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In This Issue

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EDITOR
T.K. GAHLOT
Camel Publishing House
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Email : tkcamelvet@yahoo.com
Website : www.camelsandcamelids.com • www.tkgahlotcamelvet.com • www.indianjournals.com

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Manuscripts and other related correspondence may be made to :

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

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FIRST INTERNATIONAL EXPERTS CONGRESS (ICOIEC)- ICO COLLABORATES WITH UNESCO

International Camel Organisation (ICO) deserves special appreciation for letting camel science and cultural activities on a faster pace during COVID-19 period through webinars. The one-day event, organised by ICO in collaboration with UNESCO on 29th June 2021 was aimed to highlight the connections between camel traditions and the achievement of the UN 2030 Agenda for Sustainable Development. In a historic movement First International Experts Congress (ICOIEC) was held involving more than a dozen camel experts from many countries and brain storming discussions took place in four thematic sessions. Earlier, on April 2nd, 2021 Second International Symposium was held which coincided with the World Autism Awareness Day on the Book "Camel Crazy", within the International and Cultural Symposium Programs of the International Camel Organisation - for the International Year of Camels 2024 in cooperation with the King Abdulaziz Public Library. Christina Adams shared how she had the idea that camel milk may help her son with autism. She outlined the value and wisdom of pastoralist and nomadic people, how they benefit the world by keeping camels and holding genetic resources and heritage wisdom, and how today's families can benefit from their ways. Online Third International Symposium which held on 22nd June 2021 on World Camel Day with a theme involving "Camel Surgery" and Prof. T.K. Gahlot was invited as a speaker for sharing his valuable experiences to the world. This online International Symposium was held in cooperation with the King Abdulaziz public Library as one of the programs for the International Years of Camelids 2024. Prof Abdul Raziq Kakar was also invited speaker on this occasion and spoke about genesis of world camel day and importance of camels. Dr Ilse Kohler Rollefson, LPPS, India was a moderator for the symposium.

The August 2021 issue of JCPR is rich in manuscripts based on new research. Few of these topics covered include amphotericin-B induced acute synovitis and biomarkers of camel joint structures, biosynthesised microbial silver nanoparticles, camel milk- protective effects on *Toxoplasma gondii* in mice, camelpox outbreak, camel meat-products- evaluation of the sanitary status using *Nigella sativa* and *Capsicum annuum* oils, dermatophytosis- treatment, electronic nose technology- rapid detection of adulterated camel milk powder, etio-pathology and therapeutics of pica, glycosidases in the uterine luminal fluid and blood serum, immunohistochemical localisation of mucin 1, isoniazid and rifampicin induced hepatotoxicity, meat burger quality- bactrian camel, monocytic markers, Mycobacterial infections– the current situation, ocular ultrasonography: a review, oesophageal obstruction, plasma from *Escherichia coli* and *Staphylococcus aureus* stimulated blood, *Trypanosoma evansi*-in herd in UAE and vasa recta fibrosis in kidney.

I am sure that all camel scientists and researchers would continue their support to the Journal of Camel Practice and Research which is a broadest platform of publication of camelid research.

(Dr. T.K. Gahlot)
Editor

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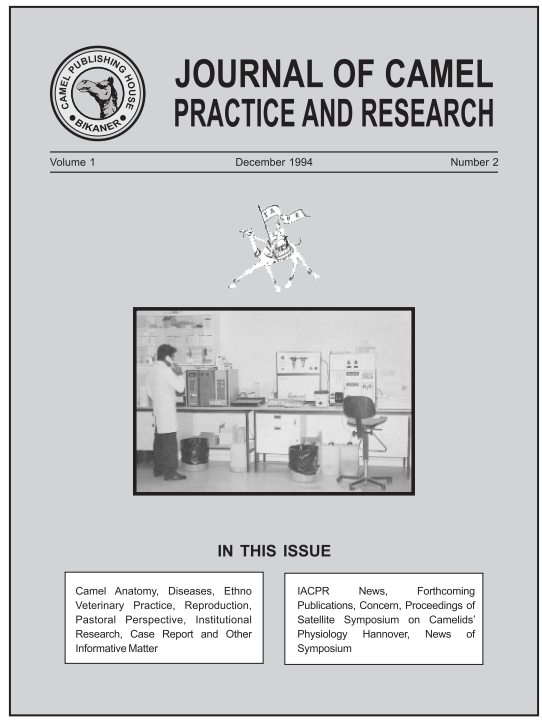

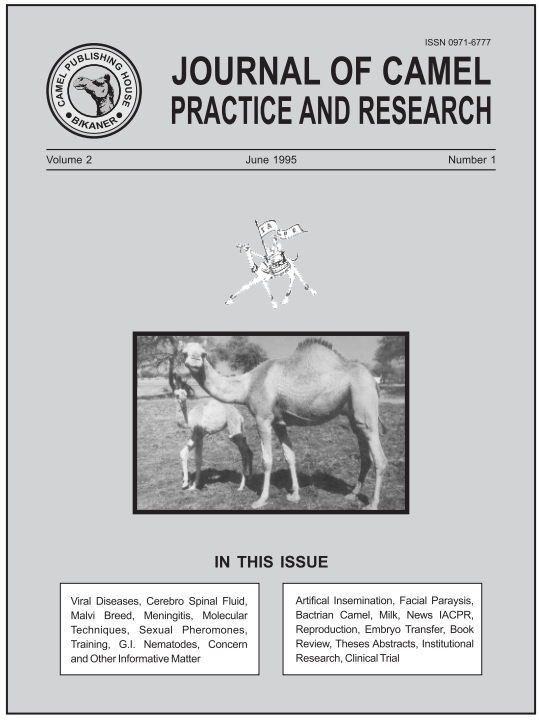

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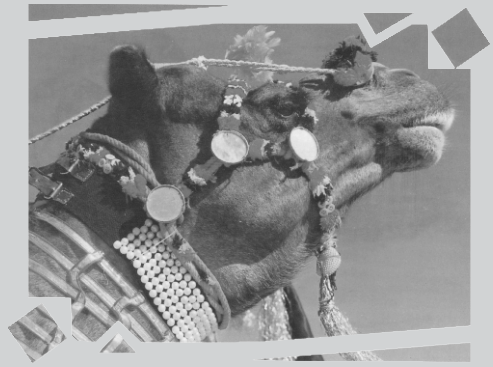

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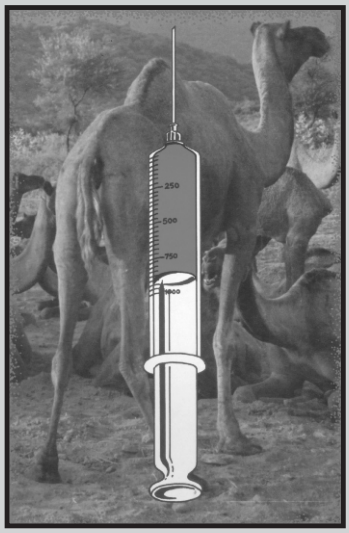

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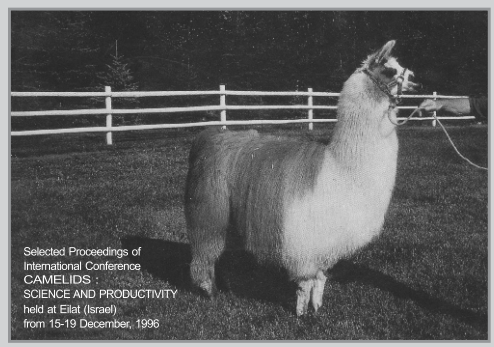

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Trypanosoma evansi IN A DROMEDARY CAMEL HERD IN THE UAE-PART II

R.K. Schuster, R. Raghavan, M. Ringu, F. Al Mheiri, M. Al Quassim and U. Wernery
Central Veterinary Research Laboratory, Dubai, UAE

ABSTRACT

Trypanosoma evansi infection was diagnosed in 15 out of 17 adult dromedaries of a breeding herd in the Emirate of Dubai. The herd had a history of abortions in the previous years. Own observations on 16 adult females and one bull lasted 12 months and revealed trypanosomosis in 15 animals in monthly haematological and serological examinations. Despite three to four injections of the trypanocides melarsamine and/ or quinapyramine, only one camel was cured. One camel aborted in the 8th month of pregnancy. Four other dams delivered healthy calves.

Key words: Aantibody ELISA, buffy coat technique, melarsomine, quinapyramine, *Trypanosoma evansi*, United Arab Emirates

Surra, a protozoal disease caused by the salivarian flagellate *Trypanosoma evansi* is the most important parasitic disease in Old world camelids. While literature on surra in Bactrian camels known also under the name Su Auru[1] is scarce, many publications on *T. evansi* infections in dromedaries are available. Most of the papers dealt with prevalence of the disease but data are difficult to compare since different diagnostic methods were used. Among direct diagnostic methods, wet blood films and blood smears are less precise. The haematocrit centrifugation technique, the so called buffy coat technique (BCT), that can be carried out in a normally equipped laboratory gives more exact results. The mini-anion exchange centrifugation is more difficult to perform and the mouse inoculation test requires animal experimentation. Detection of *Trypansosoma* DNA can be performed with polymerase chain reaction (PCR). Amongst serological tests, the card agglutination test can be performed under field conditions while the indirect immunofluorescence antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA) require well equipped laboratories (OIE, 2018).

As summarised by Wernery *et al* (2014), clinical picture of surra is multifaceted. In places where the disease has occurred for a long time, it often runs a subclinical course without obvious signs and only abortions might give evidence that trypanosomes are involved. This was the case in a camel herd kept at the outskirts of an oasis next to Dubai. The herd consisting of 18 adult dromedaries had a history of abortions and seven animals had trypanosomes in their blood while 15 were serologically positive in ELISA at the start of our observation. Only two recently purchased females were negative for both trypanosomes in BCT and *T. evansi* antibodies.

Since in the past there were claims that available trypancides were not effective anymore, the aim of this paper was investigate blood samples of camels for the presence of trypanosomes and the serum for antibodies after multiple courses of treatment with melarsamine and/or quinapyramine. This paper is the resumption of an article by Wernery *et al* (2020).

Materials and Methods

Dromedary camels

A breeding herd of 17 dromedaries with a history of abortions consisted of 16 females and one bull. Of these, two adult females were recently introduced to the herd. The camel pens are situated on a farm 25 km east of the Dubai city centre in a desert area near a settlement (Al Aweer town) surrounded by irrigated farms and gardens with date palm plantations, cultivation of forage crops and vegetables. There are several small holder farms with cattle and small ruminants in a radius of one km around the camel pens. A 2.5 ha freshwater lake is

1. In the Russian literature surra is called Su Auru. Su Auru is Kazakh and means next to water since *T. evansi* also known in elder sources as *T. ninaekohlyakimovae* is transmitted by bloodsucking flies and these insects need water or at least a muddy or damp environment for their larval development.

SEND REPRINT REQUEST TO R.K. SCHUSTER email: r.schuster@cvrl.ae

at a distance of 2.5 km. The farm on which also four Arabian horses were kept is protected from strong winds by a hedge of *Conocarpus lancifolius* trees and a 6 m high sand dune. Only the site to the west is open to a large irrigated alfalfa field. In November 2020, the whole herd was moved to a new territory in the desert, 1.5 km away from irrigated crop farms.

Observation started in June 2020 after the owner complained that pregnant camels of his herd again aborted. Abortions in the herd happened already in the previous year when a total of 15 abortions were observed. Some of the animals showed oedemas on ventral abdomen and distal legs (Wernery *et al,* 2020).

Test methods

From all 17 dromedaries blood was withdrawn from the jugular vein. The EDTA blood was analysed for haematological parameters using the automatic haematology analyzer Sysmex XN (Sysmex corporation, Japan). EDTA blood was then tested for the presence of trypanosomes with the BCT. A Giemsa stained blood smear was prepared when life trypanosomes were seen in BCT and the sample was assessed as follows:

negative: no parasites in BCT,

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

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

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

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

Sera were examined for *T. evansi* antibodies with an indirect ELISA. The antigen for coating the ELISA plates was prepared by filtration of EDTA blood of experimentally with *T. evansi* infected rats by anion exchange chromatography followed by hypotonic shock lysis. For the evaluation of the test results an optical density determined by the ELISA reader <0.3 was considered negative, 0.3-0.5 was dubious and >0.5 was positive. In May 2021 also the four camel calves that were borne during the observation period were tested for antibodies.

To exclude brucellosis as cause of abortion, all sera were also examined with the Rose Bengal Test at the beginning of our observations. All sera were negative for brucellosis.

Fly trapping

A Malaise trap and two sticky traps were set for one week next to the camel pens and were checked daily in July 2020. The trap was also set in November 2020 when temperatures had dropped considerably and lots of houseflies appeared on the farm.

Treatment

Twelve non-pregnant camels including the bull were treated with quinapyrimidine (Triquine®) at a dose of 2.5 ml/ 100 kg b.wt. s.c. while three pregnant camels received an i.m. injection of melarsomine (Cymelarsan®) at a dose of 0.25 mg/ kg b.wt. in June 2020. In a second treatment, all positive animals were treated with melarsomine at the same dosage in July 2020. Since examination of EDTA blood after the second treatment still revealed trypanosomes, all non-pregnant camels and the bull received a further treatment with quinapyrimidine and four pregnant were treated with melarsomine in August 2020 and melarsomine treatment of pregnant females was repeated the next day (Table 1).

Reproduction

During the 12 months lasting observation period camel No 9 aborted in the 8th month of pregnancy despite four treatments with melarsomine. Four other females delivered healthy calves despite showing active infection with *T. evansi* (Table 1).

Results

All camels on the farm were in fair to good condition, food and water uptake was normal. Camels Nos. 1 and 7 had oedemas that disappeared after treatment but reoccurred later on.

Parasitological examination of EDTA blood with BCT during the twelve-month observation period revealed that 15 of the 17 adult dromedaries were at least one time positive for trypanosomes despite several applications of trypanocide drugs. Fourteen animals remained all the time serologically positive. One camel (No 15) was initially diagnosed positive with trypanosomes in the blood but was cured since further testing did not reveal positive blood samples and antibodies measured as optical density faded. Two dromedaries (Nos 5 and 16) remained negative for trypanosomes over the whole observation period and remained serologically negative (Table 2, 3). Four calves that were borne in the examination period were included in the last testing in May 2021. No trypanosomes were found in BCT but two calves in an age of 8 and 11 weeks showed antibodies (optical density of 0.5).

Haematological parameters of the two negative (Nos. 5 and 16) and the cured camel (no. 15) were in

Table 1. Treatment of a camel herd against *T. evansi* and reproduction success (quina = quinapyramin, melars = melarsomine).

No.	Sex	Age (years)	Pregnant since	Treatment				Reproduction success
				8.06. 20	09.07.20	4.08. 20	5.08. 20	
1	Fem	8	no	quina	melars	quina	–	
2	Fem	10	Oct-2019	melars	melars	melars	melars	delivered 19 Novmber 2020
3	Fem	6	Jan-2020	melars	melars	melars	melars	delivered 25 March 2021
4	Fem	9	no	melars	melars	quina	–	
5	Fem	>10	no	quina	melars	quina	–	
6	Fem	10	no	quina	melars	quina	–	
7	Fem	10	Jan-2020	melars	melars	melars	melars	delivered 18 February 2021
8	Fem	9	Jan-2020	melars	melars	melars	melars	delivered 2 March 2021
9	Fem	7	Jan-2020	melars	melars	melars	melars	aborted 20 Oct 2020
10	Fem	>10	no	quina	melars	quina	–	
11	Fem	4	no	quina	melars	quina	–	
12	Fem	4	no	quina	melars	quina	–	
13	Fem	4	no	quina	melars	quina	–	
14	Fem	4	no	quina	melars	quina	–	
15	Fem	2	no	quina	melars	quina	–	
16	Fem	2	no	quina	melars	quina	–	
17	Male	4	–	quina	melars	quina	–	

Table 2. Detection of *T. evansi* in blood samples of a camel herd during a 12 month lasting observation period. (June 2020-May 2021).

No	8 Jun	7 Jul	4 Aug	26 Aug	15 Sept	15 Oct	15 Nov	15 Dec	31 Jan	24 Feb.	25 Mar	27 Apr	26 May
1	-	-	-	-	-	-	-	+	-	-	-	-	-
2	++	+	-	+	++++	-	-	++++	+++	++	-	-	-
3	-		-	-	-	+	-	-	-	-	-	-	-
4	+	+	-	-	-	-	++++	-	-	-	-	-	
5	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	+	-	-	-	-	-	-	-	-	-	-	-
7	-	-	+++	-	-	-	-	-	-	-	-	-	no blood
8	-	-	-	-	-	-	+	-	-	+++	-	-	no blood
9	++	+	-	-	+	-	+	-	++	-	-	-	no blood
10	+	-	++++	++	++	++++	++	++	-	-	-	-	no blood
11	-	-	+++	++	-	-	+	+	+	+	-	+	-
12	+	+	-	-	-	-	-	+	-	-	-	-	-
13	-	+	-	-	-	-	-	-	-	-	-	-	no blood
14	+	-	-	-	-	-	++++	-	-	-	-	-	no blood
15	+	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-
17	+	-	+	+	-	-	-	-	-	+	+	-	-

(- : no parasites in BCT,+: single parasites in BCT but not in the blood smear, ++: few parasites in BCT but occasionally in the blood smear only, +++: many parasites in BCT and single parasites in each field in the blood smear, ++++: many parasites in BCT and more than one parasite in each field in the blood smear; no blood: animals were not available)

Table 3. Optical density of an indirect ELISA for the detection of *T. evansi* antibodies in serum samples of a camel herd during a 12 month lasting observation period (June 2020-May 2021).

No	8 Jun	7 Jul	4 Aug	26 Aug	15 Sept	15 Oct	15 Nov	15Dec	31 Jan	24 Feb	25 Mar	27.Apr	26 May
1	**2**	**1.9**	**2.4**	**1.9**	**2.2**	**2.1**	**2.2**	**2**	**2.1**	**2**	**2.2**	**2.3**	**2.3**
2	**1.1**	**0.7**	**2.2**	**1**	**1.1**	**1.1**	**1.3**	**1.1**	**0.94**	**1**	**1.3**	**0.92**	**0.8**
3	**2.3**	**2.2**	**2.5**	**2.4**	**2.4**	**2.4**	**2.4**	**2.4**	**2.4**	**2.4**	**2.5**	**2.5**	**2.2**
4	**2.2**	**2**	**2.4**	**2.1**	**2.1**	**2.1**	**2.2**	**2.1**	**2**	**1.9**	**2**	**2.3**	no blood
5	0.08	0.08	0.07	0.08	0.08	0.08	0.1	0.08	0.09	0.09	0.09	0.08	0.06
6	**2.1**	**2.2**	**2.5**	**2.4**	**2.4**	**2.4**	**2.4**	**2.3**	**2.3**	**2.2**	**2.4**	**2.3**	**1.8**
7	**2**	**1.9**	**1.2**	**2**	**2.2**	**1.9**	**2.3**	**2.3**	**2.3**	**2.2**	**2.3**	**2.4**	no blood
8	**1.7**	**1.7**	**2.3**	**1.9**	**2.1**	**1.9**	**2.3**	**2.3**	**2.3**	**2.1**	**2.3**	**2.2**	no blood
9	**2.3**	**2.3**	**2.6**	**2.4**	**2.5**	**1.9**	**2.5**	**2.4**	**2.4**	**2.3**	**2.4**	**2.5**	no blood
10	**2.2**	**2.2**	**2.4**	**2.4**	**2.4**	**1.8**	**2.4**	**2.4**	**2.4**	**2.3**	**2.5**	**2.4**	no blood
11	**2**	**2.1**	**2.3**	**2.2**	**2.2**	**2**	**2.1**	**2.1**	**2.1**	**2.1**	**2.3**	**2.3**	**2.0**
12	**1.5**	**1.5**	**2.2**	**2.2**	**2**	**1.9**	**2.2**	**2.1**	**2.2**	**2.2**	**2.4**	**2.5**	**2.2**
13	**2.2**	**2.2**	**2.5**	**2.2**	**2.3**	**2.2**	**2.4**	**2.3**	**2.3**	**2.3**	**2.4**	**2.4**	no blood
14	**1.6**	**1.2**	**1.7**	**1.2**	**1.1**	**0.7**	**0.98**	**1**	**0.9**	**0.86**	**0.96**	**0.98**	no blood
15	**0.4**	**0.4**	0.3	0.09	0.07	0.1	0.1	0.1	0.1	0.1	0.08	0.11	0.01
16	0.07	0.08	0.07	0.07	0.08	0.07	0.1	0.1	0.1	0.1	0.055	0.06	0.5
17	**0.8**	**0.7**	**1.1**	**0.6**	**1.1**	**0.6**	**0.9**	**0.7**	**0.74**	**0.71**	**0.63**	**1.1**	**0.9**

(<0.3 was considered negative, 0.3-0.5 was dubious and >0.5 was positive; no blood: animals were not available).

the normal range, except for platelets that were low at few sampling dates and leucocytes for No. 16 that showed slightly enlarged numbers 15.1-18.2x10^9/l but without lymphocytosis.

For all the other camels, erythrocyte and haemoglobin values were in the normal range but platelets were below the reference value of 270 x 10^9/l at most of the sampling dates. Twelve of the trypanosome positive camels showed enlarged leucocyte counts at all collecting dates. In 10 animals, this value exceeded 20x10^9/l and reached up to 56.8x10^9/l in camel No. 3. Lymphocytosis and neutrophilopenia was observed in most of the *Trypanosoma* positive camels.

Fig 1. *Tabanus sufis*, female.

Fly trapping with a Malaise trap in July revealed seven horseflies of the species *Tabanus sufis* (Fig 1) as the only bloodsucking insect. In November four horseflies of the same species were trapped. Due to sand and dust in the air, sticky traps were not effective.

Discussion

Reoccurring of trypanosomes in the bloodstream and/ or persisting antibodies against trypanosomes in the blood serum after numerous applications of trypanocidal drugs suggest a resistance of the parasites to melarsomine and quinapyrimidine in the investigated camel herd. While quinapyramine was developed in the late 1940th and is on the market since the 1950th (Hawking and Sen, 1960) first data on the efficacy of melarsomine against *T. evansi* in naturally infected dromedaries were obtained by Zelleke *et al* (1989). A single dose of 0.3 or 0.6 mg kg b.wt. eliminated blood trypanosomes and treated animals remained negative till the end of the observation periode of 14 weeks.

It is believed by camel owners and trainers of racing camels, that an injection of a subtherapeutic

dose of melarsomine cleans the body and enhances performance of racing camels. This underdosing is the reason for resistance of *T. evansi* to melarsomine. Mass treatments using a single syringe and blood doping in racing camels can also be *Trypanosoma* infection sources.

Of the 17 dromedaries, only two were negative for trypanosomes and for antibodies throughout the whole observation period. A further camel (No. 15) had trypanosomes at the beginning of the observation and became negative after the first treatment. Serologically it became negative in August and remained negative at all further sampling dates. All other adult camels of the herd remained serologically positive and 10 out of them even showed trypanosomes in the bloodstream after treatments.

In a classical surra case in camels, the disease, if not treated, develops into a chronical course with weight loss, weakness, loss of condition, rough coat and oedema at different locations but mainly under the belly. This clinical sign develops also during the acute stage and was seen in two of the infected camels. According to textbooks, animals develop anaemia, mucous membranes are pale and changes in the haematological parameters are often significant with low red blood cell count (RBC), low haemoglobin, low packed cell volume and decreased platelets. Anaemia is often believed to be a reliable indicator of a chronic trypanosome infection, but it is not pathognomonic as mild subclinical and acute infections often show no evidence of anaemia.

Although there was a difference in haematological values of the 15 infected dromedaries in comparison to the 3 non-infected including the successfully treated camel, most of the parameters indicating anaemia were still in the normal reference range for erythrocytes and haemoglobin (7.0-10.5x10^{12}/l and 10.5-14.5 g/dl, respectively). In some of the *Trypanosoma* positive camels these parameters even were slightly above these values. This shows that it is difficult to suggest anaemia as a reliable sign of *T. evansi* infection. However, the total leucocyte counts and the share of lymphocytes were significantly increased and lay outside the reference values in serologically positive animals while platelets were decreased. It is worthwhile to mention that although *T. evansi* is a parasite, the eosinophil count was elevated only at few sampling dates in four camels.

The transmission of *T. evansi* under natural conditions is mechanically by interrupted feeding of bloodsucking flies. Horse flies (Tabanidae) with their complex mouthparts are the main vectors (Luckins, 1998). During our investigations we detected so far only one (*T. sufis*) out of 11 described species in the UAE (Ježek *et al,* 2017). A whole list of tabanids that are relevant for surra in camels is given by Wernery *et al* (2014). Both sexes of tabanids feed on plant pollen and sugars, but females need blood meals prior to oviposition. The larval development of tabanids takes place in wet or damp soil. Such conditions were present in the surroundings of the farm since forage crops in surrounding areas in this desert environment need permanent irrigation. The movement of the camels away from tabanid breeding sites reduces the chance of a natural transmission of the parasite.

The role of stable flies (*Stomoxys* spp.) as vectors for *T. evansi* is disputed and was reviewed by Baldacchino *et al* (2013) and Desquesnes *et al* (2013). During our entomological survey no stable flies were trapped but they might have been present in previous months. It is our experience that *Stomoxys calcitrans* occurs in Dubai after rainfalls in winter and reaches high population densities in March and April. With rising temperatures stable flies fade (Schuster and Sivakumar 2013a,b).

Several parasitic infections may cause abortions. According to Shaapan (2015) these are toxoplasmosis, neosporosis, sarcocystosis, trypanosomosis, tritrichomonosis and babesiosis. With regards to dromedaries, only trypanosomosis is proven to be a cause of abortion and this was the cause in the camel herd under investigation. Right from the beginning, brucellosis as another disease that has to be considered was excluded by negative results of the Rose Bengal Test. Noteworthy, four *Trypanosoma* positive dams delivered healthy calves. Low titer antibodies that were found in two of the calves most probably originated from colostrum of the mothers.

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THE ACTIVITY OF GLYCOSIDASES (β -N-ACETYLGLUCOSAMINIDASE, α-N-ACETYLGALACTOSAMINIDASE AND α L FUCOSIDASE) IN THE UTERINE LUMINAL FLUID AND BLOOD SERUM OF THE DROMEDARY CAMEL (*Camelus dromedarius*) DURING THE FOLLICULAR CYCLE

I.M. Ghoneim[1,2], M.M. Waheed[1,2], A.M.A. Meligy[1,3] and S.M. El-Bahr[4,5]

[1]Department of Clinical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia
[2]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12515, Egypt
[3]Animal health Research Institute, Agricultural Research Centre, Giza, Egypt
[4]Department of Biomedical Sciences (Biochemistry), College of Veterinary Medicine, King Faisal University, Al-Ahsa, 31982, Saudi Arabia
[5]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Egypt

ABSTRACT

The activity of three glycosidases (β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase) were estimated in the uterine fluid and serum throughout the follicular cycle of 24 slaughtered dromedary camels. These camels had ovaries bearing different size follicles (0.5-20mm) that grouped into group I (5–10 mm), group II (11–15 mm) and group III (16–20 mm). β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase were determined in the uterine fluid and serum samples using ELISA kits. Results indicated that mean concentration of β-N-acetylglucosaminidase in uterine fluid was 3.33 ± 0.26, 2.73 ± 0.15, and 6.43 ± 1.41 ng/ml in Group I, II, and III, respectively. The level of α-N-acetylgalactosaminidase in uterine fluid was 3.21 ± 0.27, 2.22 ± 0.14, and 7.24 ± 1.45 ng/ml in Group I, II, and III, respectively. α-N-acetylgalactosaminidase concentration showed statistical differences (P<0.05) among the different groups. The value of α-L-fucosidase in uterine fluid was 435.00 ± 6.94, 340.00 ± 9.82, and 362.50 ± 31.92 µmol/ml in Group I, II, and III, respectively. The maximum mean activity of α-L-fucosidase (P<0.05) was reported in-group I. In group I, the concentrations of β-N-acetylglucosaminidase and α-N-acetylgalactosaminidase were significantly (P<0.05) higher in the uterine fluid than the blood serum. In-group II, the concentration of α-N-acetylgalactosaminidase in the serum was significantly higher (P<0.05) than in the uterine fluid. In-group III, β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase concentrations were significantly (P<0.05) higher in uterine fluid than in the serum. The results proposed that β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase might play a role in carbohydrate-mediated events in the uterus of dromedary camels.

Key words: Camel; Uterine fluids; α L fucosidase, α-N-acetylgalactosaminidase; β-N-acetylglucosaminidase

Dromedary camels have been classified as induced ovulators, the ovulation mainly occurs in response to coitus (El-wishy, 1987; Ismail, 1987). In the absence of mating, the camel's oestrous cycle has no luteal activity (El-wishy, 1987; Skidmore *et al,* 1996), therefore, the oestrous cycle is described as a follicular wave pattern (Tibary and Anouassi, 1997; Skidmore *et al,* 2013). The duration of the follicular wave was recorded to extend from 17.2 to 30.0 days (Skidmore *et al,* 1996). The luminal fluids in the female reproductive tract are significant for mammalian reproduction as they provide the favourable microenvironment for passage of spermatozoa and ova, fertilisation and development of the pre-implantation embryo (Fischer and Beier, 1986; Velazquez *et al,* 2010). As the penis of the male camelids penetrates the cervical canal during copulation to deposit semen in the uterus (Vaughan and Tibary, 2006), the uterine luminal fluid is considered as initial microenvironment contacting the spermatozoa on their way to the oviduct. As the importance of uterine fluid, many studies have been investigated the different chemical constituents in

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cow (Forde *et al,* 2014; Tríbulo *et al,* 2019), ewe (Ko *et al,* 1991; Koch *et al,* 2010), saw (Zavy *et al,* 1984; Seo *et al,* 2012) and mare (Hayes e*t al,* 2012). Glycosidases are a group of hydrolytic enzymes originate from lysosome and catalyse the hydrolysis of glycoproteins, glycolipids and glycosaminoglycans (Miller *et al,* 1993; Hahn *et al,* 2001; Jóźwik *et al,* 2003). Glycosidases play an important role in different reproductive events such as cumulus cells expansion (Takada *et al,* 1994), sperm capacitation (Taitzoglou *et al,* 2007), sperm oviductal epithelial cells interaction (Lefebvre *et al,* 1997), sperm zona pellucida binding (Miranda *et al,* 2000; Zitta *et al,* 2006), polyspermy block (Miller *et al,* 1993) and early embryos development (Tsiligianni, 2018). The aim of the present study was to characterise the activity of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α L fucosidase in the uterine luminal fluid and blood serum during the follicular phase of the dromedary camel (*Camelus dromedarius*).

Materials and Methods

Experimental materials

In the present study, one hundred genitalia were recovered from clinically healthy adult (7–16 years of age) non-pregnant female camels (*Camelus dromedarius*) during the breeding season (November–April), at a local abattoir in the Eastern province of Saudi Arabia. Blood samples (10 ml/ each animal) were collected from all animals during exsanguinations into non-heparinised tubes. Pre-slaughter reproductive history of these animals was not available. Genitalia and blood samples were kept in an ice box and transported immediately (within one-hour post slaughter) to the laboratory.

Collection of uterine fluid

Upon arrival at the laboratory, the genitalia with gross pathology or those with paired ovaries bearing follicles filled with sanguineous fluid or corpus luteum were discarded from the study. Depending on ovarian follicle diameter (measured by Vernier caliper), three groups of genitalia could be distinguished: Group l (genitalia have ovary/ ovaries bearing follicle/follicles of 5-10 mm), Group ll (genitalia have ovary/ovaries bearing follicle/follicles of 11–15 mm) and Group lll (genitalia have ovary/ ovaries bearing follicle/follicles of 16–21 mm). The uterine horns of each tract of different groups were longitudinally opened with surgical scissors from the cervix. The inner uterine mucus was aspirated from both horns using a positive displacement pipette suited for viscous media (Gilson MicroMan). Uterine fluid having cloudiness or with white flakes was excluded. Consequently, of one hundred collected genitalia only 24 were eligible for this study. The samples were distributed as: 8 in Group I, 8 in Group II and 8 in Group III. Uterine fluid was centrifuged at 1250-x g at 4°C for 10 minutes to remove the cell and cell debris and the supernatant was transferred into storage vials, identified and stored at -20°*C* until further analysis. Blood sera from selected animals were separated and stored at -20°*C* until analysis.

Estimation of biochemical constituents in uterine fluid and blood sera

Commercial ELISA diagnostic kits (My Biosource) were used for determination of camel β-N-acetylglucosaminidase (Catalog # MBS094638), camel α-N-acetylgalactosaminidase (Catalog # MBS053019) and camel α L fucosidase (Catalog # MBS092780) in the uterine fluid and blood serum samples. The procedures for analysis and calculation were adopted as recommended by the manufacturer.

Statistical analysis

The data analysis of biochemical constituents in uterine fluid and blood serum was carried out using a general linear model procedure and means were compared by least significant difference using SPSS 16.0 statistical software (2007).

Results

Results for concentrations of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid of dromedary camels during the follicular phase are displayed in Table1. The mean concentration of β-N-acetylglucosaminidase was 3.33 ± 0.26, 2.73 ± 0.15 and 6.43 ± 1.41 ng/ml in Group I, II and III, respectively. The level of α-N-acetylgalactosaminidase was 3.21 ± 0.27, 2.22 ± 0.14 and 7.24 ± 1.45 ng/ml in Group I, II and III, respectively. α-N-acetylgalactosaminidase concentration showed statistical differences ($P<0.05$) among the different groups. The peak concentration was recorded in-group III while the minimum concentration was reported in-group II. The value of α-L-fucosidase was 435.00 ± 6.94, 340.00 ± 9.82 and 362.50 ± 31.92 µmol/ml in Group I, II and III, respectively. The highest mean activity ($P<0.05$) was reported in-group I. Data presented in Table 2 demonstrates the mean concentrations of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the blood serum of the dromedary camels during the follicular phase. The mean concentration of β-N-acetylglucosaminidase

was 2.00 ± 0.04, 3.07 ± 0.30 and 2.49 ± 0.13 ng/ml. in-group I, II and III, respectively. The peak concentration (P<0.05) was recorded in-group II. The mean concentrations of α-N-acetylgalactosaminidase was 1.81 ± 0.02, 3.29 ± 0.38 and 2.60 ± 0.28 ng/ml. There were statistical differences (P<0.05) among the groups. The peak concentration (P<0.05) was recorded in group II. The mean concentration of α-L-fucosidase was 560.00 ± 64.25, 310.00 ± 31.45 and 280.00 ± 8.24 µmol/ml in group I, II and III, respectively. The peak concentration (P<0.05) was recorded in group I. Table 3 displayed a comparison between the mean concentrations of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid and blood serum of the dromedary camel during the follicular phase. In-group I, the concentrations of β-N-acetylglucosaminidase and α-N-acetylgalactosaminidase were significant (P<0.05) higher in the uterine fluid than the blood serum. In-group II, the concentration of α-N-acetylgalactosaminidase in the serum was significant higher (P<0.05) than in the uterine fluid. In-group III, β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase concentrations were significant (P<0.05) higher in uterine fluid than in the serum.

Discussion

Glycosidases are engaged in several reproductive events such as cumulus cells expansion (Takada *et al,* 1994), sperm capacitation (Taitzoglou *et al,* 2007), sperm oviductal epithelial cells interaction (Lefebvre *et al,* 1997), sperm zona pellucida binding (Miranda *et al,* 2000; Zitta *et al,* 2006), polyspermy block (Miller *et al,* 1993) and early embryos development (Tsiligianni, 2018). Recently, glycosidases activity in genital tract luminal fluid is used as markers of embryo quality (Tsiligianni, 2018), embryo recovery rate (Reilas *et al,* 2000), superovulatory response (Tsiligianni *et al,* 2007). As in cattle (Tsiligianni e*t al,* 2007; Tsiligianni, 2018), sheep (Tsiligianni e*t al,* 2003; Samartzi *et al,* 2020) and mares (Reilas e*t al,* 2000), this study verified the activity of glycosidases (β-N-acetylglucosaminidase, α -N-acetylgalactosaminidase and α L fucosidase) in the uterine luminal fluid of the dromedary camel. The current study revealed obvious concentrations fluctuation of the studied glycosidases in relation to the follicle size. Parallel, glycosidases activities were described of change during different reproductive pattern such as oestrous cycle (in rats; Pizarro *et al,* 1984; ewes; Roberts *et al,* 1976b and mares; Reilas and Katila, 2002), post-partum period (in mares, Reilas and Katila, 2002) and pregnancy (in cows; Roberts and Parker, 1974). Fluctuations in glycosidase activity during the oestrous cycle are hormonally controlled (Hansen *et al,* 1985). The activity of glycosidases is recorded to be regulated by progesterone, oestrogens (Gladson *et al,* 1998; Buhi *et al,* 2000; Reilas and Katila, 2002) and pH of uterine luminal fluid (Carrasco *et al,* 2008). In cows, Mather (1975) reported cyclic pH changes of uterine fluid with the period of the cycle. The alterations of the glycosidases during the follicular cycle recorded in this study supposed its role in carbohydrate-mediated events (Rahi and Srivastava, 1983; Roy e*t al,* 1983; Carrasco *et al,* 2008). Similar with the results of Carrasco *et al* (2008) in porcine oviductal fluid, we reported maximum activities of β-N-acetylglucosaminidase and α L fucosidase in the uterine fluid at the late follicular phase. This study demonstrated significant higher concentrations of β-N-

Table 1. Concentrations (mean ± SEM) of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid of the dromedary camel during the follicular phase.

Enzymes	Group I (5–10 mm, n = 8)	Group II (11–15 mm, n = 8)	Group III (16–20 mm, n = 8)
β-N-acetylglucosaminidase (ng/ml)	3.33 ± 0.26^{ab}	2.73 ± 0.15^{a}	6.43 ± 1.41^{b}
α-N-acetylgalactosaminidase (ng/ml)	3.21 ± 0.27^{a}	2.22 ± 0.14^{b}	7.24 ± 1.45^{c}
α-L-fucosidase (µmol/ml)	435.00 ± 6.94^{a}	340.00 ± 9.82^{b}	362.50 ± 31.92^{b}

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

Table 2. Concentrations (mean ± SEM) β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in blood serum of the dromedary camel during the follicular phase.

Enzymes	Group I (5–10 mm, n = 8)	Group II (11–15 mm, n = 8)	Group III (16–20 mm, n = 8)
β-N-acetylglucosaminidase (ng/ml)	2.00 ± 0.04^{a}	3.07 ± 0.30^{b}	2.49 ± 0.13^{c}
α-N-acetylgalactosaminidase (ng/ml)	1.81 ± 0.02^{a}	3.29 ± 0.38^{b}	2.60 ± 0.28^{c}
α-L-fucosidase (µmol/ml)	560.00 ± 64.25^{a}	310.00 ± 31.45^{b}	280.00 ± 8.24^{b}

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

Table 3. Comparison among concentrations (mean ± SEM) of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine luminal fluid and blood serum of the dromedary camel during the follicular phase.

Follicles sizes	Enzymes	Uterine fluid	Serum
Group I (5–10 mm, n = 8)	β-N-acetylglucosaminidase (ng/ml)	$3.33^{a} \pm 0.26$	$2.00^{b} \pm 0.04$
	α-N-acetylgalactosaminidase (ng/ml)	$3.21^{a} \pm 0.27$	$1.81^{b} \pm 0.02$
	α-L-fucosidase (µmol/ml)	435.00 ± 6.94	560.00 ± 64.25
Group II (11–15 mm, n = 8)	β-N-acetylglucosaminidase (ng/ml)	2.73 ± 0.15	3.07 ± 0.30
	α-N-acetylgalactosaminidase (ng/ml)	$2.22^{a} \pm 0.14$	$3.29^{b} \pm 0.38$
	α-L-fucosidase (µmol/ml)	340.00 ± 9.82	310.00 ± 31.45
Group III (16–20 mm, n = 8)	β-N-acetylglucosaminidase (ng/ml)	$6.43^{a} \pm 1.41$	$2.49^{b} \pm 0.13$
	α-N-acetylgalactosaminidase (ng/ml)	$7.24^{a} \pm 1.45$	$2.60^{b} \pm 0.28$
	α-L-fucosidase (µmol/ml)	$362.50^{a} \pm 31.92$	$280.00^{b} \pm 8.24$

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

acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase in the uterine fluid than the blood serum in-group I and II. Likewise, Roberts and Parker (1974), Roberts *et al* (1976a) and Roberts *et al* (1976b) reported elevated activities of β-N-acetylglucosaminidase and α-L-fucosidase in uterine washings of bovine, human and sheep when compared with serum. This suggests that the enzyme present in uterine fluid comes mainly from within the uterus. Moreover, Hansen *et al* (1985) and Thie *et al* (1984, 1986) confirmed that luminal and glandular epithelium of the endometrium of pig mare, ewe and rabbit synthesised β-N-acetyl glucosaminidase and α-L-fucosidase.

Conclusions

In conclusion, uterine fluid displays glycosidase activity, with specific differences throughout the follicular cycle of dromedary camels, proposing that these enzymes play a role in carbohydrate-mediated events.

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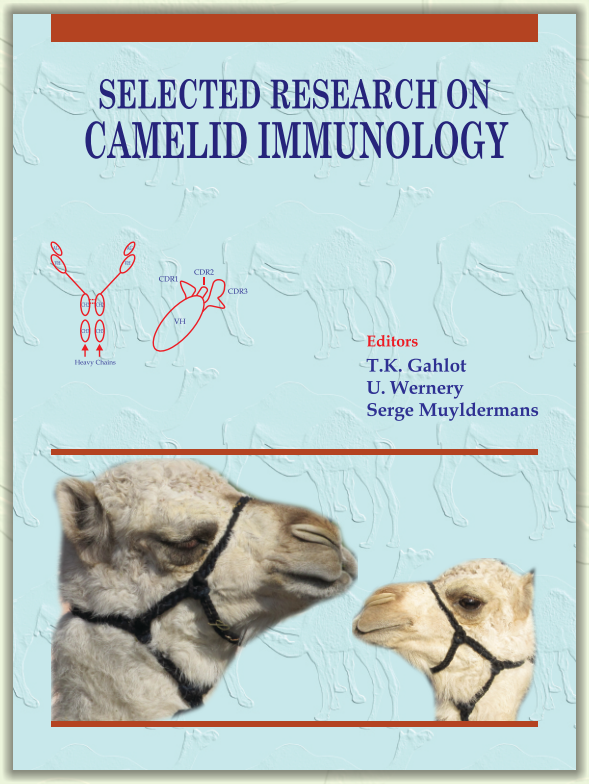
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

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

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



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PLASMA FROM *Escherichia coli* AND *Staphylococcus aureus* STIMULATED BLOOD DIFFERENTLY MODULATES THE EXPRESSION OF MONOCYTIC MARKERS ON CAMEL LEUKOCYTES

Naser Abdallah Al Humam
Department of Microbiology, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

ABSTRACT

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are two bacterial pathogens responsible for different infectious diseases in the dromedary camel with different disease outcomes. In other species, different host-pathogen interaction mechanisms have been reported for *E. coli* and *S. aureus*. To investigate the modulatory effects of the two pathogen species on the camel innate immune system, the present study used plasma samples collected from *E. coli* and *S. aureus* blood cultures for stimulation of camel leukocytes *in vitro*. Using labeling of cell markers with monoclonal antibodies and flow cytometry, the changes in the expression of several cell markers on monocytes and neutrophilic granulocytes were identified. Plasma from either *E. coli* or *S. aureus* blood cultures resulted in a significant decrease in the expression level of CD14 on blood monocytes, the decrease was, however, significantly stronger for plasma from *E. coli* than *S. aureus* blood culture. In addition, only plasma from *E. coli* blood culture was able to reduce the expression of CD14 on stimulated granulocytes. This may represent an immune evasion mechanism of *E. coli* from the CD14-mediated innate recognition of gram-negative bacteria by camel monocytes and neutrophils. No changes were observed in the expression of CD163, MHCII or CD44 on neutrophils stimulated with plasma from either of the *E. coli* or *S. aureus* blood cultures. The different effects of plasma collected from *E. coli* stimulated blood and *S. aureus* stimulated blood on monocytes and neutrophils indicates a bacterial-species-specific modulating effect on camel monocytes and neutrophils.

Key words: Camel, bacterial blood culture, blood plasma, *Escherichia coli*, monocytes, neutrophils, *Staphylococcus aureus*

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are major causative agents of different infectious diseases in the dromedary camel including metritis, mastitis in adults and respiratory and intestinal infections in newborns (Tibary *et al,* 2006; El Tigani-Asil *et al,* 2020). While *S. aureus* is mainly responsible for subclinical infections of the udder (Keefe, 1997; Bannerman *et al,* 2004; Keefe, 2012; Gunther *et al,* 2016), infection with *E. coli* results mostly in severe acute inflammatory disease with clinical signs (Bannerman *et al,* 2004).

Monocytes and neutrophilic granulocytes are innate immune cells with an essential role during the innate immune response to bacterial pathogens (Jakubzick *et al,* 2017). For their antimicrobial effects, monocytes and neutrophils are equipped with several cell surface molecules, which play key roles in pathogen detection, phagocytosis, and elimination (Ziegler-Heitbrock, 2000; Auffray *et al,* 2007). The cell surface molecules CD14, CD163, and MHCII have been proven as reliable markers of the innate function of several myeloid cells (Schwartz and Svistelnik, 2012; Thawer *et al,* 2013; Hussen *et al,* 2014; Hussen and Schuberth, 2017). Major histocompatibility (MHC) class II molecules are antigen receptors expressed on blood monocytes and B cells, and present antigens to T helper cells (Abeles *et al,* 2012). CD163 is a scavenger receptor for haptoglobin–haemoglobin complexes that is mainly expressed on monocytes and macrophages.

In other species, different host-pathogen interaction mechanisms have been reported for *E. coli* and *S. aureus*. The aim of the current study was to investigate the modulatory effects of plasma samples collected from *E. coli* and *S. aureus* blood cultures on the camel innate immune cells monocytes and neutrophils upon *in vitro* stimulation.

Materials and Methods

Blood samples were collected from healthy camels (n = 10; *Camelus dromedarius*) by venipuncture

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of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany). For the preparation of blood plasma, collected blood samples (n = 4) were stimulated with *E. coli* or *S. aureus* (two mastitis isolates) according to a previously established method (Hussen *et al,* 2013). Whole camel blood (2 ml) was diluted with 1.8 ml cell culture medium (RPMI-1640, Sigma-Aldrich, Deisenhofen, Germany) in sterile 15 ml tubes (BD Biosciences, San Jose, California, USA). Live bacterial suspension (0.2 ml; 10^7 bacteria/ml) was added to the diluted blood and the mixture was then incubated for 12 h at 37°C. A control tube containing 2 ml blood and 2 ml medium without bacteria was also included. After incubation, the tubes were then put into icy water and immediately centrifuged at 4°C for 10 min at 1000xg to collect the supernatant plasma. Collected stimulated and unstimulated plasma samples from all animals were pooled together, and the pooled plasma was used for further stimulation of camel leukocytes. All experimental procedures and management conditions used in this study were approved by the Ethics Committee at King Faisal University, Saudi Arabia (Permission number: KFU-REC/2020-09-25).

Separation of camel leukocytes and stimulation with plasma

Separation of camel leukocytes (n = 6 animals) was done after hypotonic lysis of blood erythrocytes as previously described (Hussen *et al,* 2017). Briefly, unstimulated and stimulated blood samples suspended in PBS were centrifuged at 4°C for 10 min at 1000xg and the cell pellet was suspended in distilled water for 20 sec and double concentrated PBS was added to restore tonicity. This was repeated until complete erythrolysis. Separated cells were finally suspended in RPMI medium (1 x 10^6/ ml). Separated leukocytes were stimulated in RPMI medium with plasma from control (non-stimulated blood samples), plasma from *E. coli* blood culture, plasma from *S. aureus* blood culture, or the cells were left unstimulated in RPMI medium (without plasma).

Monoclonal antibodies

Monoclonal antibodies used in this study are listed in Table 1.

Membrane immunofluorescence and flow cytometry

The expression densities of different monocytic cell surface molecules were evaluated by flow cytometry after membrane immunofluorescence (Eger *et al,* 2015). Stimulated and unstimulated leukocytes (2 x 10^5) were incubated with unlabeled primary monoclonal antibodies (mAbs) specific for the cell markers CD14, MHCII, CD163, and CD44 in MIF buffer [membrane immunofluorescence buffer consisting of PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)] (Hussen and Schuberth, 2017). After incubation (15 min; 4°C), the cells were washed twice and incubated with mouse secondary antibodies (IgG1, IgG2a; Invitrogen) labeled with FITC and PE, respectively. Washed cells were analysed using the Accurie C6 flow cytometer (BD Biosciences). At least 100 000 total leukocytes were collected and analysed with the CFlow Software, Version 1.0.264.21 (Fig 1A and Fig 2A).

Table 1. List of antibodies.

Antigen	Antibody clone	Label	Source	Isotype
CD14	TÜK4	-	WSU	mIgG1
MHCII	TH81A5	-	Kingfisher	mIgG2a
CD163	LND68A	-	Kingfisher	mIgG1
CD44	LT41A	-	WSU	mIgG2a
mIgG2a	polyclonal	PE	Invitrogen	gIgG
mIgG1	polyclonal	FITC	Invitrogen	gIgG

Ig: Immunoglobulin; m: mouse; g: goat, MHCII: Major Histocompatibility Complex class II, FITC: Fluorescein isothiocyanate, PE: Phycoerythrin.

Statistical Analyses

Statistical analysis was carried out using the software Prism (GraphPad software version 5). Results are expressed as mean ± S.E. (SEM). Differences between means were tested with one-factorial analysis of variance (ANOVA). Results were considered statistically significant at a p-value of less than 0.05.

Results and Discussion

Monocytes and neutrophils are equipped with several cell surface molecules, which play key roles in pathogen detection, phagocytosis, and elimination (Ziegler-Heitbrock, 2000; Auffray *et al,* 2007). CD14 is a membrane protein mainly expressed on monocytes, and it serves together with toll-like receptor 4 (TLR-4) as a bacterial pattern recognition receptor responsible for binding lipopolysaccharide (LPS) in the cell wall of gram-negative bacteria (Payne *et al,* 1993). In addition, camel neutrophils show a low expression level of the LPS co-receptor CD14, which is similar to bovine neutrophils (Sohn *et al,* 2007), suggesting a role for neutrophils in the sensing of gram-negative bacteria (Hussen, 2018).

In the present study, stimulation with plasma samples collected from either *E. coli* or *S. aureus*

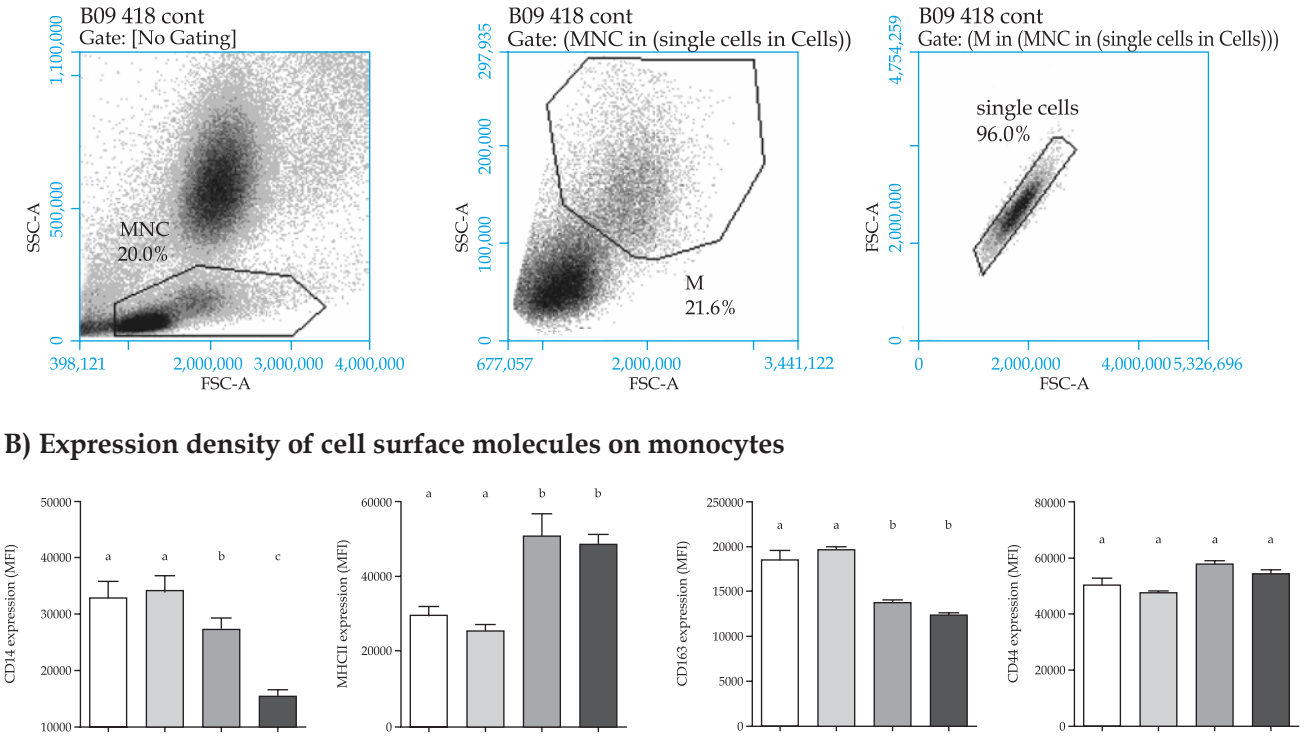

Fig 1. **A)** Gating strategy for camel blood monocytes. In a forward scatter (FSC-A) / side scatter (SSC-A) dot plot, a gate was set on mononuclear cells (MNC) a according to their scatter characteristics. After gating on MNC, monocytes were identified in a separate FSC-A/SSC-A dot plot according to their FSC and SSC properties. Duplets were excluded from the analysis by setting a gate on single cells in a FSC-A against FAC-H dot plot. **B)** The expression densities of different cell markers on stimulated and non-stimulated monocytes. The mean fluorescence intensity of the cell surface molecules, CD14, MHCII, CD163, and CD44 were calculated and presented for unstimulated monocytes and monocytes stimulated with plasma as means ± SEM. Different lowercase superscript letters indicate statistical significance ($P < 0.05$).

blood cultures resulted in a significant decrease in the expression level of CD14 on blood monocytes (Fig 1B). This decrease in CD14 on monocytes was, however, significantly ($p < 0.05$) stronger for monocytes stimulated with plasma from *E. coli* than *S. aureus* blood culture (Fig 1B). For stimulated granulocytes, only plasma from *E. coli* blood culture was able to reduce the expression of CD14 on stimulated granulocytes in comparison to cells stimulated with plasma from *S. aureus* blood culture of unstimulated blood (Fig 2B). This may indicate a suppressive effect of plasma collected from *E. coli* blood culture on the CD14-mediated innate recognition function of monocytes and neutrophils toward gram-negative bacterial pathogens.

The expression levels of MHCII and CD163 are widely accepted as markers for pro- (macrophages subtype 1; M1) and anti-inflammatory (macrophage subtype 2; M2) functional subtypes of macrophages, respectively (Hu *et al,* 2017). In the current study, plasma samples collected from either *E. coli* or *S. aureus* blood cultures induced a significantly ($p < 0.05$) higher abundance of MHCII and a significantly ($p < 0.05$) lower abundance of CD163 on camel monocytes (Fig 1B). The increased expression of the M1 marker MHCII together with the reduced expression of the M2 marker CD163 indicate a pro-inflammatory phenotype of monocytes stimulated with plasma samples collected from the two bacterial blood cultures.

In contrast to the stimulation-induced change in the phenotype of monocytes, there were no changes in the expression of the cell markers MHCII, CD163, or CD44 on neutrophils upon incubation with plasma from the bacterial blood cultures (Fig 2B). This indicates different modulatory effects of the two pathogen species on monocytes and neutrophils. Whether this is due to the existence of different immune mediators in the plasma samples collected from stimulated blood, still need to be investigated.

Conclusions

The enhanced expression of MHCII molecules together with the reduced expression of CD163

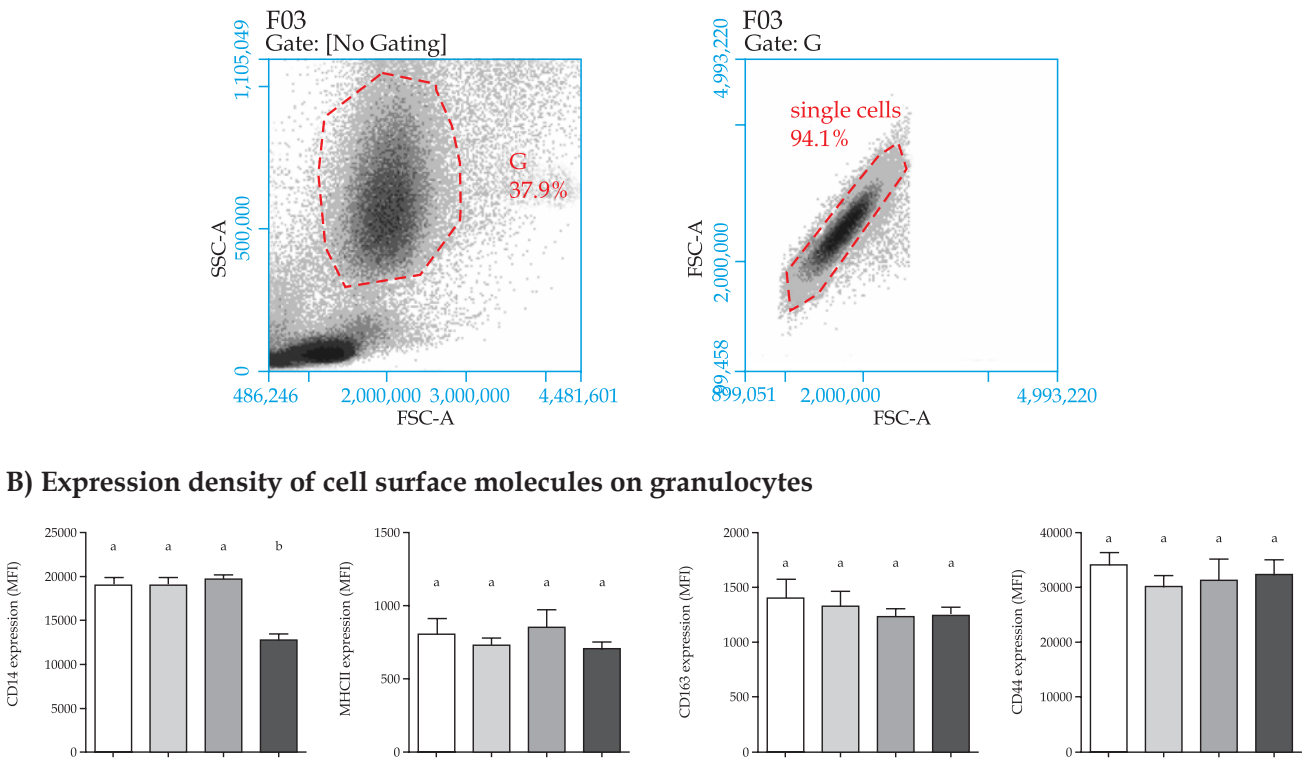

Fig 2. **A)** Gating strategy for camel blood granulocytes. In a forward scatter (FSC-A) / side scatter (SSC-A) dot plot, a gate was set on granulocytes (G) according to their scatter characteristics. After gating on granulocytes, duplets were excluded from the analysis by setting a gate on single cells in a FSC-A against FAC-H dot plot. **B)** The expression densities of different cell markers on stimulated and non-stimulated granulocytes. The mean fluorescence intensity of the cell surface molecules, CD14, MHCII, CD163, and CD44 were calculated and presented for unstimulated granulocytes and granulocytes stimulated with plasma as means ± SEM. Different lowercase superscript letters indicate statistical significance ($P < 0.05$).

molecules on camel monocytes stimulated with plasma from bacteria-stimulated blood indicates the shift of monocytes toward a pro-inflammatory phenotype. Plasma from *E. coli* stimulated blood resulted in a stronger decrease in CD14 expression on monocytes and a significant lower CD14 abundance on neutrophils, when compared with plasma from *S. aureus* blood culture. This may represent an immune evasion mechanism of *E. coli* against the CD14-mediated innate recognition of gram-negative bacteria by monocytes and neutrophils. The different effects of plasma collected from *E. coli* and *S. aureus* blood cultures on monocytes and neutrophils indicates a bacterial-species-specific modulating effect on camel monocytes and neutrophils.

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SELECTED RESEARCH ON
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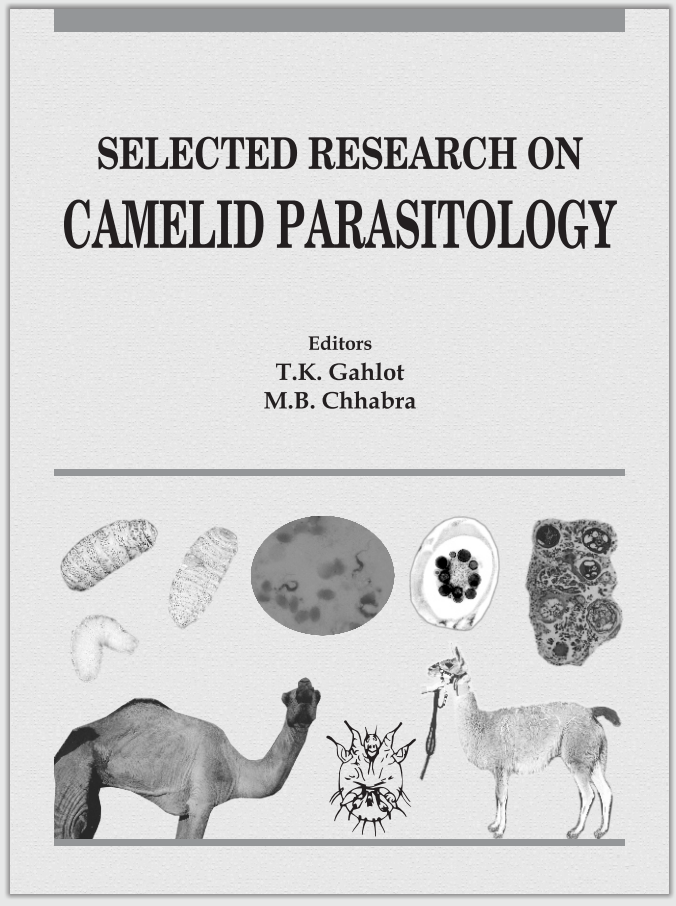
Hard bound, 291 pages, few figures coloured

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



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ACID-BASE BALANCE, BLOOD GASES AND HAEMATOBIOCHEMICAL PROFILES IN CAMELS (*Camelus dromedarius*) WITH TRYPANOSOMOSIS

Mohamed Tharwat[1,2]

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

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, 44519, Zagazig, Egypt

ABSTRACT

This study was carried out to investigate the status of acid-base balance and blood gases in camels with trypanosomosis compared to healthy camels. The haematobiochemical profiles were also reported in both groups. Forty-two camels with chronic weight loss, ventral oedema and ascites were examined. Passive haemagglutination test showed that 38 of the 42 camels (90%) were positive for *Trypanosoma evansi*. Compared to a value of 7.54±0.16 in healthy camels, the blood pH in diseased camels was 7.37±0.051. The partial pressure of carbon dioxide (PCO_2) was higher in camels with trypanosomosis than healthy camels. On the contrary, the oxygen partial pressure (PO_2) was lower in camels with trypanosomosis. The base excess (BE) was also lower in diseased camels than healthy ones. Similarly the bicarbonate (HCO_3) was lower in diseased camels. In a similar pattern, the total carbon dioxide (TCO_2) was lower in diseased than healthy group. The oxygen saturation (SO_2) decreased significantly in camels with trypanosomosis when compared to healthy group. Concerning the haematological parameters, leukocytosis, neutrophilia and lymphopenia was found in diseased camels. The RBCs count, haemoglobin and haematocrit decreased significantly in camels with trypanosomosis. Concerning, the biochemical parameters albumin and phosphorus decreased significantly and globulin and magnesium increased significantly in diseased camels. The serum activity of alkaline phosphatase, γ-glutamyl transferase and creatine kinase increased significantly in diseased camels compared to healthy camels. In conclusion, camels with trypanosomosis have metabolic acidosis, and the HCO_3 was lower than healthy camels. The PCO_2 was higher, while PO_2, BE, HCO_3, TCO_2 and SO_2 were lower in camels with trypanosomosis compared to healthy camels.

Key words: Acid-base balance, blood gases, camels, dromedary, trypanosomosis

Camel trypanosomosis (Surra) is caused by the protozoan parasite *Trypanosoma evansi* (*T. evansi*) and is a major threat to productivity and economic losses (Tehseen *et al,* 2015). Although surra is found in acute and chronic forms but chronic form is most common and is likely to present an association with secondary infection due to immuno-suppression caused by *T. evansi* infection (Olaho-Mukani *et al,* 1993; Olaho-Mukani and Mahamat, 2000; Ahmed, 2008; Eyob and Matios, 2013). The acid-base balance, blood gases and haemotobiochemical profiles may be altered in trypanosomosis in camels but these are least studied.

The effect of dehydration and exercise on the acid-base balance parameters has been investigated in dromedary camels (Abdoun *et al,* 2012; Okab *et al,* 2012). Another study has reported the influence of acid load with NH_4Cl on the acid-base status in young dromedary camels (Elkhair and Hartmann, 2010). In addition, the effect of tick infestation and stimulation by electroejaculation on the acid–base balance has also been reported in dromedary camels (Tharwat *et al,* 2014). Blood gas analysis has also been studied in healthy female dromedary camels, their calves and umbilical cord blood at spontaneous parturition (Tharwat, 2015). This study was carried out to determine acid-base balance, blood gases and haemotobiochemical status of camels with trypanosomosis.

Materials and Methods

Forty-two adult camels (*Camelus dromedarius*) were referred to the Veterinary Teaching Hospital, Qassim University, Saudi Arabia because of inappetance, loss of body condition, ventral or presternal oedema and abdominal distension. Duration of illness ranged from 3 days to 5 weeks. Clinical examination included general behaviour and condition, auscultation of the heart, lungs, rumen

SEND REPRINT REQUEST TO MOHAMED THARWAT email: mohamedtharwat129@gmail.com

and intestine, measurement of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion, auscultation of both sides of the abdomen and rectal examination (Wernery and Kaaden, 2002). Fifteen clinically healthy female camels were enrolled in this study as controls. From each camel, 10 mL blood sample were collected; 2 mL in EDTA tubes, 2 mL in heparinised tubes and the remaining 6 mL in plain tubes for serum harvesting. Using passive haemagglutination test, serum samples were tested for *T. evansi* antibodies (Omar *et al,* 1998). Sera from uninfected camel and an infected camel were used as negative and positive controls, respectively. Samples showing agglutination at 1:16 were considered positive.

Blood gas analyses and determination of haemato-biochemical parameters

The heparinised blood samples were used immediately to analyse the acid-base and blood gas parameters using a portable clinical veterinary analyser (I-STAT®, Abaxis, California, USA). In this way, blood pH, partial pressure of carbon dioxide (PCO_2), oxygen partial pressure (PO_2), bicarbonate (HCO_3), total carbon dioxide (TCO_2), base excess (BE), oxygen saturation (SO_2), and lactic acid (LA) were analysed immediately. A complete blood count [total and differential leukocytic count, erythrocyte count, haematocrit (HCT), haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] was carried out on the EDTA sample using the VetScan HM5, Abaxis, California, USA. An automated biochemical analyser (VetScan VS2, Abaxis, California, USA) was used to determine the serum concentrations of total protein, albumin, globulin, blood urea nitrogen (BUN), calcium, phosphorus, magnesium and cardiac biomarker troponin I (cTnI). The serum activity of γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK) and alkaline phosphatase (ALP) were also measured. Cardiac troponin I was measured in serum using a commercial available test (I-stat, cTnI, VetScan, Abaxis, CA, USA), using a two-site enzyme-linked immunosorbant assay (Tharwat, 2012; Tharwat, 2013; Tharwat *et al,* 2013a,b,c; Tharwat, 2020; Tharwat, 2121).

Statistical analysis

Data are presented as means ± SD and were analysed statistically using the SPSS statistical package, version 18, 2009. Student's t test was used for comparisons, and the significance was set at $P \leq 0.05$.

Results and Discussion

Definitive diagnosis of trypanosomosis in the infected camels was made on the basis of detecting antibodies in serum by passive haemagglutination test. The passive haemagglutination test showed that 38 of the 42 camels (90%) were positive for *T. evansi*. Hence, data of the 38 positive camels were used in this study. The most prominent clinical signs in the camels were weight loss, abdominal distension and ventral and subcutaneous presternal oedema. On clinical examination, ascites was detected by a fluid thrill on ballottment, by fluid sounds on percussion, or by the demonstration of excess fluid in the peritoneal cavity by abdominocentesis.

Compared to healthy control camels, the values of blood pH, PCO_2, PO_2, BE, HCO_3, TCO_2, SO_2 and LA means ± SD alongside the 25%, 50%, 75% and 99% percentiles in camels infected with trypanosomosis are summarised in Table 1. Compared to a value of 7.54±0.16 in healthy camels, the blood pH in diseased camels was 7.37±0.051, with a statistically significant difference (P=0.002). The PCO_2 was higher in camels infected with trypanosomosis than healthy camels (36.0±3.1 mmHg/L versus 30.0±8.1 mmHg, P=0.026). On the contrary, The PO_2 was lower in camels with trypanosomosis than healthy animals (25±2 mmHg/L versus 183±15 mmHg/L, P=0.0001). The BE was also lower in diseased camels than healthy ones (-4.3±2.3 mmol/L versus 2.4±5.3 mmol/L, P=0.0004). Similar, the HCO_3 was lower in diseased than healthy camels (20.9±1.6 mmol/L versus 24.9±2.9 mmol/L, P=0.0002). In a similar pattern, the TCO_2 was lower in diseased than healthy camels (22±1.8 mmol/L versus 25.7±2.9 mmol/L, P=0.022). The SO_2 decreased significantly in camels infected with trypanosomosis when compared to healthy control group (43±6.3 mmol/L in diseased camels versus 100 mmol/L in healthy camels, P=0.0001). In this study, camels with trypanosomosis had a significant decrease in the pH values compared to controls. This decrease could be easily justified by the increases in PCO_2 and additionally by the decreases in HCO_3 and BE values. The BE represents all basic components, not just HCO_3, and as such, is a more sensitive measure of metabolic acidosis than HCO_3 alone (Sigaard-Andersen and Fogh-Andersen, 1995). The decreased BE, HCO_3 and TCO_2 in this study could be explained as being due to the metabolic acidosis; this is the reason for the negative BE values. The LA concentration did not differ significantly between the 2 groups (1.46±1.4 mmol/L in diseased group versus 4.3±3.3 mmol/L in healthy group, P=0.11).

Table 1. Acid-base balance, blood gases and lactic acid concentration in camels with trypanosomosis versus healthy controls.

Parameters	Diseased camels (*n*=38)						Healthy camels (*n*=15)						*P* value
	Mean ± SD	Percentiles					Mean ± SD	Percentiles					
		25%	50%	75%	95%	99%		25%	50%	75%	95%	99%	
pH	7.37±0.051	7.35	7.38	7.41	7.43	7.44	7.54±0.16	7.42	7.50	7.70	7.80	7.80	0.002
PCO_2 mmHg	36.0±3.1	34.05	34.9	36.4	41.7	42.3	30.0±8.1	22.6	31.6	36.7	40.9	40.9	0.026
PO_2 mmHg	25±2	23.5	25.0	26.3	26.9	27.0	183±15	174	185	192	203.4	209.5	0.0001
BE mmol/L	-4.3±2.3	-6	-4	-2.5	-1	-1	2.4±5.3	0.0	1.0	7.5	10.0	10.0	0.0004
HCO_3 mmol/L	20.9±1.6	19.5	20.8	22	23.2	23.2	24.9±2.9	23.7	25.1	27.0	28.3	28.9	0.0002
TCO_2 mmol/L	22±1.8	20.8	22.0	23.3	23.9	24.0	25.7±2.9	24.8	26.0	27.3	29.1	29.8	0.022
SO2 %	43±6.3	40	41	44	50	52	100	100	100	100	100	100	0.0001
LA mmol/L	1.46±1.4	0.7	0.9	1.7	3.1	3.4	4.3±3.3	2.3	3.0	5.4	11.7	11.7	0.11

PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen; BE, base excess; HCO_3, bicarbonate; TCO_2, total carbon dioxide; SO_2, oxygen saturation; LA, lactic acid.

Table 2. Haematological parameters in camels with trypanosomosis versus healthy controls.

Parameters	Diseased camels (*n*=38)						Healthy camels (*n*=15)						*P* value
	Mean ± SD	Percentiles					Mean ± SD	Percentiles					
		25%	50%	75%	95%	99%		25%	50s%	75%	95%	99%	
WBCs ($\times10^9$/L)	30.4±20.2	15.8	23.5	37.1	62.8	69.1	16.8±3.9	15.7	17.9	18.6	21.3	22.3	0.007
LYM ($\times10^9$/L)	1.4±0.8	0.8	1.1	1.9	2.5	2.7	6.2±2.9	4.4	5.9	6.6	11.1	12.9	0.0001
NEU ($\times10^9$/L)	27.8±19.6	14.7	20.8	33.8	59.1	67.3	9.7±3.0	7.6	9.8	12.0	13.8	14.3	0.0003
RBCs ($\times10^{12}$/L)	7.5±1.0	6.9	7.4	7.8	8.9	9.2	11.3±1.4	10.4	11.5	12.0	13.5	13.6	0.0001
HB (g/dL)	11.5±1.3	10.6	12.1	12.2	12.9	13.1	16.4±2.8	14.6	16.0	18.0	21.0	23.0	0.0001
HCT (%)	20.4±2.0	19.1	20.3	21.2	23.3	24.2	28.9±2.7	27.4	29.0	30.5	33.0	33.2	0.0001
MCV (fl)	27.4±2.4	26.0	26.5	27.3	31.3	32.7	25.5±1.5	24.0	26.0	26.0	27.1	27.8	0.014
MCH (pg)	15.5±1.4	14.7	15.8	16.4	17.3	17.5	14.7±2.4	12.7	13.9	16.7	18.7	19.7	0.347
MCHC (g/dL)	56.8±7.2	51.0	57.4	62.3	65.2	66.0	57.6±9.0	50.6	53.7	64.3	74.3	74.9	0.829

WBCs, white blood cells; LYM, lymphocytes; MON, monocytes; NEU, neutrophils; RBCs, red blood cells; HB, haemoglobin; HCT, haematocrit; MCV, Mean corpuscular volume; MCH, Mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration

The means ± SD of the haematological parameters in camels infected with trypanosomosis compared to healthy camels alongside the 25%, 50%, 75% and 99% percentiles are presented in Table 2. Leukocytosis was found in diseased camels compared to healthy group (30.4±20.2 $\times10^9$/L in diseased group versus 16.8±3.9 $\times10^9$/L in healthy group, P=0.007). Similar, neutrophilia was recorded in diseased group compared to healthy group (27.8±19.6 $\times10^9$/L in diseased group versus 9.7±3.0 $\times10^9$/L in healthy group, P=0.0003). However, lymphopenia was detected in diseased camels compared to healthy ones (1.4±0.8 $\times10^9$/L in diseased group versus 6.2±2.9 $\times10^9$/L in healthy group, P=0.0001). The RBCs count, haemoglobin concentration and HCT per cent decreased significantly in camels infected with trypanosomosis when compared to healthy camels (P=0.0001). Similar findings were reported (Ahmadi-hamedani *et al,* 2014; Hussain *et al,* 2018). The MCV increased significantly in diseased camels compared to healthy camels (P=0.014). The MCH and MCHC increased in diseased group compared to healthy group but the increases were not significant (P=0.34 and P=0.82, respectively). Leukocytosis encountered in this study could be explained on the basis of the chronic nature of the disease. This denotes that camels with trypanosomosis may develop concurrent and even fatal bacterial, viral and other protozoan infections as a result of immunosuppression (Aradaib and Majid, 2006). Haematological indices showed significant reduction in the haematocrit and hemoglobin indicated that affected camels had macrocytic hypochromic anaemia in an agreement to a study reported recently (Saleh *et al,* 2009).

The means ± SD of the biochemical parameters in camels infected with trypanosomosis compared to

Table 3. Biochemical parameters in camels with trypanosomosis versus healthy controls.

Parameters	Diseased camels (*n*=38)						Healthy camels (*n*=15)						*P* value
	Mean ± SD	Percentiles					Mean ± SD	Percentiles					
		25%	50%	75%	95%	99%		25%	50s%	75%	95%	99%	
TP (G/L)	70.3±9.7	65.0	70.0	75.3	80.7	81.7	67.3±4.3	63.0	67.5	68.8	74.0	76.4	0.34
ALB (G/L)	39.5±6.4	39.0	42.0	42.5	43.7	43.9	60.39±3.0	60.8	61.5	62.0	64.3	64.9	0.0001
ALP (U/L)	94.5±90.5	35.8	62.0	120.7	204.2	220.8	6.6±2.8	5.8	6.5	8.0	10.8	12.6	0.0004
AST (U/L)	101.8±46.4	79.3	92.5	115.0	155.8	164.0	79.5±16.5	69.5	80.5	85.0	104.8	118.6	0.117
CA (MMOL/L)	2.3±0.1	2.3	2.3	2.4	2.4	2.4	2.4±0.1	2.3	2.4	2.5	2.6	2.6	0.18
GGT (U/L)	63.0±104.7	9.3	12.5	66.3	189.3	213.9	12.2±5.3	8.8	12.5	13.0	19.8	26.4	0.04
GLOB (G/L)	31.0±10.6	26.0	33.0	38.0	40.4	40.9	7.0±3.8	5.0	7.0	9.0	12.5	15.3	0.0001
BUN (MMOL/L)	9.8±9.4	5.1	6.4	11.1	21.2	24.0	6.4±1.1	5.9	6.4	6.7	8.1	8.2	0.13
CK (U/L)	240.0±92.0	183.2	253.0	309.3	321.9	324.4	139.0±21.6	127.0	136.0	148.8	171.8	178.4	0.01
PHOS (MMOL/L)	2.0±0.4	1.9	2.0	2.2	2.5	2.6	2.6±0.4	2.6	2.7	2.8	3.0	3.1	0.009
MG (MMOL/L)	0.7±0.1	0.7	0.7	0.7	0.8	0.8	0.3±0.0	0.2	0.3	0.3	0.3	0.3	0.0001
cTnI (ng/mL)	0.03±0.06	0.00	0.01	0.03	0.12	0.14	0.03±0.02	0.02	0.02	0.04	0.07	0.08	0.93

TP, total protein; ALB, albumin; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CA, calcium; GGT, γ-glutamyl transferase; GLOB, globulin; BUN, blood urea nitrogen; CK, creatine kinase; PHOS, phosphorus; MG, magnesium; cTnI, cardiac troponin I.

healthy camels alongside the 25%, 50%, 75% and 99% percentiles are presented in Table 3. Compared to healthy camels, the serum concentration of albumin decreased significantly in diseased camels (39.5±6.4 g/L in diseased group versus 60.39±3.0 g/L in healthy group, P=0.0001). Similar findings were reported (Ahmadi-hamedani *et al,* 2014; Hussain *et al,* 2018). Similar, the serum concentration of phosphorus decreased significantly in diseased camels (2.0±0.4 mmol/L in diseased group versus 2.6±0.4 mmol/L in healthy group, P=0.009). On the contrary, the serum concentration of globulin increased significantly in diseased camels (31.0±10.6 g/L in diseased group versus 7.0±3.8 g/L in healthy group, P=0.0001). Similar, the serum concentration of magnesium increased significantly in diseased camels (0.7±0.1 mmol/L in diseased group versus 0.3±0.0 mmol/L in healthy group, P=0.0001). The serum activity of ALP, GGT and CK increased significantly in diseased camels compared to healthy animals (P=0.0004, P=0.04 and P=0.01, respectively). Other biochemical parameters that included the serum concentrations of calcium, BUN, phosphorus, cTnI, and the serum activity of AST did not differ significantly compared to healthy camels (P=0.18, P=0.13, 0.93 and P=0.117, respectively). Hyperglobulinaemia encountered in this study could be explained on the basis of the chronic nature of the disease. This denotes that camels with trypanosomosis may develop concurrent and even fatal bacterial, viral and other protozoan infections as a result of immunosuppression (Aradaib and Majid, 2006).

It is concluded from this study that camels with trypanosomosis has metabolic acidosis when compared to healthy non-infected animals. The HCO_3 concentration was lower in camels with trypanosomosis than healthy camels. Changes in blood gases were remarkable where the PCO_2 was higher, while PO_2, BE, HCO_3, TCO_2 and SO_2 were lower in camels with trypanosomosis when compared to healthy camels.

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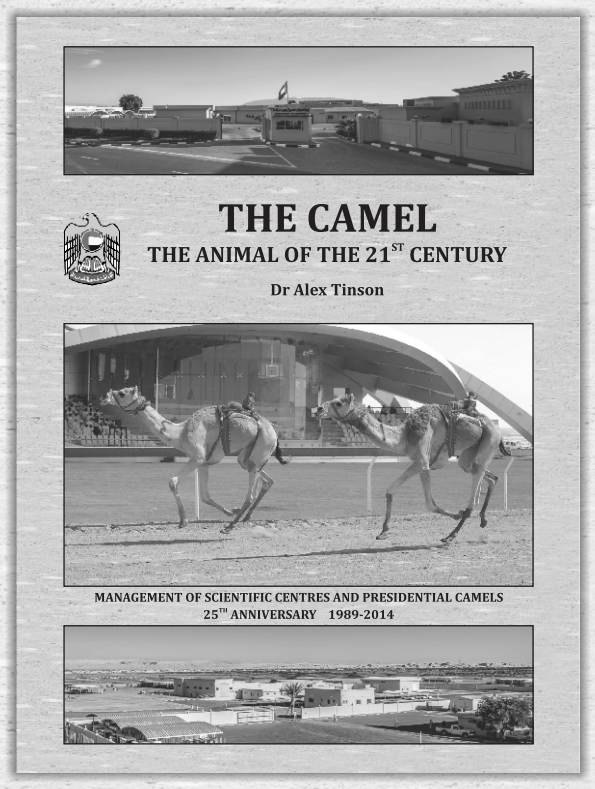
THE CAMEL

THE ANIMAL OF THE 21ST CENTURY

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



Author
Dr Alex Tinson

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ETIO-PATHOLOGY AND THERAPEUTICS OF PICA IN DROMEDARY CAMELS

R. Koted[1], F.C. Tuteja[2], A.P. Singh[1], R.K. Sawal[2], S.D. Narnaware[2] and V. Prakash[2]

[1]Department of Clinical Veterinary Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001, Rajasthan, India

[2]ICAR-National Research Centre on Camel, Jorbeer, Shivbari, Bikaner-334001, Rajasthan, India

ABSTRACT

In the present study, a very high incidence (51.66%) of pica was recorded in camels of all age groups at an organised farm. Camel calves of less than one year of age showed vices of licking the manger and corral walls along with eating floor soil. Pica was more pronounced in active growing camels between 1-5 years of age. Common vices observed were osteophagia, geophagia, lithophagia and coprophagia depending upon the availability of that particular object in the vicinity of the camels. General weakness as reflected by thinness of the hump, roughness of the hair coat, anaemia and emaciation were commonly observed symptoms. High faecal silica content in the pica affected camels suggested high silica consumption by the pica affected camels. There was a significant decrease in mean erythrocyte count, haemoglobin concentration, packed cell volume, total serum protein, serum globulin, serum calcium and phosphorus concentrations in the pica affected camel, as compared to healthy and treated camels. Decrease in zinc and iron levels were also observed in the pica affected camels as compared to healthy camels. No significant difference in serum copper, cobalt and selenium concentrations were recorded in pica affected and healthy camels. Treatment using specially designed mineral mixture at the rate of 50 gm per day per animal for 60 days was found satisfactory in terms of improving haemato-biochemical and serum mineral profiles in camels. Mineral mixture supplementation was also found satisfactory in reducing the symptoms of pica.

Key words: Camel, etipathology, pica, threrapeutics

Pica or allotriophagia associated with parasitism and deficiencies of phosphorus, salt or protein (Smith, 2015) and has been widely reported in cattle in Egypt (Elshahawy *et al,* 2016) and Iraq (Mosa *et al,* 2020). Minerals like copper, zinc, and cobalt, has been implicated in the aetiology of pica and fleece dietary pattern in sheep (Fahmy *et al*, 1980). Imbalance between the minerals either due to deficiency or interaction lowers immune status of the animals which ultimately affects its production (Judson *et al*, 1987). In camels these ailments are chronic and difficult to treat medically because of unknown etiology. Gautam and Bansal (1972) reported disease of camels known as "Mitti Khana" in India, due to heavy infections of gastro-intestinal worms with deficiencies of minerals such as calcium and phosphorus and of total proteins.

The most common cause of intestinal obstruction in camels having pica is eating hairs or plant fibres, leading to formation of phytobezoars and trichobezoars that may reach the intestine causing obstruction (Tanwar, 1985; Tharwat, 2012).

Present study was planned to record the incidence, clinical signs, serum minerals and haematological changes in camels suffering from pica along with its treatment using, specially prepared mineral mixture supplement*.

Materials and Methods

A total of 300 camels of an organised herd were included in the present study. These were given drinking water dewormers and antitrypanocidal drugs at regular intervals. These camels were maintained in the open housing system with stall feeding and allowed browsing and grazing daily for five hours in the rain fed demarcated area of the farm.

Feeding treatment trial of the selected animals: Out of these 300 camels 12 severely affected male camels aged between 2-4 years were divided into two groups comprising six animals in each group. In the feeding treatment trial six camels of group-1 were fed with specially designed mineral mixture* daily at the rate

* The composition of the designed mineral mixture (per 100 kg) was as follows: dicalcium phosphate (Ca_2PO_4) 59.00 kg; calcium carbonate ($CaCO_3$) 40.50 kg; zinc sulphate ($ZnSO_4$) 0.230 kg; copper sulphate ($CuSo_4$) 0.160 kg; manganese sulphate ($MnSO_4$) 0.030 kg and cobalt sulphate ($CoS0_4$) 0.050 kg.

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of 50 gram per animal per day, whereas 6 animals of group-2 were fed with the same basal fodder for 60 days. All these camels were observed for one hour during day time for eating of non-feed items in order to know the incidence of pica. These were also examined for associated signs and symptoms of rough hair coat, anaemia and weakness.

Two bolus (35-40 gm) of fresh faecal samples were collected separately for each camel in duplicate in polyethylene bags both prior to start of the treatment and 60 days post treatment. These faecal samples were transported to the laboratory, in a cool transport thermocol box, for further examination.

Qualitative examination of all the faecal samples was carried out to record the gastrointestinal parasitic infestation in the experimental camels by using centrifugal floatation and sedimentation techniques (Soulsby, 1982). None of the camel was severely infected therefore quantitative examination to know the eggs per gram faeces was not carried out. This might be due to the regular deworming schedule adopted at the farm.

Estimation of silica and sand content in the faecal samples: Ash is the inorganic residue left after ignition of a faecal sample in the muffle furnace at 550-600°C for 2-3 hours. The residue left after dissolving inorganic portion of total ash represented acid insoluble ash (AIA), majority containing sand and silica (Sastry *et al*, 1999).

All these faecal samples were estimated for silica content. Two boluses (35-40 gm) of faecal sample collected were taken in the pre weighed petridishes. These faecal samples were dried by keeping the petridishes in the hot air oven at 70°C for 12 hours. Five gram of the dry faecal sample was taken in the pre-weighed silica basins. These basins were kept in the muffle furnace for ignition at 550-600°C for 2-3 hour to obtain total ash. Then in each silica basin about 10 ml of dilute (1:2) HCl was added. Then the contents of silica basins were transferred to 25 ml beakers. This content was boiled for 5-10 minutes on hot plates and then filtered using Whatman no.1 filter papers in volumetric flasks (250 ml). Contents of the beaker and residue on the filter paper were made acid free by repeated hot water washing of the beaker and then pouring the residue on the filter paper. Filter paper with retained residue was transferred to a pre-weighed silica basin. Contents of these basins were again dried in the hot air oven at 70±2°C for 12 hours and then subjected to ashing in the muffle furnace at 550-600°C for 1-2 hour for decarburisation. Finally these basins were cooled in desiccators and weighed with left back AIA. In this way silica and sand content of the faecal samples were estimated by the following equation: AIA (%) = b-a/ w× 100 (Where, b= weight of silica basin with AIA, a= empty weight of silica basin, w= weight of moisture free sample taken for ashing).

Collection of blood and serum samples: Blood samples from all the 12 experimental camels were collected on day 0 (pre treatment) and then on day 15, 30, 45 and 60 (post treatment) by jugular vein puncture in sterile vacuutainers with and without anticoagulant for estimation of haematological, serum biochemical and serum mineral profiles. Blood and serum samples of 5 apparently healthy male camels of the same age group of the same herd were also collected to know the normal haemato-biochemical and mineral profiles of the herd.

Haematological examination: Blood samples were analysed for haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) using standard methods (Jain, 1986).

Biochemical analysis of serum samples was made to ascertain liver function by estimating serum total protein, serum albumin, serum globulin, alkaline phosphatase (ALKP), serum aspartate aminotransferase (SGOT) and serum alanine aminotransferase (SGPT), by the Vet Test Chemistry Analyser using kit supplied by Chema Diagnostics, Monsano, Italy. Serum globulin concentration was estimated as a difference between total protein and albumin. Albumin and globulin ratio (A: G) was derived by dividing albumin concentration with globulin concentration in g/dl.

Estimation of serum minerals (Ca, P, Zn, Cu Co, Fe): Serum samples were digested as per the procedure described by Kolmer *et al* (1951). Serum calcium, phosphorus, zinc, copper, iron, and cobalt were estimated in these digested samples by Inductively Coupled Argon Plasma Spectrometer (iCAP 7000Series) by Thermo Fisher Scientific Inc.

Statistical analysis: Mean, standard error, percentages and comparison of differences between means were calculated as per the standard statistical procedures suggested by Snedecor and Cochran (1994).

Results

A very high incidence (51.66%) of the pica was recorded in the herd studied. Vices of licking the

manger and corral walls along with eating floor soil started in camel calves of less than one year of age. Pica was more pronounced in active grower camels between 1-5 years of age (60.81%). These camels were picking and chewing long bones for longer times and were even engulfing the pieces of construction bricks. Coprophagy was also more pronounced in these age group camels (Table 1). Male camels were more affected (54.86%) as compared to female camels (49.73%) in the herd examined (Table 2).

Table 1. Incidence of pica as per age group of the camels.

Age of the camel	Total No. of the camels	Pica affected camels	Percentage of the affected camels
< 1year (young)	33	18	54.54
1-5 years (Active growers)	148	90	60.81
>5 years (Adults)	119	47	39.50
Total	300	155	51.66

Table 2. Incidence of pica as per sex of the camels.

Sex	Total No. of camels	Pica	Percentage (%)
Male	113	62	54.86
Female	187	93	49.73
Total	300	155	51.66

Table 3. Vices recorded in a total of 300 camels.

Abnormal materials	Total No. of camels	Percentage (%)
Chewing of bones 'osteophagia'	85	28.33
Sand/ soil/ mud eating 'geophagia'	47	15.67
Brick/ concrete wall eating 'lithiphagia'	28	9.33
Faeces eating 'coprophagia'	13	4.33
More than one object eating	18	6.00
Total pica affected camels	155	51.66

Common vices observed were osteophagia (28.33%) followed by geophagia (15.67%), lithophagia (9.33%) and coprophagia (4.33%), depending upon the availability of the particular objects in the vicinity of the animals. Six per cent of camels were observed to be eating more than one abnormal material (Table 3). These combinations observed were mainly of coprophagia and geophagia, osteophagia and geophagia, lithophagia and geophagia. Poor body condition as reflected by thinness of the hump, roughness of the hair coat, anaemia and emaciation were more common in pica affected compared to unaffected camels (Table 4).

Table 4. Clinical signs observed in pica affected and unaffected camels.

Symptoms	Pica affected camels (n=155)	Per cent	Pica unaffected camels (n=145)	Per cent
Weakness	89	57.42	12	8.27
Rough hair coat	25	16.12	6	4.14
Anaemia	22	14.19	0	0.00
Emaciation	9	5.81	2	1.38

Faecal samples examination for gastrointestinal parasites: Initial pretreatment faecal samples examination of the 12 experimental camels revealed minor parasitic infestations in two camels, one was positive for trichuris egg and second for strongyle egg. This reflected that pica was not due to parasitic infestation. Regular deworming schedule adopted for the farm was effective in taking care of gastrointestinal parasites. Examination of faecal samples 60 days post treatment also did not reflect any parasitic infestations in these camels.

Silica content in faecal samples: On 60 days post treatment, faecal dry matter content was observed to be higher whereas ash, acid insoluble ash and silica content of ash were lower in the treated group reflecting lower consumption of silica in the treated group compared to control group (Table 5).

Table 5. Mean+S.E of Silica content in faeces of treated and untreated control group of camels on 60 days post treatment.

Attribute	Treated group (n=6)	Untreated Control group (n=6)	P Value
Faecal DM (%)	29.11±1.19	25.32±1.01	0.067
Ash content (%)	18.47±0.52	22.65±2.36	0.174
Silica content (%)	8.51±0.60	13.43±2.97	0.200
Silica% of ash	45.72±2.04	55.22±5.63	0.210

Haematological parameters (Table 6): Significant increase in the mean TEC was recorded in the treated group compared to control group on day 30, 45 and 60 ($P \leq 0.05$), post treatment. Significant increase in mean value of TEC count was also observed on day 30, 45 and 60 post treatment compared to day 0 ($P \leq 0.05$), in the treated group. No significant difference in the mean Hb concentration was recorded between treated and control group on day-0. Mean Hb concentration in the treated group increased significantly compared to control group on day 15, 30, 45 and 60 ($P \leq 0.05$), post treatment. Significant increase in Hb was also observed on

Table 6. Mean±SE values of haematological parameters at different intervals of treatment.

Parameter	Treated group					Untreated control group				
	0 day	15 day	30 day	45 day	60 day	0 day	15 day	30 day	45 day	60 day
Erythrogram										
TEC (10^6/ mm^3)	8.06± 0.02^{a}	8.30± 0.27	8.40± 0.19^{Ab}	8.90± 0.20^{Ab}	9.20± 0.21^{Ab}	7.80±0.18	7.80± 0.20	7.70± 0.20^{B}	7.90± 0.21^{B}	8.08± 0.20^{B}
Hb (gm%)	7.30± 0.18^{a}	7.80± 0.15^{Ab}	8.00± 0.22^{Ab}	8.40± 0.22^{Ab}	8.70± 0.20^{Ab}	7.20±0.21	7.00± 0.14^{B}	7.20± 0.12^{B}	7.50± 0.17^{B}	7.50± 0.17^{B}
Range	7.0- 8.0	7.2- 8.2	7.2- 8.8	7.8 -9.4	8.0- 9.4	6.4- 7.8	6.4- 7.4	7.0- 7.8	6.8-8.0	6.8- 7.8
PCV (%)	34.16± 0.70	36.16± 0.60	36.50± 0.22	36.60± 0.42	37.50± 0.34	32.80± 0.60	34.00± 0.86	34.30± 0.61	33.80± 0.40	33.62± 0.33
TLC (10^3/mm^3)	14.38± 0.92	14.83± 0.78	13.38± 0.73^{A}	13.45± 0.64^{A}	13.95± 0.52^{A}	17.31± 2.43	18.45± 2.00	19.11± 0.98^{B}	18.95± 1.02^{B}	18.28± 0.54^{B}
DLC (%)										
Lymphocyte	53.33± 0.71	52.16± 0.65^{A}	49.0± 0.36^{A}	57.33± 1.05^{A}	55.16± 1.22^{A}	54.33± 0.61	54.33± 0.56^{B}	54.16± 0.60^{B}	63.0± 1.63^{B}	63.0± 1.26^{B}
Neutrophils	38.83± 0.60^{a}	38.8±1.05	42.66± 0.12A^{b}	40.16± 1.02	42.33± 1.05^{Ab}	37.00± 1.39	37.66± 0.33	37.50± 0.56^{B}	33.83± 1.49	33.00± 1.41^{B}
Eosinophils	5.3± 0.33^{a}	4.5± 0.50^{b}	3.8± 0.31^{b}	0.66± 0.33^{b}	0.33± 0.21^{b}	4.66± 0.33	4.0± 0.26	4.66± 0.21	2.16± 0.70	2.66± 0.49
Basophils	0.5± 0.22	0.6± 0.21	0.5± 0.22	0.16± 0.17	0.16± 0.17	1.16± 0.17	1.0± 0.00	0.83± 0.17	0.33± 0.21	0.5± 0.22
Monocytes	4.6± 0.49^{a}	3.6± 0.33	4.0± 0.26^{A}	1.6± 0.33^{Ab}	1.8± 0.60^{b}	3.6± 0.33	3.0± 0.26	2.8± 0.17^{B}	0.6± 0.21^{B}	0.8± 0.31

Note: - 1. Mean±SE bearing different superscript (A, B) between treated and control group differed significantly ($p<0.05$).

2. Mean±SE bearing different superscript (a, b) within treated group differed significantly ($p<0.05$).

day 15, 30, 45 and 60 post treatment compared to day-0 (P≤0.05), in the treated group. No significant difference in the mean PCV was recorded between treated and control group on day-0. Whereas in the treated group PCV increased significantly compared to control group on day 15, 30, 45 and 60 (P≤0.05), post treatment. Significant increase in PCV was also observed on day 30, 45 and 60 post treatment compared to day-0 (P≤0.05).

In the treated group, no significant difference in the TLC was recorded between treated and control groups on day-0. Whereas in the treated group TLC increased significantly compared to control group on day 30, 45 and 60 (P≤0.05), post treatment. No significant difference in TLC was observed post treatment compared to day-0 (P≤0.05), in the treated group. No significant difference in the mean lymphocyte count was recorded on day-0 of the start of treatment between treated and control groups. Whereas in the treated group there was significant difference in the mean lymphocyte count compared to control group on day 15, 30, 45 and 60 (P≤0.05), post treatment. No significant change in mean lymphocyte count was observed on day 15, 30, 45 and 60 post treatment compared to day-0 (P≤0.05), in the treated group. Significant decrease in lymphocyte count was observed on day 60 post treatment (P≤0.05) in the treated group compared to control group. No significant difference in the mean neutrophils count was recorded on day 0, 15 and 45 post treatment between treated and control groups. Whereas significant increase in neutrophils count was recorded on day 30 and 60 post treatment in the treated group compared to control group. Significant increase in neutrophils was also observed on day 30 and 60 in the treated group compared to day-0 of the treated group (P≤0.05). No significant difference in the mean eosinophils count was recorded on day 0, 15, 30, 45 and 60 post treatment between treated and control groups. Significant difference in mean eosinophils count was recorded on day 15, 30, 45 (P≤0.05) and 60 (P≤0.01) in the treated group compared to day-0 of the treated group. No significant difference in mean basophils count was observed between treated and control groups either pre or post treatment. No significant difference in the mean monocytes count was recorded on day 0, 15 and 60 post treatment between treated and control groups. Whereas significant difference in monocytes count was observed on day 30 and 45 post treatment

Table 7. Mean±SE value of liver enzymes, protein profile camels at different intervals of treatment.

Parameter	Treated group					Control group				
	0 day	15 day	30 day	45 day	60 day	0 day	15 day	30 day	45 day	60 day
Protein profile										
T.P. (gm/dl)	6.59± 0.34^{Aa}	8.02± 0.66	7.76 ±0.56^{A}	7.50± 0.56^{Ab}	7.99± 0.56^{Ab}	7.22± 0.16^{B}	6.61± 0.43	6.26± 0.15^{B}	5.54± 0.20^{B}	6.32± 0.30^{B}
Albumin (gm/ dl)	3.19± 0.16	2.93± 0.16A	2.94± 0.12	3.29± 0.09	3.08± 0.12	2.75± 0.29	3.46± 0.12B	3.22± 0.28	3.12±0.17	3.30± 0.14
Globulin (gm/ dl)	3.40± 0.47^{a}	5.08± 0.59^{Ab}	4.81± 0.63^{A}	4.21± 0.52^{A}	4.91± 0.61^{Ab}	4.46± 0.34	3.15± 0.49^{B}	3.04± 0.37^{B}	2.43± 0.26^{B}	3.02± 0.32^{B}
A/G (ratio)	0.93^{a}	0.57^{b}	0.61	0.78	0.62^{b}	0.61	1.09	1.05	1.28	1.09
Liver enzymes										
SGOT/AST (IU/L)	65.43± 4.28^{a}	44.40± 11.50	48.54± 6.39	50.08± 2.95^{b}	40.59± 4.40^{b}	75.64± 7.11	50.57± 13.91	55.30± 4.34	48.69± 7.36	41.40± 2.44
SGPT/ALT (IU/L)	37.89± 13.49	31.04± 14.27	41.65± 17.12	14.99± 1.15	15.60± 4.50	25.34± 2.30	18.42± 3.73	24.88± 4.26	11.29± 1.64	17.95± 4.35
ALKP (IU/L)	65.24± 10.13^{A}	78.71± 22.39^{A}	50.93± 4.03	46.51± 8.18^{A}	81.75± 14.13^{A}	133.75± 22.00^{B}	170.06± 27.99^{B}	74.36± 12.86	71.38± 4.59^{B}	37.43± 7.00^{B}

Note: - 1. Mean±SE bearing different superscript (A, B) between treated and control group differ significantly ($p<0.05$).

2. Mean±SE bearing different superscript (a, b) within treated group differ significantly ($p<0.05$).

Table 8. Mean±SE value of serum minerals in camels at different intervals of treatment

Mineral	Treated group					Control group				
	0 day	15 day	30 day	45 day	60 day	0 day	15 day	30 day	45 day	60 day
Ca (mg/dl)	6.92± 0.29^{a}	7.61± 0.41	8.45± 0.17^{Ab}	9.17± 0.27^{Ab}	10.42± 0.31^{Ab}	6.63± 0.25	7.65± 0.37	7.59± 0.29^{B}	7.66± 0.32^{B}	7.68± 0.41^{B}
P (mg/dl)	4.89± 0.20^{a}	5.25± 0.16^{b}	5.47± 0.19^{A}	5.75± 0.21^{b}	5.96± 0.18^{Ab}	4.45± 0.39	4.45± 0.33	4.27± 0.37^{B}	5.08± 0.22	4.56± 0.25^{B}
Zn (µg/dl)	37.0± 6.0^{a}	41.0± 2.0	52.0± 5.0^{b}	51.0± 4.0	58.0± 3.0^{b}	52.0± 4.0	49.0± 5.0	44.0± 2.0	52.0± 5.0	54.0± 3.0
Cu (µg/dl)	47.0± 0.4^{a}	51.0± 2.0^{b}	55.0± 1.0A^{b}	51.0± 2.2^{A}	59.0± 1.2^{Ab}	45.0± 1.5	47.0± 4.9	48.0± 2.6^{B}	45.0± 1.2^{B}	47.0± 4.0^{B}
Co (µg/dl)	0.25± 0.056^{a}	0.42± 0.19	0.48± 0.15	0.48± 0.09	0.50± 0.06^{Ab}	0.17± 0.033	0.38± 0.079	0.41± 0.079	0.36± 0.056	0.31± 0.031^{B}
Fe (µg/dl)	34.0± 4.0^{a}	53.0± 3.0^{Ab}	56.0± 3.0^{b}	60.0± 3.0^{b}	75.0± 6.0^{Ab}	32.0± 5.0	42.0± 2.0^{B}	53.0± 4.0	55.0± 5.0	50.0± 3.0^{B}
ALKP (IU/L)	65.24± 10.13^{A}	78.71± 22.39^{A}	50.93± 4.03	46.51± 8.18^{A}	81.75± 14.13^{A}	133.75± 22.00^{B}	170.06± 27.99^{B}	74.36± 12.86	71.38± 4.59^{B}	37.43± 7.00^{B}

Note: - 1. Mean±SE bearing different superscript (A, B) between treated and control group differ significantly ($p<0.05$).

2. Mean±SE bearing different superscript (a, b) within treated group differ significantly ($p<0.05$).

in the treated group compared to control group (P≤0.05).

Biochemical parameters of the camels (Table 7)**:** Significant difference was observed on day 0, 30, 45 and 60 post treatment compared to control group. Within the treated group difference was significant (P≤0.05) on day 45 and 60 compared to day-0 of the treated group. Significant difference in albumin concentration was recorded on day 15 post treatment between treated and control group. No significant difference in albumin concentrations was recorded at any other stage of the treatment. No significant difference in the globulin concentration was recorded between treated and control groups on day-0. Whereas there was significant difference (P≤0.05) on day 15, 30, 45 and 60 between treated and control groups. Within the treated group there was significant difference (P≤0.05) on day 15 and 60 post treatment compared to day-0. No significant difference in the mean AST concentration was observed between treated and control groups. This difference became significant (P≤0.05) in the treated

group on day 45 and 60 as compared to day-0, of the treated group. No significant difference in ALT concentration was recorded in both pre-treatment vs. post treatment and treated vs. control groups. There was significant difference (P≤0.05) in alkaline phosphates concentration on day 0, 15, 45 and 60 between treated and control groups. But no significant difference in treated group was recorded compared to day-0.

Estimation of serum minerals (Ca, P, Zn, Cu, Co, F) (Table 8): No significant difference in the mean serum Ca concentration was recorded on day 0 and 15 of treatment between treated and control groups. Whereas significant (P≤0.05) increase was recorded in the treated group on day 30, 45 and 60, post treatment compared to control group. Within the treated group significant (P≤0.05) increase was recorded on day 30, 45 and 60 post treatment compared to day-0. Significant (P≤0.05) increase in the mean P concentration was recorded on day 30 and 60 in the treated group compared to control group. Within the treated group significant (P≤0.05) increase was recorded on day 15, 45 and 60 post treatment compared to day-0. No significant difference in Zn concentrations was recorded in the treated group compared to control group. Within the treated group significant (P≤0.05) increase was recorded on day 30 and 60 post treatment compared to day-0. No significant difference in Cu concentrations was recorded in the treated group on day 0 and 15 compared to control group. Whereas this difference became significant (P≤0.05), on day 30, 45 and 60 post treatment compared to control group. Within the treated group significant (P≤0.05); difference was recorded on day 15, 30 and 60 post treatment compared to day-0. Significant (P≤0.05) increase in the mean Co concentration was recorded on day 60 in the treated group compared to control group. Within the treated group significant (P≤0.05) increase was recorded on day 60, post treatment compared to day-0. No significant difference in Fe concentration was recorded on day 0, 30 and 45 of the treated group compared to control group. This difference was significant on day 15 and 60 post treatment, compared to control group. Within the treated group the difference was significant (P≤0.05) on day 15, 30, 45 and 60 post treatment compared to day-0 of the treated group.

A:G ratio became low in both the treatment groups (<0.8), either because of only increase in globulin concentrations or corresponding decrease in albumin concentrations which showed rectification of compromised immunity in the treated camels. In the control groups A:G ratios were comparatively higher (>1.0) and in the healthy camels the ratio was approx. 0.8 (Table 9).

Table 9. Mean±SE of haemato-biochemical-mineral profiles of healthy camels of the herd.

Erythrogram	Healthy male camels (n=5)	
	Mean	Range
RBCs (10^6/ mm^3)	9.44±0.28	9.1-10.1
Hb (gm per cent)	9.08±0.27	8.3-9.9
PCV (per cent)	36.4±0.51	35-38
TLC ($10^3/mm^3$)	15.5±0.94	13.5-18.3
Lymphocyte	42.4±1.21	39-45
Neutrophils	51.8±1.02	49-55
Eosinophils	2.6±0.24	2-4
Basophils	0.4±0.24	0-1
Monocytes	2.8±0.37	2-3
Protein profile		
T.P. (gm/dl)	7.17±0.22	6.5-7.62
Albumin (gm/dl)	3.18±0.17	2.79-3.82
Globulin (gm/dl)	3.99±0.34	2.68-4.63
A/G (ratio)	0.79	
Liver enzymes		
SGOT/AST (IU/L)	33.58±4.23	21.73-47.73
SGPT/ALT (IU/L)	17.99±1.91	12.05-21.73
ALKP (IU/L)	43.41±5.39	31.7-57.48
Ca (mg/dl)	10.65±0.54	9.22-11.2
P (mg/dl)	6.09±0.26	5.34-6.8
Zn (µg/dl)	68.90±15.17	40.1-119.02
Cu (µg/dl)	57.12±5.62	45.77-77.0
Co (µg/dl)	0.39±0.03	0.3-0.051
Fe (µg/dl)	80.26±7.28	53.0-91.0

Efficacy of mineral mixture feeding was seen in eliminating signs of pica in camels after 60 days of treatment as these animals stopped eating non food items whereas untreated control group animals were eating non food items.

Discussion

Surprisingly very high incidence (51.66%) of the pica was recorded in the camel herd studied. Comparatively low prevalence (9.48%) has been reported in camels by Sharma (2000) in the Bikaner region. Tuteja *et al* (2018) recorded increasing incidence of pica in camels over the years, based on the surveys' carried out from 2007 to 2015, in the Rajasthan state. Kachhawaha *et al* (2013) reported 41.8 per cent camel herders of southern Rajasthan

has problem of pica in their herds. Kachhawa *et al* (2019) conducted retrospective study of diseases of camel at teaching veterinary clinical complex of RAJUVAS, Bikaner from January 2013 to December 2017. Amongst digestive disorders pica was (13.6%) a primary disorder followed by simple indigestion (12%). The high prevalence of pica and simple indigestion might have been due to poor availability of quality feed particularly deficiency of minerals like calcium and phosphorus in the region due to drought conditions.

Common vices recorded in camels were osteophagia, geophagia, lithophagia and coprophagia. Studies by previous authors report geophagia (Vernacular 'mitti khana') (Gautam and Bansal, 1972; Sharma and Satija, 1974; Shamat, 2008) and osteophagia (Dioli and Stimmelmayr, 1992; Shamat, 2008) in camels. Oesophageal obstruction in camels has been reported due to non feed items (Ramadan and Abdin-Bey, 1990).

Poor body condition as recorded by general weakness reflected by thinness of the hump, roughness of the hair coat and anaemia (pale mucous membranes) was common in pica affected camels compared to unaffected camels. Symptoms of weakness, roughness of the hair coat and emaciation in pica affected camels has already been reported (Gautam and Bansal, 1972; Kachhawaha *et al*, 2013). Shen and Li (2010) reported 'emaciation ailment' in Bactrian camels as clinical sign of pica.

Faecal dry matter was found higher in the treated camels compared to controls. Lower DM content of controls could be defense mechanism to eliminate higher contents of silica in the faeces. Consumption of some amount of soils is obvious in all the camels, being raised in the sandy arid area. Because these camels browse on desert plants with sticking sand on the plant leaves and some of the sand do remains in harvested fodders of the desert.

Significant increase in the mean TEC was recorded in the treated camels compared to untreated controls on 60 days post treatment. Decrease in TEC has been reported in pica affected camels Singh *et al,* 1986; Singh, 1993; Beniwal and Singh, 2007). Decrease of Hb concentrations in present findings in the pica affected camels was in accordance with Singh *et al* (1986) and Beniwal and Singh (2007). In pica affected camels PCV values were less than 35% whereas in healthy camels PCV values were more than 36%. In treated camels PCV values were corrected to normal (37%). Decrease in PCV in pica affected camels has also been reported (Singh *et al*, 1986; Singh, 1993; Beniwal and Singh, 2007).

In the treated group TLC count decreased significantly post treatment compared to control group. Whereas no significant difference in TLC was observed post treatment compared to day 0, in the treated group. Singh (1993) reported that in the pica affected camels TLC remained unaffected. Comparative low levels of TLC in healthy camels have been recorded (Dongre, 2000; Sharma, 2000; Mali, 2002).

In the treated group significant decrease in lymphocyte count was observed on day 60 post treatment ($P \leq 0.05$) compared to control group. Contrary to the present findings, Singh (1993) reported that in the pica affected camels lymphocytes remained unaffected. Comparative low levels of lymphocytes (<40%) in healthy camels have been recorded (Soni and Aggarwal, 1958; Banerjee *et al*, 1962; Musa and Mukhtar, 1982; Gorakhmal *et al*, 2001; Mali, 2002). In present study lymphocyte count of more than 40% was seen in healthy camels (Nassar *et al*, 1977; Rezakhani *et al*, 1997; Dongre, 2000).

Post treatment significant increase in neutrophils count was recorded in the treated group compared to control group and compared to day-0 of the treated group. Neutrophils counts of more than 50% in healthy camels have been reported (Singh *et al*, 2000; Gorakhmal *et al*, 2001; Mali, 2002). In the present study mean neutrophils count increased post treatment. Contrary to the present findings, Singh (1993) reported that in the pica affected camels neutrophils count remained unaffected.

Significant difference in mean eosinophils count was recorded on day 15, 30, 45 ($P \leq 0.05$) and 60 ($P \leq 0.01$) in the treated group as compared to day 0 of the treated group. Variations in the eosinophils counts from 1.2 to 4.53 per cent have been reported (Musa and Mukhtar, 1982; Rezakhani *et al*, 1997; Dongre, 2000; Gorakhmal *et al*, 2001; Mali, 2002) in healthy camels.

No significant difference in mean basophils count was recorded both between treated vs. control groups and treated vs. treated. Basophils count of less than one per cent in healthy camels has been reported (Nassar *et al*, 1977; Musa and Mukhtar, 1982; Singh, 1993; Rezakhani *et al*, 1997; Dongre, 2000; Gorakhmal *et al*, 2001; Mali, 2002).

Significant difference in monocytes count was observed on day 30 and 45 post treatment in the treated group compared to control group ($P \leq 0.05$). In

the treated group significant difference in monocytes count was observed on day 45 and 60 post treatment in the treated group as compared to day zero value (P≤0.05). Contrary to the present findings, Singh (1993) reported that in the pica affected camels monocytes count remained unaffected.

In the present study, mean serum total protein increased up to 7.91 gm/dl on day 60 post treatment in the treated group. Comparable protein levels in healthy Indian camels (7.17±0.22) have been reported (Bansal *et al*, 1970; Rathod, 2006). Significant decrease in total protein was recorded on day 60 post treatment compared to day-0 in the controls. Serum TP levels decrease in pica affected camels (Singh *et al*, 1986; Singh, 1993). There occurred significant increase in globulin concentrations on day 15, 30, 45 and 60 in the treated group compared to control group. Post treatment difference in TP was mainly due to increased globulin, whereas albumin concentrations remained almost unaffected.

No significant difference in the mean AST concentration was observed between treated and control groups. This difference became significant (P≤0.05) in the treated group on day 45 and 60 as compared to day-0, of the treated group. No significant difference in ALT concentration was recorded in both pre-treatment vs. post treatment and treated vs. control groups. There was significant difference (P≤0.05) in alkaline phosphates concentration on day 0, 15, 45 and 60 between treated and control groups. But no significance difference in treated group compared to day 0. The pepsinogen is a protein secreted by the stomach as precursor of the pepsin, one of the main enzyme of the digestion. An elevation in blood pepsinogen in veterinary clinics is suggestive of gastrointestinal parasitism (Faye and Bengoumi, 2018). Significant increased levels of plasma gastrin and pepsinogen in pica affected camels having sand in their third compartment of the stomach have also been reported (Kataria and Kataria, 2006).

Significant increase in Ca was recorded in the treated group on day 30, 45 and 60 post treatment compared to control and day-0 of the treated group. Serum Ca levels increased significantly and reached up to 10.42±0.31 mg/dl. Mean serum levels of more than 10 mg/dl in the healthy camels have been reported by Bhatt and Kohli (1961). In the present study, there was significant decrease in the serum Ca level in the pica affected camels. Significant decrease in serum Ca level in the pica affected camels have also been reported by Gautam and Bansal (1972) and Beniwal and Singh (2007). Mehrotra and Gupta (1989) reported seasonal variation in Ca concentration from 7.1-18.3 mg/dl in serum of Indian camels.

Serum phosphorus level increased significantly after supplementation to the level of 5.96±0.18 mg/dl. In the present study, there was significant low serum P level in the pica affected camels. Significant decrease in serum P level in the pica affected camels has also been reported (Gautam and Bansal, 1972; Beniwal and Singh, 2007). P deficiency in Australian camel was commonly manifested by bone chewing (Manefield and Tinson, 1996). Blood and Radostits (1997) mentioned that P deficiency as primary etiological factor in depraved appetite in animals.

Within the treated group significant increase in Zn concentration was recorded on day 30 and 60 post treatment compared to day-0. Comparatively high Zn levels (>80µg/dl) in healthy camels have been reported (Shekhawat, 1983; Ghosal and Shekhawat, 1992; Dongre, 2000; Dixit *et al*, 2008). Serum Zn levels become low in pica affected camels (Singh *et al* 1986; Beniwal and Singh, 2007). Manefield and Tinson (1996) mentioned that 18-20 mg of ZnSo4 per day is sufficient for good health in camels.

Cu concentration increased significantly post treatment compared to controls and day-0 of the treated group. Similar mean Cu values (60µg/dl) in camels have been reported by Faye *et al* (2005). Comparative high Cu levels (>90 µg/dl) in healthy camels have been reported (Shekhawat, 1983; Ghosal and Shekhawat, 1992; Dongre, 2000; Dixit *et al*, 2008). Decreases in serum Cu levels in pica affected camels have been reported (Singh *et al*, 1986; Beniwal and Singh, 2007). In camels mineral supplementation increased blood Cu level (Faye and Bengoumi, 1997). Mohamed (2004) observed an increase in Cu concentration with age and Cu concentrations were higher in rainy season than dry season.

Significant (P≤0.05) increase in the mean Co concentration was recorded on day 60 in the treated group compared to control group and day-0 of the treated group. In comparison to the present findings higher mean Co values (14.87±1.38 µg/dl) has been reported by Dixit *et al* (2008). Faye *et al* (2005) recorded much lower Co values (0.08µg/dl) in camels. Singh *et al* (1986) reported decrease in serum Co concentrations in pica affected camels.

No significant difference in Fe concentration was recorded on day 0, 30, and 45 of the treated group compared to control group. This difference was significant on day 15 and 60 post treatment,

compared to control group. Within the treated group the difference was significant (P≤0.05) on day 15, 30, 45 and 60 post treatment compared to day-0 of the treated group. Higher serum Fe values (>100µg/dl) in healthy camels have been reported (Dongre, 2000; Saeed *et al*, 2004; Faye *et al*, 2005; Dixit *et al*, 2008). Singh *et al* (1986) reported decrease in serum Fe concentrations in pica affected camels. Ghosal and Mathur (1992) reported five per cent of the animals having serum Fe values less than 40 µg/dl indicated subclinical deficiency in Bikaner area of Rajasthan. Haris *et al* (1995) reported large proportion of Cu circulating in plasma is combined with serum glycoprotein, ceruloplasmin, which has ferroxidase action and is required to deliver Fe to circulation, so low Fe level might result from Cu deficiency. Vegad (1995) mentioned deficiency of Cu lead to Fe deficiency anaemia which is microcytic and hypochromic.

A:G ratio becoming low in treatment group (<0.8), either because of only increase in globulin concentrations or corresponding decrease in albumin concentrations shows rectification of compromised immunity in the treated groups. In the control groups A: G ratios were comparatively higher (>1.0) and in the healthy camels the ratio was approximately 0.8.

Haematobiochemical changes in pica affected camels like eosinophilia and monocytosis along with decreased serum globulin concentrations are suggestive of bowel inflammation in pica affected camels, may be due to eating of non food items leading to inflammation which in turn may lead to disturbed absorption of other nutrients. These haematobiochemical values became normal after treatment, which is suggestive of regression of bowel inflammation, might be due to stoppage in feeding non food items. Regression of bowel inflammation and non interference of non feed items in the bowel resulted in normal absorption of various nutrients.

Mineral mixtures supplementation to pica affected camels has also been recommended by Gautam and Bansal (1972). Pica in animals is of multi-etiological origin, deficiency of essential macro or micro mineral in animals may result in low production and poor health with symptoms of pica that can be corrected by suitable supplementation of specific mineral. The concept and advantages of using area specific mineral mixture have been extensively discussed throughout the world (Prasad and Gowda, 2005; Devasena *et al*, 2010; Singh *et al*, 2016; Sahoo *et al*, 2017 and Pandey *et al*, 2018). These studies suggested that supplementation of the most deficient minerals as area specific mineral mixture improved production and reproduction of animals. Thiophanate possess a broad spectrum anthelmintic activity against Strongyle type parasites commonly associated with pica in camels (Bali *et al*, 1978).

Conclusion

A very high incidence of pica in dromedary camels of the present study along with mineral imbalance indicated importance of feeding balanced ration along with providing area specific mineral mixtures.

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IMMUNOHISTOCHEMICAL LOCALISATION OF MUCIN 1 IN MALE REPRODUCTIVE ORGANS OF DROMEDARY CAMELS DURING RUTTING AND NON-RUTTING SEASONS

Abdulhakeem Eljarah[1,2,*], Abdulrahman Alhaider[1], Marzook Al-Eknah[1] and Sabry M. El-Bahr[3,4]

[1]Department of Clinical Sciences, [3]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, P.O. Box 400, Al-Ahasa 31982, Saudi Arabia

[2]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, 22110, Jordan

[4]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

ABSTRACT

This study was aimed to investigate the immune reactivity levels of anti-MUC1 (Mucin 1) antibody in male camel accessory sex glands (prostate and bulbourethral) in addition to the testes, epididymis and ductus deferens. This is potentially useful for an insight on the role of mucous secretion during rutting and non-rutting seasons in male camel. Therefore, it is important to check the immune reactivity levels of anti-MUC1 antibody as a marker for mucous secretion activity. To achieve that, testes (proximal part, distal part and rete testis), epididymis (head, body and tail), ductus deferens (initial, middle and ampullary part, prostate (compact and disseminated part) and bulbourethral gland were collected from 12 male camel during rutting and non-rutting seasons and were subjected to immunohistochemistry. Results showed that the higher immune reactivity levels of anti-MUC1 antibody during rutting season was in rete testis, proximal and distal part of the testes, head of epididymis, ampullary part of ductus deferens, prostate (compact and disseminated part) and bulbourethral gland. Whereas, the higher immune reactivity levels of anti-MUC1 antibody during non-rutting season was in rete testis, proximal and distal part of the testes, head of epididymis, ampullary part of ductus deferens and compact part of the prostate. Thus, the distribution of mucous secretion varied between rutting and non-rutting seasons and even among the reproductive organs itself. However, highest secretory activity was found during the rutting season in the ampulla of ductus deferens, disseminated part of the prostate and bulbourethral gland.

Key words: Accessory sex glands, *Camelus dromedarius*, epididymis, MUC1, testes

The viscosity of dromedary camels' semen complicates semen assessment when trying to separate spermatozoa from seminal plasma using routine methods for spermatozoal counting (Merkt *et al*, 1990; Marai *et al*, 2009; El-Kon *et al*, 2011), as well as motility. The viscosity causes oscillatory movement of the spermatozoa (Tingari *et al*, 1984; Elwishy, 1988; Merkt *et al*, 1990; Musa *et al*, 1990; El-Kon *et al*, 2011) rather than progressive motility like other domestic animals. The existence of mucopolysaccharides in the ejaculate, according to (Mann, 1964), may explain the high viscosity of camel semen. Mucins are glycoproteins with a high molecular weight that can be found on the apical surface of glandular epithelia including the gastrointestinal, respiratory, and reproductive tracts (Lichtenwalner *et al*, 1996). Mucin 1 (MUC1) is a Type I membrane glycoprotein that is expressed on the apical cell surface of many secretory epithelial cells. Its functions include preventing adhesion, lubricating and hydrating the epithelium, and protecting it from microbial attack (Walter Bravo *et al*, 1997; Zeidan *et al*, 2001). The high viscosity of male camel spermatozoa in the female reproductive tract, on the other hand, is important for their viability (Sumar and Garica, 1986). We hypothesised that the secretory activity and amount of mucous formed by the reproductive organs of the dromedary male camel have a distinct seasonal profile. As a result, the current research investigated the localisation of MUC1 in dromedary camels' testes, epididymis, ductus deferens, prostate, and bulbourethral glands. This could help with reproductive management by

SEND REPRINT REQUEST TO ABDULHAKEEM ELJARAH, SABRY M. EL-BAHR email: selbahar@kfu.edu.sa; aeljarah@kfu.edu.sa; sabryelbahr@hotmail.com

reducing and/or avoiding mucous secretory activity during times of the year when semen can be assessed and processed efficiently for artificial insemination in this species.

Materials and Methods

Pre-experimental ethics and experimental Sampling:

All sampling and experimental management were approved by King Faisal University Animal Care and Use Committee (KFU-ACUC). Samples collected during rutting and non-rutting season from adult dromedary camel bulls (*Camelus dromedarius*) form a local slaughterhouse at Al-Ahsa- Saudi Arabia (25° 21′ 52.45″ N and 49° 33′ 55.15″ E) with an age ranges from 4-16 years. The reproductive tissues samples were obtained from testis (proximal and distal parts and rete testis), epididymis (head, body, and tail), ductus deferens (initial, middle and ampullary parts), prostate gland (compact and disseminated parts) and randomly from the bulbourethral gland.

Immunohistochemistry paraffin protocol (IHC-P) for MUC1 detection

Approximately one centimetre of testes (proximal part, distal part and rete testis), epididymis (head, body and tail), ductus deferens (initial, middle and ampullary part, prostate (compact and disseminated part) and bulbourethral gland tissue was taken from each tissue and then fixed overnight in 4% paraformaldehyde. All the fixed samples were processed for immunohistochemical examination by dehydration, clearing, infiltration, and embedding. Tissue samples were sectioned at 5 µm using a microtome (Leica, Germany) and were deparaffinised by passing the Thermo Scientific TM Super Frosted TM Plus charged slides through xylene. Two changes were made for 5 min each followed by the hydration of the sections by dipping them for 30 s in degraded alcohol (100%, 100%, 95%, 80%, 70%). The slides were then washed for 2 x 5 min in tris-buffered saline (1X TBS) plus 0.025% triton X100 with gentle agitation. The slides were then blocked in 10% normal serum with 1% bovine serum albumin (BSA) in 1X TBS for 2 h at room temperature. The slides were left to drain for a few seconds and the sections were then wiped with tissue paper. Rabbit polyclonal Anti-MUC1 antibody (Abcam, ab15481) and a mouse- and rabbit-specific HRP/DAB (ABC) detection IHC kit (Abcam, ab64264) were used to detect MUC1. Slides were counterstained by immersing the tissue sections in Mayer's hematoxylin for 5 min and washing them under tap water for 10 min. Then sections were dehydrated by dipping them in graded alcohol (70%, 95%, 100%, 100%), just for seconds, and clearing them by using 2 changes of xylene for 5 min each. Finally, the tissue sections were mounted by using DPX with cover slides (22 x 40 mm) and the staining was observed by Leica ICC50 W light microscopy under 10x and 40x magnification powers. Wi-Fi-capable digital camera detector and Leica Air Lab App software were used. Reaction reactivity was taken using image processing and analysed using an imageJ 1.52a analyzer (Wayne Rasband, National Institute of Health, USA, http://imagej.nih.gov/ij).

Results

Testes

In rutting season, the immune reactivity levels of anti-MUC1 antibody (Table 1) was intense in the proximal part (Fig 1), rete testis (Fig 2) and distal part (Fig 3). The immunohistochemical detection of MUC1 localised in seminiferous tubules with normal spermatogenesis in the proximal part (Fig 1) and distal part (Fig 3) of testes. While in non-rutting male camel testes, the immune reactivity of anti-MUC1 antibody was intense in rete testis and moderate in both proximal and distal parts of the testes as shown in Table 1. The immunohistochemical detection of MUC1 localised in the interstitial tissue of testes surrounding seminiferous tubules in the proximal part (Fig 1) and distal part (Fig 3) of testes. Whereas in rete testis, the immunohistochemical detection of MUC1 localised in interconnecting tubules in both rutting and non-rutting season as shown in Fig 2.

Epididymis

In rutting season, the immune reactivity levels of anti-MUC1 antibody was intense in the head and weak in both body and tail. The signal was strong in the basal cell (stem cell) layer and sterocelia of the columnar cell of the head of the epididymis (Fig 4) and weak positivity of the columnar cell in both body (Fig 5) and tail (Fig 6). While in non-rutting male camel epididymis, the immune reactivity of anti-MUC1 antibody was moderate in the head and very weak in both body and tail as shown in Table 1. The signal shows a moderate positivity in both basal cell and columnar cell (Fig 4) and very weak signal in columnar cell in both body (Fig 5) and tail (Fig 6).

Ductus deferens

In rutting and non-rutting season, the intense immune reactivity of anti-MUC1 antibody was only

Table 1. The semi-quantitatively immunohistochemical reactivity to Anti-MUC1 antibody in testis, epididymis, ductus deferens, prostate and bulbourethral gland.

Immunoreactivity of Rabbit Polyclonal Anti-MUC1 Antibody											
Tissue sample	Testis			Epididymis			Ductus Deferens			Prostate gland		Bulbourethral gland
	TP	TR	TD	EH	EB	ET	DI	DM	DA	PC	PD	Random part
Rutting season	+ + + +	+ + + +	+ + + +	+ + + +	+ +	+ +	+	+	+ + + +	+ + +	+ + + +	+ + + +
Non-Rutting season	+ + +	+ + + +	+ + +	+ + +	+	+	+	+	+ + +	+ +	+	+

(TP): Testis Proximal Part, (TR): Rete testis, (TD): Testis Distal Part, (EH): Epididymis Head, (EB): Epididymis Body, (ET): Epididymis Tail, (DI): Ductus Deferens initial part, (DM): Ductus Deferens Middle part, (DA): Ductus Deferens Ampullary part, (PC): Prostate Compact Part and (PD): Prostate Disseminated Part. (+) = very weak reactivity. (+ +) = weak reactivity, (+ + +) = moderate reactivity and (+ + + +) = intense reactivity.

in ampullary part of ductus deferens when compared to initial and middle parts, in which they had a very weak reactivity to anti-MUC1 antibody as shown in Table 1. The signal showed a very weak positivity in epithelium and basal cell of the initial (Fig 7) and middle parts (Fig 8). The signal showed strong positivity in epithelial columnar and basal cell in the ampullary part (Fig 9).

Prostate and bulbourethral glands

In rutting season, the highest immune reactivity of anti-MUC1 antibody was in the disseminated part of prostate and bulbourethral gland compared to moderate reaction in the compact part of prostate gland as shown in Table 1. The signal showed a moderate positivity in the acinar cells of the compact part (Fig 10) and intense signal in the acinar cells and secretory alveoli of the disseminated part of prostate (Fig 11) and bulbourethral gland (Fig 12). On the other hand, the immune reactivity of anti-MUC1 antibody in non-rutting season was very weak in disseminated part of prostate and bulbourethral gland compared to moderate reaction in the compact part of prostate gland as shown in Table 1. The signal showed a moderate positivity in the acinar cells of the compact part (Fig 10) and very weak in the acinar cell and secretory alveoli of disseminated part of prostate (Fig 11) and basal cell of the bulbourethral gland (Fig 12).

Discussion

Camelid ejaculate is highly viscous, grey to milky white in colour, with low volume and concentration of spermatozoa (Marai *et al*, 2009). Compared to stallion ejaculate; the gel fraction cannot be separated from the sperm-rich fraction making sperm cells motility assessment very difficult and highly variable (Skidmore, 2005). Moreover, the seminal plasma is considered the best media for the liveability of sperms, so great variation in seminal plasma constituents such as mucous concentration may play a major role in spermatozoal motility and liveability (Kershaw-Young and Maxwell, 2012). Mucins are categorised into membrane associated and secreted mucins and contain 17 genes: MUC 1-4, 5AC, 5B, 6-13, 15-17, 19 and 20. The large gel-forming mucins and small soluble mucins are two types of secreted mucins (Russo *et al*, 2006). Secretory mucins are released into the environment, where they can form extremely large and viscous gels, which are then cleared by net fluid flow through the lumina of various mucosa (Forstner, 1995; Hattrup and Gendler, 2008). MUC1 is a large transmembrane mucin glycoprotein that is expressed on the apical surface of a variety of reproductive tract epithelia and acts as a lubricant, hydrant of cell surfaces, and antimicrobial and degradative enzyme protectant (Brayman *et al*, 2004). Furthermore, the cytoplasmic tail of MUC1 has been found to be associated with β-catenin (Yamamoto *et al*, 1997) and other signaling molecules, such as Grb2/Sos (Pandey *et al*, 1995), implying that MUC1 can play a role in cell signaling (Gendler, 2001). We found that during rutting season, immune reactivity levels of anti-MUC1 antibody in male camel testes, epididymis, ductus deferens, and accessory sex glands were significantly higher than during non-rutting season, which was consistent with suggestion of Gendler (2001). Moreover, during rutting season, anti-MUC1 antibody immune reactivity was intense in the seminiferous tubules of the proximal and distal parts. Anti-MUC1 antibody immune reactivity

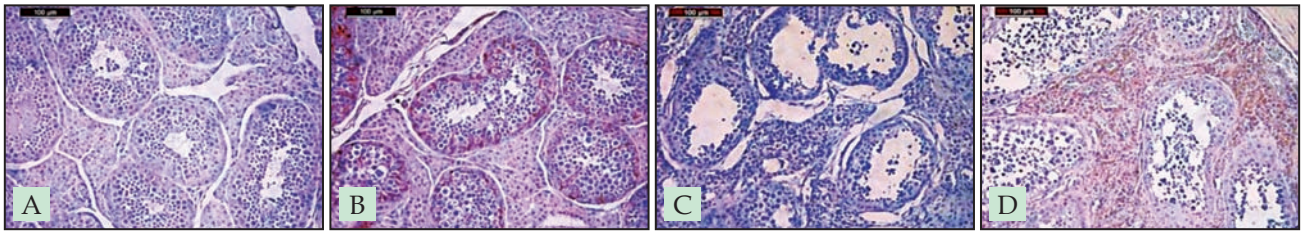

Fig 1. Chromogenic immunohistochemistry of anti-MUC1 antibody in the proximal part (TP) of the testes. (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season-mucin localised in seminiferous tubules with normal spermatogenesis. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows mucin localised in interstitial tissue of the testes. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

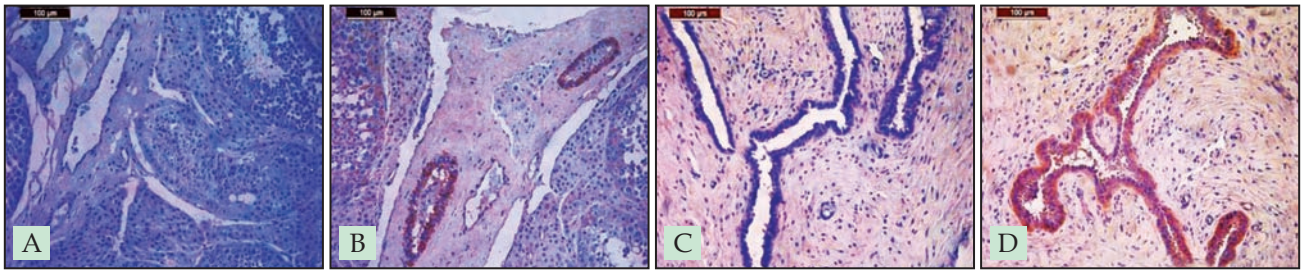

Fig 2. Chromogenic immunohistochemistry of anti-MUC1 antibody in the rete testis (TR). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong interconnecting tubules positivity. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows strong cytoplasmic positivity in myocytes in both muscle fibre and its nucleus interconnecting tubules. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

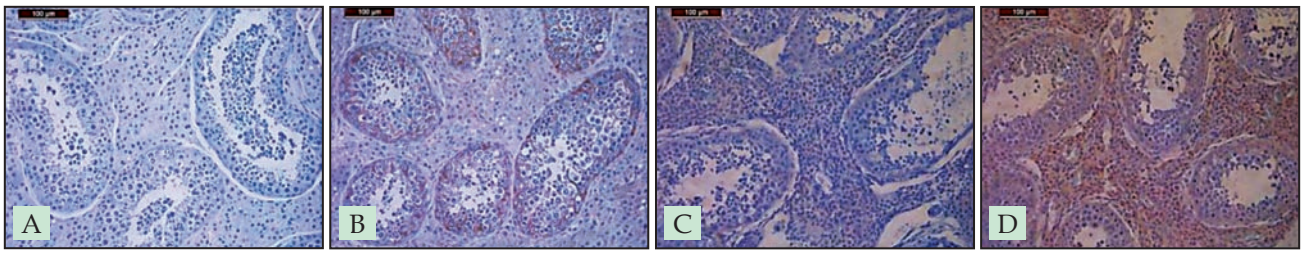

Fig 3. Chromogenic immunohistochemistry of anti-MUC1 antibody in the distal part (TD) of the testes. (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows mucin localised in seminiferous tubules with normal spermatogenesis. (**C**) Negative control of MUC1 during non-rutting season. (**D**) positive reaction of MUC1 during non-rutting season shows mucin localised in interstitial tissue of the testes. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

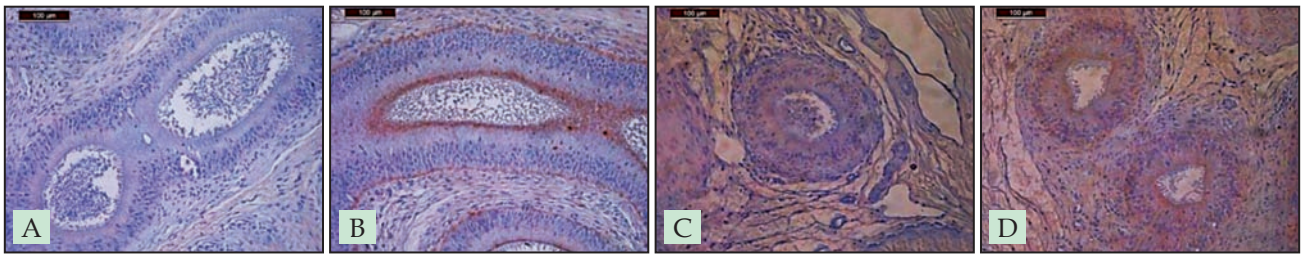

Fig 4. Chromogenic immunohistochemistry of anti-MUC1 antibody in the head of epididymis (EH). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong positivity in basal cell (stem cell) layer and sterocelia of the columnar cell. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows moderate positivity in both basal cell and columnar cell. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

was moderate in the interstitial tissue surrounding seminiferous tubules in both the proximal and distal parts during the non-rutting season. The differences in MUC1 localisation in testes during rutting and non-rutting season could be linked to the physiological state of the male in addition to steroidal hormone

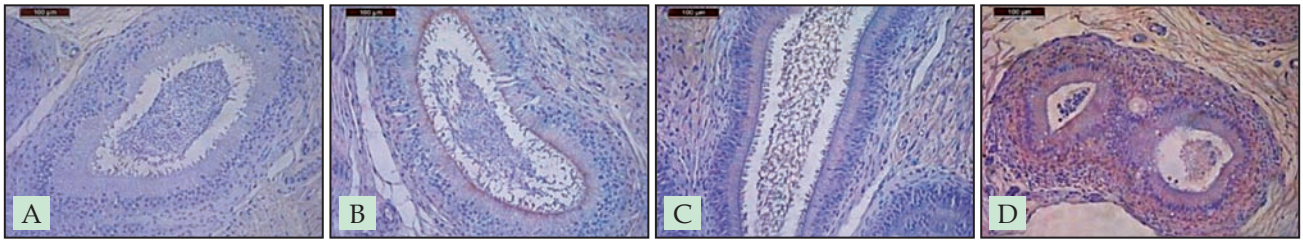

Fig 5. Chromogenic immunohistochemistry of anti-MUC1 antibody in the body of epididymis (EB). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows weak positivity sterocelia of the columnar cell. (**C**) Negative control of MUC1 in the body of epididymis during non-rutting season. (**D**) positive reaction of MUC1 during non-rutting season shows very weak positivity of MUC1 in the body of epididymis. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

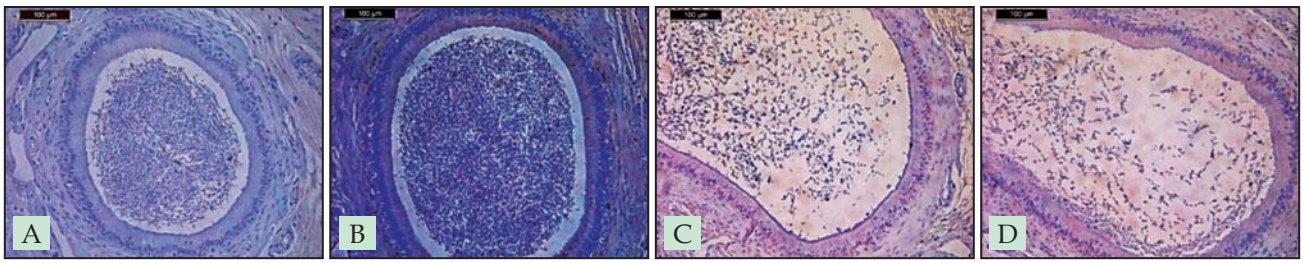

Fig 6. Chromogenic immunohistochemistry of anti-MUC1 antibody in the tail of epididymis (ET). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows weak positivity of columnar cell. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows very weak positivity of MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

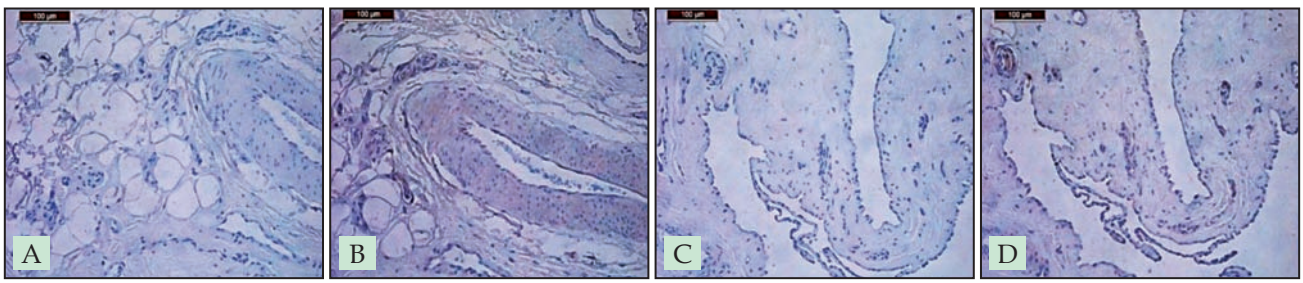

Fig 7. Chromogenic immunohistochemistry of anti-MUC1 antibody in the initial part of ductus deferens (DI). (**A**) Negative control of MUC1 during rutting season. (**B**) Very weak positive reaction of MUC1 during rutting season. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Very weak positive reaction of MUC1 during non-rutting season. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

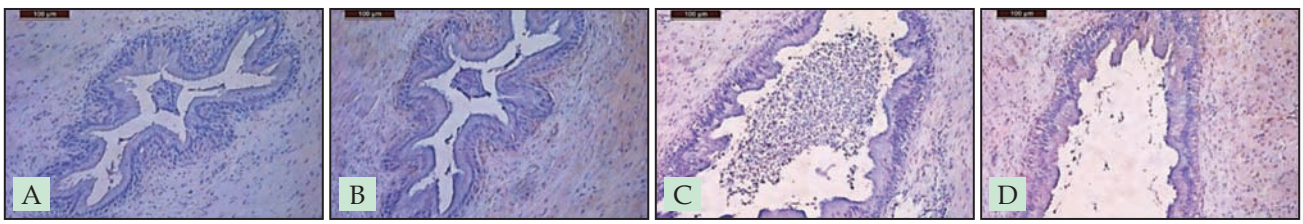

Fig 8. Chromogenic immunohistochemistry of anti-MUC1 antibody in the middle part of ductus deferens (DM). (**A**) Negative control of MUC1 during rutting season. (**B**) Very weak positive reaction of MUC1 during rutting season. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Very weak positive reaction of MUC1 during non-rutting season. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

level during rutting and non-rutting season. During rutting season, the intense immune reactivity levels of anti-MUC1 antibody within seminiferous tubules of the proximal and distal parts may be linked to increased spermatogenesis and sperm hydration. Under these conditions, the molecular mechanism of MUC1 regulation is unclear. Our findings suggest that testosterone hormone regulates MUC1 gene transcription in testicular epithelial cells during the rutting season. In this regard, further research

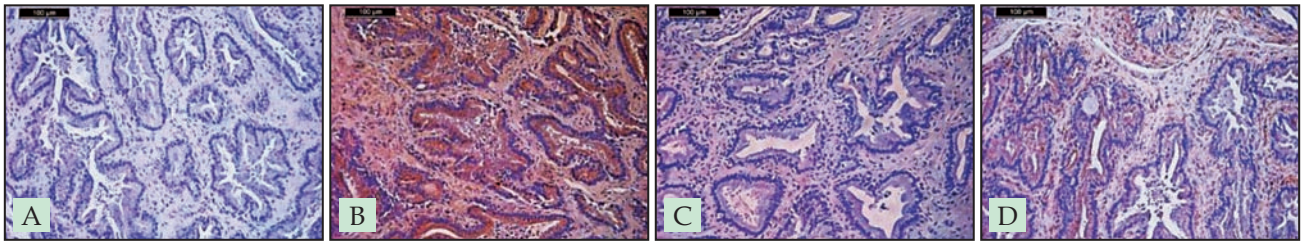

Fig 9. Chromogenic immunohistochemistry of anti-MUC1 antibody in the ampullary part of ductus deferens (DA). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong positivity of the columnar cell. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows moderate positivity of the columnar cell. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

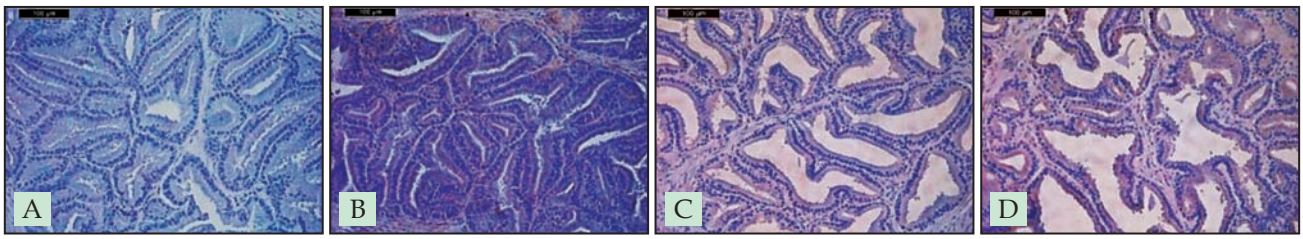

Fig 10. Chromogenic immunohistochemistry of anti-MUC1 antibody in the compact part of the prostate gland (PC). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows moderate acinar cell positivity of anti-MUC1. (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows moderate acinar cell positivity of anti-MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

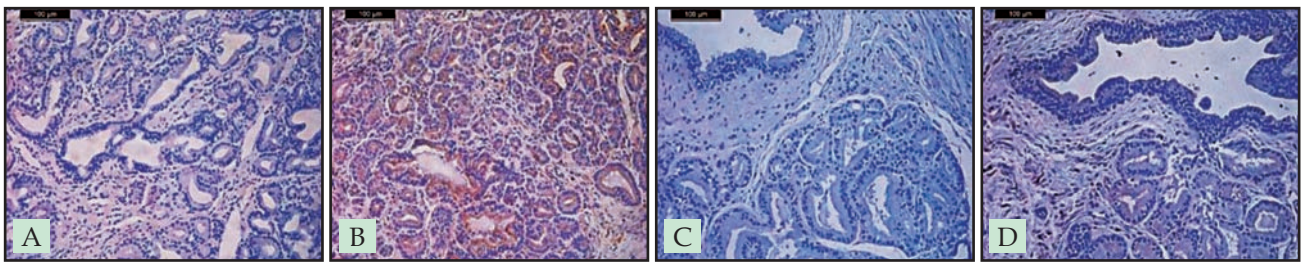

Fig 11. Chromogenic immunohistochemistry of anti-MUC1 antibody in the disseminated part of the prostate gland (PD). (**A**) Negative control of MUC1 during rutting season. (**B**) positive reaction of MUC1 during rutting season shows strong acinar cell and secretory alveoli positivity of anti-MUC1. (**C**) Negative control of MUC1 during non-rutting season. (**D**) positive reaction of MUC1 during non-rutting season shows very weak acinar cell positivity of anti-MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

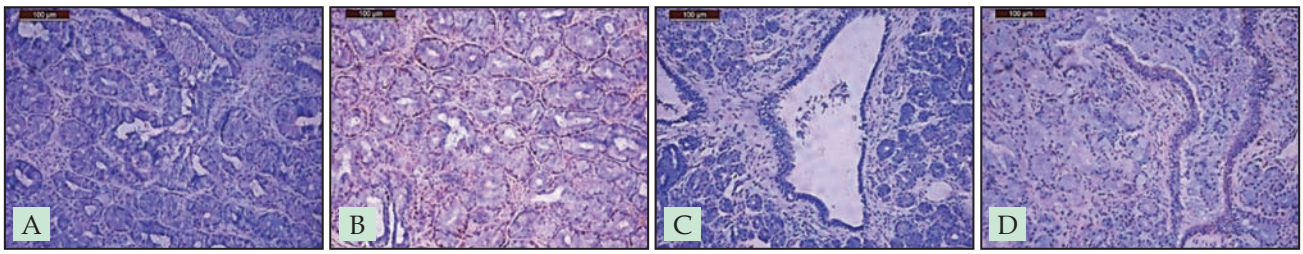

Fig 12. Chromogenic immunohistochemistry of anti-MUC1 antibody in random parts of the bulbourethral gland (BU). (**A**) Negative control of MUC1 during rutting season. (**B**) Positive reaction of MUC1 during rutting season shows strong basal cell positivity of anti-MUC1 (brown colour). (**C**) Negative control of MUC1 during non-rutting season. (**D**) Positive reaction of MUC1 during non-rutting season shows very weak basal cell positivity of anti-MUC1. MUC1 overexpression is stained by DAB/chromogen and counter stained with haematoxylin, 20x.

is needed to demonstrate and understand the mechanisms underlying steroid hormone regulation and signaling during rutting season on MUC1 and other mucin genes transcriptional regulation.

By detecting immune reactivity levels of anti-MUC1 antibody, we attempted to identify the source of MUC1 secretion in male camel prostate and bulbourethral gland. The highest immune reactivity

of anti-MUC1 antibody was found in the ampullae of ductus deferens, acinar cells, and secretory alveoli of the disseminated portion of prostate, as well as the bulbourethral gland, during rutting season. In the non-rutting season, however, anti-MUC1 antibody immune reactivity was very weak in the initial and middle part of the ductus deferens, as well as the acinar cell and secretory alveoli of the disseminated part of the prostate and bulbourethral gland. In conclusion, the main source of MUC1 secretion among male camel reproductive and accessory sex glands comes from ampullae of ductus deferens, acinar cell and secretory alveoli of the disseminated portion of prostate and the basal cells of bulbourethral gland. However, the highest secretory activity occurs during the rutting season, which could be used to reduce the effect of the gel fraction in camel semen when collected outside of the rut season, though this needs to be investigated further.

Acknowledgements

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Camel-based baby formula to hit shelves in Dubai

A UAE-based company has unveiled what it calls the world's first camel-based baby formula, an instant powder mix aimed mainly at infants allergic to cow's milk. The Emirates Industry for Camel Milk & Products, or "Camelicious," introduced the product at Gulfood 2018, an international food and beverage trade event. The milk, which it says is suitable for children ages one to three, primarily targets infants allergic to cow's milk. The company calls its latest product the world's first instant baby milk processed from camel milk. This milk genuinely complements the UAE values and traditions that are handed down from one generation to another.

(Arab News, Saturday. May 01, 2021)

Camel milk consumption drops over MERS fears

There has been a 65 percent decline in demand for camel milk in the past year, forcing some stores to stop selling the product, while many camel owners have now put a halt to this once-thriving commercial activity. This coincides with increased efforts being made by the Ministry of Health to warn the public about the serious dangers of drinking camel milk without boiling it. Scientists have linked camels and camel milk to the deadly MERS coronavirus. In spite of the Health Ministry's warnings, some camel milk consumers remain adamant. The ministry has clearly said that many are unaware that camel herders or milk consumers themselves may be carrying the coronavirus without knowing it or experiencing any symptoms. The Health Ministry warned that such infected persons could transmit the virus to one of their family members who has a poor immune system without even knowing it, putting their whole family at risk.

(Arab News, Saturday. May 01, 2021)

Dubai cafe introduces camel products on ITS menu

A Dubai cafe, trying to give a modern twist to an old Bedouin tradition, has started putting camel products on its menu. Cafe2Go, launched in September last year by an Emirati entrepreneur as part of a scheme to revive Bedouin traditions, now features camel-lattes, camel-ccinos and camel-meat fajitas. He launched Camellos — a brand name for his products derived from the Spanish word for camel. Café owner wanted our younger generation to start drinking it again by mixing it with modern drinks.

(Arab News, Saturday. May 01, 2021)

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EFFECTS OF ACUTE SYNOVITIS EXPERIMENTALLY INDUCED BY AMPHOTERICIN-B ON THE BIOMARKERS OF CAMEL JOINT STRUCTURES

Fahd Al-Sobayil[1] and Mohamed Tharwat[1,2]

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

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

ABSTRACT

The major objective of this study was to evaluate the effects of acute synovitis on the joint structures (bone, articular cartilage and synovial membrane) through the evaluation of biomarkers of bone, cartilage and synovial membrane in dromedary camels. Acute synovitis was experimentally induced by injecting amphotericin-B (20 mg in 4 ml sterilised distilled water) in the intercarpal joints of eight dromedary camels (treatment group) and other eight camels were used as a control group. Synovial fluid samples were collected prior to injection and 7, 14, 21 and 28 days post injection. Inflammatory biomarkers (Prostaglandin E_2) and biomarkers of bone (bone alkaline phosphatase, osteocalcin and pyridinum cross-links) and cartilage (Chondroitin sulfate 846 epitope, C-terminal propeptide of type II procollagen, sulfated glycosaminoglycans and hyaluronan) were determined in the synovial fluid samples. The results showed significant elevations in the inflammatory, articular cartilage and bone biomarkers in the synovial fluid from the treatment group compared to the controls. Elevations of bone resorption and formation biomarkers were late in compare to cartilage biomarkers. This study proved that amphotericin B is an appropriate drug to induce synovitis in the camel joints when injected intra-articular. According to the results of biomarker analysis, it can be concluded that cartilage degradation proceeds bone degradation in camel joint with synovitis. Within articular cartilage tissue, proteoglycan synthesis proceeds type II collagen formation.

Key words: Amphotericin-B, biomarkers, camel, joint, synovitis

Several biomarkers of bone formation and resorption have been assessed with various conditions in animals (Al-Sobayil, 2008; Tharwat *et al,* 2014; Tharwat and Al-Sobayil, 2015; Tharwat and Al-Sobayil, 2018a,b; Tharwat, 2020; Tharwat and Al-Sobayil, 2020a,b). Osteocalcin, bone alkaline phosphatase (BAP) are common examples of bone formation biomarkers. Total alkaline phosphatase (TAP) is a membrane-bound protein with enzymatic activity in hydrolysing phosphate esters. BAP is a bone formation biomarker that represents approximately 18% of total alkaline phosphatase in adult horses (Hank *et al,* 1993). With arthritic horses, BAP significantly increased with a positive correlation between the degree of articular cartilage degradation and its level in synovial fluid (Fuller *et al,* 2001). Osteocalcin is a noncollagenous protein found in bone and secreted by osteoblasts (Lian *et al,* 1985). Therefore, it is considered a specific osteoblastic biomarker produced during bone formation. With arthritis, osteocalcin levels in synovial fluid may increase (Gevers *et al,* 1988) or decrease (Garnero *et al,* 2001). The circadian rhythm of bone formation biomarkers including osteocalcin and BAP has been determined in serum of dromedary camels. Although BAP and osteocalcin are considered bone formation biomarkers, the correlation between them in camels is weak (Al-Sobayil, 2010).

In cartilage and bone, pyridinium cross-links including PYD and DPD are derived from hydroxylysine residues within the mature collagen molecule; they are considered the most promising bone resorption biomarkers (Tanimoto *et al,* 2004). DPD is found in high concentrations in collagen of bone, making it a potentially specific biomarker for bone resorption. However, patients with severe or end-stage of osteoarthritis had higher excretion of PYD compared to those with early osteoarthritis (Hellio le Graverand *et al,* 1996).

The most newly synthesised aggrecan molecules contain terminal chondroitin sulfate chains that have an epitope called CS846. CPII refers to the

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C-terminal propeptide of type II procollagen. Several studies have reported significant increase in the levels of CS486 and CPII in joints with arthritis compared to controls (Rizkalla *et al,* 1992; Nelson *et al,* 1998; Frisbie *et al,* 1999 and 2008). Sulfated glycosaminoglycans (SGAG) and hyaluronan (HA) are major biomarkers that reflect the degree of articular cartilage degradation. In addition, HA is considered a valuable biomarker for synovial membrane. It has been reported that the concentration of SGAG in synovial fluid from osteoarthritic horses was generally higher than its level compared to the controls (Frisbie *et al,* 2008). With osteoarthritis, HA levels significantly increase in human and animal joints (Bruyere *et al,* 2003; Taylor *et al,* 2006). In dromedary camels, the concentrations of SGAG and HA have been determined in normal synovial fluid (Al-Sobayil, 1997).

Prostaglandin E_2 (PGE_2) is considered one of the inflammatory mediators and causes proteoglycan depletion in the cartilage. May *et al* (1994) have assessed the concentration of PGE_2 in synovial fluid from normal and diseased joints of horses. The concentration of PGE_2 is significantly higher in arthritic joints with compared with controls (May *et al,* 1994; Spiers *et al,* 1994). The total concentrations of PGE2 in healthy dromedary camels have been recorded (Al-Sobayil, 1997).

Recently, various advanced techniques have been used to diagnose arthritis in early stages by assessing levels of joint tissue biomarkers. The aim of this study was to determine the effects of induced synovitis (by intra-articular injection of amphotericin-B) in the intercarpal joints of dromedary camels on the joint components measured by changes in levels of bone, cartilage and synovium biomarkers.

Materials and Methods

Camels

Sixteen apparently healthy adult female dromedary camels (aged 6-7 years) with normal haematobiochemistry, were used in this study. The carpal joints were ensured clinically and radiographically normal camels. The camels were randomly assigned into either treatment (n=8 camels) or control (n=8 camels) groups. The camels were fed alfalfa hay and water was given *ad lib*.

Clinical experiment

Each camel was secured in a sitting position and sedated with xylazine (0.2 mg/kg IV, Rompun 2%, Bayer Health Care, Monheim, Germany). The left carpus was aseptically prepared. The left intercarpal joints (8 joints) in the treatment group were aseptically injected with amphotericin-B (20 mg in 4 ml of sterile distal water). The left intercarpal joints of the animals in the control group (8 joints) were injected with only sterile distal water (4 ml) following the same manner as in the treatment group. Four mL of synovial fluid samples were collected from the left intercarpal joint of each camel on days 0 (prior to injection), 7, 14, 21 and 28 (i.e. 0, 1, 2, 3, 4 weeks). The synovial fluid samples were placed in plain vacutainer tube and then centrifuged at 3000 rpm for 10 minutes and the supernatant fluid was then aliquotted in a tube and immediately stored (for biomarker analysis) at -70°C.

Biomarker assays

Concentrations of osteocalcin and BAP were assessed using EIA Kit (Quidel Corporation, San Diego, CA, USA), which has been validated as biomarkers of bone formation (Lepage *et al,* 1990; Gomez *et al,* 1995). Concentrations of PYD and DPD were estimated using EIA Kit (Quidel Corporation, San Diego, CA, USA), which has been validated as markers of bone resorption (Visor *et al,* 1996; Weitz *et al,* 1999). Synovial fluid concentrations of the epitope CS846 and CPII were measured by a commercial ELISA kit (IBEX Diagnostics, Montreal, Quebec, Canada) as biomarkers of aggrecan and type II collagen synthesis, respectively (Rizkalla *et al,* 1992; Poole *et al,* 1994 and 2001; Nelson *et al,* 1998). A modified 1, 9-dimethylmethylene blue dye-binding assay was used on papain digested samples to determine SGAG concentration as a biomarker of cartilage matrix degradation (Farndale *et al,* 1986). Concentration of HA was estimated using ELISA kit (TECO medical Group, Sissach, Switzerland) and has been validated as a biomarker of cartilage matrix degradation. Concentration of PGE2 was extracted from synovial fluid and then assessed by using a commercially available high-sensitivity enzyme immunoassay kit (PGE2 ELISA, Assay Design, Ann Arbor, MI, USA) (May *et al,* 1994).

Statistical analysis

The data were statistically analysed using linear mixed model for repeated measures to evaluate the dependent variables that pertained to synovial fluid samples. The Duncan test was used to calculate multiple comparisons. The significance level was set at $P<0.05$. SPSS statistical package (2009) was used to perform all statistical calculations and statistical analysis.

Results

As expected, amphotericin-B injection resulted in a marked lameness and distention in the carpal joint. The lameness started 12 h after injection of amphotericin-B and continued through 5 days, resolving in all camels by day 10. Lameness was not seen in camels of control group. Fig 1 A and B shows the concentrations of BAP and osteocalcin in synovial fluid samples obtained from camels of both groups. A significant increase was seen in the levels of BAP at week 4 in treatment group compared to control group. The concentration of osteocalcin did not change in both groups during the time of study. Fig 1 C and D shows the levels of PYD and DPD biomarkers in the synovial samples of both the control and treatment camels. The levels of PYD significantly increased in treatment group at weeks 3 and 4 in the treatment group compared to the control group (Fig 1 C). The significant elevation of DPD was seen at week 4 in treatment group compared to the control (Fig 1 D).

Fig 2 A and B shows the levels of CS846 and CPII in synovial fluid from treatment and control groups. The concentrations of CS846 significantly increased in treatment group starting from week 2 until the end of the study. The levels of CPII significantly increased in treatment group compared to control group in the last two weeks of this study. The concentrations of SGAG and HA in synovial fluid of both groups are shown in Fig 2 (C and D). The levels of SGAG and HA were higher in treatment group compared to control group starting from week 1 until the end of the study. The levels of PGE2 significantly increased in the treatment group at weeks 1, 2 and 3 (Fig 2 E).

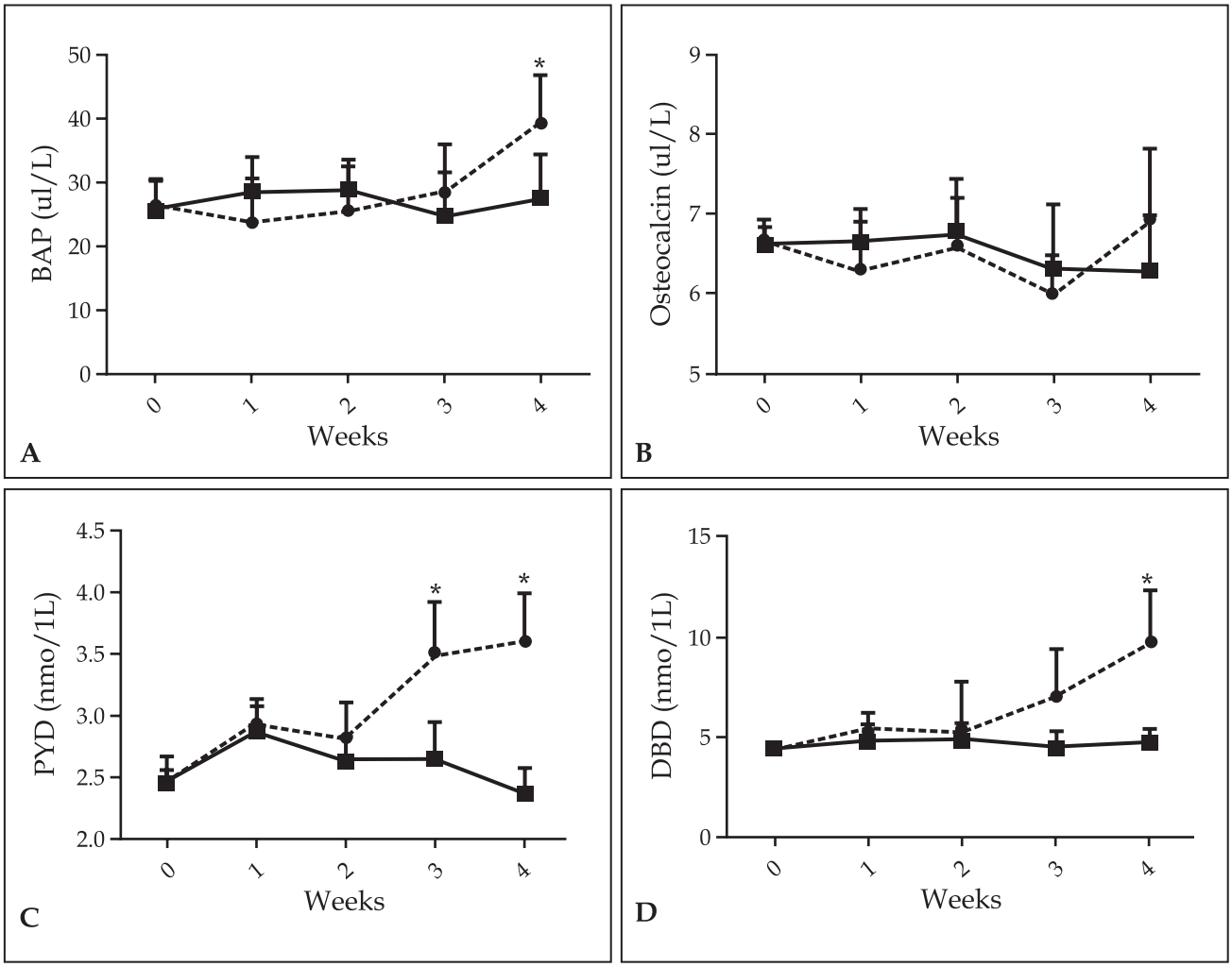

Fig 1. Concentrations of BAP (**A**), Osteocalcin (**B**), PYD (**C**) and DBD (**D**) in the synovial fluid of camels in both treatment (dotted) and control groups during weeks 0, 1, 2, 3 and 4. *Values in the treatment group significantly different from those in the control group at same time. BAP=bone alkaline phosphatase; PYD=pyridinoline; DPD=deoxypyridinoline.

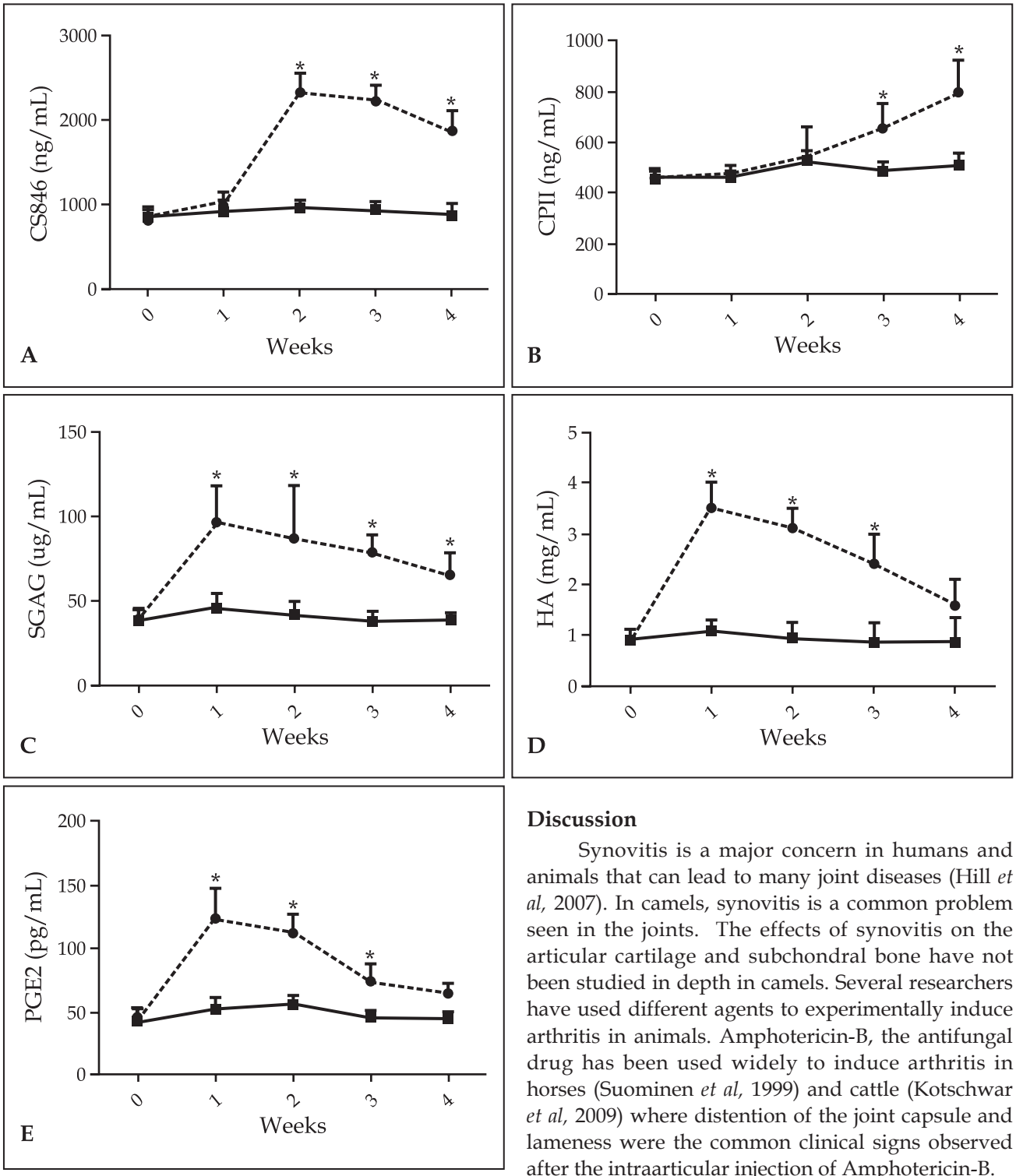

Fig 2. Concentrations of CS846 (**A**), CPII (**B**), GAG (**C**), HA (**D**) and PGE2 (**E**) in the synovial fluid of camels in both treatment (dotted) and control groups during weeks 0, 1, 2, 3 and 4. *Values in the treatment group significantly different from those in the control group at same time. CS846=chondroitin sulfate 846 epitope; CPII=C-terminal propeptide of type II procollagen; SGAG=sulfated glycosaminoglycans; HA=hyaluronan; PGE2=prostaglandin E2.

Discussion

Synovitis is a major concern in humans and animals that can lead to many joint diseases (Hill *et al,* 2007). In camels, synovitis is a common problem seen in the joints. The effects of synovitis on the articular cartilage and subchondral bone have not been studied in depth in camels. Several researchers have used different agents to experimentally induce arthritis in animals. Amphotericin-B, the antifungal drug has been used widely to induce arthritis in horses (Suominen *et al,* 1999) and cattle (Kotschwar *et al,* 2009) where distention of the joint capsule and lameness were the common clinical signs observed after the intraarticular injection of Amphotericin-B.

This study proved that the intraarticular injection of amphotericin B could be used as a model of synovitis in the camels. This study determined the effects of experimentally induced synovitis by intra-articular injection of amphotericin-B on the concentrations of bone, cartilage and inflammatory biomarkers. PGE2 is a common biomarker that has

been used as index of the level of synovitis (Frisbie *et al,* 2008). In the present study, the levels of PGE2 increased in the treatment group at weeks 1, 2 and 3 compared to the control group. This indicated that intra-articular injection of amphotericin-B induced synovitis in the intercarpal joint of camels that was significant during weeks 1, 2 and 3 post injection of amphotericin-B.

The levels of PYD significantly increased in treatment group at weeks 3 and 4. The significant elevation of DPD was seen at week 4. DPD is specific biomarkers for bone resorption because it is found in high concentrations in collagen of bone. PYD is the major cross-link found in collagens of all connective tissues including bone and cartilage. Therefore, the reason for early elevation of PYD compared to DPD might be due to the degradation occurred first in cartilage and other connective tissues (e.g. intraarticular ligaments) and then in the bone.

A significant increase was seen in the levels of BAP at week 4 in treatment group compared to control group. This indicated that bone degradation due to joint inflammation might enhance bone formation. On the other hand, the concentration of osteocalcin did not change significantly in both groups. Both osteocalcin and BAP are produced by osteoblast. However, the two biomarkers reflect different stages of osteoblast function (Bowles *et al,* 1996). BAP is secreted at early bone formation whereas osteocalcin is secreted later during bone mineralisation. Therefore, this might explain the unchanged osteocalcin concentration and at the same time a significant increase in BAP in the treatment group. Studies on camels showed that the correlation between osteocalcin and BAP is weak even though they are considered bone formation biomarkers (Al-Sobayil, 2010).

The levels of CPII significantly increased in treatment group compared to control group in the last two weeks of this study. The concentrations of CS846 significantly increased in treatment group starting from week 2 until the end of the study. The extracellular matrix of hyaline articular cartilage tissue composites of proteoglycan (i.g. SGAG and HA) and type II collagen. It has been reported that proteoglycan content of the cartilage matrix decreases in inflammed joints (Takafuji *et al,* 2002). According to this result, it might be expected that synthesis of proteoglycan in the degraded extracellular matrix of articular cartilage precedes the formation of type II collagen. The levels of SGAG and HA were higher in treatment group compared to control group starting from week 1 until the end of the study. This indicated that joint inflammation causes articular cartilage degradation.

From this study, it can be concluded that amphotericin B is an appropriate drug to induce synovitis in the camel joints when injected intra-articular. Cartilage degradation proceeds bone degradation in camel joint with synovitis. Within articular cartilage tissue, proteoglycan degradation proceeds collagen turnover. Similarly, it is expected that proteoglycan synthesis proceeds type II collagen formation during repairing of degraded articular cartilage repairing.

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Short Communication

INVESTIGATION OF THE INCUBATION PERIOD OF CAMELPOX DURING AN OUTBREAK IN A SMALL DROMEDARY HERD

Sunitha Joseph, Vijay Baskar, Nissy Annie Patteril, Joerg Kinne and Ulrich Wernery
Central Veterinary Research Laboratory, Dubai, United Arab Emirates

ABSTRACT

Our investigation showed that the incubation period of camelpox was 9 days and that vaccination with a live attenuated camelpox vaccine did not protect the dromedary camels against pox lesions, which were already in the incubation period.

Key words: Camel, camelpox, vaccination

Camelpox is a contagious viral skin disease of camelids that produce proliferative dermatitis, mainly in younger animals (Wernery *et al*, 2014). The disease occurs all over the camel-rearing areas of the world except the Australian camel population, affecting both the Old World and New World Camelids (Kinne *et al*, 1998). Both localised and generalised external pox lesions have been described. The disease is characterised by fever, local or generalised pock lesions on the skin and in the mucous membranes of the mouth, respiratory and digestive tracts (Wernery *et al*, 1997a). The causative agent of camelpox is the camelpox virus (CMLV), classified under the genus Orthopoxvirus of the family Poxviridae.

Camelpox is a zoonotic disease that was recently reported in India and Sudan (Bera *et al,* 2011; Khalafalla and Abdelazim, 2017), but according to the OIE (OIE, 2018), camelpox is of limited public health importance.

We describe here the diagnosis and incubation period of a camelpox outbreak near Dubai during the winter season.

Materials and Methods

One female camel was introduced into a small dromedary camel herd consisting of 5 adult females and 2 young males. Ten days later, this camel developed classical camelpox lesions around the head, and ten days after the introduced camel developed camelpox, the rest of the herd also developed mild camelpox around the face, although they were vaccinated on the same day with Ducapox when the introduced camel showed camelpox lesions.

From all infected camels, scabs were removed, as well as blood was taken. Scabs were tested by PCR, and virus isolation was performed on Vero cells as well as histological investigations were carried out. The sera were tested for camelpox virus antibodies with the virus neutralisation test (VNT). All tests are described by the OIE (OIE, 2018).

Results

Real-time PCR results of scabs removed from all 8 dromedary camels showed positive results. Camelpox vius produced typical cytopathic effect (CPE) plaques on Vero cells after 3-4 days' incubation at 37°C with foci of rounded cells, cell detachment, giant cell formation, and syncytia.

Typical histological alterations demonstrated a proliferative dermatitis with ballooning. Pox-like inclusion bodies were also observed in the scabs. VNT results showed no antibodies in all the affected dromedaries which had developed camelpox, but 3 weeks later.

Discussion

Ten days after an adult female dromedary camel was introduced in a small dromedary camel herd consisting of 5 adult females and 2 young males, it developed classical camelpox lesions

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around the head. This camel did not show any lesions when it was brought to the herd and was considered to be in the incubation period as the pox lesions appeared 10 days later. Nine days after the first pox alterations appeared in the index animal, the rest of the herd developed mild camelpox lesions, although they were vaccinated with the live attenuated camelpox vaccine, named Ducapox. The vaccination did not prevent the infection of the remaining 7 camels as the time between the vaccination and antibody production was too short. This is also evident from the negative camelpox antibody VNT results. However, 3 weeks later, all of them had developed VNT antibodies. It is unclear if these antibodies resulted from the infection or from the vaccination, most probably, a combined effect. Although the vaccination did not prevent camelpox in the herd, the mild form could be explained by a possible cell-mediated immunity rather than a humoral.

From the literature, it is known that the incubation period of camelpox is between 9-13 days (OIE, 2018). In our case, the incubation period was exactly 9 days because on the 10^{th} day all the camels developed camelpox. Camelpox virus can be transmitted by very close contact between the infected and non-infected animals as well as through insect vectors especially mosquitos but also ticks (Wernery *et al*, 1997b).

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EVALUATION OF THE SANITARY STATUS OF RETAILED CAMEL MEAT-PRODUCTS WITH AN IMPROVEMENT TRIAL USING *Nigella sativa* AND *Capsicum annuum* OILS

Waleed Rizk El-Ghareeb[1,2] and Hesham A. Ismail[1,3]

[1]Department of Public Health, College of Veterinary Medicine, King Faisal University, P.O. Box: 400, Al-Ahsa, 31982, Saudi Arabia

[2]Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44519, Egypt

[3]Department of Food Hygiene, Faculty of Veterinary Medicine, Assiut University, Egypt

ABSTRACT

This study was undertaken to investigate the hygienic status of the camel meat cuts in comparison with three camel meat-products namely, camel mince, camel burger and camel sausage retailed in the butchery shops and grocery stores in Saudi Arabia. Evaluation of the sanitary status of these products were done via estimation of total bacterial count (TBC), total psychrophilic count (TPsC), most probable number (MPN) of coliforms, total Staphylococcus count (TSC) and total mold count (TMC). A trial for improvement of the sanitary status of the camel mince was conducted using *Nigella sativa* and *Capsicum annuum* oils at different concentrations. The achieved results indicated unsatisfactory sanitary status of the retailed camel meat products in the study area, in terms of high microbial counts. In particular, camel mince had significantly the highest counts; while camel sausage was the lowest one. A clear and significant reduction for the microbial load was achieved after treatment of the formulated camel meatballs from camel mince with *Nigella sativa* and *Capsicum annuum* oils, particularly at 2%.

Key words: Camel meat, *Capsicum annuum*, Microbial load, *Nigella sativa*

The industry of camel meat products increased worldwide, particularly in the Middle East (Kadim *et al,* 2008; El-Ghareeb *et al,* 2019). Meat-products are popular foods for a large section of the country population, particularly among children because of their unique aroma and flavour (Fratianni *et al,* 2010).

The world annual production of camel meat reached 39,606 tonnes for the year 2017 with annual consumption of 0.42kg per capita (FAOSTAT, 2018). With its relatively lower and affordable price, camel meat is a suitable alternative to cattle meat and can significantly contributes to achieving the food security in developing countries where there is growing demand for red meat.

The contamination of the meat products may occur during processing or may be due to the use of contaminated raw material, since some bacteria that form part of the natural flora of cattle are pathogenic for humans as well (Aberle *et al,* 2001; Darwish *et al,* 2015). Therefore, there is a large need to confirm the sanitary status of the retailed meat and meat products in Saudi Arabia.

Spices represent promising tools for reducing the microbial load in meat and meat-products (Jessica Elizabeth *et al,* 2017). Spices and herbs are commonly used in the industry of the meat products for the development of their unique aroma and flavour, to give attractive colours, and for their antimicrobial activities (Aziz and Karboune, 2018). However, the effects of the black seed (*Nigella sativa*), and capsicum (*Capsicum annuum*) to improve the sanitary status and reduce the microbial load of the camel meat-products had received little attention.

In present study we investigated the sanitary status of the camel meat cuts in a comparison with three meat-products namely, camel mince, camel burger, and camel sausage. A trial for improvement of the sanitary status of the camel mince was conducted using *Nigella sativa* and *Capsicum annuum* oils at different concentrations.

Materials and Methods

Eighty samples were collected randomly and equally from camel meat cuts and three meat-

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products retailed in the butchery shops and grocery stores in Al-Ahsa, Saudi Arabia. The examined meat products were camel mince, camel burger and camel sausage (n = 20 each, each sample weighs 100 g). The collected samples were transferred cooled directly without delay to the laboratory for microbiological examination.

Organoleptic examination for the examined samples was conducted using the method of Varnam and Sutherland (1995). Samples with brick-red colour, fresh odour and firm consistency were considered normal.

Samples were prepared for microbiological examinations according to the technique recommended by APHA (2001). Total bacterial count (TBC) was estimated using the method of APHA (2001).

TBC/g = average No. of colonies × reciprocal of dilution Counted colonies expressed as log cfu/g.

For estimation of the total psychrophilic count (TPsC), the pour plate technique recommended by APHA (2001) was applied using standard plate count agar medium and incubated at 7°C for 10 days. Results were calculated and recorded in the same way as TBC. Counted colonies were expressed as log cfu/g.

Most probable number (MPN) of Coliforms was determined by three tubes most probable number (MPN) method (APHA, 2001). The most probable number of coliforms was calculated according to the recommended tables.

Total *Staphylococcus aureus* count (TSC) was determined by the method of Quinn *et al* (2011), using Baird Parker agar (Biolife, Italy) supplemented with egg yolk-tellurite emulsion (Himedia, India).

TSC/g = average No. of colonies × reciprocal of dilution.

Total mold counts were determined by the pour plate technique using Sabouraud's dextrose agar medium (Oxoid, Basingstoke, UK) supplemented with chloramphenicol 100 mg/L followed by incubation in dark at 25°C for 5-7 days. During the incubation time, the plates were examined daily for mold growth. Estimation of total mold count was obtained by direct counting of the cultured plates (Vanderzant and Splittstroesser, 2001).

TMC/g = average No. of colonies × reciprocal of the dilution.

Improvement of the microbial status of the camel minced meat was done by using *Nigella sativa* and *Capsicum annuum* oils at different concentrations. Five of the collected minced meat samples (250 g/each) were formulated as meatballs (5 balls from each sample, 50g/each). Formulated meat balls were grouped into 5 groups, namely, group 1 which was immersed in corn oil for 30 min and served as a control; group 2 which was immersed in Nigella oil at 0.5% for 30 min; group 3 which was immersed in Nigella oil at 2.0% for 30 min; group 4 which was immersed in capsicum oil 0.5% for 30 min; group 5 which was immersed in capsicum oil 2% for 30 min. Microbiological examination was conducted as mentioned before.

All values were expressed as means ± SE, and all measurements were carried out in duplicates. Microbial counts were converted into base logarithms of colony forming units per g (log cfu/g). Statistical significance was evaluated using One way analysis of variance (ANOVA), followed by the Tukey–Kramer HSD post hock test.

Results

Organoleptic examination of the collected samples revealed that all samples had normal sensory parameters. The obtained results for microbiological examination of the collected samples in this study declared that the mean values of TBC were 5.43 ± 0.26, 4.53 ± 0.15, 4.64 ± 0.19, and 4.32 ± 0.08-log cfu/g in the examined camel mince, camel meat cut, camel burger, and camel sausage, respectively (Fig 1A).

The mean concentrations of TPsC in the examined camel mince, meat cut, burger, and sausage were 3.96 ± 0.22, 3.59 ± 0.21, 3.34 ± 0.07, and 3.12 ± 0.09-log cfu/g, respectively (Fig 1B).

Most probable number of coliforms (MPN) were 2.72 ± 0.09, 2.27 ± 0.04, 2.38 ± 0.05, and 2.25 ± 0.05-log MPN/g in the examined camel mince, meat cut, burger, and sausage, respectively (Fig 2A).

The average TSC in the examined samples were 3.27 ± 0.19, 2.94 ± 0.12, 2.68 ± 0.09, and 2.51 ± 0.06-log cfu/g in the examined camel mince, camel meat cut, camel burger, and camel sausage, respectively (Fig 2B).

The average counts for the total mold in the examined camel mince, camel meat cut, camel burger, and camel sausage were 3.61 ± 0.24, 3.16 ± 0.13, 3.09 ± 0.13, and 2.62 ± 0.13-log cfu/g, respectively (Fig 3).

In an improvement trial for the sanitary status of camel mince, *Nigella sativa*, and *Capsicum annuum* oils at 0.5%, and 2% were used. The achieved results in Table 1 declared that TBC in the formulated

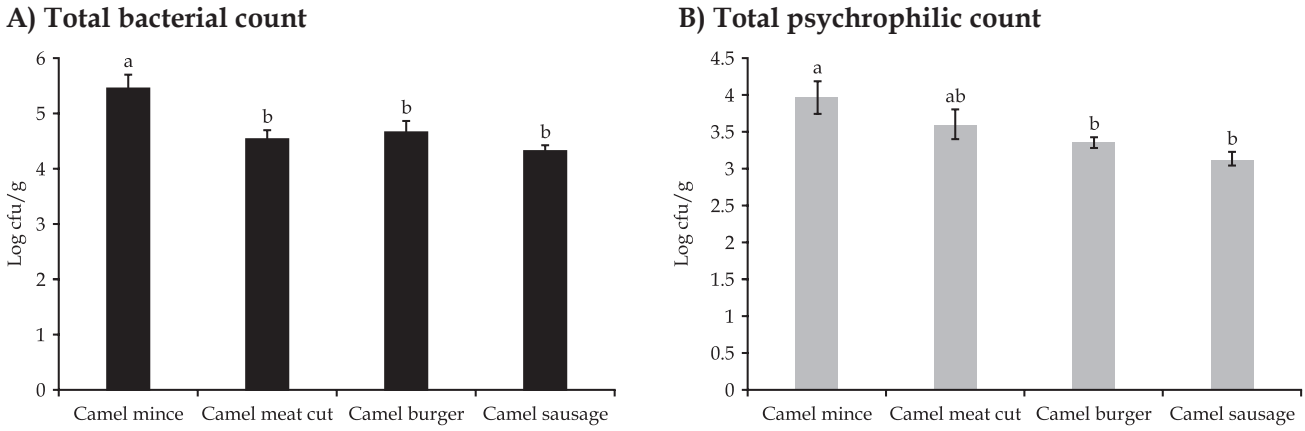

Fig 1. Total bacterial and psychrophilic counts in retailed camel meat products. **A**) Total bacterial count, **B**) Total psychrophilic count. Values represent means ± SE (Log cfu/g) of camel meat cuts, camel mince, camel burger, and camel sausage (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

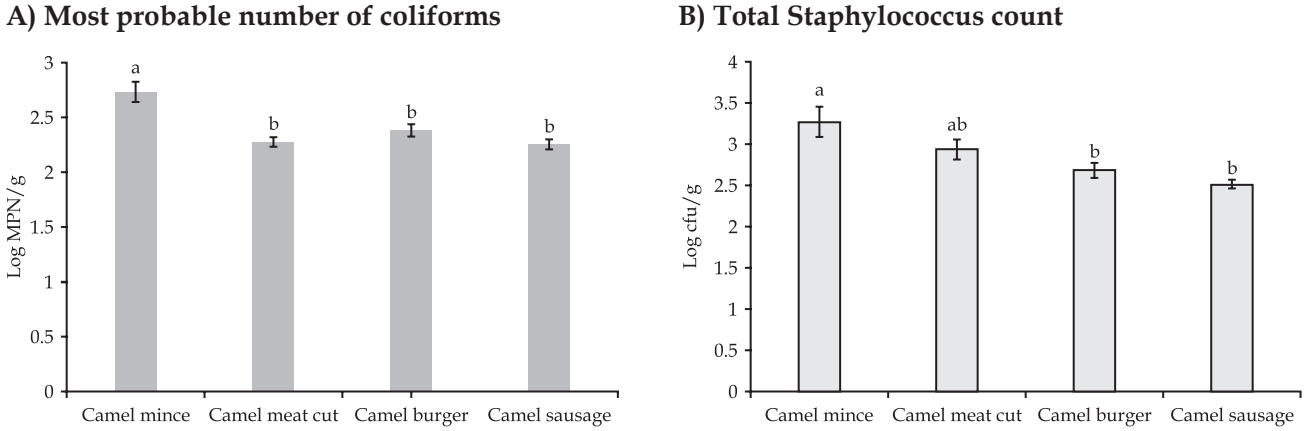

Fig 2. Most probable number of coliforms and total Staphylococcus count in retailed camel meat products. **A**) Most probable number of coliforms **B**) Total Staphylococcus count. Values represent means ± SE (Log cfu/g) of camel meat cuts, camel mince, camel burger, and camel sausage (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at $P < 0.05$.

meatballs from the camel mince was significantly reduced by 19.13%, 26.80%, 29.17%, and 38.02% after treatment with Nigella oil 0.5%, Nigella oil 2%, Capsicum oil 0.5%, and Capsicum oil 2%, respectively. Total psychrophilic counts were reduced by 25.88%, 33.34%, 28.07%, and 36.09% after treatment with Nigella oil 0.5%, Nigella oil 2%, Capsicum oil 0.5%, and Capsicum oil 2%, respectively. These treatments improved the most probable number of coliforms by 11.93%, 18.60%, 14.99%, and 26.29%, respectively; TSC by 16.33%, 20.43%, 19.23%, and 27.78%, respectively; and TMC by 10.81%, 20.61%, 14.90%, and 23.89%, respectively.

Discussion

Camel meat is considered as relatively new and emerging source for the animal-derived protein (El-Ghareeb *et al,* 2019). Meat-products such as mince, burger and sausage are preferred by a large section of the population because of their specific aroma and flavour, and their easy preparation. Sanitary status of the retailed camel meat and meat-products reflects the hygienic measures performed during handling and processing of such products and affect the microbiological quality and the shelf life of the end products (Tang *et al,* 2020). In addition, one major task for the food hygiene sector is to confirm the microbial quality of the retailed meat and meat-products. Different microorganisms have been used as indicators of the level and source of contamination of meat and meat products during animal slaughtering or meat products manufacturing. For instance, total aerobic count used to evaluate the overall degree of microbial contamination (Aberle *et al,* 2001). Coliforms, and other Enterobacteriaceae, are indicative of post-processing faecal contamination (Dogan-Halkman *et al,* 2003). Contamination of raw

Table 1. Improvement of the sanitary status of the camel mince using Nigella and capsicum oils.

	TBC		TPsC		MPN		TSC		TMC	
	Mean ± SE	Reduction %	Mean ± SE	Reduction %	Mean ± SE	Reduction %	Mean ± SE	Reduction %	Mean ± SE	Reduction %
Control	5.09 ± 0.39^{a}	0	3.79 ± 0.52^{a}	0	2.92± 0.19^{a}	0	2.94 ± 0.19^{a}	0	3.67 ± 0.06^{a}	0
Nigella 0.5%	4.12 ± 0.09^{b}	19.13	2.81 ± 0.09^{ab}	25.88	2.58 ± 0.07^{ab}	11.93	2.46 ± 0.07^{b}	16.33	3.27 ± 0.11^{b}	10.81
Nigella 2.0%	3.73 ± 0.24^{bc}	26.80	2.53 ± 0.08^{b}	33.34	2.38 ± 0.08^{b}	18.60	2.34 ± 0.04^{b}	20.43	2.91 ± 0.11^{bc}	20.61
Capsicum 0.5%	3.61 ± 0.11^{bc}	29.17	2.73 ± 0.12^{b}	28.07	2.49 ± 0.06^{ab}	14.99	2.37 ± 0.04^{b}	19.23	3.12 ± 0.07^{bc}	14.90
Capsicum 2%	3.16 ± 0.09^{c}	38.03	2.43 ± 0.11^{b}	36.09	2.16 ± 0.09^{b}	26.29	2.12 ± 0.07^{b}	27.78	2.79 ± 0.09^{c}	23.89

Values within the same column carrying different superscript letter are significantly different at P< 0.05.

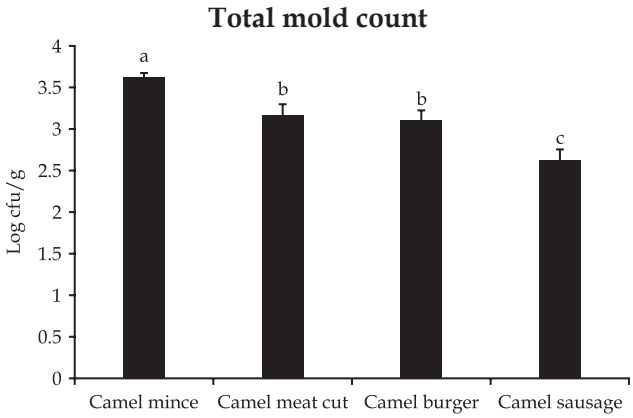

Fig 3. Total mold count in retailed camel meat products. Values represent means ± SE (Log cfu/g) of camel meat cuts, camel mince, camel burger, and camel sausage (n = 20/ each). Columns carrying different superscript letter differ significantly among examined samples at P < 0.05.

meat with *Staphylococcus aureus* is not un-common because of its inhabitance of human and animal skin as well (Adams and Moss, 1997). Thus, it is highly recommended to investigate the hygienic status of the retailed camel meat and meat-products on a regular basis. In the present work, microbial indicators for the sanitary status of the camel meat and meat-products such as TBC, TPsC, MPN of coliforms, TSC, and TMC were estimated. These indicators enables us to give correct decision about the hygienic practices adopted during product handling and processing, and subsequently accepting or rejecting the final products (Mossel *et al,* 1995).

The obtained results in the present study declared unsatisfactory sanitary status for the retailed camel meat and meat-products, in terms of high TBC, TPsC, MPN of coliforms, TSC, and TMC. In particular, camel mince had significantly (P <0.05) the highest counts followed by camel meat cuts, camel burger, and camel sausage, respectively. Similarly, unsatisfactory hygienic measures for camel meat collected from abattoir and retail houses in Jigjiga city, Ethiopia (Tegegne *et al,* 2019). Furthermore, Tang *et al* (2020) recorded higher counts for the microbial indicators in the camel meat and retailed edible offal collected from local markets in Egypt. However, Corrò *et al* (2012) reported that Enterobacteriaceae counts were below the detection limits in the slaughtered camels in the Sahrawi refugee camps located in southwestern Algeria. Camel meat was considered as a possible source for the transmission of the specific food-borne pathogens such as *Campylobacter* spp. in Iran (Rahimi *et al,* 2010), Enterohaemorrhagic *Escherichia coli* O157:H7 in Fars and Khuzestan provinces, Iran (Rahimi *et al,* 2012), Methicillin resistant and susceptible *S. aureus* in Riyadh, Saudi Arabia (Raji *et al,* 2016), *Clostridium difficile* in Iran (Rahimi *et al,* 2014), and vancomycin-resistant *S. aureus* in Egypt (Al-Amery *et al,* 2019). The achieved high microbial load may attributed to lack of hygienic conditions during different stages of meat handling including storage and processing. Cross-contamination also play a vital role in increasing the level of contamination either via worker's hands, cutting boards, knives and even water during slaughtering, evisceration and manufacturing of camel meat and meat products. Meat contaminated with mold has the potential of public health hazard because of the production of mycotoxins with their carcinogenic effect, ability to cause liver diseases and organs failure (Darwish *et al,* 2014). The high bacterial counts in the camel mince are reasonable as mincing process of meat with ingredients other than the meat itself may lead to increasing the microbiological load of the produced mince. Furthermore, mincing machine is considered as a possible source of transferring food-

borne pathogens from contaminated meat to non-inoculated ones (Papadopoulou *et al,* 2012).

In a trial to reduce the microbial load in the camel mince, *Nigella sativa* and *Capsicum annuum* oils were used. Interestingly, a significant reduction for the microbial load was achieved, in terms of reduction of TBC, TPsC, MPN of coliforms, TSC, and TMC. Both of *Nigella sativa* and *Capsicum annuum* oils improved the microbial quality in a concentration-dependent manner, with the highest reduction rate achieved with *Capsicum annuum* 2% without any change in the sensory characters of the final meat-product. *Nigella sativa* is also known as black seed or black cumin, has many pharmacological properties. Thymoquinone (TQ) is the most abundant constituent of the volatile oil of *Nigella sativa* and both of *Nigella sativa* and TQ have a broad antimicrobial spectrum including Gram-negative, Gram-positive bacteria, viruses, parasites, Schistosoma, and fungi (Forouzanfar *et al,* 2014; Tavakkoli *et al,* 2017). Furthermore, *Nigella sativa* seed extracts and oils were shown to have antimicrobial effects against fungi such as *Aspergillus fumigatus, Aspergillus flavus,* and aflatoxin production (Khosravi *et al,* 2011); and Methicillin resistant *S. aureus* (Gawron *et al,* 2019). Capsaicin is an active component of plants of the Capsicum genus, and it is commonly used as a food additive for pain relief, weight loss, body thermoregulation, and antioxidant, antimicrobial and anticancer activities (Adaszek *et al,* 2019). Capsicum extracts were also found to have antifungal activities (de Azevedo Dos Santos *et al,* 2020). The antimicrobial effects of the *Capsicum annuum* might be attributed to the high content of vitamin C, phenols and carotenoids (Al Khalaf *et al,* 2020).

In conclusion, strict hygienic precautions should be adopted during handling, processing, transportation and distribution of camel meat and meat-products. In addition, treatment of the camel meat and meat-products are of value in improving their microbial quality.

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MYCOBACTERIAL INFECTIONS IN CAMELIDS – THE CURRENT SITUATION

U. Wernery
Central Veterinary Research Laboratory, P.O. Box 597, Dubai, United Arab Emirates

It was not so long ago that camelids were thought to be resistant to tuberculosis (Fowler, 2010), which is no longer true. However, they are not highly susceptible to tuberculosis which occurs in both Old World Camels (OWCs) and New World Camels (NWCs) and in recent years a serious concern has arisen in countries where NWCs are bred away from their countries of origin. In the UK for example, which has a strong camelid society, an increase of tuberculosis has been observed in some part of the country in NWCs. This increase in the UK is associated with badger tuberculosis. NWCs which are increasingly kept on pastures in close vicinity to cattle, a spillover has been reported from cattle as well from wildlife. Tb in NWCs has also been reported in many non-GB countries. Llamas and alpacas have been detected to suffer from mycobacteriosis in Spain, Switzerland and other countries of mainland Europe where they are mainly kept as companion animals (Rhodes *et al*, 2015).

Tuberculosis is rare in OWCs when kept under nomadic condition. The disease occurs, however more frequently when camels are kept in close quarters with other camels or cattle for example in Egypt or former Russia (Wernery *et al*, 2014). As OWCs normally roam freely in the desert during the day and as coprophagus animals, they may come into contact with faeces of infected antelopes as shown by Kinne *et al* (2006) who isolated *M. bovis* (antelope clade) from such a dromedary camel.

Also in camels, tuberculosis is a chronic debilitating disease. The clinical signs of tuberculosis in camelids vary widely, but in general progressive emaciation and weight loss are the main signs. Affected camelids may live for months or even years before succumbing to disease. It is also found that some infected camelids with extensive miliary tuberculosis lesions are clinically normal. The organs most frequently affected with the pathogen are the lungs, bronchial and mediastinal lymph nodes, pleura and liver.

The tubercle bacilli are classified in two main complexes: *Mycobacterium* (M.) tuberculosis complex (MTC) and non-mycobacterium tuberculosis complex (NMTC). The "atypical" or "anonymous" or "non-tuberculosis" mycobacteria are grouped in the Runyon's classification. These bacteria are widespread in pastures, soil and water. Camelid tuberculosis is caused by tubercle bacilli of both groups: *M. tuberculosis complex* (MTC): slow growing: *M. tuberculosis, M. bovis, M. microti, M. pinnipedii;* Runyon's group (NMTC): *M. kansasii, M. aquae, M. fortuitum* and *M. smegmatis* and other which have not yet been identified (Wernery *et al*, 2014; Markey *et al*, 2013).

As the ante-mortem signs of tuberculosis are not very specific and therefore the ante-mortem diagnosis of tuberculosis in camelids is difficult. None of the tests currently available can diagnose tuberculosis in camelids with certainty, and none of the tests has been properly validated in these animal species. This refers to the classical tuberculin tests and also to serological tests. As the tuberculin skin test (TST) often gives non-specific reactions or is often negative when the animal reveals typical tuberculosis lesions at necropsy, a great number of serological tests have been developed since the 1980s for the diagnosis of tuberculosis. However, the official tuberculosis screening method for camelids traded internationally is still the tuberculin test, and this is also the only diagnostic test for tuberculosis currently recognised for use in live camelids worldwide.

Several researchers especially in the UK have used serological assyas for the diagnosis of Tb in llamas and alpacas which are also funded by the British alpaca and llama industry to evaluate their efficacy. These include the interferon-gamma assay IFNy, the STAT-PAK®, the Dual Path Platform or DPP® from Chembio and 2 ELISAs from IDEXX and Enferplex™. So far, none of them have been properly validated in NWCs nor in OWCs. At CVRL we abandoned the tuberculin test due

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to its low sensitivity and we regularly use the lateral flow test from Chembio which is a single immunochromatographic rapid test for the detection of antibodies to *M. tuberculosis* and *M. bovis*, mainly designed for elephants as there are no serological tests for camelids.

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OCULAR ULTRASONOGRAPHY IN CAMELS (*Camelus dromedarius*): A REVIEW

Mohamed Tharwat[1,2] and Omar El-Tookhy[3]

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

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, 44519, Zagazig, Egypt

[3]Department of Surgery, Faculty of Veterinary Medicine, Cairo University, Egypt

ABSTRACT

The camel eye appears to be aspheric in shape and smaller than that of cattle and horse. The axial length of the globe is shorter than the sagittal length. Corneal thickness differs significantly between the right and left eyes, gender and age. Axial and sagittal readings are significantly different between male and female camels and between the two age groups. Lens diameter is statistically significant with age. The use of diagnostic veterinary ocular ultrasonography is currently considered as a rapid noninvasive modality that provides a detailed view of the intraocular components and soft tissues surrounding the orbit. This review article is written to describe the results of ocular ultrasonography in healthy camels as well as in camels with some ocular disorders. Ocular affections in dromedaries are blepharitis, conjunctivitis, keratitis, keratoconjunctivitis, corneal wounds, panophthalmitis, corneal opacity, eye lids laceration, xerophthalmia, ruptured eyeball, prolapse of third eyelid, descemetocele, subconjunctival hemorrhage, cataract, glaucoma, retinal detachment and blindness. From the clinical point of view, by ultrasonography the clinician can get detailed information for diagnosis of various ocular problems such as keratitis, cataract, glaucoma, penetrating corneal wounds, retinal detachment and blindness.

Key words: Camels, dromedary, eye, imaging, ultrasonography

Camels suffer from diverse ophthalmic affections, which include blepharitis, conjunctivitis, keratitis, keratoconjunctivitis, corneal wounds, panophthalmitis, corneal opacity, eyelid lascerations, xerophthalmia, ruptured eyeball, prolapse of third eyelid, descemetocele, subconjunctival haemorrhage, cataract, glaucoma, retinal detachment and blindness. A wide range of ocular affections have been reported in dromedaries in India (Bishnoi and Gahlot, 2001a,b; Kumar *et al,* 2016 and Ranjan *et al,* 2016). Most affected parts found were cornea and sclera, followed by conjunctiva and eyelids. However, a retrospective study found corneal opacity, eyelid lacerations, eye injuries, excessive lacrimation, etc. in decreasing order of incidence (Kumar *et al*, 2016). In a study from Egypt, the diagnosed dromedary ocular affections were blepharitis, keratitis, conjunctivitis, keratoconjunctivitis, corneal wounds and panophthalmitis (Fahmy *et al,* 2003). Researchers from Saudi Arabia found that dromedary ocular affections were traumatic in origin and majority of these involved anterior chamber of dromedary eye (El-Tookhy and Tharwat, 2012). Moore *et al* (1999) reported congenital ocular anomalies and ventricular septal defect in a dromedary camel.

The use of ultrasonography is currently considered as a rapid noninvasive modality that provides a detailed view of the intraocular components and soft tissues surrounding the orbit (Ramirez and Tucker, 2004; Potter *et al,* 2008). Ocular ultrasonography is also proved to be an essential tool to examine intraocular structures when opacified ocular media inhibit direct examination, and to evaluate retrobulbar structures when exophthalmos is evident (Gonzalez *et al,* 2001). Hamidzada and Osuobeni (1998) conducted clinical and experimental optometry in dromedary eye and estimated ultrasound velocity in the aqueous and vitreous humours. The greatest advantage of ocular ultrasonography is the capability to evaluate ocular components when challenged with clinically opaque refractive media (Wilkie and Colitz, 2009). The intraocular components of the eye develop in a coordinated manner such that an excess in the dimension of one component is counter balanced by a reduction adjustment in another. Consequently,

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deviations from this regulated growth pattern lead to the development of refractive errors (Nelson *et al,* 1996). Recognition and familiarity with normal ocular biometry is crucial for detecting ocular abnormalities (Potter *et al,* 2008). This review article was designed to describe the results of ocular ultrasonography in healthy camels as well as in camels with some ocular disorders.

Ocular ultrasonography in camels

Ocular ultrasound is practiced in animals with ocular trauma, disparity in ocular size or with any condition that impedes visualisation of posterior ocular structures (Whitcomb, 2002). Ultrasound can be used as a tool for ophthalmological examination and to determine normal echogenicity of the main ocular structures (Kumawat and Jhirwal, 2021). The procedure is an easy, noninvasive diagnostic procedure used to evaluate ocular problems and used as a complement to traditional ocular examination. The assessment of ocular dimensions is crucial for ophthalmic surgeons that should be determined before any ophthalmic interference (Grinninger *et al,* 2010). Ultrasonography has also been used for the detection of the shape and composition of space-occupying ocular masses (Scotty *et al,* 2004), vitreous degeneration (Labruyère *et al,* 2008) and to determine ocular dimensions, corneal curvature and prediction of intraocular lens power before cataract and lens extraction (McMuller and Gilger, 2006). In addition, the technology has been used for the diagnosis of glaucoma, in evaluation of regions of the lens that are difficult to examine directly (Bentley *et al,* 2003; Wilkie and Gilger, 2004), and in the diagnosis of retinal detachment (Strobel *et al,* 2007). Most of the performed ocular studies are conducted on freshly enucleated eyes of camels by A-mode ultrasonography for measurement of optical dimensions after immersion of the eyes in distilled water kept at 20°C and the procedure is performed using a 7.5-10 MHz transducer. The measured optical dimensions include the anterior chamber depth, lens thickness, vitreous chamber depth and axial length. Generally, A-mode ultrasonography is more accurate than B-mode for estimation of intraocular measurements. Thus, A-mode ultrasonography is the procedure of choice in ocular biometry while B-mode ultrasonography is used mainly for diagnostic purposes (Abu-seida, 2016).

For ocular ultrasonography in camels, sedation is necessary by intravenous injection of Xylazine (0.2 mg/Kg). Surface corneal anaesthetic agent (Lidocaine 2%) may also be used. The camel's head should be firmly held, tilted and the eyelids are held open and a 7.5 MHz sector probe is quiet sufficient for examination. Using the direct corneal contact technique both transcorneal and transpalpebral ultrasonographic scanning techniques had been reported in camels (Abedellaah *et al,* 2019).The transducer should be placed directly on the cornea after spreading the coupling gel. Gentle pressure is then applied to maintain good contact between the transducer and the cornea. Each eye should be examined in horizontal section with the ultrasound beam running from the medial to the lateral canthi, and then the head of the transducer was rotated 90° to visualise the vertical section of the eye (Nyland and Mattoon, 1995). The aqueous and vitreous humor as well as the lens cortex and nucleus appear anechoic; however, the anterior and posterior lens capsule, sclera and iris appear hyperechoic (El-Tookhy *et al,* 2012). Similar observations were reported for the enucleated camel eyes (Kassab, 2012). The cornea generates two echoes: the first one corresponded to its epithelium and the second to its Descemet's membrane. *In vivo*, all ocular measurements are slightly increased except anterior chamber depth which is slightly decreased. Axial globe length and vitreous chamber depth are larger in she camels than male camels while the lens thickness in male camels is larger than in females (Yadegari *et al,* 2013). The cornea, anterior and posterior lens capsule and iris appear hyperechoic. The axial length vitreous chamber depth, corneal thickness, lens thickness and scleroretinal rim thickness increase with the advance of age in camels (Kassab, 2012).

Ocular ecobiometry (dimensions) in camels and normal dimensions

The cornea appears in camels to be thin at the centre and thick towards the periphery. Table 1 shows ultrasonographic measurement of the ocular structures of the right and left eyes in adult camels. Abuagla *et al* (2016) found that the different measurements of the right and left eye of dromedary camel had no significant differences ($P>0.05$). With the exception of corneal measurement, there are no significant differences between the right and left ocular components (Osuobeni and Hamidzada, 1999; El-Tookhy *et al,* 2012; Kelawala *et al,* 2015). Corneal thickness has been reported to be an important parameter in corneal surgery (Wilkie and Whittaker, 1997). In camels, corneal thickness is a common parameter which significantly differs

when compared to the eye-side, gender, or age. Measurements of the corneal thickness shows that the central part of the adult camel's cornea (CCT) is slightly thinner than the peripheral part (PCT) (CCT/PCT = 0.9/1.1mm) (El-Tookhy *et al,* 2012) which is similar to the cornea of the human (0.5/0.8 mm), dog (0.4/0.5mm), horse (1.5/1.6mm) and cow (1.6/1.7mm). The anterior chamber depth, lens thickness, vitreous depth and axial length in camels of are smaller than cattle (5.1, 19.2, 14.6, 33.6 mm) and the horse (5.9, 12, 21.9, 40 mm), respectively (McMuller and Gilger, 2006; Potter *et al,* 2008; Grinninger *et al,* 2010). In a study reported by Yadegari *et al* (2013), the means ± SD of the anterior-posterior length of the eye axis, thickness of the lens, depth of the anterior chamber and depth of vitreous were as 32.01±0.32, 11.64±0.06, 4.83±0.81 and 15.99±0.12 mm, respectively. In addition, the axial globe length and vitreous chamber depth in female camels was larger than male camels and lens thickness in male camels was larger than females. Ribeiro *et al* (2010) found that goat's ocular measurements between right and left eyes, as well as between males and females were not significantly different (P>0.05). The ultrasonographic appearances of goat eyes were very similar to those of other domestic and wild species.

Table 1. Ultrasonographic measurements (Mean ± SEM) of the ocular structures of the right and left eyes in adult camels (n=24)*.

Ocular Structure	Right	Left	P
Central corneal thickness	1.0±0.04[a]	0.8±0.06[b]	0.02
Peripheral corneal thickness	1.2±0.04[a]	1.0±0.07[b]	0.02
Anterior chamber depth	2.6±0.1[a]	2.4±0.1[a]	0.3
Lens thickness	9.7±0.1[a]	9.5±0.1[a]	0.12
Lens diameter	16.0±0.4[a]	15.7±0.3[a]	0.5
Vitreous depth	15.7±0.2[a]	15.6±0.2[a]	0.6
Axial length	30.2±0.3[a]	29.5±0.3[a]	0.1
Sagittal length	33.4±0.3[a]	32.7±0.3[a]	0.1
Anterior chamber depth/ Axial length	0.15±0.004[a]	0.15±0.004[a]	0.6
Lens thickness/Axial length	0.32±0.002[a]	0.32±0.002[a]	0.8

[a,b] Values with different superscript letters indicate significant difference (P<0.05). * (El-Tookhy *et al,* 2012).

The camel lens is long-oval in shape with average lens thickness (LT) 9.6 mm and average lens diameter (LD) 15.8 mm. The ratio between the LT and the LD was found to be 0.6; this means that camel eyes have great accommodative amplitude. The greater thickness of the lenses of camels, short vitreous depth, implied that they are more powerful and probably indicates that camel eye is adapted for far sighting (El-Tookhy *et al,* 2012). In a study designed to find the relationship between accommodative amplitude and the ratio of central lens thickness to its equatorial diameter in vertebrate eyes, it is found that vertebrates with lenses that have LT/LD ratios ≤ 0.6 have the greatest accommodative amplitudes and those vertebrates that have oval or spherical shaped lenses, like owls and most mammals have low accommodative amplitudes (Schachar *et al,* 2007).

Based on the gender, significant differences are noticed in the corneal, globe axial and sagittal measurements (El-Tookhy *et al,* 2012) (Table 2). Axial and sagittal ocular measurements differ significantly between males and females, being longer in males than females contradicting previous reports (Nyland and Mattoon, 1995). This difference is reflected on the animal's bony structure including the skull and more specifically the orbital bones. Lens thickness does not differ significantly, however; lens diameter is highly significantly with camel age. Similar findings have been reported in camels (Nyland and Mattoon, 1995; Osuobeni and Hamidzada, 1999; and Ramsey *et al,* 1999). With increasing age of the camel, there are significant increases in the corneal thickness, lens diameter, globe axial and sagittal measurements. The axial length of the camel eye is shorter than the sagittal length (El-Tookhy *et al,* 2012) (Table 3).

Table 2. Ultrasonographic measurements (Mean ± SEM) of the ocular structures adult camels in relation to gender (n=24)*.

Ocular Structure	Gender		P
	male (n= 13)	female (n=11)	
Central corneal thickness	1.0±0.05[a]	0.9±0.04[b]	0.03
Peripheral corneal thickness	1.2±0.04[a]	1.1±0.04[b]	0.03
Anterior chamber depth	2.3±0.08[a]	2.3±0.08[a]	0.8
Lens thickness	9.7±0.1[a]	9.5±0.1[a]	0.2
Lens diameter	16±0.1[a]	15.6±0.3[a]	0.1
Vitreous depth	13.9±0.3[a]	13.4±0.1[a]	0.8
Axial length	30.5±0.4[a]	29.1±0.3[b]	0.04
Sagittal length	33.7±0.4[a]	32.3±0.3[b]	0.04
Anterior chamber depth/ Axial length	0.15±0.007[a]	0.15±0.001[a]	0.6
Lens thickness/Axial length	0.32±0.003[a]	0.33±0.003[a]	0.2

[a,b] Values with different superscript letters indicate significant difference (P<0.05). * (El-Tookhy *et al,* 2012).

Table 3. Ultrasonographic measurements (mean ± SEM) of the ocular structures adult camels in relation to age (n=24)*.

Ocular Structure	Age		*P*
	5- 7year (n= 16)	> 7year (n=8)	
Central corneal thickness	0.9±0.03[a]	1.1±0.05[b]	0.0001
Peripheral corneal thickness	1.1±0.03[a]	1.3±0.04[b]	0.0001
Anterior chamber depth	2.4±0.1[a]	2.3±0.06[a]	0.5
Lens thickness	9.5±0.1[a]	9.7±0.1[a]	0.1
Lens diameter	15.6±0.3[b]	16.0±0.1[a]	0.004
Vitreous depth	10.3±0.9[a]	10.7±1.0[a]	0.7
Axial length	28.2±0.3[b]	29.5±0.5[a]	0.001
Sagittal length	31.4±0.3[b]	32.8±0.5[a]	0.001
Anterior chamber depth/Axial length	0.08±0.002[a]	0.08±0.03[a]	0.5
Lens thickness/Axial length	0.3±0.003[a]	0.33±0.002[a]	0.3

[a,b] Values with different superscript letters indicate significant difference (P<0.05). * (El-Tookhy *et al,* 2012).

Ultrasonography in ocular disorders

Keratitis

Keratitis is the most prevalent ocular condition affecting the anterior ocular segment. It may be acute, moderate or pigmentary. Keratitis may be diagnosed with or without involvement of the iris and ciliary body. Acute conditions are characterised by corneal oedema, zone of neovascularisation and hypopyon. Ultrasonographically, corneal oedema is seen as a thickened and diffusely hypoechoic area. The anterior chamber appear anechoic except when aqueous flare is present which appear as hyperechoic dots floating in the anterior chamber.

Iridocyclitis

In iridocyclitis, the iris is highly reflective than normal and in most cases is partially adhered to the cornea with a thin hypoechoic strands extending from the iris to the posterior surface of cornea. Deep keratitis with severe corneal opacity and corneal abscessation is reported where the Iris is displaced forward towards the posterior corneal surface and the ciliary body appears hyperechoic indicating iridocyclitis. Pigmentary keratitis with long branched neovascularisation surrounding a central area of corneal ulceration has been also reported (El-Tookhy and Tharwat, 2012). The iris adhered to the posterior corneal surface with hyperechoic ciliary body; corneal tissue is thickened with hyperechoic aqueous flare. Regression of symptoms of keratitis, yet partial corneal opacity, neovascularisation exists with two patches of melanin pigment deposition within the corneal stroma; clearer corneal tissue can be seen ventrally with long thin blood has been reported. Ultrasonographically, backward retraction of the iris with slight signs of iridocyclitis is reported (El-Tookhy and Tharwat, 2012).

Cataract

The incidence of cataract was seen in dromedaries (16%) and llamas (20%) (Gionfriddo *et al,* 1997); it may be unilateral or bilateral in dromedaries. The lens appears as hyperechoic mass, either in situ or displaced, with clearly defined thickened echogenic lens capsule with or without irregular margins. The interior of the cataractous lenses exhibits echogenic material. Hypermature cataract as with short, brush-like neovascularisation indicating severe deep keratitis has been reported. By ultrasound, there was an increased echogenicity of the lens with irregular lenticular borders. The cataract appear to involve the anterior capsule, nucleus and slightly affecting the posterior lens capsule (El-Tookhy and Tharwat, 2012).

Glaucoma

Glaucoma is a rare condition in dromedaries as reported in other camelids such as llamas (Gionfriddo *et al,* 1997). Clinical signs of glaucoma included buphthalmia, dilated irresponsive pupil, elevated intra ocular pressure, and chemosis. By ultrasound, the anterior chamber depth, axial and sagittal globe measurements exceeds the normal values compared to the opposite eye (El-Tookhy *et al,* 2012). Ultrasound showed that the anterior chamber contained hyperechoic material (fibrin), the lens was dislocated and vitreal hemorrhage represented by point-like echoes. Buphthalmia with severely dilated unresponsive pupil in case of glaucoma has also been reported where a dilated pupil with increased ocular dimensions was detected ultraonographically (El-Tookhy and Tharwat, 2012). An evident distention of the anterior chamber was noted similar to the findings in horses (Whitcomb, 2002). The iris and ciliary body were seen as echogenic linear structures which extend from the peripheral globe towards the cornea and the anterior chamber depth, the axial and sagittal globe measurements exceeded the normal values (El-Tookhy *et al,* 2012).

Penetrating corneal wounds

Penetrating corneal wounds occur accidentally in dromedaries due to sharp objects. The severity of damage varied from simple corneal cut with

partial iris prolapse to complete visual loss due complications involving other ocular tissues such a lens dislocation, vitreal prolapse, vitreal hemorrhage and retinal detachment. The majority of ocular problems seen in dromedary camels are traumatic with the involvement of one of more ocular tissue. Gionfriddo (2010) reported that trauma-related diseases were the most common eye problems in camelids. In cases of ocular trauma, ultrasound can be used to evaluate the integrity of the globe. Ultrasound can also be used to confirm ophthalmoscopic findings, such as retinal detachment or early cataractous changes. Old infected penetrating corneal wound shows corneal opacity, deep keratitis, prolapsed iris with purulent ocular discharge. A dislocated lens with capsular cataract, vitreal membrane represented by point-like echoes with a uniform high reflective mass seen on the fundus has also been reported (Whitcomb, 2002). Vitreal affections are best diagnosed using the ultrasound; the normal vitreal chamber is filled with anechoic fluid. In most traumatic cases, echogenic pinpoint swirling echoes are seen; these echoes represent vitreal haemorrhages or vitreal debris secondary to inflammation (Whitcomb, 2002). Partial retinal detachment and retinal folds has also been seen within the vitreous body. Relatedly, in other camelids such as llamas, it was reported that retinal diseases were infrequent (Gionfriddo *et al,* 1997). A high reflective wide-spread opacities covering the lens and occupying the vitreal space corresponding to vitreal haemorrhage has been reported (El-Tookhy and Tharwat, 2012).

In conclusion, ocular ultrasonography in dromedary camels is an important methodology for imaging the normal eye as well as for diagnosis of ocular disorders such as keratitis, cataract, glaucoma, penetrating corneal wounds, retinal detachment and blindness. Ocular imaging in the dromedaries by ultrasonography helps determining the precise diagnosis which helps in deciding the appropriate line of treatment and a possible prognosis can be drawn.

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PATHOLOGICAL ASPECTS OF VASA RECTA FIBROSIS IN KIDNEY OF DROMEDARY CAMELS

S.E. Barakat[1], M.S. Moqbel[2] and F.A. Al-Hizab[1]

[1]Department of Pathology, [2]Department of Anatomy, College of Veterinary Medicine, King Faisal University, P.O Box: 400. Al-Ahsa, 31982, Saudi Arabia

ABSTRACT

Gross and microscopic lesions of the kidneys were examined in 50 adult camels of both sexes at the point of slaughter in AL-Ahsa abattoirs. Grossly out of 50 camels examined, 17 (34%) showed some gross pathological alterations. The affected kidneys were pale, enlarged with swollen capsule. Microscopic examination after staining with Hematoxylin and Eosin, Trichrome and Congo red stains was carried out in all grossly affected renal tissues (n=17). Renal tissues stained with H&E revealed focal areas of degenerative changes in both cortical and medullary tubules. In addition, widening in the interstitial matrix at the medullary vasa recta with capillary congestion was observed in 12 (70.58 %) renal tissue samples. However, using trichrome stain confirmed vasa recta fibrosis, which is characteristic by collagen fibre deposition. Moreover, intratubular homogenous material within vasa recta were shown in 5 (29.41%) renal tissue samples. No evidence of amyloidosis was seen in renal samples after staining with Congo red stain. None of the renal tissue samples showed a defined inflammatory response. These results showed that the camels may be exposed to nephrotoxins in the study area possibly from agrochemicals or from the soil around they graze.

Key words: Dromedary camel, fibrosis, histopathology, kidney, vasa recta

The length of Henle and vasa recta loops, may be an indicator of the kidney's ability to excrete a very concentrated urine and maintain body homeostasis (Abdalla and Abdalla, 1979; Anguo, 1997; Qiusheng and Yi, 2002; Xu *et al,* 2009; Li *et al,* 2020). Pannabecker and Dantzler (2006) have evaluated the pathways and densities of descending and ascending vasa recta in rats. They concluded that the manner in which vasa recta function contribute to the concentrating mechanism depends on their three-dimensional relationships to each other and to tubular elements in the outer zone of the inner medulla. Recently, many reports have shown that camels are not exceptionally resistant to diseases and, like other animals, they can suffer from many disease conditions including those of kidneys and disorders related to dehydration and nephrotoxicity (Abbas and Omer, 2005; Kojouri *et al,* 2014). However, epidemiologic studies have revealed that fibrotic deposition which progresses silently is an important indicator of adverse renal outcomes (Genovese *et al,* 2014). It has been reported that the structure and arrangement of the vasa rectae in camel's kidney is different from that in other animals (Xu *et al,* 2009). However, in the mammalian kidney, several studies have shown that the fenestrated ascending vasa recta (AVRs) drain the interstitial fluid in this region (Bell *et al,* 1968; Fenton and Knepper, 2007; Pallone *et al,* 2003a; Pallone *et al,* 2003b; Kriz and Kaissling, 1992). The objective of the present study was to describe the pathological findings related to renal vasa recta fibrosis in camels.

Materials and Methods

A total of 50 apparently healthy adult camels (*Camelus dromedarius*) of both sexes were included in this study. After slaughtering at abattoirs of Al-Ahsa region, Kingdom of Saudi Arabia, all kidneys were thoroughly examined for gross lesions and small pieces of tissue were collected for microscopic examination.

Histopathological Technique

Renal tissue samples were fixed in 10% buffered formalin, mounted in paraffin, sectioned and stained with Hematoxylin and Eosin (H&E), Gomorri's one-step trichrome and Congo red stain saccording to the method of Bancroft and Gamble (2008).

Results

Grossly out of 50 camels examined 17 (34%) showed pathological alterations. All the affected

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kidneys were pale in colour, enlarged in size and had swollen capsule (Fig 1).

Microscopic findings

1. Renal tissues stained with Hematoxylin and Eosin:

Renal tissues stained with H & E revealed focal areas of degenerative changes in cortical tubules (Fig 2). In addition, widening of the interstitial matrix of medullary ascending and descending vasa recta (AVR and DVR) were observed in 12 renal tissue samples (70.58%) with capillary congestion. This widening of interstitial tissues of vasa recta appeared as islets in the medullary zone, characterised by presence of perivascular and intertubular eosinophilic homogenous material with fibrillar threads (fibrosis) and intratubular eosinophilic homogeneous proteinaceous material (proteinaceous casts) (Fig 3, b, c & d). No inflammatory reaction as seen in all renal tissues.

2. Renal tissues stained with trichrome:

The twelve renal tissues (70.58 %) stained with trichrome stain revealed remarkable renal fibrosis, (Fig 4, b, c&d). However, the medullary ascending and descending vasa recta (AVR & DVR), showed perivascular collagen fibre deposition (fibrosis) and congestion (Fig 4, e&f) with intratubular greenish, homogenous proteinaceous material (Fig 4, g). In addition, intratubular deeply eosinophilic homogenous material (hyalinosis) was exclusively seen in 5 renal tissue samples (29.41%) (Fig 4, h). The renal cortex showed areas of glomerular fibrosis with thickness of the Bowman's capsule basement membranes surrounded by collagen fibres. Moreover, thickening of the mesangial capillary walls (Fig 5, a&b) and mild tubulo-interstitial fibrosis were also observed (Fig 5, c).

3. Tissues stained with Congo red

Renal tissues stained with Congo red have shown no evidence of amyloidosis in the vasa recta (Fig 6 a&b).

Discussion

The results of the present study have revealed some degenerative changes in kidney cortical tubules and widening of the interstitial matrix of the medullary ascending and descending vasa recta characterised by presence of perivascular and intertubular eosinophilic homogenous material with fibrillar threads and intratubular hyaline casts after staining with H&E stain. Previous reports have described the presence of hyalinosis and progressive interstitial fibrosis associated with chronic cyclosporine nephrotoxicity in humans (Young *et al,* 1995). The prevalence of glomerular shrinkage, hyalinisation and portentous cast among the different naturally occurring kidney lesions in camels in Saudi Arabia was previously reported by Barakat *et al* (2017).

Our results have confirmed the occurrence of renal fibrosis with perivascular collagen fibres positive to trichrome stain and deposited at the descending and ascending vasa rectae. Similar sclerotic changes in camel kidneys following

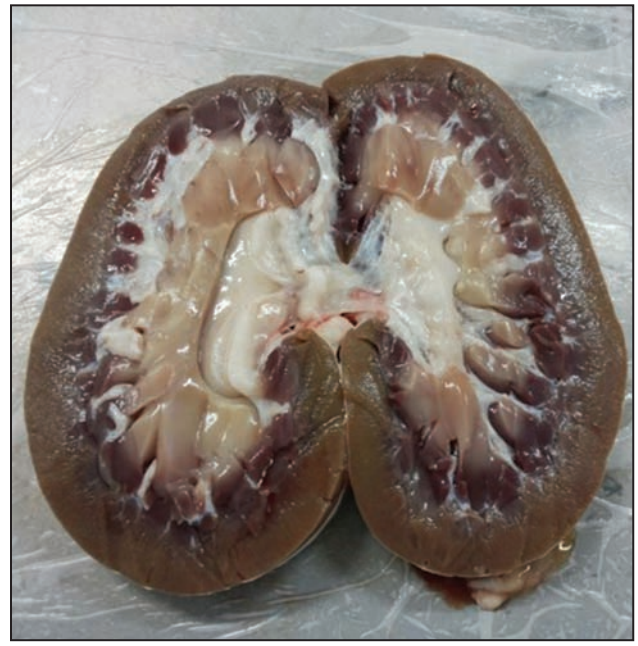

Fig 1. Kidney of camel showing enlargement in size and swollen capsule. The cortex and the medulla appearing pale in colour.

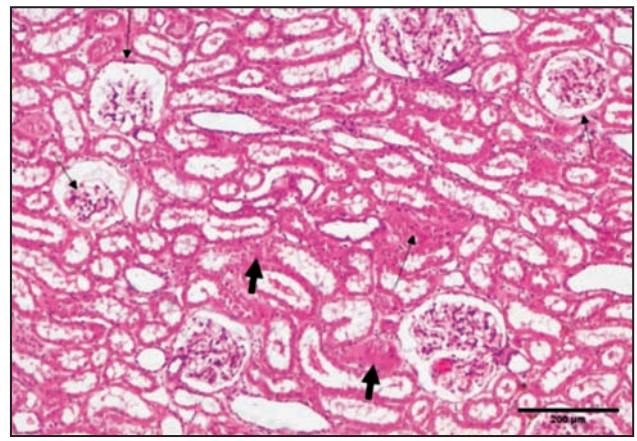

Fig 2. Camel renal cortex showing focal area of degenerative changes (thin arrows) characterised by glomerular tuft shrinkage, widening of Bowman's capsules, thickening of the glomerular membrane and tubular swelling and focal tubular necrosis (thick arrows) bar = 200 µm.

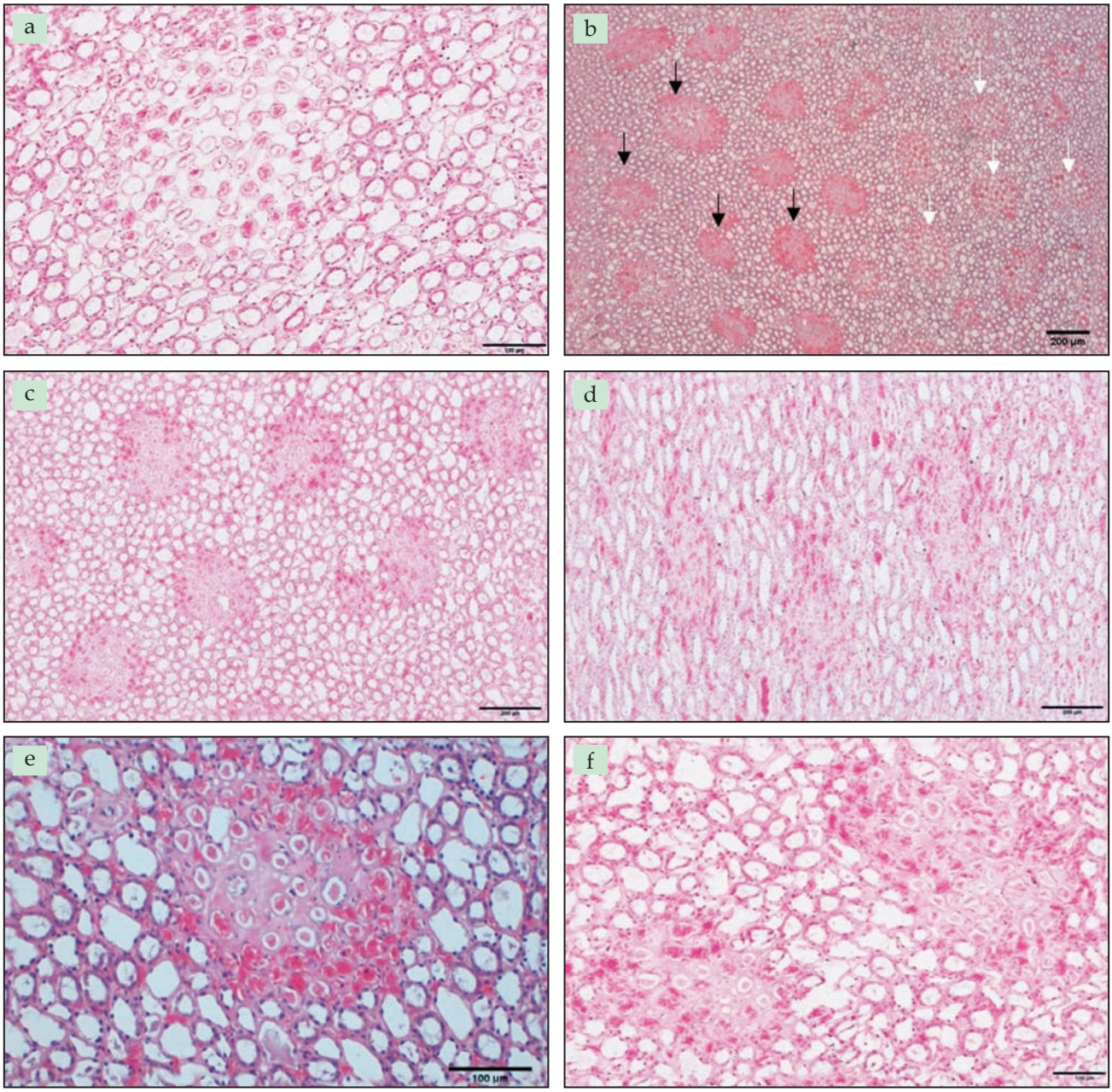

Fig 3. Camel renal tissues showing normal medullary vasa recta (a), widening of vasa recta interstitial matrix appearing as islets in medullary zone (b, black arrows) and normal vasa recta appearance (b, white arrows), presence of perivascular eosinophilic homogenous material with intravascular congestion in medullary AVR and DVR (c&d) and presence of perivascular and intertubular fibrosis and intratubular proteinaceous casts (e&f), (H&E, scale bar =100 and 200 µm).

diminazene aceturate (Berenil) toxicity were reported by Homeida *et al* (1981). Moreover, such changes may be initiated by many insults to the kidney including toxic, ischemic, infectious, paraneoplastic, congenital, genetic, endocrine, and immunological diseases (McGavin and Zachary, 2007; Snyder *et al,* 2009). It seems that many gaps exist in our understanding of the mechanism and pathogenesis of renal fibrogenesis. Efstratiadis *et al* (2009) reported that the main mechanism of renal fibrosis is the transformation of renal tubular epithelial cells to fibroblasts, which migrate to adjacent interstitial parenchyma. In addition, Liu (2011) suggested that a large proportion of interstitial fibroblasts are actually originated from tubular epithelial cells via epithelial to mesenchymal transition (EMT) in diseased kidney.

Kidney tissues stained with Congo red stain revealed no evidence of amyloidosis in all the kidney tissues included in this study. Amyloidosis is a heterogeneous group of diseases characterised by

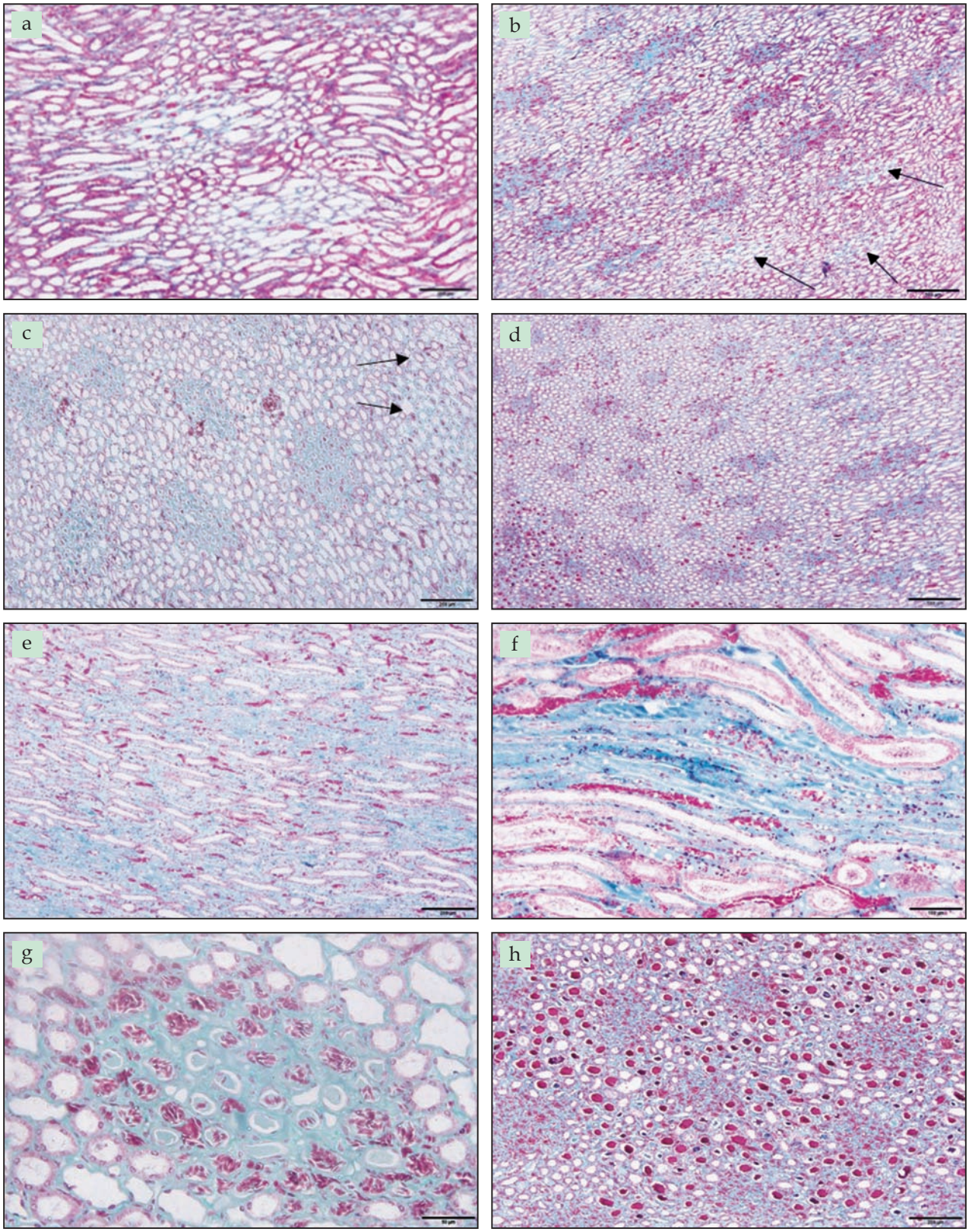

Fig 4. Camel renal tissues showing normal medullary vasa recta (a), fibrosis of vasa recta accompanied with some normal vasa recta (b&c, arrows), AVR and DVR fibrosis appeared as islets in the inner and outer medullary zone (d), perivascular and tubulointerstial fibrosis of vasa recta (e&f), perivascular collagen fibre deposition and congestion in vasa recta with intratubular greenish, homogenous proteinaceous material (g), intratubular deeply eosinophilic homogenous material (hyalinosis) (h), (Gomori's trichrome stain, scale bar =500, 200, 100 and 50 µm).

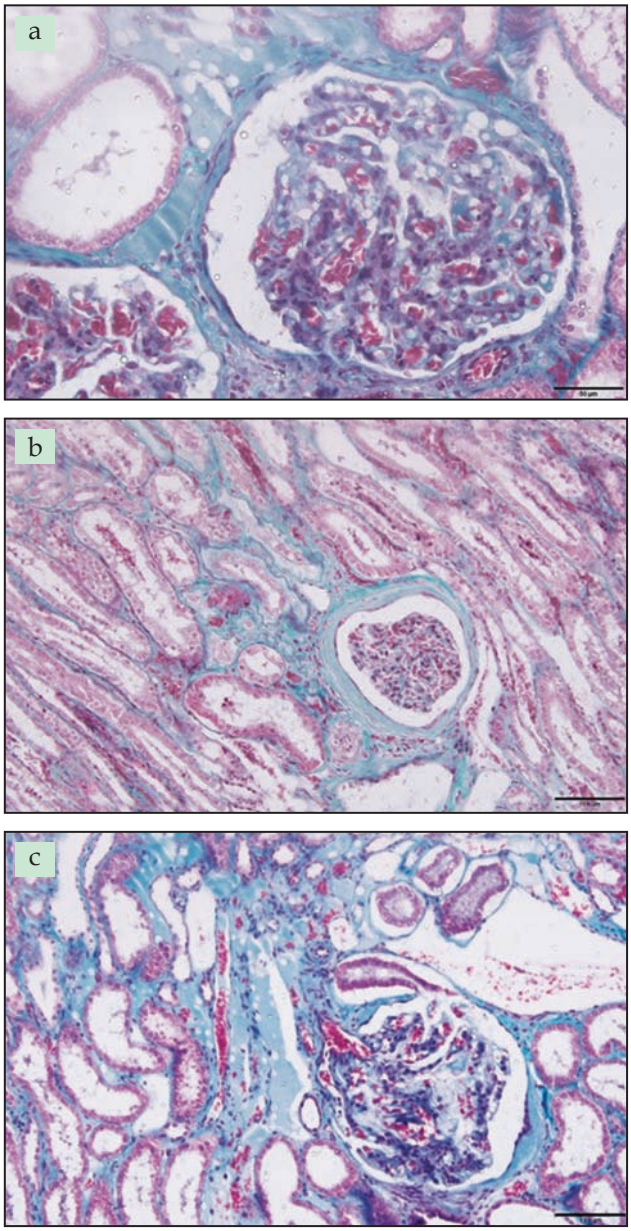

Fig 5. Camel renal cortex showing fibrotic area in the glomeruli (positive trichrome stain) characterised by thickness of the Bowman's capsule basement membranes surrounded by collagen fibres, thickening of the mesangial vessels (a&b), and mild tubulo-interstitial fibrosis and degeneration (c), (Gomori's trichrome stain, scale bar =100 and 50 µm).

deposition of an insoluble amyloid fibrils in various organs and tissues of animals and humans (Saraiva, 2002; Woldemeskel, 2012). The gross changes in kidney tissues shown in our result may be due to the degenerative changes and fibrosis. However, further microscopic examination are needed to identify and confirm the amyloidosis and differentiate it from other apparently similar extracellular deposits such as collagen and fibrin. Furthermore, analysis methods such as immunohistochemistry are more reliable

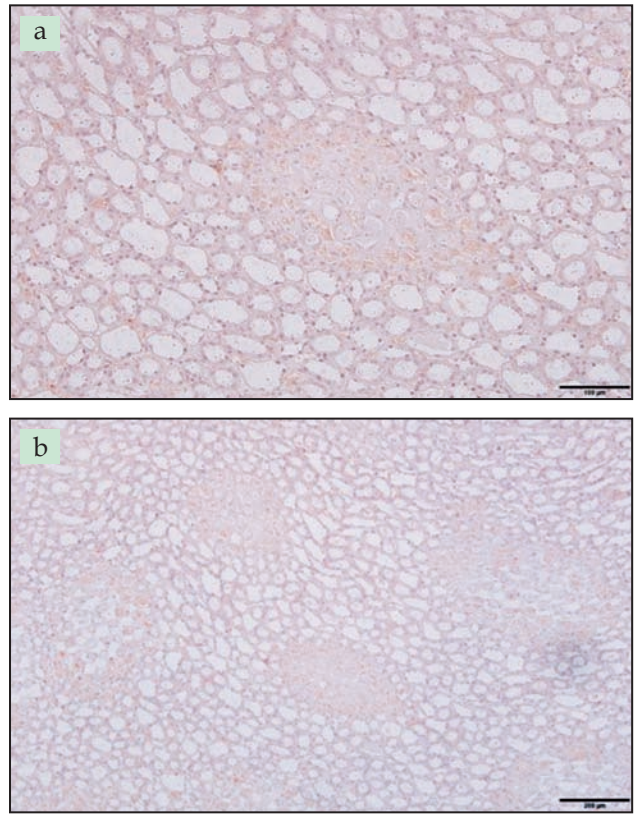

Fig 6. Camel renal vasa recta showing negative reaction for amyloidiosis (a & b), (Congo red stain, scale bar =100 and 50 µm).

procedures for accurate identification of amyloidosis (Wisniowski and Wechalekar, 2020; Iadanza *et al,* 2018).

In conclusion, vasa recta fibrosis is one of the causes of chronic renal disease (CRD), which currently is not well studied in camels.

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OESOPHAGEAL OBSTRUCTION IN DROMEDARY CAMELS: REPORT OF 4 CASES

Satyaveer Singh[1], Sakar Palecha[2], P. Bishnoi[2] and T.K. Gahlot[2]

[1]Department of Veterinary Surgery and Radiology, Post Graduate Institute of Veterinary Education and Research, Jaipur
[2]Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Bikaner
Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001, Rajasthan, India

ABSTRACT

Oesophageal obstruction and its surgical management in 4 camels is reported. The signs during clinical examination were dysphagia, salivation, and swelling at different levels of ventral aspect of the neck. The site of obstruction was confirmed by plain and contrast radiography. In 3 cases, obstruction was present at distal cervical part of oesophagus at the level of 5th cervical vertebra and in one case it was at thoracic inlet. Conservative and surgical treatment was carried out; one case was successfully recovered by conservative treatment and two cases showed the recovery after oesophagotomy and one case died after oesophagotomy.

Key words: Camel, oesophagus, oesophageal obstruction, oesophagotomy

Oesophageal obstruction is often reported in camels (Dabas *et al,* 2002; Ramadan *et al,* 1986; Anwar and Moustafa, 2014). These are caused by ingestion of food or other foreign bodies such as rags and polyethylene bags or metallic objects (Ahmed, 2011; Singh *et al,* 2008, 2011). In adult animal obstruction may be caused by bezoars migrating from the rumen or pressure against the oesophagus from the neighboring tissues such as abscess, lymph node or cysts (Ramadan, 2017). Diagnosis was based on clinical findings, passing stomach tube through the mouth, plain or contrast radiography and endoscopy was conducted in some cases (Ahmed, 2011; Ramadan, 2017). Oesophagotomy was successful to remove foreign bodies in the cervical region or even in the mediastinal region, however, rumenotomy was achieved to remove foreign bodies in the cardiac region. The operations were done with high success rate but complications were in the form of oesophageal fistula (Ramadan, 2017). Present report describes management of oesophageal obstruction in 4 dromedary camels.

Case History and Observations

Four camels; 3 females and one male, aged between 5-8 years of age were presented to the department of Veterinary Surgery and Radiology, TVCC, Bikaner with the history of dysphagia, extending neck, salivation and regurgitation of feed and water just after swallowing. All the cases had the history of obstruction between 1-5 days. On clinical examination, the oesophagus was palpated and it was found as hard swollen mass of variable thickness and location in the ventral distal cervical region of the neck. The probang was passed in the oesophagus to identify the location of the obstruction which were further confirmed by plain and contrast radiography with barium meal (Fig 1). In 3 cases obstruction was present at the distal cervical part of oesophagus at the level of 5th cervical vertebra whereas, in 1 case it was identified at the thoracic inlet.

Treatment

The obstruction was initially attempted to be removed using probang under xylazine sedation (Fig 2) and successfully cleared the obstruction in 1 case. In 3 cases, the obstruction was removed surgically by oesophagotomy (Gahlot, 2000) under xylazine hydrochloride (0.3 mg/kg b.wt) sedation administered intravenously. The camels were positioned in right lateral recumbency and the ventral neck region was prepared aseptically. The proposed incision line was infiltrated with 2% lignocaine hydrochloride (Fig 3).

The stomach tube was inserted into the oesophagus up to the site of obstruction. A linear skin incision over the obstruction site was made at the ventro-lateral aspect of the neck between jugular vein and trachea. The oesophagus was approached by bluntly separating the sternocephalicus muscles.

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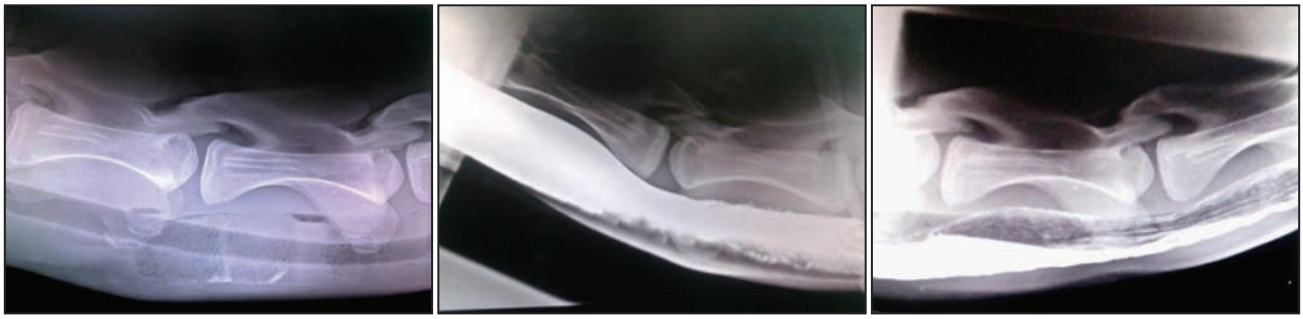

Fig 1. Plain and Contrast Radiographs demonstrating oesophageal obstruction and accumulated contrast material in oesophagus.

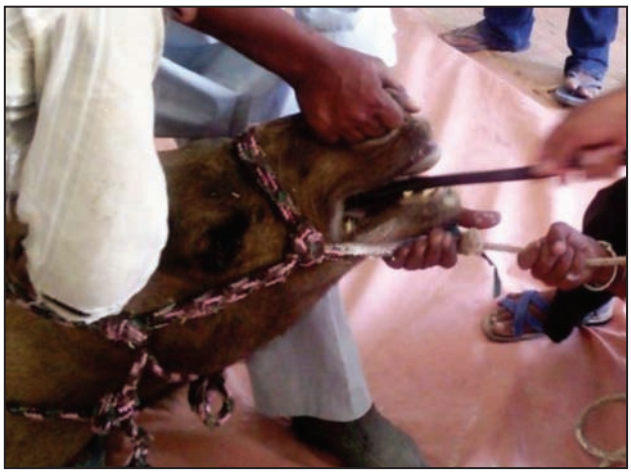

Fig 2. Relieving the obstruction by passing the probang under xylazine sedation.

The oesophagus was separated from the surrounding tissue by blunt dissection and the site was packed with sterilised gauze to prevent any tissue contamination with oesophageal luminal contents. The oesophageal wall was incised over the site of luminal obstruction. In 1 camel, 12 cm long plastic tube was retrieved from the obstructed oesophageal lumen. In 2 camels, the oesophageal lumen was impacted with sandy feed material at its distal part; feed material along with polythene bag (Fig 4). The impacted feed material was removed manually as well as, siphoning with water pressure after inserting tube from the incision towards the distal end of the oesophagus. After removal of obstruction, the oesophageal wall and the surrounding tissue at the surgical site were flushed and cleaned with normal saline. The oesophageal wall was sutured in 2 layers with Polyglactin 910 No. 1. The mucosal layer was sutured by simple interrupted suture pattern. The submucosa, muscularis and tunica adventitia layers were sutured following continuous pattern. The muscles were sutured in simple continuous pattern using chromic Catgut No. 2. The skin was closed with interrupted pattern using Silk No-2 sutures.

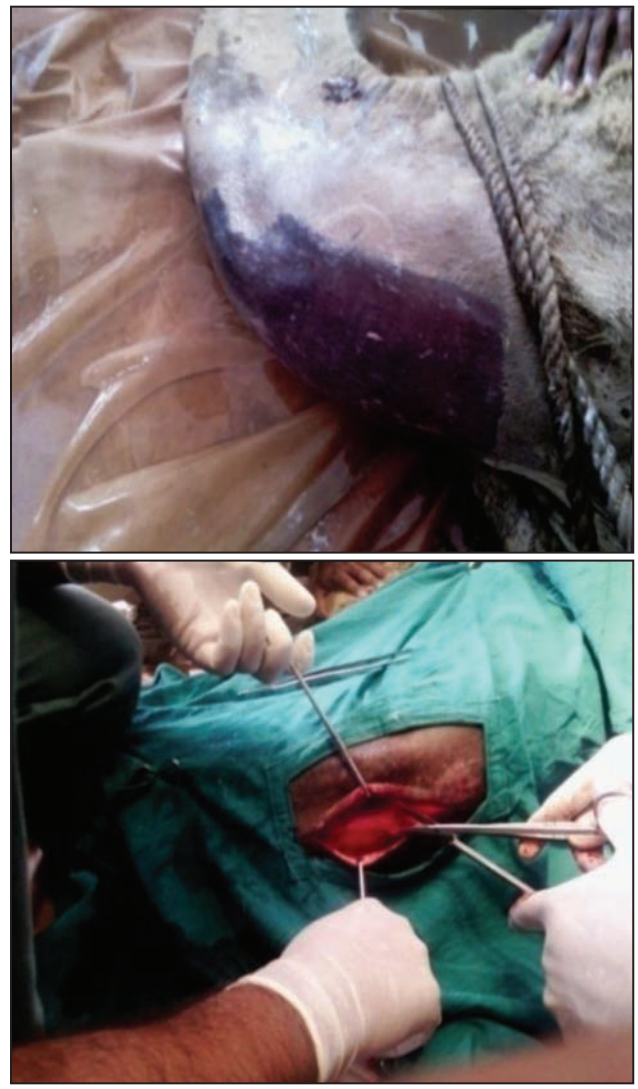

Fig 3. Surgical site and incision between trachea and sternocephalicus muscle.

Postoperatively, the camels were maintained on balanced electrolyte fluid therapy for 5-days, Inj oxytetracycline (5 mg/kg, IV), meloxicam (0.3 mg/kg, IM). The sutures were removed after 12 days. Uneventful recovery was observed in 3 cases whereas, 1 camel died on 8th day, postoperatively.

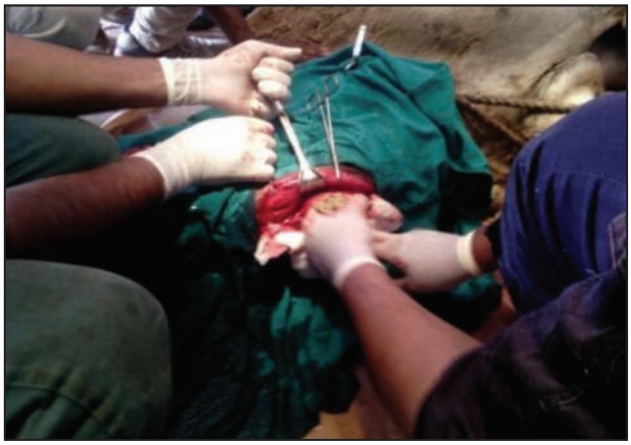

Fig 4. Incision at oesophagus and removal of impacted food material.

Fig 5. Animal after oesophagotomy operation.

Results and Discussion

In present reported cases, oesophageal obstruction or impaction was present at distal cervical region at the level of 5th cervical vertebra and near thoracic inlet which are in agreement with the observation of Ramadan *et al* (1986). Foreign bodies such as food particles, plastic bags, rags, plastic balls and cloths have been recorded to be the main cause of oesophageal obstruction in adult camels (Ramadan and Abdin-Bey, 1990). In young camels, oesophageal obstruction have been reported due to ingestion of pieces of rags, shredded polythene bags, cloth or plastic balls (Ramadan, 1994); plastic bags and sheets (Ahmed, 2011), whereas, in adult camels obstruction is caused by pieces of cloth or hair balls (Ramadan and Abdin-Bey, 1990). A small harrow piece at mid cervical region (Singh *et al,* 2011) and sewing needle have also been recovered from the oesophagus of the camel (Singh *et al,* 2008). Penetrating foreign bodies in oesophageal and paraoesophageal regions have also been reported in ruminants (Singh *et al,* 2016; Singh *et al,* 2017).

In 2 cases, oesophagus was fully impacted at the distal part with wheat straw which is not the natural feed of the camel although, they are mainly dependent on the plants of the desert. The mucosal glands present in the mucosal layer help to lubricate the feed for easy passage to 1st compartment of stomach, although, these glands gradually decrease from cranial to caudal part of oesophageal lumen wall (Nabipour *et al,* 2001), which contribute to the etiology of impaction of feed at the distal part of oesophagus. Continuous feeding of wheat straw can cause the impaction of stomach and subsequently in chronic condition dilatation and impaction of the oesophagus (Ramadan, 1994). In one case, conservative treatment has been successfully attempted with the help of probang under xylazine sedation to clear the passage of oesophagus (Marzok *et al,* 2015). In the present study, oesophagotomy was performed in 3 cases. Two cases recovered uneventfully, but 1 camel died of respiratory distress. Delay in the oesophagotomy after complete obstruction increases the post-operative complications viz. aspiration pneumonia, suture dehiscence and emphysema due to leakage of air. Aspiration pneumonia increases with an increase in duration of obstruction and it should be considered in oesophageal obstruction (Wintzer and Kraft, 1997; Niehaus, 2008) and it should be treated as an emergency to avoid the pressure on the mucosa by the obstructing material, which causes extensive tissue damage consequent to scar tissue formation, stenosis, and even oesophageal perforation (Feige, 2000). The successful recovery was reported in 3 camels with oesophageal obstruction.

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TREATMENT OF CAMEL DERMATOPHYTOSIS BY NOVEL BIOSYNTHESISED MICROBIAL SILVER NANOPARTICLES

M.S. Shathele[1], Sabry M. El-Bahr[2,3], A. FadlElmula[1] and A.M. Hereba[1,4]

[1]Department of Microbiology, [2]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

[3]Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt

[4]Medical Biophysics Department, Medical Research Institute, Alexandria University, Egypt

ABSTRACT

This study was aimed to investigate the green biosynthesis of silver nanoparticles (AgNPs) by *Escherichia coli* (*E. coli*) and *Aspergillus fumigatus* (*A. fumigatus*). The effects of AgNPs on selected microbial species including their own pathogens *in vitro* were examined. In addition, experimental infection in a healthy she camel was done by culture of *Trichophyton verrucosum* isolate from the natural infection. The animal was kept in a separate pen until the development of skin lesions. Afterwards, prepared fungal AgNPs was applied topically to cover the whole infected area. The treatment was done twice daily for six days. The current findings revealed that, AgNPs biosynthesis was fast and formed within 7 and 10 minutes for *A. fumigatus* and *E. coli*, respectively. Photometric analysis of the liquid media obtained from both microorganisms containing silver ion showed a peak around 430-450nm and 420-450nm, respectively. TEM micrographs indicated formation of well-dispersed AgNPs ranged from 3.4-26.4nm and 64.9nm for *A. fumigatus* and *E. coli*, respectively. Zetasizer analysis indicated that AgNPs produced by *A. fumigatus* and *E. coli* average was 125.3 nm and 182.9 nm, respectively. Inhibition zone diameter and minimum inhibitory concentration (MIC) of AgNPs produced by *A. fumigatus* was larger and lower, respectively than that of AgNPs produced by *E. coli* and relevant antibiotics and antifungal for all studied microorganisms. Experimental infection with *Trichophyton verrucosum* induced alopecia, erythema, numerous small subcutaneous nodules and brownish blackish crusts with hyperkeratosis of infected she camel. The treatment with current prepared fungal AgNPs induced a pronounced relieve and disappearance of lesions. The current study concluded that fungal and bacterial synthesised AgNPs have antibacterial and antifungal effect even against their own pathogens. However, *A. fumigatus* AgNPs was more efficient than that of *E. coli* due to smaller particle size and subsequent higher penetrating ability. The fungal AgNPs led to disappearance of skin lesions that resulted from experimental infection with *Trichophyton verrucosum*. Long term studies are recommended to investigate the most effective dose of fungal AgNPs against different fungal infection.

Key words: Antimicrobial, *A. fumigatus, Escherichia coli*, Green biosynthesis, silver nanoparticles

Dermatophytosis in camels is a fungal infection of the skin caused commonly by dermatophytes-*Trichophyton verrucosum*, a filamentous fungi which have ability to invade the epidermis and keratinised tissues such as hair, skin or nails (Abdalla, 2019). Dermatophytosis caused by *Trichophyton verrucosum* in a private farm of dromedary camels in Saudi Arabia had 11.5% prevalence and it was higher among camels younger than three years (22.10%). Rapid recovery was recorded in camels receiving topical application of 10% iodine ointment alongwith parenteral injection of vitamin A (400,000 IU/animal) and mineral mixture supplementation (Abdulaziz *et al*, 2016). A survey of ringworm in camels showed over 25% of young animals suffered from *T. verrucosum* infection, and fewer than 0.5% of the camels had *T. mentagrophytes* (Kuttin *et al,* 1986). Microorganisms have drawn considerable interest in synthesising nanoparticles because they can live and expand in contaminated conditions, including water and soil, because of their ability to withstand metal stress (Qiu *et al,* 2015). In the literature, there are many studies on the extracellular biosynthesis of AgNPs using bacterial cell mass (Lee *et al,* 2008; Silver, 2003) and fungi (Ahmad *et al,* 2003) or their leached cell components (Sung *et al,* 2007). These single and multicellular microorganisms are known to be environmentally friendly nanofactories for the processing of inorganic materials (Bhattacharya and Gupta, 2005). In order to reduce silver ions to

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silver nanoparticles, the cell mass and the leached components from these cells have been registered (Melaiye *et al,* 2005). Intracellular silver nanoparticles can be released via ultrasound treatment of biomass-composite nanoparticles or via reaction with appropriate detergents. However, if the metal ions exposed to the microorganisms and decreased outside the microbial (fungus and bacteria) biomass that led to the formation of metal nanoparticles in solution, it would be more effective (Mandal *et al,* 2006). Several studies have demonstrated antimicrobial effects of AgNPs, but most of the effects of bacterial and fungal Ag-NPs against the skin's own pathogens remain unknown. The current study was aimed to investigate the extracellular biosynthesis of AgNPs by *Escherichia coli* and *A. fumigatus* and of examine their microbial effects against selected microbial species *in vitro* and *in vivo*.

Materials and Methods

Bacteria and fungus used for biosynthesis of Ag-NPs

Escherichia coli ATCC 8739 obtained from culture collection of Microbiology Department, College of Veterinary Medicine, King Faisal University, Saudi Arabia. The bacterium was held at 37°C on LB agar slants and stored at −70°C in glycerol stock solutions. The fungus spores of *A. fumigatus* that had originally been isolated in laboratory of Microbiology Department, College of Veterinary Medicine, King Faisal University, Saudi Arabia from ostrich. The fungus maintained aerobically on potato dextrose agar (PDA) slants for further uses.

Preparation of Bacterial supernatants and biosynthesis of bacterial AgNPs

Bacteria grown in a 500mL Erlenmeyer flask that contained Luria–Bertani (LB) broth containing g/l Trypton 100; Yeast extract 50; NaCl 100; 1L distilled water and adjust the pH to 7.0 with 1 N NaOH (El-Shanshoury *et al,* 2011). The flasks are incubated in a shaker set at 120 rpm and 37°C for 24 hours. The culture was centrifuged at 10000 rpm after the incubation time, and the supernatant was used for AgNP synthesis. Synthesis of AgNPs carried out according to the method described previously with some modifications (El-Shanshoury *et al,* 2011). Aqueous silver nitrate solution ($AgNO_3$) (Sigma, USA, 99.9% pure) (10^{-3} M) added to bacterial supernatants (1%, v/v) and allowed to stand for 5 minutes at room temperature. Bacterial supernatant (1%, v/v) incubated without silver ions used as a control.

Preparation of fungus biomass and biosynthesis of fungal AgNPs

Fresh fungal batch that was kept in potato dextrose agar, incubated into a media containing g/L ($KH2PO_4$, 7.0; $K2HPO_4$, 2.0; $MgSO_4$. $7H_2O$, 0.1; $(NH4)_2SO_4$, 1.0; Yeast extract, 0.6; Glucose, 10.0) at 25°C on an orbital shaker (Thermo Scientific Forma Benchtop Orbital Shakers), with agitation at 150 rpm in 250mL Erlenmeyer flasks containing for 72 hours. After 72 hours incubation, mycelial biomass separated by filtration using a sterilized sieve. The filtrate washed with sterile distilled water to remove any media components. Afterwards, washed filtrate re-suspended in 100ml deionised distilled water, and incubated at 25°C on an orbital shaker, with shaking at 150rpm for 72 hours. After 72 hours, the suspension filtered through Whatman filter paper no. 42 in a 250ml Erlenmeyer flask. $AgNO_3$ solution (1 mM) mixed with 50 ml of obtained fungal filtrate and incubated at 25°C in the dark, with agitation at 135rpm for 72 hours (Bhainsa and D'Souza, 2006). Fungal filtrate incubated without silver ions was used as a control.

UV-visible spectroscopy

The formation AgNPs by the supernatant of the *E. coli* and of *A. fumigatus* in the solutions characterised by (Shimadzu model 9200 Ultraviolet visible spectrophotometer scanning the spectra between 300 and 700 nm operated at a resolution of 0.72 nm). All samples for UV/vis spectra measurement prepared by centrifuging an aliquot of culture supernatant (1.5 ml) at 10000rpm for 10 min at 25°C. For all experiments involving measurement of UV/vis spectrum, all samples were diluted 10 times. Three test tubes, the first containing $AgNO_3$ (Sigma, USA, 99.9% pure) without a supernatant, the second containing only a medium, and the third containing a 1mM concentration of $AgNO_3$ solution and supernatant, were incubated for 6, 12, 18, 24, 48 and 72 hours. The absorption spectrum of the sample were recorded. Extracellular synthesis of AgNPs by either bacteria or fungi after 6, 12, 18, 24, 48 and 72 hours of incubation, controlled by visual inspection of the test tubes for a change in the colour of the culture medium and by measurement of the peak of the UV/vis spectrum of AgNPs. Desirable UV spectra acquired by samples with high absorption intensity due to high levels of reduced silver ions or smooth curves due to better size distributions (Khosravi and Shojaosadati, 2007).

Transmission Electron Microscopy (TEM)

A filtrate sample containing the desirable UV spectrum for AgNPs was used for transmission electron microscopy (TEM; Joel, EM201, Japan) with a Gattan digital camera working at 100 kV acceleration voltage. Every sample was ultrasonically distributed to separate individual particles, and one or two drops of the suspension were mounted on holey-carbon coated copper grids and dried under an infrared light. The film of AgNPs was observed and photographed 72 hours after incubation by TEM.

Particle size distributions

Particle size distribution of the samples obtained by using Zetasizer Nano ZS (Malvern Instruments, Southborough, UK). Particle-size was performed after treatment of a 1mM solution of $AgNO_3$ with the culture supernatant at room temperature for 24 and 72 hours for the *E. coli* and *A. fumigatus*, respectively. The organisms grown in nitrate broth under incubation at 37°C for 21 hours. After the incubation time, the culture was centrifuged at10000 rpm and the $AgNO_3$ solution was reduced by the supernatant. For all experiments involving Dynamic Light Scattering (DLS) measurement, the supernatant obtained from AgNPs was diluted 10 times. The solutions were then filtered by syringe membrane filters with pores of less than 0.4µm, then centrifuged for 20 minutes at 8000 rpm.

Determination of Antimicrobial activity of AgNPs in vitro

The tested microorganisms (*Trichophyton verrucosum, Trichophyton mentagrophytes (ATCC 52015), Trichophyton rubrum (ATCC 52020). Trichophyton tonsurans, Microsporum canis, Microsporum equirum, Candida albicans, Aspergillus fumigatus, Escherichia coli and staphylococcus aureus*) were obtained from the culture collection of Bacteriology laboratory at Microbiology Department, College of Veterinary Medicine, King Faisal University, Saudi Arabia. The antimicrobial spectrum of the fungal and bacterial synthesised Ag-NPs determined by calculation of inhibition zones (disk diffusion method) and minimum inhibitory concentration compared to that of fluconazole, tetracycline and ampicillin (Birla *et al,* 2009).

Determination of Antifungal activity of prepared fungal AgNPs in vivo

Experimental infection in a healthy she camel was done by culture of *Trichophyton verrucosum* isolate from the natural infection. A three-week culture of the isolate on Sabouraud dextrose agar was scraped into sterile container, homogenized with sterile glass homogenizer and used for infection. The skin was disinfected with 70% ethanol, scratched with a scalpel until reddening but not bleeding was observed. The animal was kept in a separate pen provided with feed and water ad libitum until the development of skin lesion. Afterwards, prepared fungal AgNPs was applied topically on the lesion to cover the whole infected area. The treatment was done twice daily for six days.

Statistical analysis

All statistical analyses performed using IBM SPSS statistics 21 software for Mac OS (IBM software, Chicago, USA). Data were analysed by using One-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

Results and Discussion

Pure colonies that have been acquired and classified as *A. fumigatus* and *E. coli* based on the outcomes mentioned in Bergey's determinative bacteriology manual (Holt *et al,* 1994). Aqueous silver ions have been reduced to silver nanoparticles when added to the supernatants of *A. fumigatus* or *E. coli*, according to current findings (Mikhailova, 2020; Roy *et al,* 2019). This was observed visually by the change in colour from whitish-yellow to gray brownish (in case of *A. fumigatus*) and brownish colour (in case of *E. coli*) (Fig 1a and 1b) within approximately 7 and 10 minutes, respectively (Hemath Naveen *et al,* 2010). However, previous work stated that the time elapsed for formation of brownish colour by bacterial Ag-NPs (*Escherichia coli* ATCC 8739, *Bacillus subtilus* ATCC 6633 and *Streptococcus thermophilus* Esh1) was 5 minutes only (El-Shanshoury *et al,* 2011). The control experiments without microbial supernatants showed no formation of brown colour. The unchanged colour of the control experiment provided a strong evidence that the colour change was of microbial origin either *A. fumigatus* or *E. coli* (Fig 1a and 1b). In addition, this suggests that the reduction of silver ions to silver nanoparticles was conducted by some reducing agents that released into the cultures of studied fungus or bacteria. Previous studies using Enterobacteria support this data (Shahverdi *et al,* 2007). In this context, many hydrogen intermediates have been described as electron shuttles in the metal reduction process (Baker and Tatum, 1998; Durán *et al,* 2005). The formation of colloidal silver nanoparticles is indicated by the rapid appearance of a yellowish-brown colour in the reaction vessels.

Table 1. Inhibition zone (mm) of Ag-NPs produced from *Aspergillus fumigatus* and *E. coli* compared to fluconazole, tetracycline and ampicillin on different fungal and bacterial species.

Tested organisms	*Aspergillus fumigatus* Ag-NPs	*Escherichia coli* Ag-NPs	Tetracycline 30µg	Ampicillin 10µg	Fluconazole 25µg
Trichophyton verrucosum	23.0 ± 0.20[a]	21.0 ± 0.14[b]	–	–	18.0 ± 0.16[c]
Trichophyton mentagrophytes (ATCC 52015)	24.0 ± 0.16[a]	23.0 ± 0.21[b]	–	–	17.0 ± 0.11[c]
Trichophyton rubrum (ATCC 52020)	21.0 ± 0.15[a]	20.0 ± 0.12[b]	–	–	15.0 ± 0.18[c]
Trichophyton tonsurans	19.0 ± 0.25[a]	17.0 ± 0.22[b]	–	–	14.0 ± 0.22[c]
Microsporum canis	24.0 ± 0.23[a]	22.0 ± 0.19[b]	–	–	16.0 ± 0.12[c]
Microsporum equirum	23.0 ± 0.19[a]	22.0 ± 0.20[b]	–	–	17.0 ± 0.21[c]
Candida albicans	24.2 ± 0.13[a]	22.1 ± 0.14[b]	–	–	20.0 ± 0.21[c]
Aspergillus fumigatus	24.0 ± 0.21[a]	21.0 ± 0.12[b]	–	–	18.0 ± 0.19[c]
Escherichia coli	30.2 ± 0.17[a]	29.3 ± 013[b]	22.0 ± 0.12[c]	17.2 ± 0.11[d]	–
Staphylococcus aureus	22.0 ± 0.16[a]	21.0 ± 0.14[b]	22.0 ± 0.16[c]	15.0 ± 0.13[d]	–

[a-d] Means in the same rows with different superscripts differ significantly (P <0.05).

Table 2. Minimum inhibitory concentrations (µg/ml) of Ag-NPs produced from *A. fumigatus* and *E. coli* compared to fluconazole, tetracycline and ampicillin on different fungal and bacterial species.

Tested organisms	*Aspergillus fumigatus* Ag-NPs	*Escherichia coli* Ag-NPs	Tetracycline 30µg	Ampicillin 10µg	Fluconazole 25µg
Trichophyton verrucosum	1.50 ± 0.21[c]	12.50 ± 0.15[a]	–	–	2.50 ± 0.11[b]
Trichophyton mentagrophytes (ATCC 52015)	3.50 ± 0.11[c]	10.50 ± 0.16[a]	–	–	8.00 ± 0.21[b]
Trichophyton rubrum (ATCC 52020)	2.40 ± 0.16[c]	9.00 ± 0.11[a]	–	–	7.00 ± 0.32[b]
Trichophyton tonsurans	3.00 ± 0.14[c]	7.00 ± 0.12[a]	–	–	5.00 ± 0.12[b]
Microsporum canis	5.00 ± 0.12[c]	13.00 ± 0.20[a]	–	–	9.00 ± 0.22[b]
Microsporum equirum	3.70 ± 0.22[c]	10.00 ± 0.12[a]	–	–	12.50 ± 0.23[b]
Candida albicans	2.50 ± 0.21[c]	10.30 ± 0.19[a]	–	–	8.00 ± 0.21[b]
Aspergillus fumigatus	4.00 ± 0.20[c]	11.00 ± 0.18[a]	–	–	9.00 ± 0.22[b]
Escherichia coli	10.00 ± 0.00[d]	22.47 ± 0.06[a]	14.27 ± 0.15[b]	12.27 ± 0.09[c]	–
Staphylococcus aureus	14.27 ± 0.12[d]	24.27 ± 0.12[a]	21.33 ± 0.15[b]	18.33 ± 0.12[c]	–

[a-d] Means in the same rows with different superscripts differ significantly (P <0.05).

This may be due to surface plasmon vibration excitation, typical of AgNPs (Shahverdi *et al,* 2007). Rapid changes in colouration in case of *A. fumigatus* than that of *E. coli* may indicates rapid effect of *A. fumigatus* than that of *E. coli* as observed later in this section. Extracellular synthesis of AgNPs was carried out within hours of contact with *Fusarium oxysporum* cell filtrate and within minutes of contact with *A. fumigatus* cell filtrate (Bhainsa and D'Souza, 2006). The AgNPs synthetic method was very rapid and silver nanoparticles were produced within 10 minutes of exposure of silver ions to the Enterobacteria cell filtrate (Shahverdi *et al,* 2007). *A. fumigatus* rapid extracellular synthesis (7 minutes) has great advantages over *E. coli* (10 minutes) and protocols for intracellular synthesis because it prevents potential mycotoxin contamination. In this respect, the extracellular biosynthesis of silver nanoparticles by *A. fumigatus* and *E. coli* achieved in this study may prove to be a significant step in the right direction. This provides a great advantage from the application point of view over an intracellular synthesis process. Since the nanoparticles formed within the biomass will require additional processing steps for the release of the biomass nanoparticles by ultrasound treatment or reaction with appropriate detergents. The brown colour remained stable and further characterisation done by UV-visible spectroscopy, Transmission Electron Microscopy (TEM) and zetasizer Nano ZS. The spectra for supernatants after incubation with silver nitrate and subsequent sonication showed maximum absorbance at (430-450) for *A. fumigatus*

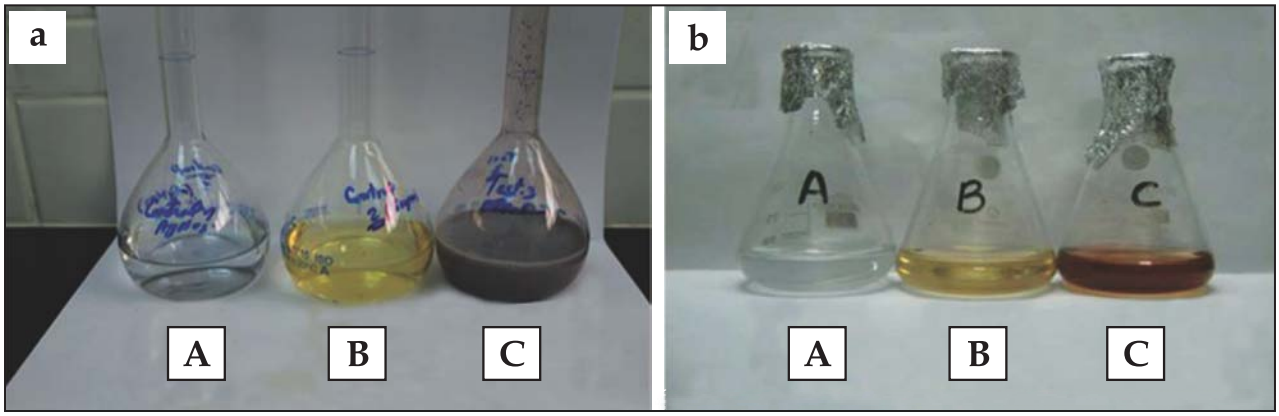

Fig 1. Visible observation of biosynthesised silver nanoparticles by (a) *Aspergillus fumigatus* and (b) *Escherichia coli*. (A) $AgNO_3$ solution without *Aspergillus fumigatus* or *Escherichia coli* supernatants for 24h (no colour change). (B) Conical flask with heat-killed *Aspergillus fumigatus* or *Escherichia coli* exposed to $AgNO_3$ solution for 24h (no colour change; media colour) (C) Conical flask with live *Aspergillus fumigatus* or *Escherichia coli* supernatants exposed to $AgNO_3$ solution for 24h (gray brownish and brownish colour, respectively).

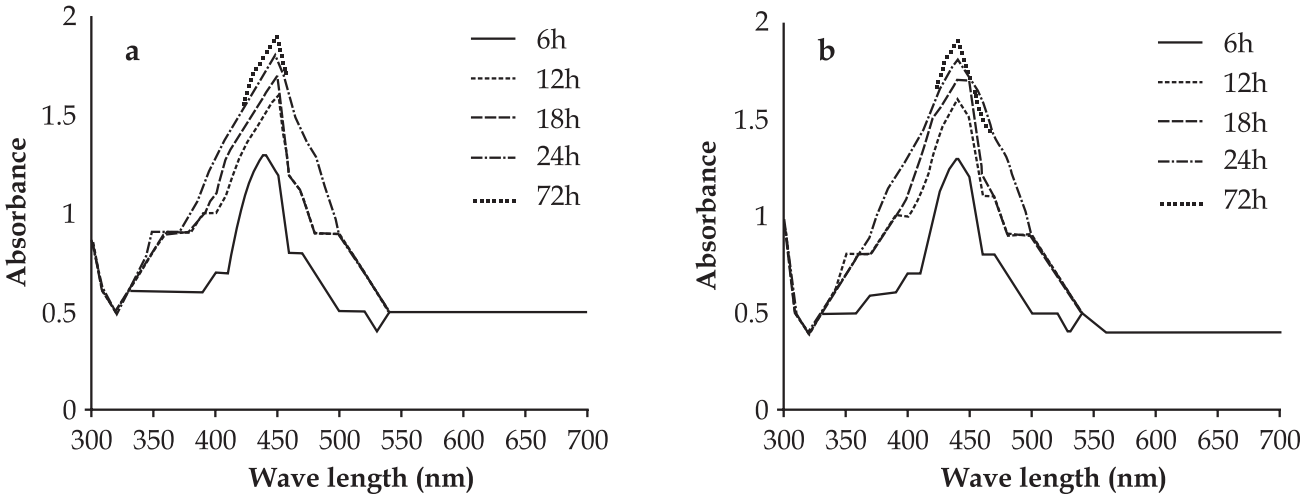

Fig 2. UV–vis spectra of (a) *E. coli* synthesised sliver nanoparticles and (b) *A. fumigatus* synthesised sliver nanoparticles recorded at different time intervals. The microbial supernatants were incubated with 1×10^{-3} M silver nitrate solution at different time intervals (6, 12, 18, 24 and 72h).

and 420-450 nm for *E. coli* which increased with the increase of incubation time (Fig 2a and 2b). Due to the reduction of silver ions present in the aqueous solution, the increase in intensity may be due to the increasing number of nanoparticles produced. Silver nanoparticles are known to have a characteristic band of surface plasmon resonance at ~430 nm that can be measured for silver nanoparticles using UV-vis spectroscopy (Rajesh *et al,* 2013). For silver nanoparticles prepared using the culture supernatant, a solid, large peak between 420 nm and 440 nm was observed in the UV-vis spectrum (Sastry *et al,* 1997; Sastry *et al,* 1998). AgNPs formed by *A. fumigatus* DSM819 and *Aspergillus fumigatus* BTCB10 (KY486782) have a characteristic band of surface plasmon resonance at 400 nm (Shahzad *et al,* 2019) and 410 (Othman *et al,* 2019), respectively. For different metal nanoparticles with sizes ranging from 2 nm to 100 nm, observation of this peak assigned to a surface plasmon is well recorded (Sastry *et al,* 1997; Sastry *et al,* 1998). A graph inset in the Figs with absorbance at 450 nm, vs. supernatants harvest time (6, 12, 18, 24 and 72 hours) indicates that there is a linear increase in the former with respect to the latter (Fig 2a and 2b) (Othman *et al,* 2019).

The TEM images of silver nanoparticles synthesised using *E. coli* ATCC 8739 and *A. fumigatus* culture supernatants were shown in Fig 3 (A and B), respectively. The results obtained from the TEM analysis (Fig 3) provide a strong indication of the nanoparticles' shape and scale. Most of the particles were spherical in shape, as seen in the picture, and

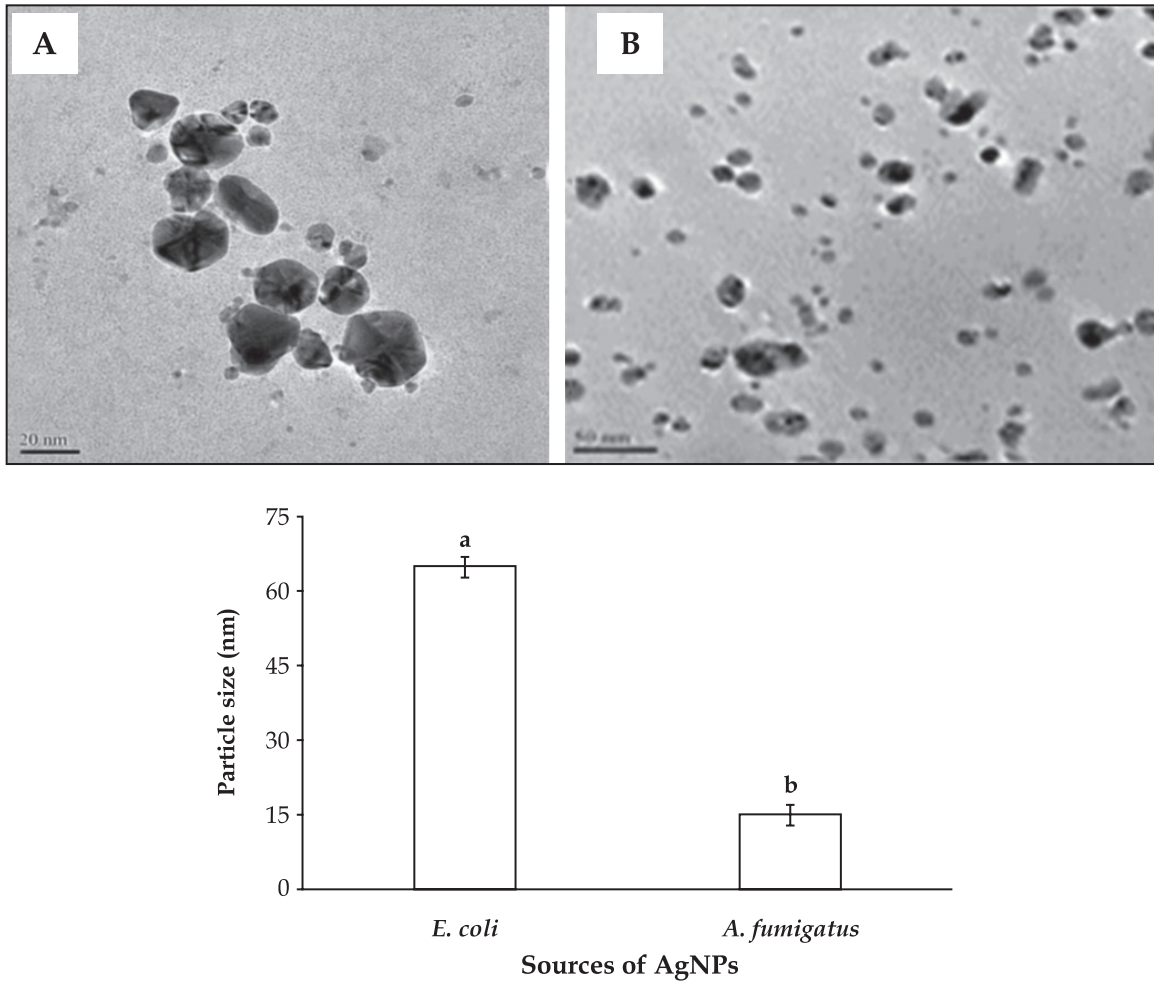

Fig 3. (A) shows TEM electron micrographs of silver nanoparticles produced by *E. coli* with average diameter of 64.9 nm and (B) produced by *A. fumigatus* with variations in diameter ranged from 3.4-26.8nm. Values are expressed as mean ± standard deviation (SD; n=5). Data provided by statistical computer system of TEM.

seemed to be fairly monodispersed (Othman *et al,* 2019). The particle size was ranged from 3.4 to 26.8 nm in diameter for *A. fumigatus* and 64.9nm for *E. coli*. Such variations in the shape and size of biologically synthesised nanoparticles are common (Bhainsa and D'Souza, 2006). Furthermore, the TEM images display at least two different regions, one with a higher contrast due to silver nanoparticles and the other with a lower contrast due possibly to insoluble silver nanocrystals, salts. The majority of silver nanoparticles were scattered, with only a few of them exhibiting aggregates of various sizes (Fig 3A and 3B). The results obtained from TEM studies (Fig 3) provide a strong indication of the nanoparticles' shape and scale. The sizes of the *E. coli* synthesised AgNPs (64.9nm) were higher than that recorded for *Bacillus licheniformis* (40 nm) (Kalishwaralal *et al,* 2008) and was approximately like that observed for *Escherichia coli* ATCC 8739 (El-Shanshoury *et al,* 2011). The sizes of the *A. fumigatus* synthesised AgNPs (64.9nm) were higher than that described earlier which was in the range of 5–25 nm (Bhainsa and D'Souza, 2006). However, the sizes of the *A. fumigatus* synthesised AgNPs (64.9nm) were lower than that of *Aspergillus fumigatus* DSM819 which was in the range of 84.4 nm (Othman *et al,* 2019).

The particle size distribution of fungal and bacterial AgNPs obtained by a zetasizer Nano ZS is shown at Fig 4. The particle average size for AgNPs produced by *A. fumigatus* and *E. coli* was 125.3 and 182.9nm, respectively. This finding indicate that the AgNPs produced by *A. fumigatus* was smaller than that of *E. coli*. This may the reason stand behind the observed rapid change in colouration of sliver nitrate in case of *A. fumigatus* than that of *E. coli*.

The antimicrobial activity of silver nanoparticles formed by either *A. fumigatus* or *E. coli* showed

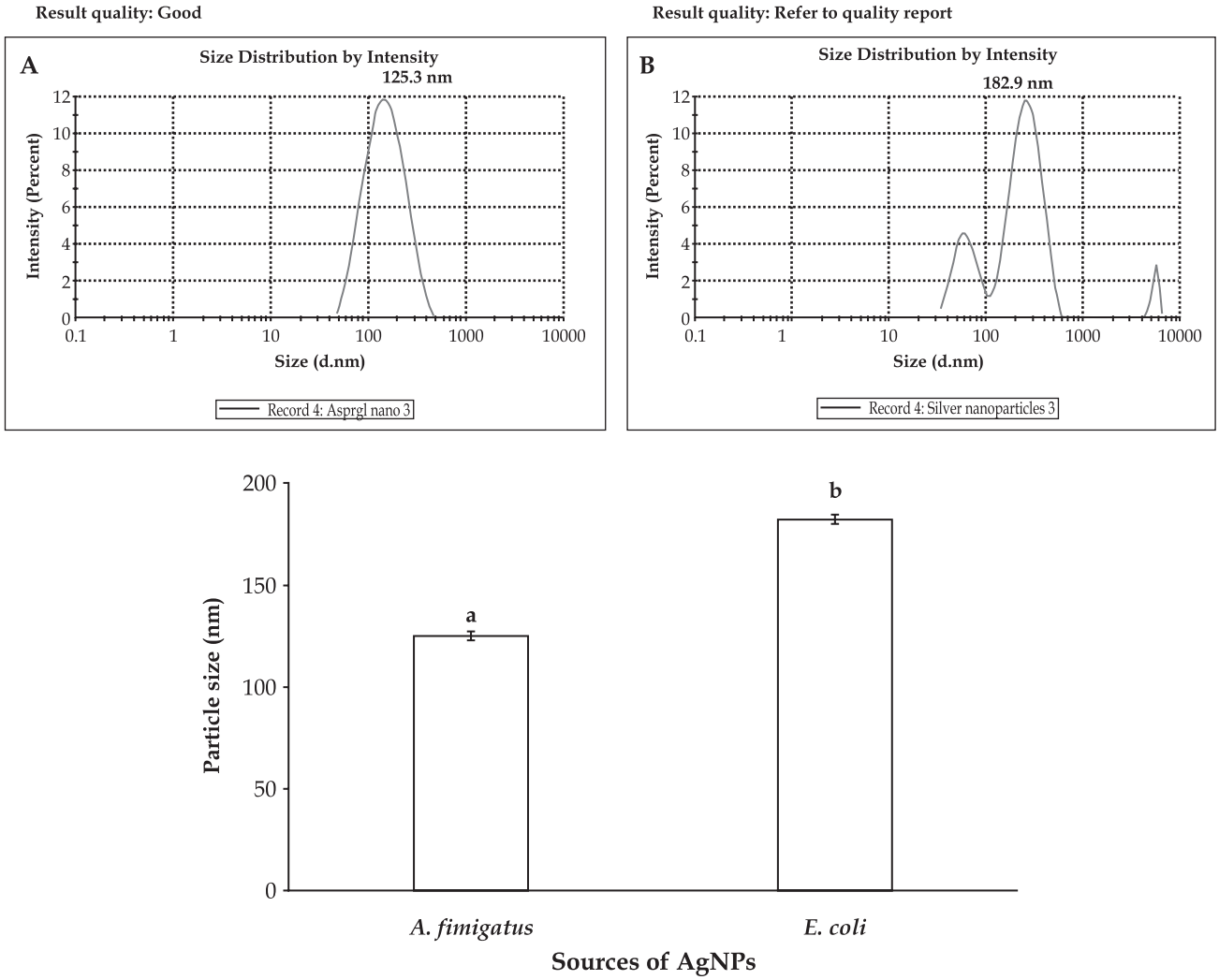

Fig 4. Size distribution of the particles was estimated using Laser particle analyser (LPA) images by measurement of diameters provided by computer programming system of Zetasizer (Malvern Instruments, Southborough, UK). (A) *A. fumigatus* and (B) *E. coli* cell filtrate reaction with 1mm $AgNO_3$ solution.

different inhibitory effect on different pathogenic fungus (*Trichophyton verrucosum*, *Trichophyton mentagrophytes* (ATCC 52015), *Trichophyton rubrum* (ATCC 52020). *Trichophyton tonsurans, Microsporum canis, Microsporum equirum, Candida albicans, Aspergillus fumigatus*) or bacteria (*Escherichia coli* and *Staphylococcus aureus*) as reflected on inhibition zone of different size (Table 1). Most of the precipitated silver nanoparticles displayed antibacterial and antifungal activity with varying magnitudes (Table 1). This dissimilarity may be attributable to the numerous interactions of nanoparticles with the studied microorganisms. The diameter (mm) of inhibition zone of AgNPs produced by *A. fumigatus* was significantly larger than that of AgNPs produced by *Escherichia coli* and relevant antibiotics (Tetracycline 30µg and Ampicillin 10 µg) and antifungal (Fluconazole 25µg) for all studied microorganisms. Minimum inhibitory concentration (MIC) of AgNPs produced by *A. fumigatus* was significantly lower than that of AgNPs produced by *Escherichia coli* and relevant antibiotics (Tetracycline 30µg and Ampicillin 10µg) and antifungal (Fluconazole 25µg) for all studied microorganisms. The efficient antimicrobial effect of AgNPs produced by *A. fumigatus* over that produced by *Escherichia coli* may be attributed to the smaller size of the former than that of the latter. Definitely smaller size of AgNPs produced by *A. fumigatus* perhaps plays a major role in the penetrating potential and subsequent antimicrobial effect. Synthesised AgNPs from *A. fumigatus* BTCB10 exhibited antibacterial activity against multidrug-resistant bacterial strains, notably, *Klebsiella pneumoniae* BTCB04, Acinetobacter BTCB05,

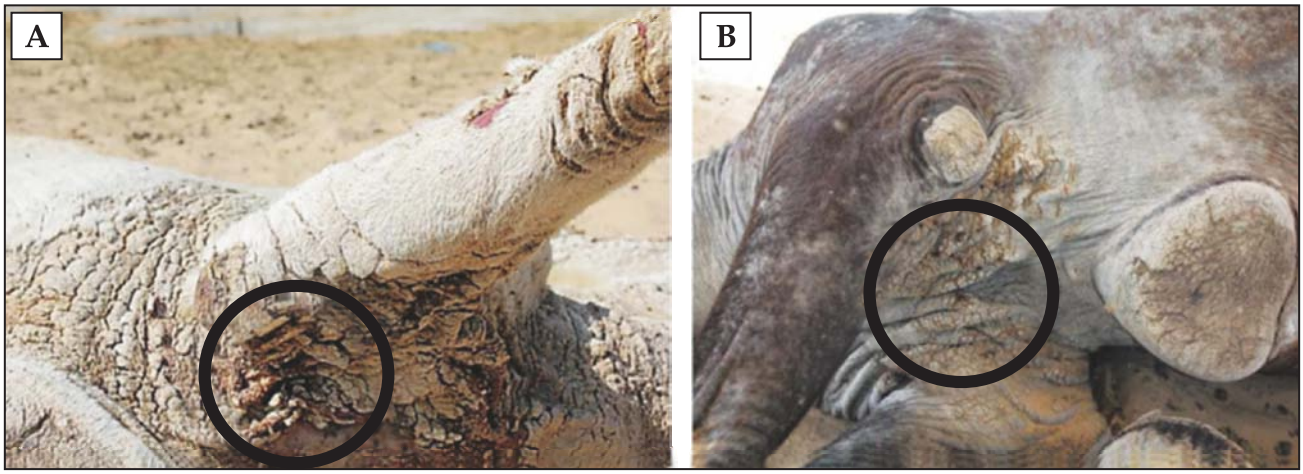

Fig 5. Experimental infection of a healthy she camel by culture of *Trichophyton verrucosum* isolated from the natural infection. (A) Alopecia, erythema, numerous small subcutaneous nodules and brownish blackish crusts with hyperkeratosis of infected she camel. (B) A pronounced relieve and disappearance of lesions after treatment with current prepared AgNPs.

Pseudomonas aeruginosa BTCB01, and *Escherichia coli* BTCB03, while maximum 7-fold was observed with Acinetobacter BTCB05 (Shahzad *et al,* 2019). The synthesised AgNPs using *A. fumigatus* mycelia extract indicated a high antibacterial activity against both Gram-positive and Gram-negative bacteria (Ghanbari *et al,* 2018). The mechanism of silver ions' inhibitory action on microorganisms is not fully clarified. DNA is believed to lose its replication capability and cellular proteins are inactivated after treatment with Ag+ (Feng *et al,* 2000). In addition, Ag+ has also been shown to bind to functional protein groups, leading to protein denaturation (Feng *et al,* 2000). A bacterial membrane displays a large increase in permeability when *E. coli* bacteria are treated with highly reactive metal oxide nanoparticles, leaving the bacterial cells unable to adequately control transport through the plasma membrane and finally causing cell death (Sondi and Matijević, 2003; Sondi and Salopek-Sondi, 2004; Stoimenov *et al,* 2002). The development of irregular-shaped pits in the outer membrane and altered membrane permeability may be caused by metal depletion. This is caused by the progressive release of lipopolysaccharide molecules and membrane proteins (Amro *et al,* 2000). Fungal and bacterial silver nanoparticles showed antifungal and antibacterial activities compared to the corresponding antifungal (fluconazole) and antibacterial (tetracycline and ampicillin) drugs. However, the best antifungal and anti-bacterial activities recorded to fungal sliver nanoparticle over the bacterial one, which may be attributed to its smaller particle size and higher surface area to volume ratio accordingly (Morones *et al,* 2005). Experimental infection with *Trichophyton verrucosum* induced alopecia, erythema, numerous small subcutaneous nodules and brownish blackish crusts with hyperkeratosis of infected she camel (Fig 5A). The treatment with current prepared fungal AgNPs induced a pronounced relieve and disappearance of lesions (Fig 5B). Fungal AgNPs was used for the treatment of experimental fungal infection because it was more efficient than that of *E. coli* due to its smaller particle size and subsequent higher penetrating ability as indicated in the current study. The synthesised AgNPs by *Aspergillus niger* were efficient in inhibiting various pathogenic organisms, including bacteria and fungi in human (Sagar and Ashok, 2012). However, long scale studies are recommended to investigate the most effective dose of fungal AgNPs against different fungal infection.

The current study concluded that *A. fumigatus* and *Escherichia coli* have shown potential for extracellular silver nanoparticles synthesis in the range of 3.4–26.8 and 64.9nm, respectively. The synthetic process of AgNPs by either *A. fumigatus* or *Escherichia coli* was quite fast and more pronounced in case of *A. fumigatus*. For the creation of a biological process for mass scale processing, rapid synthesis of nanoparticles would be sufficient. Both fungal and bacterial synthesised AgNPs has antibacterial and antifungal effect even against their own pathogens. However, *A. fumigatus* AgNPs was more efficient than that of *Escherichia coli* due to smaller particle size, which may induce higher penetrating ability. Current prepared AgNPs induced a pronounced relieve and disappearance of skin lesions that resulted from experimental infection with *Trichophyton verrucosum*.

Long scale studies are recommended to investigate the most effective dose of AgNPs against different fungal infection.

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QUALITY CHARACTERISTICS OF BACTRIAN CAMEL (*Camelus bactrianus*) MEAT BURGER AND EVALUATING ITS STABILITY DURING THE STORAGE

Dalai Siren[1,2], ZhengQi Yang[1], Li Yi[1,2], Liang Ming[1,2*] and Rimutu Ji[1,2*]

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

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

ABSTRACT

Camel meat is a kind of lean meat with a high animal protein content, which has a lower fat and cholesterol content than other animal meat. Using camel meat as raw material, we determined the optimal processing craft of the camel meat burger, followed by an analysis on the quality changes of the burger under storage conditions of 4 and -20°C. After response surface optimisation, the best formula for the camel burger was found to be a 1:5 ratio of fat to lean, with 15% ice water and 0.5% isolated soybean protein. The experimental storage results of the camel burger showed that, under the storage condition of -20°C for 3 months, the variation in the moisture activity value, pH value, thiobarbituric acid reactive substances value, colour value, flavour, etc., were smaller than that under the storage of 4°C, which could better extend the shelf life of the product.

Key words: Bactrian camel meat, burger patty, quality characteristics, shelf life

Bactrian camels are a suitable source of milk and meat for the population of east and central Aria. Bactrian camel milk has been extensively exploited for human health (Amanat *et al,* 2019), and processed into industrial dairy products such as milk powder, yoghurt, and ice cream. Abdel-Naeem and Mohamed (2016) found that addition of ginger extract and papain powder during formulation of camel burger patties can improve their physico-chemical and sensory properties. Al-Juhaimi *et al* (2018) investigated the phytochemical composition and antioxidant activity of Argel leaf powder (ALP) and its effect on the quality attributes of camel patties and found that it improved shelf life and product quality. However, there are limited reseach on bactrian camel meat, especially, the development of camel meat products in China (Park and Young, 2009).

Camel meat varies in composition according to breed type, age, sex, feeding condition, and site on the carcass (Kadim *et al,* 2006). Similar to the meat of other ruminants, camel meat is rich in moisture contents, about 70-77% moisture (Al-Owaimer, 2000), and has a good water holding capacity, hence it possess good processing properties (Babiker and Yousif, 1990) that can be recommended as an important raw material for the production of various meat products (Abdel-Naeem and Mohamed, 2016). In addition to high level of vitamins, especially vitamin B complex makes camel meat a healthy food for humans (Kadim *et al,* 2008), as well as good quality proteins, about 20-23% (Kadim *et al,* 2006), especially essential amino acids, which makes it a good source of high quality protein in arid and semi-arid regions. At the same time, camel meat contains low fat content with relatively high polyunsaturated fatty acids, and low level of cholesterol, which makes camel meat considered as a healthy option for patients with cardiovascular disease (Kadim *et al,* 2008; Raiymbek *et al,* 2019).

Recently, due to the rapid increase in consumer demand for healthy fast food, many efforts have been taken to improve the quality and stability of burgers (Papadima and Bloukas, 1999), with camel meat being one of the best candidates due to their high level of nutrition and low level of fat and cholesterol content. Some authors have reported that dromedary meat can be used to successfully in cooked burger patties (Kadim *et al,* 2008; Heydari *et al,* 2016), however, research on bactrian camel meat burger is scarce.

SEND REPRINT REQUEST TO LIANG MING; RIMUTU JI email: bmlimau@163.com; yeluotuo1999@vip.163.com

The goal of the present study was to evaluate the use of bactrian camel meat in order to produce a meat burger and to assess its stability during 3 months of storage at 4°C and -20°C.

Materials and Methods

Bactrian camel lean meat and hump fat were obtained from 3 animals (2 years old), which were slaughtered at a local abattoir (Alashan, Inner Mongolia, China). The meat and fat were vacuum packed and rapidly transported to the laboratory and maintained in freezer at -20°C until processed.

Products formulation

The main process and key influencing factors of the processing of camel meat burger patties are the proportion of lean and fat, ice water, and isolated soy protein addition. Based on previous study, the three-level-three-factor BBD (Box-Behnken designs) for the proportion of lean and fat, ice water, and isolated soy protein were carried out with the sensory score as the response value. Each group of tests was made in three parallels, and the average value was taken as the response value. The level code of each factor was shown in Table 1. The experimental design and results were shown in Table 2, in which the response value Y represents the sensory score.

Table 1. The factor level of Box-Behnken experiment.

Factors	A	B	C
Levels	Proportion of lean and fat (%)	Ice water (%)	Isolated soy protein (%)
-1	10	10	0.3
0	20	15	0.5
1	30	20	0.7

A: Proportion of lean and fat (%); B: Ice water (%); C: Isolated soy protein (%).

After the addition of the camel lean and fat, ice water, isolated soy protein, the treatment were mixed with salt (2%), sugar (1%), paprika (0.2%), ginger powder (0.2%), onion (3%), pepper (0.2%), and soy sauce (0.2%) for burger.

Burger processing and storage

Three independent replicates for burger formula were processed. After thawing overnight in a cooler (4°C), the camel meat and fat were ground through a 5mm plate grinder (sxc12/22, China), and were mixed together with water, isolated soy protein, salt, sugar, onion and other seasonings. This mixture was shaped using a commercial burger maker to obtain patties of approximately 90g, and the dimensions of 10cm diameter and 1cm thickness. Then the burger patties were placed in polyethylene packages and stored at -20°C for 3 months. For each replicate, samples were withdrawn for analysis at 1st day (0-time) and every one month.

Table 2. The experimental design and results of Box-Behnken.

Run	A	B	C	Y
1	30.00	15.00	0.70	61.00
2	20.00	20.00	0.70	69.00
3	30.00	15.00	0.30	64.00
4	20.00	15.00	0.50	88.00
5	20.00	15.00	0.50	86.00
6	20.00	15.00	0.50	87.00
7	10.00	15.00	0.70	64.00
8	20.00	10.00	0.70	67.00
9	10.00	10.00	0.50	62.00
10	20.00	15.00	0.50	87.00
11	10.00	15.00	0.30	65.00
12	20.00	15.00	0.50	87.00
13	30.00	10.00	0.50	65.00
14	30.00	20.00	0.50	61.00
15	20.00	20.00	0.30	70.00
16	10.00	20.00	0.50	68.00
17	20.00	10.00	0.30	69.00

A: Proportion of lean and fat (%); B: Ice water (%); C: Isolated soy protein (%).

Sensory evaluation

According to the experimental design (Table 2), sensory analysis was performed by 30 experienced panelists who were recruited from the staff and students of the Food Science and Engineering College, Inner Mongolia Agricultural University, Inner Mongolia, China. Panelists were selected on the basis of previous experience in consuming traditional burgers. The whole process of the sensory experiment was carried out in a sensory laboratory at the University. Rectangular pieces of approximately 1.5-2cm were prepared from the centre of burger patties and served at room temperature. The burger patty samples were provided to each panelist randomly, and three replicates of all of the experimental designs were evaluated. Tap water was provided between the samples to cleanse the palate. The evaluation considered juiciness, texture, flavour, and colour and asked participants to assign a numerical value from 1 to 25, in which the highest score of 25 expressed extremely acceptable, and 1 represented extremely unacceptable. At the end of the test, panelists were asked to give a score to each indicator (juiciness,

texture, flavour and colour), and the total score was from 0 to 100.

Burger patties analysis

The proximate chemical analysis of camel raw meat and burger patty, and microorganism indicators were determined at 0-time only. Meanwhile, water activity (Aw), pH, thiobarbituric acid reactive substances (TBARS), and colour values were evaluated at 0-time, and every month for 3 months, at the 4°C and -20°C storage temperature, respectively. In addition, the electronic tongue values and aerobic plate counts were also calculated at 0-time and every one month for 3 months at storage temperatures of 4 and -20°C, respectively. The burgers were thawed in a chiller at 4°*C* before analysis.

The proximate chemical composition

The moisture, crude protein, crude fat, and ash contents of bactrian camel meat and burger patties were determined for three replicates, according to the procedure described by the National Food Safety Standard of China. In brief, moisture was determined using the direct drying method in GB (GuoBiao, namely national standard) 5009.3-2016. The protein content was assessed using the Kjeldahl method (automatic kjeldahl nitrogen analyser, K9860, China) in GB 5009.5-2016, and the Soxhlet extraction method was evaluated for measuring the fat content (GB 5009.6-2016). Finally, the ash content was determined by ashing the samples in a muffle furnace (SX2-4-10, China) at 500°C for 24h (GB 5009.4-2016).

pH and Water activity (Aw)

The pH and water activity values were determined after processing as well as every month for 3 months at storage temperature of 4 and -20°C, through the methods of GB/T9695.5-2008 and GB/T9695.19-2008, and where three readings for each sample were obtained and the mean was calculated. Briefly, the pH value was measured with a pH meter (PB-10, China) on a suspension, resulting from blending a 15g sample with 150mL deionised water for 2min, while the water activity value was assessed in the intelligent water activity meter measuring instrument (HD-3A, China).

Lipid oxidation (thiobarbituric acid test)

The thiobarbituric acid reactive substances (TBARS) value was measured by the method described by Du and Ahn (2002), after processing and every month for 3 months at 4°C and -20°C storage temperatures, respectively. The mixture solutions (10g burger patties, trichloroacetic solution, and TBA solution) were heated for 40 min in a 90°C water bath (DK-S28, China), cooled under running water, and the supernatant was obtained at 5500rpm for 25min in a centrifuge (5811FN279354, German). The supernatant absorbance was measured at 532nm and 600nm using a UV spectrophotometer (Cambridge, U.K.), and absorbance values of A532 and A600 were recorded, respectively. Finally, the TBA value was calculated using the following formula: TBA (mg/100g) = (A532-A600)/155×(1/10)×72.6×100.

Colour evaluation

The surface colour of the burger patties was assessed using a colorimeter (TCP2, China) calibrated with a white plate and light trap supplied by the manufacturer. Three readings were taken on the burger patty surface, and a mean value was processed. The CIELAB Colour System 1976 (Allais *et al,* 2010) Colour space values (a* for redness, L* for lightness, and b* for yellowness) were assessed using a colorimeter (Konica Minolta, CR-400- Japan; Measuring aperture: 8mm; Illuminant: CIE D65; Observer angle: CIE 2◦ Standard Observer) (Mancini and Hunt, 2005).

Microbiological analysis

Microbiological analysis was completed after the samples were cooked in order to examined the hygienic quality of the burger patty processing according to the food microbiological examination from national food safety standard (China). The samples (25g) were homogenised with a 225mL phosphate buffer (Tianjin Yongda Chemical Reagent Co., Ltd., China) for 1-2min in order to obtain the liquid sample homogenate. Then, further serial dilutions were prepared for microbial determinations (GB4789.2-2010). Finally, the aerobic plate counts were determined on Plate Count Agar.

The enumeration of coliforms (*E. coli*) was counted using the multiple-tube fermentation test and was expressed as the most probable number (MPN)/g sample (GB4789.3-2010).

Salmonella testing was performed by a pre-enrichment with an apeptone water buffer and then enriched samples were applied to the 1-2 test, according to the manufacturer's directions from GB4789.4-2010.

The samples (25g) were homogenised with a 225mL sodium chloride broth (7.5%) for 1-2min, and were incubated at 36°C for 18-24h. Then, the above cultures were inoculated on Baird-Parker plates

and were cultured at 36°C for 18-24h to completed *Staphylococcus aureus* testing (GB4789.10-2010).

The samples (25g) were homogeneised with 225mL Shigella Enriched Broth (Tianjin Yongda Chemical Reagent Co., Ltd., China) for 1-2min, and were incubated in an anaerobic environment at 42°C for 16-20h. Then, the *Shigella's* enrichment solution was inoculated on a Xylose Lysine Deoxycholate (XLD) agar plate, and MacConkey (MAC) agar plate, and cultured at 36°C for 20-24h to complete the *Shigella* testing (GB4789.5-2010).

Electronic tongue

An electronic tongue (SA402B, Insent Company, Japan) was used to analyse the prepared camel meat burger patty samples, and the analysis sensor detected spicy, sweet, salty, sour, bitter, and umami in the burger patties (Charles *et al,* 2017; Schlossareck and Ross, 2019). Prior to the analysis, the sensors were hydrated in 25mL Milli-Q water for 24h. A sample of 30g of burger patty was diluted with water at a ratio of 1:5, and the fat impurities were removed through centrifugation (5000rpm, 10min). Finally, the supernatant was measured using an electronic tongue. During the analysis, the sensors were rinsed in 25mL of Milli-Q water for 10s between each sample (Charles *et al,* 2017).

Statistical analysis

The statistical data analysis for the three independent replicates was carried out using SPSS® software program version 21 (SPSS, Chicago, IL, USA) for Windows. Design of Expert 8.0.6 (DOE Version 8.0.6, StatEase. Inc, Minneapolis, MN, USA) was used for the experiment design, graph construction, and results analysis. A difference was considered significant at $p < 0.05$. Data were expressed as mean ± standard deviation.

Results and Discussion

The experimental design and regression analysis

The three-level-three-factor Box–Behnken designs (BBD) for the proportions of lean and fat, ice water, and isolated soy protein were carried out with the sensory score as the response value. The experimental design and results are shown in Table 2. Furthermore, based on the results of the BBD, the square regression analysis of the response value Y (sensory value) was implemented, and the quadratic polynomial regression equation of Y was obtained as follows:

$$Y = -121.28 + 6.425A + 11.65B + 231.25C - 0.05AB - 0.25AC + 0.25BC - 0.14125A^2 - 0.355B^2 - 234.375C^2$$

In the equation, Y was the sensory value of the camel meat burger patties, and A, B, and C represented the proportion of lean and fat, ice water, and isolated soy protein, respectively. The Y response surface regression model was extremely significant ($p<0.0001$), and the linear relationship between the dependent variable and all of the independent variables were significant ($R2=0.9987$), while the lack of fit was not significant ($p>0.05$) . The equation correction coefficient Adj $R2=0.9971$ demonstrated that the change of the response surface of 99.71% could be explained by this model, and there was a good fit between the experimental data and the regression equation. Therefore, the regression equation model was established, and it is appropriate to use this model to predict the sensory value of the camel meat burger patties. Finally, the camel meat burger patties were comprehensively optimised to be proportions of lean and fat of 20%, ice water of 15%, and isolated soy protein of 0.5%. At this time, the comprehensive evaluation of the sensory value was 88 (Table 2).

The chemical analysis of raw camel meat and burger patties

The results of the proximate chemical composition of the camel meat and camel meat cooked burger patties based on the optimal formula design were presented in Fig 1. The content of the moisture, protein, fat, and ash from the raw camel meat were 75.50%, 22.58%, 1.64%, and 1.33%, respectively. Among them, the content of the moisture, protein, and ash were similar to that of dromedary camel meat, while the content of fat was slightly lower than that in dromedary meat (Al-Owaimer *et al,* 2014), which is related to the bactrian camel meat samples collected. In this experiment, we used camel meat from a 2 year old bactrian camel, and studies have shown that the fat content of camel meat may increase with age (Kadim *et al,* 2008). Therefore, the young age of the bactrian camel leads to less fat in its meat. In the cooked camel burger patties, the protein and fat content were increased, by 40.47 and 24.30%, respectively. During cooking, some soluble proteins were separated from the meat, and the high fat content was mainly related to the content of the proportion of fat and lean in the formula. The aerobic plate counts and *E. coli* in the camel burger patties were lower than that of the national standard, which indicated our products had a good quality.

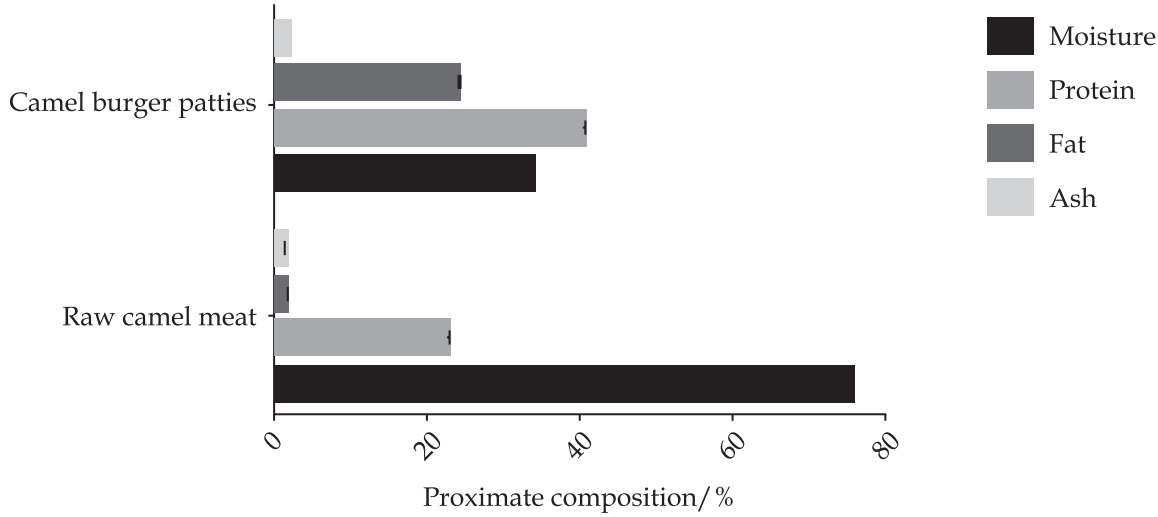
Changes in the quality of camel burger patties during storage

Water activity (Aw), pH, and thiobarbituric acid reactive substances (TBARS) values

The Aw, pH, and TBARS values were calculated after processing and monthly during storage. Under storage conditions of 4°C and -20°C, the Aw and pH values had a downward trend (Table 3). The Aw and pH values for all of the storage time were significantly ($p< 0.05$) lower than those of the control samples during the 4°C storage time; whereas, there was no significant ($p<0.05$) difference among the values of 0-time and after the first month at the -20°C storage time. The slight change in pH values of the treated burger patties may be attributed to the effect of these enzymes on the ionic strength of the meat.

Water activity (Aw) refers to the degree of water binding. There is an inverse relationship between water activity and degree of binding; the higher the water activity value, the lower the degree of binding. The value of the water activity is directly related to the growth rate of microorganisms. With the extension of the storage time, the degree of water binding and the growth rate of the microorganisms were increased in this study.

The degree of lipid oxidation is one of the indicators reflecting fat oxidation. The TBARS value can be used to indicate the degree of fat oxidation (Fernández *et al*, 1997). In present study, the TBARS values of different storage times were significantly ($p< 0.05$) lower than that of the control during frozen storage for 3 months. With the extension of the storage time, the fat oxidation increased slowly in the camel meat patties, and compared with the condition at -20°C, the degree of fat oxidation was slightly lower than that at 4°C. In addition, the rate of TBARS evolution during the storage time was



Values represent the mean of 3 independent replicates ± SD.

Fig 1. Proximate chemical composition of raw camel meat and camel burger patties.

Table 3. The value of Aw, pH and TBARS from camel meat burger patties during storage at 4°C and -20°C for 3 months.

Treatments	Storage time (days)/4°C			
	0-time	1st month	2nd month	3rd month
Aw	0.885±0.003^{a}	0.866±0.005^{b}	0.833±0.002^{c}	0.817±0.002^{d}
pH	6.460±0.000^{a}	6.280±0.010^{b}	5.960±0.010^{c}	5.817±0.006^{d}
TBARS	0.331±0.004^{a}	0.932±0.031^{b}	1.961±0.046^{c}	3.867±0.047^{d}
	Storage time (days)/-20°C			
Aw	0.885±0.002^{a}	0.884±0.002^{a}	0.872±0.002^{b}	0.867±0.002^{c}
pH	6.460±0.000^{a}	6.440±0.000^{b}	6.387±0.006^{c}	6.210±0.000^{d}
TBARS	0.331±0.004^{a}	0.597±0.009^{b}	1.014±0.059^{c}	1.621±0.016^{d}

Aw: water activity; TBARS: thiobarbituric acid reactive substances; a-b Means with different superscripts within the same row are significantly ($p < 0.05$) different. Values represent the mean of 3 independent replicates ± SD.

faster than that reported for dromedary meat stored under refrigeration conditions (Abdel-Naeem and Mohamed, 2016). It could be due to the mincing process (which favours the oxygen access).

Colour evaluation

The myoglobin and haemoglobin in the muscle mainly determine the colour quality of the meat. The colour of the surface of the meat and meat products contain L* (lightness), a* (redness), and b* (yellowness) (Kadim *et al,* 2006). The larger the value of L*, the better the brightness of the meat, and the larger the value of a*, the greater the redness of the meat. The measurement results of the changes in colour during the storage of the camel meat burgers are shown in Table 4. Under 4°C storage conditions, the different storage times had a significant ($p < 0.005$) effect on the colour value (L* and a*) of the camel meat burger patties, which indicated that a higher storage temperature had a great influence on the colour brightness and redness of meat; while the b* values of 0-time were significantly ($p < 0.05$) lower than that of the other groups (1st-3rd month), which suggested that the extended storage time had no great effect on the yellowness of the meat. Under -20°C storage conditions, during the first two months of storage, the L* value of the meat increased slowly, but there was no significant ($p > 0.005$), while a significant difference ($p< 0.05$) existed between the 3rd month and other storage times, which showed that under lower storage temperature, the colour change range of meat can be extended more efficiently.

Microbiological analysis

After the camel meat burger patties were vacuum packaged, the aerobic plate counts were calculated under the normal temperature storage, storage conditions of 4 and -20°C. After the 1st month, the higher aerobic plate counts of the normal temperature were significantly different ($p < 0.005$) compared with the other storage conditions (2nd month and 3rd month). During the second storage month, the aerobic plate counts exceeded the measurement range at the normal temperature storage conditions, while the aerobic plate counts stored at 4 and -20°C were 8.1×10^4 and 1.4×10^4, respectively. During the third storage month, the aerobic plate counts exceeded the measurement range

Table 4. The colour components of camel meat burger patties during storage at 4°C and -20°C for 3 months.

Treatments	Storage time (days)/4°C			
	0-time	1st month	2nd month	3rd month
L* (lightness)	47.673±1.685^a	50.873±1.183^b	42.620±0.040^c	35.590±0.682^d
a* (redness)	13.497±1.034^a	11.130±0.551^b	5.500±0.241^c	3.457±0.411^d
b* (yellowness)	10.727±0.827^a	15.283±1.845^b	16.117±1.102^b	17.867±0.516^b
	Storage time (days)/-20°C			
L* (lightness)	47.673±1.685^a	48.077±0.827^a	50.227±0.699^a	39.490±0.775^b
a* (redness)	13.497±1.034^a	11.573±0.432^b	11.430±0.131b^c	7.907±0.898^d
b* (yellowness)	10.727±0.827^a	13.967±0.492^b	15.480±0.624b^c	16.967±0.293^c

Table 5. Electronic tongue result of camel meat burger patties during storage at 4°C and -20°C for 3 months.

Treatments	Storage time (days)/4°C			
	0-time	1st month	2nd month	3rd month
Umami	14.580±0.010^a	12.287±0.005^{ab}	10.740±0.010^{ab}	9.333±0.005^b
Bitterness	-5.403±0.005^a	-2.953±0.005^{ab}	-1.083±0.005^{ab}	-0.897±0.005^b
Astringency	-12.183±0.005^a	-11.250±0.010^{ab}	-4.253±0.015^{ab}	-4.010±0.010^b
Saltiness	29.193±0.005^a	26.403±0.005^{ab}	14.687±0.005^{ab}	11.380±0.010^b
Sourness	-21.620±0.010^a	-20.257±0.028^{ab}	-14.960±0.026^{ab}	-12.327±0.006^b
	Storage time (days)/-20°C			
Umami	14.580±0.010^a	11.413±0.015^{ab}	12.450±0.010^{ab}	11.237±0.006^b
Bitterness	-5.403±0.006^a	-4.830±0.040^b	-2.733±0.006^c	-2.557±0.006^d
Astringency	-12.183±0.006^a	-11.247±0.006^b	-9.973±0.006^c	-6.567±0.006^d
Saltiness	29.193±0.006^a	26.397±0.006^b	17.737±0.006^c	14.047±0.012^d
Sourness	-21.620±0.010^a	-19.887±0.031^b	-18.737±0.031^c	-16.437±0.006^d

at the 4°C storage conditions. From the record of the aerobic plate counts, it can be concluded that the shelf life of our camel meat patty product was only about 30 days at normal temperature, and could be extended to 60 days under low temperature storage at 4°C, and could be extended to more than 90 days under a -20°C storage temperature.

Electronic tongue analysis

The electronic tongue was used to detect the five taste sensor signals of camel meat burger patties, namely: umami, bitter, astringent, salty, and sour during storage at 4 and -20°C (Table 5). Each sensor of the electronic tongue can respond to the camel meat burger patties in different storage periods at different storage conditions. Different signal response values indicated that the sensors have different sensitivities to camel meat burger patties in different storage periods. Among them, the signal response value of each group was the weakest at 0-time, and the signal response at the end of storage was the strongest. During the storage period of 4°C, the response value of umami gradually decreased with the increase of days, reaching 9.333 at the 3rd month; the response value of bitterness gradually increased, and finally reached -0.897; the response value of astringency increased slowly within 0-30 days, and then increased significantly after the 1st month. During the storage period, vacuum packaging and ice temperature storage can better maintain the original flavour of the product. During the storage period of -20°C, the response values of the five taste sensors changed very little. The response value of umami gradually decreased with the increase in days. The response value of the bitterness gradually increased, and the change trend was relatively stable, and finally reached -2.557. The response value of the astringent taste increased slowly; the response value of salty taste decreased from 29.193 to 14.047 after the 3rd month. During the storage period of -20°C, the change of the signal value of each sensor was less than that of 4°C, indicating that under storage of -20°C, the flavours of the camel meat burger patties can be better preserved.

Conclusions

The results from this study indicated that the manufacture of burgers from Bactrian camel meat is a viable option for an industry that has largely released its products to the fresh meat market. The results of the camel meat burger patties process conditions showed that the amount of fat and lean ratio had a greater impact on the meat texture and elasticity; the addition of isolated soy protein had a significant effect on the meat chewability; and the addition of ice water had a great effect on the hardness, taste, and shaping of the camel burger patties. After optimising the response surface, it was determined that the optimal ratio was 20% for the fat and lean ratio, 0.5% for the isolated soybean protein, and 15% for ice water. When exploring the best storage method, the water activity value, thiobarbituric acid value (TBARS) value, and colour components of the storage time within 3 month of storage at -20°C were lower than the storage of 4°C, which indicated that the storage condition of -20°C made it easier to extend the shelf life, and better for retaining the flavour of the camel meat burger patties.

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical guidelines

Ethics approval was not required for this research.

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PROTECTIVE EFFECTS OF URINE AND MILK OF CAMEL ON ISONIAZID AND RIFAMPICIN INDUCED HEPATOTOXICITY

S. Benhizia[1], N. Arzour[1], F. Bouldjenib[2] and A.B. Lakehal[3]

[1]Animal Pathology Research Laboratory PADESCA. Institute of Veterinary Sciences, El Khroub, Constantine, Algeria
[2]Department of Pathological Anatomy, [3]Department of Epidemiology,
University Hospital Centre IBN BADIS, Constantine, Algeria

ABSTRACT

The objective of the present study was to study the hepatoprotective effects of camel milk and urine in 5 groups of 8 rabbits. The first group I received saline solution (control), animals in group 2 received daily isoniazid (50 mg/Kg/d) with rifampicin (100 mg/Kg/d) orally during 10 days. Rabbits in groups 3, 4, 5 received isoniazid (50 mg/kg/day) and rifampicin (100 mg/kg/d) with added milk, urine, and a mixture of camel milk and urine, respectively. Camel milk was administered @ 33 ml, kg bodyweight, day with oral gavage using a nasopharyngeal catheter. Urine was administered @ 20 ml, kg bodyweight, day. Plasma levels of bilirubin, and activities of ALAT, ASAT and PAL were measured. Histological variations on liver tissues were also described. Group 2 rabbits showed a non-significant increase in plasma ALAT and ASAT levels and a very significant increase in bilirubin and PAL. Histological sections of liver of rabbits in group 2 showed signs of hepatocyte suffering, these biochemical changes were reversed in groups 3, 4 and 5 animals compared to the group 2. Histological variations were also reduced in animals receiving camel milk and urine mixture. Camel milk and urine thus have protective effects on hepatotoxicity induced by isoniazid-Rifampicin combination.

Key words: Camel, hepatotoxicity, isoniazid-rifampicin, milk, urine

Camel milk and urine together is used extensively in traditional medicine in Sudan and they claimed that this combination cures a lot of diseases including liver disease and jaundice (Elhag *et al*, 2017). Researchers validated these in rats with ethanol induced hepatotoxicity and beneficial hepatoprotective effects of using camel's milk and urine mixture were evidenced which could be attributed to antioxidant activity or to its chelate effects on toxicants (Elhag *et al*, 2017). Camel urine is known to contain many active components and essential inorganic elements which play a protective role as antibacterial, antifungal, antiviral and anticancer agents. Protective role of camel urine (CU) against CCl4-induced liver damage in rats was also studied. Camel urine showed to play a promising anti-oxidative and anti-free radical scavenging mechanism against hepatic dysfunction (Hany *et al*, 2019). The possible protective role of both camel milk and urine on CCL4 induced liver damage was studied and found that camel urine has protective effect against CCL4 induced liver damage more than camel milk (Khan *et al*, 2019). The thioacetamide-induced liver cirrhosis in rats was ameliorated by administration of camel milk and urine in a ratio of 2:1 (Mohamed *et al*, 2016). Histopathological and biochemical changes were also reported by rats intoxicated by carbontetrachloride and were treated by camel milk (Althnaian *et al*, 2013).

Isoniazid (INH) and Rifampicin (RMP), first-line drugs used as anti-tuberculosis chemotherapy, are associated with hepatotoxicity (Mohamed *et al,* 2016). However, hepatotoxicity has been shown to be increased in case of association with RMP. The latter playing the role of enzyme inducer (Perriot *et al,* 2011; Sahli and Rim, 2015).

In order to protect from the toxic metabolites, the liver is equipped with an endogenous protection system; antioxidant enzymes and reduced glutathione. Strengthening this system requires the use of antioxidant molecules of vegetal or animal origin.

Biochemical and histological variations related to the use of milk and urine of camels as a protective treatment against anti-tuberculosis chemotherapy has

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not been studied previously. Therefore, we studied the protective effects of camel milk and camel urine against hepatotoxicity induced by Isoniazid and Rifampicin in rabbits.

Materials and Methods

Camel milk and camel urine samples were taken from desert camels from Biskra in south-eastern of Algeria. Milk was collected in sterile vials and kept in coolers during transport to the laboratory. Milk and urine bottles were frozen at -20°C until use.

Animals

The experiment was carried out on 40 sexually mature female rabbits obtained from the Technical Institute of Livestock "ITELV" in the city of Constantine. Their live weight varied from 2 to 2.8 kg. They were individually kept stainless steel cages with a 12/12 hours light/darkness cycle. Food and drinking water were given *ad libitum*. Animals were brought 7 days before the starting of the experiment to stabilise their mood and behaviour.

Experimental protocol

The study was carried out in February 2019. Female rabbits were randomly divided into 5 groups of 8 animals on each:

- Group 01 animals represented negative controls; they did not receive treatment.
- Group 02 animals were treated with Isoniazid and Rifampicin in accordance with a standard protocol for the induction of hepatotoxicity (Bhupinder *et al,* 2007).
- Groups 03, 04 and 05 animals received treatment with Isoniazid-Rifampicin with added milk, urine and a milk/urine mixture, respectively.

Camel milk was administered at a rate of 33ml/kg bodyweight/ day by oral gavage using a nasoesophageal tube (Hassan and Emam, 2012).

Urine was administered at a rate of 20 ml/kg bodyweight/ day (Mohamed *et al,* 2014) (Table 1).

Three rabbits died during the experiment, i.e. a group 2 rabbit (on the 6th day of treatment), a group 04 rabbit (the first day of treatment following a confirmed enterotoxemia after autopsy) and a group 05 rabbit (following a false swallowing).

Blood sampling and tissue manipulation

After 10 days of treatment, 5 ml of blood were drawn directly at the time of sacrificing the animals by puncturing the jugular vein. Blood was collected in heparinised tubes, and it was quickly sent to the laboratory under cold conditions for analysis to carry out some hepatobiochemical parameters.

Table 1. Experimental protocol.

Groups	Treatment	Dose
Control 01	• No treatment (distilled water)	/
02	• Isoniazid • Rifampicin	• Isoniazid (50 mg/kg/d) • Rifampicin (100 mg/kg/d)
03	• Isoniazid • Rifampicin • Camel milk	• Isoniazid (50 mg/kg/d) • Rifampicin (100 mg/kg/d) • 33 ml milk/kg/d for 10 days
04	• Isoniazid • Rifampicin • Camel milk	• Isoniazid (50 mg/Kg/d) • Rifampicin (100 mg/kg/d) • 20 ml milk/kg/d for 10 days
05	• Isoniazid • Rifampicin • Camel milk/ urine mixture	• Isoniazid (50 mg/Kg/d) • Rifampicin (100 mg/kg/d) • 16,5 ml milk and 10 ml urine /kg/d for 10 days

Histopathological examination

Liver tissue was cut into small pieces and immersed in 10% neutral buffered formalin for 24h. The fixed tissues were treated, incorporated into paraffin, sectioned, dewaxed and rehydrated using standard techniques (Sahli and Rim, 2015). The histopathological examination was carried out at the department of pathological anatomy- University hospital centre IBN BADIS, Constantine –Algeria.

Biochemical analyses

Separated plasmas were subjected to the determination of concentrations of bilirubin, and the measurements of the activities of ALAT, ASAT and PAL.

The assay of bilirubin is carried out by a colorimetric technique with dimethylsulfoxide (DMSO) (Bergmeyer *et al,* 1978).

The evaluation of alkaline phosphatase activity is a kinetic p-Nitrophenylphosphate photometric test in accordance with the recommendations of the International Federation of Clinical Chemistry and Laboratory Medicines (IFCC) (Rosalki *et al,* 1993).

ALAT and ASAT activities are measured by enzymatic and kinetic tests with lactate dehydrogenase and malate dehydrogenase, respectively (Maloy and Evelyn, 1937).

Statistical analyses

The 2019 version of the XL-STAT software was used for statistical analysis. Data were analysed by a one-way analysis ANOVA test. The Tukey HSD

(Honestly Significant Difference) test was used to compare the means. The differences were considered significant when P-value <0,05. The results were expressed as mean ± standard deviation (mean ±SD).

Results

Biochemical analysis results

The combination of anti-tuberculosis drugs (Isoniazid and Rifampicin) caused a considerable degree of hepatotoxicity and tissue damage in the liver. There has been an increase in the activity of plasma enzymes ALAT, ASAT and PAL. We found in the present study that the group that received only suspensions of anti-tuberculosis drugs, i.e. group 02 showed a non-significant increase in the activity of ALAT and ASAT compared to the control group, a significant increase in the concentration of bilirubin (p <0,05) and a very significant increase in PAL activity (p <0,001). In the groups which were treated with milk and camel urine associated with anti-tuberculosis drugs (groups 03, 04, 05), we noticed a reversal in the levels of ALAT, ASAT, PAL and bilirubine compared to group 02 animals. However, the difference between the three groups that were treated with milk, urine, and milk/urine mixture (03, 04, 05, respectively) was not statistically significant (Table 2).

Table 2. Plasma concentrations (mean ± standard deviation) of ASAT, ALAT, PAL and Bilirubin in rabbits in the control group, intoxicated rabbits, and treated rabbits.

Group	ASAT	ALAT	PAL	Bilirubin
Control 01	69.4± 25.1	77.7± 32 .0	48.25± 17.8	0.96± 0.09
02	174± 115.9	161.01± 86.6	617± 286.4***	8.57± 7.55*
03	110.1± 62.6	97.2± 45.4	389.57± 92.0***.#	4.57± 3.91
04	61.1± 44.1#	71.14± 59.1#	392.71± 133.7***.#	5.57± 5.77
05	71.85± 55.2	68.28± 12.6#	461.14± 160.6***	4.42± 1.99

Values were expressed as mean±SD, (* , # = p<0.05 ; ** , ## = p<0.01 ; *** , ### = p<0,001) *when compared with normal control group (NC) , #when compared with treated group (03).

Histopathological analysis

Untreated rabbits liver (control group) showed a well-preserved histological architecture. Hepatocytes were arranged in radiating spans from the central lobular vein and surrounded by vascular sinusoids (Fig 1).

Liver of rabbits treated with anti-tuberculosis drugs (INH / RMP) during 10 days, showed signs of hepatocyte suffering, namely; clarification of the cytoplasm, increase in the size of the nuclei and hepatocytes, binucleation, presence of Councilman's bodies, macrovesicular and microvesicular steatosis, predominantly microvesicular, slight cholestasis and, a significant inflammatory infiltrate of lympho-plasmocytic nature with eosinophilic polynuclear cells (Fig 2, 3, 4, 5).

Liver histopathological sections of rabbits treated with anti-tuberculosis drugs (INH/RMP) associated with milk, urine and milk/urine mixture of camels showed recovery to a normal appearance. Hepatocytes with discrete microvesicular steatosis were seen for the group treated with camel urine (Fig 6, 7, 8).

Discussion

The results of our study demonstrate that the 10-days treatment with anti-tuberculosis drugs causes an alteration in the biochemical balance of intoxicated animals. These findings are in agreement with the findings of several studies (Ravinder *et al,* 2006; Bouchentouf *et al,* 2011; Shih *et al,* 2013; Vandana *et al,* 2007).

ALAT and ASAT transaminases are hepatocyte enzymes whose function is to catalyse transfer reactions of an amino group from an alpha-amino acid to an alpha-ketonic acid. ALAT is present mainly in the liver and incidentally in muscles and kidneys. ASAT has a much wider distribution, in the liver but also in the heart, skeletal muscles, kidneys and brain. PAL is found especially in liver and bones. It is increased in case of cholestatic liver damage (Sahli and Rim, 2015).

In present study, liver damage was manifested as high liver enzymes (ASAT, ALAT, PAL) and increased concentrations of bilirubin.

In animals intoxicated with anti-tuberculosis drugs, the liver parenchyma showed a less organised appearance, presence of cholestasis and signs of hepatocyte suffering. These disturbances could be due to an oxidative stress produced following the administration of Isoniazid and Rifampicin. Similar results were reported by Khan and Al Zohairy (2011) and Khan *et al* (2019) using carbon tetrachloride (CCL4) protocol in rats, by Mahrous *et al* (2016) using acetaminophen and by Elhag *et al* (2017) using ethanol in rats. Additionally, the treatment with milk, urine, and the mixture of milk and urine produced a noticeable improvement in the values of hepatic enzymes mainly of alkaline phosphatase (PAL), and also a decrease in the concentrations of bilirubin. A return to normal liver architecture was evidenced

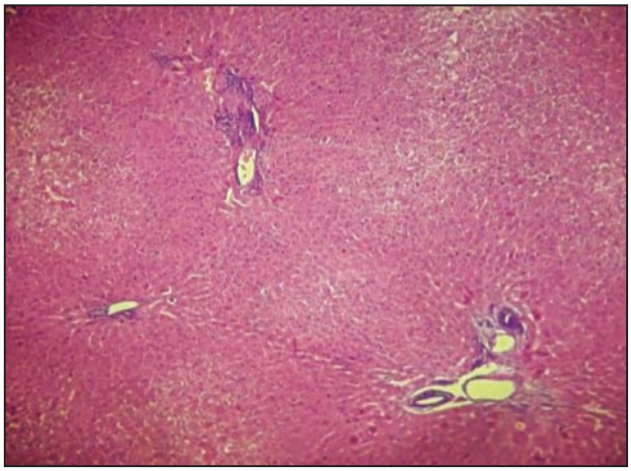

Fig 1. Normal architecture of the liver in rabbits of the control group (H&E, X40).

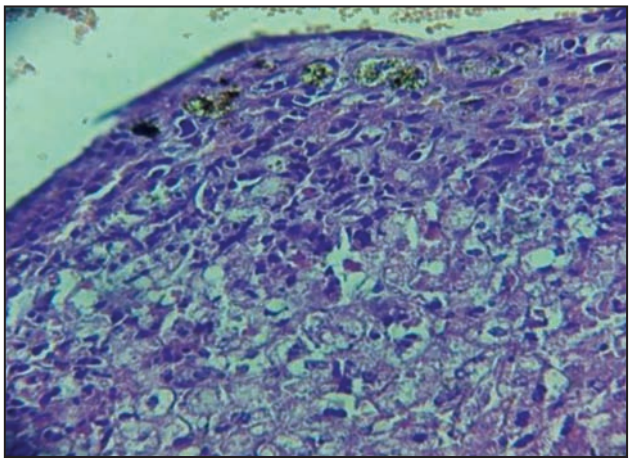

Fig 2. Cholestasis in group 01 rabbits (H&E, X100).

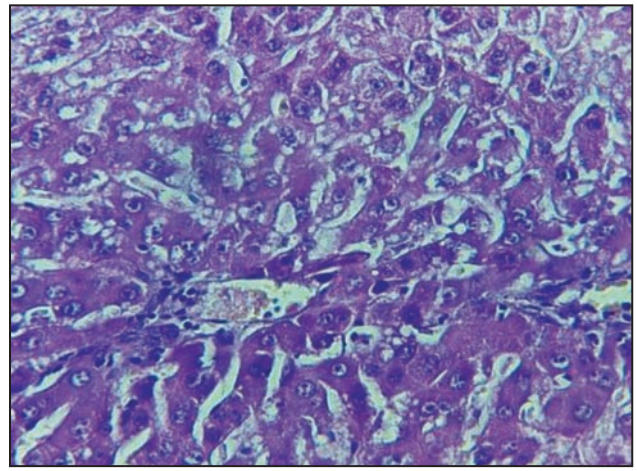

Fig 3. Clarification of the cytoplasm and microvesicular steatosis in group 01 rabbits (H&E, X400).

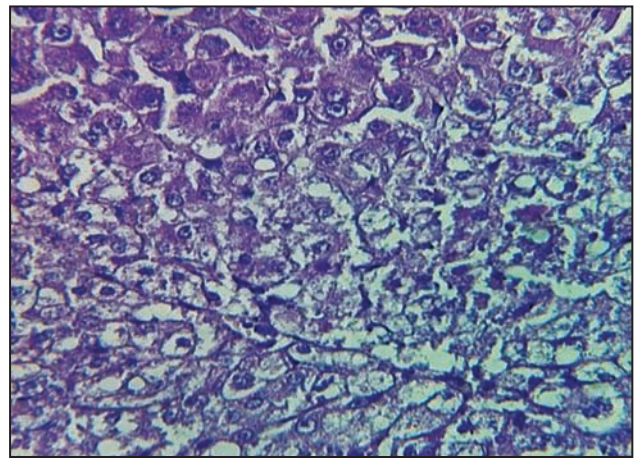

Fig 4. Presence of councilman bodies and binucleation in group 01 rabbits (H&E, X400).

that can be attributed to significant protection of the liver structure and preservation of liver function by camel milk and urine. Our results are in agreement with previous studies (Al-Hashem, 2009; Mohamed *et al,* 2016). Korish and Arafah (2013) investigated the effects of camel's milk on improving the hepatic biochemical and cellular alterations induced by a high-fat and cholesterol-rich diet. The same findings were obtained from studies with camel's urine against alcohol or CCL4 induced liver damage (Elhag *et al,* 2016; Hany *et al*, 2019).

Mahrous *et al* (2016) showed that oral administration of camel milk resulted in significant histopathological and biochemical antioxidant effect in mice, and this was indicated by a marked decrease in malondialdehyde levels. Also, ALT and AST levels were lower in the camel milk treated group.

High levels of antioxidant vitamins C, A and E and antioxidant minerals (zinc, copper and magnesium) in camel milk help reducing oxidative stress. Vitamin E and magnesium have been suggested to improve the biosynthesis of glutathione. Magnesium has been associated with the prevention of reactive oxygen species (Larrey, 2013; Wang *et al,* 2017; Mohamed, 2017; Mahrous *et al,* 2017).

Elhag *et al* (2017) showed a decrease in serum activities of liver enzymes AST, ALT and ALP especially in rats treated with camel milk and urine plus alcohol. The study suggested that camel milk intake may play an important role in ameliorating alcoholic liver injury.

Khorshid *et al* (2016) evaluated the hepato-protective effect of an extract of lyophilised camel urine in intoxicated rats.

The evaluation of the antioxidant activity of camel urine by the tests of ABTS, DPPH, FRAP and PPM showed a very important antioxidant activity (Hasni and Habita, 2015; Alebie *et al,* 2017).

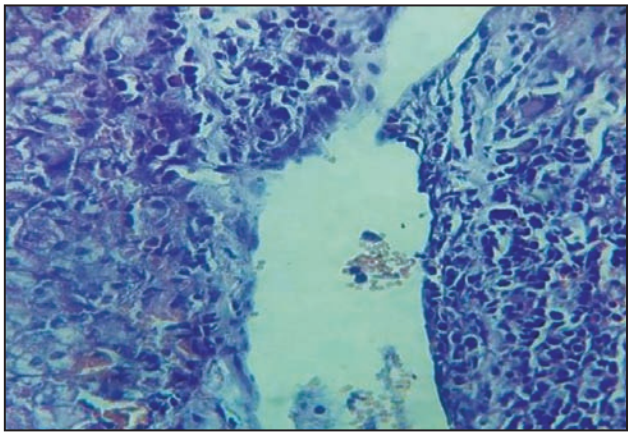

Fig 5. Portitis and lympho-plasmocyte infiltration in group 01 rabbits (H&E, X100).

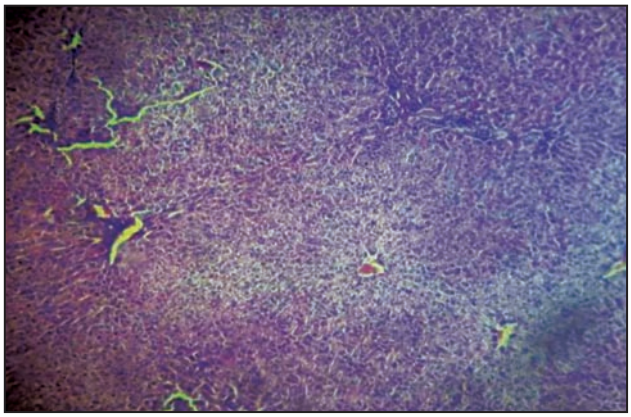

Fig 6. Portitis and lympho-plasmocyte infiltrate in group 01 rabbits (H&E, X100).

Camel urine is rich in potassium, phosphorus and magnesium, which are known for their therapeutic effects as well as by polyphenols and flavonoids. Our results were consistent with the findings of many authors (Al-Attas, 2008; Khorshid *et al*, 2016)

Conclusion

In present study, hepatotoxicity induced by the combination of anti-tuberculosis drugs was evidenced in histopathological and biochemical assessments.

Camel milk and urine administered with oral gavage were effective in reducing the severity of hepatotoxicity as reflected in histopathological lesions and biochemical changes in plasma levels of ALAT, ASAT, PAL and bilirubin.

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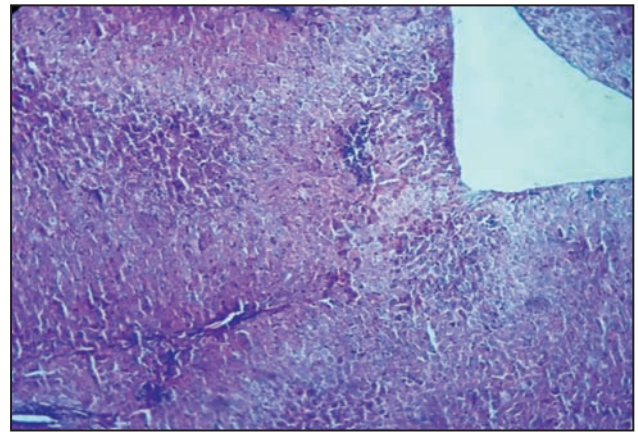

Fig 7. Discrete Microvesicular Steatosis in group 03 rabbits (H&E, X100).

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PROTECTIVE EFFECTS OF CAMEL MILK ON ACUTE AND CHRONIC INFECTION OF *Toxoplasma gondii* IN MICE

Xinlei Yan[1,3*], Wenying Han[1,3], Zhili Yang[2], Hejing Wang[1] and Ruifeng Li[2*]

[1]Food Science and Engineering College of Inner Mongolia Agricultural University, Hohhot 010018, China
[2]Department of Pediatrics, Inner Mongolia Maternal and Child Health Hospital, Hohhot 010020, China
[3]These authors contributed equally to this work

ABSTRACT

In this study, the effects of camel milk in mice infected with *T. gondii* was evaluated. We established acute and chronic infection mouse models, as well as a dexamethasone-based immunocompromised model. All mice were treated with camel milk, milk, or phosphate-buffered saline (PBS), followed by analyses of survival rate, cyst count, serum cytokine levels and brain inflammation in mice. There were significant differences in linear trend ($P < 0.05$) in the survival curve of treating by camel milk, milk and PBS. And serum levels of IL-2 ($P < 0.05$) of camel milk-treated mice were lower compared with milk group, while serum levels of IL-4 and IFN-γ ($P < 0.05$ for both, $P < 0.01$ for both) were higher of camel milk-treated mice than milk and PBS group. Additionally, camel milk reduced the extent of brain inflammation in mice with chronic *T. gondii* infection and immunocompromised mice. Importantly, camel milk alleviated the clinical symptoms of toxoplasmosis in mice. In conclusion, our findings suggest that camel milk exhibits promise for preventing or treating *T. gondii* infections.

Key words: camel milk, infection, mouse, *Toxoplasma gondii*

Toxoplasma gondii is an obligate intracellular protozoan parasite that can infect almost all warmed-blooded animals, including humans (Liu *et al*, 2015; Yang *et al*, 2013). Approximately one-third of the human population has been exposed to *T. gondii* (Zhang *et al*, 2016). In humans, *T. gondii* infections predominantly occur via consumption of water or raw meat contaminated with *T. gondii* oocysts (Dubey, 2008; Montoya and Liesenfeld, 2004). During acute infection, tachyzoites proliferate and subsequently transform into cysts to establish a chronic infection in the brain preferentially (Suzuki, 2020). Infections with *T. gondii* can be life-threatening, especially in immunodeficient patients, as they cause cerebral and ocular damage and even death (Zhang *et al*, 2016). Furthermore, reactivation of latent infection in immunocompromised individuals can result in fatal toxoplasmic encephalitis, myocarditis, and pneumonitis (Eza and Lucas, 2006 and Saadatnia and Golkar, 2012). Infections with *T. gondii* also lead to the induction of potent cellular immune responses. Upon activation, macrophages and T lymphocytes produce various cytokines, including interleukin (IL) and interferon-γ (IFN-γ), which inhibit parasite replication in haematopoietic and nonhaematopoietic cells, prevent the activation process of latent infection, and promote extracellular parasite lysis (Benevides *et al*, 2019; Khan *et al*, 1994; Kugler *et al*, 2016 and Xing *et al*, 2017).

Camel milk is an important nutritional source for pastoralists in many African and Asian countries. Camel milk has exceptionally high nutritional and medical value (Abrhaley and Leta, 2018). Camel milk is low in cholesterol and high in minerals (e.g., sodium, potassium, iron, copper, zinc cobalt, magnesium, manganese, and molybdenum) (Abrhaley and Leta, 2018 and Saini *et al*, 2007). Additionally, studies have shown that camel milk may alleviate liver conditions, malnutrition, vitamin deficiency, and allergic reactions, as well as prevent diabetes and improve immune system function (Khan *et al*, 2021; Wernery, 2006). Moreover, rats consuming camel milk have been shown to have higher IFN-γ levels compared to those consuming yak milk or cow milk (Wen *et al*, 2017).

Based on the complexity of the *T. gondii* life cycle, the diversity of its pathogenesis, and biological

SEND REPRINT REQUEST TO XINLEI YAN and RUIFENG LI email: yanxinlei1987620@foxmail.com ● 2317078007@qq.com

characteristics, there are currently no effective preventive or therapeutic agents for *T. gondii* infection (Konstantinovic *et al*, 2019). The sulfonamides are commonly used in the clinical (e.g., sulfadiazine) may alleviate symptoms in patients infected with *T. gondii*, they do not provide a cure and may cause significant toxicity (Ben-Harari *et al*, 2017). Therefore, more effective and safer therapeutic agents are urgently required. In this study, we examined the ability of camel milk to treat acute and chronic *T. gondii* infection in mice.

Materials and Methods

Animals, parasites, and drugs

Six-week-old female Kunming mice were purchased from Inner Mongolia University. Mice (five per cage) were given *ad libitum* access to food and water and housed with a 12-h light/dark cycle. Animal protocols were reviewed and approved by the Inner Mongolia Agricultural University Laboratory Animal Welfare and Animal Experimental Ethical Inspection Committee (approval no.: 2020-037). Prugniaud strains of *T. gondii* were obtained from the National Animal Protozoa Laboratory of China Agricultural University. Camel milk was obtained from Alxa Bactrian camels in Alxa Left Banner, Inner Mongolia, China. Dexamethasone was purchased from Henan Runhong Pharmaceutical Co., Ltd. Enzyme-linked immunosorbent assay (ELISA) kits for IL-2, IL-4, and IFN-γ were purchased from Shanghai Jingmei Bioengineering Co., Ltd.

Acute infection

Mice were treated daily by oral gavage with 100 µL of camel milk, milk or phosphate-buffered saline (PBS), respectively (n=12 mice per group). Five days later, the mice were intraperitoneally injected with 100 µL of PBS containing 20 cysts and the control with 0.1 mL PBS only. On 14 day post-infection (PI), the trial was over and the survival curve was drawn. Another group was treated as described above. On day 7 PI, the mice were sacrificed. Additionally, blood samples were collected and serum levels of IL-2, IL-4, and IFN-γ were measured via ELISA following the manufacturer's instructions.

Chronic infection

Mice were treated daily by oral gavage with 100 µL of camel milk, milk or PBS (n=12 mice per group). Five days later, the mice were intraperitoneally inoculated with 100 µL PBS including 3 cysts and the control injected with 100 µL of PBS alone. On 56 day PI, the mice were sacrificed, and cysts were counted. Brain tissue sections were prepared and stained with hematoxylin and eosin (H&E) to assess tissue inflammation (Afifi and Al-Rabia, 2015; Chen *et al*, 2020).

Immunosuppression

Mice were treated by oral gavage with 100 µL of camel milk, milk, or PBS (n=12 mice per group). Five days later, mice were intraperitoneally injected with 100 µL PBS including 3 cysts or 100 µL of PBS as a control. On 56 day PI, the mice were injected intraperitoneally with 3 mg/kg of dexamethasone every 2 days. The modified SHIRPA protocol was used to assess infection severity every 2 or 3 days, and on the last day of the experiment (Rogers *et al*, 1997). On 63 day PI, the mice were sacrificed by cervical dislocation, and brain tissue sections were stained with H&E.

Toxoplasma gondii cyst counting

The brains of sacrificed mice were homogenised in 2 mL of PBS. A 20-µL sample of each homogenate was absorbed to blood counting chamber and counted three times microscopically (only count intact cysts were counted). Then the mean number of cysts count per brain was calculated.

Clinical scores

Clinical examination of mice was performed using the modified SHIRPA protocol included a series of individual tests, which provided quantitative data about an individual performance (Rogers *et al*, 1997). The tests were performed in the following order, and one point was given for conformity: piloerection, abdominal writhing, weight loss, diarrhoea, lacrimation, palpebral closure, moving speed, reflexive escape from touch, spontaneous tremors, reduced grip strength, hunched posture, and changes in respiration rate (hyperventilation) (Estato *et al*, 2018). The control group was regarded as no changes in the clinical score.

Statistical analysis

GraphPad Prism software was used in plotting and data analysis. Data are expressed as the means ± standard deviations (SD). Statistical significance was determined using two-tailed t-tests. P-values ≤ 0.05 were considered to indicate statistical significance (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P < 0.001$).

Results

Effects of camel milk on the survival rate and cytokine levels of mice with acute T. gondii infection

All non-infected control mice survived. By contrast, infected mice treated with camel milk,

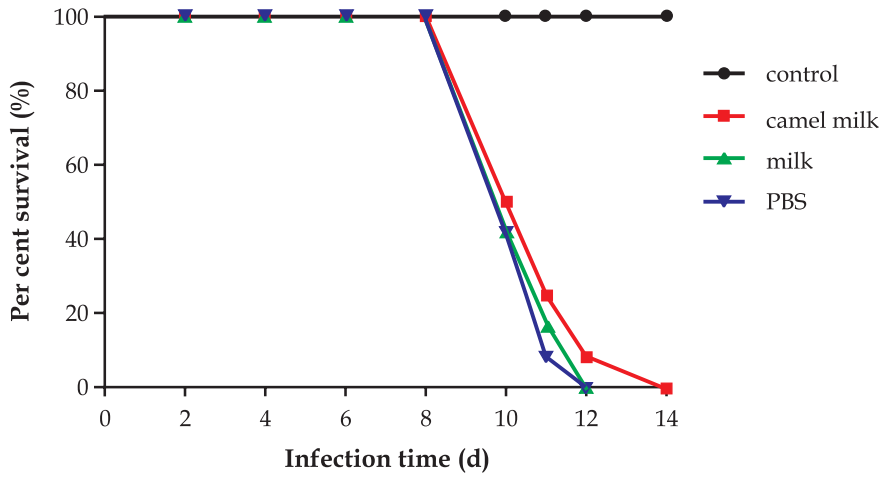

Fig 1. Survival of mice with acute *T. gondii* infection. Mice were treated daily by oral gavage with 100 µL of camel milk, milk or PBS, respectively. Five days later, the mice were intraperitoneally injected with 100 µL of PBS containing 20 cysts and the control with 0.1 mL PBS only. No deaths were observed in the control group. Camel milk treatment prolonged the survival rate of mice with acute *T. gondii* infection.

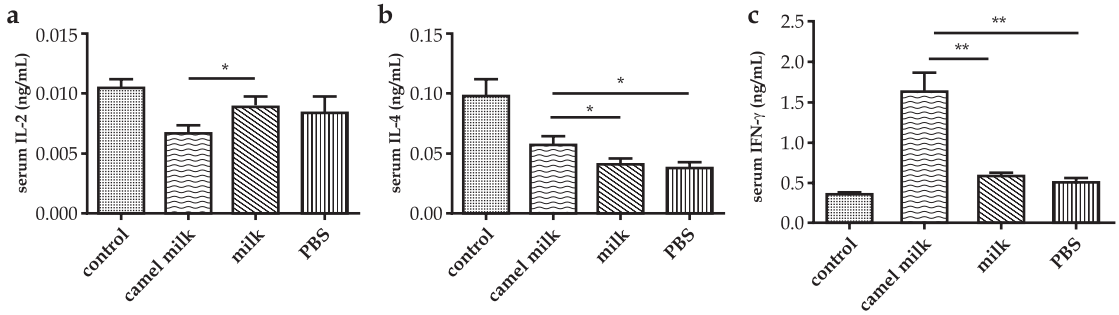

Fig 2. Serum cytokines levels in mice with acute *T. gondii* infection. Mice were treated daily by oral gavage with 100 µL of camel milk, milk or PBS, respectively. Five days later, the mice were intraperitoneally injected with 100 µL of PBS containing 20 cysts and the control with 0.1 mL PBS only. On 7 day PI, the mice were sacrificed and the levels of serum cytokines were measured. Serum levels of IL-2 (a), IL-4 (b), and IFN-γ (c). Data are shown as the means ± standard deviations (SDs; n=12 mice per group). *, P < 0.05; **, P < 0.01.

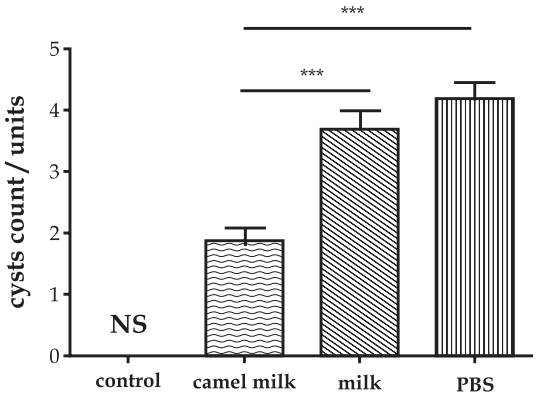

Fig 3. Effects of camel milk on the number of cysts in the brains of mice with chronic *T. gondii* infection. Mice were treated daily by oral gavage with 100 µL of camel milk, milk or PBS. Five days later, the mice were intraperitoneally inoculated with 100 µL PBS including 3 cysts and the control injected with 100 µL of PBS alone. On 56 day PI, the mice were sacrificed, and cysts were counted. Data are shown as the means ± SDs (n=12 mice per group). ***, P < 0.001.

milk, or PBS started dying at 8 day PI. The 10-day survival rate of mice treated with camel milk was 50.0%. On 11 day PI, 16.7% of the mice treated with milk were alive (Fig 1). There was no significant difference in the survival rate of mice treated with camel milk, milk, or PBS. However, linear regression analysis revealed significant differences (P < 0.05) in the survival curves of mice treated with camel milk, milk, and PBS.

The serum levels of IL-2 and IL-4 were lower in mice with acute *T. gondii* infection than in non-infected mice, whereas the levels of IFN-γ were higher. There were significant differences in the levels of IL-2 between mice treated with camel milk and those treated with milk (P < 0.05; Fig 2a). Additionally, serum IL-4 levels differed significantly between the camel milk group and the milk group, as well as between the camel milk group and the PBS group (P < 0.05 for both; Fig 2b). Compared with

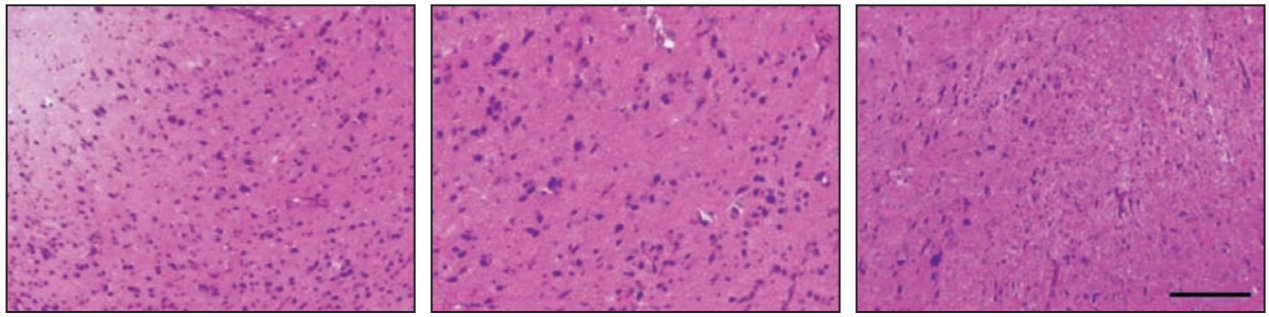

Fig 4. Effects of camel milk on brain inflammation in mice with chronic *T. gondii* infection. Mice were treated daily by oral gavage with 100 µL of camel milk, milk or PBS. Five days later, the mice were intraperitoneally inoculated with 100 µL PBS including 3 cysts and the control injected with 100 µL of PBS alone. On 56 day PI, the mice were sacrificed, brain tissue sections were prepared and stained with hematoxylin and eosin (H&E). (**a–c**) Brain tissue sections with different extents of inflammatory cell infiltration. Brain tissue section from *T. gondii*-infected mice treated with PBS (**a**), milk (**b**), or camel milk (**c**). Scale bar: 50 µm.

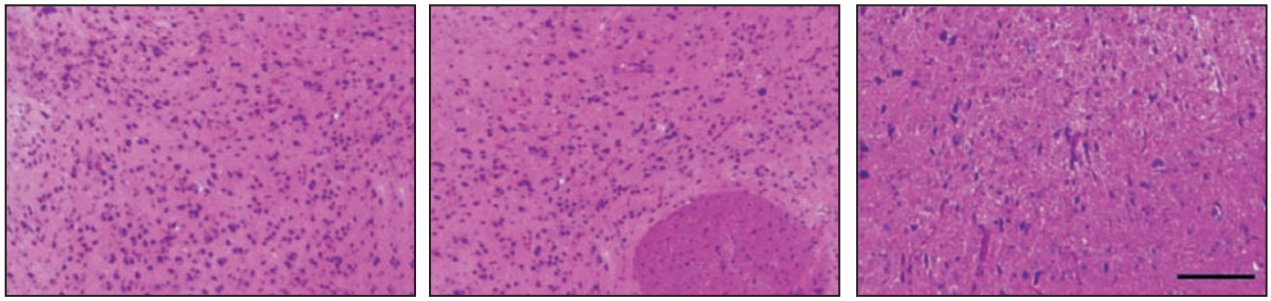

Fig 5. Effects of camel milk on brain inflammation in immunocompromised mice. Mice were treated by oral gavage with 100 µL of camel milk, milk, or PBS. Five days later, mice were intraperitoneally injected with 100 µL PBS including 3 cysts or 100 µL of PBS as a control. On 56 day PI, the mice were injected intraperitoneally with 3 mg/kg of dexamethasone every 2 days. On 63 day PI, the mice were sacrificed, brain tissue sections were prepared and stained with hematoxylin and eosin (H&E). (**a–c**) Brain tissue sections with different extents of inflammatory cell infiltration. Brain tissue section from *T. gondii*-infected mice treated with dexamethasone and PBS (**a**), dexamethasone and milk (**b**), or dexamethasone and camel milk (**c**). Scale bar: 50 µm.

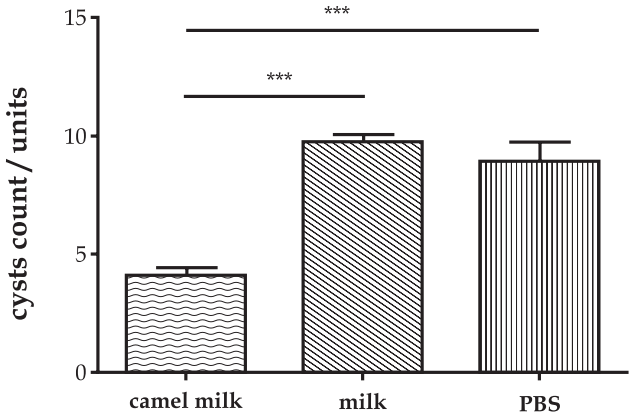

Fig 6. Effects of camel milk on clinical performance in *T. gondii*-infected immunocompromised mice. Mice were treated by oral gavage with 100 µL of camel milk, milk, or PBS (n=12 mice per group). Five days later, mice were intraperitoneally injected with 100 µL PBS including 3 cysts or 100 µL of PBS as a control. On 56 day PI, the mice were injected intraperitoneally with 3 mg/kg of dexamethasone every 2 days. The modified SHIRPA protocol was used to assess infection severity every 2 or 3 days, and on the last day of the experiment. The control group was regarded as no changes in the clinical score.

IFN-γ levels in the camel milk group, those in the milk and PBS groups were significantly lower ($P < 0.01$; Fig 2c).

Camel milk reduces the cyst counts and brain inflammation in mice with chronic T. gondii infection

As expected, no cysts were observed in the brains of non-infected mice. The cyst counts in the brains of chronically infected mice treated with camel milk, milk, and PBS were 1.86/unit, 3.72/unit, and 4.19/unit, respectively (Fig 3). The cyst count differed significantly between the camel milk and milk groups ($P < 0.001$), as well as between the camel milk and PBS groups ($P < 0.001$). These findings suggest that camel milk can reduce the number of cysts in the brain of mice with chronic *T. gondii* infection. Furthermore, we found that chronic *T. gondii* infection resulted in extensive infiltration of inflammatory cells into the brain (Fig 4a). Notably, camel milk reduced the extent of immune cell infiltration (Fig 4c).

Camel milk reduces the extent of brain inflammatory responses in immunocompromised mice

In immunosuppressed mice, *T. gondii* infection induced extensive infiltration of inflammatory cells into the brain (Fig 5a). Importantly, camel milk profoundly reduced inflammatory responses in the brain in response to *T. gondii* infection (Fig 5c).

Effects of camel milk on clinical performance in T. gondii-infected immunocompromised mice

At up to 8 days after *T. gondii* infection, we did not observe any obvious clinical signs, which was consistent with the clinical course of toxoplasmosis. Clinical signs were observed starting at 3 day PI and peaked at 12 day PI. Dexamethasone treatment improved the clinical performance of the infected mice. Severe clinical signs included palpebral closure, a hunched posture, and changes in the respiration rate (hyperventilation). Interestingly, clinical signs differed significantly between mice treated with camel milk and those treated with milk ($P < 0.001$) or PBS ($P < 0.001$) based on the Modofied-SHIRPA protocol. By contrast, there were no significant differences in clinical signs between the milk and PBS groups ($P > 0.05$; Fig 6). These results were consistent for up to 63 day PI was just showed because the mice was infected chronically all the time (chronic infection).

Discussion

We found that camel milk prolonged the survival of mice with acute *T. gondii* infection, although the differences in the survival rate among groups were not significant. This discrepancy may be explained by the high virulence and rapid proliferation of *T. gondii*. Additionally, mice with acute *T. gondii* infection exhibited significantly lower serum IL-2 and IL-4 levels and higher IFN-γ levels compared to control mice. Type 1 helper (Th1) cells induce cellular immune responses by releasing IL-2 and IFN-γ, which are key inflammatory mediators in mice infected with *T. gondii* (Yang *et al*, 2008). IL-2 further enhances IFN-γ production, establishing a positive feedback loop that induces potent immune responses (Fang *et al*, 2000). Consistent with our findings, previous in vivo studies have shown that IL-2 significantly prolongs the survival of *T. gondii*-infected mice (Cheng *et al*, 2010; Fang *et al*, 2000). However, we found that the serum levels of IL-2 were lower in mice infected with *T. gondii* than in non-infected mice, possibly due to the dual-directional regulation of IL-2 and IFN-γ production. IFN-γ has been demonstrated to prevent tachyzoite proliferation and growth by activating cerebral cells and promoting innate and T-cell-mediated immune responses (Suzuki, 2020; Suzuki *et al*, 2011); this effect of IFN-γ might have contributed to the prolonged survival of mice with acute infection. Type 2 helper (Th2) cells activate humoral immune responses by releasing IL-4 and other cytokines that induce B-cell activation. Cytokines secreted by Th1 and Th2 cells often have contradicting effects (Cheng *et al*, 2010; Vander *et al*, 2000). Although our results suggest that camel milk has protective effects in mice with acute *T. gondii* infection, the role of the Th1/Th2 balance in the protective effects of camel milk merits further investigation.

Compared with acute infection, camel milk also played an important role in inhibiting chronical infection of *T. gondii* in mice. Previous studies have shown that a suitable animal model of chronic *T. gondii* infection can be established with intraperitoneal *T. gondii* inoculation in mice (Liu *et al*, 2005). Notably, camel milk profoundly reduced the number of cysts in the brains of *T. gondii*-infected mice. Moreover, camel milk markedly inhibited inflammatory responses in the brain, pinpointing the protective effects of camel milk against chronic *T. gondii* infection.

Dexamethasone treatment in immuno-compromised mice further impairs the ability of the immune system to control *T. gondii* infection. In this study, we found that camel milk strongly inhibited inflammatory responses in the brain of *T. gondii*-infected immunocompromised mice, providing further evidence of the protective effects of camel milk. It is worth noting that compared with immunocompetent mice with chronic *T. gondii* infection, *T. gondii*-infected immunocompromised mice exhibited stronger inflammatory responses, suggesting that the severity of *T. gondii* infection may be regulated by host immunity. A previous study showed that the numbers of cysts were higher in the hippocampus and cerebral cortex of *T. gondii*-infected mice than in the brain stem and epencephalon (Bao *et al*, 2006), in accordance with the ability of *T. gondii* to impair learning and memory in mice (Witting, 1979). Interestingly, we found that mice with severe toxoplasmosis displayed palpebral closure, a hunched posture, and changes in the respiration rate (hyperventilation). Camel milk significantly alleviated these clinical signs of toxoplasmosis. In conclusion, our findings indicate that camel milk exhibits promise for preventing or treating *T. gondii* infections. The mechanisms underlying the protective effects of camel milk warrant further investigation.

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Conflict of interest

All individual authors declare that they have no conflict of interest (financial, personal or other).

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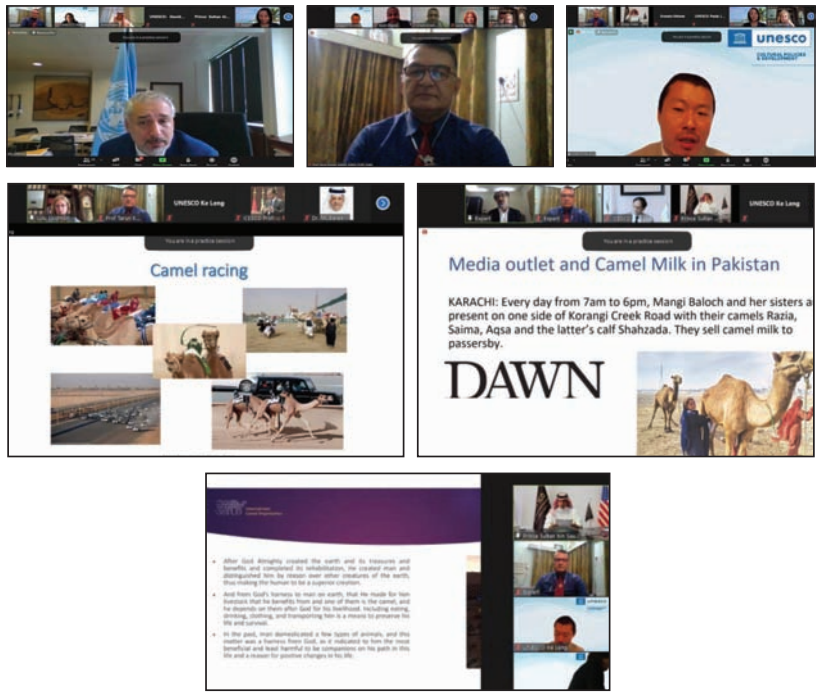
First ICO International Experts Congress (ICOIEC)

First online meeting of ICO International Experts Congress (ICOIEC) in collaboration with UNESCO on 'Cultural practices related to camel traditions to advance the SDGs' was held on 29 June 2021. Opening remarks were given by the H.E. Mr. Fahad F. bin Hithleen, Founder & President of International Camel Organization and Mr. Ernesto Ottone R. Assistant Director-General for Culture, UNESCO. There were four thematic sessions, i.e. Safeguarding the living cultural heritage enshrined in the camel traditions; Sustaining the local practices of camel herding for socio-economic development; Leveraging camel-based practices for biodiversity preservation and climate change adaptation and mitigation, and Bolstering evidence on the scientific and educational dimensions of the camelid traditions. The rapporteurs were Mr. Ahmed Skounti, Morocco; Dr. Ilse Köhler-Rollefson, LPPS, India and Germany; Ms. Hindou Oumarou Ibrahim, Chad and Dr. Laura Yereshekova, Kazakhstan. Various speakers were H.H Prince Sultan bin Saud bin Mohamed, Vice President of ICO to the State of the Headquarters, Saudi Arabia; Dr. Ed Emiri, London, Ms. Ayjarkin Kojobekova, Expert on living heritage; Dr. Hanan Abdel-Mawla, Sudan; Prof. Abdel-Razek Kakar, UAE; Prof. Hani Hayajneh, Jordan; Prof. Lulu Eskidmore, UAE, Ms. Delaram Kiramat, Uzbekistan; Prof. Abdul Malik Ibrahim Khalaf Allah, UAE and Prof. T K Gahlot, India. Closing remarks were given by the Secretary-General of ICO. The one-day event organised by ICO in collaboration with UNESCO was aimed to highlight the connections between camel traditions and the achievement of the UN 2030 Agenda for Sustainable Development, and notably their contribution to SDG 1 on reducing poverty, SDG 2 on fighting hunger, SDG 4 on education for sustainable development, SDG 8 on decent work and employment, SDG 11 on sustainable communities, SDG 12 on responsible consumption and production, and SDG 15 on environmental sustainability.



Glimpses of online presentations of First ICO International Experts Congress

First ICO International Experts Congress (ICOIEC)

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ELECTRONIC NOSE TECHNOLOGY FOR RAPID DETECTION OF ADULTERATED CAMEL MILK POWDER

Xiaoyun Wu[1], Naqin[1], Shiqi Hao[1], Rimutu Ji[1,2] and Liang Ming[1,2*]

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

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

ABSTRACT

In recent years, owing to popular use and high price of camel milk powder, it is likely to be adulterated, hence needs a quick method of detecting adulteration. This study took camel milk powder as the research object, and added 0%, 1%, 5%, 10%, 20%, 30%, 50% and 100% adulterants, such as goat milk powder, cow milk powder, protein powder and starch for sample preparation. According to the odour characteristics of adulterated camel milk, the electronic nose technology combined with principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were used for qualitative discrimination and quantitative analysis. Finally, multiple linear regression analysis (MLR) was used to verify the information of adulterated camel milk powder samples. The results showed that PCA analysis can distinguish different proportions of adulterated camel milk powder; PLS-DA model can effectively distinguish adulterated camel milk powder, and the detection limit of adulteration in camel milk powder was 1%. The correlation coefficients of linear regression analysis were all higher than 85%, and the predicted value and the actual adulteration value showed a certain linear relationship, indicating that the model had good generalisation ability. Therefore, it was feasible to use the electronic nose to realise the rapid detection method of camel milk powder adulteration.

Key words: Adulteration, camel milk powder, electronic nose, qualitative discrimination, quantitative analysis

In many countries, camel milk is popular due to its perceived health-promoting properties. Camel milk contains high amounts of the immune-active proteins, such as lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, as well as vitamin C and insulin, all of which play important roles in disease defense mechanisms (Mal and Pathak, 2010). Furthermore, camel milk has potential beneficial effects, such as anti-carcinogenic, antihypertensive and anti-diabetic ones (Marwa *et al,* 2019). However, camel milk, like any other milk, is extremely perishable, causing losses to both the farm and the market (Haileeyesus *et al,* 2018). Therefore, to preserve its physical, chemical, and nutritional properties, camel milk is usually produced and processed into camel milk powder and to, extend its shelf life, reduce transportation costs, and expand the application range (Thao *et al,* 2019).

Because of its low production, the price of camel milk is ten times that of cow milk, reaching RMB 90-120 per kilogram in China (Zhao *et al,* 2016). With the increasing demand for camel milk, adulteration of camel milk is not uncommon. (Wang *et al,* 2020). In recent years, several adulteration practices have been found in milk and dairy products, such as adding melamine (Lim *et al,* 2016), other animal milk (Liao *et al,* 2017), protein powder and starch (Tatiane *et al,* 2017). Such adulteration creates great concern for the entire production chain. The authenticity of milk powder is a big issue in China.

The existing milk powder adulteration detection technologies include near-infrared spectroscopy (Ning *et al,* 2015), high performance liquid chromatography (Jablonski *et al,* 2014), Raman spectroscopy (Qin *et al,* 2016), polymerase chain reaction (PCR) technology (Wang *et al,* 2020), fluorescence spectroscopy (Serap *et al,* 2017) and nuclear magnetic resonance (NMR) (Qiang *et al,* 2017). These instruments are expensive, and the data analysis requires specialised software and algorithms, making it time-consuming and difficult for ordinary food inspectors to master. Therefore, it is meaningful to need a simple and effective method to detect adulteration of camel milk powder.

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This study used the odour characteristics of camel milk powder through electronic nose technology to explore the lowest detection limit of the adulterated camel milk powder.

Materials and Methods

Materials

The raw camel milk powder (TF) used in this experiment was provided by Inner Mongolia Desert God Biotechnology Co. Ltd. and Zhenmu Whole Goat Milk Powder (YF), Yili Whole Milk Powder (NF), Gusong Potato Starch (DF) and By-Health Soy Protein Isolate Powder (BF) were purchased from a local supermarket.

Sample Preparation

Taking camel milk powder (TF) as the research object, adding C0, C1, C2, C3, C4, C5, C6 and C7 (0%, 1%, 5%, 10%, 20%, 30%, 50% and 100%) cow milk powder (NF), goat milk powder (YF), protein powder (BF) and starch (DF) were tested. Adulterated camel milk powder and purified water at a ratio of 1:7.2 (m/m) were stirred and mixed and then emulsified and homogenised for 15 minutes to obtain adulterated milk samples. The adulterated milk samples were brought to room temperature before being detected by the electronic nose (Ma *et al,* 2014).

For detection of E-nose, the optimised detection procedure was as follows:10mL of the milk sample was placed in a beaker of 100mL at the temperature of 25°C±3°C, and the beaker was sealed by plastic for a headspace generation time of 30 min. The headspace gas was detected by E-nose.

Detection Procedures of Electronic Nose

To collect the odour fingerprint of the adulterated camel milk powder, an E-nose of PEN 3 (Airsense Corporation, Germany) was used. The E-nose system consisted of three parts: the first was the sampling apparatus, the second was the detector unit containing of a sensor array of 10 different metal oxide sensors, and the third was pattern recognition software of Win Muster v.1.6. The nomenclature and characteristics of the 10 metal oxide sensors are listed in Table 1 (Dong *et al,* 2018). It shows that each sensor has a certain degree of affinity towards specific chemical or volatile compounds.

In order to detect adulterated camel milk powder through the electronic nose, the experimental conditions described in our previous study were used (Wu *et al,* 2021), as shown in Table 2. All the adulterated samples were detected at room temperature with 20 duplications.

Data Analysis

The response values of different sensors of the electronic nose were drawn using Origin 2019b software, principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were analysed using SIMCA 14.1 software (Umetrics, Sweden), and multiple linear regression analysis (MLR) is used Mnitab 19.0 software analysis.

Results and Discussion

Sensor selection

The electronic nose test was performed on samples with different adulteration ratios, and

Table 1. Sensors used and their main applications in PEN3 electronic nose.

Number in array	Sensor name	General description	Reference
S1	W1C	The main sensitive substances are aromatic compounds	Toluene, 10×10^{-6}
S2	W5S	The main sensitive substance is hydroxide	NO_2, 1×10^{-6}
S3	W3C	The main sensitive substances are ammonia and aromatic compounds	Benzene, 10×10^{-6}
S4	W6S	The main sensitive substance is hydrogen	H_2, 10×10^{-5}
S5	W5C	The main sensitive substances are alkanes, aromatic compounds and weakly polar compounds	Propane, 1×10^{-6}
S6	W1S	The main sensitive substance is methane in the environment	CH_3, 10×10^{-5}
S7	W1W	The main sensitive substances are sulfur-containing organic and inorganic compounds, terpenes and pyrazine compounds	H_2S, 1×10^{-6}
S8	W2S	The main sensitive substances are ethanol and some aromatic compounds	CO, 10×10^{-5}
S9	W2W	The main sensitive substances are aromatic compounds and sulfur-containing organics	H_2S, 1×10^{-6}
S10	W3S	The main sensitive substances are alkanes	CH_3, 10×10^{-5}

Table 2. Parameters of the electronic nose experiment.

Project	Parameter
Zero gas	Clean air, gas filtered with charcoal filter
The amount of sample in the vial	10mL
Vial volume	100mL
Initial injection flow	400 mL/min
Chamber flow	400 mL/min
test temperature	25°C
Presampling time	5s
Measurement time	70s
Sample interval	1s
Zero point trim time	10s
Flush time	60s

finally the response graph of the electronic nose to each sample was obtained. Fig 1A was the electronic nose sensor of unadulterated camel milk powder. The abscissa was the measurement time and the ordinate was the sensor response signal value, G/G0. G and G0 were the values reported by the sensors after exposure to the sample gas and the zero (control) gas, respectively (Xiaobao *et al,* 2018). The sensor array was composed of 10 sensors that are exposed to the headspace of the milk samples. During measurement, data were recorded every second for 70s, for a total of 700 records per sample, to allow the sensors to reach stable signal values. After sampling, the response values of the 10 sensors of the electronic nose gradually deviated from the baseline, and then gradually stabilised and most sensors began to reach a stable state in 60s. Therefore, the average value within 60-64s was selected as the characteristic value for subsequent analysis in this experiment. In addition, different sensors had different responses to camel milk powder. The S2 sensor had the largest response to camel milk powder, mainly detecting hydroxide compounds; followed by S7 and S6, mainly detecting sulfur-containing organic and inorganic compounds and methane.

In order to better compare the response of the electronic nose to camel milk powder (TF), goat milk powder (YF), cow milk powder (NF), protein powder (BF) and starch (DF), the response of the sensor was extracted and its radar chart was drwan (see Fig 1B). The radar graph showed that the response results of each sensor from each sample were different, and the response value of S7 and S6 sensors were quite different, followed by S9 and S8 sensors. Therefore, it could be distinguished based on the response difference of the 10 sensors of the electronic nose to different substances.

PCA results

PCA was used to reduce the dimension and for primary evaluation of the similarity of classes (Fardin *et al,* 2018). PCA is a mathematical algorithm that can reduce the dimensionality of the data without significant information loss and calculate the contribution of the principal components (PCs) (Stewart *et al,* 2014). The main idea of PCA is to project the information to some PCs, which can reduce the dimensionality of data while most variances are retained. A few PCs, which replace the original data, can show in a better way the variance distribution among the samples (Fei *et al,* 2017).

In order to better distinguish the adulterated camel milk powder samples, principal component analysis was performed on the electronic nose detection data, as shown in Fig 2. Based on the results of PCA analysis, the adulterated camel milk powder samples mixed with goat milk powder (YF), cow milk powder (NF), protein powder (BF) and starch (DF) (as shown in Fig 2A-2D), the two main components of PC1 and PC2 are 74.8% and 16.2%, 70.6% and 17.2%, 76.9% and 11.8%, 73.4% and 19.9%, respectively, the inter-sample variance accounted for 91%, 87.9%, 88.7% and 93.3% of the total data. The results were reliable since the first two principal components together contributed more than 85% to the cumulative variance (Rizelio *et al*. 2012). There were obvious differences between camel milk powder (TF), goat milk powder (YF), milk powder (NF), protein powder (BF), starch (DF) and adulterated camel milk powder in different proportions. Although the 10% and 20% protein powder (BF) adulteration groups partially overlapped, however, these can basically be distinguished.

PLS-DA results

A quantitative model of adulteration in camel milk powder was established by partial least squares (PLS) regression method. Partial least squares-discriminant analysis (PLS-DA) is a versatile algorithm that can be used for predictive and descriptive modelling as well as for discriminative variable selection (Lee *et al,* 2018). The main principle of PLS-DA is to first use PLS to extract the principal component of the sample, and then use the principal component as a new variable to establish a regression model between the independent variable of the

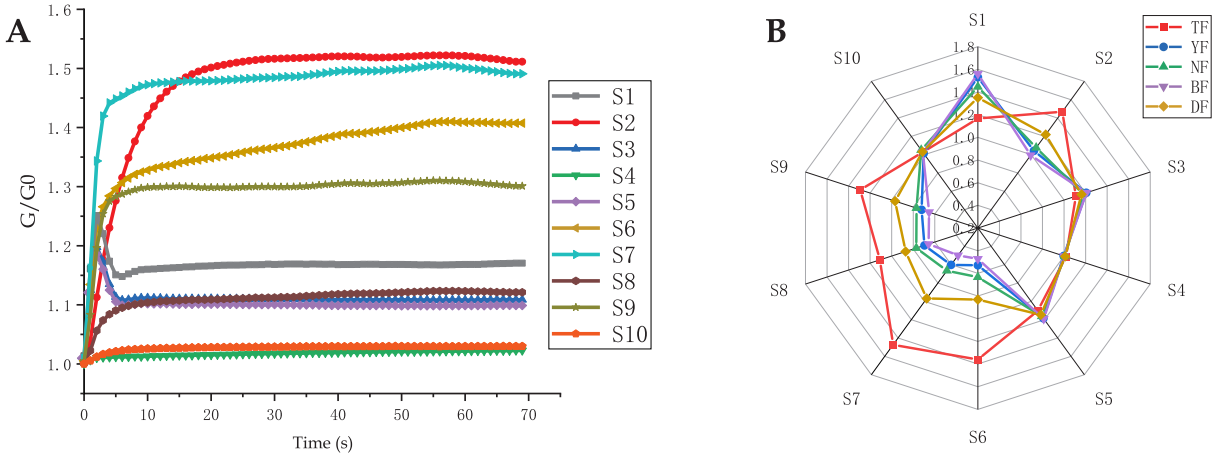

Fig 1. (**A**) Response curve of electronic nose sensor to pure camel milk powder; (**B**) Radar pattern response of electronic nose sensor.

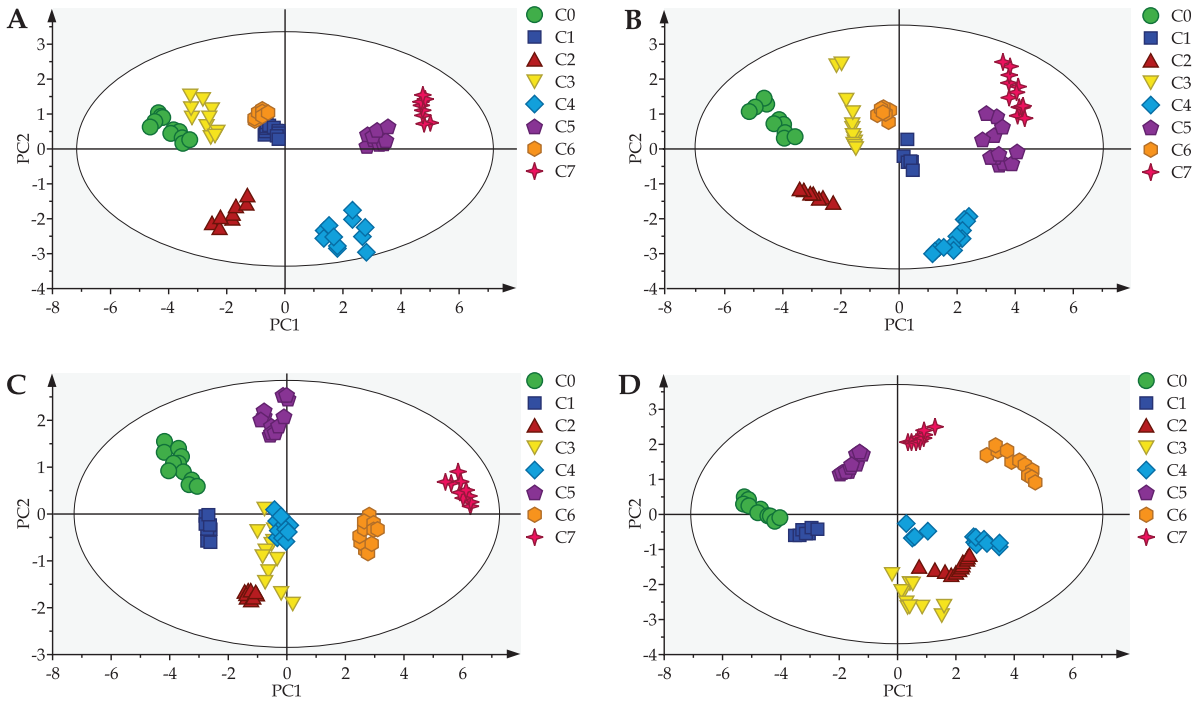

Fig 2. **(A)** PCA score chart of the electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of goat milk powder (YF); **(B)** PCA score chart of the electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of cow milk powder (NF); **(C)** PCA score chart of the electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of protein powder (BF); **(D)** PCA score chart of the electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of starch (DF).

training sample and the categorical variable, and perform discriminant analysis (Huangg, 2021). The data matrix were imported into the SIMCA statistical software and analysed it by PLS-DA (Fig 3). In the adulterated camel milk powder (TF) model was mixed with goat milk powder (YF), R2X (cum) = 0.936, R2Y (cum) = 0.601, Q2 (cum) = 0.599; incorporated into the cow milk powder (NF) model, R2X (cum) = 0.898, R2Y (cum) = 0.589, Q2 (cum) = 0.585; incorporated into the protein powder (BF) model, R2X (cum) = 0.874, R2Y (cum) = 0.975, Q2 (cum) = 0.974; incorporated into the starch (DF) model, R2X (cum) = 0.965, R2Y (cum) = 0.517, Q2 (cum) = 0.515. The value of R2Y and Q2 should be greater than 0.5, indicating that the predictive ability of the PLS-DA model is better (Xi *et al*, 2021), and the results are shown in Fig 3A-3D. The adulteration groups of different concentrations are more clearly distinguished on the PLS-DA score chart, and C0 (0%) and C1 (1%) can be better distinguished. The above

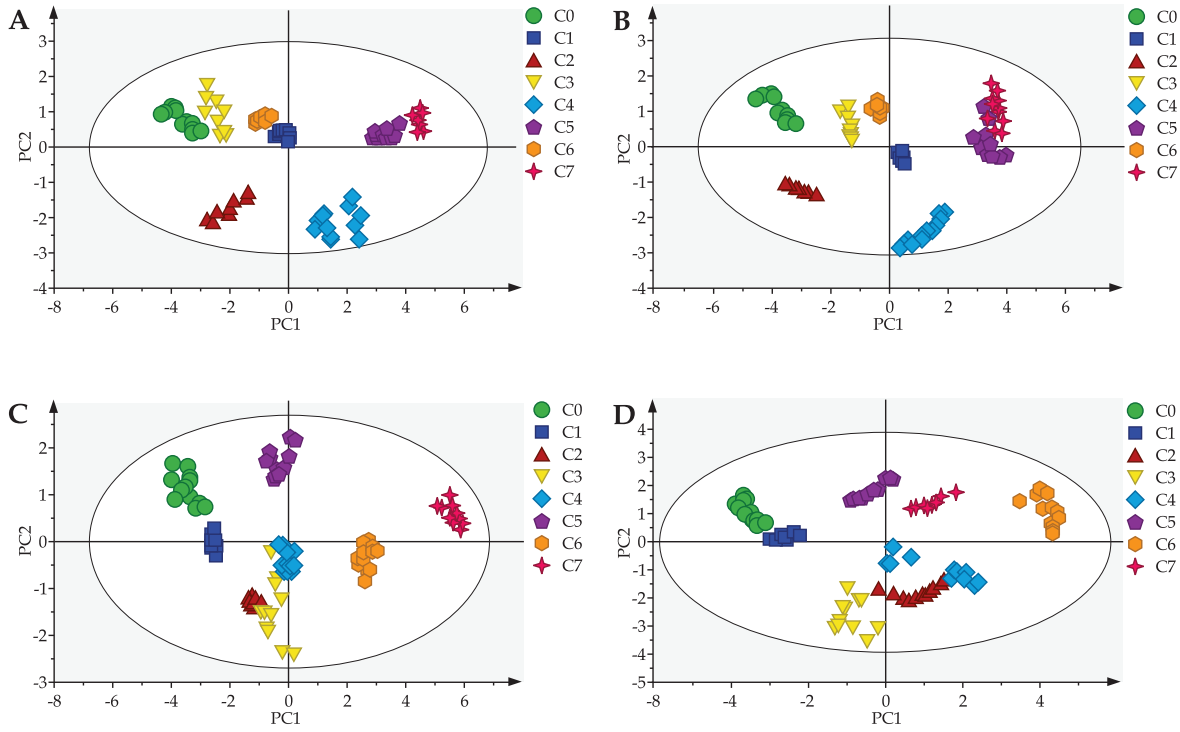

Fig 3. (**A**) PLS-DA score graph of the electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of goat milk powder (YF); (**B**) PLS-DA score graph of electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of cow milk powder (NF); (**C**) PLS-DA score map of electronic nose response value of adulterated camel milk powder (TF) mixed with different concentrations of protein powder (BF); (**D**) PLS-DA score graph of electronic nose response values of adulterated camel milk powder (TF) mixed with different concentrations of starch (DF).

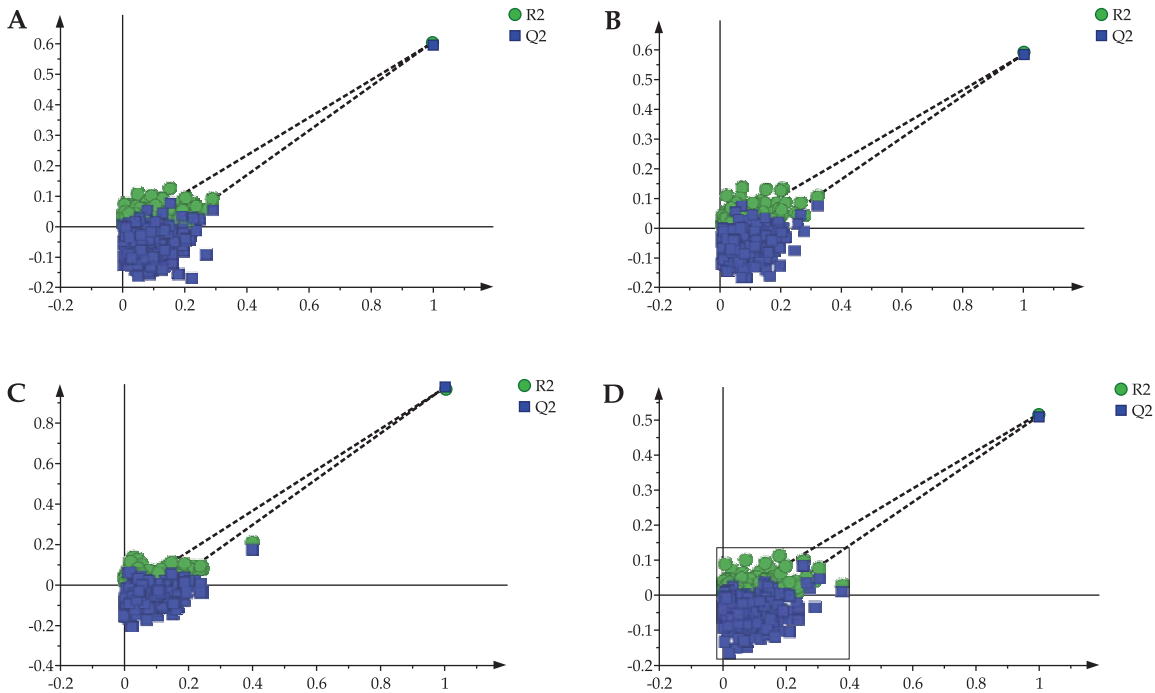

Fig 4. **(A)** The replacement inspection chart of adulterated camel milk powder (TF) mixed with different concentrations of goat milk powder (YF); **(B)** The replacement inspection chart of adulterated camel milk powder (TF) mixed with different concentrations of cow milk powder (NF); **(C)** The replacement inspection chart of adulterated camel milk powder (TF) mixed with different concentrations of protein powder (BF); **(D)** The replacement inspection chart of adulterated camel milk powder (TF) mixed with different concentrations of starch (DF).

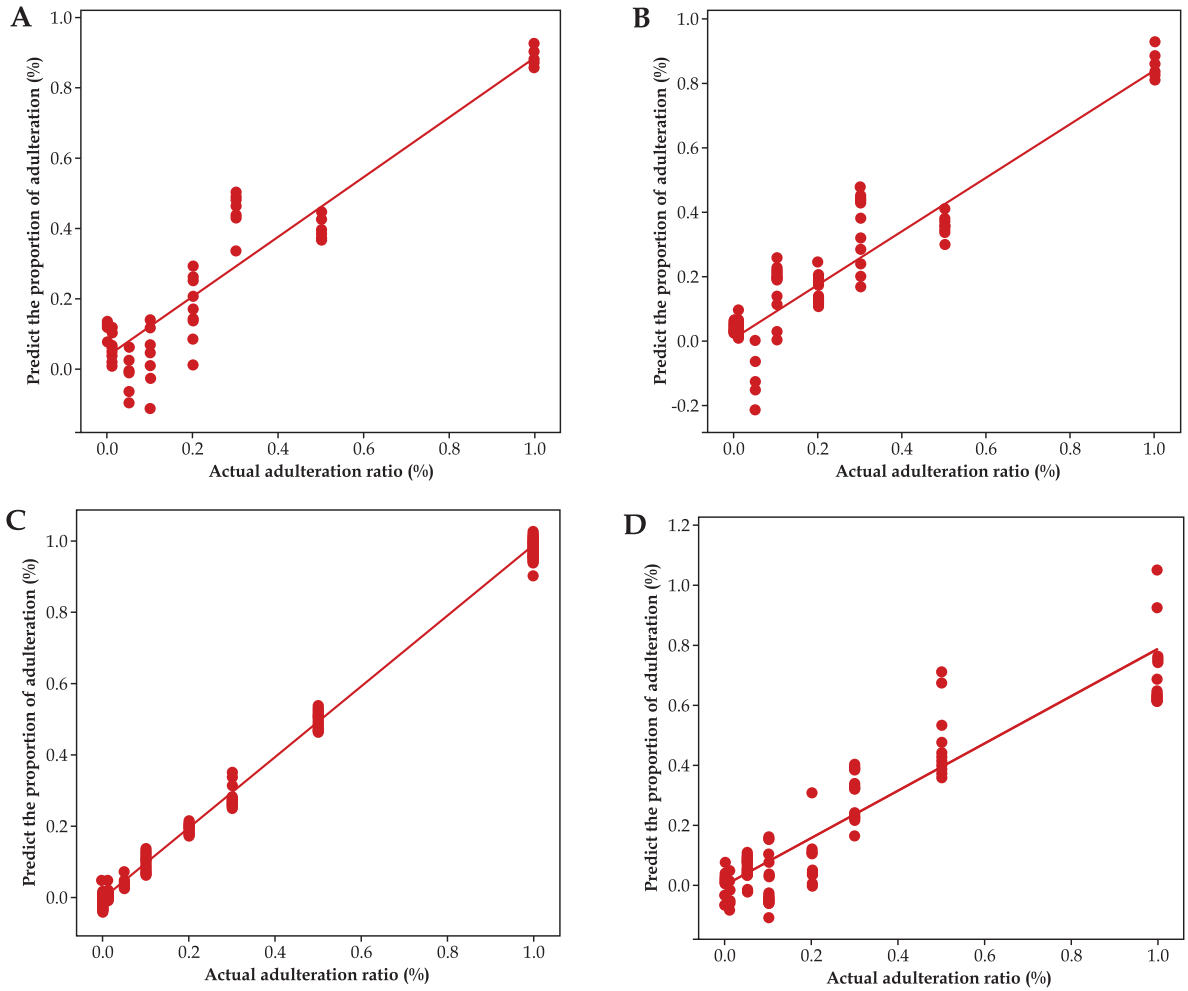

Fig 5. **(A)** MLR analysis diagram of adulterated camel milk powder (TF) mixed with different concentrations of goat milk powder (YF); **(B)** MLR analysis diagram of adulterated camel milk powder (TF) and different concentrations of cow milk powder (NF); **(C)** MLR analysis chart of adulterated camel milk powder (TF) mixed with different concentrations of protein powder (BF); **(D)** MLR analysis chart of adulterated camel milk powder (TF) mixed with different concentrations of starch (DF).

results showed that the detection limit of goat milk powder (YF), cow milk powder (NF), protein powder (BF) and starch (DF) in camel milk powder (TF) mixed with electronic nose was 1%, and it can be objectively reflect different adulteration concentrations.

In order to prevent the model from over-fitting, this study was carried out with 200 replacement verifications on the sample data (Wu *et al,* 2020). Fig 4 is a partial least squares discriminant analysis (PLS-DA) model replacement verification diagram. Among them, R2 is the cumulative variance value, and Q2 is the cumulative cross-validity, generally, Q2<0, the model is considered to be reliable, there is no over-fitting phenomenon, and the modeling is successful (Tang and Liao, 2014). The results of adulterated camel milk powder samples mixed with goat milk powder (YF), cow milk powder (NF), protein powder (BF) and starch (DF) are shown in Fig 4A-4D, Q2 is -0.115, -0.12, -0.148 and -0.0991, respectively, indicating that the original model does not have over-fitting phenomenon, and the model has good predictive ability.

MLR results

Multiple linear regression analysis is the most commonly applied statistical method of all scientific fields (Show and Gwowen, 2019). MLR is used to determine the relationship between multiple independent predictor variables and a dependent variable (Rebechi *et al,* 2015). At a first step, calibration is performed to build a mathematical model; then, the model is validated in a prediction step (Ragno *et al,* 2004 and Thomas, 1994). In this study, a linear regression fitting model was used for verification and analysis, and the size of the coefficient of determination was used to judge the degree of reliability of the regression equation estimation or the degree of fit of the regression line.

The coefficient of determination of the adulteration model with goat milk powder (YF) was 89.1%. Taking into account the influence of the number of independent variables, the coefficient of determination was corrected, and the adjusted coefficient of determination was 89%; for those with cow milk powder (NF), the coefficient of determination of the adulteration model was 89.7%, and the adjusted coefficient of determination was 89.5%; the coefficient of determination of the adulteration model with protein powder (BF) and the adjusted coefficient of determination were 99.4%; the coefficient of determination with starch (DF), the coefficient of determination of the adulteration model was 86.5%, and the adjusted coefficient of determination was 86.3%; the above results showed that the regression equation was highly reliable in estimation.

The relationship between the actual adulteration ratio of goat milk powder (YF), cow milk powder (NF), protein powder (BF) and starch (DF) and the predicted adulteration ratio of the model is shown in Fig 5A-5D. The regression equations established are as follows:

$Y = -23.20 + 2.03X_1 - 0.691X_2 - 5.37X_3 - 19.35X_4 + 28.76X_5 + 1.673X_6 + 0.299X_7 + 2.46X8 - 0.108X_9 + 10.38X_{10}$;

$Y = -53.1 + 4.61X_1 - 0.917X_2 + 0.52X_3 - 11.86X_4 + 35.80X_5 + 0.103X_6 + 2.012X_7 + 8.08X_8 - 1.556X_9 + 10.84X_{10}$;

$Y = -3.52 + 3.682X_1 - 0.2274X_2 - 2.235X_3 - 2.147X_4 - 0.82X_5 + 0.322X_6 + 0.353X_7 + 0.249X_8 - 0.1349X_9 + 3.927X_{10}$;

$Y = -54.81 - 11.32X_1 + 2.087X_2 + 27.05X_3 + 13.37X_4 + 20.7X_5 - 3.546X_6 + 1.24X_7 + 3.91X_8 - 3.409X_9 + 1.60X_{10}$;

where Y is the predicted adulteration ratio, X_1~X_{10} are the response values of 10 sensors.

It can be seen from Fig 5 that the predicted value of the adulteration ratio is relatively close to the true value, indicating that the electronic nose can better predict adulterated goat milk powder (YF), cow milk powder (NF), protein powder (BF), and starch (DF) in camel milk powder (TF). The prediction results of the electronic nose for adulteration quality scores of 0% and 1% are very close, but from the linear fitting situation, the prediction value of the electronic nose for the adulteration concentration of 1% is near the true value, indicating that the electronic nose is against the detection concentration of adulterated goat milk powder (YF), cow milk powder (NF), protein powder (BF) and starch (DF) in camel milk powder (TF) can be accurate to 1%. The MLR results once again proved that the electronic nose can be used for rapid detection and identification of adulterated odour characteristics at different concentrations, which provides a good basis for consumers and various dairy companies.

Conclusion

Based on the unique smell of camel milk powder (TF), this study used an electronic nose technology to study adulterated camel milk powder. Principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were used to qualitatively and quantitatively analyse camel milk powder with different amounts of adulteration, and successfully detected the purity and blending of camel milk powder (TF). The result analysis finally determined that the minimum detection limit of goat milk powder (NF), cow milk powder (NF), protein powder (BF) and starch (DF) mixed with camel milk powder (TF) was 1%. On this basis, a linear regression fitting model was established through the multiple linear regression analysis (MLR) method to verify the feasibility of the electronic nose technology for rapid qualitative discrimination and quantitative analysis of adulterated camel milk powder. It will help provide effective reference value for the detection of adulteration of dairy products in the market.

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Christina Adams speaks in the Second International Symposium on the Book Camel Crazy, sponsored by IOC and KAPL

Second International Symposium was held on April 02, 2021 which coincided with the World Autism Awareness Day on the Book "Camel Crazy", within the International and Cultural Symposium Programs of the International Camel Organisation - for the International Year of Camels 2024 in cooperation with the King Abdulaziz Public Library. Christina Adams shared how she had the idea that camel milk may help her son with autism, and how she tracked it down from desert Bedouins in the Middle East and flew it into America. Her son's speech, walking, paying attention, and sharing affection improved overnight after one serving. She became a camel milk expert by experimenting with the milk, spent time with camels and their keepers, travelling, and writing viral publications that helped start the camel milk industry. She connected many people to help create and grow the camel milk network worldwide. She mentors parents, and advises scientists, companies, nomadic people and farmers. She says people from different faiths and countries can work together to help others if they just reach out and connect. The science of camel milk was shared, on why the milk impacts health conditions involving inflammation, the gut-brain connection and allergic responses, such as diabetes, autism, rheumatoid arthritis, food allergies, and more. She presented patient reports on children with autism who benefitted from camel milk, who stopped showing aggression, gained more language and social skills, and healed their skin and food intolerance symptoms. She presented a patient report on a case of Type Two diabetes successfully treated with fresh camel milk. She explained the science of camelid antibodies and how they are used for snakebite, immunotherapy, vaccine development and COVID-19 treatment development.

She outlined the value and wisdom of pastoralist and nomadic people, how they benefit the world by keeping camels and holding genetic resources and heritage wisdom, and how today's families can benefit from their ways. She shared the accomplishments of the ICO under Sheikh Bin Hithleen and how the North American Members are helping people with their camels.

(Christina Adams' website is www.christinaadamsauthor.com)

Aflaj and camel racing in UAE included as a UNESCO cultural heritage

The UAE has successfully added camel racing and the Aflaj irrigation system on the United Nations Educational, Cultural and Scientific Organisation's (UNESCO) Representative List of Intangible Cultural Heritage. The Department of Culture and Tourism - Abu Dhabi (DCT Abu Dhabi) announced that these two submissions represented key elements of the UAE's heritage, and pointed out that the submissions were jointly made by the Ministry of Culture and Youth and DCT Abu Dhabi to showcase the UAE's rich cultural heritage internationally and to preserve traditions for future generations. The inclusion of Aflaj and camel races on UNESCO's Representative List of Intangible Cultural Heritage of Humanity was a great achievement that reinforced country's position as an incubator of rich heritage and national pride.

The inclusion of these two items of cultural heritage on the Representative List were awarded to the UAE at the 15th session of the Intergovernmental Committee for the Safeguarding of the Intangible Cultural Heritage of UNESCO, held virtually in Paris.

UAE has so far successfully registered 11 cultural activities and traditions on the Representative List of the Intangible Cultural Heritage of Humanity through joint national, regional, and international submissions, strengthening the country's position as an active member in the committee.

Online Third International Symposium on World Camel Day with a theme involving "Camel Surgery"

Prof. T.K. Gahlot was invited as a speaker for this symposium on "Camel Surgery" which held on 22nd June 2021, for sharing his valuable experiences to the world. Dr. Gahlot highlighted the current and future scenario of camel surgery. He showed surgery of various systems of camels out of his 4 decade experience with camel surgery.

This online International Symposium on "Camel Surgery" was held in cooperation with the King Abdulaziz public Library as one of the programs for the International Years of Camelids 2024. Prof Abdul Raziq Kakar was also invited speaker on this occasion and spoke about genesis of world camel day and importance of camels. Dr Ilse Kohler Rollefson, LPPS, India was a moderator for the symposium. She summed up the symposium through her concluding remarks and she appreaciated both the speakers who were dedicated for health and welfare of camels since a long time.

Beneficial effects of camel milk on diabetic patients: Research in UAE University

A new study by researchers at UAE University (UAEU) has shown the beneficial effects of camel milk on diabetic patients. The research, investigating the antidiabetic properties of camel milk that was published recently by the UAEU professors, revealed that the bioactive peptides extracted from camel milk have positive effects on the human insulin receptor and glucose transport in cells. The latest study was conducted by Dr. Mohammed Ayoub's laboratory from the Department of Biology, College of Science at UAEU, and Dr. Sajid Maqsood's laboratory from the Department of Food Science, College of Food and Agriculture at UAEU, with support from Zayed Center for Health Sciences, UAEU. The study was aimed at identifying the bioactive antidiabetic agent(s) from camel milk protein fractions and understanding its mode of action at the molecular level.

(Ismail Sebugwaawo / Abu Dhabi, ismail@khaleejtimes.com Filed on May 10, 2021)

New Research Finds Method to Create Safe Camel Milk Products

A research project conducted by the National Food Institute of Technical University of Denmark (DTU) has found a new formula to make safer camel milk products. Researchers aimed to find a starter culture to begin the fermentation process in camel milk. During their research, they found that two strains of lactic acid bacteria could be used as a starter culture for camel milk due to their antimicrobial properties. When used alongside other food safety practices like proper heating and cooling, these strains can inhibit the growth of pathogens in raw and pasteurized camel milk. Currently, camel milk is sold in local markets or roadside stalls in East Africa. But, if safely treated, the products' shelf life can be extended. The process would also enable producers to create and sell other camel milk products, like cheese or butter. With safer camel milk and camel milk products, camel milk farmers can reap greater economic benefits. Camel milk production and export is increasing around the world with some European supermarkets already carrying the products on their shelves. By 2024, the market for camel milk is expected to be worth more than US$8 billion.

(Foodtank: January 2021)

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

INSTRUCTIONS TO CONTRIBUTORS

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Nature of coverage: This journal is dedicated to disseminate scientific information about new and old world camelids in form of: **Original research** articles in camel health, husbandry, pastoralism, sports, specific behaviour, history and socio-economics. **Reports** on unusual clinical case(s) or unreported management of clinical case(s). Review articles will be accepted on invitation only. **Book review** directly or indirectly related to camels will be reviewed by subject-matter specialists and included if sent to the journal for this purpose. The Journal of Camel Practice and Research will occasionally contain an **invited editorial** commenting on the current research and papers in the issue.

Submission of manuscript: Manuscripts should be submitted in word files to **Dr. T.K. Gahlot,** Editor, Journal of Camel Practice and Research at tkcamelvet@yahoo.com. The figures can be submitted preferably as a high pixel JPEG or other format. The manuscript should be accompanied by a covering note and author consent letter from the author responsible for correspondence. It should also contain a statement that manuscript has been seen and approved by all co-authors. Editor and members of the editorial board are not responsible for the opinions expressed by authors and reserves the right to reject any material or introduce editorial changes. Material will be accepted for publication on the understanding that it has not been published in any other form and is not being considered elsewhere. Any material once accepted for publication may not be republished in any form without prior permission of the author.

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[1]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China

[2]Inner Mongolia Institute of Camel Research, Badanjiran, Inner Mongolia, China

[3]Alxa League Institute of Animal Husbandry, Alxa, Inner Mongolia, China

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Wilson R Trevor (2020). The one-humped camel in Eritrea and Ethiopia: a critical review of the literature and a bibliography. Journal of Camel Practice and Research 27 (3): 229-262.

For edited symposium/congress/proceedings: Abdalla HS (1992). Camel trypanosomiasis in the Sudan. Proceedings First International Camel Conference, Dubai (UAE), February 2-6, p 401-403.

Books (Personal authors): Faye B and Bengoumi M (2018). Camel Clinical Biochemistry and Haematology: Springer International Publishing. pp 275-286.

Chapter from multiauthored books: Wernery U, Kinne J and Schuster RK (2014). Unusual arboviruses and other minor viral infections. In: Camelid Infectious Disorders. OIE Book. pp 319-322.

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.

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

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

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