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JCPR GOES TRIANNUAL

Dear Readers

The Journal of Camel Practice and Research (JCPR) has attained 24 years of age and it has grown and matured remarkably. In order to reduce the wait period of manuscripts for publication, the publisher has increased its frequency and accordingly JCPR would go now triannual from 2017 and will be published as April, August and December issue. This will give advantage to the authors by enabling early publication of their papers. The size and style will remain the same. I am sure that readers will appreciate this step of Camel Publishing House.

The year 2017 is a year of conferences on camels. A special session on "Advances in Camel Science" will be organised by me in 7th International Veterinary Congress scheduled at Paris on 5th September and a full fledged camel conference entitled, "The Belt and Road: Camel Science, Industry and Culture" will take place from 22-26 September at Alxa League, Inner Mongolia, China. Both the conferences will churn-up the emerging topics of camel science in a big way and I am sure that such congregations of camel scientists will continue in the year 2018 in the forthcoming conferences at Morocco and Dubai. Marwar Camel Culture Festival will be organised from 3-5 November 2017 at LPPS campus, Sadri, Pali District, Rajasthan, India. It will have a variety of programmes and a brain storming session on the topic "What kind of Camel Science do we need?". There will be eminent penalist for this discussion.

The April 2017 issue of JCPR has incorporated the diverse spectrum of papers but noteworthy are three review papers, i.e. the one humped camel in Uganda, Bioactive properties of minor camel milk ingredients and pneumonia in dromedary camels. Another milestone paper is based on immunisation of dromedaries with live attenuated *Brucella melitensis* Rev 1 vaccine authored by Dr.U.Wernery and others from CVRL, Dubai. A good number of papers on pathology include studies on oversized follicles, kidney affections, renal cell carcinoma, uterine leiomyoma, pathogenic bacterial strains of raw camel milk and squamous cell carcinoma. Additionally, it has manuscripts on gross and imaging studies on stifle joint, identification and molecular cloning of heat shock proten-70, resistotyping of camel skin wounds associated *S. aureus*, sequence analysis and phylogenetic relationship of myostatin gene of Bikaneri camel, oesophagoscopy and endoscopic aided removal of oesophageal foreign body in camels.

I am sure that readers would like the first edition (April issue JCPR) of the year 2017 more informative and I assure you that by making it triannual we have increased the capacity of JCPR to accommodate more manuscripts in every issue.

With Best wishes

(Dr. T.K. Gahlot)
Editor

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FOR

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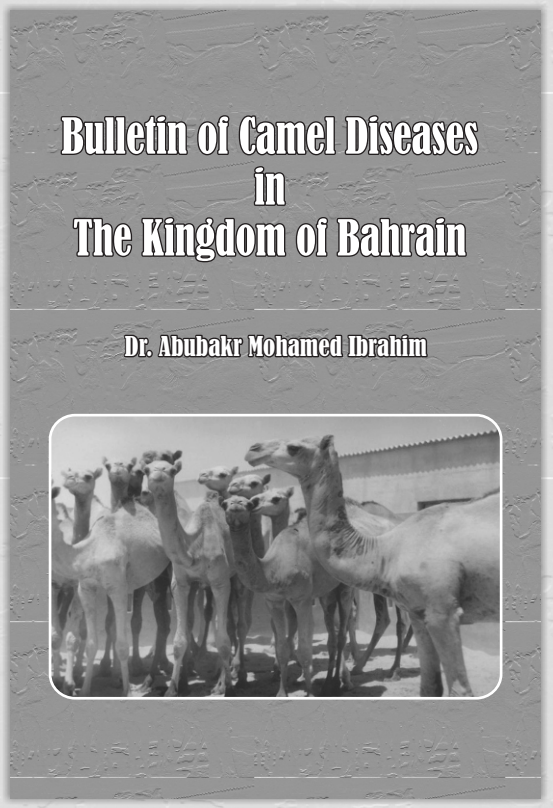
Bulletin of Camel Diseases in The Kingdom of Bahrain

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

About the Author

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



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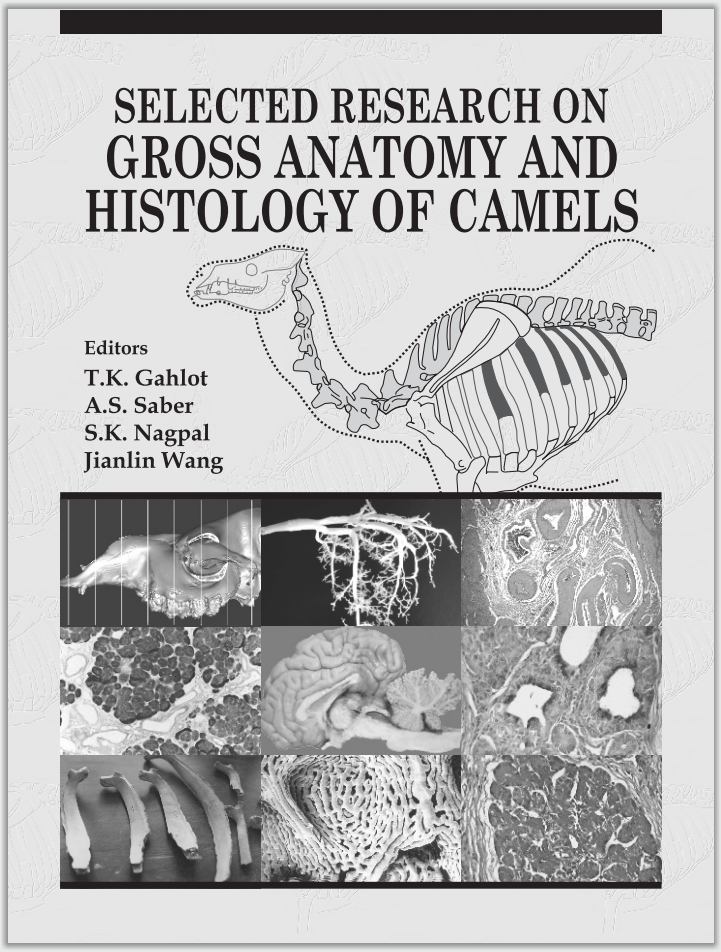
SELECTED RESEARCH ON
GROSS ANATOMY AND HISTOLOGY OF CAMELS

Hard bound, 452 pages, few figures coloured

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



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THE ONE-HUMPED CAMEL IN UGANDA

R. Trevor Wilson
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ABSTRACT

This paper provides the first comprehensive account of the presence of camels and of camel production in Uganda. Uganda is a poor East African landlocked country. Agriculture's contribution to GDP has shrunk by 23.5 per cent in the last 30 years whilst that of the service sector has increased. Commercial and food crops are the major subsectors of agriculture but livestock add almost 10 per cent to agricultural GDP. Goats are numerically the most important quadruped livestock, followed by cattle, sheep, pigs and donkeys: there are very few horses. The one-humped camel is not part of the traditional array of domestic livestock but the species started to appear in the arid northeast of the country during the 1960s/1970s. In 2008 the national camel population was about 31 000 but in 2017 may be as high as or more than 40 000. Traditional pastoral tribes have become camel keepers for the production of milk, some meat, some transport and for medical uses. Internal parasites and trypanosomes are a problem for camel health and welfare. Feed supplies in the area of camel keeping comprise many of the camel's preferred browse species. Although not a traditional species and few in number camels have the potential to contribute to food security and to the livelihoods of pastoralists in some of the remote parts of Uganda.

Key words: Animal diseases, feed resources, introductions, livestock ownership, livestock products

The Republic of Uganda is a landlocked country in East Africa. It is bordered to the east by Kenya, to the north by South Sudan, to the west by the Democratic Republic of the Congo, to the south-west by Rwanda and to the south by Tanzania. The country lies on the East African Plateau between latitudes 4°N and 2°S and longitudes 29°E and 35°E. The average altitude is about 1,100 metres (3,609 ft) above sea level. Uganda has an area of 236 040 km^2 – of which a rather large proportion comprises several lakes – and was home to 34.9 million people in 2014. For administrative purposes Uganda is divided into regions, sub-regions, districts, counties, sub-counties and parishes. The country is classified in international systems as being of low income with a Gross Domestic Product (GDP) per person per year of USD 672 in 2015 (CIA, 2016; UNSD, 2016; Nakayima *et al,* 2016).

Favourable soil conditions, good rainfall over much of the country and temperatures moderated by altitude allow continuous cultivation of perennial crops in the south and annual cropping of mainly subsistence crops in the north. The driest northeastern corner of the country supports only pastoralism. Agricultural products account for nearly all of Uganda's foreign exchange earnings and coffee alone accounts for about 25 per cent of the country's exports. Coffee, cotton and tea are the main commercial crops. Maize, plantains/bananas, cassava, beans, groundnuts, sweet potatoes, sorghum and millet are the main food crops in terms of area but plantains/bananas and cassava provide more than half of total food production. In 1980 agriculture contributed 70 per cent of the country's Gross Domestic Product but this declined to 23 per cent in 2011 as the service sector expanded to contribute 51 per cent of GDP (ADB, 2014). Agriculture, nonetheless, provided direct employment to 8.8 million people equivalent to 66 per cent of the national work force in 2011 and in total 19.3 million persons in 3.95 million households were directly supported by agriculture (MAAIF, 2011). The number of households owning livestock in 2008 was 4.5 million, this figure exceeding the number of "agricultural households" as many landless and urban people keep some livestock (MAAIF, 2009). Livestock contributed 9.1 per cent of total agricultural GDP or about 1.7 per cent of total GDP in 2011. Livestock numbers in 2008 were estimated at 12.45 million goats, 11.4 million cattle, 3.4 million sheep, 3.2 million pigs, 0.15 million donkeys, 32 870 camels and 1 590 horses: in addition there were 27.4 million poultry (MAAIF, 2009).

This paper is the first comprehensive account of camels and camel production in Uganda.

History of introductions

The only record of camels in Uganda in historical accounts is that of three baggage animals

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used by Lieutenant R. G. T. Bright in 1898 in an expedition from Uganda to Abyssinia (now Ethiopia) (Sharf, 2005). Nothing is known of the provenance of these camels nor of their subsequent fate. In more recent times the Ministry of Agriculture, Animal Industries and Fisheries has not been able to provide information on the dates that camels were introduced to Uganda (Ministry Official to Jesca Nakayima, Pers. Comm.). Anecdotal evidence from pastoralists indicates, however, a likely date in the late 1960s or early 1970s when members of the Pokot tribe living in Kenya brought in animals when visiting Pokot relatives in Uganda. The Matheniko tribal community obtained camels from Uganda Pokot and also from Turkana pastoralists who crossed from Kenya to Uganda particularly during drought periods.

The Ugandan president did not receive – or perhaps refused to accept – camels from the late President Gaddafi of Libya, in contradistinction to many of his peers in other African countries (Wilson, 2013; 2014).

Numbers, distribution and ownership

In 2008 there was an estimated 32 870 camels in Uganda (MAAIF, 2009). This number may have increased to over 41 000 in 2017. It needs to be realized, however, that cross-border movements mean that numbers are likely to be in a constant state of flux.

In 2008 Karamoja sub-region in northeastern Uganda (Fig 1) had the highest estimated number of camels at 32 030, equivalent to 97.4 per cent of all Uganda's camels: the sub-region was also home to 91.3 per cent of all national donkeys, 60.4 per cent of horses, 20.0 per cent of cattle and 16.3 per cent of goats. Within Karamoja, Nakapiripirit and Moroto were the districts with the most camels (MAAIF, 2009).[1] The climate in Karamoja is generally harsh with high rainfall variability and high evapotranspiration. Rainfall has historically been in the range 350-1000 mm per annum, with the lower end of the spectrum in the east. Precipitation is usually sporadic and falls in one rainy season. The main problem with the rainfall is its distribution rather than the total amount. The intensity and the variability, particularly the existence of sporadic intense wet periods followed by drought events, have always had debilitating impacts on the area (Egeru *et al,* 2014a). Daily temperatures exceed 30° C for most of the year and are often in excess of 40° C. Over the long term total rainfall has declined and temperatures have increased (Egeru *et al,* 2014b). In short, the local environment is more propitious to the camel than it is to other species of domestic livestock.

Tribal groups that own camels are Pokot (also known as Suk) in Amudat district and Matheniko in Moroto district, especially in Katikekile and Tapac sub-counties. The Pokot are only distantly related to the dominant ethnic Karamojong and are the most pastoral section of the Kalenjin cultural group. The Pokot extend across the border into Kenya where camels have been reared for much longer. The Kenya Pokot became camel herders via interactions with traditional camel-owning tribes such as the Samburu who inhabit a much drier part of Kenya. Interactions between the Pokot subgroups of Uganda and of Kenya led to the introduction of camels to Uganda.

The average herd size in Karamoja is 11.3 head with a median of 7.5 head, the lower median suggesting that there are some very large herds. Some herds do indeed comprise 30 to 50 camels (Nampala, 2013). Herds are slightly smaller in other areas (MAAIF, 2009). For grazing purposes the herds of several owners may be combined and herded together.

Products

The traditional pastoralist mode of production is not one of commodities as it not primarily aimed at producing for the market. The standard outputs of milk and meat are mainly for home consumption. Herd accumulation is a vital economic function not only for cash but for traditional values in the context of the extended family (being able to loan out animals), as bride price and for prestige within the community. Camel owners therefore tend to be asset rich – adult animals when sold may make 3.5 million Uganda shillings (almost 1000 US dollars) whereas a cow is worth 1.1 million Uganda shillings (300 US dollars) – whilst remaining cash poor.

Mature camels weigh up to 600 kg. Sexual maturity is achieved at 3-4 years and calves are then born at 18-month intervals. Camels may live for up to 30 years. Percentage mortality is much less than in other classes of domestic stock.

The camel value chain includes milk, meat, hides, transport and medicines. Most production is for home consumption but there is limited commercial trade in milk and meat. Milking is done

1. Nakapiripirit District has now had its eastern part excised to form Amudat District and this area, bordering on Kenya, now has the greatest number of camels.

by hand direct into containers (most often plastic) by both men and women who, because of the size of the camel, are able to stand during the process (Fig 2). Camel calves are given access to their dams to start the let-down process. Some milk is sold outside the immediate and extended family and is collected by traders in 25-litre metal containers (Fig 2). In times of plenty surplus milk is transformed to butter, which has a longer storage life than milk; this value-added product is destined for home consumption or for sale on the local market (Fig 2). Local herders claim that they milk lactating camels up to five times daily and obtain as much as 5 litres per milking.

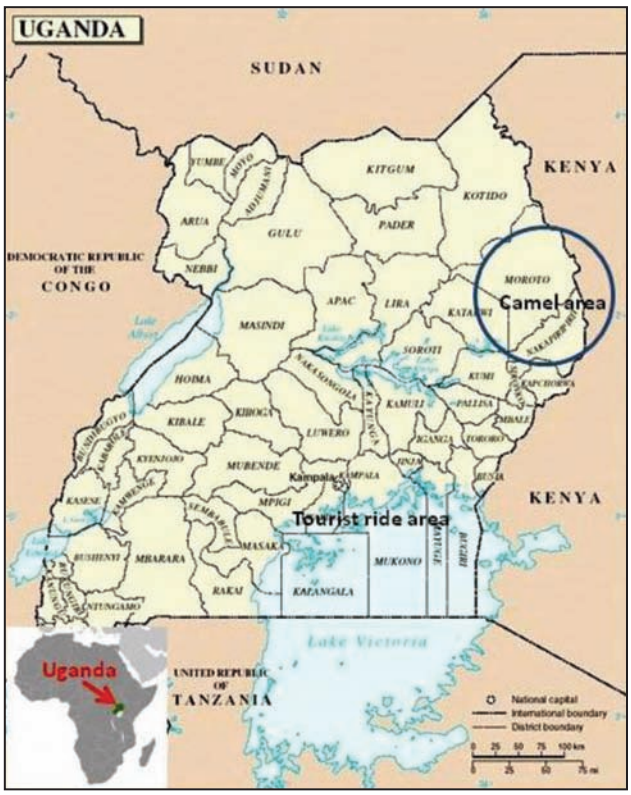

Fig 1. Area of camel herding in northeast Uganda and principal area where camels are used for tourist rides.

Most slaughtering for meat is done at the home site but occasionally an animal is sent to an abattoir. In addition to home consumption, there is an active trade in camel meat, mainly via traders of Somali origin who buy from camel owners and transport meat to the main urban areas (Fig 3). On the Kampala retail market 1 kg of camel meat is sold for 17 000 Uganda shillings (4.72 US dollars) compared to 13 000 Uganda shillings (3.6 US dollars) for beef. A recently opened camel abattoir in Kampala is testament to the development and increasing recognition of camel meat as a desirable product in the diet of the urban population.

Camels are not normally hired out to other parties by the owners for transport or agricultural purposes. They are, however, used as transport animals by the owners when the camp is moved. The use of camels for leisure as a riding animal has become increasingly popular especially in urban areas and at tourist hotels on the shores of Lake Victoria around Entebbe and other resort areas (Fig 4). Camels are also being exhibited at local carnivals and other festivities as an attraction.

In addition to providing more conventional products, the urine of this mammal is considered by some people as important in curing certain diseases, including HIV/AIDS. According to Idriss Shaban, a camel urine seller "This urine, you use three times every day, in the morning, noon and night. If symptoms persist, you must use it for four months without missing using same prescription. You then visit a doctor. If you still feel pain in that month, don't worry it will vanish." The World

Fig 2. Pokot woman assisted by her daughter milking a camel, milk being bought by a trader and butter as a value-added product for sale.

Fig 3. Meat trader's pick-up truck and logo and undifferentiated camel meat for retail sale.

Fig 4. Camel being ridden as a leisure activity

Health Organisation has urged people to refrain from drinking camel urine. It says the urine has been proven to cause the Middle East Respiratory Syndrome, a viral respiratory disease which can prove fatal. Desperate patients, however, are willing to take the risk in order to be healed. The Ugandan government has made no follow up concerning the health benefits of camel urine and will only take action if it is scientifically proven as a cure. In the meantime it remains a hope for many who overlook the risks (Africa News, 2016).

Welfare and disease

The generally good body condition of most camels and the presence of many calves and young stock in the herds are indicative of no major welfare problems (Fig 5).

Mange (referred to locally as 'emitina') is seen by the owners as the major camel health problem. A recent study involving 82 camels from Moroto and Amudat Districts was the first in Uganda to establish the parasitic worm burdens of camels. It was found that 48 camels (58.5 per cent) were infected with Strongyle eggs at a level of 1056 eggs per gram of faeces. Cestodes of the family Anoplocephalidae were found in 15 camels (18.3 per cent). The lungworm *Dictyocaulus cameli* was recorded in 24 camels (29.3 per cent) but at a very low level of infection of 1 worm per case. The coccidian *Eimeria cameli* was found in 9 camels (11.0 per cent) with a mean count of 34. Infections with one parasite species were found in 22 camels (26.8 per cent), with two parasite species in 24 camels (29.3 per cent) and with three species in 7 camels (8.5 per cent) (Nakayima *et al,* 2017).

A sample of 112 camels from Moroto District was examined for *Trypanosoma evansi* infection. The Micro Haematocrit Centrifuge (MHCT) technique was used for parasite diagnosis. Suratex® was used to detect the presence of trypanosome antigens and Enzyme-Linked Immuosorbent Assay (ELISA) was used to detect anti-trypanosomal antibodies. Parasite prevalence ranged from 0 per cent to 47 per cent in camels from three different herds, Suratex® showed positivity in the range 35-65 per cent and ELISA high antibody presence. Low haematocrit values were associated with presence of parasites and antigen-positive animals. This is the first report of *T. evansi* infection in camels in Uganda and shows that camels could be of consequence in the epidemiology of the parasite in the country (Olahu-Mukani *et al,* 1998).

Feed resources

Camel feed resources derive from four major vegetation communities: woodland; bushland; grassland; and, farmland. There is great species diversity in woodlands and bushlands, moderate diversity in grasslands and little diversity in the farmlands (Salamula *et al,* 2016). Camels are predominantly browsers and because of their size are able to procure feed from heights of up to 4 metres above the ground on resources that are not available to other domestic stock. They are, however, eclectic in their tastes and feed on a broad spectrum of fodder plants that includes thorny trees and shrubs, halophytes and aromatic species that

Fig 5. Mixed age groups of camels in night compounds constructed of thorn bushes.

Fig 6. Typical wet and dry season feed conditions for camels in Karamoja sub-region.

may be avoided by other domestic herbivores. At times, nonetheless, they compete with these latter for other types of feed including grasses and herbaceous legumes. Camels employ various feeding strategies in Karamoja, depending on the season and the available resources, using the herbaceous layer of mainly annual species in the shorter rainy season and the browse layer of perennial plants in the longer dry season (Fig 6).

In a recent study the commonest browse species recorded were *Acacia brevispica, A. nilotica, A. senegal, A. seyal, A. tortilis, A. sieberiana, Balanites aegyptiaca, Opuntia cochenillifera, Commiphora africana, Dicrostachys cinerea, Euphorbia candelabrum, Grewia mollis, Maytenus undata, Rhus natalensis, R. vulgaris, Terminalia brownii, Zanthoxylum chalybeum* and *Lannea* sp. (Salamula *et al,* 2016). Discussions with camel herders allowed the identification of preferred species (Table 1), some of which such as Euphorbiaceae and *Tribulus terrestris* were rather surprising.

Discussion

Pastoralists such as the Pokot and Matheniko have battled for centuries with adverse weather conditions and have often been more successful in adapting to changing situations than sedentary populations as they can be much more flexible in the face of changing conditions. There has, however, been widespread environmental damage from deforestation and overgrazing in recent times. Some of this damage results from increases in both human and animal populations that themselves derive from better medical care and reduced mortality. The addition of camels to the traditional domestic livestock array of the peoples of Karamoja sub-region is a logical strategy to reduce risk.

Camels have become an important part of the livelihoods of the Pokot and Matheniko but their presence in Uganda has gone largely unnoticed. Their numbers are not insignificant and they have been present for at least 50 years but the international

Table 1. Plant species indicated by local herders as preferred camel fodder in Karamoja sub-region.

Vernacular (Pokot) name	*Scientific name*
Esuguru	*Tribulus terrestris*
Eligoi/Ekilala	*Euphorbia tirucalli*
Ekorete	*Balanites aegyptiaca*
Echogorom	*Capparis* sp.
Edapal	*Opuntia cochenillifera*
Emekui	*Baleria acanthoides*
Erereng	*Cadaba farinosa*
Ekadeluae	*Capparis tomentosa*
Ekodiokodioi	*Acacia senegal*
Eregai	*A. mellifera*
Eminit	*A. tortillis*
Ekapelimen	*A. nilotica*
Amugit	*Lagenaria siceraria*
Ekaleruk	*Cucumis* sp.
Etopojo	*Lannea discolor*
Ekadeli	*Commiphora africana*

Source: Salamula *et al,* 2016

organisation charged with enumerating the world's livestock by country and species has no mention of them in its data base (FAO, 2014).

The camel lungworm *Dictyocaulus cameli* is a valid taxon but has rarely been recorded. It has been found in Iran (Ebrahimi *et al*, 2012) but the report of its presence in Uganda in this paper appears to be a first for Africa. Other diseases will certainly be found in Uganda camels in the future. For example, mastitis caused by a variety of organisms including *Staphylococcus aureus, S. epidermis, Escherichia coli, Streptococcus agalactiae* and species of *Micrococcus* and *Pseudomonas* has recently been recorded from West Pokot County in Kenya which neighbours on Nakapiripirit, Moroto and Amudat Districts (Toroitich *et al*, 2017). It is inconceivable that these organisms are not present in Uganda camels in view of the frequent interchanges across the national boundary.

Camels are better adapted to survival in areas with harsh climatic conditions than "conventional" domestic livestock species. As such the species has the potential to support the livelihoods and improve the resilience of the pastoral communities of the Karamoja sub-region and are likely to be an extremely important source of food and of improved welfare for local pastoralists.

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I am grateful to Jesca Nakayima for initial help and facilitating my contact with Jeremy Lomonyang who undertook field work on my behalf. Anthony Eguru was also extremely helpful in locating information and sharing it with me: he also read an earlier draft of this paper and made useful comments for its improvement. The Pokot camel owners, Lopongo Lokuso and Awaket Tapem, greatly assisted the production of this paper by providing information on their animals and management system. Figures 2 and 3 and the left hand part of Figure 6 were kindly provided by Anthony Eguru. Edwina Ahamize kindly allowed the use of Figure 4. Figure 5 and the right hand part of Figure 6 were produced especially for this paper.

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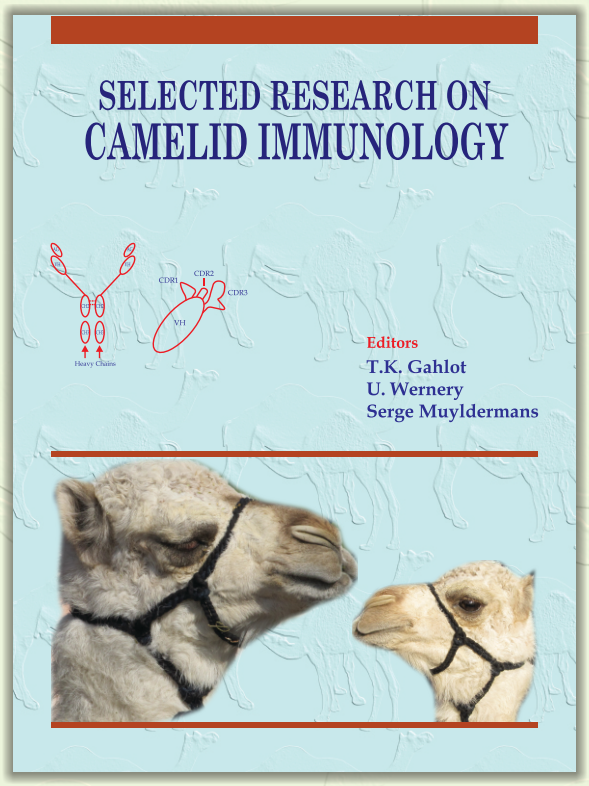
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

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

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



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LABORATORY INVESTIGATIONS AFTER EYE DROP IMMUNISATION OF DROMEDARIES WITH LIVE ATTENUATED *Brucella melitensis* REV 1 VACCINE

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ABSTRACT

The present study describes the laboratory investigations after a single right eye drop (3.1×10^9 CFU live bacteria) immunisation of 6 dromedary camels (*Camelus dromedarius*) with live attenuated *B. melitensis* Rev 1 vaccine. The experiment was conducted over a period of 5 months. The vaccine strain was isolated for 16 days from only the right eye of the vaccinated dromedaries, but not from the left eye and both nostrils. Similar pattern of results was obtained by polymerase chain reaction. It was negative for the left eye, both nostrils (except for one dromedary) and for EDTA blood and serum. All vaccinated dromedaries seroconverted from day 16 after vaccination until 4 months shown by Rose-Bengal test and slide-agglutination test. No serological reactions were found after 5 months. The complement fixation test remained negative throughout the experiment. Information about the vaccination against brucellosis in camels, the within host disperse of the vaccine strain and the serological response are scarce. The experiment provided basic data about the feasibility of Brucevac conjunctival vaccine in camels. However, to prove if the immunised dromedaries acquired a lifelong immunity against brucellosis, pregnant vaccinated dromedaries need to be challenged with a field *B. melitensis* strain. We also recommend changing the conjunctival vaccination route to subcutaneous or intramuscular to prevent accidental infection due to *B. melitensis* vaccine strain excreted by lacrimation.

Key words: *Brucella melitensis* Rev 1, dromedary brucellosis, eye drop vaccination

Brucellosis remains wide spread in domestic and wild animal populations and presents a great economic burden for tropical animal husbandry (Seifert, 1992). It is also one of the most important zoonosis in developing countries with more than 500,000 new cases annually worldwide (WHO/FAO, 1986). Infection prevalence in animal reservoirs determines the incidence of human cases (Von Hieber, 2010). Old World camels are frequently infected with brucellosis especially, with *Brucella (B.) melitensis*, particularly when they are in contact with infected small ruminants (Wernery, 2014). The disease is rare in new world camels but outbreaks with classical signs of brucellosis have been described (Fowler, 2010).

Serious efforts have been made to prevent the infection through the use of vaccines. In old world camels, both inactivated and attenuated *Brucella* vaccines have been used successfully with both *B. abortus* strain S19 (Agab *et al*, 1995) and with *B. melitensis* (Radwan *et al*, 1995). However, so far no challenge infections have been performed in pregnant vaccinated dromedaries (*Camelus dromedarius*).

We herewith, describe laboratory investigations after single eye drop immunisation of 6 dromedaries with a live attenuated *B. melitensis* Rev 1 vaccine.

Materials and Methods

Eight dromedaries were selected for this study of which 6 were immunised and two were kept as control/contact animals. The camels were kept in 2 outdoor pens of the Central Veterinary Research Laboratory (CVRL, Dubai) in shaded areas, with 4 camels in each pen. The dromedaries were of different gender and age (Table 1) and received daily alfalfa hay *ad libitum* and 2 kg of concentrate per animal.

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All dromedaries had free access to water. None of the female dromedaries was pregnant. The welfare of all experimental animals and treatment of them conducted by CVRL were reviewed and approved by the Animal Ethic Committee of CVRL and Ministry of Climate Change and Environment of the United Arab Emirates (permit number: 550353).

Table 1. Dromedaries vaccinated with *B. melitensis* Rev 1.

Camel ID	Gender	Age in Years	Trial Category
CA-1	Female	16	Control
CA-2	Female	12	Vaccinated
CA-3	Female	18	Vaccinated
CA-4	Female	14	Vaccinated
CA-5	Male	9	Control
CA-6	Male	10	Vaccinated
CA-7	Male	11	Vaccinated
CA-8	Female	10	Vaccinated

The vaccine used in the study was 'Brucevac', a freeze dried conjunctival live attenuated *Brucella melitensis* strain Rev 1 developed by JOVAC (Jordan Bioindustries Limited, Jordan). It has a titre of 3.1 × 10^9 colony forming unit (CFU) of live attenuated *Brucella melitensis* strain Rev 1 per drop. The recommended dosage is one drop per animal. The vaccine was stored refrigerated and reconstituted as per manufacturer's instructions prior to use.

Each of the 6 selected experimental dromedaries (Table 1) received a single dose of the eye drop vaccine into the right conjunctival sac. One drop consisted of approximately 40 µl. Prior to immunisation, swabs were collected from all the 8 dromedaries and thereafter on 2nd, 4th, 10th, 16th and 24th day post immunisation. Right eye, left eye, right nostril and left nostril of all 8 dromedaries were swabbed using separate sterile cotton tipped swabs. After collection, swabs were immediately placed into 100 µl of tryptic soy broth Tryptic Soy Broth (TSB), (Merck 1.05459.05000) with *Brucella* selective supplements (Oxoid SR0083A). Blood was collected in EDTA tubes before and after immunisation (on 2nd, 4th and 10th day). Serum samples were collected on 2nd, 4th, 10th, 16th, 24th day post immunisation and thereafter, monthly for 5 months from all 8 camels. EDTA blood samples were directly frozen at -80°C. The swabs and serum samples were processed on the same day of collection and were stored at -80°C.

All swabs were streaked onto 2 selective and a non-selective media: Farrell's media (Oxoid CM0169) Brain Heart Infusion agar (Oxoid CM1135 with 1% added agar) with *Brucella* selective supplements (Oxoid SR0083A) and Tryptic Soy agar (Merck 1.05459.05000 with 1.5% added agar). All plates were incubated for 12 days at 37°C in 5% CO_2 atmosphere. After 12 days the plates were examined for the presence of *Brucella* bacteria and suspicious colonies counted/graded and recorded. The suspicious colonies were preliminarily identified as *Brucella* sp. by their growth characteristics on selective agars, Gram reaction, catalase and oxidase tests. The sera were tested for *Brucella* antibodies using the OIE described test procedures; complement fixation test (CFT), Rose Bengal test (RBT) and serum agglutination test (SAT) (World Organisation for Animal Health, 2016). RBT antigen, CFT antigen and SAT antigen were purchased from Animal Health and Plant Agency, Weybridge, UK. A serum containing 30 or more IU per ml was considered to be positive in SAT (World Organisation for Animal Health, 2016). DNA was extracted from the samples with the QIAamp DNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The samples were examined for the presence of *Brucella* species with the qPCR assay targeting the bcsp31 gene using the primers bcsp31F (5'-GCT CGG TTG CCA ATA TCA ATG C-3') and bcsp31R (5'-GGG TAA AGC GTC GCC AGA AG-3'), and the probe bcsp31P (5'-FAM-AAA TCT TCC ACC TTG CCC TTG CCA TCA-BHQ1-3') (Probert *et al*, 2004). The reference strain *B. suis* biovar 2 Thomsen (ATCC 23445) was used as positive control during the examinations. All samples were run in duplicate.

Results

Detailed culture, PCR and serological results are shown in tables 2 and 3 after eye drop immunisation with a commercial *B. melitensis* Rev 1 live vaccine in dromedaries. All swab and blood samples were negative for *Brucella* by culture, PCR assays and serological tests before vaccination. Also the 2 negative control animals in pens remained negative throughout the experiment.

B. melitensis bacteria grew on all 3 culture media from the right eye from all vaccinated dromedaries from 2nd day onwards until 10th (5 animals) to 16th (animal ID: CA3) day post immunisation (p.i.). Swabs of the left eye and both nostrils remained culture negative throughout the experiment.

Swabs of the right eye examined by PCR tests became positive from 2nd day onwards until 10th (5 animals) to 16th (animal ID: CA3) day p.i. Only one swab sample of the right nostril swabs showed positivity by PCR on day 2 p.i., dromedary CA3.

Table 2. Culture and PCR results after eye drop immunisation with *B. melitensis* Rev 1 of dromedary camels.

Days Post Immunisation	Camel ID	Bacteriology - Culture				PCR –CT Values (Duplicate run)					
		Eye Swab		Nostril Swab		Eye Swab		Nostril Swab		EDTA Blood	Serum
		Right	Left	Right	Left	Right	Left	Right	Left		
1	2	3	4	5	6	7	8	9	10	11	12
Before Immunisation	CA-1 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-5 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-6	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-7	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
2 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-5 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-2	POS(+)	NEG	NEG	NEG	37.44 38.29	NEG	NEG	NEG	NEG	NEG
	CA-3	POS(+)	NEG	NEG	NEG	30.92 30.59	NEG	38.42 37.78	NEG	NEG	NEG
	CA-4	POS(+)	NEG	NEG	NEG	29.61 29.44	NEG	NEG	NEG	NEG	NEG
	CA-6	NEG	NEG	NEG	NEG	34.84 34.21	NEG	NEG	NEG	NEG	NEG
	CA-7	POS(+)	NEG	NEG	NEG	33.41 33.51	NEG	NEG	NEG	NEG	NEG
	CA-8	POS(+)	NEG	NEG	NEG	30.72 31.28	NEG	NEG	NEG	NEG	NEG
4 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-5 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-2	POS(++)	NEG	NEG	NEG	38.15 NEG	NEG	NEG	NEG	NEG	NEG
	CA-3	POS(++)	NEG	NEG	NEG	31.17 28.86	NEG	NEG	NEG	NEG	NEG
	CA-4	POS(+++)	NEG	NEG	NEG	30.94 31.75	NEG	NEG	NEG	NEG	NEG
	CA-6	POS(++)	NEG	NEG	NEG	31.9 32.17	NEG	NEG	NEG	NEG	NEG
	CA-7	POS(++)	NEG	NEG	NEG	35.06 33.82	NEG	NEG	NEG	NEG	NEG
	CA-8	POS(+++)	NEG	NEG	NEG	33.66 33.89	NEG	NEG	NEG	NEG	NEG
10 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-5 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-2	POS(+)	NEG	NEG	NEG	37.93 NEG	NEG	NEG	NEG	NEG	NEG
	CA-3	POS(+)	NEG	NEG	NEG	37.61 36.89	NEG	NEG	NEG	NEG	NEG
	CA-4	POS(+)	NEG	NEG	NEG	NEG NEG	NEG	NEG	NEG	NEG	NEG
	CA-6	POS(+)	NEG	NEG	NEG	NEG NEG	NEG	NEG	NEG	NEG	NEG
	CA-7	POS(+)	NEG	NEG	NEG	NEG NEG	NEG	NEG	NEG	NEG	NEG
	CA-8	POS(+)	NEG	NEG	NEG	NEG NEG	36.9	NEG	NEG	NEG	NEG
16 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	NEG	NEG NEG	NEG	NEG	NEG	NEG	NEG
	CA-5 (Control)	NEG	NEG	NEG	NEG	NEG NEG	NEG	NEG	NEG	NEG	NEG
	CA-2	NEG	NEG	NEG	NEG	NEG NEG	NEG	NEG	NEG	NEG	NEG
	CA-3	POS(+)	NEG	NEG	NEG	34.71 35.23	NEG	NEG	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-6	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-7	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

1	2	3	4	5	6	7	8	9	10	11	12
24 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-5 (Control)	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-6	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-7	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	CA-8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

Culture Key: + (1-50 colonies per plate), ++ (51-150 colonies per plate), +++ (> 150 colonies per plate)

Table 3. Serology results after eye drop immunisation with *B. melitensis* Rev 1 of dromedary camels.

Days Post Immunisation	Camel ID	Serology			Camel ID	Serology		
		RBT	CFT	SAT		RBT	CFT	SAT
1	2	3	4	5	6	7	8	9
Before Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA5 (Control)	NEG	NEG	NEG
	CA-2	NEG	AC*	NEG	CA-6	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	CA-7	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	CA-8	NEG	NEG	NEG
2 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	AC*	NEG	CA-6	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	CA-7	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	CA-8	NEG	NEG	NEG
4 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	AC*	NEG	CA-6	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	CA-7	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	CA-8	NEG	NEG	NEG
10 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	AC*	NEG	CA-6	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	CA-7	NEG	NEG	NEG
	CA-4	NEG	NEG	NEG	CA-8	NEG	NEG	Doubtful 26.5 IU/ml
16 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	POS (1+)	AC*	POS 80 IU/ml	CA-6	POS (2+)	NEG	POS 268 IU/ml
	CA-3	NEG	NEG	POS 80 IU/ml	CA-7	POS (4+)	NEG	POS 424 U/ml
	CA-4	POS (2+)	NEG	POS 424 IU/ml	CA-8	POS (4+)	NEG	POS 424 IU/ml
24 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	POS (2+)	AC*	POS 134 IU/ml	CA-6	POS (3+)	NEG	POS 424 IU/ml
	CA-3	POS (4+)	NEG	POS 268 IU/ml	CA-7	POS (4+)	NEG	POS 424 IU/ml
	CA-4	POS (4+)	NEG	POS 424 IU/ml	CA-8	POS (4+)	NEG	POS 424 IU/ml
43 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	POS (2+)	AC*	POS 67 IU/ml	CA-6	POS (4+)	NEG	POS 268 IU/ml
	CA-3	POS (4+)	NEG	POS 160 IU/ml	CA-7	POS (4+)	NEG	POS 268 IU/ml
	CA-4	POS (4+)	NEG	POS 320 IU/ml	CA-8	POS (4+)	NEG	POS 268 IU/ml

1	2	3	4	5	6	7	8	9
73 days Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	NEG	Doubtful 23.25 IU/ml	CA-6	POS(2+)	NEG	POS 134 IU/ml
	CA-3	POS(2+)	NEG	POS 80 IU/ml	CA-7	POS(3+)	NEG	POS 134 IU/ml
	CA-4	POS(4+)	NEG	POS 160 IU/ml	CA--8	POS(3+)	NEG	POS 186 IU/ml
3 months Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	NEG	NEG	CA-6	NEG	NEG	Doubtful 26.5 IU/ml
	CA-3	NEG	NEG	POS 46.5 IU/ml	CA-7	NEG	NEG	Doubtful 26.5 IU/ml
	CA-4	POS(1+)	NEG	POS 186 IU/ml	CA-8	POS(1+)	NEG	POS 134 IU/ml
4 months Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	NEG	NEG	CA-6	NEG	NEG	Doubtful 26.5 IU/ml
	CA-3	NEG	NEG	Doubtful 26.5 IU/ml	CA-7	NEG	NEG	Doubtful 26.5 IU/ml
	CA-4	POS(1+)	NEG	POS 80 IU/ml	CA8	POS(1+)	NEG	POS 134 IU/ml
5 months Post Immunisation	CA-1 (Control)	NEG	NEG	NEG	CA-5 (Control)	NEG	NEG	NEG
	CA-2	NEG	NEG	NEG	CA-6	NEG	NEG	NEG
	CA-3	NEG	NEG	NEG	CA-7	NEG	NEG	NEG
	CA--4	NEG	NEG	NEG	CA-8	NEG	NEG	NEG

RBT agglutination Key: + Dubious, ++ Positive, +++ Strong positive, ++++ Very strong positive

*AC :Anticomplementary reaction

Swabs of the left eye and nostril, as well as EDTA blood and serum samples remained negative by PCR throughout the experiment.

RBT and SAT showed first positivity on day 16 p.i. and remained positive for 4 months with different strength as shown in table 3. Only one camel (animal ID: CA8) was dubious in SAT already on day 10 p.i. CFT remained negative throughout the entire experimental period of 5 months.

Discussion

The information is limited about vaccination against brucellosis in camels, the optimal vaccination age, the dissemination of the vaccine strain and the serological response. Dromedaries were vaccinated with *B. abortus* strain S19 (Agab *et al*, 1995) and with *B. melitensis* in previous studies (Radwan *et al*, 1995). Agab *et al* (1995) vaccinated 5 dromedaries subcutaneously with a reduced dose (5 x 10^8 CFU/2 ml) of *B. abortus* strain S19. All 5 camels sero converted (RBT, SAT, cELISA) after 1 week and their antibody level declined after 7 weeks and the animals were tested negative 14 weeks later. Radwan *et al* (1995) vaccinated 3 month old dromedaries with a full dose (1.2 × 10^9 CFU/ml) of *B. melitensis* Rev 1 vaccine subcutaneously and adults above 10 years with a reduced dose (1.2 × 10^6 CFU/ml) subcutaneously. Both groups developed *Brucella* specific antibodies with titres between 1:25 and 1:200 using the standard USDA BPAT (made from *B. abortus* strain 1119-3), 2-4 weeks after vaccination. The antibodies receded after 8 months in young stock and after 3 months in adult camels. Similar results were obtained in our study, showing the decline of antibody level in adult camels 5 months after immunisation by RBT and SAT (similar to the USDA BPAT). The reason for the negative CFT throughout the experiment was not clarified, but it is hypothesised that the attenuation of the Rev 1 bacteria may have caused this phenomenon.

The attenuated vaccine, *B. melitensis* Rev 1 is used worldwide and it gives full immunity in sheep and goats by the conjunctival route with a dose of 1.0 to 2.0 × 10^9 CFU/animal. A slightly higher dose of 3.1 x 10^9 CFU/dromedary was used in our vaccination trial and the results showed that the *B. melitensis* vaccine strain was viable in the conjunctival sac of vaccinated animals for 10 to 16 days. Our conjunctival dromedary vaccination experiment did not include the testing to prove lifelong immunity in camelids as no challenge infections have been performed. Camels have a physiological constant lacrimation to clean their eyes from sand and dust, and by shedding the vaccine strain through their tears they may infect humans and other animals. Human infection with Rev 1 after consuming milk from vaccinated adult pregnant

animals was reported before (Bradenstein *et al*, 2002). Although, none of the control dromedaries became brucellosis positive in our study, we recommend not to vaccinate camelids through the conjunctival route but subcutaneously or intramuscularly due to the long eye excretion period. If the subcutaneous or intra muscular routes are used, great care should be taken as in some cases, human brucellosis was inflicted from accidental self-inoculation with live vaccine (Saleem *et al*, 2010).

An eradication campaign in camelids may be based on vaccination and 'test and slaughter' policy for dairy herds and 'test and no breeding' for racing herds (Wernery, 2014), because vaccinations alone would not suffice for success. The main approach in a long term control strategy of camelid brucellosis is to vaccinate only 1 to 2 year-old female replacement camels. An immunised herd could be established by this strategy without inducing abortion and excreting the vaccine strain through milk.

Conclusion

Brucella melitensis Rev 1 vaccine strain was isolated for 10 to 16 days from the right eye of 6 dromedary camels after conjunctival immunisation and may therefore be a risk for other animals as well as humans. Therefore, a subcutaneous or intramuscular immunisation is recommended.

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BIOACTIVE PROPERTIES OF MINOR CAMEL MILK INGREDIENTS - AN OVERVIEW

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ABSTRACT

Camel milk has numerous minor components which have special bioactive properties. These are present at significant concentrations and are beneficial for human diet and health e.g. lactoferrin, serum albumin, lysozyme, mono- and polyunsaturated fatty acids, vitamins B, C and E, manganese, iron, calcium and potassium, different types of immunoglobulins, as well as the hormone insulin and IGF-1. Regarding the importance of camel milk and the related health benefits of the bioactive ingredients, it must be consumed raw, fresh and should be free of pathogens.

Key words: Camel milk, bioactive substances, human health, milk composition

Milk is a complex medium containing a variety of nutrients, proteins, fats, lactose, minerals, vitamins as well as other molecules of functional or bioactive properties. Camel milk is considered one of the most valuable food sources for nomadic people in arid and semi-arid areas and has been consumed for centuries due to its nutritional values and medicinal properties (Dowelmadina *et al,* 2014; Yadav *et al,* 2015; Kaskous, 2016; Kula and Dechasa, 2016). It has high quality of composition and various bioactive ingredients, showing special properties that make it distinct and unique compared to other species milk (Wernery, 2007; Smits *et al,* 2011; Hamed *et al,* 2012; Yadav *et al,* 2015). The mean values of major components in camel milk were reported over the past 30 years: 3.5±0.1 % fat, 3.1±0.5 % protein, 4.4±0.7 % lactose, 0.79±0.07 % ash and 11.9±1.5 % total solids (Alhaj and Al Kanhal, 2010). In addition, camel milk contains high amounts of various antimicrobial and bioactive substances, e.g. lactoferrin and various classes of antibodies (Kaskous, 2016; Kula and Dechasa, 2016; Patel *et al,* 2016). Biologically, camel milk is the normal secretion of mammary gland and the most important source of nutrition for new-born camels and also considered as a mine of nutritive chemicals. Differences between camel and bovine milk lead to differences in physiological and biological properties (Jrad *et al,* 2013). Camel milk varies greatly because many external components affect its composition, such as the country or location, feeding conditions, camel breed, type of samples, milking frequency, stage of lactation, parity numbers and more (Abdoun *et al,* 2007; Dowelmadina *et al,* 2014).

Camel milk is most frequently consumed raw and hence unpasteurised, because in the raw form it retains the nutritional and immune properties. However, pasteurisation is highly recommended when the quality and safety of the camel milk is in question. Nonpasteurised camel milk can be a source for various bacteria which may lead to health hazard for humans when it is taken raw without quality or hygiene control (Elhaj *et al,* 2013) and not using clean and well managed milking procedure (Kaskous and Fadlelmoula, 2014). Investigations showed that raw camel milk is highly contaminated when camels are milked under nomadic conditions lacking proper hygiene (Wernery, 2007).

The properties of the individual bioactive camel milk components and its importance are presented and discussed.

Special properties of camel milk proteins

Camel's milk is a rich source of proteins with potential anti-microbial and protective activity. This biological beneficial effect of camel milk protein is primarily combined with minor proteins. Smits *et al* (2011) reported that characteristics of proteins in camel milk differed significantly from those in bovine milk, in terms of molecular mass and hydrophobicity. Camel milk has 21 different amino acids (Tsetsegmaa *et al,* 2008) which produced all the camel milk proteins, compared to 18 amino acids in bovines.

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However, camel milk proteins are classically grouped into 2 main classes: major milk proteins including caseins or whey proteins and minor milk proteins including lactoferrins, lysozyme, lactoperoxidase, serum albumin, whey acidic protein, peptidoglycan recognition protein, small peptides and various classes of immunoglobulins.

It is known that camelids exhibit a whole new class of immunoglobulins, which fundamentally differ from all other known antibodies. El-Agamy *et al* (2009) reported that camel milk proteins have unique patterns that are totally different from cow and human milk. The study showed that the lack of immunological similarity between camel's and cow's milk proteins may be considered as an important criterion of the nutritional physiological and clinical aspects. This leads to improved immune function after camel milk consumption. Further, camel milk consumption can also protect the organism against other external pathogens, bacteria or viruses (Agrawal *et al,* 2009; El-Fakharany *et al,* 2012; Mullaicharam, 2014; Yassin *et al,* 2016; Dubey *et al*, 2016).

Major proteins in camel milk

Caseins represent the most abundant protein fraction of camel milk (Alhaj and Al Kanhal, 2010; Hamed *et al,* 2012). It has a relative amount of 1.63-2.76% casein, representing 52-87% of total proteins compared to an average of 83% in bovine milk (Mehaia *et al,* 1995; Khaskheli *et al,* 2005; Frister, 2007; Hamed *et al,* 2012). β-Casein is the main camel milk casein with 59.40±1.04% of total casein compared with 47.77±0.35% in bovine milk casein (Hamed *et al,* 2012). α_{s1}-Casein constitutes about 23.89±0.68% of total casein compared with 38.36±0.37% in bovine milk casein (Kappeler *et al,* 1998) and 3 protein patterns named α_{s1}-Casein A, C and D in camel milk were identified (Erhardt *et al,* 2016). Only 3.48±0.29% of the total casein corresponds to κ-casein in camel milk compared with 7.20±0.41% in bovine milk casein (Hamed *et al,* 2012). Furthermore, α_{s2}-casein constitutes about 11.89±0.49% of total casein compared with 5.35±0.20% in bovine milk casein (Ribadeau-Dumas and Grappin, 1989).

Alhaj and Al Kanhal (2010) and Hamed *et al* (2012) reported that camel milk is more similar to human milk since it contains a high percentage of β-Casein. This high percentage could reflect its higher digestibility rate and lower incidence of allergy in the gastro-intestinal-tract (GIT) of infants, as β-Casein is more sensitive to peptic hydrolysis than α_s-Casein (El-Agamy *et al,* 2009). However, casein from dairy cows are metabolised incomplete in the intestines of some people. As a result, short neuroactive peptides, such as beta-casomorphins are formed which are derived from milk caseins. Beta-casomorphins has long been considered as a risk factor for autism (Woodford, 2011). It has been demonstrated that camel milk showed a therapeutic effect in the Autism disease (Shabo and Yagil, 2005; Wernery *et al,* 2012; Yagil, 2013; Al-Ayadhi and Elamin, 2013), because camel milk does not contain beta-casomorphins leading to the autism symptoms when drinking cow milk. Kappeler *et al* (1998) discovered the amino acid sequence differences of camel casein. The number of amino acid (aa) residues in the 4 major caseins subtypes were: β–Casein 217 aa; α_{s1}-Casein 207 aa; α_{s2}-casein178 aa and κ-casein 126 aa.

Whey proteins are the second major group of components of camel milk proteins and constitute 20-30% of the total proteins (Frister, 2007; Wernery, 2007; Alhaj and Al Kanhal, 2010; Hamed *et al,* 2012). The composition of camel milk whey proteins is significantly different to that of bovine milk whey (Smits *et al,* 2011). The whey protein β–lactoglobulin, which is the main allergen in bovine milk, could not be detected in camel milk, as also observed for human milk (Merin *et al,* 2001; Wernery, 2007; El-Agamy *et al,* 2009, Smits *et al,* 2011). In camel milk, whey protein α-lactalbumin is the major whey milk component (Wernery, 2007; Alhaj and Al Kanhal, 2010), whereas in bovine milk whey, β–lactoglobulin is the main component (50%) and α-lactalbumin is the second (40%) (Frister, 2007).

Minor proteins in camel milk

The milk protein lactoferrin, is an iron-binding glycoprotein, which is one of the elements essential for the proliferation of bacteria (Adlerova *et al,* 2008). Lactoferrin inhibits the growth of iron-dependent bacteria (Boretius, 1986) and is considered to be a part of the innate immune system (Adlerova *et al,* 2008). At the same time, lactoferrin also takes part in specific immune reactions in an indirect way (Legrand *et al,* 2005). Therefore, camel milk lactoferrin is called as a potent natural antibacterial and novel immune-modulator agent (Ismael *et al,* 2013) (Table 1). This natural antimicrobial property is based on a multifunctional bioactive molecule with a critical role in many important physiological pathways. The results of Ismael *et al* (2013) have shown that camel lactoferrin had a significant inhibitory effect against *Escherichia coli, Pseudomonas aeruginosa, Streptococcus agalactiae*

and *Staphylococcus aureus* and lactoferrin increased lymphocyte transformations mean values in a dose dependent manner. The highest transformations mean value was determined at 50 µg lactoferrin per ml camel milk. Anyway, camel lactoferrin have shown its biochemical similarity to human and bovine lactoferrin, as well as the cross-react with the anti-human lactoferrin antibodies (Konuspayeva *et al,* 2007). Redwan and Tabll (2007) showed that incubation of human leukocytes with camel lactoferrin and subsequent infected with HCV (Hepatitis-C-Virus) did not prevent the HCV entry into the cells, while the direct interaction between the HCV and camel lactoferrin leads to a complete virus entry inhibition after seven days incubation. Therefore camel milk with lactoferrin in higher concentrations seems to represents a primary natural produced drug against HCV infection (Redwan and Tabll, 2007). Recently, the potential of camel milk lactoferrin for its ability to inhibit the proliferation of human colon cancer cells (HCT-116) *in vitro* and the DNA damage and its antioxidant activity was evaluated for the first time (Habib *et al,* 2013). Further the antibacterial activity of lactoferrin in the camel milk was intensively researched and described in context of mastitis by Al-Majali *et al* (2007). Compared to bovine milk lactoferrin is present in large quantities in camel milk and the values varied between 0.2 and 7280 mg/ml milk (Qian *et al,* 1995; El-Gawad *et al,* 1996; Elagamy *et al,* 1996; Kappeler *et al,* 1999; Zhang *et al,* 2005; El-Hatmi *et al,* 2006; Konuspayeva *et al,* 2007; Al-Majali *et al,* 2007; Kaskous *et al,* 2012). This fluctuation of lactoferrin concentration in camel milk is influenced by many factors such as udder health. The mean concentrations of lactoferrin from mastitis camels (3.8±0.67 mg/ml milk) was significantly higher than that in normal camels (2.65±0.88 mg/ml milk). Further, the concentration of lactoferrin in 3-4 year old lactating camels showed significantly higher values than that in older camels (Al-Majali *et al,* 2007).

Cameloid Immunoglobulins (Igs)

The group of Igs of camel milk is quite unique in mammals. A further study indicated that camel milk contains a special class of cameloid immunoglobulin's (Ig). The immunoglobulins showed similar spatial structure as human immunoglobulin's (150 kDa), but only $^{1}/_{10^{th}}$ the size around 12-15 kDa (Mullaicharam, 2014). It enables cameloid IgGs an easy and quick targeting of the antigen or surface protein, and subsequently the penetration of a disease microorganism is significantly reduced, which is an advantage compared to bigger immunoglobulin's (Khamehchian *et al,* 2014). Hence it is assumed that camel milk strengthens and supports the gastrointestinal immune system (Kaskous, 2016). In the serum of camel milk, a completely new class of Ig has been discovered which is fundamentally different from all other previously known antibody classes. IgM, IgG, IgA and even IgD have been detected in camel sera. Normally, the structural configuration of the Ig in the milk is characterised by 4 polypeptides, 2 identical H-chains (heavy) and 2 identical L-chains (light) (Frister, 2007) with a size of around 150 kDa. The light chains of the camel milk Ig are completely

Table 1. Antimicrobial effect of camel lactoferrin (cLf) on *E.coli, P. aeruginosa, S. aureus* and *S. agalactiae* counts after 1, 3, 6, 12, 24 hours of incubation.

Type of Bacteria	Items	Microbial count (CFU/ml) after				
		1 hour	3 hours	6 hours	12 hours	24 hours
E. coli	Control	49.000	370.000	2.9×10^6	3.1×10^7	2.7×10^7
	cLf (1 mg/ml)	35.000	15.000	8000	500	CIG
	cLf (3 mg/ml)	CIG	CIG	CIG	CIG	CIG
P. aeruginosa	Control	1.8×10^4	2.3×10^4	1.7×10^5	2.4×10^6	2.9×10^7
	cLf (1 mg/ml)	142.000	111.000	43.000	21.000	17.000
	cLf (3 mg/ml)	107.000	93.000	17.000	950	950
S. aureus	Control	87.000	2.3×10^6	2.7×10^7	2.9×10^8	2.2×10^8
	cLf (1 mg/ml)	73.000	2.1×10^6	2.6×10^7	2.7×10^8	2.1×10^8
	cLf (3 mg/ml)	56.000	1.7×10^6	2.0×10^7	2.1×10^8	1.9×10^8
S. agalactiae	Control	0.7×10^6	2.6×10^6	3.4×10^7	2.9×10^8	3.6×10^8
	cLf (1 mg/ml)	0.4×10^6	1.8×10^6	2.3×10^5	1.8×10^4	2.1×10^5
	cLf (3 mg/ml)	2.2×10^5	1.9×10^5	1.0×10^4	1000	3300

cLf: Camel lactoferrin; CIG: Complete inhibition of growth.

missing (Hamers-Casterman *et al,* 1993). Therefore, camel Igs are significantly smaller than human or bovines and hence called 'nano-antibodies' or just 'nano-bodies'. Camel and llama 'nano-bodies' (12–15 kDa) which is less than a tenth of conventional antibodies (Mullaicharam, 2014). These unique antibodies are far superior to human antibodies as they can neutralise completely enzymes and are active against many viral or bacterial infections. The observation that camel blood is rich in such antibodies classes may explain the resistance of camels against most known animal diseases (Afzal and Sakkir, 1994).

These naturally occurring 'nano-bodies' could be a valuable tool in the control of human disease. Their small size also explains why people respond with defective immune responses so positively to camel milk. However, Camel Igs are able to penetrate into tissues and organs to fight infection and aid repair, where human or bovine Igs cannot due to their size. Katz *et al* (2008) showed that patients with IgE-mediated cow's milk allergy were only 25% tested positive by skin-prick test for cross-reactivity to camel's milk and 75% were negative. The authors suggest that the patients with proven IgE-mediated allergy to cow's milk can utilise to predict suitable alternative sources of milk. It is well known that some foods, such as cow's milk and bovine dairy products, can cause allergic reactions. El-Agamy *et al* (2009) indicated that the absence of immunological similarity between camel and cow milk proteins can be considered the key point in nutrition for children suffering cow milk allergy. In summary, Restani *et al* (1999) and El-Agamy *et al* (2009) found that IgE of children who were allergic to cow milk, do not react after consuming camel milk. They ascribed it to the phylogenetic differences between cameloids and ruminants protein and hence antigen composition (Stahl, 2005).

IgG also showed ability to recognise and inactivate Hepatitis C virus peptides with a significant titer in comparison to human IgG which failed to do it (El-Fakharany *et al,* 2012). In addition, the influence of camel's milk on the immune response in chronic hepatitis B patient has been studied and demonstrated that camel milk can enhance the cellular immune response in the patient and inhibits the replication of the virus DNA and promotes recovery of chronic hepatitis B patients (Saltanat *et al,* 2009). A recent study by El-Fakharany *et al* (2016) reported the influence of camel milk on the Hepatitis C Virus in the infected patients.

Recent research reveals further interesting characteristics in camel milk Igs. The lack of light chain, showed various unique bioactive characteristics and immune system relevant properties: (1) increased cell permeability, (2) ability to cross blood-brain barrier, (3) higher specificity with none to extremely low cross-reactivity, (4) higher thermal stability, (5) higher pH range tolerance, (6) higher water solubility without any aggregation (Niasari-Naslaji, 2010). These peculiarities grab the attention of researchers to use camel immunoglobulin for therapeutic and diagnostic purposes. If successful, the research community could lead to the development of a whole new family of vaccination against some of the biggest killer diseases of our time, such as cancer (Behdani *et al,* 2010). The scientific community speculates and postulates that camel milk Igs could even be an effective treatment against cancer, HIV/AIDS, Alzheimer's disease or Hepatitis C (Martin *et al,* 1997; Agrawal *et al,* 2003; Magjeed, 2005; Shabo *et al,* 2005; Habib *et al,* 2013). Currently, there are still many scientific workgroups are figuring out whether camel milk can also be effective prophylactic against diabetes and heart disease (Zagorski *et al,* 1998; El-Sayed *et al,* 2011; Malik *et al,* 2012; Shori, 2015).

Further immune relevant proteins in camel milk

Besides Igs camel milk contains the following immune relevant proteins in higher qualities than milk from other species, according to Hoelzer *et al* (1998), Mullaicharam (2014), Khamehchian *et al* (2014), Conesa *et al* (2008) and www.nourishinghope.com, 2011 (a selection):

(1) Peptidoglycan Recognition Protein (PGRP) is found in very high concentration in camel milk. It stimulates the hosts' immune response and has antimicrobial activity.

(2) Camel lactoferrin has higher bioactivity compared to cows or goats milk lactoferrin. Lactoferrin prevents microbial overgrowth and invading pathogens.

(3) Lysozyme is an enzyme that is part of the innate immune system that targets gram-positive bacteria.

(4) Lactoperoxidase has bactericidal activity on gram-negative bacteria like *Escherichia coli, Salmonella* and *Pseudomonas*.

(5) N-acetyl-beta-D-glucosamidase (NAGase) has antibacterial activity and is found in similar quantities in human milk.

Lysozyme is among the minor camel milk proteins that has attracted increased attention recently due to its potent antimicrobial activity against a wide

range of micro-organisms and hence potential in food preservation and safety. Lysozyme is an enzyme and it comes in the milk as part of the innate immune system. The lysozyme content in the camel milk ranged between 0.15 to 6.5 mg/l (Barbour *et al,* 1984; Elagamy *et al,* 1996). The concentration of lysozyme in the camel milk varies considerably depending on various factors such as breed, stage of lactation, nutrition, udder health and season of the year. The peptidoglycan cell wall of gram-positive bacteria can be directly attacked by lysozyme (Benkerroum, 2008). Contrary to that of gram-negative bacteria, the outer membrane may be rendered permeable by other components of the innate immune system in the milk, such as lactoferrin, so that they can be attacked by lysozyme. Apart from the direct anti-bacterial effect of lysozyme the release of peptidoglycan fragments leads to a modulation of the immune system via peptidoglycan-recognising receptors. A subset forms the calcium-ion-binding lysozyme, which include for example lysozyme from camel milk.

Lactoperoxidase is an oxidative enzyme that is found in milk of mammals including camel. However, Elagamy *et al* (1992) found that the lactoperoxidase in camel milk acts as a bacteriostatic in gram-positive bacteria strains and as bactericidal in gram-negative cultures. Bolorimoghadam *et al* (2010) also reported that, lactoperoxidase enzyme which extracted from camel milk, has a significant anti-bacterial activity on gram-positive and gram-negative bacteria. Furthermore, lactoperoxidase is destroyed when camel milk and its products are heat-treated at 75°C for 15 seconds. Therefore, this enzyme can be used as an indicator of correct pasteurisation of camel milk (Wernery *et al,* 2013).

Serum albumin in the camel milk comes from the blood. It was shown, that camel milk contained more minor protein than cow milk. This variation is primarily due to the higher content of albumin in camel milk (Al-Alawi and Laleye, 2008). However, Preeti *et al* (2014) found that camel milk has about 7 lower molecular weight bands (electrophoretic pattern), which may be of pre-albumin and other lower molecular weight proteins.

Camel milk protein also contains whey acidic protein (157 mg/l) and peptidoglycan recognition protein (107 mg/l) compared with zero values in both components in bovine milk (Wernery, 2007). Moreover, it was found that fermented camel milk has a special enzyme Angiotensin 1 converting enzyme (ACE) (Quan *et al,* 2008), which facilitates the better proteolytic digestion of the milk proteins, in particular caseins and whey proteins (Alhaj *et al,* 2006). Furthermore, it was found that camel milk and its fermented products have many bioactive peptides. Elayan *et al* (2008) demonstrated that administration of fermented camel milk has a hypo-cholesterolemic effect in rats. Hypocholesterol mechanism of camel milk is still unclear, but different hypotheses were discussed, including: (1) the interaction between bioactive peptides from camel milk and cholesterol levels is derived, which lead to cholesterol-lowering (Li and Papadopoulos, 1998) and (2) the presence of orotic acid in camel milk (arises as an intermediate in the metabolism of the nucleic acids), which is considered responsible for the lowering of cholesterol levels in rats (Rao *et al,* 1981) and in humans (Buonopane *et al,* 1992).

Based on the properties above it can be emphasised that the bioactive peptides derived from camel milk protein had higher functionality including antioxidant activity, anti-hypertension effect and antimicrobial activity comparing to bioactive peptides from bovine milk proteins and therefore, camel milk could be the super food of the future (Salami *et al,* 2010). The problem with camel milk proteins was the stability after sterilisation process, because camel milk has poor heat stability at high temperature and could not be sterilised at natural pH (Alhaj *et al,* 2011).

Properties of lipids in camel milk

Lipids in camel milk as well as their physical and chemical properties were investigated in many scientific studies (Sawaya *et al,* 1984; Abu-Lehia, 1989; Farah, 1993; Gorban and Izzeldin, 2001; Awad *et al,* 2008; Wang *et al,* 2011; Konuspayeva *et al,* 2014) (Table 2). These have shown that the content of short chain saturated fatty acids (C4-C8) is significantly higher in ruminant's milk than in the camel's milk. In addition, the proportion of saturated fatty acids in the camel milk is lower as compared to cow's milk (Hagrass *et al,* 1987; Stahl, 2005; Narmuratova *et al,* 2006; Awad *et al,* 2008). Lipids in the camel milk have a higher proportion of mono- and polyunsaturated fatty acids (Gorban and Izzeldin, 2001; Wernery, 2007; Wang *et al,* 2011; Konuspayeva *et al,* 2014), which enhance its overall nutritional quality (Konuspayeva *et al,* 2008; Ayadi *et al,* 2009). The ratio of unsaturated/saturated acid was more favourable in camel's milk compared to cow's milk or other mammalians (Konuspayeva *et al,* 2008). An advantage over the cow's milk is the ratio of saturated/unsaturated of fatty acids was 1.97 in intensive farming system (Konuspayeva *et al,* 2014). According of Gorban and Izzeldin (2001)

particularly striking is the presence of long chain fatty acids with more than 20 carbon atoms in the fat milk of camels, unlike the cattle. Furthermore, it was shown that short-chain fatty acids (C8:0 and C10:0) were higher proportion in spring and long-chain fatty acids (C17:0 and C17:1) in autumn (Konuspayeva *et al,* 2008). Wang *et al* (2011) reported that unsaturated fatty acids in camel milk were highest than the cow milk, goat milk and human milk and the value were 65.02, 40.76, 40.23 and 58.17 g/100g fatty acids respectively and C18:3 in camel milk (5.12±0.21 g/100g) was significantly higher than the cow milk (0.38 g/100g), goat milk (0.34 g/100g) and human milk (2.96 g/100g). In addition dromedary camel milk had a higher proportion of C17:0iso and C18:1 than bactrian camel milk (Konuspayeva *et al,* 2008). Another study is investigated that C18:3 in camel milk was significantly higher than the cow milk, goat milk and human milk and the value were 5.12±0.21, 0.38, 0.34 and 2.96 g/100g, respectively. Even the higher proportion of linoleic acid (C18:3) in the camel milk can be seen from a nutritional point of view as an advantage. In human, the unsaturated fatty acids play a role particularly in the prevention of cardiovascular diseases. The high content of omega-3 fatty acids and oleic acid, attributed a positive impact on health because through it the level of triglycerides and cholesterol is lowered in the blood (Carrero *et al,* 2004).

Another characteristic of the camel milk fat and its fraction were highly stable against oxidation (up to 20 days) and longer shelf-life (Awad *et al,* 2008). Furthermore, it was found that fat globule size (µm) distribution was similar in cow and camel milk (Farah and Rüegg, 1991) and an average fat globule size diameter ranged from 2.31 to 3.93 µm (Knoess *et al,* 1986). Compared with cow milk, camel milk showed a very slow creaming rate and it had no relationship between the average size distribution of fat globule and they observed poor creaming. This indicated that insufficient quantity of agglutinin in camel milk was mainly responsible for the slow rate of creaming (Farah and Rüegg, 1991).

Table 2. Unsaturated fatty acids (g/100g fatty acids) in camel, human, cattle and goat milk.

Parameter	Camel	Human	Cow	Goat
Unsaturated fatty acids (UFA)	65.02	58.17	40.76	40.23
C18:3	5.12	2.96	0.38	0.34

Properties of sugars in camel milk

Lactose is the main carbohydrate in milk. It is synthesised in the udder from galactose and glucose. The disaccharide lactose in camel milk is present in approximately concentrations of 4.8%, which is easily metabolised by persons suffering from lactose intolerance (Hanna, 2001). Mullaicharam (2014) reported that lactose-intolerant patients often easily digest camel milk. The lower lactose intolerance could be linked to the high concentration in L-Lactate in camel milk, reverse to cow milk, rich in D-Lactate (Baubekova *et al,* 2015). Therefore, camel milk can be considered as an alternative option for the individuals intolerant to lactose (Cardoso *et al,* 2010), which represents today a major market in food industry.

Properties of minerals in camel milk

Minerals at nutritional standard concentrations in foodstuffs are essential for human health. However, when these nutritional values are low or exceeded then humans may get diseases, but on the other hand the consumption of foodstuffs with high mineral contents can cause gastric irritation and diarrhoea (Blunden and Wallace, 2003). Camel milk is well known for its richness in minerals (Farah, 1993; Konuspayeva *et al,* 2008; Al-Wabel, 2008; Alhaj and Al Kanhal, 2010; Wang *et al,* 2011; Yadav *et al,* 2015). The total content of minerals in dromedary camel milk was 0.99% (Konuspayeva, 2007) and varies from 0.60 to 0.90% (Konuspayeva *et al,* 2009). The differences in concentration of minerals in the camel milk depends majorly on the feeding, but as well on breed, water intake, season and country or region (Soliman, 2005; Alhaj and Al Kanhal, 2010). However, levels of potassium, magnesium, iron, copper, manganese, sodium and zinc are higher in camel's milk than in cow's milk (Sawaya *et al*, 1984; Abu-Lehia, 1987; Yadav *et al,* 2015). Farah (1993) found low levels of potassium and phosphorus in Egyptian dromedaries. Wang *et al* (2011) found that calcium, magnesium and iron content in camel milk were highest than other milk from cows, goats and human. Al-Wabel (2008) has determined some minerals in the milk of cattle, camels, goats and sheep in Saudi Arabia and the results are shown in the table 3.

Camel's milk had the lowest concentration of zinc and there are no significant differences in the concentration of manganese and iron between cattle, camels, sheep and goats. Furthermore, camels have the highest concentration of sodium and potassium compared to other species. It is known that the mineral content of milk raised under hot and dry desert conditions, such as Saudi Arabia. Konuspazeva *et al* (2008) have determined some minerals in the camel milk in Kazakhstan and the mean values

were 1.232±0.292 g/l, 1.003±0.217 g/l and 2.02±1.24 mg/l of calcium, phosphorus and iron, respectively. These concentrations of minerals covered the most daily requirement for adult, when the consumption reached 500 ml per day of camel milk. Soliman (2005) has determined the chemical composition and the minerals in the milk of human, cow, buffalo, camel and goat in Egypt and the results showed that chemical and mineral content of the 5 studied species varied widely. Camel milk contained 0.75 % ash and it is significantly different from buffalo and human milk. Camel milk has the highest Fe, Zn, Na and Cu content than other species. The results of Halima *et al* (2012) showed that camel milk had a very high ash content (0.86 g/l) compared with human milk (0.17g/l). Furthermore, Camel milk is a rich source of chloride (Khaskheli *et al,* 2005). Mehaia *et al* (1995) reported that minerals Na, K, Fe, Cu and Mn in dromedary camel milk were higher than that in bovine milk. This camel milk is plentiful with minerals, which is necessary for the growth, development and human health.

Properties of vitamins in camel milk

Camel milk is known to be a rich source of vitamins, especially vitamin C, which 3 to 5 times higher levels than in cow milk with absolute values up to 40.9 mg/l (Farah *et al,* 1992; Stahl *et al,* 2006, Wernery *et al,* 2005; Haddadin *et al,* 2008; Wang *et al,* 2011). Raw and fermented camel milk could be a good source of vitamin C for the nomads, which are living in the desert where vegetables and fruits are not available. The mean of vitamin C in dromedary camel milk was 34.16 mg/l by Farah *et al* (1992) and 150.4±105 mg/l by Konuspayeva *et al* (2011). This concentration is dependent on many factors such as season (in dry season was 41.0±3.70 mg/l and rainy season was 33.0±4.00 mg/l) (Mohamed and Al-Rasheedi, 2013), stage of lactation (at the first 3 months of lactation was 40.10 mg/l and at 290-360 days of lactation was 44.40 mg/l) (Mohamed and Al-Rasheedi, 2013), the sampling place (Konuspayeva *et al,* 2011), breed (in bactrian camel was richer with 169±110 mg/l than the dromedary with 146±93 mg/l or hybrid with 133±129 mg/l) (Konuspayeva *et al,* 2011). However, Mohamed and Al-Rasheedi (2013) stated that the increase of vitamin C during summer season and with the advancement of stage of lactation could not be justified, and may be related to the unique glucose metabolism of camel.

The loss of vitamin C was very low (6.1%) following pasteurisation of camel milk. This fact can be considered as tremendously advantageous for the consumer in arid and semi-arid countries where vitamin sources are scarce (Wernery *et al,* 2005).

Stahl *et al* (2006) reported that vitamins A, E, B_1, and β-carotene were significantly lower in dromedary milk while vitamin C was significantly higher compared to bovine milk. However, in camel colostrum fat soluble vitamins and vitamin B_1 were higher than in mature camel milk, but vitamin C was lower in colostrum.

Furthermore, the level of niacin in camel milk was found greater compared to cow milk, but thiamine, riboflavin, folacin, vitamin B_{12}, pantothenic acid, vitamin-A, lysine and tryptophan were relatively lower as compared to cow milk (Nikkhah, 2011). Farah *et al* (1992) reported that camel milk contains substantially less vitamin-A (0.10 vs 0.27 mg/l) and B_2 (0.57 vs 1.56 mg/l), and similar vitamin E content (0.56 vs 0.60 mg/l) than cow milk, respectively. However, the content of Vitamin E was similar in camel milk (129.9±26.2 mg/l) and human milk (121.69±15.2 mg/l), but it was the highest (161.0±23.7 mg/l) in fresh cow milk.

Jrad *et al* (2013) reported that camel milk is more acidic and viscous than bovine milk and the protein and non-protein nitrogen (NPN) content in camel milk was significantly higher than that of cow milk. The fractions (NPN) have a biological importance due to their richness in acidic amino acids as well as in Vitamin B.

Table 3. Mineral content (mg/kg milk) of major elements in camels, cattle, goats or sheep milk.

Minerals	Camels	Cattle	Goats	Sheep
Zn	1.48±0.76	2.00±0.28	2.32±0.22	3.09±0.91
Mn	1.30±0.11	1.29±2.43	1.13±0.04	1.14±0.05
Cu	1.61±0.90	1.80±1.10	0.57±0.20	0.62±0.22
Fe	2.98±2.24	4.21±1.78	4.91±2.66	5.01±3.24
Ca	699.30±96.65	661.00±41.95	751.70±72.78	822.50±113.36
Na	115.87±4.99	91.60±3.45	101.30±10.71	95.40±5.47
K	133.77±5.64	113.70±5.84	123.85±9.94	127.41±1.10

Properties of selected hormones in camel milk

Camel milk contains higher average concentrations of insulin (58.67±2.01 UL) as compared to cow's milk (17.01±0.96 UL) (Hamad *et al,* 2011; Mullaicharam, 2014). Wernery (2007) has shown the same results, that insulin in camel milk was present at higher levels (40.5 µU/ml) as compared to cow milk (16.3 µU/ml). Further, camel milk remains unaffected by gastric acid and so passed to the intestine where it can be absorbed bioactive intact by the gastro intestinal tract (Abu-Lehia, 1989; Zagorski *et al,* 1998).

Usually, administration of insulin orally in diabetic patients is not effective, but it seems that insulin in camel milk may be an exception. Thus a study describes the following 3 special properties of camel milk derived bioactive insulin (Malik *et al,* 2012). (1) Camel insulin in camel milk possesses a special property that makes absorption into human circulation easier and cause resistance to proteolysis, compared to insulin from other sources (2) Camel insulin is encapsulated in nanoparticles (lipid micro-vesicles), that allows its passage through the stomach and the entry into the circulation (3) Some up to now unknown elements of camel milk make it anti-diabetic. The protein sequence of camel insulin and its predicted digestion pattern do not suggest differentiability to overcome the mucosal barriers before been degraded and reaching the blood stream. It is further reported, that camel milk contains insulin-like small molecule substances that mimic insulin interaction with its receptor. Shori (2015) concluded that camel milk has a powerful effect in reducing blood glucose levels and insulin requirement and it limits diabetic complications such as elevated cholesterol levels, liver and kidney diseases; decreased oxidative stress and delayed wound healing.

Insulin like growth factor 1 (IGF-1) and Thyroxine (T_4) hormones were determined in the camel milk. It is known, that both hormones play a major role in controlling growth and metabolism. IGF-1 was found at high level around parturition and decreased with stages of lactation, whereas T_4 levels were low at parturition and progressively increased after was (El Khasmi *et al,* 2002).

Conclusions

Regarding the macro and micronutrients of camel milk, it can be concluded that the nutritional value of camel milk is far better compared to cow milk. Camel milk is rich in various minor proteins, nano-immunoglobulins, vitamins, etc. which are not present in cow milk. Camel milk and its proteins do not seem to induce allergies, diabetes and autism as reported for cow milk. An increasing number of scientific publications focus on the nutritional importance of camel milk with its special bioactive components and its beneficial impact on consumer, especially for gastro intestinal health. To ensure complete benefits of camel milk, it must be consumed raw, fresh and free of pathogens as well as after a good and clean milking machine.

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BEHAVIOURAL, HORMONAL AND HISTOPATHOLOGICAL CHANGES ACCOMPANYING THE OVERSIZED FOLLICLES IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

This study was designed to investigate the effect of oversized follicles on the behaviour and hormonal concentrations in female dromedaries. The estrous pattern of 26 dromedaries with oversized follicles was recorded during the breeding season. Thirty–three ovarian pairs with preovulatory and oversized follicles were recovered and sectioned from slaughtered adult camels (n=33). Blood (10 ml) was collected from all females and follicular fluid from slaughtered females for estimation of reproductive hormones and nitric oxide (NO). Oversized follicles lead to infertility problems in dromedaries such as repeat breeding, nymphomania and anestrous. Serum progesterone (P_4) concentrations in repeat breeders with thin–wall oversized follicles (RB thin, n=10; 1411.50±93.39 pg/ml) and nymphomaniac with thin–wall oversized follicles (Nympho thin, n=8; 1710.00±107.74 pg/ml) were significantly ($P<0.05$) lower than that in anestrous animals with thick–wall oversized follicles (Anest thick, n=4; 2532.50±107.74 pg/ml). Serum estradiol (E_2) concentration was significantly ($P<0.05$) higher in Nympho thin (0.97±0.31 pg/ml) than Anest thick (0.30±0.08 pg/ml) camels. In Nympho–thin camels, serum testosterone (T; 39.75±4.85 pg/ml) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 173.93±9.75 pg/ml) concentrations were significantly ($P<0.05$) higher than both T concentration (17.20 ± 3.63 pg/ml) in RB thin and PG $F_{2\alpha}$ concentration (77.65±7.90 pg/ml) in RB thick camels (n=4). Serum NO concentrations in RB thin (2.49±0.03 µM) camels were significantly ($P<0.05$) higher than that in both RB thick and Anest thick camels. The oversized follicles lead to infertility problems in dromedaries, accompanied by changes in serum and follicular fluid reproductive hormones and NO concentrations.

Key words: Camel, follicular fluid, hormones, nitric oxide

In female dromedaries, the cystic ovaries were observed throughout the whole year, with variable percentages regarding both ovaries (Hussein *et al,* 2008). The follicular structures of dromedaries are classified into inactive ovaries (those containing follicles less than 3 mm in diameter), growing follicles (>3 to 9 mm in diameter), ovulatory follicles (10-19 mm in diameter) and oversized follicles (exceeding 25 mm in diameter) (range 40-64 mm), before they start regressing (Skidmore *et al,* 1996; Ali *et al,* 2010a; Skidmore, 2011). Follicles >30 mm in diameter are regarded as follicular cysts (Tibary and Anouassi, 1996). Follicle theca cysts have thin walls and fluctuate, while the contents are homogeneous and hypoechogenic (Ali *et al,* 2010a). Follicle lutein cysts or haemorrhagic cysts have thick, hard walls with non-homogenous and echogenic contents (Tibary and Anouassi, 2000). In llamas and alpacas, these oversized follicles may contain bloody fluid and are, therefore, termed haemorrhagic follicles that may become very large (up to 35 mm) and persist for a prolonged period (weeks) (Adams, 2007). The very large or haemorrhagic follicles do not appear to interfere with the growth of other smaller follicles on the same and contra-lateral ovaries and the ovarian activity may continue normally, thus, these large follicles do not constitute a major infertility problem in female camels (Tinson and McKinnon, 1992; Adams, 2007; Ali *et al,* 2010b; Skidmore, 2011). Although ovarian cysts have been described in dromedaries (El-Wishy, 1990; El-Khouly *et al,* 1990), the cystic ovary condition has not been well investigated as it has been in other domestic animals (Shawky *et al,* 2004; Ali *et al,* 2010a). In fact, the term "cystic ovaries" does not always apply to camels because a large proportion (30-40%) of females develop follicular cysts if not bred (Tibary *et al,* 2005). Ovarian cysts or oversized follicles have been found in the dromedary (El-Wishy, 1987; Tibary and Anouassi, 1996), bactrian camel (Bravo *et al,* 1993),

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llama and alpaca (Adams, 2007; Bravo *et al*, 1993). Incidence of ovarian cysts in female camels varied from 0.82 to 3.39 % (Musa, 1983; Omar *et al*, 1984; El-Wishy, 1989). The deficiency of luteinising hormone (LH) surge may be considered the main cause of cystic ovaries in camels (Jubb *et al*, 1993; Hegazy *et al*, 2004). Although some authors have suggested that ovarian cysts are a physiological variation of follicular dynamics (Tibary and Anouassi, 1997; Adams, 2007), others have claimed that camel ovarian cysts are pathological (Shawky *et al*, 2004; Ali *et al*, 2010a).

The objective of the current investigation was to study the behavioural, hormonal and histopathological alterations that accompanied the oversized follicles in the dromedary camels.

Materials and Methods

Animals and sampling

Twenty-six infertile female dromedaries (*Camelus dromedarius*) of 5-15 years of age, admitted to the Veterinary Teaching Hospital, King Faisal University, Kingdom of Saudi Arabia during the breeding season (November-April), were used in this study. Detailed previous breeding history and behaviour of the female camels were obtained. These females lived in groups with other female camels of different ages and near a mature male. A complete gynaecological examination of female dromedaries was performed via palpation and transrectal ultrasonographical (ALOKA SSD-500, Tokyo, Japan) examination (Anouassi and Tibary, 2013). These females had ovaries bearing oversized follicles of different diameters without any other ovarian structures like corpus luteum or normal–sized follicles. A 10 ml blood sample was collected via the jugular vein from each female into non–heparinised tubes and was centrifuged at 1500 g at 4°C for 10 min. Serum was harvested and stored at -20°C until analysis.

A total of 33 ovarian pairs were recovered from non-pregnant adult (7- 16 years of age) female camels (*Camelus dromedarius*) at a local abattoir in Al-Ahsa, Kingdom of Saudi Arabia. These paired ovaries were devoid of corpora lutea. Information about the reproductive status of these females was not available. A 10 ml blood sample was collected from each animal during slaughter. Immediately after collection, ovaries and blood samples were kept in an icebox and transported to the laboratory within one hour post-slaughter. Upon arrival at the laboratory, ovaries were washed twice in cooled 0.9% NaCl and left to dry. Two different follicle classes, based on follicle diameter (measured by Vernier caliper), were considered for puncture: oversized follicles of > 20 mm in diameter (n=21; Tibary and Anouassi, 1997) and preovulatory-sized follicles (dominant follicles according to the E2/P4 ratio) of 15-17 mm in diameter (n=12; Tinson and McKinnon, 1992). Follicular fluids were aspirated from all follicles using sterilised 22 gauge hypodermic needles and syringes. The follicular fluid and blood samples were centrifuged at 1500g at 4°C for 10 min. The supernatant was harvested and stored at -20°C pending analysis. Following aspiration, the oversized and preovulatory-sized follicles were sectioned and the obtained tissues were placed in 10% buffered formalin (Brandt and Manning, 1969) and processed for histopathological examination using paraffin wax. Four µm sections were cut and stained with haematoxylin and eosin (Schlafer, 2007).

Estimation of hormones and Nitric Oxide (NO) concentrations in serum and follicular fluid

Blood serum and follicular fluids progesterone (P_4) (pg/ml) were determined using EIA kits (Cayman Chemical Company, Ann Arbor, USA, Item No. 582601). The coefficients of variance (CV's) of the intra- and inter-assay were 7.3% and 16.4%, respectively. Oestradiol (E2) (pg/ml) was analysed by EIA kits (Cayman Chemical Company, Ann Arbor, USA, Item No. 582251). The CV's of the intra- and inter-assay were 7.4% and 10.7%, respectively. Testosterone (T) (pg/ml) was estimated using EIA kits (Cayman Chemical Company, Ann Arbor, USA, Item No. 582701). The CV's of the intra- and inter-assay were 4.4% and 7.7%, respectively. Human insulin like growth factor 1 (IGF-1; ng/ml) was assayed using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, USA, Catalog No. DG100, SG100, PDG100). The intra- and inter-assay CV's were 4.3% and 7.5%, respectively. $PGF_{2\alpha}$ (pg/ml) was analysed by EIA kits (Cayman Chemical Company, Ann Arbor, USA, Item No. 516011). The CV's of the intra- and inter-assay were 9.4% and 12.5%, respectively. NO (µM) was determined using Nitrate/Nitrite Fluorometric Assay kits (Cayman Chemical Company, Ann Arbor, USA, Item No. 780051). All assays were performed according to the manufacturer's directions, and the optical densities were measured using an ELISA Absorbance Microplate Reader (ELx 800TM, BioTek®, Highland Park, VT, USA) and Microplate Strip Washer (ELx800 TM, BioTek®, Highland Park, VT, USA).

Statistical analysis

Data are presented as means ± SEM. The female behaviour, hormones and NO were analysed

by analysis of variance (ANOVA). The follicular fluid hormones and NO of oversized follicles and preovulatory follicles was compared by Student's *t-test* using SPSS statistical software program (2013), version 22.0.

Results

Table 1 showed that the classification of female camel behaviour coincided with the presence of oversized follicles. This classification revealed: (a) Repeat breeder female camel (female camel failed to conceive from 3 or more regularly spaced services in the absence of detectable abnormalities; Gustafsson and Emanuelson, 2002) with low–pitched male guttural humming sound and her ovary bearing thin wall (< 1 mm) oversized follicle (RB thin; n=10, 38.4%), (b) Repeat breeder female camel with low–pitched male guttural humming sound and her ovary bearing thick wall (> 1 mm) oversized follicle (RB thick; n=4, 15.4%; Fig 1), (c) Female camel experiencing nymphomania (abnormally excessive and uncontrollable sexual desire by a female) with low–pitched male guttural humming sound and her ovary bearing thin wall (< 1 mm) and oversized follicles (Nympho thin; n=8, 30.8%; Fig 2) and (d) Anestrous female camel showing signs of pregnancy (curls her tail dorsally) with low–pitched male guttural humming sound and her ovary bearing thick wall (> 1 mm) and oversized follicles (Anest thick; n=4, 15.4%; Fig 3). The male sound was expressed all the time by female camels. The diameter of oversized follicles ranged between 2.50 and 5.22 cm.

The mean concentrations of serum hormones and NO in female dromedaries that had oversized follicles are presented in table 1. Serum P_4 concentrations in RB thin and Nympho thin camels were significantly (P<0.05) lower than that in Anest thick camels. Serum E_2 concentration was significantly (P<0.05) higher in Nympho thin camels than in Anest thick camels. In Nympho thin camels, serum T and PG $F_{2\alpha}$ concentrations were significantly (P<0.05) higher than both T concentration in RB thin camels and $PGF_{2\alpha}$ concentration in RB thick camels. Serum NO concentrations in RB thin camels were significantly (P<0.05) higher than that in both RB thick and Anest thick camels (Table 1). The comparison of mean concentrations of hormones and NO in follicular fluid between slaughtered female dromedaries having either oversized or preovulatory follicles is presented in table 2. The mean concentrations of P_4, E_2, and NO in follicular fluid of the preovulatory follicles were significantly (P<0.05) higher than that in the oversized follicles. The diameter of oversized follicles in slaughtered female dromedaries ranged between 2.30 and 7.20 cm.

Histopathology of oversized follicles in slaughtered female dromedaries showed either thick or thin walled oversized follicles. Thick–walled oversized follicles appeared when the upper layer of the cystic wall was necrotic, granulosa cells were nearly absent, and the luminal contents of fibrinous strands and bands that attached to the wall were excessive (Fig 4). Thin–walled oversized follicles appeared with highly wrinkled or wavy cystic wall with excessively congested vasculature in the granulosa cell layer and underlying fibrous theca layers (Fig 5) or appeared with the granulosa cells nearly absent, with congestion and haemorrhages from the superficial small blood capillaries (Fig 6).

Table 1. Female camel behaviour in relation to types of oversized follicles and serum concentrations (mean ± SEM) of reproductive hormones and nitric oxide.

Serum parameters	Female behaviour and types of oversized follicles			
	Repeat breeder with low-pitched male guttural humming sound and thin wall oversized follicles (n=10) 38.4%	**Repeat breeder with load male guttural humming sound and thick wall oversized follicles (n=4) 15.4%**	**Nymphomania with low-pitched male guttural humming sound and thin wall oversized follicles (n=8) 30.8%**	**Anestrus (signs of pregnancy that curls her tail dorsally) with low-pitched male guttural humming sound and thick wall oversized follicles (n=4) 15.4%**
Progesterone (pg/ml)	$1411.50^{a} \pm 93.39$	$2207.50^{ab} \pm 113.29$	$1710.00^{a} \pm 107.74$	$2532.50^{b} \pm 107.74$
Oestradiol (pg/ml)	$0.47^{a} \pm 0.09$	$0.45^{a} \pm 0.12$	$0.97^{a} \pm 0.31$	$0.30^{b} \pm 0.08$
Testosterone (pg/ml)	$17.20^{a} \pm 3.63$	$44.00^{ab} \pm 6.12$	$39.75^{b} \pm 4.85$	$15.50^{ab} \pm 5.10$
IGF-1 (ng/ml)	189.00 ± 15.45	116.65 ± 46.27	179.25 ± 6.26	208.00 ± 1.22
Prostaglandin $F_{2\alpha}$ (pg/ml)	$211.34^{ab} \pm 34.43$	$77.65^{a} \pm 7.90$	$173.93^{b} \pm 9.75$	$105.75^{ab} \pm 2.96$
Nitric oxide (µM)	$2.49^{a} \pm 0.03$	$1.91^{b} \pm 0.02$	$2.37^{ab} \pm 0.10$	$2.06^{b} \pm 0.07$

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

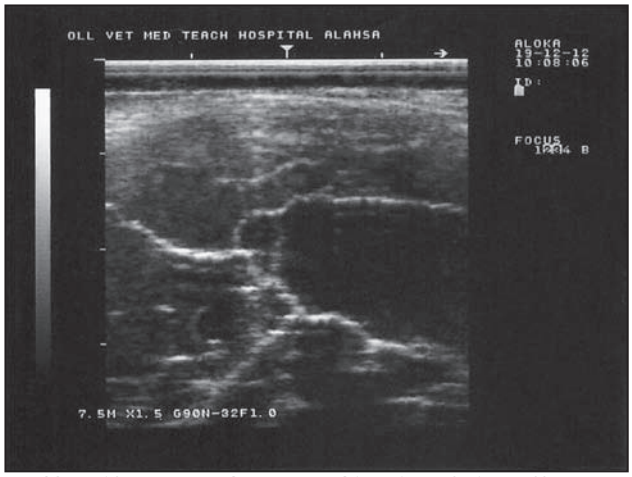

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Fig 1. Ultrasonography of oversized follicle (5.40 cm diameter) with thick wall and fibrinous strands.

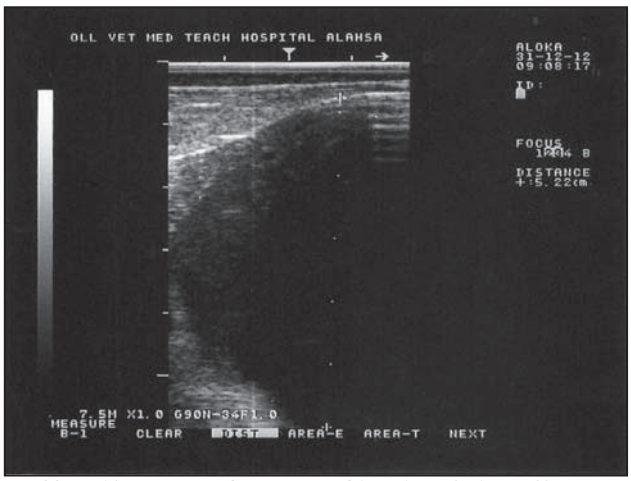

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Fig 2. Ultrasonography of oversized follicle (5.22 cm diameter) with thin wall and hypo-echoic contents.

Discussion

In the present study, the percentage of repeat breeder female camels with oversized follicles was 53.80%. On the same basis, repeat breeder syndrome has been recorded in 66.67% of female dromedaries bearing ovarian cysts (Ali *et al,* 2010a). Thin–walled oversized follicles were 69.20%. In a previous study, follicular cysts were recorded as 53.06% (Shawky *et al,* 2004). Female camels bearing thick–walled oversized follicles on their ovaries were 30.80% of studied oversized follicles. However, luteal cysts constitute 10.20% of ovarian cysts in camels (Shawky *et al,* 2004). Female dromedaries with oversized follicles and signs of pregnancy (15.40%) showed a dorsal curling up of their tails. However, this response could be also observed in animals with progesterone secreting cysts (Monaco *et al,* 2015).

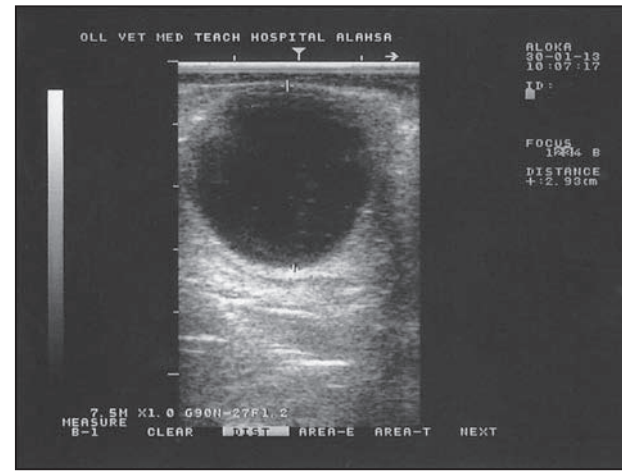

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Fig 3. Ultrasonography of oversized follicle (2.93 cm diameter) with thick wall and hypo-echoic contents.

In the present study, the repeat breeder female camels having thick–walled oversized follicles with fibrous strands on their ovaries constituted 15.40% of studied large follicles. Follicles that grow beyond 2.0 cm (4.0 – 6.4 cm) in diameter do not ovulate, but these follicles develop echogenic strands of fibrin as they degenerate (Skidmore *et al,* 1996; Skidmore, 2011). In contrast to our previous study it seems probable that these oversized follicles were pathologic and appeared to interfere with the growth of other follicles, constituting a major infertility problem in female camels in the forms of repeat breeding, nymphomania and anestrous (Ghoneim *et al,* 2013).

In the current study, serum P_4 concentrations in RB thin and Nympho thin camels were lower than that in Anest thick camels. However, serum P_4 concentrations are higher in the dromedary follicular cyst than in the luteal cyst (3.27 Vs 1.66 ng/ml, respectively; Hegazy *et al,* 2004). Lower values of

Table 2. Concentrations (mean ± SEM) of reproductive hormones and nitric oxide in follicular fluid from female camels bearing either oversized follicles or preovulatory follicles.

Parameters	Abattoir oversized follicular fluid (n=21)	Abattoir preovulatory follicular fluid (n=12)
Progesterone (pg/ml)	$2409.72^{a} \pm 64.37$	$2638.00^{b} \pm 27.10$
Oestradiol (pg/ml)	$522.69^{a} \pm 100.22$	$874.09^{b} \pm 28.53$
IGF-1 (ng/ml)	124.67 ± 8.98	150.36 ± 12.56
Prostaglandin $F_{2\alpha}$ (pg/ml)	386.97 ± 82.69	382.09 ± 91.05
Nitric oxide (µM)	$1.85^{a} \pm 0.12$	$3.91^{b} \pm 0.14$

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

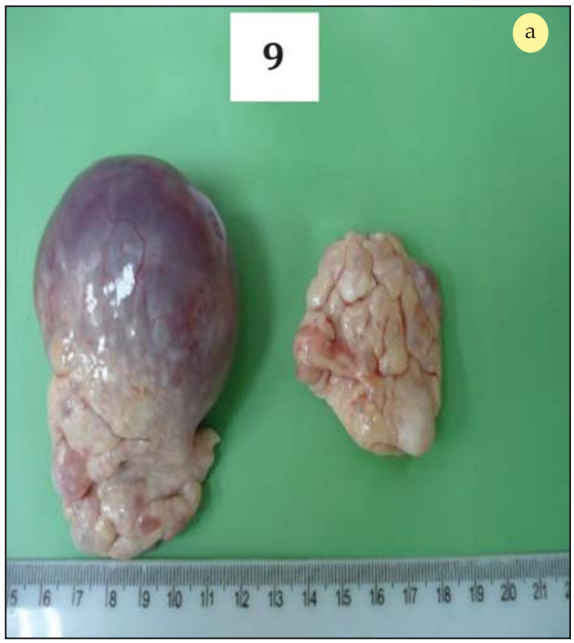

Fig 4a. Left ovary bearing oversized follicle appeared thick wall, bloody and multi-cavities with 5.7 cm in diameter. Right ovary has no structure.

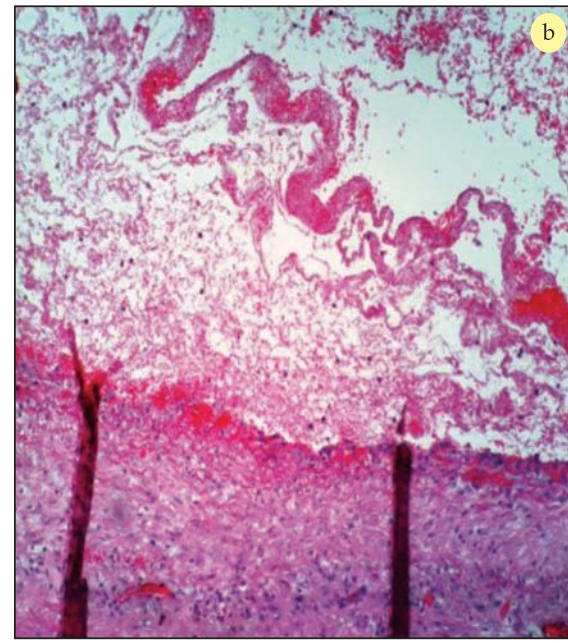

Fig 4b. Histopathology of left ovarian bloody oversized follicle (5.7 cm): The upper layer of the cystic wall appeared necrotic and nearly absence of granulosa cells and excess of luminal contents of fibrinous strands and bands that attached to the wall. H and E X=250.

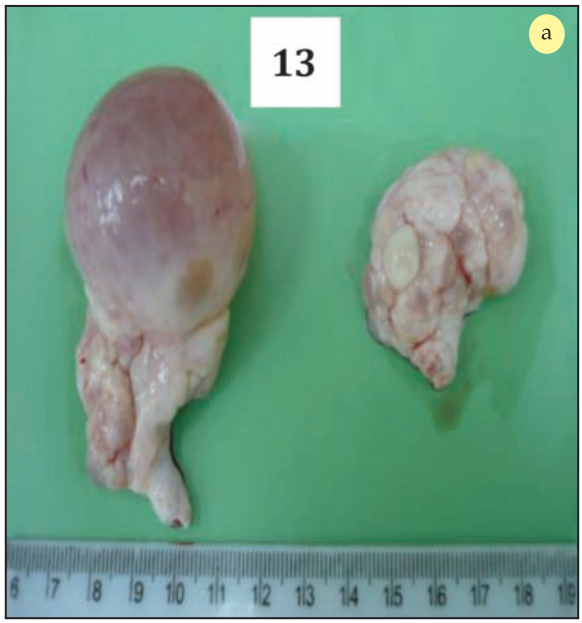

Fig 5a. Left ovary bearing oversized follicle appeared thin wall with 4 cm in diameter and light red. Right ovary has no structure.

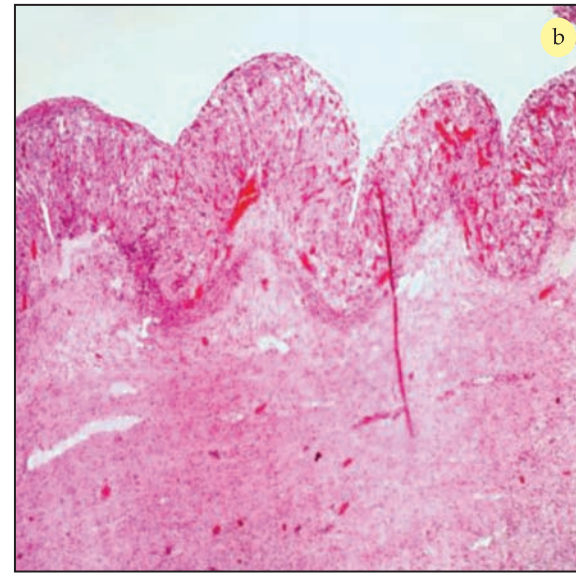

Fig 5b. Histopathology of thin walled and light red oversized follicle (4 cm) in left ovary: Highly wrinkled or wavy cystic wall appeared with excess of congested vasculature in the granulosa cell layer and underlying fibrous theca layers. H and E X=63.

serum P_4 concentrations have been recorded (0.0089 Vs 0.0093 ng/ml, respectively) for follicular and luteal cysts (Hussein *et al,* 2008) and for oversized follicles (0.53 ng/ml) (Ghoneim *et al,* 2013) in female camels. However, in sows, there is no effect of growing or decreasing number of ovarian cysts on concentrations of plasma P_4 (Szulanczyk-Mencel *et al,* 2010). In the follicular fluid, P_4 concentrations from oversized follicles were lower than that from the preovulatory follicles. However, this difference has not been found between the concentrations of P_4 in follicular fluid from cyst-like follicles and preovulatory follicles (Ghoneim *et al,* 2013). Nevertheless, in buffaloes, greater concentrations of P_4 have been reported in the cysts than normal preovulatory follicles (Goralczyk *et al,* 1992). In sows, the cystic fluid of animals with

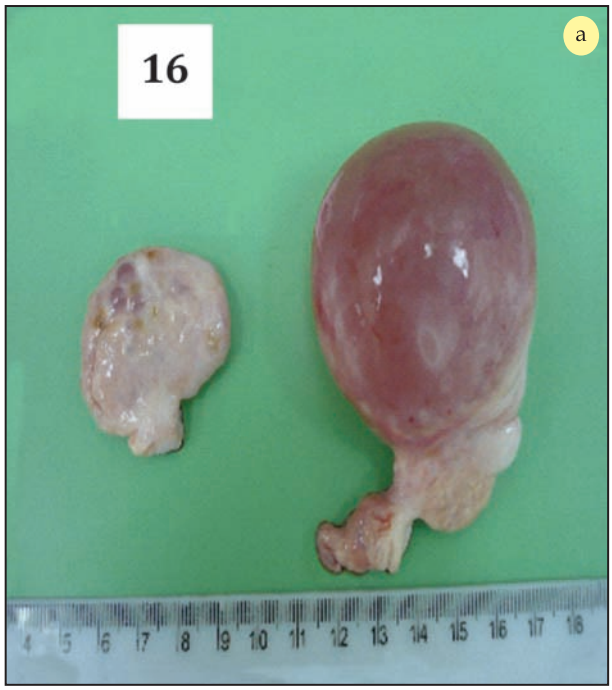

Fig 6a. Left ovary has no structure. Right ovary is an oversized follicle 5.3 cm in diameter, thin wall and reddish color.

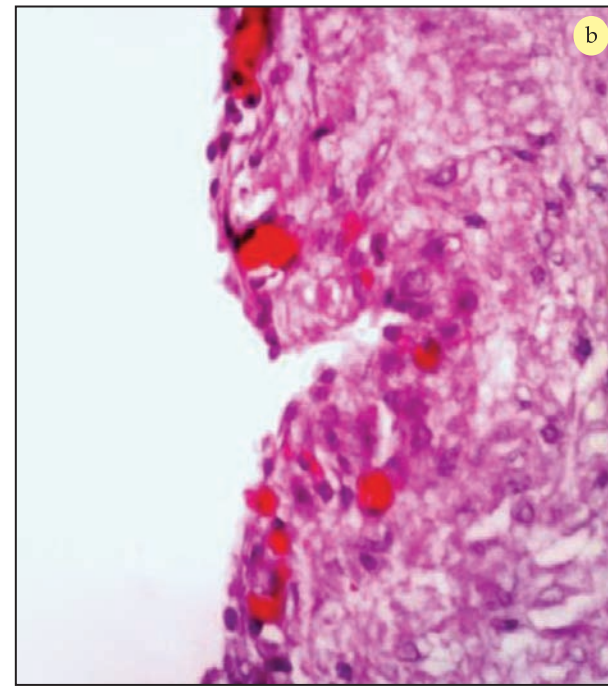

Fig 6b. Histopathology of thin walled and reddish oversized follicle (5.3 cm) in the right ovary: Nearly absence of the granulosa cells with congestion and haemorrhages from the superficial small blood capillaries. H and E X= 400.

oligocystic ovaries had a significantly (P< 0.001) higher P_4 concentration in comparison to polycystic animals (Ebbert *et al,* 2007).

In this study, serum E_2 concentration was higher in Nympho thin than Anest thick camels. However, there are no significant differences of serum E_2 between dromedary animals which have cyst-like follicles and those bearing preovulatory follicles on their ovaries (Ghoneim *et al,* 2013). Moreover, there is no line of demarcation in serum E_2 concentrations between female camels having either follicular or luteal cysts (Hegazy *et al,* 2004; Hussein *et al,* 2008). Previous studies reported no difference between blood E_2 concentrations of cystic and normal estrus in both cows (McNatty *et al,* 1984) and sows (Szulanczyk-Mencel *et al,* 2010). Follicular fluid E_2 concentrations from the preovulatory follicles were higher than those from oversized follicles. Parallel findings have been recorded in dromedary camels (Ghoneim *et al,* 2013) and cattle (Glencross and Munro, 1974; Gustafsson and Emanuelson, 2002). However, there is no significant variation in the concentration of E_2 between cystic and preovulatory follicles in buffalo (Goralczyk *et al,* 1992). Ovaries of cows with COD exhibited altered estrogen receptors expression compared with that in normal animals (Salvetti *et al,* 2007). In buffaloes with ovarian cysts, serum estrogen is significantly (P<0.5) increased, while progesterone is significantly (P<0.5) decreased (El-Sakkar *et al,* 2008).

In the present work, serum T and $PGF_{2\alpha}$ concentrations were significantly (P<0.05) higher in the Nymph thin camels than in both T concentration in RB thin and $PGF_{2\alpha}$ concentration in RB thick camels. Large amounts of P_4 are produced from granulosa and theca cells of bovine follicles which serve as a precursor for androgen and subsequently estrogen production (Homeida *et al,* 1988). The production of proteolytic enzymes is enhanced by P4 that promote the rupture of follicles at ovulation (Skidmore *et al,* 1994). In sows, T and E_2 levels in plasma and in cystic fluid of polycystic animals were significantly (P<0.01) higher in comparison to oligocystic animals, while P_4 concentration was almost the same (Szulanczyk-Mencel *et al,* 2010). In oligocystic ovaries, T in cysts exceeded the E_2 levels, whereas in polycystic ovaries the situation is reversed (P < 0.001; Ebbert *et al,* 2007).

In the present study, serum NO concentration in RB thin camels was higher than that in both RB thick and Anest thick camels. A decrease in serum NO concentrations are found in infertile cows affected with ovarian cysts (Mutlag *et al,* 2015). Serum NO values are low (P<0.01) in buffalo-cows suffering from parasitic infestation as compared to healthy animals (El-Khadrawy *et al,* 2008). The mean concentrations of NO in follicular fluid of the preovulatory-sized follicles were higher than that in

the oversized follicles. In buffaloes, follicular cysts were characterised by greater (P<0.01) concentrations of NO and P_4 than that of preovulatory-sized follicles (Khan *et al,* 2011). These greater P_4 concentrations inhibit the onset of LH surge resulting in the formation of follicular cysts. In addition, it declares the role of intra-ovarian regulators, such as NO, in development of the condition (Khan *et al,* 2011). Nitric oxide was found to be involved in the formation of hCG-induced murine follicular cysts (Nemade *et al,* 2002) and in the pathophysiology of polycystic ovary syndrome in rats (Hassani *et al,* 2012). Ovulation is a physiological process that depends on the coordinated activity of gonadotropins and steroid hormones, as well as inflammatory mediators such as NO (Khodaei *et al,* 2009). Histopathology of studied oversized follicles showed necrosis of the follicle wall, the near absence of granulosa cells, and excess luminal contents of fibrinous strands that attached to the wall. Sometimes, congestion and haemorrhages occur from the superficial blood capillaries. Histological studies of large follicles (>3 cm diameter) revealed that the granulosa cells had degenerated and become reduced to a single layer and the thecal layer thinned and became less distinct from the adjacent stroma (Skidmore, 2011). In buffaloes, the examined ovaries showed either thick walled follicular cysts or leutein cysts with luteinised granulosa cells, hyperplastic theca-interna and theca-externa besides atretic follicles and hyperplastic lining of other follicles (El-Sakkar *et al,* 2008).

In female dromedaries, the oversized follicles were accompanied with infertility problems in the form of repeat breeding, nymphomania and anestrus. Although, the peripheral blood reproductive hormones and NO concentrations were affected by the presence of the oversized follicles, the follicular fluid concentrations of P_4, E_2 and NO were significantly higher in the preovulatory-sized follicles than in oversized follicles.

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OESOPHAGOSCOPY AND ENDOSCOPIC AIDED REMOVAL OF OESOPHAGEAL FOREIGN BODIES IN CAMEL CALVES (*Camelus dromedarius*)

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ABSTRACT

In this study, endoscopy was used to confirm a presumptive diagnosis of oesophageal foreign body in 8 camel calves and it helped retrieval of the foreign bodies by an alligator forceps. The main clinical signs observed were regurgitation of food and liquids immediately after feeding, hypersalivation and inappetance. All procedures were performed under sedation. Endoscopic removal of the oesophageal foreign bodies using alligator forceps was found safe and effective.

Key words: Alligator forceps, camel, endoscopy, foreign bodies, oesophagus obstruction

Oesophageal disorders are relatively uncommon in large animals (Marzok *et al,* 2015). Oesophageal obstruction in ruminants is relatively frequent (Singh and Maghrabi, 1993; Marzok *et al,* 2015). Dry feed, rags, wool balls, polythene bags and rubber balls were the most common causative agents that have been reported in camels (Ramadan and Abdin-Bey, 1990). Ingestion of foreign bodies causing oesophageal obstruction could be attributed to pica and craving appetite (Singh and Maghrabi, 1993). Diagnosis of oesophageal obstruction is achieved in systematic manner. External palpation of the cervical region may be used to confirm the presence of an object lodged in the cervical oesophagus (Haven, 1990). Additional diagnostic tools besides the clinical signs and external palpation, may help to determine the location of an obstruction; these include oral examination, probangs or stomach tubes, oesophageal ultrasonography, oesophageal endoscopy and radiography of the cervical and thoracic oesophagus (Marzok *et al,* 2015). In comparing to ultrasonography, the endoscopic examination has the advantages of examining the oesophageal mucosa (Stierschneider *et al,* 2007). Most cases of oesophageal obstruction should be treated as an emergency as increased pressure on the oesophageal mucosa by the foreign object is likely to cause a tissue damage with consequent formation of scar tissue, stenosis, stricture and even oesophageal perforation (Feige *et al,* 2000). The high rate of complications associated with conventional oesophageal surgery was attributed to many factors. The latter include the lack of a serosal layer, physical trauma caused by food deglutition, reverse peristalsis and the nature of the segmental blood supply of the oesophagus (Meagher and Mayhew, 1978). Incisional dehiscence, stricture formation, cellulitis, oesophageal diverticulum formation, the development of fistulae and the resultant anorexia and failure to gain weight are the main post-operative complications associated with an oesophagotomy (Church *et al,* 1972; Ruben, 1977; Haven, 1990; Smith *et al,* 2008b).

In man, endoscopic removal of oesophageal foreign bodies is the gold standard and most interventions are done without major complications (Shafique *et al,* 2013). A flexible endoscope is the mainstay as it permits direct visual evaluation of foreign objects and allows assessment of oesophageal mucosa and its integrity (Arantes *et al,* 2009). However, only about 10%–20% of cases of oesophageal foreign bodies require endoscopic removal, while less than 1% of the cases require surgery for foreign body extraction and or to treat complications (Telford, 2005; Ambe *et al,* 2012). An endoscope and a forceps are generally used to visualise and remove the oesophageal foreign bodies (Seo, 1999). Flexible endoscopy is the best diagnostic and therapeutic approach for the management of foreign bodies and food bolus impaction in the upper gastrointestinal tract, with a success rate greater than 95% (Chen

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et al, 2013; Dray and Cattan, 2013; Sugawa *et al,* 2014). Endoscopic assisted removal of foreign bodies causing oesophageal obstruction in camel has not been reported previously. This study was designed to remove oesophageal foreign bodies with an alligator forceps under endoscopic guidance in camel calves.

Materials and Methods

Animals

Eight (3 females, 5 males) camel calves (*Camelus dromedarius*) aging between 14 to 120 days and body weights from 32 to 80 kg were presented to the Veterinary Teaching Hospital, College of Veterinary Medicine, King Faisal University with common clinical signs of salivation, dysphagia, regurgitation and swelling in the neck area. All animals were subjected to clinical and endoscopic examinations.

Instruments

A flexible endoscope (VetVu, a unit of Swiss Precision Products) with 8mm diameter, 110 cm long supported with an insufflation system, light source and irrigation system was used in all camel calves. To protect the endoscope from damage, a Gunther's mouth Gag (Eickeymeyer, Germany) was used to keep the oral cavity open during endoscopy procedures. A surgical suction unit (New Askir, Italy) was used to aspirate the oesophageal fluid. A grasping alligator forceps (Eickeymeyer, Germany) with 80 cm long arms (Fig 1).

Oesophagoscopy procedure

Oesophagoscopy was performed with the animal secured over a table in sternal recumbency position after sedation with xylazine (Rompun; Bayer Health Care) at the dose of 0.1mg/kg body weight. A mouth gag was placed to keep the oral cavity open in order to facilitate the entrance of the endoscope. After good restraint of the head and the neck, the endoscope was inserted via oral cavity, pharynx into the oesophagus. An insufflation and irrigation system of the endoscope were used to optimal visibility during the examination. In some animals, suction system was used to aspirate the fluid located in the oesophagus. The mucosal lumen of the oesophagus was evaluated for the abnormal findings and contents.

Removal procedure

The upper airway and the oesophagus were endoscopically examined through mouth using the video-endoscope. The endoscope was inserted through the gag into the mouth and oesophagus to identify the foreign bodies and distance from the mouth opening to foreign body was measured. The obstructive area in oesophagus was washed in some cases through the channel of the endoscope using 50 ml NaCl 0.9% solution to visualise the foreign body. The fluid was injected through the noses of endoscope and regained using fluid suction device. Thereafter, the alligator forceps was inserted to grasp the end of the foreign body and it was removed smoothly (Figs 2, 3). The procedure was repeated as many times as needed depending on the type, size and location of foreign body. Final confirmation of complete removal of the foreign body mass was made by flushing 300 ml of normal saline through the mouth.

All patients received a five day course of penicillin and streptomycin (Norbrook Laboratories, UK) at a dose rate of 1 ml/25kg body weight, Flunixin meglumine (MSD, Germany) was given intravenously at a dose rate of 2ml/50 kg body weight for three days. The camel calves were given access to suckle milk or food after 12 hours. Follow-up information was obtained for period of successive two months via telephone communication with owners.

Results

Seven out of the 8 examined animals were younger than 3 months and showed complete obstruction in the cervical oesophagus. Most of the removed foreign bodies were plastic bags (50%), clothes pieces (25%) and plastic bags mixed with sand (25%) as shown in Table 1 and Figs 4, 5, 6.

Moreover, we noticed that the location of the foreign body in the younger animals (<1 month) was in the upper third part of the neck and its distance from the mouth opening was about 60 cm, while in the elder animals (>1month) it was in the middle third of the neck and its distance was about 80 cm. The length of the removed foreign bodies ranged 16-25 cm. In most cases (87.5%) there was a complete oesophageal obstruction hence big amount of fluid accumulation proximal to the foreign body was seen. Oesophageal mucosal ulceration was observed endoscopically after removal of plastic foreign bodies.

Discussion

Despite its wide use in small animals and equine practice, oesophageal endoscopy reports in camels are very scarce (Ramadan, 2016). Oesophagoscopy is a valuable, non-invasive imaging procedure in ruminants (Franz and Baumgartner, 2002). Furthermore, conventional oesophageal surgery harbours a high rate of complications and many factors have been implicated (Haven, 1990; Ramadan, 2016). Endoscopic examination was useful to confirm the location of the

Table 1. Summary data for 8 calf camels (5 males, 3 females) with oesophageal obstructions.

Variable	Sex		Age		
	Male	Female	<1 month	1-3 Month	4 month
Type of obstruction					
Complete	5 (62.5%)	2 (25%)	4 (50%)	3 (37.5%)	0 (0%)
Incomplete	0 (0%)	1 (12.5%)	0 (0%)	0 (0%)	1 (12.5%)
Type of the foreign body					
Cloth pieces	2 (25%)	0 (0%)	1 (0%)	1(12.5%)	0 (0%%)
Plastic bags	2 (25%)	2 (25%)	2 (37.5%)	2 (25%)	1 (12.5)
Plastic bags with food materials or sand	1 (12.5%)	1 (12.5%)	1 (12.5%)	1 (0%)	0 (0%)
Total	5 (62.5%)	3 (37.5%)	4 (50%)	3 (37.5%)	1 (12.5%)

Per cent in all values is calculated from the total examined animals.

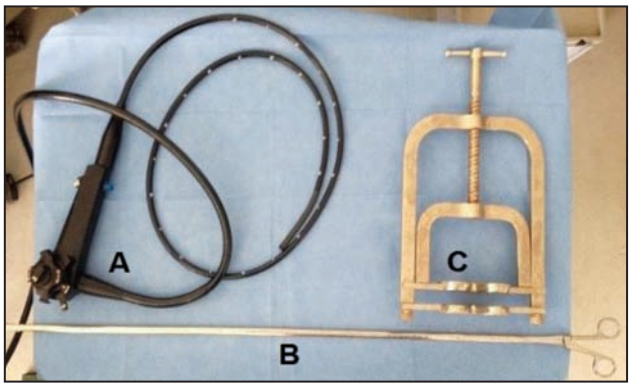

Fig 1. A: Endoscope, B: Alligator forceps, C: Gunther's mouth Gag.

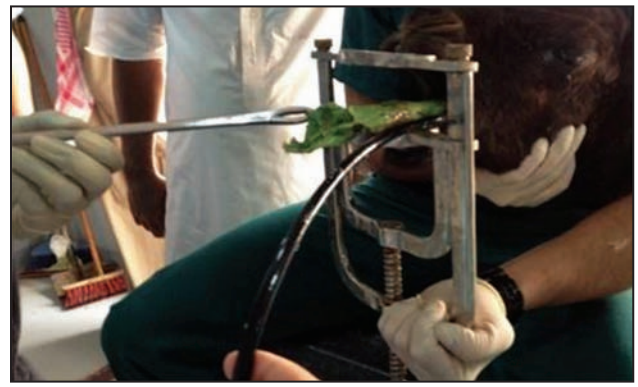

Fig 2. Endoscopic view showing a foreign body being grasped with an alligator forceps.

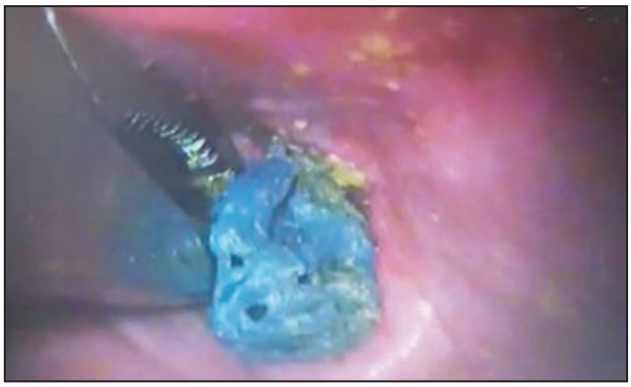

Fig 3. Performing removal of foreign body using alligator forceps under endoscopic guidance.

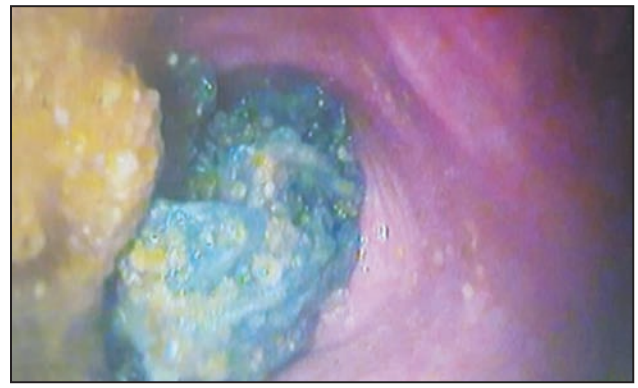

Fig 4. Foreign body (plastic bags) with sand in the cervical region of oesophagus of a 28 days old camel.

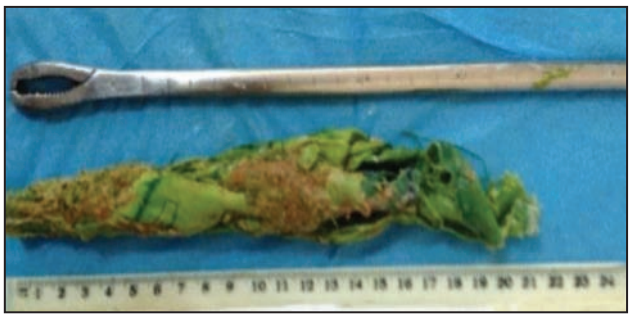

Fig 5. A cloth piece being retrieved from the oesophagus.

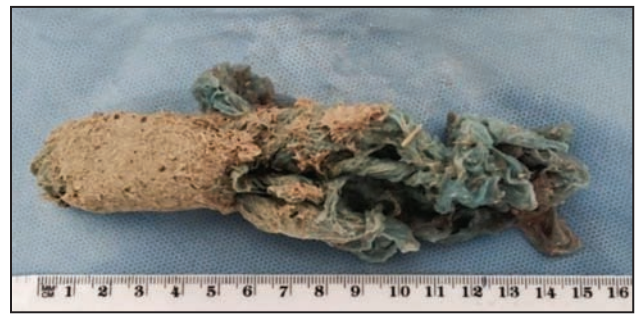

Fig 6. A plastic material mixed with food material retrieved from the oesophagus.

foreign bodies as well as the determination of abnormal anatomical changes which helps in the diagnosis and prognosis (Stierschneider *et al,* 2007). Dry feed, rags, wool balls, polythene bags and rubber balls were the most causes of oesophageal obstruction that have been reported in camels (Ramadan and Abdin-Bey, 1990). It is highly interesting that technique employs an alligator forceps under endoscopic guidance to remove oesophageal foreign body from camel calves. In the present study, it is surprising that most of the camel calves (87.5%) that had oesophageal obstruction were younger than 3 months, although these animals were suckling. The pre-dominant cause of oesophageal obstruction in this study was ingestion of plastic bags which could be present in the animal environment (Marzok *et al,* 2015). Most of obstructive objects in examined camel were either in the pharyngeal entrance (Smith, 2008a), cervical region or just at the thoracic entrance (Ramadan and Abdin-Bey, 1990). These anatomical locations ease their removal without surgical manipulation using alligator under endoscopic guidance (Ramadan and Abdin-Bey, 1990; Smith, 2008a).

Removal of most oesophageal foreign bodies were accomplished under sedation thus avoided risks associated with general anaesthesia in young animals (Gomez *et al,* 2014). Furthermore, the complications of oesophagotomy were also avoided (Haven, 1990; Ramadan, 2016).

In humans, fiberoptic-endoscopic management of oesophageal obstruction of the upper gastrointestinal tract was successful in 92% of food impactions and 76% of true foreign bodies (Vizcarrondo *et al,* 1983; Arantes *et al,* 2009). The success rates for endoscopic removal or dislodgement of oesophageal foreign bodies in dogs ranged from 26% to 85% (Gianella *et al,* 2009). Ruminal tympany was not observed in most oesophageal obstruction camel calves as these were yet to start rumination hence had a favourable effect on the prognosis of the recovery (Ramadan *et al,* 1986).

In conclusion, ingestion of foreign bodies is not uncommon in camels. However, in young camels, endoscopic removal of oesophageal foreign bodies using alligator forceps under endoscopic guidance was an effective and safe procedure.

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PROTECTIVE EFFECTS OF POLL GLAND SECRETION ON IMMUNOSUPPRESSED AND S180 TUMOUR-BEARING MICE

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ABSTRACT

The poll gland secretions (PGS) have been used traditionally for the treatment and prevention of many diseases for centuries in Inner Mongolia (China) and Mongolia. The present study was performed to evaluate the immunostimulatory activities and anti-tumour effects of PGS *in vivo* and *in vitro*. The concentration of TNF-α, IL-2, IL-6, IgG and IgM in the serum of experimental animals were measured by an enzyme-linked immunosorbent assay (ELISA) following the manufacturer's protocols and the spleen index and thymus index were calculated using the gravimetric method. The phagocytic activity of the macrophage monocytes was evaluated by a carbon clearance assay and the effect of PGS on the growth of S180 cells *in vitro* was examined by the determination of the IC50 of PGS. A moderate to high dose of PGS can elevate the spleen and thymus indices and significantly increase the serum concentrations of IL-6, IgG and TNF-α. Moreover, PGS can also enhance the phagocytic activities of macrophage monocytes in immunosuppressed experimental mice. On the other hand, PGS can significantly increase the concentration of serum TNF-α, IL-2 and IL-6 and directly inhibit the growth of solid tumours in mice. Additionally, PGS can also significantly inhibit the growth of S180 cells *in vitro*, with an IC50 of 15.63 $\mu g/ml^{-1} \pm 2.18$.PGS can significantly improve the inhibited immune function of mice induced by cyclophosphamide (CTX) and can reduce the growth of solid tumours *in vivo*. In addition, PGS can also directly inhibit the S180 cell's growth *in vitro*, as well as considerably enhance the immune function of tumour-bearing mice.

Key words: Anti-tumour effects, immunostimulatory activities, immunosuppressed mice, poll gland secretion, tumour-bearing mice

Drugs that are derived from animals have been widely used in traditional Mongolian medicine and play an important role in the treatment and prevention of certain diseases. Moreover, these drugs have historically made great contributions to mankind and some drugs still have pivotal medicinal values in modern medicine (Chen *et al,* 2004). Poll gland secretion (PGS), known as bokhi in Mongolia, is a drug of animal origin in traditional Mongolian medicine and has been commonly used in Inner Mongolia and Mongolia for the treatment and prevention of several diseases for centuries, such as uterine myoma and gastric cancer.

The poll glands are symmetrical bodies situated subcutaneously on the back of the neck and between the two ears of bull camels (Tingari and Rahma, 1981). They seem to get their name from their position in the poll region (Leese, 1927). They are present in male camels at birth and are mainly composed of sweat and sebaceous glands and no visible glands are observed in all of female camels at any age (Safwat *et al,* 2012). In addition, they are known to exhibit a cyclic activity, producing a yellowish watery secretion with a characteristics of offensive odour during the rutting season (Purohit and Singh, 1958; Lee and Schmidt, 1962; Singh and Bharadwaj, 1978; Yagil and Etzion, 1980; Taha *et al,* 1994). The glands then become atrophied during anestrus and completely shrinks in castrated bull camels. The PGS is composed of sexual hormones (e.g. progesterone, oestrogen and testosterone), as well as short chain fatty acids including acetic, propionic, isobutyric, butanoic and isopentanoic acids (Yagil and Etzion, 1980; Ayorinde *et al,* 1982; Tingari and George, 1984; Kumar and Agarwal, 1996; Rai *et al,* 1996; Rai *et al,* 1997; Yasuro *et al,* 1998). According to one report, PGS have a remarkable pheromone effect on the reproductive physiology of bull camels (Tingari and George, 1984) and attract females. The previous studies have primarily focused on the histological

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and histochemical characteristics of the poll glands, as well as ultrastructural features of the glands during the rutting and non-rutting seasons (Safwat *et al,* 2012; Tingari and George, 1984; Atoji *et al,* 1998). However, few scientific papers have been found regarding the pharmacological effects of PGS.

When PGS is used as an alternative medicine, it has a particularly important role in the treatment and prevention of many diseases, especially for certain types of tumours. These effects have been described for over 200 years in traditional Mongolian medicine, with precise therapeutic effects. Since decreased immune function is closely related to tumourigenesis and development, the immunosuppressive and tumour-bearing animal models will be established for this study. In addition, we will elucidate whether PGS possesses any positive effects on these animal models and whether it can directly inhibit the growth of solid tumour *in vivo* and S180 cells *in vitro*.

Materials and Methods

Chemicals and Reagents

Indian Ink (Xizhong, China); Cyclophosphamide (CTX, CPA) (Pude, China); ELISA kits for TNF-α, IL-2, IL-6, IgG and IgM (Boster Bioscience, China); thiazolyl blue (MTT) cell growth assay kits (Sigma); RPMI-1640 (Sigma); foetal bovine serum (TBD, China); and dimethyl sulfoxide (DMSO) (Gayload Slidell, USA).

Instruments

The primary instruments used throughout this study include, an ultraviolet–visible spectrophotometer (TU-1800PC, Persee, China); electronic analytical balance (Sartorius); Labconco Freeze Dry System/Freezone 2.5 (USA); cell counter (Cyt-1000, Japan); inverted microscope (ZXT1, Olympus); carbon dioxide incubator (Thermo forma371); automatic high pressure sterilising pot (HVE-50, Israel); multi-mode microplate reader (Synergy 4); and a refrigerated high-speed centrifuge (3-30K, Sigma).

Samples and preparation of the poll gland secretion extract

The PGS samples were collected from rutting bull camels in West Sonid, Inner Mongolia, China. The sample collection procedure was approved by the camel protection association (CPA) of Inner Mongolia for the control and supervision of experimental camels. Firstly, we sheared the hairs surrounding the poll gland, then held the poll gland with prepared gauze. When the secretion fully penetrated into the gauze, we collected the gauze and packed into sealed bags and sent them to the laboratory by cooler box. The gauze containing the secretion was steeped in 200 ml of distilled water at 37°C 3 times and then filtered. The filtration was lyophilised using the Labconco Freeze Dry System and the lyophilised PGS powder was collected.

Animals

All animal experimental procedures were performed in accordance with the guidelines of the Ethical Committee for the experimental use of animals at Inner Mongolia Agricultural University (Huhhot, Inner Mongolia, China).

Kunming mice (half male and half female, weighing 20.0±2.0 g) were provided by the Experimental Animal Centre of the Chinese Academy of Military Medical Science. The standard conditions for temperature and humidity along with the exposure to a 12h:12h light and dark cycle were maintained throughout the study. All mice were fed a standard rodent diet and were allowed to drink water *ad libitum*. Moreover, all animals were allowed to acclimatise to the experimental conditions for one week before beginning the study to minimise animal stress.

Cell lines

Mouse sarcoma S180 cell lines (ATCC-TIB66) were provided by the Chinese Academy of Military Medical Science. The S180 cell lines were maintained in the logarithmic phase of growth in the peritoneal cavity of the mice, as well as in RPMI 1640 medium supplemented with 100 IU/ml penicillin and 100 IU/ml streptomycin and 10% foetal bovine serum at 37°C under humidified air with 5% CO_2.

Induction of immunosuppressed mouse model and treatment with PGS

A total of 60 mice were randomly divided into 5 groups (n=12), with an equal number of males and females. Specific grouping and treatment protocols are presented in table 1.

Blood was collected from each animal *via* a retro-orbital puncture 24 h after the last administration of drugs and the serum was separated to detect the concentration of TNF-α, IL-6, IgM and IgG. After the blood collection, all of the mice were sacrificed, the spleen and thymus were harvested and weighed and their indices were calculated according to the following formula: the organ index = weight of organ (mg) /average body weight (g).

Table 1. Grouping of experimental mice and treatment protocols.

Groups	N	Treatments	
		Day 1~10	Day 11~14
Normal Control	12	PO NS 0.2ml $10g^{-1}$	IP NS 0.2ml $10g^{-1}$ + PO NS 0.2ml $10g^{-1}$
CTX Model	12	PO NS 0.2ml $10g^{-1}$	IP CTX 80 mg kg^{-1} + PO NS 0.2ml $10g^{-1}$
PGS Low	12	PO PGS 2.5 mg kg^{-1}	IP CTX 80 mg kg^{-1} + PO PGS 2.5 mg kg^{-1}
PGS Meddle	12	PO PGS 25 mg kg^{-1}	IP CTX 80 mg kg^{-1} + PO PGS 25 mg kg^{-1}
PGS High	12	PO PGS 250 mg kg^{-1}	IP CTX 80 mg kg^{-1} + PO PGS 250 mg kg^{-1}

Establishment of tumour-bearing mouse model and treatment with PGS

A mouse sarcoma S180 cell line was harvested and washed three times with sterilised normal saline (NS), then diluted with sterilised saline to a concentration of 1×10^7 cells/ml. Each mouse was subcutaneously inoculated into the right armpit region with 0.2 ml of the cell suspension on the first day of the experiment and the mice were randomly divided into 5 groups (n=12). The tumour model group was treated only with normal saline at 0.1ml/10 g; and the CTX-treated group was administered 20mg/kg body weight (bw) CTX. In addition, three experimental groups were treated with, i.e. a low-dose of 2.5mg/kg bw PGS; a middle-dose of 25mg/kg bw PGS; and a high-dose of 250mg/kg.bw PGS, respectively. Following 14 days of consecutive treatment of once per day, peripheral blood was collected from each mouse by a retro-orbital puncture 1h after the last administration and the serum was separated for the detection of TNF-α, IL-6, IL-6, IgM and IgG concentrations. Finally, all of the mice were sacrificed, the solid tumour was harvested from each mouse and weighed and the tumour inhibition rate was calculated according to following formula: the tumour inhibition rate = [(average tumour weight of tumour model group— average tumour weight of the experimental group) ÷ average tumour weight of the tumour model group]×100%.

Concentration of cytokines and immunoglobulins in the serum

The concentrations of TNF-α, IL-2, IL-6, IgG and IgM in the serum were determined by ELISA kits according to the manufacturer's instructions. The analytic sensitivities for these assays were 7.8 pg/ml for TNF-α, 15.6 pg/ml for IL-2 and IL-6), 0.3 µg/ml for IgG and 0.6 µg/ml for IgM, respectively.

The phagocytic activity of macrophage monocytes

Animal groups, drug delivery and the immunosuppressed mouse model are similar to mentioned above. The phagocytic activity of the macrophage monocytes was determined by the carbon clearance test. Briefly, 1h after the last dose of the drug administration, all of the mice were injected with 20% Indian ink via the coccygeal vein at a dose of 0.1ml/10 g.bw. A 20µl blood sample was collected at 2min and 20min, respectively following the injection of Indian ink, then mixed with 2 ml of 0.1% sodium carbonate solution. The absorbance of this solution was determined at 600nm by a UV spectrophotometer. The carbon clearance index k and the phagocytic index α of the macrophage monocytes were calculated using the following equation (Hafiz *et al,* 2016) :

$$k= (\lg OD_2 - \lg OD_{20}) / (T_{20} - T_2)$$

$$\alpha=\sqrt[3]{k} \times \text{body weight} / (\text{spleen weight}+\text{liver weight})$$

Direct inhibitory effect of PGS on the in vitro growth of tumour cells

The inhibition rate of the tumour cells *in vitro* following PGS treatments was performed using the MTT method (Wanpeng *et al,* 2013). The S180 cells during logarithmic growth were resuspended in serum-free complete RPMI-1640 medium to prepare a 1×10^7 cells/ml cell suspension and seeded into 96-well culture plates with 90 µl/well. All treatments were divided into 5 groups with 6 parallels in each group. A different concentration of the PGS solution was added into each experimental well to a final concentration of 1 µg ml^{-1}, 0.1 µg ml^{-1}, 0.01 µg ml^{-1}, 0.001 µg ml^{-1} and 0.0001 µg ml^{-1}, respectively. The same volume of serum-free complete RPMI-1640 medium containing the S180 cells was used as a control. The plates were then incubated for 48 h at 37°C under 5% CO_2. Next, the culture media was removed and 10 µl of the MTT solution (5 mg/ml) was added to each well. After an additional 4 h incubation, the formazan crystals were solubilised with 100 µl DMSO for 15 min. The absorbance at 570 nm was determined using a multi-mode microplate reader. The inhibition rate was calculated using the following formula:

The inhibition rate (%) = $(1 - A_{control}/A_{treated}) \times 100\%$.

Statistical analysis

All of the data in this study are expressed as the mean±standard deviation (SD). The data were evaluated by SAS 9.0 software using a one-way analysis of variance (ANOVA). The results were regarded to be statistically significant if the P value was < 0.05.

Results

The effect of PGS on the thymus index, spleen index and the phagocytic activity of macrophage monocytes in immunosuppressed mice

As shown in table 2, compared with the normal control group, the spleen index, thymus index and the phagocytic activities of the macrophage monocytes were remarkably decreased in the CTX model group ($P < 0.05$). However, a middle and a high dose of PGS were associated with a marked elevation of both spleen and thymus indices and the phagocytic activity of macrophage monocytes was also enhanced in the immunosuppressed mice. Therefore, a moderate to high dose of PGS can restore the suppressed immune function of the animals by promoting the growth of immune organs and enhancing the phagocytic function of macrophage monocytes.

Effect of PGS on serum cytokines and immunoglobulin concentrations in immunosuppressed mice

In table 3, the concentrations of IL-6, TNF-α, IgM and IgG were significantly decreased in the CTX model control group compared with the normal control group ($p < 0.05$). PGS can promote the production of IL-6 and TNF-α and their serum concentrations in immunosuppressed mice treated with middle dose and high dose of PGS reached the same or higher levels compared to the normal control group especially, the concentrations of IL-6. Additionally, the middle and high dose of PGS markedly increased the concentration of IgG in the immunosuppressed groups, while the effect of PGS on IgM levels was not statistically different. Therefore, an adequate amount of PGS can improve the suppressed immune function by promoting both cellular and humoral immune functions.

Table 2. Effect of PGS on thymus and spleen indices and macrophages phagocytic activity ($\bar{x}$±s, n=12).

Groups	Thymus Index (mg/g)	Spleen Index (mg/g)	Phagocytic Index (α)
Normal Control	2.86±0.34	3.95±0.59	6.278±0.485
CTX Model Control	1.36±0.26*	1.05±0.22*	5.540 ±0.701*
PGS Low	1.27±0.16*	0.95±0.14**	6.151±0.314
PGS Middle	1.47±0.19*	1.42±0.11$^{*\#}$	6.260 ±0.419$^{\#}$
PGS High	1.63±0.07$^{*\#}$	1.26±0.18$^{\#}$	6.651 ±0.562$^{\#}$

** Significant difference at $p < 0.01$ compared with the normal control group.
* Significant difference at $p < 0.05$ compared with the normal control group.
Significant difference at $p < 0.01$ compared with the CTX model control.
Significant difference at $p < 0.05$ compared with the CTX model control.

The effect of PGS on serum cytokines and immunoglobulin concentrations in tumour-bearing mice

The effect of PGS on the serum concentrations of IL-2, IL-6, TNF-α, IgM and IgG in tumour-bearing mice is presented in table 4 and Fig 1. Compared with the tumour model control, the IL-6, TNF-α and IL-2 concentrations in the serum of mice that received a high and middle dose of PGS were significantly increased. Additionally, some of these cytokines reached almost the same level as the CTX-treated group. These results indicate that a moderate to high dose of PGS can exert anti-tumour effects by significantly elevating pro-inflammatory cytokine and immunoglobulin concentrations in tumour-bearing animals.

In Fig 1, compared with the tumour model control, the concentrations of serum IgG in each group was not statistically different, except that

Table 3. The effect of PGS on cytokine and immunoglobulin concentrations in immunosuppressed mice ($\bar{x}$±s, n=12).

Groups	TNF-α ($pg.ml^{-1}$)	IL-6 ($pg.ml^{-1}$)	IgM ($\mu g.ml^{-1}$)	IgG ($\mu g.ml^{-1}$)
Normal Control	47.98±1.83	75.33±6.02	3.36±0.29	5.64±1.16
CTX Model Control	31.68±3.08*	63.25±5.59	2.82±0.32*	3.78±0.56*
PGS Low	35.88±8.09*	84.75±7.85$^{*\#}$	2.92±0.46*	3.24±0.77*
PGS Middle	36.68±7.04*	102.58±18.72$^{**\#\#}$	3.18±0.36	4.94±0.16$^{\#}$
PGS High	45.53±3.17$^{\#}$	100.5±9.49$^{**\#\#}$	3.10±0.13	5.42±0.82$^{\#}$

** Significant difference at $p < 0.01$ compared with the normal control group.
* Significant difference at $p < 0.05$ compared with the normal control group.
Significant difference at $p < 0.01$ compared with the CTX model control.
Significant difference at $p < 0.05$ compared with the CTX model control.

Table 4. The effect of PGS on serum TNF-α, IL-2 and IL-6 concentrations in tumour-bearing mice.

Groups	IL-6 (pg ml^{-1})	TNF-a (pg ml^{-1})	IL-2 (pg ml^{-1})
Tumour Model Control	91.83±2.56	98.56±2.80	104±19.15
CTX Treated	125.41±4.42**	196.33±10.33**	180.11±26.59**
PGS Low	99.75±4.08$^{\#}$	118.55±14.16$^{\#}$	114.72±16.03$^{\#}$
PGS Middle	115.33±7.01**	126.33±18.55$^{*\#}$	142.88±22.34*
PGS High	122.08±7.43**	165.22±21.35**	175.11±15.41**

** Significant difference at $p < 0.01$ compared with the normal control group.

* Significant difference at $p < 0.05$ compared with the normal control group.

Significant difference at $p < 0.01$ compared with the CTX model control.

Significant difference at $p < 0.05$ compared with the CTX model control.

the serum IgG content was significantly increased in the CTX treated group. However, the serum IgM concentration in the low dose PGS group was remarkably higher than that of tumour model control. Therefore, an adequate amount of PGS can promote IgM generation in tumour-bearing animals.

The effect of PGS on the growth of solid tumour in mice

The results of the effect of PGS on solid tumour weight gain and the inhibition rate are shown in fig 2. Compared with the tumour model control, the high dose of PGS remarkably inhibited the growth of solid tumour. The tumour weight decreased significantly, with a tumour inhibition rate of 48.5%. Therefore, these results suggest that PGS has a notable inhibitory effect on solid tumour in S180 sarcoma tumour-bearing mice.

Inhibitory effect of PGS on the growth of tumour cells in vitro

The cell culture time and concentration of the cell suspension was determined to be 48 h and 1×10^7/ml, respectively, according to the results of the preliminary test. The results indicated that PGS can inhibit the growth of S180 sarcoma cells *in vitro* in a dose-dependent manner (Fig 3). In addition, the half maximal inhibitory concentration (IC_{50}) was 15.63±2.18 (μg ml^{-1}), less than 30 μg ml^{-1}. Therefore, PGS has a significant direct inhibitory effect on the growth of S180 sarcoma cells *in vitro*.

Discussion

A large number of therapeutic agents derived from animal sources in traditional medicine have been used to prevent and treat various types of disease and to improve the immune functions (Choi *et al,* 2006). The poll gland is a special gland only seen in male camels and the secretions of the poll gland play a critical role in camel reproductive physiology with respect to pheromone and hormone production. Previous studies have primarily focused on the histological and histochemical characteristics of the poll glands, as well as on the ultrastructural changes of the poll glands during the rutting and non-rutting seasons of bull camels (Safwat *et al,* 2012; Tingari and George, 1984; Rai *et al,* 1996; Atoji *et al,* 1998) and the chemical components of the secretions (Ayorinde *et al,* 1982; Kumar and Agarwal, 1996). But no report has elucidated the immunostimulatory activities and anti-tumour properties of PGS. However, PGS has not only historically used in traditional Mongolian medicine for the treatment of many diseases, but is also used in modern Mongolian medicine for adjuvant therapy of immunosuppressed patients with certain types of

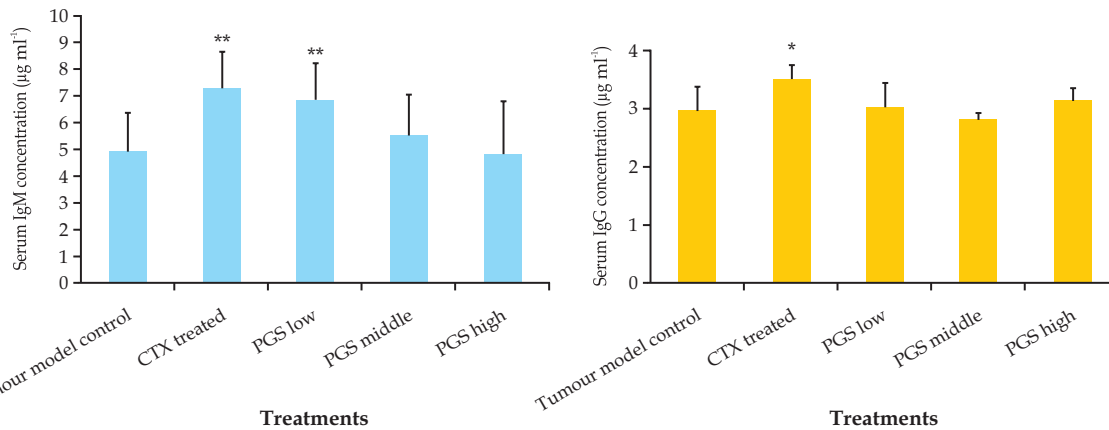

Fig 1. Effect of PGS on serum IgG and IgM concentrations in tumour-bearing mice (Note:** Significant differences at $p < 0.01$ compared with the tumour model group; * Significant differences at $p < 0.05$ levels compared with the tumour model group).

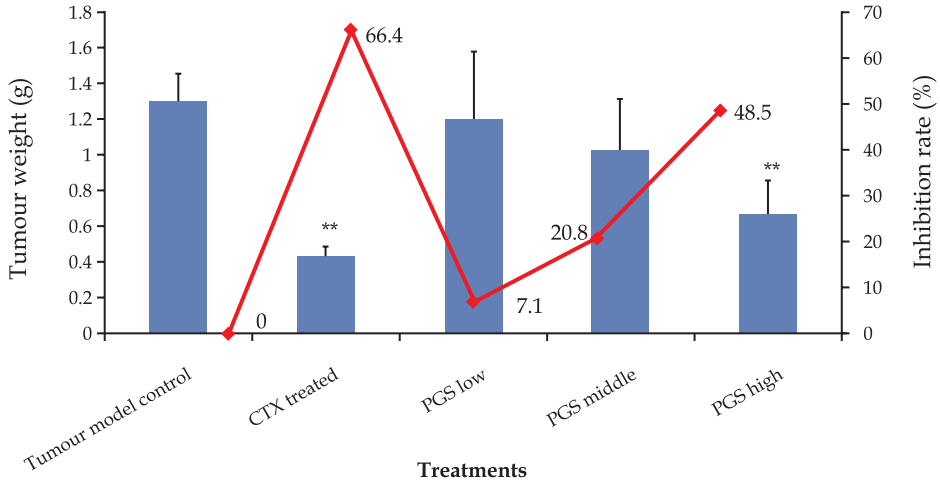

Fig 2. The effect of PGS on tumour weight and tumour inhibition rate (Note: ** Significant difference at $p < 0.01$ levels compared with the tumour model group; blue column chart indicate tumour weight; red line chart indicate tumour inhibition rate)

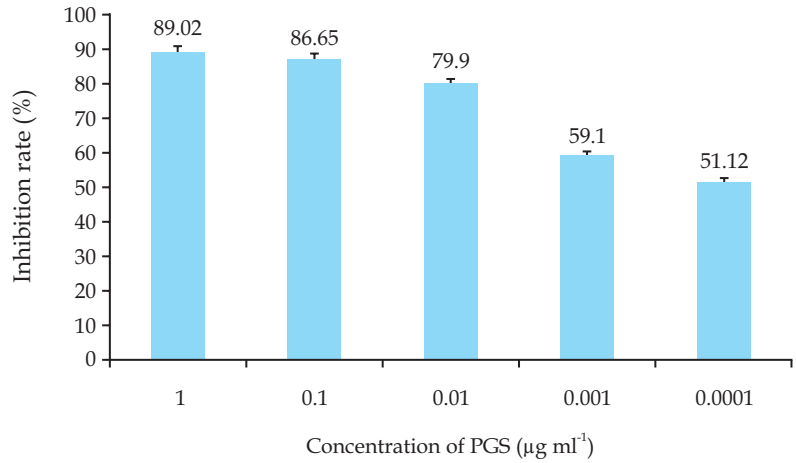

Fig 3. Effect of different concentrations of PGS on the growth of S180 cells *in vitro*

cancer. To identify and prove the pharmacological activities and potential mechanisms of PGS, we focused on the immune-potentiating activities and anti-tumour activities of PGS in present study.

The thymus and the spleen are primary and secondary lymphoid organs, respectively and the thymus index and spleen index directly reflect the nonspecific immunity of the organism (Cesta, 2006). Moreover, immunopotentiators could increase the relative weights of the thymus and the spleen (Zhang *et al,* 2013). On the other hand, macrophages are the primary phagocytes of the immune system. These cells reside in every tissue of the body (e.g., microglia, Kupffer cells and osteoclasts according to the location in the body) where they engulf apoptotic cells and pathogens, as well as produce immune effector molecules. Among these effector responses, the phagocytic function of macrophage monocytes is one of the most important indexes of nonspecific immune function and is commonly used in evaluating the non-specific immune status of animals (Andrew *et al,* 2011). Compared with the CTX model control group, the middle and high dose of PGS markedly increased both the spleen and thymus indices, as well as the phagocytic function of the monocyte-derived macrophages in our study. Therefore, we believe that PGS can restore the suppressed non-specific immune function by promoting the growth of immune organs and activating monocyte-derived macrophages.

The cytokines, including IL-2, IL-6 and TNF-α, can regulate both the cellular and humoral immune responses by affecting immune cell proliferation, differentiation and functionality, which play a critical role in combating tumour growth. IL-2 is one of the most important immune factors secreted primarily by T cells, which promotes immune cell

proliferation and differentiation. Moreover, IL-2 has been approved by the FDA for the treatment of metastatic renal cell carcinoma and metastatic melanoma (Geok and Laszlo, 2014). In addition, IL-6 is another important immune mediator that regulates diverse cellular functions, including the proliferation and differentiation of B-cells and T-cells (Sobota *et al*, 2008). TNF-α is produced at the highest levels by activated macrophages, T lymphocytes and NK cells and plays a pivotal role in the cellular immune process by aiding in the activation of macrophages following the phagocytosis of pathogens or abnormal cells (Shiro, 2011). The ability to enhance the production of these cytokines has been widely used for the evaluation of the immunostimulatory activity of immunopotentiators. Therefore, we studied the effects of PGS on the serum concentrations of IL-2, IL-6 and TNF-α, as well as, IgM and IgG in both immunosuppressed mice and tumour-bearing mice in the current study. Compared with the immunosuppressed or tumour control models, moderate to high doses of PGS could elevate the serum concentrations of IL-6 and TNF-α in an immunosuppressed mice, but could also increase the serum concentration of IL-2, IL-6 and TNF-α in tumour-bearing mice. In addition, the appropriate dose of PGS could also increase the IgG concentration in immunosuppressed mice, as well as markedly enhance the IgM content in tumour-bearing mice. Therefore, one of the most important mechanisms of the immunostimulatory and antitumour effects of PGS was to overcome the suppressed immune function by promoting both cellular and humoral immune activation.

Cancer remains one of the most common causes of death and is a disease with an infiltrative and destructive nature that has the potential to spread to various organs from its site of origin. Therefore, it is important that anticancer compounds can selectively inhibit the proliferation of tumour cells and reduce the growth of solid tumours in the body. To investigate whether PGS can directly impact tumour growth, the solid tumour inhibition rate *in vivo* and the tumour cell inhibition rate *in vitro*, respectively, were calculated in this study. Compared with the tumour control model, a moderate to high dose of PGS was found to significantly inhibit the growth of solid tumours in S180 transplanted mice, with the highest tumour inhibition rate of 48.5%. In addition, PGS was also associated with a dose-dependent inhibition of the proliferation of S180 cells *in vitro* and it's IC_{50} was 15.63±2.18 μg ml^{-1}, less than 30 μg ml^{-1}. Therefore, one of the another important mechanisms of the anti-tumour effects of PGS was the direct inhibitory effects on the growth of solid tumours in mice and the proliferation of tumour cells *in vitro*.

The experimental results of both the *in vitro* and *in vivo* experiments in the present study indicate that PGS has anticancer effects and immune activating properties. Therefore, PGS could potentially restore the suppressive immune function of animals and possess therapeutic activities against certain types of tumours.

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PNEUMONIA IN DROMEDARY CAMELS (*Camelus dromedarius*): A REVIEW OF CLINICO-PATHOLOGICAL AND ETIOLOGICAL CHARACTERISTICS

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ABSTRACT

The aim of this review article was to summarise relevant clinical, etio-epidemiological and pathological data available in the current literature regarding pneumonia in dromedary camels. Scientific resources such as Pubmed, Google scholar and Researchgate were searched for all published articles regarding bacterial and viral pneumonia in dromedary camels. The most common bacterial species isolated from lesions of pneumonic camels were *Staphylococcus aureus, Corynebacterium pyogenes, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Arcanobacterium pyogenes, Mannheimia haemolytica* and *Pasteurella multocida*. The most common viral causes of pneumonia were parainfluenza 3 (PI-3), adenovirus, respiratory syncytial virus (RSV), bovine herpes virus-1 or infectious bovine rhinotracheitis (IBR) and pestivirus or bovine viral diarrhoea virus (BVD). Clinically, pneumonic camels were reported to suffer from fever, depression, nasal and ocular discharges and coughing. Affected animals were also reported to have anaemia, leukocytosis and increased levels of serum total protein, globulin, urea, creatinine, potassium and activities of hepatic enzymes. Pathological lesions in acute pneumonia were characterised by fibrinous bronchopneumonia, oedema and congestion while lesions in chronic pneumonia were characterised by fibrosis, proliferative bronchopneumonia, pleuropneumonia and abscessation. Ciprofloxacin, cephaloridine, penicillin, ampicillin, gentamicin and tetracycline were reported as the most effective antibacterial agents against most bacterial isolates.

Key words: Bacteria, dromedary camels, gross pathology, pneumonia

Lower respiratory tract infections or pneumonia is considered as remerging health problem in dromedary camels (Buchnev *et al*, 1987; Wernery and Kaaden, 2002; Zubair *et al*, 2004; Kane *et al*, 2005; Abubakar *et al,* 2010). Although, camels are well-adapted to dry and harsh environment and resistant to many disease causing organisms, respiratory disease can still cause considerable economical losses through loss of production, cost of treatment, condemnation of carcasses and even death of affected animals (Zubair *et al*, 2004; Kane *et al*, 2005; Dia, 2006; Bekele, 2008; Abubakar *et al,* 2010). In recent literature, there are no review articles that summarise current research and knowledge about bacterial and viral pneumonia in dromedary camels. In domestic animals including camels, pneumonia is usually caused by viruses, bacteria, fungi or a parasite (Ahmed *et al*, 2015).

Risk factors

Although, most of the microbiological agents that may cause pneumonia can be found in the upper respiratory tract of normal camels, in certain circumstances, these agents can cause serious disease (Ahmed *et al*, 2015). Many of the risk factors that are known to predispose animals to pneumonia are associated with poor management conditions such as environmental stress, crowdedness, poor sanitary conditions, poor nutrition and nutritional management, extreme climatic swings and general herd health (Abubakar *et al,* 2010; Ahmed and Musa 2015). In these reviewed articles, the most commonly reported risk factors for pneumonia caused by bacteria were age and season (Al-Tarazi, 2001; Ahmed and Musa, 2015; Nahed *et al*, 2016) (Table 1). The highest incidence of pneumonia was reported in autumn in adult camels (Ahmed and Musa 2015; Nahed *et al*, 2016). Al-Tarazi (2001) on the other hand, reported that proliferative bronchopneumonia and pleuropneumonia were more frequent in older camels (about 10 years of age) while interstitial pneumonia and lung abscesses were more frequent in younger camels (6 months to 4 years of age).

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Clinico-pathological findings

Clinically, affected animals may show non-specific signs of illness such as fever, depression and anorexia (Al-Tarazi, 2001; Ahmed *et al*, 2015; Gafer *et al*, 2015; Nahed *et al*, 2016) (Table 1). Specific respiratory signs are usually nasal and ocular discharge, rapid and shallow breathing and coughing (Al-Tarazi, 2001; Ahmad *et al*, 2015; Ahmed and Musa 2015; Gafer *et al*, 2015; Nahed *et al*, 2016). Depression, ruminal atony, ataxia and decreased milk production were also detected in some cases (Nahed *et al*, 2016).

Studies also showed that affected camels may have certain abnormal findings in the haematology and serum biochemistry analyses (Abubakar *et al*, 2011; Nahed *et al*, 2016). It is reported that pneumonic camels may have anaemia, leukocytosis and increased levels of serum total protein, globulin, urea, creatinine, potassium and activities of hepatic enzymes (Abubakar *et al*, 2011; Nahed *et al*, 2016).

Bacterial pathogens

Overall, there were 9 and 6 scientific studies published in refereed journals in the last 15 years reporting different bacterial and viral species respectively, which were isolated from pneumonic respiratory samples or pneumonic lesions from dromedary camels. The most common pathogenic bacteria were *Staphylococcus aureus, Corynebacterium pyogenes, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Arcanobacterium pyogenes, Mannheimia haemolytica* and *Pasteurella multocida* (Al-Doughaym *et al*, 1999; Al-Tarazi, 2001; Abubakar *et al*, 2010; Abo-Elnaga and Osman, 2012; Wareth *et al*, 2014; Ahmed *et al*, 2015; Ahmed and Musa, 2015; Nahed *et al*, 2016) (Table 2). The most common samples that yielded bacterial isolates were nasal, nasopharyngeal, tracheal swabs and lung tissues.

Bacterial isolation in most of the reviewed studies was achieved using routine culture methods with different media such as nutrient agar, blood agar, brain heart infusion, mannitol salt agar, MacConkey agar and brilliant green agar followed by identification using morphological and biochemical characteristics of the isolated strains such as colony morphology, Gram staining, spore forming ability and acid-fast staining (Al-Doughaym *et al*, 1999; Al-Tarazi, 2001; Abubakar *et al*, 2010; Abo-Elnaga and Osman, 2012; Wareth *et al*, 2014; Ahmed *et al*, 2015; Ahmed and Musa, 2015; Nahed *et al*, 2016). Further testing was performed to determine the species of isolated bacteria such as catalase, oxidase, oxidation fermentation test (Al-Doughaym *et al*, 1999; Al-Tarazi, 2001; Abubakar *et al*, 2010; Abo-Elnaga and Osman, 2012; Wareth *et al*, 2014; Ahmed *et al*, 2015; Ahmed and Musa, 2015; Nahed *et al*, 2016). For Gram positive bacteria, species identification was performed in most of the studies using indole production, motility test, coagulase test, carbohydrates breakdown, Voges-Proskauer reaction, arginine hydrolysis, nitrate

Table 1. Risk factors and clinical signs of bacterial and viral caused pneumonia in dromedary camels.

Risk Factors	Clinical Signs	References
Bacterial Pneumonia		
- Highest incidence in autumn - Adult camels are more susceptible - Proliferative bronchopneumonia and pleuropneumonia are more frequent in older camels (about 10 years of age) - Interstitial pneumonia and lung abscesses are more frequent in young camels (6 months to 4 years of age)	- Moist painful harsh cough - Rhinitis - Congested mucous membranes - Serous or mucoid nasal discharges - Increased respiratory and pulse rates - Elevated rectal temperature - Depression - Ruminal atony - Ataxia - Decreased milk production	Al-Tarazi, 2001; Ahmed *et al*, 2015; Ahmad and Musa 2015; Nahed *et al*, 2016
Viral pneumonia		
- Young calves in BVD infections	- Fever (41.5^{o}C) - Anorexia - Listlessness - Dyspnoea - Hyperemia of the nasal mucosa - Nasal and ocular serous discharge	Gafer *et al*, 2015

Table 2. A review of the most common bacteria causing pneumonia in dromedary camels and *in vitro* most effective antibacterial agents.

Bacterial isolates	Samples	Most Effective Antibacterial	References
- *Staphylococcus aureus*	- Lung tissues - Tracheal swabs	- Ciprofloxacin - Cefazolin	Ahmed *et al*, 2015
- *Staphylococcus aureus* - *Corynebacterium pyogenes* - *Streptococcus pyogenes*	- Nasal swabs - Tracheal swabs - Lung tissues	- Penicillin - Ampicillin - Gentamicin	Al-Doughaym *et al*, 1999
- *Staphylococcus aureus* - *Escherichia coli* - *Klebsiella pneumonae*	- Lung tissues	NR	Wareth *et al*, 2014
- *Escherichia coli* - *Klebsiella pneumonae* - *Pseudomonas aeruginosa*	- Lung tissues	NR	Al-Tarazi, 2001
- *Klebsiella pneumonae* - *Staphylococcus aureus*	- Nasopharyngeal swabs - Lung tissues	NR	Nahed *et al*, 2016
- *Staphylococcus aureus* *Streptococcus pyogenes*	- Tracheal swabs - Lung tissues	NR	Ahmed and Musa, 2015
- *Staphylococcus aureus* - *Klebsiella pneumonae*	- Lung tissues	NR	Abo-Elnaga and Osman, 2012
- *Staphylococcus aureus* - *Arcanobacterium pyogenes* - *Mannheimia haemolytica* - *Pasteurella multocida*	- Lung tissue	NR	Abubakar *et al*, 2010

NR: information not reported

Table 3. A review of the most common viruses causing pneumonia in dromedary camels.

Viral agent	Samples	References
- Parainfluenza 3 (PI-3) - Adenovirus - Respiratory syncytial virus (RSV) - Pestivirus (BVD)	- Lung tissues	Muna *et al*, 2015
- Infectious bovine rhinotracheitis virus (bovine herpes virus-1) - Pestivirus (BVD)	- Nasal swabs - Lung tissues	Gafer *et al*, 2015
- Parainfluenza virus 3 (PI-3)	- Lung tissues	Intisar *et al*, 2010a
- Respiratory syncytial virus (RSV)	- Lung tissues	Intisar *et al*, 2010b
- Pestivirus (BVD)	- Lung tissues	Intisar *et al*, 2010c
- Infectious bovine rhinotracheitis virus (bovine herpes virus-1)	- Lung tissues	Intisar *et al*, 2009

reduction, growth in 6.5% NaCl broth, growth at 45°C, requirement of CO_2 for growth, sensitivity to bacitracin (0.1 unit), urease activity, gelatin liquefaction and aesculin hydrolysis (Al-Doughaym *et al*, 1999; Tarazi, 2001; Abubakar *et al*, 2010; Abo-Elnaga and Osman, 2012; Wareth *et al*, 2014; Al-Ahmed *et al*, 2015; Ahmed and Musa, 2015; Nahed *et al*, 2016). For identification of Gram negative bacteria, methods were used such as oxidase production, citrate utilisation, urease activity, growth in KCN medium, gelatin liquefaction and hydrogen sulphide production from the TSI medium, fermentation of sugars, growth at 42°C, growth on MacConkey agar, nitrate reduction, indole production, aesculin and arginine hydrolysis, Voges-Proskauer reaction and the methyl red test (Al-Doughaym *et al*, 1999; Al-Tarazi, 2001; Abubakar *et al*, 2010; Abo-Elnaga and Osman, 2012; Wareth *et al*, 2014; Ahmed *et al*, 2015; Ahmed and Musa, 2015; Nahed *et al*, 2016).

Bacterial resistance against commonly used antibiotics is being reported at an alarmingly high rate in recent literature. Antimicrobial resistance is not only important because of the high risk of treatment failures in affected animals but also because it puts human health at risk. During the last 16 years under review, there are only 2 articles that investigated antibacterial sensitivity of isolated bacterial strains (Al-Doughaym *et al*, 1999; Ahmad *et al*, 2015). The

laboratory methods of *in vitro* sensitivity tests that were used in these 2 studies were the disk diffusion methods (7 antibiotics), the broth diffusion test (4 antibiotics) and the Viteck 2 compact system (23 antibiotics) (Al-Doughaym *et al,* 1999; Ahmed *et al*, 2015). In the first study, results of the sensitivity tests showed that 87% of isolated *Staphylococcus aureus* were sensitive to ampicillin, while 83% of the isolates were sensitive to gentamicin ciprofloxacin and cephaloridine (Al-Doughaym *et al*, 1999). Ninty four per cent, 72% and 52% of *Corynebacterium pyogenes* isolates were sensitive to ampicillin, gentamicin and tetracycline, respectively (Al-Doughaym *et al*, 1999). *Klebsiella pneumonae* and *E. coli* had a similar sensitivity patterns with gentamicin and cephaloridine being the most effective (Al-Doughaym *et al*, 1999). In the second study, Gram positive bacteria were mostly sensitive to gentamicin and ciprofloxacin while most Gram negative strains such as *E. coli* and *Pseudomonas aeruginosa* were found resistant to most of the tested antibiotics (Ahmed *et al*, 2015).

Viral pathogens

The most common viruses that were found associated with pneumonia in the dromedary camel were parainfluenza 3, adenovirus, respiratory syncytial virus (RSV), infectious bovine rhinotracheitis (IBR; bovine herpes virus-1) and pestivirus or bovine viral diarrhoea virus (BVD) (Intisar *et al*, 2009; Intisar *et al*, 2010a,b,c; Gafer *et al*, 2015; Muna *et al*, 2015) (Table 3). The most common samples that yielded viral agents were nasal swabs and lung tissues.

In one camel with pneumonic lesions in Sudan, a mixed infection caused by parainfluenza 3, adenovirus, respiratory syncytial virus (RSV) was confirmed (Muna *et al*, 2015). Bovine viral diarrhoea virus (BVDV) and bovine herpes virus-1 (BHV-1) were isolated 11% and 14%, respectively from 33 clinically ill animals in Egypt confimed by immunofluorescence (IF) and immunoperoxidase (Gafer *et al*, 2015). In Sudan, out of 186 lung tissues samples examined for BVDV antigen, 13 were found positive (Intisar *et al*, 2010c). BHV-1 antigen was also detected 3 out of 186 lung tissues samples (Intisar *et al*, 2009). Parainfluenza virus 3 (PI-3) was detected in 6 out of the 281 lung samples in Sudan (Intisar *et al*, 2010a). Respiratory syncytial virus (RSV) was detected in 4 out of 280 lung tissue samples in Sudan (Intisar *et al*, 2010b).

Techniques that were used to detect pestivirus or bovine viral diarrhoea virus (BVD) and bovine herpes virus 1 were multiplex PCR assay, immunoflurescence and immunoperoxidase (Gafer *et al*, 2015). Bovine viral diarrhoea (BVD) virus was detected in serum using ELISA and positive samples were further tested using direct fluorescent antibody technique (FAT) or reverse transcriptase polymerase chain reaction (RT-PCR) (Intisar *et al*, 2010c).

For the detection of PI-3, direct immuno-fluorescent test (FAT) can be used to confirm the positive reactions for PI-3 by ELISA (Intisar *et al*, 2009). The polymerase chain reaction (RT-PCR) is also used for the detection of the PI-3 genome in lungs of camels (Intisar *et al*, 2010a). Isolation of PI-3 can also be attempted using MDBK cell culture (Intisar *et al*, 2010a). The cytopathic effect of the virus such as cell rounding, multinucleated cells, sloughing and elongation of cells and some syncytia can be observed from the 3rd to 7th day post-inoculation (Intisar *et al*, 2010a).

For the detection of respiratory syncytial virus (RSV), sandwich ELISA can be used to detect RSV antigen in lung tissues. Fluorescence antibody test (FAT) is then used to confirm the ELISA positives samples. Polymerase chain reaction (RT/PCR) can also be used for the detection of RSV genome in camel lungs (Intisar *et al*, 2010b).

Bovine herpes virus-1 (BHV-1) in camels can be detected in lung tissues of camels using sandwich ELISA technique. Direct fluorescent antibody test (FAT) is then used to confirm the BHV-1 ELISA positive samples. PCR can also be used to detect BHV-1 genome. BHV-1 can be isolated from lung tissues in MDBK cell culture (Intisar *et al*, 2009).

Pathological manifestations

Gross and histopathological lung lesions associated with bacterial pneumonia in camels have been well studied unlike that caused by viruses (Al-Tarazi, 2001; Bekele, 2008; Abubakar *et al*, 2011; Abo-Elnaga and Osman, 2012; Wareth *et al*, 2014; Ahmed *et al*, 2015; Nahed *et al*, 2016) (Table 4). In bacterial caused pneumonia, pulmonary lesions in acute pneumonia were characterised by fibrinous bronchopneumonia, oedema and congestion while lesions in chronic pneumonia were characterised by fibrosis, proliferative bronchopneumonia, pleuropneumonia and abscessation (Table 4). Fibrinous bronchopneumonia usually appears as a gray and red hepatisation with congestion of the interstitial capillaries. Suppurative bronchopneumonia is characterised by the presence of suppurative exudates in the lumen of bronchioles and

Table 4. The most common pulmonary pathological lesions associated with bacterial and viral caused pneumonia in dromedary camels.

Lesions	Causative Agent	
	Bacterial	Viral
Fibrinous pneumonia	Abubakar *et al,* 2011; Wareth *et al,* 2014; Ahmed *et al,* 2015	NR
Pulmonary abscesses	Abubakar *et al,* 2011; Al-Tarazi, 2001; Bekele, 2008; Wareth *et al,* 2014; Ahmed *et al,* 2015	NR
Suppurative bronchopneumonia	Ahmed *et al,* 2015	NR
Pleuropneumonia	Al-Tarazi, 2001; Ahmed *et al,* 2015	NR
Pulmonary emphysema	Abubakar *et al,* 2011; Wareth *et al,* 2014	NR
Interstitial pneumonia	Al-Tarazi, 2001; Abo-Elnaga and Osman, 2012; Wareth *et al,* 2014; Nahed *et al,* 2016	Ahmed *et al,* 2015; Gafer *et al,* 2015
Proliferative bronchopneumonia	Bekele, 2008; Abo-Elnaga and Osman, 2012; Wareth *et al,* 2014; Nahed *et al,* 2016	Ahmed *et al,* 2015

NR: information not reported

peribronchiolar tissues with partial replacement of the bronchiolar wall. Purulent exudates may accumulate focally to form variable sized abscesses. Adjacent areas may show variable degrees of atelectasis and emphysema in some cases. Acute interstitial pneumonia is characterised by the presence of oedema and leucocytic cellular infiltration with congestion in peri-alveolar capillaries resulting in thickening of the interalveolar septa. Chronic interstitial pneumonia is marked by thickening and fibrosis of the inter-alveolar tissues due to proliferation of fibrous tissues and lymphocytic infiltration.

Viral caused pneumonia is characterised histologically by acute interstitial pneumonia (Table 4). There is thickening of the interstitial tissues, capillary walls and alveolar septum due to mononuclear cell, red blood cells and fibroblast cell infiltration. Areas of atelectatic alveoli are present in the adjacent tissues (Ahmed *et al*, 2015; Gafer *et al*, 2015). Chronic interstitial pneumonia is marked by proliferation, hyperplasia, bronchiolitis and bronchopneumonia with accumulation of mononuclear and macrophages cell inside bronchioles (Ahmed *et al*, 2015; Gafer *et al*, 2015).

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PATHOLOGICAL AND SEROBIOCHEMICAL STUDIES ON NATURALLY OCCURRING KIDNEY AFFECTIONS IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

In the present investigation, gross and microscopic lesions of the kidney as well as some haematological and serobiochemical tests were studied in 50 adult camels. Fresh samples of blood and kidney tissues were collected from camels of both sexes at the point of slaughter in Al Ahsa abattoir, eastern region of Kingdom of Saudi Arabia. Grossly, out of 50 camels examined, 33(66%) had kidney lesions of one type or another whereas, 17 (34%) were apparently healthy. Only 2 (4%) kidneys showed hydronephrosis with clear watery fluid and hard on palpation. Renal haemorrhages and necrosis were observed in 10 (20%) of the collected samples of kidneys. Microscopically out of the 33 camel kidneys collected with naturally occurring lesions, glomerular shrinkage and hyalinisation were observed in 10 (33%) kidneys. Proteinaceous casts, (in the urinary spaces and renal tubules), were seen in 8 (27%) kidneys. Moreover, 6 (15%) kidneys showed cortical and medullary congestion whereas, tubular cell swelling, interstitial haemorrhage and thickening of the glomerular tufts were seen in 5 (15%), 2 (6%) and 2 (6%) kidneys, respectively. In addition, significant change was observed in Total Erythrocyte Count (TEC), Haemoglobin Concentration (Hb) and Packed Cell Volume (PCV) in camels with naturally occurring kidney lesions. However, the biochemical findings in camels with kidney lesions showed higher values of serum ALT, AST, GGT and creatinine, as well as, lower values of total protein. On the other hand, no change was observed in the concentration of blood urea nitrogen (BUN) in all camels. The present results indicate that the camels are exposed to nephrotoxins in the study area possibly from toxic constituents such as hazardous elements as they graze.

Key words: Camel histopathology, hydronephrosis, kidney lesion, serobiochemical changes

The kidney of the camel can conserve water by producing highly concentrated urine (Kataria *et al,* 2007; Ouajd and Kamel, 2009). The thick renal medulla with a cortico-medullary ratio of 1:4, the specialised folds of the renal pelvis (fornices) and the huge absorptive surface area of the proximal convoluted tubules combine to make the kidney of the camel very efficient in water conservation (Abdulla and Abdulla, 1979). The notion that the camel is very resistant to diseases appears to be unfounded, as the camel has been shown to suffer from many disease conditions including those that affect the kidneys (Abbas and Omer, 2005, Taha *et al*, 2007). The aetiology, epidemiology, clinical aspects pathology, diagnosis and treatment of many diseases in camelids have been extensively studied (Wernery *et al,* 2014).

Radostits *et al* (2007) reported that urolithiasis is common as subclinical disorder in animals grazing certain types of pasture. Camel diseases of the kidney e.g. nephrosis, glomerulonephritis, renal cysts, hydronephrosis, renal abscesses, urolithiasis and tumours were found coincidentally during either slaughter or postmortem examination (Fowler, 2010). Recently, dromedary camels in Saudi Arabia are implicated as possible viral reservoir for middle east respiratory syndrome coronavirus (MERS-COV) associated with severe respiratory and renal failure in infected patients (Zaki *et al,* 2012; Drosten *et al,* 2013). The present study is therefore, designed to assess the naturally occurring lesions in the kidneys of the camels and to correlate these lesions with the changes in some haematological and serobiochemical values.

Materials and Methods

The kidneys of 50 apparently healthy adult camels of both sexes were studied for gross and microscopic lesions after slaughter at Al Hofof abattoir. Blood samples were collected from the jugular vein immediately after slaughter, for haematological and serobiochemical analysis.

Total erythrocyte counts (TEC), haemoglobin concentration (Hb), packed cell volume (PCV), total

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leukocyte counts (TLC) and differential leukocyte counts (DLC) were determined using Abaxis Vetscan HM5–America analyzer.

Serum total protein, creatinine, urea and the serum enzymes alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamyle transpeptidase (GGT), were determined using Abaxis Vetscan VS2–America analyser.

Results are expressed as mean ± SD and presence of significant differences among means of the groups was determined using one way ANOVA with a Tukey-Kramer post-test for significance. Values were considered significant when P <0.05.

Kidney samples were fixed in 10% neutral formalin, mounted in paraffin, sectioned and stained with Haematoxylin and Eosin (HE) according to the method of Bancroft and Gamble (2008).

Results and Discussion

Grossly, out of 50 camels examined 33(66%) had kidney lesions of one type or another, whereas 17 (34%) were apparently healthy. Hydronephrosis with clear watery fluid and hard on palpation was observed in 2 (4%) samples (Fig 1). Renal petechial haemorrhages and necrosis were observed in 10 (20%) samples (Fig 2). Histopathological examination of the collected kidney samples revealed glomerular shrinkage and hyalinisation (Fig 3), proteinaceous casts (Fig 4), cortical and medullary congestion (Fig 5), interstitial haemorrhage (Fig 6), thickening of the glomerular tufts (Fig 7).

The haematological findings of healthy camels and camels with naturally occurring kidney lesions are given in table 1. Camels with kidney lesions showed a remarkable reduction in TEC, Hb. concentration and PCV. No significant changes were observed in TLC, neutrophils and lymphocyte count.

The serobiochemical changes in healthy camels and in camels with naturally occurring lesions are shown in table 2. Camels with naturally occurring lesions had significantly (P<0.05) lower values of serum total protein and higher values (P<0.05) for creatinine, ALT, AST and GGT whereas, no significant change was observed in the concentration of serum urea in all camels.

In this study, 66% prevalence of gross renal lesions in camels of AL Ahsa region was seen without evidence of any relevant clinical sign on antemortem examination. This is probably because renal diseases in animals, especially the camel are often subclinical (Aughey and Frye, 2001). This rate was also much higher than the 16.5% reported for some parts of Egypt (Salem and Hassan, 2007). A good reason for this high rate could be because most of the animals brought for slaughter were probably culls. There is also the possibility of the animals being exposed to nephrotoxic agrochemicals locally. Eastern province of Saudi Arabia is considered as a border of Saudi Arabia with Qatar and Bahrain. This region is characterised by petroleum industries, which might cause liberation of toxic constituents such as hazardous elements. These elements may deposit in soil then passed through the grazing plants to the different types of animals.

Table 1. Haematological parameters in healthy camels and camels with naturally occurring lesions (n=50).

Parameter	Camels with kidney lesions	Healthy camels
Total leucocyte count ($X10^6$/µl)	6.65±1.75[a]	8.81±2.50[b]
Haemoglobin concentration (g/dL)	10.11±1.02[a]	14.0±2.1[b]
Packed cell volume (%)	25.6±1.3[a]	35.3±1.4[b]
Total leucocytes count ($X10^3$/ µl)	13.3±2.1	12.9±1.8
Neutrophils (%) =7.53x10^3/ul	55.3±3.2	54.7±2.6l
Lymphocytes (%)= 5.38x10^3/ul	49.5±2.1	50.9±3.1
Monocytes (%)= 3. 38x10^3/ul	2.75±0.34	3.00±1.6
Eosinophils (%)= 1.83 x10^3/ul	1.38±0.11	1.35±0.41
Basophils (%)=0.12 x10^3/ul	0.09±0.04	0.10±0.06

Values in each row with different letters are statistically different (P<0.05).

Table 2. Biochemical parameters in healthy camels and camels with naturally occurring lesions (n=50).

Parameter	Camels with kidney lesions	Healthy camels
Total protein (mg/dl)	6.8 7±1.6[a]	9.11±1.7[b]
Creatinine (mg/dl)	1.81±0.18[a]	1.1 2±0.20[b]
Urea (mg/dl)	75.2±4.1	73.4 ± 3.6
Alanin transaminase(IU/l)	32.4±2.33[a]	12.4±3.1[b]
Aspartate transaminase (IU/l)	124.3±4.3[a]	100.1±3.1[b]
Gamma glutamyl transpeptidase(IU/l)	36.2±1.4[a]	24.2±2.4[b]

Values in each row with different letters are statistically different (P<0.05).

Minerals play a key role in the formation of the active chemical constituents present in plants and therefore they may contribute in their nutritional properties as well as toxic activities (Selvaraja *et al*, 2004, Selvaraj and Sumantha 2011). In this study, mild to moderate microscopic kidney lesions were observed in 33 (66%) of the camels studied. Previous reports have revealed that extensive damage to renal tubules

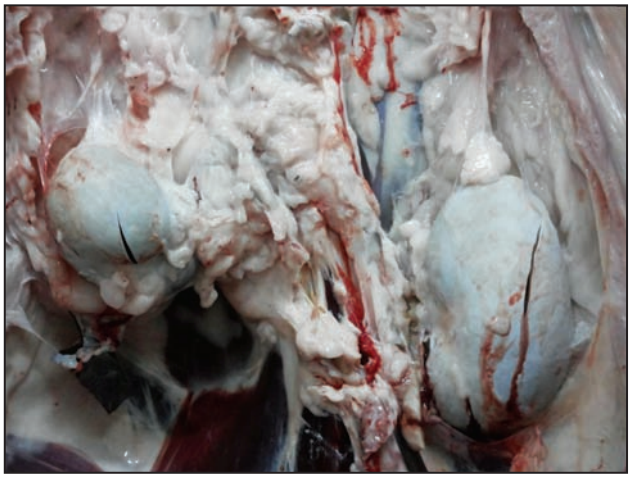

Fig 1. Left kidney of camel showing hydronephrosis.

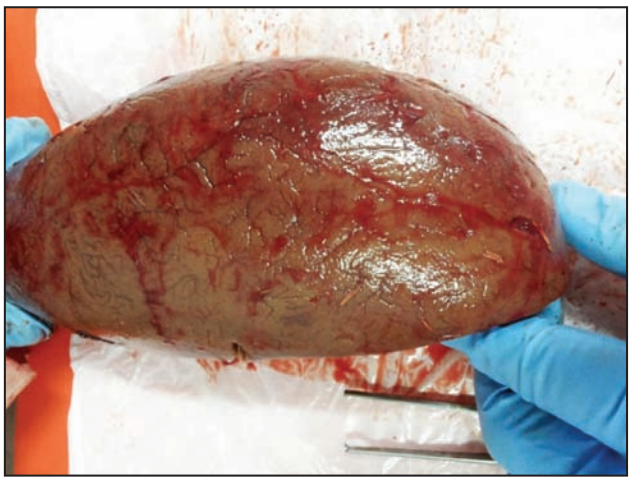

Fig 2. Kidney of camel showing sub capsular haemorrhages and necrosis.

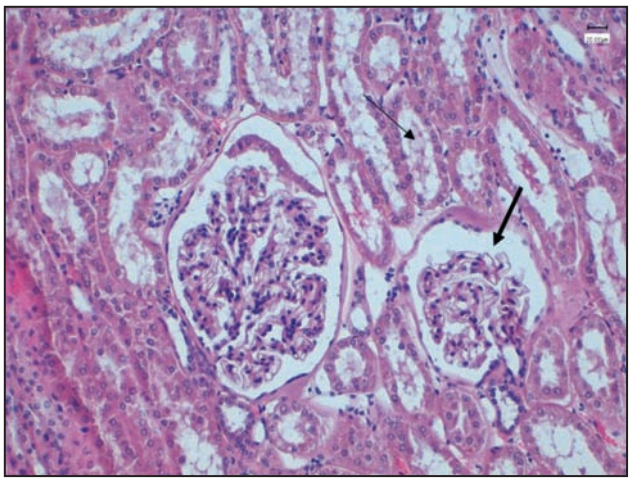

Fig 3. The kidney of camel showing glomerular shrinkage (Thick arrow) and protein casts in the proximal convoluted tubules (Thin arrow) H&E X20.

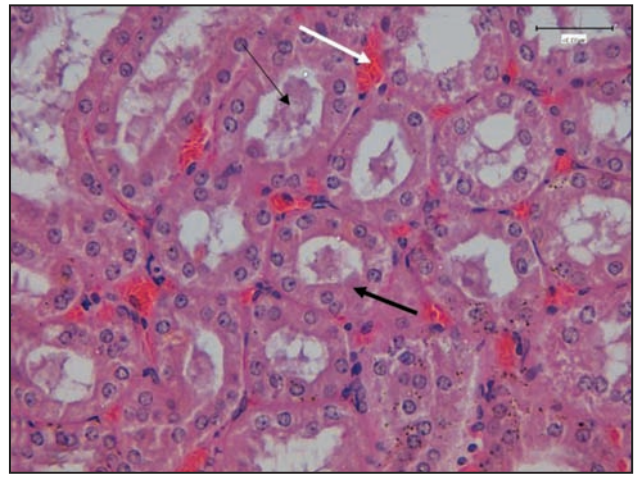

Fig 4. Kidney of camel showing tubular cell swelling (Thick arrow), protein casts (Thin arrow) and medullary congestion (White arrow) H&E X40.

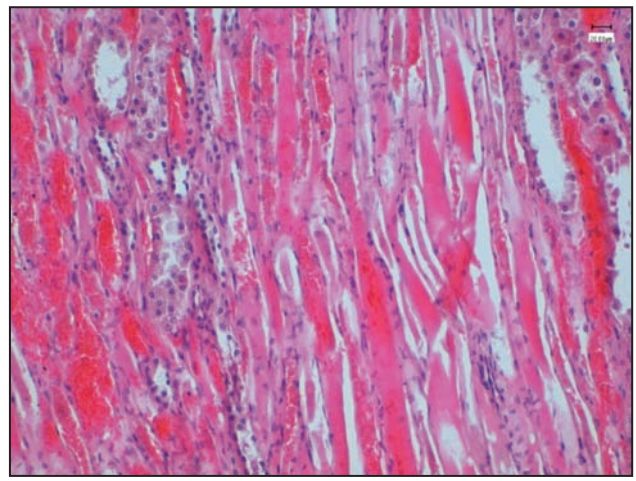

Fig 5. Kidney of camel showing interstitial haemorrhages and tubular cell necrosis H&E X20.

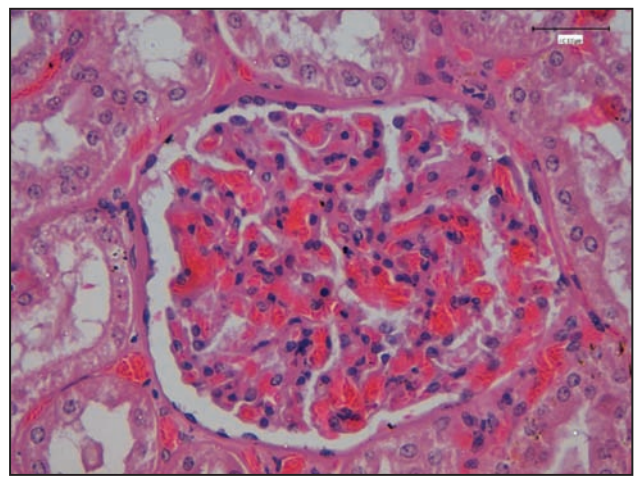

Fig 6. Kidney of camel showing cortical congestion and thickening of the glomerular tuft H&E X40.

can cause secondary atrophy of the nephron, making renal tubular degeneration and necrosis (nephrosis) a major cause of acute renal failure in man and animals (McGavin and Zachary, 2007).The Renal tubular degeneration indicates a mild to moderate reversible cellular injury that may advance to apoptosis or necrosis. The first step in the development of tubular degeneration and necrosis is the breakdown of ionic transport and the accumulation of sodium ions in the lumina of tubules, which stimulates the renin-angiotensin mechanism with subsequent vasoconstriction in the nephron (Salem and Hassan, 2007; McGavin and Zachary, 2007).

The present study showed mild alteration in in the blood constituents. A noticeable decrease in the total erythrocyte count, (TEC), haemoglobin concentration (Hb) and packed cell volume (PCV) was observed in camels with different kidney lesions. Incidence of anaemia in association with kidney diseases may be due to lack of food because of inappetence during the chronic course of kidney diseases, which may inhibit erythropoiesis (Feldman, *et al,* 2000). The obtained results showed no significant changes in total leucocyte count (TLC), neutrophils and lymphocyte count. Surprisingly, there was no evidence of a clearly defined inflammatory responses in the kidneys examined. This is not much different from the observations of Taha *et al* (2007) in which only 2% of camels examined showed mild mononuclear reactions. Salem and Hassan (2009) also reported focal mononuclear cell reaction despite acute tubular necrosis and other lesions in 2 animals out of 92. This could be partly explained by immunosuppression or unknown mechanisms that restrain inflammatory responses in order to limit tissue damage. The anti-inflammatory humoural mechanism and cytokine production was described in many species (Kelvin, 2007; Ivashkiv and Donlin, 2014). Accordingly, it seems possible to investigate this mechanism in dromedary camels.

The elevated levels of serum ALT, AST, GGT and creatinine obtained in this study suggest the occurrence of kidney damage and these serum enzymes may be a good indicators of such renal damage. However, previous reports have shown that the range of reference values of these enzymes varies considerably in dromedary camels (AL-Ali *et al,* 1988, Kataria *et al,* 2007).

Significant decrease in the level of serum protein was shown in camels with kidney lesions. It has been shown that protein casts are associated with protein loss leading to hypoproteinemia with resultant dependant edema in other animals (Aughey and Fyre, 2001). The effects of hypoproteinemia may not be apparent in the camel, because it may be able to maintain its blood pressure by adjustments in urea reabsorption and other water conservation mechanisms (Kataria *et al,* 2007; Oaujd and Kamel, 2009).

No remarkable change was observed in the concentration of (BUN) in all camels in this study. However, it has been reported that more than 50% of the renal mass must be damaged before any change in BUN is detected (Borjesson, 2003; Guyton, 2006).

Conclusion

Camels in the study area showed prevalence of renal diseases, especially those leading to chronic renal failure such as glomerular shrinkage and sclerosis with leakage of plasma proteins. Therefore, the presence of nephrotoxins in the study area is suspected.

Acknowledgement

The current research was financially supported by a grant (No. 160015) from the Scientific Research Deanship, King Faisal University, Kingdom of Saudi Arabia.

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SPECIAL SESSION ON ADVANCES IN CAMEL SCIENCE IN PARIS, FRANCE

The 7th International Veterinary Congress scheduled on September 04-05, 2017 at Paris, France with a theme: "IMPECCABLE GROWTH OF THE VETERINARY SECTOR" will have a special session on. Advances in Camel science, on 5th September and will be chaired by Tarun Kumar Gahlot, Rajasthan University of Veterinary and Animal Sciences, India and Co-Chaired by Julian .A. Skidmore, Camel Reproduction Centre, United Arab Emirates and Serge Muyldermans, Vrije Universiteit Brussel, Belgium. Special session will have its opening paper on "Camel Science- Current Scenario and Future Envision" by Tarun Kumar Gahlot, followed by other papers by Ramadan O. Ramadan, King Faisal University, Saudi Arabia; Adel Almubarak, King Faisal University, Saudi Arabia; Abdulsalam Bakhsh, King Faisal University (KFU), Saudi Arabia; Julian A. Skidmore, Camel Reproduction Centre, United Arab Emirates; Serge Muyldermans, Vrije Universiteit Brussel, Belgium; Bernard Faye, CIRAD, France; Jagdish Lal Choudhary, Maharana Pratap University of Agriculture and Technology, India; Shawaf, King Faisal University, Saudi Arabia; Surong Hasi, Inner Mongolia Agricultural University/Key Laboratory of Clinical Diagnosis and Treatment Technology in Animal Disease, China; Guleng Amu, Inner Mongolia Agricultural University, China and Amit Sangwan, Rajasthan University of Veterinary and Animal Sciences, India.

INTERNATIONAL CAMEL CONFERENCE AT INNER MONGOLIA, CHINA

The international conference "The Belt and Road: Camel Science, Industry and Culture" will held on 22-26th September 2017 at Alxa League, Inner Mongolia, China. The Chairman of Organizing committee is Batu Chaolu (Chairman, China Animal Agriculture Association of Camel). The main topics covered will be Camel Genetics and Genomics, Camel Products: camel milk, meat, hair, camel blood, leather and bones, Camel Reproduction and Management, Camel Nutrition and Metabolism, Camel Health and Diseases and Camel Culture and Tourism. Deadline for abstract submission is July 31, 2017 and for Registration it is September 22, 2017. The Conference Venue is Badanjiren Town, West Alxa, Inner Mongolia, China. The organizer will cover registration, accommodation and local transportation for invited attendants. Contact details are email (camel_2017@163.com) and Website: http://www.china-camel.com.

NEW BOOK: THE CAMEL: THE ANIMAL OF THE 21st CENTURY (ISBN 81-903140-5-X)

The hard bound book authored by Dr Alex Tinson is spread in about 300 pages and titled, "The Camel : The Animal of the 21st Century". The book is highly illustrated and informative. It's contents include 6 chapters, i.e. Title Pages and Dedications, Introduction (Early History of the Centre, World's Firsts, World Press Releases, History of Domestication and Distribution, Evolution of Camel Racing in the U.A.E. and Historical Photos the Early Days), Camel in Health and Disease (Cardiovascular, Haemopoetic, Digestive, Nervous, Reproductive, Respiratory and Musculoskeletal Systems and Infectious, Skin and Parasitic Diseases. Additionally chapters on special senses, urinary tract and nutrition are also provided. Clinical Examination and Differential Diagnosis, Special Technologies (Anaesthesia and Pain Management in Camels, Diagnostic Ultrasound and X-Ray, Assisted Reproduction in Camels, Drug and Dna Testing and Surgery) and the Future are among the remaining chapters.

(First Edition : 2017 © 2017 Camel Publishing House, ISBN : 81-903140-5-X, Printed in India and authored by Dr Alex Tinson, Director of Laboratories and Research, Head Veterinarian Hilli Embryo Transfer and Surgical Centre, Al-Ain, U.A.E.)

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RENAL CELL CARCINOMA IN A FEMALE ARABIAN CAMEL

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ABSTRACT

This report describes the clinical, haematobiochemical, ultrasonographical and pathological findings in a female Arabian camel with renal cell carcinoma. The she camel had a history of weight loss, abdominal pain and red urine. Rectal palpation revealed an enlarged mass at the right kidney which distorted its normal conformation. Centrifugation of a urine sample yielded red sediment. Alterations in haematological and biochemical parameters included a decreased hematocrit per cent, red blood cell counts, haemoglobin concentration, total protein, albumin and globulin, and increased glucose, creatinine, sodium and potassium concentrations. Increase in the serum activity of aspartate aminotransferase and creatine kinase were also detected. Ultrasonographically, a caudally protruded, large, irregular shaped, hypoechoic and cavitated mass involving the right renal parenchyma was monitored. However, the left kidney subjectively appeared normal. At necropsy, haemorrhagic, irregular shaped and cavitated tumour involving the right kidney was detected. The right kidney was mostly pelvic. Compared to a weight of 1.5 Kg of the left, the right kidney weighed 18 Kg. Histopathologically, renal cell carcinoma showing tubular differentiation with malignant epithelial lining and nuclear anaplasia was suggested. No metastasis was found in other organs.

Key words: Camels, kidney, neoplasia, renal cell carcinoma, ultrasonography

Neoplasm has rarely been reported in camelids. This may be due, in part, to a low prevalence of neoplasia within the population or a lack of presentation for clinical examination (El-Hariri and Deed, 1979; Singh *et al*, 1991; Al-Ani, 2004). Although, relatively few tumours have been reported in camelids, the basic premise is that if a determined search were made through a sufficient population of camelids, neoplasia would be noted in all organ systems (Moulton, 1978; Fowler, 1987). Many necropsies of camelids are conducted by people without specialised training in pathology. This is especially true of neoplasia that may be incidental to the actual cause of death (Fowler, 2010).

Lymphosarcoma is the most commonly reported neoplasm in camelids and has been described in Ilama, alpacas, and Arabian camels (Fowler *et al,* 1985; Underwood and Bell, 1993; Irwin, 2001; Twomey *et al*, 2008).

Recently, in dromedary camels, case reports of tumours in dromedary camels were reported. It included seminoma and granulosa cell tumour (Ali *et al*, 2013a, 2013b), chondrosarcoma (Janardhan *et al*, 2011), corneal papilloma (Kilic *et al*, 2010), multicentric schwannoma (Khodakaram-Tafi and Khordadmehr, 2011), osteosarcoma (Tuttle *et al*, 2007), mammary and pulmonary carcinoma (Bryant *et al*, 2007), vertebral osteoma (Carbonell *et al*, 2006) and ovarian teratoma (Mesbah *et al*, 2002). Squamous cell carcinoma has been reported in guanaco, ilama and Arabian camel (Altman *et al*, 1974; Cornick 1988; Rogers *et al*, 1997; Tageldin and Omer, 1986). This report describes the clinical, haematobiochemical, ultrasonographical and pathological findings in a female Arabian camel with confirmed renal cell carcinoma.

Materials and Methods

Animal, history and physical examination

A 13-year old female dromedary camel was presented at the Veterinary Teaching Hospital, Qassim University, Saudi Arabia, with a history of weight loss and abdominal pain. Voiding of red urine was reported during the past month. The animal underwent a thorough physical examination which included general behaviour and condition, auscultation of the heart, lungs, rumen and intestine, measurement of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion auscultation of both sides of the abdomen and rectal examination (Köhler-Rollefson *et al*, 2001).

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Determination of haematological and biochemical parameters

A complete blood count was carried out on the EDTA sample using the VetScan HM5, (Abaxis, California, USA). An automated biochemical analyser VetScan VS2, (Abaxis, California, USA) was used to determine the serum concentrations of total protein, albumin, globulin, glucose, blood urea nitrogen (BUN), creatinine, creatine kinase (CK), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), total bilirubin, sodium and potassium.

Ultrasonographic examination

The foreleg of the female camel was restrained in. The animal was lightly sedated using intravenous xylazine (0.02 mg/kg 10% Bomazine, Bomac Laboratories Ltd, New Zealand). Both flanks were clipped and the skin shaved. Ultrasonographic examination was carried out using 3.5 MHz sector and 7.5 MHz linear transducers (SSD-500, Aloka, Tokyo, Japan).

After the application of transmission gel to the transducer, the right and left kidneys were examined at the upper right and caudal left paralumbar fossa, 11th ICS and middle left paralumbar fossa. The left kidney was also imaged longitudinally and in a cross-sectional view, and transrectally with the 7.5 MHz linear transducer. Transmission gel was applied to the transducer which was then placed in a plastic rectal glove before being introduced into the rectum. The transducer was placed ventrally, laterally and dorsally to the left kidney (Tharwat *et al*, 2012). Because of the enlargement of the right kidney, it was also possible to scan it transrectally.

Postmortem examination and histopathology

Because of the grave prognosis the female camel was euthanised and postmortem examination was carried out. A tumour specimen was fixed in 10% buffered formalin, processed in wax, sectioned and stained with hematoxylin and eosin for routine histopathology.

Results

The main owner complaint consisted of weight loss and fits of abdominal pain (rolling). A history of haematuria was reported during the last month. Rectal palpation revealed an enlarged mass. Centrifugation of a urine sample yielded red sediment (Fig 1).

Haematological examination revealed hematocrit 21% (reference range 28.9±2.7 %), RBCs 9.32×10^6/µl (reference range $11.3\pm1.4\times10^6$/µl), haemoglobin 14.2 g/dl (reference range 16.0±2.3 g/dl), MCV 23 fl (reference range 25.5±1.5 fl), MCH 15.2 pg (reference range 14.7±2.4 pg), MCHC 66.3 g/dl (reference range 57.6±9.0 g/dl), white blood cell count 21970/µl (reference range $16.9\pm2.7\times10^9$/l), neutrophils 19120 /µl (reference range $9.8\pm3.0\times10^9$/l), and lymphocytes 2300 /µl (reference range $5.9\pm2.4\times10^9$/l).

Blood chemistry panel showed total protein 6.3 g/dl, albumin 3.4 g/dl, globulin 2.9 g/dl, glucose 182 mg/dl, BUN 13 mg/dl, creatinine 1.9 mg/dl, CK 252 U/l, calcium 9.4 mg/dl (reference range 8.6±0.7 mg/dl), AST 103 U/l, GGT 10 U/l, total bilirubin 0.4 mg/dl, sodium 136 mmol/l and potassium 4.3 mmol/l.

Transrectal ultrasonography revealed a caudally protruded, large, irregular shaped, hypoechoic and cavitated mass involving the right renal parenchyma. However, the left kidney subjectively appeared normal (Fig 2) as previously reported (Tharwat *et al*, 2012).

Necropsy findings revealed the presence of a haemorrhagic, irregular shaped and cavitated tumour involving the right kidney. The right kidney occupied mostly the pelvic cavity. Compared to a weight of 1.5 Kg of the left kidney, the right weighed 18 Kg (Fig 3).

Histopathological examination of the renal specimen revealed renal cell carcinoma showing tubular differentiation with malignant epithelial lining and nuclear anaplasia (Fig 4). No metastasis was found in other organs or even in the left kidney.

Discussion

In this report, the tumour mass involving the right kidney was detected incidentally during transrectal ultrasonography of a female camel with red urine. It was confirmed histopathologically to be renal cell carcinoma. Only another case of renal cell carcinoma was reported in the veterinary literature (Vitovec, 1982). It was identified in a dromedary camel slaughtered at the Mogadishu abattoir, Somalia. Grossly, the renal carcinoma was a large, ovoid, 21 × 12 × 12-cm expansive mass occupying most of the right caudal pole of the right kidney. The tumour was light brown on the cut surface and had a pseudolobular arrangement. This neoplasm was the only tumour found in about 13,000 camels slaughtered in the abattoir (Vitovec, 1982).

Renal carcinoma, also known as renal cell carcinoma or renal adenocarcinoma, is the most common form of renal neoplasia in humans and accounts for approximately 3% of all malignant

Fig 1. A female camel with renal cell carcinoma of the right kidney. Left image shows voiding of red urine. Middle image shows haematuria that yielded red sediment after centrifugation (right image).

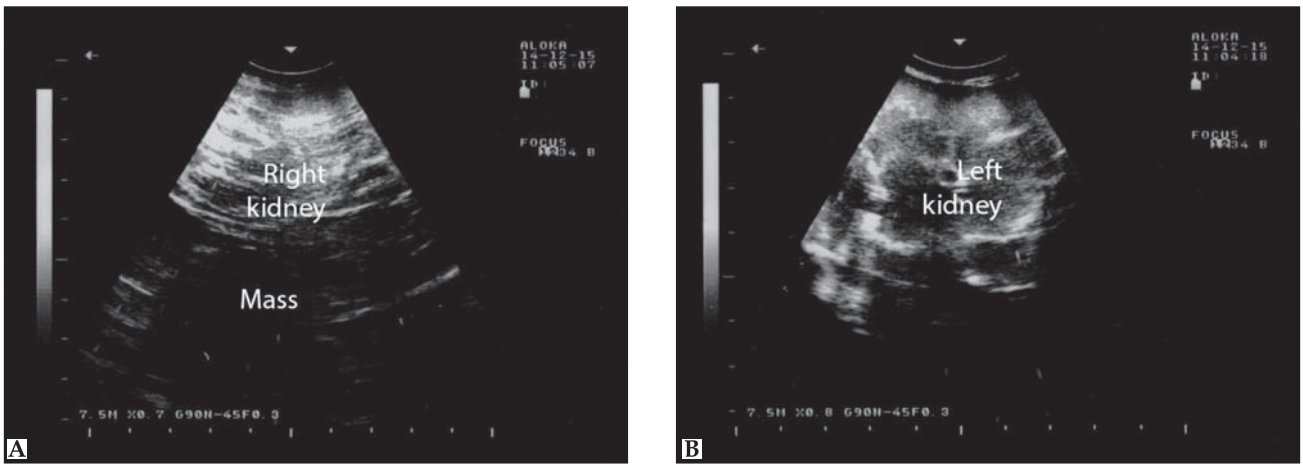

Fig 2. Transrectal ultrasonographic findings in a female camel with renal cell carcinoma of the right kidney. Image A shows a hypoechoic mass involving the right renal parenchyma while the image B shows the normal left kidney.

neoplasms in human adults (Young *et al*, 2006). In humans, the tumours often are discovered incidentally and, if diagnosed early, treatment by full or partial nephrectomy combined with immunotherapy can result in long-term survival (Flanigan, 2007). As in humans, renal carcinoma is the most common form of primary upper urinary tract neoplasia in dogs and horses (Traub-Dargatz, 1998; Bryan *et al*, 2006). Primary urinary tract neoplasia is rare in horses. In a survey of 3,633 horses examined by necropsy in New York State from 1953 to 1976, only 4 cases of renal neoplasia were identified yielding an incidence of 0.11% (Haschek *et al*, 1981).

In the present case, clinical signs included weight loss, abdominal pain and additionally red urine during the last month. These findings correlate well with findings of Wise *et al* (2009) in horses. This clinical course correlates with what is described in human medicine in that clinical signs of abdominal pain and haematuria or detection of an abdominal mass are not recognised until very late in the course of disease. Approximately 50% of renal neoplasms in humans are incidental findings discovered while imaging the abdomen (Flanigan, 2007). The blood picture and serum chemistry panel in the preset case provided little aid in obtaining a diagnosis of renal carcinoma. Our results agree with Wise *et al* (2009) in horses. On the contrary, transrectal ultrasound examination was helpful in providing information on the extent of the tumour and involvement of

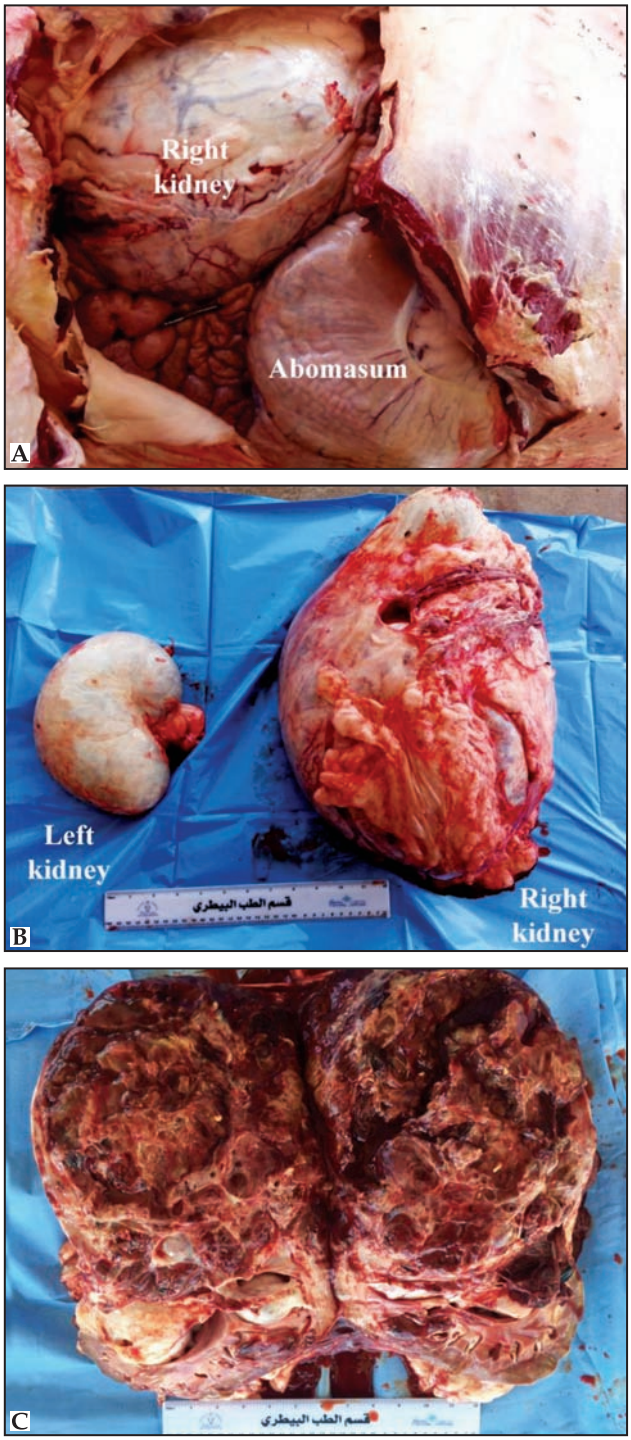

Fig 3. Postmortem findings in a female camel with tumour of the right kidney. Image A, shows that the right kidney with tumour occluding the pelvic cavity. Image B, shows 18 Kg right kidney compared to 1.5 Kg left kidney. Image C, shows cross section through the right kidney large, haemorrhagic, irregular shaped and cavitated tumour.

surrounding structures. Renal ultrasound examination is therefore, the most rewarding imaging procedure.

In this report, it was interesting to find the tumour mass involving the right kidney as Vitovec

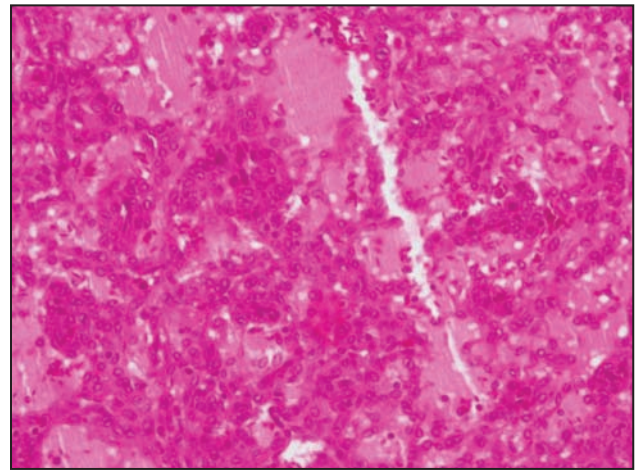

Fig 4. Renal cell carcinoma showing tubular differentiation with malignant epithelial lining and nuclear anaplasia (HE×400).

(1982) found in another camel. Wise *et al* (2009) reported that the primary renal carcinomas are most commonly unilateral and exhibit locally aggressive behaviour and metastasis. In the present case, the tumour mass was found also unilateral, however, no metastasis was found in other organs. In conclusion, the she camel presented for renal carcinoma exhibited nonspecific signs except haematuria in the late stage of the disease process. Laboratory findings were nonspecific, probably because the neoplasia was unilateral. Preliminary clinical diagnosis most often is based upon rectal examination and abdominal ultrasound examination. Histopathological examination of a tumour specimen remains the confirmatory diagnosis.

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News

MARWAR CAMEL CULTURE FESTIVAL FROM 3-5 NOVEMBER 2017

Marwar Camel Culture Festival will be organised from 3-5 November 2017 at LPPS campus, Sadri, Pali District, Rajasthan, India. It will have a variety of programmes, i.e. All-Rajasthan Camel Breeders' Meeting and Debate with Policy Makers, Visits to Nomadic Raika Camel Herds with Camel Milk Tea, Camel Cheese and Pastries with Anne Bruntse and Robert Paget, Camel Milk and Autism, Workshop with Christina Adams and Camel Milk Competition. There will be a brain storming session on the topic "What kind of Camel Science do we need?". There will be eminent penalist for this discussion. Festival will have the attractions like Rooftop Camel Café, Camel Film Festival with Clara Wieck, Doug Baum and others, Full Moon Concert of Camel Songs and Sufi Music with Rajasthani Musicians and Camel Craft Bazaar with potters from Molela, weavers, camel poo paper.

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RESISTOTYPING OF CAMEL SKIN WOUNDS ASSOCIATED *Staphylococcus aureus* ON THE BASIS OF MULTIDRUG RESISTANCE PATTERN

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ABSTRACT

The present investigation was attempted to type *Staphylococcus aureus* associated with camel skin wounds on the basis of multidrug resistance pattern against 35 antibiotics of different generations. Beta-lactamase activity was also determined for the isolates. Twenty six *S. aureus* isolates were obtained from camel skin wounds and confirmed by 23S rRNA gene ribotyping. We recorded susceptibility of 100% isolates to azithromycin, netillin, polymixin-B and rifampicin followed by susceptibility of 96.15% isolates to chloramphenicol and gentamicin, 92.30% to bacitracin, novobiocin and cloxacillin, 88.46% to clindamycin, 84.61% to tobramycin, 80.77% to erythromycin and 69.23% isolates were sensitive to ceftriaxone, methicillin, doxycycline hydrochloride, cefaclor, ciprofloxacin, norfloxacin, ofloxacin, amoxicillin, amoxyclav, sparfloxacin and trimethoprim. Nineteen different resistotypes were identified with 0.9508 discriminatory index. This higher number of resistotypes and more discriminatory index may suggest higher diversity and resistance by the isolates. The continuous local surveillance and genotypic explorations should be performed on regular basis in order to have adequate information for antibiotic resistance patterns of *S. aureus* infections.

Key words: Camel, multidrug resistance, resistotypes, skin, *Staphylococcus aureus*, wounds

The skin infections including contagious skin necrosis, dermatitis, wounds, abscesses or similar lesions is a great problem in camel. Most of the skin infections have been found to be caused by staphylococci. The disease is not fatal but due to reduced working efficiency it causes great economic losses. The skin infections are difficult to be treated medically depending on among other factors, the pathogenic quantities of the staphylococcal strain present (Wernery, 2000). The literature regarding microbiology of the skin wounds in camel is very less (Qureshi *et al,* 2002) but *Staphylococcus aureus* has been found to be most common pathogen associated with skin wounds.

Over the last few decades, there was a sudden increase in the use of antibiotics in veterinary as well as human health care not only to control disease but also as prophylactic measure for bacterial infections secondary to viral infections (Lindeman *et al,* 2013). The use of antibiotics in a frequent manner leads to development of resistance in different disease causing bacterial species. So it is very important to know about the resistance or susceptibility of the bacteria prior to administration of the treatment (Wang *et al,* 2008).

The prescription of new antibiotics to manage *S. aureus* has frequently been followed by the uprising of resistant strains (Schito, 2006). Most significantly, *S. aureus* isolates resistant to beta-lactams have become common. The ability of *S. aureus* to survive in the presence of β-lactam antibiotics remains the main problem in the therapy (Pinho, 2008). Due to various mechanisms of acquired β-lactam resistance, several resistance phenotypes have been described so far in *S. aureus* (Chambers, 1997). These include β-lactamase acquisition, modification of penicillin-binding proteins, or acquisition of low-drug-affinity penicillin-binding proteins. Beta lactams such as penicillin are the most widely used antibiotics and beta-lactamases are the greatest source of resistance to penicillins. An understanding of beta-lactamase detection is therefore valuable (Kilic and Cirak, 2006).

Presently there is growing concern among scientists in regards to increasing resistance in pathogens. The concerns are multifaceted *viz.* inaccurate diagnosis, defective dosage, indiscriminate use, development of new drugs etc. Thus the aim of this study was to assess diversification among *S. aureus* in regards to resistance patterns and to

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determine the level of drug resistance to various classes of antibiotics. This study is of significance in improving baseline data on antibiotic resistance shown by *S. aureus* isolated from camel skin wounds for the prudent use of antibiotics and to promulgate antibiotic policies in disease control programs.

Materials and Methods

Bacterial isolates

A total of 41 swabs from skin wound in camels in and around Bikaner were collected and processed for isolation and identification of *S. aureus* (Quinn *et al,* 1994). All phenotypically identified isolates were further confirmed by ribotyping based on 23S rRNA gene (Straub *et al,* 1999).

Beta-lactamase activity (Acidimetric method)

The method described by Livermore and Brown (2001) was used to demonstrate Beta-lactamase activity.

Antibiotic sensitivity test

The antibiogram of isolates against different antibiotics were determined using method of Bauer *et al* (1966). The interpretation for resistant, sensitive and intermediates was drawn as breakpoints defined by Clinical and Laboratory Standards Institute (CLSI).

Discriminatory index

The discriminatory ability of the different typing system i.e. their ability to distinguish between unrelated strains was determined by the number of types defined by the test method and the relative frequency of their types. The numerical index of discrimination was calculated using the formula given by Hunter and Gaston (1988).

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj(nj-1)$$

Where,

D = Discriminatory index, S = Total number of type used, nj = Number of strains belonging to j^{th} type, N = Total number of strains.

Results and Discussion

The antibiogram developed for 26 *S. aureus* revealed that the most effective antibiotics were azithromycin, netillin, polymixin-B and refampicin against which all the isolates were sensitive followed by chloramphenicol and gentamicin against which 96.15% of the isolates were sensitive, 92.30% isolates were sensitive to bacitracin, novobiocin and cloxacillin, 88.46% to clindamycin, 84.61% to tobramycin, 80.77% to erythromycin, 69.23% isolates were sensitive to ceftrioxane, methicillin, doxycycline hydrochloride, cefaclor, ciprofloxacin, norfloxacin, ofloxacin, amoxicillin, amoxiclav, sparfloxacin and trimethoprim. The other antibiotics were less effective. Nalidixic acid was found to be the most ineffective antibiotic. Interestingly 100% resistance was not recorded for any of the studied 35 antibiotics in the present study (Table 1). In this investigation, acidimetric method was used for detection of beta-lactamase activity and we found that only 8 (30.76%) isolates from camel skin wounds were beta-lactamase producer.

In the resistotyping, multidrug resistance was detected among all isolates except 5 (C9, C10, C15, C22 and C23) which were resistant to two antibiotics (cefalexin and nalidixic acid). Nineteen different resistotypes were detected (Table 2) with 0.9508 discriminatory index and resistance pattern against maximum 24 and minimum 2 antibiotics. The more number of resistotypes and higher value of discriminatory index indicate capabilities of resistotyping method as powerful tool to discriminate isolates. Hunter and Gaston (1988) calculated discriminatory index on the basis of total number of unrelated strains and total number of observed patterns to assess discriminatory power of typing method. It was recommended that the method with more than 0.70 discriminatory index would be considered as good discriminatory method and higher diversity among studied isolates.

The results in the present study were almost in accordance with the observations of Rathore and Kataria (2012) for azithromycin, gentamicin, norfloxacin and nalidixic acid and those of Qureshi and Kataria (2004) for gentamicin, chloramphenicol and cloxacillin who also studied *S. aureus* isolates from camel skin wounds and abscesses from the same study area. Yadav *et al* (2015) also reported similar results as in the present study for netillin, rifampicin, gentamicin, azithromycin and bacitracin from the same study area. In the present study the susceptibility of *S. aureus* to gentamicin is almost similar to that recorded by Ebrahimi and Akhavan Taheri (2009) who found 100% of the isolates susceptible to gentamicin. The continuous observations of susceptibility towards gentamicin in all the previous studies in this area suggest that this antibiotic is not being used in most of the treatment regimens in this area.

Table 1. Antibiogram for *S. aureus* isolates associated with camel skin wounds.

S. No.	Antibiotic disc	Percent (%)		
		Sensitive	Intermediate	Resistant
1	Azithromycin (AZM)	100	–	–
2	Netillin (NET)	100	–	–
3	Polymixin-B (PB)	100	–	–
4	Rifampicin (RIF)	100	–	–
5	Chloramphenicol (C)	96.15	3.85	–
6	Gentamicin (HLG)	96.15	–	3.85
7	Bacitracin (B)	92.30	7.69	–
8	Novobiocin (NV)	92.30	3.85	3.85
9	Cloxacillin (COX)	92.30	–	7.69
10	Clindamycin (CD)	88.46	11.53	–
11	Tobramycin (TOB)	84.61	7.69	7.69
12	Erythromycin (E)	80.77	19.23	–
13	Levofloxacin (LE)	69.23	26.92	3.85
14	Ceftriaxone (CTR)	69.23	23.07	7.69
15	Methicillin (MET)	69.23	19.23	11.53
16	Doxycycline hydrochloride (DO)	69.23	19.23	11.53
17	Cefaclor (CF)	69.23	7.69	23.07
18	Ciprofloxacin (CIP)	69.23	3.85	26.92
19	Norfloxacin (NX)	69.23	3.85	26.92
20	Ofloxacin (OF)	69.23	3.85	26.92
21	Amoxicillin (AMX)	69.23	–	30.76
22	Amoxiclav (AMC)	69.23	–	30.76
23	Sparfloxacin (SPX)	69.23	–	30.76
24	Trimethoprim (TR)	69.23	–	30.76
25	Cotrimoxazole (COT)	65.38	3.85	30.76
26	Moxifloxacin (MO)	65.38	3.85	30.76
27	Ampicillin (AMP)	65.38	–	34.61
28	Azlocillin (AZ)	65.38	–	34.61
29	Neomycin (N)	53.85	15.38	30.76
30	Oxytetracycline (O)	53.85	–	46.15
31	Cefotaxime (CTX)	26.92	26.92	46.15
32	Cefixime (CFM)	19.23	38.46	42.30
33	Cephalexin (CN)	11.53	–	88.46
34	Vancomycin (VA)	3.85	53.85	42.30
35	Nalidixic acid (NA)	–	3.85	96.15

In the present study, susceptibility of isolates towards nalidixic acid and vancomycin was very less but Qureshi and Kataria (2004) reported higher susceptibility towards vancomycin. The lower susceptibility of isolates in the present study towards cefixime in the present study is similar to those reported by Upadhyay and Kataria (2009), Rathore and Kataria (2012) and Yadav *et al* (2015). This antibiotic though not being used in the animals but higher resistance of isolates shows that it might have been transferred from human subjects to animals.

Sanjiv and Kataria (2006) and Upadhyay and Kataria (2009) used some similar antibiotics as in this study against *S. aureus* isolates of milk origin from cattle and goats obtained from the same area and reported higher number of isolates susceptible to cloxacillin, gentamicin, bacitracin, chloramphenicol, novobiocin as recorded in the present study. In present investigation, resistance towards methicillin was recorded in 11.53% whereas, El-Jakee *et al* (2010) recorded higher resistance (60%) by *S. aureus* isolates.

Our results are in conformity to earlier observation from same study area made by Yadav *et al* (2015) who reported 34.37% *S. aureus* isolates to be positive for beta-lactamase activity in a lot of 32 isolates obtained from cattle and buffalo mastitic milk. In a study conducted by Oberhofer and Towle (1982), 83.33% of 60 penicillin resistant and intermediate *S. aureus* isolates showed as beta-lactamase producers by acidimetric method. Still a higher percentage of beta-lactamase producing isolates were reported by Kilic and Cirak (2006) who reported as high as 84.3% to 85.5% beta-lactamase producers by using acidimetric method.

The increasing incidence of obtaining antimicrobial resistant pathogens has severe implications for the future treatments and prevention of infectious diseases in both animals and humans (White and McDermott, 2001).

The indiscriminate usage of antibiotics in domestic animals leads to treatment failure, escalated treatment costs and development of resistance to antimicrobials. Such resistance resulted in infections that are more difficult to cure. The efficacy of conventional antibiotic treatments against pathogens such as *S. aureus* is low (Wilson *et al,* 2003). Penicillin and closely related antibiotics of the β-lactam family are the best weapons against staphylococci. However, the massive usage of these antibiotics has led to a dramatic increase in pathogens that can produce an enzyme called β-lactamase that inactivates β-lactam antibiotics, thereby resulting in microbial resistance (Aarestrup and Jensen, 1998). Therefore, there is an urgent need to find new antimicrobials to treat bacterial pathogens and for maintaining optimum health state.

Table 2. Resistotypes of *S. aureus* isolates associated with camel skin wounds.

S. No.	Isolate ID	Isolate No.	Resistance pattern (Resistotype)	No. of antibiotics
1.	C17	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CTR, CIP, COX, COT, HLG, MET, MO, NA, N, NX, OF, O, SPX, TOB, TR, VA	24
2.	C6	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CIP, COT, DO, LE, MO, NA, N, NX, OF, O, SPX, TR, VA	21
3.	C20	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, COX, COT, MET, MO, NA, N, OF, O, SPX, TOB, TR, VA	20
4.	C21	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CTR, CIP, COT, MET, MO, NA, N, NX, OF, O, SPX, TR	20
5.	C5	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CIP, COT, DO, MO, NA, N, NX, OF, O, SPX, TR	19
6.	C2	1	AMX, AMC, AMP, AZ, CF, CN, CFM, CTX, CIP, COT, MO, NA, N, NX, OF, O, SPX, TR	18
7.	C3	1	AMX, AMC, AMP, AZ, CN, CTX, CIP, COT, DO, MO, NA, N, NX, OF, O, SPX, TR	17
8.	C18	1	AMX, AMC, AMP, AZ, CN, CFM, CTX, CIP, COT, MO, NA, N, NX, O, SPX, TR, VA	17
9.	C1	1	CN, CFM, CTX, NA, VA	5
10.	C7	1	CN, CTX, NA, O	4
11.	C8	1	CN, NA, NV, O	4
12.	C12	1	CN, CFM, NA, O	4
13.	C14	1	CN, CFM, CTX, NA	4
14.	C16	1	CN, CTX, O, VA	4
15.	C25	1	AZ, CN, NA, VA	4
16.	C4	1	CN, CFM, NA	3
17.	C11	1	AMP, CN, NA	3
18.	C13, C19, C24 & C26	4	CN, NA, VA	3
19.	C9, C10, C15, C22 & C23	5	CN, NA	2

The overall analysis of results of previous studies on *S. aureus* isolates from different sources revealed that the susceptibility of the organisms against the antibiotics has greatly reduced, the reason for which appears to be obvious. In this area, the awareness of farmers towards animal care has increased tremendously and they seek veterinary help promptly as and when it is required. The availability of vide variety of antibiotic regime promotes the multidrug resistance and diversification of wide resistance thus the more resistance patterns may exist among *S. aureus* isolates. It requires continuous surveillance of antibiotic susceptibility pattern of isolates. The study may further extend for genotypic characterisation of *S. aureus* isolates to explore various genetic traits involve in resistance mechanisms of organism.

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Department of Surgery & Radiology,[1,3 and 5] College of Veterinary and Animal Science,[2 and 4], College of Veterinary Sciences, CCS-Haryana Agricultural University, Hisar, 125004 INDIA.

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SEQUENCE ANALYSIS AND PHYLOGENETIC RELATIONSHIP OF MYOSTATIN GENE OF BIKANERI CAMEL (*Camelus dromedarius*)

V.K. Agrawal, G.C. Gahlot, M. Ashraf, J.P. Khicher and S. Thakur
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Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001, India

ABSTRACT

The draught potential of the Bikaneri camel depends on the gene affecting the muscular growth. Myostatin (*MSTN*) or growth and differentiation factor (*GDF8*) gene is the major regulator of myogenesis and skeletal muscle growth in mammals. Genomic *DNA* was isolated from whole blood of randomly selected Bikaneri camels (n=6) through spin column method. The *MSTN* exon-2 region of 375 bp was amplified using primers designed from homologous regions of *MSTN* gene sequence (GenBank accession number. DQ167575). The *PCR* amplified fragments of all the animals were sequenced through Sanger dideoxy chain termination method. The sequence of *MSTN* exon-2 gene was submitted to *NCBI* GenBank database to which an accession number KX863740 was assigned. A Neighborhood Joining (NJ) phylogenetic tree was constructed based on the lowest Bayesian Information Content (BIC) value. Sequence comparison of *MSTN* exon-2 gene of Bikaneri camel with homologous regions of goat, sheep, Algerian camel, buffalo, horse and pig revealed more than 95% homology. The sequence information generated for *MSTN* gene of Bikaneri camel would help in better understanding of growth traits and could support in conservation of dwindling camel population in Rajasthan.

Key words: Bikaneri camel, growth, myostatin gene, sequence analysis

The one humped Bikaneri breed of camel is the heaviest camel breed (Khanna *et al,* 2004) that is well known for its load carrying capacity and is primarily used for transport (Faye, 2015). Myostatin (*MSTN*) or GDF8 (growth and differentiation factor 8) gene is considered as candidate gene with functional and positional role in the regulation of muscular growth in different parts of the body (Tahmoorespur *et al,* 2011). The *MSTN* gene functions as a negative regulator of skeletal muscle growth in mammals (Peng *et al,* 2013). Quantitative trait loci (QTL) studies showed that myostatin gene affect the muscular development and muscle depth through alteration in amount and composition of muscle fibres (Zhang *et al,* 2012). The gene functions as a "chalone" and helps to maintain a global balance in tissue growth (McPherron and Lee, 1997). Molecular analysis of the *MSTN* gene in different species has shown that it consists of 3 exons and 2 introns (Kurkute *et al,* 2011). Mutations in the *MSTN* gene could alter its expression and may affect muscle fibre development and may cause dramatic muscularity (Mirhoseini and Zare, 2012). Endogenous myostatin mutations present in the breeding herds are difficult to detect due to highly conserved nature of myostatin gene across livestock species.

The sensitivity of earlier methods, such as single-stranded conformation polymorphism analysis (SSCP) (Kunhareang *et al,* 2009) to detect single nucleotide polymorphism (SNPs) are low (70% to 80%) and require considerable skill and labour. Direct gene sequencing is a powerful method for identifying nucleotide sequence variation in amplified DNA and is considered as the gold-standard approach for genotyping analysis and expected to have almost 100% sensitivity (Laurie and George, 2009). Sequence analysis of *MSTN* gene of Bikaneri camel and its comparison with different species or breeds would help in better understanding of muscle development and differential growth mechanisms. Such knowledge will be helpful in selection and mating strategy, development of knockout technology and understanding the structure, function and evolution of the gene. The characterisation of *MSTN* gene through gene sequence analysis would also help in the establishment of current status of Bikaneri camel.

Till now, few studies (Muzzachi *et al,* 2015) have been conducted in camel to investigate polymorphism at different myostatin loci. Thus the sequence analysis

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of *MSTN* exon-2 region in Bikaneri camel (*Camelus dromedarius*) has been undertaken in the present study.

Materials and Methods

Bikaneri camels (n=6) of unknown pedigree were randomly selected from different regions of Bikaner district of Rajasthan. The blood samples (2ml) were collected from jugular vein in vacutainer tubes containing EDTA as an anticoagulant. The genomic DNA was extracted by spin column method as per manufacturer's protocol. The quality and the concentration of DNA were checked on 0.8% agarose and nano drop spectrophotometer, respectively. Amplification primer pair was designed based on the caprine *MSTN* gene sequence (GenBank accession number. DQ167575) to amplify 375 bp fragment of exon-2 region (F-5'AAAAACCCAAATGTTGCTTCTTTA3'; R5'CAGTCCTTCTTCTCCTGGTCTGG3'). Amplification reactions for each sample was done by using the following constituents in a final volume of 25 µl containing 5X PCR buffer, 1 unit of Taq DNA polymerase, 0.2 mM each of dNTPs, 1.5 mM $MgCl_2$, 75 pMol of each primer and 100 ng of template DNA. Amplification was performed in a thermal cycler with the following program; after an initial denaturation step at 95°C for 5 min, 35 cycles were programmed as follows: 94°C for 30s, 54°C for 60s, 72°C for 60s and final extension at 72°C for 10 min. The amplified DNA fragments were stained with ethidium bromide and visualised on 1.5% agarose gel under gel documentation system.

Sequencing and Sequence Analysis of Amplicons

The amplicons for each sample were initially purified and then sequenced through Sanger dideoxy chain termination method by X celris Genomic Services (Ahmedabad, India) in both directions. Forward and reverse sequences of each gene fragment was assembled against the most closely related reference sequence of respective gene to obtain total sequence length and similarity was looked in to the non-redundant database of GenBank with BLAST algorithms (http://www.ncbi.nlm.nih.gov/BLAST/). The nucleotide sequence of the amplified fragment was submitted to NCBI database. Pair wise sequence analysis and alignment was carried out using Clustal X2 and Bioedit (v 7.0.7.1) bioinformatics tools to identify each nucleotide substitution between different samples.

Phylogenetic Analysis

Sequence analysis was further validated by multiple sequence alignment of query sequence of Bikaneri camel with *MSTN* gene sequence of different species in FASTA format using Clustal X2. Phylogenetic tree was constructed using Neighbourhood Joining (NJ) method of bootstrap test of phylogeny in MEGA7 (Kumar *et al,* 2016) to evaluate the evolutionary relationships of Bikaneri camel *MSTN* exon-2 with the *MSTN* gene of other farm animals. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and implemented with bootstrap test involving simple stepwise addition.

Results and Discussion

The present study reports for the first time the nucleotide sequence of the exon-2 region of *MSTN* gene for Bikaneri camel (*Camelus dromedarius*). The amplification band of 375 bp of *MSTN* exon-2 coding region was obtained from all the samples of Bikaneri camel (Fig 1). The sequence generated in the present study was submitted to the GenBank NCBI database and accession number KX863740 was obtained. The sequence alignment of the respective amplified products from all the 6 randomly selected camels revealed monomorphism and generated similar sequence information for 375bp fragment. The highly conserved nature of *MSTN* exon-2 gene observed in the present study in Bikaneri camel is suggestive to compare the observed sequence with other camel breeds or species to reveal the presence of any intra and inter species polymorphism.

The *MSTN* exon II gene of Bikaneri camel was compared with homologous regions of *Bubalus bubalis* (KJ123755), *Sus scrofa* (HM241657), *Capra hircus* (HM462259), *Ovis aries* (JN856459), *Equus caballus* (NM_ 001081817) and *Camelus dromedarius* Algerian population (KJ847811) which revealed that *MSTN* exon-2 region is highly conserved among livestock species. More than 95% homology of Bikaneri camel *MSTN* exon-2 with that of the buffalo, pig, goat, sheep, horse and Algerian camel sequences published in the NCBI GenBank database, was observed.

The Tamura 3 parameter with gamma distribution model (T92+G) having lowest BIC (Bayesian Information Criteria) value of 617.97 was selected for the construction of phylogenetic tree (Fig 2). The optimal NJ tree was constructed with the summed branch length of 0.3382104. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000

replicates) was shown next to the branches. Sequence of the *MSTN* exon-2 gene obtained in the present study showed 98% homology towards *Camelus dromedarius* Algerian (KJ847811), *Capra hircus* (HM462259) and *Ovis aries* (JN856459). A 96% homology was found between obtained *MSTN* exon-2 gene sequence and *Sus scrofa* (HM241657). A relatively lower homology (95%) was documented between the obtained *MSTN* exon-2 gene sequence and *Bubalus bubalis* (KJ123755) and *Equus caballus* (NM_ 001081817).

A closer relationship between Tylopoda and Suiformes was observed in the present study which is in line with previous reports (Muzzachi *et al,* 2015). Bikaneri camel and goat shared the same cluster with 98% homology, indicating that these 2 species have had a small number of mutations in this gene. The lowest homology observed between Bikaneri camel and *Bubalus bubalis* is in agreement with Muzzachi *et al* (2015). The high level of sequence conservation among all myostatin orthologs suggests the importance and conservation of its function in vertebrates (Karim *et al,* 2000). Absence of polymorphism in *MSTN* exon-2 gene of Bikaneri camel is in agreement with the reports on different *Camelus dromedarius* breeds (Shah *et al,* 2006; Muzzachi *et al,* 2015). The absence of *MSTN* diversity observed in Bikaneri camel reflects the evolution of camel from low variable wild ancestor population.

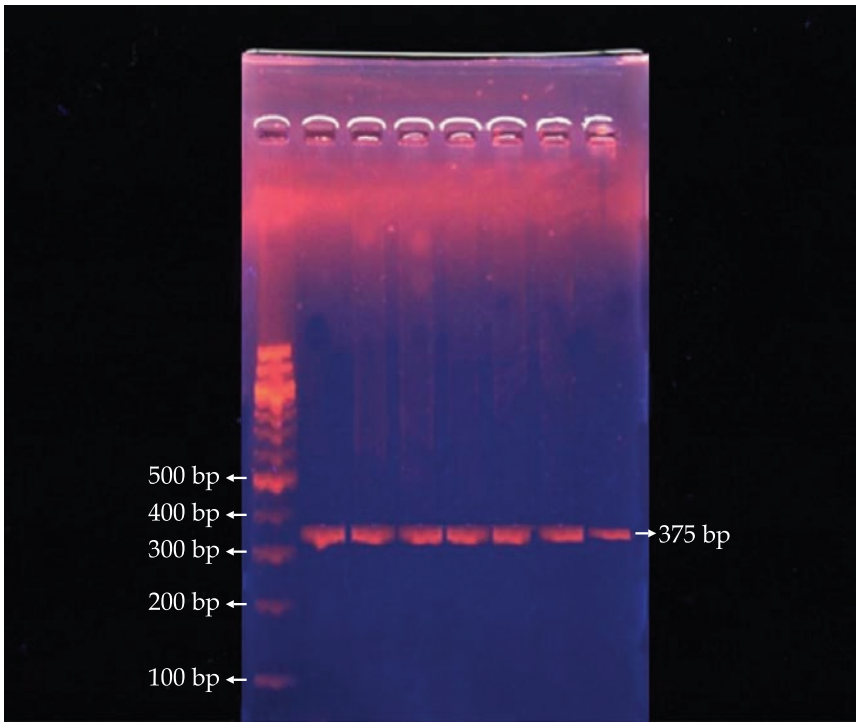

Fig 1. PCR Amplification of myostation (*MSTN*) Exon-2 Gene of Bikaner camel legends lane 1: Molecular weight marker lane 2:-8: PCR amlpcion of mystation (*MSTN*) Exon-2 Gene.

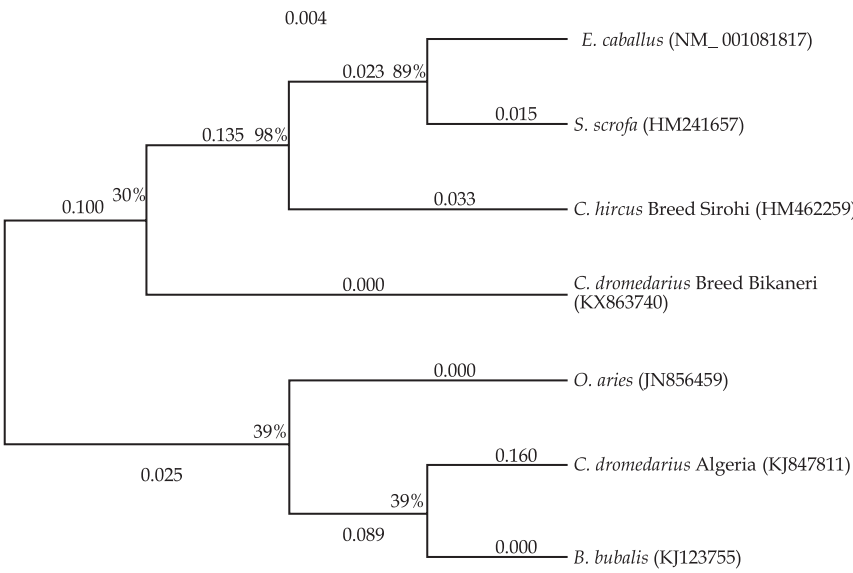

Fig 2. Phylogenetic tree showing common ancestry at *MSTN* exon-2 Gene by NJ method.

The sequence information generated for *MSTN* exon-2 gene of Bikaneri camel would help in better understanding of growth traits and provides clues for investigation of other regions of myostatin gene in Bikaneri camel for the conservation of dwindling camel population in Rajasthan (Livestock Census, 2012). Such knowledge will be helpful in further breeding and selection strategy. The sequence comparison of *MSTN* exon-2 gene of Bikaneri camel with other species could also elucidate the mechanism of diseases associated with emaciation in camels such as Trypanosomosis as over-expression of myostatin gene was

observed in muscle wasting and atrophy (Ma *et al,* 2003).

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THERAPEUTIC EFFECTS OF *Bokhi* FROM CAMELS ON UTERINE LEIOMYOMA

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ABSTRACT

Bokhi is a transparent, water-soluble, sticky and odorous liquid containing sex steroids, that is secreted by male camels from their occipital or poll glands during the mating season. We investigated whether *Bokhi* has positive therapeutic effects on the treatment of uterine leiomyoma (ULM). ULM model rats were established by multipoint subcutaneous injections of a combination of diethylstilbesterol and progesterone for 11 successive weeks. Rats were then treated by oral administration of *Bokhi* for a further 7 successive weeks. Following the 11 weeks of injections the levels of serum estradiol (E_2), progesterone (Pro), follicle-stimulating hormone (FSH), tumour necrosis factor (TNF-α) and nitric oxide synthase (NOS) in ULM rats were significantly higher than the non-ULM control rats ($P < 0.05$). This demonstrated that development of the ULM model was successful. After 7 weeks of oral treatment with *Bokhi* there was no significant difference in the levels of E_2, Pro, FSH, TNF-α, iterleukin-2 (IL-2) and NOS between the ULM rats fed high doses of *Bokhi* (HDB) and the non-ULM control rats ($P > 0.05$). This demonstrates that high doses of *Bokhi* from camels could improve ULM and that there should be further research on *Bokhi* and its potential therapeutic uses.

Key words: *Bokhi*, camel, occipital gland secretion, sex steroid, uterine leiomyoma

Uterine leiomyoma (ULM) is the most common benign tumour in the human female reproductive organs (Gambadauro *et al,* 2012) and the incidence rate is as high as 70% (Shen *et al,* 2009). There is no effective long-term medical therapy and surgery remains the mainstay of treatment for these patients (Islam *et al,* 2013). Therefore, studies to improve treatments for ULM have attracted a lot of attention in recent years.

Bokhi is the Mongolian name for the material that is secreted by male camels from the occipital or poll gland which is located on the neck midline behind the ear and is composed mainly of sweat glands and sebaceous glands. During the rutting season the occipital gland is activated; its morphological structure changes and its function is enhanced. In non-rutting season the occipital gland becomes atrophied and is completely degraded in castrated camels. *Bokhi* is a transparent, water-soluble, sticky and odourous liquid. The main components of *Bokhi* are sex steroids including sex pheromones that induce female camels into oestrus (Guo *et al,* 2013). Few studies have investigated the pharmacological role of *Bokhi*.

The main treatments for ULM are surgical or drugs. However, there are no specific drugs for the treatment of ULM and most of them have side effects. Therefore, it is of great importance to find an effective drug for the treatment of ULM. Traditionally *Bokhi* has been taken as an infusion in Mongolia for the treatment of ULM and kidney-yang-deficiency syndrome but there have been no studies to quantify any positive effects. This study evaluated the potential therapeutic effects of *Bokhi* from camels on ULM in rats.

Materials and Methods

Preparation of Bokhi

At the peak of the male camel rutting season, *Bokhi* samples were collected from the neck of mature, domesticated, bactrian camels from Alashan in Inner Mongolia. The samples were collected in sterile screw bottles and frozen for transportation to the laboratory. Each sample was placed in 200 ml of distilled water and soaked for 24 h. The resulting '*Bokhi* solution' was filtered through filter paper and the filtrate freeze-dried to produce a black solid powder.

Chemicals

Diethylstilbestrol injections were procured from Tianjin Jinyao Amino Acid Co., Ltd. (Tianjin,

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China). Progesterone injections were procured from Zhejiang Xianju Pharmaceutical Co., Ltd. (Zhejiang, China). Sodium chloride (control) injections (0.9 %) were procured from Jilin Kelun Connell Pharmaceutical Company (Jilin, China). Sildenafil citrate was procured from Pfizer Pharmaceuticals Limited (Liaoning, China). Serum E_2, Pro, FSH, TNF-α, IL-2 and NOS reagent kits were purchased from Nanjing Institute of Biological Engineering (Jiangsu, China).

Animals and treatment groups

Forty-eight healthy adult female Spague Dawley (SD) rats (weighing 200-210g, specific pathogen free) were obtained from Vital River Laboratory Animal Technology Company Limited (Beijing, China) and allowed to acclimate to the animal facility for 1 week before starting the experiment. Animals were maintained in controlled environment (room temperature of 21–23°C, relative humidity of 45–65%) in a 12h light/dark cycle with free access to food and water. All protocols were approved by the animal care and use committee at Inner Mongolia Agricultural University.

Treatment administration

Rats were randomly allocated to 4 groups as follows: control, ULM model, HDB (High Dose *Bokhi*) and LDB (Low Dose *Bokhi*), 12 rats per group. Establishment of the ULM rat model followed the recommended methods from published work (Jia *et al*, 2012). Except for the control group, the Model, HDB and LDB groups were all injected with diethylstilbestrol (0.2 mg/kg body weight) once a day and progesterone (5 mg/kg body weight) 3 times a week for 11 consecutive weeks. Rats in the control group received only multipoint subcutaneous injections of 0.5 ml/kg body weight of medical physiological saline as a control for the injection process. To determine whether the ULM model had established, blood was taken from all rats, centrifuged (3,000 g centrifugation for 10 min) and the serum isolated. The levels of E_2, Pro, FSH, TNF-α and NOS in the serum from each rat was determined using commercial assay kits according to the manufacturer's instructions. From the 12^{th} week the control and ULM model groups were given 10 ml/kg body weight normal saline by intragastric administration every day. In contrast, the HDB group was given *Bokhi* 50 mg/kg body weight daily and the LDB group was given *Bokhi* 10 mg/kg body weight daily for 7 weeks. All rats were weighed every other day and at the same time, their activity, hair gloss and shedding were observed and recorded. After the final treatment blood samples collected as described previously and all rats were sacrificed.

Statistical analysis

Data, unless otherwise indicated, was expressed as mean ± standard deviation (SD). SPSS 17.0 software was used for all statistical analysis. GraphPad Prism 7 software was used to produce all Figs. R language software was used for principal components analysis using the ggplot 2 package for data visualisation. Probability levels of <0.05 were considered significant.

Results and Discussion

Observations on the general state and condition of rats

Before implementation of the model, there were no obvious differences between the groups. Within 2 weeks of beginning model implementation rats in the ULM groups (Model, HDB and LDB) were showing signs of hair loss. With increasing time the number of rats with hair loss increased in these groups, as did the area of skin with hair loss. Rats in the ULM groups also appeared apathetic, lethargic and prone to arching behaviour. These phenomena were not observed in control group.

Following intragastric administration of *Bokhi* hair loss gradually stopped and began to grow again. The hair gradually regained luster and the apathy and lethargy decreased. With prolongation of treatment time differences between the general state of the *Bokhi* groups and the control group gradually decreased. However, there was no sign of improvement in the general state of the rats in the model group. It was apparent that treatment with *Bokhi* had a positive effect on alleviating the symptoms of ULM.

Evaluating successful establishment of the ULM model in rats

In order to determine whether the ULM model was established successfully in ULM model groups, blood samples were taken during the 11-week model establishment period and the levels of serum hormones determined (Fig 1). The levels of E_2, Pro, FSH, TNF-α and NOS in the ULM model groups (Model, HDB and LDB) were not significantly different to each other ($P > 0.05$). However, the levels of E_2, Pro, FSH, TNF-α and NOS in the ULM model groups were all significantly different to the levels in the control group ($P < 0.05$). This showed that the ULM model was established successfully.

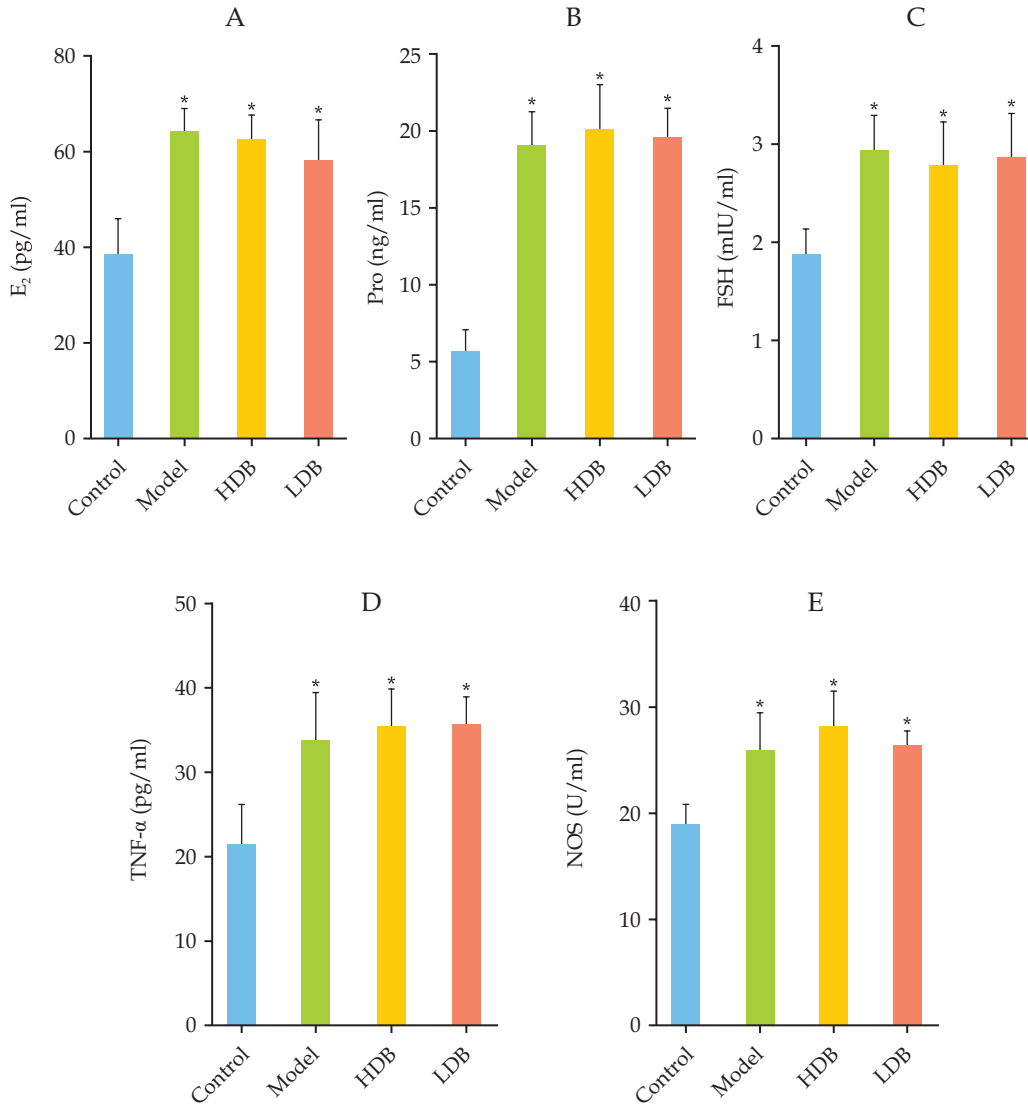

Fig 1. Levels of serum hormones E_2 (A), Pro (B), FSH (C), TNF-α (D) and NOS (E) during the establishment of the ULM model. *$P < 0.05$ compared with the Normal group.

Effects of Bokhi therapy on uterus coefficients in ULM model rats

The uterus coefficients of rats in all the *Bokhi* treatment groups were significantly different to the uterus coefficients in the model group ($P < 0.05$, Fig 2), but were not significantly different to the control group ($P > 0.05$). Following establishment of the ULM model, treatment with *Bokhi* restored uterus coefficients to the levels of the control group.

Effects of Bokhi therapy on hormones in ULM model rats

Levels of the hormone, E_2, in the HDB and LDB rats were significantly different to rats in the model group ($P < 0.01$, Fig 3 A), but were not significantly different to the control group ($P > 0.05$).

There were no significant differences in Pro between rats in the HDB group and rats in the control group ($P > 0.05$, Fig 3 B). However, there were significant differences in Pro between rats in the LDB group and rats in the control group ($P < 0.01$). Compared with the model group, there were significant difference in Pro between rats in the Model group and rats in both the HDB group and the LDB group ($P < 0.01$).

There was no significant difference in levels of FSH between rats in the HDB group and rats in the control group ($P > 0.05$, Fig 3 C). However, levels of FSH were significantly different between rats in the LDB group and rats in the control group ($P < 0.01$). Levels of FSH in rats from the HDB and LDB groups

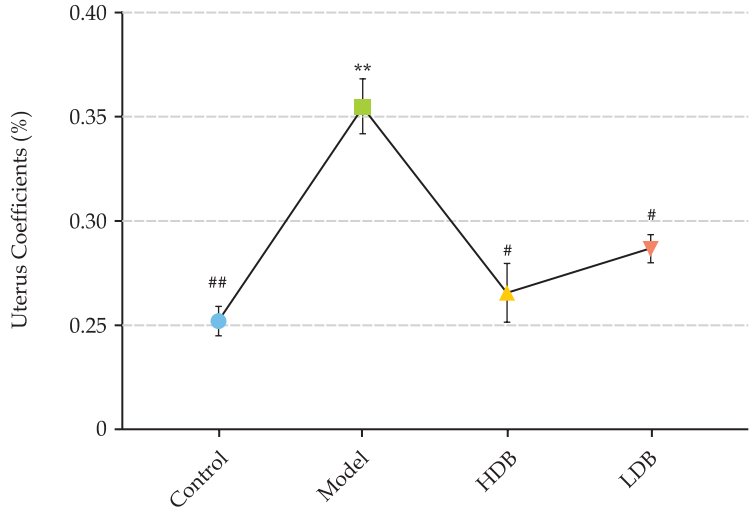

Fig 2. Effects of *Bokhi* on the level of uterus coefficients in ULM model rats compared with control rats and rats receiving *Bokhi* therapy. **P < 0.01 compared with the control group; ##P < 0.01 and #P < 0.05 compared with the model group.

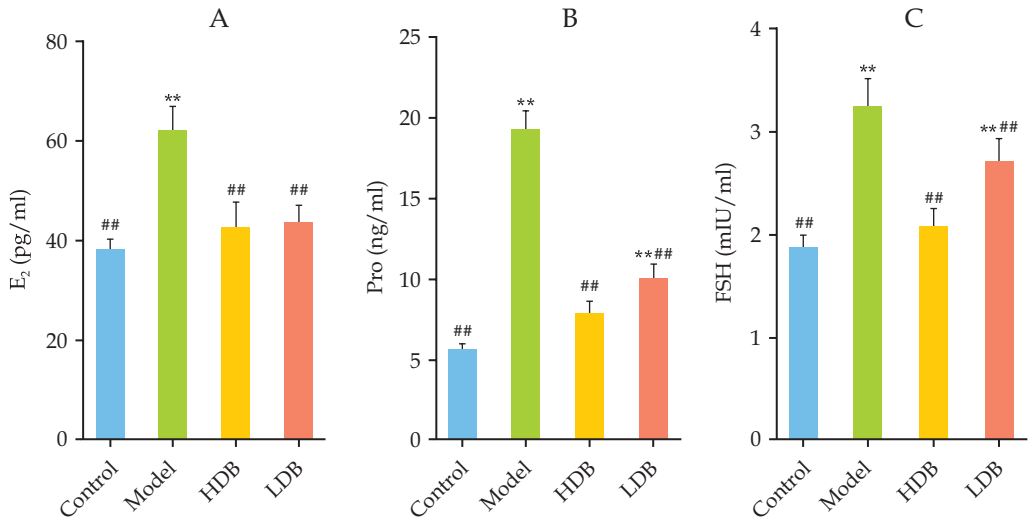

Fig 3. Effects of *Bokhi* on the level of serum hormones E_2 (A), Pro (B), FSH (C) in ULM model rats compared with normal rats and rats receiving *Bokhi* therapy. **P < 0.01 compared with the control group; ##P < 0.01 compared with the model group.

were significantly different to levels of FSH in the model group (P < 0.01).

After 7 weeks of treatment with *Bokhi*, these results showed that levels of hormones in the model group were seriously unbalanced, while in the *Bokhi* treatment groups there was a regulatory effect on E_2, Pro and FSH. The effects of HDB were the best as hormones levels returned to normal levels.

Effects of Bokhi therapy on TNF-α in ULM model rats

After 7 weeks of treatment with *Bokhi*, the level of serum TNF-α in rats from the HDB group was not significantly different to the level of serum TNF-α in rats from the control group (P > 0.05, Fig 4). However, the level of serum TNF-α in rats from the LDB group were significantly different to the level of serum TNF-α in rats from the control group (P < 0.05). Level of serum TNF-α in rats from the model group were significantly different to the level of serum TNF-α in rats from both the *Bokhi* therapy groups (P < 0.01). Thus, *Bokhi* effectively reduces the level of serum TNF-α in ULM rats.

Effects of Bokhi therapy on IL-2 in ULM model rats

There were no significant differences in the level of IL-2 in rats from the HDB and LDB groups compared with the control group (P > 0.05, Fig 5). However, there were significant differences in the

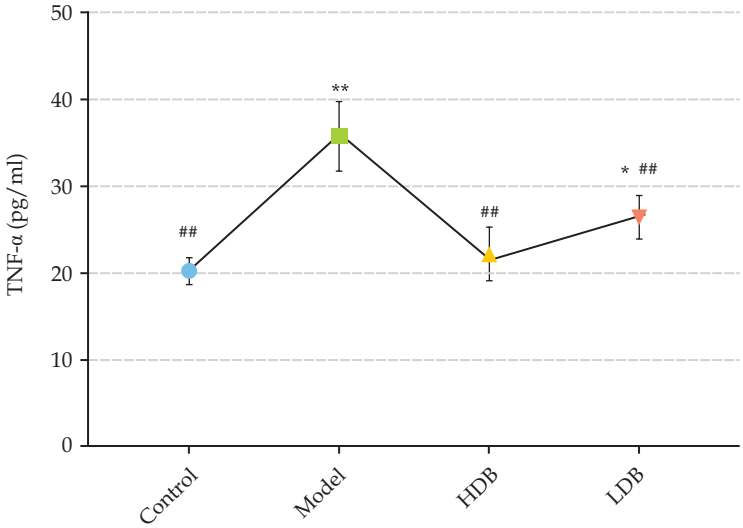

Fig 4. Effects of *Bokhi* on the level of TNF-α in ULM model rats compared with control rats and rats receiving *Bokhi* therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the control group; $^{\#\#}P < 0.01$ compared with the model group.

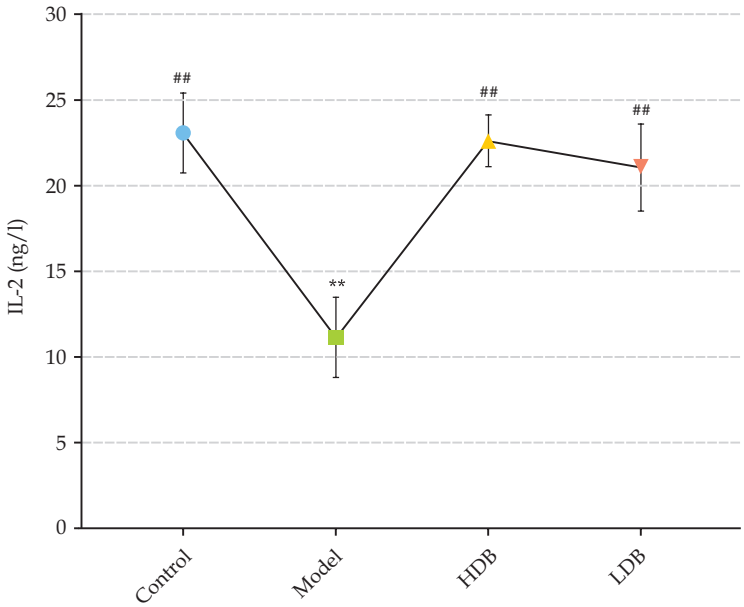

Fig 5. Effects of *Bokhi* on the level of IL-2 in ULM model rats compared with control rats and rats receiving *Bokhi* therapy. $^{**}P < 0.01$ compared with the control group; $^{\#\#}P < 0.01$ compared with the model group.

level of IL-2 in rats from the HDB and LDB groups compared with rats from the model group ($P < 0.01$).

Effects of Bokhi therapy on NOS in ULM model rats

Levels of NOS in rats from the HDB and LDB groups were not significantly different compared with levels in rats from the control group ($P > 0.05$, Fig 6). However, levels of NOS in rats from the HDB and LDB groups were all significantly different to the levels in rats from the model group ($P < 0.01$).

Principal Component Analysis (PCA)

The data for levels of E_2, Pro, FSH, TNF-α, IL-2 and NOS after therapy were used in PCA (Fig 7). The model group and the control group were very distant from each other showing that the biochemical functions of the model group had been pathologically changed. It also indicated that the establishment of the ULM model rats had been successful. The HDB group was the closest to the control group and also distant from both the Model and LDB groups. Therefore, the treatment effect achieved by high doses of *Bokhi* on ULM rats was the most beneficial.

The experimental results of therapeutic effects of camel *Bokhi* on ULM model rats showed that camel *Bokhi* had a significant regulatory effect on the levels of E_2, Pro, FSH, TNF-α, IL-2 and NOS.

Although, gonadotropins (Plewka *et al,* 2014), adipokines (Wakabayashi *et al,* 2011) and ovarian peptides (Islam *et al,* 2014) have been postulated to have some influence on fibroid onset and growth, oestradiol and progesterone are the strongest candidates for these roles (Moravek *et al,* 2015). In the pathogenesis of ULM, the effects of oestradiol and progesterone are interrelated and involve the mediation of receptors, transcription factors, kinase proteins, growth factors and numerous autocrine and paracrine factors (Ono *et al,* 2012). The blockade of pituitary gonadotropin release with gonadotropin-releasing hormone (GnRH) analogs or antagonists is an effective strategy to control fibroid symptoms and arrest their growth (Islam *et al,* 2013; Engel *et al,* 2007). Although, the inhibitory effect of these peptides might be related to their direct action on GnRH receptors in the uterus (Malik *et al,* 2016; Balkwill, 2009) or via downregulation of gonadotropin levels reducing the direct stimulus of gonadotropins on luteinising hormone (LH) and follicle-stimulating hormone (FSH) receptors within the leiomyomas (Plewka *et al,* 2014), the most probable explanation for the effectiveness of this therapy is ovarian blockade and the consequent decrease in circulating estradiol and progesterone levels. Therapeutic effects of camel *Bokhi* might be due

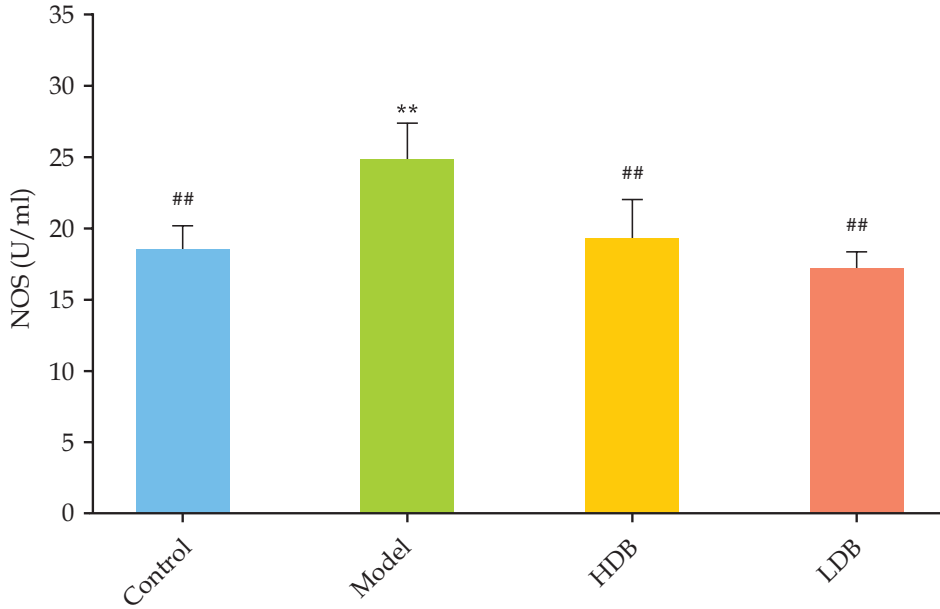

Fig 6. Effects of *Bokhi* on the level of NOS in ULM model rats compared with control rats and rats receiving *Bokhi* therapy. $^{**}P < 0.01$ compared with the control group; $\#\#P < 0.01$ compared with the model group.

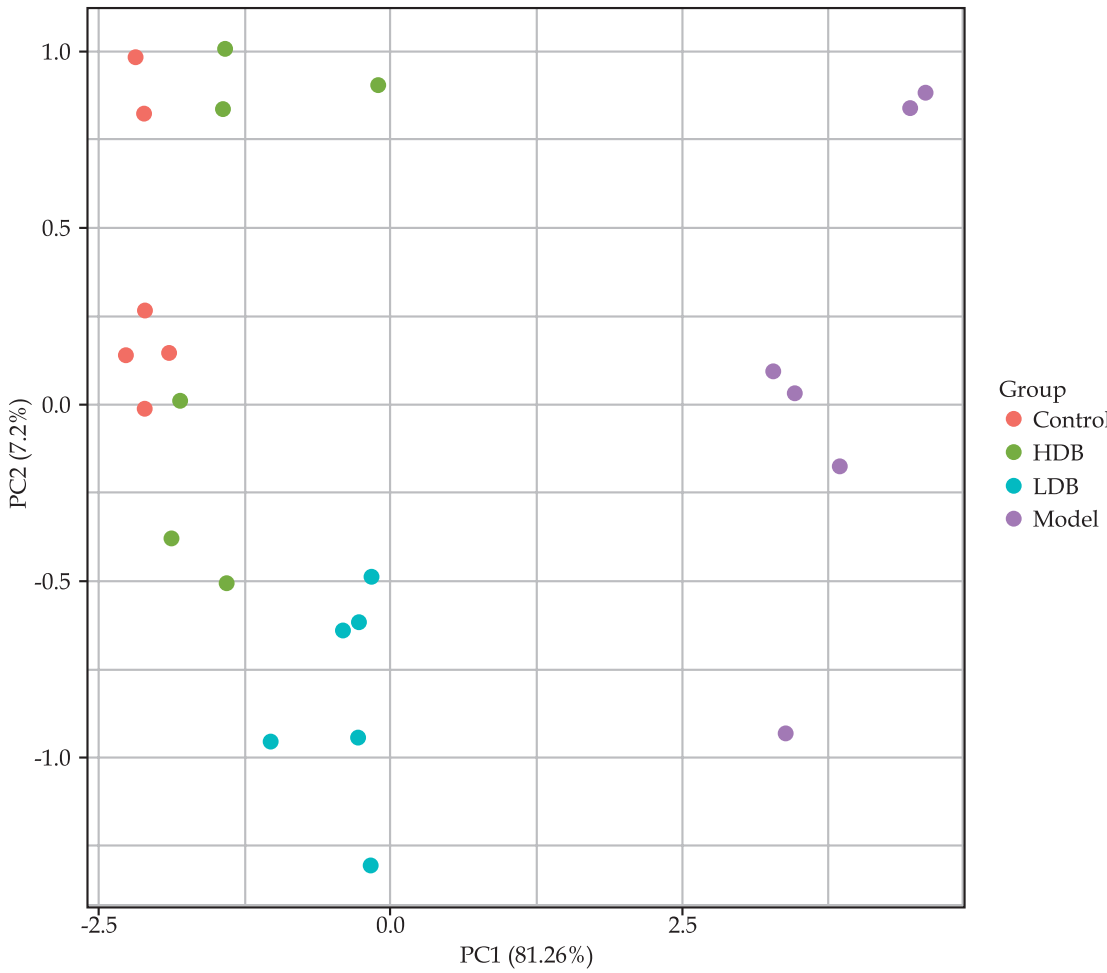

Fig 7. Effects of *Bokhi* on PCA in ULM model rats compared with control rats and rats receiving drug therapy.

to the inhibition of FSH secretion resulting in reduced levels of E_2 and Pro.

TNF-α is a pleiotropic cytokine involved in inflammation, immunity, migration, cellular homeostasis and tumor progression (Balkwill, 2009). The expression of TNF-α in ULM was higher than that in the adjacent myometrium (Kurachi *et al,* 2001). The research (Wang *et al,* 2015) reported that TNF-α upregulates matrix metalloproteinase-2 (MMP-2) expression and stimulates cell migration through activation of the extracellular signal regulated kinase (ERK) signaling pathway in leiomyoma smooth muscle cells (SMCs), but not in normal myometrial SMCs. *Bokhi* can significantly reduce the level of TNF-α and thus reduce cell migration.

IL-2 can not only promote the proliferation and differentiation of effector T cells, but also plays a key role in maintaining the stability of regulatory T cells (Treg) (Malek, 2008; Boyman and Sprent, 2012). IL-2 is also an important signal to maintain viability; if proliferation of T cells leads to the removal of IL-2 then this results in cell apoptosis (Chihara, 1998). Our experimental results showed that after treatment with *Bokhi* levels of IL-2 in ULM rats were improved, thus increasing the immunity of ULM rats.

Nitric oxide (NO) is a potent vasodilator produced by nitric oxide synthase (NOS) (Sengoku *et al,* 2001). NOS has been reported to be expressed most prominently in the uterus (Telfer *et al,* 1995; Telfer *et al,* 1997; Tseng *et al,* 1996).

Research has reported that the degree of expression of NOS was higher in the uterus of women who had ULM than in women without ULM (Oh *et al,* 2013). Therefore, by decreasing the level of NOS, *Bokhi* also reduces ULM.

The main components of *Bokhi* are sex steroids. The action of sex steroids in the myometrium are locally mediated by numerous growth factors, cytokines and chemokines. Disruption of autocrine/paracrine signaling is central to inducing healthy myometrium transformation into the leiomyoma phenotype (Ciarmela *et al,* 2011). A complete understanding of the actions of sex steroids on ULM may provide new perspectives for disease treatment with minimal interference in the systemic and physiological functions of these hormones. *Bokhi*, might act as an oestrogen receptor antagonist and thereby decrease the concentrations of growth factors within the ULM (Palomba *et al,* 2005).

Most of the drugs used in the treatment of ULM are based on animal studies. The differences in ULM mechanisms between humans and animal models are probably due to a complex interaction of different factors. One example is that the differences between rodent and primate endometrial growth (Kurita *et al,* 2005) and between oestrous and menstrual cycles, which determine obvious differences in the endometrial impact of leiomyomas in rats and humans (Bulun, 2013; Hirshfeld-Cytron *et al,* 2011). Therefore, the therapeutic effect of camel *Bokhi* on ULM needs further clinical research in humans.

Acknowledgements

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IDENTIFICATION AND MOLECULAR CLONING OF HEAT SHOCK PROTEIN-70 (*HSP*-70) GENE OF *Trypanosoma evansi* ISOLATED FROM CAMEL

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ABSTRACT

Present study was carried out to isolate the Heat Shock Protein-70 gene of *Trypanosoma evansi* using PCR. The desired amplicons of Heat Shock Protein-70 gene from genomic DNA of *T. evansi* were successfully amplified by PCR using gene specific primers at annealing temperature of 54°C. Amplified PCR product was identified on the basis of its size in agarose gel electrophoresis as 1956 bp. For cloning the purified DNA fragment was ligated to the pGEM- T Easy vector and ligated mixture was transformed into *Escherichia coli* JM109 strains. The cells containing recombinant plasmid were identified on the basis of white/blue colony selection on LB agar containing X-Gal, IPTG and ampicillin. Screening of recombinant was done by Restriction Enzyme digestion of plasmid DNAs using EcoRI and confirmed on the basis of gene size, i. e. 1956 bp for Heat Shock Protein-70 gene. Colony PCR was done for quick screening of plasmid inserts directly from *E. coli* colonies in the presence of insert specific primers.

Key words: Camel, cloning, heat shock protein-70, *Trypanosoma evansi*

Camel trypanosomosis is caused by blood protozoan parasite *Trypanosoma evansi*. It is the most pathogenic parasitic disease of camelids causing high morbidity and mortality (Luckins, 1992). Vaccine development against this organism *Trypanosoma evansi* is also hampered due to variation of surface proteins as antigen. Variant surface glycoprotein coat of *Trypanosoma evansi* is changeable when host forms antibody against it.

The major researches today are being focused on identifying various invariant protein components of trypanosome as potential drug target and basis for vaccines. Cloning and expression of mitochondrial heat shock protein 70 of *Trypanosoma congolense* and potential use as a diagnostic antigen has been studied (Bannai *et al,* 2003). Kumar *et al* (2015) studied production and preliminary evaluation of *Trypanosoma evansi* HSP 70 for antibody detection in equids. Heat shock proteins (HSPs) are a class of polypeptides powerfully induced by heat shock that mediate profound levels of stress resistance (Craig, 1985; Ellis, 2007). HSPs are molecular chaperones, binding to (holding) and refolding other cellular polypeptides (clients) with aberrant conformations (Ellis, 2007). There are a number of families of molecular chaperones (a–d), with members of class a (Hsp70, Hsp110, GRP170) and class c (Hsp90, Grp94/Gp96) thought to be of particular significance in tumour immunology (Murshid *et al,* 2011). These properties have been intensively studied for Hsp70 and Hsp90 and are largely inferred for the sibling proteins. Thus, present study was carried out to isolate the Heat Shock Protein-70 gene of *Trypanosoma evansi* using PCR and cloning of the gene.

Materials and Methods

Preparation of trypanosome strains, DNA isolation and PCR amplification

After confirmation of *T. evansi* isolates by blood smear examination, blood from infected camel was inoculated intraperitoneally in Swiss albino mice (maintained at Small Animal Laboratory, NRC on Camel, Bikaner) for propagation of trypanosomes. DEAE (Diethyl amino ethyl) cellulose column chromatography method was used for purification of trypanosomes (Lanham and Godfrey, 1970). DNA isolation from collected pellet of *T. evansi* was done as per the method utilised by Desquesnes and Davila (2002) for the preparation of animal Trypanosomes DNA from plane blood. The

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procedure for DNA isolation was same as the most commonly used procedure for DNA isolation from blood suggested by Sambrook and Russel (2001). Nucleotide primers for the amplification of HSP-70 gene of *T. evansi* were designed using the published sequence of HSP-70 gene of *Trypanosoma cruzi* (Accession No. FJ222459.1): forward primer 5′-ATGACGTACGAGGGAGCCAT-3′ and reverse primer 5′- CACTTCCTCCACCTTCGGTC-3′. PCR amplification of the HSP-70 gene was performed by cycling conditions as initial denaturation at 94°C for 4 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 60 sec, extension at 72°C for 1 min and 30 sec, and final extension for 10 min at 72°C. The PCR amplified products were checked with 1.5 kb DNA molecular weight marker in 1% agarose gel.

Cloning

The PCR products from low melting point agarose slices were purified by illustra GFX PCR DNA and Gel Band Purification Kit (GE healthcare, USA) using the manufacturer's protocol. The DNA fragment of HSP 70 gene and the pGEM-T Easy vector (Promega, USA) in which it is to be cloned were digested with T4 DNA ligase enzyme to generate compatible ends for ligation. The ligation was done (as per the Promega protocol with slight modification) in the reaction volume of 20 µl containing 10 µl of 2X Rapid ligation T4 DNA Ligase buffer [400mM Tris-HCl, 100mM $MgCl_2$, 100mM DTT, 5mM ATP (pH 7.8 at 25°C)], 6 µl PCR product, 2 µl pGEM- T Easy vector and 2 µl of T4 DNA ligase. The contents were vortexed, spun down in a micro centrifuge for 3-5 seconds and incubated for overnight at 4°C. The ligation mix was then used directly for transformation in JM109 competent cells (Promega, USA). After incubation of transformation culture 100 µl of transformation culture was plated onto antibiotic agar plates and incubated at 37°C for overnight (16-20 hr). Colonies harbouring the recombinant plasmid were inoculated into LB broth and incubated at 37°C overnight with horizontal shaking. The plasmids DNA were extracted from culture using illustra plasmid prep mini spin kit (GE healthcare, USA) according to the manufacturer's instructions. The positive clones were identified by Restriction Enzyme digestion of plasmid DNAs with *Eco*R1 and Colony PCR of plasmid colonies.

Results and Discussion

The genomic DNA was analysed in 0.8% analytical agarose gel and was found to be intact without much shearing. Gene specific forward and reverse primers were used for amplification of HSP-70 Gene and the amplicons were analysed by agarose gel electrophoresis. An intensely amplified DNA was seen in lanes B and C using genomic DNA (Fig 1). The size of the intense band was deduced from the standard curve drawn between the log molecular sizes of the marker bands against their respective mobility and found to be 1956 bp. The amplified product was purified from the LMP agarose gel and ligated with pGEM-T Easy vector (Promega). One hundred µl of transformation culture was plated onto X-gal-IPTG-Ampicillin agar plate. There were several white colonies along with a few blue colonies. The blue colonies represented the presence of vector alone but few of them contained vector with insert. The white colonies represent recombinant clones carrying insert in the plasmid. Two well separated DNA bands were seen in case of plasmid isolated from positive colonies upon digestion with *Eco*RI, the less intense lower band may correspond to the insert (Fig 2). Release of DNA fragments of around 1956 bp for heat shock protein gene was found after restriction enzyme digestion. Colony PCR was done for quick screening of plasmid inserts directly from *E. coli* colonies and amplification was found in wells of white colonies and also in blue colony (Fig 3).

Trypanosomes undergo antigenic variation that enables them to evade the host's immune system (Donelson *et al*, 1998). VSG covers the entire parasite including the flagellum presumably as a protective shield against host antibodies. This protective coat which determines the antigenic features of the parasite, is easily recognised by the host and is highly immunogenic. Heat shock proteins have been emerging as prospective drug targets (Shonhai, 2010). Drugs have been discovered to cause cellular stress resulting in the induction of heat shock proteins, ultimately improving cytoprotection (Burcham *et al*, 2012). In the present study, the amplicon size obtained was 1956 bp. Kumar *et al* (2015) identified the nucleotide sequence of 2116 bp Heat Shock Protein-70 (HSP70) from *T. evansi* proteome. Bannai *et al* (2003) examined the ability of mitochondrial heat shock protein 70 (MTP) of *Trypanosoma congolense* as a diagnostic antigen. The cDNA clone contained an open reading frame of 1,977 bp encoding a polypeptide consisting of 659 amino acids. Jose *et al* (2012) cloned, sequenced and expressed the HSP-60 gene of *Leishmania major*. Sequence analysis revealed an open reading frame of 1770 bp encoding a putative polypeptide of 589 amino acids. However, in this study, the DNA fragment amplified in the PCR

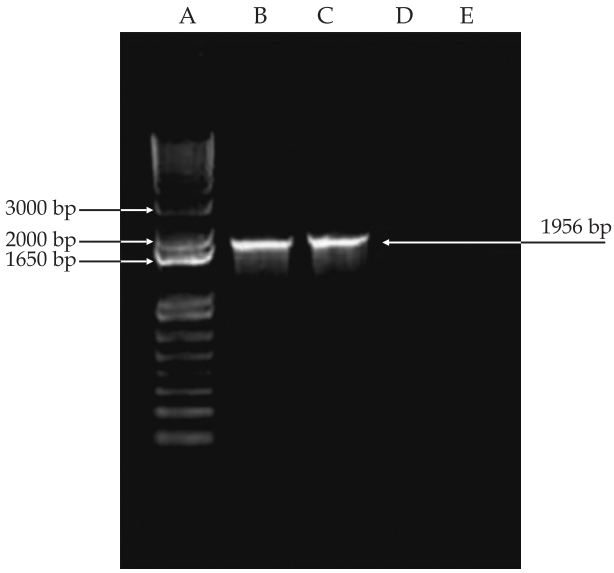

Fig 1. Amplification of *HSP-70* gene of *T. evansi* by PCR. A. 1Kb plus DNA Ladder, B-C. Amplicons.

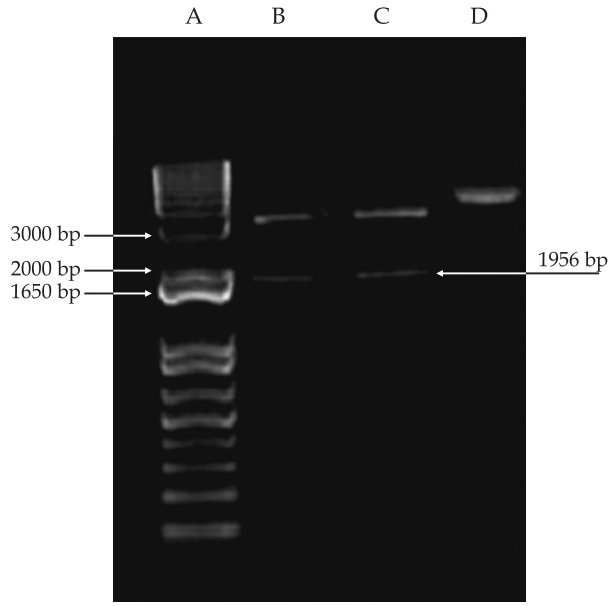

Fig 2. HSP-70 gene fragments of *T. evansi* after restriction digestion of HSP-70 gene plasmid Legends. A. 1Kb plus DNA Ladder, B – C. HSP-70 gene clone D. Uncut plasmid.

reaction was of expected size (1956bp) and highly target specific region of heat shock protein 70 gene of *T. evansi*. With the cloning and sequencing of heat shock protein gene of *T. evansi* and expression of this protein may be useful discovery of new protective antigen.

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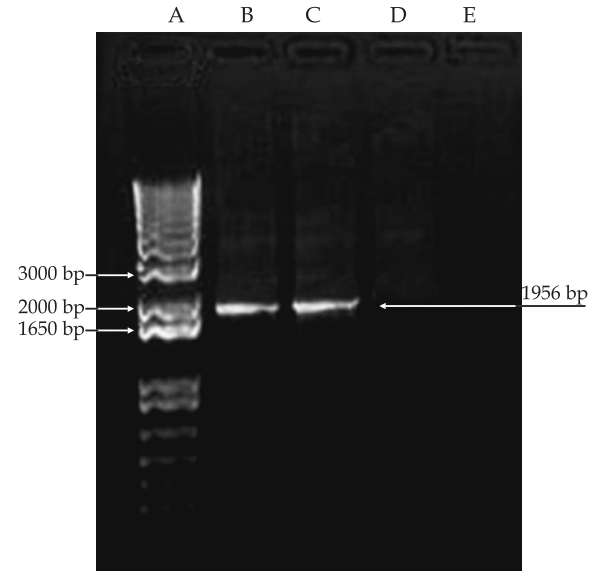

Fig 3. Amplification of HSP-70 gene of *T. evansi* by Colony-PCR. A. 1Kb plus DNA Ladder, B–C. PCR reaction with white colony shows Amplification, D-E. PCR reaction shows no amplification.

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MICROBIAL QUALITY AND MOLECULAR IDENTIFICATION OF PATHOGENIC BACTERIAL STRAINS COLLECTED FROM RAW CAMEL'S MILK IN TAIF REGION

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ABSTRACT

Sixty-four samples of raw camel's milk were collected from Jia, Oshera, Bani-Sa'ad and Al-Roduv at Taif area in different seasons. They were analysed for microbial quality. Molecular and biochemical identification for all isolated pathogenic bacteria were done. Total bacterial, yeasts and moulds counts increased significantly in summer, while they decreased in winter. The total viable counts of bacteria, yeasts and molds in Jia were 2.9×10^5 and 1.7×10^2 during Summer and 1.34×10^5 and 0.31×10^2 in winter, respectively. In Al-Roduv they were 0.9×10^5 and 0.94×10^2 in Summer, 0.65×10^5 and 0.64×10^2 in winter, respectively. The ribosmal 16S rDNA gene was completely sequenced for the isolated strains and their sequences were used with their counterparts of other related taxa to molecularly identify the isolated strains. The selected pathogenic bacteria were identified as *Proteus mirabilis, Escherishia coli, Serratia nematophila* and *Bordetella petrii* based on their morphological, biochemical and molecular characterisation. They represent 25, 35.93, 9.37 and 18.75 per cent of the total microbial count, respectively.

Key words: Bacterial count, camel, microbial quality, milk

The hyieginic conditions of the milking place, the excretion from the udder of an infected animal and quality of water used on the farm, may influence the microbiological quality of milk products (Amaral *et al,* 2003, Angulo *et al,* 2009). Physico-chemical, microbiological, hygienic and sanitation measures have been deployed by the industry to test and verify the quality of milk (Guerreiro *et al,* 2005). Raw Milk (RM) often contains microorganisms, which may cause food borne diseases (Adesiyun *et al,* 1995; Steele *et al,* 1997; Headrick *et al,* 1998). The number and types of microorganisms in milk immediately after milking are affected by animal and equipment cleanliness, season, feed and animal health (Rogelj, 2003). Bacterial contamination of raw milk can be happened from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al,* 2008). It is suggested that the differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits *et al,* 2008). Microbes like *listeria monocytogenes*, Salmonella, Campylobacter, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* and thermotolerant coliforms, especially *Escherichia coli* are the most common contaminant of raw camel's milk (Chye *et al,* 2004; Mhone *et al,* 2011).

The total viable count of bacteria is one of the main criteria used to evaluate the classification and processing of dairy products (Chye *et al,* 2004; Mhone *et al,* 2011). Although freshly drawn milk from animals may possess temporary 'germicidal' or 'bacteriostatic' properties, growth of microorganisms is inevitable unless it is processed by freezing, heat treatment or irradiation (Murphy and Boor, 2000; Saeed *et al,* 2009). Daily production, eventual marketing and sale of milk require special consideration to ensure its delivery to the market in hygienic and acceptable condition. In developing countries, outlets for the purchase of milk are numerous but most operate under unsanitary conditions and are not adequately monitored or regulated (Food and Agriculture Organisation, 1990, 2003). Under such conditions the food-borne zoonotic risk posed by milk and dairy products is of great public concern (Vanden Berg, 1988).

Several molecular studies have been conducted to identify the pathogenic bacteria found in the

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raw camel milk. As repesentatives of these studies Benmechernene *et al* (2014) identified the genomic characterisation of *Leuconostoc mesenteroides* strains isolated from raw camel milk in 2 Southwest Algerian Arid Zones. Akhmetsadykova *et al* (2015) studied the molecular biodiversity of lactic acid bacteria in raw and fermented camel's milk. Moumene *et al* (2016) studied the complete genome sequence of *Lactococcus garvieae* M14 isolated from algerian fermented milk. The present work is conducted to evaluate the microbial quality of raw camel milk from the Taif-region and isolate and identify the pathogenic strains, which are present in camel's milk using molecular and biological methods.

Materials and Methods

Milk Samples

Raw camel milk samples were collected from different places (Jia, Oshera, Bani Sa'ad and Al-Roduv) of Taif region during lactation period under aseptic conditions in a sterile screw cap tubes, processed within three hours and used for further studies.

Microbiological analysis

Milk samples (25 ml) were diluted in buffered peptone saline (225 ml, 0.5% w/v; peptone; 0.85% w/v; NaCl), mixed in stomacher bag and stomached in Seward stomacher (Seward 400, England) for 2 minutes. In order to quantify the various microbial groups, appropriate dilutions (101-105) were surface plated. Aerobic total plate count (ATPC) was carried out on plate count agar (PCA), incubated at 32°C for 72h (Marshall, 1992). For aerobic mesophilic spore count (AMSC), the milk was heat-shocked at 80°C for 10 min to destroy vegetative cells. After being cooled in an ice bath, the milk was immediately plated on plate count agar and incubated at 32°C for 48 h (Marshall, 1992). Total and faecal coliforms were determined by MPN method according to US standard method (Federal Register, 1990). Moulds and yeasts were enumerated on potato Dextrose Agar (PDA) acidified by lactic acid (Oxoid, SR21). For detection and isolate *salmonella* spp, a portion of 25 ml of milk was pre-enriched in 225 ml of buffered peptone water at 37°C for 24h. Then, 1 ml of pre-enrichment sample was incubated in 10 ml Cystine Selenite broth and Rappaport-Vassiliadis broth at 37°C for 24h. Selective enrichments were then streaked onto Bismuth Sulphite, xylose lysine Desoxycholate (XLD) and Hekton entreic agars. All selective media were incubated at 37°C for 24h. Typical colonies were examined by microscope, characteristics of growth on lysine iron agar, urease production and then tested with *Salmonella polyvalent* (Salmonella latex test, Oxoid FT0203).

Biochemical characterisation

Inculated plates were incubated at different temperature ranging from 5 to 55°C. The pH growth range was determined by streaking each inoculum onto the surface of preferred medium adjusted at different pH values ranged from 5 to 11 adjusted by HCl or NaOH. The ability to grow anaerobically was evaluated on solid medium incubated in jars with the GasPak envelops (BBL). Haemolysis was studied in solid Mueller Hinton (MH) medium supplemented with 5 % (v/v) defibrinated sheep blood. Oxidase reaction was performed according to Kovács (1956). Catalase was determined by adding 10 volumes of 3% H_2O_2 to each strain culture on their solid medium. Indole production was tested in liquid MH medium using Kovács' reagent (Kovács, 1928). Methyl red and Voges-Proskauer were tested using methyl red and Barritt's reagent (Barritt, 1936), respectively. Starch agar was used to test the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase (Srivastava and Baruah, 1986; Mishra and Behera, 2008). Cultures were inoculated into tubes of nutrient gelatin (nutrient broth, 100ml; Difco gelatin, 12 g; pH 7.0) and incubated at 37°C for 14 days. After they had been cooled to 4°C, they were observed for liquefaction of the gelatin. Casein hydrolysis was indicated by a clear zone around bacterial growth on solid MH medium plus an equal quantity of skimmed milk (Carpana *et al,* 1995). Cellulose-degrading ability of bacterial isolates was performed by streaking on the cellulose Congo-Red agar media (Lu *et al,* 2004). Chitin hydrolysis was measured by the halo diameter of enzyme diffusion on the chitinase production medium (Gao *et al,* 2015). Production of acid from carbohydrates was determined by the methods of Gordon *et al* (1974). Finally, Urea hydrolysis was detected on Christensen's medium (Christensen, 1946).

Antimicrobial susceptibility tests

Antimicrobial susceptibility of the selected bacterial strains was assayed using the diffusion agar method (Bauer *et al,* 1966). The antimicrobial compounds (MASTRING-S^{TM}) used in this study included; Ampicillin (25µg), Tetracycline (30µg), Chloramphenicol (30µg), Levofloxacin (5µg), Flucloxacillin (5mcg), Tobramycin (10mcg), Ofloxacin (5mcg), Norfloxacin (10mcg), Cefotaxime (30µg), Imipenem (10µg).

DNA Isolation from pathogenic bacteria isolate

1.5 ml of broth was taken in a microfuge tube and centrifuged at 5000 rpm for 15 min. The supernatant was discarded. To the pellet, 1.5 ml of broth was added and again centrifuged at 8,000 rpm for 10 min. 467 µl of Tris-EDTA (TE buffer), 50 µl of 0.5% lysozyme and 30 µl of 10% Sodium Dodecyl Sulfate (SDS) was added. Then 3 µl of proteinase k was added (20 mg/ml). It was mixed well and incubated for 1 h at 37°C. After the incubation, equal volume of phenol: chloroform (25:24) mixture was added and mixed well by inverting the tubes gently until the phases are completely mixed. Then it was centrifuged at 8,000 rpm for 10 min. After centrifugation, the upper aqueous phase was transferred to a new tube and 1/10 volume of sodium acetate was added. Then 0.6 volume of isopropanol was added and mixed gently until the DNA gets precipitated and then centrifuged at 8,000 rpm for 10 min. To the pellet, 70% ethanol was added to the pellet and centrifuged at 5,000 rpm for 10 min. 20 µl of TE buffer was added and stored in ice cold condition (Sambrook *et al,* 1989) and analysed by Agarose gel electrophoresis.

PCR and sequencing 16S rDNA

Both the forward primer 5`-27F 5′ (AGA GTT TGA TCM TGG CTC AG) 3′ and the reverse primer 5`-1492R 5′ (TAC GGY TAC CTT GTT ACG ACT T) 3′ were used for amplifying the complete 16S rDNA gene for the isolated DNAs of the bacterial strains. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30 µl reaction mixture by using a EF-Taq (SolGent, Korea) as follows: activation of Taq polymerase at 95°C for 2 minutes, 35 cycles of 95°C for 1 minute, 55°C and 72°C for 1 minutes each were performed, finishing with a 10- minutes step at 72°C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM Big Dye Terminator v3.1 Cycle sequencing Kit. The sequencing primers were 785F 5′(GGA TTA GAT ACC CTG GTA) 3′ and 907R 5′(CCG TCA ATT CMT TTR AGT TT) 3′. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analysed by ABI Prism 3730XL DNA analyser (Applied Biosystems, Foster City, CA).

The ribosomal 16S rDNA gene has been sequenced for the current bacterial strains. These data have been treated with Blastn program (http://www.ncbi.nlm.nih.gov) for searching for their closely related strain sequences already found in the Genbank database. Sequences with identity of 99 to 95 % to these two strains have been collected from the database. The collected data were used for phylogenetic analyses after unalienable and gap-containing sites were deleted (1422 bp in total). The aligned nucleotide sequences can be obtained from the corresponding author upon request. The analyses were done by maximum-parsimony (MP) and neighbor-joining (NJ) by heuristic searches with the TBR branch swapping and 10 random taxon additions. Bootstrap replications were adjusted for both methods to be 10000 replications. The maximum-likelihood method in PAUP* 4.0b10 (Swofford, 2003) was also used with NNI branch swapping and axis taxon additions and 200 bootstrap replications. The general reversible model (GTR+I+G) and parameters optimised by Modeltest 3.0 (Posada and Crandall, 1998) were used.

Result and Discussion

Camel's milk collected from four different ecological areas, that include Oshera, Al-Roduf, Bani Sa'ad and Alkor. Many authors described the ability of camel milk to inhibit the growth of many bacterial spp. due to the lytic action of lysozyme and lactoferrin contained in camel milk (Al- Majali *et al,* 2007; Wernery 2003; El-Agamy *et al,* 1992). Four other different pathogenic bacteria i.e., *Proteus mirabilis*, *E. coli*, *Serratia nematodiphila* and *Bordetella petrii* have also been detected. Camel milk still represents a significant source of infection for human (El-Ziney and Al-Turki, 2007; Matofari *et al,* 2007; Vanegas *et al,* 2009). Regarding to the overall prevalence of tested samples by using bacteriological isolation and biochemical identification, results revealed that an overall prevalence and hygienic quality of camel's milk were determined by total viable count of bacteria and number of yeasts and molds (Table 1).

The total viable count of bacteria varied significantly according to the site and the season of collection. Total viable count of bacteria ranged from 1.34 to 2.9 $\times 10^5$, 1.2 to 1.03$\times 10^5$, 1.9 to 1.36 $\times 10^5$ and 0.9 to 0.65$\times 10^5$ in Jia, Oshera, Bani-Sa'ad and Al-Roduv, respectively (Table 1). The highest count was obtained for Jia at summer season (Table 1). These results agree with Younan (2004) who reported that the number of bacteria ranging between 100 to 10,000 cell/ml in Kenya. On the other hand Al–Mohizea (1986) found that the aerobic plate count exceeded 10^5cfu/ml in 13 samples and averaged 2.2x10^5 cfu/ml in camel's

raw milk in Riyadh City. Moreover in Ethiopia, Semereab and Molla (2001) reported that the bacterial count ranged from $0.4x10^5$ to 10^5 cfu/ml. In addition El-Demerdash and Al-Otaibi (2012) reported that the total bacterial count of raw camel milk samples collected from different zones ranged from 1.3×10^3 to 1.3×10^6 cfu/ml. Total viable counts (TC) in milk more than 10^5 cfu/ml. These gave an evidence of the bad hygienic condition during milk production (Al–Mohizea, 1986). Our resultes indicated that the number of bacteria in summer were higher than those of Spring, Autumn and Winter. These results may be due to equipment cleanliness, season, feed and animal health (Rogelj, 2003), air, milking equipment, feed, soil, faeces and grass (Coorevits *et al,* 2008), the hygienic conditions of the milking place, the excretion from the udder of an infected animal and quality of water used on the farm (Amaral *et al,* 2003; Angulo *et al,* 2009; Torkar and Teger, 2008; Parekh and Subhash, 2008). The highest number of bacteria may be due to higher tempreature during storage and distribution in summer. Younan (2004); Swai *et al* (2002); Zelalem and Faye (2006), reported that the differentiation in bacterial count may be due to milk structure, ways of milk collection and environmental conditions. In addition, yeast and mold numbers were varied significantly according to the season and site of collection, the highest number was observed in summer at Jia, Oshera, Bani-Sa'ad and Al-Roduv, they were 1.7, 0.99, 1.24 , 0.94×10^2 cfu/ml, respectively. While the lowest values recorded at Jia and Oshera in autumn, they were 0.31 and 0.18×10^2 cfu/ml, respectively. On the other hand, yeast and mold counts were 0.19 and 0.38×10^2 cfu/ml in Bani-Sa'ad and Al-Roduv, respectively in winter. These results are in agreement with those of Karmen and Slavica (2008). Who reported that yeasts present in 95.0% of raw camel's milk samples with the mean concentration of 1.7 log10 cfu/ml. In addition, moulds were found in 63.3% of raw camel's milk samples, their mean concentration was 0.6 log10 cfu/ml. In addition they added that isolated mould strains belonged to genera *Geotrichum* (51.5%), *Aspergillus* (33.8%), *Mucor* (5.9%), *Fusarium* (2.9%) and *Penicillium* (2.9%). Numbers of yeasts and moulds in samples of camel's milk collected from Al-Ahsa area varied from 43 to 8.1×10^3 cfu/ml. Omar and Eltinay (2008), found that the rate of isolation of yeasts from all samples were 14.9%. These species were *Candida ciferri* and *Candida guilliermondii*.

Pathogenic bacteria in raw came milk samples

It has been concerned in this study to use special selective media to isolate 4 pathogenic bacteria; *Proteus mirabilis*, *Escherichia coli*, *Serratia nematodiphila, Bordetella petrii*. Raw milk often contains microorganisms, which may cause food borne diseases (Adesiyun *et al,* 1995; Steele *et al,* 1997). All milk samples tested for the presence of *Proteus mirabilis*, *Escherichia coli*, *Serratia nematodiphila*, *Salmonella* spp. and *Bordetella petrii*. Four pathogenic bacteria were isolated from camel's milk samples as shown in table 2.

The results showed that 25% of camel's milk samples contained *P. mirabilis*. These results are in agreement with FAO (2003). Results revealed different overall prevalences of microbes, i.e. *Salmonella* spp. 2.7%, *E. coli* spp. (Marth and Ryser,

Table 1. Microbiological quantity of camel's milk samples collected from Jia, Oshera, Bani-Sa'ad and Al-Roduv.

	Total bacterial count cfu×10^5/ml				Yeasts and molds cfu×10^2/ml			
Sites	**Summer**	**Winter**	**Spring**	**Autumn**	**Summer**	**Winter**	**Spring**	**Autumn**
Jia	2.9^{Aa}	1.31^{Ca}	2.1^{Ba}	1.34^{Ca}	1.70^{Aa}	0.69^{Ca}	1.18^{Ba}	0.31^{Da}
Oshera	1.2^{Ac}	0.99^{Bb}	1.04^{Bc}	1.03^{Bb}	1.24^{Ab}	0.51^{Ca}	0.8^{Bab}	0.18^{Da}
Bani-Sa'ad	1.9^{Ab}	0.95^{Db}	1.65^{Bb}	1.36^{Ca}	0.99^{Ac}	0.19^{Da}	0.49^{Cb}	0.8^{Ba}
Al-Roduv	0.9^{Ac}	0.70^{Bb}	0.76^{Bc}	0.65^{Cc}	0.94^{Ac}	0.48^{Cb}	0.48^{Cb}	0.64^{Ba}

Different superscript letters in the same row for each parameter (total bacterial count; yeast and mold) are significantly different at P≤ 0.05. Different letter in the column for each parameter are significantly different at P ≤ 0.05.

Table 2. Pathogenic bacteria detected in raw camel's milk sample.

Bacterial strains of positive samples	Number of samples	Infected samples	Per cent of infection
P. mirabilis	64	16	25
E. coli	64	23	35.93
S. nematodiphila	64	6	9.37
B. petrii	64	12	18.75

1990) 6.48% and *Listeria* spp. Chaibou (2005). Abeer *et al* (2012) stated that 1.08% of *Salmonella* spp. were detected with a prevalence rate ranging from 2.38 - 2.85%, where the lowest rate was detected in Sharqia milk samples. Many *E. coli* strains was detected with a prevalence rate ranging from 5.71 - 7.14% where the lowest rate was detected in Sharqia milk samples while the highest rate was detected with Sinai milk samples. *Listeria* spp. was detected in a prevalence rate ranging from 0- 2.85% while the negative results were detected in Sinai and Aswan milk samples. *E. coli* frequently contaminates food and considered as good indicator of faecal pollution (Dilielo, 1982; Soomro *et al,* 2002; Benkerroum *et al,* 2004). Presence of *E. coli* in milk products indicates the presence of enteropathogenic microorganisms, which constitute a public health hazard. Enteropathogenic *E. coli* can cause severe diarrhoea and vomiting in infants and young children (Anon, 1975). The negative samples of most pathogenic bacteria may be due to the activity of protective protein such as lysoyeme, lactoferrin, lactoperoxidase, immunoglobulin G and A of camel's milk, as stated by Barbour *et al* (1984).

Description of strain Proteinus mirabilis

P. mirabilis strain showed a gray colour colony, rod-shaped cells, motile and non-sporulated (Table 3). The isolate was facultatively anaerobic for growth. Optimal temperature was 30°C. The temperature range was 30-37°C. pH range was between 7.5 up to 8.5. Methyl red, haemolytic activity, catalase and nitrate reductase were positive. *P. mirabilis* tested negative for the gram reaction, oxidase, Voges-proskauer, d- arabinose, l- arabinose, mannose, maltose and manitol. It was able to utilise, starch, cellulose, urea, arginine, ornithine and gelatin as a sole carbon source. It was unable to utilise casein. The isolate tested against 10 antibiotics listed in table 3. The results obtained showed that the isolate was resistant to flucloxacillin. The antibiotics inhibited the growth of *P. mirabilis* were ampicillin, cefotaxime. levoflxacin, chloramphenicol, tobramycin, tetracycline, ofloxacin, norfloxacin and imipenem, while it was resistant to flucloxacillin.

Description of E. coli

Escherichia coli was rod shaped (coccobacillus) forms shiny, mucoid colonies which had entire margins and were slightly raised. Older colonies had a darker centre, facultatively anaerobic bacteria, non-sporulation, pigmentation was orange red and cells were motile. Optimum temperature was 35-37°C. The temperature range was 21-37°C. pH range was between 6 up to 7. Haemolytic activity, indole production, catalase, nitrate reductase, methyl red were positive. While, it showed negative for the H_2S production, gram reaction, oxidase, Voges-proskauer, acid production from maltose and phenylalanine deaminase. It utilised various sugars including d-xylose, l-xylose, d-glucose, mannose, rhamenose, sucrose, lactose, d-arabinose, l-arabinose and mannitol to produce acid. Both decomposition of starch, cellulose, tryptophan, arginine, lysine and ornithine were positive. It was unable to utilise gelatin, urea and casein as a sole carbon source. Among the tested antibiotics, *E. coli* was resistant to ampicillin and cefotaxime (Table 4).

Description of Serratia nematodiphila

S. nematodiphila has a pink-brown colony, rod shaped cells and pigmentation is red. The cells were motile. The isolate had facultative anaerobic conditions for growth. Optimal temperature was 37°C. The temperature range of growth was 5-40°C. pH range was between 5 up to 9. Optimal pH was 7.2. Gram reaction, indole production, methyl red, sporulation, H_2S production, nitrate reductase and oxidase activities were negative. It showed positive to Voges-Proskauer, haemolytic activity, catalase activity. It had the ability to produce acids from all tested sugars except L-arabinose (Table 3). It was able to utilise starch, gelatin, cellulose, casein, tryptophan, arginine, lysine and ornithine. It did not utilise the urea. Antimicrobial activity tests obtained showed that *S. nematodiphila* varied in its susceptibility to all antimicrobials used. It was resistant to Levoflxacin, Ofloxacin and Imipenem (Table 4).

Description of Bordetella petrii

As shown in table 3 *B. petrii* strain had a creamy white colour colony, pigmention was brown and had anaerobic growth. The biochemical and physiological properties of strain *B. petrii* are summarised in tables 3. It tested positive for Voges-Proskauer, catalase activity and oxidase while negative for motility, gram reaction, methyl red, sporulation, haemolytic activity, indole production and nitrate reductase. However, optimum temperature was observed at 35°C. Optimum pH was 7.9. Strain *B. petrii* unutilised all sugars in table 3 except mannose as sole carbon sources for growth and produced acids from these carbohydrates. *B. petrii* was unable to decompose or hydrolyse gelatin, urea, casein, tryptophan, arginine, lysine, ornithine, starch and cellulose. Strain *B. petrii* was susceptible to all tested antibiotics except flucloxacillin (Table 4).

Table 3. Morphological, biochemical and nutritional characteristics of the selected strains: *P. mirabilis, E.coli, S. nematodiphila, B. petrii.*

Characteristics	**Strains**			
	P. mirabilis	*E. coli*	*S. nematodiphila*	*B. petrii*
Shape	Bacillus	Rod	Rod	Rod to circular
Colony colour	Gray	Shiny	Bink-brown	Creamy white
Motility	+	+	+	-
Pigmentation	black-	Orange-	Red	Brown
Gram reaction	brown	red	-	-
Methyl red	-	-	-	-
Voges-proskauer	+	+	+	+
Sporulation	-	-	+	-
pH	-	-	5-9 (7.2)	7-9
Optimum temperature	4.8	6-7	5-40 (37)	35-37
Anaerobic growth	30-37	21-37	+/-	+/-
Haemolytic activity	+/-	+/-	+	-
Catalase	+	+/-	+	+
Indole production	+	+	-	-
Oxidase	-	+	-	+
Grow on :				
Sucrose	-	-	+	-
Mannose	+		-	+
d-xylose	-	+/-	+	-
l-xylose	+	+	+	-
lactose	+	+	+	-
l- sorbitol	-	+	+	-
Rhamenose	-	+	+	-
Glucose	+	+	+	-
d-arabinose	+	+	+	-
l- arabinose	-	+	-	-
Maltose	-	+	+	-
Manitol	-	+	+	+
Decomposition of:				
Starch	+	+	+	-
Gelatin	+	+	+	-
Urea	+	-	-	-
Casein	-	-	+	-
Cellulose	+	-	+	-
Tryptophan	-	+	+	-
Arginine	+	+	+	-
Lysine	-	+/-	+	-
Ornithine	+	+	+	-
Nitrogen reduction	+	+/-	-	-
Phenylalanine deaminase	+	-	-	-
H_2S	+	-	-	-

Molecular identification

Using PAUP program (Swofford, 2003), the aligned 1422 nucleotides among the different bacterial taxa and their related species showed 1074 constant sites and 348 variable sites. Among the variable nucleotides, 314 were parsimony-informative and 34 were uninformative under the parsimony criteria. The data exhibited consistency index (CI = 0.85), homology index (HI = 0.15), retention index (RI = 0.944) and rescaled consistency index (RC = 0.803). Excluding the uninformative characters, both consistency and homology indices showed values of 0.845 and 0.154, respectively. The base composition for the studied strains were A= 24.95%, C= 23.39%, G= 31.57% and T= 20.09%. The ML analysis for the data was conducted by the nearest-neighbour interchange (NNI) exhibiting a negative log likelihood score -lnL = 4048.80 and Akaike Information Criterion AIC= 8113.61. The best-fit model that explained the datasets was GTR+G+I showing model parameters as follows: substitution rate matrix R (a) =1.00; R (b) = 4.08; R (c) = 1.655; R (d) = 1.655, R (e) = 7.03 and R(f)= 1.00. Among-site rate variation showed the proportion of invariable sites (I) to be 0.575 and gamma distribution shape parameter to be 0.343.

A single neighbour-joining tree was shown and the bootstrap supports at the different nodes were calculated by 10000 replications for MP and NJ and by 200 replications for the ML (Fig. 1). The 8 studied strains were identified into 4 different non congeneric species which were *Proteus mirabilis* (S60, S61, S63 and S68), *Bordetella petrii* (S64) *Serratia. nematodiphila* (S66 and S67) and *Escherichia coli* (S62). Each of these well-identified strains clustered within its congeneric species with 100 % bootstrap supports. However, S62 was identified as *Escherichia coli* by NJ method only (96 % bootstrap support).

Table 4. Antibiotics susceptibility of selected strains: *P. mirabilis, E. coli, S. nematodiphila, B. petrii.*

Antibiotics	**Strains**			
	P. mirabilis	***E. coli***	***S. nematodiphila***	***B. petrii***
Ampicillin	+	-	+	+
Cefotaxime	+	-	+	+
Levoflxacin	+	+	-	+
Chloramphenicol	+	+	+	+
Flucloxacillin	-	+	+	-
Tobramycin	+	+	+	+
Tetracycline	+	+	+	+
Ofloxacin	+	+	-	+
Norfloxacin	+	+	+	+
Imipenem	+	+	-	+

\+ Inhibit the bacterial growth -Resistant to the bacterial growth

Table 5. Pairwise genetic distances calculated from the 16S rDNA data for the *different bacterial* strains studied.

	S60	***P. mirabilis***	**S64**	***B. petrii***	**S62**	***E. coli***	***S. flexneri***	**S66**	***S. nematodiphila***
S60, S61,S63, S68	-								
P. mirabilis	0.000	-							
P. vulgaris	0.009	0.009							
S64			-						
Bordetella petrii			0.004	-					
Bordetella avium			0.010	0.011					
S62					-				
Escherichia coli					0.003	-			
Shigella flexneri					0.006	0.009	-		
S66, S67								-	
Serratia nematodiphila								0.000	-
Serratia marcescens								0.001	0.001

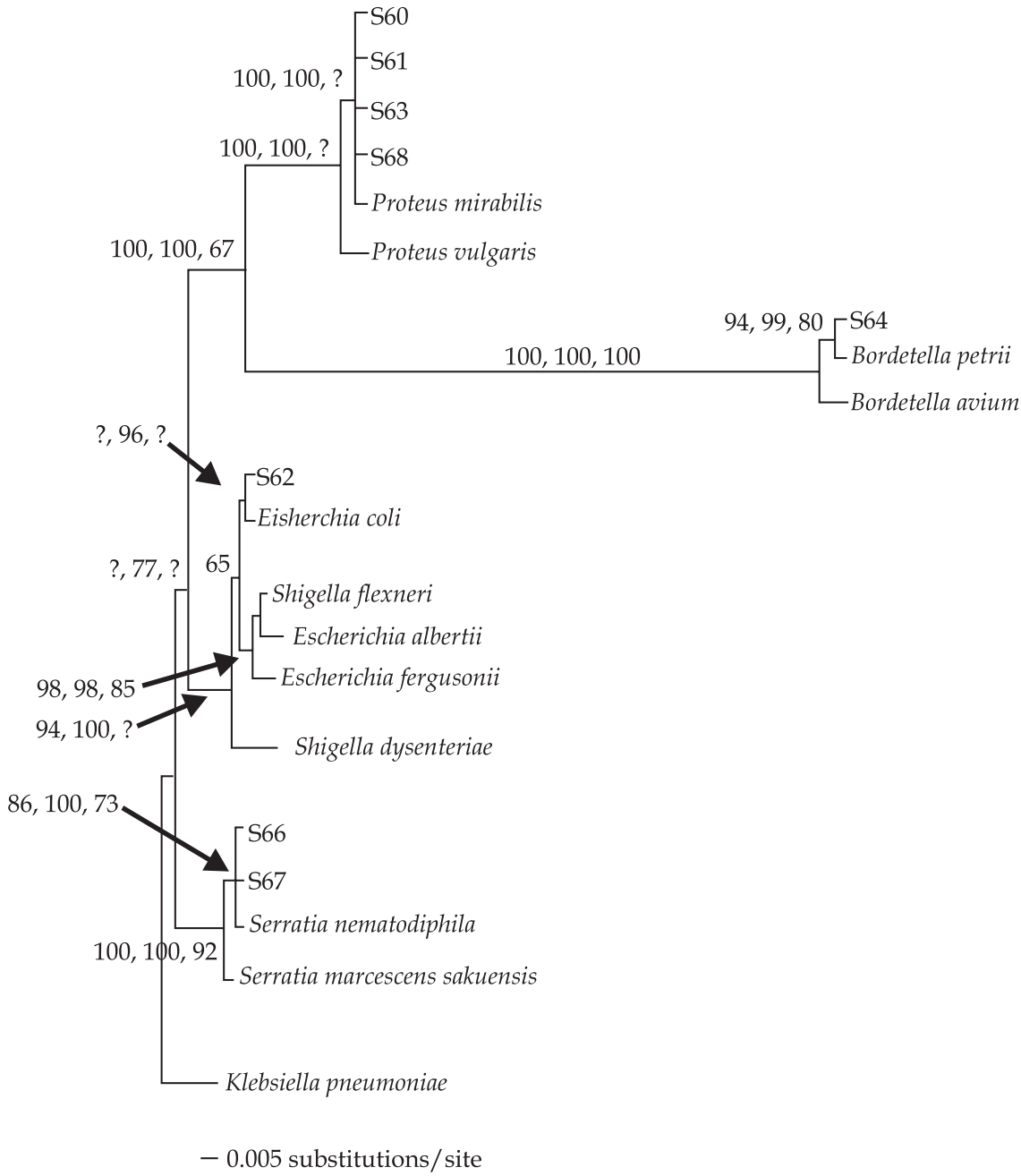

Fig 1. Neighbour-joining tree constructed from 1422 bp of 16S rDNA gene for 8 bacterial strains belonging to different 4 genera. Values at nodes represent the bootstrap support for MP, NJ and ML methods, respectively. (?) refers to that the bootstrap values are not given for the corresponding analytical method.

The aligned data were used to estimate the pairwise genetic distances among the studied strains (Table 5). In accordance to tree topology, the genetic distance showed identity between S60 and *P. mirabilis* (D= 0.000) and between S66 and *S. nematodiphila* (D= 0.000). With respect to the other strains, the smallest genetic distances were recorded between S62 and *E. coli* (D= 0.003) and between S64 and *B. petrii* (D=0.004). The smallest genetic distance exhibited between S62 and *E. coli* supports the clustering of both taxa in the tree topology. The molecular data clearly discriminated among the different strains and the tree topology is concordant with the biochemical data in constructing the relationship among the studied taxa.

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MORPHOLOGICAL AND MORPHOMETRIC STUDY ON STIFLE JOINT OF DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

Morphological and morphometric study was conducted on 10 freshly collected stifle joints from cadaver of adult dromedary camels (*Camelus dromedarius*) of local breed. In morphological study all the important anatomical structures were identified. The medial patellar ligament could not be evidenced but a medial femoropatellar ligament running between the medial epicondyle of femur and patella was found. Another strap like ligament like structure originating from medial femoral fascia and inserted on the tibial tuberosity was evidenced and it was supposed to support the patella in its normal position. The medial collateral ligament was present but its lateral counterpart could not be evidenced. The medial intercondylar tubercle of tibia was found to be somewhat elevated and larger than the lateral one. Morphometric data (length, width and thickness) of the important ligaments and anatomical structures (articular surfaces) were measured. The orientation of the medial femoro patellar ligament was at an angle of 113.8° ± 1.93° with the middle patellar ligament. There was an angle of 82.7° ± 1.77° between the meniscofemoral ligament and the ligament connecting the menisci caudally. Descriptive statistics were given as Mean ± SD (standard deviation) along with coefficient of variation to show variability of a particular dimension.

Key words: Camel, morphology, morphometry, stifle joint

Camel is an even toed ungulate, pseudo ruminant mammal and an important animal component of the fragile desert eco-system. Camel is mainly used as a drought animal in India and any affection of the stifle joint can affect the working efficiency of the animal. The incidence of hind limb lameness (54.68%) is more as compared to forelimb lameness (45.31%) in camel. Moreover the upward fixation of patella is the 3rd most common (10.71%) cause of hind limb lameness (Gahlot, 2007). The most commonly described stifle joint affections are gonitis and upward fixation of patella (Vaughan, 1965 and Wheat, 1972). The latter is the most prevalent stifle affection in camel (Krishnamurthy *et al*, 1992). Other affections of stifle joint include the rupture of cruciate ligament (Purohit *et al*, 1988a; Pearce and Hurtig, 1999 and Marriott *et al*, 1999), fibrotic myopathy of thigh muscles affecting the movement of the stifle joint (Purohit *et al,* 1988b), arthritis of stifle joint i.e. gonitis (Gahlot, 2000), poisoning of *Capparis tomentosa,* a medicinal plant leading to stiffness of stifle joint (Schwartz and Dioli, 1992). This can impose an economical set back to poor farmers making camel stifle joint an important anatomical structure for research.

Gross anatomical dissection studies on stifle joint have been done previously in camel (Siddiqui and Telfah, 2010), llama (Semevolos, 2005), cattle (Uddin *et al*, 2009), cattle and buffalo (Hifney *et al*, 2013) and ovine (Vandeweerd *et al,* 2012) with good results and excellent contribution in anatomical studies. The present study was thus planned for morphological and morphometric analysis of stifle joint of camel.

Materials and Methods

The study was carried out on 10 freshly collected stifle joints from cadaver of adult dromedary camels (*Camelus dromedarius*) of local breed. The studied specimens showed no evidence of marked bony abnormalities or degenerative disease. The study was done after carefully dissecting the skin and fascia covering the joint and nearby anatomical structures.

Morphological study

The morphological features of the stifle joint were studied as:

1) Femorotibial Articulation:

a) Ligaments

b) Articular surfaces and associated bones

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2) Femoropatellar Articulation:

a) Ligaments

b) Articular surfaces and associated bones

3) Menisci

Morphometric study

Morphometric data (length, width and thickness) of the important ligaments and anatomical structures (articular surfaces) were measured by Vernier caliper. These data were represented as Mean ± SD (standard deviation) in Table 1. The coefficient of variation was also calculated to show variability of a particular dimension. The statistical analysis was carried out with Microsoft® Excel 2007 program. The nomenclature used in the present work was adapted to the Nomina Anatomica Veterinaria (2005) as well as the available literature.

Results

I. Morphological Study

A. Femoro-tibial articulation:

This articulation was condylar type where femoral and tibial condyles articulate. Important anatomical structures evidenced on morphological study of this articulation were:

(i) Ligaments

Collateral Ligaments:

The **Lateral Collateral Ligament** was found absent (Fig 3).

The **Medial Collateral Ligament** runs from an eminence on the medial epicondyle of the femur to the medial proximal aspect of the tibia (Fig 5).

Cruciate Ligaments:

There was evidence of 2 well developed cruciate ligaments:

The **Cranial Cruciate Ligament** (Fig 1) had origin from the cranial intercondylar area of tibia and inserted on the lateral intercondylar surface of femur.

The **Caudal Cruciate Ligament** (Fig 7) had origin from caudal intercondylar area of tibia and had 2 insertions i.e. one on the cranial surface of medial intercondylar area of femur the main insertion and another more cylindrical attached lateral to it.

(ii) Articular Surfaces and Associated Bones:

Distal Femur

Condyles of femur were found to be placed at a slight angle directed caudolaterally (Fig 1). The intercondylar fossa had 3 depressions for the attachment of ligaments. The medial epicondyle (Fig 6) had a prominence for ligament attachment while the lateral epicondyle was relatively smooth.

Proximal Tibia

The tibia was triangular proximally and tapered down towards the cylindrical distal end. The 2 articular surfaces of tibia were lateral and medial tibial condyles. The lateral condyle was roughly trapezoid in shape whose narrow border end towards the lateral intercondylar tubercle. The medial condyle was larger and more rounded in shape and continued along the surface of medial intercondylar tubercle (Fig 8). The medial intercondylar tubercle was slightly higher in position than its lateral counterpart (Fig 8). There were depressions in the caudal intercondylar area for the attachment of ligaments. There was a depression between the lateral tibial condyle and the anterior tibial tuberosity. The tibial tuberosity was very well developed, irregular and directed craniodorsally (Fig 8). It was indented along its proximal surface to form a short sulcus. There was a shallow transverse depression distal to the latter.

B. Femoro-Patellar Articulation:

(i) Ligaments

This articulation was a sellar joint between the femoral trochlea and the patella. Various important anatomical structures evidenced on morphological study of this articulation were:

Middle Patellar Ligament appeared to be the only well developed ligament of patella which represents the insertion of quadriceps femoris muscle. It had origin from the craniodistal aspect of the patella and run distally as a strong wide fibrous band and inserted into the tibial tuberosity. A thick layer of adipose tissue cushion it from the joint capsule (Fig 4).

Medial Femoropatellar Ligament consists of a thin strap like ligamentous structure extended between the medial epicondyle of femur and the medial aspect of patella somewhat at mid. In addition to this or for better reinforcement of the joint there was a ligamentous band originating from the muscle fascia of the medial aspect. This band partly attaches to the medial femoral epicondyle and run in a craniodistal direction ending in the tibial tuberosity (Fig 4).

Lateral Patellar Retinaculum consists of a lateral femoropatellar ligament running between the lateral femoral epicondyle and the lateral aspect of patella at nearly distal end. It appeared to be fused

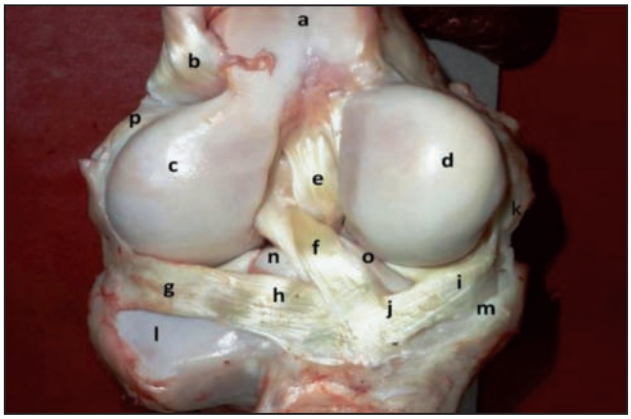

Fig 1. Gross anatomy of right stifle joint cranial view (joint capsule and patella removed).
(a) Trochlea, (b) Origin of muscle extensor digitorum longus and peronius tertius, (c) Lateral condyle of femur, (d) Medial condyle of femur, (e) Caudal cruciate ligament, (f) Cranial cruciate ligament, (g) Lateral meniscus, (h) Cranial attachment of lateral meniscus,(i) Medial meniscus, (j) Cranial attachment of medial meniscus, (k) Medial collateral ligament, (l) Lateral condyle of tibia, (m) Medial condyle of tibia, (n) Lateral intercondylar tubercle, (o) Medial intercondylar tubercle, (p) Popliteus muscle (origin).

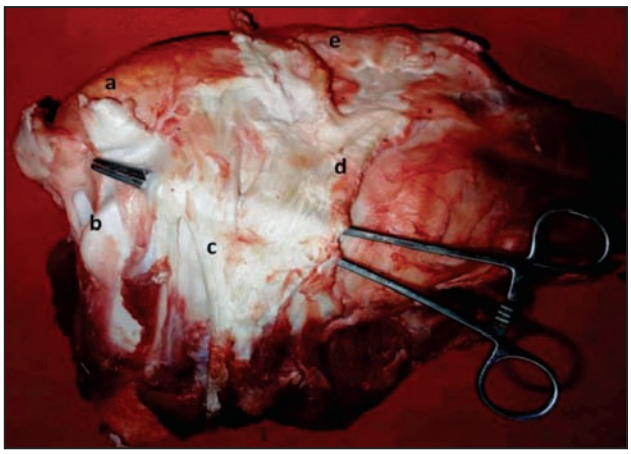

Fig 2. Gross anatomy of right stifle joint lateral view.
(a) Patella, (b) Lateral trochlear ridge, (c) Lateral femoropatellar ligament, (d) Insertion of Muscle gluteobiceps on patella and tibial tuberosity, (e) Middle Patellar Ligament.

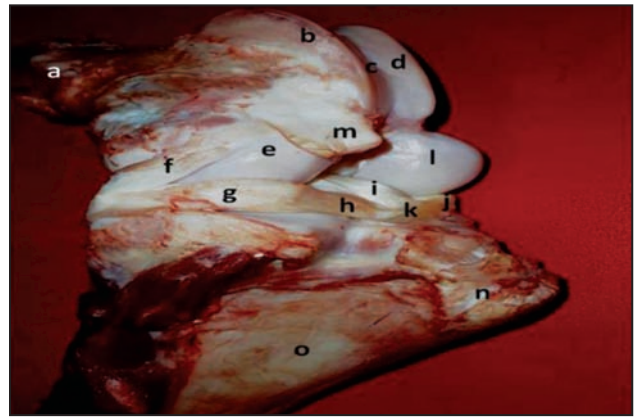

Fig 3. Gross anatomy of right stifle joint lateral view (joint capsule and patella removed).
(a) Femur, (b) Lateral trochlear ridge, (c) Femoral trochlea groove, (d) Medial trochlear ridge, (e) Lateral condyle of femur, (f) Popliteus muscle (origin), (g) Lateral meniscus, (h) Cranial attachment of lateral meniscus, (i) Cranial cruciate ligament, (j) Medial meniscus, (k) Cranial attachment of medial meniscus, (l) Medial condyle of femur, (m) Origin of muscle extensor digitorum longus and peronius tertius, (n) Tibial tuberosity, (o) Tibia.

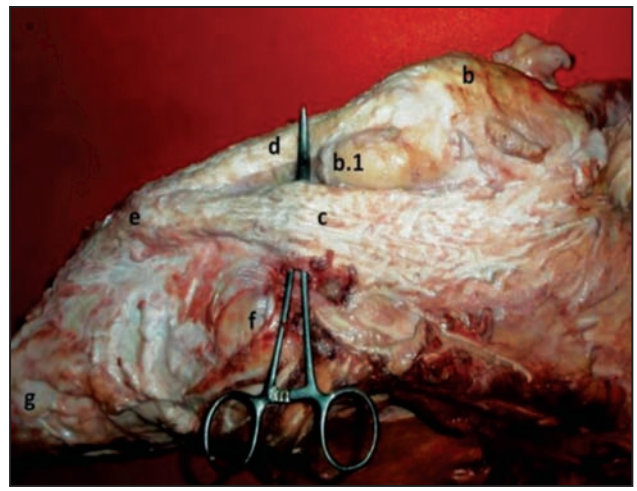

Fig 4. Gross anatomy of right stifle joint medial view.
(a) Femur, (b) Patella, (b.1) Apex of patella, (c) Ligamentous band from the medial femoral fascia, (d) Middle patellar ligament, (e) Tibial tuberosity, (f) Medial condyle of tibia, (g) Tibia.

with the middle patellar ligament at this level and also associated with the tendinous insertion of muscle gluteobiceps at patella and tibial tuberosity (Fig 2).

(ii) Articular Surfaces and Associated Bones:

a. Femoral Trochlea

The femoral trochlea consists of a gliding groove and trochlear ridges i.e. medial and lateral. The trochlea appeared to be directed distally and medially. The trochlear goove was smooth and congruent for articulation with patella. The lateral trochlear ridge was longer and prominent than the medial counterpart (Fig 3).

b. Patella

Patella was elongated bone and had a base and an apex (Fig 5). The base was blunt directed proximally and the apex was pointed directed distally. The greater curvature of the bone was convex and rough for ligaments insertion. The lesser curvature was smooth and articulate with the femoral trochlea.

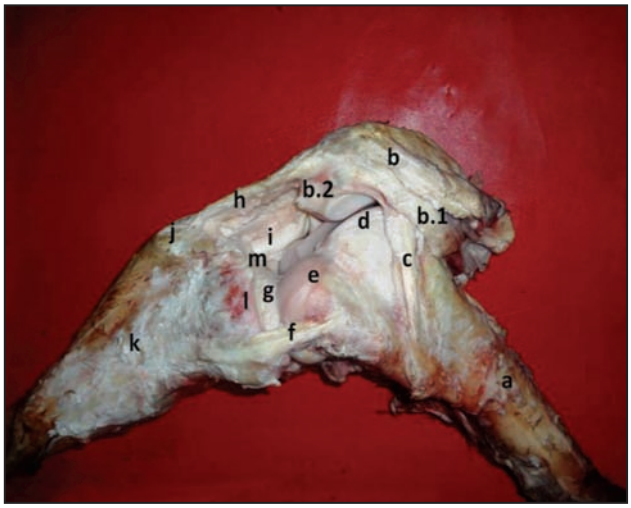

Fig 5. Gross anatomy of right stifle joint medial view I (joint capsule removed).
(a) Femur, (b) Patella, (b.1) Base of patella, (b.2) Apex of patella, (c) Medial femoro-patellar ligament, (d) Femoral trochlea, (e) Medial femoral condyle, (f) Medial collateral ligament, (g) Medial meniscus, (h) Middle patellar ligament, (i) Common tendon of origin of muscle extensor digitorum longus and peronius tertius, (j) Tibial tuberosity, (k) Tibia, (l) Medial tibial condyle, (m) Cranial attachment of medial meniscus.

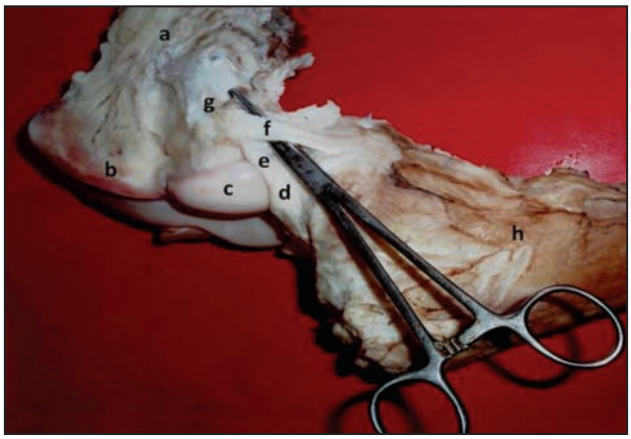

Fig 6. Gross anatomy of right stifle joint medial view II (joint capsule and patella removed).
(a) Femur, (b) Medial trochlear ridge, (c) Medial condyle of femur, (d) Medial meniscus, (e) Attachment of medial meniscus to medial epicondyle, (f) Medial collateral ligament, (g) Medial epicondyle (h) Tibia.

Menisci (Singular Meniscus)

There were two menisci, the lateral and the medial meniscus which act as cushions in the femoro-tibial articulation (Fig 1). These were almost crescent shaped, medial meniscus being somewhat more round and larger than the lateral one. These menisci were kept in place by various meniscal ligaments described as follows:

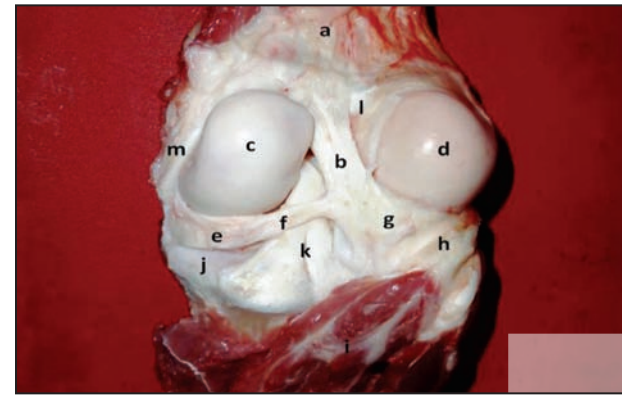

Fig 7. Gross anatomy of right stifle joint caudal view (joint capsule and patella removed).
(a) Femur, (b) Meniscofemoral ligament, (c) Medial condyle of femur, (d) Lateral condyle of femur, (e) Medial meniscus, (f) Ligament connecting menisci, (g) Lateral meniscus, (h) Popliteus muscle (origin), (i) Popliteus muscle, (j) Medial condyle of tibia, (k) Caudal cruciate ligament, (l) Cranial cruciate ligament, (m) Medial collateral ligament.

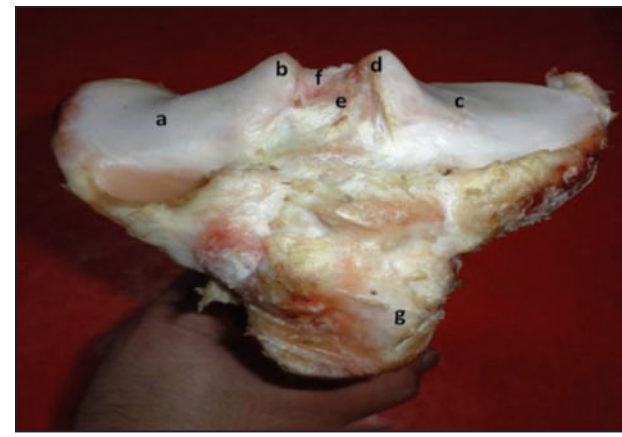

Fig 8. Gross anatomy of articular surfaces of right tibia (cranio dorsal view).
(a) Lateral condyle of tibia, (b) Lateral intercondylar tubercle, (c) Medial tibial condyle, (d) Medial intercondylar tubercle, (e) Cranial intercondylar area, (f) Caudal intercondylar area, (g) Tibial tuberosity.

Lateral Meniscus

It was **cranially** attached to the intercondylar area of tibia (Fig 1) and caudally attached to the caudal aspect of femur by meniscofemoral ligament (Fig 7).

Medial Meniscus

It was **cranially** attached to the intercondylar area of the tibia (Fig 1) and caudally attached to the caudal intercondylar area of femur and to the lateral meniscus over the caudal cruciate ligament (Fig 7).

It was medially attached to the origin of cranial cruciate ligament (Fig 1) and medial femoral

epicondyle (Fig 6) and proximally and cranially to the medial collateral ligament (Fig 5).

II Morphometric Study

Morphometric study include the measurement of ligaments and important anatomical structures in terms of their dimensions *viz.* length, width, thickness etc. These data are presented in table 1.

Discussion

The stifle joint is a compound synovial joint having femorotibial (condylar joint) and femoropatellar (sellar joint) articulation. The patella is elongated with blunt base and pointed apex (apex directed distally). It is nearly congruent and articulate with femoral trochlea. The trochlea had a gliding groove with lateral and medial ridges. The lateral ridge is longer (evidenced by morphometric study) and prominent. These findings were in consonance with the findings of Smuts and Bezuidenhout (1987), Goldblatt and Richmond (2003), Dwek and Chung (2008), Siddiqui and Telfah (2010) and Fahmy *et al* (2011). No accessory cartilage was evidenced and the finding was in accordance to Krishnamurthy *et al* (1992). However, Manefield and Tinson (1997) stated that upward fixation of patella in camel denoted a tendency for the well developed fibrocartilage hook on the patellar border to be caught on the medial trochlear protuberance.

The stability of femopropatellar joint depends upon the shape and extension of the femoral trochlea which could provide a surface for patellar motion. The joint is also supported by various ligaments, thick fascia, musculature and their tendons. These findings were supported by Dwek and Chung (2008), Goldblatt and Richmond (2003), Neyret *et al* (2002) and Simon *et al* (2000).

The middle patella ligament is well developed patellar ligament which is in accordance to the studies of Smuts and Bezuidenhout (1987) and Siddiqui and Telfah (2010).

The lateral patellar ligament is represented by the fibres running between the lateral epicondylar region of femur and distolateral surface of patella. The ligament is also found to be associated with insertion of muscle gluteobiceps on patella and runs along with it distally to end at cranial tibial tuberosity. These findings were supported by Smuts and Bezuidenhout (1987) and Siddiqui and Telfah (2010).

The medial patellar ligament could not be evidenced. There was a ligament like strap originating from the muscle fascia of the medial aspect running between the medial femoral epicondyle and tibial tuberosity. There is evidence of medial femoropatellar ligament extended between the medial epicondyle of femur and the medial aspect of patella close to the mid. These findings are in consonance with findings of Krishnamurthy *et al* (1979), Smuts and Bezuidenhout (1987), Al-Ani (2004) and Siddiqui and Telfah (2010). They described it as a poorly defined medial femoropatellar ligament. The orientation of the medial femoro patellar ligament is different and it is present at an angle of 113.8° ± 1.93218°

Table 1. Morphometric data of various anatomical structures of stifle joint of camels (*Camelus dromedarius*).

S. No.	Particulars	Coefficient of variation (CV)	Values (Mean ± SD[b]) (in cm)
1.	**Femoro-Patellar ligaments**		
	(a) Lateral Femoropatellar ligament		
	Length	1.764677	8.94 ± 0.15776
	Width	2.419535	4.11 ± 0.09944
	Thickness	9.621923	0.91 ± 0.08756
	(b) Lig. Patellae		
	Length	1.15155	15.96 ± 0.18379
	Width	2.336529	4.71 ± 0.11005
	Thickness	6.162583	1.28 ± 0.07888
	(c) Lig. Femoropatellar Mediale		
	Length	1.412131	11.66 ± 0.16465
	Width	5.250499	1.84 ± 0.09661
	Thickness	11.73631	0.88 ± 0.10328
2.	**Femoro-tibial ligaments**		
	(a) Medial Collateral Lig.		
	Length	2.84429	8.58 ± 0.24404
	Width	3.977162	2.07 ± 0.08233
	Thickness	4.689766	1.03 ± 0.0483
	(b) Lig. Cruciatum Craniale		
	Length	3.675317	4.66 ± 0.17127
	Width	6.684836	1.18 ± 0.07888
	Thickness	3.927202	1.23 ± 0.0483
	(c) Lig. Cruciatum Caudale		
	Length	2.220578	5.2 ± 0.11547
	Width	13.10514	1.57 ± 0.20575
	Thickness	7.052687	1.41 ± 0.09944
3.	**Ligaments of Menisci**		
	(I) Lateral Menisci		
	(a) Cranial Attachment		
	Length	4.072028	3.67 ± 0.14944
	Width	8.395427	1.13 ± 0.09487
	Thickness	15.05847	0.91 ± 0.13703

	(b) Lig. Menisco Femoral		
	Length	2.368912	9.13 ± 0.21628
	Width	4.451705	2.32 ± 0.10328
	Thickness	8.032982	1.09 ± 0.08756
	(II) Medial Meniscus		
	(a) Cranial Attachment		
	Length	3.458562	4.19 ± 0.14471
	Width	7.036518	1.17 ± 0.08233
	Thickness	11.71214	0.9 ± 0.10541
	(b) Caudal Attachment		
	Length	3.525821	7.2 ± 0.25386
	Width	6.588638	2.03 ± 0.13375
	Thickness	12.90746	1.02 ± 0.13166
4.	**Angle between middle patellar and medial femoro patellar lig. (in degree)**	1.697877	113.8 ± 1.93218
5.	**Angle between meniscofemoral and lig. connecting menisci caudally (in degree)**	2.136616	82.7 ± 1.76698
6.	**Menisci**		
	(I) Lateral		
	(a) Greater Curvature	2.541641	8.59 ± 0.21833
	(b) Lesser Curvature	2.713741	3.56 ± 0.09661
	(c) Max. Thickness	5.21829	2.06 ± 0.1075
	(d) Width From Centre	4.973474	2.13 ± 0.10593
	(II) Medial		
	(a) Greater Curvature	1.069014	10.62 ± 0.11353
	(b) Lesser Curvature	2.786148	4.54 ± 0.12649
	(c) Max. Thickness	5.483718	1.73 ± 0.09487
	(d) Width From Centre	4.750448	2.23 ± 0.10593
7.	**Patella**		
	(I) Length		
	(a) Greater Curvature	1.253474	15.71 ± 0.19692
	(b) Lesser Curvature	1.534022	9.62 ± 0.14757
	(II) Width		
	(a) Proximal	3.3789	4.51 ± 0.15239
	(b) Middle	2.768367	4.24 ± 0.11738
	(c) Distal	2.417706	5.25 ± 0.12693
	(III) Thickness		
	(a) Proximal	3.809706	4.47 ± 0.17029
	(b) Middle	2.472339	5.46 ± 0.13499
	(c) Distal	2.288371	5.15 ± 0.11785
8.	**Femoral Trochlea**		
	(I) Groove		
	(a) length	1.873364	7.57 ± 0.14181
	(b) width		
	(i) Proximal	2.206828	4.68 ± 0.10328
	(ii) Middle	2.983281	4.24 ± 0.12649
	(iii) Distal	2.297925	5.21 ± 0.11972
	(II) Trochlear Ridge Length		
	(a) Medial	1.397409	9.66 ± 0.13499
	(b) Lateral	1.092912	10.74 ± 0.11738
9.	**Tibial condyles**		
	(I) Lateral		
	(a) Mediolateral Dimension	1.674473	6.78 ± 0.11353
	(b) craniocaudal Dimension	2.083271	5.16 ± 0.1075
	(II) Medial		
	(a) Mediolateral Dimension	2.030873	6.25 ± 0.12693
	(b) Craniocaudal Dimension	2.188044	6.83 ± 0.14944
10.	**Femoral condyles**		
	(I) Lateral		
	(a) Mediolateral Dimension	3.312693	4.51 ± 0.149071
	(b) craniocaudal Dimension	1.865402	7.17 ± 0.133749
	(II) Medial		
	(a) Mediolateral Dimension	3.068328	4.66 ± 0.142984
	(b) Craniocaudal Dimension	2.042206	6.71 ± 0.137032

(a) Lig.- ligament
(b) SD- Standard Deviation

with the middle patellar ligament as evidenced by morphometric study.

Krishnamurthy *et al* (1992) revealed absence of medial patellar ligament in camel. However, due to similarities in texture as well as in location, the tendinous structure of the deep fascia is often mistaken for the medial patellar ligament.

Manefield and Tinson (1997) stated that the camels did not have 3 patellar ligaments as is seen in equine and the bovine, but rather a diffused band of fibrous tissue which covers the muscles on the medial aspect and inserted into the cranial tibial tuberosity.

The lateral and medial femoral condyles of femur and tibia were found nearly congruent at their respective articular surfaces of tibia. The intercondylar fossa of femur had depressions for the attachment of ligaments. The medial epicondyle had a prominence while the lateral epicondyle was relatively smooth. The fossa extensoria was deep whereas, fossa *m. poplitei* caudal to it was relatively shallow. The lateral condyle of tibia was roughly trapezoid in shape while medial condyle was larger and more rounded in shape. Margins of both condyles ended at respective intercondylar tubercle. The medial intercondylar tubercle was slightly higher in position than its lateral counterpart. There were depressions in the caudal intercondylar area for the attachment of ligaments. There was a depression between the lateral tibial

condyle and the anterior tibial tuberosity. The anterior tibial tuberosity was very well developed, irregular and directed craniodorsally. It was indented along its proximal surface to form a short sulcus. There was a shallow transverse depression distal to the latter. Similar findings were documented by Smuts and Bezuidenhout (1987).

Among collateral ligaments, only medial was evidenced and lateral was absent. Medial collateral ligament traversed from the medial femoral epicondyle to the medial proximal aspect of the tibia. There was evidence of two well developed cruciate ligaments i.e. cranial and caudal cruciate ligament found intra articularly within the joint capsule. The cranial cruciate ligament had origin from the cranial intercondylar area of tibia and inserted on the lateral intercondylar surface of femur. The caudal cruciate ligament had origin from the caudal intercondylar area of the tibia and had two insertions i.e. one on the cranial surface of the medial intercondylar area of femur and another attached laterally to it. Similar communications had been reported by Smuts and Bezuidenhout (1987).

Rooster *et al* (2006) in their study on the cruciate ligament concluded that the cranial cruciate ligament control cranial drawer motion, whereas the caudal cruciate ligament act as a major stabiliser against caudal motion. Furthermore, the latter ligament was considered to fine tune normal stifle kinetics. Goldblatt and Richmond (2003) stated that the bony architecture of the femur, patella and tibia contribute to the stability of the stifle joint, along with static and dynamic restraints of the ligaments, capsule and muscular crossing the joint. Similar conclusions might be drawn from present study.

Two crescent shaped menisci, the lateral and medial meniscus which act as cushions in the femoro-tibial articulation were evidenced along with their attachments. These findings are in consonance with the findings of Smuts and Bezuidenhout (1987).

Uddin *et al* (2009) conducted a study on cattle aimed to determine the accurate location of giving incision for medial patellar desmotomy. They analysed the measurements of patellar ligaments in stifle joint of 50 indigenous and 50 crossbred slaughtered cattle. Similarly, morphometric studies were included in present study measured in 10 stifle joints collected from fresh cadavers. Descriptive statistics were given as Mean ± SD (standard deviation) and coefficient of variation was also calculated to show variability of a particular dimension. The morphometric study could be found as a useful reference for further studies.

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Short Communication

PHYSICAL RESTRAINING TECHNIQUE FOR HIND LEG IN CAMELS

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Foot affections like puncture foot, phalangeal fractures, traumatic injuries, foot pad avulsion, digital cushion hernia, etc. are common in camels. The overall incidence of the foot disorders in camels is 10.60%. The occurrence of these affections is almost equal in both fore and hind limbs (Singh and Gahlot, 1997). Such affections of foot require appropriate restraint for proper clinical and radiographic examination and thereafter treatment. The forelimb of camel can easily be restrained in the standing position. But securing the hind limb of animal in its standing position involves risk of kicking (Gahlot, 2000). Moreover, the hindfoot of the animal cannot be stabilised for lateral and dorsoplantar radiographic examination. The traditional methods of radiographic exposure of camel foot (Singh and Peshin, 1994) might lead to damage of cassette by the animal's body weight and x-ray tube by camel. To overcome these constraints an innovative physical restraining technique was adopted for hind limb in standing position of the camel.

Six adult, male camels admitted to the Teaching Veterinary Clinical Complex with hind foot affections i.e., lymphangitis, foot oedema, lacerated wound, foot pad avulsion (2 cases) and phalangeal fracture were subjected to the new and simpler physical restraint technique using a 4 metre long thick cotton braided rope. One end of this rope was tied by using the slip knot around the distal end of canon region or distal to fetlock of the affected limb. Then the remaining length of the rope was thrown over the caudal cervical region just cranial to the withers towards the other lateral side of the animal. The attendant standing near the shoulder region on the other side pulled the rope over the withers in such a way that secured hind limb got raised cranially and dorsally with the flexion of hock and stifle joints. Once the foot is raised sufficiently above the ground surface, the pulled end of the rope was looped through the axilla around the arm of the contralateral forelimb which prevented its slipping over the withers and thus maintained the intact position of the raised limb. This prevented the caudad or lateral kicking from the secured limb. All these clinical cases were physically restrained in standing position both for examination and regular treatment by keeping the affected limb raised above the ground surface, with the foot toe directed towards the ipsilateral forelimb and its dorsal surface towards the ventral side of thoraco-abdominal surface below the chest pad. This position of foot was found suitable for clinical examination, radiographic positioning and wound dressing and bandaging. Animals did not show any resistance and discomfort with this restraining method. It enabled surgeon to position himself safely lateral to the raised foot and thus clinical examination, palpation, placing of radiograph cassette towards the ground or medial/lateral side of the foot were accomplished comfortably. Dressing and bandaging of wound also become easy, safer and faster. Further to be more safe and to prevent lateral movement of foot during examination and treatment, the raised limb can be kept pulled on the contralateral medial side by tying an another rope

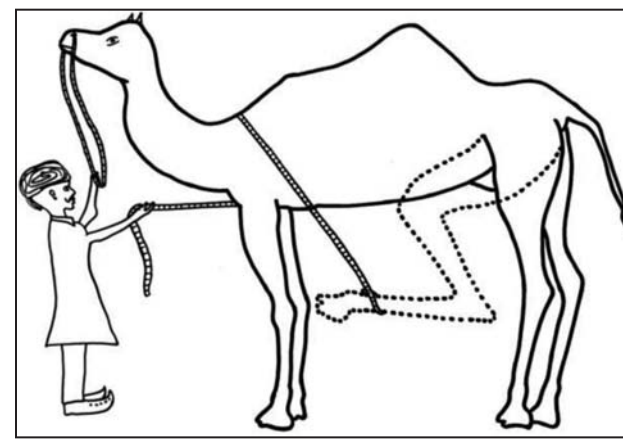

Fig 1. Raising of affected hindlimb with the flexion of hock and stifle joints.

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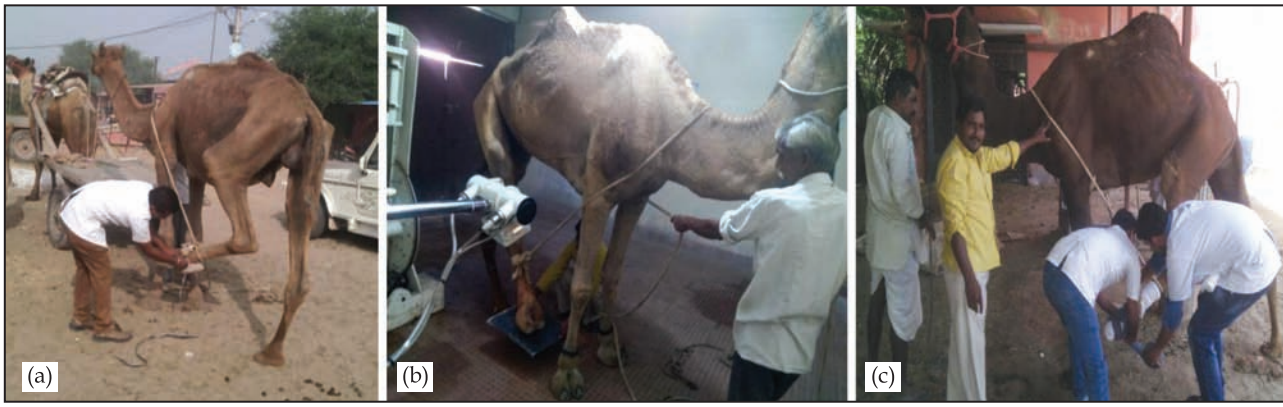

Fig 2. With the standing physical restraining technique of hind limb it was possible to (a) physically examine the affected hind foot, (b) make radiographic examination of hind foot and (c) regular wound dressing of hind foot.

at the canon region. In this manner a single person was able to keep hold the rope tied to the affected hind limb and simultaneously also held the halter rope on head of animal. Animals could easily balance their body weight on the 3 limbs. Animals were kept restrained in this standing position for a short period of time (5-10 minutes) both for examination and treatment. It enabled frequent examination and treatment of the feet lesions at the regular intervals in these cases. It offered other advantages i.e. lesser number of personnel required for animal restraining, no chemical restraining required, no injury incurred to the animal and personnel during the period of restraining and every part of the foot could easily be approached for treating any of its lesion.

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SQUAMOUS CELL CARCINOMA OF EYELID IN CAMEL: A RARE CASE REPORT

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ABSTRACT

A twelve year old camel was presented to Teaching Veterinary Clinical Complex, LUVAS, Hisar with history of swelling in the right eyelid and blurring of the eye. No other abnormality was detected in affected eye on physical examination of eye. Tmperature, pulse rate, respiratory rate were within the normal range. Excisional biopsy was performed. Impression cytology of the tissue revealed large cells with abundant basophilic cytoplasm and pleomorphic nuclei. Histopathologically, tissue section of the biopsy material showed infiltrating neoplastic squamous epithelial cells in the form of cell nests, hyperchromasia of nuclei and epithelial keratin pearl at some places. The malignant cells of cell nests revealed dense cytoplasmic immunopositive reactivity to the pancytokeratin. Based on gross examination, impression cytology, histopathology and immunohistochemical findings, the case was diagnosed as squamous cell carcinoma of eyelid. It was successfully treated with lithium antimony thiomalate @ 15ml IM 6 shots for alternate day with supportive therapy.

Key words: Anthiomaline, camel, impression cytology, squamous cell carcinoma

Neoplasm of the skin and subcutaneous tissues are the most frequently recognised neoplastic disorders in domestic animals (Singh *et al,* 1991). Squamous cell carcinoma begins from the squamous cells, (Dayananda *et al,* 2009) and is one of the main types of skin cancer. Cancer that involve the anus, cervix, head, neck and eyelid are also most often squamous cell cancer (Kari, 2012). Incidence of squamous cell carcinoma is more in females as compared to male. According to frequency, camel neoplasms can be categorised as squamous cell carcinoma, fibroma, lipoma and fibrosarcoma (Al-Sobayil and El-Amir, 2013). Sunlight is probably the most important carcinogenic stimulant for SCC and accounts for the prevalence of SCC on eyelids and conjunctiva in animals (Baniadam *et al,* 2010). Skin neoplasms have been reported scarcely in camels. However, there are very few reports of squamous cell carcinoma in eyelid of camels. Therefore the present study reports a case of squamous cell carcinoma in eyelid of camel along with successful chemotherapy using lithium antimony thiomalate (Anthiomaline, Novartis India Limited).

Materials and Methods

Case history

A twelve year old camel was presented to Teaching Veterinary Clinical Complex, LUVAS, Hisar with a history of swelling in the right eyelid and blurring of the eye. Clinical examination of eye revealed growth (1-2 cm in diameter) in right upper eyelid (Fig 1). Corneal opacity was also observed. Grossly, growth was nodular, reddish in colour and ulcerated. Respiratory rate, heart rate and blood parameters were within the normal range upon physical examination. Excisional biopsy was carried out under local anaesthesia for the confirmatory diagnosis. Impression smears from the growth were prepared, fixed with methanol and stained with Field stain for cytological diagnosis. Biopsy sample was immediately fixed in 10% buffered formalin, processed, sectioned at 5 µ and stained with haematoxylin and eosin (H & E) for histopathological diagnosis (Luna, 1968).

Immunohistochemical staining for pancytokeratin was performed in paraffin wax sections by streptavi Zdin-biotin (SAB) methods using labeled streptavidin biotin Kits (Sigma). The primary antibodies used were anti-pancytokeratin. Sections were counterstained by haematoxylin.

The camel was treated with Anthiomaline @ 15ml IM 6 shots for alternate day. Supportive therapy included ceftriazone 4.5 gm IM for 5 days and ascorbic acid 250 mg/ml Injection 3 days. Corneal opacity was treated with eye drops Neosporin for 15 days.

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Result

Cytology of impression smears revealed large cells with abundant basophilic cytoplasm, pleomorphic nuclei and atypical mitotic figures (Fig 2). Abundant neutrophils were also observed indicating ulceration which is characteristic of squamous cell carcinoma. Histopathological examination revealed nests of highly anaplastic squamous cells containing keratin pearls. Neoplastic cells revealed multiple mitotic figures with loss of polarity, marked pleomorphism and high grade of anisokaryosis in the nuclei (Fig 3). The neoplastic cells showed the downward penetration and severe infiltration of polymorphonuclear cells and some lymphocytes. On the basis of clinical signs, cytological and histopathological examination, tumour was diagnosed as well differentiated squamous cell carcinoma. Immunohistochemical staining of tumour sections revealed dense cytoplasmic immunopositive reactivity to the pancytokeratin (Fig 4). Regression of tumour was seen without recurrence in a follow up of 6 months.

Discussion

Among all species, squamous cell carcinoma may occur in young animals but the incidence increases with the age (Kashyap *et al,* 2013). In the present case, camel was in susceptible age for neoplastic growth. Increase exposure to solar radiation, chronic ocular irritation and immune suppression may participate in the development of ocular squamous cell carcinoma (Pigatto *et al,* 2010). In the present study with prolong exposure to sun light per day may have increased exposure to ultraviolet radiation. The white colour coat increased the sensitivity to such

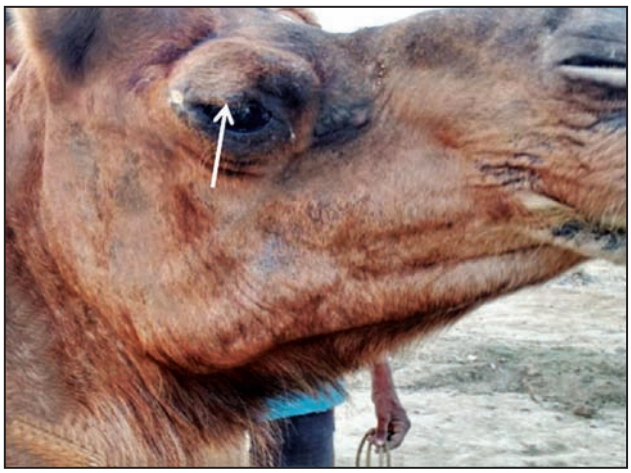

Fig 1. Camel with pea sized growth on the upper eyelid of right eye (white arrow).

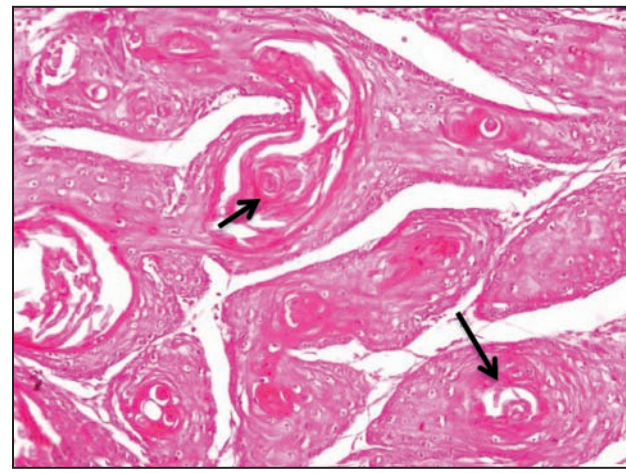

Fig 3. Showing nests of highly anaplastic squamous cells containing keratin pearls with pleomorphism (arrow) (H & E stain 200X).

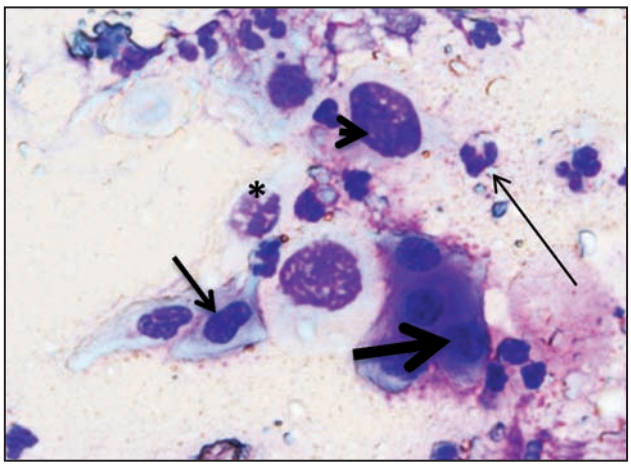

Fig 2. Large tumour cells with abundant basophilic cytoplasm (arrow), nuclear pleomorphism and mitotic figure (arrow head). Note abundant neutrophils in surrounding (Thick arrow) (Field Stain 1000X).

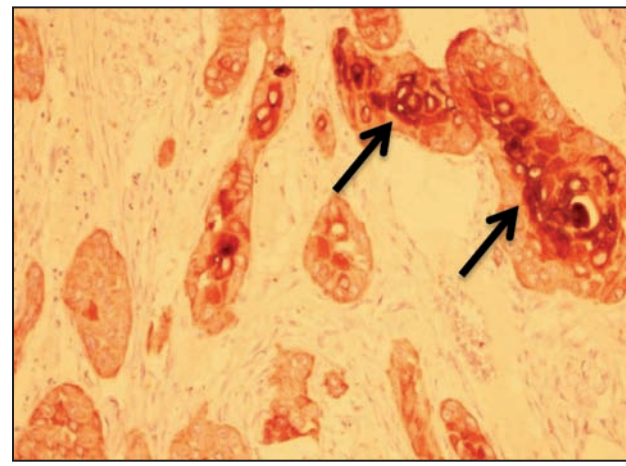

Fig 4. Showing tumour cells strongly positive cytoplasmic immunoreactivity to the pancytokeratin. Streptovidin Biotin method, counterstained with haematoxylin (H&E 400 X).

radiation with subsequent occurrence of squamous cell carcinoma (Pugliese *et al,* 2014). Cytopathological changes such as anisokaryosis, atypical mitotic figures in large neoplastic cells and polymorphonuclear cells infiltration in present case were akin to previous findings (Kane, 2007) and histopathological examination revealed well differentiated squamous cell carcinoma characterised by epithelial islands containing keratin pearls which was in accordance with the finding of Ganguly *et al* (2016). In the present study, immunoreactivity to the pancytokeratin in the cytoplasm of malignant cells was similar to the previous finding in mammary tumour of dog (Sassi *et al,* 2008). Anthiomaline is a brand of lithium antimony thiomalate which is a trivalent organic antimonial containing 16 per cent of antimony. It is extensively used in cattle and horses to treat nasal granuloma and papiliomatosis. The exact mode of action is not known but the effects produced upon the neoplasms appears through interference in their blood supply causing necrosis (www.poulvet.com/vetproduets/medicine_detail.php?mediid=1006).

It is concluded that squamous cell carcinoma of eyelid in camel can be treated with lithium antimony thiomalate.

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Thesis: Rathod Avni (2006).Therapeutic studies on sarcopticosis in camels (*Camelus dromedarius*). Unpublished Masters Thesis (MVSc), Rajasthan Agricultural University, Bikaner, Rajasthan, India.

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