JOURNAL OF CAMEL PRACTICE AND RESEARCH

CAMEL PUBLISHING HOUSE
Bikaner - 334001, INDIA

Manuscripts and other related correspondence may be made to :

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Editor, Journal of Camel Practice and Research
67, Gandhi Nagar West
Near Lalgarh Palace
Bikaner-334001, INDIA

Phone : 0091-151-2527029 (R)
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SUBSCRIPTION RATE - 2020

ANNUAL	Rs. 4500/- or US $ 450

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

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

Cover Design: T.K. Gahlot

Courtesy: Dr. Diler Singh, Veterinary Officer, Deptt. Animal Husbandry, Rajasthan State, India

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

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EDITORIAL

CAN CAMELS BE INFECTED WITH COVID-19 VIRUS?

Coronaviruses (CoV) are a family of RNA (ribonucleic acid) viruses, they exhibit a characteristic 'corona' (crown) of spike proteins around its lipid envelope. CoV infections are common in animals and humans. Some strains of CoV are zoonotic, but many strains are not zoonotic. In humans, CoV can cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (caused by MERS-CoV), and Severe Acute Respiratory Syndrome (caused by SARS-CoV). Detailed investigations have demonstrated that SARS-CoV was transmitted from civets to humans, and MERS-CoV from dromedary camels to humans. In December 2019, human cases of pneumonia of unknown origin were reported in Wuhan City, Hubei Province of China. A new CoV was identified as the causative agent by Chinese Authorities. Since then, human cases have been reported by almost all countries around the world and the COVID-19 event has been declared by the World Health Organisation (WHO) to be a pandemic. The predominant route of transmission of COVID-19 is from human to human.

Current evidence suggests that the COVID-19 virus emerged from an animal source. Genetic sequence data reveals that the COVID-19 virus is a close relative of other CoV found circulating in Rhinolophus bat (Horseshoe Bat) populations. However, to date, there is not enough scientific evidence to identify the source of the COVID-19 virus or to explain the original route of transmission to humans (which may have involved an intermediate host).

Now that COVID-19 virus infections are widely distributed in the human population there is a possibility for some animals to become infected through close contact with infected humans. Several dogs and cats (domestic cats and a tiger) have tested positive to COVID-19 virus following close contact with infected humans.

Preliminary findings from laboratory studies suggest that, of the animal species investigated so far, cats are the most susceptible species for COVID-19, and cats can be affected with clinical disease. In the laboratory setting cats were able to transmit infection to other cats. Ferrets also appear to be susceptible to infection but less so to disease. In the laboratory setting ferrets were also able to transmit infection to other ferrets. Dogs appear to be susceptible to infection but appear to be less affected than ferrets or cats. Egyptian fruit bats were also infected in the laboratory setting but did not show signs of disease or the ability to transmit infection efficiently to other bats. To date, preliminary findings from studies suggest that poultry and pigs, are not susceptible to SARS-CoV-2 infection. Currently, there is no evidence to suggest that animals infected by humans are playing a role in the spread of COVID-19. Human outbreaks are driven by person to person contact.

(Factual Excerpts verified from the websites of WHO and OIE)

(Dr. T.K. Gahlot)
Editor

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

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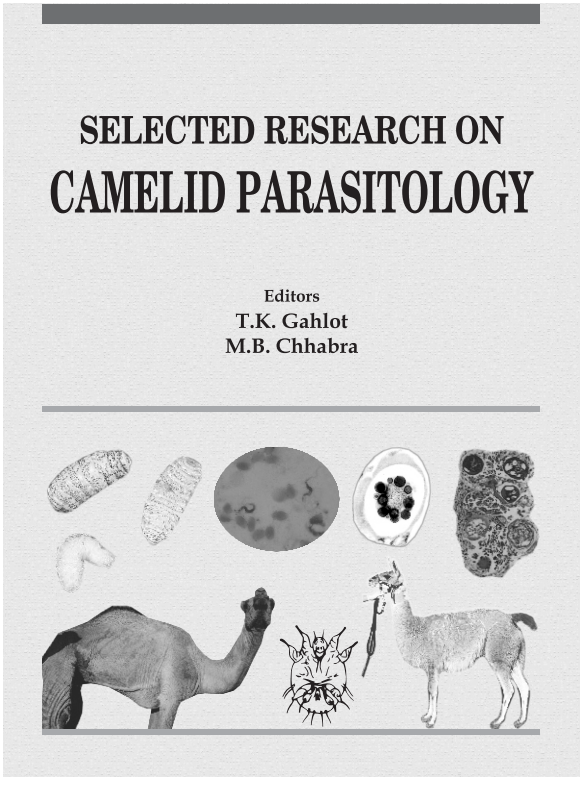

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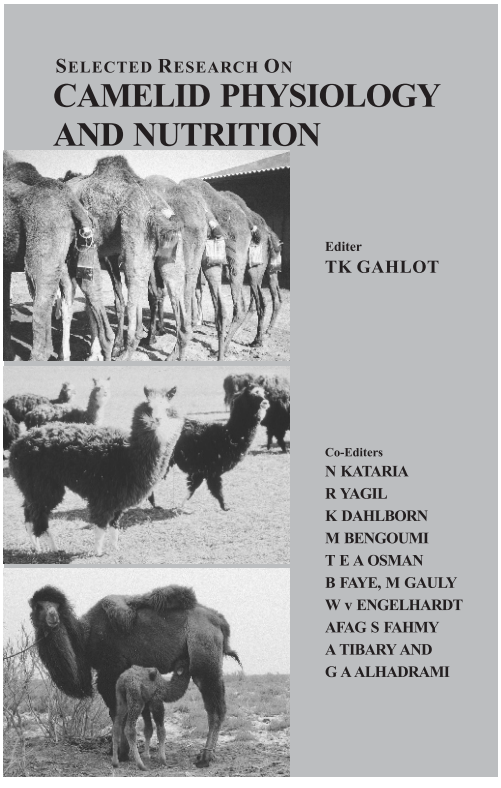

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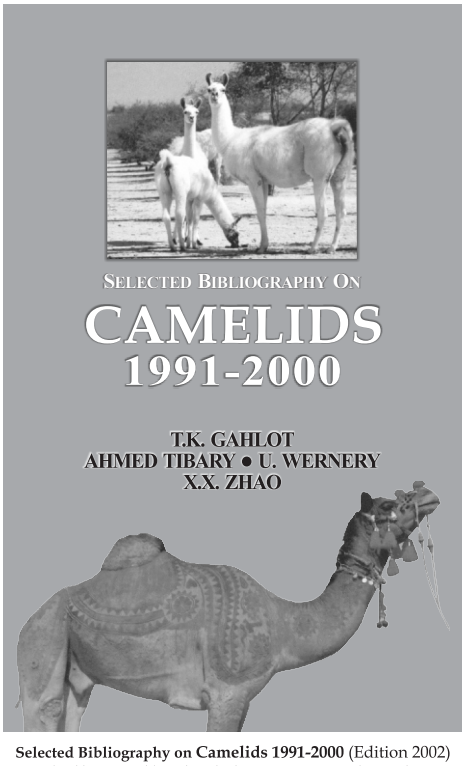

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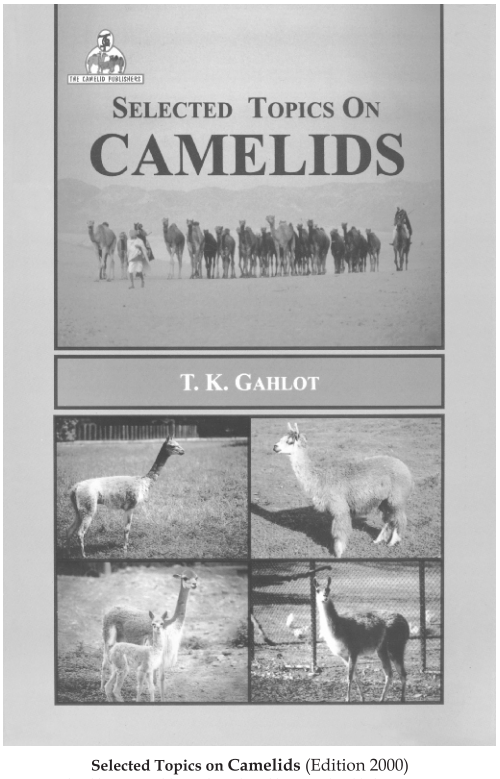
Price: US $ 90 (Abroad) INR (Rs) 950 (India)
ISBN: 81-901141-1-5



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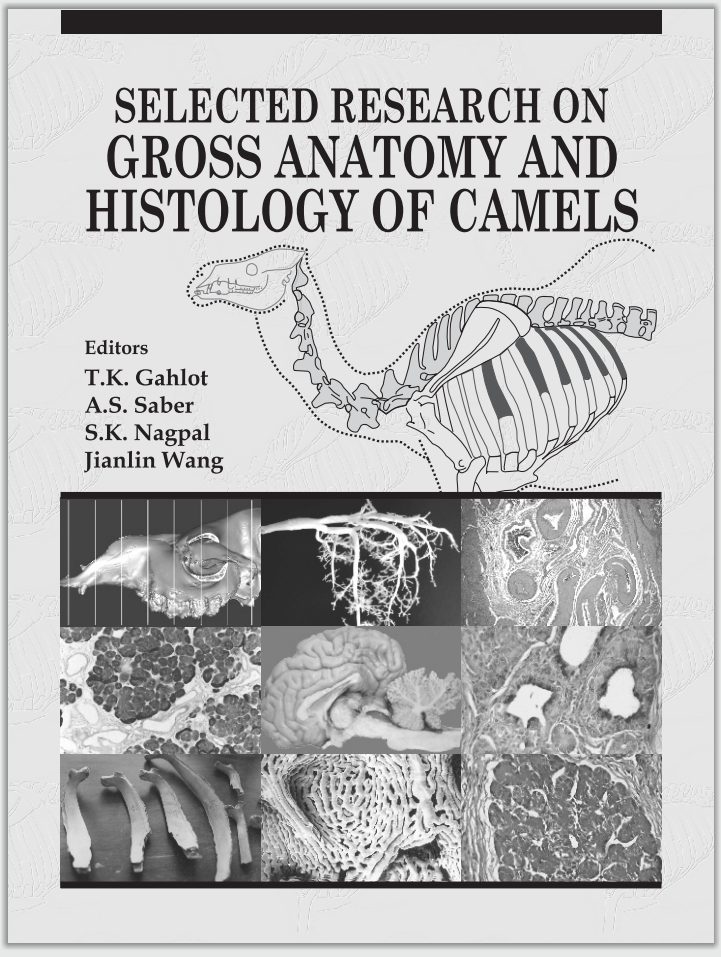
SELECTED RESEARCH ON

GROSS ANATOMY AND HISTOLOGY OF CAMELS

Hard bound, 452 pages, few figures coloured

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



Editors:
T.K. Gahlot, A.S. Saber, S.K. Nagpal and Jianlin Wang

Edition: 2011

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

Price: US $ 375 (Abroad)
INR 7500 (India)

ISBN: 81-903140-1-7

DOI : 10.5958/2277-8934.2020.00001.6 *Vol 27 No 1, p 1-12*

THE ONE-HUMPED CAMEL IN SOMALILAND

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ABSTRACT

Somaliland is a self-proclaimed republic located in the Horn of Africa. The country has adopted the same borders as the former British Somaliland Protectorate but it has had difficulties in gaining international recognition. Its area of 68 000 square miles (176 120 square kilometres) is home to about 4 million people. Sheep and goats each number more than 8 million head, camels 1.7 million and cattle 0.40 million. Livestock are the basis of the livelihoods of the majority of the nation's people. The camel has always been an icon in the culture and customs of the ethnic Somali. The British administration organised a Camel Corps to help in the maintenance of law and order in the then Protectorate but it also distinguished itself against the troops of Italian Somaliland in the World Wars of 1914-1918 and 1939-1945 before it was disbanded in 1946. As well as being important in the cultural fabric of the inhabitants of Somaliland, the camel is the major source of milk in the country, provides meat, performs transport operations and contributes its hides to the economy. Livestock in general are the major source of foreign exchange earnings to which camels contribute a considerable proportion. Feed is generally a problem and some has to be imported from Ethiopia. The presence of many diseases is a major constraint to the higher output which would be of great benefit to the welfare of the nation and of individual households and family units.

Key words: Animal diseases, camel, livestock trade, mad mullah, medicine, milk production, Somaliland ethnoveterinary

The self-proclaimed Republic of Somaliland is located in the Horn of Africa. This entity has an area of 68 000 square miles (176 120 square kilometres) and is home to about 4 million people. It claims the same borders as the former British Somaliland but is officially recognised as an independent state by very few other countries or international organisation. Its current status serves only to add to its very checkered history.

Earlier contacts and treaties from 1820 between the British and some Somali clans culminated in formal treaties between the British and the now-designated 'British Somaliland' clans were signed between 1884 and 1886. The new entity was garrisoned from Aden and administered as part of British India until 1898, then by the Foreign Office until 1905 and finally by the Colonial Office. A series of military expeditions between 1900 and 1920 known as the Somaliland Campaign (the Anglo-Somali War or the Dervish War) was conducted against the Dervishes under Mohammed Abdullah Hassan (known to the British as "Mad Mullah") with the help of Ethiopia and Italy. During the First World War (1914-1918), the Mullah was assisted by the Ottoman Empire and the Germans and for a short time from Emperor Iyasu V of Ethiopia. The conflict was eventually concluded by the British using aircraft to bomb the Dervish capital of Taleh in February 1920. This was the 5th and final expedition of the Somaliland Campaign against the Dervish forces of the Mullah. Although, most fighting took place in January 1920, the British had begun preparations for the assault in November 1919 with British ground forces and detachments of the Royal Air Force and the Somaliland Camel Corps (Jardine, 1951).

Following the Italian conquest of Ethiopia in 1936, the occupation of the greater part of Somalia by Italy, and the outbreak of the 2nd World War (3 September 1939), British Somaliland was in a invidious position. A small regular garrison was unable to defend the Protectorate which was invaded by Italy on 3 August 1940. Despite strong rearguard action by the Somaliland Camel Corps, troops of the King's African Rifles, some Indian troops and the small number of troops of the British regular army the Protectorate was evacuated. Italian troops entered Berbera in the evening of 19 August and Mussolini annexed the Protectorate to Italian East Africa as part of the Italian Empire. On 16 March 1941, the British landed a mainly Indian force and a Somali commando, supported by a naval force based at Aden, at Berbera. Due to earlier propaganda and some "misinformation", the Italians had already withdrawn most of their forces from the former

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British Protectorate. The British claimed victory on 8 April and appointed a Military Governor. British forces then advanced into eastern Ethiopia to clear out the Italians there. The Somaliland Camel Corps was reformed by mid-April and supported British forces over the next few months in flushing out Italian guerrillas still fighting in Ethiopia (Mackenzie, 1951).

The British government granted independence to its then Protectorate of British Somaliland in 1960 in order for it to unite with the Italian-administered Trust Territory of Somaliland (the former Italian Somaliland). The British Somaliland Legislative Council requested independence in April 1960 so it could unite with the Trust Territory of Somaliland that was scheduled to gain independence on 1 July. On 26 June 1960, the former British Somaliland Protectorate became independent as the State of Somaliland. The next day the Somaliland Legislative Assembly approved a bill that would formally allow for its union with the Trust Territory of Somaliland on 1 July 1960. The Italian Trust Territory of Somaliland became independent on 1 July and on that day the State of Somaliland joined with the former Italian Somaliland to become the Somali Republic. During its 5-day period of independence the State of Somaliland was recognised by 35 sovereign states. Following a series of unkept promises from the Mogadishu-dominated government and attacks by it on the northern areas the Republic of Somaliland declared itself independent on 15 May 1991 (Kaplan, 2008).

Pastoralism accounts for around 70 per cent of Somaliland's economy, employs about the same proportion of its population of around 4.5 million and generates 85 per cent of foreign exchange earnings (Muhumed and Abdiqadir, Mohamoud Yonis 2018). Goats and sheep are the primary form of capital and are fundamental to the culture. They are not just a source of milk and meat but of self-sufficiency, pride and the freedom to live a life of choice. Animals can be sold for cash or exchanged for rice, flour, medicine, water, transport or clothes. Millions of animals are shipped each year across the Gulf of Aden. A family with ten camels and a few hundred goats possesses real wealth and has access to the global economy (Hunt, 1951; Mares, 1954a; 19544b; Muhumed and Abdiqadir, 2018).

The Somaliland Camel Corps

Beginnings

The British administration was not slow to realise that a close affinity existed between the people of Somaliland and their camel charges. Establishment of the "Somali Camel Constabulary" recognised this affinity and the possibility of its use as a military force. The Constabulary, reconstituted in 1912 as the Somaliland Camel Corps, fought the Battle of Dul Madoba on 9 August 1913 against the Mad Mullah where it suffered a severe defeat. Colonel Richard Corfield, commanding the force, was among the 36 personnel of the 110-man unit that were killed: another 21 were wounded during the engagement (Irons, 2013).

As a regular unit the Somaliland Camel Corps needed a uniform and a distinctive badge (Fig 2) and, of course, they were expected to perform parades at important functions (Fig 3). The uniform was basically a standard British Army khaki drill with the addition of a knitted woollen pullover and shoulder drill patches. The lower parts consisted of shorts worn with woollen socks on puttees and *'chaplis'* (coloured sandals), boots or bare feet. Officers wore pith helmets whereas other ranks sported a *'kullah'* (cone-shaped cap inside a turban) and a *'pugree'* (a long strip of cotton or silk wound round the turban and hanging down the back as a tail). Troops were equipped with a rifle, a leather ammunition bandolier and a leather waist belt.

First World War

Hastings Lionel Ismay arrived in Somaliland on 9 August 1914 and despite pleas to be posted to Europe he stayed in the Protectorate until 1920. Ismay belonged to the 21st Prince Albert Victor's Own Cavalry (Frontier Force) (Daly's Horse) of the Indian Army and arrived with a group that was to serve as the Somaliland Indian Contingent with the King's African Rifles (National Archives, 1916)[1]. At this stage the strength of the Camel Corps was about 500 although not all fought together in all operations against the enemy. On 17 November 1914, a major assault was launched against the Mullah's fortress at Shimber Berris. In this attack Major Adrian Paul

1. General Hastings Lionel Ismay, 1st Baron Ismay KG, GCB, CH, DSO, PC, DL (21 June 1887-17 December 1965). Ismay was later (1925) an Assistant Secretary of the Committee of Imperial Defence (CID) then, on promotion to Colonel, he was military secretary for Lord Willingdon, the Viceroy of India. He returned to the CID as Deputy Secretary in 1936 before becoming its Secretary on 1 August 1938. When Winston Churchill became Prime Minister of the United Kingdom in May 1940 he appointed Ismay as his chief military assistant and staff officer in which capacity he was principal link between Churchill and the Chiefs of Staff Committee. Ismay was the first Secretary General of NATO from 1952 to 1957.

Ghislain Carton de Wiart[2] was shot twice in the face, losing his eye and also a portion of his ear. Captain Ismay was also present at this engagement and gained, along with de Wiart, the Africa General Service Medal with Clasp for Shimber Berris 1914-1915 (National Archives, 1916). De Wiart was particularly signalled out for "military reward" and, although Ismay received two Mentions in Despatches (London Gazette, 1916) it was De Wiart who got his reward in the form of a Distinguished Service Order (DSO) (London Gazette, 1915), for which Ismay had to wait several more years:

> *War Office,*
> 2nd August, 1916.
> The Colonial Office has forwarded for publication the following Despatch on military operations in the Somaliland Protectorate: —
>
> From H.M. Commissioner and Commander-in-Chief, Somaliland Protectorate.
> To the Secretary of State for the Colonies.
> The Residency, Berbera,
> 20th February, 1915.
> SIR,—I have the honour to transmit a report by the Officer Commanding the Troops, Somaliland Protectorate, on the recent military operations against the dervishes; at Shimber Berris, at the head of the Ain Valley.
>
> I also desire to put forward, for military reward, the names of the following officers, native officers, non-commissioned officers, and men : —
> Captain A. Carton de Wiart, 4th Dragoon Guards, for gallantry in charging a fort on the 19th November, though severely wounded.
>
> The following officers, non-commissioned officers and men also distinguished themselves, and are deserving of mention:—
> Captain H. L. Ismay, 21st Prince Albert Victor's Own Cavalry, made the staff arrangements in February prior to and during the operations, and with complete success, this not being an easy task in a waterless country such as Somaliland in the dry season.
>
> The following are also deserving of mention : —
> *(b) In February.*
> Captain H. L. Ismay, 21st Prince Albert Victor's Own Cavalry, made all the staff arrangements prior to and during the operations, an exceedingly difficult task in a waterless country in the dry season, and with the complete success that you witnessed.

> *War Office,*
>
> 15th May, 1915.
>
> His Majesty the KING has been graciously pleased to approve of the undermentioned rewards for Distinguished Service in the Field, in connection with the successful operations against Dervish forces at Shimber Berris, Somaliland, during the months of November, 1914, and February, 1915: —
>
> *To be a Companion of the Distinguished Service Order –*
>
> Captain Adrian Carton de Wiart, (Royal Irish) Dragoon Guards.

Not long after the attack on Shimber Berris the Camel Corps received orders from London "forbidding all offensive operations in Somaliland" due to the misfortunes of the Army elsewhere. As a result of this order,

2. Lieutenant General Sir Adrian Paul Ghislain Carton de Wiart VC, KBE, CB, CMG, DSO (5 May 1880-5 June 1963) was a British Army officer born of Belgian and Irish parents. He was awarded the Victoria Cross in 1916 after leaving Somaliland and fighting in France. Elsewhere in three wars (Second Boer War 1899-1902, sFirst World War 1914-1918, Second World War 1939-1945) he was shot in the face, head, stomach, ankle, leg, hip and ear; survived two plane crashes; tunnelled out of a prisoner-of-war camp; and tore off his own fingers when a doctor refused to amputate them.

British operations were limited for the duration of the First World War and Ismay's unit undertook no further major offensives. Ismay did, however, become Second in Command of the Camel Corps and was promoted to Temporary Lieutenant Colonel (London Gazette, 1919):

> *War Office,*
> *15th July,1919.*
> REGULAR FORCES.
> MEMORANDA.
> Bt. Maj. H. L. Ismay, Ind. Army, to be temp. Lt.-Col., for service with the Somaliland Camel Corps. 1st Apr. 1919.

Interwar period

After the end of World War I it was decided that the Mullah must be neutralised permanently. During this period the Camel Corps was very active and took part in or led many actions. Attacks against the Mullah commenced on 21 January 1920 and after several days of bombing by the Royal Air Force, Ismay was ordered to lead an assault by the Camel Corps against Hassan's fortress at Jid Ali (London Gazette 1920a; 1921a). On 7 February the Camel Corps completed a march of 150 miles in 72 hours and "animals and men now needed a rest"(!). For his service in the effort against Hassan, Ismay finally received the Distinguished Service Order (London Gazette, 1920b):[3]

> *War Office,*
> *29th November, 1920.*
> His Majesty the KING has teen graciously pleased, on the recommendation of His Excellency the Governor and Commander-in-Chief, Somaliland Protectorate, to approve of the undermentioned rewards in recognition of distinguished services rendered in connection with Military Operations in Somaliland: —
> To be dated 1st October, 1920.
> Somaliland Camel Corps.
> *Awarded the Distinguished Service Order.*
> Capt. & Bt. Maj. (T./Lt.-Col.) Hastings Lionel Ismay, 21st Cav., Ind. Army, attd. Somaliland Camel Corps.

3. In London Gazette, 1920a, two officers were promoted Brevet Lieutenant Colonel, two Brevet Major, two other officers received the DSO, one received a Bar to His Military Cross, four officers received the Military Cross and one Corporal was awarded the Distinguished Conduct Medal (the next lower decoration for other ranks after the Victoria Cross).

Ismay gained a second Clasp, Somaliland 1920, to his Africa General Service Medal and in addition to his advanced rank of Brevet Major he was made a Temporary Lieutenant Colonel (National Archives, 1922). On 5 August 1920 Captain (Brevet Major) H L Ismay was promoted to the substantive rank of Major (London Gazette, 1921b):

> *India Office,*
> *1st March, 1921.*
> The KING has approved the promotion of the following officers of the Ind. Army, Ind. Med, Service, Ind. Army Depts., and Ind. Army Res. of Officers : —
> INDIAN ARMY.
> *Captains to be Majors.*
> 5th Aug. 1920.
> H. L. Ismay, (Bt. Maj.)

During the Interwar years, the SCC was re-organised better to defend the protectorate in the event of another war. The Somaliland military garrison was progressively reduced during the 1920s and following the financial crisis of 1931 only the Somaliland Camel Corps remained, comprising 14 British officers, 400 African 'askari' and 150 African Reservists. Colonel Arthur Reginald Chater of the Royal Marines commanded the Somaliland Camel Corps from 1937 to 1940 and was then Military-Governor of British Somaliland from 1941 to 1943. In the late 1930s the Corps was allocated GBP 900 to construct defensive pillboxes and reserve water tanks.

By 1939 the Corps comprised: Headquarters and Headquarters Company, The Somalia Camel Corps based at Laferug between Berbera and Hargeisa along Route 1 Highway; 'A' (Camel) Company at Hargeisa; 'B' (Nyasa Infantry) Company at Tug Argan southwest of Laferug near Hargeisa south of Assa Hills; 'C' Company at Burao; and, 'D' Company at Tug Argan (less 2 Platoons at Sheekh). Thus, at this stage, only 'A' Company retained its camels whereas the other companies were essentially infantry units.

Second World War

A request by the Governor that 50 additional men be enlisted in the Camel Corps was approved in the spring of 1940 and two Camel Corps Companies were mechanised later in 1940 (Wavell, 1946). Following the entry of Italy into the war the

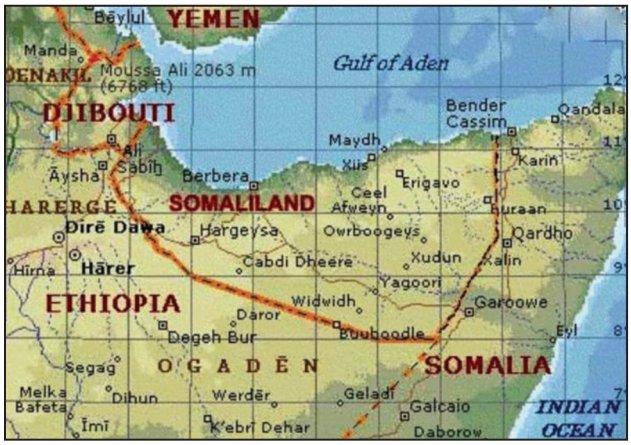

Fig 1. The Republic of Somaliland in the context of the Horn of Africa.

Fig 2. The uniform and badge of the Somaliland Camel Corps.

Fig 3. The Camel Corps on parade in 1913.

Camel Corps made a number of successful raids, usually against numerically superior forces, along the Italian Somalia and Ethiopian (now an Italian colony) borders. The Camel Corps continued to defend Somaliland almost on its own: on 6 July 1940, for example, the only other troops in the country were one battalion (Northern Rhodesia Regiment) of the King's African Rifles (K.A.R) and one Indian battalion (1st Battalion 2nd Punjab Regiment) which had arrived from Aden only a few days earlier.

Fig 4. Somali shillings to the value of USD 100 (Photo of the author and his hoard in Hargeisa in May 2003).

Fig 5. Camels awaiting export from Berbera in June 2003 (Photo by the author, June 2003).

Fig 6. Fodder imported from Ethiopia on its way to Berbera (Photo by the author, June 2003).

The total force amounted to 1475 men. A Kenya Battalion of the K.A.R and an artillery battery were said to be on the way. The situation on 1August shows the overwhelming importance of the Camel Corps: one Company Somaliland Camel Corps less one Troop in the Dobo area (forced to withdraw on 4 August by a superior Italian force); a Motor Company

of the Somaliland Camel Corps less one Troop (inflicted heavy losses on Italians on 4 August), one Troop Somaliland Camel Corps and one Company Northern Rhodesia Regiment at Hargeisa area; and one Company and one Motor Troop Somaliland Camel Corps at Burao. At Tug Argan, there was B Company Somaliland Camel Corps as well as other forces. During the fighting around Tug Argan from 11-15 August Captain Eric Charles Twelves Wilson then aged 27 kept a Vickers machine gun post in action in spite of being wounded and suffering from malaria.[4] Some of his guns were blown up by enemy artillery fire and his spectacles were smashed. He was wounded in the right shoulder and the left eye but then disappeared and was posted as missing in action and presumed killed. He had, however, been captured by the Italians, was kept in a prisoner of war camp in Eritrea and was discovered alive and released when the Italian forces capitulated in 1941. In the meantime he had been awarded a posthumous Victoria Cross. On his return to England he belatedly received his VC in July 1942. The formal citation for Wilson's VC, when he was still presumed dead, reads (London Gazette, 1940):

The War Office,

11th October, 1940.

The KING has been pleased to approve of the award of The Victoria Cross to : —

Lieutenant (acting Captain) Eric Charles Twelves Wilson, The East Surrey Regiment (attached Somaliland Camel Corps).

For most conspicuous gallantry on active service in Somaliland. Captain Wilson was in command of machine-gun posts manned by Somali soldiers in the key position of Observation Hill, a defended post in the defensive organisation of the Tug Argan Gap in British Somaliland.

The enemy attacked Observation Hill on August 11th, 1940. Captain Wilson and Somali gunners under his command beat off the attack and opened fire on the enemy troops attacking Mill Hill, another post within his range. He inflicted such heavy casualties that the enemy, determined to put his guns out of action, brought up a pack battery to within seven hundred yards, and scored two direct hits through the loopholes of his defences, which, bursting within the post, wounded Captain Wilson severely in the right shoulder and in the left eye, several of his team being also wounded. His guns were blown off their stands but he repaired and replaced them and, regardless of his wounds, carried on, whilst his Somali sergeant was killed beside him.

On August 12th and 14th the enemy again concentrated field artillery fire on Captain Wilson's guns, but he continued, with his wounds untended, to man them.

On August 15th two of his machine-gun posts were blown to pieces, yet Captain Wilson, now suffering from malaria in addition to wounds, still kept his own post in action.

The enemy finally over-ran the post at 5 p.m. on the 15th August when Captain Wilson, fighting to the last, was killed.

When the British forces evacuated Somaliland on 17/18 August, the local Somalis of the Camel Corps were given the option of evacuation to Aden or disbandment but the great majority preferred to remain in the country and were allowed to retain their arms. (Wavell, 1946). On 16 March 1941 British and Indian forces departed Aden in order to recapture British Somaliland. The Somaliland Camel Corps was re-founded and by

4. The son of a Church of England clergyman, Eric Charles Twelves Wilson (2 October 1912-23 December 2008) was commissioned Second Lieutenant in The East Surrey Regiment on 2 February 1933. Promoted to Lieutenant in 1936 he was seconded to the 2nd (Nyasaland) Battalion The King's African Rifles in 1937 and then to The Somaliland Camel Corps in 1939. He was made Captain in 1941. As a Temporary Major he was Adjutant of the Long Range Desert Group and then Second in Command, 11th (Kenyan) K.A.R which was part of 25th East African Brigade, 11th East African Division in the Burma Campaign. After aa spell in hospital with scrub typhus he commanded an infantry training establishment in Uganda. Promoted to Acting Lieutenant Colonel in June 1945 he was seconded to The Northern Rhodesia Regiment in 1946. He retired on retirement pay as a Major in 1949 but was granted the honorary rank of Lieutenant Colonel. He served in Tanganyika in the Overseas Civil Service until that country's independence in 1961. In 1962 Wilson became Deputy Warden of London House, a residence for graduates from the Commonwealth of Nations pursuing graduate studies in the UK. Promoted to Warden of London House in 1966 he retired in 1977. He died aged 96 on 23 December 2008 and was buried in the churchyard of St Mary Magdalene, Stowell, Somerset.

18 April the unit was at about 80 per cent of its former strength. Its main operations now were to harass the Italians and to resume operations against local bandits. In 1942, the Somaliland Camel Corps became fully mechanised. On 30 April 1944, members of the Camel Corps captured 53 Germans who had been forces ashore when their submarine was sunk by RAF bombers: the Germans were then interned. There were tentative plans to send the Corps to Burma but it was disbanded later in 1944 after a series of mutinies (Jama, 2000a; 2000b).

Culture and custom

Livestock have a primordial role in Somaliland not only in the household and national economies but also as intimately associated with the history and culture of the people. Livestock production and pastoralism are the bedrock of Somali life. Indeed, livestock played a prominent role in Somaliland's history where there was no centralised political authority and governance was based upon customary law ('xeer'). Among rural communities in Somaliland livestock still serves as the primary exchange market and are traded frequently by barter rather than by cash exchanges. Sheep and goats far outnumber camels which in turn are followed by cattle but in Somaliland, the camel is king. The value of any item is always equated to that of a camel:

'geel waa wixiigooyaan wa geel'
Everything equal in value to a camel can be considered as a camel

Camels have an important role in traditional social relations, such as in payment of a dowry and in compensation ("blood money", 'dia') of injured parties in clan feuds. In the latter case in the 1890s blood money for a man killed was 100 milch camels, sometimes with the addition of 4 horses but half this number was considered enough for a woman. The loss of an eye or permanent disablement of a limb required a payment of 50 camels with the loss of both eyes or disablement of both limbs the full amount as for murder was demanded. Blood loss from the head cost 30 camels and a simple bruise demanded payment of three or four camels (Swayne, 1995). In Somali culture, camel ownership (in terms of herd size) is an indication of social status. In Somali traditional economy, camels are the main reserve stock and therefore act as a store of wealth and security against drought, disease and other natural calamities (Somalilandbiz, 2019).

Somaliland's customary law ('xeer') determines daily life and is used to address a range of conflicts, including murder. This is where camels play a major role. For example: If a clan member is murdered by someone from a different clan, a council of elders from both sides will meet to hear the case and determine the punishment of the offender. In a case I became familiar with, the offender's clan had to pay the family member of the victim and his clan a total of 120 camels. This is a significant number of animals that represented the loss of meat, milk and status to the offender's clan, but symbolically addressed the loss of life of the victim for his family and clan (Hart, 2011).

Camel numbers and distribution

The country's porous international boundaries and the considerable annual and seasonal internal and cross border movement means that data on numbers (Table 1) should only be considered as estimates (MNPD, 2011)[5]. Based on these nebulous figures, camels are most important in terms of the biomass of domestic ruminants (41%) followed by goats and sheep combined (35%) and then by cattle (24%), (Wilson *et al*, 2004). Stock routes along the western border of the country move in waves into Ethiopia at the beginning of the main rainy season in April and return to Somaliland at the start of the dry season in

Table 1. Estimated numbers of livestock ('000) in Somaliland, 2005-2009.

Year	Livestock species				Total livestock
	Goat	Sheep	Camel	Cattle	
1998	6072	6909	1444	341	14766
1999	6367	7146	1476	348	15337
2000	6520	7267	1492	352	15632
2001	6676	7391	1508	356	15932
2002	6837	7517	1525	360	16238
2005	7341	7906	1578	373	17198
2006	7517	8041	1596	378	17532
2007	7698	8178	1614	384	17873
2008	7883	8317	1630	389	18219
2009	8072	8458	1646	394	18570
2010	8266	8602	1664	399	18931
2011	8464	8748	1682	404	19298
2012	8667	8897	1701	409	19674
2013	8875	9048	1720	414	20057

Source: MOL, 2006; MNPD, 2011; Too *et al*, 2015 (based on MNPD information)

5. In spite of the uncertainty the myth of an annual increase in numbers persists, based on a 1975 Somalia census of livestock population and annual growth rates 2.4 per cent for goats, 1.7 per cent for sheep, 1.7 per cent for camels and 1.2 per cent for cattle.

September. Animals normally stationed along the coast move inland at the start of the rains and head back to the coast again in December. It is nonetheless generally accepted that the Sool (in the southeast), Sanaag (in the northeast with a long coastline on the Gulf of Aden) and Togdheer (in the south centre of the country bordering Ethiopia) regions account for about 75 per cent of all livestock. Cattle are mostly found in the wetter (500 mm annual rainfall!) western part of the country whereas, camels and small stock predominate in the drier areas.

Genetic resources

A traveller at the end of the 19th century wrote that there appeared to be two distinct varieties of camel in Somaliland. The Gel Ad, or white variety, occupied the Berbera side whereas the Ayyun or dark Dankali type was common around Zeila. The Issa people admitted the superiority of the Berbera and were willing to pay a higher price for it. This author stated that Somali camels did not trot, were mainly used as transport animals and anyone wanting a faster camel was advised to buy them in Aden and import them. The Somali people named their animals individually with a good large camel being called 'maródi' (elephant) and one noted for its pace might be flatteringly called 'faras' (horse) (Swayne, 1895).[6]

In greater Somalia camels in the modern era are generally assigned to five breeds. The Somaliland is the main camel of the north. Those in the lowlands have fine sparse hair but highland camels have longer and thicker hair. Camels weigh up to 700 kg live weight. The largest Somaliland camels are owned by the Dolbahanta tribe in the southeast of the breed's range (Ahmed, 2005).

The camel of the mountainous regions is known as the 'ayuun' ot 'cayuun'. This type is slighter than the 'caroog' but is more suited to the rugged terrain of the highlands although, it is a poorer producer of milk and meat than other types. In the northern coastal strip, there is more water than in the mountains but the feed resources are not as good. The leaner camels of this area are nonetheless better milk producers than the mountain type (Rirash, 1988).

6. Harald George Carlos Swayne (1860–1940) was a British soldier who served in the Royal Engineers (eventually promoted to Colonel) and an explorer, naturalist and big game hunter. He roamed widely in the then British Somaliland , making 17 trips in the Protectorate between 1885 and 1893, sometimes with his younger brother Eric John Eagles Swayne, covering ten thousand miles with his riding and transport camels. There are 543 mentions of camels in his book of 390 pages.

Production

Camels are mainly dairy animals in Somalia although, there are no data on production. The importance of camels as milk producers results in the herd structure being 80-87% female and 13-20% male. About 23% of the herd is lactating at any one time. Camels are also used as pack animals when moving camp and to a limited extent in commercial transport. An annual reproductive rate of 0.78 young per camel in 1984 implies an interval between births of 15.4 months. This interval is shorter than most data for other countries and may at least in part be due to the bimodal rainfall pattern over much of Somalia (Wilson, 1984; Wilson *et al*, 2004).

Milk

It is estimated that some 50-60% of Somaliland's total production of milk is from camels with cattle contributing 30-40% and goats and sheep providing up to 10%. Lactation yields are estimated at 900-1100 kg. A recent study estimates lactation yields at 1450 litres and total camel milk production in 2013 of 489.4 million litres (Too *et al*, 2015).

Most milk produced is on individual holdings for family consumption but a commercial enterprise, Waayeel Camel Dairy ("Waayeel"), was established in 2014. This is a dairy production business which sells camel milk and male calves to the local markets in Burao. The company also delivers fresh milk to its customers at a kiosk in Burao and has a growing and loyal customer base for its dairy products (Somalilandbiz, 2019).

Meat

The Meat Inspection and Control Act (MOL, n.d.) regulates the slaughter of animals and the handling of them before slaughter and of their meat after slaughter. The Act has a special section on "Conditions for Halal slaughtering for Camels" although many of its stipulations are not respected where home slaughtering takes place.

The camel is the most important source of red meat and is the preferred meat type by many of the people of Somaliland. In 2013, it provided 52% of national production in Somalia as a whole, worth USD 266.1 million USDs in 2015, equivalent to 3.3% of livestock's contribution to the country's economy (Too *et al*, 2015). Somali camels weigh 500-700 kg and offtake is generally considered to be very low 1.6% (Wilson *et al*, 2004). Somalis ate 21.2 kg of camel meat in 2013 compared to 4.3 kg of goat and sheep meat combined and 4.0 kg of beef (Too *et al*, 2015).

Hides

Hides and skins, both fresh and partly cured, are an important source of export revenue in Somaliland. It is estimated that 49380 camel hides were extracted from camels in 2013 worth USD 207 396 (Too *et al*, 2015). The hide as a whole piece is rarely used when camels are killed by traditional owners but can be put to service tentage, for sandals and for making ropes (the "rawhide" of western cowboy and Indian films).

Transport

In the late 19th century, the usual load was 250 pounds (113 kg) excluding the weight of the mats used instead of pack saddles. Carrying this load, a caravan moved at a speed of 2¼ to 2¾ miles per hour (3.6 to 4.4 km/hour) for stints of 5 (4 a.m. to 9 a.m.) and 4 hours (1 p.m. to 5 p.m.) (Swayne, 1895).

It is very unusual for female camels to be used for any form of transport. In the 21st century, camels are used only to a limited extent for commercial or trade transport. They are, however, vital to the nomadic Somali way of life in transporting the tent, household goods, the elderly and frail family members and children. It takes up to 2 years to train a camel for riding or pack work starting at 4 or 5 years age (Axmed, 1987).

Trade

Before Somaliland became a British Protectorate, livestock were traded throughout the land and in coastal cities mostly by barter for food, clothes and other goods. Trade was boosted during the late 19th century as the British administration increased the demand in order to supply fresh meat to their army in Aden (Samatar *et al*, 1988).

In 1870 a British Army Officer was in Berbera to purchase transport animals for the British expedition in Abyssinia. He bought 1069 bullocks and 15 donkeys and was continuing to purchase animals of these descriptions but reported that the animal resources had been much overrated, that mules and ponies were scarce, and the few he had seen had been of the worst description. Camels, he stated, could be purchased in large numbers, but it appears he had not been authorised to buy this class of stock so none were purchased (Holland and Hozier, 1870).

Domestic animals are Somaliland's main export product with a large portion of the country's foreign exchange earnings coming from the sale of livestock (Muhumed and Abdiqadir, 2018). The number of livestock exported since the declaration of independence has, however, fluctuated wildly as a result of drought, civil unrest, urbanisation, an inadequate financial sector, poor infrastructure, the informal nature of marketing, embargoes and the policies of its main international trading partners (Table 2-4). The problems associated with this are compounded by the fact that the country suffers from extremely high levels of inflation and currency depreciation (Fig 4). This has meant that export earnings are estimated to have fallen due to the depreciation of the Somaliland shilling against the dollar (Somalilandbiz, 2019). After the Saudi Arabian ban on Somaliland's livestock was lifted in 2009, exports grew rapidly in the following years. For example, between 2009 and 2011, livestock exports more than doubled. In the meantime, livestock exports have fluctuated between 3.0 and 3.5 million animals exported each year mainly through Berbera, the principal port for Somaliland's livestock exports (Fig 5). Some 84.7% of camel exports are to Saudi Arabia, 10. 7% to Yemen and 4.6% to the UAE (Somalilandbiz, 2019).

Feed and nutrition

Most pastoral stock range freely and consume mainly low quality forage. *Acacia* are common in the mountains whereas, the main feed sources in the coastal areas are *Sueda fruticosa* and *Salvadora persica*.

Table 2. Live animal and hide exports through the ports of Bossasso and Berbera in northern Somalia, 1995-1998.

Port and year	Item exported			
	Camels	Cattle	Small ruminants	Hides (pieces)
Bossasso/a				
1996 (January-July)	15 000	6 000	570 000	363 000
1997	14 599	17 831	494 320	640 750
1998	3 938	29 492	519 020	195 060
Berbera				
1991	102	11 800	482 500	
1992	389	36 600	631 200	
1993	14 800	80 900	1 010 000	
1994	38 000	55 800	1 690 000	
1995	22 000	75 000	2 680 000	61 000
1996 (except December)	30 000	59 000	2 130 000	170 000
1997	50 598	66 939	2 814 495	33 737
1998	11 663	92 213	957 224	50 078

Note: Bossasso is in neighbouring Puntland but most camels are from Somaliland

Source: Academy for Peace and Development (2002)

Table 3. Livestock Exports through Berbera Port (1991-2000).

Year	Livestock species			Total
	Sheep/Goat	Cattle	Camels	
1991	482 508	11 756	102	494 366
1992	631 192	36 662	389	668 243
1993	1 014 921	80 861	14 824	1 110 606
1994	1 685 265	55 729	38 025	1 779 019
1995	2 713 597	75 128	21 993	2 810 718
1996	2 417 656	64 596	42 828	2 525 080
1997	2 814 495	66 939	50 587	2 932 021
1998	957 623	92 213	11 663	1 061 499
1999	2 000 335	88 939	34 840	2 124 114
2000	1 233 851	46 289	10 100	1 290 240

Source: Academy for Peace and Development (2002)

Table 4. Total livestock exports through Berbera Port (2011-2015)

Year	Livestock species			Total
	Sheep/Goat	Cattle	Camels	
2011	3 104 684	150 934	107 281	3 362 899
2012	3 219 584	190 354	102 664	3 512 602
2013	2 888 955	201 876	75 728	3 166 559
2014	3 089 592	252 397	64 578	3 406 567
2015	3 270 386	160 395	61 475	3 392 256

MNPD, 2015

Supplementary feed is rarely provided and herds of camels feed as much as 10 miles from their camp and are watered every 5 days on dry feed. The increasing number of livestock in periurban and urban areas as well as the implementation of quarantine facilities for export animals and feeding systems during shipment the fodder supply value chain is becoming an increasingly important investment. Fodder scarcity is understood to have had a direct impact on livestock production during the recent series of droughts (Somalilandbiz, 2019). One family, for example, lost 30 of 40 camels to drought in 2017 (Muhumed and Abdiqadir, 2018).

Fodder production contributes directly to increased body weight and finishing of livestock, thus creating a value-added final product. Currently, fodder availability is unreliable, which drastically affects the finishing of the livestock. This, in turn, affects the final price of the livestock for export-oriented markets. Fodder for domestic use as well as for export markets is usually sourced from Ethiopia and a vibrant fodder trade has developed over the years to sustain livestock during the long trek from the Ogaden to Hargeisa and Berbera and to allow them to achieve a better price (Fig 6). Ethiopia circulated a letter prohibiting the movement of any fodder beyond Jigjiga town.

Disease

Disease control

A Somaliland Veterinary Code (Law No 34/2006 and 2008) provides a regulatory framework that meets international standards and especially, those Office International des Epizooties (OIE, World Organisation for Animal Health). Other legal instruments pertinent to health and disease include the National Livestock Policy (in draft, 2015), the Animal Welfare Code (Ministerial Decree, 2015), Disease Contingency Plan (in draft, 2015) and Operating Procedures for Priority Diseases (Ministerial Decree, 2015).

Disease occurrence

In the late 19th century, it was written that "in the Ogádén the 'balaad', or small gadfly, is a terrible scourge to [camels], and, to a lesser extent, so is the large gadfly, or 'dúg'; they are also infested with ticks, which swell to the size of a date-stone, and are seen clinging round the eyelids.[7] While drinking, the camels often take in small leeches, which fix themselves to the root of the tongue, growing to a great size and filling the mouth with blood" (Swayne, 1895).

Trypanosomosis ('dhukan') due to *Trypanosoma evansi* and sarcoptic mange ('cadho') resulting from infestation by *Sarcoptes scabiei* are possibly the 2 most important diseases of camels (Omar and Borstein, 1991). The gathering of camel herds in the dry seasons at rivers and standing water with vegetation suitable for biting insect vectors such as tabanids and horse flies plays an important role in the epidemiology of trypanosomosis (Wilson *et al*, 2004). Other external pests and parasites include ringworm ('cambaar') and ticks ('shillin'). Internal parasites ('goryaan', 'caal') include tapeworms such as *Monezia* and *Stilesia* as well as hydatid cysts and many species of roundworm. As for other dairy animals, mastitis ('candhobarar') is a problem not only causing pain to the milking camel but to the owners in reduced milk output and the concomitant reduced quality of life.

Camel pox ('furuqa geela') caused by an Orthopox virus is widespread and although it ends to be benign in mature animals it can be very serious in young animals. Camel contagious echtyma is a

7. 'Balaad' is *Glossina morsitans*; 'dug' is a species of *Tabanus*.

benign disease that can be confused with camel pox but seems to be uncommon in Somaliland. Warts caused by a papilloma are common in young stock appearing mainly around the lips and nostrils but can be more widespread in older animals. Anthrax ('kud') is feared by the Somali herdsmen who encircle dead animals with a thorn boma to deter other stock: unlike cattle it takes a protracted course in camels with painful swelling of the lymph glands (Mares, 1954a). Other diseases that occasionally affect camels include tuberculosis ('urug', 'feero') and tetanus ('kojiso').

Somali ethnoveterinary medicine

Information on Somali ethnoveterinary practices has been documented since at least 1895 (Swayne, 1895). Camels showing stiffness were "fired, either by raising small blisters with a red-hot ramrod or spear or by striping with hoops of red-hot iron. Open sores had glowing stones strapped over them which was followed by an application of moist camel dung. When off feed, a dose of melted sheep's tail was given. Thorns were removed from the foot with the 'biláwa' or dagger and camel dung was then applied. Sore backs caused by the chafing of a load was often bitten by the camel until it festered and became invaded by maggots, the treatment for which was a strip of calico, steeped in carbolic solution, tied over the wound to protect it from attack by omnivorous birds (Swayne, 1895). Some 30 years later, a Treatise on the Camel provided additional information on ethnoveterinary medicine (Leese, 1927). Later work (Hunt, 1951; Mares, 1951; 1954a; 1954b; Peck, 1939; 1940) included descriptions of plant remedies, traditional vaccination, cautery, use of broths and salt in the form of salt bushes, salty wells and salt-rich soils. Mares (1954a; 1954b) also provided an extensive list of Somali names for livestock diseases and parasites. In the 1990s participatory techniques were used to elicit information on indigenous practices (Catley, 1996; Catley and Ahmed, 1996). More recent accounts of Somali ethnoveterinary practice show considerable agreement with the earlier work and even 40 years after the publication of Mares work, herders in northern Somalia were still using soups, cautery, and medicinal plants (Catley and Mohammed, 1995; 1996). A brief review of the literature indicates common terminology for some livestock diseases throughout Somali-occupied areas. For example, the words 'gendhi', 'dhukaan', 'caal', 'cadho' and 'cambaar' are very widely used by Somali herders from north-west Somalia to northern Kenya.

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DOI : 10.5958/2277-8934.2020.00002.8 *Vol 27 No 1, p 13-15*

Short Communication

TRICHOMONOSIS IN DROMEDARY BULLS

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ABSTRACT

During a routine health check of 12 breeding dromedary bulls using microscopy two of them were found to harbour trichomonad flagellates in their preputial washings. A PCR conducted on one of the samples did confirm trichomonads but not *Tritrichomonas foetus*. DNA sequencing identified the organism as *Tetratrichomonas* species. After repeated careful washings of the prepuce with oxidised water resampling revealed negative results.

Key words: Dromedary, PCR, sequencing, *Tetratrichomonas*, Trichomonosis

Trichomonads are small protozoan organisms which can be detected in many wildlife species and domestic animals. Usually, they are non-pathogenic commensals or cause relatively mild diseases. A clinically significant representative of this group is the species *Tritrichomonas foetus*, the causative agent of bovine trichomonosis. Trichomonosis is a venereal disease of cattle primarily characterised by early foetal death and infertility which may also result in extended calving intervals. Trichomonosis occurs worldwide (Tenter, 2006). *Tritrichomonas foetus* has three flagellas at its anterior end and an undulating membrane which makes it very motile. When cows are bred naturally by an infected bull, 30 to 90 % may become infected. Transmission of the parasite may also occur when semen from infected bulls is used for artificial insemination even when semen was frozen (Merck, 2016).

Tritrichomonas foetus is killed by drying or high temperature. Therefore, preputial washes have to reach the laboratory within 48 hours after collection. Various imidazole preparations have been used in bovines to treat bulls, but re-infection after treatment occurs. Successful treatment is measured by repeated sampling at least 3 times after treatment. No information is available of treatment in camel bulls. Literature on trichomonosis in camels is rare and there is only one report on this disease in a dromedary breeding herd from which a flagellate was isolated from 24 out of 48 dromedaries suffering from endometritis (Wernery, 1991). Here, we report a trichomonad infection in two dromedary breeding bulls.

Materials and Methods

In preparation of the 2018/2019 camel breeding season the Camel Reproduction Centre in Dubai, United Arab Emirates (UAE), performed a health check of 12 breeding bulls in late August 2018. For this, a canine urinary catheter was inserted in the distal urethra and 30 to 50 ml of a sterile PBS was introduced. After gentle massage of the prepuce the fluid was recovered with the same syringe, transferred into 50 ml Falcon tubes and sent for bacteriological and parasitological examination to the Central Veterinary Research Laboratory. At the lab the samples were centrifuged at 2,500 rcf for 3 minutes and 100 µl of sediment was microscopically examined at magnification of 100 x and 400 x for moving flagellates. In order to see morphological particularities Giemsa stained smears were examined at 600 x magnification. Isolation experiments were performed using a commercial test kit (InPouch™

Biomed Diagnostics, Inc., White City, USA) and by co-cultivation with BGM-cells (Henning and Sager, 2007).

Treatment of infected dromedary bulls was carried out by repeated careful washings of the prepuce with oxidised water (MicrocynAH, Petaluma, USA).

DNA extraction, PCR and DNA sequencing

Genomic DNA was extracted using the High Pure PCR Template Purification Kit (Roche Diagnostics, Mannheim, Germany) according to the instructions of the manufacturer. PCR for detection of

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DNA of trichomonads was carried out with primers TFR 1 and TFR 2 (Felleisen, 1997) using a modified programme. After an initial denaturation at 96°C for 60 s 35 cycles of denaturation (96°C for 15 s), annealing (67°C) and extension (72°C) followed resulting in a ca. 370 bp amplicon. Analysis was done by electrophoresis on a 1.5% agarose gel, staining with ethidium bromide and visualisation under UV light. After purification with QIAquick Gel Extraction Kit (Qiagen, Hilden Germany) the amplicon was sequenced with BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) and primers TFR 1, TFR 2, TETRI-1 (5´-TTA TTA TTT GCT TTC TGT GG-3´) and TETRI-2 (5´-CTT TGA ATG CAA ATT GCG C-3´). Sequencing products were analysed using a Genetic Analyzer ABI Prism 3130 (Applied Biosystems).

Data analysis

Species identification was carried out by BLAST search (http://www.ncbi.nlm.nih.gov/blast). Sequence of the TFR 1/2 amplicon representing part of rRNA operon was deposited at GenBank database (Acc. no. MN065772). Phylogenetic tree was constructed with Geneious Tree Builder using the Tamura-Mei Genetic Distance Model and Neighbor Joining algorithm with *Tritrichomonas foetus* as outgroup (Kearse *et al,* 2012).

Results and Discussion

Two out of 12 preputial washes in repeated samples in August and September 2018 contained quickly moving *Trichomonas* like flagellates. The oval body in Giemsa stained smears measured 7-9 x 3.5-5 µm and was equipped with an undulating membrane and with four 15 µm long anterior and a shorter 6 µm long posterior flagella (Fig 1). Long term cultivation of the trichomonads failed because of bacterial contamination. A sample which was sent to the National Reference Laboratory for Trichomonosis of Cattle, Friedrich-Loeffler-Institut in Jena, Germany was positive for trichomonads by a PCR according to Felleisen *et al*. (1998). DNA sequencing and data analysis identified the trichomonads as members of the genus *Tetratrichomonas* and excluded the presence of *Tritrichomonas foetus* in the sample.

Fig 2 shows the relatedness within *Tetratrichomonas* spezies with *Tritrichomonas foetus* as out-group. The nearest relatives of isolate 18QT0009 are trichmonads from cow, sheep and goat without valid species names. Clearly defined *Tetratrichomonas* spezies as *T. butteri, T. brumpti* or *T. gallinarum* are more distantly related.

The reproductive biology of Camelidae presents some very important particularities not seen

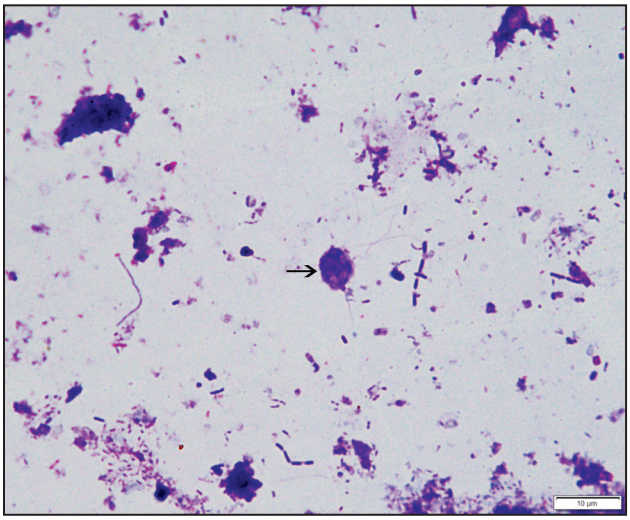

Fig 1. Giemsa stained *Tetratrichomonas* sp. (Arrow) from preputial washing of an adult dromedary bull.

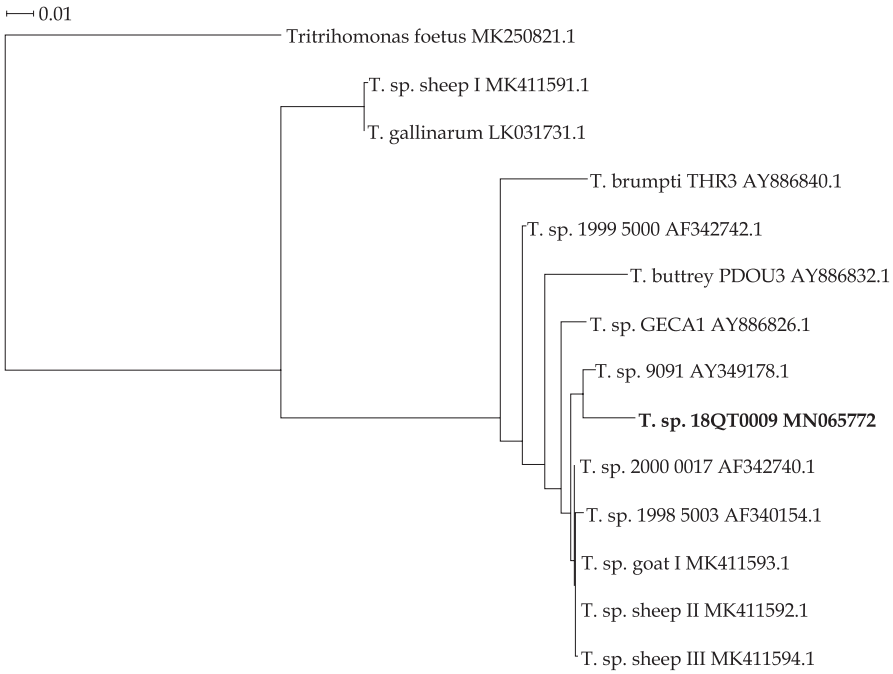

Fig 2. Relatedness within *Tetratrichomonas* isolates according to neighbour joining method.

in other domesticated ruminant species (Tibary and Anouassi, 1997; Wernery *et al,* 2014). Furthermore, infections of the reproductive tracts in Camelidae, as in other domesticated animal species, are the most commonly acquired causes of reproductive failures resulting in infertility (Tibary *et al,* 2006). Research has primarily focussed on dromedaries used for slaughtering with no information on the reproductive status of the tested dromedaries (Merkt *et al,* 1987). A great number of these investigations have been carried out in abattoirs of different countries. Interpretation of presence of bacteria from the reproductive system is difficult, especially when no history is available. In order to evaluate the role of various microorganisms in the development of uterine infections in dromedaries, Wernery *et al* (2014) proposed a classification of bacteria and protozoa as it is done for equines. Hardly, no information about the reproductive tract in camel bulls, especially about trichomonad infections, is available. During a routine inspection, a trichomonad protozoon was detected and subsequently investigated. Although camels are not ruminants the isolate 18QT0009 is closely related to trichomonads from ruminants. In this group it seems to be an own type which we have designated as "genotype camel". The presence of *Tritrichomonas foetus* in the sample could be definitely excluded.

It is not clear whether the isolated *Tetratrichomonas* species had any gynaecological relevance and may cause abortions like *Trichomonas foetus* in cows.

However, when the prepuces of both positive camel bulls flushed with oxidised water repeatedly investigated samples were negative for flagellates after that treatment.

Acknowledgement

The authors are indebted to Drs Lulu Skidmore and Clara Melo from the Camel Reproduction Centre in Nakhlee for sending the preputial washings. We acknowledge B. Hofmann and U. Pfeil at FLI for their excellent technical assistance.

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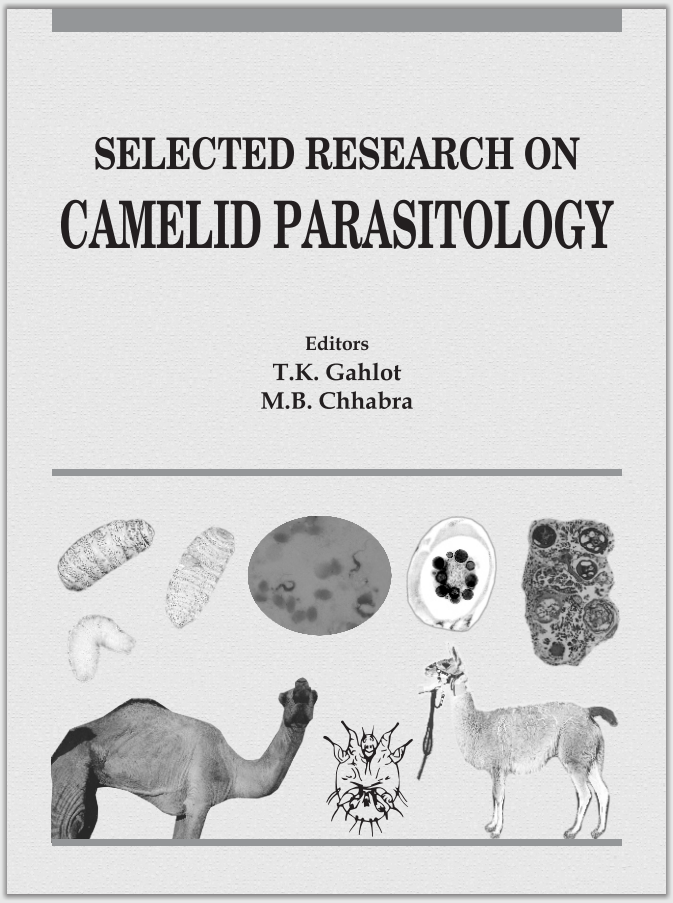

Editors:
T.K. Gahlot and M.B. Chhabra

Edition: 2009

Publisher: **Camel Publishing House**
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Bikaner 334001 Rajasthan,
India
email: tkcamelvet@yahoo.com
website: www.camelsandcamelids.com

Price: US$ 200 (Abroad)
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ISBN: 81-903140-0-9

DOI : 10.5958/2277-8934.2020.00003.X Vol 27 No 1, p 17-22

MORPHOLOGICAL AND SCANNING ELECTRON MICROSCOPIC (SEM) STUDIES OF THE PUPAE OF *Wohlfahrtia magnifica*

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ABSTRACT

Bactrian camel vaginal myiasis was caused by the larvae of *Wohlfahrtia magnifica*, and the development process of *W. magnifica* includes 3 metamorphic stages: larvae, pupae and flies. The morphological characteristics of the pupae stage of *W. magnifica* were observed by ultradepth imager and scanning electron microscopy (SEM). Through observation, the pupae eclosion process was divided into 3 stages, the 1st stage was from 1-4 days, its interior structure showing white and sticky; the 2nd stage was from 5-9 days, the head, wings, feet and abdomen of the pupa were forming and present milky white; the 3rd stage was from 10-12 days, the organs were developed and formed, and all coloured. Scanning electron microscopy (SEM) showed that the 1st, 2nd and 3rd segments of the head antennae were distinct in the 3rd stage of pupae, the 1st segment like a goat horn, the 2nd segment was trapezoidal, and the 3rd segment was rectangular with 2 aristate the outside. The antennae are covered with a large number of bristle, and the structure of bristle was flat and folded. Claw pads and claws were clearly observed on the feet, and there were a large number of bristle on its surface. The interval between the concave parts of the shell of *W. magnifica* pupae was uniform, but have not forming seven thoracic segments similar to that of the larval stage. The pupa shell was dark brown with evenly spaced segments and toughness, and not easily cut by sharp blades. Flies emerged from 13 - 15 days later. In this paper, we describe the morphological structure of pupae in its 3 development stages, which provided a biological data for the prevention and control of *W. magnifica* myiasis.

Key words: Morphology, scanning electron microscopy, ultradepth imager, vaginal myiasis, *Wohlfahrtia magnifica,* pupae

Adult *W. magnifica* feeds on plant juice, its larval stage is parasite of warm-blooded vertebrates causing myiasis (Bartel Huhe *et al,* 1994; 1995; Valentin *et al,* 1997). The mature larva is the 3rd instar leaves the host automatically, falls off to the ground, quickly burrows into the soil and metamorphoses into pupa after 1-3 days. The pupae becomes imago in the dry season after about 20 days (Wangchao, 2019) (Fig 1).

The larva of *W. magnifica* causes vaginal myiasis in Bactrian camels of Mongolia. The disease starts in early June and spontaneously stops in early October. (Schumann *et al,* 1976). Pirali Kheirabadi *et al* (2014) reported that *Wohlfahrtia* caused myiasis in Iranian camels. Xiwen *et al* (2019) reported the morphological characteristics of the larvae of Bactrian camels with vaginal myiasis.

Studies have found that the eclosion rate of pupa increases with the increase of temperature in a certain range. The high temperature and low rainfall in summer and autumn in Alxa region of China were very suitable for the survival and reproduction of *W. magnifica* (Xiwen, 2018). The incidence of this disease in camel population in Alxa region of China was 20%-30%, and the mortality rate was about 2% (Demtu Er *et al,* 2012). At present, there are only few reports on the morphological structure and living habits of the larvae, pupae and flies of *W. magnifica*. In this paper, the morphological and scanning electron microscopic (SEM) studies of the

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Co-first author: Wangchao▲ and HailingLi▲

developing pupa of *W. magnifica* collected from vaginal lesions of Bactrian camels were done.

Materials and Methods

Sample collection

The 200 3rd instar larvae (10-20 mm) were collected from vaginal lesions of Bactrian camels. In the natural environment, the 3rd instar larvae were placed in plastic tanks with a diameter of 3.5 cm and a height of 5 cm (2/3 of the soft sand from the local shade, and ventilation ports were set around them. Five 3rd instar larvae were placed in each plastic tank). After the 3rd instar larvae drilled into the sand, the cans were covered with gauze and placed in shaded place. After 2 to 3 days, the 3rd instar larvae became wrapped in brown shell and became pupae, which was regarded as the beginning of pupae emergence. In order to observe the morphological changes of pupae during eclosion, pupae samples (10-15 pupae samples) were randomly taken out every day, fixed in 70% alcohol and marked the date.

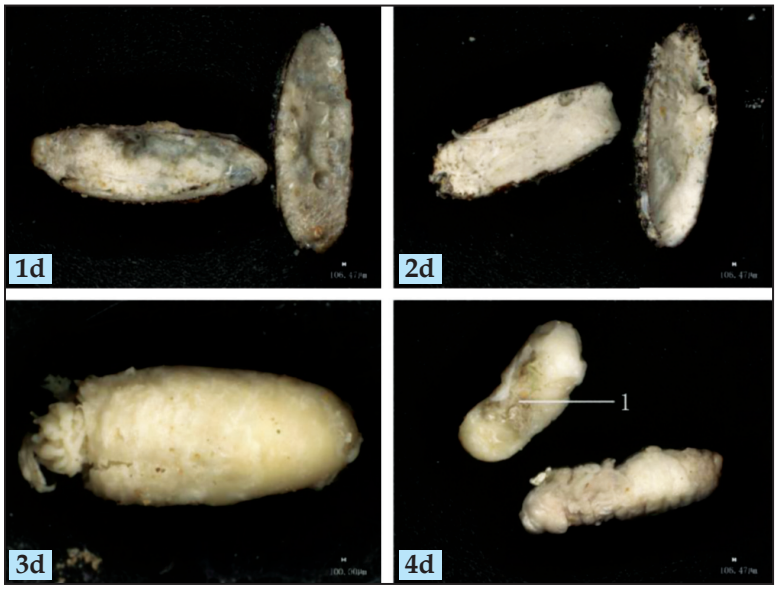

Fig 1. Developmental morphology from day 1 to day 4 of the pupa stage of *Wohlfahrtia magnifica*: 1 - Concave-convex structure.

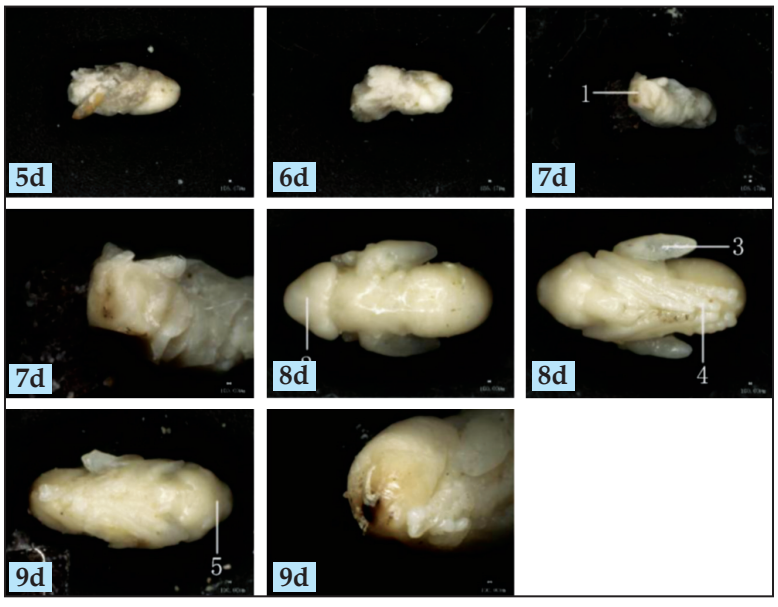

Fig 2. Developmental morphology from day 5 to day 9 of the pupa stage of *Wohlfahrtia magnifica*: 1 - anal foramen; 2 - head; 3 - wings; 4 - foot; 5 - tail.

Ultra depth imager sample preparation

Pupae samples were taken out from 70% alcohol every day during pupae eclosion. The morphological changes of pupae during eclosion were observed after the alcohol on the surface of samples completely volatilised using a KEYENCE-1000 Ultra Depth Imager.

Electron microscope sample preparation

Prior to examination, the pupae stored in 70% alcohol were cut and fixed for 12h with 2.5% glutaraldehyde solution. The fixative solution was replaced once during this period, then rinsed them six times with the phosphoric acid buffer and dehydration was carried out step by step with 55, 75 and 95% alcohol. Then, samples were dried on the critical point dryer and coated, with platinum coating on the conductive layer. Examination was done under the HITACHI-4800 Scanning Electron Microscope.

Results and Discussion

Observation results under Ultra depth imager

The 1st day of anatomical observation showed that the pupal content was a white viscous liquid, similar to egg white, without fixed shape, and could not peel off its interior with brown shell (Fig 1, 1d); the 2nd day of anatomical observation showed that the interior was white soft solid (Fig 1, 2d); the 3rd day of anatomical observation showed that the interior was white oval solid, which could peel off its interior with outer shell completely (Fig 1, 3d); and the 4th day of anatomical observation

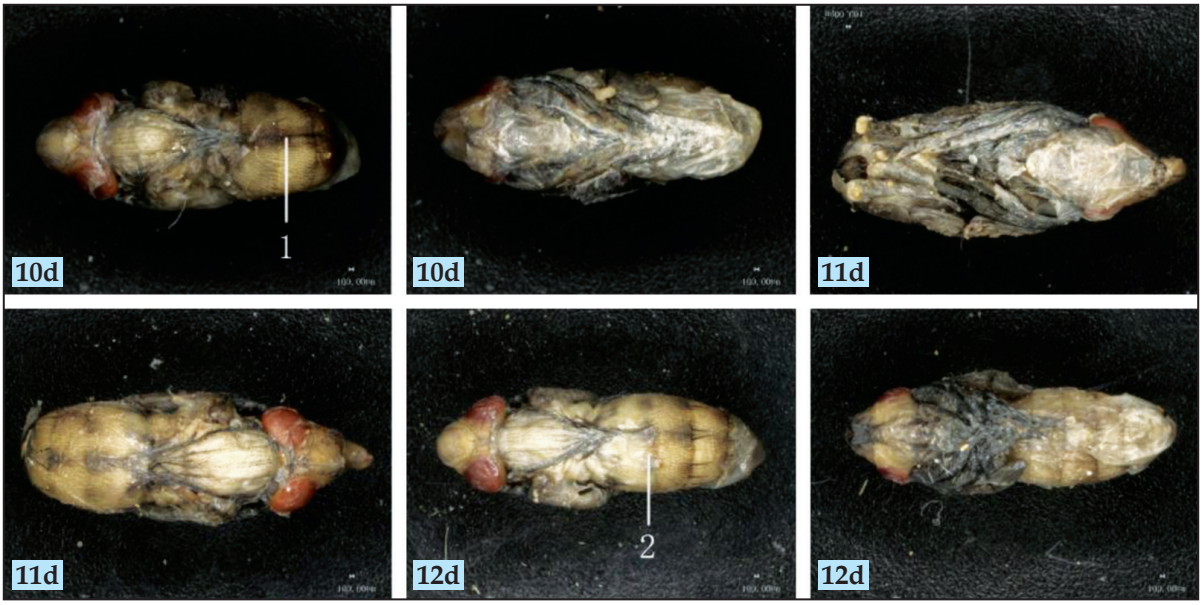

Fig 3. Developmental morphology from day 10 to day 12 of the pupa stage of *Wohlfahrtia magnifica:* 1 - mid-abdominal bristle; 2 - lateral abdominal bristle.

Fig 4. The morphological structure of the pupa stage of *Wohlfahrtia magnifica*. 1 - pupa shell; 2 - adult flies in early emergence.

showed that the interior began to have uneven structure (Fig 1, 4d).

On the 5th and 6th days anatomical observation, there was no obvious changes in the interior (Fig 2, 5d, 6d), and brown anal foramen appeared on the 7th day (Fig 2, 7d). On the 8th and 9th days, the head, wings, feet and tail of pupae had been formed, and the organs had no changes colour, all showing milky white (Fig 2, 8d, 9d).

On the 10th day of pupal development, a transparent film wrapped its interior, the colour of the head, eyes, wings, feet and abdomen had changed obviously, the colour of the black spots on the abdomen had deepened gradually, and the bristles appeared on middle and lateral margins of the abdomen; the wing chambers and veins had not been fully formed, and the wings were in a contraction state (Fig 3).

Visual observation results

The 3rd instar larvae falling to the ground became pupa. It was observed that the pupal shell of *W. magnifica* was dark brown with evenly spaced segments, and did not form 7 thoracic segments similar to the larval stage. The pupa shell was strong flexible, and difficult to cut with sharp blades, and not easily to be damaged from outside (Fig 4).

Observation results under electron scanning microscope

On the 10th day of eclosion, the antennae of the head was formed. The 1st, 2nd and 3rd segments of the antennae were clearly distinguised. The 1st segment started at the head and the 3rd is at the tip of antenna. The 1st section was curved like a goat horn at the top of the antenna, the 2nd section was trapezoidal in the middle of the antenna, the 3rd section was rectangular at the bottom of the antenna, and the 3rd parts were closely connected. There were 2 aristate on the outside of the 3rd section. A large number of bristles were found in the 1st, 2nd and 3rd segments of the antennae (Fig 5).

The eyes of the *W. magnifica* were composed of 2 smooth hemispheres by naked eye observation. The fly could not have rotated around the eyes like human eyes. It needed to observe the surrounding environment through the rotation of body and head.

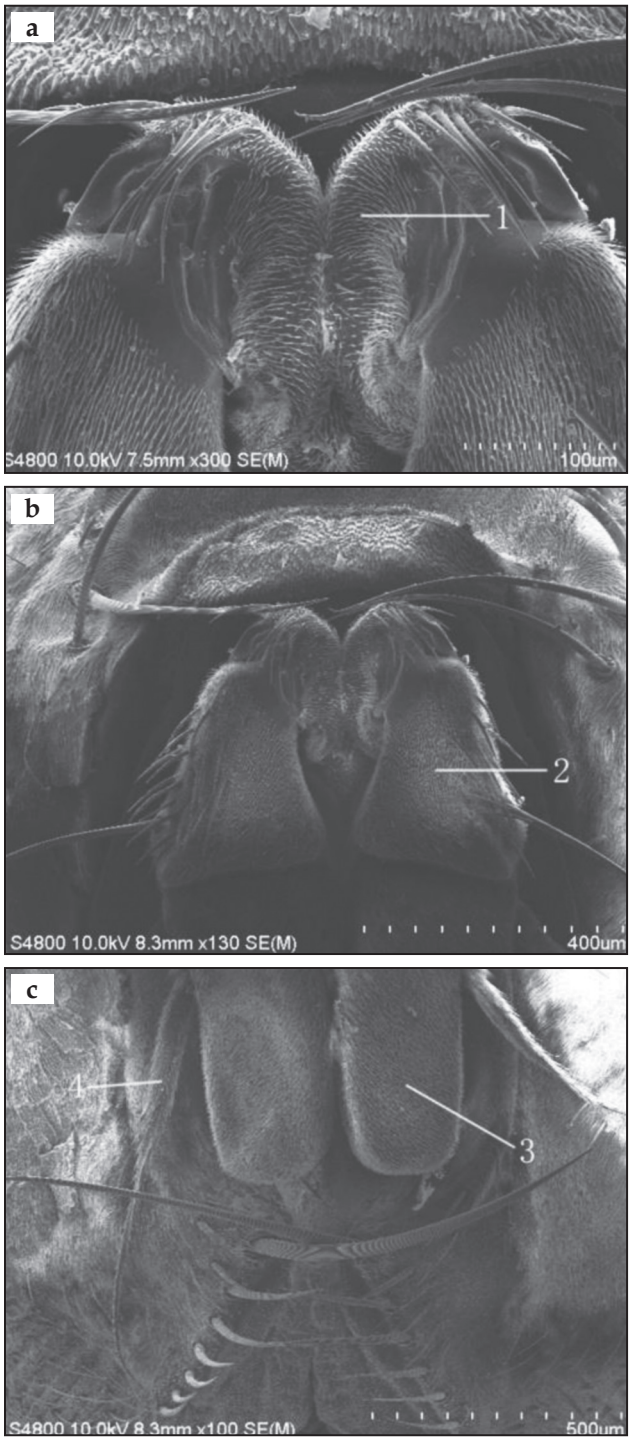

Fig 5. Electron microscopic observation of the head antennae of the pupa stage of *Wohlfahrtia magnifica*a: 1-first antennae; b: 2-antennae 2nd section; c: 3-antenna 3rd section, 4-aristate.

The outer membrane of the eyes was connected with the head. Under the electron microscope, it was found that the eyes of *W. magnifica* were composed of many small quadrangles, each of which was a small eye (Fig 6).

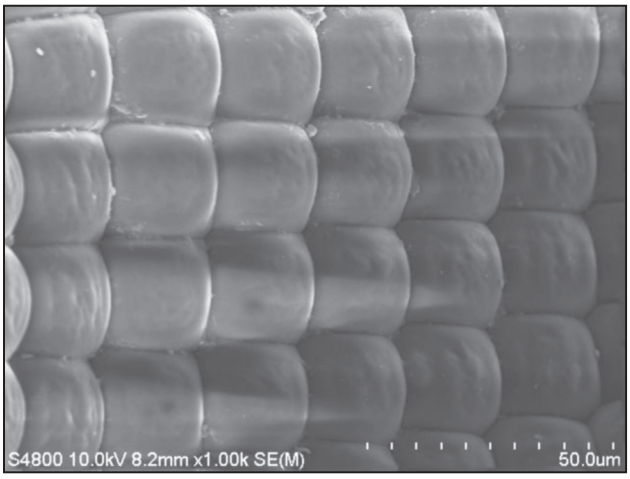

Fig 6. Electron microscopic observation of compound eyes in pupa stage of *Wohlfahrtia magnifica*.

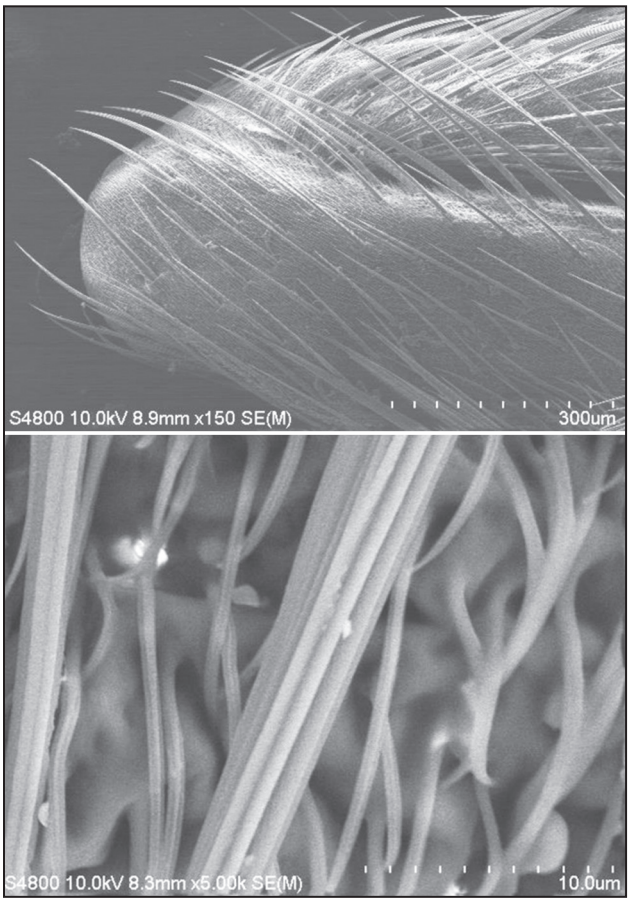

Fig 7. Electron microscopic observation of mane in pupa stage of *Wohlfahrtia magnifica*.

The structure of the bristles was found to be cylindrical needle-like with magnification of 150 times, and it was found flat and folded with 5000 times magnification (Fig 7).

Anatomical observation on the 12th day of emergence of *W. magnifica* showed that the foot had

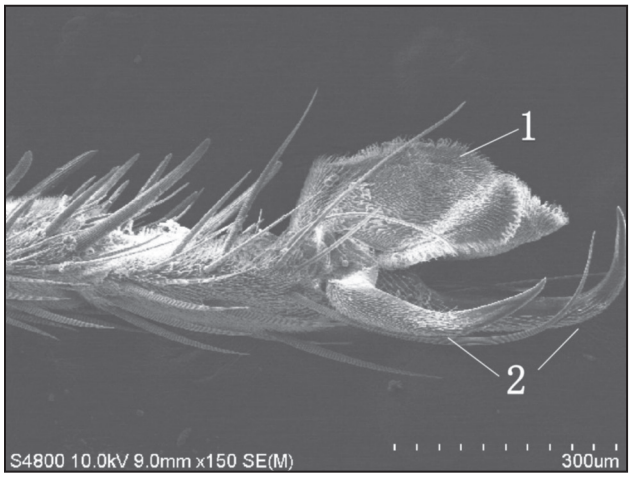

Fig 8. Observation of foot in the pupal stage of *Wohlfahrtia magnifica*: 1-claw pad; 2-claw.

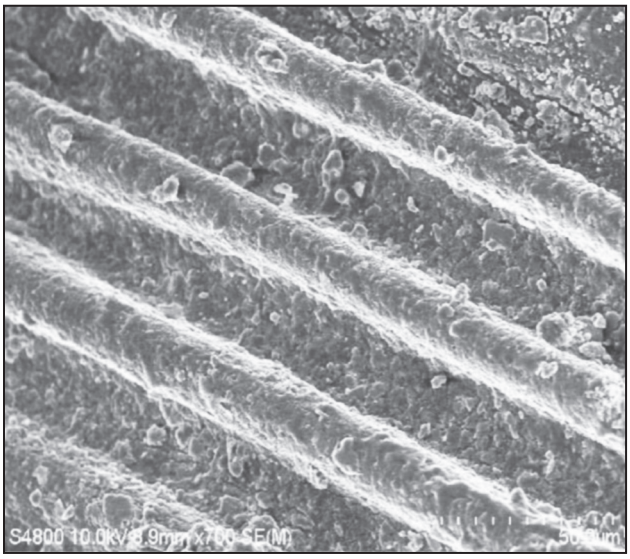

Fig 9. Electron microscope of the pupal shell of *Wohlfahrtia magnifica*.

developed and formed. The pads and claws could be seen clearly under the electron microscope, and a large number of manes were covered around them. Its claws were curved and sharp, making *W. magnifica* easy for landing in different places (Fig 8).

Under electron microscopy, the pits of the pupal shell were evenly spaced and its surface was not smooth. In the course of anatomy, the pupal shell was tough and not easily cut by sharp blades (Fig 9).

The *W. magnifica* was widely distributed in the Mongolian plateau, causing myiasis of the livestock. It is considered one of the harmful parasite in Bactrian camels causing losses to the camel industry (Guofan, 1958).

Miaomiao *et al (*2019) results showed that the pupal completes its development between 12~15 days and the development process of pupa could be divided into prepupa, 1st pupal phase, 2nd pupal phase, 3rd pupal phase before adult emergence according to the morphological characteristics. In this experiment, the pupal stage of *W. magnifica* was observed with the ultra-depth imager and it was divided into 3 stages: in the 1st stage, the pupa with white and viscous content from 1-4 days; in the 2nd stage, the organs of the imago begin to take shape from 5-9 days, and the shape of head, wings, foot and abdomen could clearly be observed under the ordinary microscope; in the 3rd stage, the internal body of the developing fly has been formed with from 10-12 days and each part was mature and coloured. From 13-15 days, the pupa developed into a fly.

The antennae of pupae head and the bristles on the antennae may be related to the odours. The antennae of adult mosquitoes carry numerous sensory structures called sensilla, which are the physical sites of odour detection (Hatem *et al,* 2018). The structure of the bristle on the antennae was flat and folded, which can increase its surface area and efficiency for receiving chemical signals from the surrounding environment. The feet pads were well developed, and its claws were curved and sharp at the end, which was convenient for the imago to land, and increases its foraging range and living space. The compound eye of *W. magnifica* was composed of many small quadrilateral eyes, which was convenient to search for hosts, food, spouses, etc, and can be used for species identification (Rafinejad *et al,* 2014). In the process of eclosion, the pigmentation of organs was carried out after the formation of organs, which may be related to the change of shell permeability and oxidation. The pupa's shell was flexible and not easily damaged by the outside, and shell surface has concave and convex segmented structure, which plays a protective role in the development of pupa during eclosion.

Acknowledgements

This work was supported by National Natural Science Foundation of China (31360591).

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DOI : 10.5958/2277-8934.2020.00004.1 *Vol 27 No 1, p 23-29*

A REVIEW ON BIOMARKERS OF BONE METABOLISM IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

Bone metabolism biomarkers of bone formation and bone resorption are released during the bone remodeling processes. In humans as well as in veterinary medicine, these bone biomarkers have attracted much attention in the last decade. However, there are few reports found in the application of bone metabolism biomarkers in camel medicine. In camels, the most commonly used bone formation biomarkers include osteocalcin (OC) and bone-specific alkaline phosphatase (b-ALP). Concerning the bone resorption biomarkers, the most commonly used one is pyridinoline cross-links (PYD). OC is synthesised mostly from by mature osteoblasts. It is believed that OC is associated with mineralisation of newly formed osteoid. Therefore it is considered as a putative biomarker of bone formation and mineralisation. b-ALP is an isoform of alkaline phosphatase and plays an important role in bone formation. With normal liver function in adults, about 50% of total b-ALP is produced from bone in serum. The PYD cross-links, indicators of type I collagen resorption, are found in the mature collagen of bone. It is not only found in mature type I collagen, which is the major type of collagen in bone tissues, but also in collagen types II and III (Eyre *et al,* 1984). Increased concentrations of PYD in the blood or urine are most commonly considered as indicators of bone resorption. This review article was written to emphasise the importance of commonly used bone formation (OC and b-ALP) and bone resorption (PYD) biomarkers in camels. It is believed that research on bone formation and bone resorption biomarkers in camels will be increasingly used in the future in the diagnosis and prognosis of bone diseases.

Key words: Biomarkers, bone formation, bone resorption, bone, camels

Early diagnosis of bone diseases constitutes a great challenge for the clinician. Although radiography is the most widely imaging technique used for the diagnosis of bone diseases, it fails to diagnose early stages of bone disorders (Al-Sobayil, 2010). It has been reported that approximately 30–40% changes in bone mineral density are required before bone changes are detected in radiography (Greenfield, 1986). Therefore, more specific, sensitive and applicable methods are needed to identify early changes in bone, assess the progression of bone damage and to monitor therapeutic response and the healing process.

Bone is a dynamic tissue, characterised by a continuously renewed through processes of bone removal parallel to bone formation and replacement, which occur in the so-called basic multicellular units. Main cells in the basic multicellular units are osteoblasts, deputed to bone formation, and osteoclasts, to bone resorption. Markers of bone metabolism are biochemical by-products that provide insight into the activity of bone cells. These biochemical markers are produced from the bone remodeling process included bone formation biomarkers and bone resorption biomarkers (Allen, 2003).

Biochemical markers of bone turnover are widely used in human clinical practice, mainly for non-invasive monitoring of bone metabolism and response to therapy of certain musculoskeletal and bone disorders (Swaminathan, 2001; Watts *et al,* 2001; Kanakis *et al,* 2004; Sabour *et al,* 2014). In animals, bone biomarkers are mostly used in preclinical and clinical studies as a rapid and sensitive method for assessment of bone response to medical treatment and surgical interventions and for the detection of musculoskeletal injuries (Allen, 2003; DeLaurier *et al,* 2004; Frisbie *et al,* 2008; Frisbie *et al,* 2010; Tharwat *et al,* 2014; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018 a,b).

The common biomarkers of bone formation include osteocalcin (OC), bone-specific alkaline phosphatase (b-ALP) and amino and carboxy propeptides of collagen type I. The non-collagenous

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protein OC, a product of the osteoblasts, is regarded as a sensitive indicator of bone formation (Pullig *et al,* 2000). The b-ALP, a glycoprotein found on the surface of osteoblasts, has also been shown to be a sensitive and reliable indicator of bone metabolism. Although b-ALP and OC are considered bone formation biomarkers, their correlation in the camel blood was reported to be weak (Al-Sobayil, 2010). The lack of a strong correlation between the two biomarkers has been attributed to the fact that each of them reflects different stages of osteoblast function (Delmas *et al,* 1990). Moreover, b-ALP represents an early osteoblast biomarker because it presents in pre-osteoblasts and osteoblasts, whereas OC is considered a later biomarker of osteoblast differentiation and bone mineralisation (Naylor and Eastell, 1999). The most common biomarkers of bone resorption include pyridinoline cross-links (PYD), deoxypyridinoline enzyme tartrate resistant acid phosphatase and amino and carboxy telopeptides of collagen type I.

This review article emphasises the importance of commonly used bone formation (OC and b-ALP) and bone resorption (PYD) biomarkers in camels. It is believed that the current review on bone formation and bone resorption biomarkers in camels will help researchers and clinicians in the future in the diagnosis and prognosis of bone diseases and their interpretation.

Bone formation biomarkers

Osteocalcin

Osteocalcin, also known as bone gamma-carboxyglutamic acid-containing protein, is a small vitamin K-dependent and calcium binding protein that contains 49 amino acids. It composes 1–20% of the noncollagenous protein of the organic matrix of bone depending on animal species, age, and site (Price, 1983; Conn and Termine, 1985). OC is synthesised by mature osteoblasts, odontoblasts and hypertrophic chondrocytes. Moreover, OC is the most abundant non-collagenous protein in bone comprised about 2% of total protein in the human body. It is believed that OC is associated with mineralisation of newly formed osteoid (Billinghurst *et al,* 2003). Therefore, it is considered as a putative biomarker of bone formation and mineralisation (Billinghurst *et al,* 2004).

Bone-specific Alkaline Phosphatase

Alkaline phosphatase is a membrane-bound enzyme that hydrolyses phosphate esters. Although total alkaline phosphatase (TAP) is not specific to bone, its levels in serum have shown correlation with bone formation rate as assessed by calcium kinetics in normal humans (Weaver *et al,* 1997). TAP levels in serum consist of several enzyme isoforms produced by bone, liver, intestine, kidney, spleen and placenta (Moss, 1987). The majority of TAP in serum is the liver and bone isoforms. Bone-specific alkaline phosphatase (BAP) is an isoform of alkaline phosphatase and plays an important role in bone formation (McIlwraith, 2005). With normal liver function in adults, about 50% of total ALP is produced from bone in serum.

In synovial fluid of active equine OA joints, the b-ALP concentrations were increased compared with normal joints (Fuller *et al,* 2001). The positive correlation between synovial fluid BAP and articular cartilage damage demonstrated a link between changes in bone and articular cartilage in OA. A correlation between cartilage damage and marker levels validates the use of synovial fluid BAP in OA assessment. In serum of horses with OC, BAP concentrations were significantly lower than in serum of normal horses. On the other hand, synovial fluid levels of BAP were significantly higher in horses with OC injury than in healthy horses (McIlwraith, 2005).

Bone resorption biomarkers

Pyridinoline cross-links

The PYD cross-links, indicators of type I collagen resorption, are found in the mature collagen of bone. It is not only found in mature type I collagen, which is the major type of collagen in bone tissues (Von Der Mark, 1999), but also in collagen types II and III (Eyre *et al,* 1984). Increased concentrations of PYD in the blood or urine are most commonly considered as indicators of bone resorption (Thompson *et al,* 1992).

Application of cardiac biomarkers in camel medicine

Similar to published results in mares (Filipovic *et al,* 2010), the serum concentrations of the bone formation biomarkers OC and b-ALP in female camels did not change significantly during the periparturient period (3wk before to 3wk after parturition). In contrast, the serum concentrations of the bone resorption biomarker PYD decreased significantly at parturition compared to 3wk before parturition and then increased significantly at 3wk after parturition (Fig 1) (Tharwat and Al-Sobayil, 2015). In mares, the concentrations of PYD in the blood plasma significantly increased around day 20 after foaling, indicating an increased rate of bone

resorption (Filipovic *et al,* 2010). An increase in the markers of bone resorption was also observed in the serum of ewes and goats in the last month of pregnancy (Liesegang *et al,* 2006, 2007). Liesegang *et al* (2006) indicated that the activity of the osteoblasts is lowered in ewes during late pregnancy.

The decreased oestrogen in camels at 3 wk after parturition (Tharwat and Al-Sobayil, 2015), could contribute to an elevated bone resorption rate. The decreased oestrogen levels post-partum may enhance osteoclast activity that, in turn, would increase bone resorption. It has been reported that a cyclical variation in bone turnover occurs over the course of the oestrous cycle in post-partum dairy cows, with decreases in plasma oestrogen below a critical threshold correlating with enhanced bone resorption (Devkota *et al,* 2012).

In a study conducted recently by our group on 20 sexually mature, healthy male dromedary camels with 8 controls, the serum concentration of OC increased significantly immediately after (electroejaculation) EEJ compared to baseline values (Tharwat and Al-Sobayil, 2018a). However, the serum concentration of b-ALP and PYD differed significantly (Fig 2). Although OC and b-ALP are considered bone formation biomarkers, their correlation in the serum of camels was reported to be weak (Al-Sobayil, 2010). The lack of a strong correlation between the two biomarkers has been attributed to the fact that each of them reflects different stages of osteoblast function (Delmas *et al,* 1990).

The non-significant changes in the serum concentration of the bone resorption biomarker PYD after EEJ may indicate that increased physical activity may have the potential to decrease the collagen resorption in male camels. This result may be influenced, in part, by a systemic suppression of collagen resorption through the systemic actions of calciotropic hormones, with emphasis on testosterones. It has been shown that increased levels of testosterone significantly reduce bone loss (Steffens *et al,* 2012; Wiren *et al,* 2012), decrease collagen and glycosaminoglycan loss in the articular tissues (Ganesan *et al,* 2008) and increase the repair strength of the ligaments and tendons (Tipton *et al,* 1975).

In a study conducted recently by our group on 23 female racing camels that participated in a 5 km race, the serum concentration of serum OC and b-ALP increased but not significantly after race. On the contrary, the serum concentration of the bone

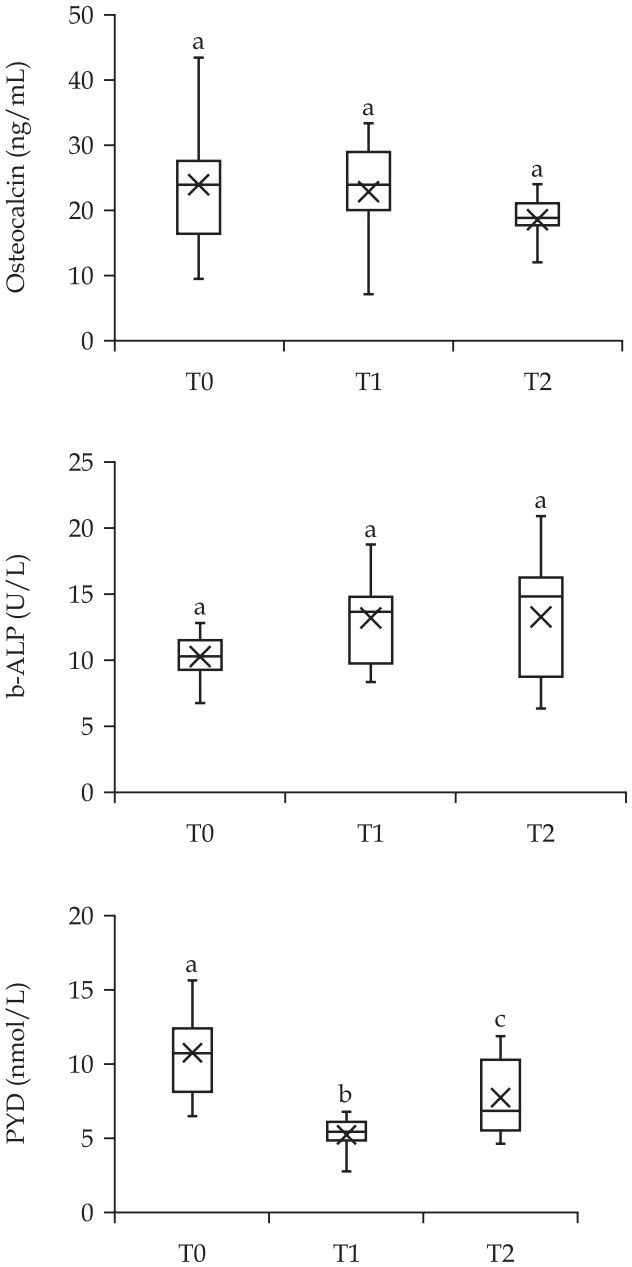

Fig 1. Box and whiskers plots of serum osteocalcin, bone-specific alkaline phosphatse (b-ALP) and pyridinoline cross-links (PYD) in camels during the periparturient period. Box represents the 75th and 25th percentiles while whiskers extend to the 95th and 5th percentiles. T0, 3 wk before expected parturition; T1, within 12h of parturition; T2, 3 wk after parturition. Values different letters differ significantly (P>0.5) (Tharwat and Al-Sobayil, 2015).

resorption biomarker PYD increased significantly after racing (Fig 3) (Tharwat and Al-Sobayil, 2018b). The non-significant elevations in the bone formation biomarker OC are consistent with findings of another exercise study involving highly conditioned Arabian horses (Porr *et al,* 1998). Similarly, in racing camels, both moderate and full-speed exercise had no effect

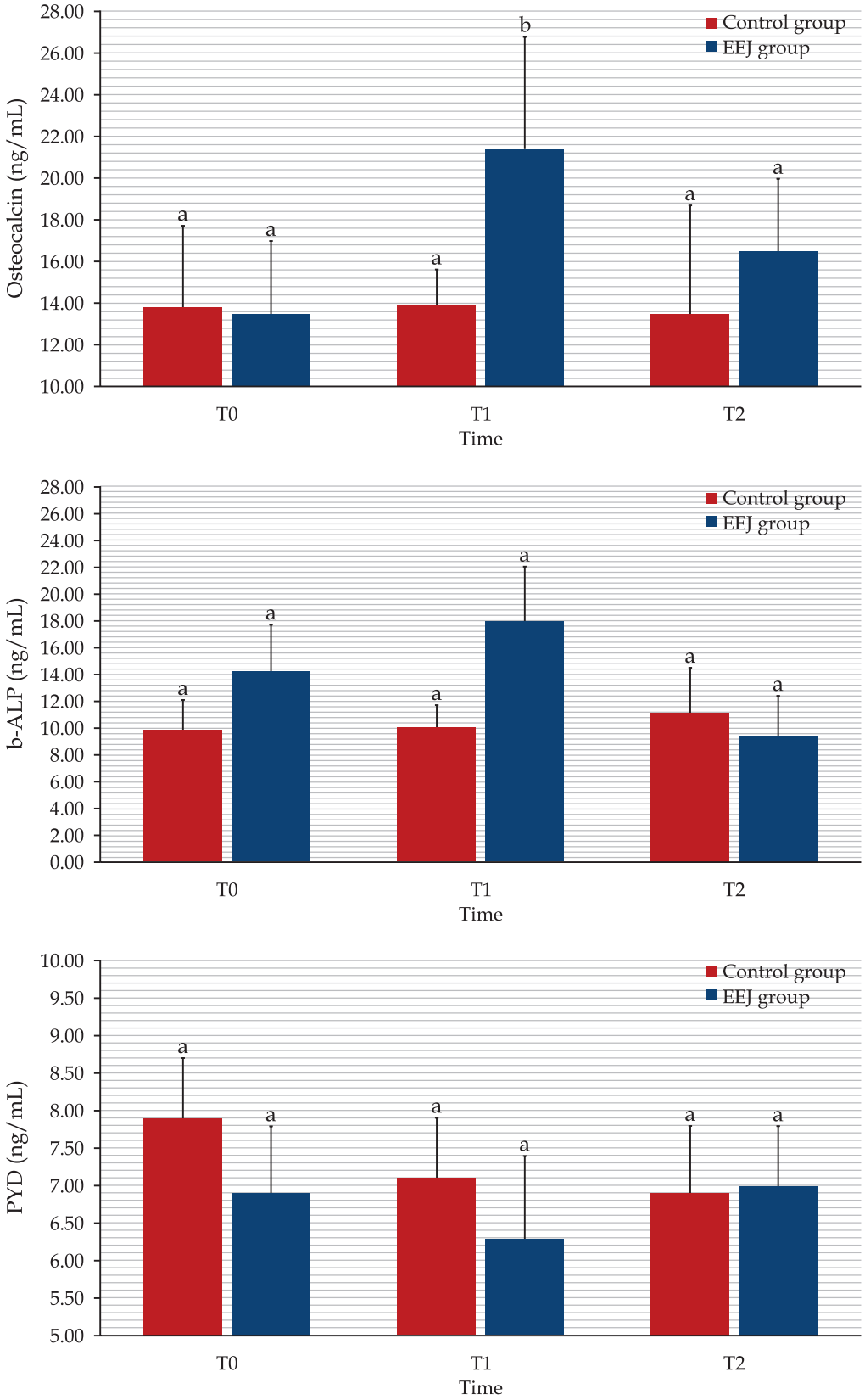

Fig 2. Effect of stimulation by electroejaculation (EEJ) on concentrations of serum osteocalcin, bone-specific alkaline phosphatse (b-ALP) and pyridinoline cross-links (PYD) in male dromedary camels (mean ± SD, n=20) compared to control group (n=10). T0: just before EEJ; T1: directly after EEJ; T2: 24h after EEJ. Values different letters differ significantly (Tharwat and Al-Sobayil, 2018a).

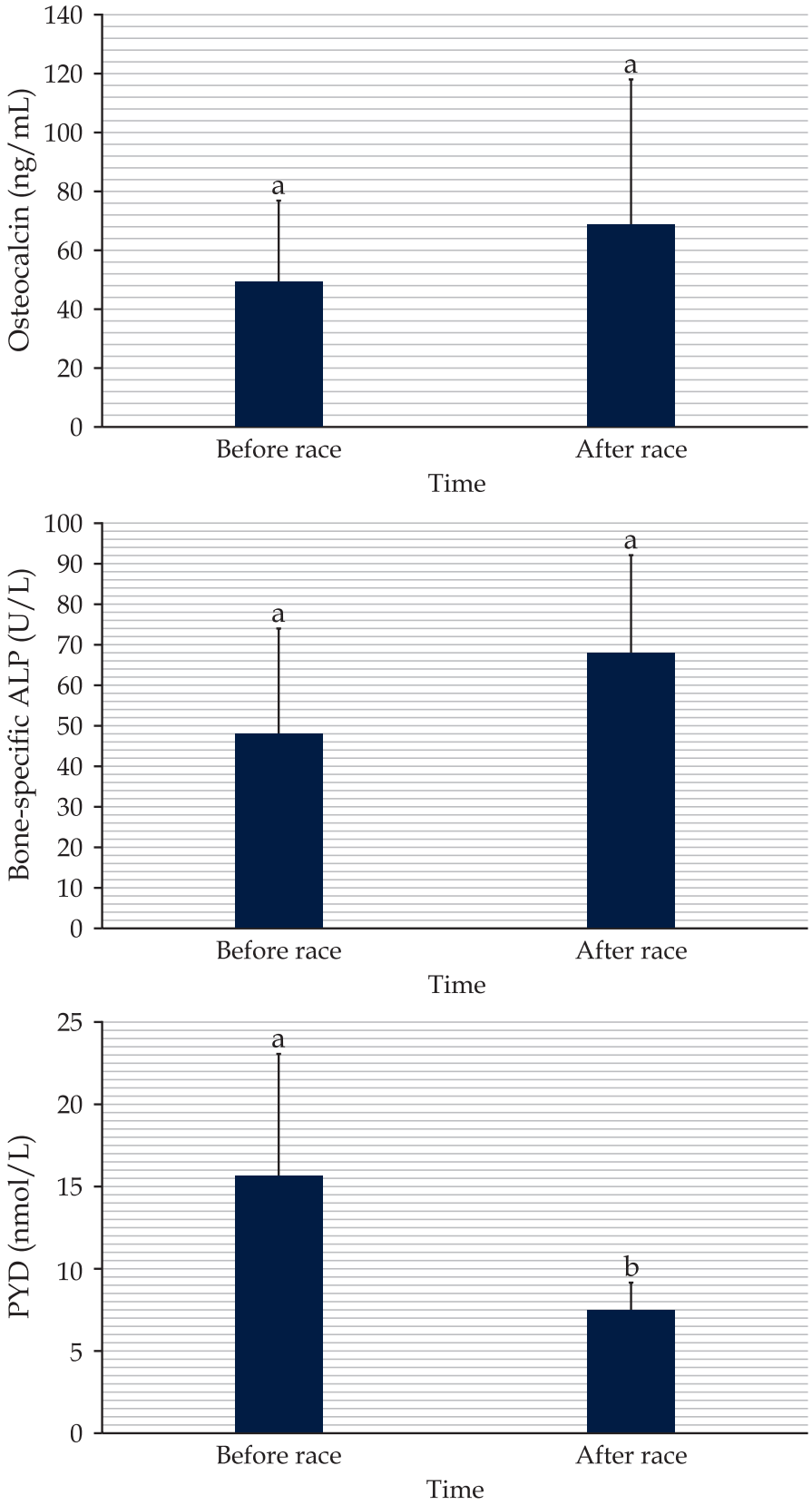

Fig 3. Serum concentration of osteocalcin, bone-specific alkaline phosphatse (b-ALP) and pyridinoline cross-links (PYD) in racing dromedary camels before and after race (Tharwat and Al-Sobayil, 2018b).

on the concentration of OC (Al-Sobayil, 2008). Rudberg *et al* (2000) reported short-lasting increases in b-ALP after 4.7 Km of jogging, a distance nearly similar ours (7 Km). It has been suggested that the increased content of b-ALP could be released from the osteoblast membranes under local factors such as changes in the pH, which triggers the release of b-ALP from the osteoblasts (Anh *et al,* 1998). The significant increases in PYD post-race in camels disagree with those of the study in horses assigned to 48-week race training (Caron *et al,* 2002), where no significant changes in serum PYD concentrations were detected post-race. It is clear from this study that the bone formation biomarkers are not influenced by the 5-km race. However, the bone resorption biomarker increased significantly. The influence of long-distance racing on these biomarkers is therefore warranted. Understanding the effect of racing on stimulation of the bone remodelling is important for the development of strategies to increase and maintain bone mass.

Conclusions

This mini review sheds light on the commonly used bone formation (OC and b-ALP) and bone resorption (PYD) biomarkers in camel medicine. These biomarkers were changed in camel serum following parturition, stimulation by electroejaculation and race. It is expected that bone metabolism biomarkers in camels would be increasingly used in the clinical studies for the diagnosis, prognosis and assessment the response of the skeleton to medical and surgical interventions.

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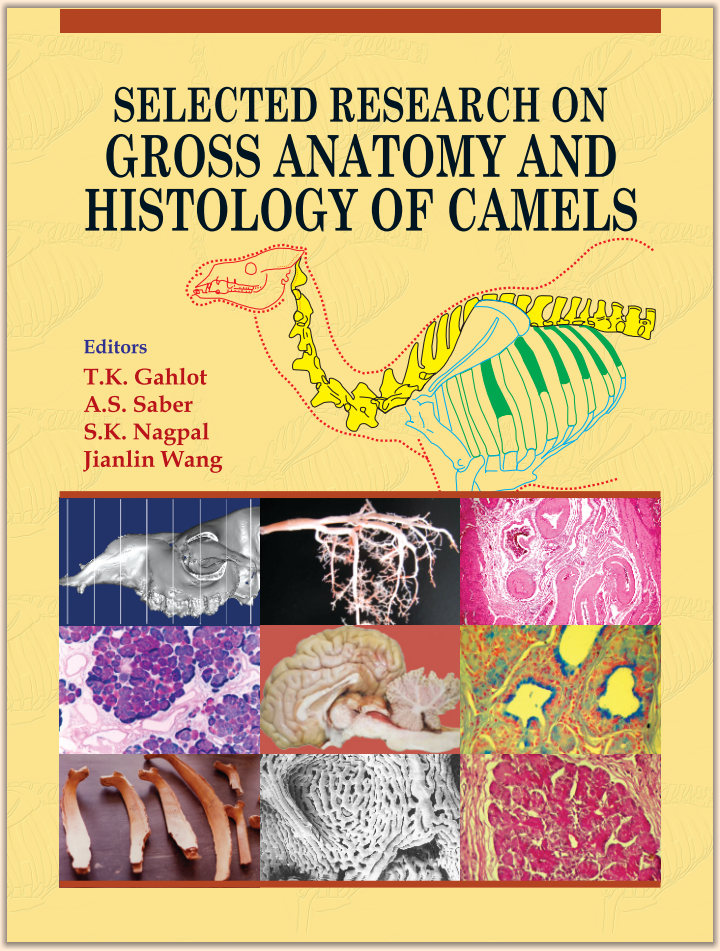

Editors:
T.K. Gahlot, A.S. Saber, S.K. Nagpal and Jianlin Wang

Edition: 2011

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

Price: US $ 375 (Abroad)
INR 7500 (India)

ISBN: 81-903140-1-7

DOI : 10.5958/2277-8934.2020.00005.3 *Vol 27 No 1, p 31-37*

EVALUATION OF MICROSATELLITE MARKERS FOR USE DURING GENOTYPING AND PARENTAGE EXCLUSION FOR DROMEDARY CAMELS IN QATAR

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ABSTRACT

This study was aimed to evaluate the efficiency of 20 previously published microsatellite markers for the determination of parentage within the racing camel population in Qatar, using multiplex polymerase chain reaction (PCR), capillary electrophoresis, and genotyping. These markers amplified 127 alleles, and 15 out of 20 loci were polymorphic among the dromedary camels in Qatar, with an average of 8.13 alleles per locus. The mean expected heterozygosity (H_e) among the studied population was 0.562 (range 0.114–0.867). The polymorphic information content (PIC) ranged from 0.107 to 0.852, with an average value of 0.516. These results indicated a low probability of identity ($2.10E^{-11}$), with a high parentage exclusion probability if either one (0.99959) or both parents (0.99999) were putative. In those study cases with parentage assignment, the 15 microsatellite loci successfully assigned 135 young calves to the correct parents, with 95% confidence. Our results demonstrated that a set of nine microsatellite DNA markers could provide highly precise individual identification and paternity assignment within the studied camel population.

Key words: Dromedary camels, genotyping, microsatellite markers, parentage assignment, pedigree

Microsatellites or short tandem repeats (STRs) have been used as widely accepted genetic markers to study DNA profiling and phylogenetic relationships between closely related genomes because they represent highly polymorphic, abundant sequences in DNA, which are inherited in a Mendelian co-dominant manner and are readily adaptable to polymerase chain reaction (PCR) methods (MacHugh *et al,* 1997; Schlötterer, 2004).

In 2014, a camel comparison test was introduced, for the first time, by the International Society of Animal Genetics (ISAG), which was able establish unique binning through the use of a core panel of 7 loci, LCA8, LCA37, LCA56, LCA65, LCA66, YWLL29, and YWLL44, and 10 additional STRs, CVRL01, CVRL04, CVRL05, LCA99, LGU49, VOLP3, VOLP32, VOLP59, YWLL08, and YWLL36. In 2016, LCA19 was added to the core panel and CVRL01, CVRL04, and CVRL05 were excluded from the backup panel.

Many researchers have used STR loci to investigate genetic polymorphisms within and between dromedary camel populations (Mahmoud *et al,* 2012; Nolte *et al,* 2005; Spencer *et al,* 2010). Few efforts have been made to use STRs during camel genotyping in Qatar (Hashim *et al,* 2014). Therefore, the present study was performed to refine the recommended ISAG panels of microsatellite markers for use during the genotyping and parentage testing of dromedary camels in Qatar.

Materials and Methods

Sampling and DNA extraction

A total of 297 blood samples (135 dams, 27 sires, and 135 calves) were collected from dromedary camels reared in Qatar. Blood samples were collected from the jugular vein and placed into tubes containing ethylenediaminetetraacetic acid (EDTA). Samples were collected utilising the pedigree records maintained by camel owners (2016-2017). Genomic DNA was extracted using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche diagnostics, #03730964001/Germany).

PCR amplification and fragment analysis

Twenty camelid microsatellite primer pairs, which were recommended by the ISAG for use

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during dromedary parentage testing, were selected for DNA amplification using PCR. Forward primers were fluorescently labelled with FAM, VIC, NED, and PET dyes (Table 1). The microsatellite markers were amplified individually and then optimised using the following three multiplex reactions: multiplex I included YWLL36, YWLL44, YWLL29, CVRL04, LCA77, VOLP59, LGU49, LCA56, and LCA24; multiplex II included LCA65, CVRL01, VOLP32, LCA37, LCA19, LCA99, and LCA66; and multiplex III included YWLL08, CVLR05, LCA8, and VOLP3.

Multiplex PCR reactions were performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems). PCR reactions were performed using a total volume of 25 µl, containing 10 ng of DNA template, 5 pmol of each primer pair, and 12.5 µl of AmpliTaq Gold™ 360 Master Mix (Applied biosystems, 4398881/USA).

The following thermal cycler programme was used: initial denaturation at 95 °C for 10 minutes, followed by 35 cycles of 30 seconds at 95 °C, 55 °C (for multiplex I), 58 °C (for multiplex II), and 60 °C (for multiplex III) for 40 seconds for annealing, 72 °C for 1 min for extension and a final extension at 72 °C for 60 minutes. Samples were maintained at 4 °C after program completion. Fragments were mixed with Hi-Di Formamide (Applied Biosystems, USA) and a Genescan Liz-600 size standard (Applied Biosystems, USA). The mixture was separated by capillary electrophoresis, on an ABI 3500 Genetic Analyser (Applied Biosystems). Finally, the fragments were analysed using GeneMapper® Software 5 (Applied Biosystems).

Statistical analysis

The number of alleles per locus (N_o), the effective number of alleles (N_e), the allelic frequencies for each locus, the observed heterozygosity (H_o), and the expected heterozygosity (H_e) were calculated using GENALEX, version 6 software (Peakall and Smouse, 2005).

Polymorphic information content (PIC) was calculated using CERVUS, version 3 software (Marshall *et al,* 1998). The Hardy-Weinberg Equilibrium (*HWE*) was estimated using GENEPOP, version 1.2 software (Rousset, 1995).

The likelihood ratio of assigning the correct parentage to a given set of parents with significant confidence was determined using CERVUS, version 3 software (Marshall *et al,* 1998). For each offspring tested, the parentage analysis module calculated likelihood ratio (LOD) scores for each candidate parent, identified the two most likely parents, calculated a corresponding delta score (Δ), as the difference between the LOD scores of the first and second most likely candidate parents, and awarded parentage, with 95% confidence. Three general probability formulae exist for parentage exclusion (PE) (Jamieson and Taylor, 1997): PE1 estimates the probability of exclusion when the genotypes of both parents are known; PE2 estimates the probability of exclusion when the genotype of only one parent is known; and PE3 estimates the probability of excluding two putative parents.

The probability of identity, sibling identity, and combined power of exclusion (CPE) values for the 20 studied loci were calculated according to allele frequencies, using GENALEX, version 6 software (Peakall and Smouse, 2005).

Results

Population structure

The genotypes of 297 individual dromedary camels were successfully generated, using 20 camelid microsatellite markers (Table 1). Fifteen loci were found to be polymorphic, and the remaining five loci (LCA19, LCA24, LCA77, VOLP59, and YWLL36) were monomorphic, among the studied group. Summary statistics for the polymorphic microsatellites are presented in Table 3. The number of alleles per polymorphic locus ranged from 2, in LCA8, LCA65, and YWLL29, to 21, in CVRL01 (Table 3), with a mean of 8.13 per locus. Ne values varied from 1.128, in LCA65, to 7.44, in LCA99, with a mean value of 3.12.

H_o values varied from 0.114 (LCA65) to 0.889 (LCA99), with a mean value of 0.555 for polymorphic loci, whereas H_e values ranged from 0.114 (LCA65) to 0.867 (LCA99), with a mean value of 0.562. PIC values ranged from 0.107 (LCA65) to 0.852 (LCA99), with a mean value of 0.516. Nine microsatellites showed increased degrees of polymorphism compared with the others, including LCA66, LCA99, LGU49, VOLP3, YWLL08, YWLL44, CVRL01, CVRL04, and CVRL05, with PIC values that ranged from 0.520 to 0.852. Four loci, LCA8, LCA37, LCA56, and YWLL29, showed intermediate PIC values (0.241–0.368). Two loci, LCA65 and VOLP32, exhibited low degrees of polymorphism, and their PIC values were 0.107 and 0.225, respectively. Eight of the 15 microsatellite markers, including YWLL44, LCA56, VOLP3, LCA8, LCA37, YWLL29, VOLP32, and LCA65, had one allele with a relatively high frequency (0.6 to 0.94), which affected their polymorphic values, as shown in Table 3.

Furthermore, the combined probability of identity (CPI) values that two randomly chosen animals would show identical genotypes were estimated to be 2.10×10^{-11}, 1.10×10^{-9} and 2.00×10^{-8} for the 15, 9, and 7 loci, respectively (Table 4). In extreme situations, such as when all individuals are full siblings, the CPI values were 4.92×10^{-5}, 3.623×10^{-4}, and 1.369×10^{-3} for the 15, 9, and 7 loci, respectively. Only one locus (LCA56) deviated significantly from the *HWE* ($P < 0.001$), whereas the other loci were consistent with the *HWE*, after Bonferroni corrections. In addition, the null-allele frequency values ranged from -0.039 (LCA66) to 0.1717 (LCA56), with an average value of 0.012 (Table 3).

Parentage analysis

The exclusion probability and the high genetic variability observed among the tested markers demonstrated the efficiency of these markers for use during paternity testing. The PE1 values of the 15 loci

Table 1. Characteristics of the 20 microsatellite loci used in this study.

Locus	Primer sequences (5′ – 3′)	Range[a] (bp)	Accession/ reference
LCA19	F: TAAGTCCAGCCCCACACTCA R: GGTGAAGGGGCTTGATCTTC	75-85	(Penedo *et al*, 1998)
LCA24	F: ACTCACGGGTGACATACAGTG R: GAGCAGTGTTTGGTTTGCATT	99-109	AF060101
YWLL36	F: AGTCTTGGTGTGGTGGTAGAA R: TGCCAGGATACTGACAGTGAT	131-141	(Lang *et al*, 1996)
VOLP59	F: CCTTCCTCAGAATCCGCCACC R: CCCGCGCACCAAGCAG	100–137	(Paredes *et al*, 2014)
YWLL08	F: ATCAAGTTTGAGGTGCTTTCC R: CCATGGCATTGTGTTGAAGAC	127-177	(Lang *et al*, 1996)
LCA8	F: GCTGAACCACAATGCAAAGA R: AATGCAGATGTGCCTCAGTT	228-240	(Penedo *et al*, 1998)
YWLL44	F: CTCAACAATGCTAGACCTTGG R: GAGAACACAGGCTGGTGAATA	85-119	(Lang *et al*, 1996)
LCA37	F: AAACCTAATTACCTCCCCCA R: CCATGTAGTTGCAGGACACG	129-141	AF060105
VOLP3	F: AGACGGTTGGGAAGGTGGTA R: CGACAGCAAGGCACAGGA	141-183	AF305228
YWLL29	F: GAAGGCAGGAGAAAAGGTAG R: CAGAGGCTTAATAACTTGCAG	203-215	(Mehta *et al*, 2007)
LCA77	F:TGTTGACTAGAGCCTTTTCTTCTTT R: GGGCAAGAGAGACTGACTGG	228-238	(Penedo *et al*, 1999)
VOLP32	F: GTGATCGGAATGGCTTGAAA R: CAGCGAGCACCTGAAAGAA	250-266	(Obreque *et al*, 1998)
LCA56	F: ATGGTGTTTACAGGGCGTTG R: GCATTACTGAAAAGCCCAGG	125-139	AF091122
CVLR05	F: CCTTGGACCTCCTTGCTCTG R: GCCACTGGTCCCTGTCATT	151-183	AF217605
LCA66	F: GTGCAGCGTCCAAATAGTCA R: CCAGCATCGTCCAGTATTCA	231-255	(Penedo *et al*, 1998)
LCA65	F: TTTTTCCCCTGTGGTTGAAT R: AACTCAGCTGTTGTCAGGGG	160-172	AF091124
CVRL04	F: CCCTACCTCTGGACTTTG R: CCTTTTTGGGTATTTTCAG	152-180	AF217604
CVRL01	F: GAAGAGGTTGGGGCACTAC R: CAGGCAGATATCCATTGAA	198-252	AF217601
LGU49	F: TCTAGGTCCATCCCTGTTGC R: GTGCTGGAATAGTGCCCAGT	214-262	(Sarno *et al*, 2000)
LCA99	F: CAGGTATCAGGAGACGGGCT R: AGCATTTATCAAGGAACACCAGC	232-334	(La Manna *et al*, 2011)

[a] Ranges differ from the original publications as per this study.

Table 2. Parentage parameters used in CERVUS parentage analysis, following allele frequency estimations and simulations.

Parameter	Value
Percentage of candidate parents typed	100%
Percentage of loci typed	100%
Error rate in likelihood calculations	0 and 1%
Number of tests performed	10000
Strict confidence level of parentage assignment	95%
Relaxed confidence level of parentage assignment	80%

Table 3. Polymorphism statistics of microsatellite loci.

Locus	N_o	N_e	FNA	H_o	H_e	PIC	PE1	PE2	F(Null)	HW
LCA08	2	1.852	0.641	0.468	0.461	0.354	0.106	0.177	-0.0086	NS
LCA37	3	1.505	0.793	0.323	0.336	0.29	0.056	0.152	0.0216	NS
LCA56	3	1.904	0.623	0.337	0.476	0.368	0.113	0.188	0.1717	***
LCA65	2	1.128	0.939	0.114	0.114	0.107	0.006	0.054	-0.0027	ND
LCA66	7	4.125	0.32	0.818	0.759	0.716	0.349	0.527	-0.0392	NS
YWLL29	2	1.389	0.832	0.269	0.28	0.241	0.039	0.12	0.0194	NS
YWLL44	8	2.331	0.601	0.525	0.572	0.52	0.172	0.328	0.0396	NS
LCA99	17	7.435	0.229	0.889	0.867	0.852	0.578	0.734	-0.0138	NS
LGU49	13	4.75	0.268	0.795	0.791	0.758	0.413	0.59	-0.0034	NS
VOLP3	10	2.421	0.616	0.579	0.588	0.561	0.206	0.388	0.0074	NS
VOLP32	3	1.347	0.848	0.263	0.258	0.225	0.033	0.113	-0.0103	NS
YWLL08	16	5.266	0.303	0.828	0.811	0.787	0.464	0.638	-0.0119	NS
CVRL01	21	5.661	0.35	0.838	0.825	0.808	0.505	0.675	-0.0107	NS
CVRL04	4	2.595	0.455	0.613	0.616	0.535	0.191	0.326	0.0038	NS
CVRL05	11	3.088	0.46	0.66	0.677	0.624	0.262	0.429	0.0096	NS
Mean±SD	8.13±6.278	3.12±1.89	0.552±0.23	0.555±0.251	0.562±0.235	0.516±0.241	0.233±0.187	0.363±0.227	0.0115±0.048	

Table 4. The cumulative exclusion probability and Multi-locus probability of identity estimations for 3 tested combination of markers.

	Excluded markers	Cumulative probability of exclusion			Overall probability of identity	
		PE1	PE2	PE3	PI	PI-Sib
15 marker set	None	0.98838	0.99959	0.99999	2.10E-11	4.92E-05
9 marker set	LCA08, LCA65, YWLL29, LCA37, LCA56, VOLP32	0.98319	0.99904	0.99999	1.10E-09	3.62E-04
7 marker set	LCA08, LCA65, YWLL29, LCA37, LCA56, VOLP32, YWLL44, VOLP3	0.97443	0.9977	0.99997	2.00E-08	1.37E-03

ranged from 0.006 (LCA65) to 0.578 (LCA99), with an average value of 0.233 (Table 3). The mean value for PE2 was 0.363 for 15 loci, and these values ranged from 0.054 (LCA65) to 0.734 (LCA99). PE1 was larger than PE2, likely because extra maternal information facilitates the exclusion of false sires. Fig 1 shows how PE values change as the number of loci increases. A very high PE level can be achieved using 7–9 of the 15 loci. The cumulative values for PE2 and PE3 were higher than 0.999, regardless of whether all 15 loci or only 9 loci were considered (Table 4). When using the 7-marker set of loci, the cumulative PE2 and PE3 values declined to 0.9977 and 0.99997, respectively. However, in cases where the genotypes of both parents were known (PE1), the use of 15 loci showed a relatively increased cumulative PE value (0.988) than that observed for the combination of both the nine- (0.983) and seven-marker sets (0.974).

CERVUS was used to estimate the success rate of parentage assignments at both a strict confidence level (95%) and a relaxed confidence level (80%) (Table 2). Parentage analyses across the 15

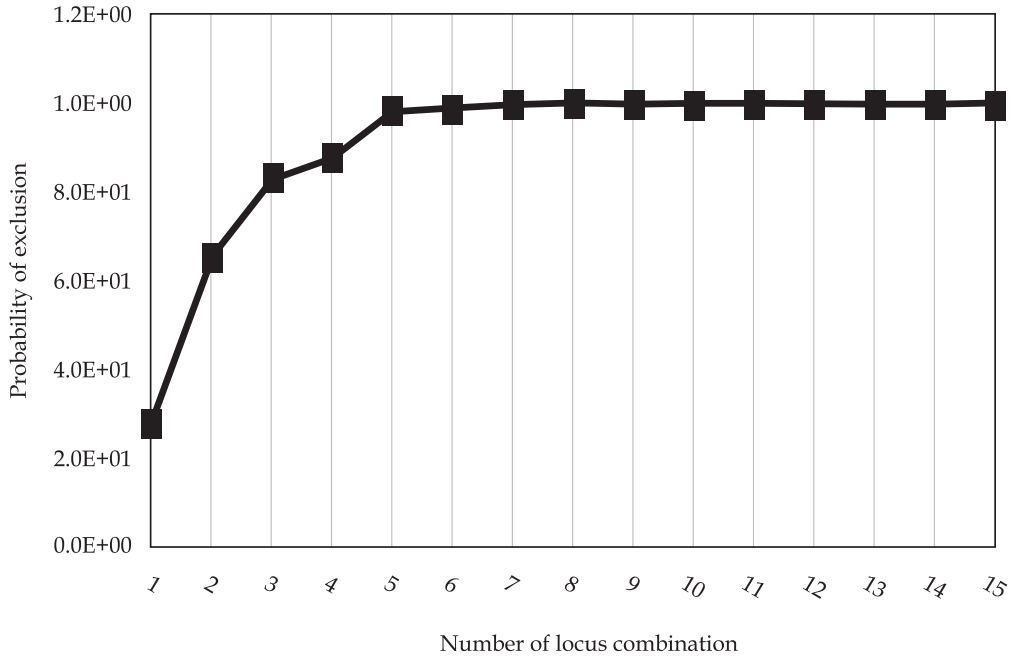

Fig 1. Probability of exclusion as a function of numbers of 15 microsatellite loci for the twenty seven bull camel families.

microsatellite markers depended on LOD and delta scores.

For maternity, the LOD values for microsatellite pairs ranged from 0.243 to 14.47, whereas the delta values for microsatellite pairs ranged from 0.243 to 14.47, which revealed the correct assignment of mothers. For paternity, the LOD values for microsatellite pairs ranged from 0.522 to 14.1, whereas the delta values for microsatellite pairs ranged from 0.522 to 14.1, denoting the correct assignment of offspring to their fathers. Moreover, the combined non-exclusion probability for a parent pair approached zero (0.0000017), providing a reliable method for correctly matching offspring with their sires and dams. However, a mismatch between 10 young calves and their candidate sires occurred during this study, likely due to mutations or null alleles in the LCA56 locus. In addition, seven offspring failed at the same loci as their corresponding candidate dams.

Discussion

DNA profiling facilitates the individual identification, parentage testing, and verification of genetic relationships among animals, helping breeders achieve their breeding goals. The parentage testing of racing camels can, therefore, enhance the efficiency of a selective breeding program.

As measures of polymorphisms, the mean number of alleles and the H_e, and PIC values were extensively studied. In the current study, which examined the Qatari racing camel population, the mean number of alleles (8.13) was comparatively higher than those reported for Omani (5.4), Pakistani (3.9) and Majaheim (7.3) dromedaries, similar to the results previously reported by Hashim *et al* (2014) for a small population containing different subtypes of Qatari camels. The mean H_o value for the Qatari dromedary population (0.555) was higher than those for Australian (0.45) (Spencer and Woolnough, 2010) and Tunisian (0.46) (Ahmed *et al,* 2010) dromedaries, and lower than those reported for Saudi Arabian (0.66) (Mahmoud *et al,* 2012) and Iranian (0.74) dromedaries (Hedayat-Evrigh *et al,* 2018) and Bactrian camels (0.676) (Ming *et al,* 2019).

The estimated mean H_e (0.562) value for the current population was lower than those reported for Sudanese (0.68) and South African camels (0.608) (Nolte *et al,* 2005). However, Kenyan (0.538) and Australian (0.544) dromedaries, studied by Spencer and Woolnough (2010), were found to have similar results. The average PIC value across the 15 loci was nearly equivalent to that reported for dromedary racing camels among 17 microsatellite loci, which was investigated by Spencer *et al* (2010).

In contrast, LCA19, LCA24, LCA77, VOLP59, and YWLL36 showed no allelic variations, similar to the results reported for Indian camel breeds, by Mehta (2014), and for a small population of dromedary

racing camels (Sasse *et al,* 2000). The monomorphic pattern observed for these 5 microsatellite loci may be attributed to the presence of higher degrees of genetic homozygosity among the studied dromedaries in Qatar. These monomorphic alleles behaved differently in Southern African (Nolte *et al,* 2005) and Australian camels (Spencer and Woolnough, 2010), where LCA77 was found to have 15 and 6 alleles, respectively.

To our knowledge, this is the first report examining the genetic indices associated with LCA99 and LGU49 among dromedary camels. The LCA99 locus produced 17 alleles, with a H_e value of 0.852, in Qatari dromedaries, whereas in Alpaca, this locus produced 11 alleles, with sizes ranging from 263-297 bp and a H_e value of 0.75 (La Manna *et al,* 2011). However, for the LGU49 marker, the N_o, H_o and H_e values in Alpaca (Sarno *et al,* 2000) were 9, 0.9 and 0.86, respectively, compared with the values found in the current study for dromedary camels, which were 13, 0.795 and 0.791, respectively. These high polymorphic indices indicated that this locus may serve as an effective marker for parentage verification in dromedary camels.

The number of detectable alleles, the number of efficient alleles, the heterozygosity and the PIC not only affect the paternity confirmation system but also affect the exclusion probability. The CPE values for our set of 15 microsatellite loci, in cases where one putative parent was excluded (0.9996), were higher than those reported for Emirati (0.9961), Australian (0.9962) and African (0.9975) dromedaries using 17 microsatellite loci, as reported by Spencer *et al* (2010). The cumulative probability for reported in that study for excluding a parent pair (0.99999) was similar to that reported by Spencer *et al* (2010).

Moreover, the 15 examined polymorphic loci conformed with *HWE* expectations, except for LCA56 (Table 3), which may be due to genotyping errors and reduced heterozygosity. The frequency of null alleles was relatively low among all loci, except for LCA56. Hence, LCA56 should be excluded from parentage verification in closely related, small populations, as suggested by Marshall *et al* (1998).

The H_o, H_e, and PIC values are inversely affected by allele frequencies larger than 0.5, according to Marshall *et al* (1998). Although YWLL44 and VOLP3 each showed one allele with a frequency exceeding 0.5, they still exhibited high degrees of polymorphism. The CPE1 and CPE2 values decreased by approximately 0.8% and 0.2%, respectively, when these two markers were excluded from the panel.

Recently, Ming *et al* (2019) identify a set of 14 polymorphic microsatellites in Bactrian camels, with an exclusion probability of 0.9999. In addition, Nouaïria *et al* (2018) stated that 12 microsatellite loci were necessary to achieve maximum exclusion in dromedary camels; however, this study found that fewer loci can provide a relatively high exclusion power. Six markers, LCA08, LCA65, YWLL29, LCA37, LCA56, and VOLP32, can easily be excluded from parentage testing, without significant reductions in exclusion power. High numbers of alleles and PIC values were observed for 9 loci, LCA66, LCA99, LGU49, VOLP3, YWLL08, YWLL44, CVRL01, CVRL04, and CVRL05, indicating that these were the most informative markers among the tested loci in this study. The use of an increasing number of loci consequently increased PE values and decreased probability of identity (PI) values. The maximum value obtained when using all 15 STRs, thus, represents an effective tool for confirming lineage.

In summary, the LCA56 locus should be interpreted with caution and should be analysed in future studies among different populations to determine whether relationships exist between this locus and any apparent traits or whether this locus is especially prone to genotyping errors. Due to the high PIC and allele frequency distribution values observed, the use of 9 loci (LCA66, LCA99, LGU49, YWLL08, YWLL44, VOLP3, CVRL01, CVRL04, and CVRL05) out of 20 markers resulted in a high degree of precision for individual identification and paternity assignment among dromedary camels within the studied population. All offspring from the 27 sire families were successfully assigned to the correct sires, dams, and parent pairs. For closely related families, and in cases where one putative parent has been excluded, increasing the number of microsatellite loci to more than the 20 ISAG-recommended loci may be necessary to increase the reliability of parentage assignment. The microsatellite analysis described in the present paper can be an efficient tool for constructing accurate breeding programs and determining the genetic merit of camel populations.

Acknowledgements

I would like to express my special thanks to Tharb camel hospital, in Leawina, Qatar, for providing financial support for this study. I would also like to thank Dr. Samantha A. Brooks, Assistant Professor of Equine Physiology, Department of Animal Sciences, University of Florida, and her

student, Catherine Roques, for their valuable manuscript revisions.

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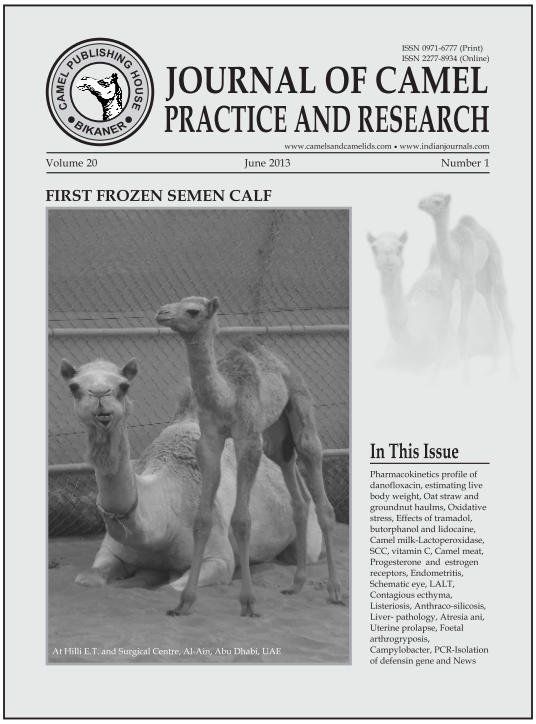

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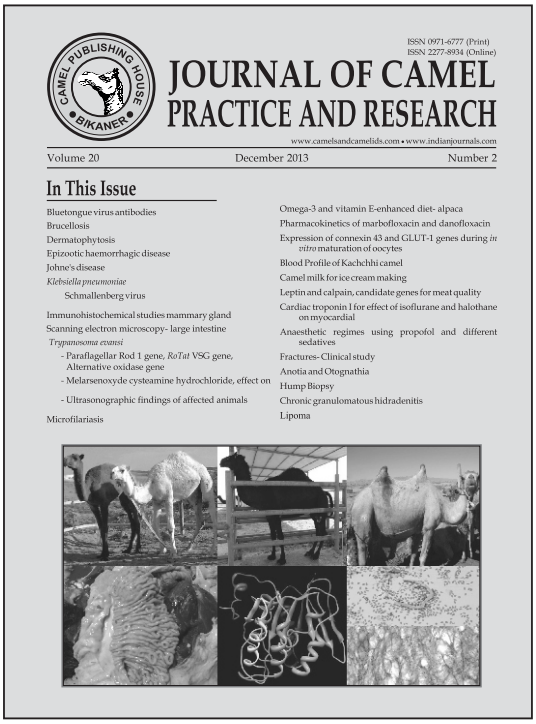

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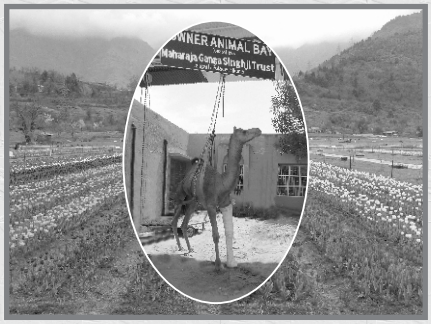

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DOI : 10.5958/2277-8934.2020.00006.5 *Vol 27 No 1, p 39-47*

SEASONAL STUDIES ON MORPHOLOGY AND IMMUNOHISTOCHEMICAL LOCALISATION OF S-100 AND ALPHA SMOOTH MUSCLE ACTIN PROTEINS IN POLL GLANDS OF DROMEDARY CAMEL

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ABSTRACT

The two poll glands rest subcutaneously behind the ear of the dromedary camel (*Camelus dromedarius*). Although they secrete a watery yellowish secretion with offensive odour during breeding seasons, their function is still largely unspecified. This study on camel poll glands morphology and immunoreactivity of S-100 and alpha smooth muscle actin (α-SMA) proteins during breeding and non-breeding seasons might shed some light on their function. During breeding season the gland was larger in size and darker in colour and its secretion increased compared to non-breeding season. No significant change was observed in the alveolar luminal diameter during seasonal reproductive cycle ($P>0.05$). However, alveolar diameter and epithelial height was significantly ($P<0.05$) increased during breeding season together with significantly increased inter-alveolar tissue thickness during non-breeding season. While S-100 reacted positively in the alveolar epithelium and inter-alveolar tissue, α-SMA reaction was negative in the alveolar epithelium and positive in the alveolar myoepithelial cells, smooth muscles and blood vessels. S-100 and α-SMA positive immunoreactivity increased during breeding season compared to non-breeding season. These results suggest that the poll gland secretory activity is correlated with male camel seasonal sexual activity. Moreover, S-100 and α-SMA are suggested to regulate cellular and muscular functions in the poll glands.

Key words: Anatomy, camel, histology, immunohistochemistry, poll gland, reproductive activity

The poll glands are symmetrical subcutaneous exocrine glands of the male dromedary camel which are located behind the ears in the neck region (Leese, 1927; Singh and Bharadwaj, 1978; Tingari and George, 1984; Ebada *et al,* 2012). The gland which has been considered as a tubule-alveolar modified apocrine sweat gland (Singh and Bharadwaj, 1978; Manivannan *et al,* 1996) is histologically similar to the mammary gland (Purohit and Singh, 1958). It is known to undergo cyclic activity, yielding a yellowish watery secretion with offensive odour, especially during the breeding season (Singh and Bharadwaj, 1978; Taha and Abdalla, 1980; Yagil and Etzion, 1980). The poll gland secretion has also been described as very copious with strong foetid smell which becomes black when exposed to the air and it dribbles down the neck of the rutting camel (Skidmore, 2004). The poll gland morphology and histochemistry have earlier been described (Purohit and Singh, 1958; Singh and Bharadwaj, 1978; Tingari and George, 1984; Tingari *et al,* 1984b; Manivannan *et al,* 1996; Atoji *et al,* 1998).

S-100 and alpha smooth muscle actin (α-SMA) are proteins suggested to affect the cellular absorption, cellular secretion and muscular contractile activities (Alkafafy *et al,* 2011b). S-100 is a calcium-binding protein which has earlier been considered specific to the nervous system as it was initially detected in brain glial cells and Schwan's cells of peripheral nerves (Bock, 1978). Later, S-100 has also been detected in other parts of mammalian body including the reproductive system (Amselgruber *et al,* 1992; Alkafafi *et al,* 2011b; Ibrahim, 2015; Ibrahim *et al,* 2017). Functionally, S-100 has many intracellular and extra cellular activities including modulation of enzymatic activity, motility and energy metabolism and cellular secretion (Heizmann *et al,* 2002). α-SMA, which has been used as a means for differentiation of normal and pathological smooth muscle cells, is mainly found in cells performing contractile functions (Skalli *et al,* 1989). In the camel epididymis α-SMA was mainly seen in the epididymal peri-tubular muscles and connective tissue as well as in the smooth

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muscles of epididymal blood vessels (Ibrahim *et al,* 2017; Abd-Elmaksoud, 2009). Immunoreactivity of S-100 and α-SMA has earlier been described in the testis and epididymis of camel and other mammalian species (Amselgruber *et al,* 1994; Abd-Elmaksoud, 2005; Alkafafy *et al,* 2011b; Alkafafy and Sinowatz, 2012; Alkafafy *et al,* 2016). Some seasonal immunohistochemical studies have been conducted on the efferent ductules (Ibrahim, 2015), testis (Alkafafy *et al,* 2016) and epididymis (Ibrahim *et al,* 2017) of the camel. According to Ibrahim *et al* (2017) S-100 and α-SMA show distinct variations in relation to reproductive activity in dromedary camel epididymis and they might change the structural and physiological states of this organ.

However, detailed immunohistochemical studies on the camel poll gland during the rutting and non-rutting seasons are scarce in the available literature. This study, therefore, was aimed to investigate the immunoreactivities of S-100 and α-SMA in the dromedary camel poll gland during breeding and non-breeding seasons. Complementary morphological investigation was also included.

Materials and Methods

Animals and tissue

Thirty adult and healthy dromedary camels (*Camelus dromedarius*) slaughtered at Buraidah Central Slaughterhouse, AL-Qassim, Saudi Arabia, were used in this study. Specimens from 15 animals during breeding season (between December and February) and from 15 animals during non-breeding season (between May to July) were used in this study. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Qassim University, Saudi Arabia.

Anatomical study

For gross anatomical study three adult camel heads during both seasons were carefully dissected and the poll glands were exposed, grossly studied and their dimensions were measured.

Microscopic study

For histological, morphometric and immuno-histochemical studies glandular samples from 12 animals during each season were quickly collected after animal slaughter and fixed in 10% buffered formalin; the samples were then dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax as described by Culling (1974). Paraffin sections (5µm thick) from each animal group were cut using a rotary microtome.

Histology

For histological observations the paraffin sections were conventionally stained with Haematoxylin and Eosin (H&E) following the procedure of Culling 35. Microscopic examination was carried out using Leica microscope (Leica DMD108-Germany).

Morphometry

Five H&E-stained sections of poll glands from each animal group were randomly chosen for measurements of alveolar diameter, epithelial height, luminal diameter and inter-alveolar connective tissue thickness. The measurements were carried out using Leica provided with Leica microscope, digital camera and measuring software (DMD108-Germany). The study only included regular transverse alveolar sections. The obtained morphometric data (expressed as means ± standard deviation) were analysed using Students T-test. $P< 0.05$ was considered as statistically significant.

Immunohistochemistry

Paraffin sections from each animal group were de-waxed using two changes of xylene and rehydrated in decreasing concentrations of ethyl alcohol. The sections were then washed in distilled water and phosphate buffer saline (PBS). Antigen unmasking/retrieval was performed using microwave heating (750 W) in 0.01 M sodium citrate buffer (pH 6.0) for 20 min. Immunohistochemical staining was carried out using Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (ab64264, Abcam, Cambridge, UK), as described by the manufacturer's instructions. Sections from both animal groups were incubated with rabbit polyclonal anti-S100 primary antibody (ab868, Abcam, Cambridge, UK), for overnight at 4°C and with rabbit polyclonal anti- α-SMA primary antibody (ab5694, Abcam, Cambridge, UK), for 3hr at room temperature. The dilutions for both anti-S100 (1:100) and anti- α-SMA (1:5000) primary antibodies were performed using a universal diluent (ab79995, Abcam, Cambridge, UK). Anti-S100 primary antibody cross-reacts with S100-equivalent protein in mouse, rat, cow, human and pig and anti- α-SMA primary antibody cross-reacts with α-SMA-equivalent proteins in rat, chicken, guinea pig, cow, dog, human and pig as stated by the manufactures. Negative control immunostaining was carried out by omitting the primary antibody. On the other hand, positive control immunoreactivity was performed according to the manufacturer's instruction for each primary antibody kit.

Results

Gross anatomy

The two poll glands rested dorsally on either side of the neck region, about 7 cm behind the occipital crest; a watery yellowish secretion with offensive odour exuded through the skin covering the gland, especially during the breeding season (Fig 1). The secretion colour changed to dark brown or black after being exposed to air. The gland was lobulated and its length ranged between 8-10 cm during rutting season and 5-8 cm during non-rutting season. Compared to non-breeding season, the glandular lobes were larger and many of them were darker in colour during breeding season, as they were filled with the glandular secretion.

Histology

The histological structure of the camel poll gland during breeding and non-breeding seasons is shown in Fig 2. The gland was surrounded by a connective tissue capsule that sent connective tissue septa which divided the gland into lobes and lobules consisted mainly of collagenous fibres, blood vessels and interlobular ducts (Fig 2a).

During breeding season (Fig 2a, b), the glandular lobules were occupied by large alveoli which were characterised by tall simple cuboidal epithelium and narrow lumina; the epithelium exhibited apical protruding blebs and it was surrounded by myoepithelial cells with dark nuclei followed by scanty inter-alveolar connective tissue. Intra-lobular ducts with tall simple cuboidal or columnar epithelium were also found in the lobules.

During non-breeding season the alveoli and intra-lobular ducts appeared smaller and their epithelium changed to simple squamous or low cuboidal, which resulted in increased luminal diameter (Fig 2c, d); the interlobular and inter-alveolar connective tissue was increased in comparison to that in breeding season.

Morphometry

The analysis of morphometric parameters of the camel poll gland during breeding and non-breeding seasons is shown in table 1.

During the breeding season, the alveolar diameter and epithelial height were significantly increased in comparison with non-breeding season ($P<0.05$). On the other hand, significantly increased inter-alveolar tissue thickness was observed during non-breeding season as compared to breeding season ($P<0.05$). However, the luminal diameter did not show any significant change during seasonal reproductive cycle ($P>0.05$).

Table 1. Seasonal morphometric measurements (µm) of the camel poll gland structures.

Parameters	Breeding season	Non-breeding season
Alveolar diameter	$64.45\pm4.04_a$	$52.34\pm6.83_b$
Epithelial height	$14.63\pm0.93_a$	$9.50\pm1.55_b$
Luminar diameter	$44.75\pm1.87_a$	$38.46\pm2.81_a$
Inter-alveolar tissue thickness	$13.80\pm1.58_a$	$32.37\pm2.78_b$

Values (mean ± SD, N = 12).
Values within the same row with different subscripts are significantly different ($P<0.05$)

Immunohistochemistry

Seasonal variations in the immunoreactivity of S-100 and α-SMA during breeding and non-breeding seasons are shown in Table 2 and Fig 3 and 4.

S-100

In the breeding season the glandular alveoli and glandular ducts exhibited numerous epithelial cells with intense S-100 immunostaining, especially in their supra-nuclear region and apical protruding blebs (Fig 3a, b). In the glandular blood vessels the S-100 immunoreaction was intense in the endothelium and moderate in the tunica media. The inter-alveolar connective tissue and myoepithelial cells appeared with weak S-100 reaction.

During non-reproductive season, decreased intensity of S-100 reaction was observed in the alveolar epithelium, ductal epithelial and blood vessel endothelium (Fig 3c). In the glandular connective tissue, blood vessel tunica media and myoepithelial cells the reaction intensity was negative to weak.

Negative control immunostaining showed negative reaction in the different parts of the poll gland during breeding and non-breeding seasons (Fig 3d).

α-SMA

During both seasons there was positive α-SMA immunoreactivity in the myoepithelial cells, peri-alveolar smooth muscles and tunica media of inter-alveolar and interlobular blood vessels. The reaction was stronger during breading season, especially in the myoepithelial cells and smooth muscles of blood vessel (Fig 4a), than that in non-breeding season (Fig 4b). However, the epithelial cells, inter-alveolar connective tissue and inter-lobular connective

Table 2. Seasonal immunohistochemical reactions of S-100 and α-SMA in the camel poll gland structures.

Protein	Season	AE	DE	MECs	IASM	CT	VE	VSM
S-100	Breeding	+++	+++	++	+	+	+++	++
	Non-breeding	++	++	+/-	+/-	+/-	++	+/-
α-SMA	Breeding	-	-	++++	++	++	+++	++++
	Non-breeding	-	-	++	+	-	+	+

Alveolar Epithelium (AE); Ductal Epithelium (DE); Myoepithelial Cells (MECs); Inter-alveolar Smooth Muscles (IASM); Connective Tissue (CT); Vascular Endothelium (VE); Vascular Smooth Muscles (VSM).Very strong (++++); strong (+++), moderate (++); weak to moderate (+/++); weak (+), weak to negative (+/-) and Negative (−) reaction.

tissue exhibited negative α-SMA immunoreactions throughout the entire reproductive cycle of the camel.

Negative control immunostaining produced negative reaction for α-SMA antibody during breeding and non-breeding seasons (Fig 4c).

Discussion

The present study showed that the paired poll gland was located dorsal to the poll (neck) region of the male dromedary camel where a yellowish watery secretion with offensive odour was observed during the breeding season. This is in line with earlier findings of a number of authors (Leese, 1927; Singh and Bharadwaj, 1978; Taha and Abdalla, 1980; Yagil and Etzion, 1980; Tingari *et al,* 1984b; Ebada *et al,* 2012). According to Yagil and Etzion (1980) the presence of androgens has been proved in the secretions of the poll glands of the rutting dromedary camel. The poll gland secretion has also been suggested to contain some types of pheromones that might be used to border a mating area for the male in the herd (Skidmore, 2004). The gross anatomical change of poll glands in this study showed darker glandular colour and increased glandular size in the breeding season. It has been mentioned that seasonal structural variations observed in the poll glands indicate their increased activity during December-March (Singh and Bharadwaj, 1978). These seasonal structural changes were considered as testosterone-dependent (Aguilera-Merlo *et al,* 2005).

The present results revealed seasonal histological and morphometric variations in the poll gland. During the breeding season the glandular alveoli showed narrow lumina and simple cuboidal or columnsar epithelium surrounded by a thin inter-alveolar connective tissue. During non-breeding season the alveoli and intra-lobular ducts were smaller and their epithelium changed to simple squamous or low cuboidal, which resulted in relatively increased luminal diameter and thicker inter-alveolar connective tissue in comparison to those in breeding season. Similar findings have also been reported by Tingari *et al* (1984b)

The histological findings in this study were confirmed by morphometric measurements which showed significantly increased alveolar diameter and epithelial height during breeding season than those in non-breeding season. Also in the breeding season, the inter-alveolar tissue thickness was significantly decreased. On the other hand, the luminal diameter did not show significant change in relation to seasonal reproductive activity. This is in accordance with records by Tingari *et al* (1984b). It has been suggested that increased poll gland activity could be indicated by higher alveolar epithelial lining and greatly reduced inter-alveolar connective tissue during December -March in winter as compared to the period between April and August in summer (Singh and Bharadwaj, 1978). Moreover, it has been noted that during peak reproductive activity in November

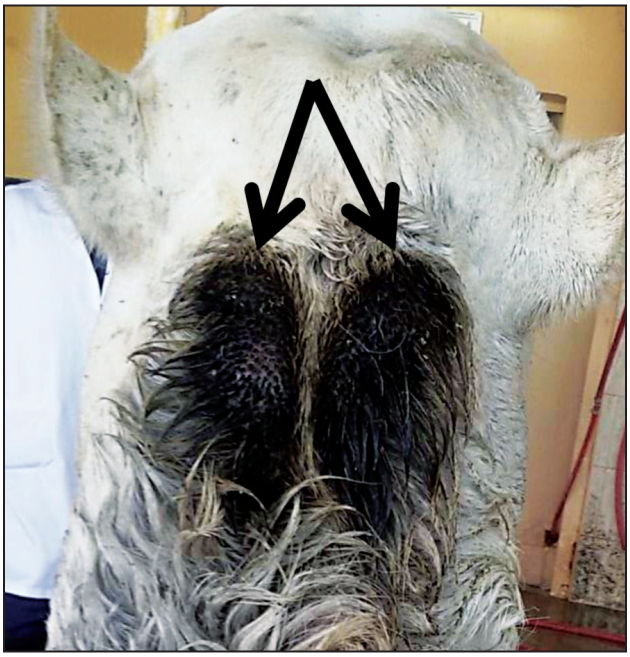

Fig 1. Dark brown secretion of the paired poll gland exuding through the skin of the rutting dromedary camel (Arrows).

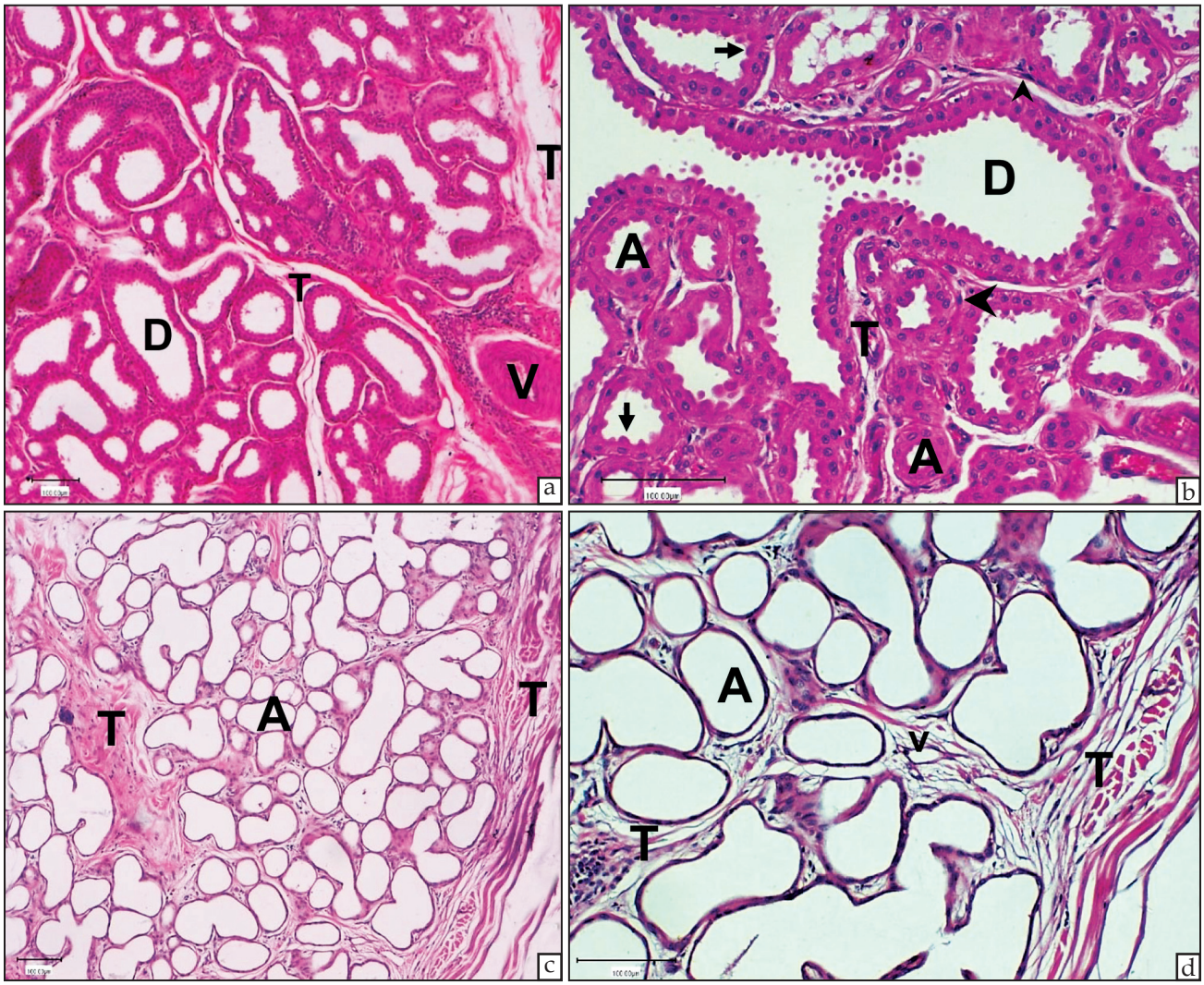

Fig 2. H & E-stained sections of camel poll gland. Fig a, b: show the glandular alveoli (A) in breeding season with apical blebs (Arrows) and myoepithelial cells (Arrowheads); note the high alveolar epithelium, narrow lumina and thin inter-alveolar and inter-lobular connective tissue (T); note the intra- and inter-lobular ducts (D) and blood vessels (V). Fig 2c,d show the glandular alveoli (A) in non-breeding season with low alveolar epithelium, wide lumina, and thick inter-tubular connective tissue (T). Scale bars: 100 µm.

and December, the poll gland lobules are separated by thin strands of connective tissue and the alveoli and intra-lobular ducts are taller and lumina are narrower than those during non-reproductive period (Tingari *et al,* 1984b). However, it has been stated that no considerably decrease was found in the connective tissue of active poll glands and the main structural difference between the active and inactive poll gland resides in the epithelial heights of their secretory units (Taha and Abdalla, 1980). Further, the poll gland secretory phase is characterised by narrow lumina and tall epithelial cells of alveoli and intra-lobular ducts (Tingari *et al,* 1984b).

The present study showed positive immunoreactivity of S-100 in the epithelial lining and connective tissue of the poll gland. A number of studies indicated S-100 localisation in the mammalian testis (Haimoto *et al,* 1987; Amselgruber *et al,* 1992; Amselgruber *et al,* 1994; Cruzana *et al,* 2003; Alkafafy *et al,* 2016), epididymis (Ibrahim *et al,* 2017; Cruzana *et al,* 2003) and efferent ductules (Ibrahim, 2015). The intensity of S-100 reaction in the current study increased during breeding season, especially in supra-nuclear region and apical protruding blebs of the alveolar epithelium. More obvious S-100 binding sites in the supra-nuclear region and apical blebs have also been reported during the rutting season in camel poll gland (Ebada *et al,* 2012). S-100 reaction in this study was also increased in the myoepithelial cells, glandular stroma and blood vessels during breeding

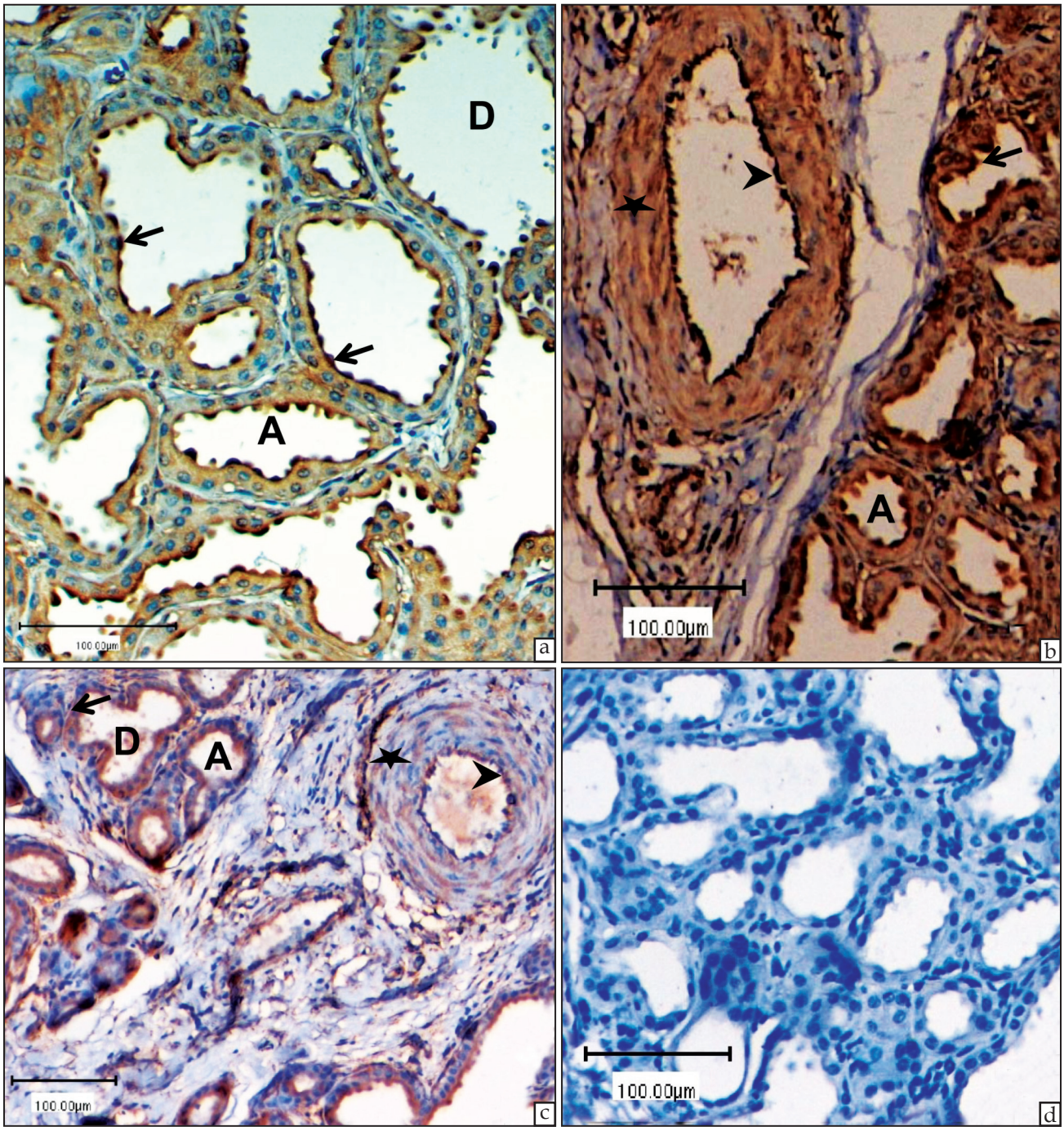

Fig 3. S-100 immuno-stained sections of camel poll gland. In breeding season (Fig 3a, b) there is strong S-100 immuno-reaction in the apical epithelium of alveoli (A) and ducts (D) (Arrows) as well as blood vessel endothelium (Arrowheads); note the blood vessels tunica media (Stars) with moderate S-100 immuno-reaction. In non-breeding season (Fig 3c) S-100 immuno-reaction shows less intense in the alveolar (A) and ductal (D) epithelium; weak to negative reaction is shown in blood vessels tunics media (Stars). Fig 3d represents the negative S-100 reaction in the control sections from breeding and non-breeding seasons. (Scale bars: 100 µm).

season than in non-breeding season. According to Ebada *et al* (2012) variable S-100 immune-reaction was observed in the glandular myoepithelial cells and peri-alveolar connective tissue, whereas weak to moderate reaction appeared in the inter-alveolar connective tissue in camel poll glands. Similarly, Kahn *et al* (1985) recorded positive S-100 immunoreaction in the myoepithelial cells of normal salivary glands using immunoperoxidase and immunofluorescence methods. Moreover, a positive reactivity of S-100

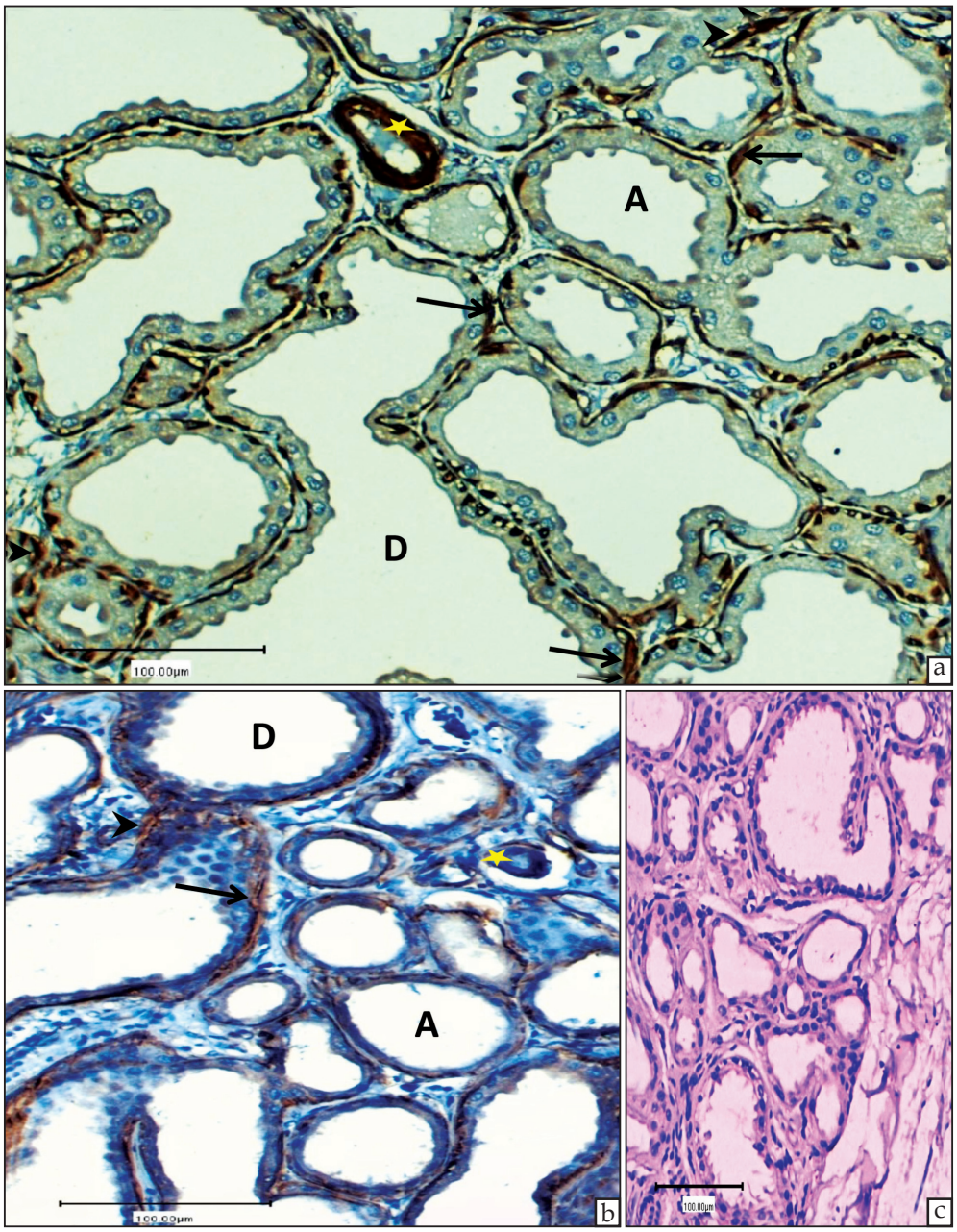

Fig 4. α-SMA immuno-stained sections of camel poll gland. In breeding season (Fig 4a) the glandular myoepithelial cells (Arrows), inter-alveolar smooth muscles (Arrowheads) and tunica media of blood vessels (Stars) show strong α-SMA immuno-reaction than those in non-breeding seasons (Fig 4b). The connective tissue (T) and epithelium of alveoli (A) and ducts (D) show negative α-SMA in both seasons. Negative control sections do not react with α-SMA in both seasons Fig 4c). (Scale bars: 100 µm).

in myoepithelial cells has also been expressed in canine sweat glands. According to Heizmann *et al* (2002) S-100 is a multifunctional protein with many intracellular and extracellular functions including motility, enzymatic activity, energy metabolism, chemotaxis, neurite extension and secretion. It has also been mentioned that S-100 protein might play a role in the absorptive and secretory activities in the testis (Amselgruber *et al,* 1994; Cruzana *et al,* 2003). Moreover, S-100 protein is considered to improve the secretory and absorptive functions in the epithelial cells and to improve smooth muscle contractility in the epididymis of rutting camels (Ibrahim *et al,* 2017). Similarly, increased S-100 immunoreaction in the poll gland epithelial cells in the breeding season observed in this study might indicate increased secretory and absorptive functions. Furthermore, increased intensity of S-100 protein immunoreactions

in the glandular myoepithelial cells and tunica media of blood vessels during breeding season recorded in this study could also indicate their increased muscular contractility. Additionally, it has been stated that positive immunoreactivity to S-100 protein in the epididymal vascular endothelium of Egyptian water buffalo indicates its contribution to the processes of transcytosis (Alkafafy *et al,* 2011b). Therefore, it could also be suggested that the transcytosis process is increased in the poll glands during breeding season as there was increased S-100 immunoreactivity in their vascular tissue.

In the present study, there was positive α-SMA immunoreaction in the myoepithelial cells and tunica media of inter-alveolar and interlobular blood vessels of poll glands. The reaction was stronger in the breeding season than in non-breeding season, especially in the myoepithelial cells. The study, however, showed negative α-SMA immunoreactivity in the epithelial cells and weak to negative reaction in the inter-alveolar connective tissue throughout the entire annual reproductive cycle. Positive α-SMA reaction in the myoepithelial cells and inter-alveolar blood vessels of camel poll glands has also been stated earlier (Ebada *et al,* 2012). Most myoepithelial cells of camel poll glands have been reported to exhibit positive α-SMA reaction (Atoji *et al*, 1998). Localisation of α-SMA has also been reported in myoepithelial cells of mammary gland of bovine (Haaksma *et al,* 2011) and human (Zancanaro *et al,* 1999) as well as in rat sweat gland (Gugliotta *et al,*1988) and apocrine tubules of Japanese serow (Atoji *et al,*1995).

According to Sato *et al* (1989), myoepithelial cells are proved to be involved in cellular contraction which results in rapid sweat excretion on stimulation of sweat glands; they added that increased luminal hydrostatic pressure in the walls of sweat glands secretory units can be relieved by the mechanical support of myoepithelial cells. These authors report that myoepithelial cells are capable of producing both nitric oxide (probably induces relaxation) and acetylcholine which induces contraction. Therefore, increased intensity of immunoreactivity of myoepithelial cells to both S-100 and α-SMA during breeding season could be an indication of increased cellular activity and contractility. They might also play a role in conduction of cellular secretion from the secretory units of poll glands to the lumen and then to the outer surface via the hair follicles. Increased contractility during breeding season has also been reported in the smooth muscle of camel epididymis (Ibrahim and Singh, 2014) and efferent ductules (Ibrahim, 2015).

Based on the morphological and immunohistochemical variations, it could be concluded that the camel poll glands are active throughout the year, though their activity increases during cold months of winter and decreases in hot months of summer; this indicates a correlation with the glandular secretion and the male camel sexual activity. Further, the S-100 and α-SMA proteins are suggested to be involved in the control of secretory activities of the poll gland.

Acknowledgements

The authors gratefully acknowledge Qassim University, represented by the Deanship of Scientific Research, on the material support for this research under the number (3359).

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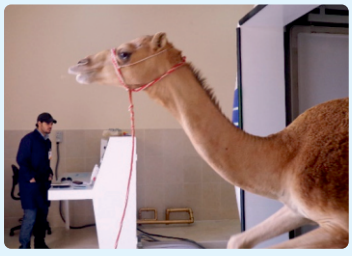
INTERNATIONAL CAMEL ORGANIZATION (ICO)

International
Camel Organization

The International Camel International is an international, non-governmental, non-profit organization. The activities of the International Camel Organization are focused on developing the culture of camel breeding through achieving the mission, principles and goals of the global camel culture and the types of nomad activities of camels and Chaired by Mr. Fahad F. Bin Hithleen.



GOALS AND FUNCTIONS OF THE ICO

- Developing the dynamics of the global camel culture, in all its forms, types, and everything related to human and camel interaction.

- Promoting mutual understanding and friendships between nations, peoples and establish societies of nature and animal lovers through various practices with camels.

- Developing and managing a code of Ethics as a set of ethical, cognitive principles, technical and fundamental skills for camel culture.

- The organization would hold and organize the International Festival of the International Camel Organization.

- Promoting and encouraging the establishment of national and regional member organizations in the International Camel Organization and organize training workshops for members of these organizations.

- Promoting research aimed at the scientific and educational development of camel culture and provide advice and opinion on everything related to camel affairs.

DOI : 10.5958/2277-8934.2020.00007.7 *Vol 27 No 1, p 49-54*

INVESTIGATION OF ALTITUDE EFFECT ON SOME PHYSIOCHEMICAL PROPERTIES OF MILK SAMPLES OBTAINED FROM CAMELS AND SMALL RUMINANTS

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ABSTRACT

This article investigated the effect of altitude on milk of camel, goat and sheep with regard to their laser induced fluorescence spectra, pH, conductivity, moisture, Total Dissolved Solids (TDS), specific gravity and ash percentage. The altitude significantly affected the specific gravity and the ash percentage of all the studied milk's samples while it with the altitude significantly affected the pH and conductivity of the camel and goat milk samples. The high altitude sheep and goat milk samples were characterised by the highest fluorescence intensity compared to their low altitude milk while the low altitude camel milk had the highest fluorescence intensity compared to the high altitude camel milk. The laser induced fluorescence (LIF) technique was very useful in differentiating between the altitude and animal source of the studied milk samples.

Key words: Altitude effect, ash, camel, conductivity, laser induced fluorescence, milk

The camel milk is composed of approximately 79% water, 11.9% total solids, 4.4% lactose, 3.5% fats, 3.1% proteins and 0.79% ash (Khaskheli *et al*, 2005; Al haj and Al Kanhal, 2010). Compared to the other animal milk, the camel milk is characterised by low sugars and cholesterol and high minerals and vitamins (Zibaee, 2015).

Goat milk has a creamy texture and is characterised by the highest buffering capacity, viscosity, surface tension and specific gravity. It has high concentration of vitamins and minerals. It is reported that the goat milk has an anti-inflammatory properties and it increases nutrients uptake, decreases blood cholesterol and boost immune system activity (Park, 2006; Lopez-Aliaga *et al,* 2005; McCullough, 2003).

Similar to the goat milk, the sheep milk has creamy texture, sweet and distinctive flavour due to its high concentration of fatty acids (Jooyandeh and Aberoumand, 2010). The sheep milk is characterised by its high content of proteins, minerals and fatty acids compared to the other mild samples. Also, the sheep milk has the lowest moisture and ash within the other types of natural milk. Worldwide, it is widely used for the production of fine and highly nutrient cheese and yoghurt (Balthazar *et al*, 2017).

Several Factors are well known to affect the physiochemical properties of milk including the milking animal and its age, genetic and environmental factors, type of nutrients, level of production, stage of lactation, season and presence of diseases (National Research Council (US), 1988). Recently, the altitude was reported to affect the physiochemical properties of milk (Quinn *et al,* 2016; Leiber *et al,* 2005).

Laser Induced Fluorescence (LIF) technique is widely used in different fields of research and industry including investigation of milk and milk products quality (Hui *et al,* 2018; Andrei *et al,* 2014; Marques *et al,* 2018; Abdel-Salam *et al,* 2015; Abdel-Salam *et al,* 2017). The best known fluorescent compounds in milk and milk products are the aromatic amino acids, riboflavin, vitamin A and nucleic acids (Sádecká and Tóthová, 2007). This article investigated the effect of milking animal and altitude on the physiochemical properties of milk of camel and small ruminants.

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Materials and Methods

Milk samples

Eighteen milk samples from camels and small ruminants, i.e. sheep and goats were collected from high (2400 metre above sea level) and low (20 metres above sea level) altitudes. Nine samples were collected from each altitude (3 each). The milk samples were analysed immediately or within one week from the sample collection and were stored at -20°C. The various physiochemical properties of milk sampled and their estimation methods are given below—

Milk pH

pH of each milk sample (20 ml) was determined using HANA pH meter (HI 8314 HANNA, Italy) after it was calibrated by two buffers with pH 4 and 7.

Milk conductivity (mS/cm)

The conductivity of the milk samples (20 ml) was determined by a calibrated Metrohm conductometer (712 conductometer, Switzerland).

Milk moisture (%)

The milk moisture was determined using the oven. The 5 gm milk sample was weighed (B) and was heated for 1 hour at 70°C and then for 6 hours at 105°C (Bradley, 2017). After heating, the milk was weighed (A) and the moisture percentage was calculated as follows:

$$Moisture\% = \left(\frac{B\text{-}A}{B}\right) \times 100$$

Total Dissolved Solids (%)

The TDS of the milk samples was measured by heating 5grams (B) of milk for one hour at 70°C and for six hours at 120°C (Bradley, 2017). The milk residue was weighed (A) and the TDS was calculated using the equation given below:

$$TDS\% = \left(\frac{A}{B}\right) \times 100$$

Specific gravity

The specific gravity of the milk is the ratio of its density to the density of the water. A 50 ml specific gravity determination bottle was used and the weight of the 50 ml milk was divided by the volume.

Ash percentage

The sample of moisture determination was heated to 600°C in a furnace oven and the weight of the ash was divided by the weight of the milk sample and multiplied by 100 to obtain the ash percentage (Marshall, 2017).

Laser Induced Fluorescence Technique

The laser excitation source of this experiment was a diode laser (Pro100 -Toptica Photonics Inc.). It provides a maximum output average power of 29 mW at wavelength 398 nm. A laser control unit was used to control the output power of the laser beam through changing the current and temperature of the diode. The output average power of this experiment was 2 mW. Each milk sample was put in a cuvette and the laser beam was focused to 2 mm onto one side of the cuvette. A lens that focuses the radiation and sends it to monochromator (ScienceTech 9055, ScienceTech lnc. Canada) was used to collect the milk emitted fluorescence. The excitation laser was blocked by a long pass filter (Thorlabs Inc. USA) that permits the emitted fluorescence radiation to pass only. The sample holder and the monochromator were arranged perpendicular to each other. The monochromator slit was opened at a width of 0.2 mm. This arrangement offers a spectral resolution of 0.2 nm. The monochrome analyses the fluorescence signal and permits it to exit through another slit to fall on a photomultiplier tube (PMT). The PMT converts the light signal to a voltage. The signal passes to the data acquisition unit connected to a computer to read and draw the signal as a wavelength change.

Statistical analysis

The ANOVA test of the SPSS statistical programme was used for the analysis of the results. The difference between the means of the parameters was considered significant if the p- value was ≤ 0.05.

Results and Discussion

General

The camel milk had the highest moisture and conductivity while the sheep milk had the highest TDS percentage and the specific gravity irrespective of the altitude. The low altitude goat milk was characterised by the highest ash percentage compared to the low altitude camel and sheep milk. The highest pH value of the low altitude milk was reported for the sheep milk. The altitude significantly affected the specific gravity and the ash percentage of all the milk, while it nonsignificantly affected the moisture and TDS percentages. The altitude significantly affected the pH and conductivity of the camel's and goat's milk (Table 1).

Table 1. Mean ± SD values of the studied parameters in the low and high altitude milk and their variation significance.

Parameters		Sheep milk	Goat milk	Camel milk
pH	High altitude	6.54 ± 0.1	6.53 ± 0.7	6.64± 0.07
	Low altitude	6.46 ± 0.002	6.37 ± 0.009	6.27± 0.04
p- value		0.23	0.031	< 0.001
Conductivity mS/cm	High altitude	4.54 ± 0.5	5.56 ± 0.14	5.97±0.8
	Low altitude	4.55 ± 0.00	7.42 ± 0.28	8.25± 0.49
p- value		0.98	0.003	0.001
Moisture%	High altitude	73.5 ± 7.9	84.3 ± 4.4	88.1±2.7
	Low altitude	74.2 ± 0.00	81.5 ± 0.14	88.75± 1.06
p- value		0.88	0.51	0.89
Ash%	High altitude	4.6 ± 0.9	3.5 ± 0.6	0.12 2.6±
	Low altitude	0.51 ± 0.001	0.58 ± 0.15	0.32± 0.06
p- value		<0.001	<0.001	0.001
Specific gravity	High altitude	1.04 ± 0.1	1.02 ± 0.05	1.01± 0.02
	Low altitude	1.03 ± 0.00	1.013± 0.004	1.015± 0.007
p- value		0.004	<0.001	0.005
TDS%	High altitude	26.5 ± 7.8	14.1 ± 6.4	12.2± 3.6
	Low altitude	25.8 ± 0.001	18.43 ± 0.25	11.21±1.09
p- value		0.88	0.37	0.829

The milk of camel and goat were more affected by the altitude than the sheep milk.

There was nonsignificant variation between the means of the studied parameters in the milk of camel and goat of the low and high altitudes. All the studied parameters except the pH of the camel and sheep milk were significantly different, irrespective of the altitude. The comparison between the goat and sheep milk showed that there was significant variation between the conductivity (p- value=0.037 and p-value< 0.001), moisture (p- value= 0.017), TDS (p-value= 0.015), specific gravity (p- value< 0.001) and ash percentage (p- value= 0.03).

The altitude may be responsible for the increased fluorescence intensity of the sheep and goats milk, while it decreased the fluorescence intensity of the camel milk (Fig 1).

According to the results of the pH, conductivity, moisture, TDS and ash, there was nonsignificant variation between the goat and camel milk (one class). The laser induced spectra showed that the pattern of flaorescence of the goat and sheep milk were similar (one class) while the camel milk was with different fluorescence pattern. According to animal taxonomy, the goats and sheep belong to one family (Bovidae) while the camel is within another family (Camelidae) (Gentry *et al,* 1999). Comparing the results of the laser induced fluorescence of the different milk samples and the animal taxonomy, it is evident that the LIF can be used as a tool for milk classification rather than the other physiochemical properties besides its ability to differentiate between low and high altitude milk. However, Sun *et al* (2019) concluded that the LIF is a very good technique for the prediction of yogurt quality. LIF was used before to differentiate between low and high altitude Switzerland cheese samples (Karoui *et al,* 2005). It was proved that the LIF is

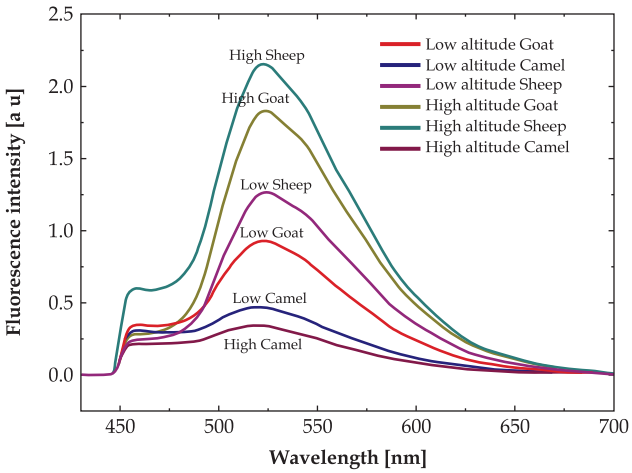

Fig 1. The fluorescence spectra of the studied milk samples. The high altitude sheep and goat milk were with high fluorescence intensity compared to the low altitude ones while the high altitude camel milk exerted low fluorescence intensity compared to the low altitude camel milk.

useful in evaluation of milk heat treatment (Birlouez-Aragon *et al,* 2002).

The results of the present study showed similar pattern of the results as observed by Sabahelkhier *et al* (2012); the camel milk had the highest moisture percentage (88.35%) compared to sheep (80.7%) and goat (88%) milk. Also, the camel milk of Sabahelkhier *et al* (2012) study was characterised with the lowest TDS (11.7%) compared to goat milk (12%) and sheep milk (19.3%). Unlike our findings the study of Sabahelkhier *et al* (2012) found that the camel milk had a higher ash percentage (0.75%) than that of the goat milk (0.7%) and lower than the sheep milk (0.85%). This study unlike results of an Egyptian study which reported that the camel milk had TDS and ash percentages more than goat milk and less than sheep milk (Hayam *et al*, 2017). The values of the studied parameters in the low altitude milk samples are much comparable to the previous studies compared to the results of the high altitude milk samples (Table 2).

Concerning the camel milk, it was characterised by high pH values with a range starting from 6.5 up to 6.7 (Sisay and Awoke, 2015). The moisture of the camel milk was in the range of 87- 90% which was comparable to the finding of this study (Singh *et al*, 2017).

The previous studies showed that the electrical conductivity of the camel milk was around 6.08 millimohs and it was mostly due to its high content of sodium, potassium and chloride ions (Yoganandi *et al*, 2014).

The mean ash percentage of the camel milk in this study was 2.6%. The results of the ash percentage disagreed with the findings of most of the previous studies such as Jilo and Tegegne (2016), Al haj and Al Kanhal (2010) and Singh *et al* (2017) who stated that the ash percentage of camel milk had a range of 0.6 - 0.9% while it was comparable to the results of Yagil (1982) who stated that the camel colostrum milk had ash percentage range of 1.44–2.80% and a mean value of 2.6%. Also, Kavas (2015) found that the mean percentage of camel milk ash was 2.932%. However, the ash percentage results reflected the effect of the lactation stage on the physiochemical properties of camel milk.

The viscosity of the camel milk was the least within the 3 milk samples (1.94 centipoise). The previous studies showed that the mean viscosity of the camel milk was 1.77 centipoise which was similar to that of buffalo milk (1.79 centipoise) and more than the viscosity of cow milk (1.54 centipoise) (Yoganandi *et al*, 2014). However, it is well known that the viscosity of milk is fat content, moisture content and temperature dependent (Bakshi and Smith, 1984).

This study revealed that the camel milk had the lowest specific gravity (1.01) which is due to the high moisture percentage of the camel milk. The previous studies showed that the specific gravity of camel milk starts from 0.96 up to 1.1 (Yagil, 1982). Yoganandi *et al* (2014) stated that the camel milk had a mean value of specific gravity of 1.029 compared to 1.029 and 1.033 in cow and buffalo milk, respectively. The TDS results had the lowest TDS value between the goat and sheep milk (12.2%). Khan and Iqbal (2001) reviewed that other researchers found the TDS of camel milk between 11.29 to 14.30%.

Table 2. The significance of the variation between the mean values of the studied parameters in the low and high altitude milk.

Parameters	High altitude Milk		p- value	Low altitude milk		p- value
pH	Camel	Goat	0.19	Camel	Goat	0.16
		Sheep	0.21		Sheep	0.02
Conductivity	Camel	Goat	0.41	Camel	Goat	0.136
		Sheep	0.02		Sheep	<0.001
Moisture	Camel	Goat	0.42	Camel	Goat	0.14
		Sheep	0.02		Sheep	0.01
Ash	Camel	Goat	0.17	Camel	Goat	0.66
		Sheep	0.01		Sheep	0.74
Specific gravity	Camel	Goat	0.12	Camel	Goat	0.41
		Sheep	≥ 0.01		Sheep	0.001
TDS	Camel	Goat	0.73	Camel	Goat	0.19
		Sheep	0.03		Sheep	0.019

There was insignificant variations between the means of the studied parameters in the camel and goat milk of the low and high altitudes. All the studied parameters except the pH of the camel and sheep milk were significantly different irrespective of the altitude. The significance was set at the level of ≤ 0.05.

In goat milk the ash percentage, TDS percentage and specific gravity were 0.71- 0.88%, 11.5- 18.68% and 1.022 - 1.026, respectively (Jenness, 1980; Clark and García, 2017). Sabahelkhier *et al* (2012) found that the mean pH value and moisture percentage of goat milk in Sudan was 6.6 and 88%, respectively. The physiochemical properties of goat milk reported by the previous studies were close to the findings of this study.

The findings of previous studies of the sheep milk pH, moisture, electrical conductivity, specific gravity, viscosity and conductivity were comparable to the findings of this study. This study reported the highest ash and TDS percentage in sheep milk; 26.5 and 4.6 compared to the previous studies which may be due to the different geographical origin or lactation stage (Balthazar *et al*, 2017; Junior *et al*, 2015; Kanwal *et al*, 2004; Hoxha and Mara, 2012; Park, 2007; Bateman and Sharp, 1928). However, high percentage of ash was reported before in camel's and cow's milk (1.44- 2.932%) (Yagil, 1982; Kavas, 2015).

Conclusions

The studied physiochemical properties of milk samples (pH, conductivity, moisture, specific gravity, TDS and ash) showed that there were insignificant variations between the camel and goat milk samples while the sheep's milk was significantly different from the camel and goat milk samples (conductivity, moisture, specific gravity and TDS).

The laser induced fluorescence intensity of the sheep and goat followed similar patterns while that of camel was with a vice versa pattern. The LIF proved its suitability to be used for the determination of the milk source and altitude. The altitude significantly affected the physiochemical properties of camel's, sheep's and goat's milk. In conclusion, more samples will be needed to confirm our findings.

Acknowledgement

The authors extend their appreciation to the deanship of scientific research of King Khalid University for funding this research under grant number (R.G.P2./25/40).

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DOI : 10.5958/2277-8934.2020.00008.9 *Vol 27 No 1, p 55-60*

ANALYSIS OF ENDOTOXIN LEVEL IN CAMEL MILK SAMPLES COLLECTED FROM VENDING OUTLETS IN SAUDI ARABIA

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ABSTRACT

The present study was done to analyse the endotoxin limits of 20 camel milk samples collected from common vending outlets, i.e. desert farms, shops etc. The milk samples were divided into 4 types (raw milk, refrigerated, boiled, stored at – 20°C) and processed for endotoxin analysis by Limulous Amoebocyte Lysate (LAL) Gel clot method. The microbial count was examined for raw milk sample as per standard protocols. Endotoxin limits observed in the raw milk ranged from 3 to more than 1200 EU/ml. Elevated levels of endotoxin were observed in refrigerated samples. There is no significant change of endotoxin found between raw and deep freezed, boiled samples. Endotoxin value of processed milk from shops were in the range of 3 – 300 EU/mL. Among 20 samples, the total microbial count ranged from 1 to 7.39 log CFU/ml and coliform count was 0 to 3.58 log coliforms CFU/ml. The findings of this report on the endotoxin limits of camel milk showed the levels to be between 3 to 9 EU/ml. However, the samples showed high endotoxin values (>1200 EU/mL) if improperly stored. Presence of high level of microbial and endotoxin in camel milk is unsafe for human consumption.

Key words: Camel milk, endotoxin limit, gel clot method, LAL test, microbial count

People of Kingdom of Saudi Arabia (KSA) consume camel's milk in relatively high quantities during festivals and celebrations (Faye *et al*,2014). Unpasteurised milk is rich in Gram-negative bacteria (GNB) and endotoxins (Kilewein, 1994). Endotoxin contamination in food products and indoor exposure are an increasing medical problem that contribute to the development and severity of asthma and other respiratory symptoms (Loss *et al*, 2011; Sipka *et al*, 2015; Kulhankova *et al*, 2016).

In the PASTURE study, endotoxin concentrations were found to be significant in cow milk samples from non-farming families compared with farming families (Gehring *et al*, 2008) and detected endotoxin levels of shop milk and farm milk samples. Another study by GABRIELA group reported that the elevated endotoxin load in farm milk may involve asthma and atopy protective effect (Loss *et al*, 2011).

There have been no studies available worldwide to analyse the endotoxin levels in camel milk to assess the hygienic quality of raw and processed camel milk.

Hence, the aim of the present work was to analyse the endotoxin levels in camel milk samples collected from vending outlets.

Materials and Methods

Camel milk samples

Random sampling of raw camel milk was done from the desert farms of Zulfi, Majmaah region, Saudi Arabia and stored milk from milking vessels, shops and directly collected from the udder of the camel between September 2018 to May 2019. About 200 ml of fresh whole milk samples were collected from each sampling point using sterile and depyrogenated screw capped bottles. All samples were tightly capped, labelled and immediately transported in an ice-cold condition to the laboratory for analysis.

These samples were classified as 4 categories (Table 1). A total of 20 camel milk samples were collected from the desert farms, among them 4 each were collected from milking container (category I) and storage container (category II). Five samples of bottled camel milk were procured from the shops (category III). Seven samples were collected directly from the camels udder (category IV) following a strict aseptic collection method.

Collected samples were split into 4 parts; the first part was considered raw milk and was immediately processed for microbiological analysis and endotoxin analysis as per standard protocol.

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The second and third parts were given different cold treatment such as 2 – 8°C and –20°C for 24 and 48 hours, respectively. The fourth part was taken for heat treatment (boiling for 10 mins) and the endotoxin limit was checked as per standard protocol.

Microbiological analysis

Total microbial count and coliform count tests were performed for raw milk samples following the international standard for examination of dairy products (EC 2001; EU 2004; ISO 14461-1:2005). Briefly, for standard plate count for total microbial count enumeration, one millilitre of the milk sample was serially diluted in 9 ml of peptone water up to six dilutions. One ml of diluted sample was poured on a sterile Petri dish and then sterile molten media (Plate Count Agar) was poured. The sample and the agar were gently mixed and left for 30 minutes. The plates were sealed with parafilm, incubated at 37°C for 2 days. Duplicates were performed for each sample and the colonies were counted using a colony counter.

For Coliform count, serially diluted samples were poured on sterile Petri dish and then sterile Violet Red Bile Agar was poured. The sample and the agar were gently mixed and left solidified for 30 minutes. Two plates were inoculated with each dilution. The plates were incubated at 37°C for 24 hours. Typical dark red colonies were considered as coliform colonies and number of colonies were recorded and tabulated.

Detection of endotoxin level

Detection of endotoxin levels in camel milk samples was done by gel-clot Limulus Amoebocyte lysate (LAL) assay method (Endosafe, Charles River, USA). All types of samples including raw, cold and heat treated samples were at room temperature and diluted with endotoxin free water or LAL water (EndoSafe, Charles River, USA). Firstly, Endotoxin test was standardised by performing 'inhibition / enhancement test' and adjusting the pH of the milk samples. All the samples were processed at a pH range between 6.0 and 8.0. The testing methodology were followed as per method outlined by the United States Pharmacopoeial Convention chapter <85> Bacterial Endotoxin Test (USP, 2012).

The test endotoxin limit was calculated based on formula

$$MVD = \text{Endotoxin limit (EU/ml)} / \lambda$$

Samples were always tested in the presence of both positive and negative controls. A quarte replicate was performed for each dilution. The minimum sample with LAL water dilution was 1:16, the maximum dilution was 1:76,800. At first, a 10-fold dilution series was prepared, followed by a two-fold dilution series. The amount of endotoxin was expressed in endotoxin units EU/ml. The lysate sensitivity of minimum endotoxin detection limit was 0.03125 EU/ml. A standard curve test was performed whenever a new lot of CSE and LAL reagent was received. Inhibition and enhancement tests were performed to detect the non interfering dilution as per standard protocol USP<85>.

Statistical methods

The significance of differences in endotoxin levels, among the various sampling categories, were analysed using Student's 't' test, a p value less than 0.05 was considered as statistically significant. Microbiological counts were approximately normally distributed after natural log (ln) transformation. Mean value of microbial counts and endotoxin levels were calculated.

Results

Results of bacterial enumeration of raw camel milk samples and endotoxin levels observed in each category of milk samples are shown in Table 2. Among the 20 samples that were tested, the total aerobic flora ranged from 1 to 7.39 log CFU/ml and coliform count was 0 to 3.58 log coliforms CFU/ml. There was no coliforms observed in category IV sample.

Category I of raw farm milk showed an endotoxin limit of more than 300 EU/ml, elevated levels of endotoxin value observed in refrigerated samples (p value 0.0412), and there was no difference observed between raw and deep freezed sample (p

Table 1. Camel milk sample details.

Type	Sample details	Time lapse between milking and sample collection
Category I	Collected from milking container	30 – 60 minutes
Category II	Collected from stored milk in milking vessel	2 – 4 hours
Category III	Processed shop milk	-
Category IV	Directly milking from udder	Not applicable

value =1). The mean total bacterial count was 4.61 and coliform count was 2.52 log CFU/ml (Table 2, 3).

In category II the sample showed a high level of endotoxin throughout all samples, mean endotoxin value of raw milk was 863 EU/ml. The microbial count (5.98 Log CFU/mL) and coliform count (3.23 Log CFU/ml) exceeded the EU reference values. An inconsistent endotoxin value in the range of 6 to 1200 EU/mL was observed in four samples of processed shop milk. There was a slight increase observed between raw and refrigerated samples, i.e. total microbial count and coliform count 4.76, 2.89 log CFU/ml, respectively. The very minimum level of endotoxin observed in category IV sample was 3 to 6 EU/ml. There was no significant difference between raw milk, cold or hot treated samples.

Overall, a range of endotoxin values of raw milk from milking vessels, storage containers, shop milk, and udder milk were 300-600 EU/ml, 1200 EU/mL, 600 – 1200 EU/ml and 3 – 6 EU/ml, respectively. One or two fold increase of endotoxin levels were observed in refrigerated samples than raw milk. There was no significant increase or decrease in endotoxin load in raw and samples which were stored at – 20°C (p value =1).

Discussion

Recent studies show that endotoxin in milk samples can have protective effects against the development of asthma and allergy (Gehring *et al,* 2002 and 2008; Loss *et al,* 2011; Illi *et al,* 2012) but such studies with camel milk are lacking. In this study, camel milk was examined using controlled individual sample collection, storage and heat and cold treatment to find out concentration of endotoxin.

Endotoxin level of raw camel's milk (category I) collected from the milking container was <600 EU/ml, however, there was a slight increase observed in refrigerated samples, this might be due to multiplication of psycrophillic Gram negative organisms which may be present in the samples. These findings match with Sipka *et al* (2016) which reports a similar suggestion with cow milk. There is no significant difference of endotoxin levels observed between raw, boiled or deep freezed milk.

Category II samples that were collected and stored for more than 6 hours showed one to two fold of endotoxin levels when compared to category I samples. This time difference was taken due to difficulties in sample collection such as milking done later in the evening and distance from the testing facility to collection place at desert farms. So these samples were considered as worst case and had higher levels of endotoxin (>1200 EU/ mL, mean 863 EU/ml) than category I. This is evident by Gehring *et al* (2008) who saw similar observations in cow's milk, and accordingly endotoxin levels in farm milk were positively associated with time duration between milking and packing.

Regarding category III milk samples, among the 4 samples analysed there was no consistent levels of endotoxin observed, two samples were < 30 and another two samples were > 600 EU/ml, this might be due to their manufacturing conditions and processing methods. Comparatively, there is no difference of endotoxin level observed in category I of raw milk and III of shop milk, this is very similar to a report in cow's milk (Gehring *et al,* 2008, Sipka *et al,* 2016). However, endotoxin levels of camel's milk collected directly from the udder (category IV) shows < 6 EU/ml, mean value of endotoxin was 4 EU/ml which is 100 to 200 times lower than other category samples. This clearly shows the true value of endotoxin levels in camel milk.

No previous published studies have analysed the endotoxin levels of camel milk. Therefore, comparison of present study results with other reports prove difficult. Although there are still very few studies available on cow's milk but recently Sipka *et al* (2016) analysed cows milk by LAL method and reported that the median value of farm cow milk was 60 EU/ml and shop milk was 102.5 EU/ml. In the PASTURE study conducted in European countries they reported that the geometric mean endotoxin value was 476, 17, 163, 459, 169 EU/ml in Austria, Finland, France, Germany and Switzerland, respectively (Gehring *et al,* 2008). The variations of endotoxin levels obtained by the available reports might be due to the nature of the milk samples, source of samplings, and the test methods employed.

Endotoxin limits were directly associated with total microbial load in particular GNB organisms, hence in the present study this was analysed and compared with total microbial and coliform count. There is no standard microbial limits for camel milk, thus the present study results were compared with European union (EU) microbiological limits (EU Regulation, 2004). Total bacterial flora was not more than 1 X 10^5 CFU/ml (5 log CFU/mL) and Coliform count <10^2 CFU/ml (2 log CFU/ml) for raw milk for human consumption. By comparing this, the mean value of TBC of category I, II and IV were in the range of EU acceptable limits for raw milk. However,

Table 2. Endotoxin level and microbial count of each sample.

Sample number	Sample types	Endotoxin level (EU/ml)				Microbiological analysis	
		Raw milk	Refrigerated#	Deep freezed*	Boiled milk$	Total count (log CFU/ml)	Lactose fermenting coliforms (log coliforms CFU/ml)
1	Category I	<600	600-1200	<600	<600	6.38	3.58
2		<300	>450	<300	<450	3.69	2.12
3		<300	300 – 600	<300	<300	3.84	1.98
4		300 -600	600	300 -600	>600	4.53	2.41
5	Category II	>1200	>1200	>1200	>1200	7.08	3.8
6		>1200	>1200	>1200	>1200	7.39	3.56
7		<600	<600	<600	>600	4.62	2.11
8		300 -600	600	300 -600	>600	4.82	2.66
9	Category III	600 – 1200	>2400	600 – 1200	>600	6.34	2.89
10		<600	<600	<600	<600	4.53	1.78
11		<300	<300	<300	<450	4.84	1.83
12		<30	>60	<30	<30	3.87	0.85
13		<6	6 to 12	<6	<6	4.07	1.95
14	Category IV	< 6	<12	< 6	< 3	2.38	0.55
15		< 3	<8	< 3	< 3	1	0
16		< 3	<6	< 3	< 3	3.65	0.12
17		< 3	<8	< 3	< 3	4.02	0
18		< 3	<6	< 3	< 3	3.99	0
19		<6	6 to 12	<6	<6	4.18	0
20		< 3	<9	< 3	< 3	4.10	0

Temperature 2 – 8°C for 24 hours * stored at – 20°C for 48 hours $ Boiled for 10 mins

Table 3. Comparison of mean microbial count and endotoxin levels of raw camel milk.

Sample Type	Total aerobic flora (Log CFU/mL)	Total coliforms (Log coliforms CFU/mL)	Endotoxin (EU/ml)
Category I	4.61	2.52	413
Category II	5.98	3.23	863
Category III	4.76	2.89	368
Category IV	3.33	0.09	3.8

samples (ID 1,5,6,9) showed a higher value than the reference limit.

The results for total count in this study were in agreement with other report from Saudi Arabia (5.0 log CFU/ml), Ethiopia (5.6 log CFU/ml) and UAE (5.22 log CFU/ml) for tests on camel milk samples (El-Ziney and Alturki, 2007; Semereab and Molla, 2001; Omer and Eltinay, 2008). The variations of total count might be due to the differences in initial microbial contamination originating from the udder surface, quality of water used for cleaning and disinfection of milking utensils and the time lapse from production to marketing. Category IV samples of milk collected directly from the udder were found to have relatively better bacteriological quality than other samples of category I, II and III. This reaffirms the microbial contamination originating from external sources.

Next moving to coliform count, the mean coliform count obtained in the present study was higher than the EU reference value 2 log coliform CFU/ml in category I, II and III. However, this is very similar to the report 2.83 log coliform CFU/ml for camel milk samples collected in UAE (Younan, 2004). In contrast, reports from Ethiopia (Abera *et al,* 2016), Morocco (Benkerroum *et al,* 2003), Algeria (Benyagoub *et al,* 2013) reported 4.03, 6.85, 6.75 log coliform CFU/ml, respectively. A mean coliform count of category IV milk was 0.09 log coliform CFU/ml, which indicates a relative increase in coliform count from udder to milking vessels to

market. This might be due to milk contamination at various levels while milk was passing through different stages of production. A high coliform count may be the reason of improper udder cleaning, preparation in pre-milking, poor hand washing practice of milker and poor quality of milking containers. The presence of high number of coliforms and other Gram-negative organisms in the milk is directly proportional to high endotoxin levels. This could evident why low levels of endotoxin were found in category IV samples.

Moving to the concern about endotoxin exposure, there are very limited studies explaining endotoxin exposure and association with asthma. Presence of elevated levels of endotoxin in farm milk may explain the asthma and atopy protective effect of farm milk noted in study reported by various researchers (Riedler *et al,* 2000 and 2001; Loss *et al* 2011; Illi *et al,* 2012). Apart from the consumption of endotoxin contaminated milk, there are various studies reported that exposure to airborne endotoxin such as home dust, workplace settings (mainly lab animal handling), waste management, and fibreglass manufacturing considered as a major risk factor for asthma, chronic rhinitis and wheezing (Gioffrè *et al,* 2012; Basinas *et al,* 2013; Salonen *et al,* 2013; Barraza *et al,* 2016). It is not clear, whether the ingestion of endotoxin has an effect on the development of asthma and allergies.

Conclusion

Endotoxin levels in camel milk were elevated in refrigerated samples compared to raw milk. Milk sample aseptically collected directly from udder was free of coliforms and had the lowest endotoxin load. However, both the concentration of endotoxin could be influenced by storage time, cleanliness of milking vessels and storage temperature. Lastly, the data indicates the consumption of raw milk might have all the risks and health hazards associated with the unpasteurised, unprocessed state. In addition, milkers keeping dairy camels have to be more aware of the importance of good hygienic conditions for the quality of milk. Finally, the base data of endotoxin in camel milk would support further research on the endotoxins role in asthma and allergy prevention.

Acknowledgement

This research work was funded by the Basic Science Research Unit, Deanship of Scientific Research at Majmaah University, Kingdom of Saudi Arabia (Project number 38/72).

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DOI : 10.5958/2277-8934.2020.00009.0 *Vol 27 No 1, p 61-68*

URIDINE 5-TRIPHOSPHATE (UTP) METABOLISING ENZYMES NUCLEOSIDE DIPHOSPHATE KINASE AND CYTIDINE TRIPHOSPHATE (CTP) SYNTHASE IN CAMELS AND *Trypanosoma evansi*

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ABSTRACT

In this work, the pyrimidine metabolic pathway Uridine 5'- Triphosphate (UTP) paths were investigated. In this context, two UTP enzymes were investigated by bioinformatics tools, nucleoside diphosphate kinase (NDK) and cytidine triphosphate (CTP) synthase. The dromedary, Bactrian and feral camels NDK showed high similarity > 97.7% to the human enzyme. The camel NDK was phylogenetically distant from eukaryotic NDK with the closest relation to prokaryotic NDK. *Trypanosoma evansi* NDK was phylogenetically distant from protozoal NDK and devoid of the histone H3 domain, which was found in eukaryotic NDK. Two isoforms of CTP synthase were retrieved from camel genome with medium homology per cent. These are replaced by one isoform in *T. evansi*. In terms of drug targets, both NDK and CTP synthase showed conserved and phylogenetically and motif distinctions. This enrolls the two targets as a choice for drug development against *T. evansi*.

Key words: Camel, CTP synthase, genome, NDK nucleotide, *Trypanosoma evansi*, UTP

Uridine 5'- Triphosphate (UTP) production process is proposed by three production routes, i.e. KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016; Ogata *et al,* 1998). UTP can be synthesised from uridine-5'-diphosphate (UDP), cytidine-5'-triphosphate (CTP) and P(1),P(4)-bis(uridin-5'-yl) tetraphosphate (UppppU) by the actions of nucleoside-diphosphate kinase, deoxycytidine triphosphate deaminase and Bis (5'-nucleosyl)-tetraphosphatase enzymes, respectively. In the catabolic pathway, the phosphorylation of UTP by thymidine-triphosphatase and/or ATP-diphosphatase or by nucleoside-diphosphate kinase can yield UDP. Whereas the reduction of UTP by ribonucleoside-triphosphate reductase and/or ribonucleoside-triphosphate reductase (thioredoxin) can yield deoxyuridine-5'-triphosphate. In addition, pyrophosphates can be removed from UTP by the action of nucleotide diphosphatase to produce uridine-5'-monophosphate (UMP). Cytidine triphosphate synthetase uses UTP as a substrate to produce cytidine-5'-triphosphate (CTP).

In this study, the enzymes involved in UTP metabolic pathways in camels were investigated. Comparisons were made between dromedary camel and human, wild camel, and eukaryotic UTP metabolising enzymes. The comparisons included homology rate, conserved domains composition, functional motifs and signatures, phylogenetic relationships and genetic composition.

Materials and Methods

The enzymes involved in metabolic pathway of UTP are given in Table 1 and those expected enzymes involved in metabolic pathway of UTP in camel and *Trypanosoma evansi* are given in Table 2 and 3, respectively.

The proposed metabolic pathway of uridine triphosphate is given in Fig 1 and those for camel and *Trypanosoma evansi* are given in Fig 2 and 3, respectively.

Retrieval of genomic data

Collection of genomic data was carried out by extracting the information from the gene

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Table 1. Enzymes involved in metabolic pathways of UTP.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	Nucleoside-diphosphate kinase
3.6.1.39	Thymidine-triphosphatase, thymidine triphosphate nucleotidohydrolase
3.6.1.5	Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
3.6.1.9	Nucleotide diphosphatase
6.3.4.2	Cytidine triphosphate synthetase
3.5.4.13	Deoxycytidine triphosphate deaminase
3.6.1.17	Bis(5′-nucleosyl)-tetraphosphatase (asymmetrical)
1.17.4.2	Ribonucleoside - triphosphate reductase (thioredoxin)
1.1.98.6	Ribonucleoside- triphosphate reductase

Table 2. The expected enzymes involved in metabolic pathways of UTP in camels.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	Nucleoside-diphosphate kinase
6.3.4.2	Cytidine triphosphate synthetase

Table 3. The expected enzymes involved in metabolic pathways of UTP in *Trypanosoma evansi*.

ID (E.C. number)	Definition (Enzyme name)
2.7.4.6	Nucleoside-diphosphate kinase
6.3.4.2	Cytidine triphosphate synthetase

database (http://www.genedb.org) (Hertz-Fowler *et al,* 2004), protein and genome databases (http://www.ncbi.nlm.nih.gov), Kinetoplastom genome resources (http://tritrypdb.org/tritrypdb/) and the Arabian camel genome project (http://www.camel.kacst.edu.sa). Information obtained from these sources included protein and nucleotide sequence information, gene annotation, paralogs and orthologs information, metabolic functions, signal peptides, transmembrane domains, predicted protein export domains, protein expression profiles, isoelectric point (pI), the number of predicted transmembrane helices, any predicted sequence motifs, and the E value of the closest PDB sequence homolog.

Searching homologues Protein sequence homologues was searched using the NCBI BLAST (Basic Local Alignment Search Tool) (Madden, 2013) or PSI-BLAST (Position- Specific Iterated-BLAST) servers (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment program for proteins and construction of phylogenetic tree was carried out using the tools available at website (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins, 2014). By the multiple sequence alignment programme (ClustalW2) the best matching among the chosen sequences was calculated. The phylogenic tree was generated from the resultant alignment, then visualised by Dendroscope phylogenic tree viewer (Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains was searched by the domain prediction programme available at (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Proteomic and genomic tools ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) were also used for analysis of nucleotide and gene sequences. Several protein parameters including the molecular weight, atomic composition, amino acid composition, theoretical pI, estimated half-life, extinction coefficient, grand average of hydropathicity and aliphatic index was searched at (http://us.expasy.org/tools/protparam.html). Protein sequences of target genes was analysed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam. PROSITE (http://www.expasy.org/proteomics/families__patterns_and_profiles) was used to search for patterns and profiles in the protein sequences of the target genes. The protein domains, families and functional sites was searched at http://prosite.expasy.org/.

Results and Discussion

Nucleoside diphosphate kinase (NDK)

Nucleoside-diphosphate kinase (NDPK; 2.7.4.6, or nucleoside 5′-diphosphate kinase; nucleoside diphosphokinase;) is a transferase enzyme catalyse the transfer of phosphates donor to acceptor nucleotide diphosphate. NDK maintains cellular homeostasis by controlling the cellular levels of nucleotides triphosphates, which are essential for many cellular activities. Moreover, NDPK is a highly conserved, multifunctional enzyme catalyses using ATP as the phosphate donor to form phosphorylated

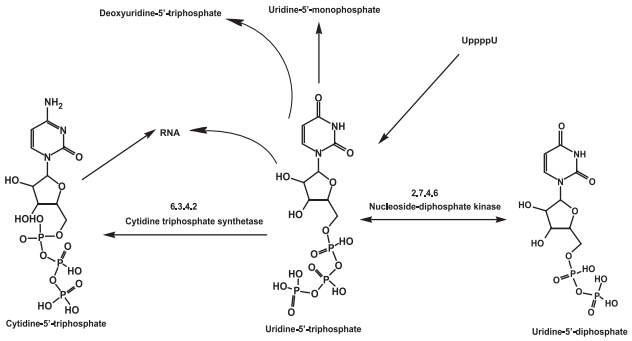


Fig 1. The proposed metabolic pathways of uridine triphosphate (UTP).

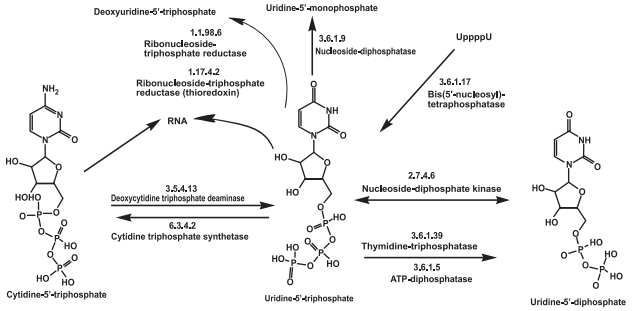


Fig 2. The proposed metabolic pathways of uridine triphosphate (UTP) in camels.

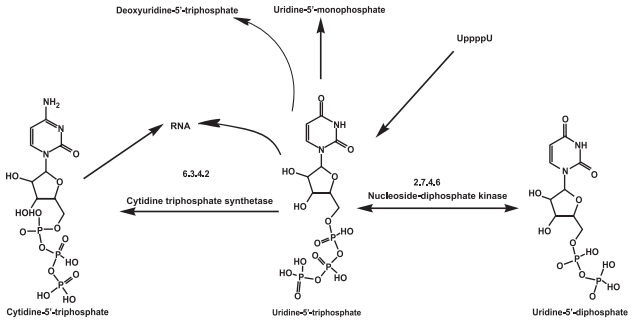
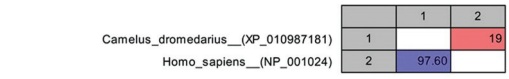


Fig 3. The proposed metabolic pathways of uridine triphosphate (UTP) in *Trypanosoma evansi*.



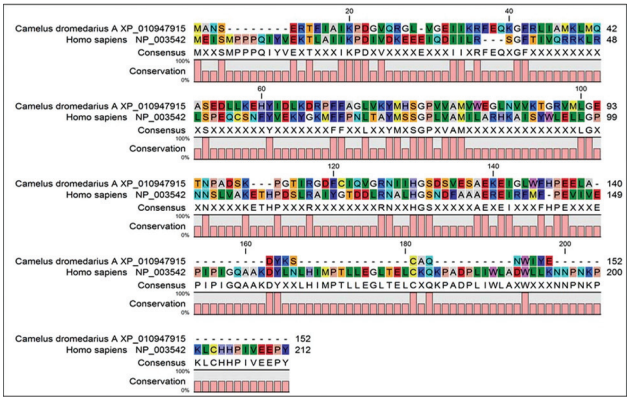


Fig 4. Multiple sequence alignment of dromedary camel and human nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

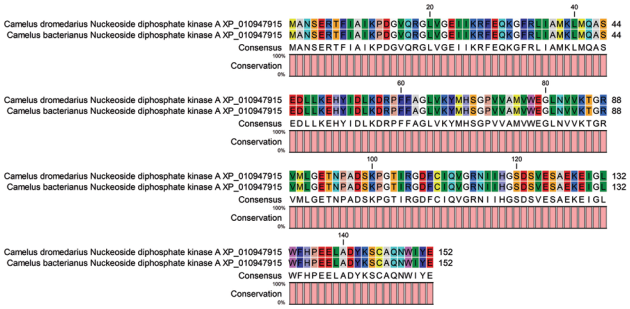


Fig 5. Multiple sequence alignment of dromedary and bactrian nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

histidine residue with high energy intermediate form (Yu *et al*, 2017). Additionally, the enzyme is nonspecific and can recognise a wide range of nucleotides and ribonucleotides (Kandeel and Kitade, 2010).

Recently, additional functions of NDPK has been appreciated. For instance, NDK was able to transphosphorylate other proteins, resembling reminiscent of bacterial two-component systems. Additional newly discovered features were the DNA-binding of NDK. The genome of the parasitic protozoon *Trypanosoma brucei* contains a single gene for NDK (Hunger-Glaser *et al*, 2000). Its sequence was conserved with high similarity with other species. NDK. Procyclic and bloodstream forms were highly expressing NDK. Immunofluorescence and immuno-electron microscopy prove that trypanosomal NDK is located in the nucleus. LY266500, a strong inhibitor of histidine phosphorylation was unable to stop the NDK activity. On the other hand, (Pereira *et al*, 2014) revealed that the immunofluorescence microscopy NDK from *Trypanosoma cruzi* enzyme NDK overexpressing parasites has a cytosolic distribution with higher sequestration at the nucleus. The *Plasmodium falciparum* NDK is characterised by broad substrate range binding all nucleotides with almost similar high efficiency (Kandeel *et al*, 2009; Kandeel and Kitade, 2010).

Camel and human NDKs showed 97.6% homology rates with about 19 residues differences

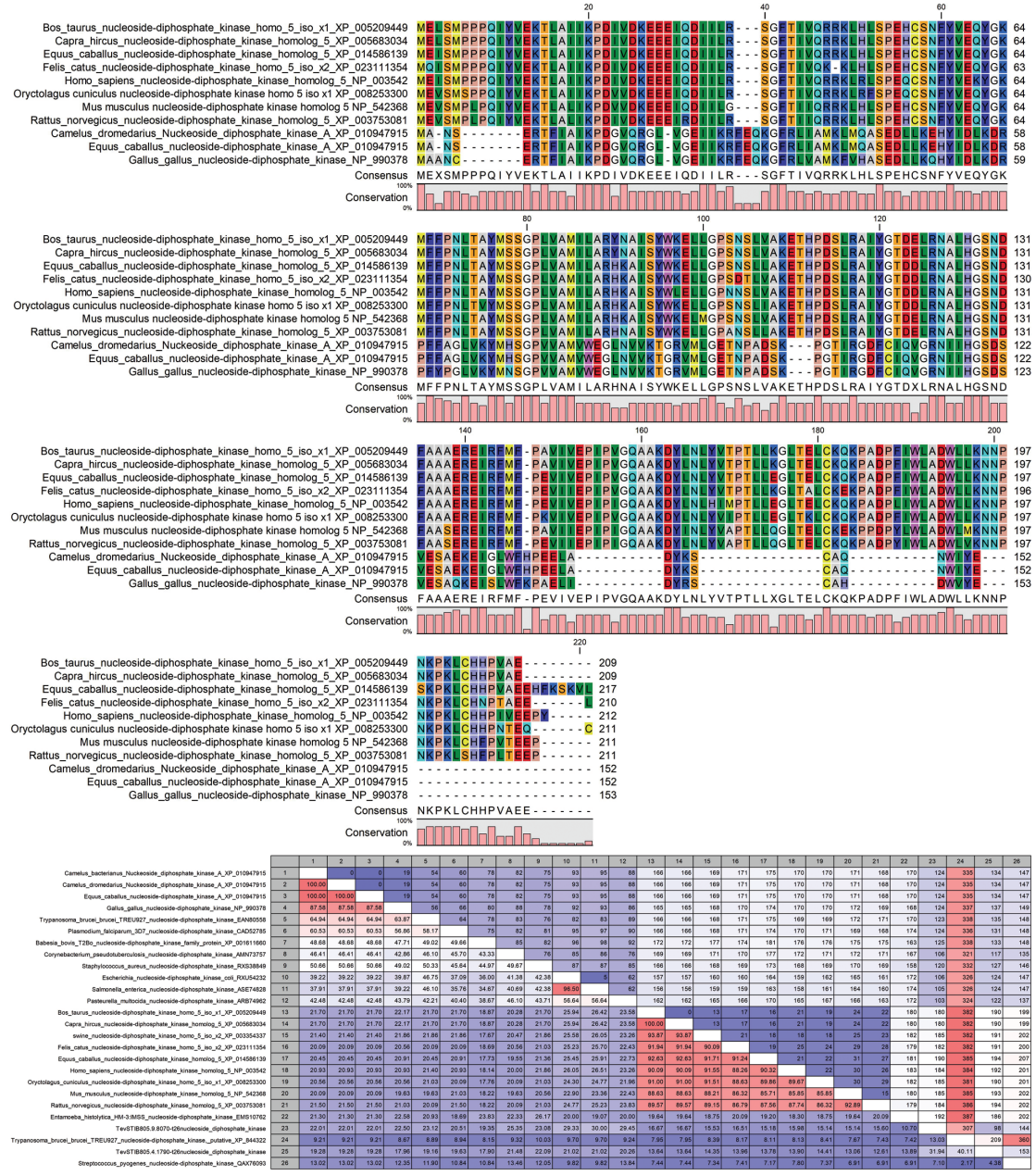


Fig 6. Multiple sequence alignment of dromedary camel and other eukaryotes nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

(Fig 4). The camel NDK showed shorter protein length of 152 residues, compared to 212 residues in the human enzyme. This was mostly due to the absence of 41 residues at the C-terminus of the enzyme.

Bactrian and feral camels showed 98.35 and 97.85% homology with the Arabian camel with 17 and 28 residues differences, respectively (Fig 5). Additionally, they also shared a short amino acids length of 152 residues. Fig 6 shows multiple comparisons of NDK from camel compared with higher eukaryotes including bovine, caprine, equine, swine, feline, mouse, rat, rabbit, chicken and human NDKs.

BLAST search of NDK using the *T. brucei* sequence against *T. evansi* database revealed two enzymes with little bit low homology rate (Fig 7). While 132-153 amino acid proteins were retrieved. The differences were 88-130 amino acid to constitute low homology rates ranging between 22.16 and 37.59%. This low rate might indicate variations in structures and functional features of NDKs in protozoa.

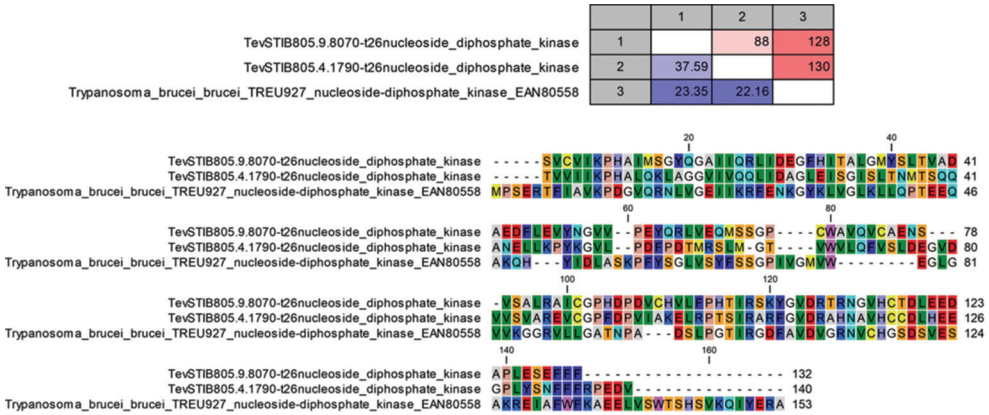


Fig 7. Multiple sequence alignment of *Trypanosoma brucei* and *T. evansi* nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

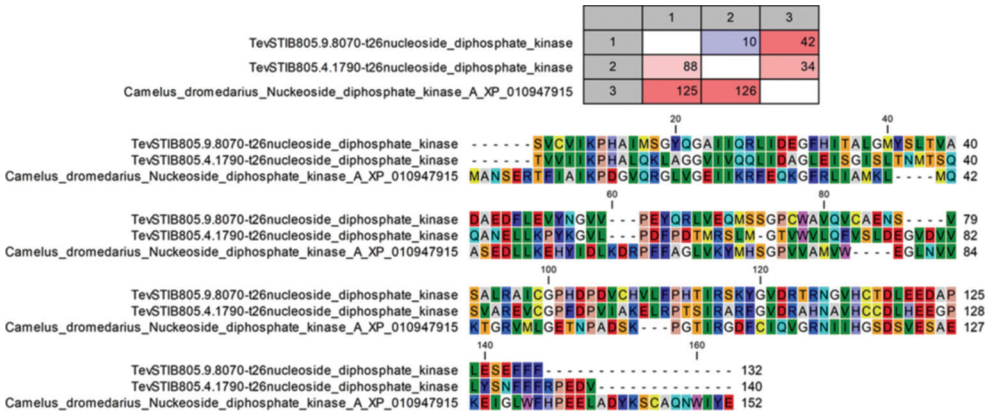


Fig 8. Multiple sequence alignment of dromedary camel and *T. evansi* nucleoside-diphosphate kinase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

In comparing camel and *T. evansi* NDKs, identity per cent was 42 and 43 (Fig 8) with about 126 and 126 differences from the two *T. evansi* enzymes.

Phylogenetic comparison (Fig 9) reveals a distant relation of camel NDK from human and most vertebrates with a closer relation to prokaryotic and protozoal enzymes. In comparison, the *T. evansi* enzymes were highly distant from the tested set of organisms. This might indicate interesting features of NDKs in both camel and *T. evansi* in comparison with other orthologous organisms.

Domains and motifs search revealed an eminent difference between camel and Trypanosoma NDKs. Camel was similar to human NDK showing two potential domains, including NDK domain and the histone H3 methylation domain (DPY30). The former domain is common with the protozoal enzymes, while the latter is unique to camel NDK.

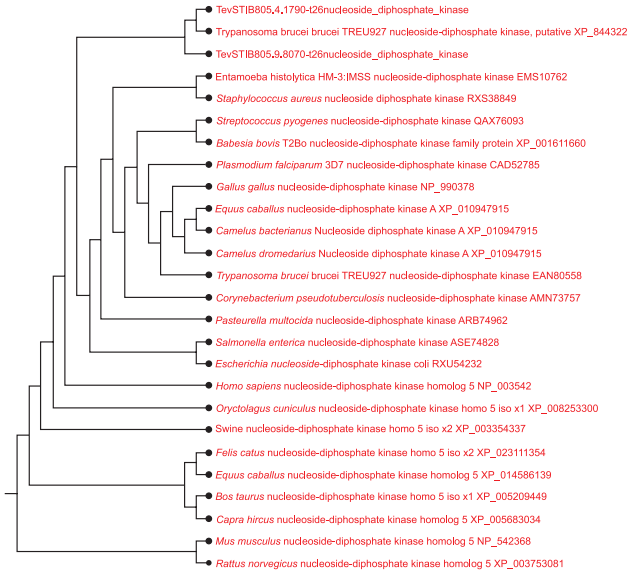


Fig 9. Cladogram of camel and *T. evansi* bifunctional nucleoside-diphosphate kinase in relation to a set of prokaryotic and eukaryotic organisms.

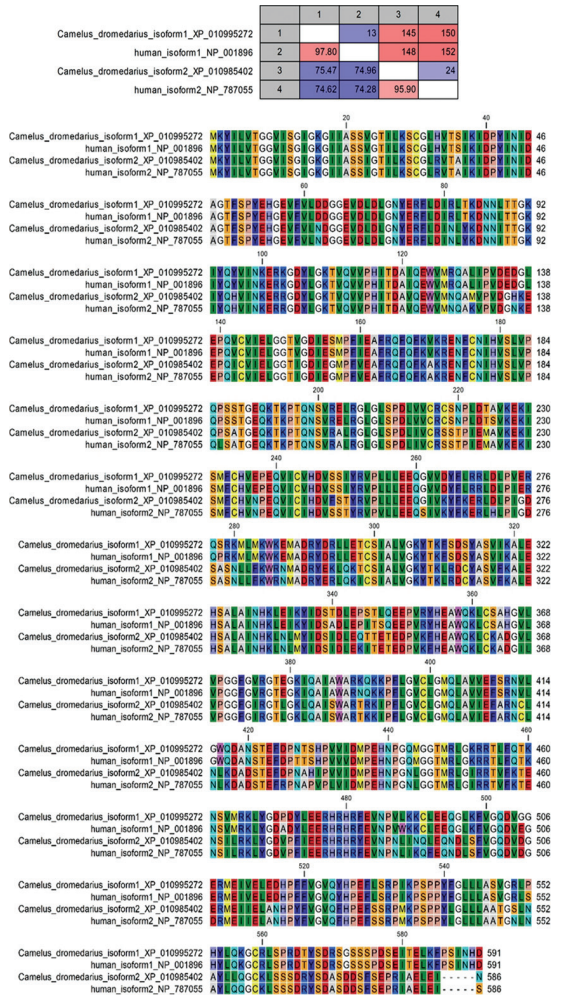


Fig 10. Multiple sequence alignment of dromedary camel and human Cytidine triphosphate synthetase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

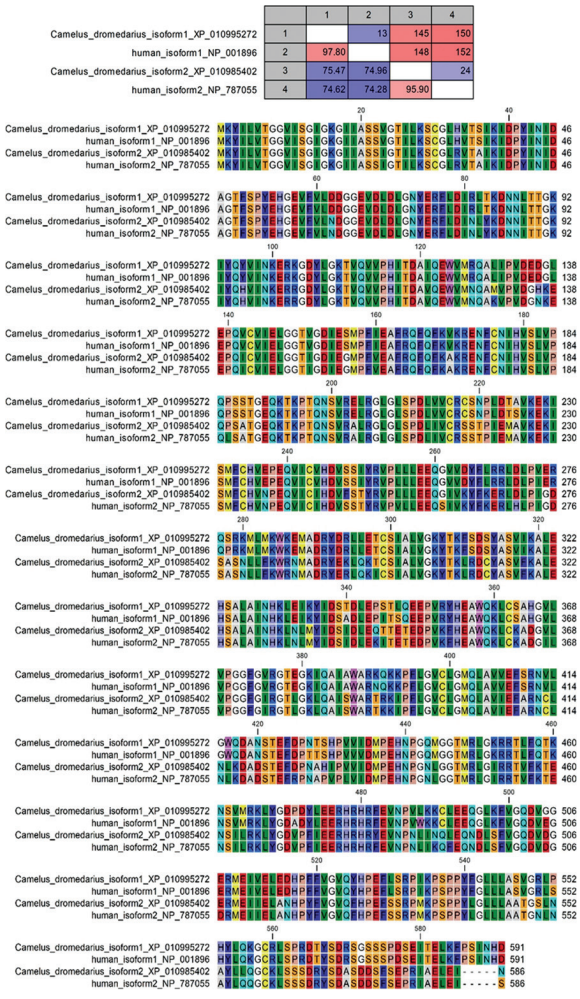


Fig 11. Multiple sequence alignment of dromedary and bacterian camels Cytidine triphosphate synthetase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Cytidine triphosphate synthetase

Cytidine triphosphate synthetase (CTP synthase; 6.3.4.2) is a ligase enzyme build carbon-nitrogen linkages catalyses the synthesis of CTP from UTP. This synthetase enzyme also known as glutamine hydrolysing; UTP—ammonia ligase; uridine triphosphate aminase; cytidine 5′-triphosphate synthetase; UTP (uridine 5′-triphosphate): ammonia ligase (ADP-forming). *Trypanosoma brucei* is protozoon parasite causing is a fatal disease known as African sleeping sickness. The concentration of CTP is very tiny in *T. brucei* comparing to mammalian cells. Moreover, the limited power for *de novo* synthesis cytidine/cytosine and the lack of salvage pathways of Trypanosomes. Therefore, the parasite CTP synthetase (TbCTPS) is rendered a prospective target for the creating of antiprotozoal agents. (Fijolek *et al,* 2007; Steeves and Bearne, 2011). Furthermore, *T. brucei* CTP synthase is stopped by purine derivatives such as GTP, guanosine, caffeine, and uric acid. Additionally, the purine ring may perform a useful structure for the construction of inhibitors of trypanosomal CTP synthase. As well, the exploitation of CTP synthetase inhibitors alone or in concoction with proper nucleosides or bases might help in management of sleeping sickness. (Hofer *et al,* 2001).

Two isoforms of CTP synthase were retrieved from the camel database of proteins (Fig 10). Isoform 1 and isoform 2 showed 97.8 and 95.9% similarity to the human isoforms, while the variation between the two isoforms extends to include 26-26% differences.

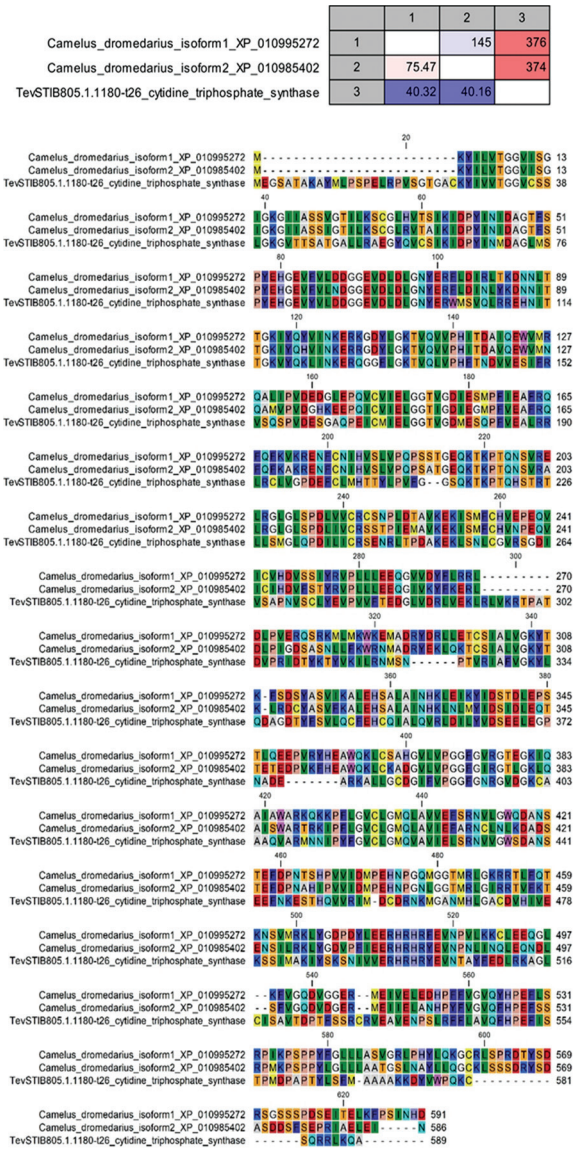


Fig 12. Multiple sequence alignment of *T. evansi* and dromedary camel cytidine triphosphate synthetases. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Similarly, comparison of dromedary and Bactrian camels revealed about 100% similarity within the same isoform and about 25% differences across the isoforms (Fig 11).

BLAST search using *T. brucei* against *T. evansi* retrieved one protein with more than 95% similarity. Furthermore, BLAST search using the two camel isoforms retrieved the same hit protein. The retrieved *T. evansi* CTP synthase showed about 40% similarity with both camel isoforms comprising average of 375 amino acids differences.

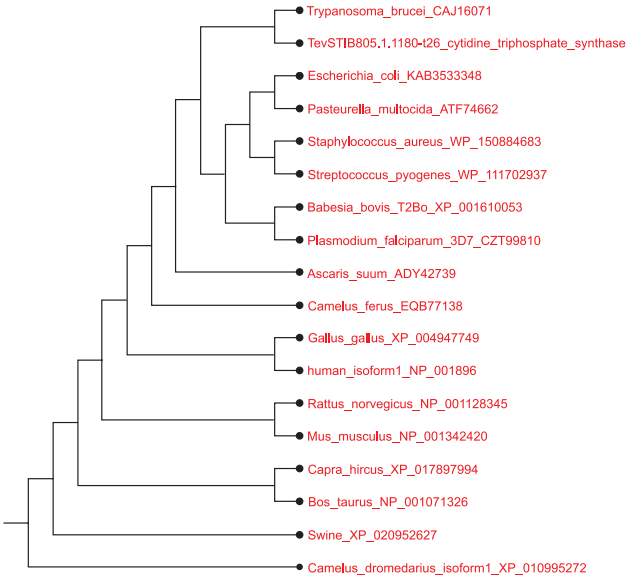


Fig 13. Cladogram of camel and *T. evansi* CTP synthetase in relation to a set of prokaryotic and eukaryotic organisms.

Phylogenetic comparison (Fig 13) reveals a close relation of camel CTP synthase from human and most vertebrates with a closer relation to prokaryotic and protozoal enzymes. In comparison, the *T. evansi* enzyme was highly distant from the tested set of organisms. This might indicate interesting features of evolutionary differences between the camel and Trypanosoma CTP synthase.

Domains and motifs search revealed almost constant and conserved domain and motifs features in both of camel and Trypanosoma. The domain content includes CTP synthase and glutamine amino transferase.

Acknowledgements

The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programmes, National Transformation Programme, under Research and Development Grants Program for National Research Institutions and Centres (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

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DOI : 10.5958/2277-8934.2020.00010.7 *Vol 27 No 1, p 69-75*

SNPs AND InDels DETECTION AND SELECTION SIGNALS IDENTIFICATION OF ALXA BACTRIAN CAMEL BY WHOLE-GENOME RESEQUENCING

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ABSTRACT

Alxa Bactrian camel has been raised as an economic and transportation animal in Alxa, Inner Mongolia, China. In a long period of evolution and artificial selection, it has formed many specific physiological characteristics that enable the production of beneficial items such as milk, meat and fur despite in the harsh environment. At the present the Bactrian camel genome is still a draft genome assembled only at scaffold level but the genetic diversity in multiple re-sequenced Bactrian camels has not been investigated yet. In this study, we evaluated the genetic features of SNPs and InDels and conducted selection signals identification to detect specific genes associated with adaptation to the environment and muscle growth of Alxa Bactrian camel by whole-genome resequencing. By next-generation sequencing technology, a total of 367.98 Gb high-quality paired-end reads were generated. On an average, 81.65% of the reference genome sequence was covered with mapping depth of 10-fold. From these data, 6,759,073 SNPs and 976,715 InDels were identified and 15,037 genes were detected, which revealed wide genetic variations and complex genetic features related to adaptation mechanism of Alxa Bactrian camel in harsh environments. By selective signals analysis, we identified 111 genomic regions, including 70 candidate genes such as BCO1, AKR1D1, SVOPL, SMS, PHEX, PCYT1B, POLA1, and MEGF10, which are potentially involved in environment adaptability and muscle growth. Generally, these results provide a framework and comprehensive insights for further genetic studies in the Bactrian camel population and research on genes, which would provide a better understanding of economically important traits and environment adaptability in Bactrian camel and further provide a scientific basis for the selective breeding of Alxa Bactrian camel.

Key words: Alxa Bactrian camel, InDels, selective signals, SNPs, whole-genome resequencing

At present, there are mainly 7 varieties of Bactrian camel in the world, distributed in the cold and desert regions, and 4 varieties are in China. They are called Sunite Bactrian camel, Qinghai Bactrian camel, Xinjiang Bactrian camel and Alxa Bactrian camel according to their breeding regions. China is the largest and most widely distributed country of Bactrian camels in the world, according to the statistics of 2016, there are about 360,000 Bactrian camels in China and 160,000 (44.92%) Bactrian camel are in Alxa, Inner Mongolia. Alxa is located in northwest China, geographically contains the vast Gobi (such as Wuliji, Yinggen) and deserts (such as Badain Jaran Desert, Tengger Desert). Through a long period of evolution and artificial selection, the appearances of the Alxa Bactrian camel living in the Gobi and the desert areas have formed their own characteristics and Alxa Bactrian camel is divided into Gobi camel and desert camel by its geographical distribution. Gobi camel is characterised with two big and erect humps, long and flat wide back waist and brown color. Desert camel is characterised with short trunk and long limb, big and round abdomen and apricot colour. At present, studies on Alxa Bactrian camel are mainly focused on its milk, meat and fat (Moshaverinia and Moghaddas, 2013). Up to date, there is no systematic study on the genome and a series of genome changes in a long period of evolution and artificial selection are still largely unknown.

Since the first whole-genome assembly of the human genome is completed in 2001, the whole-genome sequencing of mammals has been quickly completed (Venter *et al,* 2001). In recent decades, the next-generation sequencing technology has

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significantly facilitated the genetic studies of complex traits in domestic animals (Rosenthal *et al,* 2015) and has revealed natural and artificial selection footprint in many species, such as pig (Li *et al,* 2014, Ai *et al,* 2015), sheep (Yang *et al,* 2016), cattle (Sequencing and Consortium 2009) and dog (Xiao *et al,* 2014). Bactrian camel was present in the third millennium BC and subsequently spread into Central Asia (Peters and Driesch, 1997) and recent studies have focused on the divergence time between the ancestors of Bactrian camel and cattle (Sequencing and Consortium, 2012) genomic Single Nucleotide Polymorphisms (SNPs) rates, (Burger and Palmieri, 2014) the divergence time and demographic history of Bactrian camel, dromedary, alpaca and evidence of camel adaptation to desert environments (Wu *et al,* 2014) protective effect of camel milk on pathogenicity induced by *E. coli.* (Soliman *et al,* 2015). However, the whole-genome resequencing of Bactrian camel had not been utilised in the respective studies. Therefore, here we studied the whole-genome resequencing of Alxa Bactrian camel to examine the genetic variations, obtain genetic features, predict important candidate genes associated with muscle growth and development and understand the adaptation mechanism of Alxa Bactrian camel in harsh environments.

Materials and Methods

In this study, 12 Bactrian camels including 6 Gobi camels and 6 desert camels were randomly chosen to take skeletal muscle sample. Firstly, we extracted DNA from skeletal muscle, and detected the purity, potency and volume of DNA. Secondly, we created DNA library and sequenced the DNA using Illumina HiSeq platform and then filtered data and generated clean reads. Finally, we called Single Nucleotide Polymorphisms (SNPs) and short insertions and deletions (InDels), and then conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using online software.

Sample collection

Alxa Bactrian camel was sampled from Yingen Gobi and Badain Jaran Desert, where these were raised freely on local pastures. With the assistance of local herdsmen 12 Bactrian camels 8-10 years old including 6 Gobi camels and 6 desert camels, were randomly chosen at slaughter house to take skeletal muscle samples, which were snapped frozen in liquid nitrogen and stored at −80 °C. All experimental procedures were approved by the Animal Care and Use Committee of the Inner Mongolia Agricultural University, and conducted in strict accordance with the animal welfare and ethics guidelines.

Data filtering and generation of clean reads

DNA was extracted from the skeletal muscle samples with the AXYGEN Blood and Tissue Extraction Kit (Corning, USA) according to the manufacturer's instructions. Sequencing was performed on the Illumina HiSeq 2000 platform.

Data filtering and clean reads generation

First, to avoid reads with artificial bias (i.e. low quality paired reads, which mainly resulted from base-calling duplicates and adapter contamination), we removed the following types of reads: (1) Reads of joint contamination (the number of base contaminated by joints of reads > 5 bp; for paired-end sequencing, remove reads if one end is contaminated with the connector); (2) Low quality reads (the base of the mass value Q ≤ 5 in reads accounts for ≥50% of the total base; for paired-end sequencing, if one end is low-quality reads, reads from both ends); (3) Reads with N ratio > 5% (for paired-end sequencing, if one end contains N > 5%, remove the reads at both ends).

SNPs and InDels calling

Clean reads were aligned to the recently released version of the reference Bactrian genome using Burrows-Wheelser Aligner v0.7.10-r789 with default settings (Li and Durbin, 2009). The algorithm MEM was used to find the suffix array coordinates of good matches for each read. Sequence Alignment/Map tools (SAMtools)(Li *et al,* 2009) was used to convert file format from SAM to BAM and to filter the unmapped and non-unique reads. Read pairs with top mapping quality were retained. Local realignment around short InDels was performed with duplication-removed reads using Realigner Target Creator and InDels Realigner in the Genome Analysis Toolkit (GATK, version 3.3-0-g37228af) (McKenna *et al,* 2010; Depristo *et al,* 2011). SNPs and InDels were separated with GATK tool 'Select Variants' and subjected to rigorous processing to exclude false positives.

Functional enrichment analysis

We performed functional enrichment analysis within the GO and KEGG pathway terms using the Database for Annotation Visualisation and Integrated (DAVID) tool (http://david.abcc.ncifcrf.gov/) for enrichment analysis of the significant overrepresentation of GO biological processes (GO-BP), molecular function (GO-MF) terminologies, and KEGG-pathway. Only the enriched GO terms with

raw p-value <0.05 were used for further interpretation in this study. The functional relationships of the genes of interest were used in the Pathway studio program

Results and Discussion

Whole-genome sequencing, SNPs and InDels detection

General SNPs and InDels detection

Whole-genome sequence data of 12 Bactrian camels were obtained from Hiseq-2000 platform (Illumina, San Diego, CA, USA). Approximately 2,453,332,756 reads were produced (a total of 367.98 Gb paired-end sequence). Read mapping to the reference sequence was performed using BWA. About 2,003,009,380 high-quality paired-end sequences were successfully mapped to the Bactrian camel reference genome (https://www.ncbi.nlm.nih.gov/genome/10741 genome_assembly_id = 212083). On an average, 81.65% of the reference genome sequence was covered with mapping depth of 10-fold (Fig 1). These data yielded 7,735,788 variations from 12 Alxa Bactrian camels including 6,759,073 SNPs and 976,715 InDels using GATK and SAMtools. Of the identified SNPs, 4,220,597 (28.04%) were located in intergenic regions, 4,255,696 (28.27%) were located in introns, 992,658 (6.59%) and 992,658 (6.59%) and 1,007,762 (6.69%) were located in upstream and downstream, respectively. Of the identified InDels, 3,980,120 were located in intergenic, 1,902,612 were located in introns, 3,602,124 were located in upstream and downstream, respectively (Table 1).

Table 1. Summary and annotation of SNPs of Alxa Bactrian camel.

Type	Number	Percentage
UPSTREAM	992,658	6.59%
DOWNSTREAM	1,007,762	6.69%
EXON	121,018	0.80%
INTRON	4,255,699	28.27%
INTERGENIC	4,220,597	28.04%
TRANSCRIPT	4,387,197	29.14%
UTR_3_PRIME	43,408	0.29%
UTR_5_PRIME	14,532	0.10%

Specific SNPs detection

In total, 6,478,201 variations were identified from Gobi camel, including 5,770,567 SNPs and 707,633 short InDels. The number of specific SNPs was 1,494,099 in Gobi camel. In total, 5,800,250 variations were identified from desert camel, including 5,143,589 SNPs and 656,611 short InDels. The number of specific SNPs was 867,120 in desert camel. Compared to desert camel (867,120), Gobi camel (1,494,099) has more specific SNPs, indicating weaker intensive selection of desert camel (Fig 2).

Annotation and enrichment analysis

Annotation and enrichment analysis of general SNPs

In our Alxa Bactrian camel data set, 6,759,073 SNPs were detected in a total of 15,037 genes. Of the 15,037 genes, many of them matched to genes which were potentially associated with camel's system of adaptation to the environment, such as energy and fat metabolism (DGKZ), adaptation of respiratory system (FOXP3, CX3CR1, CYSLTR2 and SEMA4A), adaptation of visual system (OPN1SW, CX3CR1 and CNTFR), salt metabolism (NR3C2 and IRS1), water reservation (AQP1), osmoregulation (NFAT5 and BGTI) (Wu *et al,* 2014). As it is well known, the soil of Alxa is saline-alkali soil, therefore Bactrian camel living in Alxa has acquired specific abilities of salt tolerance. It is reported that camel can tolerate a high dietary intake of salt, and the salt tolerance level is eight times more than cattle and sheep, and the blood glucose level is twice more than other ruminants (Alali *et al,* 1988; Ali, 1994). Salt metabolism plays an important role in salt tolerance and water balance in Bactrian camel. NR3C2 and IRS1 genes play critical roles in sodium re-absorption and water balance in kidney (Sun *et al,* 1991). NFAT5 gene is the only known tonicity regulated transcription factor in mammals (Cheung and Ko, 2013). Also, Alxa is a very cold and dry region and MAPK4, NOX4, IFNGR2, SLC2A4, and PDK1 genes show significant correlations with climate variation (Lv *et al,* 2014; Yang *et al,* 2016). Additionally, we also found that ADCY4, CACNA2D1, AGT and PTGER genes are associated with adaptation of cold and dry environment. For example, ADCY4 and AC stimulated cAMP genes are involved in cAMP induced cell proliferation in cultured adrenal cells and are the key mediators of Na and water transport (Al-Hakim, 2004; Strait *et al,* 2010). These useful findings explains that Alxa Bactrian camel is well-adapted to the harsh environment.

Annotation and enrichment analysis of specific SNPs

We identified 1,494,099 and 867,120 specific SNPs, 14346 and 13132 genes of Alxa Gobi and desert camel, respectively. By analysing the annotated genes in two species of camel, 12,292 genes were

found in two species of camel at the same time. So, there are 2054 specific genes in Gobi camel and 840 specific genes in desert camel (Fig 3). In Gobi camel, Gene Ontology (GO) terms were associated with 2054 genes (Harris *et al,* 2004; Su and Zhou, 2007). GO analysis of these genes revealed enrichment in 14 GO terms in the biological processes, 14 GO terms in the cellular components and 21 GO terms in the molecular functions. KEGG enrichment analysis of these genes identified 10 pathways. The analysis showed that the genes associated with cellular components, such as nucleus extracellular exosome, cytoplasm, nucleoplasm and cytosol, were significantly enriched in Gobi camel. In desert camel, GO terms are associated with 840 genes. GO analysis of these genes revealed enrichment in 14 GO terms in the biological processes, 6 GO terms in the cellular components and 19 GO terms in the molecular functions. KEGG enrichment analysis of these genes identified 5 pathways. The analysis showed that the genes associated with cellular components, such as metal ion binding, nucleic acid binding, and protein cysteine S-palmitoyl transferase activity were significantly enriched in desert camel. These studies results suggest that the phenotypes associated with these genes may represent specific characteristics of Gobi camel and desert camel.

Selective sweep signals analysis

In order to detect genome selection signals related to evolution and meat production in Alxa Bactrian camel, our study used the sliding window method to find the highly selected genomic regions. By using 20Kb sliding window and 10Kb step to scan the whole genome of Alxa Bactrian camel to find the selected genomic signals in the process of evolution. We identified a total of 111 genomic regions under selective sweep signals analysis containing 70 candidate genes that are associated with Alxa Bactrian camel traits (Fig 4).

The annotation analysis found that these genomic regions contain 70 genes and GO annotation of these genes revealed enrichment in the biological process terms, such as cell development, cell differentiation, single-organism cellular process, single-organism developmental process, myofibrillar assembly, system development, and muscle structure development. KEGG enrichment analysis of these candidate genes identified 1 significant metabolic pathway (Fig 5). Our study results are consistent with Jirimutu *et al* (2012). They reported that these changes may underline the insulin resistance typically (Sequencing and Consortium, 2012). Candidate genes play an important role in regulation of skeletal muscle and those candidate genes associated with the growth and development of skeletal muscle were identified in several genomic regions under selective sweep signals analysis, including SMS, PHEX (NW-011517570.1), PCYT1B, POLA1, and MEGF10 (NW-011515253.1). Previous study showed that MEGF10 plays an important role in muscle stem cells and can regulate the development of skeletal muscle (Park *et al,* 2014). Because these cells are connected with the surface of muscle fibre, which is also called satellite cell, which normally are not active. Once the muscle muscle fibre is damaged the satellite cells are activated, differenced, proliferated and regenerate the muscle fibre fusion to repair the damaged muscle. PHEX also is an important

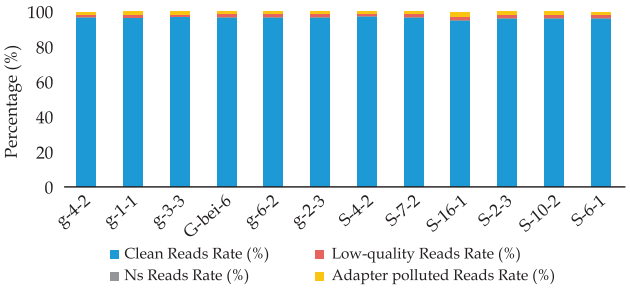

Fig 1. Raw reads statistics of Alxa Bactrian camel by whole-genome resequencing

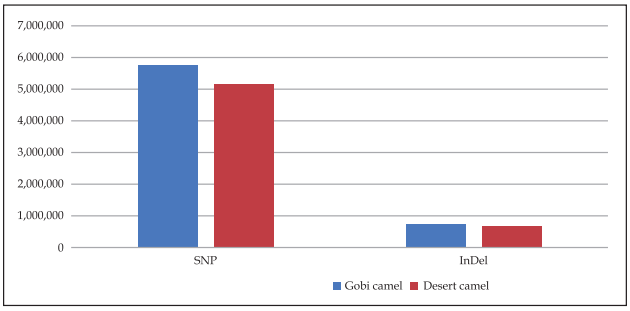

Fig 2. SNPs and InDels in Alxa Gobi and desert camel.

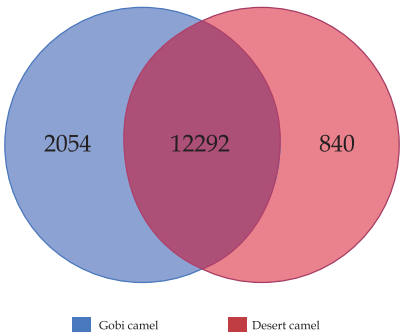

Fig 3. Common and specific genes numbers in Alxa Gobi and desert camel.

gene, which can regulate phosphate homeostasis and skeletal mineralisation (Quarless, 2003) and EZH1 can promote skeletal growth (Lui *et al,* 2016). We also found other candidate genes associated with skeletal muscle development, for example BCO1 (The enzyme β-carotene oxygenase 1) can catalyse the breakdown of provitamin A, which can be converted to vitamin A. Vitamin A plays an important role in vision (Saari, 2012), epithelium maintenance (Takahashi *et al,* 1993; Kumar *et al,* 2017), immune competence (Duriancik *et al,* 2010), reproduction, embryonic growth and development (Clagettdame and Knutson, 2011) and its neuroprotective nature (Ramani *et al,* 2017) is essential for the life of all animals. So, the BCO1 function is irreplaceable and could be efficient to regulate myogenesis and satellite cell activity *in vivo* (Praud *et al,* 2017). We also detected some genes associated with immune system, such as SASH1

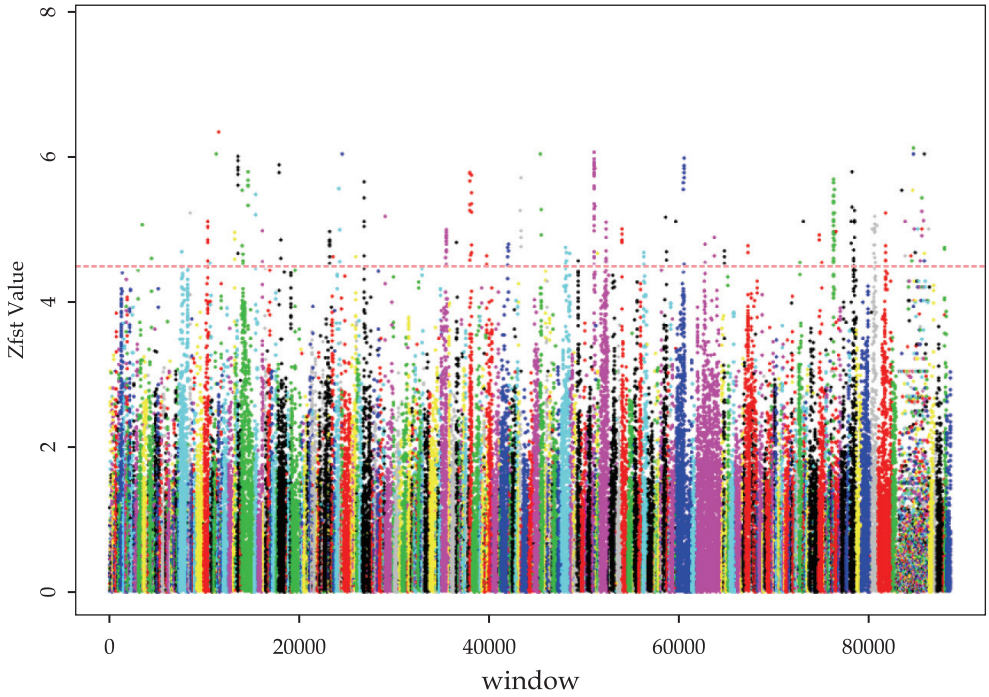

Fig 4. Selective sweep signals of Alxa Bactrian camel.

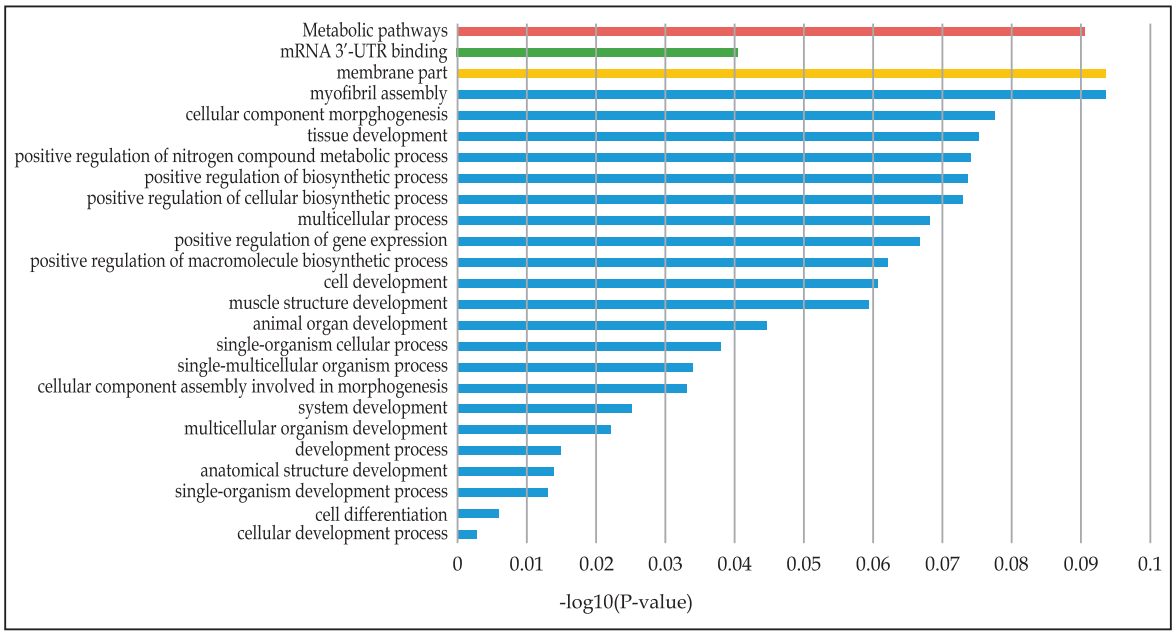

Fig 5. GO and KEGG pathways enrichment analysis of candidate genes selection of Alxa Bactrian camel.

and MS4A genes that play important roles in the development and progression of various diseases by regulating the cell growth, proliferation and apoptosis. (Liang *et al,* 2001, Lin *et al,* 2012) AKR1D1 gene can be repressed in diabetic patients (Valanejad *et al,* 2017). SVOPL gene is one of the SLC22 (solute carrier family 22) members. It is expressed in kidney and liver and regulates the uptake and excretion of environment toxins (Jacobsson *et al,* 2007). These results may explain why Alxa Bactrian camel has special functions such as high drought resistance, crude feed tolerance and adaptation to cold and dry environment compared with other animals.

Conclusion

This study is the first to explore the whole genome resequencing of Alxa Bactrian camel. We found a large number of SNPs and InDels by whole-genome resequencing between Gobi camel and desert camel. We annotated 14,346 and 13,132 genes by specific SNPs of Alxa Gobi and desert camel. These specific SNPs contained genes, such as NR3C2, IRS1 AQP1, FOXP3, CX3CR1 and CYSLTR2 are potentially involved in camel's system of adaptation to the environment. By selective signals analysis, we identified 111 genomic regions associated with environment adaptability and muscle growth. These selected genomic regions contained genes, such as BCO1, AKR1D1, SVOPL, SMS, PHEX, PCYT1B, POLA1, and MEGF10. It means these genes expressed positive selection trend in a long period of evolution and artificial selection of Alxa Bactrian camel. The detected SNPs and InDels are enough to show genetic variations between Alxa Gobi and desert camel.

Acknowledgements

The project (No. 201502069) was supported by Inner Mongolia Science & Technology Plan.

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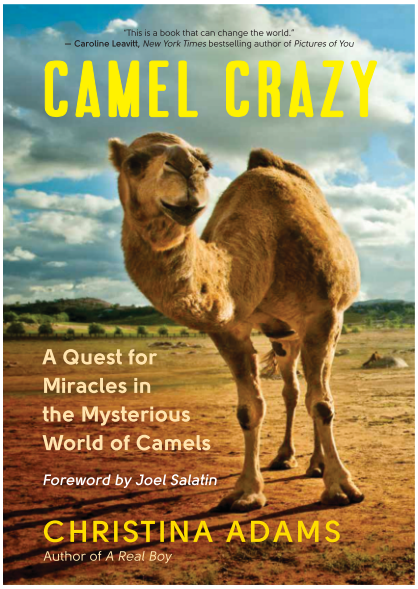
Origin and migration of domestic Bactrian camels through Whole-genome sequencing

Whole-genome sequencing of 128 camels across Asia was done by Chinese researchers. The extant wild and domestic Bactrian camels showed remarkable genetic divergence, as they were split from dromedaries. The wild Bactrian camels also contributed little to the ancestry of domestic ones, although they shared close habitat in East Asia. Interestingly, among the domestic Bactrian camels, those from Iran exhibited the largest genetic distance and the earliest split from all others in the phylogeny, despite evident admixture between domestic Bactrian camels and dromedaries living in Central Asia. Taken together, the study support the Central Asian origin of domestic Bactrian camels, which were then immigrated eastward to Mongolia where native wild Bactrian camels inhabit.

(Source: Ming, L., Yuan, L., Yi, L. *et al*, Whole-genome sequencing of 128 camels across Asia reveals origin and migration of domestic Bactrian camels. Commun Biol 3, 1 (2020). https://doi.org/10.1038/s42003-019-0734-6)

Camel Crazy author Christina Adams won a Nautilus Book Award 2019

The Nautilus Book Awards involve highly esteemed authors and publishers from across the USA, and from over 20 nations around the world. Author Christina Adams has won a Nautilus Book Award in the 2019 Nautilus Book Awards program. Camel Crazy: A Quest for Miracles in the Mysterious World of Camels has earned a silver award in the category of Health, Healing, Wellness and Vitality. Published by New World Library, the book is the story of a mother's quest to obtain camel milk for her son with autism and the resulting exploration of camel's special importance to culture, science and health for people everywhere. The book is sold internationally and has gained enthusiastic notice from general audiences, scientists, nomads and health experts in the US, India, UAE, UK and beyond. We congratulate Christina Adams for achieving this outstanding distinction as an author of her book- Camel Crazy.



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DOI : 10.5958/2277-8934.2020.00011.9 *Vol 27 No 1, p 77-79*

VIABILITY OF *Lactobacillus fermentum* CM36 AND *Lactobacillus rhamnosus* CW40 IN SKIMMED MILK DURING REFRIGERATION

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ABSTRACT

Two lactobacilli isolates namely *Lactobacillus fermentum* CM36 and *Lactobacillus rhamnosus* CW40 were used in present study. Both the lactobacilli isolates were grown in skimmed milk at 37°C for 24h. Fermented skimmed milk was stored at two different refrigeration temperatures, i.e. 4°C and -20°C. Viable cell count was determined in skimmed milk before storage and at an interval of 5 days during the total storage period of 20 days using standard pour plate method and the viability loss in percentage was calculated. Viable cell count of *L. fermentum* CM36 and *L. rhamnosus* CW40 was 52×10^9 cfu/ml and 88×10^9 cfu/ml, respectively in skimmed milk before storage. Viable cell count observed throughout the storage was in 10^9 cfu/ml range, 4°C proved to be a better storage temperature than -20°C for both the *Lactobacillus* isolates.

Key words: *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, skim milk, viability

Lactic acid bacteria, particularly those belonging to non-pathogenic and beneficial genera (*Lactobacillus*, *Pediococcus*, *Lactococcus, Streptococcus,* and *Leuconostoc*) are widely used in food industry as biopreservative agents (Arokiyamary and Sivakumal, 2011). A large number of *Lactobacillus* species are frequently used because of their antagonistic activity against pathogenic and food spoilage microorganisms (Uraz *et al,* 2001).

Health benefits of probiotic microorganisms should be documented with strains and dosage requirement (Guarner and Schaafsma, 1998). Viability of probiotic organisms is affected by low pH conditions during the process of fermentation, oxygen distribution during storage and high acidity conditions during storage or after consumption in human stomach (Shah, 2007; Kailaspathy and Chin, 2000). The present investigation was undertaken to study the viability of lactobacilli in fermented food product.

Materials and Methods

Source and Maintenance of Cultures: *Lactobacillus* used in the present investigation were previously identified using morphological, biochemical and molecular characterisation. *Lactobacillus fermentum* CM36 was isolated from raw camel milk and *Lactobacillus rhamnosus* CW40 was isolated from raw cow milk. Both the isolates showed probiotic properties such as antibacterial activity, bile salt tolerance and antibiotic resistance. Both the isolates were maintained using MRS broth, MRS agar after incubation at 37°C for 24 h and skimmed milk was used for storage of isolates.

Determination of viability in skimmed milk during storage: Each culture was grown in MRS broth and inoculated at 1% concentration in skimmed milk. Skimmed milk tubes were then incubated at 37°C for 24 h. After incubation, samples were stored in a refrigerator for 3 weeks. Viability was determined initially after 24 h which was kept as control. After that, the tubes were stored at 2 different temperatures i.e. 4°C and -20°C in refrigerator and deep freeze, respectively. Viability was determined by standard plate count method using MRS agar. Plates were incubated at 37°C for 48 h. The experiment was performed twice. The viable cell count was expressed as cfu/ml and calculated using the given formula:

$$\text{Number of cells/ ml} = \frac{\text{Number of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Results and Discussion

The viability was determined in cfu/ml and expressed in the form of percentage viability loss during storage period. The initial viable cell count of *L. fermentum* CM36 and *L. rhamnosus* CW40 after 24 h

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of incubation before any storage was 52×10^9 cfu/ml and 88×10^9 cfu/ml, respectively. During the storage period of 20 days, gradual decrease in viable cell count at both the refrigeration temperatures (4°C and -20°C) was observed. The data for the same has been presented in Figs 1 and 2, respectively.

Percentage viability loss observed in both the lactobacilli isolates is represented in Table 1. For *L. fermentum* CM36, viability loss at 4°C during 20 days of storage ranged between 11.53 to 80.76%. Residual percentage viability after the end of storage period of 20 days was 19.24%. At -20°C, viability loss ranged from 26.92 to 57.69% up to 15 days of storage and 100% loss of viability loss was observed at the end of storage period of 20 days. For *L. rhamnosus* CW40, viability loss at 4°C up to 15 days storage ranged from 77.27 to 97.72%, after 20 days of storage period 100% viable cells were lost. At -20°C, percentage viability loss ranged from 84.09 to 88.63%, 100% viability loss was observed at the end of 15 days of storage and onwards. In the case of *L. rhamnosus* CW40 viable cell count decreased sharply during storage. Problems with the stability of strains of lactobacilli in fermented milk products have been reported earlier (Gilliland and Speck, 1977 and Shah *et al,* 1995). The number of viable cells decline greatly with time indicating poor survival rate of *lactobacilli* as probiotic starter culture. Results of the present investigation were in agreement with the above mentioned studies in the case of *L. rhamnosus* CW40. The production of hydrogen peroxide by starter culture during storage may adversely affect the viability of probiotic culture (Hull *et al,* 1984), and this can be the probable reason of low viable cell count in the case of *L. rhamnosus* CW40.

Viable cells showed inverse relationship with duration of storage, as the storage period increases loss of viable cells was observed. Both the isolates *Lactobacillus fermentum* CM36 and *Lactobacillus rhamnosus* CW40 showed viable cell count at the concentration 10^9 cfu/ml in fermented skim milk during storage in refrigerator. For *L. fermentum* CM36

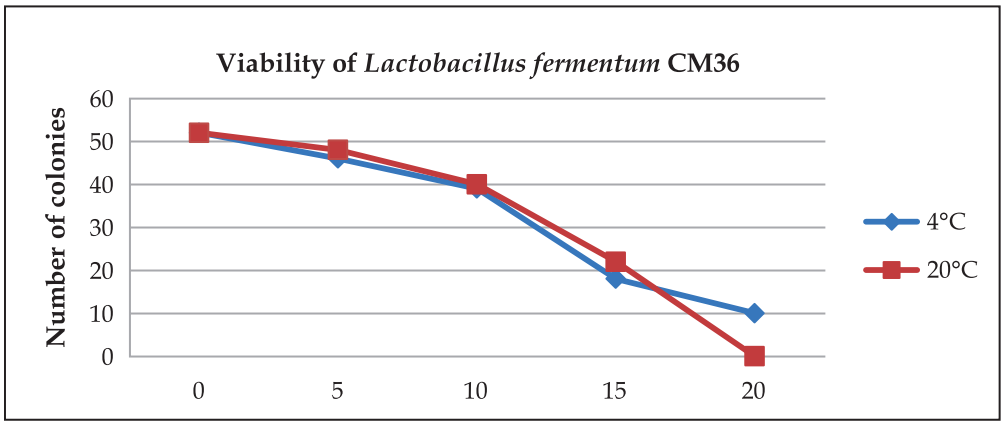

Fig 1. Number of colonies of *Lactobacillus fermentum* CM36 in skim milk during storage at 4°C and -20°C during 20 days of storage.

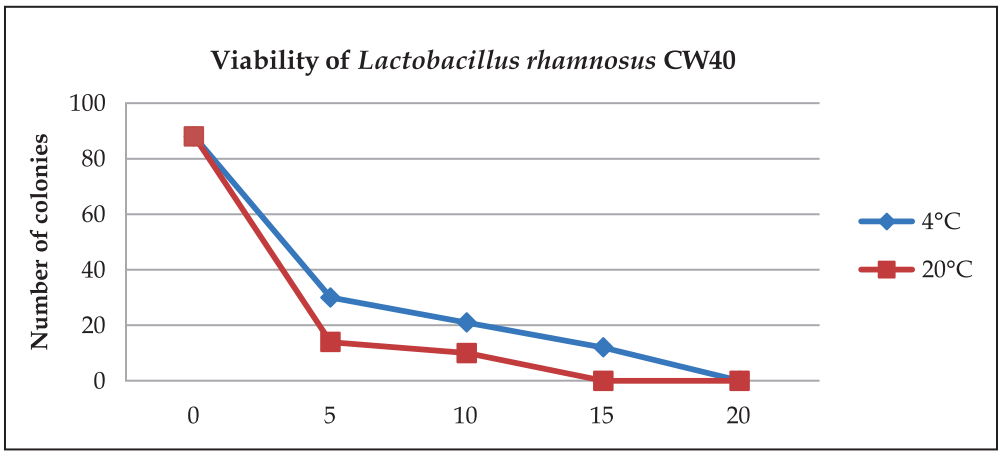

Fig 2. Number of colonies of *Lactobacillus rhamnosus* CW40 in skim milk during storage at 4°C and -20°C during 20 days of storage.

Table 1. Percentage viability loss of CM36 and CW40 during 20 days of storage at 4°C and -20°C, at a 5 days interval (Compared to the reference point of initial viable count after inoculation in skim milk and 24 h incubation without storage).

S.No.	Lactobacilli isolates	Storage temperature	Storage period (days)			
			5	10	15	20
1	*Lactobacillus fermentum* CM36	4°C	11.53%	25.0%	65.38%	80.76%
		-20°C	26.92%	42.30%	57.69%	100%
2	*Lactobacillus rhamnosus* CW40	4°C	77.27%	88.63%	97.72%	100%
		-20°C	84.09%	88.63%	100%	100%

viable cells were present at 4°C for 20 days and at -20°C for 15 days. For *L. rhamnosus* CW40 viable cells were present at 4°C for 15 days and at -20°C for 10 days. There was an adequate number of probiotic bacteria needed to be consumed in order to avail their health benefits and the minimal number of probiotic bacteria in a product should be above 10^6 or 10^7 per gram or ml to exhibit health benefits (Lahteinen *et al*, 2010). Results of the present investigation were in accordance with the above mentioned study. The rate of survival of both *L. fermentum* CM 36 and *L. rhamnosus* CW 40 in skimmed milk was significantly greater at 4°C than -20°C. Similar findings suggesting better survival rate of lactobacilli at 4°C was given by Canganella *et al* (2000).

It was concluded that *Lactobacillus fermentum* CM36 and *Lactobacillus rhamnosus* CW40 showed good viability during refrigeration and also possessed antibacterial activity, bile tolerance and antibiotic resistance. Therefore, both isolates proved to be potential strains and can be used as a starter culture for the production of fermented food products to impart health benefits to the consumer.

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World's first camel hospital at Dubai set to expand its facilities

World's first camel hospital at Dubai is set to expand its facilities by an additional 50 per cent in response to the increase in demand for its services, according to Mohammad Alblooshi, Director, Dubai Camel Hospital.

The camel hospital, built at an estimated cost of 40 million Dirhams, opened its doors in 2017 to meet the demand in the UAE for an advanced medical facility dedicated to treating camels. Since its inception, the hospital has attracted the interest of large number of camel owners or breeders. According to the veterinary hospital, the facilities have been enlarged to treat over 30 camels simultaneously. It currently has capacity for 22.

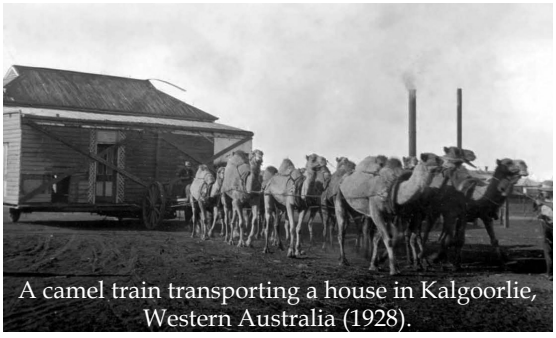
The hospital's customised equipment was adapted from equestrian medical equipment to accommodate camel treatment and the facility is also equipped with a mini-race track to rehabilitate camels after their medical procedures.

(Source: All India Radio)

The Rise and Fall of Camels in Australia

The colonial government in New South Wales initially explored the possibility of importing camels from India in 1836. But later 46 of them were to be imported from Canary Islands in October 1840. However, only one, named Harry, survived the long sea journey. Camels were in demand in dry regions of outback Australia as they could be used for travel and transportation during long journeys (even without drinking water) into the continent's interiors. By 1907, the British had imported nearly 20,000 camels into Australia, largely from India, Afghanistan and the Arab world.

From 1920s, the population of domestic camels in Australia began declining due to use of automobiles and abandoned camels were released in the open, as feral and they started multiplying rapidly. In 1969, population of such feral camels was estimated to be between 15,000 and 20,000. Feral camels became about 43,000 in 1988 in Australia. Later after the year 2000, the population started increasing every year and that rose to 6,00,000 and still more. Wildlife experts warned Australian authorities that if this population growth went unchecked, their number would double in 8-10 years. Soon, the Australian government adopted the National Feral Camel Action Plan and began massive culling operations. By 2013, the camel count was brought down to about 3,00,000.



A camel train transporting a house in Kalgoorlie, Western Australia (1928).

Today, feral camels are found in an area of 3.3 million sq km across the Western Australia, South Australia, Queensland and the Northern Territory. Thus, in the 180 years between their introduction in 1840 to the present times, camels in Australia have a come a long way: from being a valuable asset to a wild "pest".

On January 8, 2020, culling operation began in South Australia's remote areas and nearly 10,000 camels were targeted to shot down. The order was passed by the local government, Anangu Pitjantjatjara Yankunytjatjara (APY) Lands. It said the camel groups "pose threats" to communities, scarce water resources and are destroying and eating up food supplies, necessitating "immediate camel control". The damage caused by feral camels has been estimated at over $10 million each year.

(Courtesy:https://www.indiatoday.in/world/story/astonishing-story-of-australian-camels-why-thousands-of-them-are-shot-dead-routinely-1635687-2020-01-11
and https://www.feralscan.org.au/camelscan/pagecontent.aspx?page=camel_largepopulations)

DOI : 10.5958/2277-8934.2020.00012.0 *Vol 27 No 1, p 81-88*

URIDINE 5′-MONOPHOSPHATE (UMP) METABOLISING ENZYMES URACIL PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE-5′-PHOSPHATE DECARBOXYLASE/UMP SYNTHASE IN CAMELS AND *Trypanosoma evansi*

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ABSTRACT

In this study, the metabolic pathways and the enzymes involved in uridine 5′-monophosphate (UMP) were investigated in camel and the blood parasite *Trypanosoma evansi* (*T. evansi*). The pyrimidine pathway of *T. evansi* was found to be devoid of uridine kinase and uridine 5–nucleotidase. Since this can affect the de novo synthesis using uracil, salvage enzymes as uracil phosphoribosyltransferase (UPRTase) could be important for the parasite life, given that the similarity rate is not more than 32% between the camel and *T. evansi* UPRTase. The source of UMP in *T. evansi* could be from uracil by the action of UPRTase. In addition, the bifunctional orotidine 5′-phosphate decarboxylase (OMPdecase)/UMP synthase shares also in UMP homeostasis. Owing to the diverse sources of UMP in *T. evansi*, the enzymes involved in UMP metabolism are underscored for drug discovery. However, supported by the lack of uridine kinase, further studies are recommended to estimate the impact of UPRTase inhibition on *T. evansi* growth.

Key words: Camel, OPRTase, pyrimidine, *Trypanosoma evansi*, UMP, UPRTase

Bioinformatics has largely enforced drug discovery process by rapid identification of a unique target for the life of pathogens. The recent announcement of camel genome (Jirimutu *et al,* 2012) had led it feasible to compare the target genes in camels and its corresponding in the pathogens. Previously, bioinformatics were used as tools in identification and characterisation of drug targets (Kandeel *et al,* 2019a; Kandeel *et al,* 2019b).

The pyrimidine pathway is essential for every organism for the synthesis of pyrimidine nucleotides. In this work, the metabolic pathways of UMP was investigated in the camel as well as in the blood parasite *T. evansi*. The KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016) were used to map the enzymes involved in UMP binding.

At first, the standard metabolic paths are mapped followed by bioinformatics tools to find the most probable enzymes involved in these paths in camel and *T. evansi*. The comparison involved sequence similarity rate, the motif and domain content and phylogenetic mapping. These values were used in the context of comparison with human, camel species and various prokaryotic and eukaryotic enzymes.

Materials and Methods

Retrieval of genomic data

Collection of genomic data was carried out by extracting the information from the gene database (Hertz-Fowler *et al,* 2004), Kinetoplastom genome resources, protein and genome databases and the Arabian camel genome project information obtained from these sources included protein and nucleotide sequence information, gene annotation, paralogs and orthologs information, metabolic functions, signal peptides, transmembrane domains, predicted protein export domains, protein expression profiles, isoelectric point (pI), the number of predicted transmembrane helices, any predicted sequence motifs, and the E value of the closest PDB sequence homolog.

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Searching homologues

Protein sequence homologues was searched using the NCBI BLAST (Basic Local Alignment Search Tool) (Madden 2013) or PSI-BLAST (Position-Specific Iterated-BLAST) servers searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment program for proteins and construction of phylogenetic tree. It was carried out as per methods described previously (Sievers and Higgins, 2014). Multiple sequence alignment program (ClustalW2) was used to calculate the best match of the selected sequences. The resultant alignment was used to generate a phylogenic tree, which is visualised by Dendroscope phylogenic tree viewer (Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains— It was searched by the domain prediction program available at (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Proteomic and genomic tools

ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) was also used for analysis of nucleotide and gene sequences. Protein sequences of target genes was analysed for data such as pI, extinction coefficient and MW for the tagged protein sequence by PROTParam. PROSITE was used to search for patterns and profiles in the protein sequences of the target genes. The protein domains, families and functional sites was searched at http://prosite.expasy.org/.

Results and Discussion

Uridine-5′-monophosphate (UMP) production process was proposed by five production routes 1) phosphorylation of uridine 5′-triphosphate by the action of nucleotide diphosphatase, 2) the phosphorylation of uridine-5′-diphosphate by the actions of nucleoside diphosphate phosphatase and/or ATP-diphosphatase or by the action of UMP/CMP kinase and/or UMP kinase 3) from uridine by the kinase activity of uridine kinase 4) from uracil by the action of uracil phosphoribosyl transferase 5) the decarboxylation of orotidine-5′-phosphate by the action of orotidine-5′-phosphate decarboxylase as shown in Fig 1. In the catabolic direction, uridine is produced from UMP by 5′-nucleotidase. The enzymes involved in UMP pathways are shown in Tables 1-3.

Table 1. Enzymes involved in metabolic pathways of UMP.

ID (E.C. number)	Definition (Enzyme name)
3.6.1.8	ATP diphosphatase
2.7.1.48	Uridine kinase
3.1.3.5	Uridine 5′-nucleotidase
2.4.2.9	Uracil phosphoribosyltransferase
4.1.1.23	Orotidine-5′-phosphate decarboxylase
3.6.1.5	Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
3.6.1.6	Nucleoside diphosphate phosphatase
2.7.4.22	UMP kinase
2.7.4.14	UMP/CMP kinase
3.6.1.9	Nucleotide diphosphatase

Table 2. The expected enzymes involved in metabolic pathways of UMP in camels.

ID (E.C. number)	Definition (Enzyme name)
2.4.2.9	Uracil phosphoribosyltransferase
3.6.1.5	nucleoside triphosphate phosphohydrolases (Nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
2.7.4.14	UMP/CMP kinase

Table 3. The expected enzymes involved in metabolic pathways of UMP in *Trypanosoma evansi*

ID (E.C. number)	Definition (Enzyme name)
2.4.2.9	Uracil phosphoribosyltransferase
4.1.1.23	Orotidine-5′-phosphate decarboxylase
3.6.1.5	Nucleoside triphosphate phosphohydrolases (nucleoside monophosphoate-forming) or ATP- diphosphatase, apyrase, ectonucleoside triphosphate diphosphohydrolase
2.7.4.14	UMP/CMP kinase

After bioinformatics investigation, *T. evansi* was found to be devoid of uridine kinase and uridine 5′-nucleotidase (Fig 1-3). Therefore, uridine salvage is brought by uracil phosphoribosyltransferase.

Uracil phosphoribosyltransferase

Uracil phosphoribosyltransferase (EC 2.4.2.9; UMP pyrophosphorylase; UPRTase). Uridine nucleotides could be formed by energy-

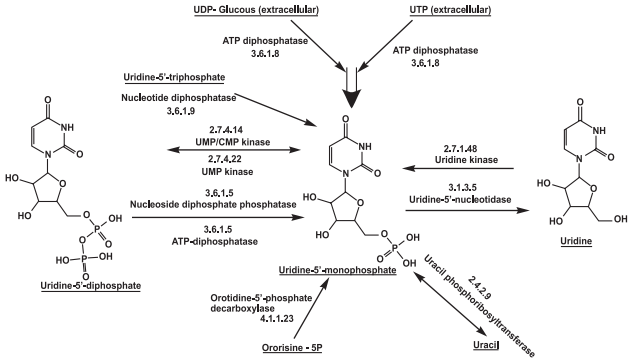

Fig 1. The proposed metabolic pathways of Uridine 5 monophosphate (UMP).

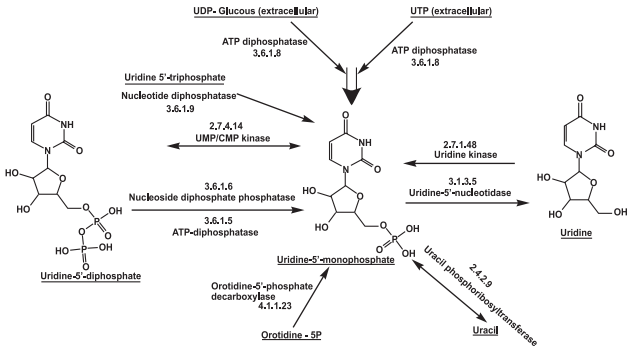

Fig 2. The proposed metabolic pathways of Uridine 5 monophosphate (UMP) in camels.

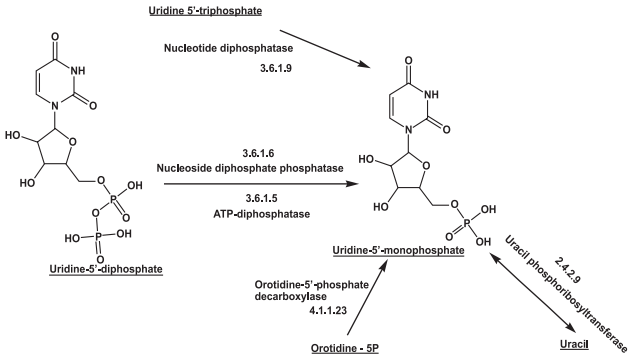

Fig 3. The proposed metabolic pathways of Uridine 5 monophosphate (UMP) in *Trypanosoma evansi*.

using pyrimidine de novo biosynthesis or by the energy-saving retrieving of nucleosides resultant of nucleotide catabolism. The UPRTs is energy-saving pentosyltransferases enzyme involved in the metabolism of uracil and related compounds that catalysing reversible salvage of pyrimidines by catalysing the formation of uridine monophosphate from uracil and 5-phospho-alpha-D-ribose 1-diphosphate (Mainguet *et al,* 2009). The UPRTase activity in *Giardia intestinalis* was remarkably increased by GTP and dGTP with no effect on the Michaelis constants. The GTP exhibited similar effect for UPRTase from other prokaryotes as *E. coli* but not from other eukaryotes (Dai *et al,* 1995). The protozoan parasite *Leishmania donovani* uracil phosphoribosyltransferase (LdUPRT), is an enzyme not found in mammalian cells, is inhibited by uracil and 4-thiouracil, nonetheless 5-fluorouracil toxicity emerges through another mechanism (Soysa *et al,* 2013).

There was high similarity rate (89%) between the camel and human UPRTase (Fig 4). Meanwhile, there was little difference of only 2-3 amino acids replacement between the three camel species, dromedary, Bactrian and feral camels (Fig 5).

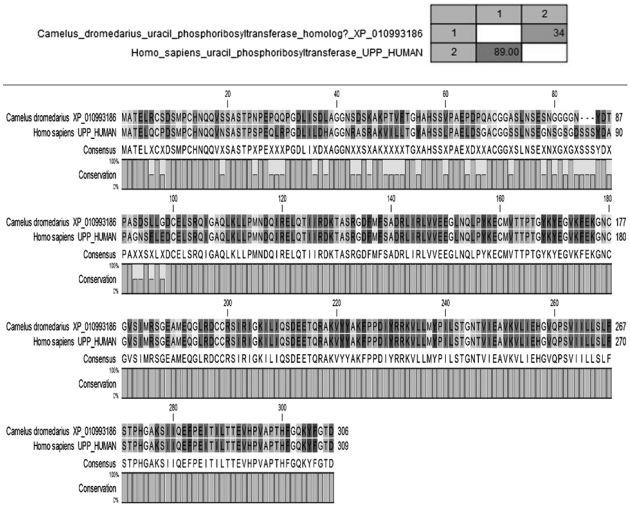

Fig 4. Multiple sequence alignment of dromedary camel and human uracil phosphoribosyltransferase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

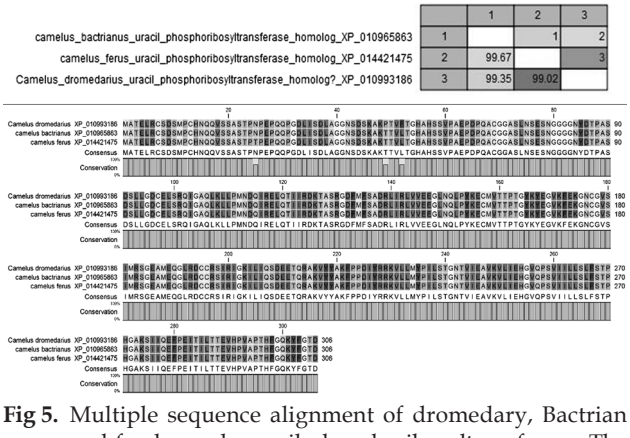

Fig 5. Multiple sequence alignment of dromedary, Bactrian and feral camels uracil phosphoribosyltransferase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences

In comparison of camel UPRTase with other vertebrate enzymes, the differences were less than 39 amino acid replacement with high similarity rate of 90-99%. There was great and obvious difference between the camel and prokaryotic and protozoal enzymes and differences were up to 227. This indicates large amount of amino acid differences between camel and lower organisms UPRTases. Interestingly, direct comparison of camel and *T. evansi* (Fig 6) revealed high rate of differences with similarity not exceeding 30.12% and 225 differences. Additionally, the camel UPRTase had 305 amino acids as compared to 240 amino acids in *T. evansi*.

The phylogenetic analysis of UPRTase in different prokaryotic and eukaryotic UPRTases revealed distant relation between the camel and *T. evansi*. The *T. evansi* UPRTase was highly related to the bacterial enzyme and very distant from the camel enzyme (Fig 7).

Domains and motifs search of UPRTase from camel and *T. evansi* revealed the presence of one domain of PRTase-1 superfamily including uracil (Tables 4 and 5).

Table 4. The motif and domain content of camel and *T. evansi* UPRTase.

Trypanosoma		
Pfam	**Position (Independent E-value)**	**Description**
Trypanosoma		
UPRTase	29..238(1.6e-68)	PF14681, Uracil phosphoribosyltransferase
Pribosyltran	139..178(4.5e-06)	PF00156, Phosphoribosyl transferase domain
Camel		
UPRTase	113..291(1.1e-53)	PF14681, Uracil phosphoribosyltransferase
Pribosyltran	223..275(0.0003)	PF00156, Phosphoribosyl transferase domain

Orotidine-5′-phosphate decarboxylase (UMP synthase) uridine 5′-monophosphate synthase

Orotidine-5′-phosphate decarboxylase (orotidine-5′-monophosphate (OMP) decarboxylase; 4.1.1.23). The OMP decarboxylase (OMPdecase) is a carboxy-lyase enzyme from higher eukaryotes and is equivalent with orotate phosphoribosyltransferase (EC 2.2.2.10). Moreover, OMPdcase is one of most competent enzymes that catalyses the decarboxylation of orotidine-5′- monophosphate (OMP) to uridine-5′-monophosphate (UMP)(Kotra and Pai, 2008). Furthermore, OMP-DC has studied as a drug target.

Table 5. The motif and domain content of camel and *T. evansi* OMPdecase.

Trypanosoma		
Pfam	**Position (Independent E-value)**	**Description**
OMPdecase	17..245(1.5e-34)	PF00215, Orotidine 5′-phosphate decarboxylase / HUMPS family
Pribosyltran	294..410(1.4e-17)	PF00156, Phosphoribosyl transferase domain
PRTase_2	363..404(0.00099)	PF15609, Phosphoribosyl transferase
UPRTase	365..413(0.00096)	PF14681, Uracil phosphoribosyltransferase
PUA	360..399(0.15)	PF014 PUA domain
Camel		
OMPdecase	252..466(1.5e-73)	PF00215, Orotidine 5′-phosphate decarboxylase / HUMPS family
Pribosyltran	44..162(1.3e-13)	PF00156, Phosphoribosyl transferase domain

Figure legends

In which a potent ligands of OMPdcase with diverse structures were examined for structural interactions with the active site of OMPdcase as trials to design novel inhibitors of OMPdcase. These ligands involve of pyrazole or pyrimidine nucleotides comprising the mononucleotide derivatives of pyrazofurin, barbiturate ribonucleoside, and 5-cyanouridine, in addition to, 1,4-dihydropyridine-based non-nucleoside inhibitors such as nifedipine and nimodipine. Binding of these ligands to OMPdecase active site displaying diverse interactions paving the way to design novel inhibitors against OMPdecase (Meza-Avina *et al*, 2010).

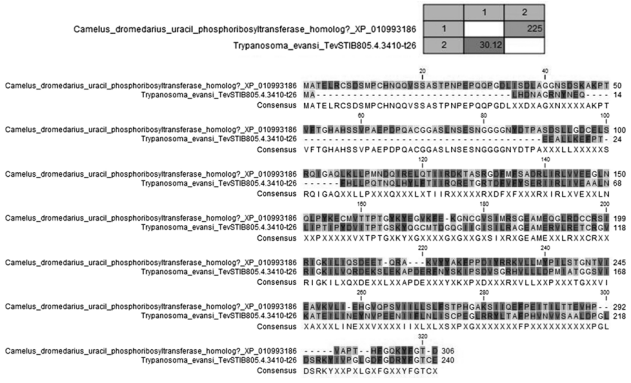

Fig 6. Pairwise sequence alignment of camel and *T. evansi* uracil phosphoribosyltransferase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences

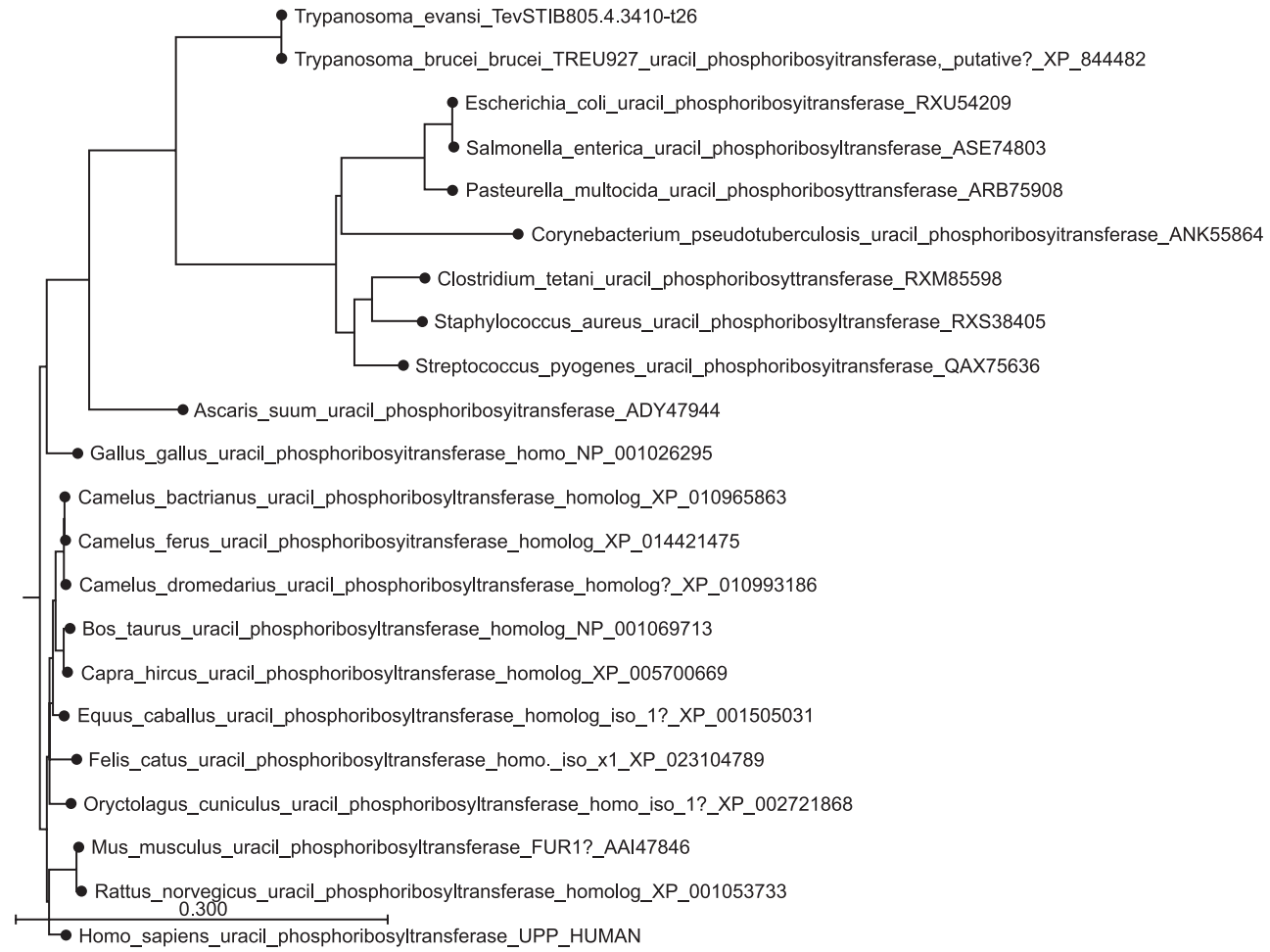

Fig 7. Phylogram of camel and Trypanosoma uracil phosphoribosyltransferase in relation to a set of eukaryotic organisms.

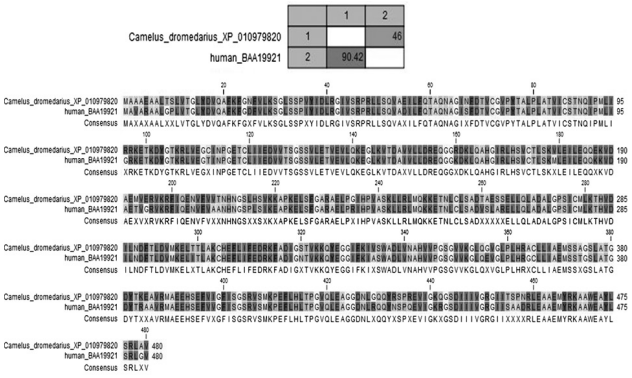

Fig 8. Pairwise sequence alignment of human and camel orotidine-5′-phosphate decarboxylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Both camel and human OMPdecase retained high similarity of 90.42% (Fig 8) with prefect complementarity of dromedary and Bactrian camels (Fig 9). The camel and *T. evansi* shared a common feature of OMPdecase length more than 450 amino acids. However, the similarity rate was as low as 18.15% (Fig 10). There was similar high similarity between the camel and other vertebrate OMPdecase with less than 67 differences (Fig 11), which extend to more than 450 differences with bacterial or protozoal enzyme.

The phylogenetic analysis of OMPdecase was having a similar pattern of UPRTase by showing distant relation between the camel and *T. evansi*. The *T. evansi* UPRTase was highly related to the bacterial enzyme and very distant from the camel enzyme (Fig 12).

The enzyme is monofunctional (about 233 amino acid) in prokaryotes, while in eukaryotes including camel and TEV the protein is about 450 amino acid and composed of two domains or bifunctional protein for UMP synthesis. Domains and motifs search of OMPdecase from camel and *T. evansi* revealed the presence of two predicted domains in camel and 5 domains in *T. evansi* (Table 5). In camel,

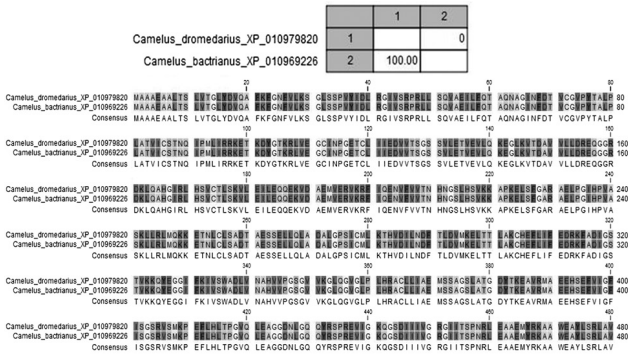

Fig 9. Pairwise sequence alignment of dromedary and Bactrian camels orotidine-5′-phosphate decarboxylase (UMP synthase). The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

the two domains include the OMPdecase and a PRTase domain. In *T. evansi*, PRTase-2 and UPRTase and confirmed with high E-values.

A recent report suggested that a null strain of UMP synthase in *T. brucei* affected the lethality of blood infection in mice (Ong *et al,* 2013). However, this was bypassed after longer culture time due to conversion of orotate to uridine by the host enzymes (Ong *et al,* 2013).

UMP-CMP kinase (UMP/CMP kinase)

A bifunctional enzyme present in eukaryotes and catalyses the phosphorylation of CMP and UMP. Prokaryyotes has two different monofunctional enzymes EC 2.7.4.25, which act as CMP kinase and EC 2.7.4.22, which act as UMP kinase.

UMP kinase

Uridylate kinase; UMPK, 2.7.4.22) has restricted kinase activity for UMP only and specific for prokaryotes. It is replaced by the bifunctional UMP-CMP kinase.

Nucleoside triphosphate phosphohydrolases

EC no. 3.6.1.5 present in vertebrates including camel and some protozoa. The enzyme was previously found by experimental procedures (Weiss *et al,* 2015), yet it was not found in the gene sequence databases after searching by accession number or name of enzyme.

Conclusions

Investigation of UMP metabolising enzymes in camel and *T. evansi* revealed the absence of uridine conversion to UMP due to the lack of

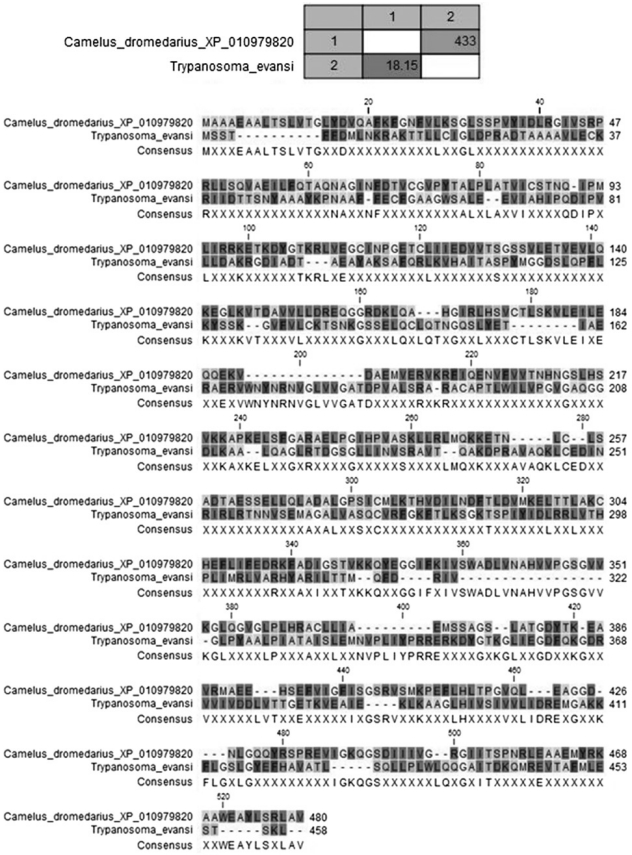

Fig 10. Pairwise alignment of dromedary camel and *T. evansi* orotidine-5′-phosphate decarboxylase (UMP synthase). The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

uridine kinase. In this context, two other enzymes are important, including UPRTase and OMPdecase. Both enzymes showed high sequence variability and distant evolutionary pattern. Inhibition of UPRTase might be of a value due to the absence of uridine kinase. However, the abundance of UMP synthetic pathways, the adaptation of parasite or compensatory mechanisms leading to UMP production and the high mw of the enzymes might affect the lab identification of these enzymes. These factors underscores using the UMP pathway as a drug target. However, more details on UPRTase inhibition in *T. evansi* is required.

Acknowledgements

The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programs, National Transformation Program, under Research and Development Grants Program for National Research Institutions and Centres (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Camelus_dromedarius_XP_010979820	1		0	0	2	1	1	0	92	92	255	235	238	241	273	273	305	310	306
Camelus_bactrianus_XP_010969226	2	0		0	2	1	1	0	92	92	255	235	238	241	273	273	305	310	306
human_BAA19921	3	46	46		2	1	1	0	92	92	255	235	238	241	273	273	305	310	306
Felis_catus_XP_003991769	4	48	48	49		3	3	2	94	94	257	237	240	243	275	275	307	312	308
Mouse_NP_033497	5	67	67	57	73		0	1	93	93	254	234	237	242	272	272	304	309	305
Rattus_norvegicus_AAH98033	6	64	64	51	68	16		1	93	93	254	234	237	242	272	272	304	309	305
Bos_taurus_AAI12873	7	67	67	77	70	87	85		92	92	255	235	238	241	273	273	305	310	306
Trypanosoma_brucei_brucei_TREU927_orotidine-5-phosphate_decarboxylase,_putative_(XP_845051)	8	428	428	431	432	431	428	432		0	257	245	280	271	285	285	315	314	312
Trypanosoma_evansi	9	428	428	431	432	431	428	432	0		257	245	280	271	285	285	315	314	312
Babesia_bovis_T2Bo_orotidine-5'-phosphate_decarboxylase_(XP_001611096)	10	457	457	462	459	461	461	463	471	471		24	247	246	190	190	158	159	161
Plasmodium_falciparum_3D7_orotidine-5'-phosphate_decarboxylase_(XP_001347509)	11	461	461	460	464	459	458	459	485	485	225		255	262	208	208	176	181	183
Clostridium_tetani_orotidine-5'-phosphate_decarboxylase_(RYU99202)	12	457	457	460	459	456	457	457	482	482	383	396		39	197	197	225	228	234
Corynebacterium_pseudotuberculosis_orotidine-5'-phosphate_decarboxylase_(RKT29909)	13	453	453	453	454	452	453	450	472	472	378	394	220		210	210	236	233	239
Escherichia_coli_orotidine-5'-phosphate_decarboxylase_(QAY44275)	14	450	450	452	453	453	453	448	483	483	347	366	354	351		0	32	39	39
Salmonella_enterica_orotidine-5'-phosphate_decarboxylase_(QBA00760)	15	456	456	456	459	459	459	454	481	481	345	364	357	354	34		32	39	39
Pasteurella_multocida_orotidine-5'-phosphate_decarboxylase_(ATN16523)	16	454	454	453	456	454	455	455	479	479	314	330	354	356	119	118		9	9
Staphylococcus_aureus_orotidine-5'-phosphate_decarboxylase_(RYV48081)	17	463	463	464	463	462	463	462	479	479	313	333	357	355	175	171	135		6
Streptococcus_pyogenes_orotidine-5'-phosphate_decarboxylase_(QBB63177)	18	466	466	463	465	463	464	464	484	484	308	332	357	352	169	169	139	104	

Fig 11. Multiple sequence alignment of Dromedary camel and *T. evansi* orotidine-5′-phosphate decarboxylase (UMP synthase) with some prokaryotic and eukaryotic enzymes. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

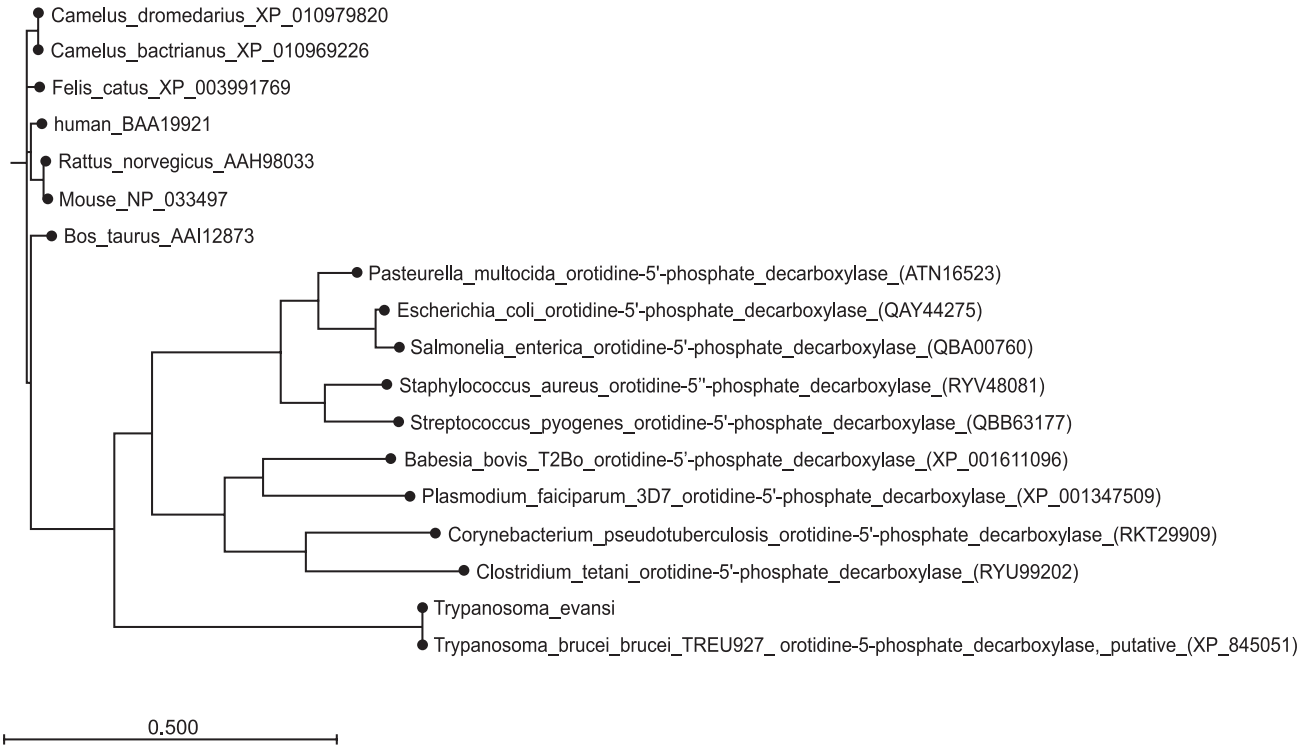

Fig 12. Phylogram of Trypanosoma orotidine-5′-phosphate decarboxylase in relation to a set of eukaryotic and prokaryote organisms.

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DOI : 10.5958/2277-8934.2020.00013.2 *Vol 27 No 1, p 89-96*

ULTRASTRUCTURAL AND MORPHOMETRIC STUDIES ON THE BULBOURETHRAL (COWPER'S) GLAND OF CAMEL (*Camelus dromedarius*)

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ABSTRACT

The bulbourethral glands of 40 dromedary camels were studied histologically, ultrastructurally and morphometrically. Three types of secretory units were encountered in the gland designated as type A, B and C. Type A cells were pyramidal or columnar in shape containing irregular nuclei. These were characterised by a cytoplasm packed with electronlucent granules. Type B units usually had dark or light cells with spherical nuclei. Type C units consisted of both type A and type B cells. Interestingly, myoepithelial cells were distinguished in this gland. Morphometric data showed that the glandular tissue occupied the bulk of the gland (54.02%) compare to the connective tissue and muscle (35.23). The ducts, blood vessels and nerves had volume densities of 6.39% and 4.36%, respectively.

Key words: Bulbourethral gland, camel, Camel Cowper's gland, morphometry, ultrastructure

The bulbourethral glands have been studied in various species, i.e. rat (Adebayo *et al*, 2015) boar (Aitken, 1960), bull (Trotter, 1959; Salisbury and Vandemark, 1961; Faulkner, 1969; Kainer *et al*, 1969; Campero *et al*, 1988), man (Riva *et al*, 1990); ram (Aitken, 1959; Khalaf and Merhish, 2010), stallion (Bradley, 1948; Bharadwaj and Calhoun, 1962), donkey (Abou-Elhamd *et al*, 2012) and water buffalo (Abou-Elmagd and Wrobel, 1989).

Nevertheless, the gland in the camel received little attention. Apart from the work of Lesbre (1903) who reported the presence of the bulbourethral glands and absence of the seminalis in the camel, only a few studies were recorded giving brief accounts on the morphology, histochemistry or morphometry of the bulbourethral gland (El-Wishy *et al*, 1972; El-Wishy, 1988; Ali *et al*, 1976, 1977; Mosallam, 1981; Badawi and Yousef, 1982; Degan and Lee, 1982; Marroni *et al*, 1982; Abou-Ahmed *et al*, 1988; Hafez and Hafez, 2001; Luo *et al*, 2016; Abdullahi *et al*, 2016).

However, ultrastructure or morphometry of the bulbourethral gland were not reported previously in camels. Current research was thus aimed to study the ultrastructure and morphometry of the bulbourethral gland of the dromedary camel.

Materials and Methods

Bulbourethral glands were collected from 40 apparently healthy mature camels, immediately after slaughtering at Tambul Slaughterhouse, Sudan.

Light Microscopy

Samples from 11 animals were used for the preparation of histological sections. Tissue pieces from the bulbourethral glands were fixed either in Bouin's fluid, 10% formal-saline, 10% formalin or Zenker formal. Sections, 5 mm^3 thick were cut from the different levels of the gland, then processed for normal paraffin techniques. Paraffin sections at 3-5µm were cut and stained with Haematoxylin and Eosin (H&E) or Masson's Trichrome.

Electron Microscopy

Material for ultrastructural studies were obtained from 9 animals. Small pieces (1mm^3) of tissues were fixed in 2.3% glutaraldehyde in 2.14% sodium cacodylate buffer pH 7.4, for 2 hours. They were then washed in 2.14% sodium cacodylate pH 7.4, postfixed in 2% osmium tetraoxide in 2.14% sodium cacodylate buffer pH 7.4, for 2 hours. They were then washed in 2.14% sodium cacodylate buffer pH 7.4 twice for 30-60 minutes. Dehydration was carried

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out in ascending grades of acetone or ethanol 50%, 70%, 90%, 95% and 100% for 30-60 minutes each. The materials were prestained in 2% uranyl acetate and 2% phospho-tungstic acid at 4°C for 20 hrs and then embedded in resin.

Semi-thin sections (0.5-1.0µm) were cut on Reichert ultramicrotome (Germany) using glass knives, stained with toluidine blue and examined with light microscope. The desired regions for electron microscopy were then selected and ultrathin sections, pale gold to silver (70-90 nm), were cut with glass knives. The sections were mounted in uncoated copper grids, double-stained with uranyl acetate for 5 minutes, washed in distilled water and placed in lead citrate for 30-45 seconds. They were then washed dried and studied in a Zeiss EM 109 electron microscope (Germany).

Morphometry

Tissue samples were collected from 20 animals. The weight and volume of the organs were measured. The volume was determined by water displacement method (Scherle, 1970).

Each gland was cut into four slices about 5 mm thick. Since the slices were more or less identical it was necessary to analyse all of them and so every second slice was taken for histological processing. The slices from each animal were used for morphometric analysis. Sections were cut at 2-5 µm thick and stained with Masson's Trichrome.

In order to determine the optimum number of points to be counted for each component of the gland, one slice was completely analysed field by field, using a 100-point integrating eye piece (Zeiss). The sufficient number of points necessary to count from each component to keep the standard error below 5% was then determined by the plots of Weibel (1963) and Dunnill (1968). Parameters like blood vessels and nerves occupied relatively small volumes and did not fall within the scope of the plots. The objective lens of X20 was used in the analysis of sections.

The volume densities of the components of the gland were taken as means of the results of analysis of all sections. The absolute volumes of these components were calculated from their volume densities (Vv) and from the total volume (V) of the gland (i.e. Absolute volume Vv. V).

The statistical analysis of the data obtained by point-counting was restricted to determination of the means and standard deviation (Weibel, 1963).

Results

Light Microscopy

The secretory units of the glands were lined with one layer of pyramid, cuboidal or columnar cells, which extended from the basal lamina to the lumen. The average cellular height was about 21 µm. There were three types of secretory units in the bulbourethral gland designated as type A, B and C. These units have been studied ultrastructurally and an account on each together with that on the basal cells herewith follows.

Electron microscopy

Type A units

The cells which constitute type A units were tall pyramidal or columnar in shape and possessed infolded plasma membrane. The lateral membranes were straight and the apposed surfaces were held together by moderate junctional complexes (Fig 1).

The nucleus was irregular flat and pushed down towards the basal cytoplasm (Fig 1). The euchromatin was evenly distributed, but heterochromatin was concentrated around the peripheral parts. One or two nucleoli, irregular in shape, were eccentrically placed in the nucleoplasm. The nucleolus had a dense band in its outer part and a lighter central portion divided into two or three parts (Fig 1).

The cell contained a large Golgi complex which was consisting of several parallel arrays of cisternae and vacuoles of various sizes and shapes. It was observed adjacent to the nucleus (Fig 2).

Mitochondria were observed possessing various forms, i.e. oval, rounded and elongated (Fig 3), but they were scattered throughout the cytoplasm nevertheless tended to concentrate in the peripheral parts. The endoplasmic reticulum consisted of branching and anastomosing tubules distributed throughout the cytoplasm (Fig 4).

The entire cytoplasm was filled with closely packed electron lucent granules of different sizes and shapes. Many of these granules coalesced with each other to from large irregularly shaped units. Small electron dense inclusions were seen frequently inside the large secretory granules (Fig 1). Few lysosomes and fat droplet were also encountered.

Type B unit

The epithelial lining of type B units contained two types of cells; dark and light (Fig 5). Some units had basal cells wedged between the columnar cells and the basement membrane (Fig 6). Myoepithelial

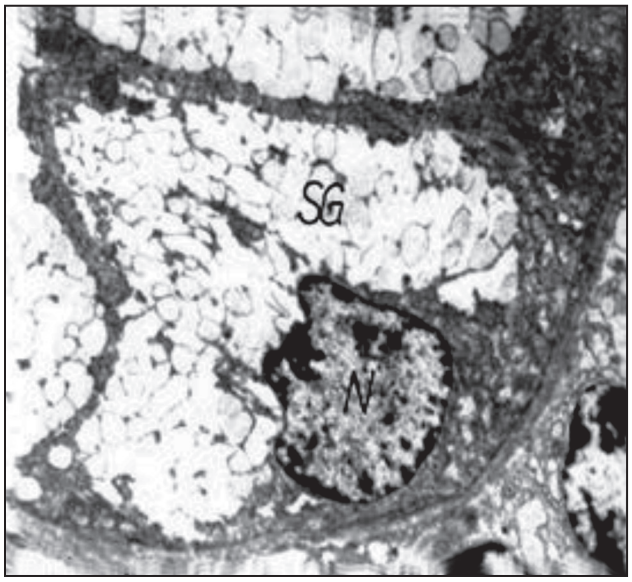

Fig 1. SEM micrograph of Bulbourethral gland. Type A unit showing pyramidal cells with flat and basal nucleus (N). Note the nucleolus. The cell is filled with electron lucent secretory granules (SG). 42200X.

Fig 3. SEM micrograph of Bulbourethral gland. Type A unit. Note the different forms of the mitochondria (M). The matrix traversed by lamellar Cristae. 60000 X.

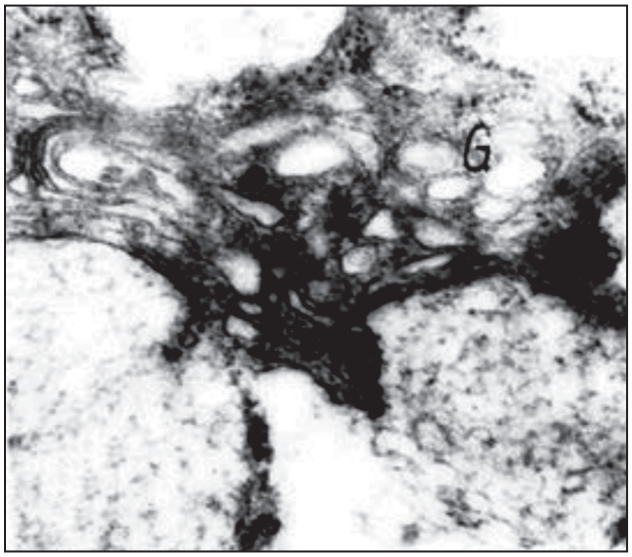

Fig 2. SEM micrograph of Bulbourethral gland. Type A unit. A large Golgi complex (G) consisting of several parallel arrays of cisternae and vacuoles of various sizes and shapes . 60000X.

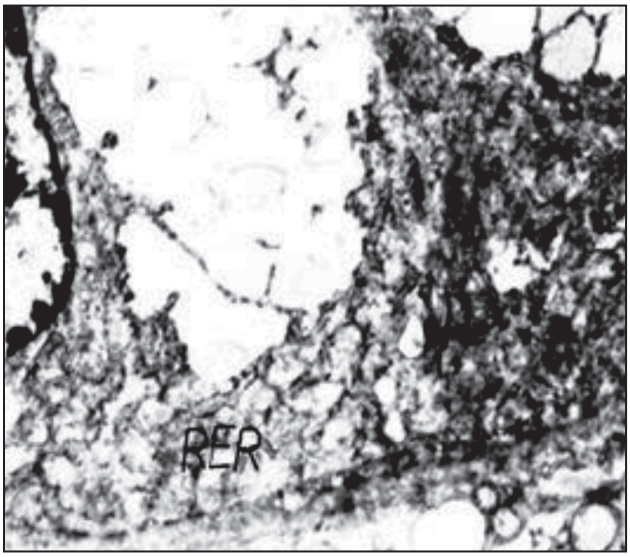

Fig 4. SEM micrograph of Bulbourethral gland. Type A unit. Rough endoplasmic reticulum (RER). 100000 X.

cells were seen in close association with outer parts of the units (Fig 7). The luminal border of the columnar cell carried long microvilli. The lateral cell membranes of epithelial cells were moderately folded and possessed desmosomes throughout their length. However, extensive folding was encountered in the basal parts.

The nucleus was spherical in shape and was disposed at different levels in the cytoplasm (Fig 8). The euchromatin was finely granular and heterochromatin was disposed peripherally. A single nucleolus could be observed and was eccentrically located in the nucleus. It has a dense band in its outer part and a lighter central portion (Fig 8).

Elements of smooth endoplasmic reticulum possibly including a Golgi complex were seen in the form of parallel arrays of cisternae and vacuoles of various sizes. They were located at different sites in the cytoplasm (Fig 9).

There were many mitochondria distributed randomly in the cytoplasm. They were in the form

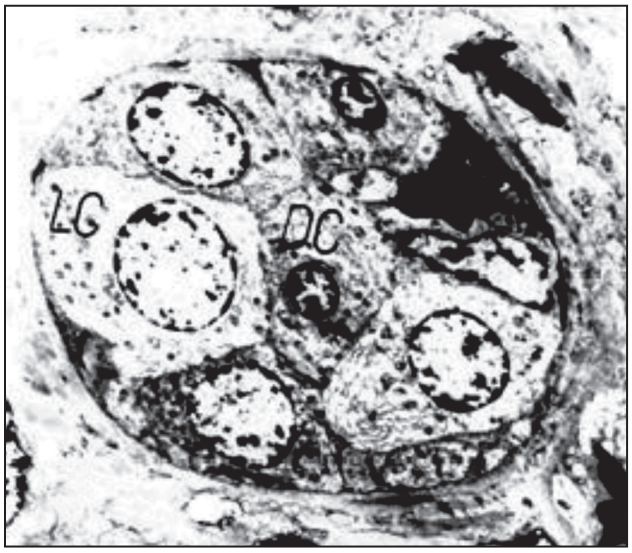

Fig 5. SEM micrograph of Bulbourethral gland. Type B unit showing dark cells (DC) and light cells (LC). 3800 X.

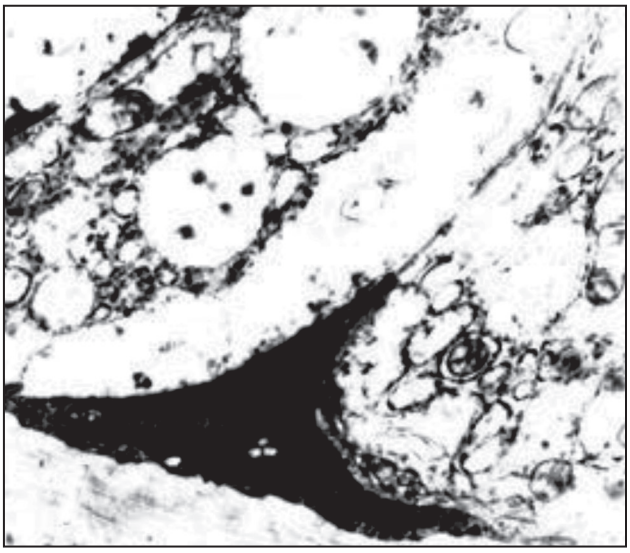

Fig 7. SEM micrograph of Bulbourethral gland. Type B unit. Myoepithelial cells. Note Myofibrils and lipid. 20000 X.

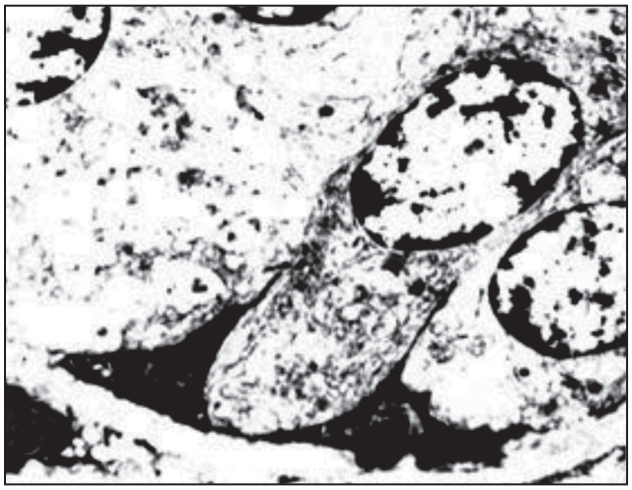

Fig 6. SEM micrograph of Bulbourethral gland. Type B unit. A basal cell (possibly two). Are wedged between the columnar cells and the basement membrane. 43200 X.

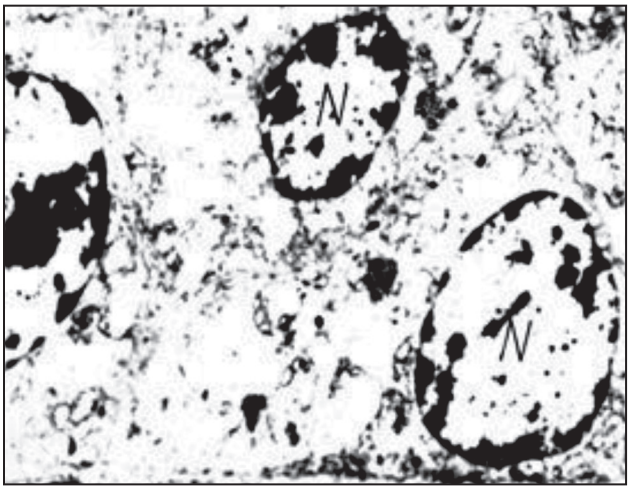

Fig 8. SEM micrograph of Bulbourethral gland. Type B unit showing spherical nucleus (N) disposed at different levels. Note the nucleolus. 44000 X.

of oval, round and elongate bodies. The matrix was traversed by lamellar cristae (Fig 10).

Type C Units

Type C of the secretory units were lined with one layer of cells consisting of both types; A and B (Fig 11, 12).

Basal cells

Basal cells were irregularly pyramidal in shape and they rested directly on the basal lamina. The cell had a large pyramidal nucleus rich in chromatin. The heterochromatin was concentrated around the periphery of the nucleus (Fig 12). The cell had a small Golgi complex, profiles of a few mitochondria, rough endoplasmic reticulum (RER) and small number of vacuoles (Fig 11, 12).

Morphometry

Tables 1, 2 and 3 are showing data and results obtained by using the point-counting technique. The mean volume of the bulbourethral gland, the total points falling on each component, the volume density of each component (Vv), the volume density percentage (Vv%) and the absolute volumes were recorded in these tables. Measurement of the volumes of the bulbourethral glands of 20 camels gave mean values of 13.49 cm^3 and those of weight of bulbourethral glands gave mean values of 14.48 gm. The components of the bulbourethral gland studied

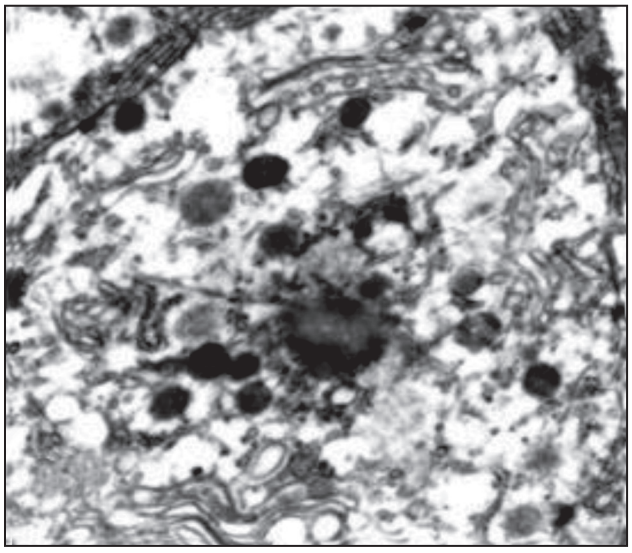

Fig 9. SEM micrograph of Bulbourethral gland. Type B unit showing elements of smooth endoplasmicreticulum including a Golgi complex. 20000 X.

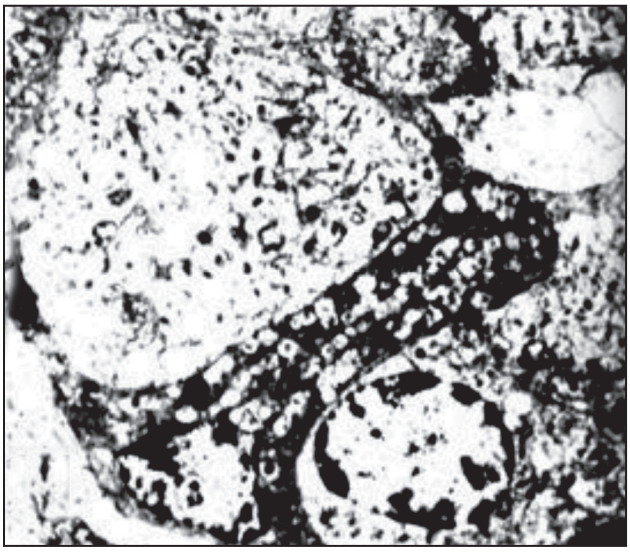

Fig 11. SEM micrograph of Bulbourethral gland. Type C unit showing type A and B in the same unit. 28600 X.

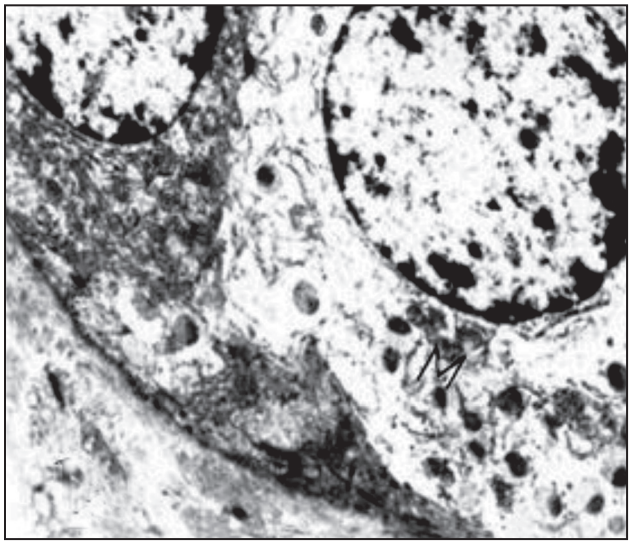

Fig 10. SEM micrograph of Bulbourethral gland. Type B unit showing many mitochondria (M) in the cytoplasm of dark and light cells. 21090 X.

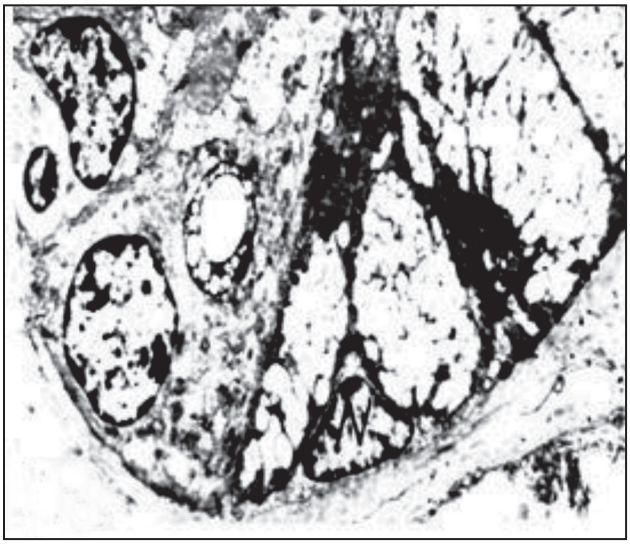

Fig 12. SEM micrograph of Bulbourethral gland. Type C unit showing a pyramidal basal cell with a nucleus (N). 24200 X.

were glandular tissue of the type A, glandular tissue of type B, glandular tissue of type C, connective tissue and muscles, blood vessels and nerves and ducts.

The greater volume of the bulbourethral glands was occupied by connective tissue and the muscle (35.23%) followed by glandular tissue type B (19.76%), glandular tissue type C (18.88) and glandular tissue type A (15.38%). The ducts, blood vessels and nerves occupied 6.39% and 4.36, respectively (Tables 1,2,3). The absolute volume of connective tissue and muscle was 5.08 cm^3 while that of glandular tissue type B, type C and type A was 2.61 cm^3, 2.55 cm^3 and 2.11 cm^3, respectively. The ducts showed an absolute volume of 0.86 cm^3 and that of blood vessels and nerves was 1.57 cm^3 (Tables 1,2,3).

Discussion

This study has shown that secretory units of the gland are lined with one layer of cells which are columnar or pyramidal in shape. These results confirm earlier observations of Ali *et al* (1977) and Mosallam (1981). The present investigation is the only study which hitherto has demonstrated the presence of the basal cells in the bulbourethral glands of the camel. The height of epithelium average is 21 µm, a similar result was reported by Ali *et al* (1977). Their

Table 1. Morphometric analysis of the Bulbourethral glands showing the number of points falling on each component counted from section 1.

No. of F.	G. T.A	G. T. B	G.T.C	C.T+M	B.VS+N	Ducts	Total
1	-	-	-	98	2	-	100
2	11	6	47	16	-	-	100
3	65	-	-	35	-	-	100
4	60	12	13	11	-	22	100
5	-	-	-	98	2	21	100
6	40	5	34	21	-	-	100
7	18	3	66	6	-	23	100
8	22	11	57	8	2	-	100
9	11	9	35	14	-	-	100
10	38	8	15	39	-	-	100
Total	**265**	**54**	**267**	**346**	**6**	**62**	**1000**

Number of field (N. of F). Glandular tissue of bulbourethral gland type A, B and C (G.T.A, G.T.B and G.T.C). Connective tissue and muscles (C.T+M). Blood vessels and nerves (B.VS+N).

Table 2. Morphometric analysis of the Bulbourethral glands showing the number of points counted (p), volume density (Vv), volume density Percentage (Vv%), and absolute volume (v) of the main components from four sections.

No. of F.	G. T.A	G. T. B	G. T. C	C.T+M	B.VS+N	Duct	Total
40	499	716	720	1821	116	128	4000
Vv	0.125	0.179	0.18	0.455	0.029	0.032	
Vv%	12.50	17.90	18.00	45.50	2.90	3.20	
Abs. v	2.44	3.49	3.49	8.87	0.57	0.63	

Number of field (N. of F). Glandular tissue of bulbourethral gland type A, B and C (G.T.A, G.T.B and G.T.C). Connective tissue and muscles (C.T+M). Blood vessels and nerves (B.VS+N).

Table 3. Morphometric analysis of the bulbourethral gland, showing the volume of the glands (v), volume density (Vv), volume density percentage (Vv%) and absolute volume (V) of the main components of 20 camels. Means ± Standard Deviations.

	G. T.A	G. T. B	G. T. C	C.T+M	B.VS+N	Ducts
Total	12304	15808	15103	28188	3486	5111
V	0.1538	0.1976	0.1888	0.3523	0.0436	0.0639
±SD	±0.294	±0.358	±0.0460	±0.0429	±0.0086	±.0132
Vv%	15.38	19.76	18.88	35.23	4.36	6.39
±SD	±2.94	±3.58	±460	±07.62	±0.86	±1.37
Abs.v	2.11	2.61	2.55	5.08	0.59	0.86
±SD	±.74	±0.66	±0.99	±2.34	±0.21	±.28

Number of field (N. of F). Glandular tissue of bulbourethral gland type A, B and C (G.T.A, G.T.B and G.T.C). Connective tissue and muscles (C.T+M). Blood vessels and nerves (B.VS+N).

study has identified three types of secretory units designated as A, B, C; this has been confirmed by the present investigation.

Type A units were lined by tall pyramidal columnar cells as described by Ali *et al* (1977) and Mosallam (1981). The cell possessed an infolded basal plasma membrane and fairly straight lateral membrane with moderate junctional complexes similar to those of the water buffalo (Abou-Elmagd and Wrobel, 1989).

The lining cells possessed a massive Golgi complex. This correlates well with the secretory function of this organelle as evidenced by the large number of the mucous secretory granules found in the cytoplasm.

The cytoplasm of the mucous cells was occupied by abundant electron lucent granules. This is in accord with results reported in the human bulbourethral glands (Riva *et al,* 1990).

Type B units were lined with one layer of cuboidal cells (Ali *et al*, 1977). Similar results were identified in the present investigation. However, the dark cells were apparently more electron dense. In the

current research a massive Golgi complex together with elements of smooth endoplasmic reticulum were randomly scattered in the cytoplasm. Nevertheless, in the water buffalo's bulbourethral glands, the Golgi complex confined to the middle part of the cell (Abou-Elmagd and Wrobel, 1989). The large size of the Golgi complex is indicative of the magnitude of secretory activity of the cells. This is confirmed by the presence of abundant secretory granules of which, some possess fine foci of electron dense material.

The secretory units of type C were lined by one layer of cells consisting of both type A and type B. Similar result was given by Ali *et al* (1977). However, in the water buffalo only type A and type B were reported (Abou-Elmagd and Wrobel, 1989). The basal cells were observed wedged among the bases of the main cells. They were irregularly pyramidal in shape with large pyramidal nucleus that was rich in chromatin. As they were poor in organelles it was concluded that they were not secretory.

Earlier studies made no reference to the presence of myoepithelial cells in the bulbourethral gland of the camel. The present study is the first to identify such cells and showed that they possess characteristic cytoplasmic myofibrils and they were interposed between the basal lamina and the alveolar cells of the secretory units of type B. Such cells were widely reported in exocrine organs of the camel, for instant the epididymis (Tingari, 1989), salivary glands (Khalil, 1989) and mammary glands of human, monkey, shrew, rat and mouse (Tsubura et al 1991), they are considered to help, through their contraction; in the empting mechanism of secretory material.

The morphometric data on the bulbourethral of the camel is virtually lacking. It was scarce for other species. From morphometric results it was clear that the glandular tissue occupied 54.02% of the volume of the gland. It was relatively larger than the volume occupied by the glandular tissue of camel prostate gland (Shaaeldin and Tingari, 2019). Within the glandular tissue type B cells have the largest volume followed by type C and then type A units. A correlation have already established between testicular weight, amount and quantity of testicular interstitial tissue, spermatogenesis and epididymis sperm content and hence testicular function on one hand and season of the year on the other hand (Maiada *et al*, 2013). It would be interesting to carry a similar study to confirm whether there is fluctuation in the amount of glandular tissue of the bulbourethral gland and to correlate the findings with the sexual activity.

Acknowledgements

This research was supported by a grant from Ahfad University for women (Sudan), The Gordon Memorial Trust (UK) and The British Council Sudan.

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DOI : 10.5958/2277-8934.2020.00014.4 *Vol 27 No 1, p 97-103*

CLONING AND BIOINFORMATICS REVEALED MOLECULAR CHARACTERISATION OF CYP2E1 GENE FROM BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

In the present study, the CYP2E1 gene from Bactrian camel (*Camelus bactrianus*) was cloned, sequenced and explored for its structural and functional aspect. The full-length sequence of CYP2E1 gene was 1754bp long, contained a coding region of 1488bp open reading frame (ORF), which was predicted to encoding 495 amino acid residues. The comparison between CYP2E1 from Bactrian camel (*Camelus bactrianus*) and other animal species showed that nucleotide and encoded amino acid sequences of the Bactrian camel CYP2E1 gene exhibited high similarity with the wild Bactrian camel (*Camelus ferus*), and the phylogenetic analysis revealed that Bactrian camel (*Camelus bactrianus*) CYP2E1 was grouped with that of wild Bactrian camel (*Camelus ferus*). The CYP2E1 protein contained a transmembrane domain which extended from 2-23 amimo acids, while 24-495 amino acids were outside the cell, and no signal peptide was found. The prediction of tshe secondary structure revealed that there had 44.04% alpha helix, 7.07% beta fold, and 48.89% random coils. Our research may be valuable for exploring the function and detoxification molecular mechanisms underlying the molecule.

Key words: Bactrian camel, bioinformatics, CYP2E1 gene

Cytochrome P450 (CYP450) is an isoenzyme encoded by superfamily genes that are closely related to structure and function in an organism. It is essential to catalyse the metabolic conversion, activation and inactivation of a wide variety of endogenous, including steroid hormones, vitamins, and fatty acid derivatives, and exogenous substances, such as drugs, xenobiotics, protoxicants, and chemicals toxicity, and carcinogens (Ohmori *et al,* 1993; Nelson, 2011). In the Bactrian camel genome there are about 17 families and 38 subfamilies, including about 63 CYP gene copies. Within them, 9 multi-gene families were found and there are more CYP2J and CYP3A copies, which may contribute to the distinct biological characteristics and metabolic pathways of Bactrian camels for adaptation to the harsh environments (Jirimutu *et al,* 2012; Hasi *et al,* 2018). CYP2E1 is an important member of the CYP450 enzyme system, and mainly located in the membrance of the endoplasmic recticulum but has also been detected in other cellular compartments such as the plasma membrane and the mitochondria (Loeper *et al,* 1990; Neve and Ingelman-Sundberg, 2001). The special importance of CYP2E1 focused on metabolising xenobiotics, high production chemicals, industrial waste as ethanol, solvents, carbon tetrachloride, benzene, and drugs such as acetaminophen, chlorzoxazone and theophyline (Song *et al,* 1986; Park *et al,* 1993; Alanazi and Saeed, 2012).

Like other mammals, several studies have focused on the camel in the CYP2E1 gene in the liver and extrahepatic tissues such as kidney, lung, spleen, tongue, and the hump (Sheikh *et al,* 1991; Raza *et al,* 2004; Alanazi *et al,* 2010). Furthermore, Mohammad *et al,* cloned the full-length CYP2E1 gene sequence from dromedary (Alanazi and Saeed, 2012). Mahmoud *et al*, determined the camel CYP2E1 and detected its evolution rate and its power to bind with various chemicals, protoxins, procarcinogens, industrial toxins and drugs were isolated (Kandeel *et al,* 2016). In the present study, Bactrian camel full-length CYP2E1 DNA were isolated and cloned and sequenced for the first time, furthermore to analyse the bioinformatics characteristics.

Materials and Methods

Experimental sample collection

The liver tissue was obtained immediately after slaughter from male domestic Bactrian camel (*Camelus*

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bactrianus) at the Alxa, Inner Mongolia abattoir under the supervision of a skilled veterinarian.

RNA extraction

The total RNA was extracted from Bactrian camel liver tissue using RNAiso Plus (TaKaRa) kit and quantified by spectrophotometry on the NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Integrity of RNA sample was assessed using formaldehyde agarose gel (1%) electrophoresis.

Primer design and synthesis

All primers used in this study were shown in Table 1, and all primers were synthesised by Sangon Biotech, Co., Ltd. Shanghai, China.

The first strand of cDNA synthesis

The reaction volume of 25μl comprised of 12.5μL of 2×GC buffer, 0.5μL of both forward and reverse primers (10μM), 0.2μL of dNTP mixture (10mM), 10.0μL of ddH_2O, 1μL cDNA template, and 0.2μL of Taq enzyme (5U/μL). The PCR reaction included an initial denaturation cycle of 3min at 95°C, followed by 33 cyclys of denaturation for 30s at 94°C, annealing for 30s at 58°C, and an extension for 90s at 72°C, with a final extension of 7 min at 72°C. The PCR products were excised from a 1.0% agarose gel, purified using Gel Extraction Kit (Sangon Biotech, B518131).

The amplification of 3′RACE and 5′RACE

The first round of 3′RACE PCR was carried out using both RC543-F1 and 5.3′ outer primers. Then, the second round of nested PCR was performed with primers RC543-F2 and 5.3′ inner using the first round of PCR product as a template. The PCR products were excised from a 1.0% agarose gel, purified using Gel Extraction Kit (Sangon Biotech, B518131). The reaction component and PCR cycling were provided in Table 2 and Table 3.

Table 2. The detail reaction component of amplification of 3′RACE.

Reaction Component	The first round (μl)	The first round (μl)
2X GC Buffer I	12.5	25
F (10 μM)	0.5 (RC543-F1)	1(RC543-F2)
R (10 μM)	0.5 (5.3′outer)	1 (5.3′inner)
dNTP (2.5 mM)	4	8
ddH20	6.3	12.5
Template	1 (cDNA)	1 (first round of PCR product)
Taq enzyme (5 U/μl)	0.2	0.5
Total	**25**	**50**

Table 3. The information PCR cycling of 3′RACE.

	The first round		The second round	
Initial denaturation	95°C	3min	95°C	3 min
Denaturation	94°C	30s	94°C	30s
Annealing	58°C	30s	58°C	30s
Extension	72°C	60s	72°C	60s
Final extension	72°C	7min	72°C	7min
Cycling	33C		33C	

According to the in vitrogen 5′RACE system manual, both RC543-RT1 and RC543-RT2 primers were used to obtain the first strand cDNA; then the first round of 5′RACE PCR was carried out

Table 1. Primers for CYP2E1 gene cloning.

Primer name	Primer sequence
Adaptor primers	5′adaptor GCTGTCAACGATACGCTACGTAACGGCATGACAGTGCCCCCCCCCCCCCCC
	3′adaptor GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTT
	5.3′outer GCTGTCAACGATACGCTACGTAAC
	5.3′inner GCTACGTAACGGCATGACAGTG
Amplification primer	RC543-92F TGGCTGTCTTGGGCATCAC
	RC543-1559R TTGGCAAACCCAGTCGCA
Specific primer of 3′RACE	RC543-F1 GTGATTCCGACACTGGACTCCGTCTT
	RC543-F2 CCAGAGAAGTTTAAGCCGGAGCACTTTC
Specific primer of 5′RACE	RC543-R2 GAACACTGGCCCGAACCGCTCT
	RC543-R1 GCGAGCCCAGGTACAGCGTGAA
	RC543-RT2 AGAGGATGTCGGAGATGA
	RC543-RT1 TAGTCAGAGTGCTTGTGGAA

Adaptor primers5′adaptor RACE: Rapid-amplification of cDNA ends

using both 5′adaptor and RC543-R1 primers, and the second nested PCR was performed with 5.3′outer and RC543-R2 primers. The reaction component and PCR cycling were provided in Table 4 and 5. The PCR products were excised from a 1.0% agarose gel, purified using Gel Extraction Kit (Sangon Biotech, B518131).

Table 4. The detail reaction component of amplification of 5′RACE.

Reaction Component	The first round (μl)	The second round (μl)
2X GC Buffer I	12.5	25
F (10 μM)	0.5 (5′adaptor)	1 (5.3′outer)
R (10 μM)	0.5 (RC543-R1)	1 (RC543-R2)
dNTP (2.5 mM)	4	8
ddH_2O	6.3	12.5
Template	1 (cDNA)	1 (第一轮PCR稀释产物)
Taq enzyme (5 U/μl)	0.2	0.5
Total	**25**	**50**

Table 5. The information PCR cycling of 5′ RACE.

	The first round		The second round	
Initial denaturation	95°C	3 min	95°C	3 min
Denaturation	94°C	30 s	94°C	30 s
Annealing	68°C	30 s	68°C	30 s
Extension	72°C	60 s	72°C	60 s
Final extension	72°C	7 min	72°C	7 min
Cycling	33C		33C	

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment was carried out using the sequence analysis software Lasergene 1 (DNASTAR Inc., Madison, WI, USA) and Clustal X program, version 1.83. A neighbor-joining tree was drawn with MEGA5.0 software (http://www.megasoftware.net), with confidence levels assessed using 1000 bootstrap replications. The sequences of reference CYP2E1 genes concluded in the multiple sequence alignment were obtained from GenBank and summarised in Table 6.

Bioinformatic analysis of CYP2E1

The basic physical and chemical properties of CYP2E1 were analysed using ProtParam online tools (https://web.expasy.org/protparam/). The hydrophobicity of CYP2E1 was predicted using ProtScale online tools (https://web.expasy.org/protscale/). The transmembrane structure of CYP2E1 was predicted using TMHMM2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). The subcellular localisation of DQA was predicted using the online software Target P1.1 server (https://www.cbs.dtu.dk//services/ TargetP/). The phosphorylation sites site of CYP2E1 were predicted using the online tools: NetPhos 3.1 server (http://www.cbs.dtu.dk/services/NetPhos/). The amino acid sequence of CYP2E1 was subjected to predict the secondary and 3D structures. The secondary structure was predicted using PredictProtein (http://www.predictprotein.org/), while the 3D conformation was predicted using the Swiss-model server (https://www.swissmodel.expasy.org/) for modeling the homologous structure.

Table 6. Sequence information used in the construction of phylogenetic trees.

Species	Accession Number
Camelus ferus	XM006178829.2
Camelus dromedarius	GU998962.1
Vicugna pacos	XM006207481.2
Sus scrofa	AY581116.1
Bos taurus	AJ001715.1
Ovis aries	NM001245972.1
Equus caballus	EU232117.1
Homo sapiens	J02625.1

Results

Molecular cloning and sequence analysis of CYP2E1 from Camelus bactrianus

Based on the RACE, a full-length CYP2E1 cDNA of 1754-bp was obtained from the camel liver tissue, and consisted of 1488-bp open reading frame (ORF). That encoded a 495-aa (32-1519bp, length: 1488bp) polypeptide of 145604 molecular weight (Fig 1). Total number of atoms was 18186, the atomic formula was C5187H8622N1754O2120S503, theoretical pI was 4.90, and instability index was 53.60, which classified the protein as unstable. Furthermore, protein sequence of CYP2E1 was predicted by EditSeq (DNAStar), which revealed that there were 59 strongly basic (+) (K, R), 56 strongly acidic (-) (D, E), 186 hydrophobic (A, I, L, F, W and V), 107 polar (N, C, Q, S, T and Y) with isolectric point at 8.304 and 5.490 charge at pH 7.0. The gene sequence comprised of 24.19% A (360), 25.47% G (379), 21.57% T (321), 28.78% C (428), 45.77% A+T (681), and 54.23% C+G (807).

The sequence analysis for the coding region of *Camelus bactrianus* CYP2E1 gene indicated a high similarity with other ungulates. We found that camel CYP2E1 showed the highest similarity with

CCCACGAGTCAGCAGACACTCAGAAGGCACC

ATG GCT GTC TTG GGC ATC ACG ATC GCT CTG CTG GTG TGG ATG GCC ACC CTG CTG CTC ATC TCC ATC TGG AAG CAC ATC TAC AGC AGC

M A V L G I T I A L L V W M A T L L L I S I W K H I Y S S

TGG AAA CTG CCC CCT GGC CCT TTC CCA CTG CCC ATC ATT GGG AAT CTT TTC CAG CTG GAT ATT AAG GAT ATT CCC AAA TCC TTA ACC

W K L P P G P F P L P I I G N L F Q L D I K D I P K S L T

AGG CTG GCA GAG CGG TTC GGG CCA GTG TTC ACG CTG TAC CTG GGC TCG CGG CGC ATC GTG GTC CTG CAT GGC TAC AAG GCC GTC

R L A E R F G P V F T L Y L G S R R I V V L H G Y K A V

AAG GAG GTC CTG CTG GAC TAC AAG AAC GAG TTT TCT GGC AGA GGA GAT AAC CCG GCA TTC CAG GTG CAC AAG AAC AAA GGA ATC

K E V L L D Y K N E F S G R G D N P A F Q V H K N K G I

ATT TTC AAC AAT GGA CCG ACC TGG AGG GAC ACC CGG AGG CTT TCC CTG ACC GTC CTC CGT GAC CTC GGG ATG GGG AAA CGG GGC

I F N N G P T W R D T R R L S L T V L R D L G M G K R G

AAC GAG GAG CGG ATC CAG AGG GAG GTC CCC TTC CTG CTG GAG GCA CTC AGG AAG ACC CAG GGC CAG CCC TTT GAC CCC ACC TTC

N E E R I Q R E V P F L L E A L R K T Q G Q P F D P T F

GTC ATT GGC TTC GCG CCC TAC AAT GTC ATC TCC GAC ATC CTC TTC CAC AAG CAC TCT GAC TAC AGT GAT AAG ACG GGC CTG AGG CTG

V I G F A P Y N V I S D I L F H K H S D Y S D K T G L R L

ATG TAT CTG TTC AAC GAG AAC TTC TAC CTG CTC AGC ACG CCC TGG ATC CAG CTT TAT AAT AAT TTC TCA AGC TGT CTA CAA TAC CTG

M Y L F N E N F Y L L S T P W I Q L Y N N F S S C L Q Y L

CCA GGA AGC CAT AGA AAA CTA TTA AAA AAT GTG TCC GAA ATA AAA GAT TAT GCT TCA GCA AGA GTG AAG GAA CAC CAG GAG TCA

P G S H R K L L K N V S E I K D Y A S A R V K E H Q E S

CTG GAC CCC AGC TGC CCC CAA GAC TTC ATA GAC AGC CTG CTG GTG GAA ATG GAG AAG GGA AAG CAC AGT GCA CAG CCT GTG CAC

L D P S C P Q D F I D S L L V E M E K G K H S A Q P V H

ACC TCG GAG GGC ATC GCC GTG ACC GTC GCT GAC CTG CTC TTC GCA GGG ACG GAG ACC ACC AGC ACC ACC CTG AGA TAT GGG CTC

T S E G I A V T V A D L L F A G T E T T S T T L R Y G L

CTG ATT CTC ATG AAA CAC CCG GAG GTC GAA GAG AAG CTT CAT GAA GAA ATT GAC AGG GTG ATC GGG CCG AGC CGA GTC CCT GCT

L I L M K H P E V E E K L H E E I D R V I G P S R V P A

ATC AAG GAC AGG CTA GAC ATG CCC TAC CTG GAT GCC GTG GTG CAC GAG ATT CAG CGA TTC ATC GAC CTC TTG CCC TCC AAC CTG

I K D R L D M P Y L D A V V H E I Q R F I D L L P S N L

CTC CAC GAA GCC ACC CAG GAC ACA GTG TTC AGA GGA TAC GTC ATC CCC AAG GGC ACG CTC GTG ATT CCG ACA CTG GAC TCC GTC

L H E A T Q D T V F R G Y V I P K G T L V I P T L D S V

TTG TAT GAC AAC CAA GAA TTC CCC GAG CCA GAG AAG TTT AAG CCG GAG CAC TTT CTG AAC GAA CAT GGG AAG TTC AAG TAC AGT

L Y D N Q E F P E P E K F K P E H F L N E H G K F K Y S

GAC TAT TTC AAG CCA TTT TCC GCA GGA AAG CGA GTG TGC GTT GGA GAA GGC CTG GCG CGC ATG GAA TTG TTT CTG TTC TTG GCC

D Y F K P F S A G K R V C V G E G L A R M E L F L F L A

GCC ATC TTG CAG CAT TTT AAC TTG AAG TCT CTC GTT GAC CCC AAG GAT ATT GAC CTC AGC CCC ATT GCG ACT GGG TTT GCC AAG ATT

A I L Q H F N L K S L V D P K D I D L S P I A T G F A K I

CCC CCC CGT TAC AAA TTC TGT GTC ATT CCC CGC TCT CAA GCG TGA

P P R Y K F C V I P R S Q A *

GGCACACGCGCTCTGAAGACCCCCGGGCACTTGATGCTGCCTGGCGGCTTCCTGCCCCACACCGTCCCCTCCGGTGGGCAGCCAGGGCTCC

ACAGGGGATCAGCTGGGCCAGGGAGGCTGCTTCCGAGTCACATTCTTCAGACAGAATTTGAAAGCAAAATCCAAAAAAAAATTGTGTCAAA

CTAATTAAACTGAATAAAGTGAACTGTGACTTCCAAAAAAAAAAAAAAAAAAAA

Fig 1. The nucleotide and deduced amino acid sequence of Bactrian camel CYP2E1. The deduced amino acid sequence is reported in one-letter code, and the stop coden is showed with asterisks.

the aminal of Camelidae family, which were wild Bactrian camel (*Camelus ferus*), alpaca (*Vicugna pacos*), and dromedary (*Camelus dromedarius*), (99.9%, 98.5% and 98.4%); followed by goat (*Ovis aries*) and cattle (*Bos taurus*) (87.8% and 87.4%); the similarity with pig (*Scrofa microsomal*) and horse (*Equus caballus*) were 87.0% and 85.8%, respectively. The deduced amino acid sequences similarities ranging from 79.9% to 94.7% as compared to other ungulates, such as 94.7% with dromedary (*Camelus dromedarius*), 87.3% with goat (*Ovis aries*), 85.5% with cattle (*Bos taurus*), 83.4% with pig (*Sus scrofa*), and 79.9% with horse (*Equus caballus*). Furthermore, the phylogenetic tree was constructed using neighbor-joining method, and selected 1000 bootstrap repetition (Fig 2). The result showed that *Camelus bactrianus* and *Camelus ferus* formed one monophyletic clade, meanwhile that from other species except *Equus caballus* formed a

separate clade. The *Camelus bactrianus* CYP2E1 gene was grouped closely with that of *Vicugna pacos* and *Camelus dromedarius* (Fig 2).

Molecular Characterisation of CYP2E1 gene from Camelus bactrianus

Analysis of Hydrophilic and Hydrophobic

The hydrophilicity and hydrophobicity were analysed of CYP2E1 protein based on the protscale, and Hphob./Kyte & Doolittle were selected as the prediction standards. The highest score was 3.211, at position 451Leu (L), represented maximal hydrophobicity; while the lowest score was -2.678, at position 190Asp (D), represented strongest hydrophilicity. Since more than half of the amino acid sequence were hydrophilic residues, the entire polypeptide chain was considered to be a hydrophilic residue (Fig 3).

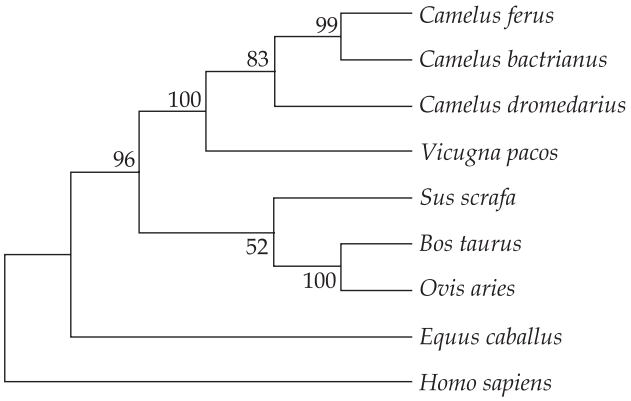

Fig 2. CYP2E1 genetic phylogenetic tree between bactrian camel and other species. The bootstraping value at the nodes and the bold refered to sequencing of this study

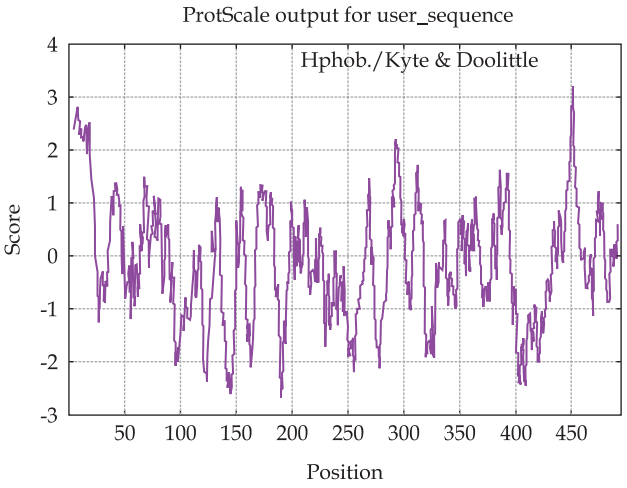

Fig 3. The hydrophilicity profile of *Camelus bactrianus* CYP2E1 protein. Y-axis displays the hydrophilic index: a positive number indicates hydrophobicity, the greater the value, the greater the hydrophobicity; negative numbers indicate hydrophilicity, smaller values indicate stronger hydrophilicity. The x-axis displays the position of CYP2E1 amino acids.

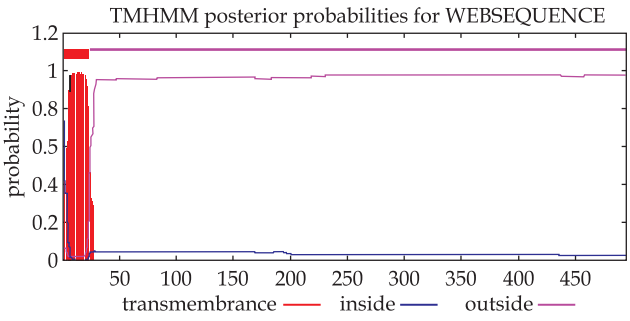

Fig 4. Prediction of transmembrance region of CYP2E1 protein from *Camelus bactrianus*.

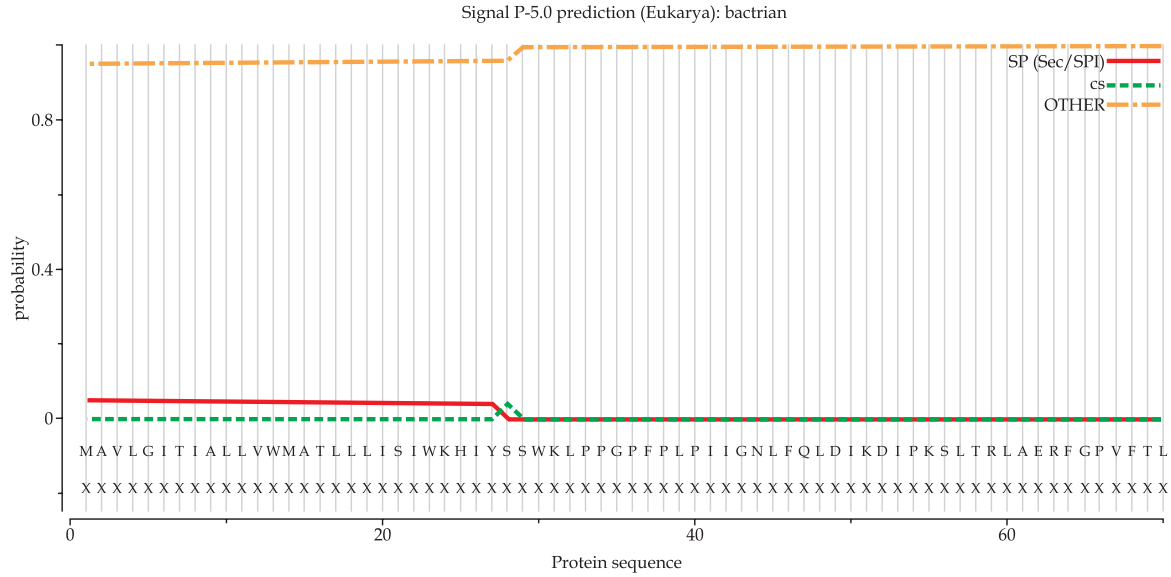

Fig 5. Prediction of signal peptide of CYP2E1 protein from *Camelus bactrianus*.

Analysis of Transmembrane Helical Structure and Signal Peptide

The transmembrane helical structure was predicted of CYP2E1 protein based on the TMHMM 2.0 software (Fig 4). The protein contained a transmembrane domain with extended from 2-23 amimo acids, while 24-495 amino acids were outside the cell. Furthermore, signalP 4.1 server was used to predict the signal pepetide of CYP2E1 protein (Fig 5), and no signal peptide was found in this protein, and it did not have signal peptide recognition function.

Prediction of Phosphorylation site

NetPhos 3.1 server predicted the potential phosphorylation site of CYP2E1 from *Camelus bactrianus* (Fig 6), including 15 Thr (threonines), 11 Tyr (tyrosines), and 28 Ser (serines) above 0.5 threshold.

Subcellular Localisation and Domain Prediction

The Target P1.1 server result showed a signaling pepetide (SP) of CYP2E1 gene from *Camelus bactrianus* (Fig 7); SMART program predicted that 2-23 aa was the transmembrance region.

Advanced structure prediction of CYP2E1 protein

Predict Protein software was used to predict the secondary structure of Bactrian camel CYP2E1. The result showed that the CYP2E1 protein was composed of 44.04% alpha helix, 7.07% beta fold, and 48.89% random coils. Furthermore, the tertiary structure of the DQA protein was predicted using the SWISS-MODEL Server, that indicated that the alpha helix, beta folding, and random crimp was in agreement with the predicted secondary structure of CYP2E1 protein (Fig 8).

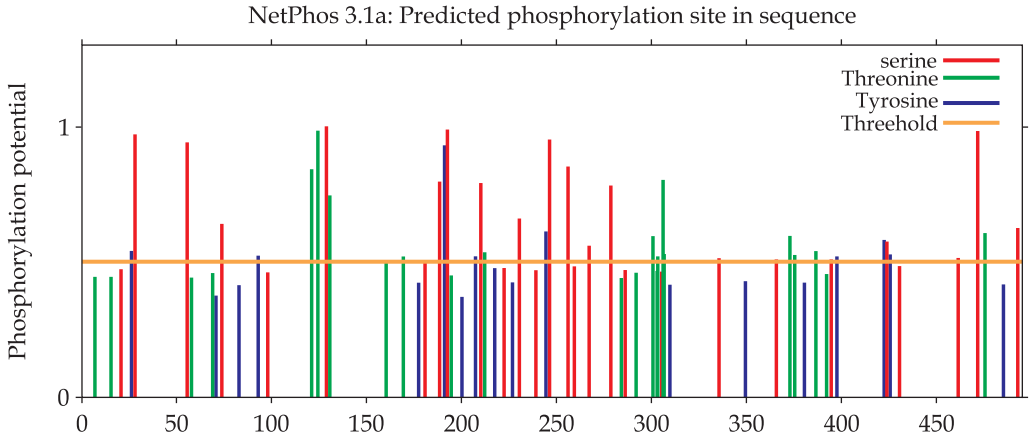

Fig 6. Prediction of phosphorylation sites of CYP2E1 protein from *Camelus bactrianus*.

targetp v1.1 prediction results
Number of query sequences : 1
Cleavage site predictions not included.
Using NON-PLANT networks.

Name	Len	mTP	SP	other	Loc	RC
Sequence	495	0.015	0.993	0.021	S	1
cutoff		0.000	0.000	0.000		

Fig 7. The subcellular location analyses of *Camelus bactrianus* CYP2E1.

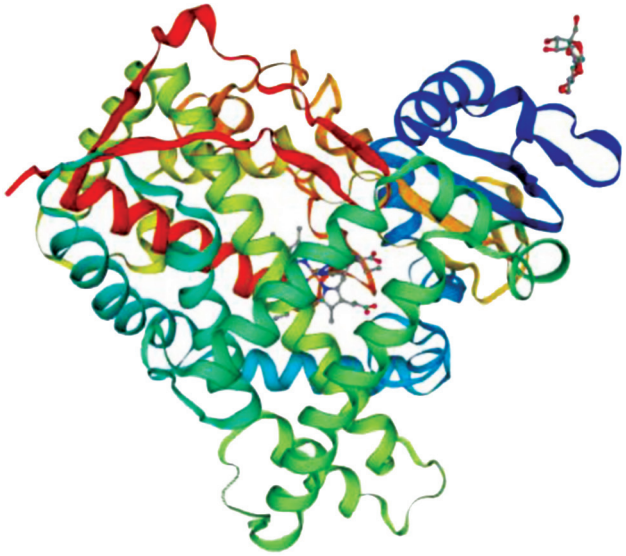

Fig 8. Prediction of the tertiary structure of CYP2E1 protein.

Conclusion

The *Camelus bactrianus* CYP2E1 has an open reading frame of 1488bp, and the cDNA encodes a protein of 495 amino acid residues with a molecular weight of 145.6kDa. The deduced amino acid sequence of Bactrian camel CYP2E1 showed the highest identity with *Camelus dromedarius* (94.7%), *Ovis aries* (87.3%), *Bos taurus* (85.5%), *Sus scrofa* (83.4%), and *Equus caballus* (79.9%). the phylogenetic analysis revealed that Bactrian camel (*Camelus bactrianus*) CYP2E1 was grouped with that of wild Bactrian camel (*Camelus ferus*). The CYP2E1 protein contained a transmembrane domain which extended from 2-23 amimo acids, while 24-495 amino acids were outside the cell, and no signal peptide was found.

Acknowledgements

This work was supported by grants from the High-level Talents Introduction to Scientific Research Start-up Project (NDYB2017-28), the Inner Mongolia Natural Science Foundation Project (2018BS03017), the Inner Mongolia Autonomous Region Science and Technology Innovation Guide Project (KCMS2018048), and the Double-class discipline innovation team building (NDSC2018-14).

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DOI : 10.5958/2277-8934.2020.00015.6 *Vol 27 No 1, p 105-110*

EFFECT OF HEAT TREATMENTS ON ANTIOXIDANT PROPERTIES AND INSULIN CONTENT OF CAMEL MILK

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ABSTRACT

The aim of this study was to evaluate the changes in antioxidant activity and insulin content of camel milk after thermal treatment. Camel milk samples were collected from dairy farm (ICAR-National Research Centre on Camel, Bikaner, India). The samples were processed with two heat treatment methods: High Temperature Short Time (HTST) and Low Temperature and Long Time (LTLT) and fresh camel milk (untreated) was used as control. The antioxidant activity of the milk was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) radical scavenging activity. Insulin concentration was determined using ELISA kits in the different milk samples. DPPH radical scavenging activity was significantly lower ($p<0.05$) in LTLT heat treated (pasteurised) milk compared to fresh milk (Control). However, no significant difference was found between HTST group and control. Similar results were observed for ABTS radical scavenging activity of the milk samples. There was also no significant ($p>0.05$) difference in the insulin content in heat treated groups when compared with control group. Thus, data suggested that the milk processing by HTST pasteurisation preserves the antioxidant activity as well as insulin content in camel milk.

Key words: ABTS, antioxidants, camel milk, DPPH, insulin, pasteurisation

Significant health benefits of camel milk in human disease conditions have been suggested by several researchers (Agrawal *et al,* 2004; Magjeed, 2005; Meena *et al,* 2016). The antioxidant and antimicrobial properties of pure and hydrolysed camel milk proteins is also reported (Salami *et al,* 2009; Kumar *et al,* 2016b, 2016c & 2017). The anti-diabetic effect of camel milk is the most studied area in the human health benefits. However, effect of heat treatment of camel milk regarding preservation of its antioxidant properties as well as insulin content and thus for the management of different disease conditions has not been explored.

Antioxidants protect the cells against over production of reactive oxygen species (ROS) and prevent oxidative tissue damage (Jackson *et al,* 2002). Though, oxidative metabolism is essential for the cell survival and various regulatory processes, its negative impact may lead to generation of free radicals or reactive oxygen species (ROS). Excess level of free radicals or ROS may have detrimental effect by oxidizing membrane lipids, proteins, DNA etc. which can further damage the normal cellular processes. Antioxidants in milk are known for preventing the damages caused by Reactive Oxygen Species (ROS) (Lindmark-Mansson and Akesson, 2000). Milk contains a number of enzymatic as well as non enzymatic antioxidant like glutathione peroxidase, superoxide dismutase, catalase, vitamin E, vitamin C, β- carotene, which may protect newborn calves against ROS at the early stage of life and during oxidative stress (L'Abbe and Friel, 2000; Scheibmeir *et al,* 2005). Very limited data is available regarding the comparative evaluation of antioxidant property of fresh and heat processed milk from different species.

Milk not only contains antioxidants but also contains an array of bioactive substances including insulin hormone (Read *et al,* 1984; Hamosh, 2001). Effect of heat treatment, pasteurisation on insulin concentration of camel milk has been studied (Wernery *et al,* 2006b).

Present study was therefore aimed to evaluate the effects of thermal treatment on antioxidant

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potential and changes in the level of insulin hormone in camel milk.

Materials and Methods

Chemical and Reagents

Insulin ELISA Kit was procured from RayBiotech (USA), and chemicals such as 2, 2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were procured from SRL Pvt Ltd (India). Other required analytical chemicals were also procured. All solutions, prepared in Milli Q water, and were kept at 4°C for further use.

Collection and processing of camel milk samples

Fresh milk was collected from Camel Dairy Farm, ICAR-National Research Centre on Camel, Bikaner in sterile containers and chilled immediately to 5°C. The samples were defatted by cream separator and skimmed milk was used for different thermal treatments. Two thermal treatments: Low Temperature Long Time (LTLT, 63°C for 30 min.) and High Temperature Short Time (HTST, 72°C for 15 sec.) were given to milk. The untreated (control) and processed milk samples were stored at -20°C till further analysis.

Antioxidant activity

2-2-azinobis-3 ethylbenthiazoline-6-sulphonic acid (ABTS+) radical scavenging activity

ABTS assay is based on the reduction of ABTS+ radicals by antioxidants present in the milk. The ABTS+ radical scavenging activity of camel milk samples was determined by method as described by Salami *et al* (2009) with slight modifications. Briefly, ABTS stock solution was prepared by mixing equal volume of 2.45 mM potassium persulphate ($K_2S_2O_8$) to produce ABTS radical cation. Before use, this mixture was kept in the dark at room temperature (37°C) for 16 h. The working solution was obtained by diluting the stock solution of the ABTS radical cation with ethanol to obtain an absorbance of 0.70 ±0.005 at 734 nm. After that 1ml of ABTS+ working standard solution was mixed with 20 µl of milk samples. The reaction mixture was mixed gently and was passed through 0.2 µm syringe filter, absorbance was measured after 20 min (t_{20}) at 734nm using UV-VIS spectrophotometer (Smart Spec Plus Spectrophotometer, BioRad, USA). The activity of ABTS+ was calculated by using formula:

$$\textit{ABTS+ activity (\% inhibition)}=\left(\frac{0.7\text{-OD at t20}}{0.7}\right)X100$$

DPPH radical scavenging activity

The ability to scavenge 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical of milk samples were determined using the method of Brand-Williams *et al* (1995) with a slight modifications. One ml of DPPH working solution (100µM) was mixed with 250 µL of Tris-HCl buffer (pH 7.4) and milk samples (25 µL) in the test tube for 30 min at room temperature (37°C). The reaction mixture was mixed gently and was passed through 0.2 µm, absorbance was measured at 517 nm immediately at time t=0 (t0) using UV-VIS spectrophotometer (SmartSpec Plus Spectrophotometer, BioRad, USA), the reaction mixtures were incubated for 30 min in dark at room temperature (37°C). The absorbance (t30) was again measured at 517 nm and the ethanol was used as blank. The activity of DPPH was calculated by using formula:

$$\textit{DPPH activity (\% inhibition)}= 100 - \left(\frac{\text{OD at t30}}{\text{OD at t0}}\right)X100$$

Determination of Insulin Concentration

Insulin concentrations in milk samples were analysed using an immunochemical insulin analysis with insulin ELISA Kit (Raybiotech, USA). The kit is based on Sandwich-based technique using colorimetric method of detection. The analysis was performed according to the manufacturer's instructions. Briefly, all the kit reagents were brought to room temperature (37°C) before use and dilutions were prepared as per manufacturer's instruction. 100 µL standard or samples was added to each well and incubated for 2.5 h at room temperature (37°C). The solution was discarded from the ELISA plate and washed 4 times with wash solution. 100 µL of biotin antibody was added to each well and incubated for 1 h. The solution was discarded and washed 4 times with wash solution. 100 µL of streptavidin solution was added and incubated for 45 min at room temperature (37°C). 100 µL of TMB one-step substrate reagent was added to each well and incubated for 30 min. 50 µL stop solution was added and immediately OD was recorded at 450 nm using plate reader (infinite 200 Pro Tecan, Switzerland).

Calculation

Standard curve (scattered chart) was prepared with insulin standard using Microsoft Office-EXCEL. The final insulin concentration of the respective samples was calculated from the standard curve.

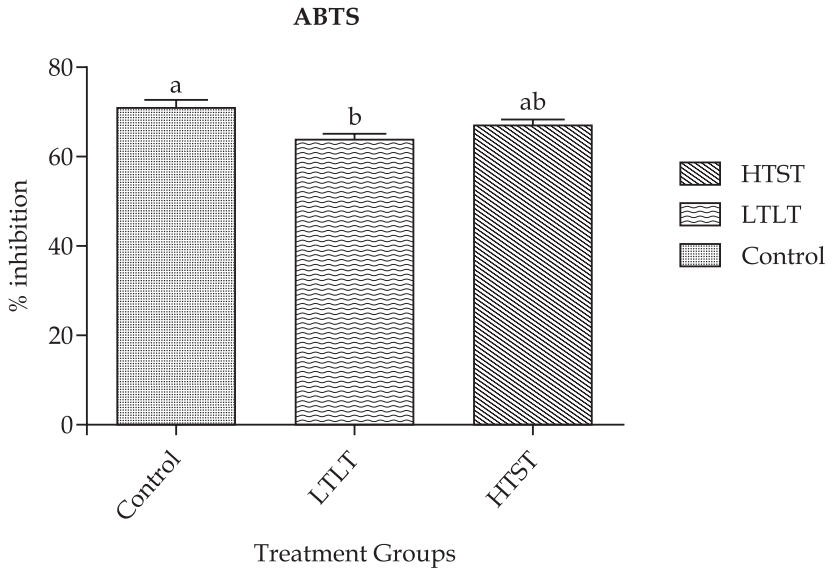

Fig 1. ABTS (% Inhibition) of raw and heat treated camel milk samples.

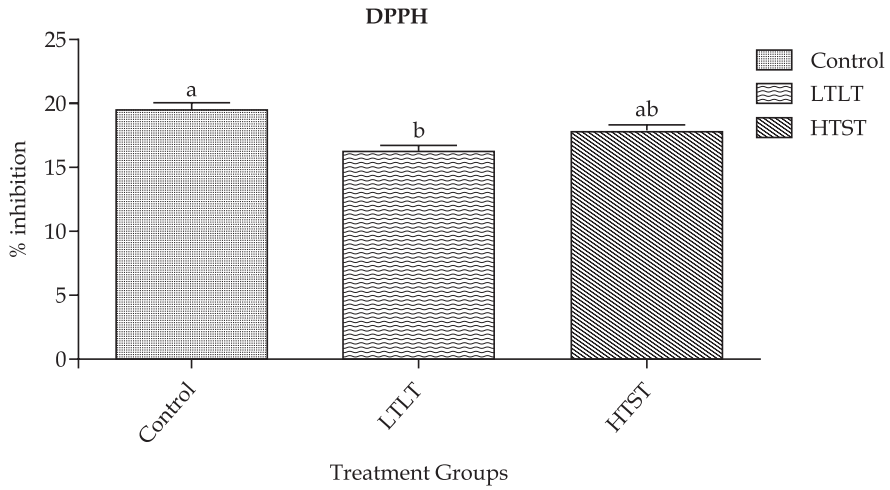

Fig 2. DPPH (% Inhibition) of raw and heat treated camel milk samples.

Statistical Analysis

All the experiments were repeated three times and parameters were analysed in duplicate (n=6). One-way analysis of variance (ANOVA) was done by comparing the means at 95% confidence level using GraphPad Prism software. Data were expressed as means with standard error.

Results and Discussion

Current study focused on the comparative evaluation of antioxidant activity/potential and insulin hormone of camel milk under different pasteurisation methods.

Antioxidant activity of thermal treated camel milk

Antioxidant activity of control (fresh camel milk) and thermally treated (LTLT and HTST) camel milk was determined by ABTS and DPPH assays, compared and discussed in this section.

The cationic radical scavenging activity of ABTS+ is mostly utilised to measure antioxidant activity of food ingredients and processed food products. Since, the reagents are dissolved well in both aqueous hydrophilic and organic solvent hydrophobic groups, this assay measures both the hydrophilic and lipophilic antioxidants. Its efficiency depends upon the number of aromatic rings, nature of hydroxyl groups and molecular weight (Hagerman *et al*, 1998). Hence, it is required to verify the antioxidant activity of food ingredients by conducting different assays, because the mechanism of action in one assay differs from another and also influence the end results.

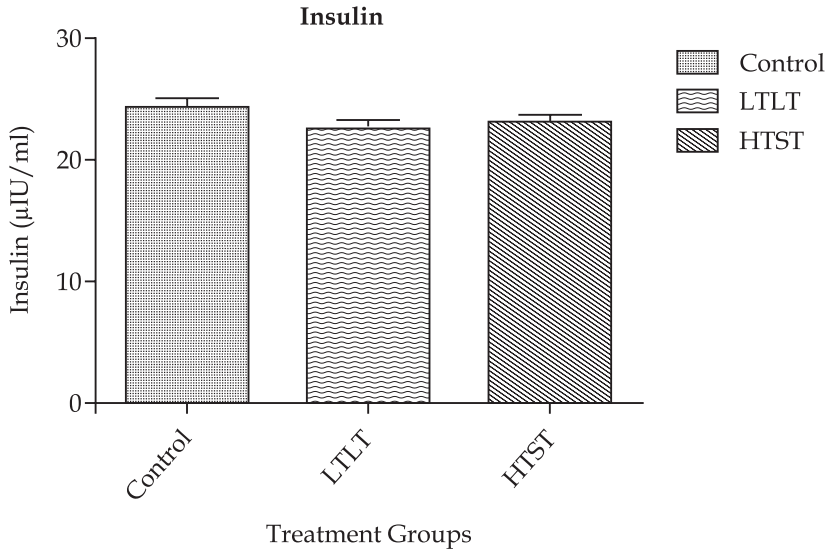

Fig 3. Insulin concentration of raw and heat treated camel milk samples.

A negative correlation was found between thermal treatment of camel milk and antioxidant activity. Both the assays (ABTS and DPPH) evidenced the lower antioxidant activity of pasteurised milk compared with control (fresh). The ABTS activity (% inhibition) of this experiment is presented in Fig 1. Very little information is available about the effect of heat on the antioxidant activity of camel milk. In this study the ABTS radical scavenging activities of raw camel milk and thermally treated camel milk was compared. The ABTS activity (% inhibition) of control, LTLT and HTST milk samples were 71.12±1.26, 63.81±1.22 and 66.88±1.207, respectively. The decrease in ABTS activity was significantly ($p \leq 0.05$) lower in LTLT milk samples; however, it was not statistically different from HTST samples. The ABTS activity of control samples were highest among three groups, however, it was comparable to that of HTST milk samples. The decrease in radical scavenging activity of thermally treated milk might be due to partial degradation of bioactive components responsible for antioxidant activity upon different level of heat application. In LTLT method, although the temperature applied is lower than the HTST method, but longer heat exposure might be responsible for reduced antioxidant activity. Yilmaz-Ersan *et al* (2018) also compared the ABTS activity of raw and pasteurised cow and ewe milk and reported reduction in antioxidant activity of both types of milk after pasteurisation. The significant reduction in antioxidant activity and reduction in total vitamin C content upon pasteurisation was also reported for human milk (Moltó-Puigmartí *et al,* 2011). The Holder pasteurisation (63°C for 30 min) induced a significant ($p < 0.05$) loss of total antioxidant capacity, while total antioxidant capacity was same in both the fresh milk samples and in the samples treated to HTST-pasteurisation (75°C for 15 sec) (Silvestre *et al,* 2008) similar results was found in the current study.

DPPH has been commonly used for the analysis of antioxidant activity for the primary characterisation of the scavenging potential of proteins/peptides. The DPPH scavenging activity was assayed as an additional measure for assessing antioxidant activity of camel milk. The DPPH activity (% inhibition) of fresh and heat treated samples is presented in Fig 2. The DPPH activity of fresh (control), LTLT and HTST milk samples were 19.47±0.4564, 16.14±0.49 and 17.70±0.55, respectively. The DPPH activity of LTLT samples were significantly lower ($p \leq 0.05$) as compared to control samples; however, it was comparable to HTST samples. The HTST samples had higher DPPH activity as compared to that of LTLT samples; however, the differences were non-significant ($p > 0.05$). Hilario *et al* (2010) reported that pasteurisation negatively affects the antioxidant components like total phenolic concentration in goat milk. Reduced DPPH scavenging activity of pasteurised and boiled milk in cow and buffalo has been reported by Khan *et al* (2017). Similar results were also reported by Yilmaz-Ersan *et al* (2018) for DPPH activity of raw and pasteurised cow and ewe milk.

Vitamins like with vitamin-C and reduced glutathione are some of the compounds which contribute to the antioxidant system of the milk. Camel milk is rich in with vitamin-C which is also an antioxidant and contributes its antioxidant activity in the milk (Farah *et al,* 1992). It is reported that the vitamins especially vitamin-C thermally degraded after thermal treatment (Munyaka *et al,* 2010). Lysozyme concentration is also high in camel milk than cow milk (Korhonen, 1977; Duhaiman, 1988; Singh *et al,* 2017) and may also contribute to the higher antioxidant capacity as it is rich in amino acid lysine which shows antioxidant activity (Ahmad *et al,* 1996). Casein is probably a major ABTS+ scavenger in the milk (Chen *et al,* 2003) as it has a high content of potential antioxidative amino

acids like tyrosine, tryptophan, histidine, lysine and methionine (Uchida and Kawakishi, 1992). Similar observation was also reported by Wernary *et al* (2005). They postulated that with vitamin-C and reduced glutathione are the most heat susceptible component in camel milk when pasteurised at 63°C. However, in another study Wernery *et al* (2003) reported that different components of camel milk are more heat resistant than those in cow milk by which it has tremendous advantage over other milk in relation to the commercial production and processing.

Changes in Insulin in camel milk

Insulin content of control (fresh) and heat treated (LTLT and HTST) camel milk was estimated with insulin ELISA kit. There are very few reports available regarding the effect of heat treatment on milk insulin content and as per best of our knowledge this the first with report which compares the effect of HTST and LTST on antioxidant activity with insulin content of camel milk. The insulin content in different groups is presented in Fig 3. In the present study the insulin content of raw milk and heat treated milk was compared. Concentration of insulin in control and both heat treated milk samples were 24.35±0.59, 22.51±0.633 and 23.01±0.61 µIU/ml, respectively. There was a non significant ($p>0.05$) decrease in insulin content was found after pasteurisation (Fig 3). In earlier reports, the mean concentration of insulin was reported in camel milk was 41.9±7.38 µIU/ml (Wernery *et al,* 2006a, 2006b), 45 to 128 µIU/ml (Singh *et al,* 2006), and 58.67±2.01 µIU/ml (Hamad *et al,* 2011). Different concentration of insulin was also reported in human milk (Young *et al,* 2017). In the present study the mean insulin concentration was 24.35 µIU/ml in fresh camel milk. This variable insulin content might be due to the difference in protocol/technique used in estimation and due to breed/region as well as lactation effects. In a study, Wernery *et al* (2006b) reported that pasteurizing camel milk at 72°C for 5 min and boiling at 98°C for 5 min resulted in significant reduction in milk insulin concentration. However, in another study Ollikainen (2013) found no changes in insulin concentration in bovine milk after pasteurisation at (63°C/ 30 min) and 72°C; though the losses in immunochemical activity were reported at higher temperatures.

Conclusion

The present study suggested that the fresh camel milk is having high antioxidant potential or free radical scavenging activity and pasteurisation by heat treatment (both by HTST and LTST) does not significantly affect the insulin content; though the antioxidants activity is lowered by LTLT treatment. Thus, HTST method of pasteurisation can be used for effective preservation of camel milk without much compromising on antioxidant activity and insulin content.

Acknowledgement

Authors are thankful to director(s) of ICAR-Indian Veterinary Research Institute, Izzatnagar and ICAR-National Research Centre on Camel, Bikaner, India for financial support for this study.

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DOI : 10.5958/2277-8934.2020.00016.8 *Vol 27 No 1, p 111-119*

BIOINFORMATICS OF URIDINE/DEOXYURIDINE PATHS IN *Trypanosoma evansi* REVEALED TARGETING URIDINE PHOSPHORYLASE AND CYTIDINE DEAMINASE

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ABSTRACT

The differences between the host and parasite metabolic paths could be a hot spot for discovery of antiparasitic targets. In this work, the metabolic paths of uridine and deoxyuridine in camels and the blood protozoan *Trypanosoma evansi* (*T. evansi*) were investigated by bioinformatics tools. While a set of de novo and salvage enzymes of uridine were found in camels, *T. evansi* was lacking uridine kinase and the source of UMP comes through salvage of uridine conversion to uracil. Therefore, inhibition of uridine phosphorylase (UPase) or cytidine deaminase (CDa) will affect the downstream UMP, dUMP and thymidylate synthesis by lowering the levels of uracil and uridine inside the parasite, respectively. Given the presence of two UPases in camel, low sequence similarity between camel and *T. evansi* UPases and CDase, targeting these enzymes might be without deleterious effects on the host cells.

Key words: Camel, cytidine deaminase, deoxyuridine, pyrimidine, *Trypanosoma evansi*, uridine phosphorylase

The drug discovery process depends heavily on computational approaches, which help to edit the genome sequences. Sequences in a host and target parasite can be compared to find new parasite-specific pathways and proteins that are unique to parasite life. In the last few years, the research in camel was revolutionized by the decoding of camel genome sequence (Jirimutu *et al,* 2012). In this context, computational tools had been used in approval of drug targets and their validation (Kandeel *et al,* 2019a; Kandeel *et al,* 2019b).

In this article, the uridine and deoxyuridine metabolic pathways were compared in camels and the blood protozoan, *T. evansi*. The KEGG maps (Kanehisa *et al,* 2007; Kanehisa *et al,* 2016) were used to explore the enzymes involved in uridine and deoxyuridine metabolism. Comparisons were made between dromedary camel and human, wild camel, and eukaryotic uridine metabolising enzymes.

In this work, by using bioinformatics tools, we show that uridine and deoxyuridine pathway is not well developed in *T. evansi* as in camels. The deficiency of uridine kinase renders this pathway interesting for further studies.

Materials and Methods

Retrieval of genomic data— Collection of genomic data was carried out by extracting the information from the gene database (http://www.genedb.org) (Hertz-Fowler *et al,* 2004), Kinetoplastom genome resources (http://tritrypdb.org/tritrypdb/) and protein and genome databases at (http://www.ncbi.nlm.nih.gov).

Searching homologues— Protein sequence homologues was searched using the NCBI BLAST (Basic Local Alignment Search Tool) (Madden, 2013) or PSI-BLAST (Position- Specific Iterated-BLAST) servers (http://blast.ncbi.nlm.nih.gov/Blast.cgi) searching against the non redundant (nr) database, with filtering of low complexity regions.

Multiple sequence alignment program for proteins and construction of phylogenetic tree— It was carried out using the tools available at (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers and Higgins, 2014). Multiple sequence alignment program (ClustalW2) was used to calculate the best match of the selected sequences. The resultant alignment was used to generate a phylogenic tree, which is visualized by Dendroscope phylogenic tree viewer

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(Huson *et al,* 2007) or CLC genomics workbench (Sequencing, 2011).

Putative domains— It was searched by the domain prediction program available at (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer *et al,* 2005). The genomic and molecular information was obtained from Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/).

Proteomic and genomic tools— ExPASy Proteomics tools (http://us.expasy.org/tools/) (Gasteiger *et al,* 2003) and tools available at the website of the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/) (Labarga *et al,* 2007) were used.

Results and Discussion

Uridine

Uridine production process was proposed by four routes 1) dephosphorylation of uridine 5′-monophosphate by the action of uridine 5′-nucleotidase 2) from uracil by the action of uridine phosphorylase 3) by deamination of cytidine by the action of cytidine deaminase 4) the 3′-nucleotidase uses 3′-UMP to yield uridine as shown in Fig 1. In the catabolic direction, uridine was used by uridine phosphorylase or uridine nucleotidase to give uracil (Fig 1). In the de novo pathway of pyrimidine synthesis, UMP was produced by the kinase activity of uridine kinase.

Uridine phosphorylase (EC 2.4.2.3; pyrimidine phosphorylase); UrdPase; UPH; UPase. It is a glycosyltransferase enzyme which participates in pyrimidine ribonucleosides catabolism and salvage pathways in which, it catalyses the reversible phosphorolysis of uridine to generate uracil and alpha-D-ribose 1-phosphate so that the nucleotide base may be recycled. In addition, mammalian UPase also receives 2′-deoxyuridine (Liu *et al,* 1998). Unfortunately, Information about de novo pyrimidine biosynthesis in parasitic protozoa is seriously restricted. Moreover, de novo pyrimidine biosynthesis vs. salvage differs from organism to organism and even from one growth stage to another. The first characterisation of UPase from *Trypanosoma brucei* viewed that it is a homodimeric (Larson *et al,* 2010). Additionally, *Trypanosoma cruzi* UPase reaction mechanism of is defined as steady state that revealed no rate-limiting step after formation of reaction products (Silva and Schramm, 2011). Furthermore, UPase has an expanded S(N)2 character transition state (Silva *et al,* 2012).

After bioinformatics investigations, both camels and *T. evansi* shared common features of the absence of 3′-nucleotidase and uridine nucleotidase. Uridine kinase and uridine 5′-nucleotidase were detected in camels but not in *T. evansi*. Therefore, cytidine deaminase and UPase are the only enzyme of uridine pathway that is present in both of camel and *T. evansi* (Fig 1-3 and Tables 1-3).

Table 1. Enzymes involved in metabolic pathways of uridine.

ID (E.C. number)	Definition (Enzyme name)
3.1.3.6	3′-Nucleotidase
3 2.4.2.3	Uridine phosphorylase
3.2.2.3	Uridine nucleosidase
3.5.4.5	Cytidine deaminase
3.1.3.5	Uridine 5′-nucleotidase
2.7.1.48	Uridine kinase

Table 2. The expected enzymes involved in metabolic pathways of uridine in camels.

ID (E.C. number)	Definition (Enzyme name)
3 2.4.2.3	Uridine phosphorylase
2.7.1.48	Uridine kinase
3.5.4.5	Cytidine deaminase

Table 3. The expected enzymes involved in metabolic pathways of uridine in *Trypanosoma evansi*.

ID (E.C. number)	Definition (Enzyme name)
3 2.4.2.3	Uridine phosphorylase
3.5.4.5	Cytidine deaminase

T. brucei was found to be devoid of uridine kinase activity (Hammond and Gutteridge, 1982). In this study, there was no route of converting uridine to UMP in *T. evansi* (Fig 3). In addition, the sole route of generating uridine was by deamination of cytidine. Therefore, the expected route of UMP biosynthesis is through the salvage of uridine to the direction of uracil by the action of uridine phosphorylase, which is then converted to UMP by the action of uracil phosphoribosyl transferase.

Uridine phosphorylase

Two uridine phosphorylases were found in camels, UPase 1 and UPase 2. BLAST search of these 2 enzymes against *T. evansi* database did not get any significant hits. A previous study of crystal structure of a putative protein in *T. brucei* revealed its content of uridine phosphorylase domain and activity. Despite sequence search and analysis revealed it is a nucleoside phosphorylase, the determined structure and biochemical activity revealed that

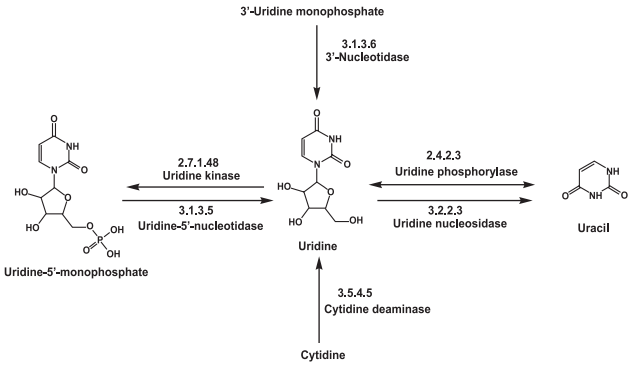

Fig 1. The proposed metabolic pathways of uridine.

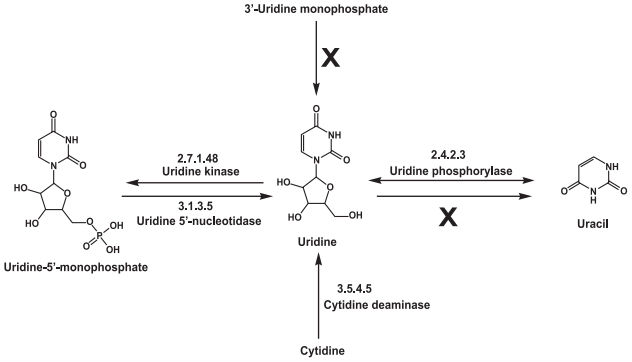

Fig 2. The proposed metabolic pathways of uridine in camel.

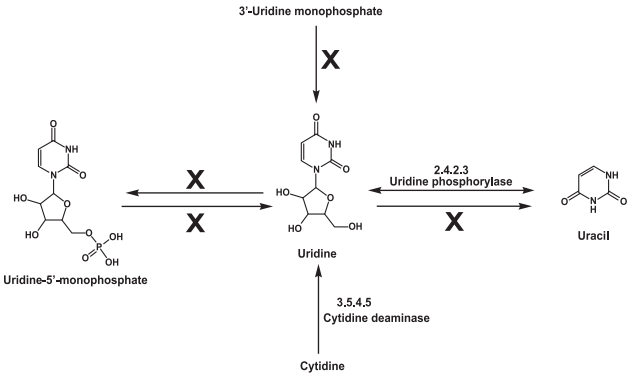

Fig 3. The proposed metabolic pathways of uridine *Trypanosoma evansi*.

it's a purine nucleosidase as it catalysed reaction involving uridine only and was nonspecific for other nucleosides (Larson *et al,* 2010). BLAST search of *T. evansi* database using the characterised enzyme from *T. brucei* retrieved a gene with 100% similarity to the *T. brucei* enzyme (Fig 4).

Comparison of human and camel uridine phosphorylases 1 and 2 is given in Fig 5. There was 49% similarity between camel uridine phosphorylases 1 and 2. While the similarity between human and camels was 65.1% for uridine phosphorylases 1 and 73.74% for uridine phosphorylases 2 and 78-100% similarity for uridine phosphorylases 1. This

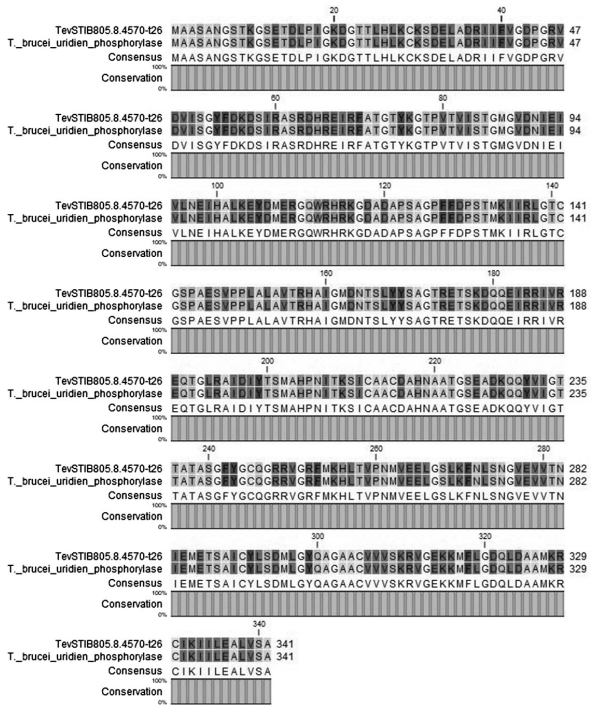

Fig 4. Pairwise sequence alignment of *T. evansi* and *T. brucei* uridine phosphorylase.

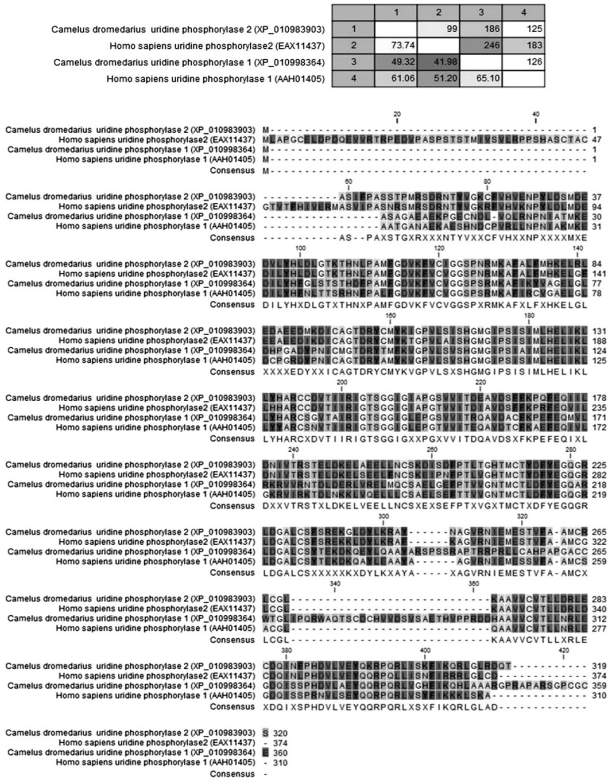

Fig 5. Multiple sequence alignment of dromedary camel and human uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

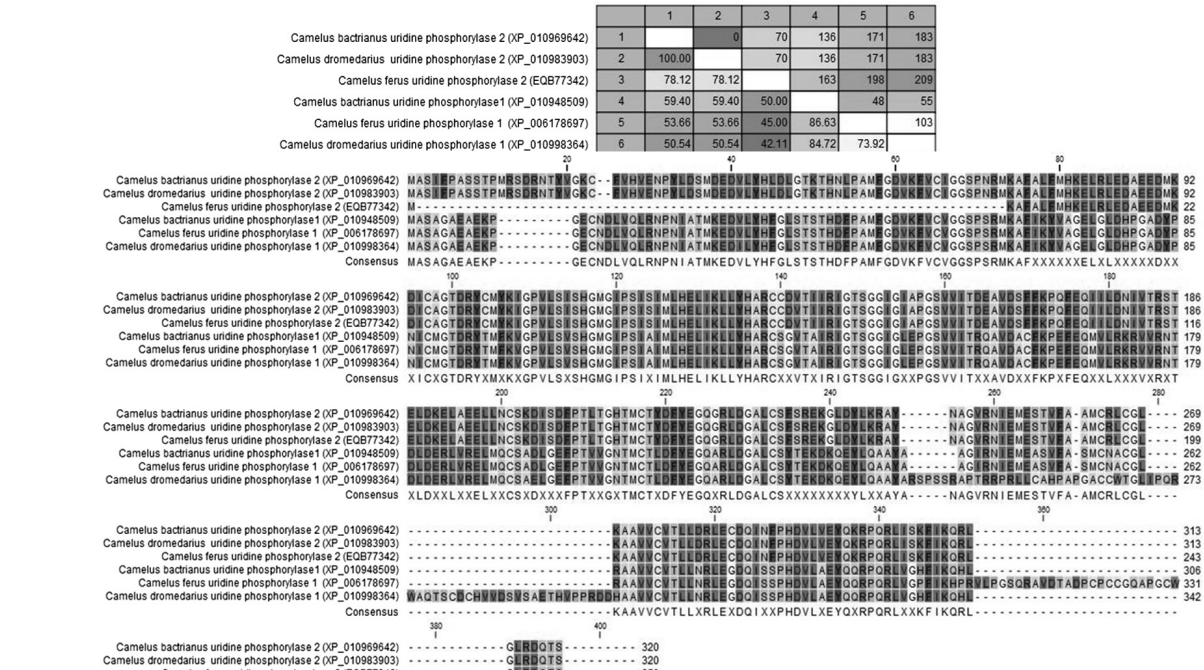

Fig 6. Multiple sequence alignment of dromedary, Bactrian and feral camels uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

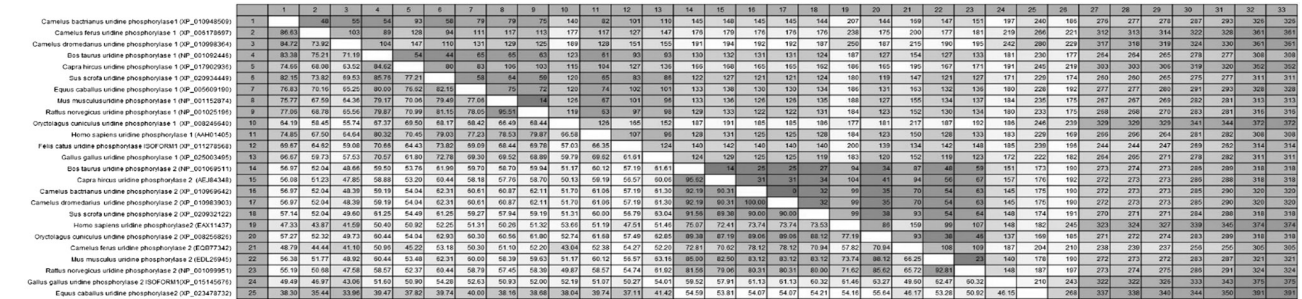

Fig 7. Dromedary camel, *T. evansi* and other prokaryotes and eukaryotes uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

was affected by the long sequence insertions in the dromedary camel sequence (Fig 6).

Multiple sequence alignment (Fig 7) and phylogenetic analysis (Fig 8) with a set of prokaryotes and eukaryotes uridine phosphorylases revealed close relation of the single bacterial and protozoal UPase with the eukaryotic UPase 1, which were distant from UPase 2. The similarity rate was 66-86% within UPase 1, which lowers to the range of 43-56% after comparison with uridine phosphorylase 2. The prokaryotic and Trypanosoma UPase showed very low similarity rates of 15.7-18.8% compared with the eukaryotic uridine phosphorylases.

Comparison between *T. evansi* and camel UPase showed that the trypanosomal enzyme shared 18.64 and 15.39% similarity with the camel UPase 2 and 1, respectively (Fig 9). The domain prediction tool at NCBI predicted that the retrieved sequence is similar to the confirm uridine phosphorylase from *T. brucei* (Fig 10).

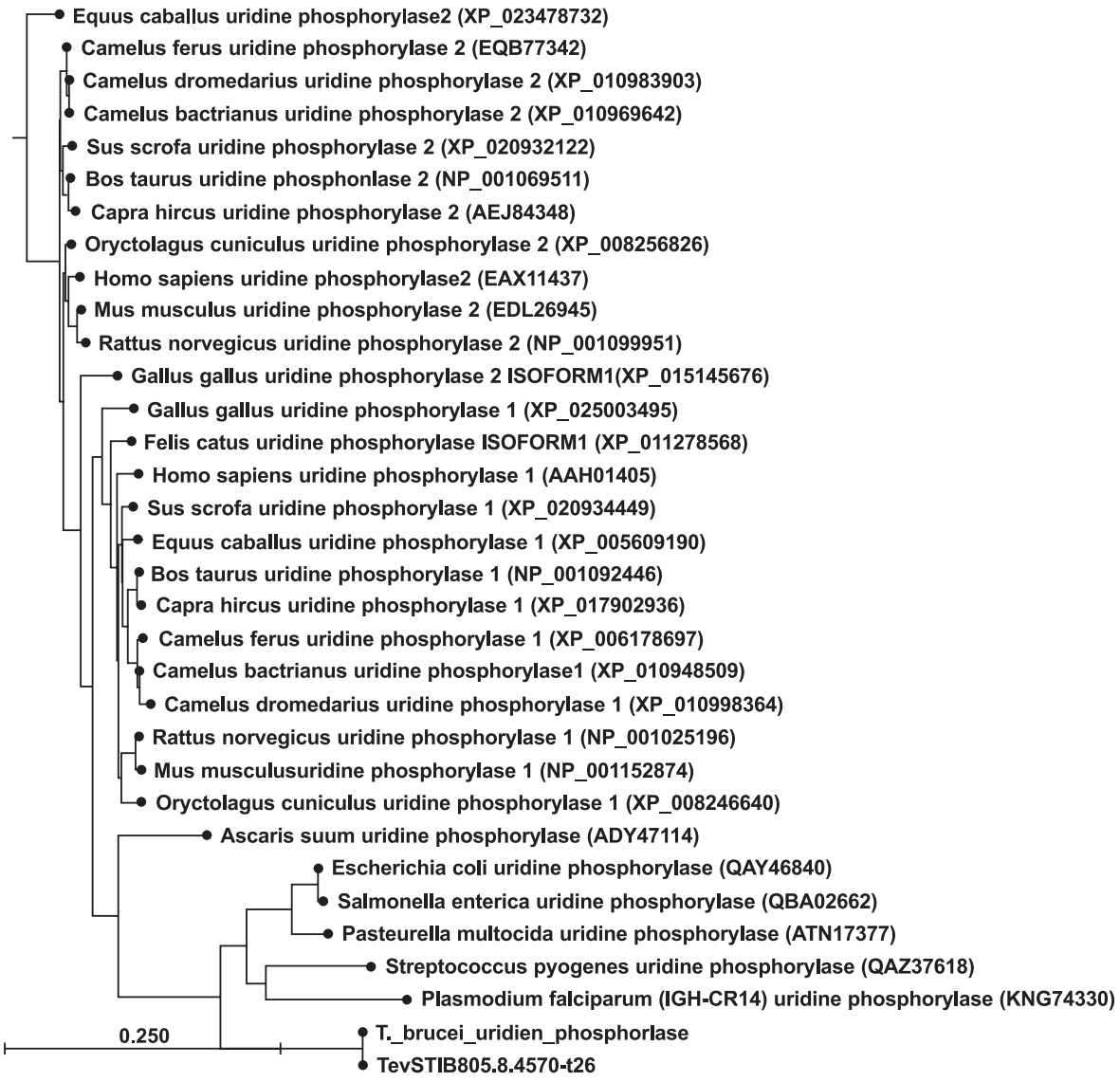

Fig 8. Phylogram of camel and *Trypanosoma evansi* uridine phosphorylase in relation to a set of eukaryotic and prokaryotic organisms.

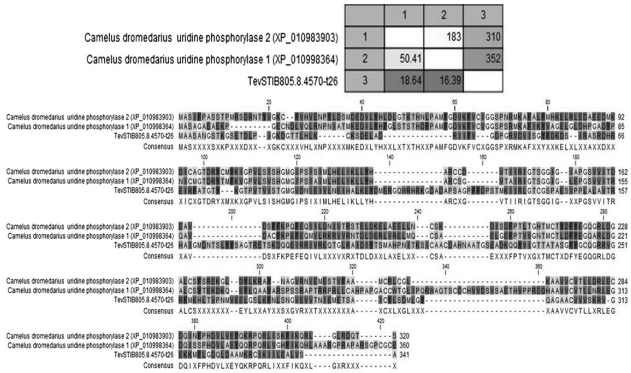

Fig 9. Pairwise sequence alignment of dromedary camel and *Trypanosoma evansi* uridine phosphorylase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

Cytidine deaminase

Cytidine deaminase (EC 3.5.4.5, CDA). A hydrolase metalloenzyme (contains zinc), broadly disseminated amongst organisms, acting on carbon-nitrogen bonds, not peptide bonds. The enzyme implicated in salvage of both exogenous and endogenous cytidine and 2′ deoxycytidine for UMP synthesis. Likewise, it catalyses the deamination of cytidine and 2′-deoxycytidine with analogous effectiveness. Interestingly, the activity of the plant enzyme is almost very analogous to that of the human (Vincenzetti *et al,* 1999). *Crithidia fasciculata* (a mosquito parasite) and *Trypanosoma cruzi* (a human pathogen) have cytidine deaminase enzyme. The enzyme from *C. fasciculata* deaminated both cytidine and deoxycytidine, the affinity of CDA for cytidine being much lower than deoxycytidine. Meanwhile,

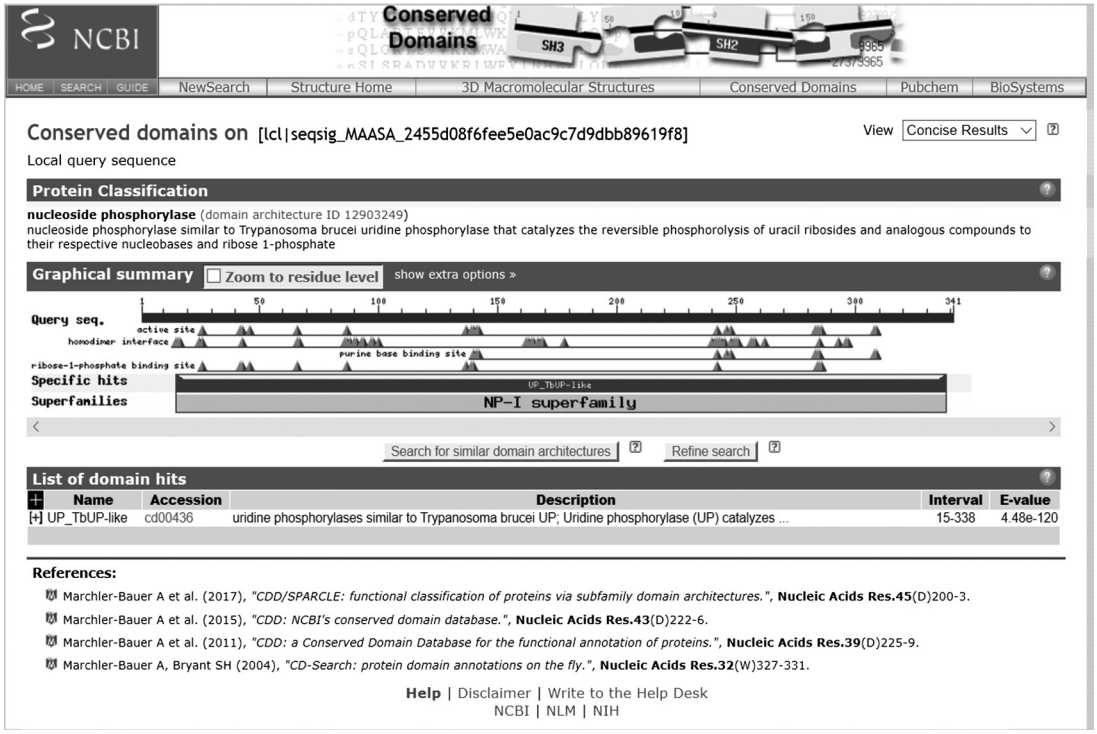

Fig 10. The expected uridine phosphorylase domain in *T. evansi*.

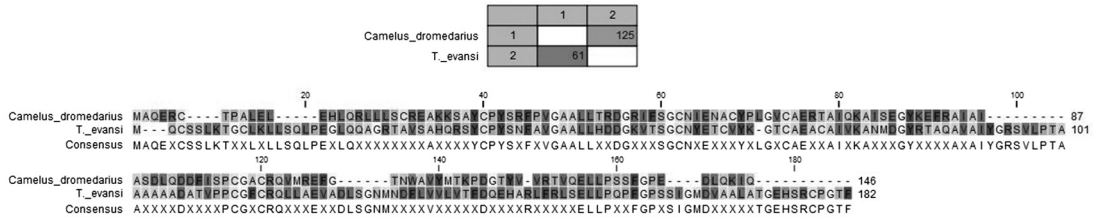

Fig 11. Pairwise sequence alignment of dromedary camel and *T. evansi* cytidine deaminase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

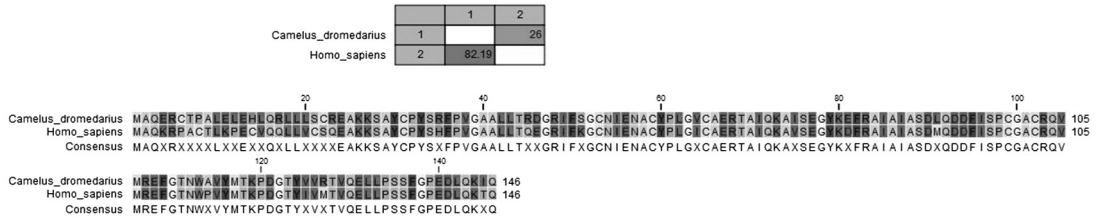

Fig 12. Multiple sequence alignment of dromedary camel and human cytidine deaminase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

the *T. cruzi* enzyme has identical affinities for both substrates. Pyrimidine nucleosides as cytidine, uridine, 5-bromouridine, thymidine, and orotidine significantly triggers the production of the enzyme in *C. fasciculata*. Cytidine is the only nucleoside stimulated enzyme production in *T. cruzi* (Kidder, 1984).

It was previously reported that CDa is essential for the life of *T. brucei* (Moro-Bulnes *et al,* 2019). Additionally, CDa is the major source for uridine (Leija *et al,* 2016), which is important for dUMP and thymidylate synthesis. In *T. brucei*, CDa was found to be a tetrameric enzyme, which is similar to the

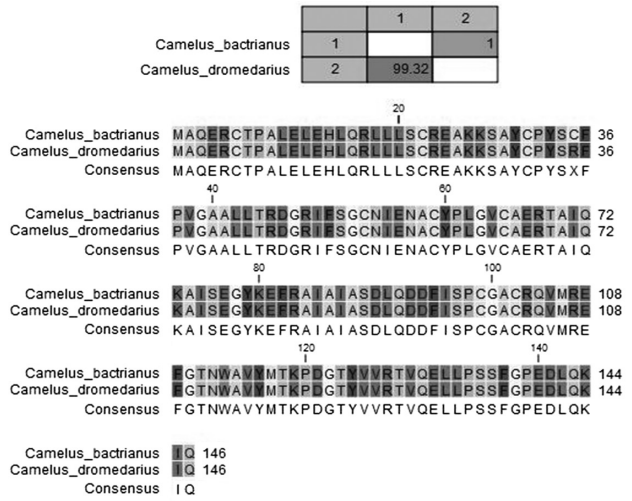

Fig 13. Multiple sequence alignment of dromedary and Bactrian camels cytidine deaminase. The upper panel represents sequence comparison statistics. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two sequences.

eukaryotic and vertebrate CDa. In addition, CDa showed more important role in synthesis of dUMP and thymidylate synthesis than dUTPase (Moro-Bulnes *et al,* 2019).

Pairwise comparison of camel and Trypanosoma CDa showed that the protozoal enzyme is about 40 peptides longer than the camel enzyme with about 61% similarity (Fig 11). The camel and human CDa shared a high similarity rate, more than 82% Fig 12). Camel species showed very high similarity of 99.32% (Fig 13). Multiple sequence comparison and phylogenetic tree of camel and *T. evansi* cytidine deaminase in relation to a set of eukaryotic and prokaryotic organisms are provided in Fig 14 and 15. The *T. evansi* CDa was closely related to higher vertebrate CDas and distant from the prokaryotic and other protozoal enzymes. The prokaryotic and protozoal enzymes showed 5-13% similarity with the camel and higher vertebrates. While in some protozoa including *T. evansi* and helminths, the rate showed medium similarity values of 30-55%. In comparing the domain and motif content in camel and Trypanosoma, the tetrameric form was readily detectable with camel CDa (Fig 16) but not with *T. evansi*, which contained CDD cytidine deaminase superfamily nucleotide metabolism and transportation.

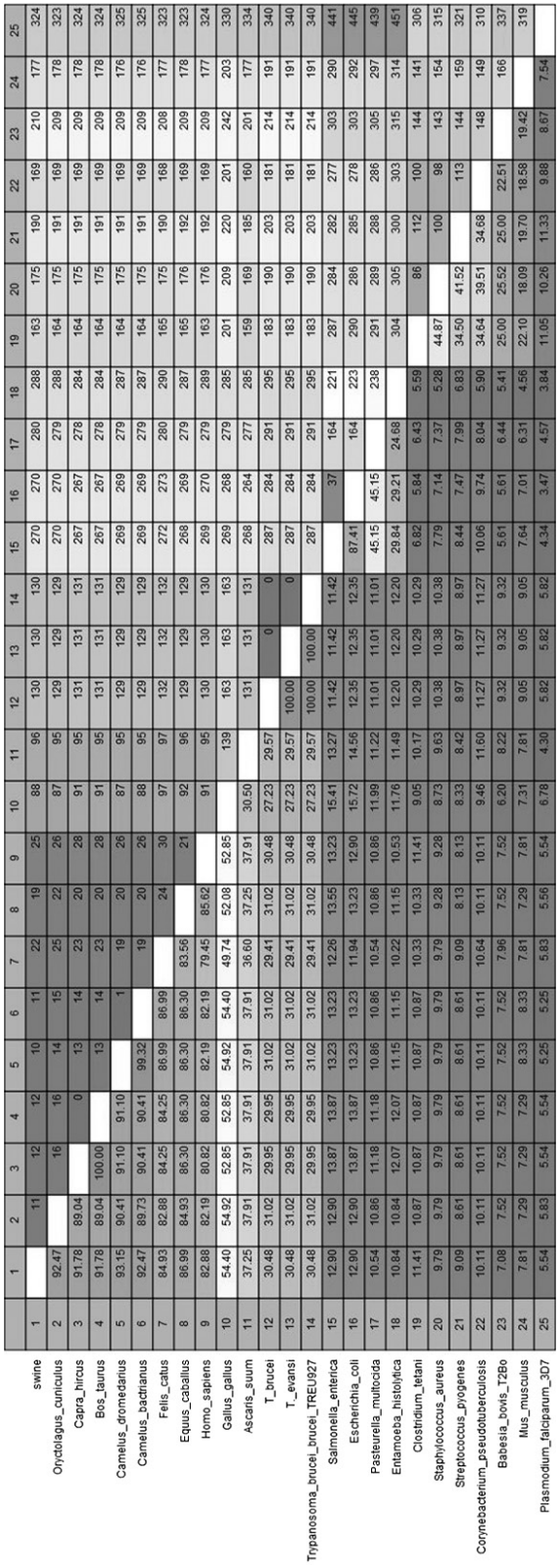

Fig 14. Multiple sequence alignment camel and *Trypanosoma evansi* cytidine deaminase in relation to a set of eukaryotic and prokaryotic organisms. The upper right diagonal region is explains the number of differences between two sequences, while the lower left diagonal region explains the per cent of identity between two

Deoxyuridine

Deoxyuridine production process is proposed by three routes (Fig 17) 1) dephosphorylation of

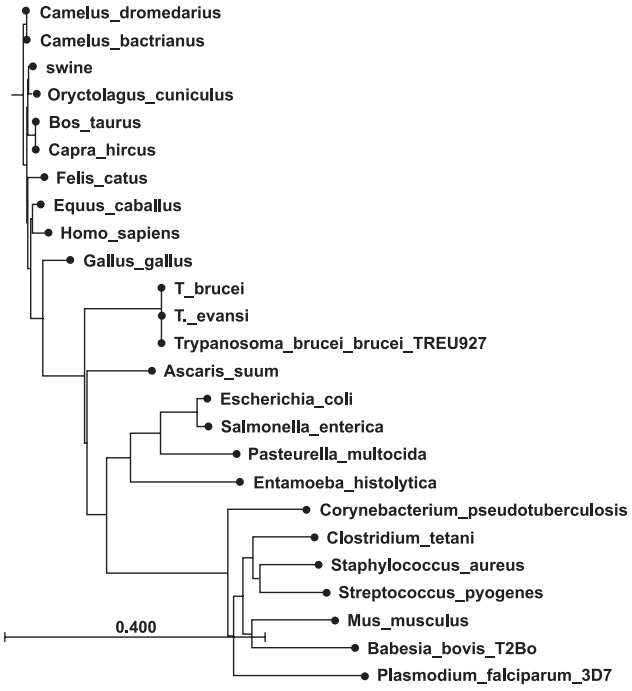

Fig 15. Phylogram of camel and *Trypanosoma evansi* cytidine deaminase in relation to a set of eukaryotic and prokaryotic organisms.

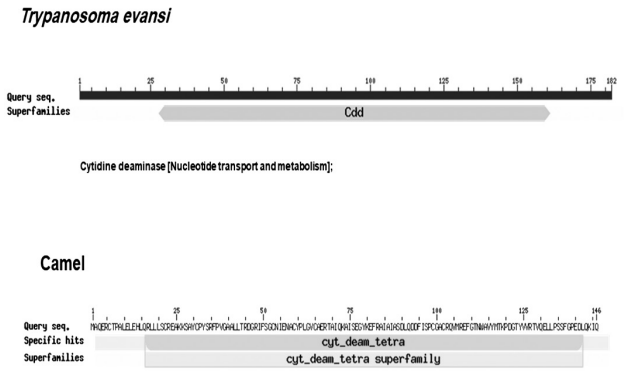

Fig 16. Motif and domain search of camel and *Trypanosoma evansi* cytidine deaminase.

uridine 5′-monophosphate by the action of uridine 5′-nucleotidase or 5′-deoxynucleotidase 2) from uracil by the action of uridine phosphorylase or pyrimidine nucleoside phosphorylase 3) by deamination of deoxycytidine by the action of cytidine deaminase. In the catabolic direction, deoxyuridine is used by uridine phosphorylase or nucleotide phosphorylases to give uracil (Fig 17). In the de novo pathway of pyrimidine synthesis, dUMP is produced from deoxyuridine by the kinase activity of thymidine kinase. Camels maps obeys the general described paths for deoxyuridine. In contrast, *T. evansi* showed similar profile with exception of the lack of dUMP degradation to give deoxyuridine (Fig 18, 19).

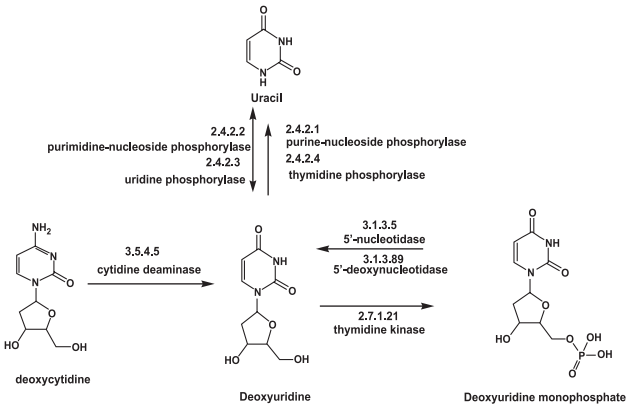

Fig 17. The proposed metabolic pathways of deoxyuridine (dU).

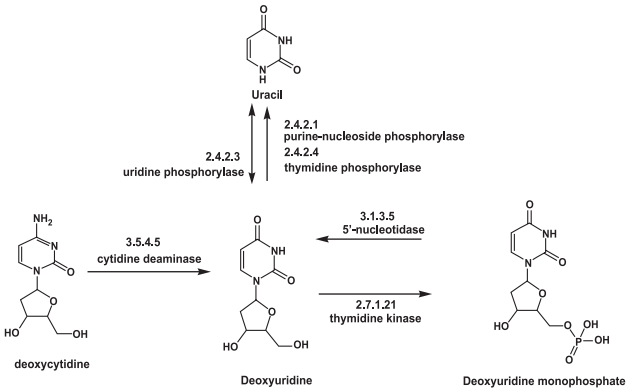

Fig 18. The proposed metabolic pathways of deoxyuridine (dU) in camels.

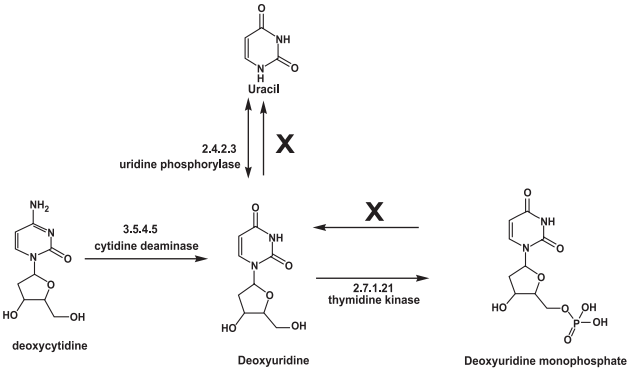

Fig 19. The proposed metabolic pathways of deoxyuridine (dU) in *Trypanosoma evansi*.

Conclusions

The uridine metabolic pathway in *T. evansi* is an important target in *T. evansi*. The lack of uridine kinase in *T. evansi* had led to dependence on conversion of uridine to uracil to help in getting UMP by the salvage of uracil by uracil phosphoribosyl transferase. The obtained results from bioinformatics investigations suggests targeting UPase and CDa as drug targets by affecting uridine and uracil paths.

Acknowledgements

The authors acknowledge the financial support of this project by King Abdul-Aziz City for Science and Technology (KACST), Basic Research Programs, National Transformation Program, under Research and Development Grants Program for National Research Institutions and Centres (GPURC), Kingdom of Saudi Arabia (Grant No. 2-17-04-004-0001).

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Chapter from multiauthored books: Chawla SK, Panchbhai VS and Gahlot TK (1993). The special sense organs-Eye. In: Ruminant Surgery, Eds., Tyagi RPS and Singh J. Ist Edn., CBS Publishers and Distributors, Delhi, India. pp 392-407.

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

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

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DOI : 10.5958/2277-8934.2020.00017.X Vol 27 No 1, p 121-128

THE CARDIAC BIOMARKERS TROPONIN I AND CREATINE KINASE MYOCARDIAL BAND IN CAMELS (*Camelus dromedarius*)– A REVIEW

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ABSTRACT

Cardiac biomarkers are helpful in the early detection, diagnosis and prognosis of cardiac and non-cardiac diseases. Cardiac troponin I (cTnI), one of these biomarkers, is a highly sensitive and specific marker for myocardial injury in humans and in veterinary medicine. cTnI elevates in serum after acute myocardial injury because of leakage from the damaged myocardial cells. Creatine kinase myocardial band (CK-MB), another cardiac biomarker, has been found high following exercise. With chest pain in humans, the level of CK-MB increases and subsequently declines to normal range. In animals, however, a rise in CK-MB is not always indicative of acute myocardial infarction. cTnI therefore is currently the preferred cardiac biomarker in human medicine for assessing myocardial damage, with absolute specificity and higher sensitivity. The degree of increase in cTnI has been shown to correlate with the extent of myocardial damage and with survival in humans and animals. In camels, the evaluation of cardiac disease can be challenging; the patient history, clinical data and routine blood examination are often nonspecific. Therefore, blood-based biomarkers that are capable of detecting and staging cardiac disease are a subject of considerable interest. Myocardial damage, as demonstrated by elevated cTnI in blood, appears to be a common sequel to a wide variety of both primarily cardiac disease and of other diseases that do not primarily involve the cardiovascular system. This review was written to shed light on the commonly used cardiac biomarkers in camel medicine cTnI and CK-MB and its clinical significance.

Key words: Camels, cardiac biomarkers, cardiac troponin I, creatine kinase–myocardial band, heart diseases

Biomarkers can indicate physiological (such as growth and aging), or pathophysiological processes that occur with disease (e.g. cardiac damage and heart failure). Among these biomarkers, cardiac biomarkers can be helpful in the management of cardiac and non-cardiac diseases (Jesty, 2012). In humans, cardiac biomarkers aid in the early detection, diagnosis and prognosis of cardiac diseases (Ginsburg and Haga, 2006).

Among cardiac biomarkers, cardiac troponin I (cTnI), is a highly sensitive and specific marker for myocardial injury in humans (Ladenson, 2007; Reagan *et al,* 2013) and in veterinary medicine (Wells and Sleeper, 2008; Fonfara *et al,* 2010; Tharwat, 2012; Tharwat *et al,* 2012; Tharwat *et al,* 2013a,b,c,d; Tharwat and Al-Sobayil, 2014a,b,c; Tharwat *et al*, 2014a,b; Tharwat, 2015; Tharwat and Al-Sobayil, 2015). The serum concentration of cTnI elevates after acute myocardial injury because of leakage from the damaged myocardial cells (O'Brien *et al*, 2006). In veterinary medicine, cTnI has also a high sensitivity and specificity in animals with diseases of cardiac and noncardiac origin (O'Brien *et al,* 2006; Wells and Sleeper, 2008). The degree of increase in cTnI has been shown to correlate with the extent of myocardial damage and with survival in humans (Stanton *et al*, 2005) and animals (Oyama and Sisson, 2004; Fonfara *et al*, 2010).

Creatine kinase myocardial band (CK-MB) is another cardiac biomarker that has been reported to increase with exercise (Mamor *et al,* 1988; Rahnama *et al,* 2011). With chest pain in humans, the level of CK-MB reaches its peak at 10-24 hours subsequent to the initial injury and declines to normal range within 72-96 hours (Volz *et al,* 2012). Chronic occlusion of the coronary artery significantly increases the serum levels of CK-MB (Sharkey *et al,* 1991). However, a rise in CK-MB is not always indicative of myocardial damage; it has been elevated in patients with acute skeletal muscle trauma, dermatomyositis,

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polymyositis, muscular dystrophy and renal failure (Erlacher *et al,* 2001). Therefore, cTnI is nowadays the superior biochemical parameter in humans for assessing myocardial damage, with absolute specificity and sensitivity than CK-MB (Alpert *et al,* 2000; Collinson *et al,* 2012).

In camels, heart diseases include pericarditis, vegetative valvular endocarditis, hypertrophic cardiomyopathy necrotic myocarditis, congenital defects including septal defects, patent ductus arteriosus, transposition of the aorta and pulmonary artery, persistent aortic trunk, and persistent right aortic arch and sarcocystosis (Fowler, 2010). These are mostly diagnosed at slaughterhouses or incidentally discovered during postmortem (Fowler, 2010). The diagnosis of camel heart disease is a challenging task especially when typical clinical signs of heart failure are absent. Therefore, blood-based biomarkers capable of detecting and staging diseases of cardiac and non-cardiac origin are a subject of considerable interest in camels.

This review was designed to shed light on the commonly used cardiac biomarkers in camel medicine cTnI and CK-MB as indicators of cardiac injury.

Cardiac troponins

Troponins are 3 distinct myofibrillar proteins (I, C, and T) that regulate the calcium-mediated interaction between actin and myosin in both cardiac and skeletal muscle (Babuin and Jaffe, 2005). Of troponins, cTnI is the only one that is expressed in the myocardium. The amino acid sequence for cTnI is highly conserved among mammalian species so human kits can be used; however, the references and values can differently depend on the analyser used since they can give different results (Apple *et al,* 2008). Mildly elevated concentrations of cTnI alone will unlikely lead to a definitive diagnosis, but together with the clinical presentation and findings on ECG and echocardiography it can become an important marker for myocardial disease. On the other hand, marked elevations of cTnI alone could be considered as a strong indication of myocardial disease (Nostell and Haggstrom, 2008). However, A constant elevation of cTnI indicates persistent damage to the cardiomyocytes (O'Brien *et al,* 2006; Wells and Sleeper, 2008), and the degree of elevation has been shown to be correlated with the extent of myocardial damage and with survival in humans (Stanton *et al,* 2005) and in animals (Fonfara *et al,* 2010; Tharwat, 2012; Tharwat and Al-Sobayil, 2014a).

Low to non-detectable cTnI levels have been found in healthy mammals (Baker *et al,* 2011). Elevated cTnI has been reported in calves (Peek *et al,* 2008), cattle (Varga *et al,* 2009; Mellanby *et al,* 2009), horses (Kraus *et al,* 2010; Holbrook *et al,* 2011), foals (Slack *et al,* 2005), dogs (Herndon *et al,* 2002; Spratt *et al,* 2005) and lambs (Gunes *et al,* 2010) indicating that elevations in the blood would serve as useful biomarkers of myocardial injury.

Creatine kinase myocardial band

Creatine kinase is a dimeric enzyme found primarily in brain and muscle tissue. Three isoforms are known for creatine kinase: BB, MM, and MB. The isoform BB is found in the brain. The second isoform MM is found primarily in skeletal muscles. Cardiac muscles also primarily contain the MM isoform, but with higher amounts of MB, typically around 20% of CK activity (Moss and Henderson, 1994). In humans, serum from healthy individuals typically contains the MM isoform and a small amount of the MB isoform. CK-MB can be released into the bloodstream by a number of actions, including skeletal muscular injury and myocardial damage.

Cardiac biomarkers in camel medicine

In humans, nonprimary cardiac diseases can induce myocyte damage leading to increased serum troponin concentrations (Mahajan *et al,* 2006). For example, study of 144 patients with increased cTnI concentrations identified a wide range of diseases that can be associated with increased cTnI concentrations including sepsis, collagen vascular disease, gastrointestinal bleeding, pulmonary embolism, diabetic ketoacidosis, and chronic obstructive pulmonary disease (Mahajan *et al,* 2006). Other studies in dogs with gastric dilatation and volvulus and in dogs and cats with azotaemia renal failure and in dogs with non-cardiac systemic disease had increased cTnI concentrations indicating cardiomyocyte degeneration and necrosis (Schober *et al,* 2002; Porciello *et al,* 2008). Similar findings have been found in cattle with noncardiac and intrathoracic diseases, even though no gross cardiac abnormalities were detected at postmortem examination (Mellanby *et al,* 2009). Most of these studies concluded that the heart may be a non-target tissue bystander in these processes that leads to elevations in cTnI, but there is little strong data to definitively identify the mechanism.

In recent years, our research group has observed significant elevations of cTnI in camel blood

following prolonged recumbency (Tharwat, 2012), general anaesthesia (Tharwat *et al,* 2013a), long road transportation (Tharwat *et al,* 2013b), racing (Tharwat *et al,* 2013c), tick infestation (Tharwat and Al-Sobayil, 2014) and after stimulation by electroejaculation (Tharwat *et al,* 2014a) and following parturition stress (Tharwat, 2015). In humans, studies on the prognostic significance of cTnI concentrations in patients with non-primary cardiac disorders have found that cTnI can predict disease outcome. Recently, in cattle with haemolytic anaemia, long-term follow-up of serum cTnI concentrations was valuable in assessing the relationship between anaemia and myocyte damage (Fartashvand *et al,* 2012). In addition, an elevated serum concentration of cTnI has been used as a poor prognostic indicator in goats with pregnancy toxaemia (Tharwat *et al,* 2012), in downer camels (Tharwat, 2012) and in camels infested with ticks (Tharwat and Al-Sobayil, 2014a).

In a study published recently in camels with tick infestation (Tharwat and Al-Sobayil, 2014a), 14 recovered out of 15 camels (93.3%) had a serum concentration of cTnI lower than 1.0 ng/ml, and the remaining camel (6.7%) had a higher cTnI concentration (1.65 ng/ml). In the same study, all 8 died camels had a serum concentration above 1.22 ng/ml, with a maximum value of 5.22 ng/ml (Fig 1). Therefore, it was assumed that the increased serum concentration of cTnI above 1.0 ng/ml at initial examination was a bad prognostic indicator in the camels with tick infestation. Elevated serum concentration of cTnI has been reported in cattle with theileriosis (Fartashvand *et al,* 2013).

In camels infected with *Trypanosoma evansi* (n=74), the values of cTnI and CK-MB were significantly higher in *T. evansi* infected camel compared to controls (n=20) (El-Deeb and Elmoslemany, 2015). Successfully treated camels (n=43) had lower levels of cTnI and CK-MB compared to camels with treatment failure. cTnI showed better sensitivity and specificity than CK-MB. Similar in cattle, serum concentration of cTnI was significantly higher (P=0.003) in cattle with theileriosis (mean: 0.028 ng/mL; range: 0.005–0.21 ng/mL) compared to controls (mean: 0.011; range: <0.005–0.09 ng/mL) (Fartashvand *et al,* 2013). Anaemia followed by hypoxia and increased oxygen consumption by the myocardium during a prolonged period of tachycardia will possibly cause myocardial injury and subsequent increased serum concentration of cTnI in animals with parasitic infestation (Fartashvand *et al,* 2012; Tharwat and Al-Sobayil, 2014a).

In a study carried out on 33 long-standing recumbent camels (Tharwat, 2012), marked elevations of cTnI in the downer camels was considered as a strong indication of myocardial damage and was used to predict treatment outcome and mortality (Fig 2). In the same study (Tharwat, 2012), the serum concentration of cTnI in the 11 cured camels was 0.05±0.02 ng/ml. In the remaining 22 camels that did not recover, the serum concentration of cTnI was 0.53±0.64 ng/ml. A recent study in dairy cows with downer cow syndrome concluded that cTnI concentrations could help to rapidly identify cows that have poor chances of recovery and would benefit from a more aggressive treatment or euthanasia (Labonte *et al,* 2018).

In 25 camels transported for a 5km round trip, the mean cTnI concentration was 0.032±0.023 ng/mL comparing to resting values of less than 0.08 ng/mL

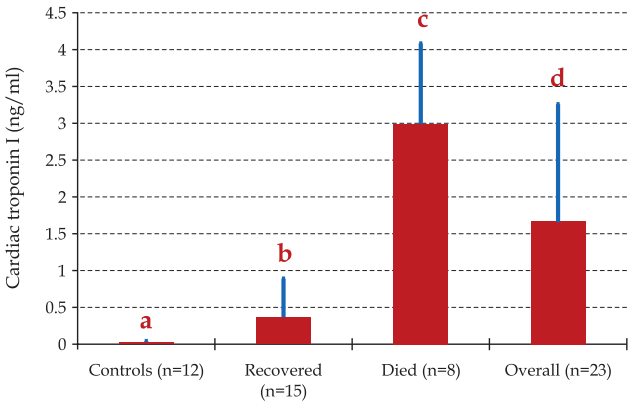

Fig 1. Mean serum concentrations of serum cardiac troponin I in camels with tick infestation. [a,b,c,d] Differ significantly (Tharwat and Al-Sobayil, 2014a).

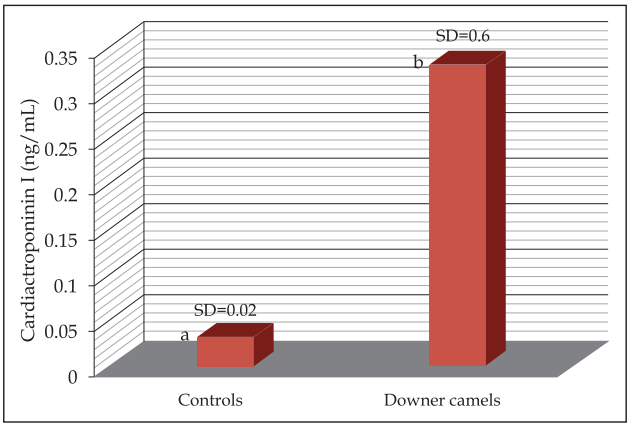

Fig 2. Cardiac troponin I values in downer camels compared to control healthy camels. [a,b] different letters indicate a significant difference (P=0.019). SD = standard deviation (Tharwat, 2012).

(Tharwat *et al,* 2013b). The cTnI concentration was significantly higher (P<0.001) in all the 25 camels compared to values before transportation. The CK-MB concentration in the same camels was 0.19±0.05 ng/mL compared to resting values of less than 0.33 ng/mL. Only in 3 of the 25 camels (12%), the CK-MB values were above values before transportation and no statistical differences were recorded (Fig 3). Transportation is a well-known stressor that has adverse effects on livestock production and health including muscular damages, generating concerns of an economic as well as a welfare-related nature (Tharwat *et al,* 2013b).

Following a 5 km race in 23 camels, 91.3% of the camels had increases in serum cTnI concentrations, while concentrations remained unchanged in 8.7% (Tharwat *et al,* 2013c). The cTnI concentration (median 0.06 ng/mL; range, 0.03–0.15 ng/mL) was significantly higher (P<0.001) than the pre-race values (median 0.04 ng/mL; range, 0.01–0.07 ng/mL). Twenty-four hours post-race, the cTnI concentrations had returned very nearly to their pre-race values (median 0.04 ng/mL; range, 0.00–0.09 ng/mL) and were not significantly different (P=0.35) from the pre-race values (Fig 4). Following the 5 km race, increases in CK-MB mass were seen in 17.4% of the camels, with no changes in 4.3% and decreases in 78.3%. The CK-MB mass (median 0.41 ng/mL; range, 0.19–0.60 ng/mL) did not differ significantly (P=0.84) when compared to the pre-race values (median 0.42 ng/mL; range, 0.32–0.55 ng/mL). Twenty-four hours post-race, the CK-MB mass concentrations (median 0.41 ng/mL; range, 0.15–0.55 ng/mL) did not differ significantly (P>0.05) compared to pre-race or immediate post-race values (Fig 4). Post-exercise cTnI release and clearance were also reported in normal Standardbred racehorses. All horses experienced an increase in cTnI post-exercise, with peak occurring 2-6 h post-exercise (Rossi *et al,* 2019). In a study carried out on 32 racing greyhounds following a 7 km race, 31/32 greyhounds showed increases in cTnI concentrations which were significantly higher than the pre-race concentrations (P<0.0001). cTnI concentrations dropped back 24h post-race to values were not significantly different from the pre-race concentrations. Only 5/32 greyhounds showed mild increases in CK-MB concentrations but these were

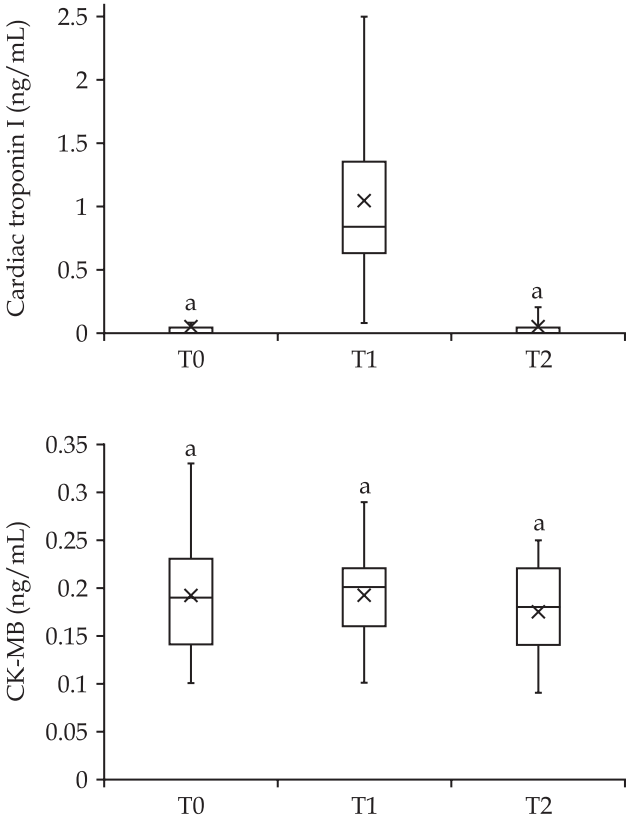

Fig 3. Box and "whisker" plots of cTnI and CK-MB values in camels before (T0), within 2h of transportation (T0) and 24h after transportation (T2). Values with different letters differ significantly (P<0.001) (Tharwat *et al,* 2013b).

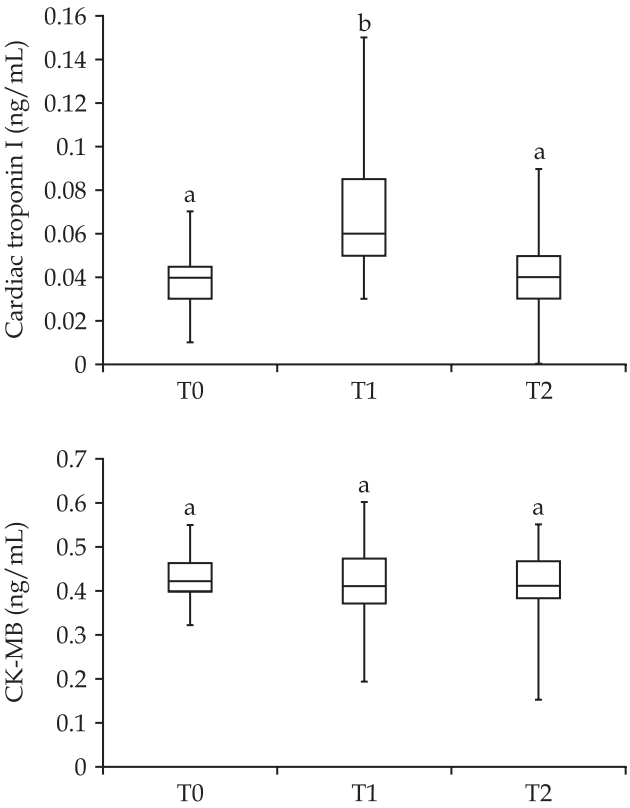

Fig 4. Cardiac troponin I values in camels before (T0), 2h after (T1) and 24h after (T2) a 5 km race. [a,b] different letters indicate a significant difference (P<0.05) (Tharwat *et al,* 2013c).

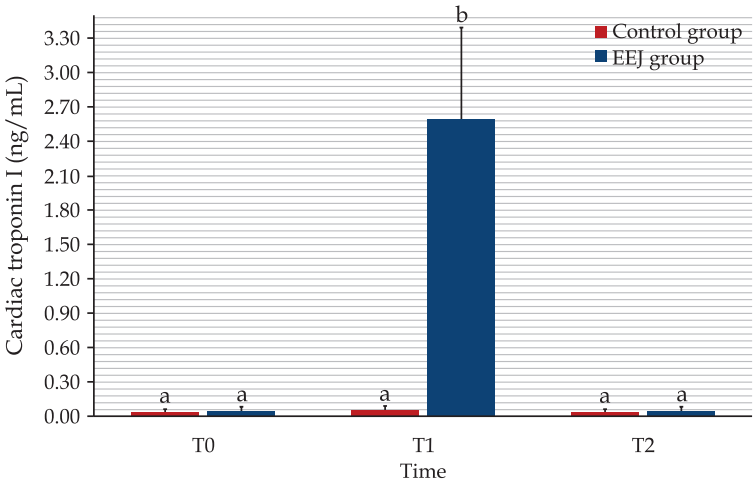

Fig 5. Effect of stimulation by electroejaculation (EEJ) on cardiac troponin I in male dromedary camels (mean ± SD, n=20) compared to control group (n=10). T0: just before EEJ; T1: directly after EEJ; T2: 24h after EEJ. [a,b] Values differ significantly (P=0.0001) (Tharwat *et al,* 2014a).

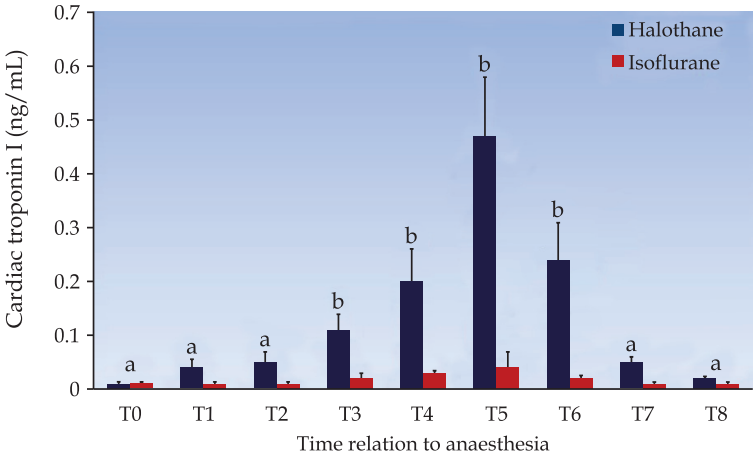

Fig 6. Pre-anesthetic, anesthetic and post-anesthetic serum concentration of cardiac troponin I (means ± SEM) in camels (n = 6) undergoing isoflurane and halothane anesthesia.T0, immediately before anesthesia; T1, 20 min after xylazine administration; T2, 20 min after ketamine administration; T3, 60 min during inhalation anesthesia; T4, 40 min of recovery; T5, 80 min of recovery; T6-T8, 24 h, 48 h and 72 h after anesthesia. bDiffers significantly between the two anesthetic agents at P<0.05 (Tharwat *et al,* 2013a).

not significantly different from the pre-race values (Tharwat *et al,* 2013e).

After EEJ (electroejaculation) in 20 male camels, the mean serum concentration of cTnI had increased significantly in all camels following EEJ, but not in controls (Fig 5) (Tharwat *et al,* 2014a). However, at 24h post-EEJ, the serum concentration of cTnI did not differ significantly compared to baseline values. Because the serum concentration of cTnI increased significantly in the EEJ camels, it is therefore recommended that the status of the cardiovascular system of the camel be checked prior to applying the EEJ technique. In another study, the serum concentration of cTnI has been increased significantly (P=0.0001) in 18 male camels with erectile dysfunction compared to 10 healthy controls (Derar *et al,* 2017). The rise of cTnI in the males with erectile dysfunction is probably indicative of myoctitic damage which support the concept that failure to erect the penis or maintain an erection is primarily related to the inability to maintain a closed blood circuit at the penile tissue (Barassi *et al,* 2015)

Cardiac injury had been reported in camels with halothane and isoflurane general anaesthesia (Tharwat *et al,* 2013a). In this study, camels had mildly and significantly elevated cTnI with isoflurane and halothane anaesthesia, respectively; however, in the isoflurane group the upper limit for the camel reference range was not exceeded (Fig 6). The cause of the cardiac cell compromise during halothane anaesthesia was likely due to extreme changes in heart rate and blood pressure, and the increased arterial concentration of PCO_2. Based on the results of this study, it was concluded that isoflurane is superior to halothane as an inhalation anaesthetic in camels especially in those with suspected cardiac diseases. The influence of general anaesthesia on serum concentration cTnI in healthy dogs has also been studied (Verbiest *et al,* 2013). Fifty-five percent of the dogs had a post-anaesthetic increase of cTnI concentration relative to their pre-anesthetic cTnI concentration, whereas a decrease was observed in eleven percent of the dogs.

Conclusions

In camel medicine cardiac biomarkers are an exciting and growing science. The most established applications involve the use of cTnI to help detect early myocardial injury following prolonged recumbency, after general anaesthesia, secondary to long road transportation, following racing, as an influence of parasitic infestation and after semen collection by electroejaculation. The cTnI assay helps to rapidly determine the prognosis in camels and thereafter deciding either continuing treatment or euthanasia. CK-MB is a less sensitive biomarker

for myocardial activity when compared with cTnI in detecting myocardial injury. Finally, cardiac biomarker tests are complementary to existing cardiac diagnostic testing and should be interpreted in the context of the overall clinical picture rather than being used as a stand-alone test.

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DOI : 10.5958/2277-8934.2020.00018.1 *Vol 27 No 1, p 129-134*

ULTRASONOGRAPHY OF THE DIGESTIVE TRACT IN YOUNG CAMEL CALVES UNTIL THE AGE OF 100 DAYS

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ABSTRACT

This study was designed to obtain the normal imaging pictures of the gastrointestinal tract (GIT) including the gastric compartments and small and large intestines in camel calves until the age of first 100 days of life. The GIT was examined at day 1, day 20, day 40, day 80 and day 100 by ultrasound in 15 clinically healthy camel calves (from 1 day until 100 days of age) and the normal imaging patterns were recorded and analysed. The results of ultrasonography as well as the imaging of the gastric compartments and small and large intestines are summarised. Ultrasonography could be used as a noninvasive diagnostic tool in order to detect GIT diseases in the camel calves.

Key words: Abdomen, camel calves, digestive, gastrointestinal, ultrasonography

The ultrasonographic picture of the gastrointestinal tract (GIT) including the gastric compartments and small and large intestines has been reported for adult dromedaries (Tharwat *et al,* 2012a). In diseased camels, on the other side, diagnostic ultrasonography has been applied for the evaluation and determining the prognosis of camels with gastrointestinal disorders such as abdominal distension (Tharwat *et al,* 2012b), paratuberculosis (Tharwat *et al,* 2012c), abdominal disorders (Tharwat and Al-Sobayil, 2016), gastrointestinal tumours (Tharwat *et al,* 2018) and chronic peritonitis (Tharwat, 2019).

In cow calves, ultrasonography of the reticulum, rumen, omasum and abomasum has been reported (Braun *et al,* 2013; Braun and Gautschi, 2013). In growing camel calves, however, the ultrasonographic picture of the dramatically changed GIT during the first 100 days of age has not been reported. The purpose of this study was therefore designed to periodically examine the GIT including the gastric compartments and small and large intestines in camel calves until the age of 100 days to gain detailed information about normal ultrasonographic imaging patterns of the GIT. It is believed that knowledge of the ultrasonographic appearance of the normal GIT in camel calves will provide a reference for the interpretation of the GIT in camel calves with suspected digestive abnormalities.

Materials and Methods

Animals and physical examination:

For obtaining the normal imaging patterns of GIT, 15 clinically healthy camel calves were used (from day 1 until 100 days of age). Calves were kept in the Veterinary Teaching Hospital, Qassim University, Saudi Arabia. Camel calves underwent a thorough physical examination including general behaviour and condition, auscultation of the heart, lungs, stomach and intestine, detection of heart and respiratory rates and rectal temperature (Köhler-Rollefson *et al,* 2001). All camel calves were considered clinically healthy based on physical and laboratory evaluation (complete cell blood count and chemistry panel), and they had full access to feed and water before and after examination.

Ultrasonographic examination

Ultrasonographic examination of the GIT was carried out in the camel calves at day 1, day 20, day 40, day 80 and day 100. Ultrasonography of the GIT was carried out in camel calves as described for adult camels (Tharwat *et al,* 2012b) in sternal recumbency. Ultrasonographic examination was carried out using a 3.5 to 5.0 MHz sector transducer (SSD-500, Aloka, Tokyo, Japan). After the application of transmission gel to the transducer, the animals were examined beginning at the caudal abdomen and extending

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forward to the level of the sternal pad. The gastric compartments, i.e. (first compartment (C1), second compartment (C2) and third compartment (C3) and small and large intestines were scanned and images were stored.

Results

At day 1 of age, the contents of C1 were anechoic and it was best seen in the left caudal paralumbar fossa. In the later region, the large caudodorsal sac was visualised close to the spleen and left kidney. The wall of C1 was smooth and echogenic. Reverberation artifacts running were seen in the region of the dorsal gas cap. Because of its gaseous nature, the contents of C1 could not be seen at that time (Fig 1).

Motility of the C1 was seen as shifting, retreating, and eventual replacement of portions of the wall during gastric contraction cycles. Because of the lack of penetration and large volume of the C1, it was not possible to measure the size of the C1. At day 20, C1 was imaged from left and right sides of the abdomen and the contents of the C1 were seen with disappearance of the reverberation artifacts. The contents of the C1 were visible as hyperechoic echoes interrupted with hypoechoic contents (Fig 2).

At day 40, the contents of the C1 were anechoic with appearance of the longitudinal groove splitting the C1 compartment into dorsal and ventral sacs (Fig 3).

From day 40, the water sacs were visualised in all camel calves and it appeared as series of hyperechoic, semicircular protrusions, curving away from the ventral body wall (Fig 4).

At imaging the C1 at day 80, it was found occupying the entire left abdomen. Imaging of the C2 was seen in only three of the calves at day 40 with weak biphasic contractions. At day 80 and 100, the C2 contractions were stronger and longer. The first contraction was incomplete and was followed by an interval of incomplete relaxation; this was followed immediately by a second complete contraction where the C2 could not be seen on the ultrasound monitor. An interval of complete relaxation follows, in which the C2 returned to its initial position. The wall of C2 is thick and appeared as a half-moon-shaped structure with an even contour (Fig 5).

The C3 was imaged in 3, 5 and 8 calves at day 1, 20 and 40, respectively. At day 80 and 100, it was imaged in all calves through the 6^{th} to 8^{th} intercostal spaces on the right side. It was visible as a tubular structure extending between these intercostal spaces and coursing along the body wall approximately parallel to the long axis of the camel. The contents of the first part of C3 were moderately imaged. It appeared largest in the 7^{th} intercostal space and decreased in size cranially and caudally from this point. An active motility was visualised in the first part of C3, but in a shorter duration than what has noticed in the C2. The most important imaging pattern of the first part of C3 was the fine mucosal folds that appeared as a fine hyperechogenic folds protruded from the mucosa (Fig 6). The folds of the last part of C3 appeared coarse and elongated when compared with that of the first part of C3 (Fig 7).

Small intestinal structures were best seen low in the right paralumbar fossa. Its contents were almost hypoechoic, heterogeneous and it contracted every few seconds. Boluses of hypoechoic fluid ingesta could be seen, but were rarely present more than those few seconds before the intestine contracted. Individual segments of intestine were difficult to discern in areas of collapsed intestine because of the lack of contrast between wall and lumen; gas shadowing was not seen. Because of the absence of the gallbladder in camels, it was very difficult to identify and image the duodenum in any of the examined cases. Therefore, the duodenum, jejunum and ileum could not be differentiated from one another ultrasonographically. Several loops of small intestine were imaged adjacent to one another from the lower right flank and lateral abdominal wall and from the 9^{th} to 11^{th} intercostal spaces (Fig 8).

The large intestine was usually easy to differentiate from the small intestine based on its marked gas content and relatively large diameter. Because of the gas, only the wall of the large intestine close to the transducer was imaged where it appeared as a thick echogenic line. The wall of the large intestine could not be imaged. The cecum was imaged chiefly in the caudal right flank. The tip of the cecum could also not be imaged because of its caudal position. Owing to the presence of gas, the content of the cecum could not be imaged in any of the camel calves (Fig 9).

Segments of ascending colon could be seen in the right paralumbar fossa. The spiral colon was confined in all calves to the caudal ventral half of the abdomen. It appeared as structures with thick echoic lateral walls with a number of echogenic arched lines next to each other (Fig 10).

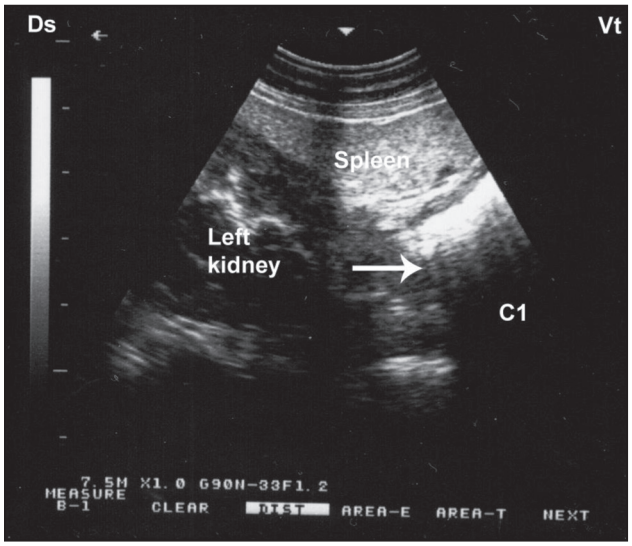

Fig 1. Ultrasonography of the C1, spleen and left kidney in a 1-day-old camel calf. Image was taken from the caudal left paralumbar fossa. The contents of the C1 could not be seen. Reverberation artifacts running parallel to the C1 wall were seen in the region of the dorsal gas cap (arrow). C1, first gastric compartment; Ds, dorsal; Vt, ventral.

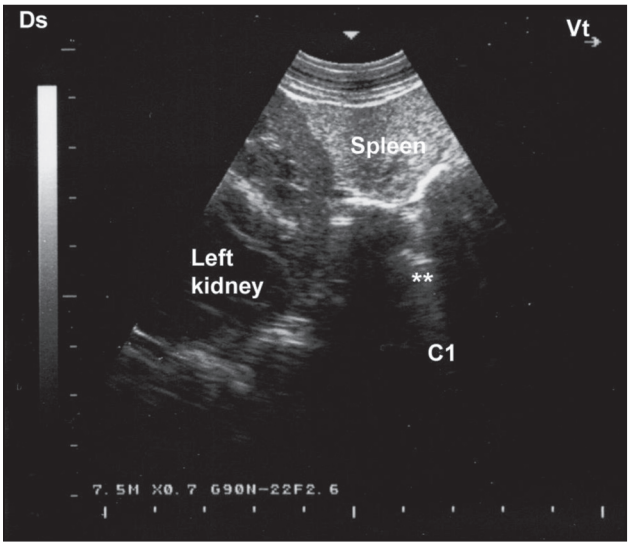

Fig 3. Ultrasonography of the C1, spleen and left kidney in a 40-day-old camel calf. Image was taken from the caudal left flank. The contents of the C1 were anechoic. The longitudinal groove (**) were imaged as a mucosal fold dividing the C1 into dorsal and ventral sacs. C1, first gastric compartment; Ds, dorsal; Vt, ventral.

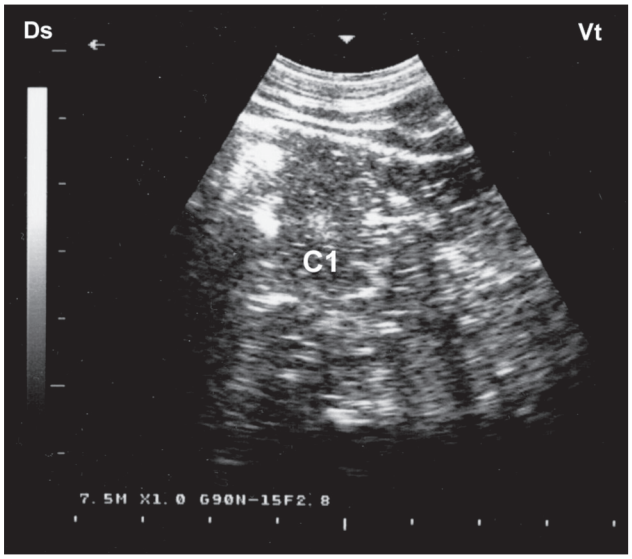

Fig 2. Ultrasonography of the C1 in a 20-day-old camel calf. Image was taken from the lower left abdomen. The contents of the C1 were visible as hyperechoic echoes interrupted with hypoechoic contents. C1, first gastric compartment; Ds, dorsal; Vt, ventral.

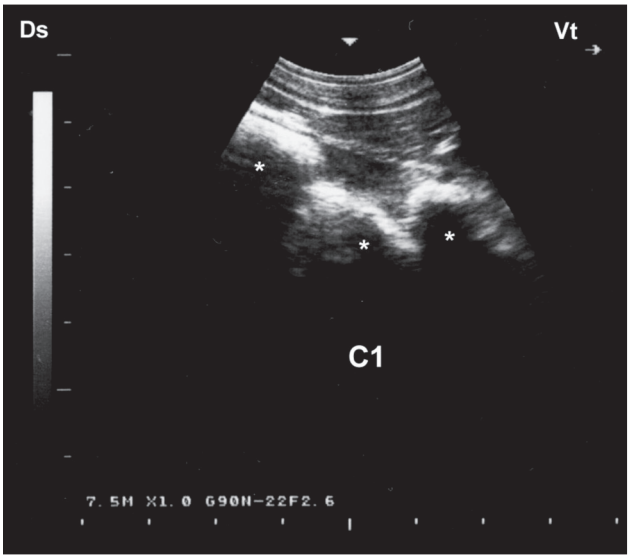

Fig 4. Ultrasonography of the water sacs in a 100-day-old camel calf. Image was taken from the left abdomen at the level of the 10th intercostal space. Water sacs appeared as series of hyperechoic, semicircular protrusions, curving away from the ventral body wall (stars). C1, first gastric compartment; Ds, dorsal; Vt, ventral.

Discussion

To the best of the author's knowledge, this is the first report describing the imaging patterns of the GIT of camel calves until the age of 100 days. In cow calves, ultrasonography of the GIT has been carried out in milk-fed calves from birth to 20 days of age (Jung, 2002) and in 90-day-old hay-fed calves (Gautschi, 2010). When the ultrasonographic findings of the two studies were compared, great differences were detected. The reason why such differences were recorded is that the GIT change dramatically during the first few months of life when milk is replaced by hay, which leads to an increase in the size of the rumen (Braun *et al,* 2013).

In this study, the size of the C1 was considerably smaller during the first 40 days of life. When examined at day 80, the C1 was imaged

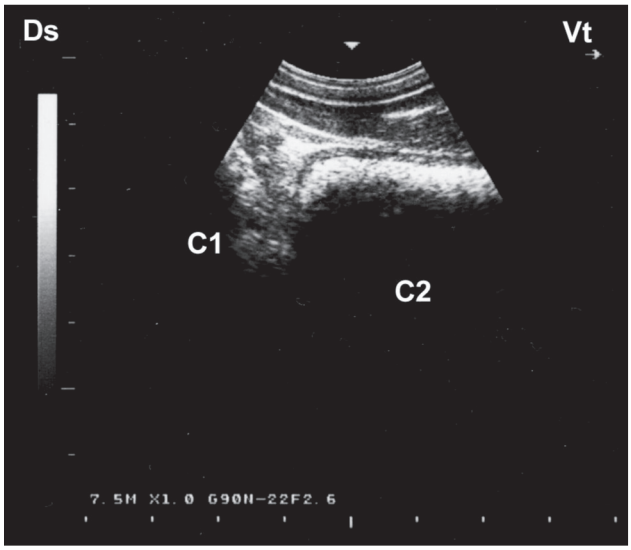

Fig 5. Ultrasonography of the C2 in a 40-day-old camel calf. Image was taken from the ventral left abdomen at the level of the 6th intercostal space. The C2 wall was thick that appeared as a half-moon-shaped structure with an even contour. C1, first gastric compartment; C2, second gastric compartment, Ds, dorsal; Vt, ventral.

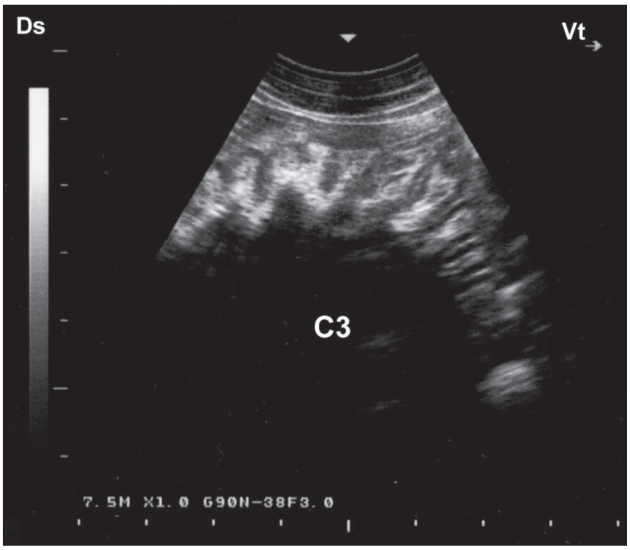

Fig 7. Ultrasonography of the last part of C3 in a 100-day-old camel calf. Image was taken before feeding at the level of the 8th intercostal space on the right side. The folds appeared coarse, hyperechoic and elongated and protruding from its mucosa. C3, third gastric compartment; Ds, dorsal; Vt, ventral.

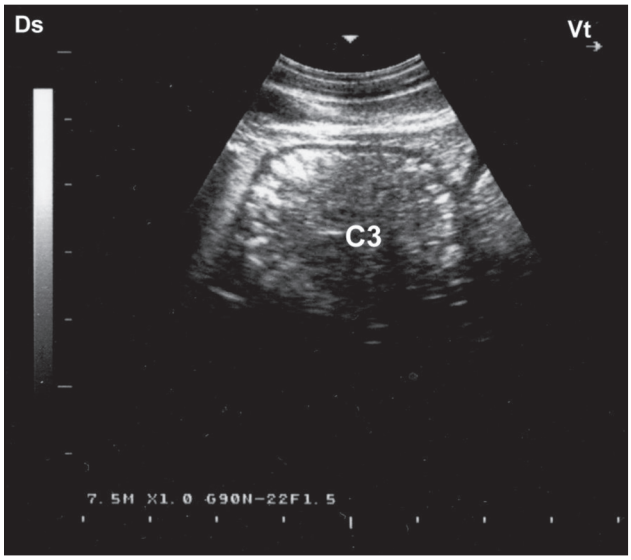

Fig 6. Transverse section in the first part of C3 in an 80-day-old camel calf. Image was taken at the level of the 7th intercostal space on the right side. Note the hyperechoic fine folds protruded from its mucosa. C3, third gastric compartment; Ds, dorsal; Vt, ventral.

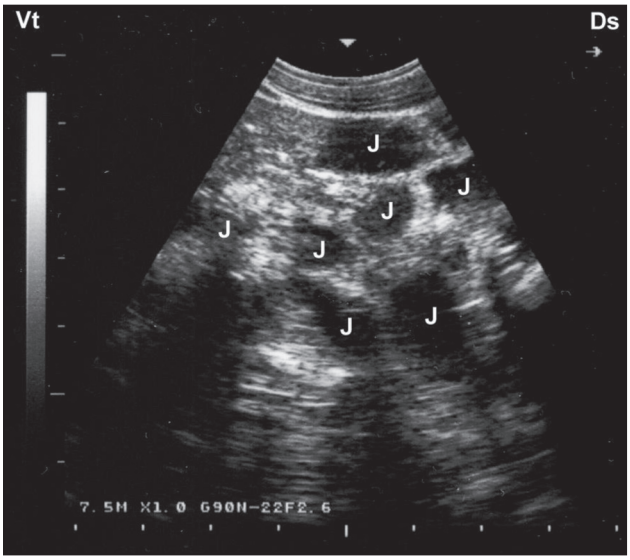

Fig 8. Transverse section showing several loops of jejunum (J) adjacent to one another in an 80-day-old camel calf. Image was taken from the lower paralumbar fossa at the level of the 10th intercostal space on the right side. Ds, dorsal; Vt, ventral.

occupying the entire left abdomen. The C2 contractions were only noticed in 3 of the 15 calves at day 40 (20%) and the contractions were weak and short. At day 80 and 100, on the other hand, C2 contractions were recorded in all camel calves and were stronger and longer. The nature of feeding hay at this age may be the cause of these stronger and longer contractions. Similar findings were reported in cow calves (Jung, 2002; Gautschi, 2010; Braun *et al*, 2013). In a study of Braun *et al* (2013), the reticulum was identified in only one of the newborn cow calves; thereafter it was visible in all calves and had typical biphasic contractions.

The C3 was imaged in 3, 5 and 8 calves at day 1, 20 and 40, respectively. At day 80 and 100, it was imaged in all camel calves through the 6th to 8th intercostal spaces on the right side. In an ultrasonographic study of 10 cow calves several

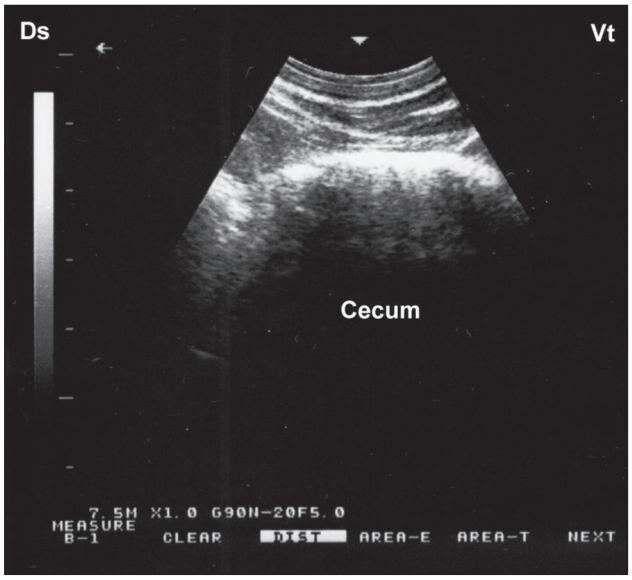

Fig 9. Ultrasonography of the cecum in an 80-day-old camel calf. Diameter of the cecum was large and its wall appeared thick and hyperechoic and the contents appeared anechoic. This image was captured at the caudal right flank. Ds, dorsal; Vt, ventral.

days after birth, the omasum was seen in only one 14-day-old calf (Jung, 2002). In cow calves that were an average of 20 days of age, the omasum was seen in 9 of 10, and by 90 days of age, the omasum could be seen in all 10 calves (Gautschi, 2010). In this study, the C3 appeared as a tubular structure and the contents were moderately imaged with an active motility. The most important imaging pattern of the C3 was the mucosal folds that appeared as hyperechogenic folds protruded from the mucosa. Jung (2002) was able to see the omasal leaves in their entirety in the cow calves, whereas Gautschi (2010) reported seeing only the base of the omasal leaves. The mucosal folds of the last part of C3 appeared in camel calves coarser and more elongated when compared with that of the first part of C3. In newborn cow calves, the abomasum extended caudally as well as to the left and right side of the abdomen with the volume of milk ingested (Wittek *et al,* 2005). Immediately after ingestion of milk, the contents of the C3 appeared echogenic in this study. The milk rapidly forms large echoic milk clot, which was broken down over the course of several hours resulting in liquefaction of the abomasal content (Miyazaki *et al,* 2009; Gautschi, 2010).

Ultrasonography of the GIT in camel calves was helpful in differentiating between the small and large intestine. The duodenum could not be imaged in any of the examined camels. In cattle, presence of the gallbladder facilitates the identification of the duodenum; however because of the absence of the

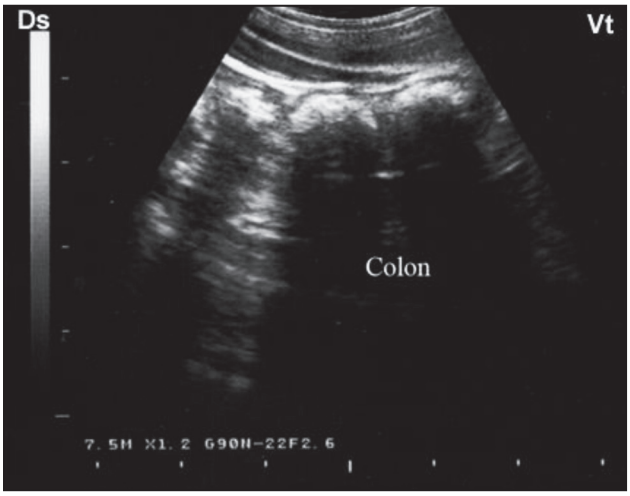

Fig 10. Ultrasonography of the colon in a 100-day-old camel calf. Image was taken in the right paralumbar fossa. Its wall appeared thick and hyperechoic and the contents appeared anechoic. Ds, dorsal; Vt, ventral.

gallbladder in camels, it was difficult to differentiate the duodenum from the jejunum and ileum. The jejunum and ileum were most often seen in the ventral region of the right flank. This is in contrast to cattle in which these were always seen in the 11th and 12th intercostal spaces (Braun and Marmier, 1995). Because of intraluminal gas, only the wall of the large intestine closest to the transducer could be imaged as a thick echoic line.

In conclusion, this study provides the normal imaging pictures for the GIT in camel calves during the first 100 days of life. Results of this study demonstrated that the GIT in camel calves is easily accessible to ultrasonography. The C1 is best seen in the left flank; its contents are anechoic and its wall is smooth and echogenic. The C2 has biphasic contraptions; its wall is thick and appeared as a half-moon-shaped structure with an even contour. The C3 is visible as a tubular structure; its first and last parts have fine and coarse mucosal folds, respectively. Small intestinal structures are best seen low in the right flank, its contents are hypoechoic, heterogeneous and it contracted every few seconds. The large intestine is easy to differentiate from the small intestine based on its marked gas content and relatively large diameter. Finally, ultrasonography could be used as a noninvasive diagnostic tool in order to detect GIT diseases in the camel calves.

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DOI : 10.5958/2277-8934.2020.00019.3 *Vol 27 No 1, p 135-138*

GROSS AND SCANNING ELECTRON MICROSCOPIC STUDIES ON OESOPHAGUS OF CAMEL (*Camelus dromedarius*)

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ABSTRACT

The present study was conducted on 10 oesophagus of adult camels (*Camelus dromedarius*). Oesophagus of camel was musculo- membranous tube extending from *aditus oesophagi* of pharynx to junction of C1 and C2 compartment of stomach. It was divided into cervical part (larger) and thoracic part (smaller). No abdominal part was observed. At the junction of pharynx and oesophagus, a transverse fold of mucous membrane was present in mid ventral portion of the wall and extending to dorsal region. The SEM studies, the surface of oesophagus was undulating and wavy and covered with square or hexagonal cells.

Key words: Camel, oesophagus, Scanning Electron Microscopy

Regurgitation of partly digested contents of forestomach for remastication is a tough task of the oesophagus (Lechner-Doll and Hoffrogge, 2011). The length of oesophagus, presence or absence of glands as well as type of musculature of the wall largely affected the passage of food inside the oesophagus (Abass, 2009). The cervical and thoracic course of the oesophagus of llama has been described by the Sukon *et al* (2009) and about the musculature and glands by Jamdar and Ema (1982). The present study describes the gross and scanning electron microscopic structure of oesophagus of dromedary camel.

Materials and Methods

The present study was conducted on oesophagus obtained from recently dead 10 camels irrespective to age and sex brought at Clinics of Teaching Veterinary Clinical Complex, RAJUVAS, Bikaner. These were free from any pathological condition of tongue, mouth and GIT. All samples were subjected to gross anatomical study and 4 samples were used for electron microscopic studies.

The length of dissected out oesophagus was measured from *aditus oesophagi* to the thoracic inlet for the cervical part, and from thoracic inlet to *hiatus oesophagi* for thoracic part, respectively. The diameter of oesophagus was measured at *aditus oesophagi*, middle of cervical part, middle of thoracic part and at atrium ventriculi by Vernier caliper. The gross and topographic anatomical study of oesophagus was done.

The ultra structural surface morphology of oesophagus was studied by Scanning Electron Microscope (SEM) at Department of Microbiology, College of Veterinary & Animal Sciences, Bikaner, RAJUVAS, Bikaner. The samples were processed by the technique as described by Bozzola and Russell (1999).

Tissue section (2 – 3 mm) were taken from representative areas and were gentally washed with isotonic buffer and were primarily fixed in Karnovsky's fixative (mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0.1M Phosphate buffer) at 4°C. After primary fixation, tissue were washed with 0.1M phosphate buffer (3 changes, each of 15 minutes) followed by post fixation in 1% solution of Osmium tetraoxide, and again washed with 0.1M phosphate buffer (3 changes, each of 15 minutes). The tissues were chemically dried by acetone (3 changes, each of 15 minutes) and dry acetone (2 changes, each of 15 minutes). Up to this, all steps were carried out at 4°C. The samples were then dried in critical dryer (Biostag, New Delhi) at 31.5°C and 1100 Psi. The samples were mounted on stub, gold coating using gold target in sputter coater (Polalis, South Korea) and viewed by Scanning Electron Microscope (Genesis – 1100, Emcraft, South Korea).

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Data generated from the study were analysed using standard statical methods as described by described by Kaps and Lamberson (2004).

Results and Discussion

Gross examination

Shape and Colour

Oesophagus of camel was musculo-membranous tube extending from *aditus oesophagi* of pharynx to junction of C1 and C2 compartment of stomach (Fig 1 & 2) which was in accordance with studies of Raghavan (1964) and Frandson (1981) in ox. The complete course of oesophagus was divided into cervical part (larger) and thoracic part (smaller) (Fig 1), the similar findings was reported by Raghavan (1964) in ox, Nabipour *et al* (2001) in camel and Sukon *et al* (2009) in llama. Abdominal part oesophagus was not observed, however, Nabipour *et al* (2001) found a short abdominal part in oesophagus of camel. In fresh state the colour of external surface of oesophagus was reddish brown and luminal surface was pink.

Size

The mean length of oesophagus at cervical region was 121.5 ± 1.514 cm and mean value of circumference at *aditus oesophagi* was 15.24 ± 0.193 cm and at mid cervical part it was 8.61 ± 0.213 cm. The mean length of oesophagus at thoracic region was 72.8 ± 1.1 cm and mean circumference at mid thoracic was 8.28 ± 0.186 cm and at *hiatus oesophagi* it was 15.53 ± 0.189 cm. The total mean length of oesophagus was 194.3 ± 2.55 cm, whereas according to Sisson and Grossman (1958), average diameter of oesophagus was about 2 inches, and its total length in an ox of medium size was about 3-3.5 feet, according to Raghavan (1964) total length was about 0.75 to 1 metre and its diameter was about 50 mm in ox, according to Nabipour *et al (*2001) total length was 165 - 215 cm in camel, according to Sukon *et al (*2009) total length was 121 cm, with the cervical portion 80 cm and the thoracic portion 40 cm and oesophageal outer diameter began in the cervical portion at 2.5 cm and gradually enlarged throughout the length to 3.9 cm at the caudal thorax in llama. According to Abass (2009) total length of the oesophagus appeared to be in the range of 110-120 cm, where in the cervical portion was 68-70 cm and the thoracic portion was 50 cm. Both the protions had 8-10 cm diameter. According to Hussein *et al* (2016) length of the oesophagus of camel was 148±2.3 cm. The oesophageal outer diameter at cervical portion was 2.6 ±0.5 cm which gradually enlarged to 4±0.2 cm at thoracic inlet.

Topography and Course of oesophagus

The cervical portion of oesophagus commenced at *aditus oesophagi*, dorsal to cricoid cartilage of larynx and extended caudally on the dorsal aspect of trachea up to the level of 3rd cervical vertebrae (Fig 1), which was in congruence with the findings of Raghavan (1964) in ox, Nabipour *et al* (2001) and Hussein *et al* (2016) in camel. Later it traversed to the left side of trachea and extended caudally on the left side of trachea, slightly inclined and entered in the thoracic inlet (Fig 1), whereas in musk-ox calf (Sack and Ballantyn, 1965) opposite the sixth cervical vertebra, which was approximately 5 cm cranial to the cranial border of the first rib, the oesophagus deviated sharply to the left from its previous midline position and came to lie lateral to the trachea. However, according to Nabipour *et al* (2001) and Hussein *et al* (2016), at the level of sixth cervical vertebra oesophagus again slopes to the dorso-median of the trachea in camel.

The complete thoracic part was laid in the mediastinal space, which was also reported by Smuts and Bezuidenhout (1987) in camel. The thoracic part started at 1st rib and it gained the dorsal aspect of trachea at 2nd thoracic and continued it up to the bifurcation of trachea. In the middle at mediastinum, the aortic arch pushed it slightly towards the right side of midline. In the caudal mediastinum, the oesophagus extended caudally, inclined upward. It entered into abdominal cavity through *hiatus oesophagi* of the diaphragm. It opened immediately into C1 compartment of stomach (Fig 1). Similar findings were observed by Raghavan (1964) in ox. Abdominal part was not observed in camels of present study which was in conformity with the findings of Sisson and Grossman (1958) in ox and Abass (2009) in camel, whereas it was contrary to the findings of Sack and Ballantyn (1965) in musk-ox calf and Nabipour *et al* (2001) and Hussein *et al* (2016) in camel and Sukon *et al* (2009) in llama.

Interior of Oesophagus

At the junction of pharynx and oesophagus, a transverse fold of mucous membrane was present in mid ventral portion of the wall and extended to dorsal region (Fig 3). At the terminal part, oesophagus was dilated as also reported by Abass (2009) in same species. The mucosa of empty oesophagus exhibited the transitory longitudinal folds (Fig 4).

Scanning Electron Microscopy

The surface of oesophagus was undulating and wavy and covered with square or hexagonal cells

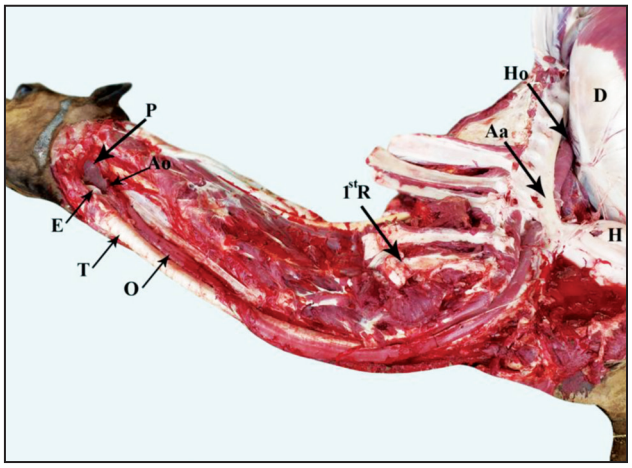

Fig 1. Photograph showing oesophagus of camel. P - Pharynx, E - Epiglottis, Ao - *Aditus oesophagi*, T - Trachea, O - Oesophagus, 1st R - 1st Rib, Aa - Abdominal aorta, Ho - Hiatus oesophagi, D - Diaphragm, H – Heart.

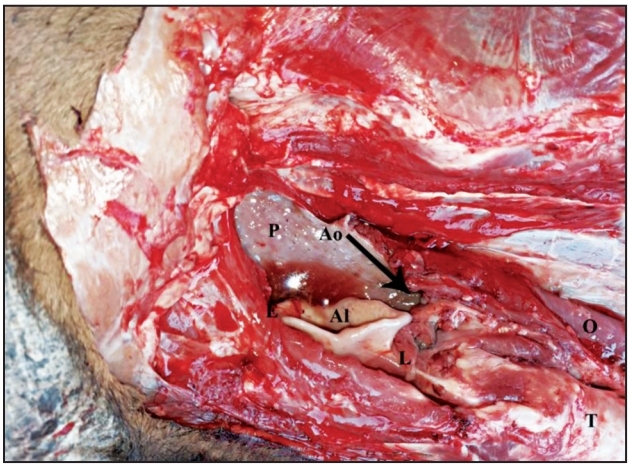

Fig 2. Photograph showing pharynx of camel. P - Pharynx, E - Epiglottis, Al - Aditus laryngus, O - Oesophagus, L - Larynx, Trachea.

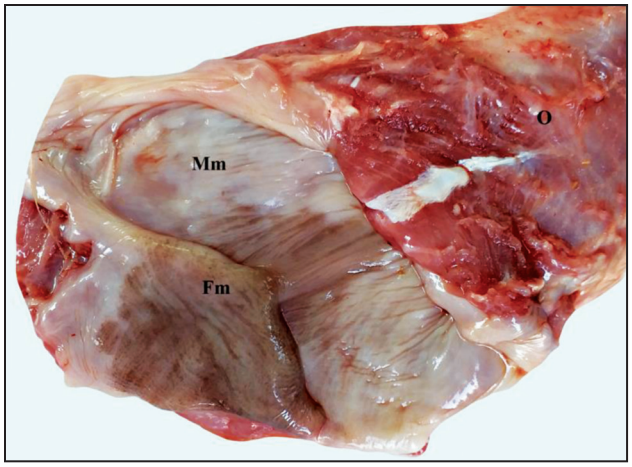

Fig 3. Photograph of oesophagus at the opening of pharynx. Fm - Fold of mucous membrane at the junction of pharynx and oesophagus at ventral part. Mm - Mucous membrane, O - Oesophagus.

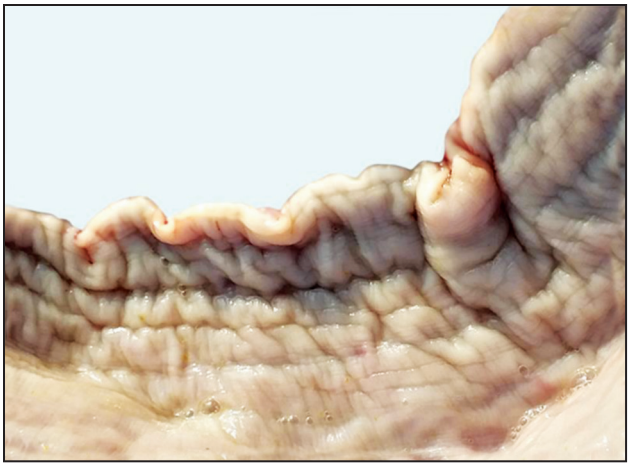

Fig 4. Photograph of oesophagus of camel showing folds of mucous membrane . Lfm -Longitudinal folds of mucous membrane.

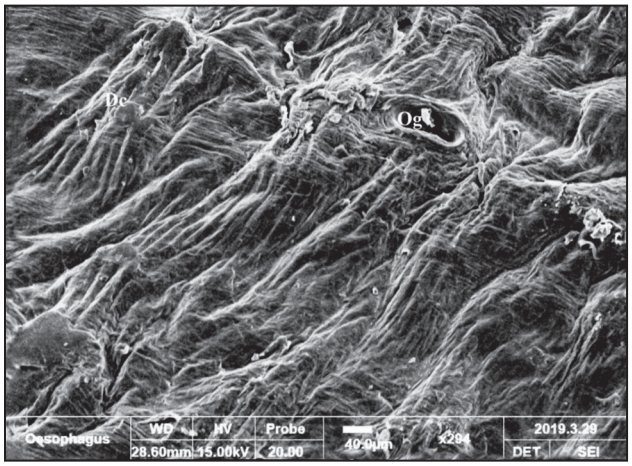

Fig 5. Scanning electron micrograph of oesophagus of camel showing wavy and undulating surface. Og - Opening of gland, Dc - Desquamating cell. (40.0 µm x 294).

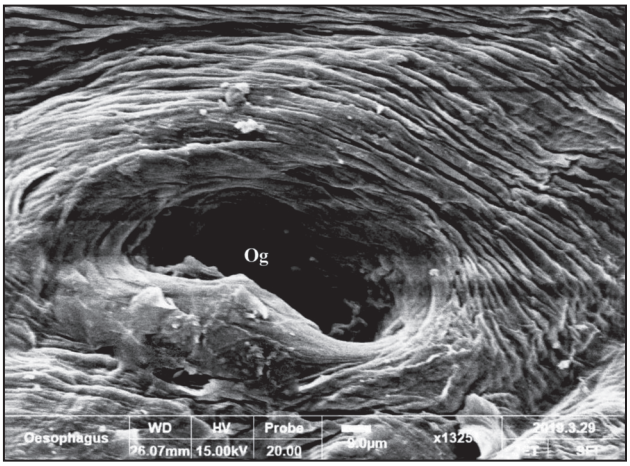

Fig 6. Scanning electron micrograph of oesophagus of camel showing micro ridges. Og- Opening of gland (9.0 µm x 1325).

which had well defined, fairly straight boundaries. The sloughing of cells was observed and edges were raised (Fig 5), which was in accordance with the findings of Carr *et al* (1974) in human. The openings of the glands were seen on the surface (Fig 5 & 6). The micro ridges were found on surface of the superficial cells of all parts of the oesophagus. The ridges were arranged parallel and the intervals between them were greater at many places (Fig 6). These findings were in close agreement with the findings of Gardner and Scott (1976) in ovine oesophagus. However, Kathleen *et al* (1978) reported small microvillous processes in human oesophagus. The whorl-like arrangement of ridges was observed around the openings of the glands and these became more numerous as the cells approached the junction (Fig 6), which was supported by the findings of Henk *et al* (1986) in dogs.

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DOI : 10.5958/2277-8934.2020.00020.X Vol 27 No 1, p 139-140

Short Communication

CASTRATION OF CAMELS THROUGH PRESCROTAL MIDLINE INCISION

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ABSTRACT

Five dromedary male camels were castrated through prescrotal midline incision method in lateral recumbency under general anaesthesia by using Xylazine HCl, Ketamine HCl and Butorphanol Tartarate, intravenously. Prescrotal midline incision was given and both testicles were excised from a single incision one by one and ligation of spermatic cord was done by chromic cat gut no. 2. Surgical wound was closed in two layers, using Vicryl and skin was closed by using intra dermal suture pattern. No complications were observed post-surgery except slight swelling on scrotum on next day which subsided when camels were allowed little exercise. All 5 camels recovered well and intra dermal suture pattern left no scar at incision site.

Key words: Camel, castration, ketamine stun, prescrotal midline

Castration is done in camels in standing position (Tibary and Anouassi, 2004) and recumbent position (Telfah *et al* 2012). The routine castration process involves incision over scrotum at both sides and removing testicle after ligation of vascular portion of the spermatic cord and wounds are left open for postoperative care to completely heal like in horses (Gahlot, 2000). Pre-scrotal midline incision method does not require any postoperative care (Telfah *et al*, 2012).

In this study all 5 camels were castrated in lateral recumbency by using prescrotal midline incision method under the anaesthesia by using intravenous ketamine recumbent stun technique (Abrahamsen, 2008).

Materials and Methods

Anaesthesia and Surgical Technique

Five single humped adult camels were kept off feed for 18 hours (water was allowed to drink) before surgery. Camels were anaesthetised by using intravenous Ketamine recumbent stun which is cocktail of injection Xylazine HCl @ 0.025mg/kg of body weight, Ketamine HCl @ 0.5 mg/kg of body weight and Butorphanol Tartarate @ 0.05 mg/kg of body weight. Camels were restrained physically for giving intravenous injection, once cocktail was injected, animals became anaesthetised within 1-2 minutes. The camels were secured into lateral recumbency and head and neck were kept on a soft pillow to protect eyes which were close to the ground and drooling out of excessive saliva. Inj. Phenylbutazone @ 4.4 mg/kg of body weight and Inj. Streptopenicillin @ 10mg/kg of body weight were administered before the surgery. All vital physiological parameters i.e. heart rate, respiratory rate, rectal temperature were recorded before surgery and were found in normal range. Surgical site was prepared aseptically and Inj. 2% Lignocaine hydrochloride was infiltrated at the surgical site. A 2-inch linear incision was made at prescrotal midline and one testicle was squeezed out from the incision, then vascular and non-vascular parts were separated, ligated and testicle was removed. In same way, other testicle was also exteriorised from the same incision and and removel in the same manner. Ligation of the spermatic cord was done by using chromic cat gut number 2. After removal of both the testicles, surgical wound was closed by using Vicryl number 2 in two layers. Skin wound was closed by using intra dermal suture pattern. Camels were taken in sternal recumbency. Camels recovered from anaesthesia after 30 minutes, without any assistance. Antibiotic and NSAID course was repeated for next 5 days.

Results and Discussion

Pre scrotal midline incision method used in animal of present study, complications i.e., haemorrhages, accumulation of inflammatory fluids, etc. were not observed except mild oedematous

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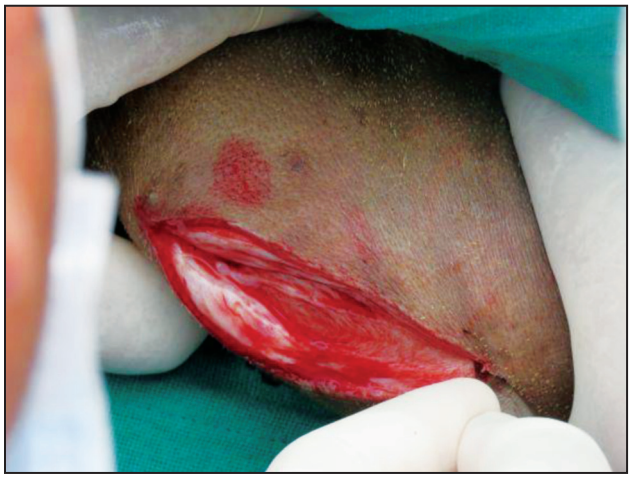

Fig 1. Pre scrotal midline scrotal incision.

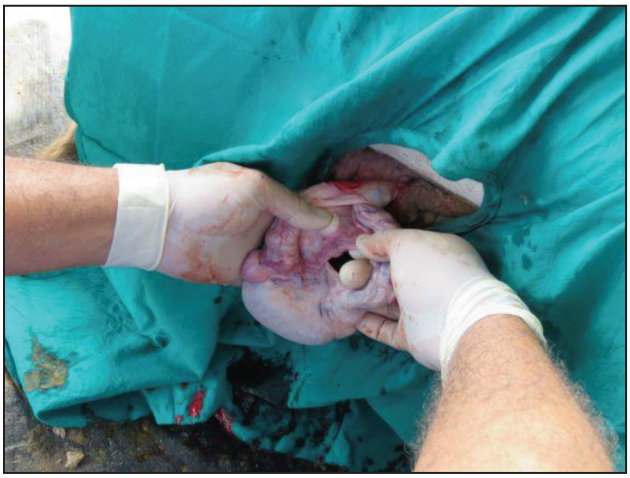

Fig 2. Separation of vascular and non-vascular part.

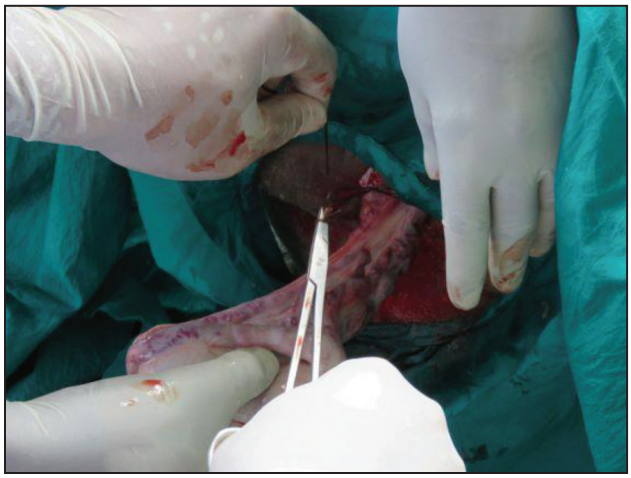

Fig 3. Ligation of vascular part.

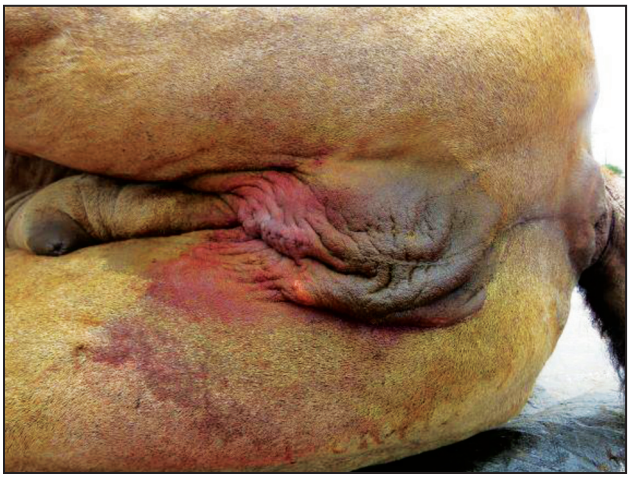

Fig 4. Complete closing of surgical wound using intradermal pattern.

swelling was seen on scrotums in 4 camels out of 5 on next day, which later subsided.

In this method all 5 camels recovered uneventfully. The intradermal sutures placed for closing of skin gave a cosmetic view at surgical site. Incision scar was not visible post-operatively.

Castration of camels under general anaesthesia by using intravenous recumbent Ketamine stun proved satisfactory to carryout castration. Gahlot (2000) performed castration in camels under epidural anaesthesia with either xylazine or 2% lignocaine hydrochloride. A single or double transfixation ligature were used with or without emasculator. An open wound healing was allowed without any complications. However, in animals of present study also similar observations were noted in healing but emasculator was not used.

Ramadan (2016) performed castration under 2% xylazine (0.1-0.2mg, kg) sedation along with local infiltration anaesthesia at scrotum.

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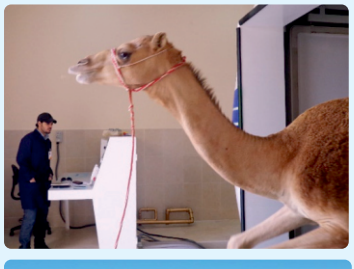
INTERNATIONAL CAMEL ORGANIZATION (ICO)

International
Camel Organization

The International Camel International is an international, non-governmental, non-profit organization. The activities of the International Camel Organization are focused on developing the culture of camel breeding through achieving the mission, principles and goals of the global camel culture and the types of nomad activities of camels and Chaired by Mr. Fahad F. Bin Hithleen.



GOALS AND FUNCTIONS OF THE ICO

- Developing the dynamics of the global camel culture, in all its forms, types, and everything related to human and camel interaction.

- Promoting mutual understanding and friendships between nations, peoples and establish societies of nature and animal lovers through various practices with camels.

- Developing and managing a code of Ethics as a set of ethical, cognitive principles, technical and fundamental skills for camel culture.

- The organization would hold and organize the International Festival of the International Camel Organization.

- Promoting and encouraging the establishment of national and regional member organizations in the International Camel Organization and organize training workshops for members of these organizations.

- Promoting research aimed at the scientific and educational development of camel culture and provide advice and opinion on everything related to camel affairs.

- Achieving excellence by all members of the organization, organizers, practitioners and participants in camel activities in all fields, such as technical equipment, cultural, social and sporting events, talent development in the field of camel breeding and its activities, industry and science.

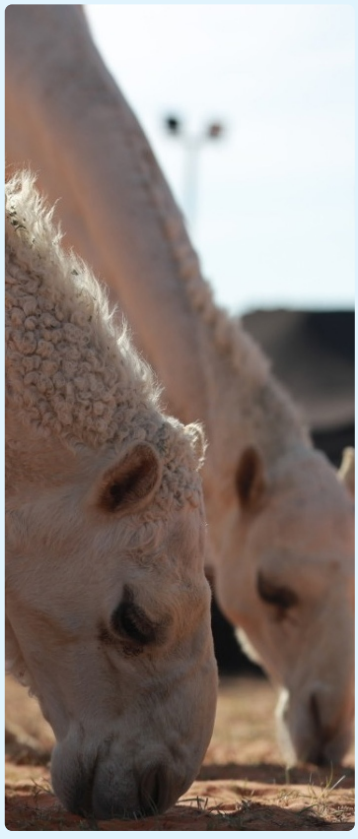
- Merging the standards of friendly organizations and institutions concerned with national, regional, continental and international measures in the International Organization for Living Organisms that feed their activities in the field of camel breeding.

- Publishing and expanding the base of practice, equipment, breeding sites and camel farms, improving implementation methods and searching for suitable sites in coordination with the competent authorities in each country.

- Maintaining the international rules and principles for the practice of camel breeding and issue the necessary controls in accordance with the statute of the International Camel Organization, and the instructions and principles established by the relevant international organizations.

- Evoke history, create new competitions for passengers, create new roads and convoys, and travel and trips.

- Issuing camel registration certificates.



VISION AND MISSION OF THE ICO

Encouragement of Scientific Research
The ICO encourages scientific research and written scientific research works in the field of camel breeding and enrichment of the global library about camels, as well as providing support for authors, translators and publishers.

Principle of humane attitude towards camels
The ICO cooperates maintenance of the highest level of safety and security during various practices of camel events, holding courses and seminars, establishing regulations aimed at improving safety of camels and all members of the ICO.

Cooperation with international organisations
The ICO cooperates with international organisations, develops and optimizes normative acts relating to practices of camel activities in order to dedicate the culture of camel breeding to serving humanity and struggling for peace.

Fair competition and doping
The ICO pursues a fair competition policy, ensures awareness of the ICO members on the dangers of doping, and ages a fundamental struggle against doping.

Organisation of ICO World Festivals
The ICO organises World Festivals ("ICO WF") as the highest level of demonstration of the achievements of the WCM.

ICO Vision: The ICO recognises the unique role of the camel breeding culture in the history and development of the nomadic civilisation and humanity, as well as a necessity to preserve, revive and develop in conditions of globalisation of the modern world by consolidating the activities of various organisations, prominent government, political and public figures for restoration, revival, preservation, development and promotion of the culture of camel breeding in the world, the ICO contributes to development of special friendly relations between peoples and countries.

Mission of the ICO: To contribute to preservation of ethno-cultural heritage and originality of peoples of the world through development of the camel breeding culture.

Representation in the ICO: The ICO has the right to be represented in the work of the General Assembly ("GA"), as well as in departments, committees, commissions of the ICO, as well as regional, Asian, African, European and other international organisations of the ICO.

Promotion of international rules and principles
Promotion of international rules and principles of practice of the camel activities, establishment of necessary rules and regulation is carried out in accordance with instruction and principles established by the ICO and corresponding to international organisations.

ACHIEVEMENTS AND ACTIVITIES

The 1ST International Conference for Central Asian Countries with the theme "Camel Culture in Central Asia: Historical Heritage and Prospects" was held on August 27th-30th 2019 at Bishkek – Kyrgyzstan. The conference will be held annually in one of its countries, with the expansion of the conference programme by including camel competitions and scientific seminars. A scientific journal on the activities of the International Camel Organization will be released.

Recent events

- The 1st European Camels and Camelids Fair, September 14th – 18th 2019, Janvry, South Paris – France. - The 1st European Forum for Camel Ranch Owners, October 25th – 27th 2019, Zurich – Switzerland.

Establishment of the First European Association of Camel Owners took place, urging countries to establish specialized technical administrative bodies in European Ministries of Agriculture to oversee the development of camel breeding and it was agreed to hold a camel festival in Zurich from 29th – 30th August 2020.

EUROPEAN CAMEL RANCH OWNERS ASSOCIATION - ECROA