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JOURNAL OF CAMEL PRACTICE AND RESEARCH

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Volume 29 December 2022 Number 3

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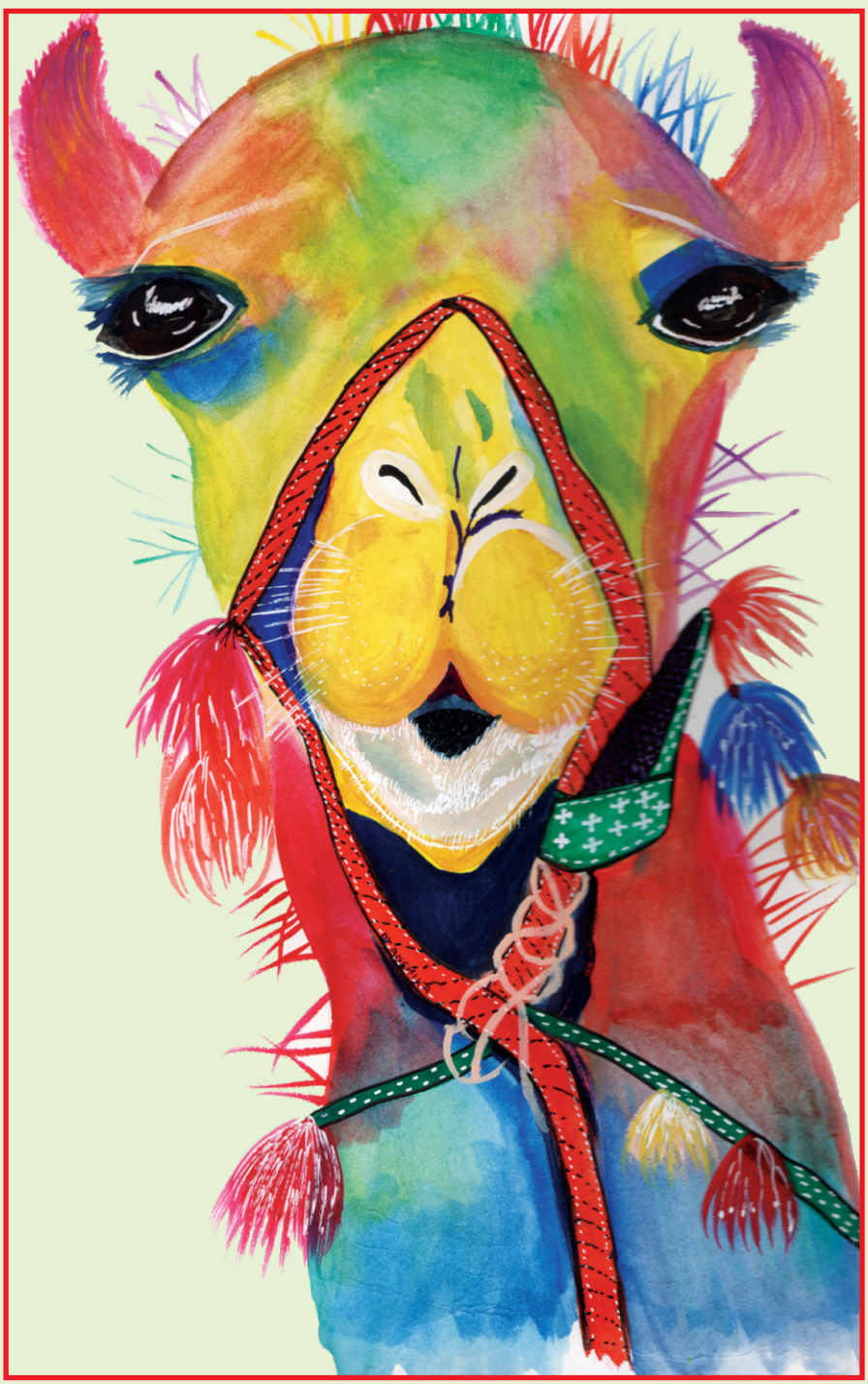

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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Scope of Journal of Camel Practice and Research

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

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THERAPEUTIC POTENTIAL OF CAMEL WHARTON JELLY MESENCHYMAL STEM CELLS (CWJ-MSCS)

Therapeutic potential of Camel Wharton Jelly Mesenchymal Stem Cells (CWJ-MSCs) in canine chronic kidney disease (CKD) model has been explored by the Egyptian scientists. Umbilical cord specimens were obtained fresh from a healthy pregnant camel for further processing. Isolated CWJ-MSC was identified by morphology and flow cytometric analysis. The serum creatinine level was significantly decreased at the 4th week just after the 1st stem cells injection. Such research opened avenues for use of the tissue of camels for stem cell preparation and this could be useful to treat many diseases in animals. Saudi scientists carried out research on natural camel milk products and found that unique properties of camel milk may reduce oxidative stress in the consumers, ameliorating many conditions, including those of the CNS, such as autism spectrum disorders (ASDs). Treatment of ASD with raw and boiled camel milk resulted in significantly lower Childhood Autistic Responsiveness Scale (CARS) scores.

Last quarter of the year had many activites and conferences based on camels. Camel Charisma and LPPS (Lokhi Pashu Palak Sansthan) jointly organised the Godwar Camel Cheese Fest from 23-24 November 2022 at the site of the Kumhalgarh Camel Dairy near Ranakpur-Sadri, India. JANVRY, second festival of camelids was organised in France. The festival (17-18 September 2022) included conferences, camel show, degustation and selling of camelid products (pasteurised and powder milk, kefir, cheese, sweet, cosmetic, wool). The Saudi Veterinary Medical Society held its first international conference from 11-13 October at King Faisal University, Al-Hasa, Saudi Arabia. It included many lead and research papers based on camels. First International Conference on the safety of camels was also organised by the Interanational Camel Organisation on 27th July at Riyadh.

The year 2022 had 52 manuscripts published on various aspects of dromedary and Bactrian camels in the Journal of Camel Practice and Research. Highest number of research manuscripts were based upon immunology (15.38%) followed by diseases (13.6%), parasitology (11.5%), reproduction and anatomy (9.61%), production (7.69%), imaging (5.77%), physiology, microbiology, pharmacology, lameness and genetics (3.8%, each), pathology and surgery (1.92%, each). This indeed shows a current trend of research in camel science.

The current issue of JCPR has two review papers which are related to brucellosis and immune system of camels. It has another 3 manuscripts based on parasitology and 2 manuscripts based on MERS disease and nutrition, each. Interesting manuscript on antidiabetic, anticolitis and anticancer activities of camel milk has given a new confidence for an advantage to the human health although more validation of such results are needed.

I wish Merry Christmas and a Happy New Year 2023 to all the members of the editorial board of JCPR and camel scientists worldover. I am sure that JCPR will make new strides in the new year 2023.

(Dr. Tarun Kumar Gahlot)
Editor

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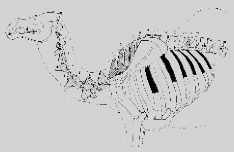
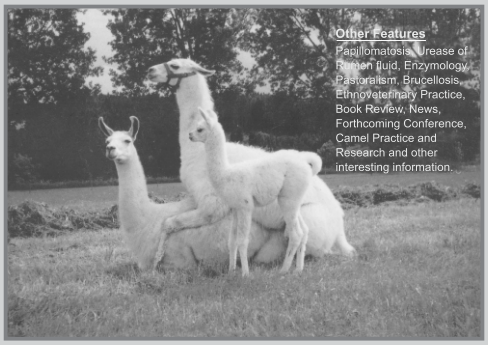


ANATOMY SPECIAL

Archaeological, Gross, Histology, Histochemical, Radiological studies, Development of teeth, Dentition, Anatomy of posterior choanae, Nasal cavity, Salivary gland, Tongue and Rumen papillae, Pancreas, Kidney, Ovary, Placenta, Spinal cord, Stifle joint etc.



Other Features

Papillomatosis, Urease of Rumen fluid, Enzymology, Pastoralism, Brucellosis, Ethnoveterinary Practice, Book Review, News, Forthcoming Conference, Camel Practice and Research and other interesting information.

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JOURNAL OF CAMEL PRACTICE AND RESEARCH

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In This Issue

Camel's Immunoglobulins, Immunodiagnosis and/or other laboratory diagnosis methods for viral, bacterial, fungal, parasitic, systemic and metabolic diseases and toxicity. Physiological studies on digestion and rumen fluid, regular columns etc. Also look inside for a new book and a new vaccine for camelids.

Laboratory Diagnosis Special

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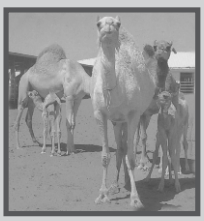


In This Issue

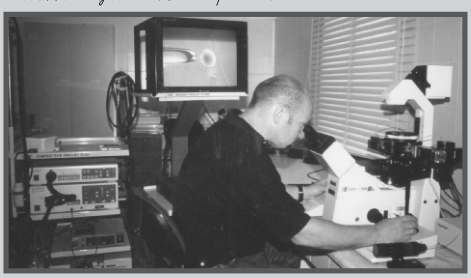
Predominance :

CAMEL : Physiology / Parasitology

Additionally :
Manuscripts based on camel nutrition, diseases, microbiology surgery and reproduction.

Other features :
Camel Practice and Research, News of Camel Science and Forthcoming Conferences, Concern, Ethnoveterinary Practice etc.

Al Ain : Marching Ahead In Camel Reproduction



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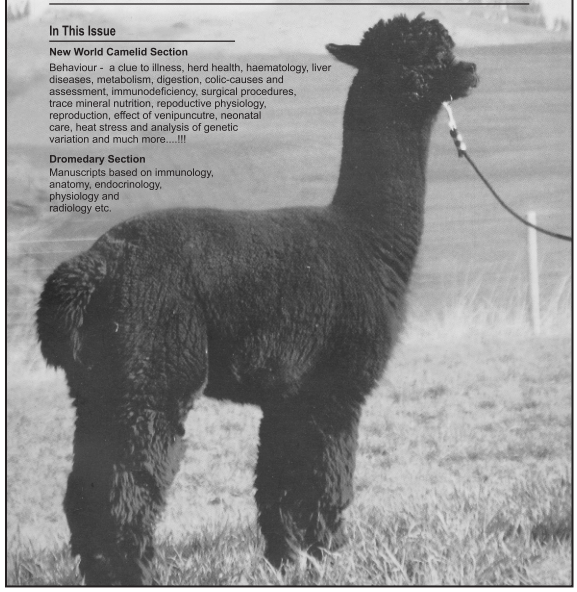
Dromedary Section

Manuscripts based on immunology, anatomy, endocrinology, physiology and radiology etc.



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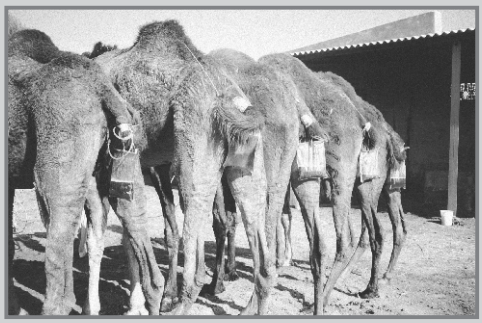
Manuscripts based on camel physiology, husbandry, parasitology, immunology, pharmacology, anatomy, news, book review etc.

Physiology Special



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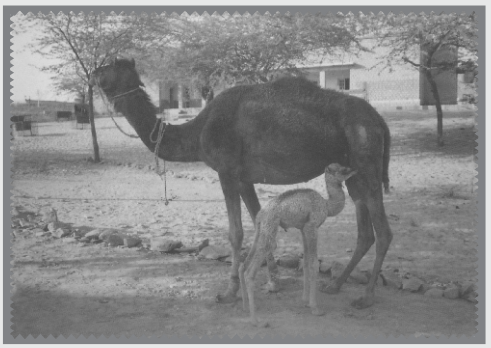
Look for important VACANCY also inside



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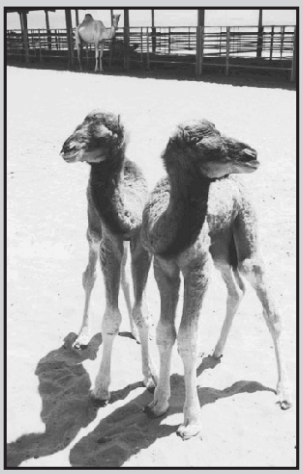

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CAMELID BRUCELLOSIS - CLINICAL FEATURE, EXCRETION PATTERN, SEROLOGICAL AND BACTERIOLOGICAL DIAGNOSIS : REVIEW*

U Wernery[1], R Raghavan[1], N M Paily[1], Sh M Thomas[1], B Johnson[1] and Sh Jose[1]
[1]Central Veterinary Research Laboratory, P O Box 597, Dubai, UAE

Human brucellosis remains one of the most common zoonotic diseases worldwide with more than 500.000 new cases annually (Wernery, 2016). Infection prevalence in animal reservoirs determines the incidence of human cases. That means, where there is animal brucellosis also human brucellosis is diagnosed.

Clinical Feature

In general, *Brucella*–infected animals do not show any clinical signs and therefore it is very difficult to convince the owner, that his animal has a severe disease and has to be culled. This is one of the big challenges veterinarians face, at least with dromedary camels on the Arabian Peninsula. Most of the dromedaries are used for racing and are very valuable animals. The owner will not agree to euthanise a serological positive animal. Compensation does not exist.

CVRL proposed to the government the following:

- Chip all positive dromedaries and keep a record
- Castrate positive bulls
- Never breed with positive females

The WOAH (World Organisation for Animal Health) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals – earlier known as the OIE Manual – is the bible of infectious diseases and it has been upgraded since many years. The 2022 online version of the manual is now available and contains the amended Brucellosis chapter which includes *B. melitensis, B. abortus* and *B. suis*.

This chapter was revised by experts and it gives the newest scientific research results. It is a great piece of work and more or less all answers to any question, will be found in this chapter. WOAH chapters, are regularly revised by experts mainly from WOAH reference laboratories, but also by others, now in total 9 times.

Camelid brucellosis is now in the general chapter of cattle, sheep, goats and swine. CVRL approached the WOAH to also add a separate brucellosis chapter on camelids, but this was declined for the moment.

Brucellosis in canines has become a concern over the years as a zoonotic disease and will be added in future in a special chapter.

Brucellosis has been reported in dromedary camels (*Camelus dromedarius*), the two humped or Bactrian camels (*Camelus bactrianus*) as well as in South American New World camels: Llama, alpaca, guanaco and vicuna. They contract infection when intermingling mainly with small ruminants infected with *B. abortus* and/or *B. melitensis*, but mainly *B. melitensis* (OIE, 2012).

Brucellosis in camelids occurs in all of the known forms described in ruminants. Abortion is the most obvious manifestation. Infections also result in still born calves, retained placenta and reduced milk yield. Hygromas are rare (Fig 1).

Orchitis and epididymitis have been described. Pathological lesions have been rarely described but they include lymphocytic and histiocytic placentitis with oedema and necrosis. Aborted foetuses show subcutaneous oedema, interstitial pneumonia and liver degeneration.

In general, abortions occur only in the first pregnancy and infected dams are healthy. Abortion can occur at any pregnancy stage.

Interestingly, camel calves born to infected dams always remain sero negative (Von Hieber, 2010) although the pathogen is intermittently excreted through milk.

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*Presented on 15.09.2022 at EURL Brucellosis Workshop, Italy 14.09.2022 – 15.09.2022

Infection occurs via the mucous membranes: oral - nasopharyngeal, conjunctival, genital mucosa, also through cutaneous abrasions (handlers), because *Brucella* are tiny bacteria.

Excretion Patterns

Camels become infected through:

- Contaminated feed
- Colostrum and milk
- Contaminated water
- Licking or sniffing at placenta and aborted foetuses
- Very rarely through semen

Shedding of the pathogen is through placenta, aborted foetus and milk, not through nasal discharge, urine or faeces. Through placenta, *Brucella* bacteria are excreted in the hundred millions (10^{12} to 10^{13}) (Juhasz *et al,* 2019).

Camelids do not ingest their placentas (placentophagy) as many other animal species do (Juhasz *et al,* 2019). The placenta dries and wind can bring the pathogen to other farms 500m away. Airborne infections are well known also in laboratories.

Serological diagnosis

An important sentence in the WOAH Manual chapter is the following statement, which is cited herewith:

"No single serological test is appropriate in each animal species and therefore each test should be validated for its fitness in the corresponding animal species".

This means, that the existing brucellosis tests have to be evaluated for use in camelids, which is very important, as camelids possess a special immune system devoid of light chains, named *nanobodies*. During one of the meetings at the WOAH Headquarters in Paris, WOAH representatives asked CVRL to perform such an evaluation.

For the evaluation of *Brucella* serological tests in dromedaries, H.H. Sheikh Mohammed Bin Rashid Al Maktoum, Vice President and Prime Minister of U.A.E, Ruler of Dubai donated 14 *Brucella* negative dromedaries which were infected intranasally and intratracheally with a *B. melitensis* strain, previously isolated from a dromedary camel (Figs 2 and 3).

Over a period of 12 months serological and culture methods including PCR were used for the diagnosis of these 14 experimentally infected *B. melitensis* dromedaries. The details are shown in Table 1.

The development of *Brucella* antibodies in the experimentally infected dromedaries over a period of 12 months by comparing 15 different serological tests (Soellner *et al,* 2018) showed the following results after hundreds of test results were analysed:

Only 2 serological tests were characterised by a high degree of sensitivity for the diagnosis of brucellosis in dromedaries. These 2 tests are:

- Rose Bengal Test (RBT) from Vircell, Spain
- Competitive ELISA from Ingenasa, Spain

The RBT from Vircell produced for the diagnosis of human Brucellosis gave the best results as the concentration of the antigen was ideal, not too thick and nor too thin. If the concentration of the antigen is too thick the 1+ reaction will be missed.

Bacteriological diagnosis

Seven of the *B. melitensis* experimentally infected dromedaries were euthanised and properly investigated. In total, from each dromedary 43 samples were taken and examined (Johnson *et al,* 2018).

- Method of isolation:

1. Direct culture method from tissue samples.
2. Concentration method includes mincing, maceration and homogenising the samples in PBS and using the centrifuged sediment for culture.
3. Enrichment method in which the sediment from concentration method is inoculated into Tryptic Soy Broth (TSB) with Brucella supplement.

Farrell's agar, Brain-Heart infusion Agar (BHI) with *Brucella* supplement and Tryptic Soy agar

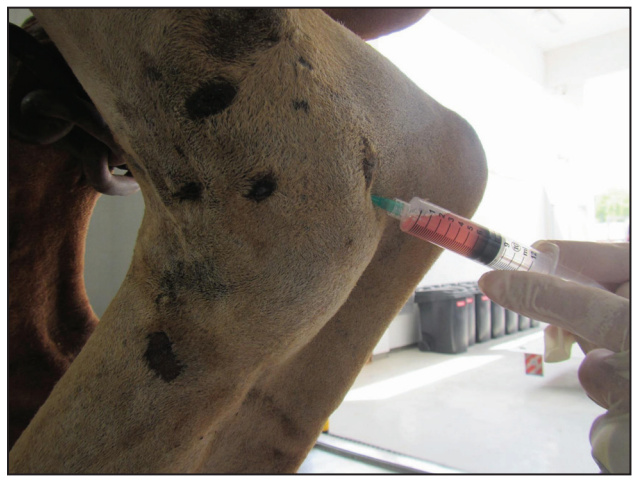

Fig 1. Hygroma caused by *B. melitensis*.

Fig 2. Intranasal infection of a dromedary camel with *B. melitensis*.

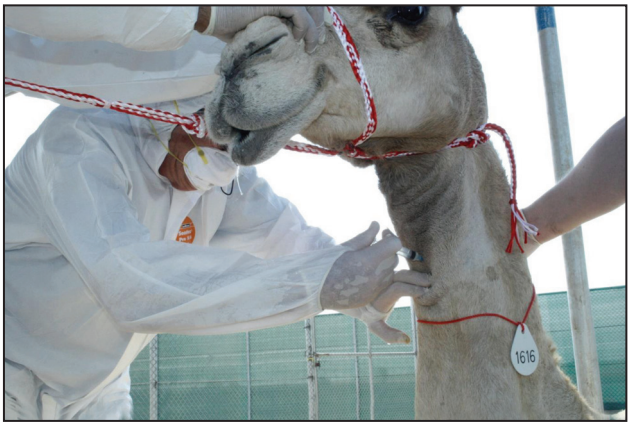

Fig 3. Intratracheal infection of a dromedary camel with *B. melitensis*.

Table 1. Overview of all 15 serological tests used in the diagnosis of brucellosis in experimentally infected dromedaries.

Test	Name/Antigen	Conjugate	Species	Company	Country
RBT	Rose Bengal Antigen	-	Animals	APHA Scientific	UK
RBT	BENGATEST	-	Animals	Synbiotics Europe/ Zoëtis	France
RBT	Pourquier®Rose Bengal Ag	-	Animals	IDEXX	USA
RBT	Rose Bengal	-	Humans	Vircell	Spain
CFT	*Brucella abortus* antigen	-	Animals	APHA Scientific	UK
SAT	*Brucella abortus* antigen	-	Animals	APHA Scientific	UK
Lateral flow test	*B. melitensis/abortus/suis*/ Antigen	Monoclonal anti-camel-IgG	Animals	MEDLINK, MSA™	UAE
Lateral flow test	*Brucella* sLPS (*B. melitensis/ abortus/suis*)	Protein G	Bovine, cattle, sheep, goat & human	Genomix	India
i-ELISA	*Brucella abortus* antigen	Anti-ruminant	Cattle	IDEXX	USA
i-ELISA	*Brucella* LPS	Anti-multi-species-IgG-HRP conjugate	Bovine, ovine, caprine, porcine	ID. vet	France
i-ELISA	Anti-*Brucella* ELISA Camel (IgG), *Brucella* LPS	No Information	Camel	EUROIMMUN	Germany
i-ELISA	Argentina Antigen	Protein A	Animals	Inhouse	-
c-ELISA	*Brucella abortus* antigen	Goat anti-mouse IgG antibody	Bovine, ovine, caprine, porcine	Svanova Boehringer Ingelheim	Sweden
c-ELISA	*Brucella abortus* antigen	Monoclonal antibody specific to the epitope C of LPS of *Brucella*	Small ruminants, bovine, porcine	Ingenasa	Spain
c-ELISA	*Brucella melitensis* LPS extract	Monoclonal anti-*B. melitensis* LPS antibody	Cows, sheep & goats	APHA Scientific	UK

without supplement are inoculated with samples from the above mentioned methods and incubated for 6 days in 5% CO_2 at 37°C. BHI is the preferable agar as after 6 days of incubation the colonies are usually bigger and easier differentiated compared to others.

The results were as follows:

- The isolation of *Brucella* organisms is the gold standard, PCR often failed when less than 3 colonies grew on culture media.
- The organs for the culture of *B. melitensis* are lymph nodes but which ones is unpredictable. However, the pathogen was mostly found in pre-scapular lymph nodes, udder lymph nodes, lung-associated lymph nodes, submandibular and pharyngeal lymph nodes. Interestingly the pathogen was not found in the uterus.
- The remaining infected camels are still serologically positive even after 5 years, may be lifelong.

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TISSUES AND ORGANS OF THE IMMUNE SYSTEM OF DROMEDARY CAMEL (*Camelus dromedarius*): A COMPARATIVE REVIEW

Saeed Y. Al-Ramadan
Department of Anatomy, College of Veterinary Medicine, King Faisal University, Saudi Arabia

ABSTRACT

The immune system is formed of sets of tissues and organs that have the ability to discern self-substances from non-self (antigen) substances and to destroy or inactivate the latter. This review presents a literary survey of what has been written on the subject of the histology of the immune system in dromedary camels and compared the structure of these tissue and organs to that of ruminants.

Key words: Camel, comparative, histology, immune, organs, system, tissue

Camelids, until now, considered as a case of evolutionary innovation, represent a new mammalian model particularly useful for understanding the role of diversity in the immune system function. Studies on selected aspects of camel immune system and immune responses has been done. The anatomical and histological details of the camel's immune organs have shown a structure which differs from other ruminants, in particular the thymus, tonsils, and peyer's patches (Al-Ramadan *et al,* 2021). Camels show strong resistance to many viral and microbial infections, including tetanus, foot-and-mouth disease and mad cow disease (or bovine spongiform encephalopathy) which is attributed to its special immune system (Jassim and Naji, 2001).

A compilation of research on camel immune system; determinates of innate immunity, cells, organs and tissues of immune system; antibodies; immunomodulation; histocompatibility; sero-prevalence, diagnosis and immunity against infections; application of camel immunoglobulins and applications of immune mechanisms in physiological processes is published (Gahlot *et al,* 2016).

Some research and review papers have been published recently on the camel's immune system (Al-Ramadan *et al,* 2021; Hussen and Schuberth, 2021). However, there is no organised compilation of the microscopic structure of all known tissues and organs of the immune system. Therefore, this paper is aimed to project an overview of the histological characteristics of different tissues and organs of the immune system of dromedary camel.

The tissues and organs of the immune system of dromedary camel are described as the central and peripheral lymphatic organs.

The Central Lymphatic Organs

These organs are referred to as the primary lymphoid organs where the lymphocytes develop and mature.

Bone Marrow:

The bone marrow is where pluripotent haematopoietic stem cells reside (Gurkan and Akkus, 2008). In addition to its function as a generator for blood cells, bone marrow represents the antigen independent phase of B-lymphocyte development in many animal species (Boes and Durham, 2017). Paradoxically, despite the importance of the marrow for immunity, the camel's bone marrow has not been studied in-depth compared to other species. The procedure goes through several complex steps, including decalcification of the bone, which has made many investigators reluctant to study the bone marrow *in situ* and use bone marrow aspiration instead. However, the bone marrow is formed of haematopoietic cells, fat cells, blood vessels, and a connective tissue framework. The haematopoietic cells occur in various stages of formation and maturation (Travlos, 2006; Samuelson, 2007; Boes and Durham, 2017). A network of sinusoidal capillaries permeates the myeloid tissue of bone. These blood sinusoids are wide, irregular vessels, the walls of which consist of fenestrated endothelium, thin basal lamina, and

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some scattered adventitia, allowing the newly formed cells to squeeze between the spaces to and from circulating blood. The framework of bone marrow is represented by reticular cells, osteogenic cells, and fat cells. The reticular cells are branched cells with large pale nuclei, while the osteogenic and fat cells have ordinary cell structure (Travlos, 2006; Samuelson, 2007).

Smears taken from bone marrow showed that the mean myeloid/erythroid ratio was 1.21. In percentiles, the mean erythroid percentage was 42.7%, and the mean myeloid percentage was 52.0%; the percentage for other cell types was 5.2% (Nazifi *et al,* 1998).

Thymus Gland:

Thymus gland originates from the 3rd and 4th pharyngeal pouches and descends to ventral part of the cervical region, thoracic inlet, and cranial mediastinum as a bilobed organ. The thymus gland started as an epithelial structure; during its ontogenesis, it was infiltrated with lymphocytes that originated in the bone marrow (Varga *et al,* 2011). As more lymphocytes accumulated in thymus, the epithelial cells became loosely arranged in the reticulum and are called epithelial-reticular or epithelioreticular cells; hence, the thymus gland is described as a lymphoepithelial organ (Roballo *et al,* 2019).

In dromedary camel, the thymus gland descended back and finally positioned itself at the ventral aspect of the neck and the thoracic cavity, at the anterior superior mediastinum in front of the heart and behind the sternum (Jarrar and Faye, 2013). It might extend to the caudal fourth segment of the neck, where it can be seen between the trachea and left external jugular vein (Smuts and Bezuidenhout, 1987; Al-Ramadan *et al,* 2021). In dromedary camel, a thin connective tissue capsule surrounds the thymus gland. Arising from the capsule, thin connective tissue trabeculae extend deeply into the parenchyma, dividing the lobes into incompletely separated lobules. The thymic lobules have irregular outlines, and each consists of an outer dark cortex and inner light medulla (Fig 1).

The cortex is highly populated by T-lymphocytes (thymocytes), rendering this peripheral part of the lobule darkly stained. In addition to T-lymphocytes, epithelioreticular cells could be detected in the camel's thymus gland. However, these cells were highly outnumbered by thymocytes, and they had a stellate-shape with extending cell processes (Jarrar and Faye, 2013). This histological appearance in the dromedary camel has also been reported in other domestic animals where the thymic lobule has a distinct medulla and cortex. The cortex has been described as densely packed with small lymphocytes, rendering the cortex darkly stained, while the medulla is occupied by fewer lymphocytes and is thus lightly stained (Aughey and Frye, 2001). Within the medulla, acidophilic bodies 20–100 µm in diameter could be detected. These structures are called thymic corpuscles and are composed of concentric whorls of epithelioreticular cells (Pearse, 2006; Samuelson, 2007). As with the camel, the epithelioreticular cells are irregular with large branching cytoplasmic processes. These processes connect to each other by means of desmosomes, forming a blood-thymus barrier (Aughey and Frye, 2001). Moreover, the epithelioreticular cells have large, oval, lightly stained nuclei (Banks, 1993). In cattle calves, the lobules are surrounded and interconnected by interlobular connective tissue that contains blood and lymph vessels (Gasisova *et al,* 2016). In camel, however, although a similar description has been mentioned, numerous granulated cells of different shapes and sizes were observed in the interlobular connective tissue and occasionally adjacent to the cortex (Ismail and Ali, 2015). According to morphology and function, two distinct types of lobules have been identified: (a) passive and (b) active; the active lobules are larger and have a clear demarcation between the cortex and medulla. In addition, they differ from each other by cell density and mitotic activity (Gasisova, 2016). No such description has been found in the camel except for the ordinary description of thymic lobules as having a dark cortex and lightly stained medulla (Ismail and Ali, 2015).

The Peripheral Lymphatic Organs

Mucosal Associated Lymphatic Tissue (MALT)

Sporadic unencapsulated lymphatic tissue:

These cells are scattered throughout the body, mainly in the connective tissues of mucous surfaces. Their presence is more noticeable in the organs of digestive, respiratory, and urogenital systems that are continually subjected to noxious agents. This lymphoid tissue appears as a rather loose aggregate of cells and shows no distinct demarcation from the surrounding tissue with which it gradually merges (Cesta, 2006). According to the anatomical location, several groups of MALT have been described (Casteleyn *et al,* 2011a; 2011b; Girgiri and Kumar

2020). The two groups most extensively described are bronchus-associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT). However, conjunctiva-associated lymphoid tissue (CALT) and teat-associated lymphoid tissue have also been described (Casteleyn *et al,* 2011a).

BALT

Bronchus associated lymphoid tissue is not a constitutive structure in all domestic animal species. While it is absent in dogs (Peeters *et al,* 2005), it has been reported in sheep (Liebler-Tenorio and Pabst, 2006; McNeilly *et al,* 2008), goats (Rodriguez *et al,* 2001; Choudhary and Das, 2019), cattle (Chase and Kaushik, 2019), and equines (Hannant, 2002; Liebler-Tenorio and Pabst, 2006). In this consideration, Patches of lymphocytic aggregations represent BALT in the camel are formed of lymphoid nodules that are scattered along the bronchial tree in the entire lung. These are of variable sizes and seen under the epithelial lining of bronchi and bronchioles in the lamina propria and submucosa (Fig 2) (Elhussieny *et al*, 2017).

Recently, Elhussieny and Zidan (2021) provided temporospatial characteristics of BALT in the dromedary camel. BALT of the extrapulmonary bronchi of young camels (younger than 2 years) was rarely detected. In middle-aged camels (from 3 to 7 years), however, BALT was represented by well-developed nodular and diffuse internodular lymphocytes, which involuted in camels older than 12 years to be in the form of a few diffusely distributed lymphocytes. BALT of intrapulmonary bronchi of young and middle-aged camels was very well developed and formed of organised lymph nodules and internodular lymphocytes. In older age camels, BALT was faded out, and only few lymphocytes were detected in the lamina propria (Fig 2) (Elhussieny and Zidan, 2021).

In this respect, BALT of the Bactrian camel is represented by isolated aggregates of lymphoid nodules in the bronchial tree. However, the density of BALT increased from trachea to the lower graded branches where most density was seen at bronchioles, which then decreased at the level of respiratory bronchioles (He *et al,* 2019). In bovines, both organised lymphoid nodules and unorganised aggregates of lymphocytes were seen in the bronchi as well (Liebler-Tenorio and Pabst, 2006). Whereas, BALT of young goats was represented by aggregations of lymphocytes that were seen in diffused form rather than nodular form (Choudhary and Das, 2019), BALT could not be detected in the neonatal goats (Barman *et al,* 1996). The authors suggested that the absence of any lymphoid tissue in the bronchial tree in the neonatal goats and the presence of it at later ages suggest postnatal evolution of BALT similar.

GALT

GALT comprises diffuse and isolated lymphoid nodules that are scattered throughout the intestinal tract (Cesta, 2006). However, in some locations, the nodules in the gut aggregate to form patches, which will be discussed in the section on nodular lymphatic tissue because of their immunological significance.

With the exception of pigs, gastric GALT is not very well developed as in other farm animals (Liebler-Tenorio and Pabst, 2006; Mazzoni *et al,* 2011). Gastric GALT in Bactrian camels has been described in several publications (Wang, 2003; Xu *et al,* 2010; Zhang *et al,* 2012a); in dromedary camels, the gastric lymphoid tissue was reported only in the 4th compartment of the stomach (Naser *et al,* 2011). The latter authors could detect several lymphoid nodules with massive cellular components at the pyloric region of the fourth compartment (Fig 3).

In this respect, the gastric GALT in Bactrian camel was located only in the cardiac glandular area of the 3rd stomach compartment, forming a long triangle-like region, and distributed along the ventral walls of the stomach neck, the beginning of the cranial enlargement, and along the lesser curvature, forming lymphoid nodules (Wang, 2003; Gahlot *et al,* 2016). However, Xu *et al* (2010) and Zhang *et al* (2012a) found that the number of these nodular aggregations changed with age, with peak at the age of puberty and decreasing as the animals aged. A large number of lymphocytes in the form of lymphoid aggregations were observed in torus pyloric region of the caprine abomasum (Mahesh *et al,* 2017).

CALT

In the camel, CALT was detected at the palpebral surface of the eyelids in the form of several lymphoid follicles. These follicles formed aggregates near the medial canthus (Al-Ramadan and Ali, 2012; Al-Ramadan, 2015) and morphologically appeared as basophilic patches of a single follicle or as a group of two or more nodules (Fig 4). CALT is a constant component of conjunctiva of several domesticated animals. The CALT is formed of solitary or aggregates of lymphoid nodules in Bactrian camel (Yang and Wang, 2015; Gahlot *et al,* 2016). In cattle, sheep, and pigs, isolated lymphoid nodules that were

predominantly localised at the palpebral surface of the conjunctiva represent the CALT (Chodosh *et al,* 1998.

Nodular lymphatic tissue:

Populations of lymphatic nodules are organised into compact, somewhat spheroidal or oval structures of MALT. It may occur anywhere in connective tissue but is prominent along the digestive and respiratory tracts. In mammals, some of these nodule aggregations at locations such as pharynx are called tonsils, and in the small intestine called Peyer's patches or intestinal tonsils (Banks, 1993; Liebler-Tenorio and Pabst, 2006; Samuelson, 2007).

Tonsils:

Tonsils are aggregates of lymphatic nodules at the entrance of digestive and respiratory systems. The surface facing the cavity is covered by irregular epithelium, and underneath these are partially encapsulated with dense connective tissue. The epithelial irregularities could be in the form of crypts or sinuses, resulting in two classes of tonsils: (a) tonsils with crypts, which have deep invagination of the surface epithelium, and (b) tonsils without crypts (Banks, 1993; Casteleyn *et al,* 2011b). Further classification based on the anatomical locations of tonsils has classified the tonsils into three groups: (a) oropharynx tonsils, (b) nasopharynx tonsils, and (c) laryngopharynx tonsils. The oropharynx group is formed of lingual, palatine, and velar (soft palate) tonsils; the nasopharynx tonsils are subdivided into the pharyngeal and tubal tonsils; and the laryngopharynx type is formed of the paraepiglottic tonsil only (Cocquyt, 2008; Perry and Whyte, 1998). In a comprehensive study using the dromedary camel, Achaaban *et al* (2016) described tonsils within each of the 3 groups.

Oropharynx group:

A. *Palatine tonsil:* This tonsil could be described as comprising spherical masses located within a tonsillar fossulae and extending between the palatoglossal and palatopharyngeal arches. Microscopically, these spherical masses consist of a large number of lymphatic cells arranged into heavily cellular ovoid or spherical nodules and internodular areas. The mucous membrane covering the palatine tonsil is formed of a thick layer of nonkeratinised stratified squamous epithelium. As shown in Fig 5, the epithelium is reflected inside the tonsil, forming a blind-ended crypt that is usually infiltrated with lymphocytes to form reticular epithelium (Zidan and Pabst, 2009; Achaaban *et al,* 2016; Al-Ramadan and Alluwaimi, 2018). In the Bactrian camel, this tonsil is very similar to that of the dromedary camel, except that the crypt in the Bactrian camel is often branched with wide lumen (Yang *et al,* 2011). The palatine tonsil of cattle is formed of a large ovoid lymphoid structure with a central sinus, giving the tonsil a bean-shaped appearance with an entrance visible at the oropharyngeal surface (Effat and Milad, 2007; Casteleyn *et al,* 2011a). Opening into this tonsillar sinus are numerous crypts in the center between the tonsillar nodules. More than one sinus could be detected in the palatine tonsil of bovines (Casteleyn *et al,* 2011a). Similar to that in camel, the crypt in bovines comprises nonkeratinised stratified squamous epithelium and is often infiltrated by lymphoid cells (Velinova *et al,* 2001; Liebler-Tenorio and Pabst, 2006; Palmer *et al,* 2009; 2011). The palatine tonsil of sheep is a hazelnut-sized ovoid structure that could be detected at both sides of the oropharynx. This tonsil has 1 to 3 entrances between the palatoglossal and palatopharyngeal arches. Each one of the entrances leads to the underlying crypts. In turn, these crypts are centrally located between the lymphoid nodules (Cocquyt *et al,* 2005; Casteleyn *et al,* 2011a). Within the crypts is overlying nonkeratinised stratified squamous epithelium. The epithelium is irregularly modified into a reticular epithelium as a result of heavy infiltration by lymphoid cells (Casteleyn *et al,* 2011a). In goats, the palatine tonsil is larger than that in sheep. It is easily detected at the lateral oropharyngeal wall. Moreover, it is characterised by few crypt openings leading to large diverticula (Indu *et al,* 2018). The lymphoid tissue of goat tonsils is formed of many secondary follicles and interfollicular lymphoid tissue. The tonsil is covered by nonkeratinised stratified squamous epithelium, and the epithelium that lines the crypt is transformed into follicle-associated epithelium, which is characterised by the absence of goblet cells and cilia, a reduced number of cell layers, and infiltration of a large number of lymphocytes (Casteleyn *et al,* 2011a).

B. *Lingual tonsil:* This tonsil is located at the root of the tongue and made up of a cluster of spheroidal lymphoid masses which protrude into the oropharynx. Achaaban *et al* (2016) and Zidan and Pabst (2020) agreed that the lingual tonsil in dromedary camel is visible at the root of the tongue as several spherical macroscopic nodules protruding into the oropharynx; however, they disagreed about the presence of the crypt of this tonsil. Achaaban

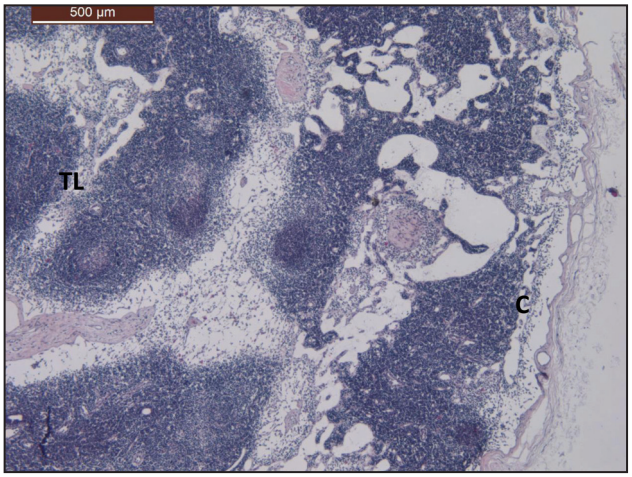

Fig 1. Thymus gland of dromedary camel showing capsule (C), Thymic lobule (TL) (H&E). Bar= 500µm.

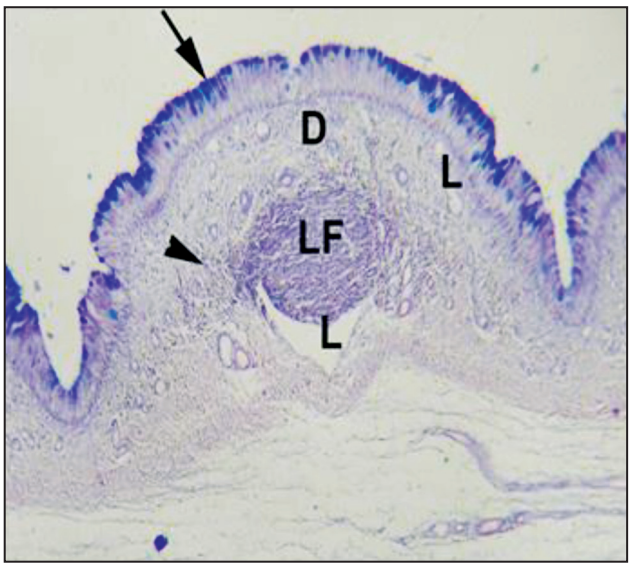

Fig 2. The BALT of the extrapulmonary bronchus is localised in dome like area (D) and formed from lymphoid follicle (LF) and diffused lymphocytes (arrowhead). The covering epithelium (arrow) is rich in goblet cells. L= lymphatics. Alcian blue- PAS stain. (El-hussainy *et al,* 2017).

et al (2016) mentioned that the crypt is not clearly visible in the lingual tonsil of the dromedary camel, but Zidan and Pabst (2020) described more than one crypt in the dromedary camel's lingual tonsils. Microscopically, lingual tonsil dromedary camel consisted of a cluster of lymphoid nodules and internodular tissue. These nodules were covered by a keratinised stratified squamous epithelium continuous with similar epithelium of the lingual surface (Fig 5). In this respect, Yang *et al* (2011) found that lingual tonsil of the Bactrian camel is formed of aggregations of lymphoid cells at the dorsum of the tongue, extending 2 cm rostral to last vallate papilla on each side. In bovines, the lingual tonsil is

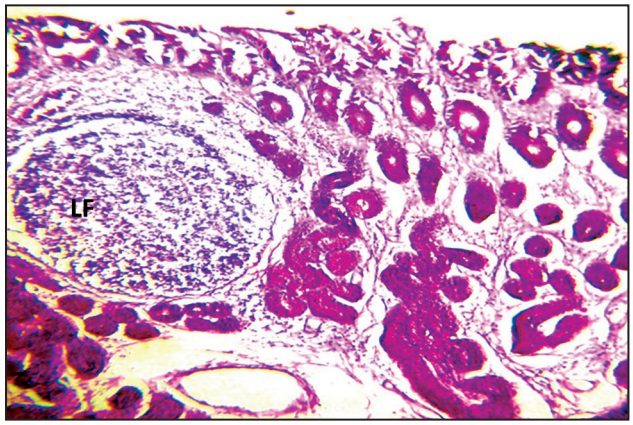

Fig 3. GALT, Lymphoid Follicle (LF) at the submucosa of the fourth compartment of camel stomach. (PAS stain). (Naser *et al,* 2011).

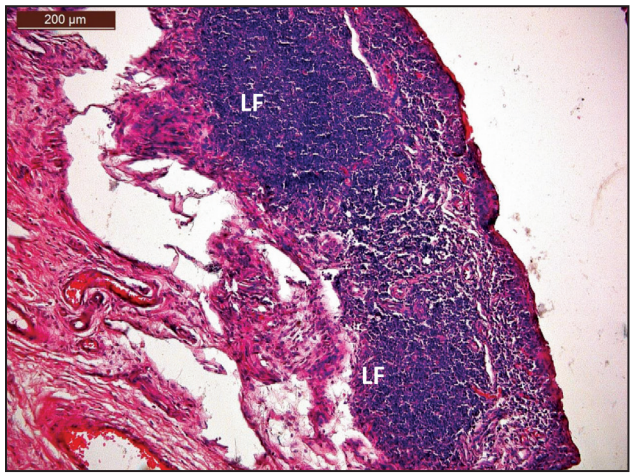

Fig 4. Conjunctiva of camel showing lymphoid follicles (LF) in the lamina propria. (H&E; bar = 200 µm). (Al-Ramadan, 2015).

represented by macroscopic rows of tonsillar fossules at the dorsolateral surface of the tongue's root, caudal to the circumvallate papillae (Liebler-Tenorio and Pabst, 2006; Cocquyt *et al,* 2008; Breugelmans *et al,* 2011; Casteleyn *et al,* 2011a). Moreover, the lingual tonsil of cattle is organised in the form of lymphoid nodules and internodular regions around crypts (Breugelmans *et al,* 2011). However, some aggregations of lingual lymph nodules without crypts have also been detected in cattle (Cocquyt *et al,* 2008). The crypt is covered by keratinised stratified squamous epithelium, which is often infiltrated by lymphoid cells (Casteleyn *et al,* 2011a). In contrast to camels and cattle, the lingual tonsil of sheep is not grossly visible, but small aggregates of lymphoid tissue could be detected at both sides of the dorsal part of the tongue (Cocquyt *et al,* 2005). In addition, Casteleyn *et al* (2011a) described some lymphoid tissue dispersed within the core of the vallate papillae

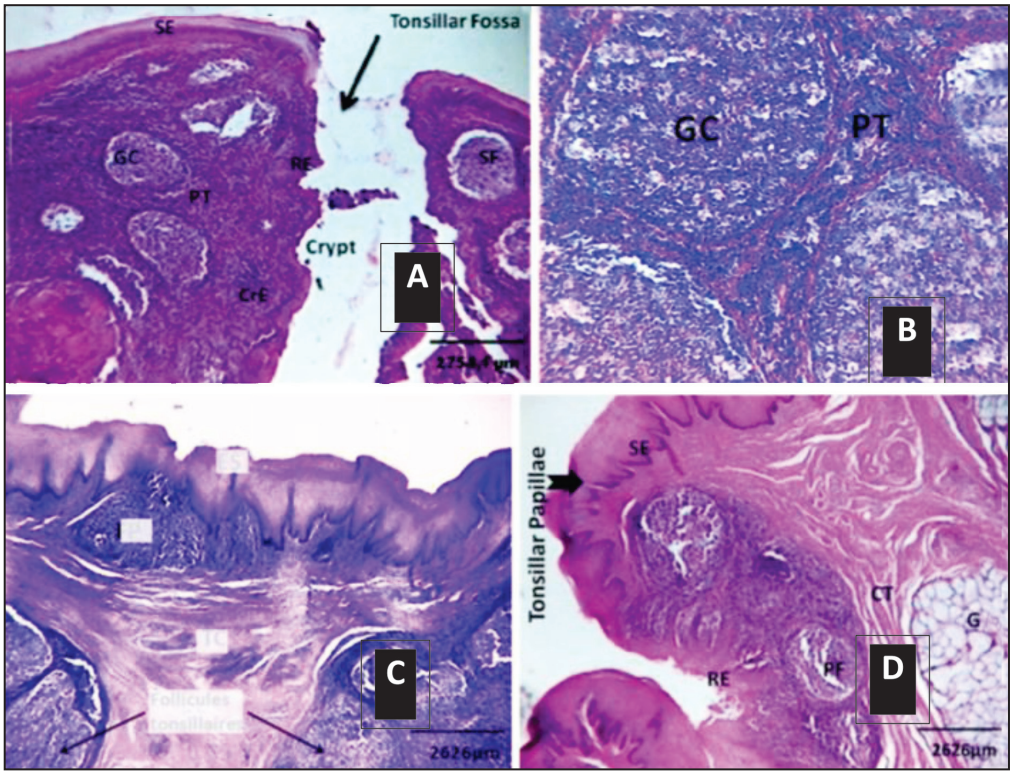

Fig 5. Tonsils of camel: (A & B) palatine tonsil; (C&D) Lingual tonsil: showing squamous epithelium (SE), primary follicle (PF), secondary follicle (SF), parafollicular tissue (PT), germinal center (GC), lingual gland (G), reticular epithelium (RE), connective tissue (CT), and crypt epithelium (CrE). (H&E) (Achaaban *et al*, 2016).

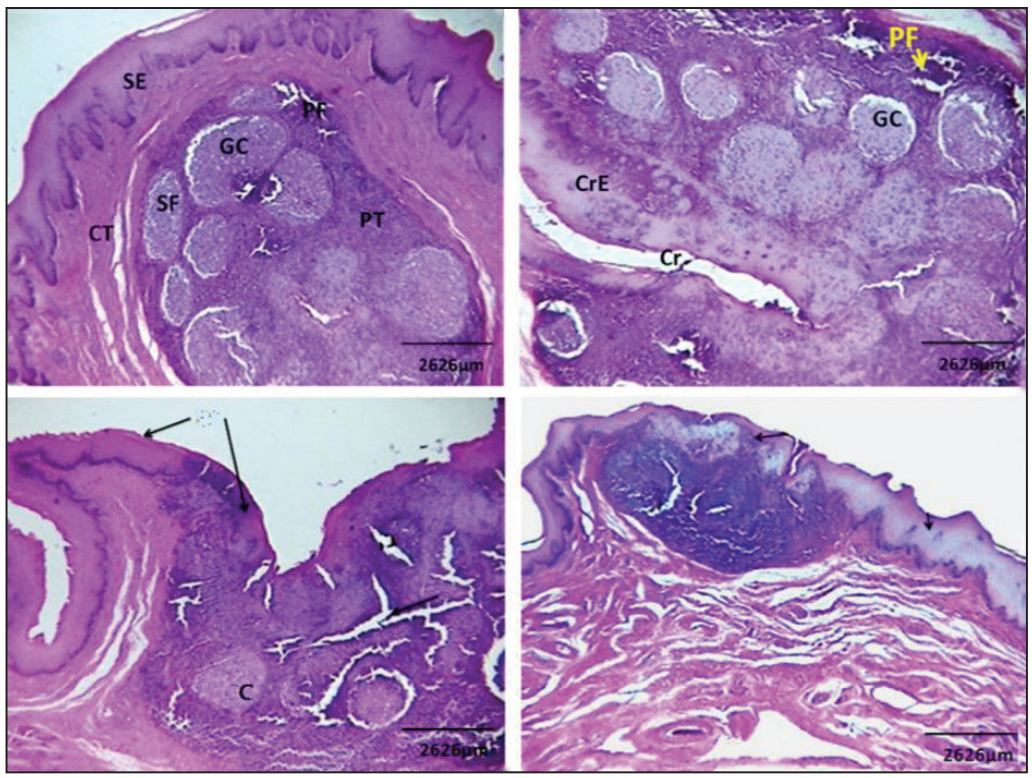

Fig 6. Velar tonsil of camel showing squamous epithelium (SE), primary follicle (PF), secondary follicle (SF), parafollicular tissue (PT), germinal centre (GC), lingual gland (G), connective tissue (CT), crypt (Cr) and crypt epithelium (CrE). (H&E) (Achaaban *et al*, 2016).

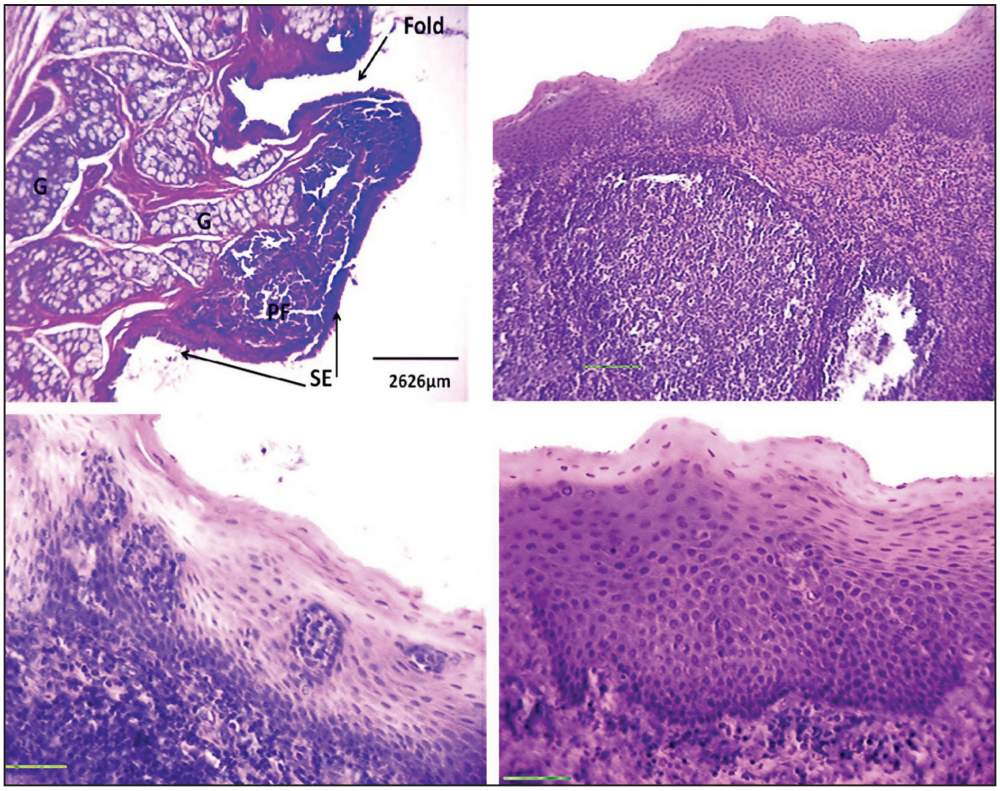

Fig 7. Tonsils of camel: Pharyngeal tonsil of camel showing squamous epithelium (SE), primary follicle (PF), secondary follicle (SF), parafollicular tissue (PT), pharyngeal gland (G). (H&E) (Achaaban *et al*, 2016).

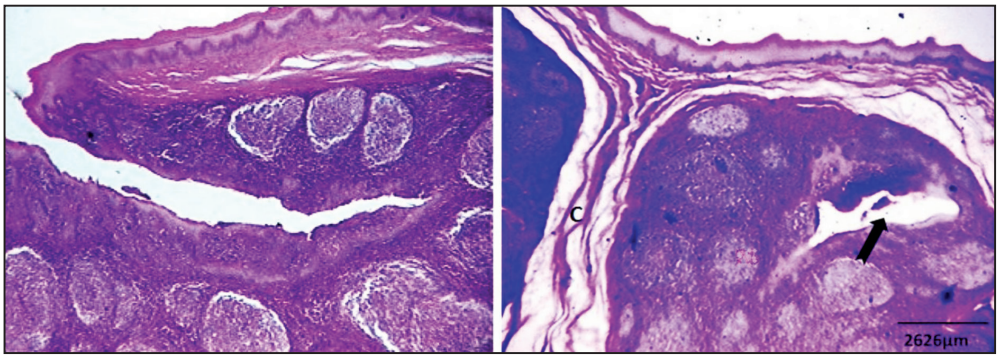

Fig 8. Tonsils of camel: Paraepiglotic tonsil of camel showing squamous epithelium (SE), secondary follicle (SF), parafollicular tissue (PT), reticular epithelium (RE), crypt fossulae (arrow). (H&E) (Achaaban *et al*, 2016).

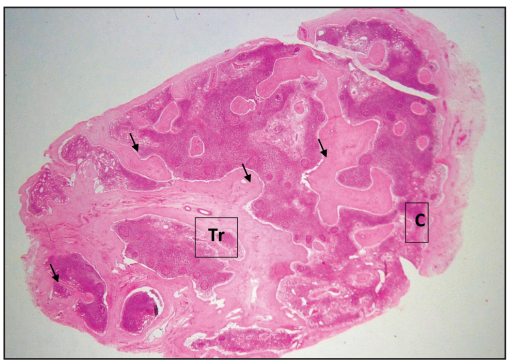

Fig 9. Lymph node of camel showing capsule (C), Trabeculae (Tr) lymphoid nodules (arrows). Dissecting microscope (H&E).

and the location of the caprine lingual tonsil at the root of the tongue disseminated from just underneath the lingual epithelium through the lingual muscle. Some lymphoid tissue was detected with connective tissue core of the vallate papillae (Casteleyn *et al*, 2011a). However, the conventional description of the tonsil is not applicable to lingual tonsil of the small ruminants that have only small aggregations of lymphoid cells that do not form lymph nodules (Casteleyn *et al*, 2011b).

C. *Velar (soft palate) tonsil:* This tonsil is located at the oropharyngeal (oral) surface of the soft palate. It is found more densely in area close

to the junction with hard palate (Achaaban *et al,* 2016). Microscopically, the velar tonsil is formed of small disseminated nodules. These nodules could be differentiated into primary (without a germinal center) and secondary (with a germinal center). The lymphoid nodules are separated by paranodular lymphoid tissue and are distributed within the connective tissue of the soft palate. Moreover, the velar tonsil is covered by keratinised stratified squamous epithelium (Achaaban *et al,* 2013; Achaaban *et al,* 2016). A crypt lined with a nonkeratinised stratified squamous epithelium has been described in this tonsil as well (Fig 6). The crypt is infiltrated with some lymphoid cells (Achaaban *et al,* 2016). In contrast to the dromedary camel, the Bactrian camel showed no macroscopic signs of velar tonsil at the oral surface of the soft palate, although it was visible after treating the soft palate with acetic acid on the aboral (nasopharyngeal) surface (Yang *et al,* 2011). Accordingly tonsil is formed of scattered lymphoid nodules that might number up to 150. In cattle, diffuse primary and secondary lymphoid follicles and interfollicular lymphoid tissue represent the velar tonsil. Similar to in the Bactrian camel, these lymphoid structures are located on the aboral surface of the soft palate underneath the respiratory epithelium (Casteleyn *et al,* 2011a). In sheep, the lymphoid tissue of the velar tonsil is unequally disseminated on the subepithelial tissue of the oral and aboral surfaces of the soft palate. While the lymphoid cells form dense aggregations on the aboral surface of the soft palate, the velar is represented by very few lymphoid cells on the oral surface (Casteleyn *et al,* 2007; Girish *et al,* 2020). Similar to cattle, the caprine velar tonsil was detected on the aboral surface of the soft palate only, where it presented in the form of primary or secondary lymphoid nodules; no aggregation of lymphoid tissue has been detected on the oral surface (Casteleyn *et al,* 2011a; Indu and Lucy, 2016).

The nasopharynx group

A. *Pharyngeal tonsil:* In the dromedary camel, this tonsil is located within the median part of the dorsal wall of nasopharynx behind the nasal septum. The tissues of this tonsil are arranged as a set of epithelial foldings, which are more like crypts. Histologically, this tonsil is formed of lymphoid nodules with diffuse lymphocytes in-between. Keratinised stratified squamous epithelium is present over this tonsil (Fig 7) (Achaaban *et al,* 2016). Likewise, the pharyngeal tonsil of the Bactrian camel is represented by a large number of primary and secondary lymphoid nodules on the caudal part of the pharyngeal septum. Over this tonsil in the Bactrian camel is folded keratinised stratified epithelium (Yang *et al,* 2011). Conversely, Casteleyn *et al* (2011a) mentioned that the pharyngeal tonsil in bovines is large, and the mucous membrane that covers this tonsil forms several folds and sometimes forms crypts. Microscopically, the lymphoid tissue consists mainly of secondary lymphoid nodules separated by internodular lymphoid tissue. This lymphoid tissue is organised around a central connective tissue core. The pseudostratified columnar ciliated epithelium is often reticulated because of infiltration by lymphocytes (Casteleyn *et al,* 2011a). Among the other tonsils, the pharyngeal tonsil is considered to be the largest tonsil in sheep (Breugelmans *et al,* 2011; Girish *et al,* 2020). It is formed of numerous lymphoid nodules and a large internodular zone and is covered by pseudostratified columnar ciliated epithelium arranged in longitudinal folds (Breugelmans *et al,* 2011; Casteleyn *et al,* 2011a). Similar to in bovines, deeper within the sheep pharyngeal tonsil, there is a connective tissue core rich in blood and lymph vessels (Casteleyn *et al,* 2011a). In this respect, the caprine pharyngeal tonsil is similar to that of sheep in terms of location and structure (Casteleyn *et al,* 2011a).

B. *Tubal tonsil:* This tonsil could be detected at both lateral walls of the nasopharynx, close to the opening of the auditory tube. Dorsally, tubal tonsil continues with the pharyngeal tonsil (Achaaban *et al,* 2016). Histologically, it is formed of lymphoid nodules, which could be detected beneath the folding squamous epithelium, similar to that observed in the pharyngeal tonsil, while the nonnodular part was arranged within the epithelial folds of nasopharynx (Achaaban *et al,* 2016). As a result of its location, the tubal tonsil is often confused with the pharyngeal tonsil (Achaaban *et al,* 2016), which might be the reason that tubal tonsil was not detected in the Bactrian camel, as mentioned by Yang *et al* (2011). Similar to the dromedary camel, the tubal tonsil in cattle is macroscopically distinguishable and microscopically consisted of primary and secondary lymphoid nodules and internodular lymphoid tissue (Casteleyn *et al,* 2011a). Only after 4 hours fixation could this tonsil be seen in sheep (Casteleyn *et al,* 2011a). However, other authors have mentioned that the nasopharyngeal tonsil consists of a raised mass covered with irregular epithelium that is deeply furrowed, but they did not specified whether it was pharyngeal or tubal tonsil (Kumar and Nagpal, 2007). Microscopically, the lymphoid tissue of the tubal

tonsil was scattered and varied from aggregated lymphoid cells to primary and secondary lymphoid follicles (Casteleyn *et al,* 2011a). Similar to sheep, this tonsil is not visible in fresh specimens from goats; however, primary and secondary lymphoid follicles and diffuse lymphoid tissue containing scattered lymphoid cells were observed on histological examination (Casteleyn *et al,* 2011a; Indu *et al,* 2015).

Laryngopharynx group

Paraepiglotic tonsil: In dromedary camels, this tonsil is situated on both sides at the base of the epiglottis. It forms circular clustered lymphoid nodules with crypt. This tonsil is formed histologically of smaller aggregates of lymphoid nodules molded together (Fig 8), constituting a very compact tonsil (Yang *et al,* 2010; Achaaban *et al,* 2016). In the Bactrian camel, this tonsil is readily located at a similar location as that of the dromedary camel. Yang *et al* (2010; 2011) mentioned that the paraepiglotic tonsil consisted mainly of secondary lymphoid nodules encapsulated in dense connective tissue. Each tonsil formed of up to 3 to 8 nodules shared 2 to 3 entrances which leads to crypts. These lymphoid tissue might serve as a connection between tonsil and BALT (Yang and Wang, 2013). In cattle, this tonsil could not be detected before immersing the laryngeal tissue in 2% acetic acid for 24 hours (Casteleyn *et al,* 2008a). However, a few lymphoid nodules could be detected microscopically that did not form aggregates. In sheep, the paraepiglotic tonsil has a similar location to that in the dromedary camel, and the nodular tissue of this tonsil could be seen as mucosal elevations separated by deep invaginations. Microscopically, it consisted of dense aggregations of lymphoid cells and primary and secondary lymphoid nodules (Casteleyn *et al,* 2011a). In contrast to sheep, this tonsil was found only in a minority of goats at the microscopic level. When it was present, it contained primary and secondary lymphoid nodules separated by internodular regions (Casteleyn *et al,* 2011a).

Peyer's Patches:

Peyer's patches (PP), also called intestinal tonsils, are aggregations of lymphoid nodules located along the antimesenteric side of the small intestine. The number, size, and distribution of PP vary according to species. In term of PP distribution, animals have been categorised into 2 groups. Group 1 includes species in which the PP are distributed all over the intestine in an unequal manner, and PP are much more numerous in ileum than in any parts of the intestine. This group includes cattle, sheep, goats, horses, pigs, and dogs. In Group 2, however, the PP are equally distributed between the jejunum and ileum, as in rabbits and rodents (Alluwaimi *et al,* 1998). In the dromedary camel, the presence of PP is confined to the ileum only (Zidan and Pabst, 2008). However, isolated follicles could be detected microscopically, which might compensate the function of jejunal PP in other species. Brandtzaeg and Pabst, 2004 and Zidan and Pabst, 2008). In this respect, PP of the Bactrian camel were detected in 3 parts of the small intestines and extended behind down to the colon (Qi *et al,* 2011; Zhaxi *et al,* 2014).

In young dromedary camels, the PP were cup-shaped masses elevated about one cm above the luminal surface. Each of these masses was formed as an aggregate of lymphoid nodules located in the submucosa, some of which extended deeply to the lamina propria. These nodules were distributed around the lateral borders and the bottom of the patches (Alluwaimi *et al,* 1998). The number of nodules per patch ranged between 25 and 27 in the cranial portion and 31 to 38 in the caudal portion. The diameters of nodules ranged between 500 and 900 µm. In adult dromedary camel, the number and size of PP were less than that of young camels (Alluwaimi *et al,* 1998). However, in a later study, Zidan and Pabst (2008) could not find any age-related variation. As is the case with other domestic species, the mucosa covering the PP was devoid of any intestinal villi (Alluwaimi *et al,* 1998; Soni *et al,* 2006).

In the Bactrian camel, four different types of PP have been described: (a) nodular, (b) faviform, (c) cup-shaped, and (d) cystic form (Qi *et al,* 2011). The nodular type has been detected in the duodenum. The faviform-shaped PP were located at the jejunum and first part of the ileum. The cup-shaped PP were mainly distributed in the mid-portion of ileum. The cystic form PP were distributed in the distal portion of the ileum (Qi *et al,* 2011).

According to Yasuda *et al* (2002) and Beyaz and Asti (2004), two types of PP were recognised in small intestine of cattle: (a) the jejunal (JPP) and (b) the ileal (IPP). In bovines, JPP were represented by several patches that could be seen along the antimesenteric side of the jejunum, while the IPP were represented by a single long patch that might reach up to 3 metres (Liebler-Tenorio and Pabst, 2006). The lymphoid nodules of JPP were small and pear-shaped with extensive internodular areas and domes. The IPP, however, had long, oval lymphoid nodules and narrow internodular areas (Liebler-Tenorio and Pabst, 2006). A similar distribution and histological

appearance to that of cattle were reported in small ruminants (Liebler-Tenorio and Pabst, 2006).

The lymph nodes:

The lymphatic centers:

The lymph nodes are grouped into lymph centers that constantly occur in same region of the body. The centres of lymph nodes in the dromedary camel could be topographically grouped as follows: (a) lymph nodes of the head, including parotid, the mandibular, and retropharyngeal lymph centers; (b) lymph nodes of the neck, including superficial and deep cervical lymph centers; (c) lymph nodes of front limb, including axillary lymph center; (d) lymph nodes of thorax, including dorsal thoracic, ventral thoracic, mediastinal and bronchial lymph centers; (e) lymph nodes of abdomen, including lumbar, coeliac, and cranial mesenteric and caudal mesenteric lymph centers; and (f) lymph nodes of pelvic regin and hind limb, including iliosacral, iliofemoral, inguinofemoral, ischiatic, and popliteal lymph centres (Smuts and Bezuidenhout, 1987).

Histology of lymph nodes:

Stroma

The nodes are encapsulated oval lymphoid structures designated to filter lymph that originates in the interstitial spaces of most of the body's tissues. Each lymph node has afferent vessels penetrating the convex capsular surface and is drained by an efferent lymph vessel emerging from a depression (hilum) along with blood vessels (Samuelson, 2007). Traditionally, histology of lymph nodes could be described as having three compartments: (a) cortex, (b) paracortex, and (c) medulla (Willard-Mack, 2006; Jalkanen and Salmi, 2020). Outside the lymph nodes is a collagenous capsule from which fibrous connective tissue trabeculae extends and divides the subcapsular area into several cortical compartments (lobules). These trabeculae branch and anastomose at the medulla. A network of reticular fibres supports the cellular parenchyma of the lymph node (Willard-Mack, 2006; Jalkanen and Salmi, 2020).

With regard to stroma of the lymph node, there have been various descriptions of the capsule in dromedary lymph node. Zidan and Pabst (2012) mentioned that the capsule is formed of two layers; an outer thicker layer of connective tissue and an inner thinner layer of mainly smooth muscles. Abdel-Magied *et al* (2001) mentioned that the presence of smooth muscle in the capsule was not detected in all examined animals. Later publications mentioned that the nodular capsule is formed of a single layer which is made up of dense, fibrous connective tissue with collagen, a small amount of elastic and reticular fibres, and smooth muscle cells (Gavrylin *et al,* 2013; Rahmoun *et al,* 2020). In sheep and goats, however, no smooth muscle layer was described, and the capsule was formed of fibroelastic connective tissue composed of collagen, elastic and reticular fibres (Senthilkumar *et al,* 2019). While in the cattle, lymph node capsule composed of connective tissue with interspersed smooth muscle cells from which thin trabeculae extended into the cortex (Casteleyn *et al,* 2008b).

From the capsule, the trabeculae extend toward center of the lymph node, dividing parenchyma characteristically into incomplete lobules (Soliman and Mazher, 2005; Zidan and Pabst, 2012). In this respect, two types of trabeculae have been mentioned in dromedary camel: (a) Type I, formed of two layers of connective tissue and muscle in which branches of blood and lymphatic vessels are located, and (b) Type II, formed of smooth muscles only (Garvilin *et al,* 2017).

In the dromedary camel, the lymph node receives one or two large afferent lymphatics that penetrate the capsule. In some camels, these may extend to the connective tissue trabeculae and be drained by 4 or 5 efferent lymphatics (Abdel-Magied *et al,* 2001; Gavrylin *et al,* 2013).

Parenchyma:

The conventional description of parenchyma of the dromedary camel's lymph node as being composed of an outer cortex and inner medulla is not applicable to lymph nodes of other farm animals (Samuelson, 2007). The parenchyma in dromedary camel is composed of lymph nodules that are regularly distributed in the parenchyma, and in between them is a nodular, dense lymphoid tissue and diffuse lymphoid tissue (Fig 9) (Abdel-Magied *et al,* 2001; Soliman and Mazher, 2005; Zidan and Pabst, 2012; Garvilin *et al,* 2017; Rahmoun *et al,* 2020). The parenchyma of lymph node in Bactrian camel is composed of lymphatic nodules, dense anodular lymphoid tissue and diffuse lymphoid tissues (Ye *et al,* 2014; Gahlot *et al,* 2016).

Spleen

Among lymphatic organs of domestic animals, spleen is the largest (Zidan *et al,* 2000a; Samuelson, 2007). In the dromedary camel, it is crescent-shaped and is located at the dorsocaudal aspect of rumen

and extend to the greater omentum (Smuts and Bezuidenhout, 1987; Mohamed, 2020). The spleen is supplied by splenic artery that arises from the coeliac trunk and penetrate spleen at different points of the dorsal end to the middle of the caudal margin (Nawal and Maher, 2018). The splenic artery then branched into cranial and caudal branches in most of the cases, however, middle branch is not uncommon (Radmehr, 1997).

The spleen is surrounded by a thick connective tissue capsule covered externally by mesothelial cells (Alshammary, 2010). The capsule could be easily subdivided into outer and inner layers; the outer layer was composed of connective tissue, including collagen, elastic fibres, and fibroblasts in addition to a few smooth muscle cells whereas, the inner layer was composed mainly of smooth muscles supported by connective tissues (Alshammary, 2010; Maina *et al,* 2014). The trabeculae, either vascular or avascular, extended from capsule to the parenchyma. The vascular trabeculae contained nerve fibres and arteries without veins, but the avascular trabecvlene could be further subdivided into primary and secondary trabeculae. The primary trabeculae were composed mainly of smooth muscle cells supported by reticular, collagen, and elastic fibres (Zidan *et al,* 2000b; Maina *et al,* 2014). The secondary trabeculae were formed of smooth muscle cells with reticular fibres among them (Zidan *et al,* 2000b).

As with other domestic species, the parenchyma of spleen in the dromedary camel is formed of white and red pulps. The white pulp is formed of periarterial lymphatic sheath (PALS) and lymphoid nodules (Zidan *et al,* 2000b). The lymphoid nodules are spherical and sometimes indented on one side. These nodules consist mainly of B-lymphocytes; hence, they represent the B-dependent zone in the spleen (Zidan *et al,* 2000b; Maina *et al,* 2014). The PALS contains 3 to 4 arteries surrounded with T-lymphocytes and is therefore, considered as the T-dependent zone (Zidan *et al,* 2000b).

The red pulp represented by subcapsular blood sinuses which are connected to the peritrabecular sinuses. Furthermore, the red pulp is divided by secondary trabeculae resulting into smaller compartments, the splenic cords. Each cord formed of a reticular network that contains different blood cells (Zidan *at el,* 2000b).

To the best of the author knowledge, no morphological and histological study has been performed on the spleen of the Bactrian camel. In the previous literature, domestic ruminants have similar structural components, in terms of spleen stroma and parenchyma, as that of the dromedary camel (Samuelson, 2007; Suri *et al,* 2017; Gnanadevi *et al,* 2019).

Haemal Nodes

Haemal nodes are encapsulated haematopoietic and lymphoid organs that have been reported in some mammals such as humans, rats and ruminants (Zhang *et al,* 2012b). They were described as spleen-like structures located retroperitoneally along the vertebral column (Banks, 1993). In camels, the hemal nodes are brown to dark-red and are spherical or kidney-shaped embedded in the fat of the abdominal and pelvic cavities. Their size varied from 2 to 12 mm in diameter (Zidan and Pabst, 2004). However, Hussin (2016) described their shape as conical with convex base and apex. Histologically, each node consisted of a capsule enclosing the parenchyma. They may have more than one hilum where arteries and nerves enter and veins and lymphatics leave the node. The parenchyma of the haemal node is composed of a cortex and a medulla supported by reticular fibres. The cortex is formed of lymphoid nodules and diffuse internodular lymphocytes (Zidan and Pabst, 2004; Hussin, 2016). Interestingly, Zidan and Pabst (2002) detected several apoptotic cells within the germinal centers of the lymphoid nodules.

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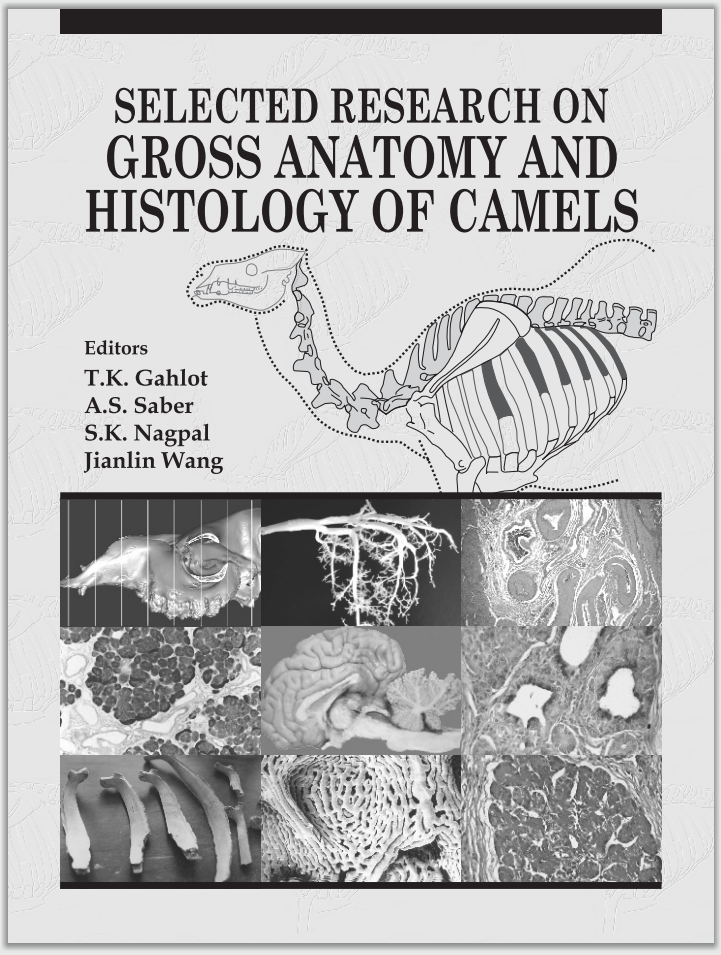

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UPREGULATION OF CORONAVIRUS (MERS-COV) RECEPTOR DIPEPTIDYL PEPTIDASE 4 ON CAMEL LEUKOCYTES AFTER BACTERIAL STIMULATION *IN VITRO*

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ABSTRACT

Middle East respiratory syndrome coronavirus (MERS-CoV) is an emerging zoonotic pathogen which causes high mortality rate in humans. Dromedary camels may play a central role in virus transmission to humans. Dipeptidyl peptidase-4 (DPP4), a transmembrane protein located on the cell surface of many epithelial and endothelial tissues was identified as the receptor for MERS-CoV. The current study investigated the possibility that bacterial stimulation of camel blood could affect the expression level of DPP4 on camel leukocyte subpopulation, which in turn may contribute to the higher susceptibility of camels with bacterial infection to MERS-CoV infection. DPP4 expression was evaluated by membrane immunofluorescence and flow cytometry. Stimulation of camel blood with the bacterial species *S. aureus* or *E. coli* resulted in the upregulation of DPPV on both monocytes and granulocytes, while *S. agalactiae* did not significantly modulate DPPV expression on either of the immune cells ($p > 0.05$). None of the bacterial species could induce a change in DPPV expression on lymphocytes from stimulated blood. Collectively, the present study showed an enhancing effect of bacterial stimulation on DPPV expression on camel monocytes and granulocytes.

Key words: Bacterial stimulation, coronavirus, dipeptidyl peptidase 4, leukocytes, MERS-CoV, Middle East respiratory syndrome

According to recent studies, dromedary camels play a pivotal role in Middle East Respiratory Syndrome Coronavirus (MERS-CoV) transmission to humans (Reusken *et al,* 2013; Adney *et al,* 2014; Reusken *et al,* 2014a; 2014b; Gossner *et al,* 2016; Haagmans *et al,* 2016; Harcourt *et al,* 2018; Te *et al,* 2022a). Members of the camelids family are currently discussed as a research model to study the pathogenesis of the MERS-CoV in human (Gultom *et al,* 2022; Te *et al,* 2022a; 2022b).

Dipeptidyl peptidase-4 (DPP4), which is also called CD26, is a transmembrane protein located on the cell surface of many epithelial and endothelial tissues in several organs such as kidneys, lungs, liver, and intestine (Lambeir *et al,* 2003; Klemann *et al,* 2016). Its expression levels and activity were found to be altered in several diseases, including inflammatory and infectious diseases and tumours (Hildebrandt *et al,* 2000; Dang and Morimoto, 2002; Klemann *et al,* 2016).

As the Middle East respiratory syndrome-coronavirus (MERS-CoV) emerged in 2012 (Zaki *et al,* 2012), DPP4 was identified as its receptor (Raj *et al,* 2013). After binding of the spike (S) protein of the virus on DPP4, virus entry is initiated by receptor mediated endocytosis (Lu *et al,* 2013; Wang *et al,* 2013). The expression level of CD26 on the surface of cells of respiratory tract has been found to be correlated positively with susceptibility to MERS-CoV infection (Cai *et al,* 2014; Meyerholz *et al,* 2016). CD26 Monoclonal Antibody was also able to inhibit MERS-CoV infection *in vitro* (Ohnuma *et al,* 2013)

In the current study, we investigated the upregulation of corona virus (MERS-CoV) receptor DPP4 on camel leukocyte subpopulation after bacterial stimulation *in vitro*.

Materials and Methods

Animals and blood sampling

Blood samples were collected from 3 male and 3 female dromedary camels (*Camelus dromedarius*) aged between 9 and 11 housed at the Camel Research Centre, King Faisal University, Al-Ahsa, Saudi Arabia. All animals were fed on hay and barley in addition to a mineral supplement. Water was available *ad libitum*. Blood was obtained by jugular venipuncture into

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vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany). Collected blood samples were stimulated *ex vivo*.

Bacterial stimulation of camel blood samples

Whole camel blood was stimulated with *S. aureus*, *E. coli*, or *S. agalactiae*. Whole blood (1 ml) was diluted with 0.9 ml medium (RPMI-1640, Sigma-Aldrich, Deisenhofen, Germany) in sterile 12x75 mm glass tubes (BD Biosciences, San Jose, California, USA) (Hussen, 2021). Live bacterial suspension (0.1 ml; 10^7 bacteria/ml) was added to the diluted blood and the mixture was then incubated for 6 h at 37°C. A negative control tube containing 1 ml blood and 1 ml medium without bacteria was also included. After incubation, the tubes were then put on ice and immediately centrifuged at 4°C for 10 min at 1000xg. After removing the supernatant, the cell pellet was suspended in PBS.

Leukocytes separation from camel blood

Separation of blood leukocytes was performed after hypotonic lysis of blood erythrocytes (Hussen *et al,* 2017; Hussen *et al,* 2021). After centrifugation at 500 × g for 10 min (4°C) with break, the cell pellet was resuspended. The erythrolysis was repeated (usually twice) until the complete removal of red blood cells. Subsequently, the cells were suspended in 10 ml PBS and washed two times (250 × g and 100 × g for 10 min each) and finally adjusted to 5 x 10^6 cells/ml in MIF buffer (PBS containing bovine serum albumin (5 g/L) and NaN3 (0.1 g/L)).

Cell labeling with DPPV antibodies and flow cytometry

Separated camel blood leukocytes (4 x 10^5) were incubated with monoclonal goat IgG antibody (R & D Systems) specific for CD26 molecules (Pierson *et al,* 2008; van Doremalen *et al,* 2014) in PBS containing bovine serum albumin (5 g/l) and NaN3 (0.1 g/l). After 30 minutes incubation (4°C), cells were washed twice and incubated with a Alexa Fluor 488a-labelled rabbit F(ab')2-anti-goat IgG (H+L) secondary antibody. After incubation (30 minutes; 4°C), labelled cells were washed twice and analysed on the flow cytometer (Accuri C6, Becton Dickinson Biosciences, San Jose, California, USA). At least 100 000 cells were collected and analysed with the CFlow Software, Version 1.0.264.21 (BD Biosciences).

Statistical Analyses

Statistical analysis was performed with Prism (GraphPad). Results are presented as means ± S.E. of the mean (SEM). Differences between means were tested with one-factorial analysis of variance (ANOVA) and Bonferroni's correction for normally distributed data. Results were considered significant at a p-value of less than 0.05.

Results and Discussion

The present study investigated the impact of *in vitro* stimulation with different bacterial species on the expression level of DPP4 whose expression was evaluated by membrane immunofluorescence and flow cytometry using a goat polyclonal antibody to human DPP4, which is reactive with camel DPP4 (Widagdo *et al,* 2016). After the exclusion of cell doublets, gates were made on the different subsets of camel leukocytes (Fig 1A). DPP4 expression on cells from unstimulated blood and cells from blood stimulated with *Staphylococcus aureus* (*S. aureus*), *Streptococcus agalactiae* (*S. agalactiae*) and *Escherichia coli* (*E. coli*) was evaluated as the mean fluorescence intensity (Fig 1B).

The comparison between leukocytes populations regarding the basic expression level of DPPV confirms the higher expression level on monocytes compared to lymphocytes and granulocytes. However, unstimulated granulocytes and lymphocytes showed a comparable expression level of DPPV ($p<0.05$). Stimulation of whole camel blood with the bacterial species *S. aureus* or *E. coli* resulted in the upregulation of DPPV on both monocytes and granulocytes ($p<0.05$), while *S. agalactiae* did not modulate DPPV expression significantly on either of the immune cells ($p>0.05$). None neither of the bacterial species induced a change in DPPV expression on lymphocytes from stimulated blood, ($p>0.05$).

In a previous report, the expression level of DPP4 on monocytes increased significantly after stimulation with LPS (Al-Mubarak, 2018). In the humans, a similar enhancement of DPP4 expression was observed upon *in vitro* activation of human monocytes as well as on activated alveolar macrophages in human patients with chronic lung diseases (Zhong *et al,* 2013; Meyerholz *et al,* 2016).

The increased expression of DPPV on cells from the upper respiratory tract of human has been linked to their higher susceptibility to MERS-CoV infection compared to dromedary camels (Widagdo *et al,* 2016). The results from the present study indicated a possible role of bacterial infections in increasing the susceptibility of camels to MERS infection. Further studies are needed to confirm the findings

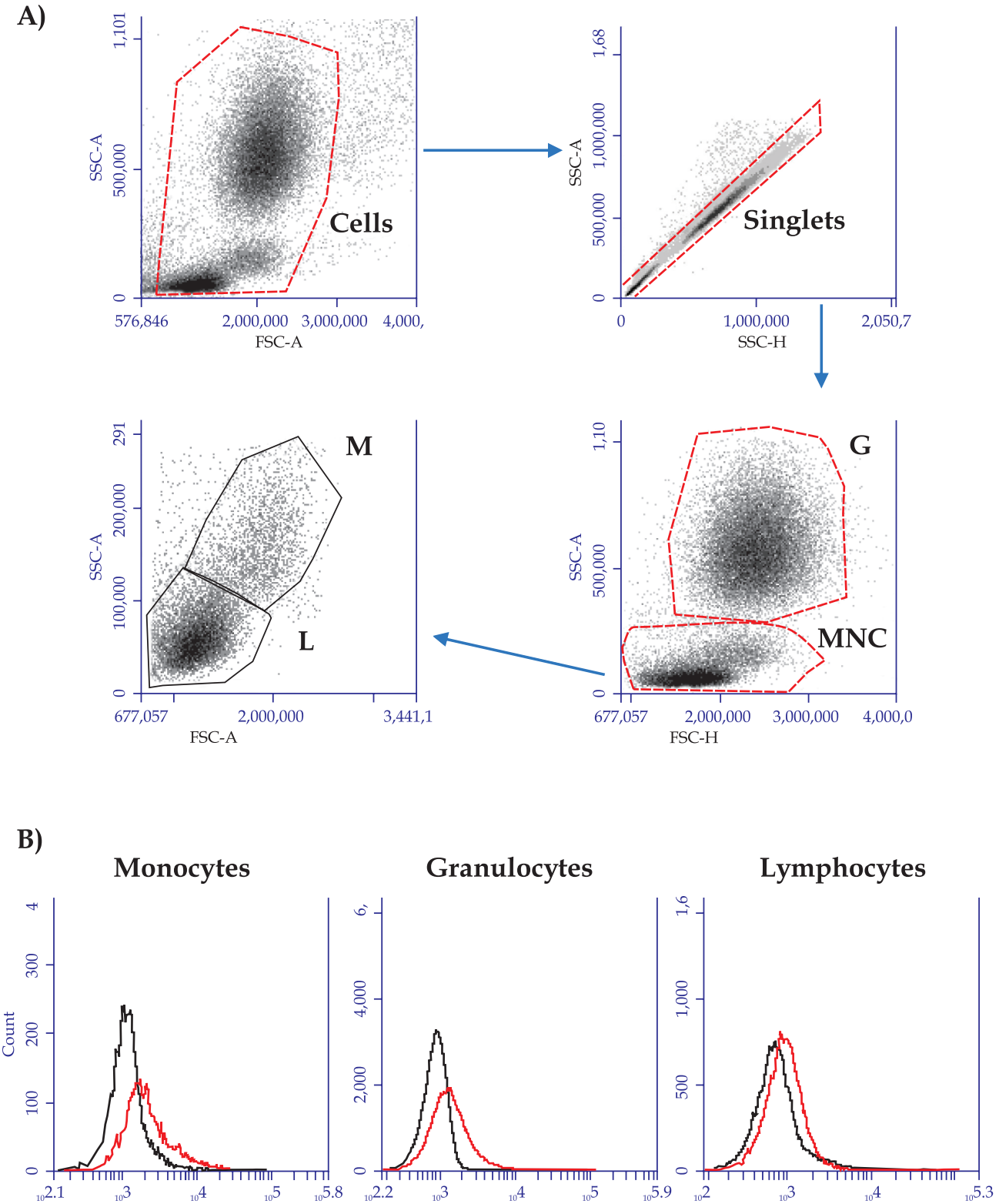

Fig 1. Flow cytometric analysis of DPP4 expression on the main leukocyte populations in stimulated and unstimulated peripheral blood of dromedary camel. Camel leukocytes were separated by hypotonic lysis of erythrocytes and the separated cells were labeled with polyclonal antibody to DPP4 and analysed by flow cytometry. A) After the exclusion of cell duplicates using a SSC-H/SSC-A dot plot, a SSC/FSC dot plot was used to identify granulocytes (G), monocytes (M) and lymphocytes (L) according to their forward and sideward scatter characteristics. B) After setting a gate on monocytes, granulocytes, and lymphocytes, the mean fluorescence intensity (MFI) of DPP4 for stimulated (red lined histogram) or unstimulated cells (black lined histogram) was presented in overlay histograms (data presented is for blood stimulated with *S. aureus*).

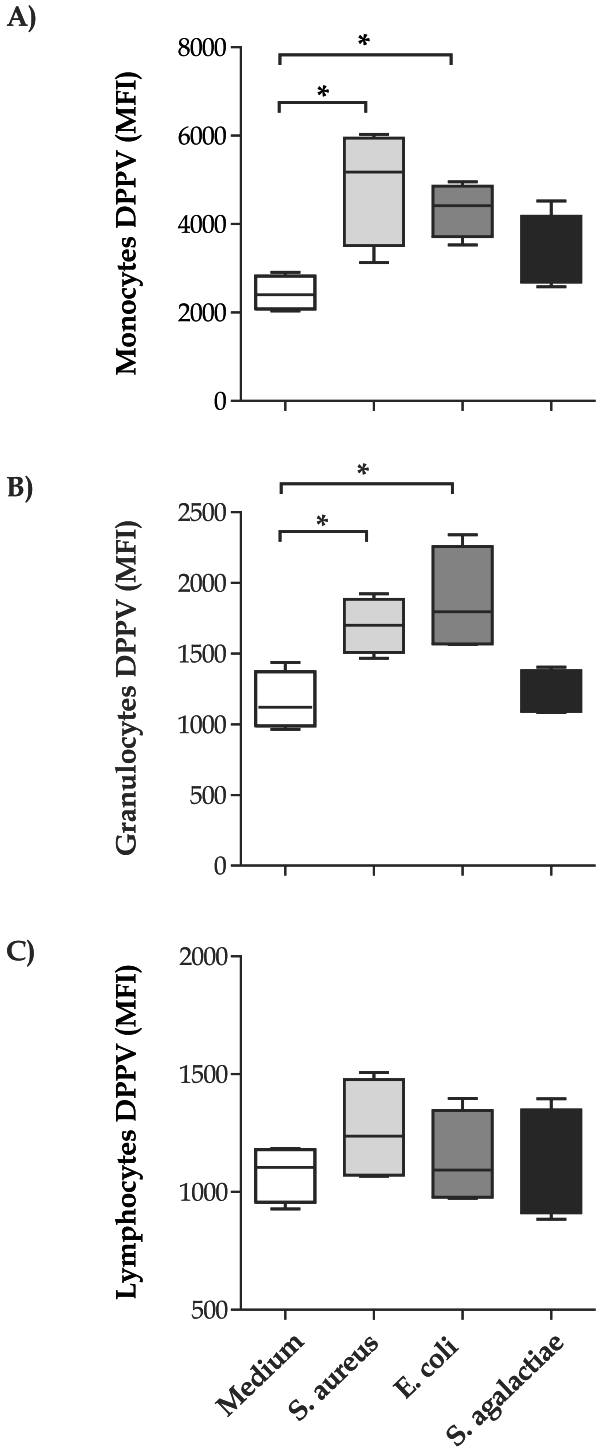

Fig 2. The effect of stimulation with live bacteria of camel blood on the expression level of DPP4 on the main leukocyte populations. Camel whole blood was stimulated for 4h with *S. aureus*, *E. coli*, or *S. agalactiae*. Separated leukocytes were labeled with polyclonal antibody to DPP4 and analysed by flow cytometry. After setting a gate on granulocytes, monocytes and lymphocytes, the mean fluorescence intensity (MFI) of DPP4 expression on granulocytes (G), monocytes (M) and lymphocytes (L) for stimulated and unstimulated leukocytes was calculated and presented graphically as mean +- SEM (* = $p<0.05$).

of the current study by the evaluation of disease susceptibility in healthy camels and camels with bacterial infections.

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Conflict of interest:

None.

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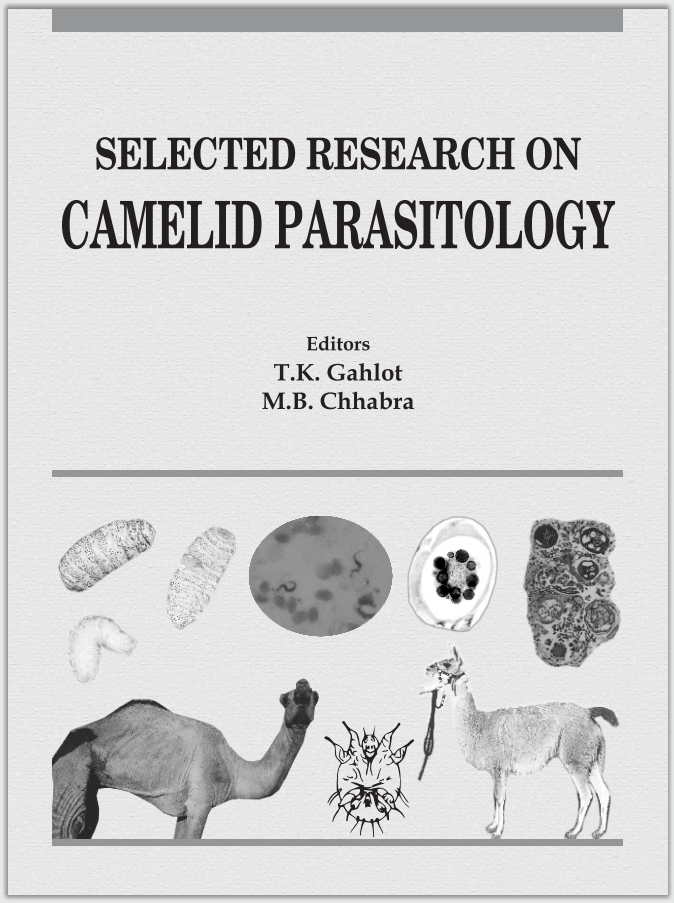

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Short communication

DURATION OF MERS – CORONAVIRUS ANTIBODIES IN A SMALL CLOSED DROMEDARY CAMEL HERD IN DUBAI

U. Wernery, S. Joseph, M. Rodriguez, N.M. Paily, S.M. Thomas and R. Raghavan
Central Veterinary Research Laboratory, Dubai, UAE

ABSTRACT

This investigation shows that MERS-CoV ELISA antibodies remain in dromedary camels at least for 9 years, most probably lifelong. The viral infection was most likely acquired at a young age when maternal antibodies have receded between 4 to 8 month of age. Additionally, in the investigated herd, the 4 ELISA antibody negative dromedary camels remained negative also for 9 years, indicating, that no MERS-CoV was circulating in the herd. Until today, the specific source of MERS-CoV infection for young dromedaries remains unknown.

Key words: Mers – Coronavirus, dromedary camel

MERS-Coronavirus is a zoonotic virus, that can be transmitted from dromedary camels to human beings, in which it causes severe respiratory disease with a high case fatality. Dromedary calves may develop no or only mild respiratory signs and adult camels are free of the disease (Wernery *et al,* 2017). Seroepidemiological studies have found high levels of MERS-CoV antibodies in dromedary camels in the Middle East and Africa (Muller *et al,* 2014; Mackay and Arden, 2015), but not in Bactrian camels in their home country (Chan *et al,* 2015).

Unlike SARS-CoV which was eradicated from the globe by closing animal markets in China, where the palm civet cat was sold, MERS-CoV will remain in many countries where dromedary camels are reared. However, the incidence has dropped significantly over the last years. A serological MERS-CoV survey was conducted in a dromedary herd over a period of nearly 10 years, the results of which are presented here.

Materials and Methods

Twenty-two adult dromedary camels aged between 8 to 25 years and of different gender belonging to the Central Veterinary Research Laboratory (CVRL) were tested for MERS-CoV antibodies with the "Anti-MERS-CoV ELISA Camel (IgG)" from Euroimmun over a time frame of 9 years. Blood was regularly withdrawn from the jugular vein of the camels from 2013 and last in 2022. The blood was centrifuged for 5 minutes at 4000 rpm, and serum stored at -20°C until tested. Not every time blood was collected from all dromedary camels as the test kit was not always available. However, in August 2022 blood was withdrawn from 21 animals and tested. One dromedary camel had died in 2021. All dromedary camels were donated to CVRL more than 15 years ago.

The results were evaluated semi quantitatively by calculating a ratio of the extinction value of the control or sample over the extinction value of the calibrator (provided in the test kit). According to the manufacturer of the ELISA, a ratio of < 0.8 was negative, a ratio of ≥ 0.8 to < 1.1 was borderline and a ratio of ≥ 1.1 was positive.

Results

The results of the ELISA are shown in Table 1.

In total, over 9 years the results of MERS-CoV antibodies of 22 (21) dromedary camels remained the same: 4 animals remained negative, 18 (17) remained positive.

Discussion

Twenty-two adult dromedary camels of different gender were tested over a period of 9 years for MERS-CoV antibodies with a commercial antibody (IgG) ELISA. Eighteen of the camels

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Table 1: MERS-CoV antibody results of 22 dromedary camels tested over 9 years.

No.	Camel ID	Date of Sampling & Ratio (ELISA)							MERS-CoV Antibody ELISA results
		18.06.13	22.10.15	19.09.17	16.07.19	14.12.19	29.12.19	16.08.22	
1	A44 CORINA	0.20	0.08	0.10	0.07	0.05	0.05	0.09	Negative
2	973 Alia	3.70	3.10	2.50	2.09	1.63	1.44	1.59	Positive
3	26	ND	ND	ND	2.97	2.50	3.04	1.98	Positive
4	Tiny 136	ND	4.10	3.70	4.00	3.37	3.44	Died	Positive
5	6A5	0.06	0.08	0.08	0.06	0.06	0.07	0.13	Negative
6	DBO	0.09	0.07	0.12	0.07	0.08	0.09	0.25	Negative
7	O5E	0.20	0.12	0.10	0.05	0.07	0.08	0.22	Negative
8	1	ND	4.00	4.30	4.11	4.18	4.44	4.89	Positive
9	7	ND	ND	ND	3.42	2.55	2.76	2.84	Positive
10	3	ND	ND	ND	4.59	4.60	4.91	5.90	Positive
11	5	ND	ND	ND	2.48	2.23	4.72	2.66	Positive
12	4	ND	3.80	ND	3.22	2.54	5.35	4.40	Positive
13	10	ND	ND	ND	4.62	ND	ND	3.20	Positive
14	1702	ND	ND	4.50	ND	ND	ND	6.00	Positive
15	1704	ND	ND	4.40	ND	ND	ND	5.70	Positive
16	1705	ND	ND	3.20	ND	ND	ND	2.40	Positive
17	1053	ND	ND	ND	ND	ND	ND	4.60	Positive
18	24	ND	ND	ND	ND	ND	ND	3.80	Positive
19	9	ND	ND	ND	4.56	ND	ND	5.40	Positive
20	22	ND	ND	ND	4.92	ND	ND	6.70	Positive
21	29	ND	ND	ND	4.22	ND	ND	4.50	Positive
22	28	ND	ND	ND	4.46	ND	ND	5.10	Positive

ND - Not done

remained MERS-CoV antibody positive and 4 remained negative through the entire testing period. The animals were kept in different groups in 7 pens and had constant contact to each other. The result shows 2 interesting facts. No circulating MERS-CoV exist in this herd as all 4 negative dromedary camels remained negative for 9 years. The MERS-CoV ELISA antibody level remained more or less stable with few variations in the ratio, indicating that an infection at young age may produce lifelong immunity. Over many years from serological positive MERS-CoV dromedaries greater than 4 years of age, which died from different diseases and were necropsied at CVRL, no MERS-CoV had been recovered from many organ specimens (Wernery *et al,* 2017). However, MERS-CoV has been isolated from nasal swabs of young necropsied camels (Wernery, 2014), most probably when maternal antibodies disappeared between 4 and 8 month of age, permitting infection to occur during the seronegative period (Bin *et al,* 2016).

Until today, the specific source of infection for young dromedaries remains unknown, although it is likely to be from other dromedaries.

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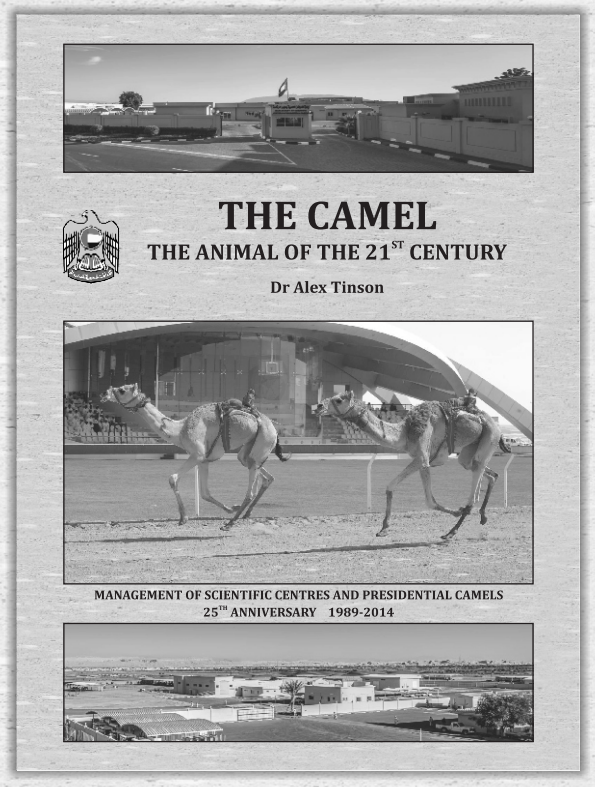
THE CAMEL

THE ANIMAL OF THE 21ST CENTURY

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



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GENETIC DIVERSITY OF THE MONGOLIAN BACTRIAN CAMEL BASED ON MITOCHONDRIAL SEQUENCES

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ABSTRACT

Current genetic diversity of 5 local breeds of Mongolian camels and 2 samples of *Camelus ferus* was studied and compared their levels of variability. DNA was extracted using Qiaamp® DNA kit (Qiagen) and amplified mtDNA D-loop control region from 36 blood samples of Mongolian Bactrian camels and 2 from Mongolian *Camelus ferus*. Three common haplotypes (H1, H2, H6) and 4 unique haplotypes (H3, H4, H5, H8) revealed in the population of domestic camels based on the mitochondrial DNA region. For the further study we carried out best result 26 sequences. We did not find high inbreeding levels in the different breeds. The phylogenetic analysis suggested that domestic and wild camels have two distinct lineages.

Key words: Camel, genetic diversity, mitochondrial DNA, Mongolian camel breeds

The distribution of Mongolian camels is limited and there are relatively few differences between local camels in terms of body size, shape, colour and productivity. These are bred mainly in Tugrug and Tonkhil soums of Gobi-Altai province, Khanbogd soum in Umnugovi province and Mandal-Ovoo soum in Umnugovi province.

Scientists have identified 3 breeds which are based on their productivity, colour and morphological differences. The "Galbyn Gobi Red" and "Khanyn Hetsiin Khuren" were approved as breed while "Tuhum tungalagiin khuren" was approved as breeding group (Baldan *et al,* 2015).

Battsetseg (2018) studied 2 species from genus *Camelus* and domestic camel populations in Mongolia using 18 mitochondrial and nuclear microsatellite markers to detect genetic patterns and evolution and to assess genetic diversity. The genetic characteristics of the camel breeds of the Galbyn Gobi Red, the Khanyn Hetsiin Khuren and the Tuhum tungalagiin khuren were identified, revealing differences in the main genetic parameters of the population, such as allele number, frequency, heterozygosity and genetic distance. Based on neutral markers, little population structure in Mongolian Bactrian camels were observed and the phenotypic differentiation was mainly due to recent anthropogenic selection, which would change allele frequencies in selected genes rather than in neutral markers.

Wild Bactrian camel is similar to Bactrian camel, but morphologically have different characteristic features (Andrei and Bat-Erdene, 1998; Adiya, 2017).

There is a lack of genetic study about the Mongolian Bactrian camel population, however, a comparative study between the genetic diversity of two Mongolian camel populations from China and the area bordering China was conducted (Jianlin *et al,* 2004).

Mongolian camel herders have traditionally selected camels based on morphological and productivity traits for milk, meat and wool (Chuluunbat *et al,* 2014). Considering this morphological characterisation, we investigated the genetic diversity among the described breeds and expected breed-specific population differentiation by analysing nucleotide sequence of the control region in mitochondrial DNA.

Materials and Methods

In total, 36 camels were sampled from 7 different localities of Mongolia (Fig 1). Blood, skin and hair samples of Galbyn Gobi Red camel from Khanbogd soum of Umnugovi province, Khanyn Hetsiin Khuren camel from Mandal-Ovoo soum,

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Tuhum tungalagiin khuren camel from Tugrug soum of Gobi-Altai province and local Mongolian camels populations from Bayantooroi bagh of Tsogt soum of Gobi-Altai province, Aldarkhaan soum of Zavkhan province, Bugat soum of Gobi-Altai province and Baruunturuun soum of Uvs province were collected and stored in the -20°C.

DNA extraction from blood

DNA extraction from EDTA-preserved blood was performed with the Qiaamp® DNA kit (Qiagen). Hair samples were digested with a non-commercial lysis buffer (Pfeiffer *et al*, 2004) and DNA was extracted with the NucleoSpin® Tissue Kit (Macherey-Nagel). PCR amplification using the primer combinations: Pro FCCACCACCAGCACCCAAAGCTG Phe RGGC CAGGTGCCCATCCAGGCAT. The amplification was carried out in a 30 µl reaction volume containing 1 µl of the extracted DNA, 1µl of the 25 mM of dNTPs, 1µl of the each primers (10 pmol), 0.5 units of Dream taq (Thermofisher scientific) and 1xPCR buffer (includes 20 mM $MgCl_2$). The PCR conditions were the following: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 69°C for 1 min and extension at 72°C for 1 min. The final extension was at 72°C for 10 min.

Results and Discussion

The study included a total of 36 samples, which consisted of 6 samples from each population such as Besreg, Khanyn Khetsiin Khuren, local Mongolian camel, wild Bactrian camel, Galbyn Gobi Red and Tuhum tungalagiin khuren. The 1100 base pairs of the mitochondrial DNA control regions were amplified and analysed (Fig 2). For the further study we carried out best result 26 sequences.

The 657bp sequences of the control region in 26 samples were aligned with the reference sequences (NC 009628.2 *Camelus bactrianus*, NC 009629.2 *Camelus ferus*) in MEGA v.10.0.4. A total of 9 haplotypes were identified using DnaSP v.5.10 software (Rozas *et al*, 2017) to determine the haplotype of the DNA control region, but no common haplotypes were found between a wild camel and domestic camel populations. Two samples of wild camels were included in one haplotype (H7), while the domestic camel population was divided into 8 haplotypes.

Burger (2012) and Silbermayr *et al* (2009) have sequenced the cyt b gene and D-loop in the mitochondrial genome of Bactrian camels and identified 6 haplotypes (D1-D6) from 41 domestic

Fig 1. Geographic distribution of the domestic Bactrian camel samples analysed in the present study. 1. Umnugovi province, Han-bogd: Galbyn Gobi Red camel; 2. Umnugovi province, Manal- ovoo: Khanyn Hetsiin Khuren camel; 3. Gobi-Altai province, Tugrug: Tuhum tungalagiin khuren camel; 4. Gobi-Altai province, Tsogt: Mongolian camels; 5. Zavkhan province, Aldarkhaan: Mongolian camels; 6. Gobi-Altai, Bugat: Mongolian camels; 7. Uvs province, Baruunturuun: Mongolian camels.

camels and 2 haplotypes from 27 wild camels (W1, W2) were detected. Based on this, domestic and wild camels have many common haplotypes. Previous studies have also shown that the mitochondrial cyt b gene has a slower evolution rate than the D-loop. In other words, because camel species are less susceptible to evolution, some genes in the mitochondrial structure, such as the mitochondrial cyt b gene, are relatively conservative, while a relatively large number of mutations have accumulated in the D-loop, hence is more appropriate for detecting genetic differences in camel species.

A total of 9 haplotypes were detected in the nucleotide sequences of the mtDNA control regions in the 26 samples. In order to avoid duplication of nucleotide sequences in the mtDNA control region, one sample was selected as representative from the samples with a common haplotype in each haplotype and a total of 9 newly discovered haplotypes were registered with the gene bank (NCBI) (Table 1).

A total of 16 nucleotide substitutions were observed in domestic camels and wild camels when compared with the reference sequence from the gene bank (NC 009629.2 *Camelus ferus*, NC 009628.2 *Camelus bactrianus*) (Tables 1 and 2). In them, 12 nucleotides were replaced in wild camels and 5 nucleotides were replaced in domestic camels. The absence of a

common haplotype between domestic camels and wild camels (H9) indicates that there is a significant genetic difference. In the domestic Bactrian camel, all 3 main haplotypes were identified - H1, H2 and H3 (which is often found in Chinese, Russian and Mongolian Bactrian camels). Haplotypes specific to a particular geographical region (H6, H4, H5, H8) were also found.

A phylogenetic tree from all the detected haplotypes was constructed by neighbour joining method based on the Tamura-Nei model using 1000 bootstrap replications in MEGA v.10.0.4. The haplotype of the wild camel was in the same cluster with the reference sequence of wild camels from the gene bank (NC 009629.2 *Camelus ferus*) and the other domestic camels were clustered together with *Camelus bactrianus* from the gene bank (NC 009628.2 *Camelus bactrianus*) (Fig 3).

Genetic diversity was averaged over nucleotide diversity, haplotype diversity and nucleotide differences (Table 3). Domestic camel populations had high genetic diversity according to an mtDNA control region.

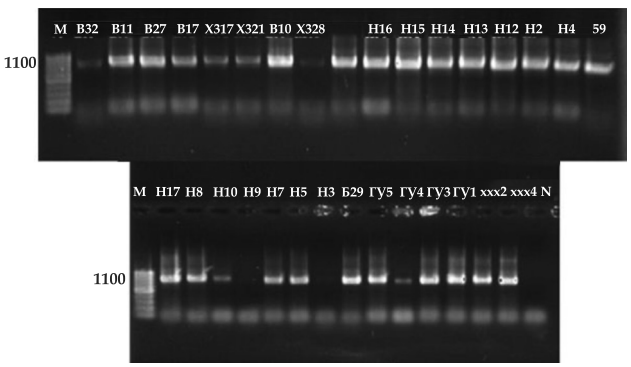

Fig 2. PCR product of control region mtDNA in 1% agarose gel electrophores. M- 100bp marker, N-negative control, Б-Besreg, Х3- Tuhum tungalagiin khuren, Н- local Mongolian camel, ГУ- Galbyn Gobi red camel, XXX- Khanyn khetsiin khuren.

Although, the genetic diversity of the Galbyn Gobi red and local Mongolian camel populations is greater than that of other populations, it may have depended on the number of samples. However, the nucleotide diversity of the Bearded camel is lesser than that of the Khoz Zogdort populations where sample numbers are the same. Due to the small number of wild camel samples, genetic diversity could not be determined.

At the end of *cyt b* gene and the beginning of the control region of mitochondrial DNA in Mongolian camel breeds and local Mongolian camel populations, the molecular diversity index showed high haplotype diversity, averaging H_d = 0.725 (± 0.044), nucleotide diversity was π= 0.00195 and the average number of genetic differences was k = 1,566 (Battsetseg, 2018).

In present study, the haplotype diversity was H_d = 0.6-0.93, the nucleotide diversity was π= 0.0009-0.002 and the average number of nucleotide differences was k = 0.6-1.6.

When calculating the genetic distance between two species and populations of camels, there was a significant genetic difference between wild camel populations and domestic camels. However, there were not many genetic differences between domestic camel populations (Table 6).

When comparing to the nucleotide sequences of 15120-15923 site from mitochondrial DNA, Mongolian camel Khanyn Khetsiin Khuren, Tuhum tungalagiin khuren, Galbyn Gobi Red, local Mongolian camel populations and wild camel were compared based on Tamura-Nei model in MEGA v6 and the genetic distance between Tuhum tungalagiin khuren - Khanyn Khetsiin Khuren was 0.0063, Galbyn Gobi Red-Khanyn Khetsiin Khuren was 0.0025, Galbyn Gobi red- Tuhum tungalagiin khuren was 0.0037, local Mongolian camels- domestic camel breed was

Table 1. The haplotype of domestic and wild camels and the gene bank accession number.

Populations	Numbers of Samples	Haplotype	Sample	NCBI GenBank Accession number
Domestic camels	7	H1	Б11, Б9, Б10, Х315, Х36, ГУ1, ГУ33	MT582437
	9	H2	Б27, Б17 Х310, Х326, НУ14, НУ2, ГУ5, ГУ34, ХХХ2	MT582438
	1	H3	НУ13	MT582439
	1	H4	НУ15	MT582440
	1	H5	НУ16	MT582441
	3	H6	НУ12, ХХХ4, ХХХ13	MT582442
	1	H7	ГУ3	MT582443
	1	H8	ГУ50	MT582444
Wild camel	2	H9	ХАВТГАЙ 9, ХАВТГАЙ 8	MT582445

Table 2. Nucleotide substitution in the control region of mtDNA.

Sample of camels	Name of population	Haplotype	Nucleotide of substitution																	
NC 009629.2 *Camelus ferus*	Reference suence		G	T	T	.	T	G	C	.	T	G	C	.	T	A	T	A	T	T
NC 009628.2 *Camelus bactrianus*			.	.	.	.	.	.	.	C	.	.	.	.	.	.		.	C	C
b11	Besreg	H1	A	C	.	A	C	A	A	T	C	A	T	A	C	C	C	C	-	-
b27		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
b17		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
b9		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
b10		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
hos zogdort 15	Khos zogdort	H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
hos zogdort 10		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
hos zogdort 26		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
hos zogdort 6		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Nutgiin temee 13	Domestic camels	H5	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	-	-
Nutgiin temee 15		H4	.	.	.	G	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Nutgiin temee 14		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Nutgiin temee 16		H3	.	.	.	.	T	.	.	.	.	.	.	.	.	.	T	A	-	-
Nutgiin temee 12		H6	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-
Nutgiin temee 2		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Hawtgai 9	Wild camels	H9	G	T	T	.	T	G	C	.	T	G	C	.	T	A	T	-	-	-
Hawtgai 8		H9	G	T	T	.	T	G	C	.	T	G	C	.	T	A	T	-	-	-
Galbiin ulaan 5	Galbyn gobi red camel	H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Galbiin ulaan 3		H7	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Galbiin ulaan 1		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Galbiin ulaan 50		H8	.	.	.	.	T	.	.	.	.	.	.	C	.	.	T	A	-	-
Galbiin ulaan 33		H1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-
Galbiin ulaan 34		H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Haniin hets 2	Khanyn khets	H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	A	-	-
Haniin hets 4		H6	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-
Haniin hets 13		H6	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-

low (0.0025-0.0050) and wild Bactrian camel-Bactrian camel was high (0.3369-0.3432) (Battsetseg, 2018). The nucleotide sequence of the mtDNA control region in the study, the genetic distance between Galbyn Gobi Red-Khanyn Khetsiin Khuren was 0.00297, local Mongolian camels-domestic camel breed was low (0.00153-0.00297) and wild Bactrian camel-Bactrian camel was high (0.01584-0.01652).

From the mid-90s to 2014, the population of Mongolian Bactrian camels declined from 700,000 to less than half of their previous population, according to the census. The consequences of such a severe reduction could have implications for the future sustainable use and conservation of this important livestock species. To assess the impact of this recent demographic event on genetic variability, a comprehensive study of mitochondrial and nuclear genetic diversity was conducted in three phenotypically different Mongolian domestic breeds compared to ordinary local Mongolian camels (Battsetseg *et al,* 2014). They concluded that Mongolian Bactrian camels have a genetic diversity comparable to Chinese Bactrian camels and dromedaries. They found neither a high level of inbreeding in various Mongolian breeds nor signs of a bottleneck. In recent years, due to economic needs (wool), over the past 10 years, the actual number of Mongolian Bactrian camels has increased by 68%.

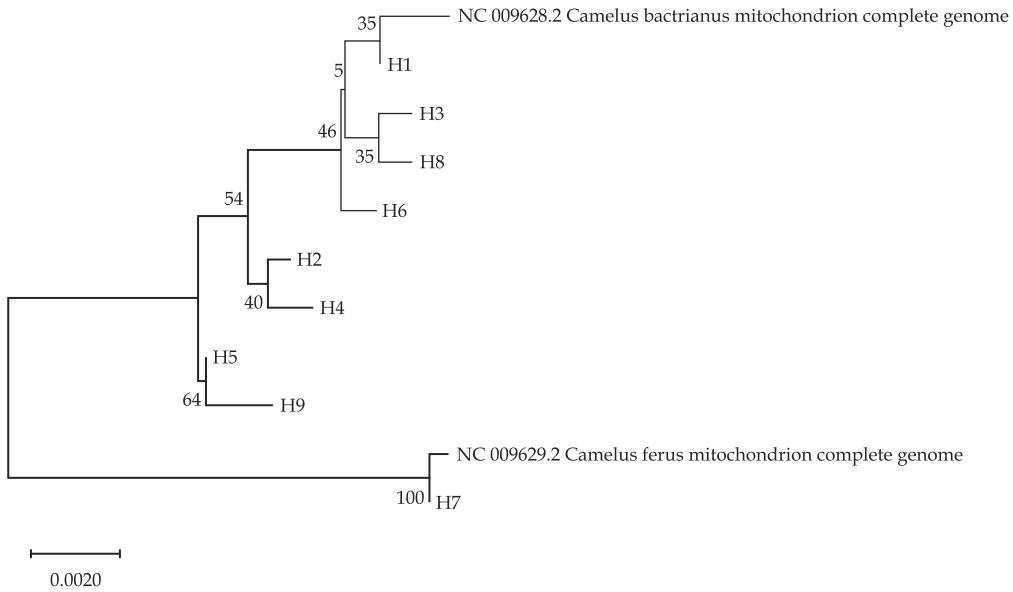

Fig 3. Phylogenetic tree established using haplotype of mtDNA control region. The evolutionary history was inferred using the Neighbour-Joining method.

Table 3. Genetic diversity comparison of mtDNA control regionNote: N-sample number; H-haplotype number; H d - haplotype diversity; Number of S-variable sites; π-nucleotide diversity; κ- Average number of nucleotide difference, SE-standard error.

Population	N	H	S	Hd±SE	π±SE	K
Wild camel	2	1	-	-	-	-
Galbyn Gobi red	6	4	4	0.86	0.002	1.6
Besreg camel	5	2	1	0.6	0.0009	0.6
Khanyn Khetsiin brown	3	2	2	0.66	0.002	1.3
Tuhum tungalagiin khuren	4	2	1	0.66	0.001	0.6
Local Mongolian camel	6	5	3	0.93	0.002	1.6

Table 6. Domestic camel populations and wild camels genetic distance of control region of mtDNA.

	Besreg	**Tuhum tungalagiin khuren**	**Local Mongolian camel**	**Wild camel**	**Galbyn gobi red**
Besreg					
Tuhum tungalagiin khuren	0.00153				
Local Mongolian camel	0.00290	0.00280			
Wild camel	0.01652	0.01636	0.01584		
Galbyn Gobi Red	0.00229	0.00229	0.00314	0.01663	
Khanyn Khetsiin khuren	0.00244	0.00254	0.00297	0.01558	0.00297

Mitochondrial DNA analysis has proven to be a useful tool for rapid screening of wild populations for maternal hybridisation and is also suitable for noninvasively collected samples, such as hair flakes from bushes (Lucas Lipp, 2013).

Based on the mtDNA D loop region, a small population structure in the Mongolian Bactrian camel could be seen. Three common haplotypes (H1, H2, H6) and 4 unique haplotypes (H3, H4, H5, H8) were found in the population of domestic camels. This suggests that the presence of unique haplotypes in the local populations of the Galbyn Gobi red indicates greater genetic diversity than in other populations. We assume that this diversity of haplogroups is related to the history of Bactrian camels (along trade routes between Asia and Europe). When compared with the populations of Galbyn Gobi Red and Khanyn Khetsiin Khuren, the value of the genetic distance shows an increase (from 0.0025 to 0.00297), indicating greater genetic diversity than in other populations.

As for local Mongolian camels and domestic camel breeds, the value of the genetic distance shows a decrease (from 0.0025-0.0050 to 0.00153-0.00297), which means the low genetic diversity of populations.

Wild Bactrian camels showed lower levels of nucleotide diversity and haplotype diversity, which may be due to the extremely small effective population size of the wild Bactrian camel (Ming, 2016). In our result, the value of the genetic distance between Wild and Bactrian camels was higher (0.3369-0.3432 to 0.01584-0.01652).

Conclusion

In present study, 9 haplotypes were identified in the Mongolian camel population from 16 haplotypes genus of Camelus, such a variety of haplogroups is probably related to the history of Bactrian camels (along trade routes between Asia and Europe). The Galbyn Gobi red and Khanyn Khesin Khuren has a the greater genetic diversity than in other populations. In contrary, low genetic diversity of populations has been shown for local Mongolian camel breeds.

Camelus bactrianus and *Camelus ferus* evolved as 2 distinct lineages and it is assumed that these do not have the same maternal origin. *Camelus ferus* showed lower levels of nucleotide diversity and haplotype diversity, which might be due to the extremely small effective population size of wild camels. On the other hand, our results and other research emphasises the importance of preserving the current variability of wild camel populations as a very valuable species in the desert.

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MOLECULAR CHARACTERISATION AND *in silico* ANALYSIS OF NOD-LIKE RECEPTOR P12 (NLRP12) IN DROMEDARY CAMEL

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ABSTRACT

In the present study, complete coding sequence of camel Nod-like receptors P12 (NLRP12) was sequenced and analysed using various tools and softwares. The coding sequence had 3454 bp long contig and 3281 nucleotide long open reading frame (ORF) which translated into 1093 amino acid long protein sequence. The Camel NLRP12 protein sequence had maximum similarity with that of Pig (87.37%) while minimum similarity was found with mouse (80.20%). The protein domain prediction of camel NLRP12 showed the characteristic N-terminal PYRIN domain, a central NACHT domain associated with FISNA domain and eleven C-terminal LRR domain which was found conserved in all studied species. However, one LRR domain was found missing in cattle NLRP12. The NLRP12 of camel was phylogenetically closely related to pig and distantly related to mouse NLRP12. The equal numbers of LRR domain, compared to other species except cattle, may suggest similar ligand binding capacity in camel NLRP12 in order to activate immune responses.

Key words: Camel, domain architecture, innate immunity, NLRP12, phylogeny, sequence analysis

The Nod-like receptors belong to a family of cytoplasmic pathogen sensors and are found in macrophages, lymphocytes, dendritic cells and also in non-immune cells like epithelial cells (Franchi *et al,* 2009). Members of the NLR family of proteins share many common structural features involved in agonist sensing or ligand binding (Zhong *et al,* 2013). They are subdivided into 4 subfamilies (NLRA, NLRB, NLRC and NLRP) (Brahma *et al,* 2015). NLRA or Class II transactivator (CIITA) contains an acid transactivation domain, NLRBs or neuronal apoptosis inhibitor proteins (NAIPs) possess a baculovirus inhibitor of apoptosis protein repeat (BIR). NLRCs have a caspase-recruitment domain (CARD) and NLRPs a pyrin domain (PYD) (Zhong *et al,* 2013). On the basis of these domains, variable numbers of NLRs present in different species have been characterised, i.e. 22 members in human and 33 in mice (Franchi *et al,* 2009). Among NLRs, NOD1, NOD2 and NALP3 are functionally well defined members of protein family. However, NLRP2, NLRP6, NLRP7, NLRP12 were found with less characterised inflammasome structures (Radian *et al,* 2013).

The subfamily of these receptors that is NLRP comprises with 14 members, distinguished by the presence of an N-terminal pyrin (PYD) effector domain (Stutz *et al,* 2009). They were found with conserved motif in more than 20 human proteins, with functions in apoptotic and inflammatory signaling (Kim *et al,* 2016 and Stutz *et al,* 2009). These NLRPs recognise various ligands originated from microbial pathogens (PGN, flagellin, viral RNA, fungal hyphae etc.), host cells (cholesterol crystals, uric acid etc.) and environmental sources (alum, asbestos, silica, alloy particles, UV radiation, skin irritants etc.) (Kim *et al,* 2016).

One of the NLR family members of i,e NLRP12 appears to be found as a negative regulator of immune response by interfering with NF-B activation (Allen *et al,* 2012; Fontalba *et al,* 2007; Zaki *et al,* 2011). The previous studies on animal models have shown its potential role in development of colitis and colorectal tumorigenesis (Allen *et al,* 2012; Ye *et al,* 2008). Moreover, mutations in the NLRP12 gene have been associated with auto-inflammatory diseases such as atopic dermatitis (Macaluso *et al,* 2007) and hereditary periodic fever syndromes (Jéru *et al,* 2008; 2011). In present study, the full length protein coding sequence of NLRP12 in dromedary camel was generated and compared it with some of the other species.

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Materials and Methods

Ethics committee approval and collection of blood samples

All animal procedures were conducted according to the guidelines for the care and use of animals established by the Institutional Animal Ethics Committee (IAEC).

Blood samples were randomly collected from the jugular vein of dromedary camel admitted to the Veterinary Clinical Complex of the College of Veterinary and Animal Sciences, Bikaner. Approximately 5 ml of blood was collected in anticoagulant containing vacutainers and immediately transported to the laboratory on ice and processed the same day.

Isolation of RNA and cDNA synthesis

Total RNA was extracted from blood by using a Gene Jet Whole blood RNA purification kit (Thermo scientific), following the manufacturer's instructions; both the quantity and quality of RNA were assessed at OD260 and OD280 using Nanodrop (Implen NanophotometerTM Pearl device). An aliquot of total RNA was reverse transcribed using the RevertAid First strand c-DNA synthesis kit (Thermo scientific) as per the manufacturer's protocol.

Sequence retrieval and designing of oligonucleotide primers

Primers were designed for camel NLRP12 from orthologous genomic sequences of a camel retrieved from NCBI. Orthologous sequences of NLRP12 were retrieved by using Human and Mice NLRP12 sequences under the accession no. NM_144687.4 and NM_001033431.1 (Ref.), respectively, as a query from TSA, SRA and NCBI database search. The obtained sequences which have >80 % query coverage and >50% similarity were used for selection of camel NLRP12 sequence. Partial coding sequences were not used for the selection of camel NLRP12 sequence. After the selection of NLRP12 sequence, primers were designed by using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/ primer3 _www.cgi) and synthesised at Integrated DNA Technologies (IDT), India. These Primer sequences with their amplicon length and the respective annealing temperature have been listed in table 1.

Amplification cDNA of camel NLRP12

The conventional PCR method was used to amplify NLRP12 cDNA using specific primers (Table 1) designed by Primer3 software. A total of 8 primer pairs were taken, covering the entire coding sequence. Cycling conditions were denaturation at 94°C for 5 minute, annealing at 58-61°C for 30 second to 1 minute and extension at 72°C for 1 minute and a final extension at 72°C for 10 min. Annealing temperatures for different primer pairs are given in table 1. Amplified PCR products were analysed on 1.5% agarose gel and were sequenced using Sanger's sequencing method.

Sequence analysis and bioinformatics approach

The obtained PCR products were sequenced at the sequencing facility of Delhi University (South Campus). The sequence reads were checked and edited in BioEdit sequence alignment editor. Sequence reads were filtered by removing primer sequences and low quality base call of nucleotides showing spurious intensity (not depicted single peak for particular nucleotide) of the peak in the chromatogram which further verified in overlapping reads. After the quality check and filtering, Contig Assembly program (CAP contig) of BioEdit software was used to create contigs of sequence reads in order to make complete sequence assembly of camel NLRs. The nucleotide sequences of NLRP12 were translated into protein sequences through Expert protein analysis system (ExPASY) software and searched for homology against the various animals such that swine, sheep, goat, cattle, buffalo, human, gorilla, horse, mouse, wild bactrian camel, double humped camel and rabbit by analysing per cent similarity and per cent identity with the help of the BLASTp (Basic Local Alignment Search tool for Protein) algorithm.

Evaluation of physicochemical properties of the NLRs protein

To determine the physicochemical properties of camel NLR proteins an open access ExPasy server-ProtParam (https:// web.expasy.org/protparam/) tool was used. Protein sequences of NLRP12 were submitted in the query search in order to compute various parameters such as molecular weight, instability index, aliphatic index, GRAVY (Grand average hydropathy value), extinction coefficient, theoretical pI, the half-life of the protein.

Determination of domain architecture of NLRs

The domain structure analysis was executed with the deduced amino acid sequence of camel NLRP12 and other reported species in the database, using Simple Modular Architecture Research Tool (SMART). The normal mode of SMART was used for all protein domain searches and predictions, along

Table 1. Primers used for PCR amplification of NLRP12 cDNA of dromedary camel.

S. No.	Primer name	Sequence of primer	Amplicon Size	Annealing temp.°C
1.	NLRP12F1	AGTGAGTGGTCAAGGGAGTG	700	58
	NLRP12R1	GAATTTCCTGCGGACGTAGT		
2.	NLRP12F2	TGGGAGAGAGGACAGAGAGA	649	61
	NLRP12R2	ATGGCTTGAGCTTGTCAAAG		
3.	NLRP12F3	TCTTCATTATCGACGGCTTT	569	59
	NLRP12R3	CTGCTCCTCAAACAGGATCT		
4.	NLRP12F4	CCTCTTCACCCTGTGCTTC	594	59
	NLRP12R4	CACTCCAACAGTTCCACCTT		
5.	NLRP12F5	TGTCTCCTCCTTCCTCAACA	696	60
	NLRP12R5	AGGGCATTGCTGTACAAAAC		
6.	NLRP12F6	GAACACATGGTTTCCTCGTT	571	61
	NLRP12R6	GTGCATTTCCTGTGAGATCC		
7.	NLRP12F7	CTGACAGCCAACAAACACTT	595	58
	NLRP12R7	CCTCACACAGCAACCACAT		
8.	NLRP12F8	GAGCTGGATCTCACAGGAAA	740	59
	NLRP12R8	TCCTCCTCCATACTGACTGC		

with signal peptides and internal repeats. SMART tool was used to reveal changes in protein domain architectures under the influence of amino acid variation in camel NLRs with respect to other animal species.

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of camel NLRP12 was performed using ClustalW programme of Bioedit software. The Fasta file of camel NLRP12 protein sequence and the orthologous sequences in different species were taken as an input file in the software. ClustalW programme was executed to align multiple sequences were saved in mega file format. The alignment files were used as an input to construct phylogenetic tree by using MEGA7 software. The neighbour –joining method algorithm, a distance based method was used to infer phylogenetic relationship among all the species.

Results and Discussion

The present study was conducted to illustrate the NLRP12 receptor family member in camel genome which provided the opportunity to examine their coding sequence, genomic location, adaptation and evolution and domain organisation. Camel NLRP12 was identified in *Camelus dromedarius* genome in the present study, against the sequence retrieved from human and mouse under the accession no. NM_144687.4 and NM_001033431.1, (Zhang *et al,* 2020; Coulon *et al,* 2019), respectively.

Molecular characterisation

The PCR for the amplification of NLRP12 gene was designed such that overlapping fragments of cDNA were obtained. Such overlapping fragments help during the sequence analysis. In the amplification reactions specific PCR products of predicted sizes of camel NLRP12 gene were obtained. The obtained products were found to be free from primer dimerisation, smearing or any nonspecific amplification (Fig 1).

Sequence characterisation

Obtained PCR products of camel NLRP12 cDNA were sequenced and assembled into a 3454 bp long contig. This contig was found to have 3281 nucleotide long open reading frame (ORF) through ORF finder. The sequence was submitted to NCBI under the GenBank accession no. OK504508. The NLRP12 gene was found to be present on chromosome 9 and has 10 exonic parts was found to be involved in NLRP12 gene expression when ENSEMBLE genome browser was used for the study.

On translation of predicted open reading frame (ORF) of camel NLRP12, 1093 amino acid long protein sequence was obtained. The total amino acids in NLRP12 of other species as found in database have less or greater number of amino acids. The camel NLRP12 was found to consist of 20 different types of amino acids. Leucine (184) was found to be in maximum numbers, whereas tryptophan was found in least (17) numbers (Table 2).

Table 2. Amino acid composition of camel NLRP12 protein.

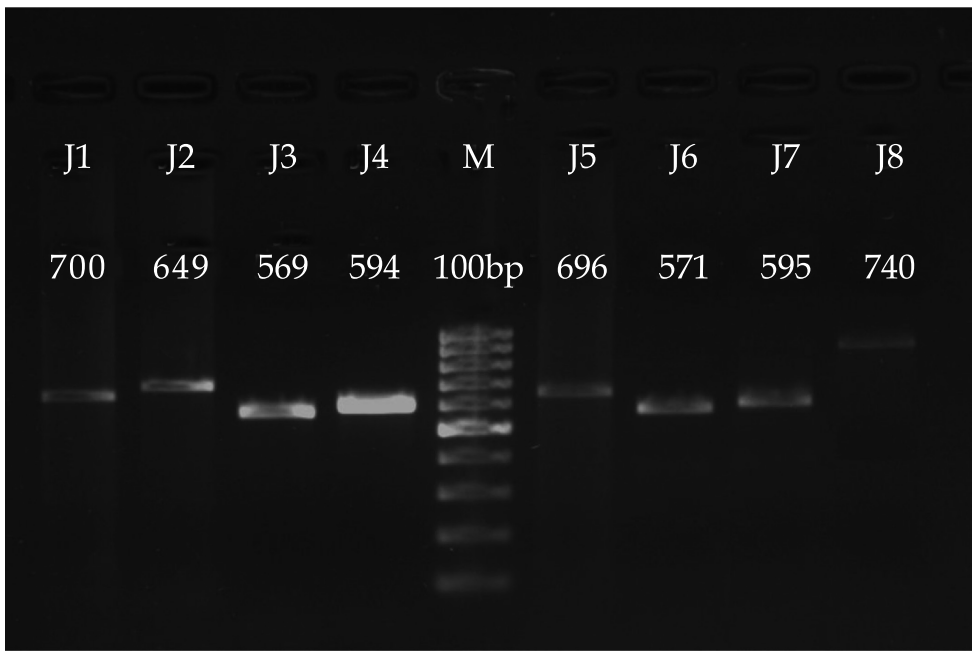
S.No.	Amino acid	Number of Amino acids (%)
		NLRP12
1.	Ala (A)	6.80%
2.	Arg (R)	6.90%
3.	Asn (N)	3.10%
4.	Asp (D)	4.10%
5.	Cys (C)	3.80%
6.	Gln (Q)	4.80%
7.	Glu (E)	7.10%
8.	Gly (G)	6.90%
9.	His (H)	2.70%
10.	Ile (I)	2.30%
11.	Leu (L)	16.80%
12.	Lys (K)	4.30%
13.	Met (M)	2.40%
14.	Phe (F)	3.50%
15.	Pro (P)	5.00%
16.	Ser (S)	6.50%
17.	Thr (T)	4.30%
18.	Trp (W)	1.60%
19.	Tyr (Y)	2.20%
20.	Val (V)	4.90%

Physicochemical characterisation of camel NLRP12 protein

The physicochemical property of camel NLRP12 was evaluated and presented in this study (Table 3). The molecular weight of this protein was found 112.73 kda, whereas, the isoelectric point value 7.40 shows slightly basic in nature. The instability index was found 53.69, which shows instability of this protein as computed instability index was detected >40. The instability index provides an estimate of the stability of protein in a test tube not in the cell. It predicts the stability on the basis of certain dipeptides, which is significantly different in the unstable proteins (Guruprasad *et al,* 1990). Moreover, the half-life of the camel NLRP12 protein was found to be 30 hours in mammalian reticulocytes, *in vitro*; more than 20 hours in yeast and more than 10 hours in *E. coli*, *in vivo* which was assessed by identifying N-terminal residue (Bachmair *et al,* 1986). In this study, the grand average of hydropathicity (GRAVY) was observed as -0.043, depicts hydrophilic nature of this protein while positive value of GRAVY displays the hydrophobic nature of the protein (Kyte and Doolitte, 1982). The aliphatic index was found 101.14, value depicted >100, which suggest increased thermo stability of this proteins. The relative value of occupied aliphatic side chains (alanine, valine, isoleucine and leucine) is a positive factor for the increase of thermostability of globular proteins (Ikai, 1980). These values will help in isolation and purification leading to further characterisation of camel NLRP12 protein.

Homology study of camel NLRP12

To evaluate the homology of camel NLRP12, the percent amino acid similarity and nucleotide



(M = DNA marker-100bp)

Fig 1. PCR amplification of overlapping fragments of camel NLRP12 gene coding region.

identity were obtained and depicted in table 4. The polypeptide so obtained was compared with amino acid sequence of other 12 vertebrates *viz* wild Bactrian camel, double humped camel, cattle, buffalo, goat, horse, gorilla, human, rabbit, sheep, mouse and pig and per cent similarity of 99.45%, 96.34, 86.18%, 81.45%, 86.45%, 86.11%, 85.81%, 86.23%, 82.37%, 86.27%, 80.20% and 87.37%, respectively was found with these species. The high degree of nucleotide identity and amino acid similarity of camel NLRP12 was noticed with that of pig, where as, minimum nucleotide identity and amino acid similarity was found with that of mouse. The amino acid sequence similarity of NLRP12 of different vertebrates was observed to be 80% or more in all species whose sequences are there in the database and show that the NLRP12 is conserved in all these vertebrate species.

Table 3. Physicochemical parameters of camel NLRP12 protein.

S.No.	Physiochemical properties	NLRP12
1.	Molecular weight (kDa)	112.73
2.	Theoretical pI	7.40
3.	Instability index	53.69
4.	Aliphatic index	101.14
5.	GRAVY (Grand average of hydropathicity)	-0.043

Protein domain architecture of camel NLRP12

Online tool Simple Modular Architecture Research Tool (SMART) was used for further analysis of deduced amino acid sequence of camel NLRP12. Camel NLRP12 protein was found with characteristic N-terminal PYRIN domain, a central NACHT domain and 11 C-terminal LRR domain (Fig 2) which was found in accordance to human NLRP12 (Inohara *et al,* 2000; Nembrini *et al,* 2009; Travassos *et al,* 2010). The N-terminal PYRIN domain consisted with total number of 82 amino acid residues at position (44-126) of amino acid sequence. Fish-specific NACHT associated domain also found in camel NLRP12, which is frequently found associated with the NACHT domain in fish and other vertebrates (Stein *et al,* 2007). This domain consisted with total number of 73 amino acid residues at position (163-236) of amino acid sequence. The central NACHT domain consisted with total number of 170 amino acid at position (246-416) of camel NLRC3 protein followed by eleven LRR domains, which consisted with total number of 303 amino acid residues at position 744-771, 773-800, 801-828, 830-857, 858-885, 887-914, 944-971, 972-999, 1001-1028 and 1029-1056, respectively, shown in fig 2 and table no. 5. The characteristic ATP/GTP-binding site motif also called Walker A motif/ (P-loop) was found at 252-259 position which help to bind ATP (Brahma *et al,* 2015).

The comparative analysis of NLRP12 domain architecture was represented in the fig 3. The PYRIN, FISNA and NACHT domain in camel NLRP12 were found conserved when compared to other species such as wild Bactrian camel, double humped camel, cattle, buffalo, dog, horse, gorilla, human, sheep, mouse pig, rabbit and goat. The LRRs domain was also found conserved in *Camelus dromedarius* with other species. However, in cattle NLRP12 one LRR domain was found absent as compared to other studied species. The equal number of LRRs in species suggest their similar ligand sensing capabilities.

Table 4. Nucleotide sequence identity and amino acid sequence similarity of dromedary camel NLRP12 coding sequence with other vertebrates.

S. No.	Host	NCBI accession no	Query cover (%)	Nucleotide (% Identity)	Amino acid (% similarity)
1.	*Camelus ferus* (Wild Bactrian Camel)	XM_014554582.2	100	99.65	99.45
2.	*Camelus bactrianus* (Double Humped Camel)	XM_010961832.1	92	99.59	96.34
3.	*Equus caballus* (Horse)	XM_014730410.2	95	85.50	86.11
4.	*Bos taurus* (Cattle)	NM_001192551.2	96	84.33	86.18
5.	*Bubalus bubalis* (Buffalo)	XM_025269546.1	96	84.52	81.45
6.	*Capra hircus* (Goat)	XM_018063018.1	93	84.89	86.45
7.	*Ovis aries* (Sheep)	XM_042232368.1	96	84.60	86.27
8.	*Gorilla gorilla* (gorilla)	XM_019014916.2	94	83.46	85.81
9.	*Homo sapiens* (Human)	NM_144687.4	94	84.17	86.23
10.	*Mus musculus* (Mouse)	NM_001033431.1	56	77.54	80.20
11.	*Oryctolagus cuniculus* (Rabbit)	XM_008251651.2	92	80.56	82.37
12.	*Sus scrofa* (Pig)	AK347328.1	92	87.76	87.37

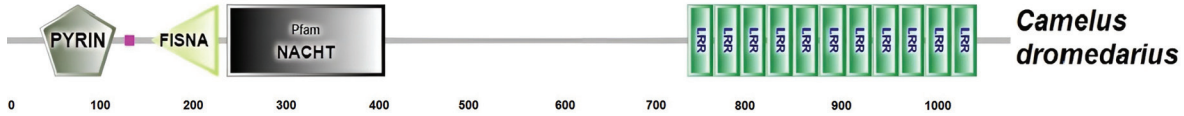


Fig 2. Diagrammatic representation of domain organisation in Camel NLRP12 by utilizing SMART tool (N-terminal PYRIN domain, Fish-specific NACHT associated domain, central NACHT domain and C-terminal LRR domain in green box).

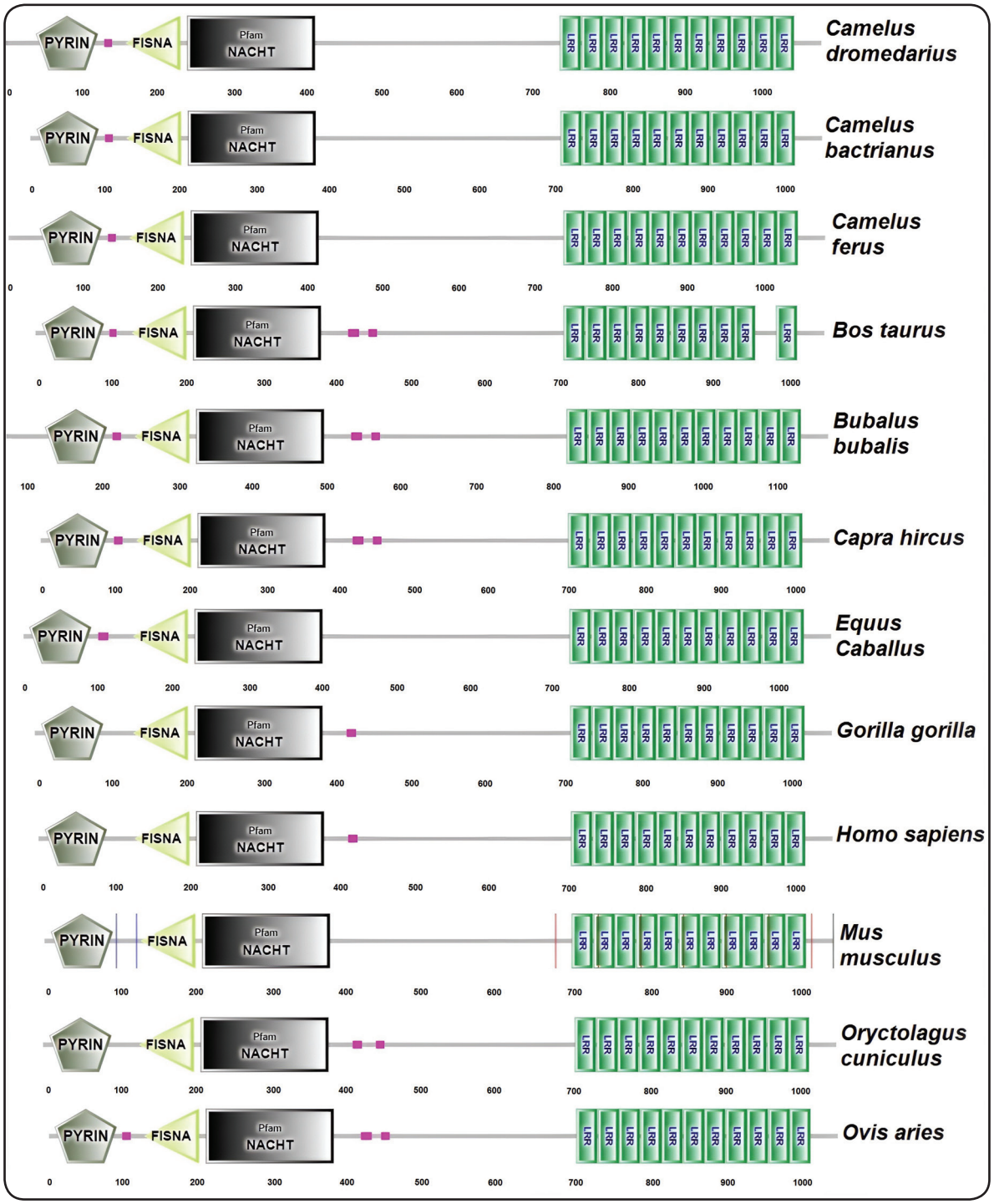


Fig 3. Comparative diagrammatic representation of domain organisation in camel NLRP12 along with other vertebrate species (N-terminal PYRIN domain, Fish-specific NACHT associated domain, central NACHT domain and C-terminal LRR domain in green box).

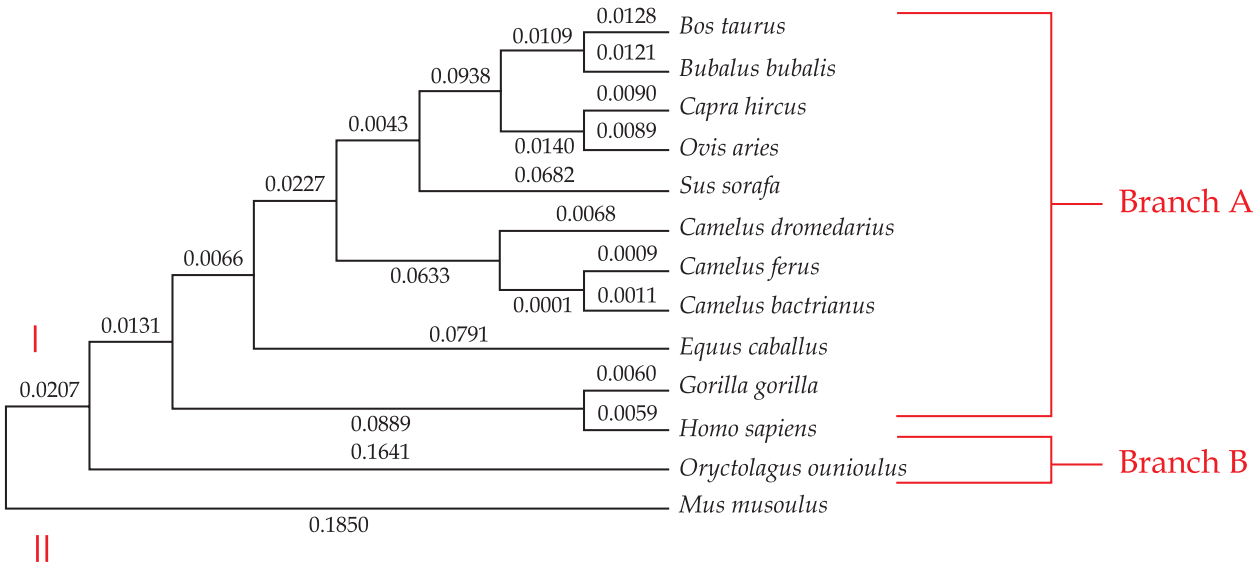


Fig 4. Phylogenetic analysis of camel NLRP12 at amino acid level by Neighbour-Joining method.

Table 5. Protein domain prediction with their respective positions in *Camelus dromedarius* NLRP12 using SMART tool.

Name	Start	End	E-value
PYRIN	44	126	6.01e-29
low complexity	135	145	N/A
FISNA	163	236	2.54e-18
Pfam: NACHT	246	416	2.4e-51
LRR	744	771	0.0000988
LRR	773	800	7.25
LRR	801	828	0.00000154
LRR	830	857	2.64
LRR	858	885	0.00295
LRR	887	914	0.588
LRR	915	942	0.00000873
LRR	944	971	6.36
LRR	972	999	0.0000284
LRR	1001	1028	0.499
LRR	1029	1056	0.000521

Phylogenetic analysis

Neighbor-joining method algorithm was used for the construction of phylogenetic tree. This tree was constructed by using deduced amino acid translated from coding sequence of NLRP12. Branch I and II two major branches were obtained in this study. Most of the species lies in Branch I whereas, Branch II contains only one species, i.e. mouse. Similar type of clustering was found in buffalo NOD-like receptor1 gene (Mishra *et al*, 2019). Branch I further divided in two sub branches namely branch A and B. Branch A cluster had buffalo, cattle, sheep, goat, horse, gorilla, human, camel and pig, whereas branch B had only one species that is dog. These findings suggest that pig NLRP12 contains closest orthologous sequence to camel NLPR12 gene with cluster A and they share a common ancestry where as, it was found distantly related to Branch II that having mouse NLRP12 sequence (Fig 4).

This study comprises of identification and characterisation of full-length ORF of the cDNA sequence of *Camelus dromedarius* NLRP12. Comparative sequence analysis showed that camel NLRP12 sequence share high similarity to pig, goat, sheep and human. Comparative domain analysis suggest conserved nature of functional domains and further work is required to determine whether less number of LRRs have significant contribution in cattle immune responses. The determination of domain architecture and phylogenetic analysis may be useful for studies on evolutionary lineages and immune responses for various infections in camel.

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THE CAMELS, HUMANS AND BOVINES HAEMOGLOBIN: IN SILICO AND MOLECULAR DYNAMICS PERSPECTIVE AND BINDING POTENCY WITH HAEME

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ABSTRACT

This study compared the molecular dynamics (MD) of haemoglobin (Hb) bound to haeme in humans, camels, and bovines. The camel haemoglobin alpha and beta subunits showed larger amino acid differences, compared with bovine and human Hb. The bovine Hb was more phylogenetically related to human Hb than camel Hb. The camel haemoglobin structure complexed with haeme showed the highest stability by showing the lowest root mean square deviations (RMSD) and root mean square fluctuations (RMSF), compared with bovine and human structures. The Molecular Mechanics/Poisson Boltzman Surface Area (MM/PBSA) method was used to estimate the binding potency of haeme with the studied Hbs. The binding free energy of haeme was calculated to be -688.062 for bovines, -897.019 for camels, and -585.291 for humans. As a consequence, camel Hb had the highest binding potency, followed by bovines, and then humans. The structure and binding features of camel Hb contributes to its role in adaptation to dehydration and the harsh environment by adopting of higher affinity for haeme.

Key words: Camel, haemoglobin, haeme, molecular dynamics

Erythrocytes, red blood cells (RBCs), contain a significant amount of the haemoglobin molecule. It is a type of oxygen-carrying protein that is responsible for giving blood its distinctive red colour. Haemoglobin in adult vertebrates is made up of four different protein chains, two of which are alpha chains and the other two being beta chains (Storz, 2018). In camel haemoglobin from Asian and African camels, the amino acid His2 binds to 2,3-diphosphoglycerate (DPG) and substantially decreases the oxygen affinity. However, in camel haemoglobin from Andean camelids, a replacement of the amino acid at second position of β-chain (His2-to-Asn) increases affinity for oxygen by suppressing binding of DPG. This aids in adaptation of those small camelids to high altitudes. The vicuna is the camelid species native to greatest elevational zone in the Andes (4,500–5,000 m), and it also has the highest blood oxygen affinity (P50 = 17.5) of all the Andean camelids. A mutation from *Ala* to *Thr* at position α-Ala130 and a substitution from *His* to *Asn* at position β-His2 are thought to be responsible for exceptionally high oxygen affinity of vicuna haemoglobin (Storz, 2007). This may be the reason why camels prefer to dwell in lowland areas.

Camelus dromedarius haemoglobin is an intriguing case study of adaptability to live in deserts at extremely high temperatures. Camelids that live at different altitudes survive in a variety of climates and environments. The structural analysis of camelid haemoglobin allows researchers to link oxygen affinity to adaptation to extremely high and dry settings (Balasubramanian *et al*, 2009).

Erythrocytes in animals have a spherical or concave form, which has been extensively proven. Camels, on the other hand, have very enucleated, ovaloid erythrocytes that are exceedingly tiny, flat, and in vast numbers. The RBC of a camel is a great example of an organism's ability to adapt to harsh environments. It is important to note that haemoglobin found in camels is very enormous for an animal of its size (camel haemoglobin is around the size of a pea). Camel RBCs are alls exceptionally resistant to osmotic haemolysis and that they may grow to 240% of their usual volume before rupturing (Adah *et al,* 2016; Al-Bassam *et al,* 2007; Oyewale *et al*, 2011).

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This study was conducted to compare the molecular dynamics of human, camel and bovine Hb bound with haeme. The aspects of comparison comprise the structure stability, Hb backbone fluctuations, structure compactness, the number of hydrogen bonds between Hb and haeme during simulations and strength of binding between Hb and haeme in different structures. The results of this study will shed new insights into the differences in interactions of Hb with haeme in humans, camel and bovine.

Materials and Methods

Retrieval of haemoglobin chains sequences

The sequences utilised in this work were obtained from the GenBank database and the protein database, which are found at (https://www.ncbi.nlm.nih.gov/). Human, dromedary camel, and bovine sequences were retrieved. CLC genomics software was used to import the alpha and beta chain sequences (Qiagen software, Denmark).

Multiple sequence alignment and phylogenetic tree

The sequences of alpha and beta Hb chains were aligned by the sequence alignment tool and the tree was generated by CLC genomic software.

Molecular dynamics simulations

Molecular dynamics simulations set up and conditions were performed as previously described with slight modifications (Kandeel *et al,* 2018; 2021). GROMACS simulation package (GROMACS 2020.4) was used to perform molecular dynamic dynamics simulations. MD simulation of alpha chain of Hb was carried out for 100 ns in water using CHARMM36 forcefield; trajectory and energy files were written every 10 ps. The system was solvated in a truncated octahedral box containing TIP3P Water molecules. The protein was centered in the simulation box within a minimum distance to the box edge of 1 nm to satisfy the minimum image convention efficiently. Potassium/chlorine ions were added to the complex to neutralise the overall system. Minimisation was carried out for 5000 steps using Steepest Descent Method, and the convergence was achieved within the maximum force < 1000 (KJ mol-1 nm-1) to remove any steric clashes. All systems were equilibrated at constant- volume and constant- temperature (NVT) and isobaric–isothermal (NPT) ensembles for 100ps (50,000 steps) and 1000ps (1,000,000 steps), using time steps 0.2 and 0.1 femtoseconds (fs), respectively, at a temperature of 300 °K to ensure a fully converged system for the production run which was carried out at a constant temperature of 300 °K and a pressure of 1 atm or bar (NPT) using a weak coupling velocity-rescaling (modified Berendsen thermostat) Parrinello-Rahman algorithms, respectively. Relaxation times were set to τ T = 0.1 ps and τ P = 2.0 ps. All bond lengths involving the hydrogen atom were kept rigid at ideal bond lengths using the Linear Constraint Solver (lincs) algorithm, allowing for a time step of 2 fs. The Verlet scheme was used for the calculation of non-bonded interactions. Periodic Boundary Conditions (PBC) were used in all x, y, z directions. Interactions within a short-range cutoff of 1.2 Nm were calculated in each time step. Particle Mesh Ewald (PME) was used to calculate electrostatic interactions and forces to account for a homogeneous medium outside the long-range cutoff. The production was run for 100ns for the complex.

Binding energy calculations

Molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) was used in the study of biomolecular interactions and computational drug design. MM-PBSA binding energy of chosen molecules was calculated *via* g mmpbsa. This tool calculated the enthalpic components of MM-PBSA interaction using GROMACS and APBS packages.

Results and discussion

Comparison of humans, dromedary camel and bovine Hb

Multiple sequence alignment was generated for the Hb sequence from humans, camels and bovines (Figs 1 and 2). In the alpha subunit (Fig 1), the camel Hb beta subunit showed 21 and 23 amino acid differences with bovines and humans Hb, respectively. This accounts for 87.2 and 83.6 identity%. In the beta subunit (Fig 2), the camel Hb alpha subunit showed 25 and 24 amino acid differences with bovines and humans Hb, respectively. This accounts for 84.1 and 82.8 identity%. In both subunits, the camel proteins were more distant to humans, compared with the bovine proteins.

Several studies have found that the distal histidine residues α:His58 and β:His63 play an important role in haeme binding (Ali *et al*, 2022; Jameson *et al*, 1980). They are also important in controlling the rate and affinity of oxygen binding to human haemoglobin. These residues were conserved in human, camel and bovine Hb (Figs 1 and 2).

Root mean square deviations (RMSD)

RMSD was calculated for the complex based on 'Backbone' atoms using GROMACS program.

RMSD graph (Fig 3, Column A) for protein complex showed that the structure remained stable throughout the simulation time with some fluctuation within the range of ~1 Å, which is a normal behaviour of globular protein. Ligand RMSD was calculated for the ligand-based on the ligand's atoms using GROMACS program and it is shown in Fig 3, column A. RMSD of ligand remained reasonably stable and throughout the simulation for all 3 complexes. The camel structure complex showed highest stability by showing the lowest RMSD, compared with bovine and human structures (Fig 3A). The RMSD profile of Haeme was lower than the protein during all simulation time, which was lower than the protein RMSD, indicating stable binding with the examined proteins.

These findings agree with the previously reported camel Hb structure stability. At different conditions of temperature and salt, camel Hb was more stable than the human structure (Ali *et al*, 2022). The camel Hb showed higher stability over bovine and human structures.

Root mean square fluctuations (RMSF)

RMSF was calculated for protein complex based on 'C-alpha' atoms using GROMACS program. Overall, the fluctuation intensity remains below 2.5 Å except for some residues which represent a loop or turn in the protein (Fig 3, Column B). In this context, the camel protein residues showed the lowest RMSF value indicating the stability of complexes with Haeme.

Radius of gyration (ROG)

The radius of gyration was calculated for the complex based on 'C-alpha' atoms using GROMACS program. The slight fluctuation within the 1 Å Rog value during the MD simulation time indicates a slight opening and closing of the N and C terminal domains (Fig 3). This indicates the general compactness of the examined systems.

Hydrogen bonds (Hb-haeme)

The total number of hydrogen bonds formed between ligand and protein during 100 ns of the

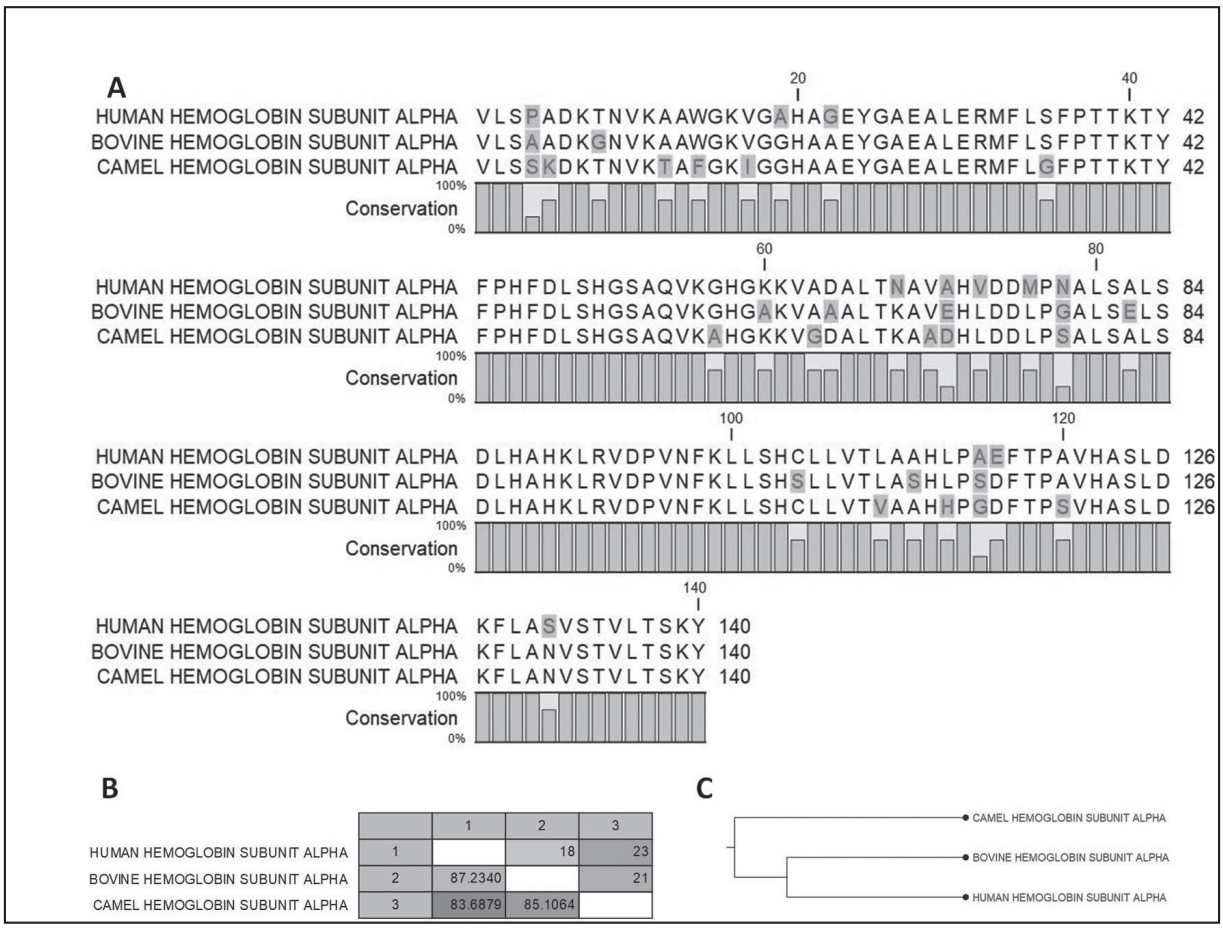

Fig 1. Sequence comparisons of Bovine, Camel and Human HB Alpha subunit. A) Pairwise sequence alignment. The different residues are highlighted. B) Pairwise sequence comparison. The upper right panel is the number of differences. The lower left panel is the percent identity. C) Phylogenetic relations of Bovine, Camel and Human HB Alpha subunit.

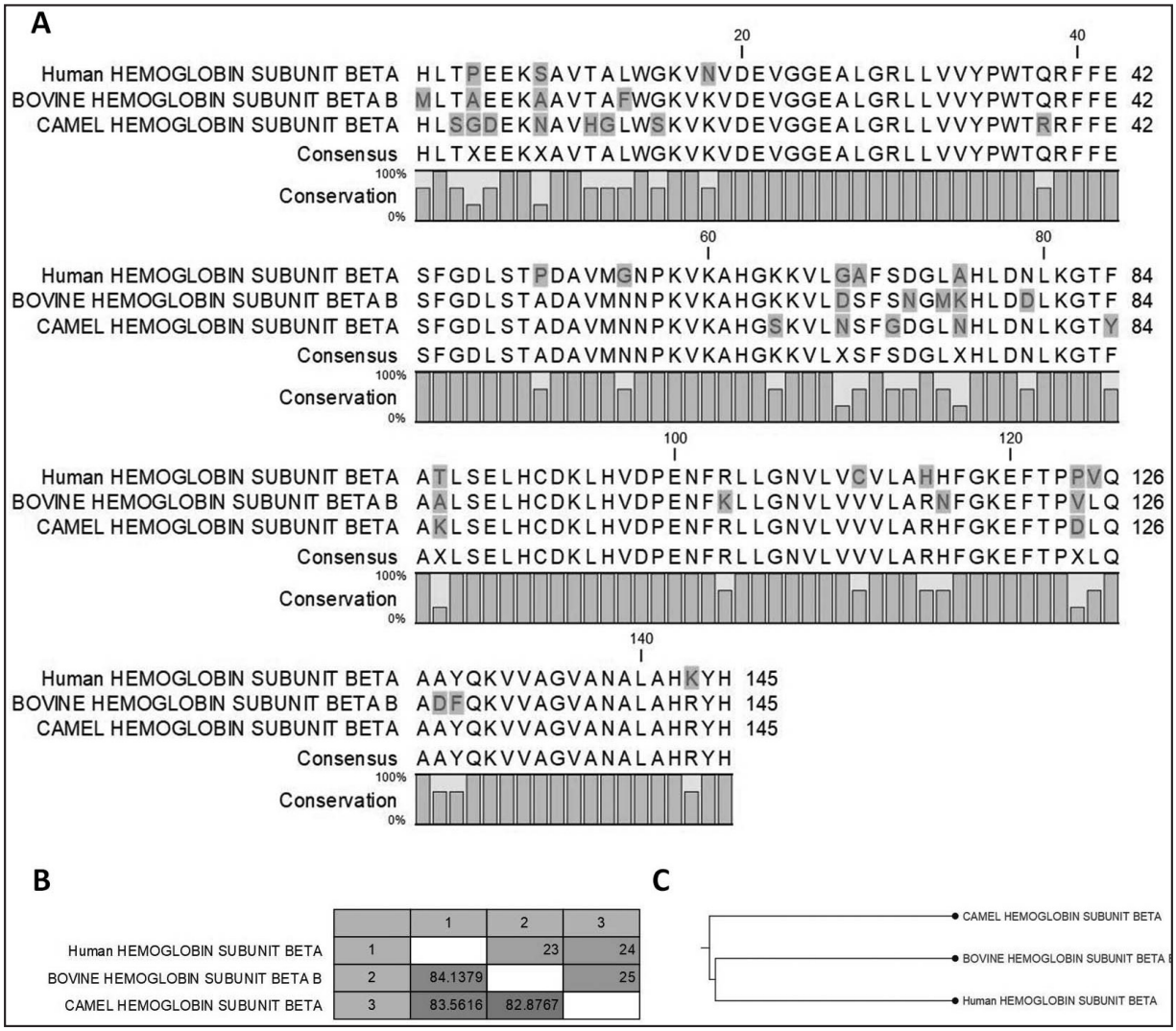

Fig 2. Sequence comparisons of bovine, camel and human HB Beta subunit. A) Pairwise sequence alignment. The different residues are highlighted. B) Pairwise sequence comparison. The upper right panel is the number of differences. The lower left panel is the percent identity. C) Phylogenetic relations of Bovine, Camel and Human HB Alpha subunit.

simulation time is shown in (Fig 4). A conserved average of one Hbond was observed in all examined structures. Therefore, there are almost conserved features of the binding space around haeme in the studied structures.

Average center-of-mass distance

The average center-of-mass distance between ligand and protein during 100 ns of the simulation time is shown in (Fig 4). There was no observable difference in human, camel and bovine structures.

Contact frequency (CF) analysis

To further evaluate the binding between the protein and the ligands, a contact frequency (CF) analysis was performed using contact free.tcl module in Visual Molecular Dynamics (VMD) with a cutoff of 4 Å. The residues with higher CF% are shown in (Fig 5). The camel protein showed higher contact frequency with PHE98, HIS45, ASN97 and LEU129. There was almost conserved contact time with important residue α:His58, implying almost conserved binding with this residue. The residues α:Phe43 and α:Phe98 showed higher contact frequency with haeme in camels than in human or bovine Hb (Fig 5). This agrees with the previously described role of these residues in sustained interaction with haeme at extreme salinity and temperatures (Ali *et al*, 2022).

Potential energy, pressure and temperature

The potential energy, pressure, and temperature of the system during 100 ns of MD simulation as obtained from GROMACS edr file are shown in (Fig 6). The graph shows converged potential energy,

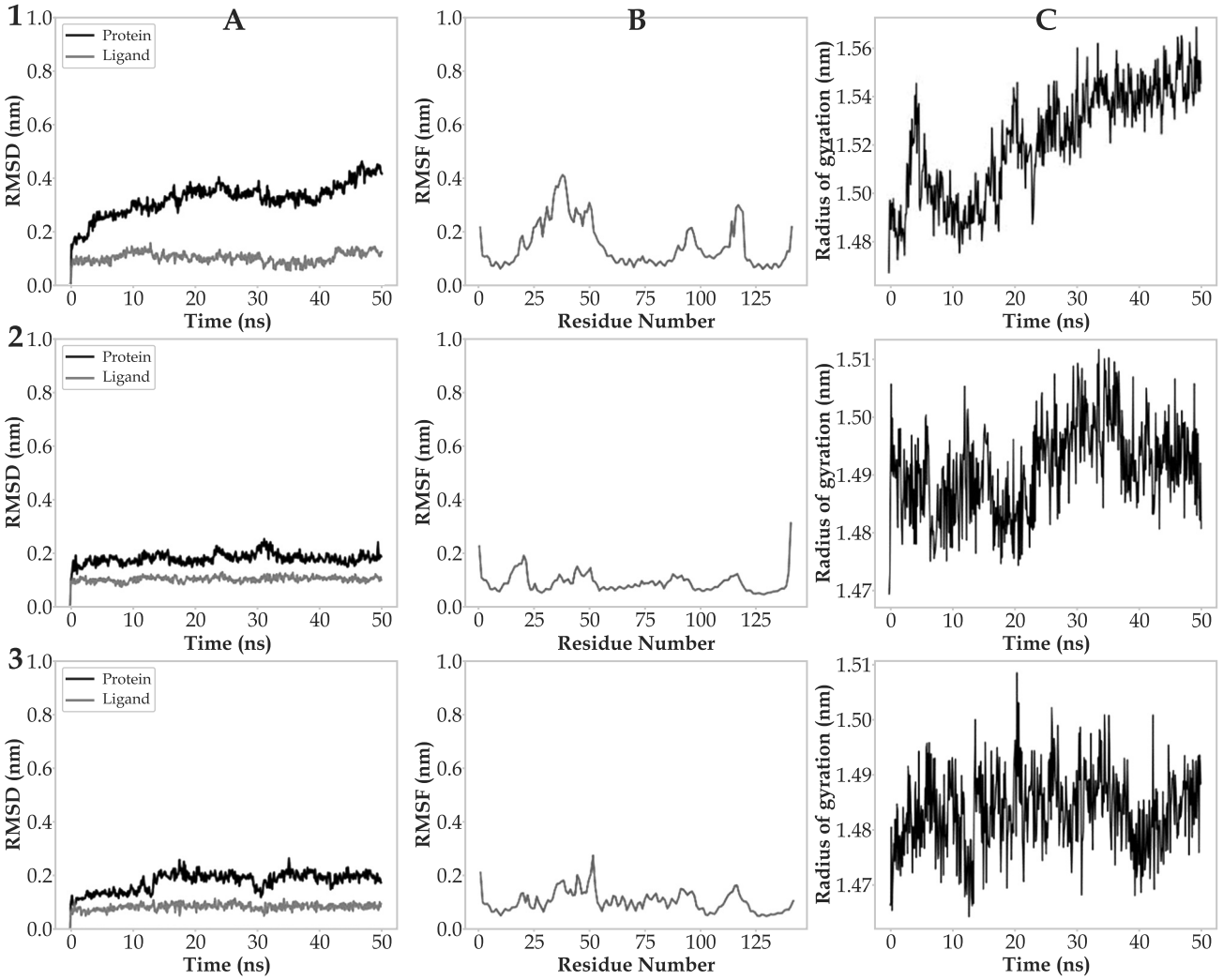

Fig 3. RMSD of the bovine, camel and human HB bound with Haeme. From left to right: (A) RMSD, (B) RMSF and (C) Radius of gyration of the complexes during 100ns MD simulation. Bovine (top), Camel (middle) and Human (bottom).

pressure, and temperature throughout the 100ns simulations.

MM/PBSA binding energy

MM/PBSA method was selected for rescoring complexes because it is the fastest force field-based method that computes the free energy of binding, as compared to the other computational free energy methods, such as free energy perturbation (FEP) or thermodynamic integration (TI) methods. The MM/PBSA calculation was performed using g-mmpbsa software. The calculated binding free energies are shown in table 3.

The binding capacity of the camel protein with haeme was found to be the highest. It was estimated that the binding free energy of haeme was -688.062 ± 58.805 for bovine, -897.019 ± 42.708 for camel, and -585.291 ± 70.592 for humans, respectively. As a result, the binding potency was recurod highest in camel Hb, followed by bovines, and then humans.

In conclusion, after 100 ns simulation, the camel Hb showed lower backbone RMSD, RMSF and higher binding potency with haeme. The stronger haeme binding with Hb indicates stronger oxygen binding power, higher oxygen transport and storage capacity and higher tissue oxygen delivery. These features support the higher structural competency of camel Hb to adapt to adverse conditions.

Acknowledgements

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Table 3. Calculated binding free energies of Haeme binding with Bovine, Camel and Human HB [kJ/mol].

Complex	ΔG	van der Waal energy	Electrostatic energy	Polar solvation energy	Solvent-accessible surface area (SASA) energy
Bovine	-688.062 ± 58.805	-246.185 ± 14.942	-857.242 ± 171.840	443.664 ± 127.511	-28.298 ± 1.935
Camel	-897.019 ± 42.708	-264.881 ± 4.544	-862.527 ± 82.713	257.009 ± 78.213	-26.619 ± 0.844
Human	-585.291 ± 70.592	-257.899 ± 10.052	-552.638 ± 174.680	251.729 ± 106.498	-26.482 ± 1.453

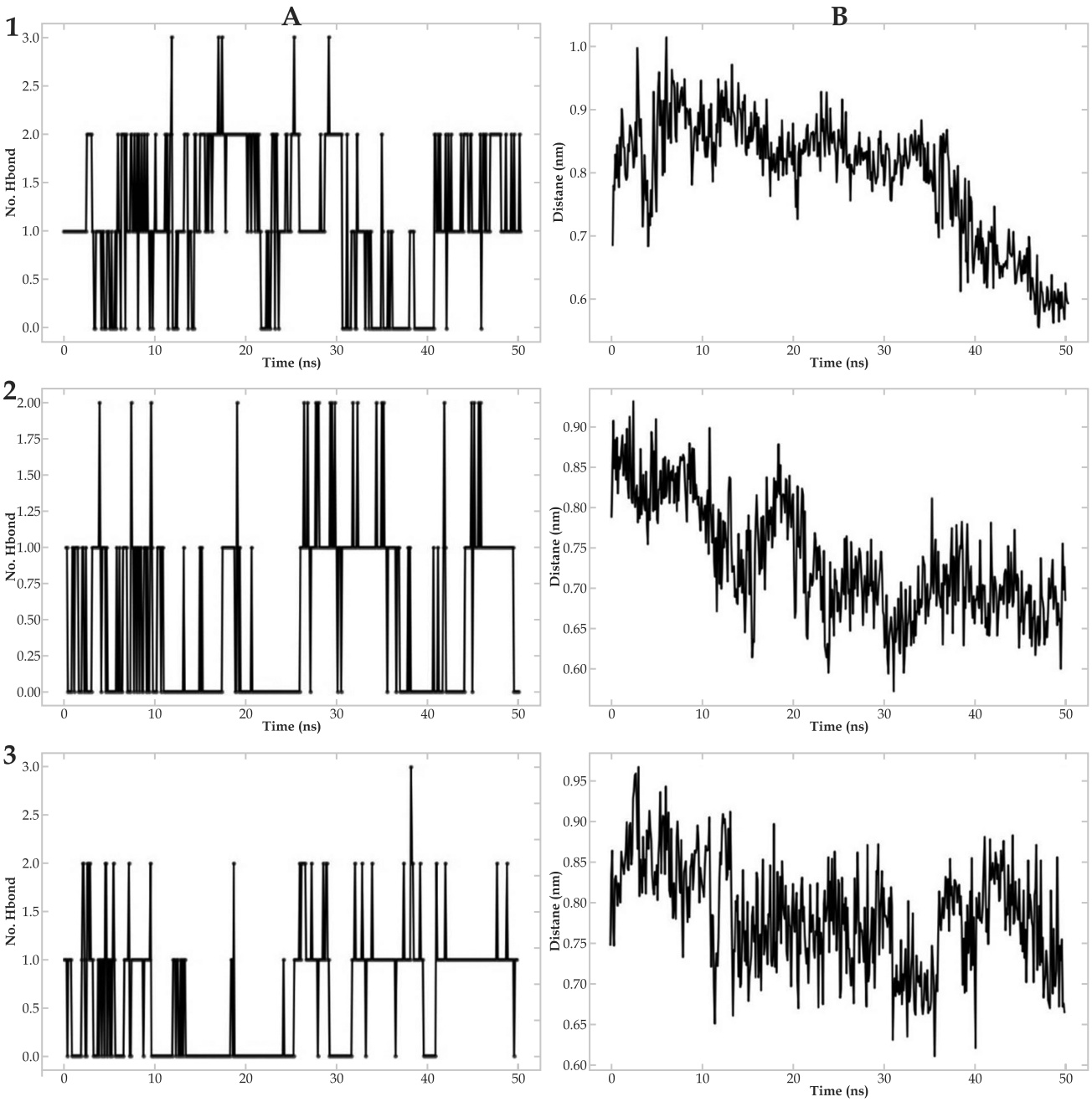

Fig 4. Hydrogen bonds and distance of the bovine, camel and human HB bound with haeme. (A) Hydrogen Bonds (Protein-ligand) and (B) Average distance between ligand and the protein for of the complexes during 100ns MD simulation. Bovine (top), Camel (middle) and Human (bottom).

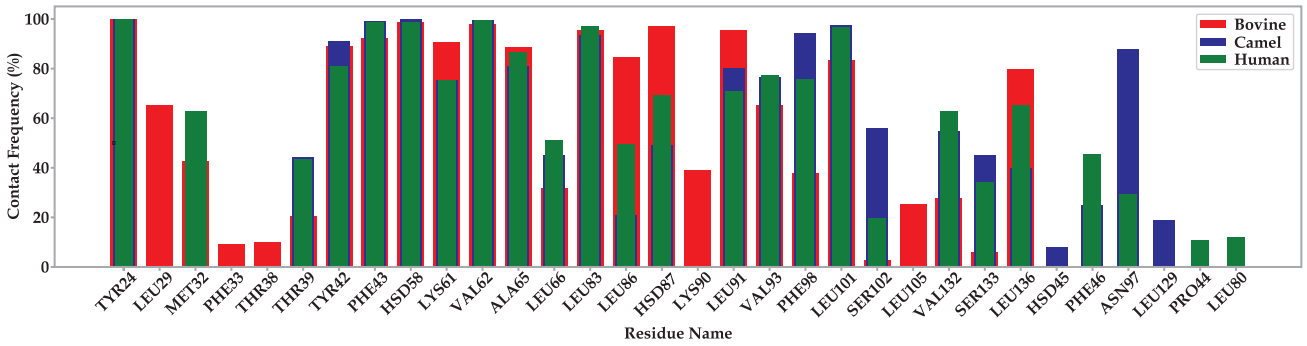

Fig 5. Contact frequency (CF) analysis of Bovine, Camel and Human HB with Haeme.

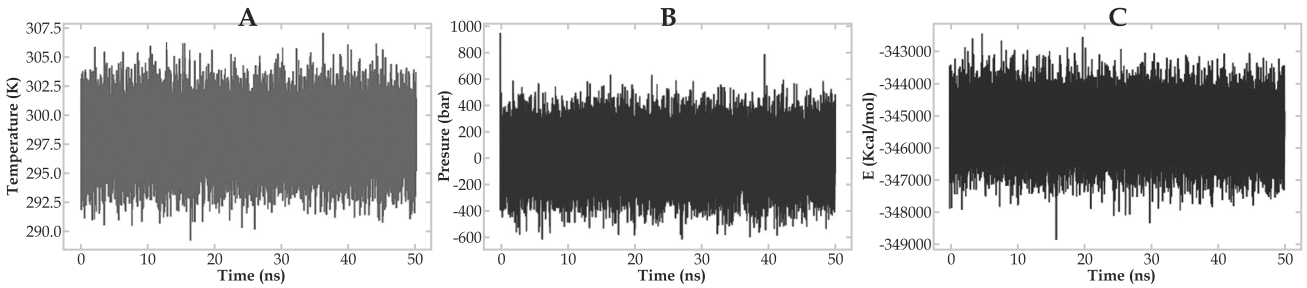

Fig 6. Potential energy, pressure and temperature during MD simulation. (A) temperature, (B) pressure and (C) potential energy during the 100ns MD simulations.

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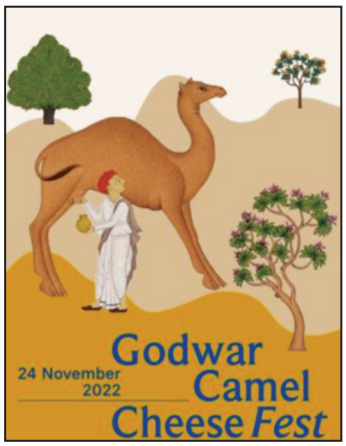
JANVRY, SECOND FESTIVAL OF CAMELIDS IN FRANCE

The festival of camelids is a public event organised for the second time in a small village of Essonne (France) gathering all the actors of the camelid (small and large) sector, not only in France, but also in Europe (Spain, Italy, Austria, Belgium), in USA and some "camel countries" as Morocco, Algeria, Mauritania, Chad, Niger, Libya, Saudi Arabia, Kazakhstan, Mongolia, and others…. The festival included conferences, camel show, degustation and selling of camelid products (pasteurised and powder milk, kefir, cheese, sweet, cosmetic, wool). In addition, many exchanges occurred between large public and camel farmers, veterinarians, and camel scientists involved in different European projects as CAMELMILK or CAMELSHIELD. Among the conferences, 3 were particularly focused on generic research with different topics as "Is the camel conquering the world?" (B. Faye, France), "The secrets of the camel genetic" (E. Ciani, Italy), "Truth and legends on camel milk" (G. Konuspayeva, Kazakhstan). Several thousand visitors came on the festival which had an important mediatic covering.

GODWAR CAMEL CHEESE FEST HELD IN SADRI, INDIA

Camel Charisma and LPPS (Lokhi Pashu Palak Sansthan) jointly organised the Godwar Camel Cheese Fest from 23-24 November 2022 at the site of the Kumhalgarh Camel Dairy near Ranakpur-Sadri. This was aimed to showcase the culinary potential and diversity of camel dairy products. It included meeting of camel breeders from all over Rajasthan to understand and discuss their prespective. Second day included a visit to the nomadic herd to have a taste of fresh camel milk in oak leaf and to enjoy the camel milk tea brewed over the fire. Later in day, camel cheese tasting was planned. The concept of a cruelty free dairy was also planned to discuss. The event was organised by the Hanwant Singh Rathore and Dr. Ilse. (www.camelcharisma.com).



LEAD SPEAKER IN THE CONFERENCE OF SAUDI VETERINARY MEDICINE SOCIETY

Dr T.K. Gahlot Editor JCPR was Invited as a keynote speaker in the conference of Saudi Veterinary Medicine Society at King Faisal University in Al Ahsa, Saudi Arabia from 11-13 October 2022. He delivered a lead paper on "An Overview of Camel Surgery" in inaugural session of the conference. He also made a visit to the College of Veterinary Medicine, KFU, Al Ahsa.

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DIAGNOSTIC AND PREDICTIVE SIGNIFICANCE OF ACUTE PHASE RESPONSE AND NEOPTERIN LEVELS IN LAME RACING DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The lameness of racing camels is a major health concern and a financial burden for many camel owners. This study was aimed to investigate different acute phase proteins, cytokines (CYTs), and neopterin (NPT) in the blood of racing lamed camels to highlight their role in disease diagnosis, pathogenesis and monitor treatment response. A 35 out of 315 racing camels exhibited clinical lameness. The mean serum levels of serum amyloid A (SAA) and CYTs (TNF-α, IL-1α, IL-1β, and IL-6) in lame dromedary camels with different detected causes (punctured foot and traumatic injury) were remarkably over than those detected in control healthy dromedary camels. It was found that lame dromedary camels had non-significant changes in serum levels of haptoglobin (HP) and NPT when compared with control healthy camels. A dramatic decline was detected in serum levels of SAA and CYTs of lame camels after 10 days of treatment whereas, the levels of HP and NPT remain at the same levels without any significant changes. The ROC curves were created for the tested biomarkers. The AUCs were calculated to evaluate each variable's accuracy to differentiate diseased from healthy camels. Based on the ROC curves and AUCs, both the SAA and CYTs provide similar and highly accurate diagnostic accuracy (AUC > 0.8) and monitoring of lameness treatment response.

Key words: Camel, cytokines, haptoglobin, lameness, neopterin, serum amyloid A

Lameness in camels can be caused by a variety of factors and extensively diverse causes including direct trauma, nutrition, fractures, punctured feet, abnormal limb conformation, and infection (Gahlot, 2007; Al-Juboori, 2013). Gahlot (2007) diagnosed lameness in camels, clinically manifested as partial or non-weight bearing by one or more limbs, swelling over joints, pain on palpation, toe-out postures, shivering of hind quarters while sitting, semi-flexed hocks in sitting postures, and an asymmetrical pelvis. Camels with lameness suffer substantial economic losses in the form of decreased milk production, decreased reproductive performance, and growth retardation, culling from competition or farms, decreased physiological vitality of the camels and additional treatment and care costs (Al-Juboori, 2010). Compared to cattle and horses, camels have a different pattern of lameness attributed to its peculiar anatomy, biomechanics, geo-climatic adaptation, and use (Gahlot, 2000).

Acute-phase response (APR) is a seditious response of the host in response to tissue damage. APR acts through pro-inflammatory cytokines (CYTs) such as interleukin-6 (IL-6) and acute phase proteins (APPs), confining microbial growth and maintaining homeostasis (Murata *et al,* 2004). During APR, acute phase proteins help the humoral and cell-mediated immunity elements from causing gratuitous damage to the host cells (Rossbacher *et al,* 1999). Because of various diseases, higher levels of bovine and ovine APPs (serum amyloid A; SAA and haptoglobin; HP) have been detected in the blood of diseased animals. These diseases include lower respiratory tract disease (El-Deeb and Elmoslemany, 2016; El-Deeb *et al,* 2020), mastitis (El-Deeb, 2013; El-Deeb *et al,* 2021) and hoof disorders (Ilievska *et al,* 2019; Kontturi *et al,* 2019). Moreover, APR was previously detected in camels with urinary tract infection (El-Deeb and Buczinski, 2015), *Trypanosoma evansi* infection (El-Deeb and Elmoslemany, 2015) and camels infected with *Coxiella burnetii* (El-Deeb *et al,* 2019). There was also evidence of higher cortisol levels, and a higher cortisol to dehydroepiandrosterone ratio in lame cows, which

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has been linked to inflammation (Almeida *et al*, 2008). A common method of testing the degree of inflammatory processes that may result from physical trauma, cardiovascular disorders, cancer, bacterial, parasitic, and viral infections is to measure neopterin concentrations in the urine and blood (Melichar *et al*, 2017).

The study of APR and neopterin (NPT) levels in camels against lameness is poorly understood. Accordingly, this study was aimed to investigate different APPs and NPT in blood of racing lame dromedary camels to highlight their role in disease diagnosis, pathogenesis, and to monitor treatment response.

Materials and Methods

Animal selection and sampling protocol:

A total of 315 racing dromedary camels, aged 5.5-8.1 years, were investigated between January and September 2019 in one herd in the eastern region, Saudi Arabia. Thirty-five of these camels exhibited recent acute clinical lameness. Punctured foot and traumatic hoof injuries were diagnosed as lesions accountable for clinical lameness in these racing camels. Bovine hoof tester and walk on sandy, pebbled, or hard tracks (Gahlot, 2000; 2007) were used to diagnose the camel foot lameness. Moreover, 20 clinically healthy racing dromedary camels with no lameness signs were included in control group. Whole blood samples were collected from the jugular vein of clinically lame dromedary camels (N = 35) and control healthy group (N= 20). Blood samples were centrifuged, serum separated and stored at -20°C for further biochemical analysis. Lame dromedary camels were subjected to treatment protocol including the use of non-steroidal anti-inflammatory drugs (NSAIDs) (2.2mg flunixin per kg body weight, IV injection), cleaning and disinfection of lesions for 5 consecutive days with complete rest.

Acute-phase proteins (APPs): Test kits (Tridelta Development Ltd., Kildare, Ireland) were used to measure haptoglobin (HP) and serum amyloid A (SAA) in serum samples.

Proinflammatory cytokines (CYTs): In order to estimate CYTs concentrations (TNF-α, IL-1α, IL-1β, and IL-6) in serum, commercial ELISAs (MyBioSource, San Diego, USA) were used according to the manufacturer's recommendations.

Neopterin (NPT): Commercial ELISA kit for measuring NPT concentrations in camel serum (Bovine NPT (Neopterin) ELISA Kit, Fine Test, Wuhan Fine Biotech, Wuhan, China) were used.

Data Analysis:

Comparisons in mean were performed by Kruskal–Wallis ANOVA on Ranks followed by Dunn's multiple comparisons. The different means were significant at $P<0.05$. Statistical analysis was performed using JMP software version 11.0.0 (SAS Institute, Cary, NC, USA). Graphpad Prism v5 software (Graphpad Software, Inc., San Diego, CA) was used to draw the figures. The correlation between parameters was evaluated using Spearman's rank correlation test. Each assay's diagnostic accuracy was evaluated by creating the ROC (receiver operator characteristics) curve and determining the area under the curve (AUC). An AUC of 0.7 to 0.9 was considered moderately accurate, an AUC of >0.9 highly accurate, and an AUC of 1 perfect (Gardner and Greiner, 2006).

Results and Discussion

The mean serum levels of SAA and CYTs(TNF-α, IL-1α, IL-1β, and IL-6) in lame dromedary camels with different detected causes (punctured foot, traumatic injury) were remarkably ($P < 0.0001$) over than those detected in control healthy dromedary camels (Fig 1). However, it was found that lame dromedary camels had non-significant changes in serum levels of HP ($P < 0.37$) and NPT($P < 0.75$) when compared with control healthy camels (Fig 1). The serum levels of APPs, NPT, and CYTs markers in lame dromedary camels' pre-and post-treatment was measured in this study. A dramatic decline ($P < 0.0001$) was detected in serum levels of SAA and CYTs of lame camels after 10 days of treatment, whereas the levels of HP ($P < 0.72$) and NPT ($P < 0.82$) remain at the same without any significant changes (Fig 2). Spearman's correlation was estimated for the study biomarkers in clinically lame camels and healthy ones and in lame camels before and after 10-days of treatment (Table 1). A positive significant correlation exists between SAA and all tested CYTs (TNF-α, IL-1α, IL-1β, and IL-6).

The diagnostic test characteristics of APPs and CYTs parameters in camel with lameness were presented in table 2 while the ROC curves were created as presented in Fig 3. The AUCs were assessed to evaluate the accuracy of each variable to distinguish diseased and healthy dromedary camels. Based on the ROC curves and AUCs; SAA ($AUC\geq0.957$) and CYTs (AUCs ranged from 0.872 for IL-1α to0.927 for IL-1β) were highly diagnostic and predictive for treatment response in lame camels. Conversely, HP and NPT showed a poor diagnostic

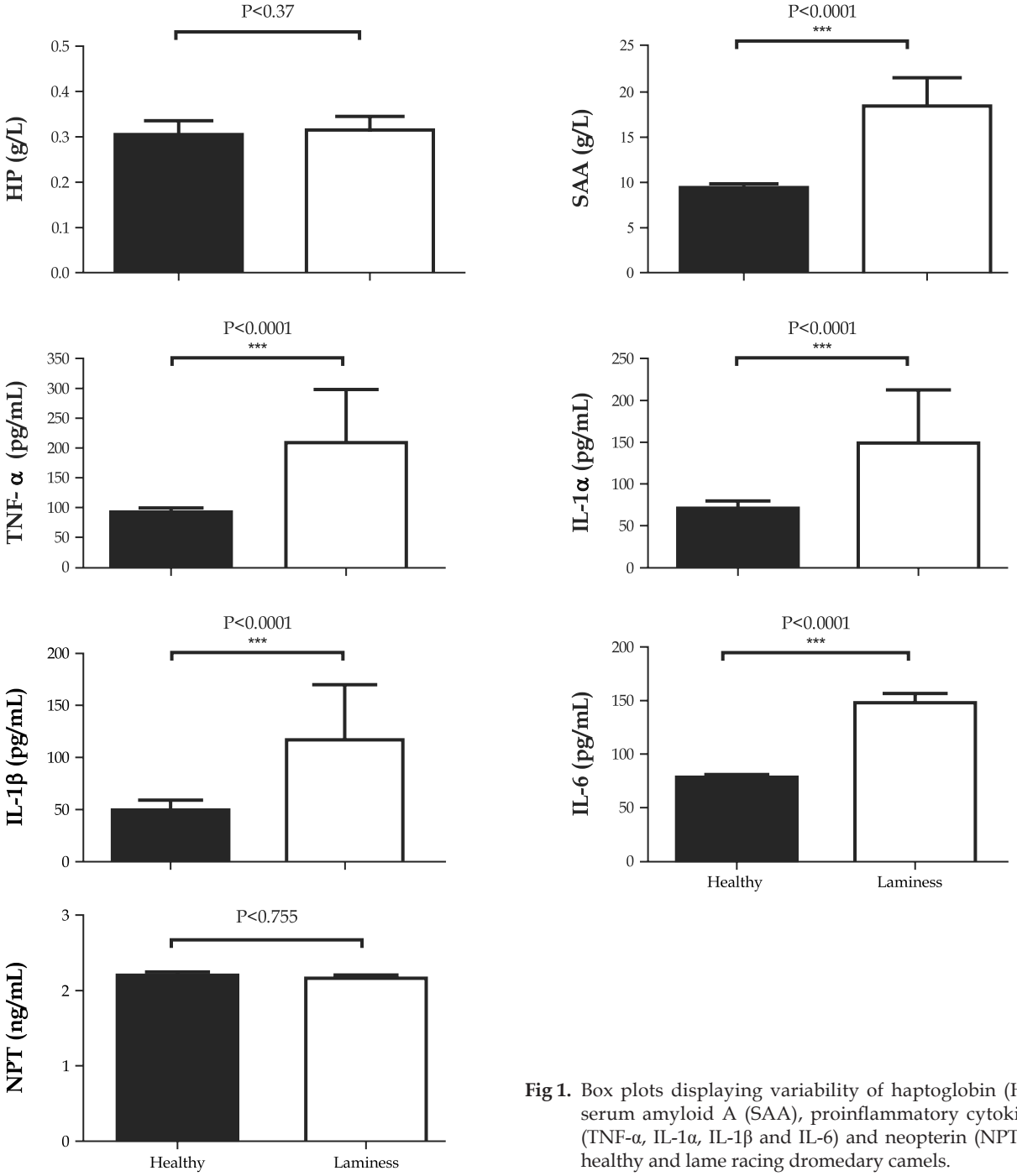

Fig 1. Box plots displaying variability of haptoglobin (HP), serum amyloid A (SAA), proinflammatory cytokines (TNF-α, IL-1α, IL-1β and IL-6) and neopterin (NPT) in healthy and lame racing dromedary camels.

ability to distinguish lame dromedary camels from control healthy ones and to evaluate the treatment response.

APR is a seditious response of the host in response to tissue damage. In this study, The SAA of the lame dromedary camels was the only APPs measured at higher levels than that of the control camels. Our findings are in harmony with other research studies that have also reported elevated SAA values for lame cows as well (Kujala *et al,* 2010; Tóthová *et al,* 2011). The levels of SAA were returned to normal levels 10 days after treatment with non-steroidal anti-inflammatory drugs and other lameness treatment protocols. It was suggested that mere pain may lead to an elevation of SAA concentration in the circulation (Tóthová *et al,* 2011), and this was supported by research work on human SAA (Zhang, 2007). In light of these results, we propose that lameness in camels can cause systemic APR, evident by the higher SAA levels. It was previously reported

that blood SAA concentration is related to the degree of tissue damage (Murata *et al,* 2004).

Monitoring, the healing advancement of lameness in dromedary camels is laborious. However, in present study, the alterations in tested biomarkers were estimated after 10 days of treatment. A marked decline in SAA was reported in lame racing camels after treatment protocol toward normal control values. Consequently, SAA could be used to monitor the healing process, the treatment of lame dromedary camels were evaluated and adjusted in a better way.

Unexpectedly, in this study, HP showed non-significant changes in the serum levels of lame dromedary camels when compared with control ones. The levels of HP in control healthy camels are in harmony with those reported by Nazifi *et al* (2006) and El-Deeb *et al* (2019). In contrast to findings in the present study in racing lame camels, HP has been recognised as a valuable biomarker of ruminant diseases where it offers further data to the classical haematological studies (Skinner *et al,* 1991). HP has been evenly recognised in calves' serum and broncho-alveolar lavage with experimental and clinical pasteurellosis (Katch *et al,* 1999; El-Deeb *et al,* 2020). In the same concern and in contrast to our results, high HP levels were detected at higher serum levels in 60 dairy cows with clinical lameness due to septic pododermatitis, pododermatitis circumscripta, interdigital necrobacillosis, and papillomatous digital dermatitis lesions (Smith *et al,* 2010). The authors concluded that lameness because of claw infections could be allied with a significant systemic APR and higher serum HP levels in cattle. Moreover, the authors mentioned that HP levels seemed effective for all claw disorders except for pododermatitis

Table 1. Correlation matrix among different acute phase proteins (HP and SAA), cytokines and neopterin in healthy and lame dromedary racing camels (20 control and 35 lame camels).

Parameter[a]	HP	SAA	TNF-α	IL-1α	IL-1β	IL-6	NP
HP	1.000						
SAA	0.197	1.000					
TNF-α	0.179	0.375	1.000				
IL-1α	0.008	0.411	0.440	1.000			
IL-1β	0.069	0.572	0.586	0.458	1.000		
IL-6	0.147	0.397	0.667	0.461	0.523	1.000	
NP	0.159	-0.042	-0.045	0.173	-0.063	-0.012	1.000

[a]HP, haptoglobin; SAA, serum amyloid A; TNF-α, tumour necrosis factor alpha; IL-1α; interleukin 1-alpha; IL-1β, interleukin 1-beta; IL-6, interleukin 6; NPT, neopterin.

Table 2. Diagnostic test characteristics of acute phase proteins, cytokines and neopterin in racing dromedary camels with clinical lameness.

Parameters [a]	Threshold	Diagnostic characteristics (%)		
		Se (95% CI)	Sp (95% CI)	AUC
HP	≥ 0.35	0.20 (0.08% to 0.36%)	0.80 (0.56% to 0.94%)	0.569
SAA	≥ 12.75	0.91(0.76% to 0.98%)	1.00 (0.83% to 1.00%)	0.957
TNF-α	≥ 114.80	0.80 (0.63% to 0.91%)	0.85 (0.62% to 0.96%)	0.907
IL-1α	≥ 108.30	0.77 (0.59% to 0.89%)	1.00 (0.83% to 1.0%)	0.872
IL-1β	≥ 88.74	0.74 (0.56% to 0.87%)	0.95 (0.75% to 0.99%)	0.927
IL-6	≥ 115.8	0.82 (0.66% to 0.93%)	0.90 (0.68% to 0.98%)	0.912
NP	≤ 2.70	0.08 (0.01% to 0.23%)	0.95 (0.75% to 0.99%)	0.515

[a] HP, haptoglobin; SAA, serum amyloid A; TNF-α, tumour necrosis factor alpha; IL-1α; interleukin 1-alpha; IL-1β, interleukin 1-beta; IL-6, interleukin 6; NPT, neopterin.

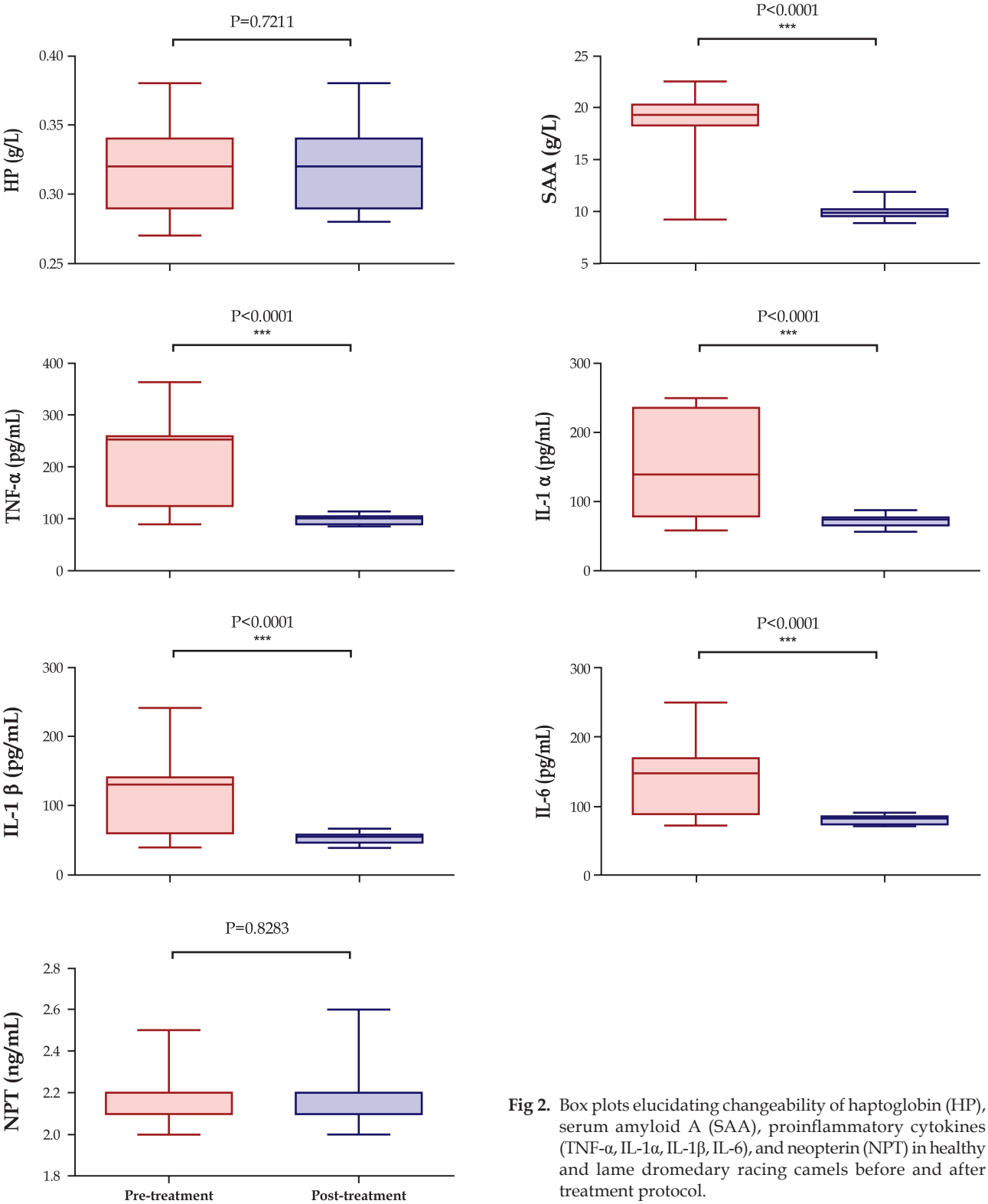

Fig 2. Box plots elucidating changeability of haptoglobin (HP), serum amyloid A (SAA), proinflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6), and neopterin (NPT) in healthy and lame dromedary racing camels before and after treatment protocol.

circumscripta. In a similar study, significantly higher fibrinogen, HP, and SAA levels in dairy cows suffering from limb diseases as compared to control cows were also reported (Jawor *et al,* 2008). Other studies have also shown SAA and HP as the major positive APPs, which can increase several folds after tissue injury in cattle (Murata *et al,* 2004).

The difference between the present findings and previous research work regarding HP levels might be attributed to the time of sampling and

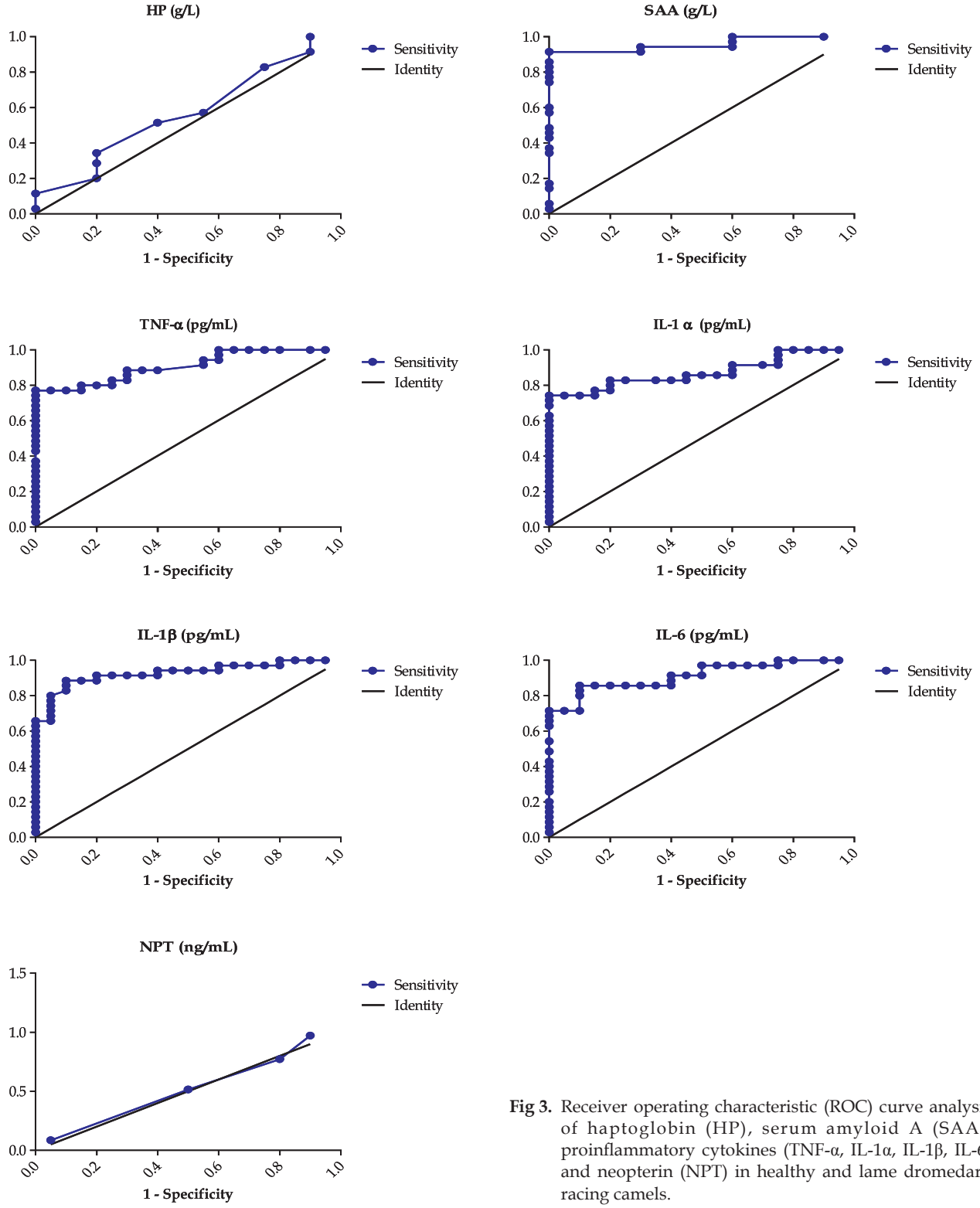

Fig 3. Receiver operating characteristic (ROC) curve analysis of haptoglobin (HP), serum amyloid A (SAA), proinflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6) and neopterin (NPT) in healthy and lame dromedary racing camels.

the non-infectious nature of lameness in racing camels under investigation (Ossent and Lisher, 1998). Moreover, the APR in camels toward lameness might be different from that of cattle. Consequently, further investigations of APR in lame camels at different time interval are recommended. Comparable to our assumption, Jacobsen *et al* (2004) and Petersen *et al*. (2004) proposed that synthesis of HP and SAA are controlled by dissimilar means. It appears that HP is not a very sensitive biomarker for this type of

lameness in racing dromedary camels. HP is related mainly to bacterial infection and not to non-infectious problems (Skinner *et al,* 1991).

Inflammatory responses in the peripheral and central nervous systems play key role in the development and persistence of many pathological pain states (Watkins *et al,* 2003). Certain inflammatory cytokines in the spinal cord, dorsal root ganglion (DRG), injured nerves or skin are known to be associated with pain behaviours and with the generation of abnormal spontaneous activity from injured nerve fibres or compressed/inflamed DRG neurons. Inflammatory reactions are up-regulated by proinflammatory cytokines, which are produced predominantly by activated macrophages. Numerous studies have demonstrated that CYTs such as IL-1β, IL-6, and TNF-α contribute to pathological pain (Watkins *et al,* 2003).

As evident in the results, the level of TNF-α, IL-1α, IL-1β, and IL-6 increased in the lame group of dromedary racing camels when compared to control camels. The reason behind this might be that CYTs appear to peak in blood in response to an inflammatory process (El-Deeb and Buczinski, 2015; El-Deeb *et al,* 2019; El-Deeb *et al,* 2020). However, following NSAIDs and other treatment protocols, a significant decline in TNF-α, IL-1α, IL-1β, and IL-6 indicates the improvement in lameness in dromedary camels after 10 days of treatment. The NSAIDs perform their action by hindering the production and release of prostaglandins and consequently resulting in decreased pain sensation and inflammatory process (Weissmann *et al,* 1987). Jawor *et al* (2008) assessed the levels of APPs at selected time points throughout the treatment protocol of cows with limb disorders with an aid to guide the treatment success and as a primary predictive biomarker of probable complications. The authors reported that sole ulcer, white line disease, and arthritis, were the most frequently diagnosed problems in cattle. Higher levels HP, SAA, and fibrinogen were reported at the beginning of the treatment protocols as also detected in lame camels in this study. In cattle, in which the treatment protocol went without clinical complications, a high gradual fall in APPs levels was detected. In this study, the fall in SAA and CYTs after treatment ascertained that the selected treatment protocol was appropriate and that it contributed towards decreasing the inflammatory process in lame racing dromedary camels. Based on these findings, lame camels which had higher APPs (SAA) or CYTs values post treatment had not been completely cured, and consequently, treatment process should be sustained.

The NPT marker is part of cell-mediated immunity system derived from monocyte/macrophages during the inflammatory conditions. In urine, serum, or plasma, NPT can be easily quantified (Rokos *et al,* 1985). It is detected before specific antibodies are formed, and often detected before disease states are fully manifested (Werner *et al,* 1990). Based on its ease of quantification and biochemical aspects in relation to the activation of the cellular immune system, NPT has gained increased use in human medicine as a sensitive and specific biomarker of disease activity in clinical situations including neoplasia (Weiss *et al,* 1993), autoimmune disease, viral, bacterial and parasitic infections (Facer, 1995), Crohn's disease (Judmaier *et al,* 1993), and HIV-1 (Fahey *et al,* 1990). In this study, the NPT levels did not altered in lame racing camels when compared to control healthy camels is also comparable to those detected in llamas (Stang and Koller, 1998). The normal levels of NPT in lame camels may be attributed to these injuries may not be allied with any detectable Th1-immune reaction. The AUCs were assessed for APPs, CYTs, and NPT to evaluate the diagnostic accuracy of each variable to distinguish lame and healthy camels. Based on the ROC curves and AUCs, SAA and CYTs provide highly accurate diagnostic accuracy and monitoring of treatment response for lame racing dromedary camels which are in agreement with the diagnostic accuracy guidelines (Gardner and Greiner, 2006).

According to this study, lame dromedary racing camels were linked to significant alterations in SAA and CYTs (TNF-α, IL-1α, IL-1β, and IL-6) indicators with non-significant changes in HP and NPT levels. Likewise, this study found that lame racing camels have higher levels of SAA and CYTs (TNF-α, IL-1α, IL-1β, and IL-6) biomarkers than healthy camels. It is concluded that in addition to clinical examination of lame camels, SAA and CYTs levels could be a useful diagnostic and predictive tool for lameness in racing dromedary camels.

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PREDICTED PHARMACOKINETIC PARAMETERS IN CAMELS OBTAINED BY ALLOMETRIC SCALING FROM OTHER SPECIES IS ACCURATE

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ABSTRACT

The objective of the present study was to evaluate how accurate the predicted pharmacokinetic (PK) parameters of some drugs are in camels, obtained by allometric scaling from animal species when compared to observed values established by us previously. The PK parameters tested were plasma clearance (Cl) and volume of distribution at steady state (Vss). The drugs evaluated were amikacin, gentamicin, kanamycin, tobramycin, antipyrine, flunixin, ketoprofen (R), ketoprofen (S), meloxicam, phenylbutazone, theophylline, and tramadol. The PK parameters scaled well for all drugs tested except meloxicam, where the predicted Cl was 12.5 fold greater than the observed value.

Key words: Allometry, camels, clearance, volume of distribution

Camels have not received much research attention in the field of pharmacokinetic (PK) compared to other animals. In most cases, the doses used in horses and cows were extrapolated to camels without clear scientific basis. In most veterinary preparations, manufacturers do not site dose regimens for camels; if they do, however, one does not find supportive experimental data. We have faced this issue several times when performing PK, pharmacodynamics (PD) and metabolism studies in camels. We had to rely on dose regimens used in bovine and horses. Our experiments covered different classes of drugs, including antipyrine (Wasfi *et al,* 1998a), non-steroidal anti-inflammatory drugs (Al Katheeri *et al,* 2000; Wasfi *et al,* 1997; 1998b; 2012), aminoglycosides (Hadi *et al,* 1994; Wasfi *et al,* 1992; 1993; 1999a), theophylline (Wasfi *et al,* 1999), tramadol (Elghazali *et al,* 2008) and others. While some pharmacokinetic studies in camels were carried out by other researchers, the total number of medicines investigated in camels is incredibly modest when compared to the list of drugs commonly used in veterinary medicine. As a result, it seems appropriate to use interspecies allometric scaling principles to predict pharmacokinetic parameters in camels when camel-specific PK data is lacking (Mahmood, 2006). Predicting PK parameters between species with differences in body weight of several orders of magnitude has been done effectively using allometric scaling (Martinez *et al,* 2006; Boxenbaum and Dilea, 1995; Lave *et al,* 1999; Obach *et al,* 1997; Mahmood, 2005). Tang and Mayersohn (2005), however, stressed the significance of species selection for the accuracy of allometrically predicted pharmacokinetic parameters in humans. Interspecies allometric scaling was extensively used for clearance (Cl) and volume of distribution (Vss). It is important, however, to acknowledge the factors that can influence the accuracy of the predictions of the PK parameters or else extrapolations can lead to sub-therapeutic or toxic doses (Mahmood *et al,* 2006). For this reason, correction factors or the use of other allometric equations have been suggested to improve the accuracy of drug clearance predictions (Mahmood *et al,* 2006). In its simplest form the pharmacokinetic parameter (Y) and body weight (W) are transformed logarithmically and fitted by linear regression to the equation:

$$\log Y = c + b \log W$$

Where Y is the PK parameter of interest, W is the body weight, while b and c are the slope and the intercept, respectively. Coefficients of determination (r^2) and P-values could be calculated for each correlation to evaluate the goodness of fit. The following allometric equation can then be applied:

$$Y = a\, W^b$$

Where (a) is the antilogarithm of the intercept (c).

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The objective of this study was to evaluate the accuracy of predicted pharmacokinetic parameters of some drugs in camels, obtained by allometric scaling from other mammalian species, when compared to observed values previously reported in camels.

Materials and Methods

Allometric functions for the drugs of interest were obtained from the report of Huang *et al* (2015). In this report, the authors obtained their initial PK data from the FARAD database from which they calculated the allometric functions. From these functions, we scaled the PK parameters (Vss and Cl) to camels for all the drugs reported in this study (12 drugs for Cl and 11 drugs for Vss). In addition, the allometric functions for aminoglycosides reported by Dinev (2008) was also used for scaling Vss and Cl parameters of the four aminoglycosides reported in this study. Based on both studies as well as data obtained from our previous studies, only data obtained by the intravenous route were used. Vss was expressed in litres and Cl was expressed in mL/minutes. The per cent error (PE) of predicted to observe value was calculated as:

$$PE\% = \frac{\text{Predicted value} - \text{Observed value} \times 100}{\text{Observed value}}$$

Results and Discussion

Tables 1 and 2 summarises the predicted PK parameters when the coefficients and the exponents of the allometric scaling were obtained from Huang *et al* (2015). The PE% for the aminoglycosides for both Vss and Cl was reasonably accurate and ranged from -10.16% to 41.60% and from -30.10% to 39.14% for Cl and Vss, respectively. The PE% of the predicted Vss and Cl of these aminoglycosides was also accurate when the coefficients and the exponents of the allometric scaling were obtained from Dinev (2008) (Table 3). Aminoglycosides were known to be almost completely eliminated by renal excretion without metabolism. They also had small Vss due to limited tissue distribution as they are highly polar compounds. These properties were therefore expected to make them scale well among species based on body weight. It was stated that simple allometric approach was not applicable for drugs in certain situations one of which if a drug was significantly metabolised (Huang *et al,* 2015). Such an example is antipyrine, which was used as a test drug to investigate hepatic drug metabolism. Antipyrine offered the advantage of negligible plasma protein binding, low hepatic extraction ratio, and was metabolised almost entirely by the liver (Brodie and Axelrod, 1950). Huang *et al* (2015) found that the predicted Cl of antipyrine in humans was 6.6-fold higher than the observed Cl. It was reported that in humans more than 90% of the antipyrine dose administered was excreted into the urine in the form of metabolites, namely, norantipyrine, 4-hydroxyantipyrine and 3-hydroxymethylantipyrine (Schmid *et al,* 1995). At least six CYP isoforms were involved in the metabolism of antipyrine (Engel *et al,* 1996). The multiplicity of the CYP isoforms involved in the formation of antipyrine metabolites in humans, was probably the cause of the poor prediction of its Cl when scaled from animals. However, in the present study we found that antipyrine predicted Cl in camels

Table 1. Observed vs predicted Cl (ml/min) scaled from mammalian data to camels. The coefficients and the exponents of the allometric scaling were obtained from Huang *et al* (2015).

Compound	a	b	W (Kg)	Predicted	Observed	% PE	Reference
Amikacin	2.1	0.843	270	235.30	261.90	-10.16	Wasfi *et al* (1999a)
Gentamicin	3.809	0.78	270	300.00	243.00	23.46	Wasfi *et al* (1992)
Kanamycin	3.16	0.891	270	463.23	327.15	41.60	Wasfi *et al* (1993)
Tobramycin	3.725	0.786	270	304.01	243.00	25.11	Hadi *et al* (1994)
Antipyrine	7.7535	0.8496	400	1259.52	986.66	27.65	Wasfi *et al* (1998a)
Flunixin	1.4818	0.9607	475	552.45	680.83	-18.86	Wasfi *et al* (1998b)
Ketoprofen (R)	3.3113	0.8881	400	677.40	413.30	63.90	Wasfi *et al* (2000)
Ketoprofen (S)	1.7713	1.0056	400	732.70	464.00	57.91	Al Katheeri *et al* (2000)
Meloxicam	0.5037	0.9643	450	182.25	14.55	1152.57	Wasfi *et al* (2012)
Phenylbutazone	0.8474	0.7146	400	61.31	32.66	87.72	Wasfi *et al* (1997)
Theophylline	1.57	0.9249	385	386.54	437.60	-11.67	Wasfi *et al* (1999b)
Tramadol	10.351	1.1474	300	7198.60	9700.00	-25.79	Elghazali *et al* (2008)

W : body weight in Kg; a: coefficient; b: exponent; PE: per cent error of predicted value compared to observed value

Table 2. Observed vs predicted Vss (l) scaled from mammalian data to camels. The coefficients and the exponents of the allometric scaling were obtained from Huang *et al* (2015).

Compound	a	b	W(Kg)	Predicted	Observed	PE	Reference
Amikacin	0.1727	0.9826	270	42.30	60.52	-30.10	Wasfi *et al* (1999a)
Gentamicin	0.2724	0.9782	270	65.10	57.11	13.99	Wasfi *et al* (1992)
Kanamycin	0.0551	1.3509	270	106.09	83.54	27.00	Wasfi *et al* (1993)
Tobramycin	0.1947	1.071	270	78.23	56.22	39.14	Hadi *et al* (1994)
Antipyrine	0.5242	1.0911	400	361.91	263.60	37.30	Wasfi *et al* (1998)
Flunixin	0.3954	0.9053	475	104.77	158.65	-33.96	Wasfi *et al* (1998)
Ketoprofen (R)	0.2392	0.9452	400	68.90	60.40	14.08	Al Katheeri *et al* (2000)
Ketoprofen (S)	0.2759	0.915	400	66.32	61.60	7.66	Al Katheeri *et al* (2000)
Meloxicam	0.294	0.9006	450	72.08	41.76	72.61	Wasfi *et al* (2012)
Phenylbutazone	0.0371	1.2961	400	87.48	228.00	-61.63	Wasfi *et al* (1997)
Theophylline	0.6023	1.0249	385	268.94	415.80	-35.32	Wasfi *et al* (1999b)

W : body weight; a: coefficient; b: exponent; PE: per cent error of predicted value compared to observed value

Table 3. Observed vs predicted Cl and Vss scaled from mammalian data to camels. The coefficients and the exponents of the allometric scaling were obtained from Dinev (2008).

Vss (l)	a	b	W	Predicted	Observed	PE
Amikacin	0.44	0.86	270	54.25	60.52	-10.35
Gentamicin	0.37	0.91	270	60.36	57.11	5.69
Kanamycin	0.46	0.87	270	59.99	83.54	-28.19
Tobramycin	0.13	1.1	270	61.44	56.22	9.28
Cl (ml/min)	**a**	**b**	**W**	**Predicted**	**Observed**	**PE**
Amikacin	2.99	0.84	270	329.62	261.90	25.86
Gentamicin	1.21	1.01	270	345.51	275.00	25.64
Kanamycin	2.41	0.92	270	415.79	327.15	27.09
Tobramycin	6.41	0.63	270	218.08	243.00	-10.25

W: body weight; a: coefficient; b: exponent; PE: per cent error of predicted value compared to observed value. References of the observed values as in table 1.

scaled well from other species. We have previously found that the activity of mixed function oxidases estimated by *In vivo* antipyrine clearance, was similar in horses and camels (Wasfi *et al,* 1998a).

We observed a 12.5 fold increase of predicted meloxicam Cl in camels than the observed value (Wasfi *et al,* 2012). Meloxicam, however, was known to be extensively metabolised to four inactive metabolites in humans (Türck *et al,* 1996). In most species, however, meloxicam was cleared almost exclusively by metabolic mechanisms (Schmid *et al,* 1995). The prominent P450 enzyme involved in the phase 1 biotransformation of meloxicam in humans are CYP 2C9, with a minor contribution of CYP 3A4 (Chesne *et al,* 1998). We have previously shown that camel CYP2C and CYP3A subfamilies were both well expressed *in vitro* together with a high glucuronidating capacity (Al Katheeri *et al,* 2006). We recently reported the presence of methylhydroxy meloxicam in urine following intravenous administration of meloxicam (Wasfi *et al,* 2012). However, we have not investigated the presence and extent of other meloxicam metabolites in camels. Extensive metabolism of meloxicam in camels might be the cause of its poor Cl allometric prediction.

It is generally recognised that allometric scaling does not always work well and occasionally fails to predict PK values (Huang and Riviere, 2014). These "failures" frequently point to species-specific drug bio-disposition processes that inhibit proper scaling. For example, after administration of racemic ketoprofen to camels, we have observed a predominance of R-KP, a phenomena that was shared with sheep (Alkatheeri *et al,* 2000; Delatour *et al,* 1993). This, however, contrasts with the findings reported in other species like humans, rats, rabbits, calves, dogs,

cats, monkeys, horses and elephants (Hunter *et al*, 2003) where S-KP was predominant and had been attributed to unidirectional inversion of R-KP to S-KP. Interestingly, Hunter *et al* (2003) generated allometric equations for Vd, Clp, and t½ of each enantiomer versus body weight for 8 or 9 mammalian species and found that none of the pharmacokinetic parameters was scalable allometrically across the range of mammalian species evaluated. Interconversion of ketoprofen enantiomers was different between species and this was possibly the cause of the large PE% observed in predicted Cl in camels.

They argued that when the log-log transformation was used to plot Cl vs. body weight, a constant variance was surrounding camel metabolism, serum albumin, and adaptability when compared to other species, yet the limited data present in this study demonstrated that the allometric equations were in general suitable for predicting pharmacokinetic parameters for certain drugs in camels and are useful for estimating a first time in camel dose. However, for drugs which were extensively metabolised, one should take scaled parameters with caution. Hunter and Isaza (2008) evaluated three methods of drug scaling, the third method of which, allometric scaling, was pertinent to this study, where the authors pointed to practical considerations when scaling between species. For example, the authors pointed to the question about the use of the correlation coefficient (R^2) as an indicator of accuracy. They argued that when the log-log transformation was used to plot Cl vs body weight, a constant variance was assumed to be present around the Cl parameter; if this assumption was false, allometry should not be used. Since it was common practice to predict PK values between species with body weight variations of several orders of magnitude, a constant variance around the Cl parameter might not be anticipated. The calculation of the linear regression tends to be heavily influenced by the data at the high end of the calibration curve. The reason for this was that for heavier body weights, the absolute variation was greater. At the bottom of the curve, this frequently results in excessive error. However, It was possible to correct for this error and improve the experimental data's fit to the calibration curve by weighing the data inversely to body weight. According to the authors, the chosen species for allometic scaling, should preferably be closely related, share the bulk of physiological functions, and only vary in size. They further acknowledged that, medications with limited hepatic metabolism and blood-flow dependent clearance were the best candidates for allometric scaling. Our findings corroborated this idea, showing that the aminoglycosides, which had blood-flow dependent clearance, scaled better than the extensively metabolised meloxicam.

Data availability statement

All data necessary for calculations are presented in tables.

Author contributions

Both authors contributed in drafting and interpretations of the manuscript.

Animal welfare and ethics statement

Not applicable

Declaration of Competing Interest

The authors have no competing interests to declare.

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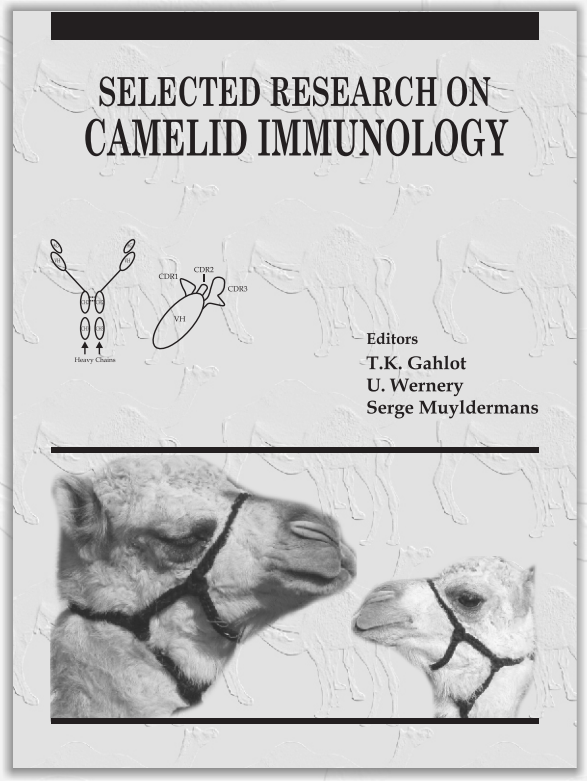
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

(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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SURRA IN THE UAE: DO WE HAVE DRUG RESISTANT *Trypanosoma evansi*?–PART 1

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ABSTRACT

An experimentally *Trypanosoma evansi* infected dromedary (intravenous injection of 3 million parasites) was under investigation for 200 days. Already after 24 h, living trypanosomes were detected in the blood. On day 6, parasites reached a high density and the host showed first clinical signs. Several treatments with melarsomin on day 6 and in combination with quinapyramin on day 23, 68, 102 and 198 decreased the number of trypanosomes in the blood but did not eliminate the parasite indicating a drug resistant *T. evansi* strain. While throughout the observation period there was no signs of anaemia, the number of leucocytes increased shortly after infection and remained high later on. Antibodies indicating a positive surra infection appeared on day 20 and remained on a high level.

Key words: Dromedary, melarsomin, quinapyramin, Surra, *Trypanosoma evansi*

Of eight world-wide used veterinary trypanocides for animals (suramin, homidium bromide, homidium chloride, isometamidium chloride, quinapyramine sulfate, quinapyramine sulfate:chloride diminazene aceturate, melarsomine dihydrochloride) (Giordani *et al,* 2016), six products are registered for the use in camels in the UAE. These are Trypanosoma^R (isometamidium), CymelarsanR (melarsomine dihydrochloride = melaminylthioarsenate) and the quinapyramines Triquin^R, Triquin inj. 2.5 g^R, Interquin^R and Asypur^R. A further product PiroplasminR that contains diminazen aceturate is registered only for horses and dogs and has a claim also for Babesia and Trypanosoma infections in these hosts.

Quinapyramine was developed in 1950 and has long metabolic half-life also thus bears a prophylactic effect (Curd and Davey, 1950). The large scale use of quinapyramine has been interrupted because of serious drug resistance problems in cattle trypanosomosis in Africa in the mid-1970s. Quinapyramine is now produced mainly for the treatment of surra in camels and horses, in particular where there is resistance of *T. evansi* to suramin (Uilenberg, 1998).

The phenanthridinium, isometamidium chloride (Samorin^R), a conjugate of the homidium (ethidium) and part of the diminacene molecule launched in the 1960s, is used exclusively as a veterinary trypanocide, and it is used both prophylactically and therapeutically.

Melarsomine dihydrochloride, a member of the triazines family is the latest drug that was produced by combining melarsenoxide with two cysteamine equivalents. Melaminophenylarsenicals were already used in the late 1940s to treat Rhodesian sleeping sickness and melarsopol (Mel B^R) was the drug of choice in human medicine. It was less toxic than melarsenoxide (De Koning, 2020). Melarsomine was developed in the 1980s and was commercially available in 1992 for the treatment of surra in camels.

Complaints of camel owners in the UAE led to a survey where a small camel herd was monitored over a period of 12 months including repeated treatments with melarsomine and quinapyramine (Wernery *et al,* 2020; Schuster *et al,* 2021). As a result, only one camel was successfully treated and all other positive animals remained serologically positive and trypanosomes were detected in their blood at least once after the last treatment.

In order to find out if this was a result of drug failure or reinfection, an infection trial was carried out and melarsomine and quinapyramine were administered to the experimentally infected camels kept at Central Veterinary Research Laboratory in Dubai.

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[1]It is noteworthy that in our treatment trials we used Triquin^R that is a combination of quinapyramine sulfate and quinapyramine chloride.

Materials and Methods

The Central Veterinary Research Laboratory in Dubai keeps 24 adult dromedaries for experimental and teaching purposes. Three to four dromedaries are accommodated in 225 m^2 (15x15 m) partially shaded pens on sandy grounds. The animals received hay and crushed cereals twice a day and drinking water *ad libitum* through automatic drinkers.

The Trypanosoma strain that was used in the trial originated from a camel of the farm of a previous investigation (Schuster *et al,* 2021). Trypanosomes were injected into the jugular vein in a dose of 3 million parasites. Prior to the start of our investigations, all dromedaries at CVRL were haematologically examined with negative results for trypanosomes. Blood sampling of the experimentally infected animal was done daily for the first 31 days and later in weekly intervals.

Haematological examination

The EDTA blood was analysed for haematological parameters using the automatic haematology analyser Sysmex XN (Sysmex Corporation, Japan) and was examined for the presence of trypanosomes with the buffy coat test (BCT). Giemsa stained blood smears were prepared when life trypanosomes were seen in BCT and the sample was semi-quantitatively assessed as follows:

negative : no parasites in BCT

\+ : single parasites in BCT but not in the blood smear

++ : few parasites in BCT but occasionally in the blood smear

+++ : many parasites in BCT and single parasites in each field in the blood smear

++++ : many parasites in BCT and more than one parasite in each field in the blood smear.

Serological examination

Serum samples were examined for *T. evansi* antibodies with an indirect ELISA. The antigen for coating the ELISA plates was prepared by filtration of EDTA blood of experimentally with *T. evansi* infected rats by anion exchange chromatography followed by hypotonic shock lysis. For evaluation of the test results, an optical density determined by the ELISA reader <0.3 was considered negative, 0.3-0.5 was dubious and >0.5 was positive. The commercially available CATT test was also employed.

The experimentally infected female dromedary No 26 was treated several times with melarsomin and quinapyramine (Table 1) and blood samples were taken prior and 3 days after treatment of the subsequent treatment.

Results

The buffy coat test

The infection dose of 3 million parasite cells was relatively high and there was no surprise, that trypanosomes were already seen 24 h after infection. Already on day 5, the number of trypanosomes reached ++++ in our semi quantitative assessment. On day 6, the camel showed clinical signs (depression, inappetence). An effect of the treatment was seen on day 9 when the concentration of trypanosomes dropped to +. On day 15, the number of trypanosomes reached again ++++.

Knowing that melarsomin is quickly excreted, for the second treatment on day 23, quinapyramin and melarsomin was given. The effect was seen between day 25 and day 27 when only single trypanosomes remained in the buffy coat. However, on day 39, again many trypanosomes were observed in the Giemsa stained blood smears. It seemed that the concentration of trypanosomes got less when the examination of blood on day 67 revealed

Table 1. Treatment of an experimentally with *T. evansi* infected dromedary. The dose route of application of the trypanocides was according to recommendations of the producer.

Treatment		**Haematology result (presence of *T. evansi*)**			
Trypanocide drug	**At day**	**Day prior treatment**	**Days after treatment**		
			1	**2**	**3**
melarsomin	6	++++	+++	++	+
melarsomin + quinapyramin	23	++++	++++	+	+
melarsomin + quinapyramin	68	++	+	+	+
quinapyramin	198	+++			
melarsomin	199		-	+	+

only occasional findings of trypanosomes in microscopic fields in Giemsa stained blood smears (++). A combined treatment of quinapyramin and melarsomin on day 68 decreased the number of trypanosomes again to (+) but did not eliminate the infection, although, no trypanosomes were seen at some occasions, later on. When the concentration of *T. evansi* reached high values (++++) again, quinapyramine was given on day 198, followed by melarsomin the next day. The haematological examination on the day after treatment gave a negative result but later, *T. evansi* occurred again (Table 1) and stayed until the end of observation.

Antibodies

The first measurable ELISA antibodies against *T. evansi* were detected on day 20, and by day 27, they reached a value of 1.0. Further testing revealed rising concentrations of antibodies to 1.3 on day 67, 1.8 on day 90 and 2.3 on day 196.

Haematology

Contrary to the number of erythrocytes and the concentration of haemoglobin that remained in the normal range, there were changes in the number of leucocytes that rose quickly after infection and remained on a high level between 20 and 25 x 10^9/ L and reached values of 30 x 10^9/ L at the end of the observation period. A severe neutrophilia and lymphopenia was observed in the initial phase of the infection but at the end of the observation period neutrophils and lymphocytes reached normal values. The concentration of monocytes was in the normal; range at the beginning of the trial but doubled starting from day 8 (two days after the first treatment) and remained at a high level and reached a maximum of 25% on day 25. Later, this value declined but still with values slightly above the norm. Eosinophile and basophile leucocytes were in normal range throughout the whole observation period.

Discussion

Following the introduction of a latently Trypanosoma infected dromedary to the small camel herd of CVRL in winter 2009/2010, 4 of the resident dromedaries got infected. It required 2 treatments with melarsomine to eliminate the infection. While trypanosomes and trypanosoma DNA disappeared after the 2nd treatment, antibodies remained up to 10 months. This small trial showed that melarsomine has been an effective drug.

According to Holmes *et al* (2004), trypanocide resistance occur under large-scale drug use, by using inadequate dosing and by using correct dosing with drugs that are slowly eliminated from the body.

Our treatment trials showed that the tested *T. evansi* strain could not be eliminated neither with melarsomine nor in combination with quinapyramine. While melarsomine levels quickly reach a maximum level in the blood stream, this drug is also quickly eliminated from the body (Kasozi *et al,* 2022). Contrary to this, quinapyramine unfurls a prolonged action. TriquinR, used in the trial is a combination of quinapyramine dichloride and quinapyramine sulfate. While dichloride salt is slowly adsorbed and protects the host of up to 2 months from susceptible trypanosomes, the sulfate is easily dissolved in water and subsequently resorbed but the protection lasts only for 2 weeks (Steuber and Kroker, 2002).

The mode of action of the melamino-phenylarsenical substance, melarsomine, is the neutralisation of enzymes and the interruption of the ATP generation of the trypanosomes. As other chemicals of this group, it irreversibly binds to sulfhydryl groups on the enzyme pyruvatekinase and leads to disrupting energy production. In addition, the trypanothion reductase is inhibited, and this causes death of the trypanosomes (Steuber and Kroker 2002). Resistances are subsequently related to the adenine-adenosine transporter, P2, due to point mutations within this transporter. Adenosine and adenine and the transport inhibitor dipyridamole are able to block the trypanocidal activity of melarsen oxide that has a similar mode of action as melarsopol and melarsomine. The P 2 encoding gene, TbAT1, and an allele bearing multiple polymorphisms were found to be responsible for the resistance. In case of resistance, use of isometamidium is recommended (Kasozi *et al,* 2022).

Quinapyramin interferes with DNA synthesis and suppression of cytoplasmatic ribosomal activity in the mitochondria of the trypanosomes. Resistance to quinapyramin is due to variations in the potential of the parasite's mitochondrial membrane (Kasozi *et al,* 2022). Quinapyramin chloride is slowly adsorbed and protects infection for up to 2 months and therefore, has a prophylactic effect too. The disadvantage is that resistance occurs when infection takes place at a time when the drug becomes sub therapeutic concentration. Also, for the management of resistance, isometamidium is recommended.

Another trypanocide is isometamidium. It freely crosses the plasma membrane of the parasite by facilitating diffusion and is subsequently

actively accumulated into the mitochondria using the mitochondrial potential as a driving force. In resistant parasites with a low mitochondrial potential, isometamidium rapidly diffuses from the cell when placed in isometamidium-free medium. In sensitive *T. congolense*, a similar amount of isometamidium diffuses from the cell under these conditions but a large proportion of the drug, sequestered in the mitochondrion, is retained (De Konig, 2001).

Diminazene aceturate has a claim for treatment of trypanosomosis, the effective dose for the treatment of *T. evansi* is 5 – 10 mg/kg body weight, however, this dose is toxic for camels (Homeida *et al,* 1981; Peregrine and Mamman,1993).

Resistance mechanisms of trypanosomes to trypanocides are well investigated but there are only speculations about the genesis of *T. evansi* resistance in Dubai camels. It is a fact that trypanocides can be purchased without prescription and it is believed among camel owners that melarsomin enhances racing performance of camels. Since the active substance is quickly eliminated from the body, it cannot be detected in forensic examination after the race. It is also believed by camel owners that a subtherapeutic dose of melarsomin "cleans" the body of the camel. Thus, frequent applications and underdosing might be the reasons for the loss of efficacy.

Except for the 1st week after infection when the camel was depressed and lost appetite, it did not show obvious clinical signs. This agrees with our experience in a naturally infected camel herd (Schuster *et al,* 2021) and confirms that the used *T. evansi* strain causes mild clinical signs. With regards to haematological values, anaemia is often described in Trypanosoma infected dromedaries but was not observed in present study. However, observations in this study lasted only 200 days.

The antibody titre measured by the CVRL in house ELISA became positive at day 20. This is in agreement with Luckins and Dwinger (2004) who stated that antibodies against *T. evansi* occur 14 to 21 days after infection.

Contrary to the number of erythrocytes and the concentration of haemoglobin that remained in the normal range, there were changes in the number of leucocytes that rose quickly after infection and remained on a high level. Platelets were already low prior to infection and stayed low over a longer period but reached normal values at the end of observation period. A severe neutrophilia and lymphopenia was observed in the initial phase of the infection but at the end of the observation period neutrophils and lymphocytes reached normal values.

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Trypanosoma evansi AS A CAUSE OF OCULAR DISORDERS IN DROMEDARY CAMEL (*Camelus dromedarius*) IN THE UNITED ARAB EMIRATES: A CLINICAL REPORT

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ABSTRACT

Ocular disorders characterised by corneal opacity, hyperaemia, oedema, excessive lacrimation and ocular discharges were detected in 4 female dromedary camels naturally infected with the *Trypanosoma evansi* in the dairy camel farm in the United Arab Emirates. Animals with ocular lesions also showed clinical signs of emaciation, pyrexia, anorexia, ataxia and decreased milk yield. *T. evansi* antibodies were detected in the serum of affected animals by card agglutination trypanosomosis test (CATT/ *T. evansi*) and by the enzyme - Linked immunosorbent assay (ELISA). The presence of the parasite in the blood was confirmed in one animal by the use of the haematocrit technique.

Key words: Dromedary camel, ocular disorders, *Trypanosoma evansi*, trypanosomosis, UAE

Camel trypanosomosis caused by *Trypanosoma evansi*, is a widely prevalent insect- borne disease of camels in Africa, Asia and Middle East (Enwezor and Sackey, 2005; Desquesnes *et al*, 2013). *Trypanosoma* spp are mainly present in the blood of infected animals but may also localise extravascularly in many other tissues including the central nervous system (CNS), aqueous humour, heart, lung, liver, kidney and spleen (Sudarto *et al*, 1990; Tuntasuvan *et al*, 1997, 2000; Rodrigues *et al*, 2009). The clinical signs of the disease are nonspecific and not sufficiently pathognomonic but the most prominent signs include dullness, emaciation, and paleness of mucous membranes, brisket oedema and oedema of the eyelids (Derakhshanfar *et al*, 2010; Padmaja, 2012). Neurological manifestations may also occur in advanced stages when the parasite invades the central nervous system. In addition, ocular disorders particularly corneal opacity and loss of vision were occasionally reported in man and several species of domestic animals including cattle, buffaloes, sheep, goats, dogs and cats (Ikede, 1974; Morales *et al*, 2006; Bal *et al*, 2014; Lisulo *et al*, 2014; Rjeibi *et al*, 2015). The *Trypanosoma* - induced ocular lesions in infected animals are apparently attributed to the immunosuppressive effect of the parasite (Reddy and Sivajothi, 2017; Sivajothi and Reddy, 2018). However, previous reports on ocular disorders associated with camel trypanosomosis are apparently lacking and the present communication describes their occurrence in 4 dairy camels naturally infected with *T. evansi* in the United Arab Emirates (UAE).

Materials and Methods

The present investigation was carried out at the Dairy Camel Farm owned by the Emirates Industry for Camel Milk and Products (EICMP), Dubai, United Arab Emirates. The farm contains the largest herd of dairy camels in the world with a total population of 8500 heads of dromedaries. The camels are kept under intensive management system. Their general health condition, environment and nutrition are closely supervised by well-trained veterinary medical staff. Heparinised and non-heparinised blood samples were collected from animals showing observable clinical signs and ocular disorders. The haematocrit centrifuge technique described by Woo (1971) was used for microscopic detection of trypanosomes in a buffy coat of blood of suspected animals. In addition, card agglutination trypanosomosis test (CATT/ *T. evansi*;

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Institute of Tropical Medicine, Antwerp, Belgium) and enzyme-linked immunosorbent assay (Surra Ab - EISISA in-house Method) (Laha and Sasmal, 2009) were further used for detection of *T. evansi* antibodies in the serum of camels with clinical signs and ocular involvement. Affected camels received medicinal treatment with a systemic trypanocidal drug (Triquin injections; Vetoquinol-India. 0.03ml suspension/kg body wt subcutaneously) combined with supportive vitamin therapy (AD_3E-Vitamin A 100000 IU, Vitamin D_3 50000 IU and Vitamin E 50mg; Dana Pharmaceuticals, given intramuscularly as a single dose of 10 ml). These animals also received topical ophthalmic treatment with combined antibiotics and anti-inflammatory eye drops (TobraDex: Tobramycin 0.3% and Dexamethasone 0.1%, Novartis, Switzerland) 3 drops twice daily for 1 week.

Results

Table 1 and Figs 1-4 summarise ocular disorders, laboratory tests and treatment response in *T. evansi* infected camels in the dairy farm owned by the Emirates Industry for Camel Milk & Products, Dubai, UAE.

Ocular disorders were detected in 4 camels showing systemic clinical signs including pyrexia, anorexia, ataxia decreased milk yield, emaciation and lacrimation. The ophthalmic involvements were bilateral in 2 camels and unilateral in the other 2. The affected eyes showed corneal opacity in all of the 4 cases. Moreover, hyperaemia, oedema, excessive lacrimation and either seromucoid or purulent ocular discharges were also observed. Some affected eyes showed corneal granulation and change of the normal colour of the lens to white, red or dark blue with complete response defect of pupil and eyelids. The vision of the affected eyes which was partially lost in 2 camels started to improve within 48 hours after treatment with systemic and local medication. Gradual improvement in the vision was concomitant with decrease of the intensity of the corneal opacity in the treated animals. On the other hand, the remaining two camels showed complete lack of response to medical treatment with consequent development of permanent corneal opacity (cataract) and partial blindness.

The results of the serological tests CATT/*T. evansi* and ELISA were positive in the 4 cases which showed detectable clinical signs and ocular disorders.

In addition, *T. evansi* was further detected in the buffy coat smear prepared from the blood of one infected camel.

Discussion

Trypanosoma species such as *T. evansi*, *T. cruzi* and *T. vivax* were occasionally found to cause various types of ocular disorders in man and several species of domestic animals including cattle (Bal *et al*, 2014), sheep (Ikede, 1974), goats (Morales *et al*, 2006) and dogs (Lisulo *et al*, 2014; Rjeibi *et al*, 2015). No reports are currently available on the incidence of ocular lesions in *Trypanosoma* – infected camels.

The existence of camel trypansomosis in the UAE has recently been confirmed by various methods including direct microscopy, CATT/ *T. evansi* and PCR (Habeeba *et al*, 2022). The disease was also diagnosed in female camels with recent history of abortion without mentioning any type of ocular involvement in infected animals (Wernery *et al*, 2020; Schuster *et al*, 2021). The present communication, however, describes the occurrence of ocular disorders in 4 dairy camels naturally infected with *T. evansi* in the largest dairy camel farm in the United Arab Emirates. Infection with *T. evansi* was confirmed by serological tests (CATT/*T. evansi* and ELISA) and demonstration of the parasite in the blood stream by the use of haematocrit method. The incidence of *T. evansi* infection in camels described in the present report is mostly attributed to the presence of the vector of the parasite (*Stomoxys* and *Tabanus*) in the area of study and to the fact that all camels in the farm were imported from an area of the South Central Asia where the disease is endemic.

Table 1. *T. evansi* ocular disorders in camels: laboratory tests, eye lesions and treatment response.

Camel ID	Buffy coat smear	CATT/ *T. evansi*	Surra Ab ELISA	Ocular lesions	Location	Treatment Response
4301	-ve	+ve	NA*	+v	Bilateral	Permanent corneal opacity and partial blindness
3229	+ve	+ve	+ve	+v	Bilateral	Permanent corneal opacity
7079	-ve	+ve	+ve	+v	Unilateral	Cured
6776	-ve	+ve	NA*	+v	Unilateral	Cured

* Not available (NA)

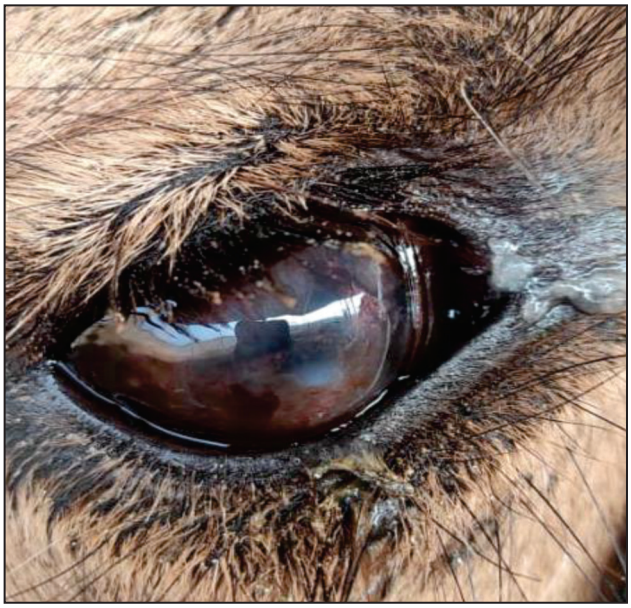

Fig 1. Right eye of camel no. 4301 showing corneal opacity. Note lacrimation and colour change.

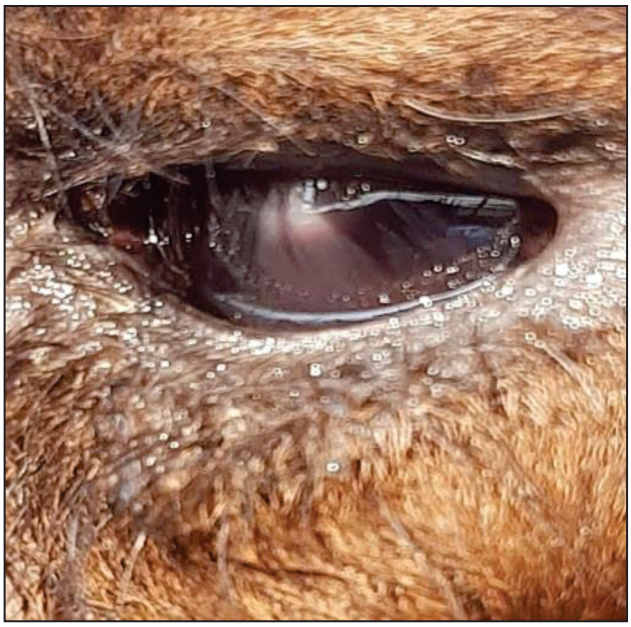

Fig 2. Right eye of camel no. 3229 showing corneal opacity affecting sizable portion of the cornea.

Fig 3. Left eye of camel no. 7079 showing change of colour of the affected cornea.

Fig 4. Left eye of camel no. 6776 showing corneal opacity and change of colour.

Ocular disorders characterised by corneal opacity, corneal ulceration, oedema, conjunctivitis, blepharitis, keratitis and partial or total loss of vision have occasionally been reported in camels as a results of traumatic, nutritional or infectious causes other than *Trypanosoma* spp (Bishnoi and Gahlot, 2001, 2004; Tharwat and El-Tookhy, 2021; Ranjan *et al*, 2016; Kumar *et al*, 2016; Abdella, *et al*, 2018). The present report therefore, confirms the occurrence of ocular disorders in *T. evansi* - infected camels. Further investigations are essentially required to establish the possible role of *T. evansi* in the development of ocular lesions in the dromedary camel.

Acknowledgement

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DETECTION OF POLYMORPHISM IN EXON-3 REGION OF LEPTIN GENE IN BIKANERI CAMEL

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ABSTRACT

The study was undertaken on 40 Camels (*Camelus dromedarius*) of Bikaneri breed to identify the single nucleotide polymorphism (SNP) in leptin gene. Nucleotide sequence of *Lama glama* was used to design primers to amplify selected region of leptin gene in Bikaneri camel. A 540bp fragment was amplified which covered Partial intron-2, exon-3 and partial 3′ UTR region of leptin gene in Bikaneri camel. The genetic variation in partial ntron-2, exon-3 and partial 3′ UTR (540bp) region of leptin gene was studied utilising single strand conformational polymorphism (SSCP) technique followed by nucleotide sequencing. SSCP analysis revealed monomorphic pattern as only AA genotype in studied region of leptin gene was observed. For verification and validation of observed results of SSCP, amplified PCR products of 6 animals were randomly selected for sequence analysis to ascertain the sequence pattern and existence of point mutations in the selected region. All the 6 animals had same sequence pattern. Monomorphic pattern showed the conserved nature of leptin gene in dromedary camel.

Key words: Bikaneri camel, exon-3, Leptin gene, *Lama glama*, polymorphism

Leptin, the product of the LEP gene, is a 16-kDa protein synthesised by adipose tissue and is involved in the regulation of feed intake, energy balance, growth, fertility, production traits and immune functions. Delavaud *et al* (2013) observed vital role of plasma leptin in adaptation of camel in harsh environmental conditions such as food and water shortages. Leptin and camel milk plays a significant role to abate oxidative stress (Hamzawy *et al*, 2018). PCR based single strand conformational polymorphism (SSCP) is more useful and easy technique to detect single nucleotide polymorphism (SNP) in large population. SSCP analysis has benefits over restriction fragment length polymorphism (RFLP) due to revealing more polymorphisms at various positions in DNA fragments (Orita *et al*, 1989). SSCP detects single nucleotide polymorphism by uncharacteristic electrophoretic passage of one or both single strands in a non-denaturing polyacrylamide gel (Gruszczynska *et al*, 2005). SNPs detection using SSCP is appropriate marker for the removal of sequencing errors during high density mapping (Vignal *et al*, 2002).

The leptin gene is comprised of 3 exons and 2 introns of which only exon 2 and 3 are translated into protein (Bruce *et al*, 1997). The sequence pattern of the leptin gene is known for many livestock species. However, very few entries are available for the camel leptin gene in the NCBI nucleotide database repository so designing of primers were done using nucleotide sequence of leptin gene in *Lama glama*. Considering the limited information available for the camel leptin gene, the objective of this study was to reveal the genetic polymorphism in exon-3 region of leptin (LEP) gene in Bikaneri camel breed of India.

Materials and Methods

Animals and sampling

All animal experiments were performed after approval of Institutional Animal Ethics Committee (IAEC). Forty camels (*Camelus dromedarius*) of Bikaneri breed were selected as representative sample to identify the single nucleotide change in leptin gene. Out of these, 28 were lactating females and 12 were male progenies of selected females. The Bikaneri breed of camels for the trial were selected from ICAR-NRCC Bikaner, Rajasthan, India.

About 10 ml of blood was collected aseptically through jugular vein puncture into anticoagulant

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EDTA containing vacutainer tube from selected animals and stored at 4°C till isolation of genomic DNA.

DNA extraction

Genomic DNA was isolated from the blood samples by 'Phenol Chloroform extraction method (Sambrook and Russel, 2006) with slight modification. The integrity and quality of extracted DNA were checked on 0.8% agarose under horizontal gel electrophoresis and visualised under gel documentation system.

Gene amplification

The leptin gene sequence of *Lama glama* available online in the GenBank (www.ncbi.nlm.nih.gov) (Acc. No KC295539.1) was used for primer design due to unreported genomics sequence in camel. In this study, 540 bp fragment length primer was designed to detect polymorphism which covered partial intron-2, exon-3 and partial 3' UTR region of leptin gene using primer-3 software (Untergasser *et al*, 2012). Forward "TGATGTGGAAGGGAGAAAGG" and Reverse "AGACACCTGGAAGCTCAGGA" primer sequences (5' to 3') were used to amplify the desired region. For amplification, tubes with a final volume of 25 µl containing 2 µl (50ng/µl conc.) of template DNA, 10 mmol/µl conc. of each primer, 13 µl GoTaq® Green Master Mix, 2X (PROMEGA, USA) and 7 µl of nuclease free water were inserted in applied biosystem gradient thermocycler. The amplification program consisted of an initial denaturation at 95°C for 5 min, then 34 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 45 seconds, elongation at 72°C for 45 seconds, and a final extension of 72°C for 10 min. Amplified samples were held at 4°C for 5 min for better stability and condensation.

Analysis of PCR products

The quality and size of the PCR amplicons were assessed on 1.5% agarose gel. The size of the amplified DNA fragments was assessed by comparison with standard molecular weight marker as shown in Fig 1.

Leptin gene polymorphism

PCR based single strand conformational polymorphism (SSCP) technique was used for analysis of leptin gene polymorphisms. Each PCR product was diluted in a denaturing solution (95% formamide, 0.025% xylene cyanol and 0.025% bromophenol blue, 25 mM EDTA), denatured at 98°C for 10 min, immediately chilled on ice and resolved on 10% denaturing urea polyacrylamide gel. The electrophoresis was performed at 4°C temperature for 8h using refrigerator in a vertical mini gel electrophoresis system. The gel were stained with 1% ethidium bromide solution and visualised under UV light gel documentation system. Representative PCR products of electrophoresed SSCP patterns sequenced for both direction in a commercial laboratory (X celris Genomics Pvt. Ltd, Ahmedabad, India) through Sanger dideoxy chain termination method (Sanger *et al*, 1977). The nucleotide sequence was submitted to the GenBank database with accession no. MT103545.1. Elbers *et al* (2019) submitted nucleotide sequence of leptin gene of Arabian camel to the NCBI GenBank database with gene ID 105085360.

Results and Discussion

A 540bp fragment of leptin gene was amplified. PCR-SSCP of the amplified leptin fragment was performed to detect any mutation. Several combinations for concentration of acrylamide:bisacrylamide in urea polyacrylamide gel mix for SSCP were tried to get optimum resolution and migration of the amplified products.

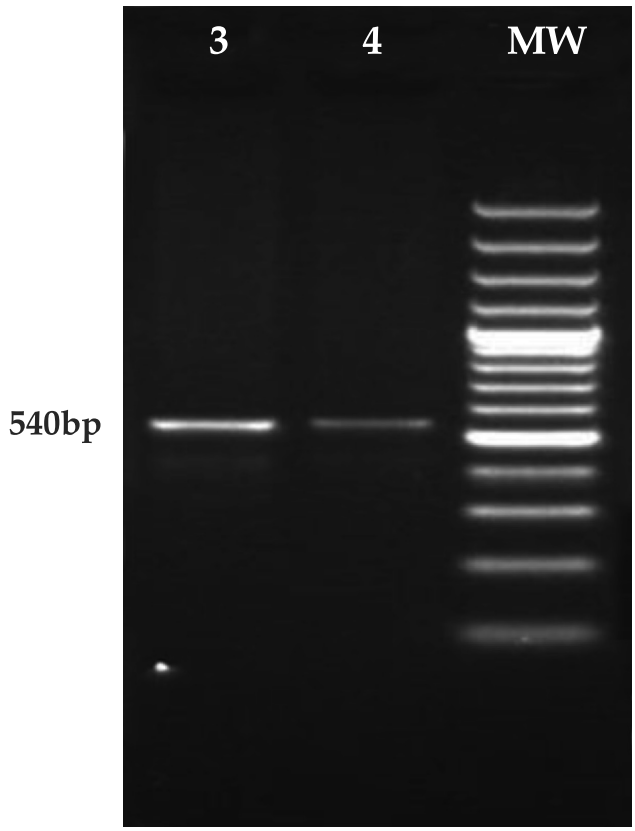

Fig 1. Amplification of camel leptin gene, partial intron-2, exon-3 and partial 3' UTR region (540 bp, lane 3-4), MW: Molecular weight marker (100 bp).

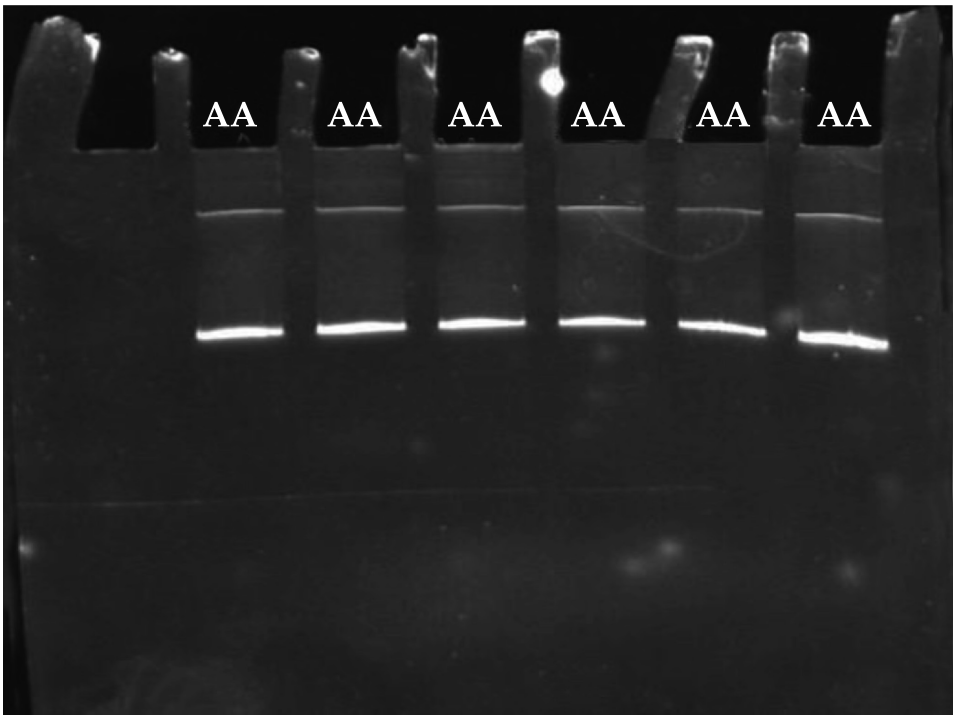

Fig 2. PCR-SSCP for partial intron-2, exon-3 and partial 3′ UTR region (540 bp) of leptin gene in Bikaneri breed camel.

In all 40 Bikaneri camels same band pattern denoted as AA, was observed as shown in Fig 2. However, the polymorphism in the exon 3 of the LEP in sheep was reported by Zhou *et al* (2009) using PCR-SSCP technique. For the verification and validation of observed results of SSCP, amplified PCR products of 6 animals were randomly selected for sequence analysis to ascertain the sequence pattern and existence of point mutations in the selected region. After sequencing in both direction of PCR product of amplified fragment and its editing, a single contiguous of 544bp length was confirmed. In initial sequence analysis, all the 6 animals has same sequence pattern and SNP was not revealed in exon-3 region of leptin gene. Al-Sharif *et al* (2022) reported no variation in coding region of leptin gene in dromedary she-camels. Piri *et al* (2018) reported transitional substitutions during comparative nucleotide sequence analysis of Sistan and Baluchistan population of camel. Nobari *et al* (2021) observed length and the difference on sequence of exons of the leptin gene when Turkmen camel gene sequence compared with other types of camels. However, Mahmoud *et al* (2014) observed two non-synonymous SNPs in exon-3 region of leptin gene in Najdi sheep.

This study revealed that studied region lacks SNP and thus monomorphic in nature. Monomorphic pattern showed the conserved nature of leptin gene in dromedary camel. Tahmoorespur and Shojaei (2013) amplified 471bp fragment of leptin gene and also reviewed by Ramadan and Inoue-Murayama (2017). They observed no genetic variation among the 25 Iranian native camels, and no polymorphism in dromedary and bactrian camels. However, Dubey *et al* (2012) reported 3 patterns in both exon-2 and exon-3 of leptin gene using PCR-SSCP followed by nucleotide sequencing in Mithun (*Bos Frontalis*). Mahrous *et al* (2020) revealed monomorphic pattern using PCR-RFLP followed by sequence analysis reported 4 SNPs in Egyptian river buffaloes. It is concluded that polymorphisms reported in other species which have association with economic traits of animals in previous referenced studies whereas no polymorphism is reported in present studied region of leptin gene. Further studies are required with recommendation of large sample size to investigate the relationship between polymorphisms of exon-3 region of leptin gene and the performance traits that may be useful in marker assisted selection (MAS).

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LIGHT AND ELECTRON MICROSCOPY OF BUCCAL SALIVARY GLANDS OF THE DROMEDARY CAMEL

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ABSTRACT

The study was aimed to investigate the light and electron microscopic structure of buccal salivary glands in the dromedary (*Camelus dromedarius*). Anatomically, the dromedary camel buccal glands were well-developed and were located on the lateral side of the cheek. Three parts (groups) of the buccal glands could be observed: dorsal, middle and ventral parts. Histologically, the secretory units were tubule-acinar, surrounded by myo-epithelial cells and supported by connective tissue. Whereas, the dorsal glands were purely mucous and the ventral glands were purely serous in secretion, the middle ones were muco-serous with predominant mucous cells. Ultra-structurally, the glandular cells of the serous secretory units (type I cells) contained numerous microvilli, many electron-dense secretory granules, abundant rough endoplasmic reticulum and mitochondria. On the other hand, the mucous-secreting cells (type II cells) appeared with numerous electron-translucent supra-nuclear granules and flat basal nuclei; dilated tubules of rough endoplasmic reticulum; fewer mitochondria and Golgi bodies were also observed. The obtained results have been compared with the previous reports on salivary glands of camel and other mammalian species.

Key words: Buccal glands, dromedary camel, histology, microscopy, ultra-structure

The mammalian species have two types of salivary glands, i.e. major and minor salivary glands (Grossman and Yousem, 2010; Som and Brandwein Gensler, 2011; Kessler and Bhatt, 2018; Maher *et al,* 2020). Several morphological studies have been carried out on major salivary glands of the dromedary camel (Nawar and El-Khaligi , 1975; Khalil, 1989; Al-Asgah *et al,* 1990; Mansouri and Atri, 1994; Nabipur *et al,* 2003; Mursal *et al,* 2016; Rezk and Shaker, 2017). However, minor salivary glands of the camel have received little attention (Taib and Jarrar, 1989; Nabipour, 2011). Therefore, the current study is an attempt to reveal morphological structure of the buccal salivary glands in the adult dromedary camel, using light and transmission electron microscopy.

Materials and Methods

The general histology and ultrastructure of buccal glands was done in 9 healthy camels obtained from Alsallam slaughterhouse, Omdurman, Sudan. For histological study, samples were quickly taken and washed in normal saline and were fixed in 10% buffered formalin. Samples were later dehydrated in a series of graded, alcohol, cleared in xylene and embedded in paraffin wax. Sections (5µm thick) were stained by haematoxylin and eosin (H&E), Toluidine blue and Masson's Trichrome as described by Culling (2013) to study the histological features.

Transmission electron microscopic study was performed on small tissue pieces (0.3 cm X 0.3 cm) taken from buccal salivary glands; these were fixed in 2.5% glutaraldehyde in Millonig's phosphate buffer (pH 7.4). Later, these were post-fixed in 1% osmium tetroxide for 1 hour, washed in Millonig's buffer, dehydrated in graded ethanol and propylene oxide series and embedded in Epon. Ultrathin sections of 50- 90 nm were cut using a diamond knife, mounted on uncoated grids and stained first with uranyl acetate and then with lead citrate (Robinson and Gray, 1990). The image examination was carried out in Philips XL transmission electron microscope.

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Results and Discussion

Anatomical findings

The buccal salivary glands in dromedary camels were found well-developed and composed of 3 parts or groups, i.e. dorsal, middle and ventral parts and were located on the lateral side of the cheek (Fig 1). These were bordered by the dorsal buccal branch of the facial nerve dorsally, the ventral buccal branch of the facial nerve ventrally, labial salivary gland rostrally and the maxillary vein and mandibular salivary gland caudally and caudo-ventrally, respectively. The middle buccal part is the largest of the 3 parts of buccal glands and is located between the dorsal buccal part, ventral buccal part, maxillary vein and mandibular salivary gland dorsally, ventrally, caudally, and caudo-ventrally, respectively.

Histological findings

Each part of the buccal glands was surrounded by a dense connective tissue that sent connective tissue septa dividing it into lobes and lobules (Fig 2A). Each lobule contained tubulo-acinar secretory units with simple cuboidal epithelial lining was surrounded by myoepithelial cells (Fig 2B). The duct system started with the intercalated ducts which drained the secretory units and were lined by simple squamous epithelium; the excretory ducts were connected to the striated ducts that were lined by simple cuboidal or columnar epithelium; striated ducts lead to larger interlobular ducts in the connective tissue septa that had stratified cuboidal or columnar epithelium and wide irregular lumen (Figs 2A).

There were 2 main types of glandular secretory units detected in the camel buccal glands: the mucous secretory units and serous secretory units. The mucous secretory units had relatively short cells with light-stained basophilic cytoplasm, flat basal nuclei and wide lumen. The serous secretory units consisted of tall (pyramidal) cells with dark acidophilic cytoplasm, spherical central nuclei and a narrow lumen. Generally, the dorsal part of buccal glands had mucous secretory cells (Fig 3A); a few secretory units, however, showed serous cells arranged as serous demilunes (Fig 3A). The secretory units of the middle part were muco-serous with predominant mucous cells (Fig 3B). The secretory units of the ventral part were serous in nature (Fig 3 C).

Ultrastructural findings

Two main types of cells were identified in the camel buccal glands secretory units depending on their electron-density of cytoplasmic granules:

Type I:

This type contained small and rounded electron-dense granules that were concentrated in the apical cytoplasm and lumen; numerous microvilli projecting from the apical cell membrane into the lumen were also observed in this type. Diffuse rough endoplasmic reticulum and mitochondria with different shapes and sizes were present around the nuclei were also observed. The nucleus was eccentric and round with diffuse chromatin scattered among heterochromatin. This cell type was present in the serous secretory units of the ventral part and in the serous demilune and secretory units of the middle part of the buccal salivary glands (Fig 4).

Type II:

It contained numerous mucous electron-translucent supra-nuclear granules of different sizes and round shapes. The nucleus was pushed by the mucous granules towards the basal part of the cells. Dilated tubules of rough endoplasmic reticulum were located laterally, basally or between the mucous granules and the nucleus. A few mitochondria, supra-nuclear rough endoplasmic reticulum and sparse Golgi bodies were also observed. This cell type was found in the secretory units of the dorsal parts and the mucous secretory units of the middle parts of the buccal salivary glands (Fig 5).

In the middle part of the buccal salivary glands, many cells showed many electron-translucent granules with different sizes and shapes filling the upper part of the cells; several rounded electron-dense granules were also observed between the light granules and the basal nucleus (Fig 6).

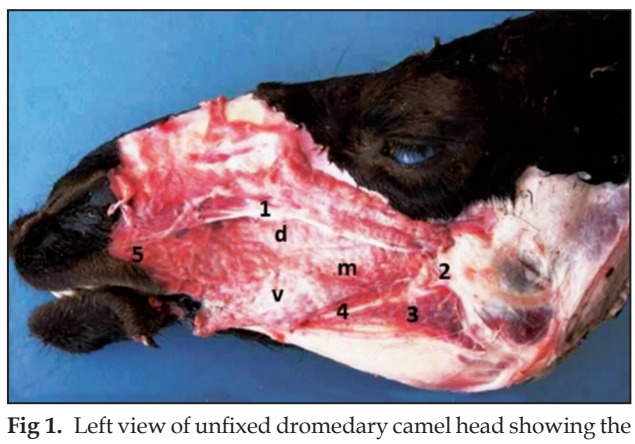

Fig 1. Left view of unfixed dromedary camel head showing the three parts of the buccal salivary glands: dorsal part (d), middle part (m), ventral part (v). Note the dorsal buccal branch of the facial nerve dorsally (1), the maxillary vein (2) and mandibular salivary gland (3), the ventral buccal branch of the facial nerve ventrally (4) and labial salivary gland (5).

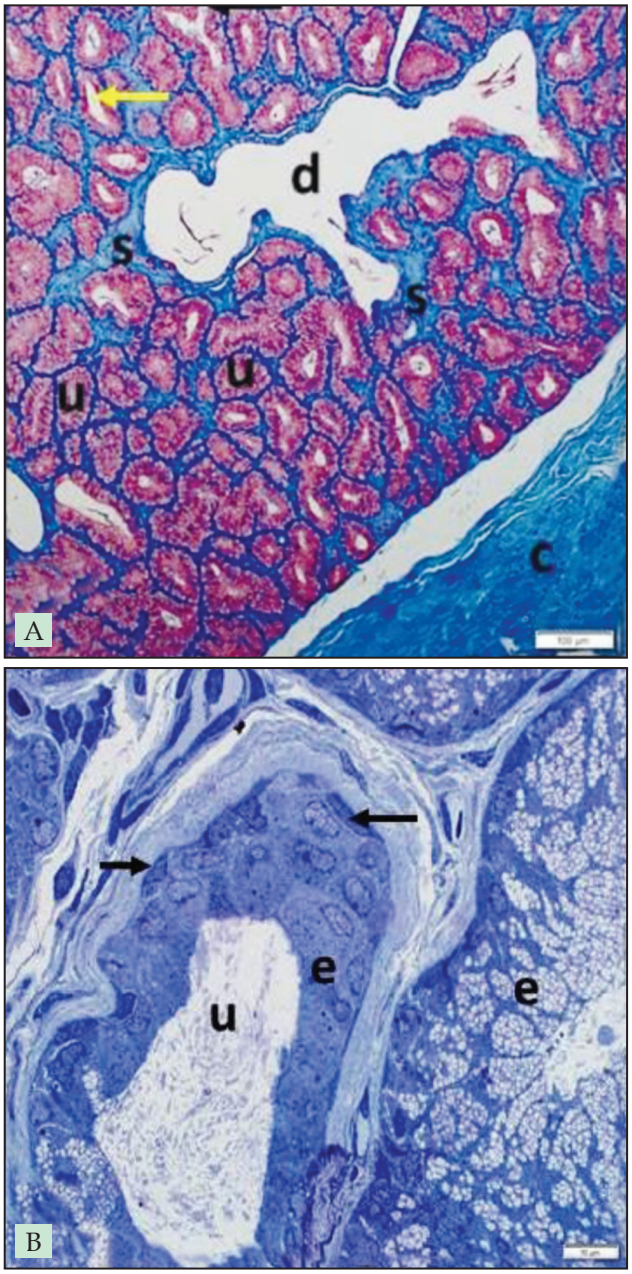

Fig 2. **A.** Photomicrograph of the ventral buccal glands surrounded by connective tissue (t) with septa (s), tubule-acinar secretory units (u), intercalated duct (black arrow), striated duct (yellow arrows) and interlobular duct (d). Masson trichrome stain. Image bar= 100µm.

B. Photomicrograph of the middle buccal glands showing secretory unit (u) lined by simple cuboidal epithelium (e). Note the myoepithelial cells (arrows) between the basement membrane and basal cell membrane (arrows). Toluidine blue satin. Image bar= 20µm. bar= 10µm.

Several morphological studies have been performed on the minor salivary glands in the different mammalian species (Parida and Das, 1991; Frith and Townsend, 1997; Stinson and Calhoun, 1993; Hand *et al,* 1999; Mohammadpour, 2010; Sadi, 2013).

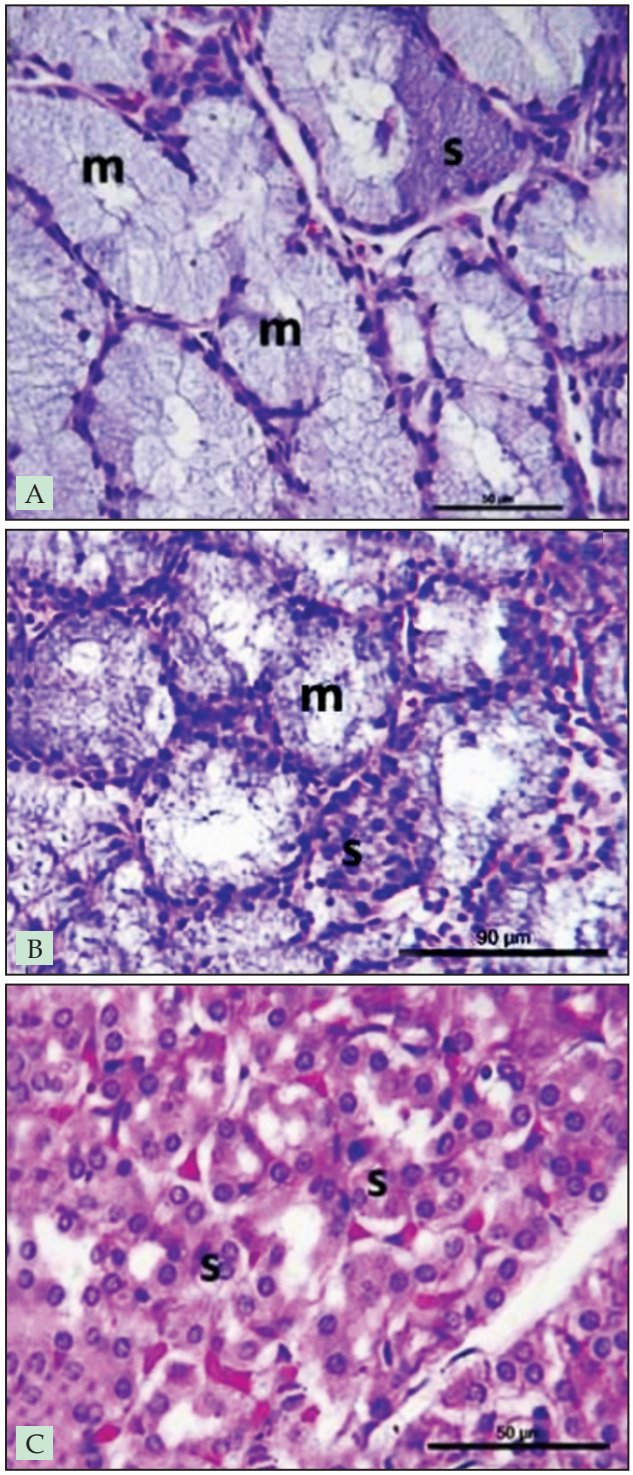

Fig 3. **A.** Photomicrograph of the dorsal buccal glands showing mucous secretory units (m). Note the serous demilune (s). H&E stain. bar= 50µm.

B. Photomicrograph of the middle buccal glands showing mucous secretory units (m) with light fells and flat basal nuclei narrow lumina, and serous secretory units with dark cells and round nuclei H&E stain. bar= 90µm.

C. Photomicrograph of the ventral buccal glands showing secretory units (s) of serous units lined by dark cells with round nuclei H&E stain. bar= 50µm.

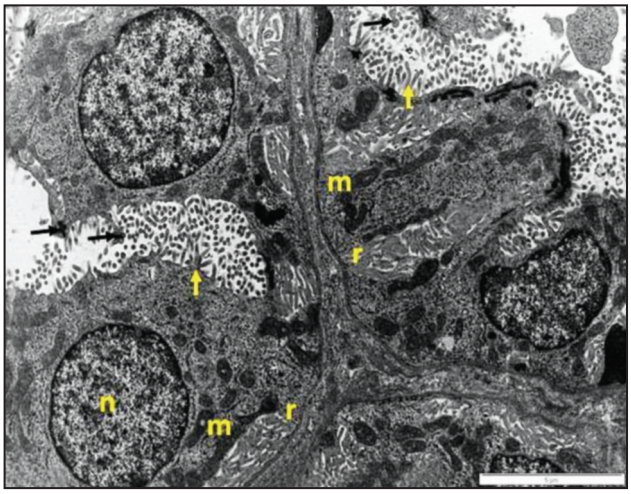

Fig 4. Transmission electron micrograph of the ventral buccal glands showing numerous microvilli (yellow arrows) projecting in the lumen and dark and small secretory granules (black arrows) in the apical cytoplasm and lumen. Note the numerous mitochondria (m) and diffused rough endoplasmic reticulum (r) around the round and central nuclei (n). Image bar= 5 µm.

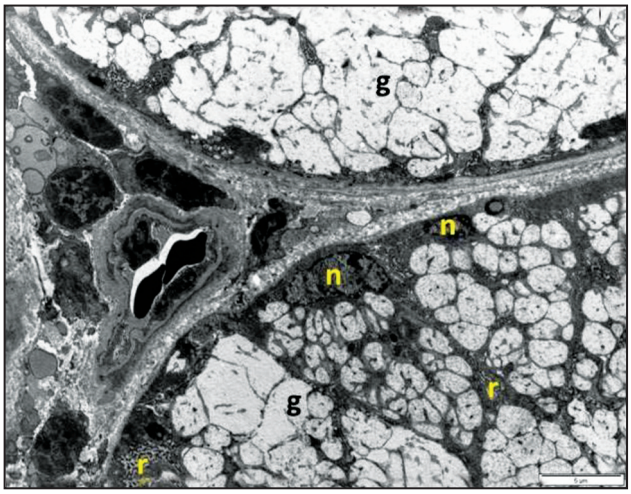

Fig 5. Transmission electron micrograph of the dorsal buccal glands showing light granules (g) filling the upper part of the cells pushing the flat nuclei (n) towards the basal part of the cells. Note the diffused rough endoplasmic reticulum (r) between the granules. Image bar= 5 µm.

The current anatomical results confirmed the earlier findings in camels (Smuts and Bezuidenhout, 1987), domestic ruminants (Parida and Das, 1991; Singh *et al,* 2011) and horse (Stinson and Calhoun, 1993) and accordingly the buccal salivary glands have three parts, i.e. dorsal, middle and ventral. In contrast, van Lennep (1957) reported that the buccal glands of dromedary camels had two parts, i.e. dorsal and ventral.

Histologically, the present study revealed that each part of the buccal glands of dromedary camel was

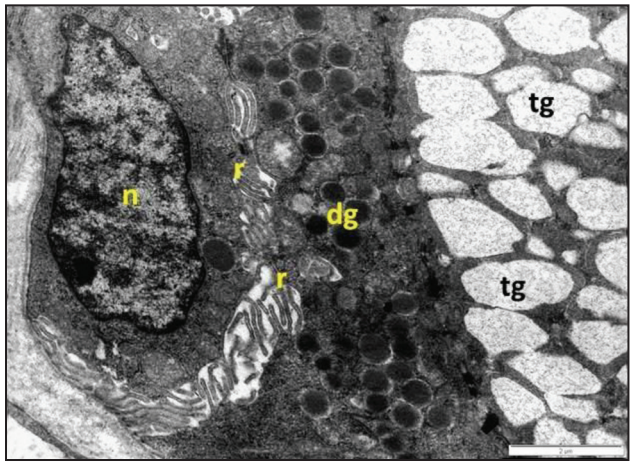

Fig 6. Transmission electron micrograph of the middle buccal gland showing electron-translucent granules (tg) occupying the upper part of the cells. Small and rounded electron-dense granules (dg) are concentrated between the electron-translucent granules and the basal nucleus (n). Note the oval nucleus (n), well-developed supra-nuclear rough endoplasmic reticulum (r) and Golgi body (g). Image bar= 2 µm.

covered by a dense connective tissue and supported by connective tissue septa to divide it into lobes and lobules; the lobules were occupied by tubulo-acinar secretory units with myoepithelial cells. These findings are in line with those reported by Taib and Jarrar (1989) and Nabipour (2011) in the buccal glands of camels. The present results showed that the secretory units of camel buccal glands were generally mucous, mixed and serous in the dorsal, middle and ventral parts, respectively. Similar findings have also been stated in the buccal glands of dromedary camels (Nabipour, 2011) and other mammals (Kumar, 2014). However, a few serous demilunes were observed in some secretory units of the dorsal part. Moreover, Taib and Jarrar (1989) claimed that the dorsal part of the glands contains muco-serous acini. In the present study, the dorsal part of the buccal glands in the camels was mucous and the middle part was muco-serous with predominant mucous-secreting cells. Moreover, van Lennep (1957) found that the ventral buccal glands in the same species have a mucous secretion in their upper part. Thus, it could be suggested that the secretion of the buccal glands in dromedary camels is mainly mucous, which might help in lubrication and swallowing of food, protection of oral mucosa and preservation of water. This fact might be of great importance for this animal species which is living in a desert environment that is characterised by scarcity of water and few food.

The two main types of cells (type I and type II) in the secretory units of the camel buccal glands.

Type I, which was found in serous secretory units of the ventral part and middle buccal glands, contained electron-dense granules, numerous microvilli, abundant rough endoplasmic reticulum and mitochondria. These cellular characteristics have also been described in bovine (Shackleford and Wilborn, 1969), rats (Igbokwe, 2018) and camel (Mansouri and Atri, 1994) parotid glands which have purely serous secretory units. Shackleford and Wilborn (1969) have also mentioned that the ultrastructural appearance of camel parotid gland is similar to that of bovine parotid gland. Projection of microvilli into the acinar lumen and presence of intercellular canaliculi could suggest increased secretory surface of the glandular cells; these characteristic features have been considered as an indication of increased production of saliva (Shackleford and Wilborn, 1968, 1969; van Lennep *et al*, 1977).

Type II or mucous cells detected in this study contained numerous electron-translucent supra-nuclear granules and flat basal nuclei; dilated tubules of rough endoplasmic reticulum, a few mitochondria and Golgi bodies. Similar ultrastructural observations have also been reported in the bovine submandibular glands (Shackleford and Wilborn, 1970). In the present study, the serous demilunes showed similar characteristics of the serous secretory units that included many electron-density granules, well-developed rough endoplasmic reticulum and sparse Golgi body. The structure and organelle distribution in the demilunar cells presented in this study has also been reported in other mammalian mandibular salivary glands (Shackleford and Wilborn, 1970; Pinkstaff, 1980).

It could be concluded that most of the histological and ultrastructural features of the buccal salivary glands in dromedary camels resemble those of other mammalian species.

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THE INFLUENCE OF THE CORPUS LUTEUM LOCATION ON HORMONAL AND VITAMIN C COMPOSITION OF FOLLICULAR FLUID AND SERUM IN DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The aim of this study was to compare hormonal composition of the predominant antral follicle coexisting with or without a corpus luteum (CL) in dromedary camels. Forty-seven genitalia and blood samples were collected from clinically healthy adult (7–12 years of age) non-pregnant female camels during the breeding season at a local abattoir. Follicles (0.7–1.8 cm in diameter) did not coexist with a CL were classified as follicles F1 and their blood serum labeled F1S (n=12), follicles coexist with CL on the same ovary were labeled F2 and their blood serum labeled as F2S (n=9), and follicles with contralateral CL on the other ovary labeled F3 and their blood serum named F3S (n=11). Follicular fluid (FF) and sera were subjected to biochemical and hormonal analysis. Results revealed greater ($P<0.05$) concentrations of progesterone in the FF of F1 follicles and F1S serum than that found in F3 follicles and F3S serum. The concentrations of cortisone and T3 were higher ($P<0.05$) in FF of F1 and F3, and serum of F1S and F3S in comparison to FF of follicles type F2 and serum of F2S. The thyroxine and vitamin C concentrations in FF from F1 and F2 follicles, and serum of F1S and F2S were higher ($P<0.01$) in comparison with the FF from F3 and serum of F3S. The presence of CL might exert a local effect on hormonal composition of FF and could indirectly influence follicular development and oocyte quality. It is better to use oocytes originated from ovaries without a CL in *in-vitro* fertilisation (IVF) in dromedary camels.

Key words: Antral follicles, camel, corpus luteum, hormones

The camels' oestrous cycle is described as a "follicular wave pattern" (Tibary and Anouassi, 1996; Skidmore *et al,* 2013). Camels are induced ovulators and thus ovulation and CL formation normally occur in response to mating, and the CL that develops has a lifespan of only 10-12 days (Skidmore, 2011). In camels, failure of conception and early embryonic death could occur in the presence of the CL due to low peripheral progesterone concentrations (Mostafa *et al,* 2017). In the application of assisted reproductive techniques, the salutary roles of follicular fluid (FF) on *in-vitro* maturation (IVM), IVF and subsequent embryo development have been investigated in several species (Da Silva, 2008; El-Shahat *et al,* 2019). The FF contains hormones and metabolites, which are locally synthetised and easily pass through the basal lamina to enter the antrum or escape toward circulating blood (Gérard *et al,* 2002; Blaszczyk *et al*, 2006; Fahiminiya *et al,* 2011). It has been reported that FF is rich in steroid reproductive hormones, such as oestradiol-17β (E2) and progesterone (P4) in cattle (Bearden and Fuquay, 2003), and mares (Bashir *et al,* 2016; Młodawska *et al,* 2018; Satué *et al,* 2019). There are scarce reports about comparing the effect of the absence or presence of a corpus luteum (CL) on FF hormonal composition (Kor, 2014). The composition of FF is modified according to the absence or presence of CL in the ovary in mares (Da Silva, 2008), cows (Kor *et al,* 2013), ewes (Karami *et al,* 2010) and camels (El-Shahat *et al,* 2019). These modifications could

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indicate the existence of a possible local effect of CL on the dynamic development of follicles. Corpora lutea affected ovarian follicular dynamics in both ovaries by a systemic effect with evidence for a local ipsilateral effect (Contreras-Solis *et al,* 2008). The cortisol in ovarian FF inhibits the ovarian steroidogenesis (Michael *et al,* 1993) and stimulate oocyte maturation (Fateh *et al,* 1989; Jimena *et al,* 1992). T3 concentrations were high in the FF collected from predominant follicles existing in absence of CLs (Tabatabaei *et al,* 2011). Thyroxine affects the follicular development and oocyte maturation (Fedail *et al,* 2014), and ovarian steroidogenesis (Spicer *et al,* 2001). In human, the quality of oocyte maturation and embryos is better in lower levels of vitamin C (Saffari *et al,* 2015).

As changes in the hormonal and vitamin C (Saffari *et al,* 2015) composition of FF may influence the maturation and quality of the oocyte, the aim of the present study was to compare some hormonal and vitamin C composition of the predominant antral follicle coexisting with or without a CL in dromedary camels.

Materials and Methods

Experimental materials

A total of 47 genitalia were collected from clinically healthy adult (7–12 years of age) non-pregnant female camels (*Camelus dromedarius*) during the breeding season (November–March) at a local abattoir in the eastern province of Saudi Arabia. The reproductive history of those animals was unknown. Blood sample (10 ml) was collected from each animal during exsanguinations into plane vacutainer tubes. After slaughtering, the clinically normal genitalia were transported to the laboratory in an icebox within one-hour post-slaughter. In the laboratory, ovaries were washed twice in cooled 0.9% NaCl and blotted dry. Paired ovaries bearing oversized follicle (>20 mm in diameter; Tibary and Anouassi, 1997; Ghoneim *et al,* 2013) or ovarian cyst were excluded from the investigation. Predominant antral follicles without a corpus luteum (CL; Fig 1; F1), follicles coexist with CL on the same ovary (F2), and follicles with contralateral CL on the other ovary (Fig 2a and 2b; F3) were considered for measuring using a Vernier caliper. The follicular diameter ranged from 0.7–1.2 cm (Skidmore, 2011). Follicular fluid (FF) of the predominant follicles was aspirated using sterilised 22-gauge hypodermic needles and syringes. The FF and blood samples were centrifuged at 1500 g at 4°C for 10 minutes. The supernatant fluid and serum were harvested and stored at -80°C until analysis. Serum samples were labeled as F1S (Serum of camels bearing follicles without CL; n= 20), F2S (Serum of camels bearing follicles coexist with CL on the same ovary; n= 12), and F3S (Serum of camels bearing follicles with contralateral CL on the other ovary; n= 15).

Hormonal and Vitamin C analyses

The follicular fluid and serum concentrations of camel oestradiol 17-Beta-Dehydrogenase (E2; pg/ mL; Catalog No. MBS9381137), camel progesterone (P4; ng/ mL; Catalog No. MBS7606970), camel cortisone (ng/ mL; Catalog No. MBS062065), camel insulin-like growth factor-II (IGF-II; ng/mL; Catalog No. MBS058122), camel ultra-sensitivity triiodothyronine (T3; nmol/L; Catalog No. MBS056436), general thyroxine (T4; ng/mL; Catalog No. MBS289276) and vitamin C (µg/ml; Catalog No. MBS2700398) were estimated using Enzyme immune assay kits My BioSource® (USA).

The laboratory reported intra- and inter-assay coefficient of variances of the studied hormonal concentrations were 4.4% and 5.1% for E2, 2.3% and 4.8% for P4, 4.4% and 5.1% for cortisone, 3.1% and 3.9% for IGF-II, 2.3% and 4.1% for T3, 3.2% and 5.3% for T4, 3.9% and 7.4% for vitamin C, respectively. The sensitivity of assays for E2, P4, cortisone, IGF-II, T3, T4 and vitamin C were 0.1 ng/ml, 0.188 ng/ml, 1.0 ng/ml, 1.0 ng/ml, 0.1 nmol/L, 0.0975 ng/ml and 173.5 µg/ml, respectively. All measurements were carried out according to the manufacturers' guidelines. The optical densities were measured using an ELISA reader (Absorbance Microplate Reader ELx800TM, Bio Tek®, Highland Park, VT, USA and Microplate Strip Washer (ELx800 TM, Bio Tek®, Highland Park, VT, USA).

Statistical analysis

The data of follicular fluid hormonal and vitamin C compositions are presented as means ± SEM. Analyses were conducted by ANOVA using SPSS statistical software programme (2013), version 22.0.

Results and Discussion

The mean concentrations of E2 in FF of F3 follicles were higher ($P<0.01$) compared with that of F2 follicles (Tables 1 and 2). The concentrations of P4 were higher ($P<0.05$) in the FF harvested from F1 follicles than that collected from F3 follicles (Tables 1 and 2). The concentrations of cortisone and T3 were higher ($P<0.05$) in FF harvested from F1 and F3, in comparison to FF of follicles type F2 (Tables 1 and

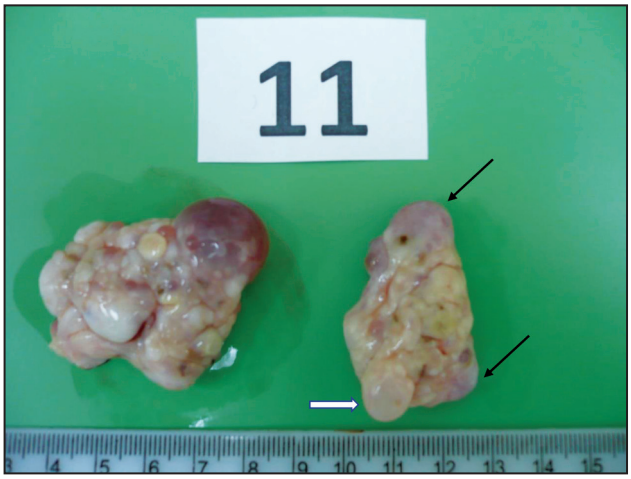

Fig 1. Congested follicle (1.8 cm in diameter) showed on the left ovary and regressed CLs (Black arrows) and predominant antral follicle (0.8 cm in diameter; White arrow) present on the right ovary.

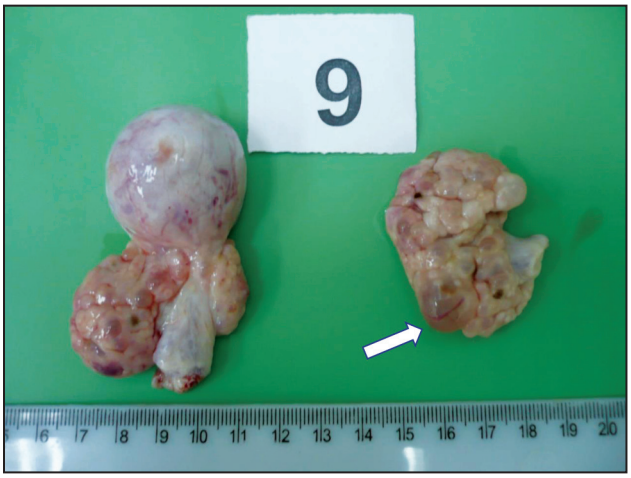

Fig 2. A. Mature CL showed on the left ovary and predominant antral follicle (0.8 cm in diameter) present on the right ovary.

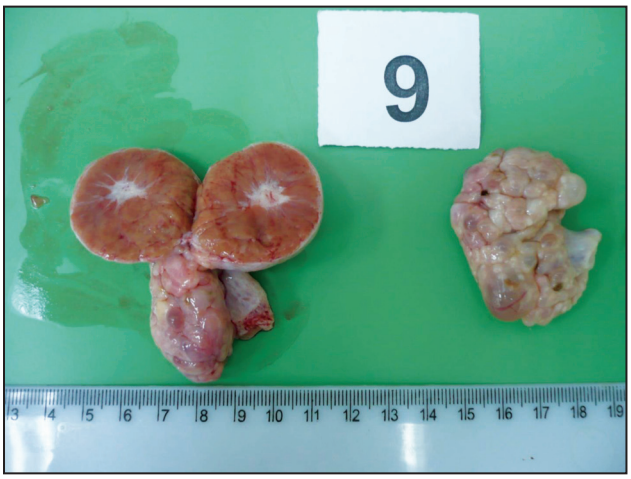

Fig 2. B. The mature CL present on the left ovary after dissection.

2). The IGF-II concentrations in the FF from F1 and F3 were lower (P<0.001) compared with that of FF from F2 (Tables 1 and 2). As shown in tables 1 and 2, the T4 concentrations in FF from F1 and F2 follicles were higher (P<0.01) compared with the FF from F3. Vitamin C concentrations in the FF harvested from F1 follicles were higher (P<0.001) than the concentrations present in F2 and F3 follicles (Tables 1 and 2).

The mean concentrations of E2 in serum of F3S were higher (P<0.01) compared with that of serum of F2S camels (Tables 1 and 2). The concentrations of P4 were higher (P<0.05) in serum of F1S camels than that present in F3S serum (Tables 1 and 2). The concentrations of cortisone and T3 were higher (P<0.05) in serum of F1S and F3S in comparison to serum of F2S (Tables 1 and 2). The IGF-II concentrations in serum collected from F1S and F3S were lower (P<0.001) compared with that serum from F2S (Tables 1 and 2). As shown in Tables 1 and 2, the T4 concentrations in serum of F1S and F2S were higher (P<0.01) compared with serum of F3S. Vitamin C concentrations in serum of F1S camels were higher (P<0.001) than the concentrations present in serum from F2S and F3S (Tables 1 and 2). The hormonal and vitamin C concentrations in FF were many folds higher than those of serum, and there was no correlation in these concentrations between the FF and peripheral circulation.

The assessment of some hormonal and vitamin C composition of the predominant antral follicles coexisting with or without CL were the goals of this study. FF holds different biochemical metabolites that are derived from serum or synthesised locally in the follicles and shared in the metabolic activities of follicular cells (Edwards, 1974; Gérard *et al,* 2002). The constituents of the FF change through the growth and development of each follicle (Wise, 1987). This study reported a significant increase in the concentrations of E2 of FF from F3 and serum of F3S compared with FF from follicles F2 and serum F2S. The ability of antral follicles to produce large amounts of E2 is a distinctive sign of health status of the follicle (Beck *et al,* 2003; Kobayashi *et al,* 2006). Granulosa and theca cells secret large amounts of progesterone which act as a precursor for androgen and subsequently oestrogen production (McNatty *et al,* 1984). It has also been suggested that E2 is important in the oocyte acquisition of fertilisation competence (Yoshimura *et al,* 1987; Dode and Graves, 2003). In mares, intrafollicular E2 concentrations were significantly higher in non-CL-bearing ovaries than in CL-bearing

ones (Satué *et al,* 2019). However, in bovine, location of follicles relative to the CL had no influence on oestradiol-17 beta concentrations (Brantmeier *et al,* 1987).

In the prsent study, P4 concentrations in both FF of follicles F1 and serum F1S were higher than these concentrations in FF of F3 and serum of F3S. The source of the significant high peripheral P4 concentrations reported in camels bearing predominant follicles without CL may be the adrenal cortex (Asher *et al,* 1989). Moreover, in mares, FF P4 concentrations of ovaries not bearing CL were higher than that present in FF of the CL bearing ones. Thus, CL modifies the intrafollicular P4 concentrations

Table 1. Hormonal and vitamin C compositions of follicular fluid in presence or absence of the corpus luteum in camels (Mean ± SEM).

Hormones & vitamin C	Follicles without CL [F1] (n= 20)	Follicles coexist with CL on the same ovary [F2] (n= 12)	Follicles with contralateral CL on the other ovary [F3] (n= 15)	P-value
Camel oestradiol 17-Beta-Dehydrogenase (E2; pg/ml)	0.290^{ab} ± 0.038 C. I.* 0.205 – 0.375	0.131^{a} ± 0.028 C. I. 0.069 – 0.193	0.347^{b} ± 0.063 C. I. 0.203 – 0.490	P<0.01
Camel progesterone (P4; ng/ml)	0.280^{a} ± 0.006 C. I. 0.267 – 0.295	0.253^{ab} ± 0.015 0.220 – 0.286	0.224^{b} ± 0.019 0.182 – 0.267	P<0.05
Camel cortisone (ng/ml)	0.236^{a} ± 0.033 C. I. 0.161 – 0.311	0.080^{b} ± 0.019 C. I. 0.036 – 0.124	0.245^{a} ± 0.055 C. I. 0.122 – 0.368	P<0.05
Camel IGF-II (ng/ml)	41.140^{a} ± 1.288 C. I. 38.227 – 44.053	57.271^{b} ± 1.041 54.915 – 59.627	45.810^{a} ± 2.331 40.537 – 51.083	P<0.001
Camel Ultra sensitivity triiodothyronine (T3; nmol/L)	3.849^{a} ± 0.023 C. I. 3.797 – 3.901	3.591^{b} ± 0.055 3.466 – 3.716	3.872^{a} ± 0.111 3.620 – 4.124	P<0.05
General Thyroxine (T4; ng/ml)	9.409^{a} ± 0.010 C. I. 9.387 – 9.431	9.491^{a} ± 0.406 8.572 – 10.410	8.349^{b} ± 0.187 7.927 – 8.771	P<0.01
Vitamin C (µg/ml)	26.920^{a} ± 0.081 C. I. 26.738 – 27.102	19.921^{b} ± 0.519 18.748 – 21.094	19.511^{b} ± 1.020 17.203 – 21.819	P<0.001

Means with different superscripts are significantly different.

*Confidence Interval.

Table 2. Serum hormonal and vitamin C concentrations in presence or absence of the corpus luteum in camels (Mean ± SEM).

Hormones & vitamin C	Serum of camels bearing follicles without CL [F1S] (n= 20)	Serum of camels bearing follicles coexist with CL on the same ovary [F2S] (n= 12)	Serum of camels bearing follicles with contralateral CL on the other ovary [F3S] (n= 15)	P-value
Camel oestradiol 17-Beta-Dehydrogenase (E2; pg/ml)	0.053^{ab} ± 0.019 C. I.* 0.011 – 0.095	$0.0.024^{a}$ ± 0.014 C. I. 0.004 – 0.049	0.063^{b} ± 0.032 C. I. 0.011 – 0.124	P<0.01
Camel progesterone (P4; ng/ml)	0.139^{a} ± 0.012 C. I. 0.111 – 0.167	0.126^{ab} ± 0.030 0.091 – 0.162	0.111^{b} ± 0.038 0.076 – 0.151	P<0.05
Camel cortisone (ng/ml)	0.018^{a} ± 0.008 C. I. 0.001 – 0.037	0.006^{b} ± 0.005 C. I. 0.0002 – 0.015	0.019^{a} ± 0.013 C. I. 0.001 – 0.044	P<0.05
Camel IGF-II (ng/ml)	61.014^{a} ± 1.713 C. I. 57.138 – 64.890	84.938^{b} ± 1.384 82.082 – 87.830	67.940^{a} ± 3.100 60.591 – 75.245	P<0.001
Camel Ultra sensitivity triiodothyronine (T3; nmol/L)	4.118^{a} ± 0.098 C. I. 3.895 – 4.341	3.842^{b} ± 0.234 3.555 – 4.135	4.143^{a} ± 0.473 3.713 – 4.589	P<0.05
General Thyroxine (T4; ng/ml)	7.832^{a} ± 0.160 C. I. 7.470 – 8.194	7.900^{a} ± 0.406 6.821 – 9.045	6.950^{b} ± 0.292 6.308 – 7.621	P<0.01
Vitamin C (µg/ml)	24.712^{a} ± 0.976 C. I. 22.504 – 26.920	18.287^{b} ± 0.254 15.779 – 20.952	17.911^{b} ± 2.290 14.479 – 21.672	P<0.001

Means with different superscripts are significantly different.

*Confidence Interval.

(Satué *et al,* 2019). On the contrary, there were significant higher P4 concentrations in the FF collected from pregnant camels (presence of a CL) than those obtained from non-pregnant ones (El-Shahat *et al,* 2019; Fawzy *et al,* 2021). The presence of the CL on the ovaries could play an important role in follicle growth, development, and control the concentrations of biochemical metabolites and hormonal profiles in the FF of dromedary camels (El-Shahat *et al,* 2019). Moreover, in sheep (Dufour *et al,* 1972; Rexroad and Casida, 1977) and cattle (Kor, 2014), the higher P4 concentration in FF of ovaries bearing CL than the FF of ovaries have no CL may relate to the presence of the CL. There is a relationship between the development of the CL and the development of follicles (Dufour *et al,* 1972; Rexroad and Casida, 1977). Corpora lutea affect the dynamics of follicles in both ovaries by a systemic effect with evidence for a local ipsilateral effect (Contreras-Solis *et al,* 2008).

Similar to the data reported in this study, the serum concentrations of E2, P4 and cortisol are many folds lower than those of follicular fluid in dromedary camels (Rahman *et al,* 2008) and mares (Satué *et al,* 2019). There are no correlations between the serum and FF of P4 in the preovulatory period or transitional mares (Bøgh *et al,* 2000; Satué *et al,* 2020).

In the present study, the cortisone concentrations in the FF of F1 and F3, and serum of F1S and F3S camels were higher than those present in FF of F2 and serum of F2S. Glucocorticoids in follicular fluid are derived from the general circulation. The glucocorticoid status of ovarian FF is prognostic to oocyte quality (Lewicka *et al,* 2003). The 11 beta-hydroxysteroid dehydrogenases convert cortisol to its inactive metabolite cortisone and *vice versa* (Quinkler *et al,* 2003). Cortisol inhibits the ovarian steroidogenesis (Michael *et al,* 1993) and stimulate oocyte maturation (Fateh *et al,* 1989; Jimena *et al,* 1992). During the LH surge, an increase in total and free cortisol occurs in the ovarian follicle (Harlow *et al,* 1997; Yong *et al,* 2000). Formation and function of the CL benefits from a high local concentration of free cortisol, whereas the surrounding developing follicles may experience negative effects (Andersen, 2002).

In the present study, camel IGF-II concentrations in the FF of F2 and serum of F2S were higher than those concentrations in the FF of F1 and F3 and serum of F1S and F3S. The IGFs play a significant role in follicular and luteal development in the bovine ovary, and locally produced IGF-II is probably an important regulator of follicular growth in cattle (Perks *et al,* 1999). In primate species, IGF-II is the predominant circulating and intraovarian form of IGFs (Giudice, 2001; Tkachenko *et al,* 2021). Besides, IGF-II stimulates granulosa steroidogenesis (Giudice, 2001).

The T3 concentrations in the FF of F1 and F3, and serum of F1S and F3S camels were higher than those found in FF of F2 and serum of F2S. Parallel with these results, T3 concentrations were significantly high in the FF collected from predominant follicles existing in absence of CLs (Tabatabaei *et al,* 2011). T4 concentrations were high in the FF of F1 and F2 and serum harvested from F1S and F3S. However, the majority of thyroxine present in follicular fluid appear to be derived from peripheral blood and enter follicles through theca interna cells (Cai *et al,* 2019). Thyroid hormones control the growth, differentiation, and metabolism in almost all somatic tissues (Ingbar and Wieber, 1981). Moreover, thyroxine affects the follicular development and oocyte maturation (Accardo *et al,* 2004; Ashkar *et al,* 2010; Verga Falzacappa *et al,* 2012; Zhang *et al,* 2013; Fedail *et al,* 2014), and ovarian steroidogenesis (Cecconi *et al,* 1999; Spicer *et al,* 2001). T3 appears to synergise with follicle-stimulating hormone (FSH) to induce development of granulosa cells (Shabankareh *et al,* 2013). The concentrations of T3 and T4 in the FF were higher in absence of CL when compared with those in follicles coexist with CL on ovaries (Kor, 2014). In this study, Vitamin C concentrations were high in the FF of F1 and serum of camels' type F1S when compared with those concentrations in the FF of F2 and F3, and serum of dromedaries' type F2S and F3S. The ovaries store high amounts of ascorbic acid within the granulosa, thecal, and luteal cells (Deane, 1952). Ascorbic acid endorses steroidogenesis (Sanyal and Datta, 1979), acts as an antioxidant (Goralczyk *et al,* 1992; Luck *et al,* 1995) and assets in remodeling the basement membrane during follicular growth (Murray *et al,* 2001). However, in human, the quality of oocyte maturation and embryos is better in lower levels of vitamin C compared to higher levels (Saffari *et al,* 2015).

The presence of CL might indicate the existence of a local drastic effect on hormonal and vitamin C composition of FF and could indirectly influence follicular development and oocyte quality.

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EFFECT OF PHYTOCHEMICAL-RICH PELLETED COMPLETE FEED ON HAEMATO-BIOCHEMICAL PARAMETERS IN CAMEL CALVES

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ABSTRACT

To study the effect of phytochemical-rich pelleted complete feed on haemato-biochemical parameters of camel calves, 98 days feeding trial was conducted on 15 camel divided into 3 equal groups *viz.*, T_1 , T_2 and T_3. The experimental feed was iso-nitrogenous prepared with concentrate to roughage ratio 60:40. Treatments were: (T_1) a basal diet (control) consisted of roughage (groundnut straw and guar straw) and concentrate mixture, (T_2) the basal diet supplemented with *Prosopis cineraria* (20% of roughage moiety), (T_3) the basal diet supplemented with *Ziziphus nummularia* (20% of roughage moiety). There was no significant difference in the mean haemato-biochemical parameters *viz.*, haemoglobin, packed cell volume, blood glucose, total serum protein, urea, AST and ALT. Results indicate that *P. cineraria* and *Z. nummularia* leaves supplementation of diets of Indian dromedary camel calves at 20% level in roughage moiety of pelleted complete feed does not affect physiological health status of camel calves.

Key words: Camel, haemato-biochemical, pelleted complete feed, phytochemical

The phytochemicals contained in plants are a part of the herbivore diets and these bioactive compounds have rumen modifying capabilities (Wallace *et al,* 2002). The phytochemicals or plant secondary metabolites act as natural feed additives to improve the efficiency of rumen fermentation such as enhancing protein utilisation efficiency, decreasing methane production, reducing nutritional stress such as bloat, and improving animal health and productivity (Singh and Sahoo, 2004; Patra *et al,* 2006; Benchaar *et al,* 2007). Camel has a unique ability to convert the scanty plant resources of the desert into milk, meat and fibre. Its unique physiological system aids this animal to fill important niche in desert ecosystem (Ahmad *et al,* 2010). The conventional sources of fodder seem inadequate and diminishing gradually and thus, it is imperative to identify other non-food plant resources and unconventional forages to assist the animal feed industry and improve livestock productivity (Sahoo and Sawal, 2021). The multipurpose tree leaves and shrubs have been proclaimed as a solution to feeding of ruminants in the tropical areas, especially as supplementary feeds to low-quality forages containing low levels of crude protein and fermentable energy (Singh and Sahoo, 2004; Patra and Saxena, 2009; Sharma and Sahoo, 2017). The objective was to evaluate the effects of tanniferous *P. cineraria* and saponiferous *Z. nummularia* supplementation in pelleted complete feeds of Indian dromedary camel calves on haemato-biochemical parameters.

Materials and Methods

Dietary treatments and feeding plan

The present experiment was conducted at ICAR-National Research Centre on Camel, Bikaner, Rajasthan, India. Fifteen Indian dromedary camel calves (5-6 months old; initial body weight 143±2.7 kg) were randomly assigned to one of three dietary treatments in a 98 days completely randomised design experiment. The experimental feed was iso-nitrogenous prepared with concentrate to roughage ratio 60:40. Three treatments given were: T1- a basal diet (control) consisted of roughage (groundnut straw and guar straw) and concentrate mixture, T2- the basal diet supplemented with *Prosopis cineraria* (20% of roughage moiety), T3- the basal diet supplemented with *Ziziphus nummularia* (20% of roughage moiety). Animals were housed in individual semi open pens.

All diets were mixed mechanically and pressed to form pelleted complete diets. Diets were offered individually twice daily (10:00 and 16:00 h), while feed residues, if any, were removed and weighed

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once daily before morning feeding. Fresh water was available *ad lib*.

Haemato-biochemical assay

Blood samples were collected at fortnightly intervals for assessing the haemato-biochemical status of camel calves.

Haemoglobin and packed cell volume (PCV) were determined using Sahli-Hellige haemoglobinometer and microhematocrit method, respectively, while mean corpuscular haemoglobin concentration (MCHC) was calculated as the ratio between the two and expressed as percentage. Serum was separated from another blood sample to estimate the biochemistry parameters, *viz*. blood glucose, serum urea, total protein, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by Biotron BTR-830 photometer using standard kit supplied by AGD Biomedicals (P) Ltd., India.

Statistical analysis

The data on various haemato-biochemical profile were analysed using analysis of variance (ANOVA) by applying the general linear model (GLM) with two principal variables treatments and periods of observation (SPSS, 2011; version 20.0). The mean values were compared by employing Tukey's multiple comparison and significance was declared at $P < 0.05$.

Results and Discussion

The nutrient composition of tree leaves in the pelleted feeds differed with respect to polyphenolic composition and was due to addition of phytochemical rich tree leaves at 20% replacement to conventional groundnut straw and guar straw mixture that was offered to the control group. This deviation was due to higher tannin content in *Khejri** (*P. cineraria*) leaves and higher saponin content in *Pala leaves*** (*Z. nummularia*) (Pal *et al,* 2015; Singh *et al,* 2005).

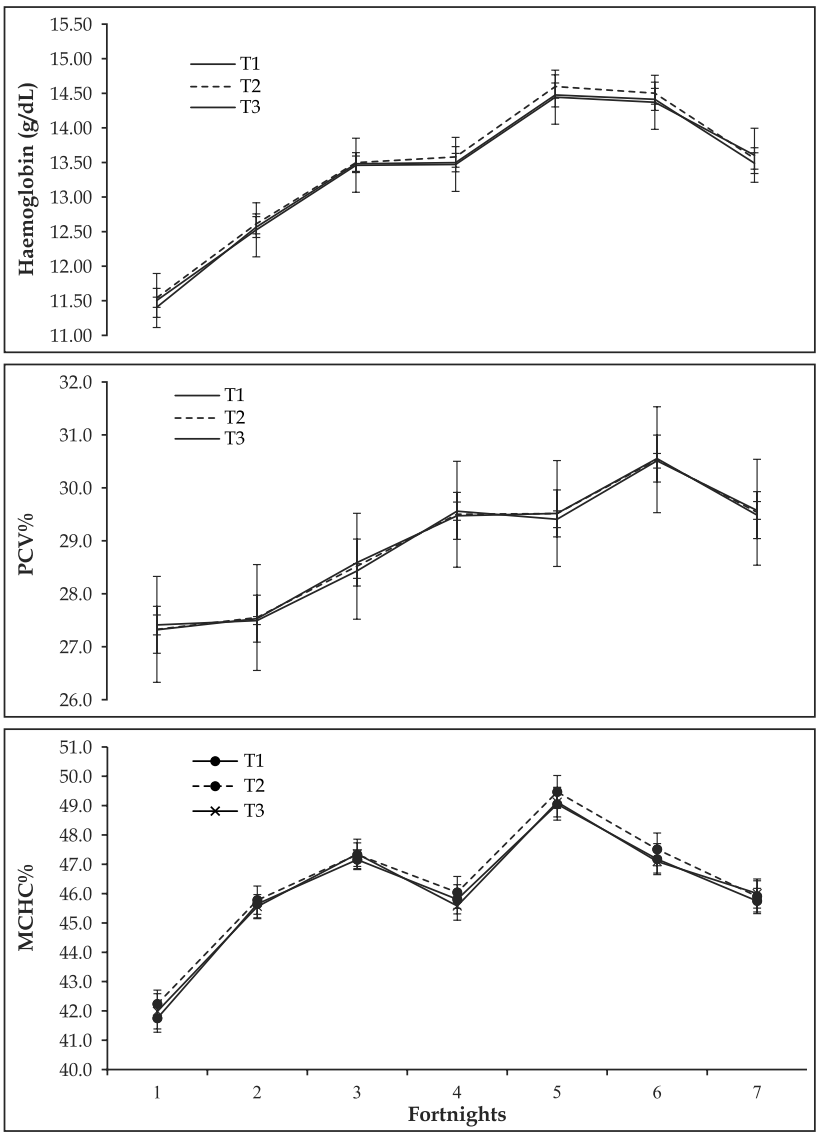

Fig 1. Haemoglobin, PCV and MCHC concentration of camel calves in different groups during the pre-weaning periods.

Haematological parameters

There was no significant effect of phytochemical-rich dietary variation on the haematological parameters *viz*., haemoglobin (P=0.536), PCV (P=0.983), MCHC (P=0.615) between the groups, but periodic alteration (P<0.001) with non-significant treatment × period interaction was seen (Fig 1). Invariably, all the three parameters were highest during the 5th fortnight and lowest during the first fortnight. It was revealed that the observed values in all the 3 dietary groups were well within the normal range and were in line with other reports (Dey *et al,* 2015; Pathak *et al,* 2016; Sireesha *et al,* 2021). The present haematological observations in preweaning camel calves may be useful as reference values for camel calf health and metabolic profile in correlating to nutritional status or

* and ** Vernacular names.

different disease conditions.

Blood-biochemistry

The blood biochemical constituents *viz.* glucose, urea, total proteins were found similar amongst the groups (Table 1).

Serum enzymes

The serum concentration metabolising enzymes, ALT and AST did not differ between the treatments, periods or treatment × period interaction in camel calves during the pre-weaning periods (Table 2).

In the present study, glucose, urea, total protein, AST and ALT levels in all the 3 dietary groups were found within the physiological range indicating that inclusion of phytochemical-rich pelleted feeds did not elicit any adverse effect on these parameters and agree with previous findings of Yadav and Bissa (1998) and Bogin (2000). Raghuvansi *et al* (2007a) also reported similar non-significant effect of inclusion of tree leaves in the diet of sheep on blood biochemical constituents

Table 1. Effect of phytochemical-rich pelleted feeds on blood biochemical parameters of camel calves during the pre-weaning periods.

Attributes	Fortnights							Pooled	SEM	Significance		
	1	2	3	4	5	6	7			Treatment	Period	Treatment × Period
Glucose (mg/dL)												
T1	127.4	127.8	128.1	126.2	125.0	128.8	127.2	127.2	0.48	0.128	0.882	0.900
T2	127.9	126.9	128.7	129.4	127.7	126.3	126.4	127.6	0.44			
T3	126.5	126.1	125.8	127.3	126.1	124.7	124.9	125.9	0.34			
SEM	0.41	0.49	0.88	0.94	0.78	1.19	0.67					
Plasma urea (mg/dL)												
T1	19.47	19.44	17.44	18.65	18.59	19.31	18.78	18.81	0.27	0.579	0.122	0.747
T2	19.76	18.34	17.33	19.56	19.24	16.85	16.59	18.24	0.50			
T3	19.11	19.91	18.35	18.90	18.55	16.62	18.25	18.53	0.38			
SEM	0.20	0.47	0.35	0.27	0.20	0.85	0.67					
Serum protein (g/dL)												
T1	4.27	4.31	4.39	4.30	4.36	4.29	4.32	4.32	0.022	0.203	0.340	0.210
T2	4.34	4.30	4.27	4.26	4.31	4.29	4.24	4.29	0.013			
T3	4.26	4.35	4.33	4.23	4.30	4.34	4.36	4.31	0.021			
SEM	0.004	0.030	0.028	0.031	0.026	0.033	0.005	0.034				

T1, pelleted feed with conventional roughages; T2, pelleted feed containing 20% *P. cineraria* leaves; T3, pelleted feed containing 20% *Z. nummularia* leaves.

Table 2. Effect of phytochemical-rich pelleted feeds on serum enzymatic profile indicative of the liver function of camel calves during the pre-weaning periods.

Attributes	Fortnights							Pooled	SEM	Significance		
	1	2	3	4	5	6	7			Treatment	Period	Treatment × Period
Aspartate transaminase activity (IU/L)												
T1	53.5	52.9	54.3	55.9	50.4	51.3	55.4	53.4	0.77	0.906	0.953	0.954
T2	53.9	53.1	53.3	52.2	52.6	52.6	56.5	53.5	0.55			
T3	51.4	54.7	51.4	56.3	52.0	50.9	51.2	53.2	0.79			
SEM	0.78	0.57	0.85	1.31	0.66	0.51	1.41					
Alanine transaminase activity (IU/L)												
T1	10.16	10.22	11.87	10.81	10.68	11.93	12.18	11.12	0.32	0.932	0.241	0.999
T2	10.12	11.31	10.51	11.20	11.72	11.34	12.47	11.24	0.29			
T3	10.86	10.56	11.61	11.16	10.69	10.54	12.05	11.07	0.22			
SEM	0.25	0.32	0.43	0.13	0.33	0.41	0.12					

T1, pelleted feed with conventional roughages; T2, pelleted feed containing 20% *P. cineraria* leaves; T3, pelleted feed containing 20% *Z. nummularia* leaves.

and found no health disorders during the period of study. Likewise, Raghuvansi *et al* (2007b) also found no effect of foliage supplementation on haemato-biochemical parameters of sheep.

In the present study, haemato-biochemical parameters that were assessed and monitored fortnightly as a measure of nutritional and health wellbeing of camel calves in response to feeding on phytochemical-rich diets did not alter.

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CHARACTERISATION OF SALIVARY GLAND PROTEINS AND P-18 GENE OF CAMEL TICKS FROM BIKANER

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ABSTRACT

The main objective of this study was to investigate the various salivary gland proteins and gene analysis of camel ticks *Hyalomma dromedarii* found on one-humped camels (*Camelus dromedarius*) in Bikaner city, Rajasthan, India. For this purpose, Salivary glands were collected from 5 adult ticks (engorged females). Various protein analysis from salivary gland using SDS-PAGE for determination of protein bands and their molecular weights for each fraction was done. The molecular weight of resolved major bands ranged from 14.4 to 96.0 KDa. Out of them large majority of protein molecular weight was around 18 KDa. This low molecular weight band of around 18 KDa was detected in the all 5 fractions of salivary gland of *Hy. dromedarii* ticks. The protein gene P–18 of *Hy. dromedarii* of present study had 90.4% sequence identity with that of *Hy. asiaticum* from China.

Key words: Camel *Hyalomma dromedarii*, P-18 Gene salivary gland proteins

Hyalomma dromedarii ticks were the object of several studies seeking to characterise the molecules isolated from salivary gland extracts and saliva. In order to avoid host defenses, ticks secrete saliva at the bite site that contain many biologically active molecules that display anticoagulation, antiplatelet, vasodilatory, anti-inflammatory and immunomodulatory activities (Kazimirova and Stibraniova, 2013; Chmelar *et al,* 2012; Simo *et al,* 2017).

The tick saliva has hundreds of different proteins (Chmelar *et al,* 2016) which are multipotent and had pharmacological features (Steen *et al,* 2006). Accordingly, several transcript and protein profiles of tick salivary glands were carried out in different stages of development, for both genders and feeding behaviour (Francischetti *et al,* 2011; Tirloni *et al,* 2014; Tan *et al,* 2015). The field of vector–host interaction has gained tremendously by high–throughput analysis of salivary gland transcripts and proteomes, collectively called the sialome (Ribeiro and Francischetti, 2003). More interestingly, sialo transcriptomic analyses improved proteomic studies of unknown genome species that seek to identify pharmaceutically active proteins (Evans *et al,* 2012; Mudenda *et al,* 2014).

Only few reports have explored *Hy. dromedarii* salivary glands. The characterisation of salivary gland proteins of *Hy. dromedarii* at protein level was done (Kandil and Habeeb, 2009). The molecular characterisation of Bm86 orthologue from *Hy. dromedarii* was carried out (Ben Said *et al,* 2012). Compared to other haematophagous parasites, relatively little information exists about the molecular composition of *Hy. dromedarii* salivary glands (Ibrahim and Masoud, 2018; Marzouk and Darwish, 1994).

In present study the various salivary gland proteins and gene analysis of camel ticks *Hyalomma dromedarii* found on one-humped camels (*Camelus dromedarius*) in Bikaner city were investigated.

Materials and Methods

Collection of ticks from camels

Five engorged adult female ticks were collected from the ground of camel pens at herd of National Research Centre on Camel (NRCC), Bikaner, India. These were morphologically identified (Apanaskevich *et al,* 2008) and kept individually in plastic tubes, incubated at 26°C, 75% RH and photoperiod of 12:12 (L: D). The ticks were cleaned several times in sterile 1 x PBS (pH 7.2).

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Isolation of salivary glands and proteins analysis

Salivary glands of collected *Hyalomma dromedarii* (engorged females) were isolated as per the described by EL–Kammah *et al* (2005). These were placed into phosphate buffer saline (PBS) (pH 7.4) and opened along their dorsal surface. Salivary glands were removed, dissected free of other tissues, placed into PBS at 4°C. The salivary gland proteins were analysed using SDS-PAGE for determination of protein bands and their molecular weights for each fraction as described by Hames (1987).

RNA Isolation and cDNA Synthesis

Total cellular RNA was extracted from tick salivary glands using Total RNA isolation kit–Cells and Tissues (Bangalore GeNei Pvt. Ltd, India). Using total cellular RNA extracted from the tick salivary glands as a template, cDNA was synthesised by Easyscript First Strand cDNA Synthesis Kit according to the manufacturer's instructions from the resultant cDNA synthesised.

Characterisation of salivary gland protein gene (P-18)

The P–18 protein gene of *Hyalomma dromedarii* was amplified using the forward primer of 5′ GAG CGG ATC CAT GAT TTT ATG GGC GCT TTG 3′ and reverse primer of 5′ CGC GCT CGA GTT ACC ACT CAA TCT TGA CTG 3′. The sequences of the primers were deduced from the Gen Bank Accession No. EU000252. PCR amplification was performed with following thermal profiles: initial denaturation of 94°C for 3 min. followed by 35 cycles of denaturation at 94°C for 1 min., 57°C for 1 min for P–18 gene and extension at 72°C for 1 min. and final extension at 72°C for 10 min. The PCR amplified products were checked on 1% agarose gel.

Cloning and sequencing of P-18 Gene

The purified amplicons corresponding to genes encoding P–18 of *Hyalomma dromedarii* were cloned into pGEM–T Easy vector (Promega Corp., Medison, USA). The ligated mixtures for P-18 gene was individually transformed into *Escherichia coli* DH 5α (Sambrook *et al,* 1989). The positive clones were confirmed by colony PCR using gene–specific primers and restriction analysis with EcoRI. The positive clones were sequenced at the sequencing facility, Delhi University (South campus), Delhi. Since pGEM–T easy vector was used for the cloning purpose, universal T7 and SP6 primers were used for the sequencing of recombinant clones. The primer sequences used for the sequencing were based on respective promoter sequences.

Sequence analysis of P-18 Gene

Nucleotide identity and comparison of sequences with published sequences of members of *Ixodidae* available in the GenBank database were carried out using the computer software Bio Edit version 7.0.9. These sequences were compared in Clustal X (Thompson *et al,* 1997) and a phylogenetic tree was constructed based on the amino acid sequences by the neighbour–joining method using Mega 4 (Molecular Evolutionary Genetics Analysis software with bootstrap values calculated for 1, 000 replicates (Tamura *et al,* 2007).

Results

The salivary glands isolated under light microscope were significantly larger and were observed having white folds and normal acini.

Salivary glands protein (P-18) analysis by SDS-PAGE

The protein analysis from salivary gland using SDS-PAGE for determination of protein bands and their molecular weights for each fraction. A total number of 11 major bands of salivary gland proteins with molecular marker were seen in the gel (Fig 1). The molecular weight of the resolved major bands ranged from 14.4 to 96.0 KDa. Out of them, the intense band of protein having the molecular weight of around 18.0 KDa was observed in all the salivary glands of five ticks examined.

Genes encoding P–18 of *Hyalomma dromedarii* were cloned and the length of the P–18 gene sequenced was 461 bp (Fig 2).

Sequence comparison of P-18 Gene

BLAST search analysis in NCBI database showed that it closely matched with P–18 gene of *Hyalomma asiaticum* (Accession No. EU000252), which

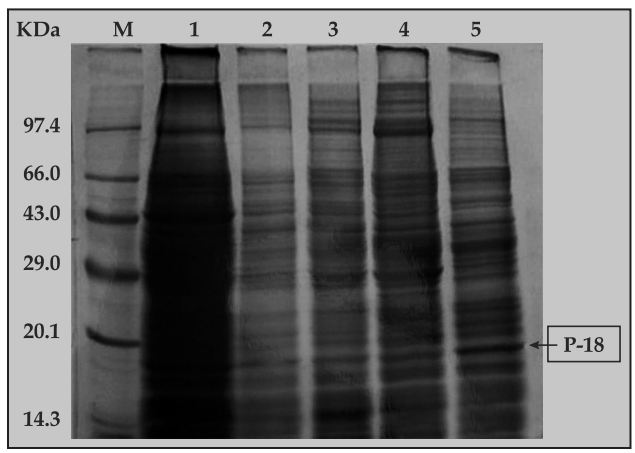

Fig 1. SDS-PAGE photograph showing protein profile of salivary glands of *Hyalomma dromedarii.*

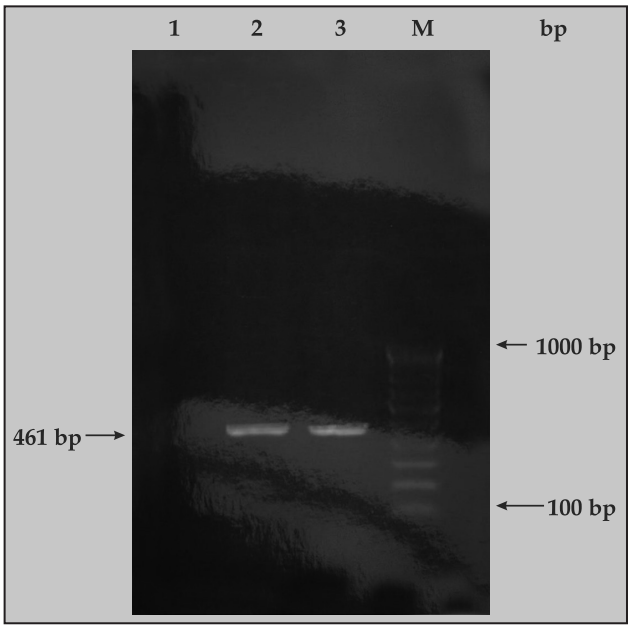

Fig 2. Ethidium bromide stained 1.2% agarose gel showing amplification of salivary gland protein (P18) gene of *Hyalomma dromedarii*.

Lane M-100 bp DNA. Ladder Lane 1- Negative control. Lane 2-3-Desired amplicons.

is the only one available sequence in NCBI database. Pair–wise comparison of these 2 sequences showed that 461 bp of *Hy. dromedarii* from India was matching with the nucleotide base, 26 to 486 of P–18 gene of *Hy. asiaticum* from China (Fig 3). These partial 461 bp gene sequences compared with the corresponding nucleotide sequences of P–18 gene of *Hy. asiaticum* (Gen Bank Accession No. EU000252) revealed that they had 90.4 % sequence identity with *Hy. asiaticum*. The nucleotide sequences of the salivary gland protein (P–18) gene of *Hy. dromedarii* were submitted to GenBank, NCBI database and assigned accession number HM051110.

Discussion

Hyalomma dromedarii is a very characteristic tick closely associated with camels and widely distributed in desert and steppes, wherever, camels are found.

Several investigations have successfully induced host resistance by administration of tick antigen derived from salivary gland (Wikel, 1996; de la Fuente *et al,* 1998; Mahmoud *et al,* 2005). The use of concealed tick antigens was considered the first

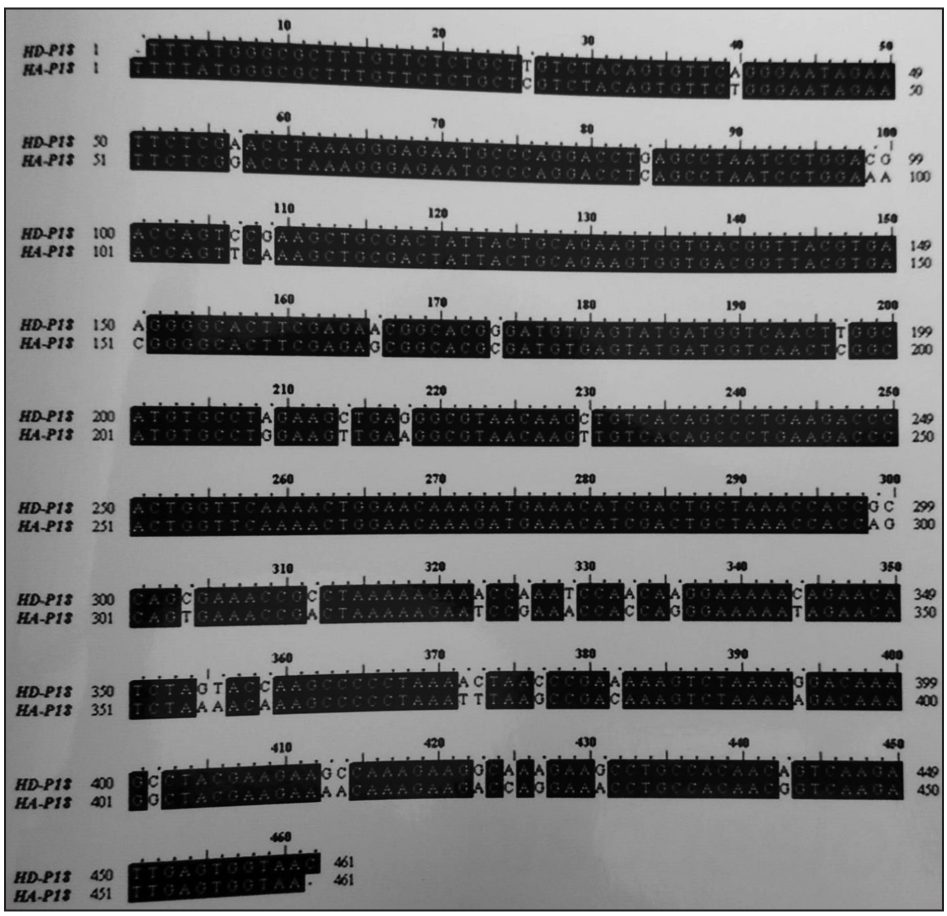

Fig 3. Multiple alignment of nucleotide sequence of P18 gene of *Hy. dromedarii* from India and *Hy. asiaticum* from China.

step for the basis of a commercial vaccine. Therefore, several workers have reported development of acquired immunity against tick by artificial immunisation with tick salivary gland antigens of *Boophilus microplus* (Parmar *et al*, 1996); *Hy. anatulicum anatolicum* (Sran *et al*, 1996) and *Hy. dromedarii* (El-Kelesh, 2002; Kandil and Habeeb, 2009).

The first proteomics study informed by transcriptomics to identify *Hy. dromedarii* salivary gland proteins in both genders using LC-MS/MS was reported by Bensaoud *et al* (2019). Only few reports have explored *Hy. dromedarii* salivary glands.

The nucleotide sequences of the salivary gland protein (P–18) gene of *H. dromedarii* were submitted to GenBank and the assigned accession number was HM051110.

The baseline information about the salivary gland protein P–18 gene of the present study would help the exploration of sialomics of *Hy. dromedarii* and other ixodid ticks from different geographical areas of India and thereby the development of a new generation vaccine for control of ticks would be feasible in India.

Acknowledgements

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Conflict of Interest

The authors declare that they have no conflict of interest.

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NATURAL CAMEL MILK PRODUCTS TO TREAT AUTISM-SPECTRUM DISORDERS

Camel milk contains essential vitamins, minerals, and immunoglobulins, providing the milk with antioxidant, antibacterial, and antiviral properties. These properties may reduce oxidative stress in camel milk consumers, ameliorating many conditions, including those of the CNS, such as autism spectrum disorders (ASDs). Scientists performed a meta-analysis of randomized controlled trials (RCTs) in which camel milk administration (boiled or raw) was examined as an ASD treatment intervention. The primary endpoint was participants' total autism scores, determined using the Childhood Autistic Responsiveness Scale (CARS). Treatment of ASD with raw and boiled camel milk resulted in significantly lower CARS scores than the placebo. Researchers were of the opinion that the development of larger, more populated RCTs is required to establish camel milk's overall potential as a therapeutic intervention for CNS disorders.

(Source: Kandeel M, El-Deeb W. The Application of Natural Camel Milk Products to Treat Autism-Spectrum Disorders: Risk Assessment and Meta-Analysis of Randomized Clinical Trials. Bioinorg Chem Appl. 2022 May 27;2022:6422208. doi: 10.1155/2022/6422208. PMID: 35669459; PMCID: PMC9166988.)

THERAPEUTIC POTENTIAL OF CAMEL WHARTON JELLY MESENCHYMAL STEM C ELLS (CWJ-MSCS) IN CANINE CHRONIC KIDNEY DISEASE (CKD) MODEL

Recently, Egyptian scientists found out therapeutic potential of Camel Wharton Jelly mesenchymal stem cells to treat chronic kidney disease in canines. Umbilical cord specimens were obtained fresh from a healthy pregnant camel and placed in sterile saline at 4°C. Isolated CWJ-MSC was identified by morphology and flow cytometric analysis. The transplantation of CWJ-MSCS in the CKD model induced in dogs showed improved kidney function tests after one week of stem cells injection and by the end of the experiment, they got classified as stage 2 of CKD according to the staging system. The histological section of the kidney from the treated CKD model with CWJMSCs (G2) showed great improvement of glomerular lesions and tubular injury recorded in the CKD model. The lesion score of cortical and medullary glomerular lesions and fibrosis were significantly lower than the CKD group but was still significantly higher than the normal group. The serum creatinine level was significantly decreased at the 4th week just after the 1st stem cells injection. The value decreased from 3.22 to 2.59 and consequently reached 1.86 at the end of the experiment.

(Source: El Miniawy, H.M.F., Farghali, H.A., Khattab, M.S. *et al*. The therapeutic potential of Camel Wharton jelly mesenchymal stem cells (CWJ-MSCs) in canine chronic kidney disease model. Stem Cell Res Ther 13, 387 (2022). https://doi.org/10.1186/s13287-022-03076-8)

AUTHORS RECEIVED THE AWARD OF THE FRENCH VETERINARY ACADEMY

Face to the emergence of camel farming development in Western countries, not only for touristic attractions but also for agricultural activities as milk production, the "new camel farmers" are looking for practical guide allowing to understand the physiology, health, and management of camels in such new context. It was one of the objectives of the book, "l'élevage des grands camélidés" written by Dr Bernard Faye, Dr Gaukhar Konuspayeva and Dr Cecile Magnan and Published in French by QUAE editions. For this book, the authors received the Award of the French Veterinary Academy. Turkish, Arabic and Spanish versions of this handbook are already available for dairy camel farm management (in the frame of the European project CAMELMILK) and a more complete English version will be ready soon edited by Springer. German, Italian, and Kazakh versions are also expected.

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ANTIDIABETIC, ANTICOLITIS AND ANTICANCER ACTIVITY IN CAMEL MILK: A SYSTEMATIC ANALYSIS

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ABSTRACT

Camel milk is a nutrient-dense diet with anti-inflammatory, immune-regulating, gut microbiota-maintaining effects, a protein-rich vehicle with a wide range of therapeutic applications in gastrointestinal affections such as colitis, diabetes and cancer. In addition, the global awareness of camel milk and its products is now increasing with expected large camel products economic growth. Camel milk exosomes and camel albumen complexes with oleic acid showed promising anticancer actions. Camel milk ameliorates oxidative stress by increasing the expression of antioxidant genes as well as reducing angiogenesis and tumour growth factors in cancer. Camel milk is equipped with noticeable antidiabetic power comprising 52 insulin U/L, rich in β–cells nourishment and enhancing mediators and proteins. Camel milk boosts the expression of carnitine palmitoyltransferase type I, insulin receptor substrate type 2 and fatty acid synthases, which aids in insulin production and regulation and helps diabetic patients improve and normalise blood glucose, lipid profile, total glycerides and high-density lipoprotein levels. Camel milk has a unique quality as a powerful antimicrobial system made up of H_2O_2, lysozymes, immunoglobulins, antioxidants and low-weight molecular antibodies. Camel milk showed more nutritional and medicinal properties than other ruminant milks, hence numerous western countries are becoming more interested in raising camels for milk and byproducts.

Key words: Anticancer, bioactive components, camel milk, colitis, diabetes

Camel milk is high in insulin, lactoferrin, minerals and protein such as iron, vitamins, sodium, magnesium, iodine and potassium (Mabood *et al,* 2017). Autism, jaundice, hepatitis, anaemia, asthma, lactase deficiency and breast cancer are just a few of the conditions for which camel milk has shown to be helpful (Rahim *et al,* 2020). Camels' milk may be stored for extended periods without refrigeration (Mullaicharam, 2014). Combination of camel milk and urine were effective in treating breast and prostate cancers (Gupta *et al,* 2021). Camel milk dramatically inhibited cell growth, viability and migration. The rise of microtubule-associated protein1 light chain 3 (LC3-II) protein, together with a decrease in the expression of autophagy proteins, suggested autophagy induction. Confocal microscopy revealed (GFP)-LC3 puncta, which showed autophagosome production in response to camel milk therapy (Krishnankutty *et al,* 2018). The capacity of alpha-lactalbumin (α-LA), a tiny milk calcium-binding globular protein, to form complexes with oleic acid has been shown to have significant anticancer effect, especially breast cancer (Uversky *et al,* 2017).

β-Lactoglobulin is naturally missing in camel milk, making it similar to human milk and avoiding the major allergic protein in cows' milk. Whey protein/casein and β-casein/αs-casein ratios are greater in camel milk than in bovine milk. One of the key reasons for human milk's simple digestion in babies has been attributed to its high whey protein content and β-CN dominance. Camel milk also includes significant levels of proteins such as lactotransferrin, peptidoglycan recognition protein and whey acidic protein, which have beneficial functions in the immunological system. Camel milk's distinct physiochemical qualities make it a potential alternative to bovine milk for daily dairy consumption and newborn formula basis. Camel milk has piqued the curiosity of both academics and industry throughout the world in recent years. Although the amount of protective proteins in camel milk has been thought to contribute to its health and therapeutic advantages, little is known about how these bioactive proteins operate. This research investigates the applications of camel milk as a therapeutic tool with a focus on its anticolitis, antidiabetic and anticancer properties.

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Materials and Methods

The PRISMA checklist and guidelines were used to implement the analysis (Page *et al,* 2021).

Search strategy

The search engines Google Scholar, PubMed and Web of Science were used to retrieve the data. The range was between January 2013 and January 2021. The search approach comprises combination of several keywords e.g. Camel milk, composition, therapeutic, biological functions and benefits. The keywords that were used are shown below in Table 1.

Table 1. The keywords that were used in searching databases.

Search context	keywords
Camel milk	Composition of camel milk, Nutritional Value of camel milk, Benefits of camel milk, biological functions of camel milk, camel milk and its products.
Diseases	Therapeutic effects of camel milk, Camel milk and diabetes, camel milk and colitis and camel milk as anticancer.

Inclusion criteria

To collect high-quality research, journals having an impact factor were chosen. The papers focused on camel milk, its composition and its medical benefits of camel milk were included. The publications considered in the study were about diseases such as cancer, diabetes and colitis, as well as the influence of camel milk on reducing the effects of diseases on the human body.

Exclusion criteria

The papers omitted from the analysis did not fulfill the study's inclusion criteria. This study disregarded articles that give hetero-analysis on the selected issues and also excluded studies that were not in English or not accessible. Studies that were not primarily focused on the qualities of camel milk were also omitted. Finally, studies that did not address any intervention or outcomes of interest were excluded.

Results and Discussion

Identification and data input

About 988 papers were obtained from databases and 254 relevant articles were retrieved from various websites, organisations and pre-existing citations in the databases. The papers that did not fall under eligibility criteria were eliminated from the study (n= 176). Abstracts and titles were examined to sort the relevant themes and complete articles were retrieved and evaluated based on eligibility criteria if the abstract and title offered adequate information. After retrieving the articles, filtration based on the accessibility, language and overall merit, a total of 22 articles were selected which are shown in Fig 1 and Table 2.

The included studies

After extensive filtration and deliberation, 22 articles were selected as the best fit for evaluating camel milk. The studies were published between 2013 and 2021. The studies evaluated throughout the investigation were conducted in various territories. Every study and review included in the analysis discovered a connection between camel milk and its composition, as well as camel milk and therapeutic benefits.

The therapeutic effect of camel milk

Camel products and byproducts are gaining popularity across the world as a source of essential nourishment and a way to improve human health. When compared to other milk products, camel milk has more therapeutic, immune-restorative and nutrient-rich potential, making it a superior animal of the twenty-first century. Camel milk protein's bioactive peptides are one of the most essential features in maintaining human health since they assist to prevent many microbial infections and increase the immune system's normal function. Bioactive peptides are formed during the digestion of camel milk protein and serve as hypoglycemic and anti-obesity agents (Mudgil *et al,* 2018). Even though it is accessible in pasteurised and fresh raw milk at room temperature, bovine milk has a shorter shelf life than camel milk (Khalifa and Zakaria, 2019). Camel milk has a unique quality as a powerful antimicrobial system (Muhialdin and Algboory, 2018). This dynamic antimicrobial system is made up of H_2O_2, lysozymes, Immunoglobulins, antioxidants and low-weight molecular antibodies and it has a long shelf life. Camel milk, like bovine milk, is substantially supplemented with vital nutritional components; yet, in allergic reaction sensitive babies, camel milk is better since it includes β-casein and lactalbumin whey protein in comparable proportions to human milk (Ali *et al,* 2019; Solanki *et al,* 2017). Camel milk has an elevated concentration of mesophiles (Lactobacilli and Leuconostoc), as well as some bacteria such as *E. coli* and salmonella and their accumulation might be attributable to harsh scorching circumstances in drought-stricken areas. Although these organisms have both positive and negative effects on human health, their primary benefit is to improve intestinal digestibility (Issa and Tahergorabi, 2019).

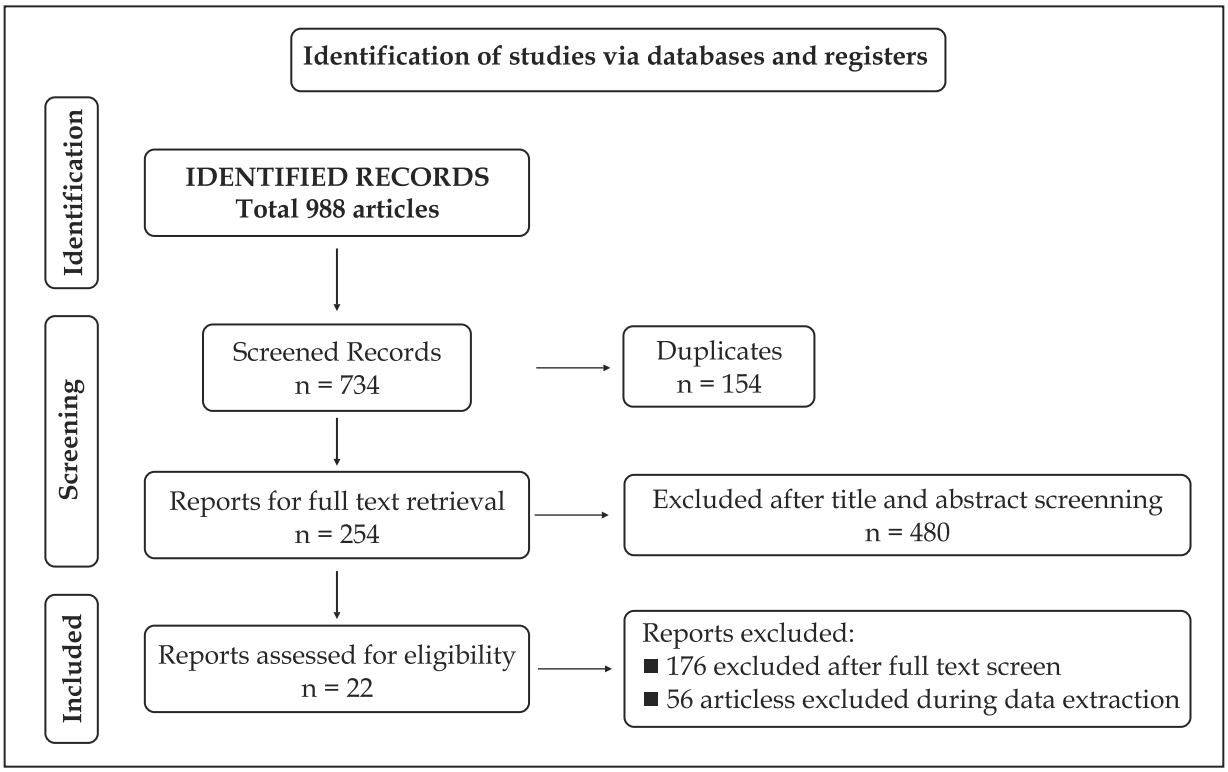

Fig 1. The PRISMA flow chart of article selection, screening and output.

Table 2. The selected studies on camel milk after search, identification and filtration.

Variables	Topic	References
Effectiveness of camel milk on diabetes	— The short-term therapeutic efficacy of camel milk vs. buffalo milk in alloxan-induced diabetic rabbits — A comparison of the hypoglycemic and antithrombotic (anticoagulant) effects of whole bovine and came milk in rats with streptozotocin-induced diabetes mellitus — Characterisation and identification of novel anti-diabetic and anti-obesity peptides derived from camel milk protein hydrolysates — A review of camel milk's role in the treatment of type 2 diabetes	• (Deeba *et al*, 2020) • (Khan *et al*, 2013) • (Korish *et al*, 2020) • (Mudgil *et al*, 2018) • (Shahriari *et al*, 2018)
Effectiveness of camel milk on cancer	— Camel milk reduces inflammatory angiogenesis in mice by downregulating proangiogenic and proinflammatory cytokines — *In vitro* and *in vivo* therapeutic effect of camel milk and its exosomes on Mcf7 cells — The use of human, bovine and camel milk albumins in oleic acid-based anticancer complexes — Research on camel milk's antimicrobial and antioxidant properties, as well as its role as an anti-cancer and anti-hepatitis agent. — The antigenotoxic and anticytotoxic effects of camel milk in cisplatin-treated mice	• (Alhaider *et al*, 2014) • (Badawy *et al*, 2018) • (El-Fakharany *et al*, 2018)
Effectiveness of camel milk on colitis	— Camel's milk ameliorates TNBS-induced colitis in rats via downregulation of inflammatory cytokines and oxidative stress.	• (Arab *et al*, 2014) • (He *et al*, 2022)
Nutritional property and Consumer acceptance of camel milk	— The effects of some milk heat treatments on the compositional, rheological and organoleptic qualities of camel milk labneh. — Composition of camel milk: a health blessing.	• (Desouky *et al*, 2013) • (Ali *et al*, 2019) • (Fufa and Haile, 2020)
	— A review of camel milk production and composition and its beneficial uses. — A review paper on the quality and therapeutic aspects of camel milk. — A review of camel milk and its associated health claims. — A review of the role of camel milk and milk products in domestic diet and therapeutic advancement.	• (Sakandar *et al*, 2018)

Fermentation of camel milk is beneficial for improving digestibility, ensuring milk preservation and keeping microbes at bay (Solanki and Hati, 2018). When compared to camel milk, bovine milk contains fewer bioactive peptides (ACE-inhibitory peptides and anti-oxidative peptides) generated by lactic acid bacteria (Moslehishad *et al,* 2013). Gariss (fermented camel milk) is a kind of camel milk that has been fermented (Sulieman *et al,* 2006).

Camel milk has a great reputation in the cosmetics industry because it contains a lot of alpha-hydroxyl acid and liposomes, which help to reduce wrinkles and dryness in the skin, giving it an anti-aging effect (Choi *et al,* 2014; Mohan *et al,* 2020).

Colitis treatment by camel milk

The antioxidant and anti-inflammatory characteristics of camel milk were thought to be beneficial in lowering signs of colon affection. Camel milk was administered to adult mice with and without drug-induced colitis using 2,4,6-trinitrobenzene sulfonic acid (Arab *et al,* 2014). Camel milk (10 ml/kg b.i.d. via oral gavage) successfully reduced the degree of colon injury, as demonstrated by improvements in macroscopic damage, colon weight/length ratio, histological changes, leukocyte influx and myeloperoxidase activity. The administration of camel milk reduced the levels of TNF- and IL-10 cytokines in the colon. The attenuation of camel milk to colon damage was also connected with suppression of oxidative stress by a decrease of lipid peroxides and nitric oxide, as well as enhancing antioxidant defenses via colon glutathione restoration and overall antioxidant capacity. Caspase-3 activity, an apoptotic marker, was also suppressed. As a result, camel milk may be an intriguing supplementary method to colitis treatment. Bacillus amyloliquefaciens-enriched camel milk was able to alleviate chemicals-induced colitis in mouse models (Khalifa *et al,* 2022). The colitis disease index was lowered and body weight and colon length were improved. Furthermore, therapy reduced Myeloperoxidase and pro-inflammatory cytokines. Furthermore, the inflammatory process mRNA and protein markers nuclear factor kappa B, phosphatase and tensin homolog, proliferating cell nuclear antigen, cyclooxygenase-2 and occludin were down-regulated.

The preventive impact of camel milk in mice with colitis caused by dextran sodium sulfate was assessed (He *et al,* 2022). Camel milk can prevent body weight loss, lower the disease activity index and minimise colon tissue damage. Furthermore, camel milk has been shown to lowers inflammatory factor overexpression and limits apoptosis of intestinal epithelial cells. Furthermore, camel milk effectively regulated intestinal microbiota in mice with colitis by increasing the diversity of the gut microbiota, increasing the abundance of beneficial bacteria and decreasing the number of harmful bacteria (Fig 2).

Camel milk and cancer

The exosomes from camel milk showed a higher apoptosis rate, inhibition of oxidative stress, lower inflammation rate and improved immune response. Overall, delivery of camel milk-derived exosomes had a greater anticancer impact but a lower immunological response than camel milk therapy. Furthermore, local injection of exosomes resulted in greater results than oral treatment. These data imply that camel milk and its exosomes have an anticancer impact on the tumour microenvironment, perhaps by inducing apoptosis and inhibiting oxidative stress, inflammation, angiogenesis and metastasis. As a result, camel milk and its exosomes have the potential to be used as an anticancer agent in cancer treatment (Badawy *et al,* 2018). Compared to the complexes of oleic acid with human and bovine albumins, the complex of camel albumin with oleic acid demonstrated the most powerful anti-tumour effects (El-Fakharany *et al,* 2018). Various diseases, including cancer and hepatitis, have been linked to oxidative stress. Camel milk may decrease oxidative stress by increasing antiproliferative effects and regulating antioxidant genes during cancer and hepatitis (Khan *et al,* 2021).

Camel milk has been shown to have a regulatory effect on parameters of the primary components of inflammatory angiogenesis, providing insight into the possible therapeutic advantage underpinning the anti-cancer properties of camel milk. Camel milk treatment reduced wet weight, vascularisation, collagen deposition, macrophage recruitment and levels of vascular endothelial growth factor, interleukin and transforming growth factor (Alhaider *et al,* 2014).

The antioxidant, anticancer, antihypertensive and anti-angiotensin-converting enzyme activities of fermented camel milk with fermented bovine milk in a lab setting were compared (Ayyash *et al,* 2018). Fermented camel and bovine milks were made using the probiotic strain of *Lactococcus lactis* KX881782 (Lc. K782) found in camel milk and the control strain of *Lactobacillus acidophilus* DSM9126 (La.DSM) found in bovine milk. Water-soluble extract (WSE) proteolytic

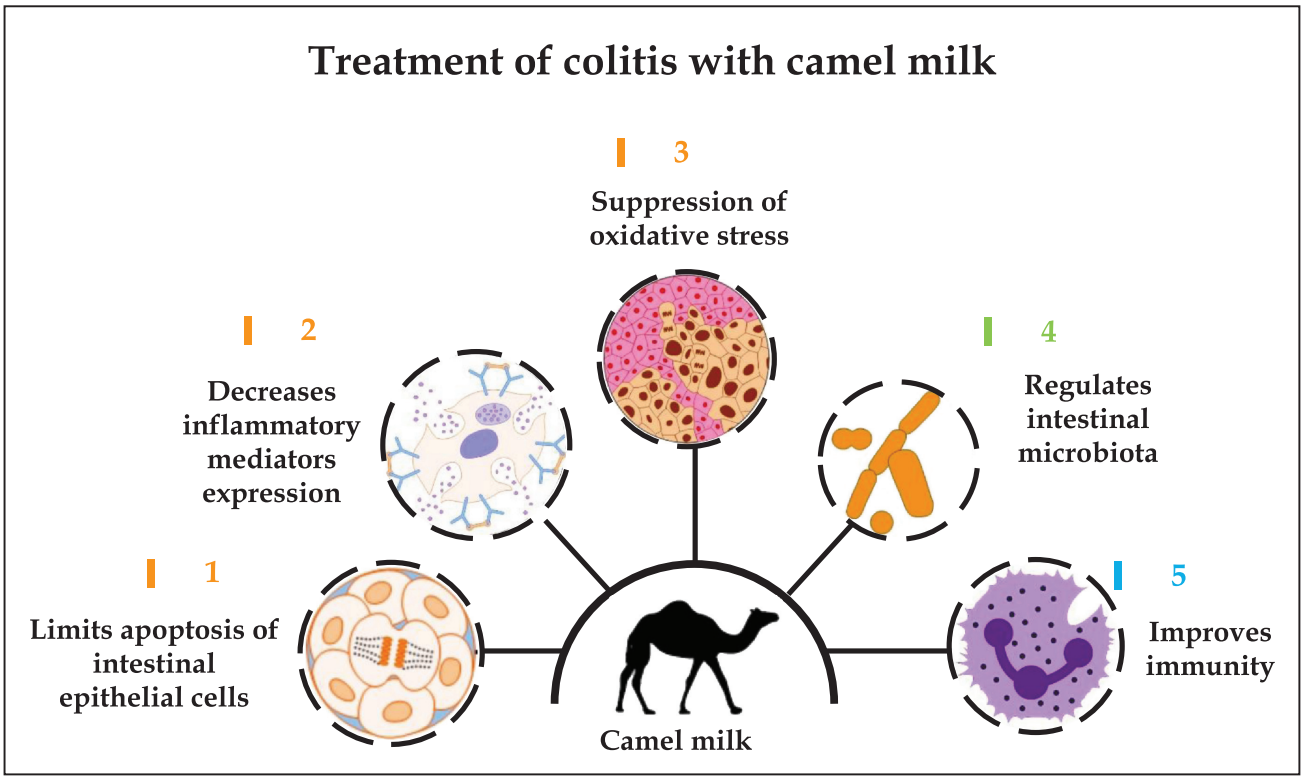

Fig 2. Outline of the mechanism of action of camel milk in treatment of colitis.

activities in all fermented camel milk were higher than those in fermented bovine milk. The maximum antioxidant activity was found in fermented camel milk. When fermented camel milk extracts were used to treat cancer cells, the proliferation of Caco-2, MCF-7 and HELA cells was significantly reduced (Ayyash *et al,* 2018).

Camel milk and diabetes

The anti-diabetic activities of camel milk and bovine milk were studied in streptozotocin-induced diabetic rats by oral feeding for 8 weeks. Camel milk is more successful in improving body weight, lowering blood glucose levels and lowering glucose tolerance (Korish *et al,* 2020). Camel milk protein contains bioactive peptides that the inhibit enzymes responsible for causing diabetes and obesity as well. Camel milk contains RQ-8 peptide and acts as an antioxidant property, which lowers the oxidative stress that is associated with type-II diabetes (Shahriari *et al,* 2018). In a trial to investigate the potential anti-diabetic benefits of camel milk in diabetic rats induced by streptozotocin, for 30 days, the rats were fed fresh camel milk which considerably lowered blood glucose, urea, uric acid and creatinine levels while increasing albumin, albumin/globulin ratio and restoring all liver function marker enzymes and lipid profile to near control levels (Khan *et al,* 2013).

In a study on the therapeutic applications of camel milk in the management of type 1 diabetes, there were significant reductions in HbA1c (7.8 1.38 - 6 0.96; p 0.001), MBI (17 4.4 - 19.7 2.97; p 0.001) and required insulin dose (32 12 - 17.88 12.40; p 0.005) as compared to baseline values (Agrawal *et al,* 2005). The synthetic peptides produced and discovered from camel milk whey hydrolysates and lactoferrin isolated from milk may be suitable candidates for testing *in vivo* for their hypothesised effects on insulin-dependent responses, either alone or in conjunction with insulin (Anwar *et al,* 2021).

Camel milk boosts the expression of carnitine palmitoyl transferase type I, insulin receptor substrate type 2 and fatty acid synthases, which aids in insulin production and regulation and helps diabetic patients improve and normalise blood glucose, lipid profile, total glycerides and high-density lipoprotein levels. As a consequence, frequent use of camel milk can help to reduce the risk of diabetic complications (Aqib *et al,* 2019).

Short-term therapeutic efficacy of camel milk vs buffalo milk in alloxan-induced diabetic rabbits showed improved hematological (RBC, MCV, Hb, MCH) and serological markers (AST, ALT, creatinine, BUN, TPs and TOS) with camel milk treatment. Camel milk with glibenclamide significantly lowered

blood glucose levels compared to buffalo milk, while kidney function improved significantly. Camel milk and glibenclamide helped to return the liver and kidney pathological exam to near normal values (Deeba *et al,* 2020).

Camels milk may be utilised for a variety of beneficial domains, including medicine and sustenance. Camel dairy and meat production on a large scale has gained popularity as the general public becomes more aware of the benefits of camels and their products. Camel milk production and animal health improve when camels are maintained in a semi-intensive setting rather than on the conventional camel rearing method (Abdel Fattah and Roushdy, 2016). Camel milk's nutritive and therapeutic values are proved to be better than other ruminant species, hence several western nations are increasingly interested in rearing camels for milk and its byproducts. Furthermore, these countries have made significant contributions to the development of milk-based products, as well as consumer education and awareness of these products.

Camel milk consumption has been linked to lower fasting blood glucose levels and better lipid profiles (Mohammadabadi, 2019). Raw camel milk contains three times more insulin and/or insulin-like proteins (52 units of insulin per liter) than cow milk. These proteins interact directly with insulin-sensitive tissues and insulin receptors, potentiating insulin action and signaling (Abdulrahman *et al,* 2016). Camel milk proteins and bioactive peptides act directly or indirectly on particular cellular pathways and influence insulin generation and release by pancreatic β–cells. Camel milk is rich in lactoferrin. Lactoferrin reduces hyperglycemia through increasing insulin sensitivity, anti-inflammatory responses and activating insulin receptors. It is possible to prevent diabetes-related liver and kidney diseases, as well as enhance wound healing, by drinking camel milk. The action of camel-milk insulin was eliminated by pepsin, indicating that it is vulnerable to digestion by digestive enzymes (Abou-Soliman *et al,* 2020). Pepsin entirely abolishes the action of camel-milk insulin hence it was construed that camel's milk's anti-diabetic activity is not due to insulin or insulin-like protein alone. It has been hypothesised that insulin in camel milk is resistant to proteolysis. Camel milk lipid vesicles have also been proposed to help in the absorption of camel milk insulin into circulation by encapsulating and preserving the insulin from stomach digestion (Malik *et al,* 2012). Furthermore, insulin-like proteins in camel milk may be able to imitate insulin's interaction with its receptor. Bioactive peptides are presumably generated following the digestion of camel milk proteins, which are tiny and quickly absorbed into the bloodstream. The existence of small chemical compounds with anti-diabetic potential in camel milk is currently under investigation.

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Data availability statement

All data are within the manuscript

Funding statement

This work was supported through the Annual Funding track by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Project No. AN00055].

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SUBJECT INDEX

INSTRUCTIONS TO CONTRIBUTORS

(Effective from year 2023)

(Journal of Camel Practice and Research - triannual -April, August and December issues every year)

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PROTEOMIC CHARACTERISATION OF SERUM DURING THE BREEDING CYCLE IN MALE BACTRIAN CAMELS

Le Hai[1], Rendalai Si[2], Fu-Cheng Guo[1], Jing HeI, Li Yi[1], Liang Ming[1], Jun-Wen Zhou[3], La Ba[3], Rigetu Zhao[3] and Rimutu Ji[1,2]

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Commercial booklets: Anonymous/Name. Conray-Contrast Media. IIIrd Edn., 1967; pp 12-15, May and Baker Ltd., Dagenham, Essex, England.

Magazine articles: Taylor D. The Constipated Camel. Reader's Digest. Indian Edn. RDI Print & Publishing (P) Ltd., Mehra House, 250-C, New Cross Road, Worli, Bombay, India. 1985; 126:60-64

News paper articles: Christina Adams. Camel milk: a miracle cure for children with autism?. Gulf News, Published: April 09. 2014.

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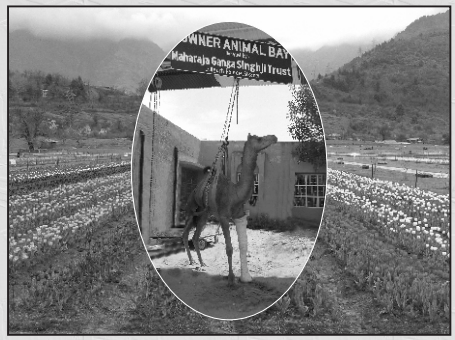

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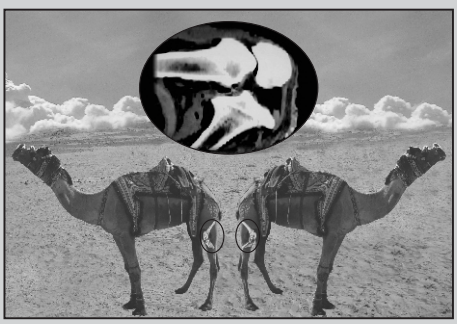

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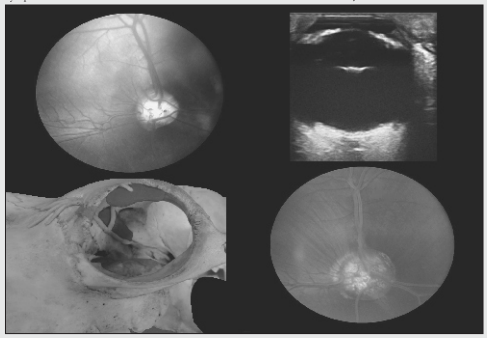

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