ranges can vary with time and geographic location, which may affect the validity of the analysis. Therefore, we used a control group from the same herds; these animals had been reared under similar feeding systems, management and environmental conditions throughout the study period. Here, the affected camels with anaplasma alone showed a significant reduction in the total RBC count, HGB concentration, HCT and MCV. These results indicated that the infected camels might suffer from haemolytic anaemia (Ismael *et al,* 2014) and partly similar to that described by other workers (Alsaad, 2009; Rabana *et al,* 2011). Anaplasmosis is primarily an anaemia, the degree of anaemia varying with the proportion of erythrocytes which are parasitised. This infection leads to continued erythrocyte destruction which resulted in the development of mild to severe anemia and icterus without haemoglobinemia and haemoglobinuria. The first appearance of the anaplasma in the blood coincides with a fall in the hematocrit and erythrocyte levels, the appearance of immature erythrocytes in blood smears and the development of fever (Mohammed *et al,* 2007; Radostits *et al,* 2007). Significant increases in total WBC count, lymphocytes %, MCHC and platelets were observed as earlier reported in Iraq and Nigeria (Alsaad, 2009; Rabana *et al,* 2011). The high MCHC value for infected camels may be due to erythrocyte destruction releasing haemoglobin into the plasma. The leukocytosis and lymphocytosis could be attributed to stimulation of lymphoid tissues and stem cells in the bone marrow by anaplasma infection (Mahran, 2004). However, the mixed infection considerably affected on some haematological parameters which was in accordance with Rabana *et al* (2011) who reported that packed cell volume was severely affected in camels infected by both haemoparasite and helminths compared to those infected by either type of parasite alone.

The mean values of the biochemical parameters in clinically healthy camels lie within the normal ranges previously reported (Ayoub *et al,* 2003). In present study, the increase in GGT, AST, ALT and total bilirubin levels in infected camels compared with healthy animals might indicate hepatic dysfunction in anaplasma positive camels. Similar findings were observed by Alsaad (2009). In addition, the hyperbilirubinaemia could be attributed to excessive destruction of RBCs and the indirect hepatocellular damage. The increased level of creatinine and blood urea nitrogen may indicate indirect damage of renal tissue and the presence of globin catabolites liberated from haemoglobin lysis by the reticulo-endothelial system through the process of erythrophagocytosis (Kataria and Bhatia, 1991; Qarawi, 1999). The increased level of LDH in this study may indicate damage to the skeletal or heart muscles and hepatic tissues (Kataria and Bhatia, 1991). The mixed infection considerably affected some biochemical parameters which was similar to that previously determined (Ismael *et al,* 2014).

In present study, the applied control measures were succeeded in controlling anaplasmosis in the affected herds (Kocan *et al,* 2000). Vaccination with killed *A. marginale* vaccine is not used because the studied regions are not enzootic areas. In addition, attention should be given to prevent iatrogenic transmission with instruments used for injections or surgical operations by disinfection after use on each animal.

It was concluded that anaplasmosis has a deleterious effect on the health of camels and affects their haematological and biochemical parameters. Our study represents the first success of *Anaplasma marginale* control in dromedary.

Moreover, it represents the first description and diagnosis of anaplasmosis in dromedary in Saudi Arabia. Additionally, it measured some of the haematobiochemical parameters in camel anaplasmosis that were not previously reported and could serve as the basis for subsequent studies in dromedarius under natural and experimental conditions. Additional studies are needed to clarify the pathogenesis of *A. marginale* in dromedary.

Acknowledgements

The authors extended their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through the research group project RGP-267.

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DOI : 10.5958/2277-8934.2016.00015.1 Vol 23 No 1, p 101-102

Short Communication

PREVALENCE OF SARCOCYSTOSIS IN DROMEDARY CAMELS FROM INDIA

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Sarcocystis species are intracellular protozoan parasites with a requisite 2 host life cycle based on a prey-predator (intermediate- definitive) host relationship (Fayer, 2004). The disease is zoonotic and has been reported worldwide (Hamidinejat *et al,* 2013). *Sarcocystis cameli* is the only species of sarcocysts reported from camel for which the dog remains the final host (Boid *et al,* 1985). The *Sarcocystis* infections in livestock are usually self-limiting, of short duration, and often asymptomatic. However, acute infections can result in loss of weight, anaemia, haemorrhages in skeletal and heart muscles and abortion (Dubey *et al,* 1989). Studies in India point to a vast reservoir of infection with high prevalence rates in cattle and other livestock species (Chhabra and Samantaray, 2013). However, *Sarcocystis* infection in camels has remained totally unexplored so far in India.

Materials and Methods

The camels of the present study were from an organised camel herd having total herd strength of 350 camels and located at the Bikaner district (Rajasthan State, India). These camels were raised under semi-intensive system of management in outdoor facilities and fed with pellet feed, hay, and water *ad libitum*. These camels were regularly sent for grazing in nearby field area inhabited by stray dogs. During the study period of 5 years (2010-2015), total 92 camels were presented for routine post-mortem which includes 39 males and 53 females. These camels were categorised into 3 age groups i.e. 2 years and below (n= 31), 3-9 years (n= 35) and 10 years and above (n= 26). After observation and recording of gross lesions, the heart tissues were 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 haematoxylin and eosin (HE) stain. The degree of association between each risk factor and the occurrence of sarcocystosis was assessed using the Pearson Chi-square ($\chi 2$) test in SPSS 16 statistical software.

Results

No macroscopic sarcocysts were found in cardiac muscle tissues during carcass examination, but *Sarcocystis* bradyzoites were found by microscopical examination of histological slides in 33 (35.87%) out of 92 investigated camels. The incidence of sarcocystosis was not significant between the camels of age group 10 years and above (57.14%) and 3-9 years (50%) whereas, none of the calves (below 2 years) were found infected. Sex wise there was no significant difference between occurrence of sarcocystosis in male (30.76%) and female (39.62%) camels.

Grossly, there were no significant gross changes in the heart of affected camels except incidence of hydropericardium in 5 (15.15%) of the infected camels. Microscopically, the sarcocysts were thin walled and dark blue coloured in HE stain (Fig 1). There was no significant pathological reaction in cardiac muscles surrounding to sarcocyst in majority of the cases. However, mild eosinophil and

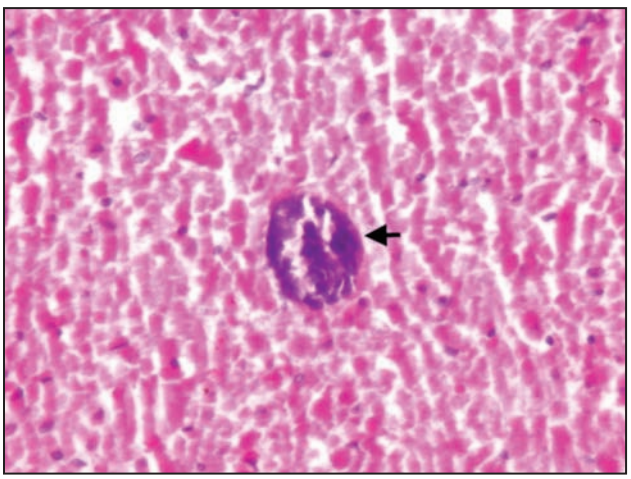

Fig 1. A sarcocyst in the cardiac muscle (arrow) (Haematoxylin & Eosin stain. 400X).

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mononuclear infiltration was observed in 12 (36.36%) and myocardial necrosis was observed in 9 (27.27%) infected camels.

Discussion

The results of the present study revealed a moderate prevalence of *Sarcocystis* infection among camels of the present study. This prevalence is comparable to those reported from other parts of the world such as Afghanistan (47.3–66.3%) (Kirmse and Mohanbabu, 1986) and Iran (52.3% and 51.5%) (Shekarforoush *et al,* 2006; Hamidinejat *et al,* 2013). However, comparatively higher prevalence rates were reported in Iraq (91.6%) (Latif *et al,* 1999) and Saudi Arabia (88.4 %) (Fatani *et al,* 1996). In India, the studies in cattle reported prevalence rate of sarcocysts as high as 80% (Jain and Shah, 1987). Differences between the previously reported infection rates may be due to different husbandry management systems in these countries, as well as use of different diagnostic methods and examination of different tissues for infection. The tissue distribution of *Sarcocystis* in different organs of camels reported by different investigators is also variable. The previous studies in camels detected high prevalence in oesophagus (Shekarforoush *et al,* 2006), diaphragm (Fatani *et al,* 1996) and tongue (Hussein, 1991).

There was no significant difference in frequency of sarcocystosis between male and female camels of the present study. Lack of relationship between sex and infection rates has shown in similar studies on camels before (Shekarforoush *et al,* 2006; Valinezhad *et al,* 2008; Hamidinejat *et al,* 2013). In the present study, higher infection rate was observed among adult and aged camels, which is in accordance with previous studies (Shekarforoush *et al,* 2006; Hamidinejat *et al,* 2013).

The moderate incidence of the sarcocyst in camel population of the farm indicated major role of carnivores invading the grazing areas of camels. Since camels are the browsing animals, the infection through pasture contaminated with dog faeces may be the important transmission source for Sarcocystosis in camels of the present study. The key to control sarcocystosis is in the interruption of the life cycle by preventing the carnivore definitive hosts from eating raw meat or offals of slaughtered/ dead animals, and contaminating the feed and water of livestock with their faeces (Bhatia *et al,* 2010). Therefore, an effort should be made to control the transmission of sarcocystosis by the safe disposal of infected offal and the control of stray dog population from the camel grazing areas.

The histopathological findings in heart muscles of camels of the present study were in agreement with those described by Valinezhad *et al* (2008). The present study showed that the examined camels have infected only with microscopic form of *Sarcocystis* which is in agreement with previous studies (Shekarforoush *et al,* 2006; Valinezhad *et al,* 2008; Hamidinejat *et al,* 2013).

In conclusion, the incidence of sarcocystosis in Indian camels showed important role of camels in the continuation of the *Sarcocystis* life cycle.

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DOI : 10.5958/2277-8934.2016.00016.3 Vol 23 No 1, p 103-107

EVALUATION OF A CONTINUOUS RATE INFUSION OF PROPOFOL-KETAMINE FOR TOTAL INTRAVENOUS ANAESTHESIA IN ONE HUMPED CAMELS (*Camelus dromedarius*) AFTER XYLAZINE PREMEDICATION: A CLINICAL CASE SERIES

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ABSTRACT

Seven adult dromedary camels were anaesthetised to undergo surgical procedures. All patients were premedicated with an intravenous (IV) administration of 0.2 mg kg^{-1} xylazine. Anaesthesia was induced with 1.0 mg kg^{-1} propofol (P) and 0.8 mg kg^{-1} ketamine (K) given IV and was maintained with a a continuous rate infusion (CRI) of 4 mg kg^{-1} $hour^{-1}$ P and 3.3 mg kg^{-1} $hour^{-1}$ K. Heart rate, respiratory rate, arterial blood pressure and quality of anaesthesia were recorded before and after xylazine administration (XA) as well as at 5 minutes after induction and every 10 minutes until the end of the procedure. Mean anaesthetic duration was 82.9 ± 16.0 minutes. Mean heart rate increased after induction and remained at relative constant levels during maintenance. Respiratory rate dropped after XA, but quickly returned the baseline level. Mean arterial blood pressure significantly decreased below baseline level after XA, but rose within anaesthesia maintenance, without reaching baseline values though. The mean recovery time was 37.6 ± 24.2 minutes. A very good level of surgical anaesthetic depth was achieved and maintained during all procedures, and all animals could be discharged safely after a smooth and uneventful recovery. This P-K CRI after XA seems to be clinically safe and effective in dromedary camels and provides very good operating conditions for major surgeries in this species. Still, further studies are necessary to evaluate more cardiorespiratory and haematological parameters to confirm the safety of this new technique.

Key words: Anaesthesia, camel, ketamine, propofol, TIVA

Major surgeries in dromedary camels frequently carried out under total intravenous anaesthesia (TIVA). There are few published studies related to the use of modern anaesthetic agents in this species, limiting clinicians to using older anaesthetic agents administered as repeated boluses. Propofol, an alkyl phenol derivative, is a short acting non-cumulative intravenous (IV) anaesthetic agent, used in many animal species and characterised by a rapid induction, acceptable haemodynamic stability, as well as a fast and smooth recovery (Watkins *et al,* 1987; Weaver and Raptopoulos, 1990). Respiratory depression represents its most relevant clinical side effect (Quandt *et al,* 1998). It can be used alone (Duke *et al,* 1997) but its poor analgesic properties mean that it is preferable to use in combination with agents such as xylazine (Kim and Jang, 1999) or ketamine (Flaherty *et al,* 1997). The cardiac and respiratory depressant effect of propofol may be reduced by combining it with ketamine, which also allows a reduction of the propofol dose (Flaherty *et al,* 1997; Guit *et al,* 1991; Nolan *et al,* 1996). However, only very few studies have investigated the effect of propofol in camels (Fahmy *et al,* 1995; Al-Mubarak 2008), and to the authors' knowledge, there is no published data describing the use of propofol and ketamine combination in this species. The objective of this report was to evaluate the effectiveness and practicability of a propofol/ketamine continuous rate infusion (CRI) for TIVA in camels which have been premedicated with IV xylazine.

Case Histories and Management

Seven dromedary camels of two breeds, four Magateer and three Majaheem, five males and two females, were admitted to the Veterinary Teaching Hospital of the King Faisal University for surgical indications. Mean age ± SD of the animals was 6.5 ±

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3.8 years, and their weight was 547 ± 239 kg. Types of surgeries carried out were four jaw fixations, two mastectomies, and one laparotomy for intestinal surgery. Food, but not water, was withheld for 48-72 hours before surgery. Camels were restrained manually in sternal recumbency before an initial physical examination. All camels received IV xylazine (Ilium-Xylazil-20, Troy Laboratories, Australia) at 0.2 mg kg^{-1} as pre-anaesthetic medication. Camels were then positioned as required for surgery (laterally for mastectomy and laparotomy, and sternal for jaw fixation). The hairs over the area of surgery were shaved, and the skin was prepared for aseptic surgery. A 14 gauge catheter (Braunüle MT Luer Lock, B. Braun Melsungen AG, Germany) was placed in jugular vein prior to induction of anaesthesia. Anaesthesia was induced with a combination of 1.0 mg kg^{-1} propofol (Recoofol 10mg mL^{-1}, Leiras Oy, Finland) and 0.8 mg kg^{-1} ketamine (Ketamil, Troy Laboratories, Australia) administered IV as a single bolus. Five special syringes (60 mL; BD, NJ, USA) mounted on two syringe pumps, were used for continuous drug administration. They were connected to the IV catheter by a syringe pump connector to maintain anaesthesia. A multi infusion pump (Stoelting Syringe Pumps, IL, USA) infused propofol at a constant rate of 4 mg kg^{-1} $hour^{-1}$, and another infusion pump (AP 14, Ascor S.A, Poland) delivered ketamine at a constant rate of 3.3 mg kg^{-1} $hour^{-1}$. Once the surgery was completed, infusions were disconnected. Baseline (before pre-anaesthetic medication) heart rate and respiratory rate were assessed by auscultation. Baseline indirect blood pressure values were assessed by oscillography using a cuff placed around the base of the tail and connected to a patient monitor (Infinity Delta XL, Drager Medical, Germany). These parameter values were further recorded at 5 minutes and 10 minutes after xylazine premedication and every 10 minutes until the end of surgery. Electrocardiogram recording was started immediately after induction of anaesthesia, using the same monitor as above. Clinical signs of anaesthesia, including the quality of induction, presence or absence of spontaneous movement within the maintenance, and palpebral reflexes were evaluated in each animal at 10-minutes intervals. Time from disconnection of infusions to sternal posture with the ability to support and raise the head was recorded, and quality of the recovery subjectively assessed. Mean time ± SD from induction of anaesthesia until disconnection from anaesthetic infusion was 83 ± 16 minutes. Induction of anaesthesia was generally very smooth and rapid. A satisfactory depth of anaesthesia adequate for the surgery performed was achieved in all camels. Spontaneous movement was noted once in one camel, 45 minutes of induction of anaesthesia, and an extra dose 0.8 mg kg^{-1} of ketamine was administered IV. Eyes remained open and palpebral reflex was positive throughout the anaesthesia period in all cases. Heart rate initially decreased after injection of xylazine, but then continuously rose above base line values. Respiratory rate also decreased after sedation, but then returned to values similar to the base line before further rising (Table 1). The mean values of arterial blood pressure decreased during anaesthesia (Table 1). Body temperature decreased slightly during the anaesthesia but remained within the normal physiological limits. The mean time ± SD until the camels were able to support and raise their head in sternal posture was 38 ± 24 minutes (range 10-60 minutes) after anaesthesia was discontinued. The quality of recovery was evaluated as good in all patients, as it was smooth and uneventful.

Discussion

In previous trials, the effect of propofol administration alone, without further analgesia, was judged to be not satisfactory for clinical situations in camels (Al-Mubarak, 2008). The doses of propofol that had been used in that report were 2.5-3 mg kg^{-1} IV given in bolus. This current report describes the use of propofol-ketamine bolus combination for induction and for maintenance using a CRI in seven camels, which underwent operations of various types and durations. The quality of induction and maintenance of anaesthesia was generally very good. This is in agreement with the results of studies in other species (Lerche *et al,* 2000; Umar *et al,* 2006), who demonstrated that the addition of ketamine improves the quality of anaesthetic produced by propofol. This combination has also been shown to reduce the dose of propofol (1.0 mg kg^{-1} compared to 2.5-3.0 mg kg^{-1} in the above study) required to achieve satisfactory TIVA in camels.

Mean heart and respiratory rates in this study decreased initially after premedication with xylazine, a typical effect of alpha-2 adrenergic agonists (Maze and Tranquilli 1991; Wagner *et al,* 1991; Mama *et al,* 1996). However, heart rate then increased continuously above the baseline after induction and during the maintenance of anaesthesia, while respiratory rate remained within ranges close to baseline values. Similar results were observed in other studies when ketamine or propofol administration

Table 1. Mean values ± SD of respiratory rate (fR) breaths minute^{-1}, heart rate (HR) beats minute^{-1}, temperature (Temp.), mean arterial blood pressure (MAP), systolic arterial blood pressure (SAP), and diastolic arterial blood pressure (DAP) at the baseline (BL), at 10 minutes after premedication with xylazine (AX), and 5-100 minutes after the induction of anaesthesia. N; number of animals.

Variable	Time (minutes)												
	BL N=7	**AX N=7**	**5 N=7**	**10 N=7**	**20 N=7**	**30 N=7**	**40 N=7**	**50 N=7**	**60 N=6**	**70 N=6**	**80 N=6**	**90 N=4**	**100 N=1**
RR	18.9 ±8.2	16.7 ±7.5	11.4 ±4.4	13.3 ±8.6	15.9 ±9.3	16.3 ±9.3	18.4 ±8.4	17.1 ±9.2	22 ±13.1	20 ±10.9	21.3 ±10.3	25 ±13.3	16
HR	45.4 ±8.1	40 ±10.1	68.7 ±20.8	73.6 ±34.1	86.1 ±17.8	83 ±17.4	89.3 ±17.3	88.4 ±18.4	87.5 ±17.0	90.8 ±24.7	88.2 ±27.5	101.8 ±28.1	80
Temp.	37.4 ±0.8	37.3 ±0.5	37.3 ±0.9	37.1 ±0.7	37.1 ±0.7	37 ±0.8	36.7 ±0.8	36.6 ±0.8	36.3 ±0.5	36.3 ±0.5	36.3 ±0.5	36.3 ±0.5	36
MAP	172 ±29.3	133 ±28.4	113.1 ±27.9	136 ±38.7	136 ±36.0	155.7 ±31.8	155.1 ±38.4	152.1 ±33.3	143.2 ±35.4	136.5 ±34.6	128.8 ±34.6	119.3 ±22.6	155
SAP	206.4 ±35.2	175.5 ±36.7	133 ±32.1	153 ±44.6	159.1 ±44.2	175.1 ±37.3	176.2 ±43.3	178.4 ±39.0	177 ±24.7	166.3 ±19.0	158.8 ±30.0	144.3 ±29.1	193
DAP	±140.7 29.1	109.4 ±31.6	101.4 ±23.7	118 ±41.2	119.1 ±39.5	136.7 ±31.7	133.7 ±40.3	128.3 ±34.8	117.8 ±41.1	116.2 ±40.1	110 ±36.2	101.3 ±25.9	122

counteracted the changes in heart and respiratory rates induced by xylazine premedication (Marntell and Nyman, 1996; Nolan and Hall 1985; Mama *et al,* 1995). The increases in heart rate recorded in this study could also be a compensatory response and reflect an increase in sympathetic activity associated with an increasing $PaCO_2$ as reported in other species (Mama *et al,* 2001), although $PaCO_2$ was not measured in this study. Apnoea is frequently observed after ketamine injection in sheep (Thurmon *et al,* 1973), and also recorded after propofol injection in dogs and cats (Watkins *et al,* 1987; Morgan and Legge 1989). Pablo *et al* (1997) reported apnoea in goats after propofol injection and suggested that this was because of rapid administration, which results in a higher plasma concentration in a short period of time. In contrast, Rolly *et al* (1985) using different rates for IV propofol injection in humans, found that apnoea was not related to the administration rate. In this current study, post-induction apnoea was observed once in this study in one camel lasting for 3 minutes, which is a major concern, as manual intubation into the trachea to provide positive support for respiration is fairly difficult due to narrow oropharynx space, elongated soft palate and very sharp teeth in this species. Arterial catheterisation is also problematic in this species, due to their thick skin and muscle layers, and so monitoring arterial blood gases and direct arterial blood pressure measurement was not carried out in this clinical study. Non-invasive blood pressure devices can be a very useful means of early warning of impending problems during anaesthesia (Sawyer *et al,* 2004). The method of indirect oscillometry for blood pressure measurement used in this study provides useful information for most horses, but may produce erroneous values in a small number (Hall *et al,* 2001). Following administration of alpha-2 adrenoceptor agonists and propofol, hypotension resulting from a decrease in cardiac output and systemic vascular resistance often occurs (Wagner *et al,* 1991; Keegan and Greene, 1993), but ketamine increases cardiac output and mean arterial pressure (Clark *et al,* 1982; Kim *et al,* 2004). In this study, mean arterial blood pressure initially decreased after injection of xylazine, and then increased during induction and maintenance of anaesthesia, but still remaining below baseline value, although the camels never became hypotensive.

A rapid recovery is desirable in ruminants as extended recumbency enhances the risk of tympany, hypoxaemia and aspiration pneumonia (Prassinos *et al,* 2005). Propofol is associated with rapid recovery time due to its rapid clearance. In the previous study of propofol TIVA in camels, recovery time was 10-13 minutes (Al-Mubarak, 2008). In this current time study, the recovery time was 38 ± 24.2 minutes (range 10 to 60 minutes), presumably due to the added action of ketamine. However, recovery was smooth and uneventful in all camels.

In summary, in this case series, the technique of a CRI of propofol and ketamine using a multi-syringe infusion pump was assessed as practical, easy to manage and effective to maintain adequate anaesthesia. The multi-syringe pump is necessary for these large animals due to the available concentration of propofol (10mg mL^{-1}). Monitoring was non-

invasive, but within its limitations, other than one case of apnoea at induction, cardiorespiratory parameters appeared to be maintained adequately. Recovery, although smooth was longer than desirable, and further controlled studies, using direct monitoring techniques are required to elucidate further the cardiorespiratory status, and also if lower doses of ketamine would be satisfactory, potentially shortening speed of recovery.

Conclusion

This combination provided very good operating conditions for major surgeries in dromedary camels. However, further work needs to be done in a larger numbers of subjects, and with evaluation of other cardiorespiratory parameters, including invasive blood pressure measurements, blood gas analysis and continuous capnography recording. Moreover, investigating pharmacokinetics of this combination is also necessary to establish its safety in camels.

Acknowledgement

The authors gratefully acknowledge Prof. KW Clarke for her help in proofreading and editing the manuscript, and the Deanship of Scientific Research in King Faisal University for the financial support.

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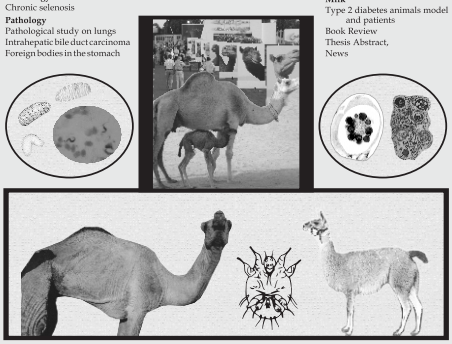

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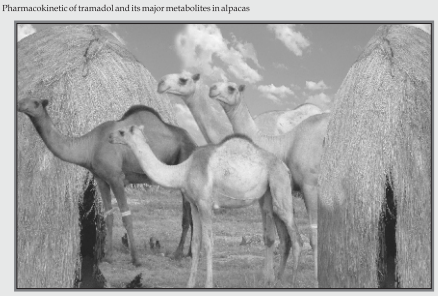

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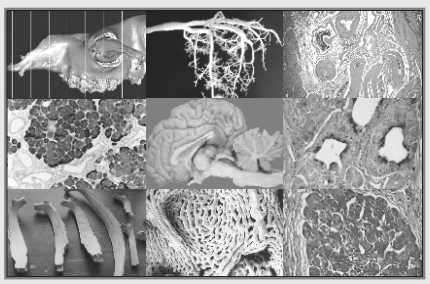

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DOI : 10.5958/2277-8934.2016.00017.5 Vol 23 No 1, p 109-110

Short Communication

A COMPARATIVE STUDY ON HAEMATOLOGICAL AND BLOOD BIOCHEMICAL PROFILE OF DOUBLE HUMPED (*Camelus bactrianus*) AND SINGLE HUMPED CAMEL (*Camelus dromedarius*)

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The bactrian camels (*Camelus bactrianus*) of Central Asia, China, and Mongolia are important to the indigenous people of this region. A small population of bactrian camel exists in the Nubra valley of Ladakh and at present, only about 210 camels are left (Ranjan *et al*, 2015). They are strong built animals, used mainly for pack and draught purposes. They appear quite resilient to changes in weather conditions, can withstand the very low temperatures (often close to -20°C) in winter and moderately high temperature (up to 35°C) in summer prevailing in these regions. The present study was aimed to investigate the normal haematological and blood biochemical profile of double humped camel of Ladakh region during winter season and to compare the values with the single humped camel (*Camelus dromedarius*).

Materials and Methods

Six healthy adult non pregnant female double humped camels and 6 non pregnant apparently healthy female single humped camels were randomly selected for this study. Double humped camels were from Nubra valley of Ladakh district reared under extensive system, while single humped camels were maintained at the ICAR- National Research Centre on Camel, Bikaner in semi-intensive management practices. Blood samples were collected in winter season for analysis of different haematological and biochemical parameters. Blood haematological and biochemical parameters were estimated by standard laboratory methods. The biochemical parameters were estimated using commercially available kits (Span diagnostics™). The data was analysed using SPSS statistical software.

Results

The results of haematolgical and biochemical parameters are given in tables 1 and 2, respectively. The haematological parameters revealed significantly ($P<0.01$) lower total leukocyte count in double humped camels compared to single humped camels. Whereas, the neutrophil per cent in double humped camels was significantly ($P<0.05$) higher than single humped camels. Other haematological parameters did not show any significant difference between the 2 groups.

Table 1. Haematological parameters (mean±S.D.) in dromedary and bactrian camels.

Parameter	Single humped (Dromedary) camel	Double humped (Bactrian) camel	P value
Haemoglobin (g/dl)	11.06±0.65	11.65±1.42	0.383
TEC (million/µl)	7.75±0.95	8.04±0.36	0.500
TLC (per µl)	10800±1277.49	7375±453.59	0.000
Neutrophil (%)	58.66±2.94	63.66±3.72	0.027
Lymphocyte (%)	33.5±2.25	31.5±2.42	0.170
Monocyte (%)	4±2.09	2.16±1.94	0.147
Eosinophil (%)	3.83±1.72	2.83±1.16	0.267

Table 2. Blood biochemical parameters (mean±S.D.) in dromedary and bactrian camels.

Parameter	Single humped (Dromedary) camel	Double humped (Bactrian) camel	P value
Total protein (g/dl)	6.50±0.77	6.51±0.48	0.976
Albumin (g/dl)	3.92±0.20	3.91±0.32	0.925
SGOT (IU/L)	82.44±7.13	108.55±5.54	0.000
SGPT (IU/ L)	11.16±4.31	12.49±2.36	0.522
Creatinine (mg/dl)	1.09±0.91	2.54±0.50	0.000
Triglycerides (mg/dl)	19.27±4.47	42.36±15.53	0.006
Magnesium (mg/dl)	6.01±1.61	2.53±0.16	0.000
Calcium (mg/dl)	11.82±3.44	11.03±1.05	0.603

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The serum biochemical parameters revealed significant (P<0.01) increase in SGOT, creatinine and triglycerides in double humped camels compared to single humped camels; whereas, serum magnesium was found significantly (P<0.01) decreased in double humped camels in comparison to single humped camels. The other biochemical parameters showed no significant variation between single and double humped camels.

Discussion

Investigations of blood constituents can provide valuable benefit and indication about the general health of animals. In bactrian camels, haemoglobin concentration and total erythrocytic count were slightly higher than dromedary camels, though the difference was statistically non-significant. Similar observations were also recorded by Mal *et al* (2001). This may be due to adaptive changes in bactrian camel to withstand the harsh climate of the high altitude and lower available atmospheric oxygen. Similar to the findings of the present study, higher neutrophil per cent in double humped camels as compared to single humped camels was recorded in previous studies (Mal *et al,* 2001; Raghvendar *et al,* 2000). Lower total leukocyte count, but higher neutrophil percentage indicated better disease resistance capacity in bactrian camel as neutrophils provide the first line of defence against invading microorganisms, tissue trauma or any inciting inflammatory signal (Weiss and Wardrop, 2010). The stress hormones particularly adrenaline and cortisol released in response to the hypoxic stress of high altitude are well known for their ability to increase WBC count and neutrohil per cent (Benschop *et al,* 1996). However, continuous altitude residence results in a return of WBC and leucocyte subset numbers towards baseline sea level values (Hannon *et al,* 1969). Hence, in present study leukocytosis might be result of increased cortisol release during sample collection, as these animals mostly remain under semi-wild conditions.

Similar to the findings of the present study no significant difference was recorded in biochemical parameters *viz*., total protein, SGPT and albumin in a study by Mal *et al* (2001). Whereas, no variation was recorded in values of GOT, triglycerdies and serum magnesium in single and double humped camel in previous studies (Mal *et al,* 2001; Raghvendar *et al,* 2000). These differences may be attributed to difference in feeding habits and geographical location of these animals. Also, the bactrian camels of the present study were grazing in the areas where there was sparse vegetation due to winter season. It has been studied in human subjects that residents of high altitude usually have higher levels of serum creatinine and increased oxidative stress as compared to those at sea level (Jefferson *et al,* 2004). This may be the possible reason for increased serum creatinine in bactrian camels of the present study.

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DOI : 10.5958/2277-8934.2016.00018.7 *Vol 23 No 1, p 111-116*

A HISTOLOGICAL AND HISTOCHEMICAL STUDY OF THE SMALL INTESTINE OF THE DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

This study was aimed at the investigation of the histological and histochemical features of the small intestine in the one-humped camel (*Camelus dromedarius*), in view of the metabolic characteristics of this species. For this purpose, the duodenum, jejunum and ileum of 6 healthy adult camels were used. Microscopic examination revealed that the length of the mucosal folds (villi) decreased progressively from the duodenum to the ileum. The deepest crypts were observed in the duodenum ($P<0.001$) and the longest villi were detected in the jejunum ($P<0.001$). Villi with the largest crypt diameter were observed in the ileum ($P<0.001$). The small intestine epithelium consisted of 3 parts: the tip of the *villus intestinalis*, the villus-crypt space and the crypt base. These parts were examined for their histochemical features, and data were evaluated subjectively. Goblet cells were rich in neutral carboxylic acidic mucosubstances and poor in sulfated acidic mucosubstances. While the concentration of the neutral mucosubstances was higher at the tip of the villi, the concentration of the acidic mucosubstances was higher in the crypts. The present study describes the histological/histochemical structure of the small intestine in the one-humped camel (*Camelus dromedarius*) and thereby, provides an opportunity for the comparison of findings obtained in camels and other ruminant species.

Key words: Camel, histochemistry, histology, mucosubstance, small intestine

The camel is a pseudo-ruminant, and its stomach (Eerdunchaolu *et al,* 1999) and duodenum (Althnaian *et al,* 2012; Althnaian *et al,* 2013) differ morphologically and histologically from those of other ruminants. The primary functions of the small intestine are digestion and absorption. This is achieved by the mixing of food with digestive enzymes secreted from the endocrine glands (Guyton and Hall, 2006). In domestic animals, the small intestine, i.e. the duodenum, jejunum and ileum share a common histological pattern with some specific characteristics of their own (Mescher and Jungueira, 2010).

The intestinal mucosa is densely populated with microorganisms (both commensal and pathogenic) capable of intense metabolic activities, such as the fermentation of complex carbohydrates contributing to the host metabolism (Macfarlane *et al,* 2006). The gastrointestinal epithelium is covered by a protective mucous gel composed predominantly of mucin glycoproteins that are synthesised and secreted by goblet cells (Specian and Oliver, 1991). Goblet cells reside throughout the length of the small and large intestines and are responsible for the production and maintenance of the protective mucous blanket by synthesising and secreting high-molecular-weight glycoproteins known as mucins (Deplancke and Gaskins, 2001; Kim and Khan, 2013). The major function of this mucous layer is to lubricate and protect mucosal epithelia from damage caused by food, digestive secretions, and microorganisms. Mucous also serves as a selective barrier for absorption across the small intestine (Schrager, 1970; Guyton and Hall, 1997). Glycoconjugates are important constituents of the intestinal mucosal barrier, which are involved in digestion and the absorption of nutrients, as well as in the protection of the gut mucosa against possible damage from ingested material and interactions between cells and pathogens in the intestinal lumen (Chae, 1997; Damjanov, 1987; Gelberg *et al,* 1992). Some glycoproteins contain no acid groups (neutral glycoproteins), whereas, others have limited amounts of carboxyl or sulfate radicals (acidic mucous substances). The carbohydrate moiety of proteoglycans and glycoproteins, containing sulfate and carboxyl groups, reacts strongly with the alcian blue dye. Neutral glycoproteins can be identified in

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tissue sections by their reaction with the periodic acid-Schiff (PAS) reagent (Kleessen *et al,* 2003). Acidic mucins are more resistant to degradation by bacterial glycosidases and host proteases, and show higher viscosity and acidity, compared to neutral mucins (Deplancke and Gaskins, 2001).

In present study the histological and histochemical features of the small intestine in the one-humped camel (*Camelus dromedarius*) were investigated.

Materials and Methods

The small intestines, i.e. duodenum, jejunum and ileum of 7 healthy adult slaughtered camels (*C. dromedarius*) of both sexes were dissected, and the tissue samples were fixed by immersion in 10% neutral formal saline for 24 h. Subsequently, the tissue samples were dehydrated, cleared and embedded in paraffin.

Histochemistry: Conventional histochemical techniques were used for the identification of glycoconjugates in goblet cells in the small intestine of *Camelus dromedarius*. Sections (6µ-thick at 90µ-intervals) were stained with periodic acid-Schiff (PAS) for glycogen and neutral mucosubstances, alcian blue pH 2.5 for the carboxyl group of acidic mucosubstances, combined PAS-alcian blue pH 2.5 (PAS/AB) for neutral and acidic mucosubstances, aldehyde fuchsin for the sulfate group of acidic mucosubstances, and combined aldehyde fuchsin-alcian blue pH 2.5 (AF/AB) for the sulfate and carboxyl groups of acidic mucosubstances (Table 1).

Histometry: The histometric sections (6µ-thick at 90µ-intervals, a total of 6 sections) of duodenum, jejunum and ileum were stained with Crossmon's modification of Mallory's trichrome method (Denk *et al,* 1989). Images of the microscopic fields were digitised with a 20X objective, and the height and diameter of the villi, and depth of the crypts were measured using a Leica DMLB light microscope equipped with a Leica DC200 CCD camera and by use of the Q-win image analysis software. The height of 3 villi (HV), the depth of 3 crypts (DC) and the diameter of 3 villi were measured in each section. Histometric data (villus length, crypt depth, villus diameter) were analysed by ANOVA with Duncan's multiple-range test (Statistic Packet of Social Science (SPSS) 14.01 serial: 9869264). Differences between the least squared means were analysed by orthogonal contrast and considered significant at P<0.001.

Table 1. The procedures and references for various types of glycoconjugates.

Procedures	References	GCs revealed
PAS	(McManus, 1963)	Neutral GCs
PAS/ AB (pH 2.5)	(Mowry, 1956)	Neutral and/ or acid rich GCs
AB (pH 2. 5)	(Lev and Spicer, 1964)	Acidic GCs with carboxylated and sulphated esters
AF/ AB (pH 2.5)	(Spicer and Mayer, 1960)	Acidic GCs with carboxylated and sulphated esters
Best's Carmine	(Strous and Dekker, 1992)	Acidic GCs with carboxylated and sulphated esters

GCs: Glycoconjugates, **AB:** Alcian blue, **PAS:** Periodic acid/ Schiff, **AF:** Aldehyde fuchsin

Results

Histology and Histometry

The small intestine of the dromedary camel was divided into 3 regions, namely, the duodenum, jejunum and ileum. Although, these regions were histologically similar, specific minor differences permitted their identification. The luminal surface of the small intestine was modified to increase its surface area. Microscopic studies revealed the presence of macroscopic and microscopic folds (*villi intestinalis*) throughout the small intestinal mucosa. It was determined that the length of the macroscopic folds decreased progressively from the duodenum to the ileum (Fig 1).

Camel intestinal villi are outgrowths of the mucosa projecting into the lumen of the small intestine. The longest villi were detected in the jejunum (P<0.001) and the largest villus diameters were observed in the ileum (P<0.001). The villi opened into simple tubular glands (crypts). The deepest crypts were observed in the duodenum (P<0.001).

The mucosa of the camel small intestine was lined by simple columnar epithelium consisting of surface absorptive cells and goblet cells. A fewer number of goblet cells were observed at the tip of the villi. A thick tunica muscularis, which was composed of an inner circular layer and an outer longitudinal layer, existed. The thickness of the inner circular layer of the tunica muscularis was greater in the Peyer's patches. The tunica serosa was observed as a thin layer of connective tissue.

Means in the same column with different superscripts are statistically different, as demonstrated by one-way ANOVA followed by Duncan's test (P<0.001) (Table 2).

Histochemistry

The gut epithelium was divided into 3 parts: the tip of the *villus intestinalis*, the villus-crypt space and the crypt base. These parts were examined for their histochemical features and data were evaluated subjectively. The results of the histochemical staining reactions are summarised in Table 2. While the goblet cells at the tip of the villi showed strong PAS positivity (Fig 2), those within the crypts showed strong AB (pH 2.5) positivity (Fig 3). When the sections were stained with PAS/AB (pH 2.5), goblet cells stained pink at the tip of the villi, purple in the villus-crypt space and blue at the crypt base (Fig 4). Glycogen-containing cells were not observed in the epithelium of the small intestine. Goblet cells were observed to be rich in neutral and carboxylic acidic mucopolysaccharides and poor in sulfated mucopolysaccharides. While the concentration of the neutral mucopolysaccharides was higher at the tip of the villi, the concentration of the acidic mucopolysaccharides was higher in the crypts (Tables 3, 4, and 5).

Discussion

In the present study, the mucosal structure and carbohydrate character of the goblet cells were determined in the duodenum, jejunum and ileum of the dromedary camel. Crossmon's modification of Mallory's trichrome method allowed the histometric analysis of the gut mucosa. Estimations of the mucosal structure, length and diameter of the villi, and depth of the crypts were made in the cross sections.

Digestion and the absorption of nutrients and water occur, to a great extent, in the small intestine. Enzymes in the small intestine break down nutrient molecules into their building blocks (Guyton and Hall, 2006). In agreement with previous research, in

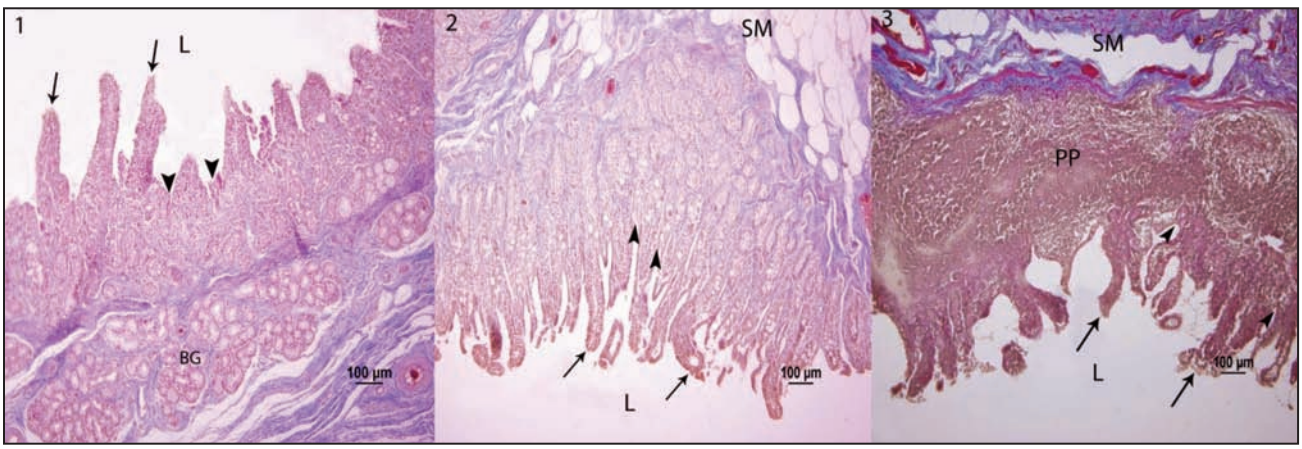

Fig 1. Histological stain: 1. Duodenum: **L:** lumen, **BG:** Brunner Gland, **arrow:** *villus intestinalis*, **arrow head:** crypt 2. Jejunum : **L:** lumen, **SM:** Submucosa, Triple Stain **arrow:** *villus intestinalis*, **arrow head:** crypt 3. İleum : **L:** lumen, **PP:** Peyer patches, **SM:** submucosa, **arrow:** villus intestinalis, **arrow head:** crypt. Mallory's trichrome method X 20.

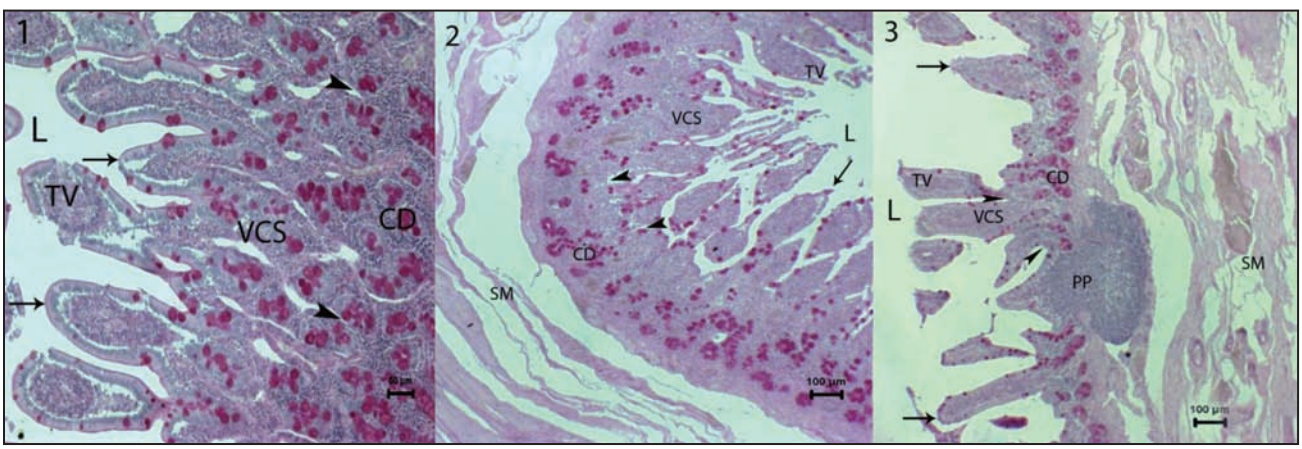

Fig 2. Histological stain: 1. Duodenum: **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt 2. Jejunum : L: lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt **SM:** Submucosa 3. İleum : **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt **PP:** Peyer patches. PAS stain X 20.

Table 2. Various parameters of different parts of small intestine, i.e. VL: villi length, VD: villi diameter, CD: crypt depth (X±SX).

Group	VL	VD	CD
Duodenum	336,67±8,4[a]	101,57±3,7[a]	285,13±8,8[b]
Jejunum	411,53±11,8[b]	100,43±2,3[a]	237,41±5,9[a]
Ileum	338,74±6,9[a]	121,81±3,1[b]	230,11±6,5[a]
P	***	***	***

***: P<0,001

Table 3. Histochemical staining properties in the goblet cells in duodenum of camel small intestine.

Staining reaction of duodenum

Procedure	Tip of villi	Villus-crypt space	Crypt depth
PAS	2	3	3
PAS/ AB pH 2.5	3 (R)	4 (P)	4 (P/B)
AB pH 2.5	2	3	3
AF/ AB pH 2.5	1 (B)	2 (B)	3 (B)

Staining intensity is indicated by; **4,** very strong; 3, strong; **2,** moderate; **1,** weak, **0,** negative. **R,** red; **B,** blue; **P,** Purple

the present study, it was determined that the camel small intestine was composed of 4 layers, from the inner to the outer surface, namely, the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The tunica mucosa was composed of 3 layers: the lamina mucosa lined by a simple columnar epithelium), lamina propria, and lamina muscularis (Althnaian *et al,* 2013; Mescher and Jungueira, 2010). The inner surface of the small intestine is covered with finger-like projections referred to as villi, which increase the surface area available to the absorption of nutrients from the gut content (Mescher and Jungueira, 2010). By increasing the surface area of the small intestine, villi increase the chance of a food particle encountering a digestive enzyme and being absorbed across the

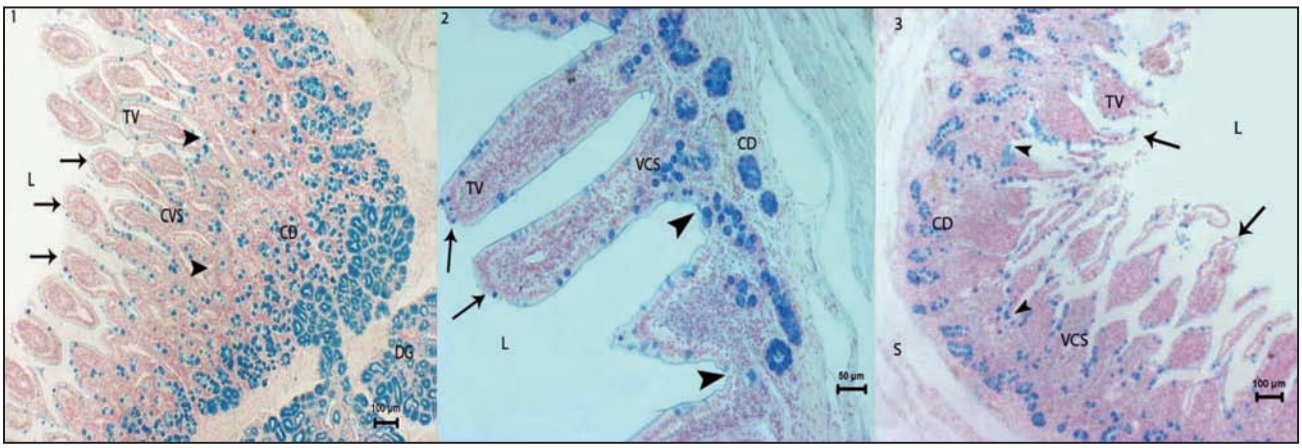

Fig 3. Histological stain: 1. Duodenum: **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt, **BG:** Brunner gland. 2. Jejunum : **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt. 3. İleum : **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt, **SM:** Submucosa. AB stain X 20.

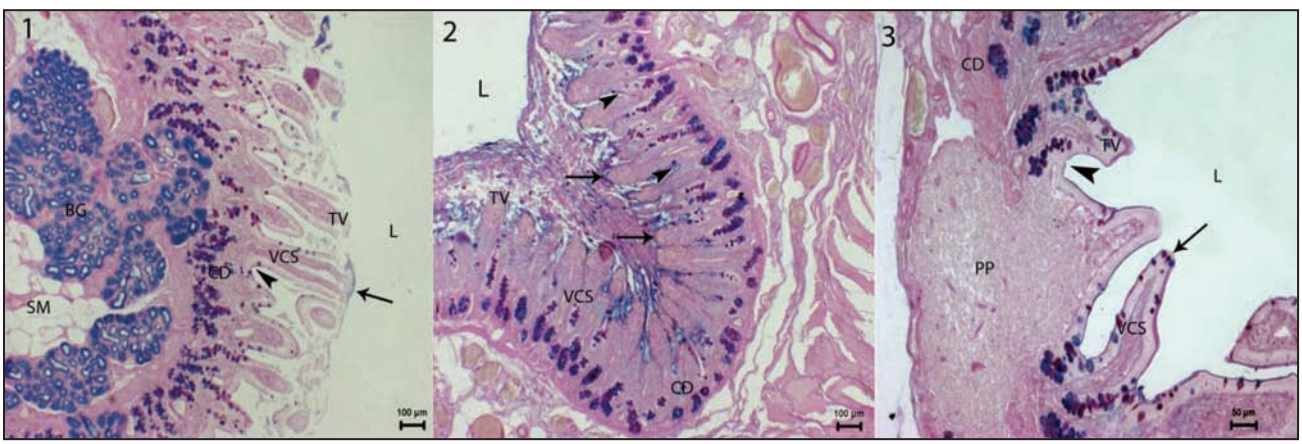

Fig 4. Histological stain: 1. Duodenum: **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt, **SM:** Submucosa. 2. Jejunum : **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt. 3. İleum: **L:** lumen, **TV:** Tip of villi, **VCS:** Villus-crypt space, **CD:** Crypt depth, **arrow:** *Villus intestinalis*, **arrow head:** Crypt **PP:** Peyer patches. PAS/AB stain X 20.

Table 4. Histochemical staining properties in the goblet cells in jejunum of camel small intestine.

Staining reaction of jejunum

Procedure	Tip of villi	Villus-crypt space	Crypt base
PAS	2	3	4
PAS/ AB (pH 2.5)	2 (R)	3 (P)	4 (P/B)
AB (pH 2.5)	2	3	3
AF/ AB (pH 2.5)	1 (B)	2 (B)	3 (B)

Staining intensity is indicated by; **4,** very strong; **3,** strong; **2,** moderate; **1,** weak, **0,** negative. **R,** red; **B,** blue; **P,** Purple

Table 5. Histochemical staining properties in the goblet cells in ileum of camel small intestine.

Staining reaction of ileum

Procedure	Tip of villi	Villus-crypt space	Crypt base
PAS	2	2	3
PAS/ AB (pH 2.5)	2 (R)	3 (P)	4 (R/P)
AB (pH 2.5)	2	2	2
AF/ AB (pH 2.5)	2 (B)	2 (B)	3 (B)

Staining intensity is indicated by; **4,** very strong; **3,** strong; **2,** moderate; **1,** weak, **0,** negative. **R,** red; **B,** blue; **P,** Purple

epithelium and into the blood stream. Crypts are formed by secretory epithelial cells (Guyton and Hall, 2006). In this study, it was ascertained that the villi in the jejunum were much longer than those in the duodenum and ileum. Furthermore, the deepest crypts were found to be located in the duodenum, and the largest villus diameter was observed in the ileum. All these findings demonstrated that, in the small intestine of the dromedary camel, the highest rate of absorption occurred in the jejunum, and the highest rate of digestion took place in the duodenum.

The mucous gel layer is an integral structural component of the intestine, which acts as a medium for the protection and lubrication of the mucosa, and for transport between the luminal content and epithelial lining (Forstner *et al,* 1995). The presence of neutral mucosubstances and carboxyl-rich non-sulfated glycoconjugates has been demonstrated in the enterocyte brush border of the lamb duodenum (Pedini *et al,* 2001). On the other hand, it has been reported that in adult sheep, sulphomucin was absent in the duodenal gland cells and goblet cells (Ohwada and Suzuki, 1992). It was reported that the duodenum, jejunum and ileum showed a higher percentage of goblet cells containing acidic mucins, compared to neutral mucins in cattle (Machado-Neto *et al,* 2013). Acidic mucins provide protection against bacterial translocation as sulfated mucins, in particular, are less degradable by bacterial glycosidases and host proteases (Fontaine *et al,* 1996). Neutral mucins occur in greater quantities in the gastric mucosa, whereas, acidic mucins predominate in the intestinal epithelium (Deplancke and Gaskins, 2001). In the present study, the level of acidic mucosubstances decreased progressively from the duodenum to the ileum. It was determined that carboxylic acidic mucosubstances were of the highest concentration in the camel small intestine. Given that acidic mucosubstances have a bactericidal effect, the findings obtained in the present study suggest that defence against bacteria is stronger in the duodenum, which is the entry site of the gastric content into the intestines. It was determined that the concentration of neutral mucosubstances was higher in the ileum, and that intestinal goblet cells were poor in glycogen and sulfated acidic mucosubstances.

This study was aimed at describing the intestinal structure and mucin histochemistry of the intestinal goblet cells of the dromedary camel.

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DOI : 10.5958/2277-8934.2016.00019.9 Vol 23 No 1, p 117-119

A STUDY ON TEAR FLUID SECRETION RATE IN DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

This study was aimed at determining the normal reference range for tear fluid secretion rate in different Indian breeds of dromedary camel (*Camelus dromedarius*). Tear fluid secretion was measured in 81 apparently healthy eyes (left eye) of 81 camels of Jaisalmeri (n= 19), Kachchi (n = 19), Bikaneri (n= 17) and Mewari (n= 26) breeds using the Schirmer Tear Test 1 (STT 1). Animals were divided into three groups on the basis of age, i.e. Gr. I: Less than 5 years (n = 29); Gr. II: 5 years to less than 10 years (n = 28) and Gr. III: 10 years and above (n = 24). Tear fluid secretion rate varied from 14.5 to 30.5 mm/ minute with overall mean (± S.E.) value of 21.89±1.01 mm/ min. The mean values in different breeds (Jaisalmeri, 22.39±1.06; Kachchi, 21.55±1.28; Bikaneri, 22.12±1.25 and Mewari, 21.63±1.01) were statistically comparable. The mean tear fluid secretion rate in different groups within a breed did not differ significantly. Likewise, values in male (20.81±0.74 mm/ min) and female (22.95±0.81 mm/ min) camel were also statistically comparable. From the present study, it can be concluded that breed, age and sex do not have significant effect on normal tear fluid secretion rate in dromedary camel. The results of the study may be used as normal reference range for STT 1 and may assist veterinarians in diagnosis of ocular diseases and syndromes affecting the tear film dynamics in dromedary camel.

Key words: Camel, dromedary, eye, reference-range, tear-fluid, tear-secretion

Dromedary camel (*Camelus dromedarius*) survives in the extremely harsh desert conditions comprising a long, hot (temperature sometimes exceeding 50°C), dry (near zero relative humidity) and dusty (sand storms) summer seasons (Chen *et al,* 2011). The camel's eyes are protected from blowing sand and dust by a double row of eyelashes and three eyelids on each eye. The extra eyelid also helps protect against the blazing sun, and protects them from getting blind. The camel eye shows many distinct features, few resembling horses, cattle and sheep, whereas others resembling lower mammals such as the rabbit (Rahi *et al,* 1980). Moreover, the camel tear has some unique components that provide stabilisation of tear film under the harsh environmental conditions (Chen *et al,* 2011; Shamsi *et al,* 2011). Nevertheless, ocular problems do occur in dromedary camel, though scientific reports are meagre (Fahmy *et al,* 2003). Several studies have been conducted to explore the normal anatomy of the eye (Yadegari *et al,* 2013) as well as to characterise the tear components of the dromedary camel (Chen *et al,* 2011). However, to our knowledge, no study has been conducted so far to determine the normal reference range for STT I values in dromedary camel (*Camelus dromedarius*). The normal reference values for STT I and II in llamas (*Lama glama*), another member of family Camelidae, were reported recently (Trbolova *et al,* 2012). The present study aimed to determine the reference range for STT I in four Indian breeds of dromedary camels to help veterinarians in diagnosis and management of ocular diseases in this species.

Materials and Methods

Study animals

The study was conducted in 81 (40 male and 41 female) adult apparently healthy dromedary camels of Jaisalmeri (n= 19), Kachchi (n = 19), Bikaneri (n= 17) and Mewari (n= 26) breeds free from any systemic or ocular disease. Animals were maintained in the animal farm of ICAR- National Research Centre on Camel, Jorbeer, Bikaner, India. Selected animals were divided into three groups i.e. Group I: less than 5 years (n = 29); Group II: 5 years to less than 10 years (n = 28) and Group III: 10 years and above (n = 24).

Measurement of Tear Fluid Secretion Rate

The tear fluid secretion rate was estimated by Schirmer tear test I (STT I). The STT I was performed using a commercial STT strip (Opstrip, Ophtechnics Unlimited, Gurgaon, India) after placing it in the left eye for one minute during day time (between 9.00 to 12.00 a.m.) without use of any chemical restraint (Maggs *et al*, 2008; Fig 1).

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Statistical analysis

The data obtained were analysed using statistical software. STT I values (mm/min) were expressed as mean±SE and evaluated using Duncan's multiple range test. The statistical analysis were carried out using Statistical Product and Service Solutions (SPSS), version 16.0 (SPSS Inc., China) statistical software. P< 0.05 was considered statistically significant.

Results

The results of the present study are summarised in Table 1, 2 and Fig 2. The tear production rate varied from 14.5 to 30.5 mm/ min with overall mean 21.89 ± 0.56 mm/ min and median 20.00 mm/ min. The STT I values in Jaisalmeri (22.39±1.06 mm/ min), Kachchi (21.55±1.28 mm/ min), Bikaneri (22.12±1.25 mm/ min) and Mewari (21.63±1.01 mm/ min) camels did not differ significantly from each other (Table 1). Likewise, no significant difference was observed in the STT I values between groups I, II and III in all four camel breeds (Fig 1). The overall mean values in male (20.81±0.74 mm/ min) was lower than female (22.95±0.81 mm/ min), though the difference was statistically non-significant (Table 2).

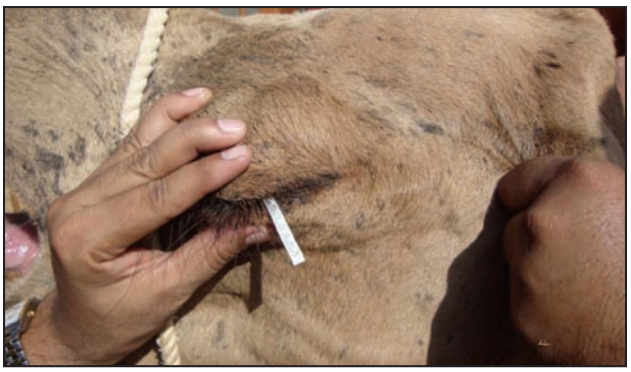

Fig 1. Measurement of tear production rate in dromedary camel.

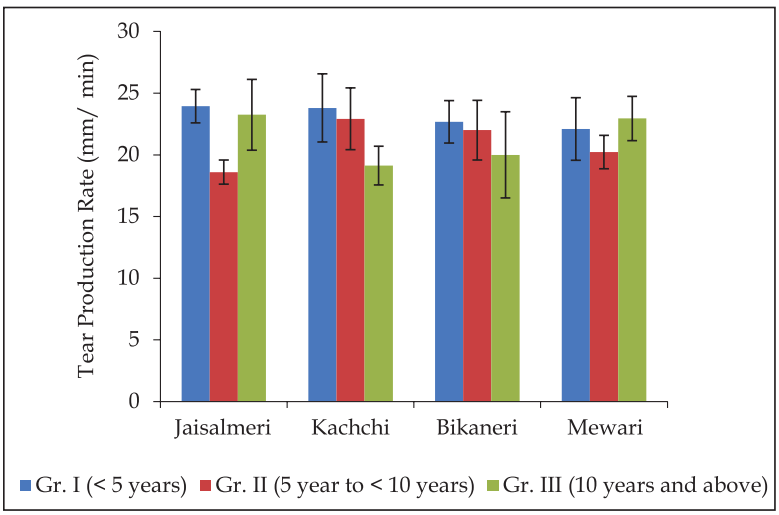

Fig 2. Tear production rate in different age groups of camel

The Schirmer tear test (STT) is a semi-quantitative method of measuring production of the aqueous portion of the precorneal tear film (Maggs *et al,* 2008). STT is further classified into two types. STT I measures both basal and reflex tear secretion rate and is determined by applying Schirmer tear test strips in sensitive eye. For measurement of STT II, corneal sensation is abolished with topical anaesthetic. The STT II values are usually lesser than STT I values as afferent limb of the reflex path is blocked and reflex secretion by the lacrimal and nictitans glands is reduced. The aqueous layer of precorneal tear film provide lubrication and supply the cornea with nutrients including oxygen, amino acids, vitamin A, growth factors and antibodies and remove metabolic waste products. Deficiency of the aqueous phase of the precorneal tear film leads to xerosis and keratoconjunctivitis sicca (KCS). Determination of STT helps in diagnosis of KCS in domestic animals.

Discussion

The present study reports the mean values and ranges of STT I in four different Indian breeds of dromedary camel (*Camelus dromedarius*). STT I value in dromedary camel was higher than llama (17.3±1.1 mm/ min), another member of the Camelidae family (Trbolova *et al,* 2012). Other domestic ruminants like sheep (18.5±2.5 mm/ min) and goat (15.8±5.7 mm/ min) have lower STT values (Broadwater *et al,* 2007; Ghaffari *et al,* 2010). STT I value was reported to decrease with age in dogs (Hartley *et al,* 2006). However, in our study a generalised trend of non-significant decrease in STT I value with age was recorded only in Kachchi and Bikaneri breed. In Jaisalmeri and Mewari camels, values within the breed were high, albeit non-significant in camels older than 10 years. Sex of the animal appeared to have no significant effect on tear production rate, as STT I values in male and female were statistically comparable. Likewise, no significant effect of age, season, environment and sex on STT I values in horses was reported in the past (Beech *et al,* 2003).

Conclusions

From the present study it can be concluded that breed, age and sex do not have significant effect on normal

Table 1. Tear production rate (mm/min) in different breeds of camel.

Parameter	Breed			
	Jaisalmeri (n = 19)	Kachchi (n = 19)	Bikaneri (n = 17)	Mewari (n = 26)
Range	15.00-30.00	14.50-30.50	15.00-30.00	15.00-30.00
Median	20.00	20.00	22.00	20.00
Mean ± S.E.	22.39±1.06	21.55±1.28	22.12±1.25	21.63±1.01

Note: Mean ± S.E. values between different breeds were statistically comparable.

Table 2. Tear production rate (mm/min) in male and female camel.

Parameter	Male (n = 40)	Female (n =41)	Overall (n= 81)
Range	14.5-30.00	15.00-30.50	14.5-30.5
Median	20.00	20.50	20.00
Mean±S.E.	20.81±0.74	22.95±0.81	21.89±0.56

Note: Mean ± S.E. values between male and female were statistically comparable.

tear fluid secretion rate in different Indian breeds of dromedary camel. The results of the study may be used as normal reference range for STT I and may assist veterinarians in diagnosis of ocular disorders affecting the tear film dynamics in dromedary camel.

Acknowledgements

Authors are thankful to Dr. Sanjay Kumar, Scientist, Shri Radhakishan (Technical officer) and Shri Mohan Singh (Technical officer) and for the assistance extended during the study.

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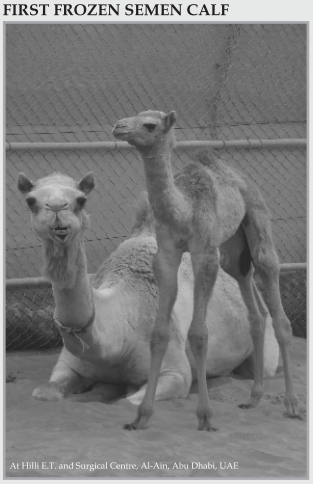

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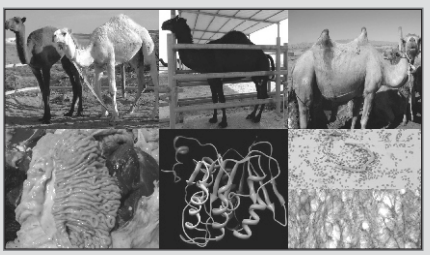

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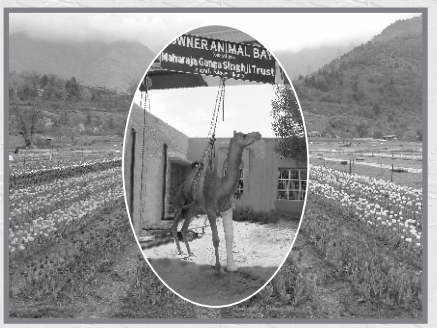

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DOI : 10.5958/2277-8934.2016.00020.5 Vol 23 No 1, p 121-125

ANATOMICAL AND HISTOCHEMICAL FEATURES OF THE BULBOURETHRAL GLANDS IN BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

Anatomical and histochemical features of bulbourethral glands in the Bactrian camel were investigated by gross-anatomical, histological and histochemical methods, and also by transmission microscope. The bulbourethral glands are compound tubuloalveolar glands, surrounded by a capsule of dense connective tissue. The lobules of the glands are formed by secretory units and excretory ducts, which are both lined by a single epithelium of mucous cell with a basal nucleus. Three types of secretory unit, designated A, B and C, were observed in the glands. Type A is lined with high columnar cells and the cytoplasm contains lots of secretory granules, which are PAS-positive, Alcion Blue-positive but Toluidine blue-negitive. Type B is lined with pyramidal or cuboidal cells and the cytoplasm shows PAS-positive, Alcion Blue-positive and Toluidine Blue-positive. The ultrastructures of epithelial cell shows that amount of granules with different electron density occupy the most space in the cytoplasm. Secretory granules can contain round inclusion. The shape of the epithelium varies according to the different stage of the secretory cycle.

Key words: Anatomy, bactrian camel, bulbourethral glands, histochemical feature

The development, composition of the secretion of bulbourethral glands, and the contents of the secretion in semen varies considersably in the different mammal species (Nielsen *et al,* 1977 and Setchell *et al,* 1993). Recent studies found that in boars and goats, the bulbourethral glands secretion may play an important role in sperm metabolism (Badia *et al,* 2005; Lasson *et al,* 1976; Gupta and Singh, 1963; Yamada, 1985). However, among Camelids, the histological studies of bulbourethral glands of dromedaries (Ali *et al,* 1978) and alpacas (Haidong *et al,* 2007) have been reported but it is lacking for bactrian camels.

The purpose of this study was to investigate the histological and histochemical characteristic of bulbourethral glands in bactrian camels by using light microscope and transmission electron microscope.

Materials and Methods

Samples of prostate glands were collected from 3 adult bactrian camels during rutting season (November, 2008).

For light microscopy study, the samples were fixed in Bouin's fluid or 10% neutral formalin solution for 72h, and processed for paraffin sections, 7μm thick. Then these sections were stained using following methods: haematoxylin-eosin (H-E) for general observation, Mallory as a trichrone stain for collagenous and muscle fibres, toluidime blue for metachromatic substances, periodic acid-Schiff (PAS) for neutral glycoconjugates, alcian blue (AB) for acid glycoconjugates, and a combined reaction of alcian blue and periodic acid-Schiff to show both acid and neutral mucosubstances (Badia *et al,* 2006).

The samples for electron microscope were fixed in 2.5% glutaraldehyde and then sent to School of Basic Medical, Lanzhou University.

Quantitative measures

The diameters of the endpieces and the height of the epithelium in endpieces and excretory ducts were measured in 25 transverse sections under the light microscope. The values were expressed as the mean±S.D. (n=3).

Results

The bulbourethral glands of the Bactrian camel were paired located on the dorsolateral aspect of the pelvic urethra, above the ischial arch. The glands were nearly spherical and covered by striated muscle in dorsal surface and part of the lateral surface.

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Light microscopy

Bulbourethral glands were compound tubuloalveolar glands, covered with dense connective tissue. Part of the connective tissue left the capsule and divided the parenchyma in lobules. Each lobule consisted of several end pieces and excretory ducts (Fig 1).

The end pieces were oval, with an average size of 32.45±8.47×23.15±7.01μm^2. The end pieces were lined by a single layer of epithelial cells, 9.30±1.41μm high. Most of the cells were high columnar epithelium, some were pyramidal epithelium. According to the shapes of the epithelium, the end pieces could be divided into three types, designated A, B and C. Type A is lined with a layer of high columnar cells, and had obvious acinar cavities. Type B was lined with a layer of pyramidal or cuboidal epithelium, and had no lumen. Type C had both kinds of epithelium in type A and B (Fig 2). The nuclei of all these cells were oval and basal. The cytoplasmic contained a large number of secretory granules.

The excretory ducts were lined by a single layer of columnar cells, irregular in shape and have developed lumen. The ducts between lobules were lined by transitional epithelium.

The interstitial tissue surrounding the endpieces and ducts was scarce and contained amount of collagen fibres and smooth muscular fibres. In the interstitial tissue, there were abundant venules and capillaries (Fig 3).

Transmission electron microscopy

The epithelium of end pieces and ducts in bulbourethral glands extended from the basal membrane to the luminal surface of the cells. The nuclei were oval, at the bottom of the cells. The chromatin was homogeneously and condensed close to the nuclear membrane.

The cytoplasm of epithelial cells contained amount of oval secretory granules, most of the granules had distinct membranes as a boundary. The secretory granules showed different electron densities, most of which had low electron densities. Sometimes the granules fused with each other (Fig 4). Occasionally, the secretory granules containing round inclusions were observed. In addition, the secretory granules in the apical cytoplasm were found occasionally fused the plasma membrane.

There wee amount of rough endoplasmic reticulum in the superanuclear region, which contianed electron-dense granules (Fig 5). Mitochondria were observed near the nucleus. The lateral plasma membranes were joined by typical junctional complexes (Fig 6). But no protruding structures were observed on the luminal surface of the secretory epithelium.

Histochemistry

The epithelial cells of the end pieces in bulbourethral glands were stained intensely with PAS. Meanwhile, the positive reaction in high columnar epithelium was much stronger than that in cuboidal epithelium (Fig 7). Besides, the epithelial cells had reactions with alcian blue (AB), and AB combined with PAS obviously, in which the high columnar epithelium was stained deeper than the other two types of epithelium. The ducts had no staining reactions with AB (Figs. 8 and 9).

The staining reaction with toluidine blue showed that the cytoplasm in pyramidal cells was metachromatic, but the high columnar cells was non-metachromatic (Fig 10).

Discussion

According to the reports on the bulbourethral glands in dromedary, different types of end pieces relate to their physiological state. Type A end pieces, which were particularly abundant during rutting seasons, were at a high level of physical activity, while type B and C end pieces were at a lower level of physical activity, which were rich in non-rutting seasons (Perk, 1962). The samples used in this study we collected in December, which was during the rutting season, and thus a large number of type A end pieces could be observed.

In the histochemical studies, type A units were stained intensely with PAS and AB. Type B units contained a large number of metachromatic granules by using toluidine blue staining, but type A units contained almost no metachromatic granules. According to the histochemical analysis that the neutral glycoconjugates showed a visible reactivity with PAS, but no reactivity with AB, while the acid glycoconjugates showed a reactivity with AB, but no reactivity with PAS (Evcrson Pcarso, 1980), the type A end pieces in bulbourethral gland of bactrian camel consisted of amount of neutral and acid glycoconjugates, but no metachromatic substances, type B units contained neutral mucopolysaccharide and a large number of metachromatic acidic polysaccharides. Type A and B both had certain activities, which was not consistent with the previous reports that type B units were inactive. However, the number of type B units was far less than type A units as reported before (Perk, 1962).

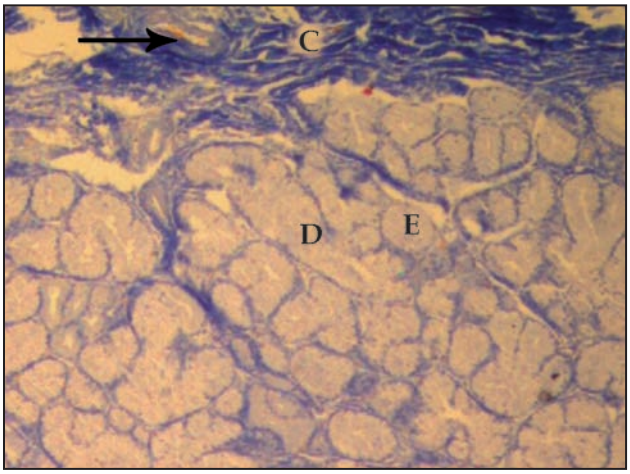

Fig 1. The whole view of bulbourethral glands in bactrian camel. C, capsule of dense connective tissue; E, endpieces; D, ducts; Arrowhead (→) shows the vein in connective tissue. Mallory's trichrome stain X 100.

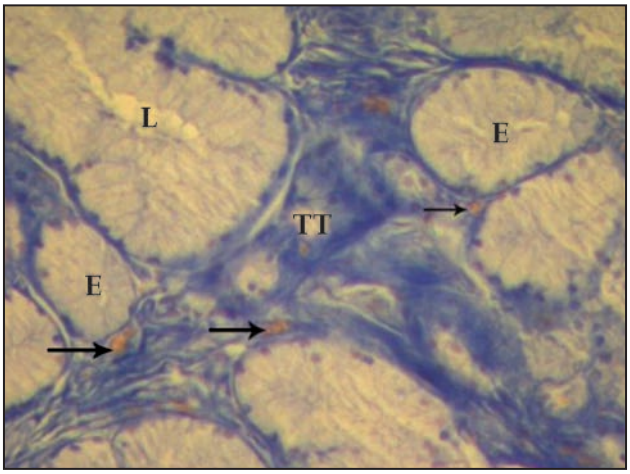

Fig 3. The interstitial tissue and capillary between the lobules of bulbourethral in bactrian camel. E, end pieces, D, ducts, IT, interstitial tissue. Arrowhead (→) shows the capillary. Mallory's trichrome stain X 400.

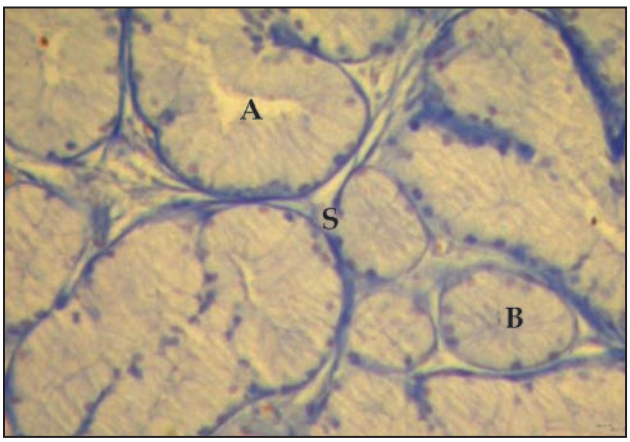

Fig 2. Different types of secretory unit in bulbourethral glands in bactrian camel. Type A is lined with one layer of tall columnar cells. Type B is lined with one layer of pyramidal cells. Mallory's trichrome stain X 400.

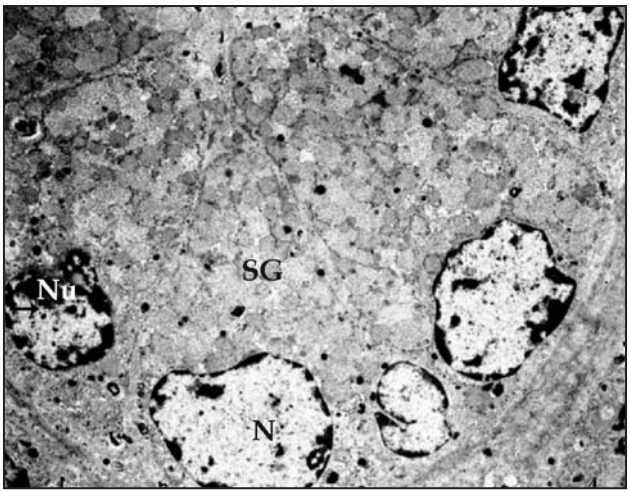

Fig 4. Ultrastructural view of the secretory epithelium of bulbourethral glands in bactrian camel The abundance of secretory granules in the cytoplasm causes the displacement of the nucleus at the cell base. N, nucleus; Nu, nucleolus; SG, secretory granules X 3,000.

In this study, no sulfated acidic muco-polysaccharide has been found in epithelial cells. In constract, the bulbourethral glands of rats are rich in sulfated acid mucopolysaccharide (Nielsen, 1976). In goats, the two types of secretion cells were differentiated by PAS and AB staining (Tsukise and Yamada, 1987). But in bactrian camels, the distribution of the epithelial cells with neutral or carboxylated acid mucosubstances cannot be distinguished strictly. Besides, the distribution and the proportion of the products that secreted by type A and B end pieces could not be determined.

The epithelium contained abundant secretory granules which were different in electron-density, when observed under the transmission electron microscope. No evidence of distributing rules of these granules was found in this study. The differences of the secretory granules contained high columnar epithelium and pyramidial epithelium was not found either. Some secretory granules contained round inclusions, but no membranous inclusions were observed in bactrian camel bulbourethral glands (Badia *et al,* 2006; Wong *et al,* 1988).

In this study some granules were found fused with the cell membranes at the apical part of the cell, and released into the glandular lumen. The fusion of these granules indicated a merocrine type of secretion, which was in agreement with the reports in boars (Nielsen *et al,* 1977). The presence of the secretory granules exocytosis and abundance of secertory products in lumen indicated that the secretions of bulbourethal glands released into the

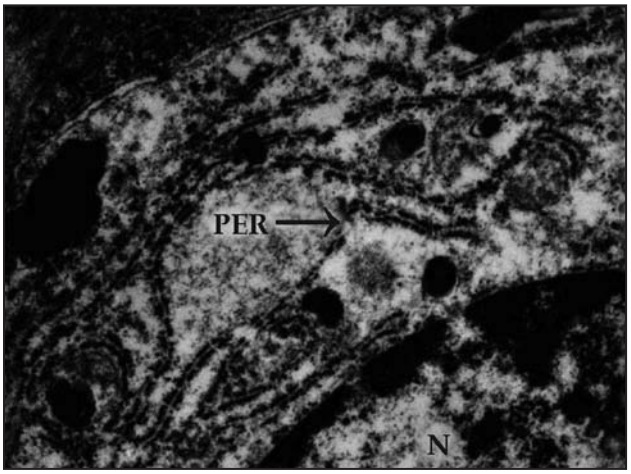

Fig 5. Ultrastructural view of the secretory epithelium. The abundance of rough endoplasmis reticulum occupies the supranuclear region. RER, rough endoplasmic reticulum; N, nucleus X 20,000.

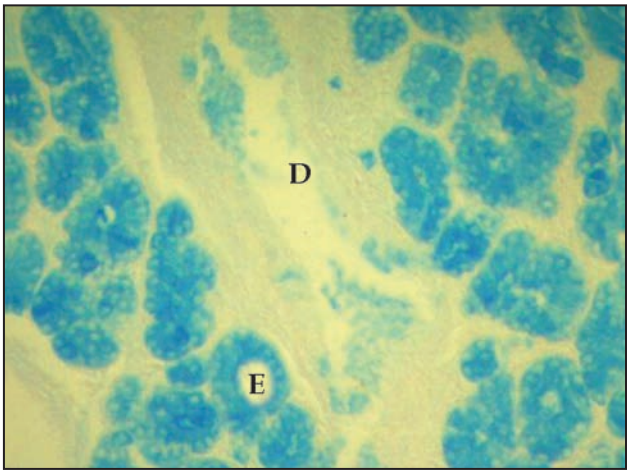

Fig 8. Bulbourethral glands of bactrian camel with Alcian blue stain. E, end pieces; D, ducts X 400.

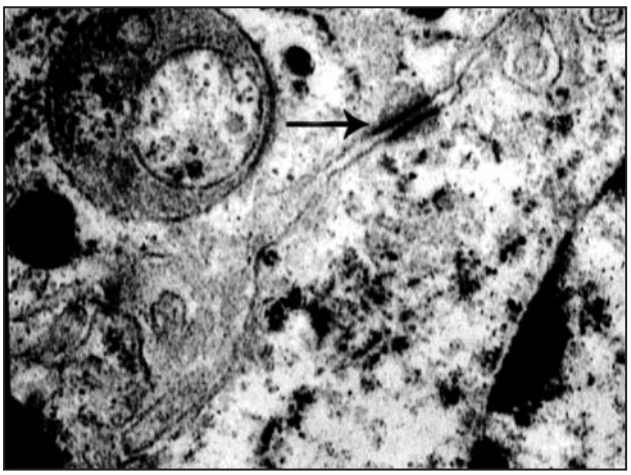

Fig 6. Ultrastructral view of the secretory epithelium. Arrow (→) shows the boundary between two adjacent cells X 40,000.

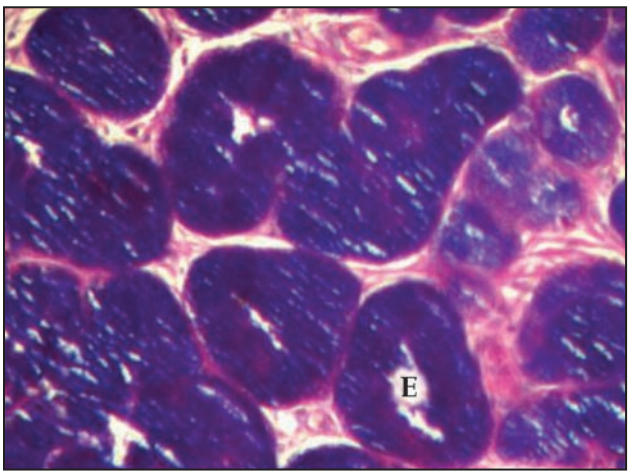

Fig 9. A conbined reaction of Alcian blue and PAS in bulbourethral glands of bactrian camel. E, end pieces X 400.

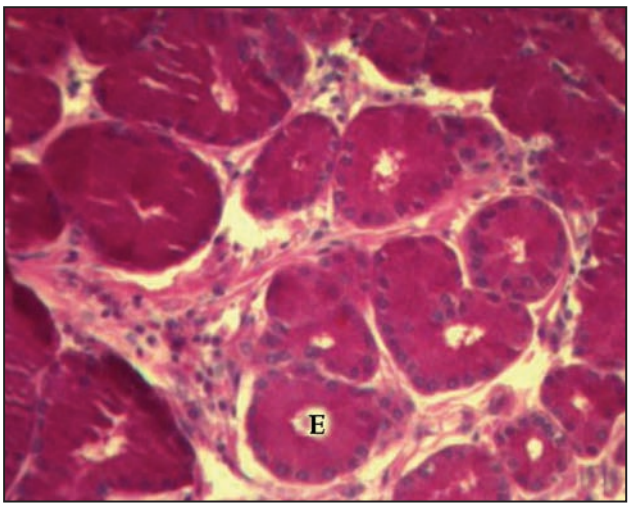

Fig 7. Bulbourethral glands of bactrian camel with PAS stain. E, end pieces X 400.

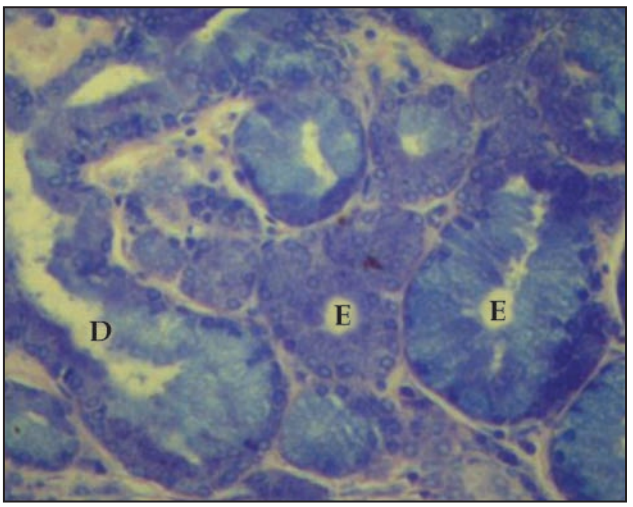

Fig 10. Bulbourethral glands of bactrian camel with Toluidine blue stain. E, end pieces; D, ducts X 400.

lumen continuously, and they could accumulate in lumen before being expelled.

In summary, the present study indicated that the two types of secertory eptithelial cells of bulbouretral glands in bactrian camel perform the same function, but were different in activities. But certain compositions of the secretion were not clear enough. This study identified that the neutral and carboxylated acid mucopolysaccharide was contained in the cells of secretory epithelium and demonstrated the existence of metachromatic acid polysaccharides. The different kinds of secretions in bactrian camel bulbourethral glands must play different roles during reproductive process. The exact functions of each kind of secretions still need further studies.

Acknowledgements

We thank Wenling Ye, Guofen Wu and all other members in our laboratory for their selfless help. This study received financial supports from National Natural Science Foundation of China (81472541, 81171954), Innovation Fund For Technology Based Firms of China (12C26216207052) and Central University Basic Scientific Research Fee of Lanzhou University, China (lzujbky-2014-253).

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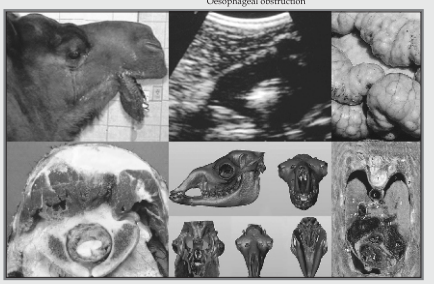

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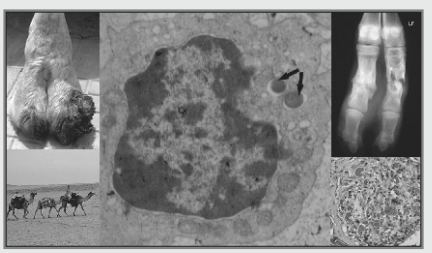

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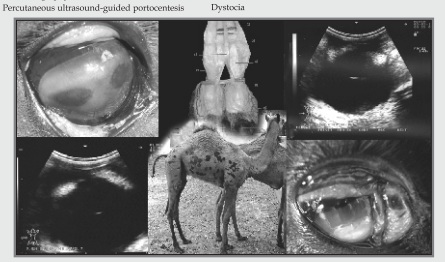

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DOI : 10.5958/2277-8934.2016.00021.7 Vol 23 No 1, p 127-131

APPLIED ANATOMY OF THE MAXILLOFACIAL AND MANDIBULAR REGIONS OF THE DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

The study involved some osteometric parameters of the upper jaw and mandible of 6 apparently healthy adult camels without any apparent skeletal disorders. A total of 21 head measurements and indices were recorded in the present study. The supraorbital foramina distance, infraorbital foramina distance, skull length, skull width, cranial length, nasal length and skull width of the Indian one-humped dromedary camel were 6.35±0.047 cm, 8.41±0.076 cm, 48.75±0.244 cm, 22.66±0.108 cm, 32.73±0.484 cm and 16.89±0.283 cm, respectively. The skull index was 46.51±0.29. In addition, the distances from facial tuberosity to the infra-orbital canal and from the latter to root of the 1st upper premolar tooth were 2.91±0.068 cm and 3.21±0.078 cm, respectively. The length and height of the mandible were 42.98±0.624 cm and 22.58±0.287 cm, respectively. Furthermore, the distances from the lateral alveolar root to mental foramen and from the mental foramen to caudal mandibular border were 9.22±0.059 cm and 32.12±0.165 cm, respectively. In the present study, the distances from mandibular foramen to the base of mandible as well as from caudal border of mandible to below the mandibular foramen were 8.84±0.085 cm and 6.32±0.048 cm, respectively. Also, the distances from the base of mandible to condyloid fossa and from the latter to the maximum height of mandible were 18.38±0.15 cm and 4.175±0.046 cm, respectively. Finally, the distance from caudal border of mandible to mandibular foramen and from the latter to mandibular angle were 5.88±0.055 cm and 8.29±0.079 cm, respectively.

Key words: Anatomy, camel, mandibular, maxillofacial, regional anaesthesia

Anatomy of skull of dromedary is studied previously (Smuts and Bezuidenhout, 1987). Various nerve blocks and regional anaesthesia of the head region of dromedary have been reported, i.e. retrobulbar blocks (Hassanein *et al,* 1984), auriculopalpebral nerve blocks (Zabady and Elnady, 2004), facial nerve block (Arnautovic *et al,* 1970; Stanic *et al,* 1972), infraorbital nerve block (Ahmed, 1978) and mental nerve block (Ramadan, 2014). The clinical application of these nerve blocks in diverse surgical disorders of head region has been well documented (Gahlot, 2000; Ramadan, 1994 and 2014). The osteometry of skull of dromedary camel is scarcely reported. Present study was therefore undertaken to study osteometric parameters of maxillofacial and mandibular region of skull of dromedary camel.

Materials and Methods

This study involved some morphometric parameters of the upper jaw and mandible of 6 apparently healthy adults Indian one-humped dromedary camel (*Camelus dromedarius*) without any apparent skeletal disorders. A total of 21 morphometric measurements were done in the upper jaw and mandibles using scale, thread and digital calipers and data were presented as means±SD.

The various parameters studied are described below and shown in Figs 1-4.

A. Skull Length; from the dorsal lateral nasal cartilages to the external occipital protuberance; sub-divided into cranial length (A1) and nasal length (A2).
B. Skull width; maximum distance between two zygomatic arches.
C. Skull/cephalic index (SI): Skull width/ Skull length X 100 (Miller *et al,* 1964).
D. Supraorbital foramina distance; greatest width between the supraorbital foramina.
E. Infraorbital foramina distance; facial width between the supraorbital foramina.
F. Facial tuberosity to the infra-orbital canal; from the level of the most lateral bulging of the facial tuberosity to the mid level of the infra-orbital canal.
G. Infra-orbital canal to the root of alveolar tooth; the measurement was taken vertically from the

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mid-level of the infra-orbital canal to the root of the alveolar tooth.

H. Distance between supraorbital foramina and infraorbital foramina.

I. Distance between orbital rim to supraorbital foramina.

J. Distance between orbital rim to infraorbital foramina.

K. Lateral alveolar root to mental foramen; from the mental foramen to the lateral extent of the alveolar root of lower incisor.

L. Mental foramen to the caudal mandibular border; from the level of the mental foramen to the extreme caudal border of the mandible.

M. Mandibular length; from the level of the cranial extremity of the alveolar root of the incisor to the level of the caudal border of the mandible.

N. Maximum mandibular height measured from the basal level of the mandible to the highest level of the coronoid process.

O. Mandibular foramen to base of mandible; vertical line from the ventral limit of the mandibular foramen to the base of the mandible.

P. Caudal border of mandible to below mandibular foramen; length from the caudal most border of the mandible to the vertical line produced by a description of the measurement of the mandibular foramen to the base of the mandible.

Q. Condyloid fossa to the height of the mandible; from the maximum height of mandible to the condyloid fossa.

R. Condyloid fossa to the base of the mandible.

S. Caudal border of mandible to the level of mandibular foramen.

T. Mandibular foramen to mandibular angle; shortest distance from the mandibular foramen to the extreme caudal border of the angle of the mandible.

U. Height and width of the supraorbital foramina.

These parameters of the mandible were measured and subjected to routine statistical analysis (Snedecor and Cochran, 1994). The photographs of the skull of dromedary camel were taken by the Nikon D3200 digital SLR camera and labeled with Adobe Photoshop CS6 extended version 13.0.1.

Results

In the present study, the supraorbital foramina distance, infraorbital foramina distance, skull length, skull width, cranial length and nasal length of the Indian camels were 6.35±0.047 cm, 8.41±0.076 cm, 48.75±0.244 cm, 22.66±0.108 cm, 32.73±0.484 cm and 16.89±0.283 cm, respectively (Table 1).

The distance between supraorbital foramina and infraorbital foramina was 4.485±0.046 cm, while the distance between rim of the orbit to the supraorbital foramina and infraorbital foramina was 5.66±0.051 cm and 5.87±0.053 cm, respectively in Indian native camels.

The skull index was found to be 46.51±0.29. The distance from the facial tuberosity to the infra-orbital canal and from the latter to the root of the alveolar tooth directly ventral to it were 2.19±0.068 cm and 3.21±0.078 cm, respectively in camel (Table 1). The data are of clinical importance because the facial tuberosity is very prominent even in live animals as a guide for tracking the infra-orbital nerve, and necessary for its desensitisation during the manipulations in the skin of the upper lip, nostril and face at the level of the foramen. The injection of local anaesthetic agents within the canal via the infra-orbital foramen will also lead to analgesia of the incisor, canine and first two premolar teeth.

The distance between the lateral ends of the alveolus of the 3rd incisor tooth to the mental foramen was 9.22±0.059 cm in dromedary camel (Table 1) which is an important landmark for achieving the location of the mental nerve for the regional nerve block in camel. In the anterior aspect of the mandibular canal, injection can be made through the mental foramen to desensitise mental aspect of the mandibular nerve. The distance from the mental foramen to the caudal mandibular border was 32.12±0.165 cm.

The length and height of the mandible were 42.98±0.624 cm and 22.58±0.287 cm, respectively in camel. The distance between the condyloid fossa to the height of mandible and condyloid fossa to the base of the mandible were 4.175±0.046 cm and 18.38±0.15 cm, respectively in camel. The distance between the vertical line drawn downward from the caudal border of mandible (R) and the vertical line drawn from the mandibular foramina downwards (O) was (P) 6.32±0.048 cm.

The distances from the mandibular foramen to the base of the mandible, caudal border of mandible to the level of mandibular foramen and the mandibular foramen to the border of mandibular angle were 8.84±0.085 cm, 5.88±0.055 cm and 8.29±0.079 cm, respectively. The morphometric parameters of the mandibular foramina in the present study is helpful in open reduction and internal fixation of mandibular fracture under regional

Table 1. The measurements of upper jaw and mandibles of dromedary camel (*Camelus dromedarius*).

Sr. No.	Different Parameters	Mean+SD
A.	Skull length	48.75±0.244
	Cranial length (A1)	32.73±0.484
	Nasal length (A2)	16.89±0.283
B.	Skull width	22.66±0.108
C.	Skull/cephalic index	46.51±0.29
D.	Supraorbital foramina distance	6.35±0.047
E.	Infraorbital foramina distance	8.41±0.076
F.	Facial tuberosity to the infra-orbital canal	2.19±0.068
G.	Infra-orbital canal to the root of alveolar tooth	3.21±0.078
H.	Distance between supraorbital foramina and infraorbital foramina	4.485±0.046
I.	Distance between orbital rim to supraorbital foramina	5.66±0.051
J.	Distance between orbital rim to infraorbital foramina	5.87±0.053
K.	Lateral alveolar root to mental foramen	9.22±0.059
L.	Mental foramen to the caudal mandibular border	32.12±0.165
M.	Mandibular length	42.98±0.624
N.	Mandibular height	22.58±0.287
O.	Mandibular foramen to base of mandible	8.84±0.085
P.	Caudal border of mandible to below mandibular foramen	6.32±0.048
Q.	Condyloid fossa to the height of the mandible	4.175±0.046
R.	Condyloid fossa to the base of the mandible	18.38±0.15
S.	Caudal border of mandible to the level of mandibular foramen	5.88±0.055
T.	Mandibular foramen to mandibular angle	8.29±0.079

anaesthesia using mandibular nerve block in Indian native camels.

Discussion

In the present study, the supraorbital foramina distance, infraorbital foramina distance, skull length, skull width, cranial length and nasal length of the Indian camels were 6.35±0.047 cm, 8.41±0.076 cm, 48.75±0.244 cm, 22.66±0.108 cm, 32.73±0.484 cm and 16.89±0.283 cm, respectively. However, the supraorbital foramina distance, infraorbital foramina distance, skull length, cranial length and nasal length were 18.3 cm, 6.43 cm, 46.2 cm, 32.5 cm and 13.3 cm, respectively in the Iranian one-humped camels. Zhu *et al* (2014) also reported skull length, skull width and cranial length in donkey were 44.307±5.35 cm, 16.90±1.76 cm and 20.782±2.22 cm, respectively.

The values of supraorbital foramina distance, skull length, cranial length and nasal length of the dromedary camel were relatively higher than the results obtained from the immature one-humped camel in Nigeria (Yahaya *et al,* 2012). It is may be due to the existence of significant differences in the some skull morphometric indices between adult and young animals.

The distance between supraorbital foramina and infraorbital foramina was 4.485±0.046 cm, while the distance between rim of the orbit to the supraorbital foramina and infraorbital foramina was 5.66±0.051 cm and 5.87±0.053 cm, respectively in dromedary camel.

The skull index was 46.51±0.29 in Indian camel, whereas it was 38.23±0.85 in donkey (Zhu *et al,* 2014) and 46.12±0.12 in blackbuck (Choudhary and Singh, 2015b).

The distance from the facial tuberosity to the infra-orbital canal and from the latter to the root of the alveolar tooth directly ventral to it were 2.19±0.068 cm and 3.21±0.078 cm, respectively in dromedary camel while in West African Dwarfs goats were 1.6-1.8 cm and 1.3-1.6 cm (Olopade and Onwuka, 2005); in Gwembe Valley dwarf goat were 2.06±0.14 cm and 1.13±0.11 cm (Kataba *et al,* 2014); in Iranian native cattle were 2.8 cm and 2.5 cm (Monfared, 2013b) and in blackbuck were 2.37±0.009 cm and 0.72±0.008 cm (Choudhary and Singh, 2015a). Uddin *et al* (2009) also reported same measurements for Black Bengal goat and these were 1.85±0.14 cm and 1.75±0.19 cm.

The distance between the lateral end of the alveolus of the 3rd incisor tooth to the mental foramen was 9.22±0.059 cm in dromedary camel while it was 1.6±0.22 cm in West African Dwarfs goat (Olopade and Onwuka, 2005), 2.0±0.3 cm in Red Sokoto (Maradi) goat (Olopade and Onwuka, 2007), 4.74 cm in Iranian one-humped camels (Monfared, 2013a) and 2.45±0.008 in blackbuck (Choudhary and Singh, 2015a).

In the anterior aspect of the mandibular canal, injection can be made through the mental foramen to desensitise mental aspect of the mandibular nerve. This will ensure the loss of sensation of the lower incisors, premolar and lower lip on that side (Hall *et al,* 2000). The distance from the mental foramen to the caudal mandibular border was 32.12±0.165 cm. However, the distance from the mental foramen to the caudal mandibular border was 13.43±0.081 cm in Iranian one-humped camels (Monfared, 2013a).

The length and height of the mandible were 42.98±0.624 cm and 22.58±0.287 cm, respectively in camel which was higher than the value obtained for West African Dwarfs goats of Nigeria as 12.00±1.89 cm and 6.90±1.09 cm, respectively (Olopade and Onwuka, 2005). However, the length and height of the mandible were 27.4 cm and 15.88 cm in Iranian native cattle; 39.9 cm and 9.92 cm in Iranian one-humped camels (Monfared, 2013a) and 16.53±0.128 cm and 10.69±0.024 cm in blackbuck (Choudhary *et al*, 2015b).

The distances between the condyloid fossa to the height of mandible and condyloid fossa to the base of the mandible were 4.175±0.046 cm and 18.38±0.15 cm, respectively in camels of present study. Whereas, the distances between the condyloid fossa to the height of mandible and condyloid fossa to the base of the mandible were 3.09±0.008 and 7.57±0.024 cm, respectively in blackbuck (Choudhary and Singh, 2015a).

The distance between the vertical line drawn downward from the caudal border of mandible (R) and the vertical line drawn from the mandibular foramina downwards (O) was 6.32±0.048 cm (P), while same distance was 1.85±0.011 cm in blackbuck (Choudhary and Singh, 2015a). The mandibular fracture was most common, followed by tibial fracture in camels (Ahmed and Al-Sobayil, 2012).

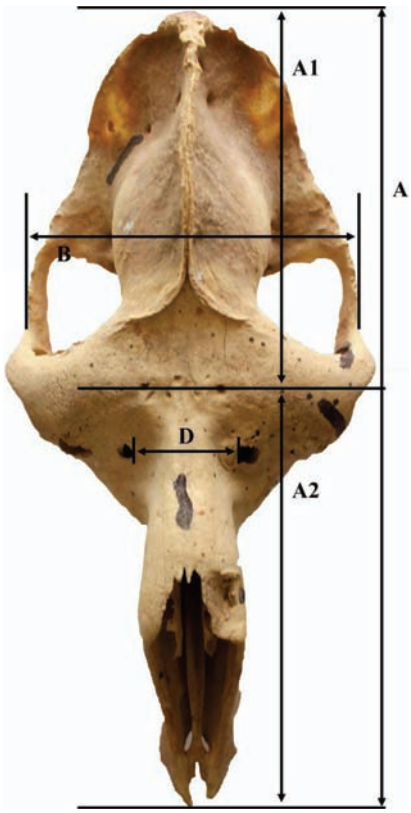

Fig 1. Measurements of the skull of dromedary camel showing skull length (A), cranial length (A1), nasal length (A2), width of skull (B), distance between supraorbital foramina (D).

The most frequent technique failure in anaesthesia of the inferior alveolar lies the inappropriate setting of the needle, due to the inaccurate location of anatomic parts (Hetson *et al*, 1988), i.e. mandibular foramen.

Fig 2. Measurements of the skull of dromedary camel showing distance between facial tuberosity to the infra-orbital canal (F), infra-orbital canal to the root of alveolar tooth (G), distance between supraorbital foramina to infraorbital foramina (H), supraorbital foramina to rim of orbit (I), infraorbital foramina to rim of orbit (J).

Fig 3. Measurements of the mandible showing distance from lateral alveolar root to mental foramen (K), mental foramen to the caudal mandibular border (M), mandibular length (N).

Fig 4. Measurements of the mandible showing maximum mandibular height (N), distance from mandibular foramen to base of mandible (O), caudal border of mandible to below mandibular foramen (P), condyloid fossa to height of mandible (Q), condyloid fossa to the base of the mandible (R), caudal border of mandible to the level of mandibular foramen (S), mandibular foramen to mandibular angle (T).

The distances from the mandibular foramen to the base of the mandible, caudal border of mandible to the level of mandibular foramen and the mandibular foramen to the border of mandibular angle were 8.84±0.085 cm, 5.88±0.055 cm and 8.29±0.079 cm, respectively. However, the distance from the mandibular foramen to the base of the mandible, caudal border of mandible to the level of mandibular foramen and the mandibular foramen to the border of mandibular angle were 4.18±0.014 cm, 1.36±0.010 cm and 3.07±0.006 cm, respectively in blackbuck (Choudhary and Singh, 2015a). Equivalent figures for West African Dwarfs goats of Nigeria were 1.57±0.44 cm, 2.58±0.34 cm, respectively for caudal border of mandible to below mandibular foramen and the mandibular foramen to the base of the mandible (Olopade and Onwuka, 2005). In horse and dogs, the distance between the mandible foramen and the base of the mandible was 3 cm and 1.5 to 2 cm, respectively (Hall *et al,* 2000). The parameters studied would help in establishing landmarks for diverse nerve blocks and regional anaesthesia of head region.

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Continued on page 138

DOI : 10.5958/2277-8934.2016.00022.9 Vol 23 No 1, p 133-137

OSTEOMETRIC EVALUATION OF THE METAPODIAL BONES IN ONE-HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

The osteometric evaluation was done on 40 macerated metapodial bones of 10 male adult one-humped camels by using 16 linear measurements. There was no difference between the right and left metapodial bones both in the fore and hind limbs. Means of the most parameters in the metacarpus were greater than those of the metatarsus. In all metapodial bones, means of the greatest length of the lateral side were greater than those of the medial ones, which are similar to other reported domestic and wild ruminantes. Current findings may be helpful for biomechanical and osteoarcheological studies.

Key words: Camel, metapodial bones, morphometry, osteometry

The ability of camel to easily transverse through sandy and rough terrain is due to the special anatomy of its foot (Gahlot, 2000). In unguligrade quadrupeds, the bones of their metapodia are among the important limb long bones (Paral *et al,* 2004). The 3rd and 4th metacarpal bones in the thoracic and pelvic limbs are the only remaining of the metapodial bones and united to form cannon bones before birth except for the distal 1/5th. The metapodial bones of the dromedary camel exhibit 2 unique features including absence of rudimentary 2nd and 5th metapodial bones (dew claws) and presence of the long incisure at the distal part of cannon bone (Smuts and Bezuidenhout, 1987). The later anatomical trait through a common sole of both digits may aid camels to have wider foot for passing sandy desert.

There are few studies in the current literature regarding the morphometry of the metapodial bones of camels. Metapodial bones of a male juvenile dromedary camel were studied by Bani Ismail *et al* (2008) as a morphometric survey on the distal limb bones. Topographic anatomy and morphomtery of the cannon bones and digital bones of the adult bactrian camel and one humped camel were described by She *et al* (2007), Badawy (2011), El-Shafey and Sayed-Ahmed (2012) and El-Shafey and Kassab (2013).

Some authors declared that the medial and lateral digits slightly differ in length in several domestic and wild ruminants (Keller *et al,* 2009; Muggli *et al,* 2011; Nourinezhad *et al,* 2014). This difference may be due to higher length of the lateral condyles of the metapodial bones. The difference in digit length not only helps animal's coordination at standing and locomotion; but also, predisposes lateral digit to have more fluency to disease for having more pressure (Muggli *et al,* 2011). Previous researches about the difference in length between the lateral and medial metapodial condyles of the dromedary camel were not traceable in available literature. Moreover, the current study is the continuation of our previous researches in terms of the dromedary distal limb structures (Nourinezhad *et al,* 2011; 2015) aimed to find, if there is any asymmetry in the metapodial bones which may be useful for osteoarcheological purposes.

Materials and Methods

Twenty metacarpus and 20 metatarsus of apparently 10 healthy male adult one-humped camels aged 6-10 years and body weight 340-640 kg were collected from a local slaughterhouse. The bones were successively prepared by boiling, cleaning, degreasing, washing, and drying based on techniques of Nourinezhad *et al* (2014 and 2015). All metapodial bones after maceration were measured by 2 calipers (200 and 500 mm) with an accuracy of 0.01mm. Sixteen linear measurements (Fig 1) were used *viz.* width of proximal end (Bp), width of distal end (Bd), and smallest width of diaphysis (SD) (Von den Driesch, 1976), the greatest length of the medial part (ML) and

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greatest length of the lateral part (LL) (Nacambo *et al,* 2007), mid-shaft width of the diaphysis (d), mid-shaft depth of the diaphysis (e), cranio-caudal diameter of the internal trochlea of the medial condyle (DIM), cranio-caudal diameter of the external trochlea of the medial condyle (DEM), cranio-caudal diameter of the internal trochlea of the lateral condyle (DIL), cranio-caudal diameter of the external trochela of the lateral condyle (DEL), medio-lateral width of the medial condyle (WCM), and medio-lateral width of the lateral condyle (WCL) (Davis, 1996). The greatest lateral length (GLL) and greatest medial length (GLM) were considered in order to calculate the difference in length of two condyles (λ). For measuring GLL and GLM, the distance between the highest point of the proximal end of the metapodial bones and the distal point of the lateral and medial condyles were measured. The highest point for the metacarpus was the medial articular surface of the proximal end and for the metatarsus was the plantar process of its proximal end. All parameters were measured 3 times and the mean values were documented. The mean parametric values were established by use of SPSS 16.0. An independent student t-test was applied to analysis whether there was any difference between the mean values of the measurements of the right and left sides as well as the lateral and medial sides of the bones. Parametric values were shown as

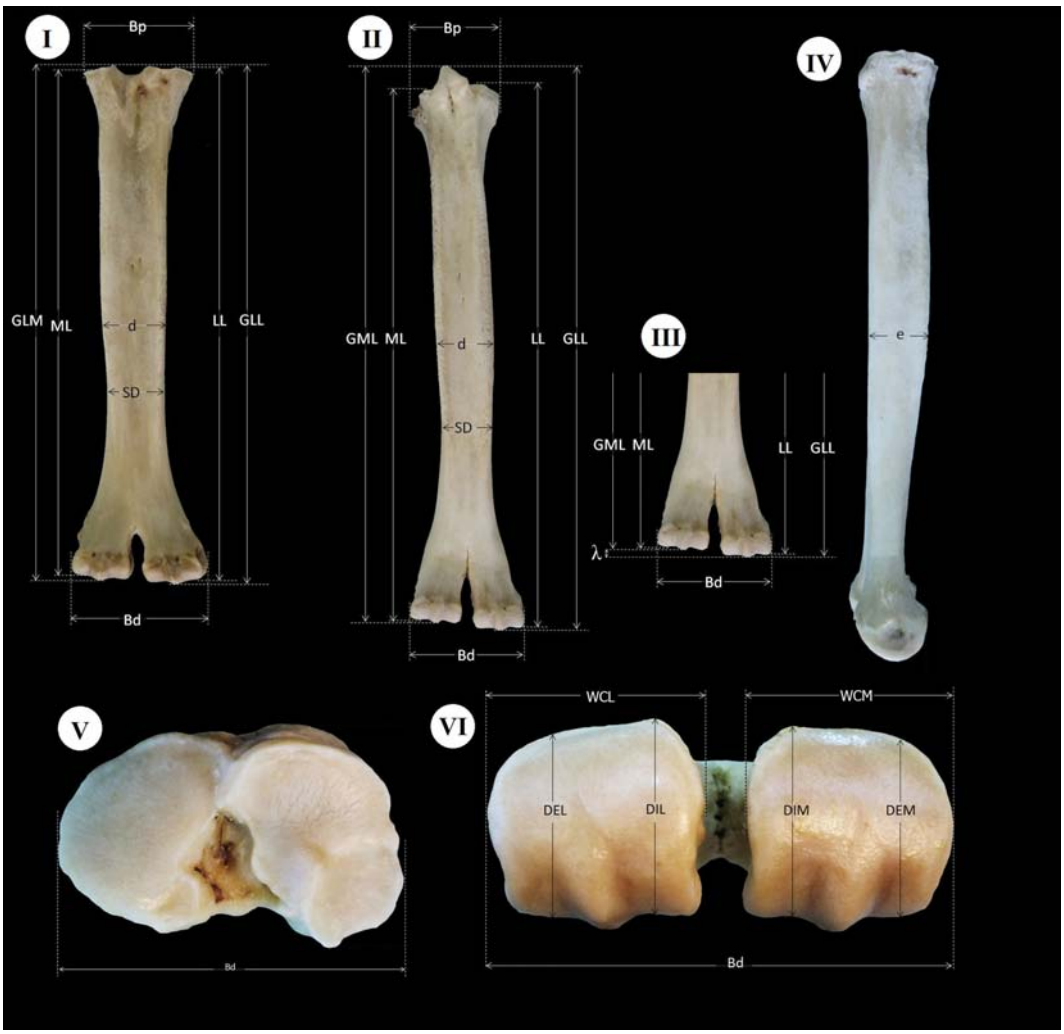

Fig 1. Palmar view(I), plantar view(II), distal part of I (III), medial view (IIII), proximal view (V), and distal view (VI) of the right metacarpal bone of the one- humped camel showing where the parameters were measured. Width of proximal end (Bp), width of distal end (Bd), and smallest width of diaphysis (SD), the greatest length of the medial part (ML) and greatest length of the lateral part (LL), mid-shaft width of the diaphysis (d), mid-shaft depth of the diaphysis (e), cranio/caudal diameter of the internal trochlea of the medial condyle (DIM), cranio/caudal diameter of the external trochlea of the medial condyle (DEM), cranio/caudal diameter of the internal trochlea of the lateral condyle (DIL), cranio/caudal diameter of the external trochlea of the lateral condyle (DEL), medio-lateral width of the medial condyle (WCM), and medio-lateral width of the lateral condyle (WCL), the greatest lateral length (GLL), greatest medial length (GLM), the difference in length of two condyles (λ).

mean, standard division, minimum, and maximum of metacarpal and metatarsal bones in right and left limbs. The p value of less than 0.05 was taken as significant.

Results

The mean parametric values of the right and left metapodial bones are presented in table 1.

There was no difference between the right and left metapodial bones both in the fore and hind limbs.

Means of the most parameters such as Bp, d, Bd, SD, WCM, WCL, DIM, DIL, DEM, and DEL in the metacarpus were greater than those of the metatarsus. Means of GLL, GML, and e in the metatarsus were significantly greater than those of metacarpus.

Means of GLL were greater than those of GLM in all samples, which indicate that the lateral condyles were longer than the medial ones. This discrepancy (λ) was significantly greater in metatarsus than that of metacarpus.

Means of LL were greater than those of ML in 67% of all metapodia (83% in the metacarpus and 56% in the metatarsus). Means of ML were greater than those of LL in 28% of the specimens (16% in the metacarpus and 35% in the metatarsus). Means of LL and ML were equal in 8% of the specimens (0% in the metacarpus and 8% in the metatarsus).

Discussion

Based on our findings, the hypothesis that the lateral condyle of the metapodial bone is longer than the medial ones was confirmed, which is in agreement with findings of previous studies reported by Nacambo *et al* (2007), Keller *et al* (2009) and Muggli *et al* (2011) in domestic and wild ruminants. Nacambo *et al* (2007) studied the metapodial bones of 42 calves and 10 dairy cows. They found a significant difference between lengths of the condyles of these bones. Nearly all of the lateral condyles were longer in the metatarsal bones (98.8%), but the lateral condyles of the metacarpal bones were longer than the medial ones (52.4% of specimens). Such results were obtained in our study regardless of the percentages. Keller *et al* (2009) also found the length asymmetry between the 2 epiphysis of metacarpal and metatarsal bones

Table 1. Mean values of parameters (mm) of the metapodial bones (n=40) of the dromedary camels.

Parameters	Fore limbs (Means±SD)		Hind Limbs (Means±SD)	
	Right	Left	Right	Left
GLL	369.25 ± 10.47^{B}	367.82 ± 11.42^{B}	384.86 ± 13.50^{A}	391.57 ± 11.71^{A}
GLM	367.18 ± 10.63^{B}	365.87 ± 11.28^{B}	380.60 ± 12.33^{A}	387.47 ± 12.01^{A}
Λ	2.07 ± 0.18^{B}	1.95 ± 0.13^{B}	4.26 ± 0.52^{A}	4.10 ± 0.50^{A}
LL	366.73 ± 09.79^{b}	364.58 ± 10.54^{b}	374.81 ± 13.58^{a}	377.62 ± 10.43^{a}
ML	356.50 ± 10.33^{b}	363.73 ± 10.28^{b}	369.07 ± 12.32^{a}	373.61 ± 10.72^{a}
Bp	72.12 ± 3.12^{a}	71.76 ± 3.49^{a}	59.30 ± 3.55^{b}	61.29 ± 3.41^{b}
d	39.26 ± 2.01^{a}	39.85 ± 1.23^{a}	34.47 ± 1.42^{b}	35.16 ± 1.58^{b}
SD	39.64 ± 1.38^{a}	39.08 ± 1.74^{a}	31.55 ± 2.31^{b}	32.18 ± 2.52^{b}
e	33.55 ± 2.14^{B}	34.61 ± 2.16^{B}	36.05 ± 3.27^{A}	37.30 ± 3.01^{A}
Bd	96.22 ± 3.11^{a}	95.92 ± 3.37^{a}	81.50 ± 3.04^{b}	82.65 ± 2.14^{b}
WCM	42.78 ± 1.68^{a}	42.88 ± 2.13^{a}	35.30 ± 2.06^{b}	36.23 ± 1.26^{b}
WCL	43.55 ± 1.90^{a}	43.51 ± 1.96^{a}	35.88 ± 1.94^{b}	36.05 ± 1.30^{b}
DIM	42.48 ± 1.80^{a}	42.38 ± 1.85^{a}	35.99 ± 1.38^{b}	36.78 ± 1.27^{b}
DIL	43.04 ± 1.79^{a}	42.82 ± 1.81^{a}	37.82 ± 2.04^{b}	37.77 ± 1.75^{b}
DEM	42.14 ± 1.51^{a}	41.79 ± 1.59^{a}	39.73 ± 1.23^{b}	35.68 ± 1.11^{b}
DEL	41.47 ± 1.64^{a}	41.18 ± 1.57^{a}	35.42 ± 1.38^{b}	35.41 ± 1.53^{b}

Width of proximal end (Bp), width of distal end (Bd), and smallest width of diaphysis (SD), the greatest length of the medial part (ML) and greatest length of the lateral part (LL), mid-shaft width of the diaphysis (d), mid-shaft depth of the diaphysis (e), cranio/caudal diameter of the internal trochlea of the medial condyle (DIM), cranio/caudal diameter of the external trochlea of the medial condyle (DEM), cranio/caudal diameter of the internal trochlea of the lateral condyle (DIL), cranio/caudal diameter of the external trochlea of the lateral condyle (DEL), medio-lateral width of the medial condyle (WCM), and medio-lateral width of the lateral condyle (WCL), the greatest lateral length (GLL), greatest medial length (GLM), the difference in length of two condyles (λ).

Means in a row with different large superscript letters (A,B) are statistically different between the metacarpal and metatarsal bones ($p<0.01$).

Table 2. Comparison between means of the greatest length (Gl) and width (d) of the metapodial bones among various ruminants.

Authors	Species	Metatarsus		Metacarpus	
		Gl	d	Gl	d
Berteaux and Guintard (1995)	Old cattle	205.4	34.8	233.3	29.6
Paral *et al* (2004)	New cattle	220	–	240	–
Nourinezhad *et al* (2015)	Water buffalo	211	41.40	240.90	34.40
She *et al* (2007)	Bactrian camel	325	37	–	–
Bani Ismail *et al* (2008)	Juvenile dromedary camel	318. 5	–	320	–
Present study	Adult dromedary camel	369	39.26	384.86	34.47

*Empty cells marked with (-) were not reported by authors

in some wild ruminants, including european moose, bison, and fallow deer. In addition, Muggli *et al* (2011) described that the lateral digits in 40 cattle of different ages were slightly longer than the medial ones. This asymmetry in the young animals was due to longer lateral proximal and distal phalanges, and in older ones is mainly because of longer lateral condyle and proximal phalanx.

Surprisingly, Altrib *et al* (2013) reported that the medial condyle of the 3rd metapodial bone in horse was wider than the lateral condyle, but the lateral condyle was longer than all metapodial bones of 23 horses of 3 groups studied. Although, the horse has only one main metapodial bone on each limb, this difference in length may help in coordination.

Bani Ismail *et al* (2008) described morphometric measurement of the metapodial bones in juvenile male camels. The variables were Bp, Bp, SD, Gl, and d. They did not calculate the length difference between the lateral and medial condyle of the metapodial bones, but they indicated that the means of B p, Bp and SD in the metacarpal bone were greater than those of the metatarsal ones, which corresponded with our findings.

In clinical perspective, a fracture of the lateral condyle of the right metacarpus of a 10 year old camel has been noted by Singh and Gahlot (1997). This fracture may be related to longer length of the lateral condyle, which leads to having more loads.

Based on table 2, the means of Gl and d differed among the large ruminants. In dromedary camel, the means of d in the metatarsus were greater than those of the metacarpus. In other ruminants the means of Gl in the metatarsus were greater than those of the metacarpus. The metacarpal and the metatarsal bones are nearly equal in length among different ages i.e. seven months old calf (Grossman, 1960), juvenile dromedary camel (Bani Ismail *et al,* 2008); and adult one humped camel (Smuts and Bezuidenhout, 1987). However, in present study, the metatarsal bone was somewhat longer than metacarpal ones. But this disparity is not as much as other animals. It may have additional advantages in terms of walking ability.

Although, longer metatarsal bone (Gl) in the dromedary camel is due to the presence of the plantar process on the plantar surface of the proximal extremity which may not contribute in total length of the pes, closer examination of the pes of all specimens revealed that manus is longer than the pes. Digital bones are the mainly responsible for that asymmetry between the pes and the manus (Nourinezhad *et al,* 2015).

Furthermore, it is clearly obvious that the length of the metapodial bone in the dromedary is greater than that of other reported ruminants. Thus it seems that such an anatomical difference in camel metapodia may play an important role to have longer steps and better walking ability. Current findings may be helpful for biomechanical and osteoarcheological studies.

Acknowledgements

The author would like to thank Prof. Dr. M. Daram of the Department of English Language and Literature of Shahid Chamran University of Avaz for editing the manuscript. The authors also appreciate the Research Affairs of Shahid Chamran University. We are grateful to Mr. R. Fathi for his assistance in preparing the bones and Mr. MR Ahi for preparing the figures.

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DOI : 10.5958/2277-8934.2016.00023.0 *Vol 23 No 1, p 139-146*

A SYSTEMIC REVIEW ON ULTRASONOGRAPHIC APPLICATIONS IN CAMELS

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ABSTRACT

Ultrasound is widely accepted as a safe noninvasive diagnostic imaging technique in animals and human. This review aims to shed light on the current applications and future prospect of ultrasonography in camels. To date, ultrasonography has been used efficiently to study the ovarian status in she camels such as; follicular wave, spontaneous ovulation, optimum time for mating, ovarian vasculature, superovulatory response, ovarian follicular dynamics, ovarian follicular wave synchronisation and follicular deviation. Moreover, it has been applied for collection of cumulus oocyte complexes, pregnancy diagnosis, foetometry, foetal sexing, embryo transfer programmes, assessment of somatic cell nuclear transfer and evaluation of the quality and developmental ability of dromedary embryos. Uterine involution and various reproductive disorders such as; early embryonic death, endometritis, vaginal adhesions, ovarian cysts and ovarian hydrobursitis have been diagnosed by ultrasound. In male camels, ultrasonography is a useful tool in studying the developmental changes of testes and pelvic genitalia including; bulbourethral gland, prostate, and pelvic urethra and predicting puberty and future fertility. Normal pleura, heart, fore stomach, liver, small and large intestines, kidney, eye, udder and teat, foot, carpal and tarsal joints have been successfully imaged. However, very limited affections of these structures including; infectious pleuropneumonia, peritonitis, trypanosomiasis, John's disease, intestinal obstruction and ruptured urinary bladder have been diagnosed ultrasonographically in camels. Therefore, ultrasonographic application in camels, compared to other farm animals, is still limited. In conclusion, ultrasonography is untapped in camel practice however, it can offer veterinarians the opportunity for more precise diagnosis and treatment of numerous disorders.

Key words: Camel, dromedary, foetometry, ovum pick-up, pregnancy diagnosis, ultrasonography

Ultrasonography is used extensively as a safe and non-invasive diagnostic technique in veterinary medicine (Fouad *et al,* 2000; Elnahas, 2008; Mostafa *et al,* 2014a; Abu-Seida *et al,* 2015) and as a method of choice for detecting reproductive disorders in large domestic animal species (Ali *et al,* 2013; Nagy *et al,* 2015). It is ideal for dairy production because it does not emit radiation that can be harmful to the pregnant herd and does not require anti-radiation shields, vests and building which can cause additional farm expenses.

Camel has several anatomical adaptations to survive the dry and arid climates. Therefore, numerous ultrasonographic differences have been recorded between camel and other ruminants especially on the foot, udder, optic dimensions and reproductive physiology.

In large animal practice, ultrasonography has been widely used with a great reliability for identification of several physiological and pathological conditions (Abu-Seida, 2012; Mostafa *et al,* 2014b and Mostafa *et al,* 2015).

The literature on ultrasonography in normal and diseased camels is quite scarce. Therefore, this review aims to shed the light on the current applications of ultrasonography in various organs of camels in a trial to find out the points of strength and weakness and future prospect of ultrasonographic applications in camels.

Ultrasonographic examinations in camels:

In camels, ultrasound has been applied to identify the following organs

1. The reproductive organs:

Transrectal ultrasonography has been used to study the ovarian status in she camels such as; spontaneous ovulation (Nagy *et al,* 2005), follicular wave, optimum time for mating, ovarian vasculature (Rawy *et al,* 2014), superovulatory response (Vyas *et al,* 2004a and Nowshari and Ali 2005), follicular dynamics (Manjunatha *et al,* 2012a&b); follicular wave synchronisation for a timed breeding in both dromedary (Skidmore *et al,* 2009; Nagy and Juhasz,

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2012 and Manjunatha *et al,* 2015) and bactrian camels (Nikjou *et al,* 2008), follicular deviation (Manjunatha *et al,*2014) and pre-ovulatory follicle during non-breeding season (Vyas *et al,* 2004b).

Both transrectal colour-Doppler and B-mode ultrasonography are used to detect changes in the ovarian structures and blood vasculature. Three phases of follicular development, those of growth, maturation, and regression, are observed during each follicular wave (Skidmore *et al,* 1996). The optimum time to mate or attempt to induce ovulation in she camels is when the growing follicle measures 0.9-1.9 cm in diameter.

Transvaginal ultrasound guided ovum pick-up (OPU) technique is carried out in dromedary camels. For collection of cumulus oocyte complexes (COCs) the transducer is introduced through the vulva into the most cranial portion of the vagina. Then a 17-gauge, 55 cm single-lumen needle is placed in the needle guide of the ultrasound probe and advanced through the vaginal fornix and into the follicle. Follicular fluid is aspirated using a regulated vacuum pump into tubes containing embryo-flushing media. These aspirates are searched for COCs using a stereomicroscope and they are then denuded of cumulus cells by hyaluronidase and repeated pipetting. The developmental response, to chemical activation, of *in vivo* matured oocytes collected by ultrasound guided transvaginal (OPU) is better than *in vitro* matured oocytes obtained from slaughterhouse ovaries (Wani and Skidmore, 2010).

Ultrasonography is used in pregnancy diagnosis of mated dromedary and South American camelids (Wright *et al,* 1998 and Ali *et al,* 2013). Ultrasonographic foetometry is a helpful tool for evaluation of foetal development, gestational age, and prenatal foetal sexing in camels. Intrauterine fluid accumulation is detected between the second and third weeks of pregnancy and the embryo is seen properly between the third and fourth weeks. Organisation and ossification of the embryo is first visualised at the 6^{th} to 7^{th} weeks and 7^{th} to 9^{th} weeks, respectively. The accessibility for crown-rump length, biparietal diameter, abdominal diameter, ruminal length, and eyeball diameter during the total gestational period are 10.6%, 10.6%, 12.8%, 12.8% and 38.3%, respectively. The best window for foetal sexing is found during the 11^{th} week of pregnancy with an overall accuracy of 91.7% (Ali *et al,* 2013).

The conceptus is always observed by ultrasound through the left caudal abdomen approach (above the base of the udder) at the 6^{th} to 12^{th} week. Between the 13^{th} and 27^{th} week, the foetus is well visualised through the middle abdominal approach (from the base of the udder to the umbilicus). While from the 28^{th} to 52^{nd} week, the conceptus is mainly detected through the left cranial abdominal approach (from the umbilicus to the xiphoid cartilage) (Ali *et al,* 2015a). Moreover, several foetal parameters including orbital diameter, biparietal diameter, abdominal diameter, chest depth and ruminal diameter are measured by ultrasound. Also, ultrasonography is used during the embryo transfer programmes (Skidmore and Billah, 2005; Khatir and Anouassi, 2006 and Skidmore and Billah, 2011), interspecies embryo transfer (Niasari-Naslaji *et al,* 2009), assessment of somatic cell nuclear transfer (Khatir and Anouassi, 2008 and Khatir *et al*, 2009) and assessment of the quality and developmental ability of dromedary embryos obtained by IVM/IVF, *in vivo* matured/IVF or in vivo matured/fertilised oocytes (Khatir *et al,* 2007). Moreover, ultrasonography is a beneficial tool for comparison of pregnancy rates in dromedary camels after deep intra-uterine versus cervical insemination (Skidmore and Billah, 2006).

Uterine involution is completed from 25 to 30 days postpartum and follicles (>/=1.0 cm diameter) can be imaged by ultrasound in only 52.7% of the examined she camels from 34 to 70 days postpartum. Half of these she camels had a confirmed pregnancy at 60 days after mating with virile studs (Vyas and Sahani, 2000 and Derar *et al,* 2014).

Regarding uterine pathologies, ultrasound has been used for diagnosis of various reproductive disorders such as; early embryonic death, endometritis, vaginal adhesions, ovarian cysts, ovarian hydrobursitis and during treatment with intrauterine therapies (Tibary and Anouassi, 2001; Ali *et al,* 2010a&b; Nagy *et al,* 2015 and Ali *et al,* 2015b). Ultrasonographically, ovarian hydrobursitis, is characterised by a collection of anechoic fluid within the ovarian bursa and hyperechoic encapsulation of the ovary (Tibary and Anouassi, 2001).

In male camels, ultrasonography is a useful tool in studying the developmental changes of the testes and pelvic genitalia including; bulbourethral gland, prostate, and pelvic urethra and predicting puberty and future fertility. Ultrasonographic testicular measurements including; testicular length, breadth, and depth as well as epididymal head and tail. All of these testicular and epididymal measurements show significant increase with age (Derar *et al,* 2012).

The normal ultrasonic appearance and seasonal changes in the testicular parenchyma in camels has been scanned by using a B-mode real time ultrasound scanner connected with a 7.5-MHz linear-array transducer. The testicular tunics appear as hyperechoic lines surrounding homogenous, moderately echogenic testicular parenchyma. The mediastinum testis is seen as hyperechoic central line and as a spot in longitudinal and transverse scans, respectively. In winter, the testicular parenchyma appears as hyperechoic with a thin hyperechoic mediastinum testis. During spring, moderate echogenic parenchyma and a relatively thick hyperechoic mediastinum are scanned. In summer and autumn, less echoic testicular parenchyma and thick mediastinum are visualised (Pasha *et al,* 2011).

Moreover, testicular ultrasonography can afford veterinarians the opportunity for more precise diagnosis and treatment of numerous dromedary infertility disorders (Waheed *et al,* 2014).

2. The respiratory organs (the lung and pleura)

Ultrasonographically, the different layers of thoracic wall appear as narrow bands of variable echogenicity in normal camels. Pulmonary parenchyma cannot be imaged due to its gas contents. Mostly, the right and left pulmonary surfaces are visualised at 5^{th}-10^{th} intercostal spaces (ICSs). Sometimes, it can be scanned at 4^{th} and 11^{th} ICS. The length of ventral lung border is largest at the 4^{th} ICS and smallest at the 11^{th} ICS. Moreover, the coastal and the parietal pleurae appear as echogenic line of 1-4mm thickness on the surface of the lung (Tharwat, 2013).

Abdominal and thoracic ultrasonography show severe bicavitary effusion, peripheral lung consolidation and intestinal hypomotility in a camel calf suffering from infectious pleuropneumonia and peritonitis (Stoughton and Gold, 2015).

3. The heart

Echocardiography is a helpful tool for determination of morphological and functional status of the heart. On the right side of camels, the caudal long-axis four-chamber view of the ventricles, atria, and the interventricular septum is imaged when the probe is placed in the right 5^{th} or 4^{th} ICS and when the probe placed more cranially in the 4^{th} ICS, the caudal long-axis four-chamber view and the caudal long-axis view of the left ventricular outflow tract (LVOT) are scanned. The short-axis view of the ventricles is obtained at the 4^{th} ICS when the transducer is rotated between 0° and 25°. When the transducer at the 3^{rd} ICS, visualisation of the right ventricular outflow tract (RVOT) is achieved (Tharwat *et al,* 2012a).

On the left side, a four-chamber view is obtained when the probe is placed in the 5^{th} or 4^{th} ICS. The LVOT and RVOT are imaged from the 4^{th} ICS and 3^{rd} ICSs, respectively.

Echocardiography was helpful for diagnosis of a persistent ventricular septal defect (VSD) in a camel calf (Moore *et al,* 1999) and hypertrophic cardiomyopathy in a 9-year-old dromedary camel that showing thickening of left ventricular free wall and interventricular septum and small left ventricular lumen (Gutierrez *et al,* 2000).

4. The digestive organs:

Ultrasound has been used firstly to visualise the gastrointestinal tract and liver in healthy camels. On ultrasound examination, the rumen of normal camels has a smooth and echogenic ruminal wall.

The reticulum appears as a half-moon-shaped structure with a thick echogenic wall (1.17±0.27 cm) and a biphasic contraction. Mostly, the ventral part of the reticulum can be imaged from left and right paramedian region just behind the sternal pad.

The omasum can be viewed at the right 8^{th}-6^{th} intercostal spaces with a wall thickness of 1.1±0.7 cm and a transverse diameter of 8.74±3.4 cm.

The abomasum can be visualised at the right 9^{th}-7^{th} intercostal spaces (Tharwat *et al,* 2012a)

Small intestines can be imaged at the ventral part of the right paralumbar fossa and has a thin wall (0.43±0.14cm) and a diameter of 2.62±0.47cm. The caecum is imaged mainly in the caudal area of right flank. It has a thin wall (0.37±0.05 cm) and a diameter of 13.8±1.6cm. The proximal loop of the large colon appears as thick, echogenic, slightly curved and continuous lines. It has a thin wall (0.51±0.08cm) and a diameter of 3.5±0.8cm. The spiral colon can be imaged at the caudal ventral half of the abdomen and appears as a structure with thick echogenic walled and several echogenic arched lines next to each other. Mostly, free anechoic peritoneal fluid pockets can also be imaged in camels (Tharwat *et al,* 2012b). Percutaneous ultrasound-guided aspiration of peritoneal fluid (PF) is performed in healthy camels to study its constituents. Free anechoic PF is imaged in the triangular space between the dorsal ruminal sac and reticulum and 10-cm cranial to the umbilicus (Tharwat *et al,* 2013).

Ultrasonography is a useful imaging tool for evaluation of abdominal distension in camels

caused by trypanosomiasis, intestinal obstruction and ruptured urinary bladder.

Camels with trypanosomiasis show accumulation of large amount of hypoechoic abdominal fluids with floating liver, intestine, kidney, spleen and urinary bladder. Mostly, no detectable abnormal ultrasonographic findings are imaged in liver, heart, major blood vessels and kidneys. The main ultrasonographic findings in camels suffering from intestinal obstruction include; distended intestinal loops, markedly reduced or absent intestinal motility and hypoechoic fluid with or without hyperechoic fibrin between intestinal loops (Tharwat *et al,* 2012c).

Ultrasonographic findings in camels with Johne's disease are clumps of echogenic tissue interspersed with anechoic fluid pockets between the intestinal loops, various degrees of intestinal wall thickening and corrugation, excessive anechoic peritoneal fluid, severe enlargement of mesenteric lymph nodes with hypoechoic, heterogenic and echogenic contents, increased hepatic brightness, pericardial and pleural effusions. Sensitivity values of ultrasonography for detecting intestinal lesions and enlarged mesenteric lymph nodes are 95% and 84%, respectively (Tharwat *et al,* 2012d).

Hepatic ultrasonography can be carried out at the right 11^{th} to 5^{th} intercostal spaces (ICSs). The distance between the dorsal liver margin and the midline of the back is shortest (39.1 ± 7.4 cm) at the 11^{th} ICS and increases cranially to 5^{th} ICS. (Tharwat *et al,* 2012e&f). Sternal recumbancy position was the most suitable, practicable and safe position for liver ultrasonographic examination in camels. The long axis of liver extended from caudodorsal to cranioventral in the right lateral side of the abdomen. The distance between the transverse process of the 2^{nd} lumbar vertebra and the dorsal liver margin in camels ranged from 12.9 ± 3.9, 19.2 ± 4.4, 27.7 ± 6.2, 36,6 ± 7.2, 45.6 ± 6.7 and 52.7 ± 9.3 cm in the 11^{th}, 10^{th}, 9^{th}, 8^{th} 7^{th} and 6^{th} intercostal spaces, respectively. The distance between the transverse process of the 2^{nd} lumbar vertebra and the ventral margin of the liver in the 11^{th}, 10^{th}, 9^{th}, 8^{th}, 7^{th} and 6^{th} intercostal spaces was found to be 21.9 ± 10.1, 24.7 ± 6.2, 36.9 ± 10.4, 42.6 ± 7.1, 49.9 ± 7.5 and 55.8 ± 9.2 cm, repectively. The normal camel liver parenchyma consisted of numerous medium echoes homogenously distributed over the entire area of the liver in all intercostal spaces. Fissures in the hepatic visceral liver surface were always observed in the 8^{th} intercostal space and sometimes in the 10^{th}, 9^{th} and 7^{th} intercostal spaces (Elnahas, 2008).

5. The urinary organs

As regards the application of ultrasound in the urinary system of camels, little information are available. Hence, further studies concerning the ultrasonographic diagnosis of urinary disorders in camels are recommended.

In camels, the right kidney is visualised from the right 10^{th} and 11^{th} ICSs and upper right flank. While the left kidney is imaged from the caudal left flank (Tharwat *et al,* 2012e). Ultrasound clearly differentiates the renal cortex from medulla.

Camels with ruptured urinary bladder show ruptured and collapsed urinary bladder, echogenic blood clots inside the urinary bladder and peritoneal cavity, thickened bladder wall, floating intestines in hypoechogenic fluid and sometimes echogenic urethral calculi (Tharwat *et al,* 2012c).

6. The eye

Transcorneal ultrasonographic scanning of camels is performed using a 7.5-10 MHz transducer. Most of the performed studies are conducted on freshly enucleated eyes of camels by A-mode ultrasonography for measurement of optical dimensions after immersion of the eyes in distilled water kept at 20°C. The measured optical dimensions included the anterior chamber depth, lens thickness, vitreous chamber depth and axial length. Generally, A-mode ultrasonography is more accurate than B-mode for estimation of intraocular measurements. Thus, A-mode ultrasonography is the procedure of choice in ocular biometry while B-mode ultrasonography is used mainly for diagnostic aims. Compared to the average A-mode values, B-mode overestimates corneal thickness and anterior chamber depth and underestimates lens thickness, vitreous chamber depth and axial length (Hamidzada and Osuobeni, 1999).

In vitro, the average values of the anterior chamber depth, lens thickness, vitreous chamber depth and axial length are 5.27 mm, 10.93 mm, 14.85 mm and 31.05 mm, respectively. The uncorrected average corneal thickness is 0.76 mm (Osuobeni and Hamidzada, 1999). *In vivo*, all ocular measurements are slightly increased except anterior chamber depth which is slightly decreased. Axial globe length and vitreous chamber depth are larger in she camels than male camels while the lens thickness in male camels is larger than in females (Yadegari *et al,* 2013).

The cornea, anterior and posterior lens capsule and iris appear hyperechoic. The axial length,

vitreous chamber depth (VCD), corneal thickness, lens thickness and scleroretinal rim thickness increase with the advance of age in camels (Kassab, 2012)

The average velocity of ultrasound through aqueous and vitreous humour samples derived from normal camels is 1,499 ± 23 m/s and 1,497 ± 24 m/s, respectively. These values are similar to that of cattle and pigs but slower than in humans (Hamidzada and Osuobeni, 1998). These ultrasonographic findings in normal camel's eye are valuable for comparative ocular anatomy and ultrasonographic evaluation of ocular diseases in the future.

7. The udder and teats

Ultrasonography of udder and teats provides a good assessment of both normal and diseased udder (Kotb *et al*, 2014). In lactating camels, B-mode ultrasonographic examination of the udder in the water-bath was performed by using 6.5-8.5 MHz linear array transducer (Abshenas *et al*, 2007). The streak canal, teat sinus, gland sinus and lactiferous ducts are imaged easily. The teat has a hyperechoic outer layer, a hypoechoic thicker middle layer and a less hyperechoic inner layer. The intercisternal wall of each teat can be divided into 3 layers: two outer thin hyperechoic layers and a thicker middle hypoechoic layer.

8. The musculoskeletal system

Several studies have been conducted on the normal carpal joint (Kassab, 2008), tarsus (Hagag *et al*, 2013) and foot (Abu-Seida *et al*, 2012) in camels. However, no available studies concerning ultrasonographic diagnosis of lameness are reported in camels.

The extensor carpi radialis, extensor digitorum communis and extensor digitorum lateralis tendons are easily identified by ultrasound at the dorsal aspect of the carpus and distal radius. Meanwhile, the extensor carpi obliqus tendon is difficulty identified and the ulnaris lateralis tendon is seen laterally. Moreover, the flexor carpi radialis, flexor digitorum superficialis and flexor digitorum profundus tendons can be observed at the palmar aspect (Kassab, 2008).

Ultrasonography is a highly impressive cross sectional diagnostic imaging in camel's digits. Transverse and sagittal ultrasonographical examinations are carried out on digits using a 6-8 MHz linear transducer. On the saggital scan at the dorsal aspect of fetlock joint, common digital extensor tendon appears as a hyperechoic band of 9-15 mm width then bifurcates into medial and lateral hyperechoic branches of 8-10 mm width. Hyperechoic thick superficial digital flexor tendon of 15-20 mm width and thickness splits just below the fetlock joint to pass the hyperechoic deep digital flexor tendon (DDFT). On sagittal scan, DDFT appears as hyperechoic band while it appears as hyperechoic oval structure surrounded with anechoic synovial fluid and hyperechoic tendon sheath in transverse scan. The DDFT has 2 hyperechoic enlargements at the fetlock joint and under the second phalanx. The camel's foot has three digital cushions (DC). The largest one is the middle digital cushion (MDC) that appears on sagittal scan of the solar aspect as a fish-like high echogenic homogenous structure. The abaxial (Abx DC) and axial digital cushions (Ax DC) are visualised as thin echogenic bands over the middle digital cushion. On transverse scan of the solar aspect, digital cushions appear as 3 high echogenic homogenous structures which are surrounded with a common hyperechoic capsule. The MDC appears as an oval or rounded echogenic structure surrounded by 2 cresentric echogenic Ax DC and Abx DC. The MDC is larger in the forelimb than hind limb and the Ax DC is thicker than Abx DC

Transverse scan of the solar aspect at the interdigital notch shows 2 cresentric echogenic Ax DC of both claws which are separated by anechoic interdigital septum and 2 rounded echogenic MDC of both claws. The sole consisted of hyperechoic thin keratinised layer and thick anechoic layer. The thickness of the sole was 9-12 mm cranially and decreased gradually backward to be 6-7 mm at heel (Abu-Seida *et al*, 2012).

Tarsal ultrasonography is conducted in four planes including; dorsal, medial, lateral and plantar using a 7.5 MHz convex transducer to visualise all tarsal structures in camels (Hagag *et al*, 2013).

Conclusions:

Compared to other farm animals, ultrasonography is untapped in camel practice, however, it can provide veterinarians the opportunity for more precise diagnosis and treatment of numerous dromedary disorders.

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DOI : 10.5958/2277-8934.2016.00024.2 Vol 23 No 1, p 147-150

PREVALENCE RATE AND COMPOSITION OF BLADDER STONES IN CAMEL (*Camelus dromedarius*)

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ABSTRACT

Urolithiasis is an important disease of food animals and a few cases are reported in camels. Camel is an animal which anatomically and physiologically adapted itself to weather condition of desert and it can produce urine twice more than viscosity of sea water. This investigation was undertaken to study prevalence of urolithiasis and determination of calculi composition in camel in Najaf-Abad slaughter house, Iran. Therefore, total, 600 urinary bladders of camel were studied. This survey showed 4 urinary stones in four 5-7 years old male camels. The first stone was cream, 0.5-0.7 millimeter diameter, 0.35 gram weight, rough and uneven with Calcium carbonate, ammonium carbonate and Calcium phosphate. The second stone was cream, 0.8-0.9 millimeter, 0.65 gram, smooth with Calcium carbonate, Calcium hydrogen phosphate, Magnesium ammonium phosphate plus other ingredients. The third stone was cream, 0.2-0.3 millimeter, 0.15 gram with rough and uneven surface. Chemical ingredients were similar to first one. The fourth stone was cream, 0.2-0.5 millimeter, 0.25 gram with rough and uneven surface. Chemical ingredients were similar to first one. Urinary stones might be known as calcite (Calcium carbonate) and the prevalence of disease was 0.66%. There was significant correlation between sex and age group with the prevalence of urinary bladder stone.

Key words: Bladder stone, camel, Iran, Najaf-Abad, prevalence rate

Among farm animals, bladder and urethral diseases are more common and more important than diseases of the kidneys (Radostitis *et al,* 2007; Kojouri *et al,* 2014). The urinary bladder in camel is relatively small and it is limited to the pelvic cavity. Partial or complete obstruction of the urethra by urinary calculi are almost exclusively in male, the most common site of urinary stones is outlet of urine and penile sigmoid flexure especially at its tail end (Smith, 2009). The combination of urinary stones that has been reported is different according to their geographic location and the stones made of silicon, phosphate and calcium carbonate were observed (Radostitis *et al,* 2007). Prevalence rate of bladder stones and chemical composition in camels has not been reported previously, hence present study was undertaken.

Materials and Methods

Sampling (The slaughter house survey)

The present study was conducted in Najaf-Abad slaughter house (Isfahan province, Iran) from April 2012 to February 2013. Three hundred female and 300 male camels (*Camelus dromedarius*), were inspected. During antemortem examinations, each camel was given an identification number and age, sex, diet and origin of animals were recorded. The age of the animals were recorded according to dental formula (Al-Ani, 2004). The camels were classified in four different age groups 1 to 3 (25%), 3 to 5 (25%), 5 to 7 (25%) and up to 7 years old (25%), ratio of males and females was equal in each groups. All camels were studied in terms of bladder stones. Following slaugther each bladder was removed and the bladders were emptied of urine on clean gauze. Bladder was then incised to see presence of calculi, ulceration and hyperaemia. Stones or calculi were placed in plastic containers with lids and these were sent for laboratory analysis.

Physical analysis

The samples were dried in air and its colour, weight were noted and size was determined by Vernier caliper.

Chemical analysis

The stones were powdered by mortar for chemical analysis using kits (Darman Kave Biochemical co., Diagnostic kits. Iran). A snapper of homogeneous white powder from the stone was dissolved in sulfuric acid. Distilled water was added to increase the volume to 50 ml. This solution was used to measure different parameters and

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composition of stone or calculi. Carbon dioxide was evaporated during dissolving the sample in sulfuric acid to see carbonate in stone. 5 ml of solution of the desired stone were transferred to a marked vial of kit, then 2 drops of sodium hydroxide and one snapper of chalcone carboxylic acid were added, respectively and shaken gently, then added Titriplex® III (ethylenedinitrilotetra acetic acid disodium salt dihydrate) gently till colour of sample changed from red to blue. Droplets were added to till 5 per cent of calcium was obtained. 5 ml of solution of the desired stone was transferred to a marked vial of kit, then 2 drops of Borate buffer, 3 drops of ferrous III and 3 drops of sulfosalicylic acid were added and shaken, then 2 minutes later compared colour of sample with coloured chart of the kit till oxalate concentration was determined. 5 ml of solution of the desired stone was transferred to a marked vial of kit, then added, 3 drops of potassium - mercury iodide, 3 drops of sodium hydroxide and compared colour of sample with coloured chart of the kit till ammonium concentration was determined. 5 ml of solution of the desired stone was transferred to a marked vial of kit, then added, 5 drops of ammonium molybdate, 5 drops 4-Aminomethyl phenol sulfate, then 2 minutes later compared colour of sample with coloured chart of the kit till phosphate concentration was determined. 1 ml of solution of the desired stone was transferred to a marked vial of kit, then added, 4 ml distilled water, 10 drops of borate buffer, 10 drops of colour-causing reagent, then 1 minute later compared colour of sample with coloured chart of the kit till magnesium concentration was determined. 5 ml of solution of the desired stone was transferred to a marked vial of kit, then 5 drops of molybdatophosphoric acid was added, 2 minutes later 3 drops borate buffer was added, then compared the colour of sample (The colour is not stable and disappears in 15 seconds) with coloured chart of the kit till Uric acid concentration was determined. 5 ml of solution of the desired stone was transferred to a marked vial of kit, then added 5 drops of ammoniac 10%, a snapper of sodium sulphite, 1 minute later added a snapper of sodium nitroprusside, then compared colour of sample with coloured chart of the kit till cystine concentration was determined. Thus, appropriate and possible anion for each cation was found, then a computational ruler was used to obtain the chemical composition of the stone.

Results

In total, bladder stones were found in four castrated 5 to 7 years old male camels (Fig 2). The characteristic of each stone is placed in Table 1. In this study, the incidence of bladder stones was too meagre (0.66%). In addition, the results of qualitative analysis of the samples showed that the most obtained stones were made of calcium carbonate. Occurrence of bladder stones was seen in male camels. Their composition is shown in Fig 1.

Discussion

Calculi are produced by an animal's disturbed metabolism, often resulting from dietary and vitamin deficiency or glandular imbalance (Duffin *et al,* 2013). Urethra, triangular-shaped area of the bladder wall, ureter and pelvis are the most common sites of urinary stones. The etiology of urinary stone disease is multi factorial and not completely well understood (Jeong *et al,* 2011). Recent studies have shown that obesity is associated with unique changes in serum and urinary chemistry such as increased urinary excretion of calcium, citrate, sulfate, phosphate, oxalate, uric acid and cystine contribute in stone

Table 1. The physical and chemical characteristics of stones obtained of urinary bladder in four camels.

Number of sample	Total	Colour	Size (mm)	Surface	Weight (Gram)	Composition
1	One	Cream	0.5 × 0.7	Rough	0.35	Calcium carbonate 70% Ammonium carbonate 20% Calcium phosphate 10%
2	One	Cream	0.8 × 0.9	Smooth	0.65	Calcium carbonate 40% Calcium hydrogen phosphate 35% Magnesium ammonium phosphate 20% Other compounds 5%
3	One	Cream	0.2 × 0.3	Rough	0.15	Calcium carbonate 75% Ammonium carbonate 10% Calcium phosphate 15%
4	One	Cream	0.2 × 0.5	Rough	0.25	Calcium carbonate 75% Ammonium carbonate 5% Calcium phosphate 20%

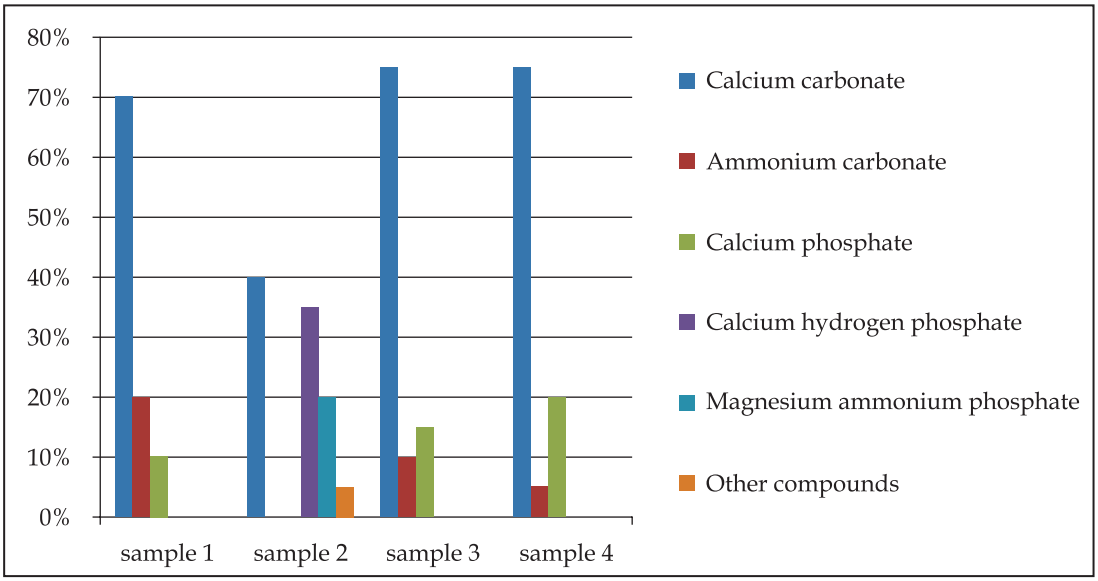

Fig 1. Content of various chemical compositions in four stones recoverd in present study.

formers (Duffey *et al,* 2008; Negri *et al,* 2008; Asplin, 2009; Eisner *et al,* 2010). Some of the predisposing factors for formation of urinary stones are high concentrations of urine, deprivation of water for long time and urinary retention.

Camels can produce urine with twice the concentration of sea water (Wernery and Kaaden, 2002). One of the more important components of the diet and formation of urinary stone in camels is salt. Intake of salt in the camels diet is relatively high and often the plants that they eat such as *Atriplex* spp. (family: Amaranthaceae) kept on their own much salt. However, the role of salt in the physiology of the camel is not clear Khaki *et al* (2006) studied bladder of 140 camels in slaughter houses around Tehran and announced in the bladder of an adult male camel had two stones and the incidence of urinary stones was reported to be 0.71%. The composition were calcium carbonate that is consistent with obtained results in this study. Kock (1985) reported two cases of urinary stones in male camels and found that the infection, metabolic disease, malnutrition and climatic stress are involved in the formation of urinary stones. Gutierrez *et al* (2002) examined the sensitivity of the six castrated male camels in four groups that each group received different amounts of salt in the diet, ten month later control group animals did not show obstruction and retention, hence opined that supplementation of 52 grams of salt in the daily ration can prevent formation of urinary stones in equatorial climates (Dorman, 1986). Two cases of urinary obstruction caused by silicate stones have been reported in camels at a farm in Iceland by

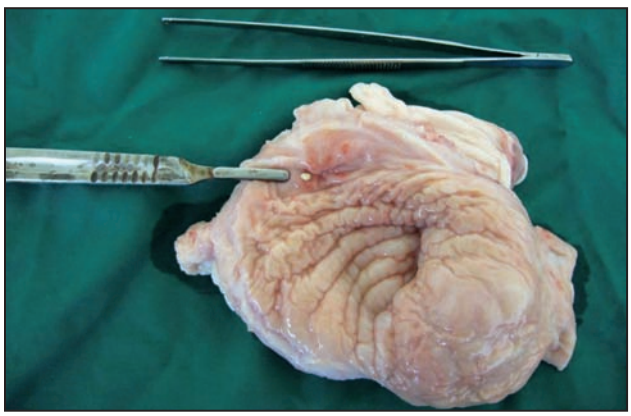

Fig 2. Hyperaemia and calculi in the urinary bladder.

Gutierrez *et al* (1999) and opined that early castration and reducing salt in daily diet are causes of stone formation. It seems that plants and water of desert areas of Iran that have large amounts of salt and fed with *Atriplex* spp. (Frandson *et al,* 2009; Laudadio *et al,* 2009) are presumably causes of the low prevalence of urinary stones in these animals. Detection of stones in male is related to castration possibly due to reduction in diameter of urethra.

In conclusion, this study demonstrated that in the geographic regions in desert of Iran, the occurrence of bladder stones in the camels is low and appears to be associated with castration; also the most obtained stones were composed of calcium carbonate.

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DOI : 10.5958/2277-8934.2016.00025.4 Vol 23 No 1, p 151-154

CHOROID PLEXUS PAPILLOMA IN ONE-HUMPED CAMEL (*Camelus dromedarius*) IN SUDAN

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ABSTRACT

The present article describes choroid plexus papilloma in a 6-year-old male dromedary camel. The main clinical manifestations were stiff gait and incoordination followed by paresis then complete paralysis of hind quarters. Postmortem revelated a mass of 2 centimetres in diameter located in a dilated 4th ventricle. Hydrocephalus was evident by multiple cystic cavities, varying in size in brain stem and cerebral hemispheres. Histopathological sections of the mass displayed proliferation of densely packed arborising papillary fronds of fine fibrovascular core covered with single to multilayer and cluster or nest of cuboidal to columnar epithelial cells. The neoplastic epithelial cells showed positive immunoreactivity to cytokeratin 7. The gross, microscopic and immunohistochemistry findings were consistent with choroid plexus papilloma (C.P.P.).

Key words: Camel, choroid plexus, papilloma, Sudan

Choroid plexus tumour (CPT) is an uncommon intraventricular papillary neoplasm derived from choroid plexus epithelium. There are benign and malignant variants, typically classified as choroid plexus papilloma (CPP) and choroid plexus carcinoma (CPC) (Moulton, 1978; Rickert and Paulus, 2001; Koestner and Higgins, 2002; Maxie and Youssef, 2007; Gobal *et al,* 2008). These tumours occur primarily in dogs and to a lesser extent in cats (Koesnter *et al*, 1999; Koestner and Higgins, 2002); infrequently in cattle (Lunginbuhl *et al,* 1968; Yamda *et al*, 1998; Hoenerhoff *et al,* 2006; Sant'Ana *et al*, 2009) and as an individual report in a horse (Pirie *et al,* 1998) a goat (Klopfeisch *et al*, 2006) and ferret (Van Zeeland *et al,* 2009). The available literature has revealed reports of 4 intracranial neoplasms including astrocytoma in a llama (*Llama glama*) (Garlick *et al,* 1990); teratoma in an Alpaca (*Vicugna pacos*) (Hill and Mirams, 2008), histocytic sarcoma and meningioma in bactrian camels (Molenaar *et al,* 2009). However, present report describes occurrence of choroid plexus papilloma in a dromedary camel but CPT is not reported previously.

Materials and Methods

Case history

During a field investigation of dromedary herds, owned by nomadic pastoralist, northern Darfur State, western Sudan for neurological diseases in 2010, a 6-year-old dromedary camel was subjected to clinical examination. The animal had a history of gradual onset of hind-quarters paresis, inco-ordination, paralysis and recumbency. It was then sacrificed for humane reasons and brain was visually examined and fixed in 10% formalin, processed for paraffin wax sections, 5-6 µ sections were cut and stained with haematoxylin and eosin (H&E). For immunohistochemistry, paraffin wax embedded sections were stained with striptavidin-biotin (SAB) methods using labelled striptavidin-biotin kits (LASB). The primary antibodies used were monoclonal mouse antihuman cytokeratin-7, polyclonal rabbit anti-glial fibrillary acid protein (GFAP), (Dako, Denmark).

Results

Postmortem examination did not reveal any gross lessions in all organs except brain. Brain lesion was in form of a well-circumscribed mass, about 2 cm in diameter, hairy forming pinkish to grey fib-pip-like structure granular papillae in the dilated 4th ventricle. The mass was associated with hydrocephalus as evident by cystic cavities of varying diameters (0.5-3 cm) in the cerebral hemispheres, cerebellum and spongiosis of brain stem (Fig 1). Other central nervous system (CNS) gross lesions include oedema and brownish-yellow gelatinous necrotic areas in the

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lumbo-sacral spinal nerves (LSPN). The cerebrospinal fluid was clear and abundant.

Histopathologically, the choroid plexus mass displayed non-capsulated branching arboriform and papillary structure, comprised delicate fibro-vascular stromal connective tissues that in places were wider, well vascularised and infiltrated with mononuclear cells (Fig 2). It was covered with a single layer of well-differentiated cubo-columnar epithelial cells that often proliferated forming clusters and nests (Figs 3, 4). The cytoplasm of the neoplastic cells was moderately amorphous eosinophilic and their luminal surfaces were straight, as apposed to hobnail appearance of normal choroid plexus epithelium (CPE), devoid of cilia and blepharoplasts. Most of the nuclei of the neoplastic cells were monomorphic, round to oval, hyperchromatic with evenly distributed chromatin and basely located. Infrequently, slightly elongated nuclei were observed. There was no evidence of tumour in the neutrophils or meningeal implantation. Other changes in the brain included multifocal areas of vacuolation or malacic changes. The malacic lesions were associated with glail proliferation, peri-vascular cuffing of mononuclear cells around stenosed blood vessels, neuronal degeneration and necrosis.

Immunohistochemical staining of CPE showed diffused brown staining of cytoplasm to cytokeratin-7 antibodies, and negative staining to GFAP antibodies.

Discussion

Choroid plexus papillomas (CPPs) are uncommon, usually slow growing neoplasms. The diagnosis is occasionally difficult because the morphohistological and immunohistochemical features overlap with other intra-ventricular localising papillary neoplasms (Gaudio *et al*, 1998; Koestner and Higgins, 2002).

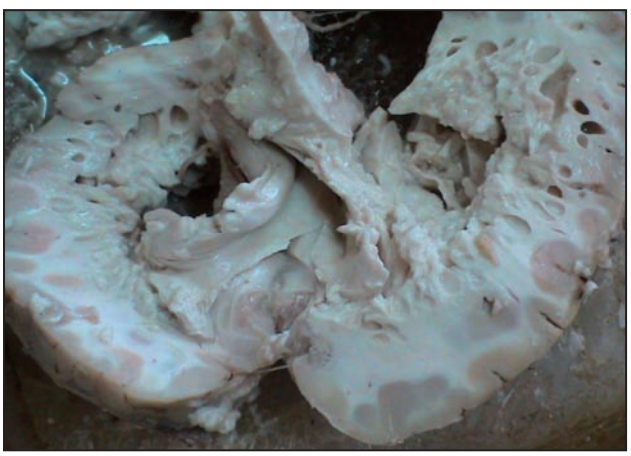

Fig 1. The cavities and vacuolations (red arrows) in the brain tissue.

In the present study, an intraventricular mass was diagnosed as CPP based on the presence of arboriforming and papillary projections of well differentiated cubocolumnar epithelial cells mimicking those of normal CPE, supported by modest fibro-vasccular stroma. These histomorphological changes were considered as a diagnostic feature of CPPs (Bonnin, 1987; Ribas *et al*, 1989; Yamada *et al*, 1998; Koestner *et al,* 1999; Aguzzy *et al,* 2000; Koester and Higgins, 2002; Hoenerhoff *et al,* 2006; Pant *et al,* 2007). As aforementioned, the pathological diagnosis of CPPs may be quite difficult in certain cases. The papillary ependymoma (PE) is often included in the differential diagnosis. Both CPPs and PEs have papillary structures and intra-ventricular location. Differentiation is based on that the epithelial cells of CPPs resemble those of normal CPE, devoid of cilia and blepharoblast in their luminal surface, which is a common feature of the ependymoma. Furthermore, true rosette and peri-vascular pseudo-rosette is present in PE, which also is positive for GFAD and negative for cytokeratin immunostaining (Lewis, 1967; Carrigan *et al*, 1996; Koestner and Higgins, 2002). The stromal connective tissue of CPPs is composed of connective tissue elements; whereas the PE has stroma consisting of fibrillary neuroglia (Kurtz and Hanlon, 1971; Zaki and Nafe, 1980; Koestner and Higgins, 2002). Rubinstein and Brucher (1981) and Taratuto *et al* (1983) have shown local ependymoma differentiation in choroid plexus tumours (papilloma and carcinoma). Kitada *et al* (2001) showed that choroid plexus epithelium cells may undergo differentiation into other type of glial cells *in vivo*, and suggest that focal ependymal differentiation may occur in the CPP, thus, confounding previous immunohistochemical diagnosis. (Rubinsten and Bruches, 1981; Taratuto *et al,* 1983).

Internal hydrocephalus encountered in the present study confirms earlier observation in dogs

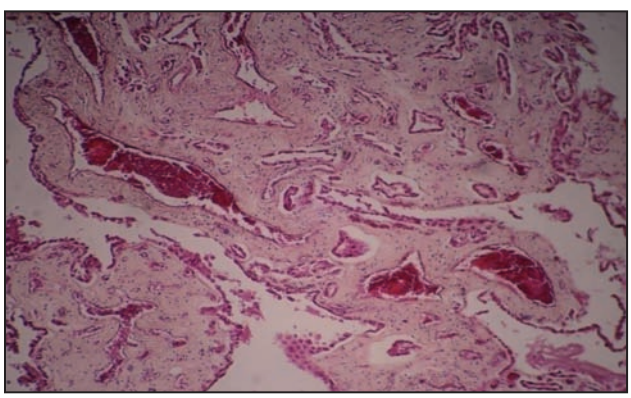

Fig 2. Histopathology of choroid plexus papilloma revealed a well vacuolated connective tissue. (H&E) x 10.

(Kurtz and Hanlon, 1971; Wilson *et al*, 1989; Cantile *et al*, 2002; Koestner and Higgins, 2002; Pastoello *et al*, 2010) and in man (Fairburn, 1960; Fortuna *et al*, 1979; Tomasello *et al*, 1981; Boyd and Steinbok, 1987; Gaudio *et al*, 1998). Many explanations have been given for the development of hydrocephalus in patients with choroid plexus tumour. Obstruction of CSF pathway and/or overproduction of CSF by tumour itself are the most common reasons, especially with tumours originating from the 3rd or 4th ventricle (Gaudio *et al*, 1998; Rickert and Paulus, 2001; Koestner and Higgins, 2002). Furthermore, CPPs have been proved to cause hydrocephalus by overproduction of CSF (Milhorat *et al*, 1976).

Our results of multifocal malacic lesions and glial proliferation confirm typify reported in brain of dogs. (Wilson *et al*, 1989; Cantile *et al*, 2002; Pastoello *et al*, 2010). In addition, Pastoello *et al* (2010) observed compression of white matter adjacent to the tumour, mild angioedema and moderate multifocal haemorrhage.

Acknowledgements

The authors would like to thank all members of the Department of Pathology and Diagnosis, Veterinary Research Institute for their technical assistance.

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DOI : 10.5958/2277-8934.2016.00026.6 Vol 23 No 1, p 155-156

MULTINODULAR THYROID GLAND HYPERTROPHY IN A CAMEL

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ABSTRACT

This case report include the clinical, haematology, radiology, ultrasound and cytology results of the multinodular thyroid hyperplasia in a camel. A fourteen-year-old, Tulu breed, male camel was presented with the complaint of respiratory sounds and gradually enlarging a swelling under the neck for one year. Clinical examination revealed a massive swelling, which was bilateral, symmetric, and located ventral and 1/3 cranial part of the neck and suspected to be the cause of the respiratory sounds. Routine haematology of the camel was normal but thyroid function tests had low TSH, high level T3 and T4. Radiology revealed peritracheal soft tissue opacity and tracheal deviation. Ultrasonographic examination revealed the heterogenic mass and numerous different echogenities (anechoic, hyperechoic) in the parenchyma (multinodular appearance). Ultrasound guided fine needle aspiration and then cytology was performed. Cytology pointed to thyroid hyperplasia. Iodine therapy was planned. After two months, the swelling regressed to about 30% rate and abnormal respiratory sounds also disappeared.

Key words: Camel, multinodular thyroid hyperplasia

The thyroid gland plays an important role as endocrine organ that secretes thyroglobulin, triiodothyronine and thyroxine hormones (Kausar and Shahid, 2006). Histologically, the gland contains follicular and parafollicular endocrine cells for synthesis the specific hormones for processing of metabolism (Adbel-Magied *et al,* 2000). A comparative thyroid status of camel, sheep, goat and cattle has been studied previously (Eltom and Abdalla, 1981). Thyroid gland pathologies can be congenital among the young or acquired in adult camels (Schlumberger, 1955). As acquired thyroid pathology, nodular hyperplasia was reported in 3% healthy camels during slaughter (Yadegari *et al,* 2014). A case of multinodular thyroid hypertrophy is reported herewith which includes the clinical, laboratory, radiological, ultrasonographic and cytological results.

History

A 14-year-old, Tulu breed, male camel was presented to Uludag University, Faculty of Veterinary Medicine Clinics with the complaint of gradually enlarging a swelling under the neck over last one year with associated respiratory sounds.

Result and Discussion

Clinically, a bilateral massive moveable swelling was symmetrical and was located on the ventral 1/3 cranial part of the neck (Fig 1). It was painless and non-inflammatory and considered as a cause of the respiratory sounds. Routine haematology of the camel had normal values, but thyroid function tests were low TSH (0.3 mIU/L), high level T3 (0.05 nmol/L) and T4 (159.03 nmol/L).

A lateromedial radiographic view of neck revealed peritracheal soft tissue opacity and tracheal ring compression and deviation (Fig 2). The mass appeared heterogenic in appearance and had numerous different echogenities (anechoic, hyperechoic) in the parenchyma (multinodular appearance) ultrasonographically (Fig 3). Ultrasound guided fine needle aspiration and then cytology was performed.

Cytology revealed abundant erythrocytes, some naked nuclei, a few neutrophils and numerous round and slightly basophilic epithelial cell nests, which had distinct borders, round-oval nuclei with finely stippled chromatin pattern. Epithelial cells had uniform appearance, and there was no colloidal material between epithelial cells. Based on the results, a nodular thyroid gland hypertrophy was diagnosed in the camel, and parenteral iodine therapy was administered in the camel. After two months, the swelling was seen regressed about 30% rate, and there was no respiratory sound.

Location of the thyroid gland is around the first tracheal ring and it consists of two lobes on both

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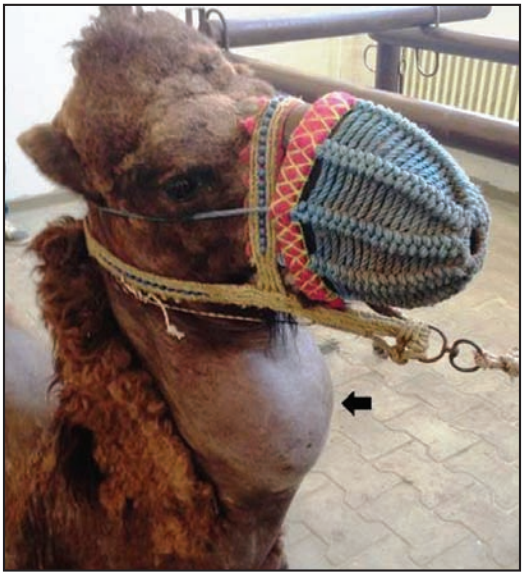

Fig 1. A bilateral symmetrical swelling (arrow) on the cranio-ventral part of neck of camel.

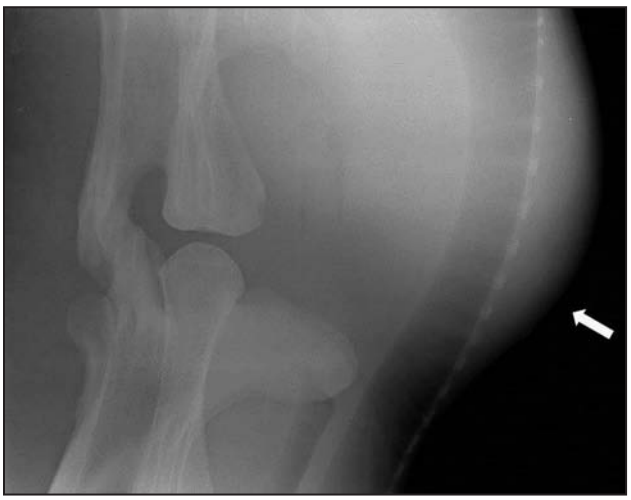

Fig 2. Latero-medial radiographic view shows the peritracheal opacity and tracheal ring compression (arrow) and soft tissue enlargement.

the sides (Kausar and Shahid, 2006). It's enlargment due to pathology, can compress the carotid arteries and the other organs around it (Schlumberger, 1955). Radiological and ultrasonographical examination helped confirming the thyroid pathology and hyperplasia was confirmed on cytological examination in animal of present study which was in accordance to the diagnostic approach adopted for thyroid gland by Yadegari *et al* (2014) in camel. Ultrasonographic and cytological results of present case were compatible with the multinodular thyroid gland hypertrophy.

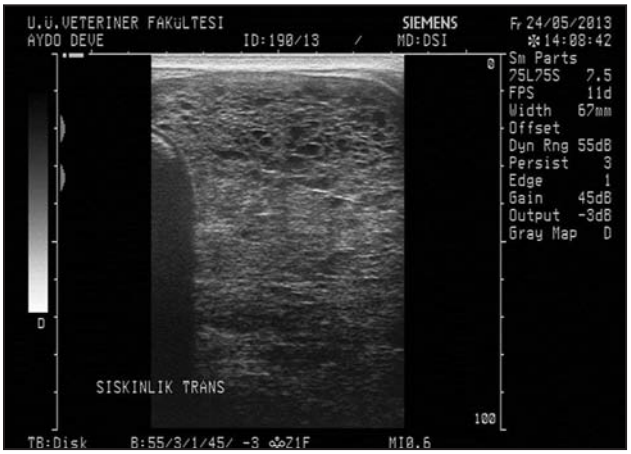

Fig 3. Ultrasonographic scan showing multinodular echogenic view in transversal section of the swelling.

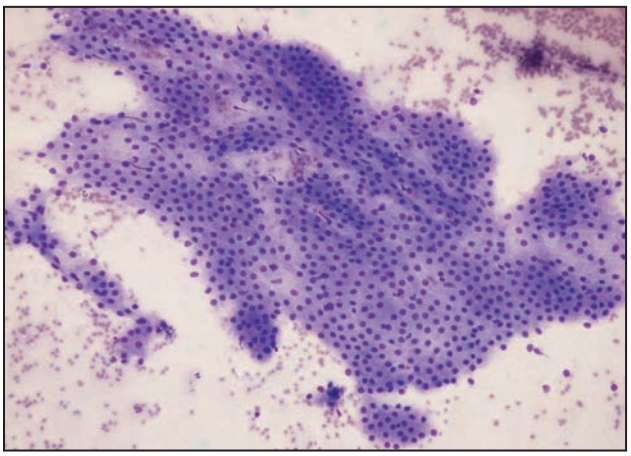

Fig 4. Cytology revealed numerous epithelial cell nests which had uniform appearance. Hemacolour X 200.

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DOI : 10.5958/2277-8934.2016.00027.8 *Vol 23 No 1, p 157-162*

PREVALENCE OF INFLAMMATORY CONDITIONS OF UPPER GASTRO-INTESTINAL TRACT OF THE CAMEL (*Camelus dromedarius*) IN WESTERN RAJASTHAN

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ABSTRACT

The present investigation was carried out to study the prevalence of various inflammatory conditions which affect the upper gastro-intestinal tract of camel from February 2014 to January 2015, in western Rajasthan. During this period, postmortem examination of 246 camels were conducted and samples of the upper gastro-intestinal tract of camel irrespective of age, sex and breed were collected and examined grossly. Samples showing frank macroscopic lesions were used for further histopathological examination by paraffin embedding using acetone and benzene technique. The prevalence of inflammatory conditions in upper gastro-intestinal tract was 20.32%. The incidence of various inflammatory conditions was observed as chelitis (6.0%), gingivitis (10.0%), stomatitis (8.0%), glossitis (4.0%), pharyngitis (4.0%), oesophagitis (4.0%), acute rumenitis (28.0%), acute reticulitis (6.0), chronic reticulitis (8.0%) and abomasitis (22.0%), respectively. In the whole investigation, the most common findings reported were acute rumenitis and abomasitis.

Key words: Camel, gastro-intestinal tract, inflammatory conditions, Rajasthan

Inflammatory conditions of gastrointestinal tract may be due to gastro-intestinal helminths (Bekele, 2002), foreign bodies (Ahmed *et al,* 2000), oesophageal obstruction (Ramadan *et al,* 1986), infection, toxemia, haemorrhagic diseases, poisoning etc. (Al-Ani, 2004). Parasitic gastro-enteritis in camelids is associated with various nematode species in the abomasum and intestines (Wernery *et al,* 2014). In view of scarce literature available on pathological studies of upper gastrointestinal tract, present investigation was undertaken to study the inflammatory conditions of this region in camels.

Materials and Methods

Upper gastro-intestinal tract of 246 camels irrespective of age, breed and sex were examined during February 2014 to January 2015, from carcass of camels subjected to postmortem examination at veterinary hospitals of various districts of western Rajasthan, dead camels from municipal corporation, carcasses of camel submitted to the department of Veterinary Pathology, College of Veterinary and Animal Science, Bikaner for routine post-mortem examination. Samples were taken from 50 camels showing gross lesions of upper gastro-intestinal tract and were preserved in 10% formalin for histopathological examination.

Histopathological examination

Processing of tissue was done by paraffin embedding using acetone and benzene technique (Lillie, 1965). The tissue sections of 4-6 microns were cut and stained with haematoxylin and eosin. Results were described on the basis of gross and histopathological examination.

Results and Discussion

In the present investigation, out of 246 camels, 50 camels (20.32%) showed frank macroscopic lesions of upper gastro-intestinal tract. The prevalence of various inflammatory conditions found in upper GIT are depicted in table 1 and described below. The prevalence is given in parenthesis.

Chelitis (6%)

Grossly, the affected lip appeared swollen with hyperaemic mucosa. The inner surface was eroded and had irregular reddened ulcerative patches, were in agreement with Narnaware *et al* (2013). Microscopically, diffused and focal cellular infiltration of neutrophils and lymphocytes were seen in lamina muscularis and submucosa (Fig 1). Infiltration around orbicularis oris muscles was also evident. Similar observations were described by Kitching (2002) and Sharma (2014). Above findings might be due to viral infections or due to trauma.

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Table 1. Prevalence of various inflammatory conditions of upper gastro-intestinal tract in camel.

S. No.	Name of Condition	No. of cases	Percentage (%)
1.	Chelitis	3/50	6.00
2.	Gingivitis	5/50	10.0
3.	Stomatitis	4/50	8.00
4.	Glossitis	2/50	4.00
5.	Pharyngitis	2/50	4.00
6.	Oesophagitis	2/50	4.00
7.	Acute Rumenitis	14/50	28.0
8.	Acute Reticulitis	3/50	6.00
9.	Chronic Reticulitis	4/50	8.00
10.	Abomasitis	11/50	22.0
	Total	50/50	100

Gingivitis (10%)

Grossly, the gums showed ulceration and hyperaemic mucosa. Inflammation was seen around the tooth (Fig 2). Microscopically, this condition was characterised by desquamation of stratified squamous epithelium of gums along with infiltration of neutrophils and lymphocytes in the mucosa and submucosa of gingivae. Similar observations were described by Nagahata *et al* (1993), Muller *et al* (1994) and Moshaverinia *et al* (2013). Above findings might be due to viral infections, nutritional deficiencies or traumatic injuries.

Stomatitis (8%)

Grossly, pathological changes were erosive and showed few ulcerative patches on buccal mucosa which were associated with congested and haemorrhagic oral mucosa. Similar signs were also described by Williamson *et al* (2008) and Maclachlan *et al* (2009). Microscopically, epithelium was markedly desquamated and the mucosa and submucosa was infiltrated by polymorphonuclear and mononuclear cells mainly neutrophils and lymphocytes (Fig 3). Stratified squamous epithelium exhibited focal areas of basal cell degeneration in lamina propria and these were in agreement with the findings of Nagahata *et al* (1993) and Brown *et al* (2007).

Above findings might be due to physical agents (trauma and heat), chemical agents (corrosive), infection and nutritional deficiencies.

Glossitis (4%)

The main gross finding was breach in superficial layer, swelling and ulceration along with haemorrhages. Similar findings were also described by Yeruham *et al* (1998), Fava *et al* (2000) and Brown *et al* (2007) in cattle. Microscopically, tongue showed desquamation of stratified squamous epithelium along with mild infiltration of neutrophils and lymphocytes in lamina propria of mucosa and submucosa. Leucocytic infiltration was also evident in between striated muscles at the core of the tongue (Fig 4). Such changes were also described by Sharma (2014) in sheep. This condition might occur due to infectious diseases, traumatic injuries or nutritional deficiencies.

Pharyngitis (4%)

Grossly, pathological changes were erosive patches with congestion and massive haemorrhages on mucosa. Microscopically, cellular infiltration of polymorphonuclear and mononuclear cells mainly neutrophils and lymphocytes along with congestion and haemorrhages (Fig 5) were observed which were in agreement with findings of Sharma (2014) in sheep.

Musa *et al* (1989) found severe deterioration of nasopharyngeal mucosa in camels caused by *C. titillator* larvae in a slaughter house. Two leeches (*Limnatis nilotica*) were found attached to the pharyngeal mucosa (Manefield and Tinson, 1997). Thus pharyngitis could be aggravated by parasites also in camels.

Oesophagitis (4%)

Grossly, swollen and congested esophageal mucosa with erosive and ulcerative spots were seen which were in agreement with finding of Giles *et al* (1980) and McGavin *et al* (2001). Similar changes were also described by Sastry and Rao (2011). Microscopically, desquamation of stratified squamous epithelium of esophagus with infiltration of mononuclear and polymorphonuclear cells mainly neutrophils and lymphocytes in submucosa were seen (Fig 6). Marked congestion in submucosa seen in persent study were in agreement with finding of McCrindle *et al* (2001) in cattle. This condition might occur due to physical agents (trauma and heat), chemical agents (corrosive), viral infections or traumatic injuries by stomach tube or foreign bodies.

Acute ruminitis (28%)

Grossly, hyperaemia and massive haemorrhages were seen on dorsal non-glandular part of Compartment 1 (C1) (Fig 7). In some cases the glandular ventral part of C1 was enlarged and contained turbid, foul smelling watery fluid, hypereamic ruminal surface was also seen, along with sloughing of mucosal layer (Fig 8). Similar findings were observed by Panciera *et al* (2007). Microscopically, tissue section showed necrosis and massive haemorrhages in submucosa of C1 with

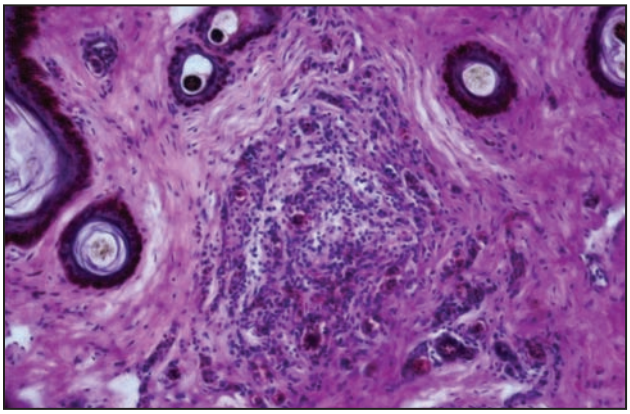

Fig 1. Microphotograph of lip showing heavy focal cellular infiltration mainly neutrophils and lymphocytes in lamina muscularis and submucosa. H&E 200X.

Fig 2. Photograph of gingival mucosa showing ulceration and congestion.

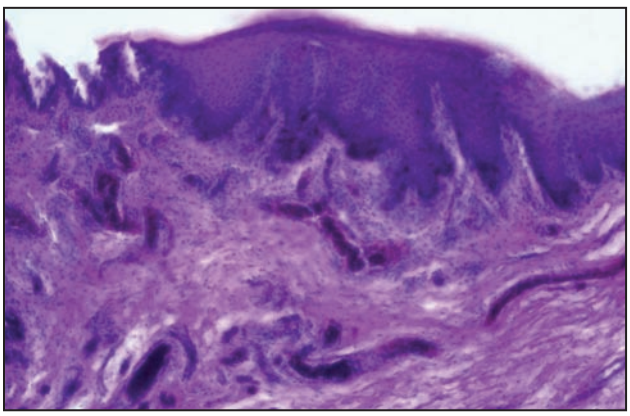

Fig 3. Microphotograph of oral mucosa showing desquamation of epithelium and cellular infiltration of neutrophils and lymphocytes in mucosa and submucosa. H&E 100X.

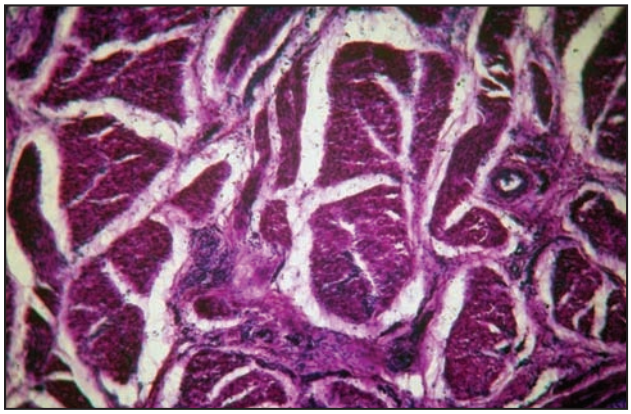

Fig 4. Microphotograph of tongue showing focal infiltration of neutrophils and lymphocytes in between striated muscles of tongue. H&E 400X.

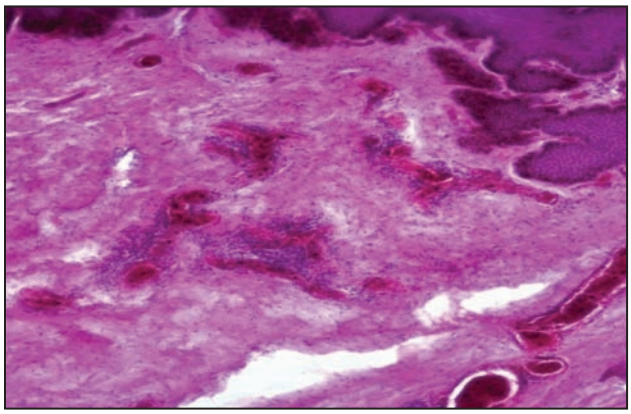

Fig 5. Microphotograph of pharynx showing cellular infiltration of neutrophils and lymphocytes along with congestion and haemorrhages. H&E 100X.

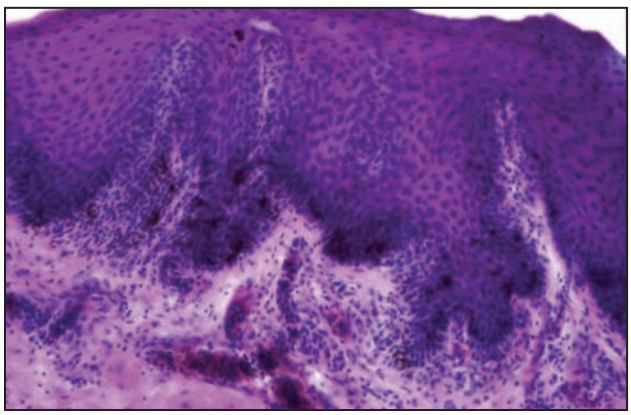

Fig 6. Microphotograph of oesophagus showing infiltration of polymorphonuclear cells and lymphocytes in mucosa and submucosa along with congestion. H&E 100X.

diffusely infiltrated red blood cells and infiltration of polymorphonuclear cells mainly neutrophils and lymphocytes in mucosa and submucosa. Similar findings were reported by Dzhurov (1975). In some tissue sections desquamation of epithelium and infiltration of neutrophils and mononuclear cells were reported with marked congestion in blood vessels of submucosa (Fig 9). It was also reported by Kharalambiev *et al* (1976), Brown *et al* (2007) and Sharma (2014) in cattle. This condition might occur due to grain engorgement, acidosis or excess milk feeding.

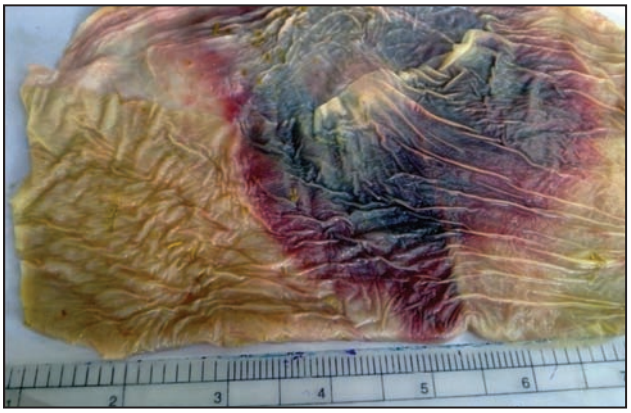

Fig 7. Photograph of nonglandular part of Compartment 1 showing congestion and haemorrhages.

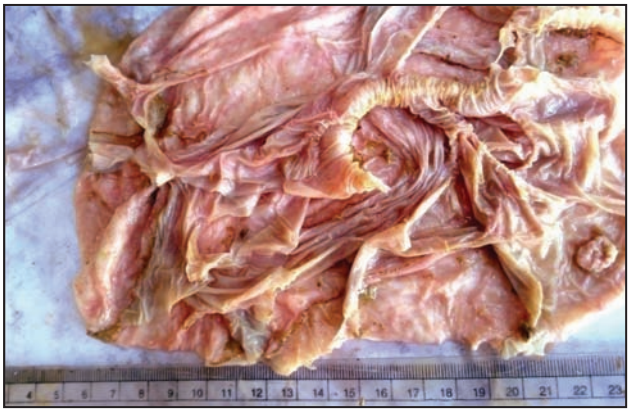

Fig 8. Photograph of glandular part of Compartment 1 showing sloughing of mucosa and hyperemia.

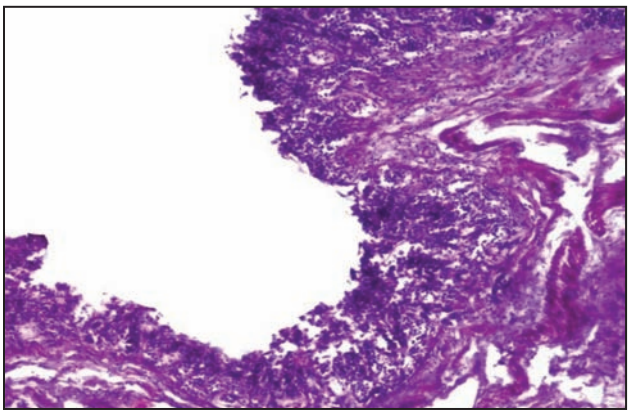

Fig 9. Microphotograph of Compartment 1 showing desquamation of epithelium and heavy cellular infiltration of neutrophils and lymphocytes in mucosa. H&E 100X.

Manefield and Tinson (1997) described occurrence of foreign body penetration in rumen which might cause obstruction.

Acute reticulitis (6%)

Grossly, Compartment 2 (C2) showed rupture of mucosa, congestion and haemorrhages (Fig 10). Microscopically, tissue section showed microvacuolation

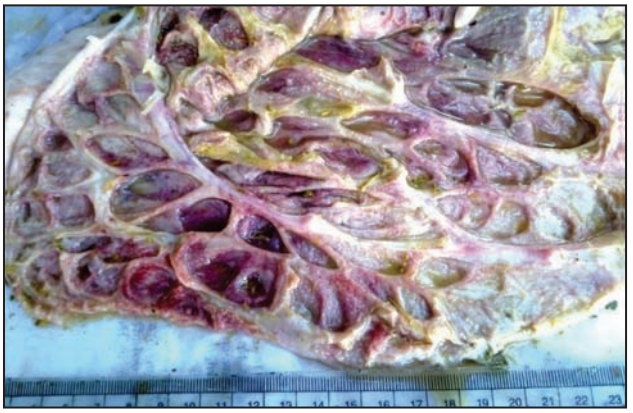

Fig 10. Photograph of Compartment 2 showing congestion and haemorrhages.

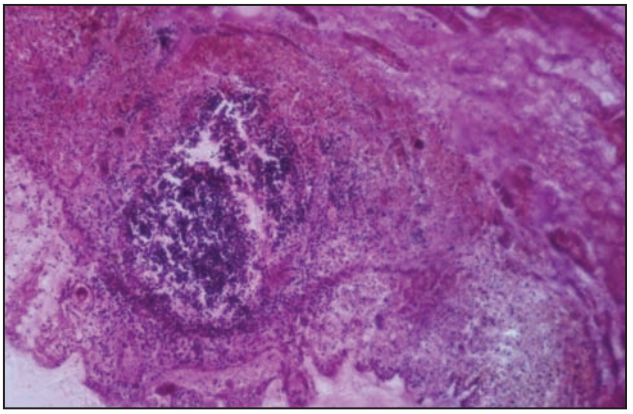

Fig 11. Microphotograph of Compartment 2 showing microvacuolation and infiltration of polymorphonuclear cells with congestion and haemorhages in mucosa and submucosa. H&E 100X.

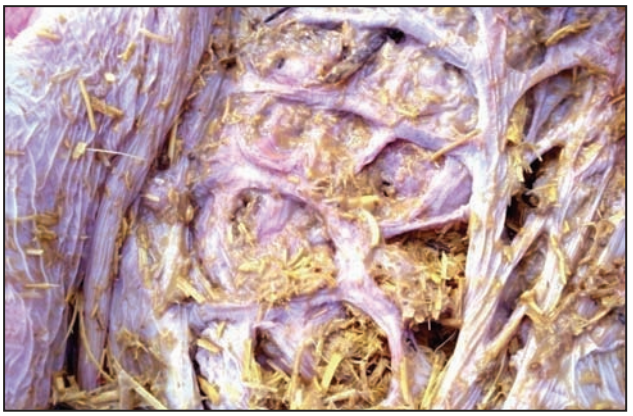

Fig 12. Photograph of showing metallic foreign bodies in sacs of Compartment 2.

and infiltration of polymorphonuclear cells mainly neutrophils in mucosa and submucosa along with congestion and haemorrhages (Fig 11). Such changes were also described by Sharma (2014) in cattle. This condition might be due to acidosis.

The occurrence of traumatic reticuloperitonitis is very rare in camels, possibly due to anatomical and

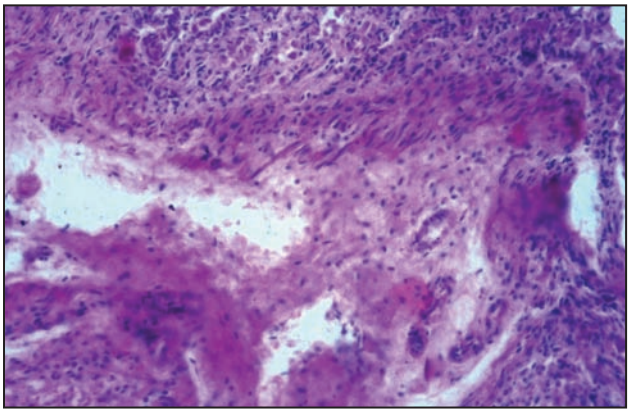

Fig 13. Microphotograph of Compartment 2 showing proliferation of fibroblast cells along with infiltration of lymphocytes and neutrophils. H&E 100X.

Fig 14. Gross appearance of fundic region of Compartment 3 showing congestion and haemorrhages.

functional reasons but foreign body trauma is more commonly seen in rumen (Manefield and Tinson, 1997).

Chronic reticulitis (8%)

Grossly, metallic foreign bodies (nails) were present in reticular sacs (Fig 12) and C2 showed hardness of reticular walls and mild haemorrhages. Such finding were in agreement with Ahmed *et al* (2000). Microscopically, this condition was characterised by fibrotic changes as marked proliferation of fibroblast cells in lamina propria of reticular folds. Connective tissue bands of the C2 between lamina propria and submucosa became thickened and fibrocellular. There was infiltration of lymphocytes in submucosa, beneath the damaged epithelium (Fig 13). Similar observations were also described by Vegad and Swamy (2010). This condition might occur due to persistence of chronic irritants (foreign bodies) and infectious diseases.

Abomasitis (22%)

The main gross finding was thick mucosal folds along with congestion and haemorrhages

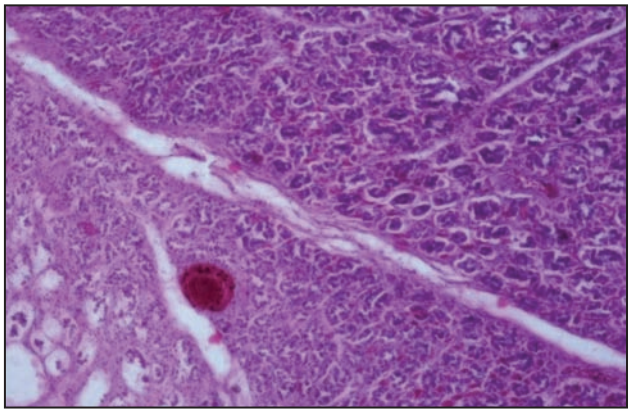

Fig 15. Microphotograph of Compartment 3 showing infiltration of polymorphonuclear and mononuclear cells between gastric glands along with congestion and haemorrhages. H&E 100X.

in Compartment 3 (Fig 14). These findings were in agreement with those observed by Welchman and Baust (1987) in veal calves. Microscopically, Compartment 3 (C3) showed microvacuolation and infiltration of polymorphonuclear cells mainly neutrophils in mucosa and submucosa along with congestion and haemorrhages (Fig 14). In some cases the fundic region of abomasum showed cellular infiltration of polymorphonuclear and mononuclear cells between the abomasal glands along with congestion in lamina propria (Fig 15), similar to those observed by McCrindle *et al* (2001) and Panciera *et al* (2007) in cattle. Similar histopathological observations were also described by Kruiningen *et al* (2009) in dairy calves. This condition might occur due to bacterial and viral infection.

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DOI : 10.5958/2277-8934.2016.00028.X Vol 23 No 1, p 163-167

SWEAT-GLAND-TUMOUR WITH OSSEOUS METAPLASIA "CHONDROID SYRINGOMA" IN THE ONE-HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

A pleomorphic adenoma of sweat gland is a rare cutaneous adnexal neoplasm in domestic animals. We diagnosed such a tumour in a 12-year-old-male camel. The mass which occurred on the lateral part of the left masseter region was hard, infiltrative and covered by an intact skin. Grossly, following surgical excision the mass was grayish in colour. Histologically, the neoplasm displayed irregular tubules of varying shape, size and surrounded by thin connective tissue. These lobules were composed of either solid sheet or formed glandular structure with multilayer tubules. The neoplastic cells of both solid cells and glands were polygonal with indistinct boundaries and abundant faint pink cytoplasm that was vacuolated. The nuclei of these cells were small, vesicular with marginated chromatin. Areas of malignant transformation were encounterd, where the neoplastic cells revealed cellular and nuclear pleomorphism and moderate mitotic activity. Interspersed among the neoplastic tubules were osteoid trabeculae. The neoplastic cells of solid mass and tubule revealed dense and diffuse cytoplasmic immunoreactivity with S-100 protein, whereas only the epidermal epithelium showed immunopositive reactivity to cytokeratin.

Key words: Camel, chondroid syringoma, dromedary, neoplasm, sweat gland

Mixed tumour of sweat glands (synonymous: mixed tumour of skin, chondroid syringoma, pleomorphic adenoma) has been categorized into either apocrine or eccrine types (Goldschmidt and Hendrick, 2002; Ginn *et al,* 2007; Kazakov *et al,* 2011). Tumour of the apocrine glands are more common than the eccrine glands and occur frequently in dogs, occasionally in cats and rarely in other domestic animals (Goldschmidt and Hendrick, 2002; Ginn *et al,* 2007). Three documented reports of mixed apocrine tumour in bovine (Garma–Avina *et al,* 1981; Piercy *et al,* 1994; Gulbahar *et al,* 2002) and two in horses (Cotchin, 1960; Anderson *et al,* 1990). Mixed tumour of eccrine sweat glands are rare intracutaneous neoplasm, mostly reported as isolated cases in man (Kazakov *et al,* 2011). This tumour occurs rarely in all domestic animal species. (Goldschmidt *et al,* 1998; Goldschmidt and Shofer, 1998 ; Goldschmidt and Hendrick, 2002; Ginn *et al,* 2007). Malignant counterpart is extremely rare and too few cases have been reported in footpads of cats and dogs where these glands are normally located (Goldschmidt *et al,* 1998; Goldschmidt and Hendrick, 2002).

Materials and Methods

Case history

A twelve-year-old male dromedary camel was admitted to Veterinary Teaching Hospital, King Feisal University for investigation and treatment. The animal had a firm infiltrated mass, covered by an intact healthy skin on the lateral part of the left masseter region (Fig 1). The animal was sedated with xylazine hydrochloride at the dose 0.2 mg/kg/.body weight. The growth was excised from the subcutaneous tissues and the wound was closed with polyglactic 910 (Vicryl). Grossly, following excision the mass was grayish, lobular hard in consistency and had a gritty sensation.

Represented portion of tumour specimens were immediately fixed in 10% formal saline after surgical removal, processed, sectioned at 5-6 µ and stained with haematoxylin and eosin (H&E), periodic-acid-schift (PAS) and immunohistochemical staining for cytokerratin and S-100. This was performed in paraffin wax sections by streptavidin-biotin (SAB) methods using labeled streptavidin biotin Kits

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(LASB) (Dako Denmark). The primary antibodies were anti-cytokeratin and S-100.The sections were counterstained by iorn haematoxylin.

Results

The epidermal layers covering the neoplastic lesion displayed superficial ulceration, slight acanthosis, hydropic degeneration and necrosis with micro vesicles formation. This was accompanied with leukocyte infiltration in subepithelial area (Fig 1).

The dermis and subcutaneous connective tissues were infiltrated by disorderly distributed proliferated cellular mass which was composed of irregular lobules of varying shape and dimensions bound by thin fibrous tissue (Fig 2). These lobules consisted of either solid nests or formed tubules with multilayer cell. The arrangement of these layers was disorganised that appeared as pseudo-stratification. At the periphery, just below the epidermis the neoplastic cells of both solid nests and multi tubules were cuboidal to polyhedral with faint pink vacuolated cytoplasm or clear cells, undefined bounderies and interspersed with spindle, shaped cell morphologically mimicking myoepthelial cells. The nuclei of these proliferated cells were vesicular with marginated chromatin, some nuclear pleomorphism and scarce mitotic figs. In serial section and moving to the centre of lesion continuing with areas exhibiting carcinomatous changes, implying malignant transformation, which formed the bulk of the region. In these areas the neoplastic cells arranged in diffuse or expanded sheets of pleomorphic cells with nuclear atypia and moderate mitotic figs Coexisting with these cells, mostly at the periphery of the sheets, were loosely arranged cluster of spindle shaped cells (Fig 3). In addition, these areas displayed extensive tumour necrosis particularly at the lumina of the adenomatous tubules which often had comedo-necrosis appearance. The necrotic tissue composed of disassociated neoplastic cell, spindle shaped cells, leucocytes (Fig 3) and/or eosinophlic material. Furthermore, superficial and intradermal infiltration of solid neoplastic nests and leukocytes were encountered forming pustules-like structure (Fig 4).

Most of the proliferated neoplastic cells stained faint purplish with PAS. Only the epidermal epithelial cells showed strong immunoreactivity with cytokeratin. All tumour cells in lobules showed dense and diffuse immunoreaction with S-100 protein (Fig 5).

Interpressed among the tubules were osteoid tissues in a form of irregular trabeculae with areas of multinucleated osteoclast small areas of keratinised and unkeratinised undifferentiated epithelial cells forming hair bulb-like structure, blood vessel and lymphatic vessels, which contained varying amount of amorphous pink material. The interstitial stroma which had pale pink homogenous appearance was irregularly dispersed, in some areas it was abundant and more cellular (Fig 6)

Discussion

Syringoma are benign adenexal neoplasms exhibiting feature of eccrine ducts (Elder *et al,* 1999), were first described by Goldman *et al* (1956). These neoplasms, which commonly defined rare, occur more frequent than expected in man (Hassab-el-Naby *et al,* 1989; Yavuzer *et al,* 2003; Nair, 2008), but rare in domestic animals (Goldschmidt and Hendrick, 2002). The malignant counter-part is extremely rare and only few cases have been reported, in footpads of dog and cat, to know their biological behaviour (Goldschmidt and Hendrick, 2002). Similarly, in man the low incidence of above mentioned tumour makes difficult to collect sufficient number of specimens to know much about these (Giorgini *et al,* 2012).

Morphologically, the mass in current study was composed of mixture of glandular component (epithelial and myoepithelial cells) and mesenchymal (stromal and osteoid tissue) element which was considered characteristic feature of mixed tumour (Weiss and Frese, 1974; Hampe and Misdrop, 1974; Goldschmidt *et al,* 1998; Goldschmidt and Hendrick, 2002 and Misdrop, 2002).

The neoplasm in the present study was located intradermally in the face, which did not contain apocrine gland, hence it was eccrine gland type. The differential diagnosis from apocrine gland tumour was based on the anatomical location of apocrine gland confined to the axillae and ano-genital regions, where as the eccrine gland remains distributed in the skin of most of parts of the body with exception of areas such as margins of the lips and the glans penis (Wheater *et al,* 1982). The surfaces of luminal cells of apocrine gland contain blebs (decapitation secretion) which are typical normal apocrine feature (Anderson *et al,* 1990; Gulbahar *et al,* 2002; Morandi *et al,* 2005) and these were not detected in the present study. The tubular branching that were considered (Requena *et al,* 1994; Takamitsu and Shinchi, 1997; Srivastava *et al,* 2008) as expression of apocrine differentiation was not encountered herein. Moreover, in man the typical body location

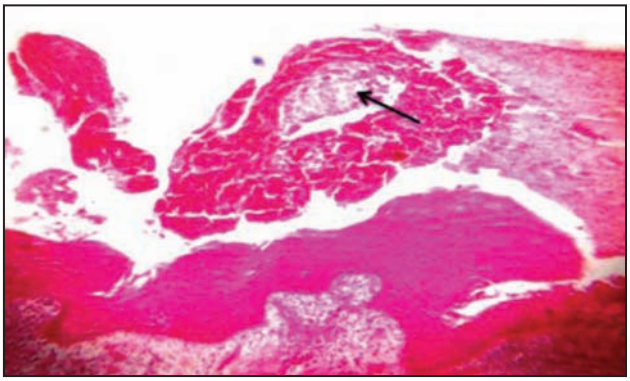

Fig 1. Section of skin tumour. Note tumour nests in the superficial ulcer (arrow), infiltration of leukocytes in the subepithelial area. H&EX40.

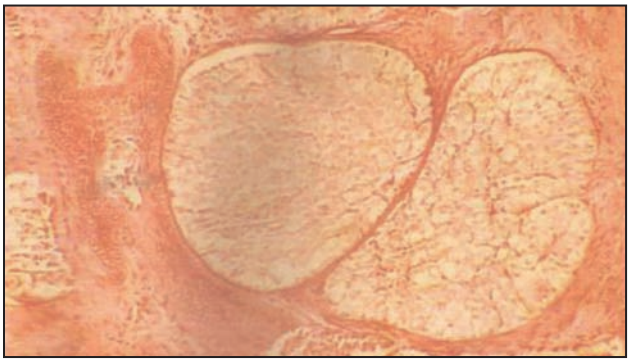

Fig 2. Section of tumour. Note the irregular- shaped lobules encompassed by delicate connective tissue, tumour cells were faint pink vacuolated.

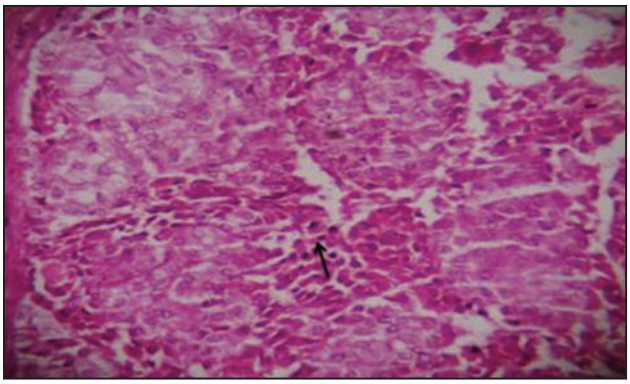

Fig 3. Cluster of sweat glands showing cellular nuclear pleomorphism (arrow). H&EX100.

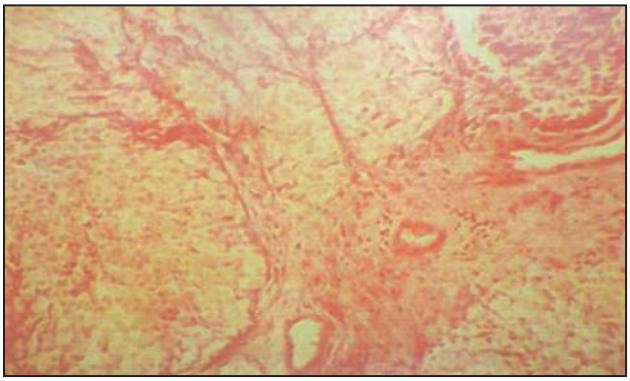

Fig 4. Section of tumour. Note large lobules subdivided by delicate fibrous connective tissue and thick fibro vascular connective tissue between the lobules. H&EX10.

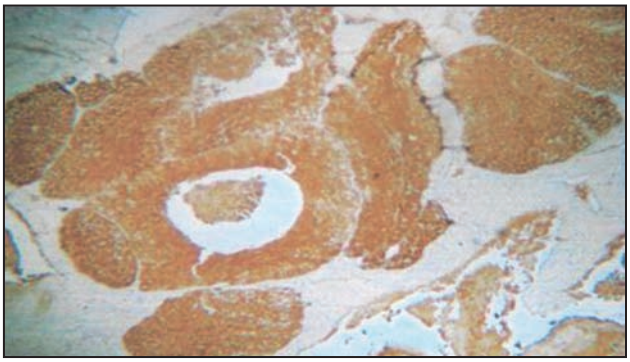

Fig 5. Section of skin. Note tumour cells were strongly positive for S-100. Stre toviodin Biotin method, counterstained with haemato line. H&EX40.

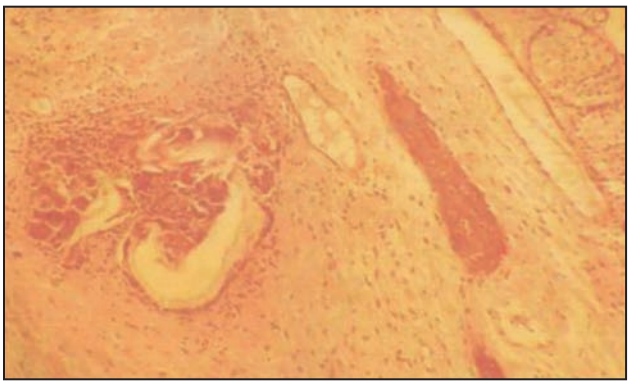

Fig 6. Section of skin. Note tumour cells were strongly positive for S-100. Stre toviodin Biotin method, counterstained with haemato line. H&EX40.

of eccrine neoplasms gland masses were found at sole, palm, face, neck and trunk (Ackerman and Abenoza, 1990; Storm and Seykora, 2002; Mebazaa *et al,* 2006). Chondroid syringoma without specification (eccrine- apocrine) have been diagnosed in human face (Trown and Heenan, 1994; Mathiasen *et al,* 2005); hand and arm (Webb and Stott, 1975; Medina *et al,* 2001; Walarai *et al,* 2011) and in foot (Redon *et al,* 1982; Barnett *et al,* 2000).

Present study reported benign mixed tumour with areas of malignant transformation without clinical manifestation other than local infiltrative nodule with non-defined margins and border was thick. Similar areas of malignant transformation in pre-existing lesion has been described in eccrine spiradenocarcinoma (Storm and Seykora, 2002). Metastasis was not identified because necropsy was not performed. The diagnosis of malignancy was based on the mass which was immobile; the growth was infiltrative and not delineated with capsule, increased cellularity, loss of cellular polarity, cellular and nuclear pleomorphism, necrosis and mitotic figs.

Immunohistochemically, all adenomatous tissue (solid nest and tubules) demonstrated dense and diffuse immunoreactivity to S-100 protein. This is consistent with findings of Bates and Baithun (1998). In addition, our investigation showed that these stromal cells stain positive to S-100, all tumour tissue stained negative to cytokeratine. This suggests that the proliferated cells were myoepithelial rather than epithelial. This is in agreement with findings of Iglesias *et al* (1990) and Souza *et al* (2011) who found that the stromal cells and solid nests stained to the outer layer of tubuloglandular components. They suggest that the myoepithelial cells and stromal elements may be drived from the outer layer of the tubuloglandular component and that the myoepithelial cells have important role in the histogenesis of these tumours. In contrast, the investigation of Hassab-el-Naby *et al* (1989) on 64 specimens of mixed tumour of sweat gland (eccrine and aprocrine) showed that the polygonal cells resembling epithelial cells in the stromal tissue stained positive to S-100 protein, keratin but negative for actin. They favour the hypothesis that these cells were epithelial rather than myoepithelial.

Acknowledgement

We would like to acknowledge the Deanship of Scientific Researches, King Faisal University for providing a grant for this research.

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ADVANCES IN SURGERY AND DIAGNOSTIC IMAGING OF THE DROMEDARY CAMEL

(Soft bound, 403 pages, First Edition 2016, R.O. Ramadan (Ed), All coloured Figs)

Dr. R.O. Ramadan, Professor of Surgery and Radiology at King Faisal University, Al-Hasa, Saudi Arabia has authored a new book titled, "Advances in Surgery and Diagnostic Imaging of the Dromedary Camel" bearing a foreword of eminent scientist and author. The book contains about 408 pages and contains 12 chapters bearing a support of 550 pictures. Various chapters included in the book are well classified in pertinent sub-topics and give reader a convenience to select out the reading material. The advancement in the field of camel surgery, anaesthesia, diagnostic imaging and orthopedics are depicted in different chapters through colourful illustrations, radiographs and tables. Majority of cases represented in the book are personal experiences of Dr. Ramadan who has a long chequered career in this field. His endeavour to putforth his knowledge amassed during this period in form of this book would benefit camel surgeons in a big way. All the chapters bear a list of pertinent references which would certainly help researchers in tracking the review on various aspects of camelid surgery and radiology.

It is worthwhile to point out strengths of the book. The general chapter contains a good description of ageing and estimation of body weight. Both these parameters are important in judging, racing and trading of camels. A short description of intravenous and inhalation anaesthesia generates an insight on these scarcely studied fields of camel anaesthesia. Diagnostic imaging chapter starts with proper anatomic directional terms used in radiography. A large number of radiographs of different parts of camels are shown with their line sketches which helps understanding radiographic anatomy and pathology. Digital radiography, computed radiography and magnetic resonance imaging are also well covered. Ultrasonography has also been dealt with a detailed version of various ultrasonograms taken. The chapters on general surgery and essentials of surgery have given a detailed account of surgical instruments, sutures etc and management of different types of wounds, sinus, fistula and cysts. It has also given a detailed account of bursitis, dislocation and fractures. Management of hernias and onco-surgery has been given with good details. The important surgeries of upper respiratory, gastrointestinal and urogenital systems covers majority of soft tissue surgery including important affections like impaction of dulla, disorders of forestomachs, retention of urine and urolithiasis with associated complications and their management. The female urogenital system has also been given due attention by describing ovariectomy, hydrobursitis, caesarean sections, prolapses of vagina and uterus and rupture of perineum. Camel being a milch animal, surgical affections of the mammary gland are given in a separate chapter. The lameness, conformation and diverse affections of musculoskeletal system are given in a separate chapter which helps understanding orthopedics of camels. Surgical affections of special sense organs are very important for camels and these has good incidence, hence these are discussed in a separate exclusive chapter.

This book will prove a good reference book on camel surgery and radiology to the researchers, clinicians and specifically camel-vets.

(ISBN: 9960-08-120-6)

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DOI : 10.5958/2277-8934.2016.00029.1 *Vol 23 No 1, p 169-174*

OBSERVATIONS ON SEMEN COLLECTION AND SUITABILITY OF DIFFERENT MODIFICATIONS OF ARTIFICIAL VAGINA FOR DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The present investigation was carried out to study the behavioural features at collection and to compare 7 modifications of bovine AV for semen collection from camels. During the period of study 197 attempts for semen collection were made and 178 ejaculates were obtained using different types of the AV. Reaction time, mating duration, interruption of mating and physical attributes of the ejaculate collected were recorded. Behavioural and seminal parameters were compared with different modifications of the AV used, to decide which is the best one for routine usage.

Quantity and quality of semen samples collected from different AV types are described. Bovine artificial vagina of shorter length fitted with the usual smooth rubber liner was adequate for semen collection in camels. The need for special liners and cervix imitations were not found essential. However, shortening of the rubber cone and usage of wide-mouthed and shorter collection vials appeared beneficial to increase the quality and quantity of the ejaculate being collected. Physical conditions required for the AV were also more or less the same as that for bovine, except, the need for smearing lubricants on the liner was not deemed essential. It is concluded that a bovine artificial vagina with minor modifications is sufficient for collecting fairly good quality ejaculates from dromedary camels.

Key words: Artificial vagina, camel, mating behaviour, semen collection, semen

Semen collection from camels is comparatively difficult (Tibary and Anouassi, 1997; Waheed *et al,* 2011) owing to behavioural features of the species such as mating in sitting position, long mating periods, prolonged phase of ejaculation (Skidmore *et al,* 2013), lack of clear external indications for ejaculation and the chance of falling on to the sides after mating. Seasonality of the libido and the aggressive nature of male camels during the period of rut are additional factors making semen collection difficult (Hemeida *et al,* 2001). Most male camels resist restraining devices on the head, making them difficult to handle and take to special collection yard. Hence semen collection has to be performed close to their housing and very often on the sand floor itself.

Like any other farm animals, semen collection using a bovine artificial vagina (AV) is being used in most of the earlier studies (Morton *et al,* 2011 ; Skidmore and Billah, 2006; Skidmore *et al,* 2010). However, in order to facilitate the collection process in camels and to increase the quantity and quality of semen collected, various modifications of the AV are reported by earlier workers. These modifications include shortening the cylinder (Marai and Zeidan, 2007), usage of special liners and connecting cone (Bravo *et al,* 2000 ; El-Behrawi, 2010; Medan *et al,* 2008), providing cervix imitation (Skidmore, 2005), water jacketed collection vials (Skidmore *et al,* 2013) and usage of lubricants. Even with these modifications, semen quality and yield has been reported to be highly variable (Tibary and Anouassi, 1997) and remains the major hurdle for semen preservation studies and insemination trials. Hence the present study was carried out to study the behavioural features of camels at semen collection and to compare various modifications of the AV to enhance yield of semen as well as the convenience of semen collection from camels.

Materials and Methods

The study was carried out on 9 adult male camels belonging to Omani (1), Pakistani (4) and Hazmi (4) breeds. The animals were aged from 8 to 14

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years with 550 to 850 Kg body weight. Starting from October, camels were trained for semen collection using a female camel as the mount. Collection from each camel was attempted in the morning hours and regular collection at intervals of 3 to 7 days was continued from the end of November to middle of May consistent with the report of Deen and Sahani (2000). Various aspects of semen collection with an emphasis on improving yield of semen were observed and salient points were recorded.

Different modifications of bovine AV as described in table 1 were tried for semen collection and those found usable after the initial trials, based on the convenience and yield of semen, were selected for further trials. Each modification was tried in different animals and those that failed in three consecutive attempts were discontinued from further usage and others were continued to use to identify the type most congenial to camels.

Structural components altered between types of AV included length of rubber cylinder (Medan *et al,* 2008), length of the connecting cone, nature of the rubber liner, type of collection vial, provision of cervix imitation inside the AV (Bravo *et al,* 2000) and the nature of cervix imitation provided (Hemeida *et al,* 2001). Physical conditions of the AV such as temperature, pressure and lubrication remained more or less the same except for minor variations made according to the response of the camels. AV temperature was maintained between 35-42°C (Vyas *et al,* 1998; Wani *et al,* 2008) and pressure was regulated to obtain adequate bulging of liner ends.

Response of the animal to the collection process was assessed based on behavioural parameters such as reaction time (time taken from reaching the mount animal until mounting and onset of exploratory thrusting to locate the vaginal passage), mating duration (time taken from entry of penis into the AV until dismounting or complete arrest of thrusting movements) and interruption of mating as evidenced by penis withdrawal, abrupt dismounting and/or cessation of mating.

Collected ejaculates were subjected to evaluation of various physical and microscopic parameters of semen quality. The inner aspect of the AV liner as well as the connecting cone were inspected for the extent of seminal fluid as well as froth sticking on them. Observations such as mating duration, semen yield and quality parameters were compared to decide among the modifications of AV for camels. Further, collections were repeated using the selected models and the results are discussed.

Results and Discussion

Altogether 197 collection attempts were made from 9 camels during the period of November to May to obtain 178 ejaculates excluding 12 attempts when the camel refused to mount and 7 attempts when there was lack of ejaculation even after mounting and thrusting. The success percentage of obtaining semen ejaculate in the present study (90.35%) was more than the report of 74.6% out of collection attempts using AV by Deen *et al* (2003). The number of collections taken during each month of the breeding season is given in Figre No 1. Variation in collection numbers does not reflect libido of the camels except during the last 2 months, because of the involvement of various management reasons affecting frequency and regularity of collections.

Collections were taken in the premises of male camel housing. Two docile females were used alternatively as the mount. The area of collection was provided with a carpet during the early attempts of collection in order to minimise contamination. But, later on collection was continued without a carpet, since there was no advantage felt from using the carpet, because of the chances of contamination with loose dung voided by the mount, as well as camels moving out of the carpet during the collection process.

Even though collection was attempted from both sides in initial occasions, the left side was preferred for most of the collections. Providing AV from left side appeared to be better since the spirally coiled tip of the penis directed towards the left side. Most of the animals showed dismounting towards right which might be due to collection attempts from the left side. Risk of dismounting on to the side of collection was further checked by the attendant made to stand on the left side beside the collecting person.

Reaction time: It is an indicator of libido or sexual desire of the male animal since better libido is reflected by shorter reaction time. Reaction time was up to 30 seconds in 161 cases of collection and more than 30 seconds in 23 collection attempts. The maximum recorded was two minutes, excluding cases of no mounting at all. This is shorter than the reaction time reported by Alfuraiji (1999) in dromedary camels who observed a maximum time of 4.4 minutes. Reaction time was slightly longer for older animals and towards end of the breeding season, attributable to decrease of libido towards end of the season.

Duration of mating: Mating duration ranged from 1 – 11 minutes which is in agreement with the

time reported by Bravo *et al* (2000) using a modified AV with cervix imitation and the average mating duration recorded in 154 cases was 5.08 ± 0.14 minutes similar to the value reported by Hemeida *et al* (2001). Duration of mating varied between camels, being slightly longer for younger than older animals. Similarly mating duration appeared shorter towards end of the breeding season. The yield of semen was found to be positively correlated with duration of mating (0.307, $P < 0.01$).

Interruptions: Mating was interrupted by abrupt dismounting in some cases, while complete withdrawal and reinsertion of penis once or more times was noticed in 59 collections. Completion of mating was manifested by dismounting or falling down in a lateral position on to the side of the mount. Two of the male camels occasionally fell onto the back upon completion of the mating, while one young male was reluctant to dismount during most of the collections, even after complete cessation of thrusting movements, unless forced to dismount. However, even that animal started dismounting by its own towards the end of breeding season

Mating process: Adoption of the mating posture was done within a few seconds of approaching the mount. However, less experienced camels sometime adopted abnormal positions such as sitting on the neck region, or sitting perpendicular from sides of the mount, refusing to get up, sitting with a wide gap between pelvic regions, and so on. The mating process was characterised by protrusion of the penis immediately after adoption of posture and onset of exploratory thrusting. Since the Artificial Vagina was offered soon after protrusion of the penis, actual time taken for intromission could not be recorded.

Soon after intromission there were weak thrusting movements, which became stronger along with bodily movements such as bringing the pelvis closer, interaction with the mount, adoption of a more crouching posture and repositioning of the hind limbs. After some time physical movements became

Table 1. Types of AV tried and major features of each type.

Type of AV	Major features
Bovine AV (Long)	Typical bovine AV, with rubber cylinder - 40 cm, inner diameter of the cylinder - 6 cm, Rubber cone - 20-25 cm, and long collection vial of 15 ml graduation
AV with plastic sleeve	Long bovine AV provided with thin plastic sleeve inside the rubber liner to avoid semen contact with rubber liner, collection vial attached to the plastic sleeve and retained inside the AV at the distal end.
Bovine AV (Short)	Bovine AV with cylinder length of 30 cm, liner, cone and collection vial as described for long AV
Normal AV with wide mouthed collection vial	Bovine AV (short) provided with wide mouthed collection vial (Tulip shaped) having graduated bottom portion and shortened connecting rubber cone with effective length of 5-10 cm.
AV with rough liner	Short AV with wide mouthed collection vial provided with rough surfaced rubber liner and shortened cone
AV with silicone liner	Short AV with soft silicone liner and wide mouthed collection vial attached to protruding end of the liner itself without a connecting cone.
AV with cervix imitation	Short AV with wide mouthed collection vial, provided with cervix like portion made of sponge fixed inside the liner at the distal end of AV
AV with twisted liner	Short AV and wide mouthed collection vial provided with a 180 degree twist for the inner liner to have a constriction like cervical opening
AV with Rubber ring	Short AV and wide mouthed collection vial provided with narrow rubber ring around rubber liner at distal 1/3 of the AV length

Table 2. Number of collections taken using each type of AV and drawbacks noticed.

Type of AV	Number of collections	Major drawbacks
Bovine AV (Long)	3	Ejaculation within the liner, Poor yield of semen and more heavy
AV with plastic sleeve	5	Poor yield of semen, more froth formation, difficult to maintain
Bovine AV (Short)	14	Need more time for semen to reach the vial, More area of contact with the cone
Normal AV with wide mouthed collection vial	53	Need to transfer large volume ejaculates into other vials for measuring the volume
AV with rough liner	8	Not preferred by most males and less yield of semen
AV with silicone liner	10	Difficulty to prepare and maintain, no added advantages seen
AV with foam cervix	18	Difficult to maintain, less yield of semen
AV with twisted liner	29	Difficult to maintain, less yield of semen
AV with Rubber ring	46	Same drawbacks of wide mouthed vial as with normal AV

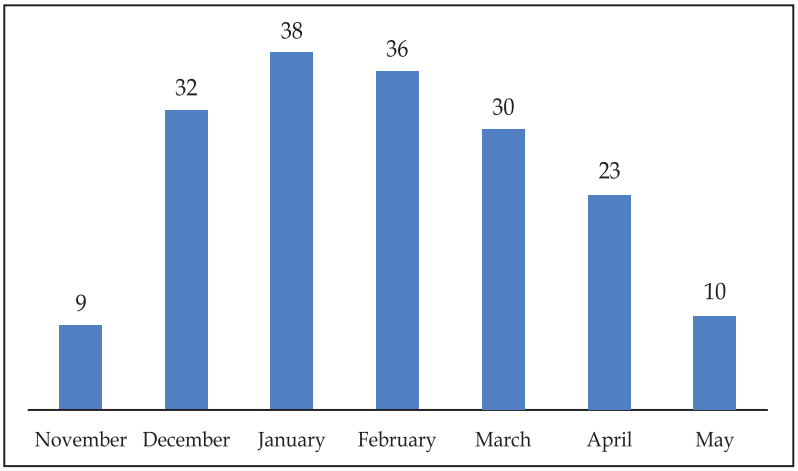

Fig 1. Number of collections during the months of breeding season.

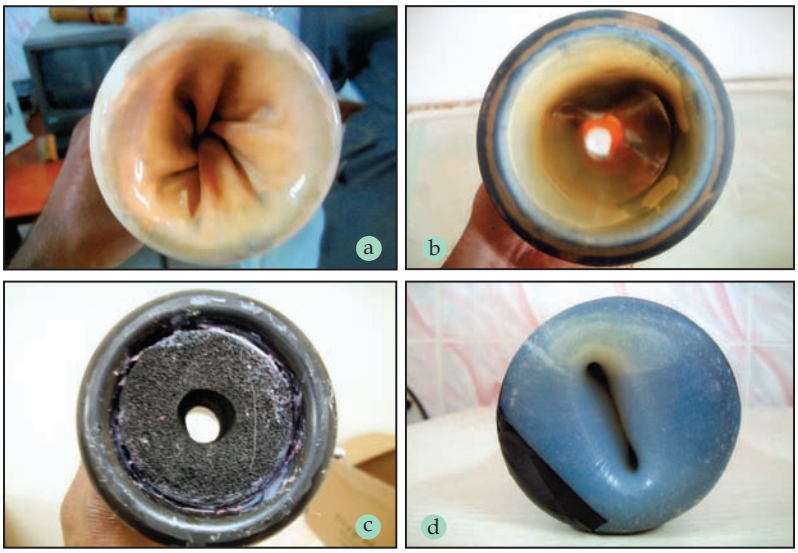

Fig 2. Modifications inside the lumen of artificial vagina to imitate cervix feeling (a) Twisted liner (b) Rubber band inserted inside the liner (c) Cervix like portion made of sponge (d) soft and smooth liner made out of silicone gel.

less frequent even though strong rotatory movements of the penis was felt over the prepuce. It appeared that strong thrusting movements were taking place until the penis enters into the cervix and rotatory movement of the penis continues thereafter along with intermittent phases of rest. This is in agreement with the observation made by Bravo *et al* (2000). Ejaculation was characterised by pelvic thrusts of low amplitude and increased frequency, sometime felt in the form of tremors of the pelvic region. Mating posture, the nature of physical movements and behaviour during mating varied between animals.

Suitability of AV modifications: The number of collections using different types of AV and major drawbacks are shown in Table 2. Convenience or adequacy of each type of AV is reflected by the numbers of collection taken using each of them. AV modifications such as a silicone liner (Fig 2d), plastic sleeve insertion, long AV, AV with rough liner and bovine AV with long connecting cone and collection vial were discontinued after very few trials for lack of noticeable advantages and other reasons such as dislike shown by the animal, smearing of semen over a larger area of liner and consequent low yield of semen. Skidmore *et al* (2013) made a similar observation that a disposable plastic inner liner in the AV is not accepted well by male camels.

AV with cervix imitation (Fig 2c) was used for taking 18 collections. Mating duration was rather low (3.33 min) and the volume of semen obtained was more than that from a normal AV (4.19 versus 3.84 ml). This is more than the value reported by Morton *et al* (2011) who obtained 2.85+/-0.37ml using a bovine AV with an imitation cervix but less than the value reported by Bravo *et al* (2000) using the same type of AV. However, the proportion of samples having higher initial oscillatory movement was low (22.22%) and average sperm concentration was only 21.67 millions / ml for the samples collected by this type of AV, which is far lower than the concentration reported by Hammadi *et al* (2008) and El-Hassanein *et al* (2010) who used a modified bovine AV with imitation cervix.

Out of the total 178 collections, maximum numbers were taken using the normal AV (28%) (Fig 3) followed by the AV with rubber ring (25%) (See Fig 2b) and twisted liner (16%) (See Fig 2.a). Relative resistance for penetration of penis through lumen of these AV types varied from low, medium and high, respectively. Mean mating duration in minutes was 4.6, 4.96 and 4.9 with normal, rubber ring and twisted liner types of AVs, (n= 56, 45, 26, respectively) while the mean volume of semen was 3.84, 4.46 and 5.16 ml, respectively. Even though the mating duration was not much different between the three types of AV, there appears to be a direct relationship between the volume of semen and the resistance offered by the AV liner.

The relationships between the three most commonly used types of AV and semen quality

Fig 3. Normal AV (Short bovine AV with tulip shaped collection vial and short cone).

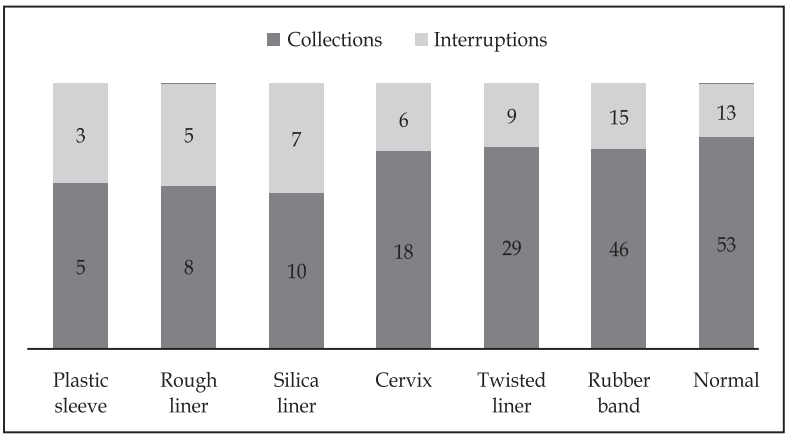

Fig 4. Number of collections with types of AV and interruptions.

parameters are showed in Table 3. While volume of semen and amount of froth increased with types of AV that provided more resistance inside the lumen, other parameters such as sperm availability, initial oscillatory movement and sperm concentration showed a decreasing trend. Thus it can be inferred that increased effort for penetration leading to an increase of froth formation and expulsion of more seminal plasma, results in increased volume together with lowered sperm availability.

Interruption with types of AV: The mating process was interrupted by interim dismounting or penis withdrawal in 38% of the cases, even though mounting and intromission were repeated soon after and the mating process continued further. The number of interruption was found to be influenced by the type of AV used for collection. Numbers of interrupted collections among the total number of collections using each type of AV are shown in Fig 4. The proportion of collections involving interruption was lowest for the normal type AV (24.53%) compared to the AV with rubber band (32.61%), twisted liner (31.03%) and cervix imitation (33.33%).

Number of interruptions can be considered as a sign of dislike shown by the animal towards the type of AV, even though other factors also involved. Proportions of interruptions recorded were much higher for AV with a silicone liner (70%), rough liner (62.50%) and plastic sleeve (60%). These AVs were discontinued from use after a few collection attempts. However, the higher figs for proportions of interrupted collection with these AV s could be exaggeration since only few numbers of collections were taken using them.

Contamination: Withdrawal of the penis and reinsertion often carry sand and other extraneous particles into the AV leading to contamination of the semen (Deen *et al,* 2003; Skidmore *et al,* 2013; Tibary and Anouassi, 1997) . Out of 178 collections taken, 50.6% were without any visible extraneous particles, while 32.6% and 16.3%, respectively had minor and gross contamination, and the contaminants were mainly sand and vegetative particles. Even for the few samples collected on carpeted floor, there was not much difference in the occurrence of contaminants. Hence there is need to resort to other measures to reduce contamination such as better cleaning of the animal's body prior to collection, modification of the floor, minimisation of the factors causing interruptions of the mating process and so on.

Table 3. Relationships of AV type with mean values of semen quality parameters.

AV type	Volume of semen (ml)	Amount of froth (cm)	Proportion of sperm rich samples	Samples with better initial oscillatory motility	Sperm Concentration Millions /ml
Normal	4.05 +/- 0.37	0.91	58.5 %	35.8 %	305.5
Rubber ring	4.46 +/- 0.47	1.04	43.47 %	30.43 %	143.1
Twisted liner	5.16 +/- 0.42	1.41	44.82 %	13.79 %	79.66

It was concluded that Normal AV (Short bovine AV with shortened connecting cone and wide mouthed collection vial) gave better quality semen than the various other types tried. It was not essential to have special liners and cervix imitations for the AV. Possible measures to reduce interruption of mating leading to contamination and possible measures to improve quantity and quality of camel semen has to be explored further.

Acknowledgement

The authors express their sincere gratitude to Khalifa Al Ali, Executive Director of ADFCA, and the whole organisation - Abu Dhabi Food Control Authority, for providing the opportunity, facilities and support for conducting this study.

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DOI : 10.5958/2277-8934.2016.00033.3 *Vol 23 No 1, p 175-178*

ESTIMATION OF SOMATIC CELL COUNT, AS GOLD STANDARD TO DETECT SUBCLINICAL MASTITIS, IN DROMEDARY CAMEL

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ABSTRACT

The objective of the present study was to estimate cut-off point of somatic cell count (SCC) to detect subclinical mastitis using mathematical modelling in dromedary camel. Camel milk samples were collected from individual quarters (n=243) of 95 milking camels. Approximately five minutes prior to milking, camel received oxytocin (20 I.U, IM), the teat was washed and the camel calf was released to stimulate the dam. After 2 minutes, the suckling was interrupted and the teat was dried with tissue and sampling was conducted to perform CMT, and to collect milk for SCC. The range of SCC corresponding with CMT scores of 0, T, 1, 2 and 3 were 0-51000, 57000-108000, 116000-306000, 342000-1830000 and 2129000-8435000, respectively. The threshold values for SCC to detect subclinical mastitis in camel were calculated by considering two different approaches: frequentist analysis (306000 cells/ml) and Bayesian analysis (390000 cells/ml). In conclusion, SCC values beyond 306000 cells/ml could be considered as subclinical mastitis in camel.

Key words: Dromedary camel, SCC, subclinical mastitis

Prevalence of mastitis in camel was assumed to be low due to the thin streak canal, covering udder to restrict suckling (Manefield and Tinson, 1996; Wernery and Kaaden, 2002), the least contact of udder to contaminated bed throughout rest period (personal observation), the low density of population due to scattered individuals throughout the pasture and finally the common practice of hand milking rather than machine milking. Although machine milking has been adopted for camel in very few countries (Yagil, 1982; Nagy *et al,* 2013), dairy camel industry still depends on hand milking in most countries worldwide. It is expected that machine milking can predispose camels to mastitis, particularly in the subclinical form.

The prevalence of subclinical mastitis in this species varied among different studies (15-67.4%; Bhatt *et al,* 2004; Abera *et al,* 2010; Seifu and Tafesse, 2010; Alamin *et al,* 2013). Part of this variation could be due to the variety of methods used to identify subclinical mastitis including CMT, SCC, and bacteriological investigation in this species. Although, SCC has become the gold standard to measure milk quality, there is no cut-off point estimated for somatic cell count (SCC) to detect subclinical mastitis in dromedary camel. The objective of this study was to investigate the cut-off point of SCC, as a gold standard, to detect subclinical mastitis in dromedary camel.

Materials and Methods

The present study was conducted during 2012 and 2014 between months May and June, in Golestan Province, the main region for dairy camel industry in I. R. Iran. Dromedary milking camels (n=95), 7-11 years of age, 2-4 months after calving, with the average daily milk production of 6 kg, were used in this study. They were milked manually trice daily (5:00, 16:00, 21:00) and maintained on pasture throughout the day.

California mastitis test was carried out according to the method described previously (Schalm and Noorlander, 1957) for cow. In brief, at camel-side, each quarter milk sample was placed in one clean well of a special plastic test paddle and mixed with an equal volume of commercial CMT

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solution (Kruuse, UK). As the plate was rotated gently, any changes in the colour and consistency were observed and interpreted: Scores were given within the range of 0-3; with 0 for no reaction, T for a trace (slight thickening that tends to disappear with continued movement of the paddle), 1 for a weak positive (distinct thickening, but no tendency toward gel formation), 2 for a distinct positive (mixture thickens immediately) and 3 for a strong positive (adhered gel). Accordingly, scores T and 1 were considered as suspicious and scores 2 and 3 were considered as positive for subclinical mastitis.

Somatic cell count was done from milk samples collected into the tube with potassium dichromate (Floka, Boches, Switzerland) and were counted using Fossomatic machine (Fossomatic 5000, Fossomatic Company, Denmark). Standard sample consisting of 389000 cells was used to calibrate the machine before the SCC. Samples of 25 ml volume were assigned into special racks and allowed to be automatically homogenised and counted individually by the detector.

Experimental design

Camel milk samples were collected, at morning time, from individual quarters (n=243) of 95 milking camels, without any observable disease and clinical signs of mastitis. Five minutes prior to milking, camel received oxytocin (20 I.U, IM; Aburaihan Pharmaceutical Co., Iran), the teat was washed and the camel calf was released to stimulate milk let down from the dam. After 2 minutes, the suckling was interrupted and CMT was performed. After discarding the first few squirts of milk, about 50 ml of milk were collected into sterile bottle. Samples were kept on ice block and transported to the laboratory and examined within 8 hrs after milk collection for SCC measurement.

Statistical model

A threshold from both the frequentist and the Bayesian perspectives was investigated. In frequentist analysis, a summary and quantile of the data were used to identify outliers and exhibit the form of the distribution. Then, Maximum Likelihood (ML) method was used to estimate the parameters of the model (Lehmann and Casella, 1998). Bayesian analysis of extreme events was used to estimate the Bayesian interval for threshold (Behrens *et al,* 2004). In this mixture model, a parametric form for the centre and a Generalised Pareto Distribution (GPD) for the tail of the distribution were used with all observations to infer about the unknown parameters from both distributions (Behrens *et al,* 2004). Then the algorithm based on Markov Chain Monte Carlo (MCMC) was used to make inferences about the posterior distribution. All of the computer programs and MCMC simulations were performed using R Statistical software.

Results

According to CMT results, out of 243 quarters of 95 dairy camels, 122 quarters (50.2%) were negative, 77 quarters (31.7%) were suspicious (CMT scores of T and 1) and 44 quarters (18.1%) were positive (CMT scores of 2 and 3) for subclinical mastitis (Table 1).

The range of SCCs for CMT 0, T, 1, 2 and 3 were 0-51, 57-108, 116-306, 342-1830 and 2129-8435 (x1000) cells/ml, respectively (Table 1).

Table 1. Relationship between CMT and SCC of milk samples in dromedary camels

Number of quarters	California Mastitis Test score	Somatic Cell Counts (cells/ml)
122	0	0-51,000
39	T	57,000-108,000
38	1	116,000-306,000
28	2	342,000-1,830,000
16	3	2,129,000-8,435,000

Distribution function of the data could be estimated by considering a distribution function that belongs to the family of extreme value theory. After fitting distribution to data, it was revealed that a Frechet distribution would be an appropriate choice. Selection criteria were based on rank of statistics in three goodness of fit tests, including Kolmogorov Smirnov, Anderson Darling and Chi-Squared tests (Lehmann and Casella, 1998). The ML method estimates the parameters of the Frechet distribution. These data are well approximated with a distribution which belongs to the family of Generalised Extreme Value (GEV distributions). Accordingly, upper percentiles of the data between 80-97.5% were considered to elucidate a possible threshold value for somatic cell counts and to suggest that SCC greater than 306000 could be considered as abnormal.

In Bayesian analysis, after the outliers were excluded, 100 chains were simulated and in each chain 1000 data were generated. After chain convergence, the mean of threshold value and 83.75% of the Highest Density Region (HDR) were calculated. Accordingly, the lower bound of the HDR was considered as a Bayesian point estimate for the threshold value of 390000 cells/ml.

Discussion

Using two different mathematical models: frequentist and Bayesian approaches, the threshold value for SCC to detect subclinical mastitis in camel was suggested to be either 306000 or 390000

cells/ml, respectively. Defining the cut-off point is one of the most important steps in controlling subclinical mastitis in camel, which is estimated using mathematical modeling in the present study.

CMT and SCC have been used as diagnostic tools to detect subclinical mastitis in camels (Abdurahman *et al,* 1995; Schepers *et al,* 1997; Almaw and Molla, 2000; Younan *et al,* 2001; O'Mahony *et al,* 2006). In the present study, the range of SCC in relation to CMT was presented for the first time in camel, which revealed that the relationship between the range of SCC and CMT in camel was different from that reported in cattle (Dohoo and Meek, 1982) and goat (Perrin *et al,* 1997). The range of SCC with the CMT scores of 0, T and 1 was between 0 and 306000 cells/ml whereas, with the same CMT scores, the SCC has been reported to be ≤1000000 cells/ml in cattle (Dohoo and Meek, 1982) and ≤750000 cells/ml in goat (Perrin *et al,* 1997). In this study, SCC of ≥342000 cells/ml had CMT scores of 2 and 3. This has been indicated to be >1000000 and >750000 cells/ml in cattle and sheep, respectively (Dohoo and Meek, 1982, Perrin *et al,* 1997). As a result, CMT scores (≤1 or ≥2) represents considerably lower values of SCC in camel compared to those in cattle and sheep. Any explanation for such biological difference would be the subject of further research.

If the cut-off point of 306,000 cells/ml is used as the gold standard to detect subclinical mastitis in camel, corresponding to CMT scores of 2 and 3, the apparent prevalence of subclinical mastitis in the present study was 9.46% (23/243 quarters). There has been considerable variation among studies in terms of the prevalence of subclinical mastitis in camel, partly due to the substantial variation in defining criteria and lack of a well-defined cut-off point to determine the prevalence of subclinical mastitis in camel. In this context, using CMT as a criteria to detect subclinical mastitis, the prevalence of subclinical mastitis has been reported to be 36.87% (59/160 camels; Suheir *et al,* 2005) and 15% (9/60 quarters; Alamin *et al,* 2013) in Sudan, 15.8% (80/505 quarters; Abera *et al,* 2010), 20.7% (30/145 camels; Abera *et al,* 2010), 22% (43/195 camels, Almaw and Molla, 2000), 67.4% (433/642 quarters; Seifu and Tafesse, 2010), 39.4 % (137/348 camels, Regassa *et al,* 2013) in Ethiopia, 11.67% (21 /180 camels; Ibrahim *et al,* 2011) in Saudi Arabia, 38% (57/150 camels; Sibtain *et al,* 2012) in Pakistan, and 41% (41/100 quarters) and 72% (18/25 camels) in India (Bhatt *et al,* 2004). Indeed, this variation perpetuate unless we define an appropriate cut-off point for determination of subclinical mastitis in camel. The prevalence of subclinical mastitis in sheep (Bergonier and Berthelot, 2003; Contreras *et al,* 2003; Contreras *et al,* 2007) and cow (Plozza *et al,* 2011) were 5-30 and 11-43%, respectively. Therefore, the prevalence of subclinical mastitis in camel is within the range of subclinical mastitis in other food animals.

The prevalence of mastitis was relatively low in the present study. It is well known that susceptibility to mastitis is determined by a combination of factors including bacterial virulence, environmental conditions (housing, management, feeding and milking technique) and animal-related factors (milk yield, genetics). These factors are interdependent to each other and their impact depends on the type of pathogen (Burvenich *et al*, 2003). The streak canal is relatively thin in camel which could play a role in low prevalence of mastitis in this species (Manefield and Tinson, 1996). Moreover, the cover used to prevent the calf from suckling has been suggested as a reason for low rate of mastitis in camel (Manefield and Tinson, 1996; Wernery and Kaaden, 2002). It is believed that the cover protects the animal from mechanical traumas. Yet it should be considered that the cover could be moistened with milk and become contaminated with bedding, and consequently predispose the animal to intra-mammary infections. Nevertheless, given no research has been conducted in this regard, any hypothesis requires to be tested by a well-designed controlled study. In addition, machine milking is uncommon in camel, which might have contributed as an additional reason for low prevalence of mastitis in this species. The other suggested factors for low prevalence of mastitis in camel are the type of resting, few contact of mammary glands with the bedding, low density of animals in the pasture and the dryness of the bedding. Finally, one of the potential factors in this context is the antimicrobial components of camel milk (El-Hatmi *et al,* 2007; Salami *et al,* 2010). Further studies are warranted to investigate the underlying mechanisms for low prevalence of mastitis in camel.

In conclusion, the present study revealed that there is low number of SCC for different scores of CMT in camel as compared with corresponding figures in other ruminants. Additionally, using frequentist and Bayesian approaches, we defined cut-off point for detection of subclinical mastitis in dromedary camel.

Acknowledgements

We acknowledge UNESCO Chair on Interdisciplinary Research in Diabetes, University Tehran, Tehran, Iran and organisations, scientists, the authorities in Golestan province and dairy camel producers for their valuable support.

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DOI : 10.5958/2277-8934.2016.00030.8 *Vol 23 No 1, p 179-184*

CLINICAL FINDINGS AND REPRODUCTIVE PERFORMANCE OF FEMALE DROMEDARY AFFECTED WITH VAGINAL AND CERVICAL ADHESIONS AND STENOSIS

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ABSTRACT

The present study was conducted in order to investigate clinical findings, histopathology and efficiency of treatment in female camels affected with vaginal adhesions (n = 57), and cervical stenosis (n = 17) and cervical adhesions (n = 5). Breeding history, ultrasonography and vaginal exploration were undertaken. The female camels with vaginal and cervical adhesions were treated by manual breakage. The females affected with cervical stenosis were treated by administration of Dinoprost (25 mg im). The pregnancy rate was calculated 45 d after mating. Results showed that refused-mating was the main recurring complaint (72.15%). The majority of cases (89.9%) showed an accumulation of fluid in the uterus. At the time of examination, a corpus luteum (CL) was present in all the cases with cervical stenosis. The culling rate was greater in the females with vaginal adhesions (54.4%) than in those with cervical adhesions (20%) and cervical stenosis (11.8%). The pregnancy rate was higher in the females with cervical stenosis (73.3%). It was concluded that vaginal and cervical adhesions, characterised by refused mating and accumulation of fluid in the uterus, constitute a long-standing reproductive problem in dromedaries, and consequently result in a high culling rate.

Key words: Cervical stenosis, female dromedary, pregnancy rate, vaginal adhesion

Vaginal adhesion is the second major cause of infertility in female camels, usually ending with reproductive failure and the culling of animals with permanent infertility (Ali *et al*, 2010a). The pathogenesis of this affection is not clear. Chronic vaginitis, overbreeding, aggressive mating practice, injuries during parturition, increasing parity could be suggested as factors which contribute to the problem (Tibary *et al*, 2001). Compared to other domestic species, Camelidae seem to be reproductively unique in their susceptibility to severe secondary strictures and adhesions of the vaginal vault (Tan and Dascanio 2008). The condition of vaginal adhesions in camels (Ali *et al*, 2010a and b; Tibary *et al*, 2006) and alpacas (Vaughan, 2008) has been reported in the literature; however, despite its importance, the condition has not been discussed separately and has been referred to only as a cause of infertility. Factors that have been suggested as contributing to the problem include chronic vaginitis, overbreeding, aggressive mating practices, injuries during parturition, increasing parity and intrauterine infusion with caustic solutions (Tibary and Anouassi, 2000).

Other acquired anomalies of the cervix and vagina include adhesions or lacerations resulting from a complication of birth or excessive trauma during manipulation. The condition of vaginal adhesions has been reported in the literature (Tibary *et al*, 2006). The present study was aimed to examine the breeding history, clinical and histopathological findings, and possibility of treatment of vaginal and cervical adhesions and stenosis in female dromedary camels.

Materials and Methods

Animals and management

Female dromedary camels with vaginal adhesions (complete occlusion of the vaginal passage, n = 57), cervical adhesions (complete occlusion of the cervical passage, n = 5) and cervical stenosis (narrowing of the cervical canal, n = 17) were used in this study. The animals were generally healthy with no systemic illness. The majority of animals were kept

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free in desert areas and fed mainly on alfalfa hay and barley seed concentrate or formulated rations. These were continuously exposed to fertile males during the breeding season.

Breeding history and clinical examinations

The age, parity, clinical manifestation, duration of infertility, condition of the last parturition and body condition score on a scale of 1 to 5 (Sghiri and Driancourt, 1999) were recorded for each animal on a special form at the animal admission section of the Qassim University Veterinary Teaching Hospital in Saudi Arabia. Dystocia was considered when the dams were assisted during their last parturition with external help by excessive traction, correction and traction or foetotomy. Gynaecological examinations were performed using the standard transrectal, vaginal, and ultrasonographic techniques (Aloka SSD-500, equipped with 5 to 7 MHz linear-array transducer, Aloka Co., Ltd., Tokyo, Japan). The vaginal adhesions were graded after modification of a previous report (Irkorucu *et al*, 2009) in degree as: light (very small, irregular, medium-intensity and easily separable adhesions composed of a mesh of breakable strands resembling a spider web); mild (regular, intense and not easily separable adhesions); and severe (very intense, homogeneous and not easily separable adhesions accompanied by collapse of the vaginal walls). The following criteria was used to differentiate between congenital occlusion and adhesion of the posterior genital tract; 1) animals were mated before at least once without failure of penile intromission; 2) persistent hymen is defined as a single membrane which is found only in nullipara and lacks additional tissues behind. Once punctured, the vaginal lumen is patent and allows fluids of variable viscosity and volume to exude outside the vulvar lips. Sites of the vaginal adhesions were determined and classified into anterior (caudal to the external cervical orifice), middle (half way between the external urethral opening and the posterior cervical orifice) and posterior (just cranial to the external urethral orifice and including the whole vagina proper). Cervical adhesions were considered when the cervical canal found non patent due to presence of connective tissue membrane or web in the cervical lumen despite the manual advancement through the first or second cervical annular rings. Cervical stenosis or failure of dilatation was considered when the cervical rings were found resistant to manual dilatation during the vaginal examination.

Histopathology

Specimens from the adhered vagina were obtained using sterile tissue forceps and surgical scissors. Moreover, a tissue specimen was taken from the normal, healthy vagina of a pluriparous, non pregnant, non parturient female camel just after slaughtering at a local abattoir. The specimens were immediately fixed in 10% neutral buffered formalin, then dehydrated in ascending grades of ethanol alcohol, cleared in xylol, cast and blocked in paraffin, sectioned at 2 to 5 µm, dewaxed and stained with Hematoxlin and Eosin.

Treatment trials

The female camels with vaginal and cervical adhesions were treated by manual breakage of the adhesions (Fig 1C), local applications of antibiotics and anti-inflammatory suspensions (to prevent re-currence) consisted of procaine penicillin 100 mg; streptomycin sulphate 100 mg; neomycin sulphate 100 mg; prednisolone 10 mg (Multiject, Norbrook Laboratories Ltd., Northern Ireland) on the disrupted areas, in addition to administration of PGF2α 25 mg im (Dinoprost, Lutalyse, Pharmacia and Upjohn, NY) to induce luteolysis and uterine contraction and drainage and daily, for 5 days, phenylbutazone 4.4 mg/kg im (Phenylarthrite, Vetoquinol Veterinary Pharmaceuticals, Lurecedex, France). After evacuation, the uterus was irrigated with 10% povidone-iodine solution. The treatment was repeated after 10 days and animals were examined for uterine clearance and patency of the posterior tract. The females affected with cervical stenosis or failure of dilatation were treated by manual dilatation (if possible), administration of Dinoprost 25 mg im and daily, for 5 d, phenylbutazone 4.4 mg/kg im followed by uterine irrigation with 10% povidone-iodine solution. Treatment was repeated with PGF2 α thrice every 3 days and animals were examined for cervical canal patency and uterine clearance. One week after treatment, the females were re-examined in preparation for mating. Animals which were found unresponsive for treatment or developed adhesions after treatment or affected with incurable adhesions (severe, blind, occupying the whole proper vagina, membranous and highly vascular adhesions and those associated with bilateral ovarian hydrobursitis). The treated recovered females were mated naturally by fertile males when their genital tract found clinically sound on rectal and ultrasonographical examinations. The pregnancy rate (number of pregnant females/total number of mated females) was calculated 45 d after mating.

Data analysis

Data related to breeding history and clinical findings were presented in numbers and percentages. Correlation between age, parity, body condition score and duration of infertility of the affected females and incidence of adhesions was calculated, and analysed statistically using the SPSS statistical package, version 18 (2009). Chi square was used to compare mating (animals recovered and mated relative to the total number of affected animals), pregnancy and culling rates. Results were considered significant at $P<0.05$.

Results

Breeding history

The mean ± SEM age of the animals was 8.91 ± 3.3 y and ranged from 4 to 20 y. The mean body condition score was 3.28 ± 0.60. The mean duration of infertility was 52 ± 4.41 months and ranged from 24 to 72 months. Most of the affected females were pluriparous (79.75%). A history of difficulty during the last parturition was recorded in 34.69% of the cases. Refused mating was the main clinical manifestation in females with vaginal and cervical adhesions (72.15%), while repeat breeding and refused mating were recorded for those with cervical stenosis (Table 1). Age, parity, body condition score and duration of infertility of the affected females did not correlate with the incidence of adhesions in these animals.

Clinical findings

Clinical findings and responses to treatment are presented in Table 1. Vaginal adhesions were found mostly in the posterior third of the vagina (Fig 1B). Most cases of vaginal and cervical adhesions were of severe degree. The majority of cases showed ultrasonographically a distended uterus with hypo- or hyperechogenic fluid (89.9%) (Fig 1A). A mature corpus luteum (CL) was detected in all cases with cervical stenosis and in a few cases with vaginal adhesions, but in none of the cases with cervical adhesions. Ultrasonography was of little value in detecting the site of the adhesions and was not indicative in detecting their occurrence.

Histopathology

The vaginal adhesions were composed mainly of fibrous connective tissue invaded by fibroblasts and characterised by ulceration, oedema of the lamina propria, congestion and hemorrhages (Fig 2).

Culling and pregnancy rates

Due to poor response to treatment, the culling rate was greater in females with vaginal adhesions than in those with cervical adhesions and cervical stenosis ($P = 0.001$). Pregnancy rates were higher in females with cervical stenosis than in those with vaginal and cervical adhesions ($P = 0.001$).

Discussion

The results revealed that vaginal and cervical adhesions, characterised by occlusion of the genital tract, refused mating and accumulation of fluid in the uterus are serious long-standing reproductive problems in dromedaries, and thus result in a high culling rate. The condition was more frequent in the pluriparous animals. Likewise, other studies have reported that vaginal adhesions in female dromedaries (Tibary *et al*, 2008; Ali *et al*, 2010a), mares (Ammary, 2013), and in female llamas and alpacas (Tan and Dascanio, 2008) were more frequent in multiparous animals.

In the present study, refused mating was the most predominant sign associated with adhesions. It is well known that a curled and erect tail is an external sign of pregnancy and refusal of the male (Skidmore, 2011). Fluid accumulated in the uterus mimics pregnancy, which may explain the refusal of the males in most of the affected females. A tightly-closed cervix or vagina causes retention of uterine exudates which sets up an inflammatory reaction in the uterine lining (Cozens, 2009). The uterine tissue recognises these fluids as foreign material and white blood cells appear to clean up (Hughes *et al*, 1979) by releasing lysosomal enzymes, which are acidic. This acid environment damages the uterine lining, causing inflammation and fluid buildup (Watson, 1994). Unlike in cattle, pyometra in female camels is not usually associated with a retained CL (Ali *et al*, 2010a). Earlier reports indicated that persistence of luteal function (high progesterone concentrations) is rarely due to maintenance of the corpus luteum but rather to the luteinisation of hemorrhagic follicles (Tibary and Anouassi, 2000). However, in the present study, a CL was found in all the animals affected with cervical stenosis and 9.37% of females affected with vaginal adhesions. The persistence of the CL may be attributed to the inability of the damaged endometrium to produce prostaglandin F2α to destroy the CL at the proper time of the reproductive cycle. The infertility accompanying these conditions was partly due to the mechanical hindrance of the copulation process represented by the adhesions and partly to the complete loss of the endometrium and underlying submucosa due to the long standing inflammatory processes (Marai *et al*, 2009).

Table 1. History, clinical findings and culling and pregnancy rates in female dromedaries affected with vaginal/cervical adhesions and cervical stenosis.

	Vaginal adhesions N=57	Cervical adhesions N=5	Cervical stenosis N=17
History			
Refused mating	80.7%	80%	41.2%
Repeat breeding with irregular heat interval	8.8%	20%	47.1%
Repeat breeding with regular heat interval	1.8%	–	11.7%
Failure of penile intromission	3.4%	–	–
Bleeding during coitus	5.3%	–	–
Site of vaginal adhesions			
Anterior third	19.30%	–	–
Middle third	24.56%	–	–
Posterior third	56.14%	–	–
Degree of adhesions			
Light	5.26%	20%	–
Mild	14.04%	–	–
Severe	80.70%	80%	–
Associated genital diseases			
Fluid–filled uterus	89.47%	100%	88.13%
Ovarian hydrobursitis	10.53%	–	11.87%
Ovarian findings			
No follicles, no corpus luteum	31.25%	60%	–
Follicles <1 cm	15.62%	40%	–
Follicles 1-2.5 cm	31.25%	–	–
Follicles > 2.5 cm	12.51%	–	–
Corpus luteum	09.37%	–	100%
Efficacy of treatment			
Culling	54.4%	20%	11.8%
Mating	45.6%	80%	88.2%
Pregnant	11.5%	–	73.3%

Mating in camels typically takes 7 to 20 min (Novoa, 1970; Skidmore, 2011) or 14 to 36 min (Zeidan and Abbas, 2003; Marai *et al*, 2009). The aggressive mating during the ''wrong'' phase of follicular developmental phase has been reported as a cause of severe uterine inflammation (Tibary *et al*, 2001; Vaughan and Tibary, 2006). Other management errors include breeding with a young male, overuse of males, and lack of verification of intromission during copulation (Tibary and Anouassi, 2000). Since breeding is quite invasive in camelids, frequent mating can cause trauma to the cervix and uterus and increases the risk of uterine insult. This makes camelids more susceptible to uterine infections (Tibary and Anouassi, 2002). Herdsmen have claimed that 29.82% of males in the present study refused to dismount females, despite completing their sexual task. Instead, they continued playing in the copulatory organs of the receptive female or obliged to re-service the same female several times at the same occasion during the period of sexual receptivity to ensure conception. Bleeding was a sequel of this aggressive behaviour or bad management during copulatory act. As a result, adhesions may occur after mating and sometimes after conception. It is interesting to know that the authors have recorded 4 cases of vaginal adhesions in female dromedaries at the time of parturition (Unpublished data).

The severe degree of adhesions was the most frequently encountered type in this study. Adhesion formation and adhesion-free re-endothelialisation are alternative pathways. Both begin with coagulation, which initiates a cascade of events resulting in the buildup of a fibrin gel matrix which, if not removed, serves as the progenitor to adhesions by forming a band or bridge (Holmdahl *et al*, 1997). The degree of adhesions intensifies with time. All the animals in this study had been infertile for more than 24 months, which may explain the prevalence of the severe degree of adhesions found here. It is worthy to note that these adhesions were highly vascular and could have been life-threatening if handled roughly (Irkorucu *et al*, 2009). The treatment of these kinds of adhesions is ineffective and not recommended.

Dystocia has been considered as a contributing factor for the occurrence of adhesions in female camels (Ali *et al*, 2010a and Tibary *et al*, 2006) and

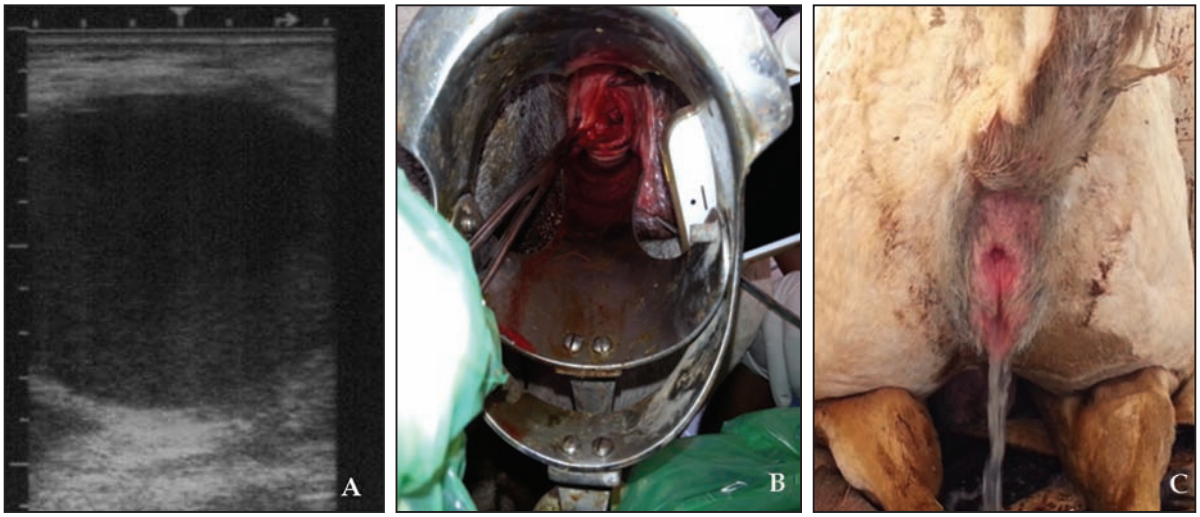

Fig 1. Sonogram of fluid-filled uterus (A); vaginal examination to monitor adhesions (B) and manual dehiscence of adhesions and evacuation of the uterus (C) in female dromedaries affected with adhesions of the vagina.

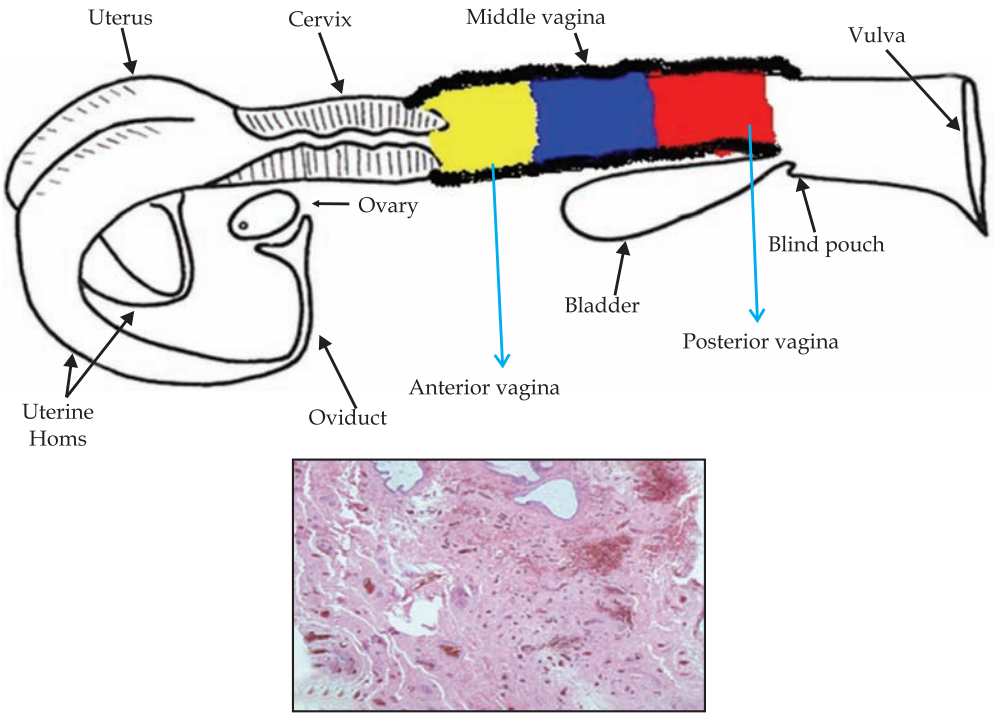

Fig 2. Histopathology of vaginal adhesions showing fragment of congested fibrovasular tissue, no epithelial covering, congested fibrous connective tissue, oedema and vascular proliferation (H&E).

in mares (Ammary, 2013). More than one third of the animals with vaginal adhesions had a history of difficult parturition. Herdsmen tend to help their animals unnecessarily during parturition. Furthermore, some ethno-veterinary practices present a dilemma in camel husbandry and are implicated in the development of adhesions, especially cervical ones. Non-licensed veterinary practitioners resort to placing some odd materials (dates, margarine and other biological matter) inside the cervix and uterus or to cutting part of the vaginal folds in order to treat infertility, and these practices may additionally predispose the animal to adhesions. It is worth mentioning that quite a large number of the affected animals in this study originated from the same herd, were reared in the same area with the same quality of nutrition and were frequently served by the same male.

It appears that treatment of the majority of cases of vaginal and cervical adhesions is not promising and frequently unrewarding due to recurrence of adhesion formation after treatment. In addition,

the loss of the vaginal vault tubularity makes the manual reconstruction of its former shape difficult. Similar results were seen in mares after trials to treat vaginal and cervical adhesions (Ammary, 2013). The main treatment strategy for adhesions is to break them down and prevent their recurrence. However, this strategy probably depends mainly on long-term follow-up with manual breakage/anti-adhesive materials or the involvement of adhesion barriers. Recently, phosphatidylcholin and tissue plasminogen activators have been used to minimise adhesions in rats (Irkorucu *et al*, 2009) and rabbits (Jin *et al*, 2013) with fairly good results. Until these techniques are practiced in large animal medicine, the fact remains that the treatment of adhesions is frustrating and the prognosis seems to be very poor. On the other hand, cervical stenosis in the absence of fibrous adhesions responded well to treatment with PGF2α.

It was concluded that vaginal and cervical adhesions, characterised by occlusion of the genital tract, refused mating, and accumulation of fluid in the uterus, constitute a serious, long-standing reproductive problem in dromedaries, and thus result in a high culling rate.

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DOI : 10.5958/2277-8934.2016.00031.X Vol 23 No 1, p 185-186

DYSTOCIA IN SHE CAMEL AND ITS CORRECTION WITH PERCUTANEOUS FOETOTOMY - A CASE REPORT

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ABSTRACT

A case of dystocia in a Bikaneri she camel with foetal presentation of dorsal deviation of neck and bilateral flexion of both fore limbs at shoulder joint. Dystocia was relieved by performing percutaneous foetotomy of neck and both legs using thygesons foetotome. Remaining foetus was delivered by traction through the birth canal lubricated with liquid paraffin. The post-operative administration of antibiotics, anti-inflammatory, multi-vitamin and fluid therapy was followed and the animal was discharged in healthy condition.

Key words: Camel, dystocia, foetotomy

The incidence of dystocia is low in camels (Arthur and Al-Rahim, 1982; Tibary and Anouassi, 1997). Dystocia of foetal origin (foetal maldisposition) is more common in camels as compared to maternal dystocia (Purohit *et al,* 2011; Purohit, 2012). Because of the exceptionally long neck and extremities when dystocia occurs, it is difficult to manage (Purohit, 2012) and flexions of the limbs and or neck deviations are the common causes (Van Straten, 2000; Purohit *et al,* 2011). Foetopelvic disproportion and monstrosities were considered rare in camel (Arthur *et al,* 1999). It is estimated that approximately 5% of all camelid births will require some assistance and ~2% will require advanced obstetrical expertise (Tibary *et al,* 2008). Manual correction of flexion and deviation is possible within 12 h of 2nd stage of labour (Purohit, 2012). Partial foetotomy of head and limbs is possible in camels using a thygesons fetotome used in cattle (Purohit *et al,* 2011; Purohit, 2012). When camels are presented beyond 48 h of 2nd stage of labour, foetotomies are considered less rewarding (Purohit *et al,* 2011). In this case report percutaneous foetotomy of neck and both fore limbs are described.

Case history

A 8 year old female camel in its 2nd parity was presented to the Department of Veterinary Gynaecology and Obstetrics with a history of dystocia for 12 hours at the time of presentation. Both the water bags had ruptured and no foetal extremities were protruding through the vulva. The animal was alert and active. The rectal temperature of the animal recorded was 97.9°F and the respiration rate was 12 per min.

Correction of dystocia

The animal was sedated by intravenous administration of 140 mg of xylazine. Per vaginal examination revealed that the foetus was in anterior presentation with dorso-sacral position and neck was deviated in dorsal side and both fore limbs were flexed at the shoulder joint. Foetus was dead and foetal extremities were more cranially deviated. It was decided to correct the dystocia through percutaneous foetotomy using thygesons fetotome (Fig 1). Foetotomy of neck was done followed by amputation of legs at shoulder joint one by one. A krey-schottler obstetrical hook was inserted in the birth canal and placed at the remaining part of the neck near thorax of the foetus. Traction was applied on the krey-schottler obstetrical hook after adequate lubrication with liquid paraffin (Fig 2) and the foetus was delivered (Fig 3). She camel was treated with antibiotics (ceftriaxone), multivitamin, NSAID and fluid therapy (4 litres of 5% dextrose, 400 ml of calcium borogluconate and 2 litres of Ringer's lactate). Antibiotics were also administered intrauterine. An uneventful recovery was observed.

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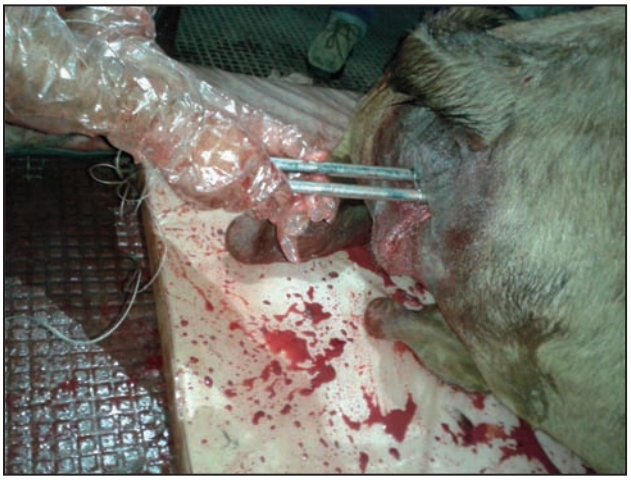

Fig 1. Partial percutaneous foetotomy of head and both fore limbs performed using thygesons fetotome in a camel.

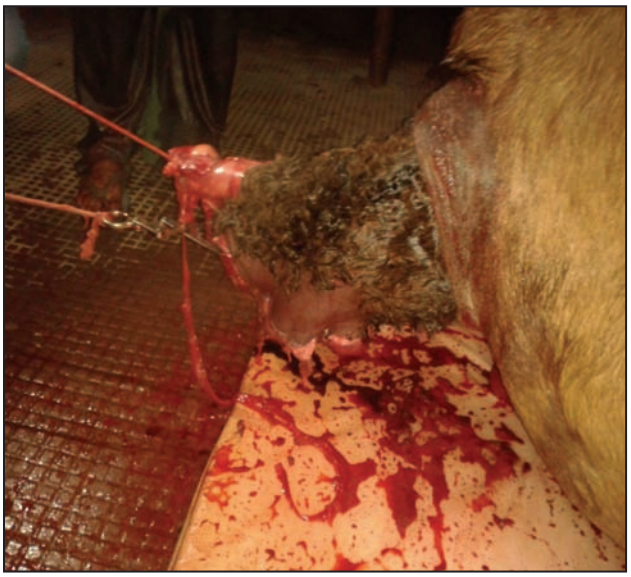

Fig 2. Application of traction on remaining part of neck by krey-schottler obstetrical hook.

Discussion

Foetotomy appears to be difficult and less rewarding in camels (Purohit, 2012) owing to the exceptionally long extremities and neck. It has been mentioned that foetotomies should be carefully performed in camels (Tibary *et al,* 2008; Purohit *et al,* 2011) as they can lead to laceration in the birth canal with resultant fatal haemorrhages. Subcutaneous foetotomy can be performed in camels with dead foetus (Kumar *et al,* 2012). More cranially placed foetal extremities may be at times beyond the reach of the clinician hand and thus difficult to correct manually. In present case, the dorsal deviation of neck and flexion of legs were severe and the neck could not be

Fig 3. Dead foetus of camel delivered after percutaneous foetotomy.

brought in the birth canal. Thus it was concluded that percutaneous foetotomy can be performed in camels with dead foetus to relieve dystocia.

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DOI : 10.5958/2277-8934.2016.00032.1 Vol 23 No 1, p 187-191

SURGICAL MANAGEMENT OF FRACTURES AND LUXATIONS OF THE TARSUS IN THE DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

Thirty eight camels were presented with tarsal lameness; 15 animals had avulsion of the tuber calcis while another 6 suffered from fracture of the fibular tarsal bone at its base. Fractures of central, second & third and fourth tarsal bones occurred in three animals. Lameness in the remaining 14 animals was due to luxation of the tarso-metatarsal, tibio-tarsal or the first inter-tarsal joints. Out of these animals, 6 avulsion fractures were treated with Steinmann's pin and tension band wiring. Four animals were treated with modified inverted L plates. Two animal were treated with cortical stcew and tension band wiring. Plate and screws were used to treat two comminuted fractures of the fibular tarsal bone.

Subluxation of the tarso-metatarsal joint (3 cases) or of the proximal inter-tarsal joint was stabilised by plate and screws. Three animals were managed conservatively. Follow-up cases showed that tarso-metatarsal subluxations had a good prognosis, while results with luxation of the tibio-tarsal joint had poor prognosis.

Key words: Dromedary camel, Fractures, Luxation, Tarsus

Tarsal injuries cause lameness and may lead to fracture of individual bones or to luxation of the joint or joints associated with the tarsus (Ramadan, 1992). Among such lesions, fractures are the most serious injury (Purohit *et al,* 1983; Gahlot and Chouhan, 1994). Apart from fractures, other causes of tarsus lameness include luxations and subluxations of the joints. Similar injuries were reported in dogs (Hickman and Walker, 1980; Campell *et al,* 1976; Phillips, 1979) and the horse (Jakovljevic *et al,* 1982; Scott, 1983; Stashak, 1987; Sullin, 2002; Butler *et al,* 2008; Bonilla and Smith, 2012). Depending on the injury involved, variety of surgical techniques have been proposed. These include open reduction followed by stabilisation using Steinmann's pins or plates and screws (Scott, 1983; Gahlot and Chouhan, 1994; Ramadan, 1994) or by fiberglass casting with or without tension band wiring (Siddiqui and Telfah, 2010). Luxation of the tibiotarsal, tarso-metatarsal joint and of inter-tarsal joints are well recognised in dogs (Campbell *et al,* 1976; Brinker *et al,* 1990), they are also reported in the horse (Butler *et al,* 2008) but they are rare in the bovine (Singh and Tayal, 2002).

The paucity of information available on the diagnosis and treatment of tarsal lameness encourage us to report the outcome of fractures and luxations involving this joint in the dromedary camels.

Materials and Methods

Record prospective for camels diagnosed with fracture or luxation of the tarsus at Veterinary Teaching Hospital over the last 15 years were examined. Inclusion in the study required that the diagnosis of fracture or luxation was made on the basis of clinical and radiological examination using latero-medial, dorsoplantar and occasionally on the latero-medial oblique views (Hassanein *et al,* 1985; Ramadan, 2014).

Results

The hospital database search revealed 38 animals that fulfilled the inclusion criteria. The distribution of lesions are given in table 1.

Clinical examination

Fractures

Fractures are caused by severe violence such as automobile accidents, injury by other animals, or trapping the leg in fence or rat-hole (Purohit *et al,* 1983).

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Table 1. Sex and age of animals affected with fractures and dislocations.

Fractures	Age years		Sex		Dislocation	Age years		Sex	
	<5	>5	M	F		<5	>5	M	F
Fibula tarsal (Avulsion)	13	2	12	3	Tibia-tarsal	0	4	3	1
Fibula tarsal (Body)	0	6	5	1	Inter-tarsal (first)	0	3	2	1
					Inter-tarsal (second)	0	0	0	0
Central tarsal	0	1	1	0	Central	0	2	2	0
Second & Third tarsal	0	1	1	0	Second & Third tarsal	0	0	0	0
Fourth tarsal	0	1	0	1	Tarso-metarasal	0	5	4	1
Total	24		24		Total	14		14	

Tarsal fractures result in severe lameness with some animals remaining in the sternal position. The diagnosis of fractures in all animals reported here was based on radiographic findings. Fracture of os-calcis occurring at or near the growth plate, was characterised by signs similar to achilles tendon rupture (Fig 1). On radiograph, a small piece was separated from the tuber of os calcis and pulled along with the tendon of the gastrocneous muscle (Fig 2). The Achilles tendon was flaccid, the tarsus showed mild swelling but the animal occasionally walked on the plantar aspect of the metatarsus region flexing the tarsus and extending the stifle joint. When the fracture occurred in the body (no=6), the swelling was prominent and crepitus sound could be heard on palpation. There were 2 comminuted and 4 simple non-displaced fractures (Fig 3).

Chip fractures of the tarsal bones were of insidious nature. The owners brought their animals for examination after a lapse of days or week after injury. The tarsus joint was associated with secondary degenerative changes.

Luxations

Luxations of the tibio-tarsal joints (n=3) and proximal inter-tarsal joints (n=3) were of sudden onset, the animals were acutely lame, they carried the limb in mild flexion of the tarsal joint. The tarsal joint was distorted and locked in semi flexion. Radiography confirmed the type and site of luxation. In luxation of the tarso-metatarsal joints (n=5) (Fig 4), the range of movement increased and lameness is less severe. There was excessive cranio-caudal motion at the joint.

Closed reduction was not successful in all tibio-tarsal luxations but those of the tarso-metatarsal and first inter-tarsal joints were treated with arthrodesis.

Management of tarsal fractures

Fibular tarsal bone fractures

Avulsion of tip of os calcis

Surgery was performed in 12 animals with os calcis avulsion fractures. The remaining 3 animals were treated conservatively (stall rest for 6 months). Before surgery, each animal was sedated with Xylaxine Hydrochloride (Ilium-Xylazil-20, Troy Laboratories, Australia) intravenously given at the dose of 0.2 mg/kg body weight. It was then anaesthetised with Ketamine Hydrochloride (Ketamil, Troy Laboratories, Australia) administered intravenously at the dose of 1 mg/kg body weight. Additional analgesics were infiltrated into the proposed site of operation. The surgery was performed via a curved skin incision at the lateral part of the bone. The Achilles tendon was isolated by blunt dissection and the displaced piece of os calcis bone was identified. It was managed by the use of reverse pinning. The pin was placed so that it emerged at the tendon of insertion of the gastrocnemius muscle. In 2 animals, a 4.5 mm cortical screw was used. This passed first through the proximal avulsed fragment, the fracture was then reduced and then the screw was driven into the distal portion of the bone (Lawson, 1963). The use of tension band wiring was used in 8 of 12 occasions. When used Steinmann pin was driven through the fragment and after reduction of the fracture in alignment, as described above, the fracture was then supported by tension band wiring.

In other fibular tarsal bone fractures, a tension band wire was used to support bone plates or the cortical screws. Fractures in 4 animals were fixed using an inverted L plate fitted on the plantar surface of the fibula tarsal bone (Fig 5). A Steinmann pin was driven through the bone fragment that was then dragged and placed on the os calcis. The plate was then secured in place using 4.5 mm cortical screws driven through the holes of the plate.

Four non-displaced fractures of the fibula tarsal bones were treated by simple reduction and supporting the tarsal joint with external support. Two comminuted fractures of the fibula tarsal bone were treated with bone plates fixed on the lateral part of the bone. Fracture of the named tarsal bones were treated conservatively.

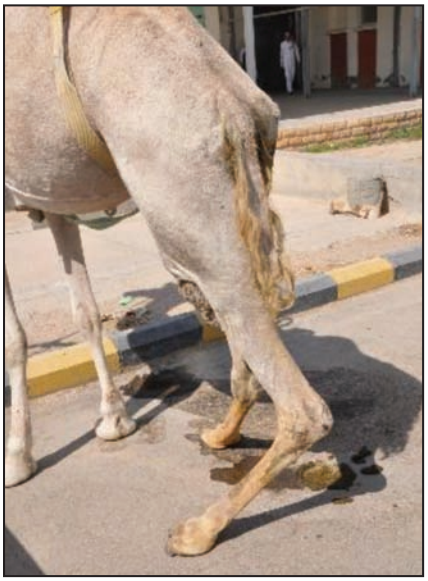

Fig 1. A camel with avulsion of os calcis in a stance similar to rupture Achilles tendon. Extended stifle and flexed tarsal.

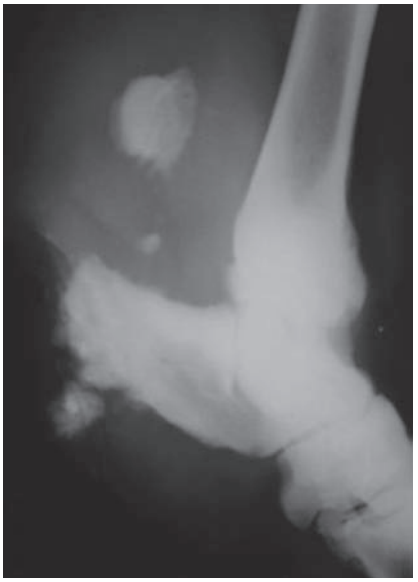

Fig 2. Lateral radiograph of the tarsus showing typical avulsion joint of os calcis.

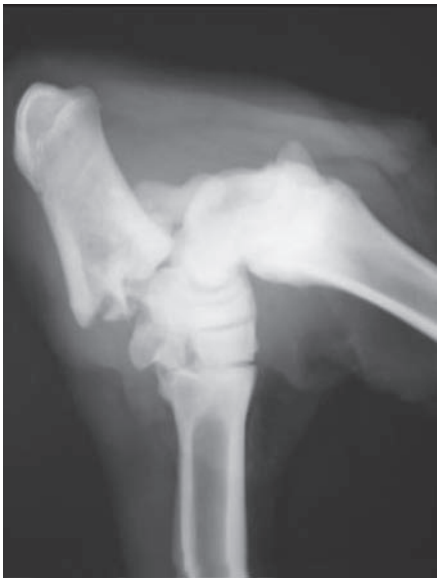

Fig 3. Lateral radiograph showing displaced fracture at the base of fibula tarsal bone.

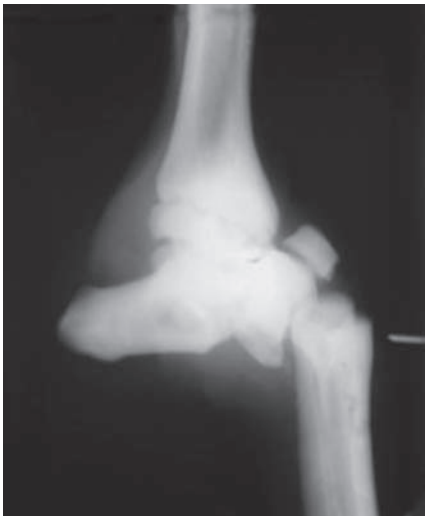

Fig 4. Lateral radiograph showing dislocation of tarsometatarsal and central tarsal bone.

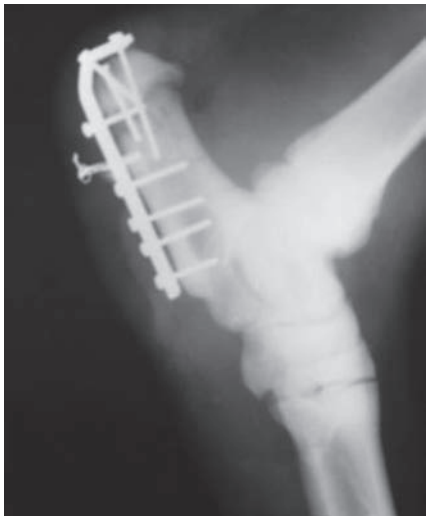

Fig 5. Lateral radiograph showing inverted L plate on plantar surface of tarsus.

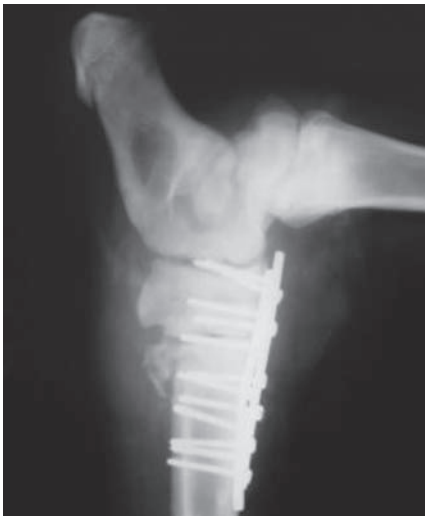

Fig 6. Lateral radiograph showing stabilisation of a tibio-tarsal luxation with plates and screws to induce arthrodesis.

Of the 21 camels with fracture of the fibular tarsal bone, 15 had physis related injuries in the regions consistent with the growth plate of the os calcis and 6 had fractures in the body either simple (n=4) or comminuted (n=2). Three animals with avulsion fractures were treated conservatively either because they were too young (2) or were older than 5 years (1). There were 2 comminuted and 4 simple non-displaced fractures. At follow-up, there was improvement of gait in those animals treated by tension band but curved plates were superior. One animal treated with Steinmann pin and tension band wiring showed rotation of the Steinmann pain and increased new bone formation after two months of surgery.

Other fractures

Fractures of the named tarsal bones were treated conservatively. Chip fractures of the tarsal bones were of insidious nature. These were associated with secondary degenerative changes and therefore were treated conservatively.

Management of tarsal luxations and dislocations

Tarso-metatarsal subluxation and dislocation of the first inter-tarsal joints were treated through

arthrodesis. The articular surface was curetted and plates were applied through cranial part or lateral part of the bone (Dowling *et al,* 2000) (Fig 6). Dislocation of the tibiotarsal joint was difficult to reduce even under general anaesthesia.

Discussion

Fracture of os calcis has a reported prevalence 2.78 and 4.73 per cent (Ramadan 1992; Gahlot and Chouhan, 1994). It is associated with automobile accident, trauma from the fences or of falling with vigorous twist. Trauma to the tarsal region usually initiated the fracture and the strenuous pull by tendon Achilles which is sufficient to displace the fragment proximally. Spontaneous avulsion or traction fracture of the os-calcis result from direct trauma or powerful contraction of the tendo-achilles during exercise, jumping or racing (Siddiqui and Telfah, 2010). The condition is seen in young camels (beyond the age of five years) and rarely in older camels beyond the age of five. If occur in either sex, however, the majority of the fractures occurred in males (Gahlot and Chouhan, 1994). The most likely explanation is that males are more active than females.

Tarsal fractures are either, simple or comminuted (Purohit *et al,* 1983; Gahlot and Chouhan, 1994; Ramadan, 1994). In cases reported here radiographical examination helped in assessment of fragment size, established that the fracture has occurred, and helped to anticipate progress of healing (Ramadan, 2014).

ln the present work, attempts have been made to compare four different techniques to augment repair of fractures including avulsion fracture of the os calcis.

Where the fracture was not displaced, external support with fiberglass cast or plaster of Paris casting proved to be a good option. Minimally displaced fractures may be left to heal without further intervention provided restricted activity can be assured (Siddiqui and Telfah, 2010). Some authors have previously successfully, considered manual reduction followed by external support (Gahlot and Chouhan, 1994). But other authors advocate the use of Steinmann pins (Purohit *et al,* 1983; Gahlot and Chouhan, 1994). Non-displaced fractures can also be treated with plates contoured to fit the surface of the bone. Avulsion fractures of os calcis are managed in a different manner. In camels, Ramadan (1994) and Siddiqui and Telfah (2010) found it necessary to perform a surgical reduction and fixation with tension band wiring techniques. Tension band wiring may be supplemented with a bone plate applied to the lateral side of os calcis. In horses, tension plates fixed on the plantar aspect of the fibular tarsal bone was superior in returning a horse after a tarsal fracture (Scott, 1983). Currently, we modified such plates to an inverted L shape and have used them in four patients. The technique gave good clinical outcome with minimum complications and should be considered in the patient with avulsion fractures.

Fracture of os calcis can be plated and plastered in pet animals and those of central tarsal received great attention since decades (Hickman and Walker, 1980; Denny, 1982).

Conservative treatment of avulsion fractures did not cause any improvement and one animal was unable to ambulate for six months. That individual occasionally kneeled to feed and drink, but the swelling subsided. The other two animals treated conservatively were aged between 1-2 months and the owner did not inform us about the response to treatment.

Fractures of the named small tarsal bones are frequently reported in racing dogs (Brinker *et al,* 1990). In addition, there are few examples of these fractures in cattle, where the fused second and third tarsal bones have torn completely free in the joint cavity (Adams and Fessler, 1974).

In the present investigation, chip fractures of the individual named tarsal bones were treated conservatively. This approach was prompted by the suggestions of Jakovljevic *et al* (1982) that surgical excision of chip fracture of fibular tarsal bone in the horse should not be attempted unless the fragments enter the tarsocrural joint. Also because chip fractures often are quite small and may not cause lameness. Slab fractures may require the placement of bone screws. In the dog fractures could be treated by fixation with cortical screws (Brinker *et al,* 1990). Even when fixation was not ideal the fractures could be treated satisfactorily by inter-tarsal ankylosis (Guillard, 2000).

The plating used in present report using a curved plate was suitably screwed into position and offered the best means of immobilising the fracture after reduction but in practice the accurate fitting of the plate to the frequently complicated contour of this bone is difficult and unrewarding task. The intramedullary pin, on the other hand, is generally inserted easily but it does not prevent rotation of the distal fragment, (Purohit *et al,* 1983; Gahlot and Chouhan, 1994).

The prognosis for luxation of the tibio-tarsal joint is poor, but open reduction and arthrodesis has been found successful in treating luxations of the tarsometatarsal and proximal inter-tarsal joint (Butler *et al*, 2008).

Acknowledgements

We are grateful to the Deanship of Scientific Research for providing a grant for this study. Thanks are due to Professor Fintan Mc Evoy Faculty of Health and Medical Science, University of Copenhagen Denmark for his help with the manuscript.

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Molecular genetics of Old World camelids

Old World camels are unique in their morphological and physiological characteristics and capable of providing vital products even under extreme environmental conditions. The evolutionary history of dromedary and Bactrian camels traces back to the middle Eocene (around 40 million years ago, mya), when the ancestors of Camelus emerged on the North American continent. While the genetic status of the two domestic species has long been established, the wild two-humped camel has only recently been recognised as a separate species, Camelus ferus, based on molecular genetic data. The demographic history established from genome drafts of Old World camels shows the independent development of the three species over the last 100,000 years with severe bottlenecks occurring during the last glacial period and in the recent past. Based on the now available whole genome drafts, specific metabolic pathways have been described shedding new light on the camels' ability to adapt to desert environments. These new data will also be at the origin for genome-wide association studies to link economically relevant phenotypes to genotypes and to conserve the diverse genetic resources in Old World camelids.

(Source: Trop Anim Health Prod. 2016; 48: 905–913)

Genetic history of camels- New Research

Nottingham University in Britain has managed to reveal the genetic history of camels. Researchers analysed genetic information from a sample of 1,083 living dromedaries from 21 countries across the world. The findings showed that these were genetically very similar, despite populations being hundreds of miles apart. Centuries of cross-continental trade caused this "blurring" of genetics, the researchers explained. Our analysis of this extensive dataset actually revealed that there is very little defined population structure in modern dromedaries. It is believed that this is a consequence of cross-continental back and forth movements along historic trading routes, said Olivier Hanotte, Professor at Nottingham University in Britain. The results point to extensive gene flow which affects all regions except East Africa where dromedary populations have remained relatively isolated.

"Selected Research on Camelid Immunology"- New Book

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

Camel Publishing House has brought out yet another important publication- "Selected Research on Camelid Immunology" edited by T.K. Gahlot, U. Wernery and Serge Muyldermans. This book is a unique compilation of research papers based on "Camelid Immunology" and published in Journal of Camel Practice and Research between 1994-2015. Research on this subject was done in 93 laboratories or institutions of 30 countries involving about 248 scientists. In terms of number of published papers in JCPR on the immunology the following countries remain in order of merit (in parenthesis), i.e. Iran (1), India and UAE (2), China and Saudi Arabia (3), Sudan (4), Kenya and Belgium (5), USA (6), Germany (7) and so on. The book contains 11 sections and is spread in 384 pages. The diverse sections are named as overview of camel immune system; determinates of innate immunity, cells, organs and tissues of immune system; antibodies; immunomodulation; histocompatibility; seroprevalence, diagnosis and immunity against bacteria, viruses, parasites and combination of other infections; application of camel immunoglobulins and applications of immune mechanisms in physiological processes. The camelid immunology has to go a long way in its future research, therefore, this reference book may prove quite useful for those interested in this subject. Book can be seen on www.camelsandcamelids.com.

(ISBN:81-903140-4-1)