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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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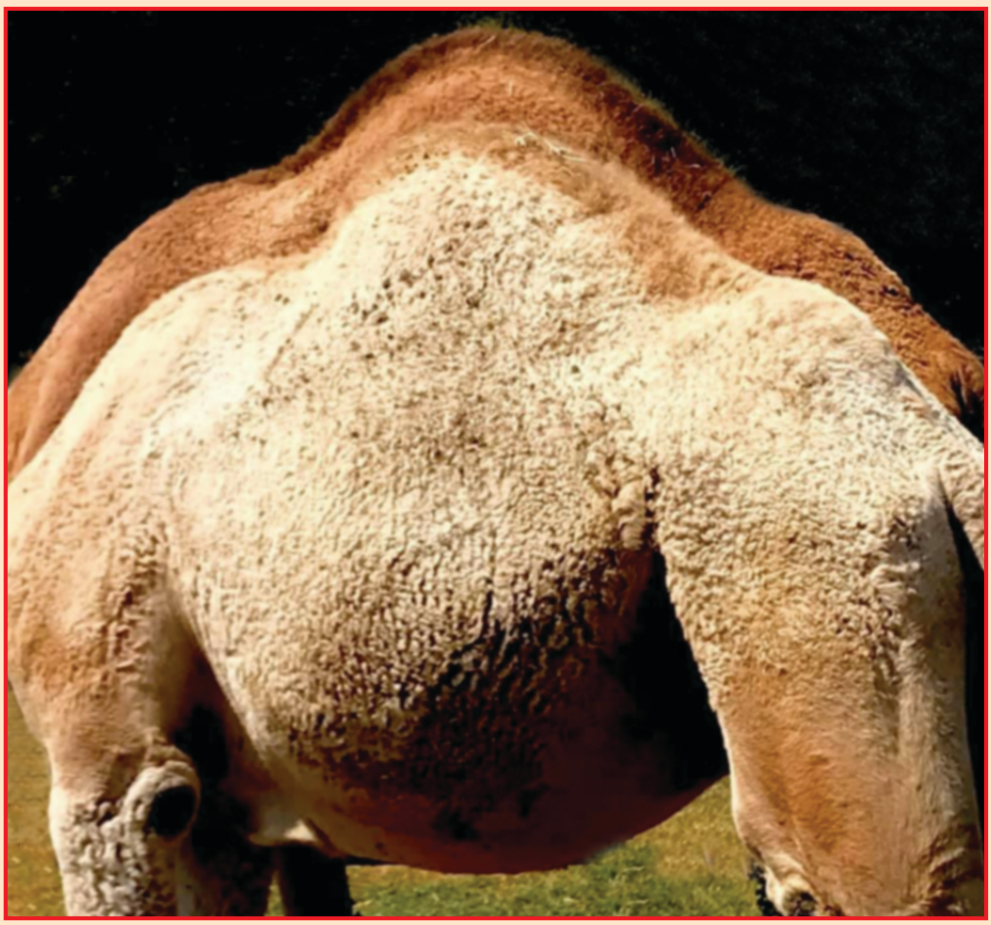
Volume 26 August 2019 Number 2

In This Issue



JOURNAL OF CAMEL PRACTICE AND RESEARCH

CAMEL PUBLISHING HOUSE
Bikaner - 334001, INDIA

Manuscripts and other related correspondence may be made to :

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SUBSCRIPTION RATE - 2019

ANNUAL	Rs. 4000/- or US $ 400

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

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

Cover Design: Aligned humps of dromedary camels

Courtesy: Doug Baum

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

CONTENTS

Volume 26 **August 2019** **Number 2**

TRIANNUAL JCPR IMPROVES IMPACT FACTOR IN 2018

Journal of Camel Practice and Research was made triannual since 2017 and seen then it had many positive effects. The wait list of manuscripts was shortened and number of manuscripts published in a year was increased which in-turn increased the citation of its published papers. Its reported IF (Impact factor) in the year 2018 became 0.168 with 188 cites (https://www.bioxbio.com/journal/J-CAMEL-PRACT-RES).

Camel science is marching ahead with a rapid pace. The reproduction scientists of UAE compared the pregnancy rates with transfer of *in vivo*-produced embryos using multiple ovulation and embryo transfer (MOET) with *in vitro*-produced embryos by somatic cell nuclear transfer (SCNT) in dromedary camels. Another group of scientists carried out genomic research on molecular diversity of MHC (major histocompatibility complex) class II genes and found that these are significantly lower in Old World camels than in other mammals and the major part of the diversity resides in the DQB gene, which is not very well annotated in the camel genome.

First Conference of International Camel Organisation (ICO) was held on 28-29 August 2019 at Bishkek, Kyrgyzstan on the theme- Camel Culture in Central Asia- Historical Heritage and Prospects. The ICO is an international organisation created as per the decision of the organisation's Constituent Assembly, with the participation of special organisations from 36 countries of the world, on 22 March 2019 at Riyadh, Saudi Arabia.

The current issue starts with serological studies on camels vaccinated with *Brucella abortus* RB51 and *Brucella melitensis* Rev 1 Vaccines in UAE. Important parasitological studies include hepatic cysticercosis and acaricide resistance in *Hyalomma dromedarii*. Immunohistochemical studies on neuropeptides in the pancreas of dromedary camel and evaluation of preslaughter stress responses during waiting time at lairage are other important manuscripts. Studies on Bactrian camels include three manuscripts, i.e. identification of microsatellites and parentage testing development; comparative transcriptome analysis of liver tissues and study on physical properties of camel cashmere. Teat characteristics, chronic peritonitis, endometritis, assessment of trace elements in meat, hump and liver, mandible fractures using interdental wiring (IDW) and surgical management of soft palate haematoma are other noteworthy manuscripts of this issue.

I am sure that great support of all camel scientists will continue and would help JCPR increasing its Impact Factor in future as well.

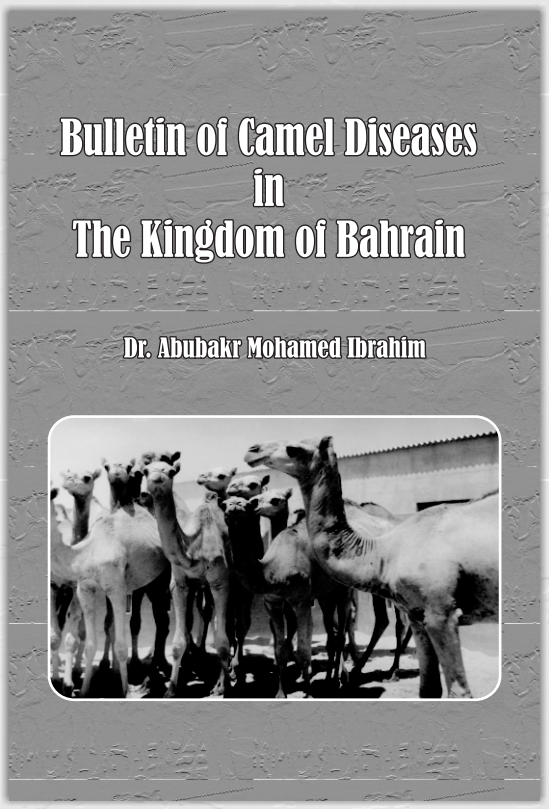
(Dr. T.K. Gahlot)
Editor

Bulletin of Camel Diseases in The Kingdom of Bahrain

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

About the Author

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



Editor:
Dr. T.K. Gahlot

Edition: 2014

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

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

Price: US $ 90
INR 1000

ISBN 81-903140-2-5

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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

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Volume 20 June 2013 Number 1

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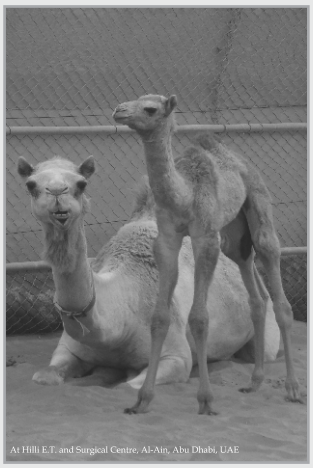

At Hilli E.T. and Surgical Centre, Al-Ain, Abu Dhabi, UAE

In This Issue

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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com • www.indianjournals.com

Volume 20 December 2013 Number 2

In This Issue

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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com • www.indianjournals.com

Volume 21 June 2014 Number 1

In This Issue

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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com • www.indianjournals.com

Volume 21 December 2014 Number 2

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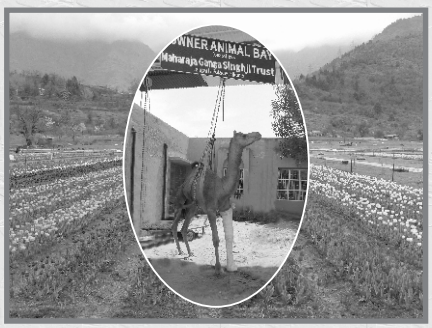

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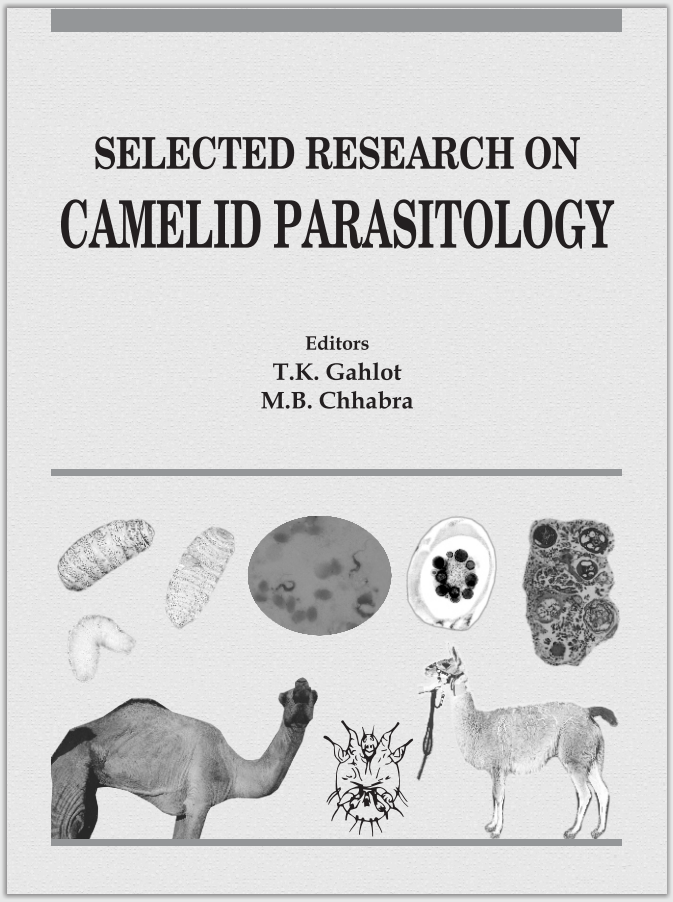
SELECTED RESEARCH ON
CAMELID PARASITOLOGY

Hard bound, 291 pages, few figures coloured

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



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

Edition: 2009

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

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

ISBN: 81-903140-0-9

BACK ISSUES OF JCPR AVAILABLE

ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 18 June 2011 Number 1

In This Issue

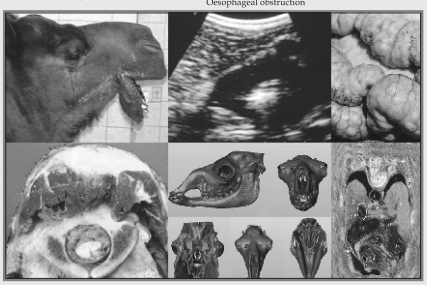
ISSN 0971-6777

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 18 December 2011 Number 2

In This Issue



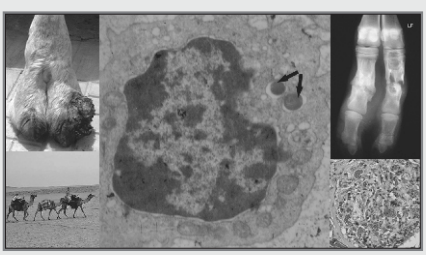
ISSN 0971-6777 (Print)
ISSN 2277-8934 (Online)

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

Volume 19 June 2012 Number 1

In This Issue



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

JOURNAL OF CAMEL PRACTICE AND RESEARCH

www.camelsandcamelids.com

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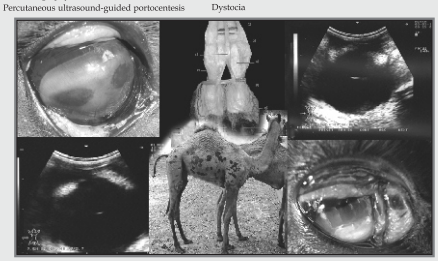

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DOI : 10.5958/2277-8934.2019.00018.3 Vol 26 No 2, p 121-124

SEROLOGICAL RESPONSE OF DROMEDARY CAMELS VACCINATED WITH *Brucella abortus* RB51 AND *Brucella melitensis* REV 1 VACCINES

U Wernery, Rodriguez Caveney M, Sh Jose, B Johnson, R Raghavan, J Christopher, G Syriac, Sh M Thomas and N M Paily

Central Veterinary Research Laboratory, P O Box 597, Dubai, UAE

ABSTRACT

A vaccination experiment was conducted in dromedary camels at CVRL. Six adult dromedaries received 2 ml of the *Brucella abortus* vaccine RB51 subcutaneously and 6 camels 1ml of the *B. melitensis* Rev 1. Four different serological tests were conducted to follow the seroconversion post vaccination. None of the 6 dromedaries vaccinated with RB51 produced antibodies, measured for 1 year, due to lack of lipopolysaccharide O - side chains in the vaccine. However, antibodies appeared in all 6 dromedaries 30 days post vaccination with *B. melitensis* Rev 1, which declined slowly over time. Three hundred thirty days post vaccination ELISA antibodies were still present in 5 of the 6 dromedaries as well as RBT, SAT and CFT antibodies in some of them. This is a disadvantage as all serological methods for the diagnosis of brucellosis cannot differentiate between vaccine or natural infection titres.

Key words: Antibodies, *Brucella abortus* RB51, *Brucella melitensis*, serology, vaccination against brucellosis

Brucellosis is a contagious disease caused by bacteria of the genus Brucella. Taxonomically, the genus is divided into ten classified species and subdivided into biovars. Recently, Brucella strains have been isolated from numerous marine mammal species. Brucellosis is one of the most important zoonoses in developing countries. Old World Camels (OWCs) are frequently infected with brucellosis, particularly when they are in contact with infected small ruminants. The disease is rare in New World Camels (NWCs), but outbreaks with clinical signs of brucellosis have been described (Wernery, 2016).

Vaccination of camelids to protect them against brucellosis has been reported only by few researchers, but no challenge infection after vaccination has been performed. It was proposed by Wernery *et al* (2017) not to use the intra conjunctival vaccination route with *Brucella (B.) melitensis* Rev1, as the vaccine strain is excreted via the eye for several days due to the camels' physiological constant tear flow, which may put camel handlers in danger to become infected.

Both, inactivated and attenuated Brucella vaccines have been used in OWCs and their antibody development were described. Dromedaries were vaccinated with Strain 19 and with Rev1 subcutaneously (Radwan *et al,* 1995). Three month-old dromedaries received a full dose of the Rev1 vaccine and 10 year-old a reduced dosage. Both groups developed antibodies which receded after 8 months in young stock and 3 months in adult camels. A similar experience was made by Wernery *et al* (2017) with the Rev1 eye drop vaccine. Agab *et al* (1995) vaccinated 5 dromedaries with a reduced dose (5×10^8 cfu/2ml) of Strain 19. All 5 camels sero converted after one week and their antibodies vanished 7 weeks later.

The aim of our study was to investigate the antibody development of 6 dromedaries each after a single subcutaneous vaccination with a full dose of *B. abortus* RB51 strain and *B. melitensis* Rev1.

Materials and Methods

Dromedary camels

In total 12 adult dromedaries were used for this experiment conducted at CVRL. All camels received Timothy hay *ad libitum* and 2 kg of concentrate on a daily basis. They were kept in 2 separate pens and had access to fresh water through automatic drinkers. Six camels in one pen which included 4 females and 2 castrated males were vaccinated against *B. melitensis* and 6 female camels were vaccinated against *B. abortus*.

Vaccines

For the immunisation against *B. abortus*, the attenuated freeze-dried RB51 vaccine strain was used. It contains 10-34 $\times 10^9$ cfu (CZ Veterinaria, Spain). For the immunisation against *B. melitensis* the attenuated

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freeze-dried Rev1 vaccine strain was used. It contains 0.5-4.0 x 10^9 cfu (Brucevac, Jordan). Each dromedary received subcutaneously 2 ml of the RB51 and 1ml of Rev 1 reconstituted vaccines on the left side of the neck, only once.

Serology

Four different serological tests were performed on the serum samples following the procedures laid down by the OIE (2018). They included:

- Rose Bengal Test (RBT) with antigen from Vircell, Spain
- Complement Fixation Test (CFT) with antigen from Weybridge, UK
- Serum Agglutination Test (SAT) with antigen from Weybridge, UK
- Indirect ELISA from Ingenasa, Spain

Blood was regularly withdrawn from the camels' jugular veins, centrifuged and serum frozen until tested.

Results

After immunisation with *B. abortus* RB51 strain of 6 dromedaries and *B. melitensis* Rev1 strain of another 6 dromedaries, the following serological results were achieved shown in Table 1.

Vaccination of 6 dromedaries with RB51 did not produce any antibodies. Vaccination with Rev 1 produced antibodies with all 4 serological tests 30 days post vaccination (pv) which slowly declined over time. Three hundred thirty days pv ELISA antibodies in 5 of the 6 vaccinated dromedaries were still present and also RBT, SAT and CFT antibodies in few.

Discussion

Two vaccine types are available for brucellosis control: attenuated vaccines which are the preferred vaccines and inactivated (killed) vaccines. Vaccination against animal brucellosis as the sole means of disease control has been very effective. In many countries, brucellosis treatment is prohibited and strictly restricted to human cases.

The fact that brucellosis is associated with a chronic debilitating disease in human beings and reproductive disorders in domestic and wild animals leading to severe social and economic repercussions, should put the eradication and control of this zoonosis on the priority list of national health authorities and international organisations like FAO and OIE. An official estimate of animal losses for example in Latin America due to bovine brucellosis amounts around 600 million US $ (Acha and Szyfres, 2003) per year. In brucellosis endemic countries like the United Arab Emirates (UAE) an eradication programme should be established which includes testing, slaughter of reactors and vaccination of animals which can also be initiated by zoning and regionalisation. First, herds must be tested at regular intervals until two or three successive tests are negative and brucellosis reactors eliminated. These non-infected herds must then be protected by vaccination. As the greatest danger comes from replacement animals, those animals should be isolated for 30 days and serologically tested before negative animals are added to the herd after vaccination. Commercial serological tests have been evaluated by Soellner *et al* (2018) and Soellner (2020) for use in dromedary camels. They are now available.

In cattle which mainly harbor *B. abortus*, cases of cross-infections with *B. melitensis* are regularly observed in herds mixed with sheep and goats in southern Europe, Israel, Kuwait and Saudi Arabia. In some South American countries *B. suis*, biovar 1 has also become established in bovines (Saegerman *et al,* 2010). *B. melitensis* and *B. abortus* have both been cultured from Old World Camels (OWCs) (Wernery *et al,* 2014). However, *B. melitensis* is prevalent in Arabia and East Africa, whereas *B. abortus* occurs in East Asian camelids including in the old Russian States.

Vaccination against *B. abortus* is performed with Strain 19 or RB51. A small percentage of vaccinated animals, however, develop antibodies to Strain 19 and may persist for years and confuse diagnostic test results. To minimise the problem, animals in different countries for example in the USA are mainly vaccinating with a vaccine of Strain RB51. This is a rough attenuated strain which does not cause production of vaccine antibodies measurable by classical serological tests, regardless of age, dose or frequency of injections, because it is devoid of the lipopolysaccharide (LPS) O-side chains. RB51 is given subcutaneously to bovine calves aged between 4 to 12 months at a full dose of 1.0 - 3.4 x 10^{10} cfu. It does not cause abortion when used at 60 days of pregnancy.

Our vaccination experiment in dromedaries showed the same results, as 6 dromedaries vaccinated with RB51 vaccine did not produce any measurable antibodies using conventional serological methods.

In bovines, *B. abortus* Strain 19 vaccine induces protection against both *B. abortus* and *B. melitensis* without persistent antibody response. However, when using *B. abortus* Strain RB51 vaccine, it is unknown if this vaccine protects against *B. melitensis* infection in cattle as well (OIE Manual, 2018), but it is certainly not effective against *B. melitensis* in sheep. This important fact has not yet been investigated in camelids.

Table 1. Serological results of 6 dromedaries each vaccinated with *B. abortus* RB51 and *B. melitensis* Rev 1.

Camel ID	Vaccine	Before Immunisation				After Immunisation															
						30 days				60 days				90 days				120 days			
		RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA
1	*B. abortus* RB51	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
4		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
1	*B. melitensis* Rev 1	Neg	Neg	Neg	Neg	++	1:160+++	1:32++++	96.4%	++	1:80+++	1:32++++	96.1%	++	1:80+++	1:32+++	96.2%	++	1:80+	1:16++++	95.8%
2		Neg	Neg	Neg	Neg	++	1:80++	1:8+	83.6%	+	1:40++	1:8+	81.3%	Neg	1:20++++	1:4++++	74.0%	Neg	Neg	1:4++++	74.7%
3		Neg	Neg	Neg	Neg	++	1:160++	1:4++	94.9%	++	1:80+	1:4++++	92.7%	+	1:40++	1:4+	91.3%	+	1:20++	1:2++++	84.6%
4		Neg	Neg	Neg	Neg	++	1:320++	1:32+++	96.0%	++	1:160+++	1:16++++	94.9%	++	1:80+++	1:8++++	93.6%	++	1:80+	1:8+	88.2%
5		Neg	Neg	Neg	Neg	+++	1:320+++	1:32++	91.3%	++	1:320+++	1:64+	89.7%	++	1:320++	1:64+	89.7%	+++	1:160+++	1:32++++	86.5%
6		Neg	Neg	Neg	Neg	+++	1:320++	1:128+++*	96.5%	+++	1:160+	1:64++++	96.4%	++	1:80+	1:16++++	96.1%	+	1:40+++	1:8++++	94.8%

Red = Positive results Green = Dubious results

Camel ID	Vaccine	After Immunisation																			
		150 days				180 days				210 days				240 days				270 days			
		RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA
1	*B. abortus* RB51	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
4		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
1	*B. melitensis* Rev 1	++	1:80+	1:16++++	95.8%	+	1:40+++	1:16+	94.5%	+	1:20++++	1:8++++	95.7%	+	1:20++++	1:8++	94.3%	++	1:20++++	1:8++++	94.9%
2		Neg	Neg	1:4+	74.2%	Neg	Neg	Neg	69.7%	Neg	Neg	Neg	73.5%	Neg	Neg	Neg	58.5%	Neg	Neg	Neg	50.8%
3		+	Neg	Neg	85.7%	Neg	Neg	Neg	79.6%	Neg	Neg	Neg	86.2%	+	Neg	Neg	76.4%	+	1:20++	Neg	71.1%
4		++	1:40++++	1:4++++	87.5%	+	1:40++	1:4++++	83.0%	+	1:20++++	Neg	87.3%	+	1:20++++	Neg	79.4%	+	1:20++++	Neg	73.4%
5		++	1:160+++	1:16++++	88.2%	++	1:160+++	1:16+	89.5%	+	1:80+++	1:8++++	90.7%	++	1:80++	1:8+	84.7%	++	1:40+++	1:8+	82.4%
6		+	1:40++	1:4++++	92.7%	Neg	1:20+++	1:4+	89.5%	Neg	Neg	Neg	93.0%	Neg	Neg	Neg	87.5%	Neg	Neg	Neg	87.5%

Red = Positive results Green = Dubious results

Continued Table 1.....

Camel ID	Vaccine	After Immunisation							
		300 days				335 days			
		RBT	SAT	CFT	ELISA	RBT	SAT	CFT	ELISA
1	*B. abortus* RB51	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
2		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
4		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
6		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
1	*B. melitensis* Rev 1	+	1:20++	1:4++++	82.9%	+	1:20+++	1:4++++	88.8%
2		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3		Neg	Neg	Neg	60.5%	Neg	Neg	Neg	51.6%
4		Neg	1:20++++	Neg	57.9%	Neg	1:20++++	Neg	63.4%
5		+	1:40++	1:4++	66.5%	+	1:40++	1:4+++	71.0%
6		Neg	Neg	Neg	66.9%	Neg	Neg	Neg	66.5%

Red = Positive results Green = Dubious results

In countries, where *B. melitensis* infection is endemic, vaccination with Rev1 strain is common in small ruminants. No vaccination experiments, however, have been reported showing efficacy of Rev1 against *B. melitensis* infection in cows. Additionally, the safety of Rev1 vaccine is unknown in cattle and is therefore not recommended for use in bovines. Rev1 strain is an attenuated strain of *B. melitensis* and is pathogenic for people.

Our experiment shows that dromedaries subcutaneously vaccinated with the full dose of Rev 1 strain produced different antibodies which remained in some dromedaries for 12 months. Five of the 6 dromedaries tested for ELISA antibodies remained positive for more than a year. This is a severe disadvantage as all serological methods for the diagnosis of brucellosis cannot differentiate between vaccine or natural infection titres. Long lasting serological reactions can be expected among vaccinated camels because of antibodies cross-reacting with wild strain infections. So far, no challenge infection has been conducted in vaccinated camels and therefore the vaccine efficacy is unknown. However, if a Brucella vaccine is used in camelids it should be RB51 strain, but before this is done, further research has to be conducted.

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DOI : 10.5958/2277-8934.2019.00019.5 Vol 26 No 2, p 125-128

ANOTHER CASE OF HEPATIC CYSTICERCOSIS CAUSED BY *Cysticercus tenuicollis* IN A DROMEDARY

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ABSTRACT

The necropsy of an adult female dromedary revealed a large number of calcified rice grain to cherry sized bodies in the liver. Larger structures were calcified cysts containing a partially calcified cysticercus with an evaginated scolex. The morphology of rostellar hooks matched with both *Taenia hydatigena* and *T. hyaenae*. However, molecular techniques identified the bladderworms as *Cysticercus tenuicollis*.

Key words: *Camelus dromedarius*, *cysticercus tenuicollis*, liver cysticercosis, United Arab Emirates

Cysticercus tenuicollis, the larval stage of *Taenia hydatigena* is a quite common parasite in domestic small ruminants, pigs and a variety of wild ungulates (Scala *et al,* 2015). Textbooks and reviews (Abuladze 1964; Dakkak and Ouheli, 1987; Fassi-Fehri, 1987; Troncy *et al,* 1989; Kaufmann, 1996; Parsani *et al,* 2008; Abu-Samra 2015) mentioned also camels as intermediate hosts, but there were hardly any reliable original references. In 2015 we published the finding of a metazoan parasite in the liver parenchyma of a three months old camel calf. The bilaterally flat structure with an invagination on one pole suggested the presence of early development stages of a metacestode and molecular examination revealed *T. hydatigena* (Schuster *et al,* 2015). Another recent paper (Omar *et al,* 2016) reported the finding of *C. tenuicollis* in one out of 103 camels slaughtered in abattoirs in Upper Egypt but authors failed to give details about the number of cysticerci and their location. *Cysticercus dromedarii*, the larval stage of *Taenia hyaenae*, is predominantly located in the liver of dromedaries but is also found in heart, skeleton muscles and brain. It was also detected in cattle and wild ruminants in African countries. Number and sizes of rostellar hooks of both species overlap according to literature data (Table 1). Therefore, a clear differentiation based on the morphology is difficult.

Case report

A fresh carcass of an adult female, not pregnant and not lactating dromedary in fair condition (371 kg) was sent for necropsy to the Central Veterinary Research Laboratory in November 2018. The animal originated from one of the camel reproduction centres of Dubai where it has been kept for the past two years. There was no record of the origin of the animal. The carcass was infested with many ticks between hind legs and on udder, predominantly nymphal stages and few adult *Hyalomma dromedarii*. Multiple ulcera in abomasum that led to massive bleedings into the alimentary tract were the main pathological findings and cause of death. The 7.3 kg liver showed multiple rice grain to cherry seed sized calcified cysts under its capsule (Fig 1). Cutting the organ into 0.5 cm slices revealed a total of 233 calcified structures in sizes of 3 to 12 mm. No further bladder worms were found either in omentum, heart, tongue, masseters or in the brain of the animal.

Parasitological examination

While smaller cysts were totally calcified, 18 larger cysts were surrounded by a calcified capsule, contained some white jelly (Fig 2) and a partly calcified white cysticercus with a 3-5 mm sized liquid filled bladder and an evaginated scolex (Fig 3). Fifteen cysticerci with an apparently complete set of rostellar hooks were put for 12 hours into 30% lactic acid. The scolices were then cut off, transferred on a glass slide, covered with a cover slip and were microscopically examined. The following parameters were established and measured: size of scolex, diameter of suckers and rostellum, number and size of hooks.

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Molecular examination

Genomic DNA was extracted from 4 individual cysticerci using G-spinTM Total DNA Extraction Kit (iNtRon Biotechnology, Korea). Two target sequences of mitochondrial DNA coding for the partial cytochrome c oxidase 1 (cox1) (~391bp) and NADH dehydrogenase subunit 1 (ND1) (~471bp) were amplified by polymerase chain reaction (PCR). PCR was performed using primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (5'-AAAGAAAGAACATAATGAAAATG-3'), JB11 (5'-AGATTCGTAAGGGGCCTAATA-3') and JB12 (5'-ACCACTAACTAATTCACTTTC-3') for cox1 and ND1, respectively, as previously described (Boufana *et al*, 2015). PCR products were purified using a Nucleospin Gel and PCR Clean Up (Macherey-Nagel GmbH & Co. KG, Düren, North Rhine-Westphalia, Germany) and sent to an external sequencing service (Eurofins Genomics, Germany). PCR products were commercially sequenced by MWG-Biotech (Ebersberg, Germany). Generated nucleotide sequences were edited in MEGA 6.0 (Tamura *et al*,, 2013) and compared to those available in the National Centre for Biotechnology Information database (NCBI) using the basic local alignment search tool (BLAST).

Results

Parasitological examination

Details of measurements of scolices are summarised in Table 2. The nearly square scolices are armed with 28-36 rostellar hooks arranged in two circles (Fig 4). In larger hooks measuring 171-207 µm, the handle was slightly bigger than the blade while smaller hooks with a length of up to 144 µm had a slightly bigger blade. The handle of some smaller sized hooks were not fully developed (Fig 5). The tip of the smaller hook's guard was clearly divided (Fig 6).

Molecular examination

Of the 4 *C. tenuicollis* included in this study, 4/4 (100%) were successfully amplified for the (cox1 386 bp) and ND1 (452 bp) mitochondrial genes, respectively. Molecular analyses of cox 1 and ND1 mitochondrial genes, respectively shown

Table 1. Morphometrical data of 15 intact scolices from a dromedary liver.

Number of examined	Scolex width (µm)	Suckers diameter (µm)	Rostellum diameter (µm)	Hooks						
				number	large			small		
					average	from	to	average	from	to
1	865	275	435	30	200	195	208	137	131	143
2	875	310	430	30	193	188	202	129	121	135
3	925	300	405	32	185	183	189	115	109	120
4	850	310	420	30	192	188	195	137	127	142
5	870	330	400	30	179	174	186	127	116	135
6	830	290	440	32	192	187	198	121	86	130
7	900	360	350	30	179	171	186	119	109	128
8	850	330	410	28	177	173	182	124	109	135
9	785	260	440	32	195	189	202	123	63	134
10	850	300	400	32	193	182	197	131	128	136
11	830	320	410	32	202	197	207	130	125	144
12	870	280	450	30	186	175	191	114	102	134
13	850	275	425	36	192	189	199	123	113	130
14	820	300	400	32	196	193	202	129	87	140
15	915	360	450	32	187	179	192	111	99	118
Average:	**860**	**307**	**418**		**190**			**125**		

Table 2. Number and sizes of rostellar hooks of *T. hyaenae* and *T. hydatigena* according to different authors. ([a] brown hyaena, [b] dromedary, [c] sheep, [d] dog).

Hooks	*T. hyaenae*				T. hydatigena			
	Baer (1926)[a]	Pellegrini (1945)[b]	Vester (1969)[a]	Loos-Frank[a] (2000)	Abuladze (1964)[c]	Vester (1969)[d]	Loos-Frank (2000)[d]	Radfar *et al.* (2005)[c]
Number	32-38	28-40	28-36	28-36	34-44	28-38	28-36	28-34
Large (µm)	223	185-210	191-218	191-218	187-212	218-242	202-242	199+10.9
Small (µm)	127	126-160	118-143	118-143	112-137	142-162	128-159	135+12

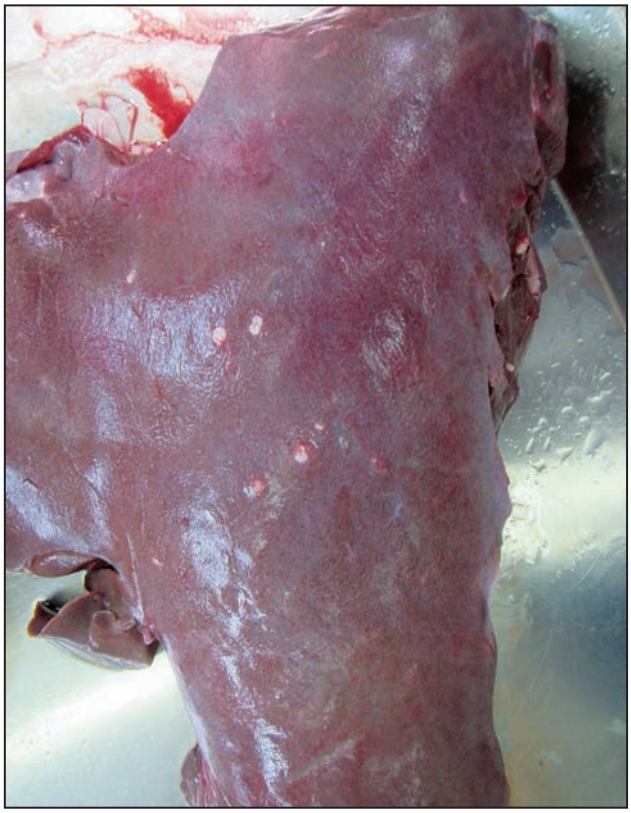

Fig 1. Liver of a dromedary with calcified cysts under capsule.

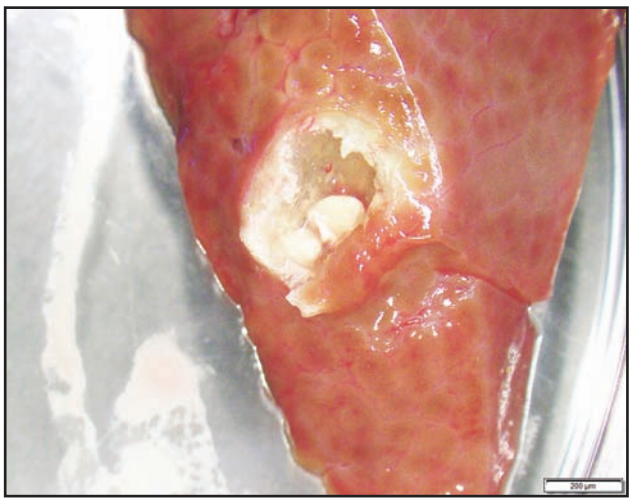

Fig 2. Dromedary liver with a calcified cyst containing gelly like substance and a cysticercus.

an homology of 99% with *T. hydatigena* sequences deposited in GenBank (accession numbers AB792722 and JN831270).

Discussion

T. hyaenae was described by Baer (1926) from material collected by Theiler from a brown hyena in South Africa. This tapeworm was also reported from spotted hyenas in Kenya (Baylis, 1937). Its larval stage, *C. dromedarii*, was first found in dromedaries

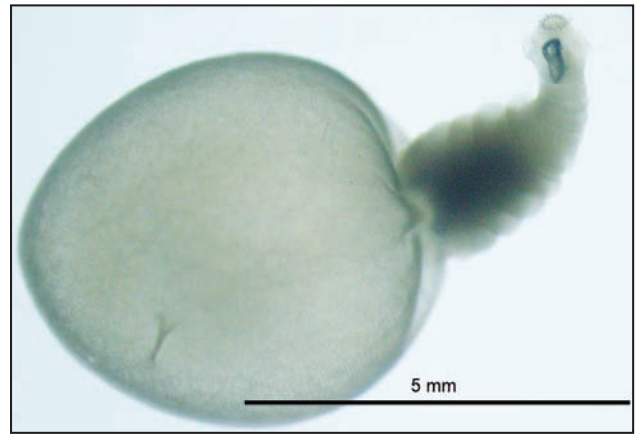

Fig 3. Custicercus with an evaginated scolex removed from a calcified liver cyst of a dromedary.

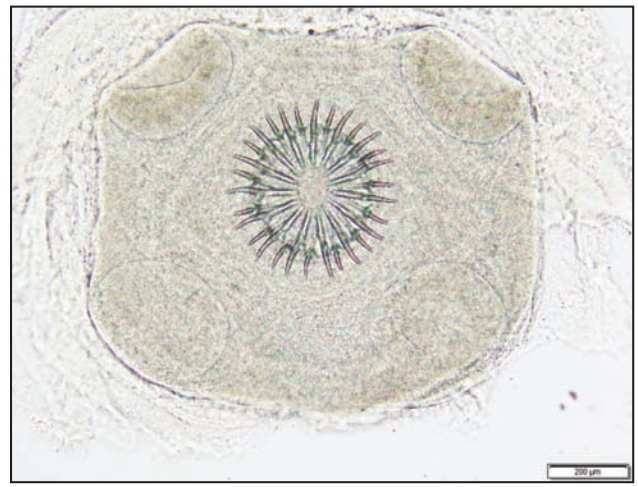

Fig 4. Isolated scolex, apical view.

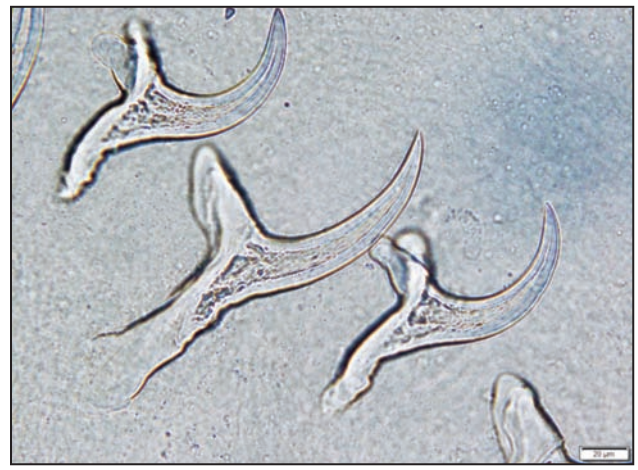

Fig 5. Large and small rostellar hooks. The guard of the small rostellar hooks is divided.

in Somalia by Pellegrini (1945). According to the author, *C. dromedarii* is mainly located in the liver but can be found also in muscle tissues, lungs, spleen lymphnodes and brain and form oval or round cysts of up to 13 mm in diameter. Aging cysts tend to

calcify and getting filled with a white material. In our case most of the structures removed from liver tissue were completely calcified; only larger ones contained white masses and an already dead, partially calcified cysticercus with an evaginated scolex. According to the morphology of the rostellar hooks, the bladder worms could belong to the above mentioned species.

Molecular results however, revealed that the infection was caused by *C. tenuicollis*, the larval metacestode stage of *T. hydatigena*. Data available on the occurrence of *C. tenuicollis* in camels are scant. In particular, there are only two cases of *C. tenuicollis* in camels described in the literature referred at findings in a camel calf in Dubai and in a camel in Upper Egypt (Schuster *et al,* 2015; Omar *et al,* 2016). Moreover, there are little genetic informations about the ubiquity of this parasite in ruminant populations (Ghaffar, 2011; Rostami *et al,* 2015; Omar *et al,* 2016). In the present report we found mostly degenerated cysts. This finding and the fact that there are few cases of *C. tenuicollis* in camels is in agreement with our previous study in which we suggested that camels may be aberrant and/or poor host for *C. tenuicollis* (Schuster *et al,* 2015).

Dogs are the main final hosts for *T. hydatigena* and become infected when they feed on offals or on carrion (Varcasia *et al,* 2011). Due to strict veterinary–sanitary rules in Dubai that allows slaughtering of animals only in abattoirs with proper meat inspection it is quite unlikely for dogs to become infected with the adult cestode. It is more likely that the camel with liver cysticercosis was imported from abroad. Official figures of FAO (FAOSTAT, 2016) showed that the camel population in the UAE in 2016 consisted of 443,568 heads. Of these, 67,109 camels were imported.

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DOI : 10.5958/2277-8934.2019.00020.1 Vol 26 No 2, p 129-132

DETECTION OF THE STATUS OF ACARICIDE RESISTANCE IN *Hyalomma dromedarii* FROM NOMADIC CAMELS OF NORTH GUJARAT

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ABSTRACT

The present study was undertaken to evaluate the efficacy of commonly used acaricides *viz.* deltamethrin, cypermethrin, flumethrin and fipronil against ticks infesting camels of north Gujarat. Larval packet test was conducted using field isolates of *Hyalomma dromedarii* for determination of 50 and 95% lethal concentration of deltamethrin, cypermethrin, flumethrin and fipronil. LC_{50} and LC_{95} values of deltamethrin (3.82, 35.36 ppm) and cypermethrin (6.59, 140.01 ppm) indicated the susceptibility of *Hyalomma dromedarii* larvae to these chemicals. Flumethrin and fipronil were found to be highly toxic to the larvae and thus, the LC_{50} and LC_{95} values were as low as $2.92x10^{-8}$, 0.02 and 0.06, 1.62 ppm, respectively. Resistance characterisation revealed very low resistance factor in the ticks studied, *i.e.* 0.33 for deltamethrin, 0.03 for both cypermethrin and fipronil, respectively. Flumethrin recorded more than 99% mortality even at its lowest concentration. Multi-host life cycle and tick management practices without the use of chemicals might have protected these ticks from development of resistance.

Key words: Acaricide resistance, camel, *hyalomma dromedarii*, pyrethroids

Gujarat is the state in India where substantial camel population exist and they are primarily reared for transportation, agricultural operation in addition to the secondary utility of milk and hair production (Saini *et al,* 2006). This species has significantly contributed in rural economy and livelihood of desert dwellers of Gujarat. The problem of tick and tick borne diseases has been ranked high in terms of their adverse impact on the livelihood of poor farming communities in developing countries including India. The tick, *Hyalomma dromedarii* is an obligate parasite affecting camels and considered as the most important obstacle to camel production. Relatively little information is available on the role of hard ticks (Acari; Ixodidae) as disease vectors in camels. *Theileria camelensis* (also referred to as *T. dromedarii*), has been reported from India (Rao *et al,* 1988) based on the presence of the erythrocytic piroplasm stage of the parasite.

In an integrated tick control programme, the control measures varied from manual removing of ticks from the camels (as practiced by nomadic people) to the application of chemical acaricides. The most widely used method for controlling tick populations are based on the application of synthetic acaricides both in the environment and to animals. However, continuous and indiscriminate use of these substances has led to resistance and residues in the environment. In this study, we evaluated the efficacy of deltamethrin, cypermethrin, flumethrin and fipronil against ticks infesting camels.

Materials and Methods

Tick collection and laboratory maintenance

The ticks were collected from Bhagalin of Banaskantha district located in northern part of Gujarat. Camels were kept under traditional management practices. Live engorged adult female ticks were handpicked under close scrutiny from the body surfaces of the camel and sandy areas near camel dwellings. Ticks were kept in vials covered with muslin cloth and these were brought to Entomology Laboratory, Department of Veterinary Parasitology, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India. Identification of ticks was done both by gross examination and with microscope using tick identification sources (Geevarghese and Dhanda,

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1987; Walker *et al,* 2014). Ticks were then washed, cleaned, labelled and were kept for egg laying at 28 ± 1˚C and 85 ± 5% relative humidity. Ticks were kept up to 10-15 days for oviposition and subsequent hatching. Further, larvae were kept for 12–14 days before performing experimental bioassay.

Commercial synthetic pyrethroids

For dose dependent bioassay, commercially available preparations of deltamethrin (1.25%) and cypermethrin were diluted in distilled water whereas flumethrin (1%) and fipronil (0.25%) was diluted in 25% acetone to make different concentrations, namely, 12.5, 25, 50, 100 and 200 ppm for deltamethrin, 100, 200, 400, 800 and 1600 ppm for cypermethrin 50, 100, 200, 400 and 600 ppm for flumethrin and 8, 10, 12, 16, 18 and 24 ppm for fipronil.

Larval packet test (LPT)

Larval packet test was conducted according to FAO (2004) guidelines with minor modifications (Shyma *et al*, 2015). Filter paper rectangles (3.75 cm x 8.5 cm) (Whatman filter paper no. 1) were impregnated with 600 µl of different concentrations of deltamethrin, cypermethrin, flumethrin and fipronil. The impregnated paper was dried by keeping the same for 30 minutes in incubator at 37˚C. The rectangles were folded in half to form an open-ended packet and sealed on the sides with adhesive tapes, to place tick larvae. Approximately 100 larvae were inserted and the packet was sealed with adhesive tape. Total three packets filled with larvae were kept for each concentration of drugs. These packets were then placed in desiccators in BOD incubator maintained at 28 ± 1˚C and 85 ± 5% RH. The packets were removed after 24 h and larval mortality was calculated after counting the number of live and dead larvae. Control packets impregnated with water and 25% acetone were also prepared for each series of concentration to be tested.

Statistical analysis

Dose–response data were analysed by probit method (Finney, 1972) using Graph Pad Prism 4. Regression curves of mortality of larva were also plotted against values of acaricide concentrations by log dosage probit mortality analysis for determination of LC_{50} and LC_{95} values of both the synthetic pyrethroids.

Results

The ticks collected in the present study were identified as *Hy. dromedarii*. The effect of different concentrations of deltamethrin (12.5, 25, 50, 100, 200 ppm), cypermethrin (100, 200, 400, 800, 1600 ppm), flumethrin (50, 100, 200, 400, 600ppm) and fipronil (8, 10, 12, 16, 18ppm) on larval stages of *Hy. dromedarii* by larval packet test (LPT) are presented in Table 1. Graphs were plotted between log concentrations of acaricides and probit mortality for determination of LC_{50}, LC_{95}, slope and coefficient of determinations (R^2) values (Fig 1). The mean mortality of larvae treated with various concentrations of deltamethrin ranges from 86.52 to 99.86%, 93.68 to 99.82% with cypermethrin, 99.64 to 99.85% with flumethrin and 99.14 to 99.86% with fipronil. Insignificant mortality was recorded in larvae of control group treated with distilled water and acetone. LC_{50} and LC_{95} values of deltamethrin (3.82, 35.36 ppm) and cypermethrin (6.59, 140.01ppm) by LPT indicated the susceptibility of *Hy. dromedarii* larvae to these chemicals. Flumethrin and fipronil showed to be highly toxic to the larvae and thus, the LC_{95} values were lower than 0.02 (0.01-0.03 CI) and 1.62 (1.38-1.92 CI) ppm, respectively. The goodness of fit (R^2) data of different tick isolates ranged from 0.79 to 0.96 showing 79-96% correlated response with log doses of acaricides.

Discussion

Ticks are obligate sanguivorous parasites of terrestrial vertebrates, distributed worldwide,

Table 1. Response of *Hyalomma dromedarii* against various acaricides by LPT.

Chemical	Slope	R^2	LC_{50} (95% CI)	LC_{95} (95% CI)	RF	RL
Deltamethrin	1.71	0.92	3.82 (3.62-4.04)	35.36 (31.64-39.51)	0.33	S
Cypermethrin	1.24	0.96	6.59 (6.11-7.10)	140.01 (119.90-163.50)	0.03	S
Flumethrin	0.29	0.79	$2.92x10^{-8}$ ($2.11 x10^{-8}$-$4.04 x10^{-8}$)	0.02 (0.01-0.03)	–	S
Fipronil	1.17	0.88	0.06 (0.058-0.068)	1.62 (1.38-1.92)	0.03	S

Susceptible=RF < 1.4; level I=1.5 < RF < 5; level II=5.1 < RF < 25; level III=26 < RF < 40; level IV=RF > 41; S=susceptible.

causing anaemia, skin injury, tick worry and some times tick paralysis (Wall and Shearer, 2001). In India, almost all livestock species are suffering from tick infestations throughout the year. *Hy. dromedarii* commonly known as 'The camel Hyalomma' is closely associated with camels. To deal with the tick challenge, farmers rely on acaricides for chemical control of ticks. There is a huge demand and market for acaricides and moreover, it is easily accessible to the farmers. Incorrect dilutions, application methods and increased acaricide pressure leads to acaricide resistance. Increased reports of treatment failure to control ticks raises serious warning of possible emergence of acaricide resistant ticks in the state. Several methods have been proposed for detection of acaricide resistance like larval packet test (LPT), larval tarsal test (LTT) and adult immersion test (AIT) (FAO, 1984). *In vitro* bioassays are relatively simple to perform, incur a low cost and have few requirements for specialised equipment (Scott, 1995). Tick resistance to various classes of acaricides has been reported by the senior author in one host tick, *Rhipicephalus* (*Boophilus*) *microplus* (Shyma *et al,* 2013, 2015) and a few in multi host tick *Hyalomma anatolicum* (Shyma *et al,* 2012). No experimental data is available with regard to the susceptibility or resistance status of *H. dromedarii*. The present study demonstrates the comprehensive information on the status of resistance/susceptibility in *Hy. dromedarii* to commonly used synthetic pyrethroids and phenylpyrasole compound, fipronil using bioassay.

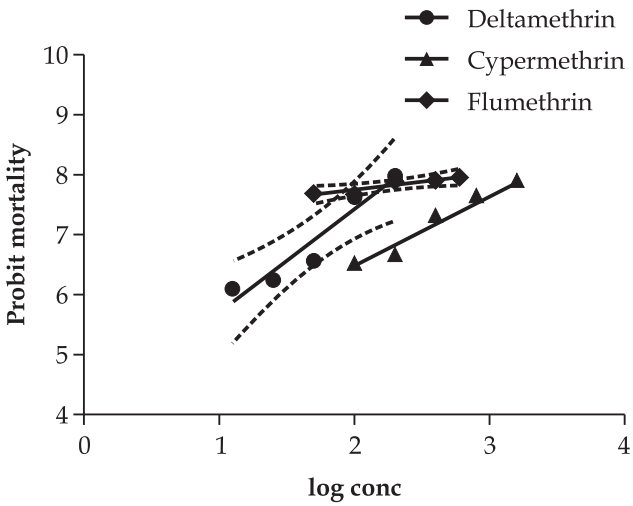

Fig 1. Dose dependent mortality curve of *Hyalomma dromedarii* against deltamethrin, cypermethrin and flumethrin.

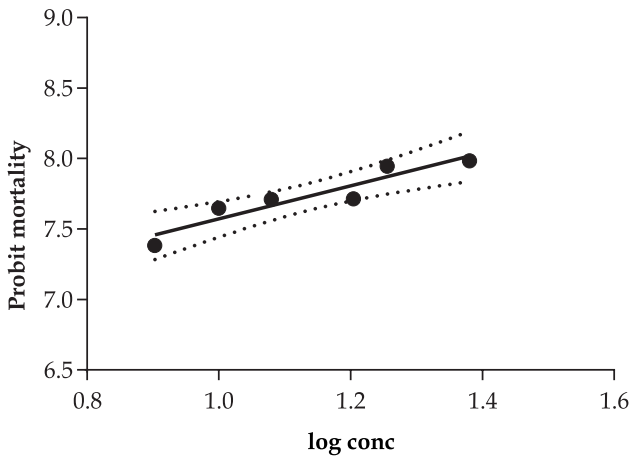

Fig 2. Dose dependent mortality curve of *Hyalomma dromedarii* against fipronil.

The results of the current study advocate that deltamethrin, cypermethrin, flumethrin and fipronil were effective on larval stages of *Hy. dromedarii* at its recommended dosage. Resistance factors (RF) for field tick isolates were worked out by the quotient between LC_{50} of field isolates and LC_{50} of reference *Hy. anatolicum* IVRI-II line (Shyma *et al,* 2012; Castro-Janer *et al,* 2009). The LC_{50} of reference *H. anatolicum* IVRI-II line against fipronil was not available, hence compared with that of LC_{50} of reference susceptible IVRI-I line of *Rh.* (*B.*) *microplus* to determine the RF (Kumar *et al,* 2015). On the basis of RF, the resistance status in the field population of *Hy. dromedarii* was classified as susceptible (RF ≤ 1.4), level I resistant (RF = 1.5–5.0), level II resistant (RF = 5.1–25.0), level III resistant (RF = 26–40) and level IV resistant (RF ≥ 41) (Kumar *et al,* 2011). Resistance characterisation revealed very low resistance factor in the ticks studied. It was estimated as 0.33 for deltamethrin, 0.03 for both cypermethrin and fipronil, respectively and therefore, these were categorised under susceptible. Resistance factor of ticks against flumethrin could not be determined owing to lack of data on reference tick line. However, flumethrin recorded more than 99% mortality even at its lowest concentration (50 ppm). In the current study, commercially available acaricides were used to assess the efficacy of these widely used drugs which could not have been possible with the use of analytical grade acaricides as commercial products are prepared with many proprietary ingredients and it is difficult to assess the responses due to individual components of the formulations (Haque *et al,* 2014). Only few records of resistance are available in multi host ticks to synthetic pyrethroids (Nolan, 1990) which could be due to later introduction of these acaricides in field or slower development of acaricide resistance in multi host ticks.

Hy. dromedarii behave as a three, two, or one-host species, whereas Hoogstraal *et al* (1981) found

out that the two-host life cycle is the most common for this species. In global basis, the incidence of acaricide resistance is highest in one host ticks of the genus *Boophilus* (Wharton and Roulston, 1970). This is because a much larger fraction of the total population of such species remains under chemical challenge at any one time than multi host ticks. In addition, multi-host tick has extended generation interval compared to *Boophilus* (Harley, 1966). The immature stages of the multi host ticks often feed on small wild animals, even if the adults tend to prefer large domestic animals. These could also be contributory factors which protect these ticks from exposure to chemicals. The ticks in the present study were collected from nomadic camels which are reared by particular community of Gujarat. These farmers have never opted for the acaricides and preferred for the botanicals for the control of ticks in camels. It seems these ticks have never been exposed to chemical acaricides and this might be another reason for its susceptibility. It is appreciative that the acaricides which are being used for decades are still working against *Hy. dromedarii*.

Acknowledgement

We would like to express our gratitude to Director of Research, Sardarkrushinagar Dantiwada Agricultural University and Dean, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University for providing all the necessary facilities for carrying out this work.

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DOI : 10.5958/2277-8934.2019.00021.3 Vol 26 No 2, p 133-142

IDENTIFICATION OF MICROSATELLITES AND PARENTAGE TESTING DEVELOPMENT OF BACTRIAN CAMEL (*Camelus bactrianus*)

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ABSTRACT

This report is aimed to identify polymorphic microsatellites from Bactrian camel transcriptome sequences and apply microsatellite multiplex systems for parentage testing. 1,820 simple sequence repeats (SSRs) were identified from 23,624 transcriptome sequences, representing an average density of SSR/26.57 kb. The most abundant SSR, the trinucleotide repeat motif, accounts for 30% of all SSRs. Tetra-, penta-, di- and hexanucleotide repeats account for 29%, 22%, 17% and 2% of SSRs, respectively. Based on sequence redundancy and PCR amplification, we selected 14 polymorphic microsatellites for Bactrian camel parentage testing analysis in a population of 117 unrelated camels from China and Mongolia. We identified a total of 149 alleles, with 5-23 alleles per locus (10.64 ± 5.472) and an average heterozygosity (HE) of 0.676 (range: 0.380-0.888). Based on only the genotype of the offspring, a parentage exclusion probability of 0.9999 was calculated when excluding a candidate parent from parentage of an arbitrary offspring. When excluding a candidate parent from parentage of an arbitrary offspring and based on both the genotype of the offspring and the other parent, an exclusion probability of 0.9999 was calculated. We selected 15 baby camels, 20 sires and 20 dams for parentage testing. According to the parentage assignment, the microsatellite panel assigned all 15 offspring with high confidence. This core set of 14 microsatellites represent a powerful and efficient method for determining parentage in domestic Bactrian camels.

Key words: Multiplex, parentage exclusion probability genotyping, simple sequence repeats

The Bactrian camels mainly provide milk, meat, wool and contributes to the tourism industry. This lead to a continuously growing number of camels in recent years. With the increasing demand for camel products, individual identification of camels is necessary and the importance of DNA testing to ensure an accurate identification and parentage assignment in bactrian camel breeding, which is essential for a reliable breeding programme aimed at genetic improvement of productive traits in bactrian camels. Over the past decade, several methods have been employed to identify individual camels, including blood typing, biochemical polymorphism, protein electrophoresis, randomly amplified polymorphic DNA (RAPD) and DNA fingerprinting. However, these all methods carry significant limitations.

Simple sequence repeats (SSRs), also referred to as microsatellites, are short repeat motifs (1-6 base pair) present in the genomes of higher organisms (Gupta *et al,* 1996; Tóth *et al,* 2000). They are very powerful genetic markers, due to their simplicity, abundance, genetic codominance and multi-alleles dispersal throughout the genome (Schlötterer and Tautz, 1992; Li *et al,* 2002). Microsatellites are broadly used in genetic mapping and molecular evolution research and are considered the markers of choice in the molecular characterisation of livestock genetic resources (Yan *et al,* 2008; Amoli *et al,* 2018; Chen *et al,* 2018). In the past few years, many SSR loci have been characterised in camelids (Penedo *et al,* 1999; Mariasegaram *et al,* 2002; Evdotchenko *et al,* 2010; Lang *et al,* 2010; Amoli *et al,* 2017); and several have been used for parentage testing in alpaca and dromedary (Lang *et al,* 2010). However, there are currently no reports of parentage testing in Bactrian camels. Here, we searched and identified new microsatellite loci from Bactrian camel transcriptome sequences optimised for parentage testing.

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Materials and Methods

Camel transcriptome sequences

23,624 camel transcriptome sequences were directly obtained from the National Centre for Biotechnology Information (NCBI) (ftp.ncbi.nlm.nih.gov/genomes/all/ GCA_000767855.1_Ca_Bactrianus_MBC_1.0). All the sequences were saved separately in FASTA-Formatted text files which were then used for further analysis in this study.

Detection of SSRs

SSR motifs were identified using the MicroSatellite identification Perl tool (MISA) available at http://pgrc.ipk-gatersleben.de/misa/misa.html. Transcriptome sequences following five combinations of di-, tri-, tetra-, penta- and hexanucleotide repeats were investigated. The minimum repeats number criteria for dinucleotide was 7, 6 for tri-nucleotide, 5 for tetranucleotide, 4 for pentanucleotide and 3 for hexanucleotide (Yan *et al,* 2008). Single nucleotide repeats are not considered to be effective polymorphic markers and were therefore not used.

Sample collection and DNA extraction

Blood samples from 117 unrelated Bactrian camels of both sexes representing 6 Bactrian camel populations were collected from private breeding herds in different localities comprising three regions of China (Qinghai Province (n=16), Inner Mongolia (n=57) and Xinjiang Province (n=15), three regions of Mongolia (Hanbogd soum (n=10), Mandal-Ovoo soum (n=10) and Tugrug soum (n=9). Additionally, to test the microsatellite panel on parentage assignment, 20 sires, 20 dams and 15 offspring registered in the herdsman's record pedigree book as having parent-offspring relationships were used. Blood samples were drawn by venipuncture into EDTA-containing tubes and stored at -20°C. DNA was extracted from 400 µL of blood using the DNA Extraction Kit (QIAamp DNA Blood Mini kit -Qiagen) and following standard procedures. The quality and quantity of genomic DNA was determined with a NanoDrop spectrophotometer.

Primer design

First, based on sequence redundancy, identical SSR sites were deleted, leaving 56 SSRs sites. For each microsatellite-containing transcriptome, primers were designed using Primer Premier 5.0 and synthesised by Shanghai Sangon Biotech, China (Supplemental Table 1). Among the 56 SSR markers, fourteen were considered as high polymorphic SSR markers and easy to identify and repeatable and furthermore, fourteen primer pairs were selected based on amplification results with clear amplification fragments and then fluorescently labelled. Five primers were labeled with FAM (Applied Biosystems), five primers with FTAM (Applied Biosystems) and four primers with HEX (Applied Biosystems) (Supplemental Table 2).

Table 1. Summary of Bactrian camel transcriptome sequences.

Parameter	Number
Total size of examined sequences (bp)	48349611
Total number of transcriptome sequences	23624
Total number of SSRs identified	1820
Number of sequences containing more than 1 SSR	218
Number of SSRs present in compound formation	98
Total number of SSR containing sequences	1550

PCR amplification and capillary electrophoresis

Touchdown PCR was performed with a total reaction volume of 25 µL: approximately 1 µL of DNA 55ng, 10×PCR Buffer 2.5µL (Mg^{2+} free, 100 mM Tris-HCl pH 8.8 at 25°C; 500 mM KCl, 0.8% (v/v) Nonidet), 25 mM MgCl2 2.0µL, 10 mM dNTP, 5 U/µl Taq polymerase and 10 µM of each primer (forward primer labelled with fluorochrome at its 5′ end) 0.5µL. PCR conditions for all reactions consisted of an initial denaturation at 95°C for 3 min, followed by 10 cycles of 30 s at 95°C, 30 s at 63°C and 30 s at 72°C and a final cycle of 6 min at 72°C. Then, a 3 min cycle at 95°C, 20 cycles of 30 s at 95°C, 30 s at 58°C and 30 s at 72°C and a final cycle of 6 min at 72°C.

Fragments were combined with loading mix containing Hi-Di Formamide (Applied Biosystems) and GeneScan LIZ-500 Size Standard (Applied Biosystems). Fluorescently labelled DNA fragments were run on the 3730xl DNA Analyser (Applied Biosystems). DNA fragments were scored with the aid of GENEMARKER software (v1.9, Soft Genetics).

General statistical analysis

Genotypic data were initially manipulated and checked for errors using Microsoft Excel (Heyen *et al,* 2015). The total number of alleles (direct count) and the mean number of alleles per locus (mean±SD), expected heterozygosity $\left(H=1-\sum p_i^2\right)$, observed heterozygosity, exclusion probabilities (Jamieson and Taylor, 2015), Likelihood Ratios (LOD scores) and polymorphism information content (PIC) were calculated for each marker by CERVUS v3.0 (Kalinowskl *et al,* 2007) software. Inbreeding coefficients (Weir and Cockerham, 1984) were calculated using FSTAT v.2.9.3.

Table 2. Genetic variability of 14 microsatellite loci amplified from 117 Bactrian camels.

Locus	N_A	Ne	H_O	He	PIC	PE[1]	PE[2]
XM_006194104_5177-5222	8	4.797	0.378	0.796	0.759	0.591	0.413
XM_006191380_1034-1055	9	4.404	0.611	0.764	0.725	0.631	0.452
XM_006186221_4890-4933	18	4.150	0.549	0.762	0.732	0.610	0.430
XM_006185105_1253-1274	5	2.277	0.056	0.563	0.504	0.835	0.687
XM_006184182_1654-1697	18	7.639	0.639	0.873	0.859	0.400	0.249
XM_006183662_2012-2041	7	1.607	0.111	0.380	0.357	0.924	0.787
XM_006182535_1080-1115	14	2.670	0.465	0.620	0.597	0.761	0.571
XM_006182052_4595-4622	7	4.089	0.584	0.759	0.721	0.639	0.457
XM_006180554_882-917	8	2.117	0.085	0.530	0.466	0.853	0.717
XM_006177721_2115-2144	11	4.382	0.491	0.775	0.740	0.609	0.431
XM_006177716_989-1008	6	1.940	0.435	0.483	0.410	0.882	0.767
XM_006173968_3405-3428	8	3.062	0.349	0.680	0.612	0.754	0.597
XM_006173207_3661-3682	7	2.467	0.158	0.594	0.535	0.811	0.658
XM_006172972_1548-1587	23	8.640	0.714	0.888	0.874	0.373	0.229
Mean ± SD	10.64± 5.472	3.874±2.094	0.402	0.676	0.635		
[3]Total exclusion probability(PEtotal)	-	-	-	-	-	0.999999	0.999991

Note: N_A: Allele number; Ne: Effective number of alleles; H_O: Observed heterozygosity; He: Expected heterozygosity; PIC: polymorphism information content; PE1: [1]Exclusion probability; PE2: [2]Exclusion probability;

The exclusion probabilities and inbreeding coefficient were calculated as follows:

[1]Exclusion probability (PE1):

$$P_i = 1 - 4\sum_{i=1}^{n} p_i + 2(\sum_{i=1}^{n} p_i^2)^2 + 4\sum_{i=1}^{n} p_i^3 - 3\sum_{i=1}^{n} p_i^4.$$

[2]Exclusion probability (PE2):

$$P_i = 1 - 2\sum_{i=1}^{n} p_i^2 + \sum_{i=1}^{n} p_i^3 + 2\sum_{i=1}^{n} p_i^4 - 3\sum_{i=1}^{n} p_i^5 - 2(\sum_{i=1}^{n} p_i^2)^2 + 3\sum_{i=1}^{n} p_i^2 \sum_{i=1}^{n} p_i^3.$$

[3]Total exclusion probability (PEtotal):

$$PE_{total} = 1 - \prod_{k=1}^{kn} [1 - p_k]$$

[4]Exclusion probability (PE3): Parentage exclusion, given two parents and one offspring: exclude both parents

$$P = 1 + 4\sum_{i=1}^{n} p_i^4 - 4\sum_{i=1}^{n} p_i^5 - 3\sum_{i=1}^{n} p_i^6 - 8(\sum_{i=1}^{n} p_i^2)^2 + 8(\sum_{i=1}^{n} p_i^2)(\sum_{i=1}^{n} p_i^3) + 2(\sum_{i=1}^{n} p_i^3)^2$$

Inbreeding coefficient [F_{IS} = (mean H_E - mean H_O)/ mean H_E]

Where Pk = Exclusion probability of k locus; p_i = allele frequency; H_E = expected heterozygosity; H_O = observed heterozygosity.

Results

Characterisation of microsatellite sequences

MISA identified 1,820 SSRs from 23,624 sequences in the Bactrian camel transcriptome, representing an average density of one SSR/26.57 kb. The identified SSRs are summarised in Table 1. The SSRs sequences account for 7.7% of the total camel transcriptome. Among them, the trinucleotide repeat motifs comprised the largest proportion (30%), followed by tetra- and pentanucleotide repeats (29% and 22%, respectively) and hexanucleotide repeats (2%). SSR transcriptome frequencies are presented in supplemental Fig 1.

The possible combinations of different repeat motifs are demonstrated in Supplemental Fig. 1-5. 4 types of dinucleotide repeat motifs, 44 trinucleotide, 23 tetranucleotide, 137 pentanucleotide and 267 hexanucleotide were identified. 316 loci contain dinucleotide repeats, with AC/TG as the most abundant type, accounting for 50%, while GC/CG motif only accounts for 1%. The tri-, tetra-, penta- and hexanucleotide combination were detected in 549, 41, 393 and 521 loci, respectively.

The selection effective SSR loci and analysis of genetic diversity

Fifty-six primer pairs were first selected (Supplemental Table 2). Then, SSR sites with perfect repeats were selected and fourteen primer pairs which displayed clear amplified fragments were used to test Bactrian camel parentage. The detailed information of the fourteen microsatellite markers, primer sequences and repeat motifs is summarised in Supplemental Table 2.

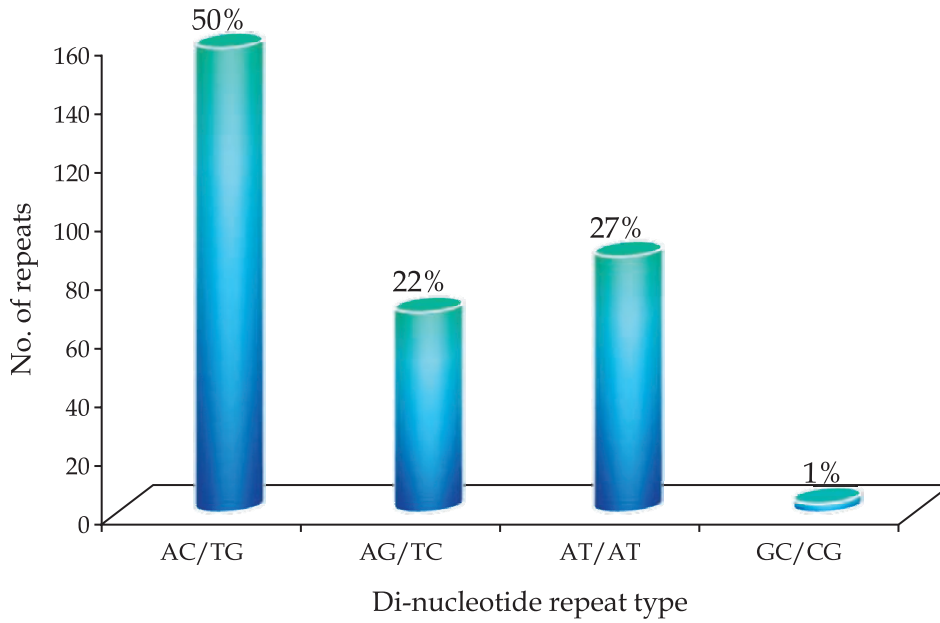

Fig 1. Frequency distribution of 4 dinucleotide repeats in transcriptome sequences of Bactrian camel. The number on the bars indicates the percentage of the 4 dinucleotide repeats in all dinucleotide repeat types.

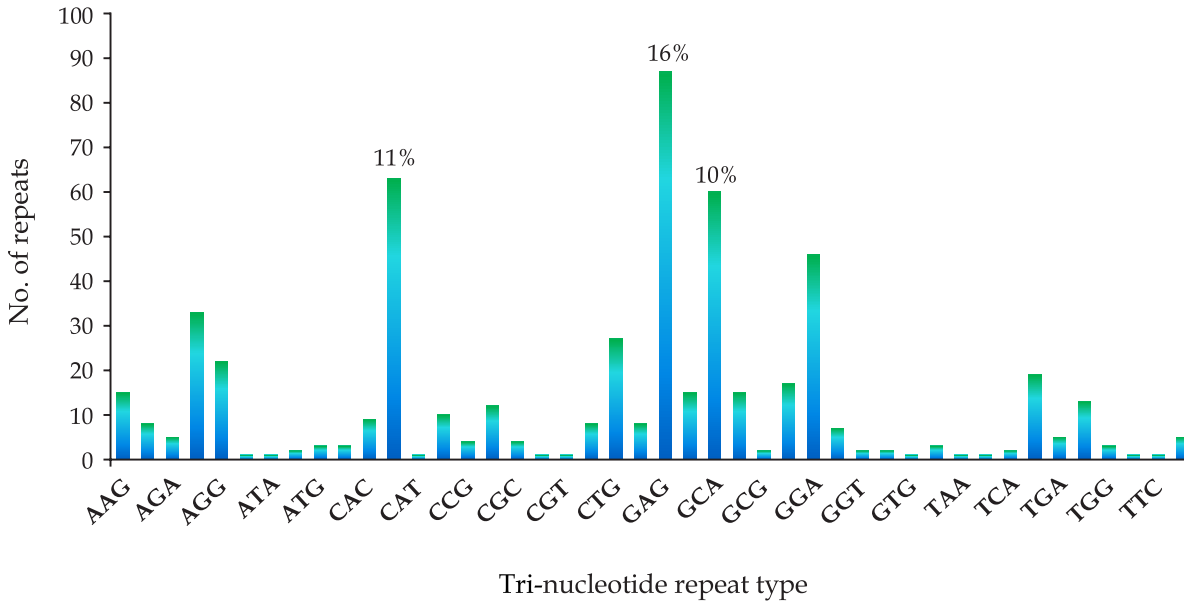

Fig 2. Frequency distribution of 44 trinucleotide repeats in transcriptome sequences of Bactrian camel. The percentage of CAG, GAG and GCA in all trinucleotide repeats is indicated on the graph.

The genetic diversity among 117 Bactrian camel individuals from two regions (China and Mongolia) was analysed with the new developed SSR primer pairs. All samples were amplified and about the 9.3% of missing data for each marker (Supplemental Table 2). The genetic parameters including allele number (N_A), effective number of alleles (Ne) and observed/expected heterozygosity were estimated for all 14 polymorphic microsatellites (Table 2). A total of 149 alleles (10.64 ± 5.472) were detected and all loci were polymorphic in both populations. Observed heterozygosity (HO) for the 14 loci ranged from 0.056 (XM_006185105_1253-1274) to 0.714 (XM_006172972_1548-1587), with an average of 0.402. The expected heterozygosity (HE) estimates at each locus ranged from 0.380 (XM_006183662_2012-2041) to 0.888 (XM_006172972_1548-1587), with a mean of 0.676. The polymorphism information content (PIC) ranged from 0.410 (XM_006177716_989-1008) to 0.874 (XM_006172972_1548-1587), with an average PIC value of 0.635 (Table 3). Inbreeding coefficient for China (F = 0.344) and Mongolia (F = 0.435) suggest

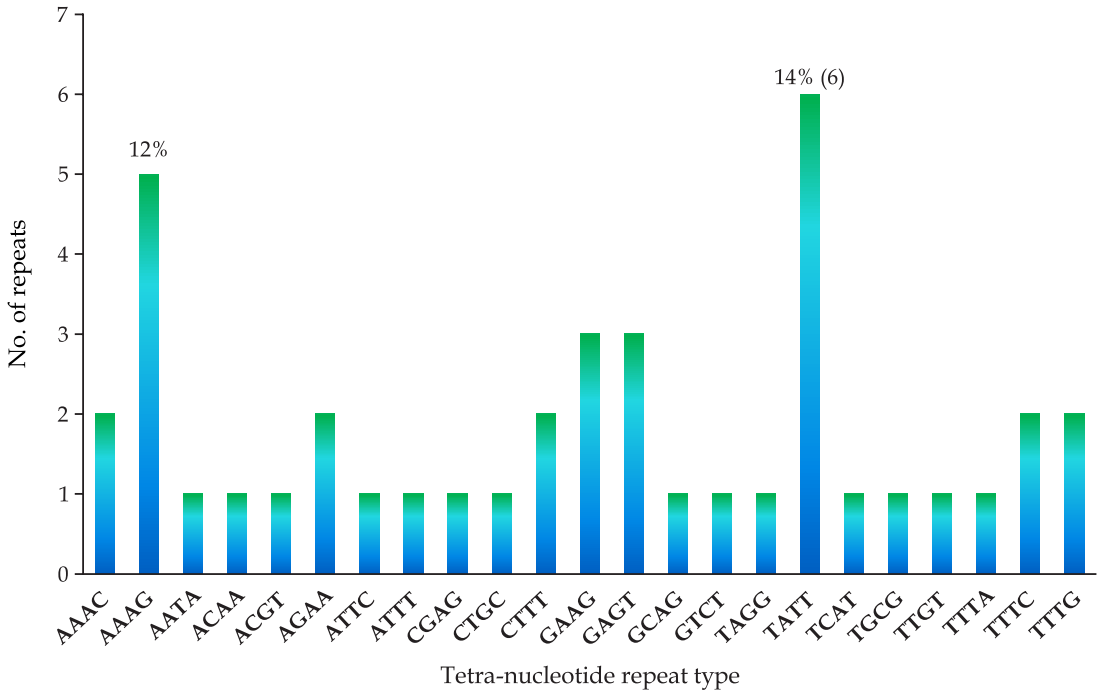

Fig 3. Frequency distribution of 23 tetranucleotide repeats in transcriptome sequences of Bactrian camel. The percentage of AAAG and TATT in all tetranucleotide repeats is indicated on the graph.

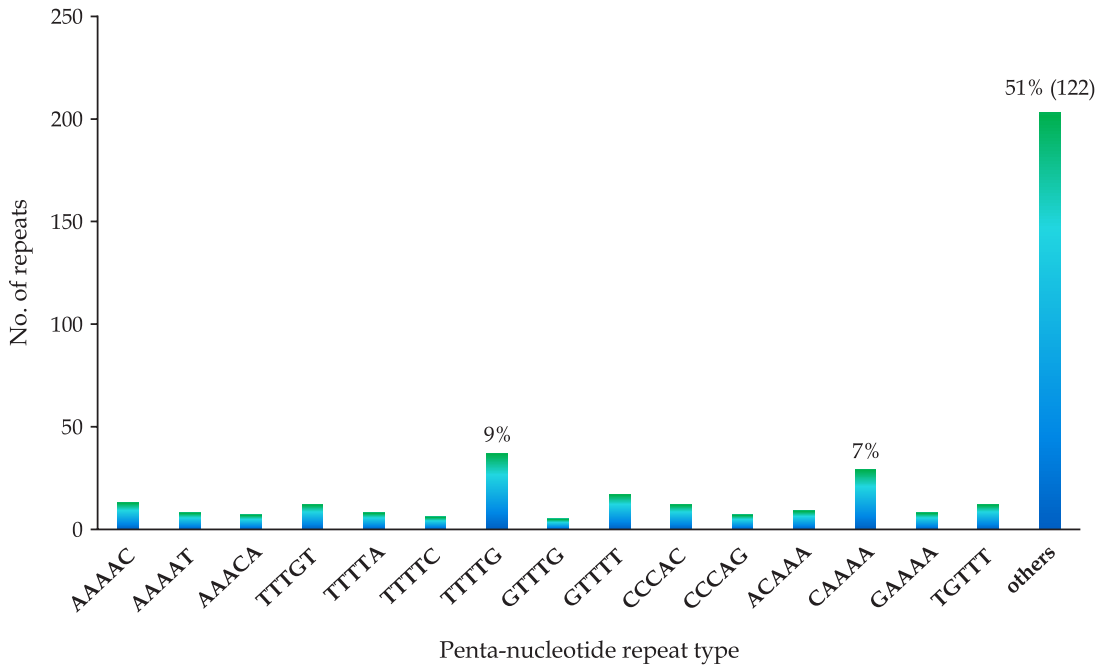

Fig 4. Frequency distribution of 137 pentanucleotide repeats in transcriptome sequences of Bactrian camel. The percentage of TTTTG and CAAAA in all pentanucleotide repeats is indicated on the graph. The "others" category contains 122 different pentanucleotide repeat types.

no heterozygote deficit across loci in the population samples.

The analysis of parentage exclusion probability

We estimated the parentage exclusion probability (PE1 and PE2) at each locus (Table 2) by CERVUS. PE1 ranged from 0.924 (XM_006183662_2012-2041) to 0.373 (XM_006172972_1548-1587). If the genotype of an alleged parent and an offspring was known, but the genotype of a confirmed parent was unknown (parentage exclusion, PE1), the total parentage exclusion probability for the 14 loci was

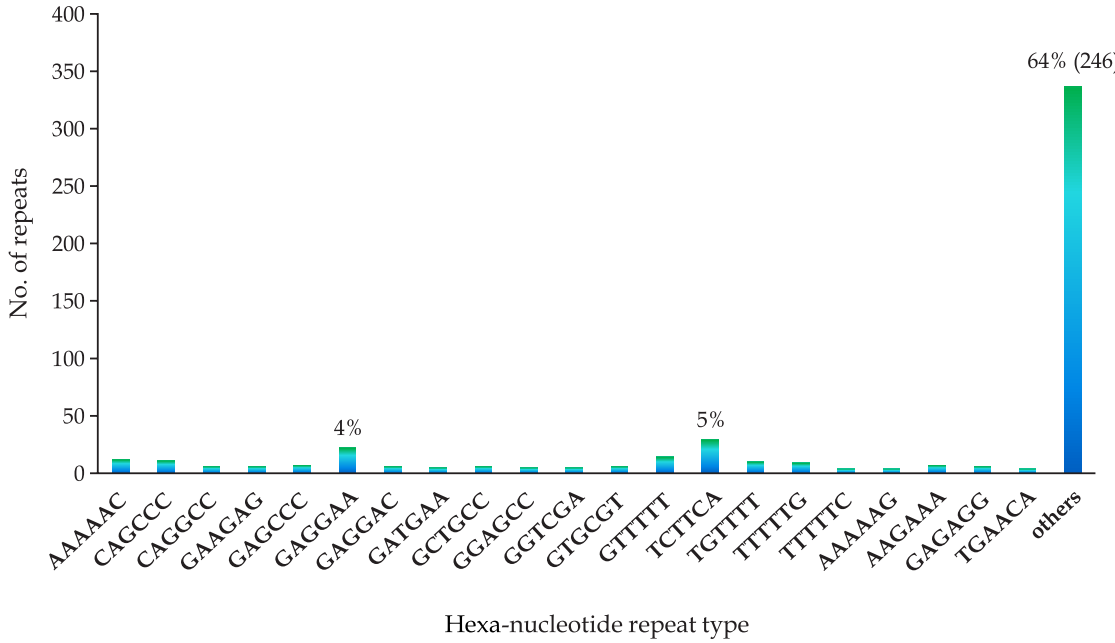

Fig 5. Frequency distribution of 267 hexanucleotide repeats in transcriptome sequences of Bactrian camel. The percentage of GAGGAA and TCTTCA in all hexanucleotide repeats is indicated on the graph. The "others" category contains 246 different hexanucleotide repeat types.

0.999999. When the genotypes of the offspring and both the alleged and confirmed parent were known (parentage exclusion, PE2), the total parentage exclusion probability was 0.999991 (Table 2). These results are in line with reports on other camelids, including dromedary (Spencer *et al,* 2010) and alpacas (Agapito *et al,* 2008). The high exclusion power (PEtotal = 0.99999) of the 14 microsatellite multiplex system implies that the newly discovered markers are useful for parentage testing in Bactrian camels.

Parentage inference

15 known parentage cases from inner Mongolia and China were used to check the inheritance of the new 14 microsatellite markers (Table 3). All 15 offspring assigned successfully to the most likely sires (LOD, 2.3-9.31) and dams (LOD, 3.9-12.6), with no errors in parentage assignment. These results further confirm that the 14 microsatellite markers can successfully identify parentage assignment in Bactrian camels, which is essential for breeding and genetic improvement.

Table 3. Genetic diversity and measures of probability exclusion.

	Origin	
	China	**Mongolia**
Sample size	88	29
Number of loci typed	14	14
Total number of alleles (N)	133	87
Number of alleles (NA)	9.500±4.879	6.214±3.556
Number of effective alleles (Ne)	3.361±1.724	3.812±2.137
Observed heterozygosity (HO)	0.40741±0.238	0.385±0.225
Expected heterozygosity (HE)	0.627±0.186	0.694±0.134
Inbreeding co-efficient (F)	0.344	0.435
Mean polymorphic information content (PIC)	0.584	0.628
Probability of exclusion when the genotypes of both parents are known (PE1)[1]	0.9909	0.9958
Probability of exclusion when the genotypes of only one parent is known (PE2)[2]	0.9997	0.9999
Probability of exclusion two putative parents (PE3)[4]	0.9999	0.9999

Discussion

In recent years, molecular markers have been used to evaluate animal genetic variability. Many genetic studies (Mehta *et al,* 2007; Reed and Chaves, 2008; Groeneveld *et al,* 2010; Mahmoud *et al,* 2012) and parentage testing on dromedary (Spencer *et al,* 2010) and alpaca (Agapito *et al,* 2008) have been carried out by using microsatellite markers. However, no studies using microsatellite markers have been performed on the Bactrian camel. In this study, we performed MISA to identify microsatellite markers from the Bactrian camel transcriptome. Fourteen polymorphic microsatellite markers, which proved

Supplemental Table 1. The characterisation of 56 microsatellite markers, primer sequences and repeat motifs.

NO.	Locus	Primer (5'-3') forward and fluorescent dye	Core repetitive
1	XM_006194610_1303-1328	F:GGCAGTGCTTGCTGTTTG R:ATCACAGAGCACCTAACA	$(TG)_{13}$
2	XM_006194512_29-58	F:AGAAGACTCTGGAACAACTG R:GGCATCGCATACACTGATA	$(CT)_{15}$
3	XM_006194346_171-227	F:CCTTACGCTCAGGGCTTGG R:GCCGTCGTAAGAGTCTGTGAGT	$(GCA)_{19}$
4	XM_006194104_5177-5222	F:GAAACAAGCCTTACTCAT R:AAGGTGCTATACTTCTGTGA	$(AC)_{23}$
5	XM_006193424_372-413	F:TCCGCTGATCTTAAAGACAT R:CAGGACTCAGATGCTCCAAT	$(GCA)_{14}$
6	XM_006193308_19-52	F:GCTGTGAGGAGAAGTTGT R:CGGAATGTGCTGAATGAC	$(CA)_{17}$
7	XM_006192341_1630-1661	F:AGTATCGTCACCGCAAGA R:GTTGAGCAGGAAGCAGAC	$(TC)_7\,(CT)_9$
8	XM_006191865_4159-4192	F:TGCGAAGAATCTCATCTGT R:GCTGCTGTCTATGTCACT	$(AT)_8\,(GT)_9$
9	XM_006191818_876-911	F:AGCCTCCTATCCCATGTCCTA R:GCAGTCGGCAGAGTTGAAGG	$(CCA)_{12}$
10	XM_006191432_206-253	F:TGATGTCCCAGGCACTGAT R:AGTTCCTGAGCGGCTGTTA	$(AGC)_{16}$
11	XM_006191380_1034-1055	F:GTCATCACGACGGACTGC R:CAAGGAGACGCAAGCATT	$(CA)_{11}$
12	XM_006190640_102-131	F:GAGCAGGCACAGCAGGAGGA R:GCGTGTTAGGCATCTGAGTTT	$(GCA)_{10}$
13	XM_006173283_3058-3099	F:GACCTGCCTTCTACTTCTG R:ACGCTCACTGATGTTATCTT	$(CA)_{21}$
14	XM_006190432_1533-1552	F:CTCAAGCAGTAGTTCAGTCT R:GCCAGCATCAGTAAGGTAA	$(AT)_{10}$
15	XM_006190324_6290-6333	F:GGACGACCAGAAGGAAGA R:CAAGTGCCGAGACAGAAG	$(TG)_{15}\,(CG)_7$
16	XM_006188059_1-38	F:GAATCTAACACGGCACTTG R:CCTCATCTAATAACACCTCATC	$(GA)_{19}$
17	XM_006187877_2476-2507	F:TCAGGAGTTGAAGACGCTAA R:TGAGGTAGGTGGGTCAGAGT	$(GT)_{16}$
18	XM_006186888-1748-1785	F:ACAGCATGGACCCAGTTA R:CACGGAGTGAGCAGTTTT	$(TG)_{19}$
19	XM_006186221_4890-4933	F:TTCTGGGCAATGATGGGATG R:AGGAAGGAGGTTCTTTGTCT	$(AC)_8\,AT(AC)_{13}$
20	XM_006186214_1-52	F:TACGGCTCACAGAAGACA R:GAGACACCGCAGTTACAA	$(AGAA)_{13}$
21	XM_006186084_5319-5354	F:AGGATGCCTCAGTTCTTA R:TGACTCGCCCAAGAAACT	$(GT)_{18}$
22	XM_006186063_678-725	F:CCCAGCATCTAACTAACA R:CTTCACAATACTGTACCCTAAT	$(TTTTTG)_8$
23	XM_006185788_2571-2600	F:GTGTTGCTAAGTATAGGGAGTT R:GTTTGGGTCTTCCATCAC	$(AC)_{15}$
24	XM_006185701_4240-4270	F:TCCGAAGATTTGGCAACG R:GAGGGCAAAGTTAGGAAT	$(CT)_7\,C(CT)_8$
25	XM_006185524_2092-2125	F:CCCAGTAAGGAAGGGTTT R:CTGGTGCCATTTATCTTT	$(TG)_{17}$
26	XM_006185105_1253-1274	F:TGACCACCAAGGAGAAGC R:CCACGATGGCATAAGGAA	$(TA)_{11}$
27	XM_006184931_7022-7058	F:GTGCTTCCCTTCATCCTT R:GCAGTGGAATGAGGGATA	$(AT)_8\,GTGTG(TA)_8$

28	XM_006184735_250-291	F:CGTGGTGACTGAGAAAGG R:CGCTGTCATCATCGGAAT	$(GAG)_8$ $(GATGAA)_3$
29	XM_006184676_1-40	F:GGCACTTACTCGTTCATTAG R:CCACAAGGTTAGGAAGAGAA	$(GT)_{20}$
30	XM_006184277_253-288	F:CTTCACCTGTGCCTCCCA R:GTTCCTCGGAGTCGCTGT	$(GCT)_{12}$
31	XM_006184223_843-914	F:CGGAGGGCTCTGGCATTA R:TGTCCTGGTGGCTCTTGC	$(CAG)_{24}$
32	XM_006184182_1654-1697	F:CAATCCCGCCTGATTCCT R:CATTGAGAAGAATAGAGGTGGA	$(TG)_{22}$
33	XM_006183827_1395-1499	F:CGGCTACCAGGTTCCAGT R:CCTCTGCTCCCTGAACAATA	$(GCA)_{35}$
34	XM_006183663_2744-2771	F:ACTTCACATCGCTTGTAC R:TGACAGTGAAATGCTCCC	$(TG)_{14}$
35	XM_006183662_2012-2041	F:AACGTCTGCGTGTATGGC R:CCCTAAAGTAAAGCAAACCC	$(TG)_{15}$
36	XM_006182569_1307-1344	F:GACATCGCTGAATACCTCTA R:TATCCTGCTCCTCCACTC	$(TA)_{10}$ TTTC$(AT)_7$
37	XM_006182535_1080-1115	F:CAGATAATCAGACCAGCGTTAC R:AAGCCTTCAATAGTCTTCATC	$(GT)_{18}$
38	XM_006182496_1210-1278	F:AGGCCAGGTATGGCACTT R:TGGCTGAGTAGGTGGTGC	$(CAG)_{23}$
39	XM_006182351_38-87	F:CGGACCAGTGTAGAGATTC R:TGATACGCTGCTAGTGATG	$(TC)_{25}$
40	XM_006182350_12220-12265	F:ATGCTACAACTGATGCCTTA R:TGATACGCTGCTAGTGATG	$(CAC)_9$ T$(ACC)_6$
41	XM_006182052_4595-4622	F:AGTTCAAATCAGTCGTCCTT R:TGGTAACTCCCTTTGGTA	$(CA)_{14}$
42	XM_006181405_1069-1118	F:TTGCCACCAAAGACAGAC R:ACAGCCTCAGTGTCTCCA	$(AGC)_6$ AA$(CAG)_{10}$
43	XM_006180604_1198-1254	F:AGCAGTGAGACCAAGCCCAAGC R:TCATTGTCAGACCACCATCC	$(AGC)_{19}$
44	XM_006180554_882-917	F:AAGCAGGTGTTTGATTTGT R:CCAGGGAGAATGGAGAATA	$(GT)_7$ TT$(TG)_{10}$
45	XM_006180397_1557-1592	F:GGATGGAGGCAGTGACTA R:TGGTGATGAGCAGATTGAG	$(GCA)_{12}$
46	XM_006179120_6522-6570	F:GGCGAGTATGAACCCACA R:CTGCTGGAACTGGCTGTG	$(GCA)_8$ A$(CAG)_8$
47	XM_006177721_2115-2144	F:TCTGTGCCACGCCCTTAC R:AAGGACACCAGGGAAACTAG	$(GT)_{15}$
48	XM_006177716_989-1008	F:ACTGGCACAACTGGAAGC R:ATTGGTGGAAACAGAACA	$(TG)_{10}$
49	XM_006177711_1068-1097	F:ACACCACTGGCTCCCTTTC R:TGTAGGGTTATGTCGTTC	$(TGC)_{10}$
50	XM_006177371_4929-4970	F:TCCCACTTAGTTACTGTTT R:AAGTAGGACCACCTTTCA	$(TA)_{14}$ $(CA)_7$
51	XM_006176817_1425-1460	F:ACAGGCAAGTCTGGTAAA R:GTGCTTCCTGTGCCCAAT	$(AC)_{18}$
52	XM_006173968_3405-3428	F:CCTGGACGGCAGTGATAG R:TAATCCCTTACTCCCTGAAAA	$(GT)_{12}$
53	XM_006172939_3609-3630	F:TCCTCTTCTGCGTTACCA R:GATCACTTAGCACCAACGA	$(AT)_{11}$
54	XM_006173232_1196-1215	F:AACTGATGGAGGAATGAAG R:AGGGCAAGAAAGGTGGTC	$(CA)_{10}$
55	XM_006173207_3661-3682	F:ATAGAAGCATGTCGGTAG R:CCCTCTGCTGGCACTGAA	$(TA)_{11}$
56	XM_006172972_1548-1587	F:CGCTGGGACAGCTAAGAC R:CTTTCTGGTAAAGGCAAC	$(CA)_{20}$

Supplemental Table 2. The detailed information of fourteen microsatellite markers, primer sequences and allele size.

NO.	Primer (5'-3') forward and fluorescent dye	Reverse	Repeat motif	Size range/bp
4	FAM-GAAACAAGCCTTACTCAT	AAGGTGCTATACTTCTGTGA	(AC)23	207-219
11	TAM-GTCATCACGACGGACTGC	CAAGGAGACGCAAGCATT	(CA)11	334-357
19	HEX-TTCTGGGCAATGATGGGATG	AGGAAGGAGGTTCTTTGTCT	(AC)8AT(AC)13	203-243
26	TAM-TGACCACCAAGGAGAAGC	CCACGATGGCATAAGGAA	(TA)11	314-320
32	FAM-CAATCCCGCCTGATTCCT	CATTGAGAAGAATAGAGGTGGA	(TG)22	170-196
35	HEX-AACGTCTGCGTGTATGGC	CCCTAAAGTAAAGCAAACCC	(TG)15	237-249
37	HEX-CAGATAATCAGACCAGCGTTAC	AAGCCTTCAATAGTCTTCATC	(GT)18	256-296
41	TAM-AGTTCAAATCAGTCGTCCTT	TGGTAACTCCCTTTGGTA	(CA)14	307-315
44	FAM-AAGCAGGTGTTTGATTTGT	CCAGGGAGAATGGAGAATA	(GT)7TT(TG)10	128-153
47	FAM-TCTGTGCCACGCCCTTAC	AAGGACACCAGGGAAACTAG	(GT)15	181-192
48	FAM-ACTGGCACAACTGGAAGC	ATTGGTGGAAACAGAACA	(TG)10	251-256
52	TAM-CCTGGACGGCAGTGATAG	TAATCCCTTACTCCCTGAAAA	(GT)12	389-410
55	TAM-ATAGAAGCATGTCGGTAG	CCCTCTGCTGGCACTGAA	(TA)11	321-378
56	FAM-CGCTGGGACAGCTAAGAC	CTTTCTGGTAAAGGCAAC	(CA)20	170-233

efficient for parentage testing, were characterised using 117 domestic Bactrian camels representing two regions. These revealed 149 total alleles, with 5 to 23 alleles per locus. These results are in line with studies performed in alpaca with 10 microsatellite markers, where the range was 6-28 alleles (Agapito *et al,* 2008) and in dromedary with 17 microsatellite markers, where the range was 5-26 alleles (Spencer *et al,* 2010). The calculated PIC value, which reflects polymorphism, indicated higher polymorphism (average value = 0.635), which is lower than in the alpaca (0.7951) and higher than in the dromedary (0.510). Furthermore, the total parentage exclusion probability measured in domestic Bactrian camel for these new loci are comparable with other studies, including cattle (Schnabel *et al,* 2000; Heyen *et al,* 2015; Strucken *et al,* 2017), yak (Pei *et al,* 2018), goat (Luikart *et al,* 1999), sheep (Tortereau *et al,* 2017), horse (Tozaki *et al,* 2001), dromedary (Spencer *et al,* 2010) and alpaca (Agapito *et al,* 2008).

Mosconi *et al* (2005) established a new standard for parentage testing through combined polymorphic microsatellite loci and fluorescent genotyping.

The core set of 14 microsatellite markers presented in this study represent a powerful battery of markers for parentage testing. These are crucial to accurate identification of pedigrees in the Bactrian camel.

Conclusion

The polymorphic microsatellites from Bactrian camel transcriptome sequences and apply microsatellite multiplex systems for parentage testing were identified. Based on sequence redundancy and PCR amplification, we selected fourteen polymorphic microsatellites for Bactrian camel parentage testing analysis in a population of 117 unrelated camels from China and Mongolia. At last, we selected 15 baby camels, 20 sires and 20 dams for parentage testing. According to the parentage assignment, the microsatellite panel assigned all 15 offspring with high confidence. This core set of 14 microsatellites represent a powerful and efficient method for determining parentage in domestic Bactrian camels.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

We gratefully acknowledge support and funding received from the Science and Technology Major Project of Inner Mongolia Autonomous Region, Inner Mongolia Natural Science Foundation Project (2018BS03017) and the project of high-level talents introduce scientific research (NDYB2017-28). We thank, the researchers at the Animal Husbandry Workstation of West Sunid, Shiliingol Aimag, Inner Mongolia, for providing the samples and facilitating their identification. We would like to thank the native English speaking scientists of Elixigen Company (Huntington Beach, CA, USA) for editing our manus.

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DOI : 10.5958/2277-8934.2019.00022.5 Vol 26 No 2, p 143-147

NEUROPEPTIDES IN THE PANCREAS OF DROMEDARY CAMEL: IMMUNOHISTOCHEMICAL LOCALISATION

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ABSTRACT

The persent study is aimed to identify and localise some of the neuropeptides that may be present in the camel pancreas using the immunohistochemical techniques. The specimens from 5 camels (*Camelus dromedarius*) of both sexes at different ages (2-12 years) were used. The neuropeptides, calcitonin gene-related peptide (CGRP), Substance P (SP), Vasoactive Intestinal Polypeptide (VIP) and Cholecystokinin (CCK-8) were detected in the camel pancreas by light microscope immunohistochemistry. The current findings indicated that, the dromedary camel pancreas contains several neuropeptides including CGRP, SP, VIP and CCK-8. The CGRP immunoreactivity was located in nerve fibres of the parenchyma and some ganglion cells. The SP stained cells in the pancreatic islets were located at the peripheral parts and the VIP immunoreactive nerve fibres were distributed throughout the exocrine and endocrine portions of the camel pancreas. The CCK-8 was found in varicose nerves innervating acinar cells and the blood vessels of the pancreas and also found in the endocrine cells. In conclusion, the pancreatic tissues and islets contained several neuropeptides which are probably involved in the regulation of pancreas locally.

Key words: Dromedary camel, immunohistochemistry, neuropeptides, pancreas

A large number of neuropeptides including CGRP, VIP, SP and CCK-8 have been found in the pancreatic innervation (Sundler *et al,* 1978; Larsson, 1979; Bishop *et al,* 1980; Sternine and Brecha, 1986). In the pancreas of various species, CGRP immmunoreactivity is located in nerve fibres in the intra- and interlobular spaces, in or around the islets and near blood vessels. They are also found in single or clustered endocrine cells (Sternine and Brecha, 1986; Ahren and Sundler, 1992; De Giorgio *et al,* 1992; Adeghate, 1999). Adeghate (1999) also reported CGRP in gangllion cells of rat pancreas and in nerve fibres innervating CGRP–negative gangllion cells. In camel, CGRP immmunoreactivity has been observed only in fine varicose nerve fibres which are situated in perivascular and periacinar regions of the exocrine pancreas (Adeghate and Pallot, 1996). Functionally, CGRP is demonstrated in rats to be localised with and stimulate somatostatin, an inhibitor of insulin secretion (Hermansen and Ahren, 1990) and may be involved in the regulation of insulin and glucagon secretion of the porcine pancreas (Rasmussen *et al,* 1998). VIP immunoreactive nerve fibres are distributed throughout the exocrine and endocrine portions of the pancreas and in the wall of the blood vessels. VIP immunoreactivity is also observed in the peripheral part of the islets, in neurons in the exocrine pancreas and as a varicose fibres on the wall of the pancreatic ducts (Adeghate and Donath, 1990; De Giorgio *et al,* 1992; Adeghate, 1999; Myojin *et al,* 2000). The VIP stimulates the exocrine pancreatic secretion in rat and pig (Jansson, 1994; Kiela *et al,* 1996), modulating the release of pancreatic polypeptide, but had no siginificant effect on the release of either panreatic glucagon or insulin in calf (Edwards *et al,* 1997). SP immunoreactivity observed in nerve fibres in the connective tissue between the lobules, around the intrinsic ganglia and islets of Langerhans and associated with blood vessels. However, there is little SP associated with pancreatic ducts (Sharkey *et al,* 1984; De Giorgio *et al,* 1992; Adeghate, 1999; Myojin *et al,* 2000). SP is also reported in neurons at the interlobular areas of the rat pancreas (Adeghate, 1999) and in some endocrine cells of the bovine

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pancreas (Myojin *et al,* 2000). SP may regulate both the exocrine and endocrine secretion of the pancreas in rats and mice (Katoh *et al,* 1984), dog (Iwatsuki *et al,* 1986) and in bovine (Myojin *et al*, 2000). However, SP may modulate the secretion of the pancreatic polypeptide and glucagon in the normal conscious calf but doesn't affect the secreation of insulin (Edwards and Bloom, 1994). In rats and mice, CCK-8 immunoreactivity is seen in varicose nerves innervating the blood vessels and acinar cells of the pancreas. It is also observed in few neurons scattered in the perivascular and periacinar regions of the pancreatic tissue (Larsson, 1979; Adeghate, 1999). Simillar pattern of distribution is observed in camel (Adeghate and Pallot, 1996; Adeghate, 1997). It is also reported that, CCK-8 producing cells were present in the adult rat islets and have potentiation effect on the islet hormones (Shimizu *et al,* 1998; Singh *et al,* 1999). The CCK-8 is a potent and specific pancreatic vasodilator in dog (Nakajima *et al,* 2001) and can also stimulate the exocrine pancreas secretion in pig (Kiela *et al,* 1996). Few studies are available regarding localisation of neuropeptides in the pancrease of dromedary camels. Therefore, the persent study was undertaken to identify and localise some of the neuropeptides that may be present in the camel pancreas.

Materials and Methods

Animals and tissues preparation

Specimens of the pancreas were collected from 5 one-humped camels (*Camelus dromedarius*) of both sexes, from Al-hasa slaughterhouse and from the Camel Research Centre, King Faisal University, College of Veterinary Medicine, Saudi Arabia. The specimens were apparently normal and free from gross pathological changes. The age of the animals ranged from 2 to 12 years (Ramadan, 1994). The specimens were fixed in 4% paraformaldehyde at room temperature for 24 hours. The tissues were dehydrated by increasing concentration of ethanol, cleared in xylene and embedded in paraffin and cut at 5–7 µm using a Reichert rotatory microtome. The sections were floated on warm water bath (45°C) before being mounted onto chrome alum gelatin coated slides. Deparaffinised sections were hydrated through graded series of alcohol then washed in phosphate buffer saline, incubated in 0.08% hydrogen peroxide in methanol for 5–10 minutes to reduce endogenous peroxidase, then rinsed in phosphate buffer saline.

Staining procedure

In the current study, we used two alternative methods for demonstration of neuropeptides; the streptavidin biotinylated horseradish peroxidase complex method and Dako labelled streptavidin biotin plus kit. In case of streptavidin biotinylated horseradish peroxidase complex method, blocking was carried out by incubating the prepared sections in 5% normal goat serum for 10 minutes after that the normal goat serum was drained and the sections were incubated in the primary antiserum for 18 hours at 4°C in the humidified petri dish, and then washed in phosphate buffer solution 3 times for 10 minutes each. Thereafter, sections were incubated in the secondary antiserum (1:50) for 1 hour at room temperature, then washed in phosphate buffer solution 3 times for 10 minutes each. After rinsing, sections were incubated at room temperature with streptavidin biotinylated horseradish peroxidase complex (1:50) for 1 hour. After rinsing in 0.2 acetate buffer, sections were immersed in glucose oxidase–DAB-nickel method (which includes diaminobezidine in 50 ml distilled water containing 2.5 g nickel ammonium sulfate, 0.2M acetate buffer, B-D glucose, ammonium chloride and glucose oxidase) for 5-10 minutes. In case of Dako labelled streptavidin biotin plus kit method, after the incubation in the primary antiserum, enough drops from secondary antisera were applied and sections were incubated for 15 minutes, and then placed in fresh phosphate buffer solution bath. Then enough drops from (streptavidin) were applied to the specimens for 15 minutes, and placed in fresh phosphate buffer solution bath. The sections were immersed in the substrate of chromogen solution and incubated for 5 minutes. The sections were stained with haematoxylin or 1% alcoholic solution of eosin, after that mounted in DPX and examined under the light microscope. In the control, omission of the primary antisera followed by Streptavidin biotinylated horseradish peroxidase complex method or Dako Labelled Streptavidin Biotin plus kit, peroxidase staining. In addition, in the control the primary anti-sera has been replaced with normal goat serum. The control incubation were carried out on sections adjacent to those used in the normal Dako Labelled Streptavidin Biotin plus kit, peroxidase or Dako Labelled Streptavidin Biotin plus kit, peroxidase. The primary antisera used in this investigation were shown in table 1. The secondary antiserum (biotinylated goat anti-rabbit IgG) and Streptavidin biotinylated horseradish peroxidase complex were purchased from Amersham Pharmacia Biotech. Generally, the immunolabelling of the control experiments of the current study was not observed.

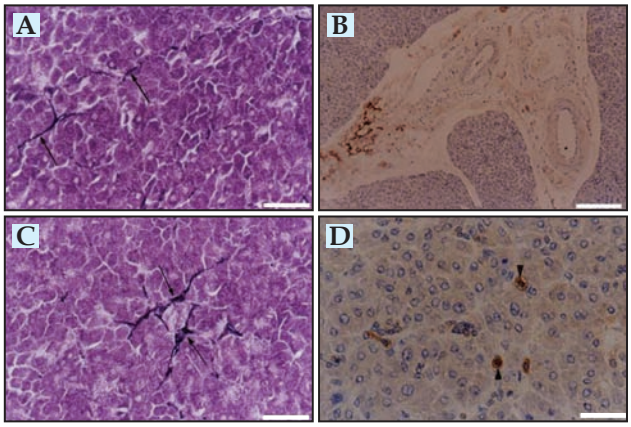

Fig 1. Localisation of CGRP in camel pancreas. (A-D): **A:** CGRP-immunoreactive nerve fibres in the interlobular septa (arrows) X400; **B:** perivascular fine CGRP-immunoreactive nerve fibres X100; **C:** CGRP-immunoreactive nerve fibres surrounding CGRP-negative cell (arrows) X400; **D:** CGRP-positive cells in the parenchyma (arrowheads) X400.

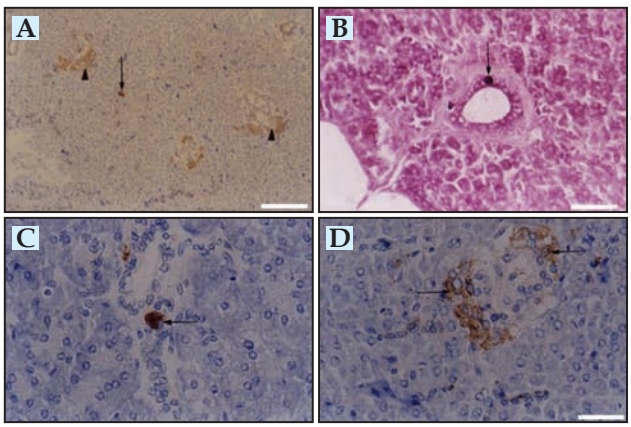

Fig 2. Localisation of SP in camel pancreas. (A-D): **A:** SP-immunoreactivity in exocrine (arrow) and endocrine (arrowheads) portions X100; **B:** SP-immunoreactive cell present in the duct epithelium (arrow) X400; **C:** SP-immunoreactive cell (arrow) in the connective tissue of the duct X400; **D:** SP-immunoreactive cells are peripherally located in an islet of Langerhans (arrows) X400.

Table 1. The primary antisera

Antibody	Dilution	Source
Rabbit anti-CCK-8	1:100	Serotec, UK
Rabbit anti-CGRP	1:100	Serotec, UK
Rabbit anti-VIP	1:100	Serotec, UK
Rabbit anti-Substance P	1:100	Serotec, UK

Results and Discussion

Calcitonin gene-related peptide (CGRP)

The present findings indicated that, CGRP immunoreactivity was located in nerve fibres throughout the parenchyma of the pancreas.

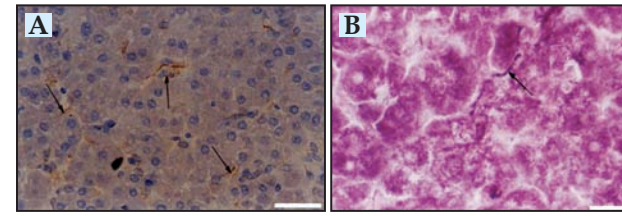

Fig 3. Localisation of VIP in camel pancreas (A-B). **A:** immunoreactive VIP nerve fibres throughout the pancreas (arrows) X400; **B:** VIP varicose nerve fibres (arrow) X1000.

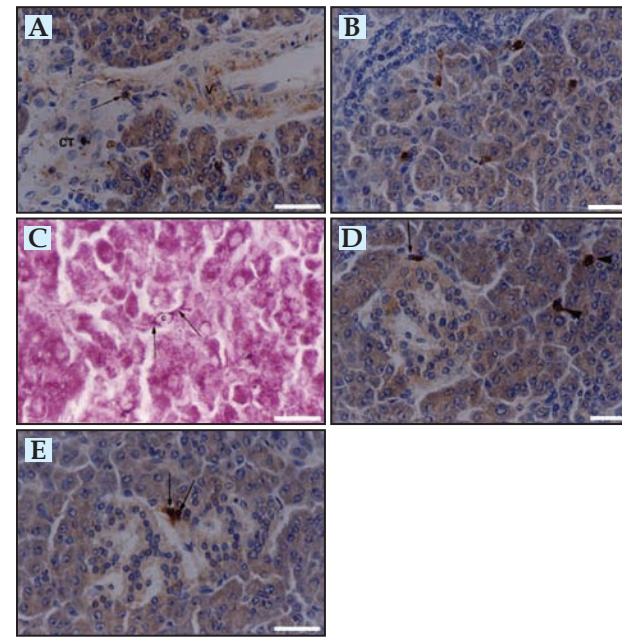

Fig 4. Localisation of CCK-8 in camel pancreas (A-E). **A:** Immunoreactivity for CCK-8 in a blood vessel wall (V), interlobular connective tissue (CT) and perivascular cells (arrow) X400; **B:** periacinar CCK-8 positive cells X400; **C:** CCK-8 immunoreactive nerve fibres (arrows) surrounding unstained cell (C) X1000; **D:** few CCK-8 immunoreactive endocrine cells at the periphery of the islet (arrow) and in the parenchyma (arrowheads) X400; **E:** Two CCK-8 immunoreactive endocrine cells at the periphery of a pancreatic islet (arrows) X400.

It was detected in nerve fibres in the intra- and interlobular connective tissue septa, in perivascular fine nerve fibres (Fig 1A, B) and in nerve fibres innervating CGRP–negative cells (Fig1C). CGRP immunoreactivity was also observed in some cells of the pancreas (Fig 1D) and around the islets.

In the persent study, SP immunoreactivity was distributed in exocrine and endocrine portions of the pancreas (Fig 2A). It was observed in the very fine varicose nerve fibres in the connective tissue between the lobules. It was also detected in single endocrine cells within the parenchyma, in the duct epithelium (Fig 2B) and near the ducts (Fig 2C). In pancreatic

islets, SP stained cells were located in the peripheral parts (Fig 2D).

The VIP immunoreactive nerve fibres were distributed throughout the exocrine and endocrine portions of the pancreas (Fig 3A). The nerve fibres were fine and varicosed (Fig 3B).

The CCK-8 immunoreactivity was found in varicose nerves innervating the blood vessels and acinar cells of the pancreas (Fig 4A), and in some cells that scattered in the perivascular and periacinar regions of the pancreatic tissue (Figs 4A,B). In addition CCK-8 immunoreactivity was demonstrated in nerve fibres innervating CCK-8 unstained cells (Fig4C) and occasionally observed in a few cells at the periphery of the islets (Figs 4D, E).

Discussion

The camel pancreas contains several neuropeptides including CGRP, SP, VIP and CCK-8. The CGRP immunoreactivity was located in nerve fibres throughout the parenchyma of the pancreas. It was also detected in the nerve fibre in the intra- and interlobular connective tissue septa and in the perivascular fine nerve fibres. Similar pattern of distribution was observed in other animals (Sternine and Brecha, 1986; Ahren and Sundler, 1992; De Giorgio *et al,* 1992; Adeghate, 1999). CGRP immunoreactivity was also observed in some ganglion cells of the pancreas, resembling the results in rat pancreas (Adeghate, 1999). In contrast, Adeghate and Pallot (1996) have stated that, in camel pancreas CGRP immunoreactivity was observed only in the nerve fibres. SP immunoreactivity was distributed in exocrine and endocrine portions of camel pancreas; a finding which has not previously been mentioned in the camel pancreas. It was observed in very fine varicose nerve fibres in the connective tissue between the lobules, in the duct epithelium and near to the ducts. Similar findings were reported in other animals (Sharkey *et al,* 1984; De Giorgio *et al,* 1992; Adeghate, 1999; Myojin *et al,* 2000). SP was also detected in single endocrine cells within the parenchyma similar to previous results in bovine (Myojin *et al,* 2000). The findings have been presented for the first time that, the SP stained cells in the pancreatic islets were located at the peripheral parts. Moreover, the current findings presented for the first time that, VIP immunoreactive nerve fibres were distributed throughout the exocrine and endocrine portions of the camel pancreas. These nerve fibres were fine and varicose, more or less as reported in the other animals (Adeghate and Donath, 1990; De Giorgio *et al,* 1992; Adeghate, 1999; Myojin *et al,* 2000). The CCK-8 immunoreactivity was found in varicose nerves supplying the blood vessels, the acinar cells of pancreas, and in some neurons that scattered in the perivascular and periacinar regions of pancreatic tissue. This confirms the previous pattern of distribution that has been observed in camels (Adeghate and Pallot, 1996; Adeghate 1997). Similar findings were also observed in rats and mice (Larsson, 1979; Adeghate, 1999). The CCK-8 was found in the islets at the periphery, which is similar to that observed earlier in rats (Shimizu *et al,* 1998; Singh *et al,* 1999). The pancreatic tissues and islets contained several neuropeptides which are probably involved in the regulation of pancreas locally.

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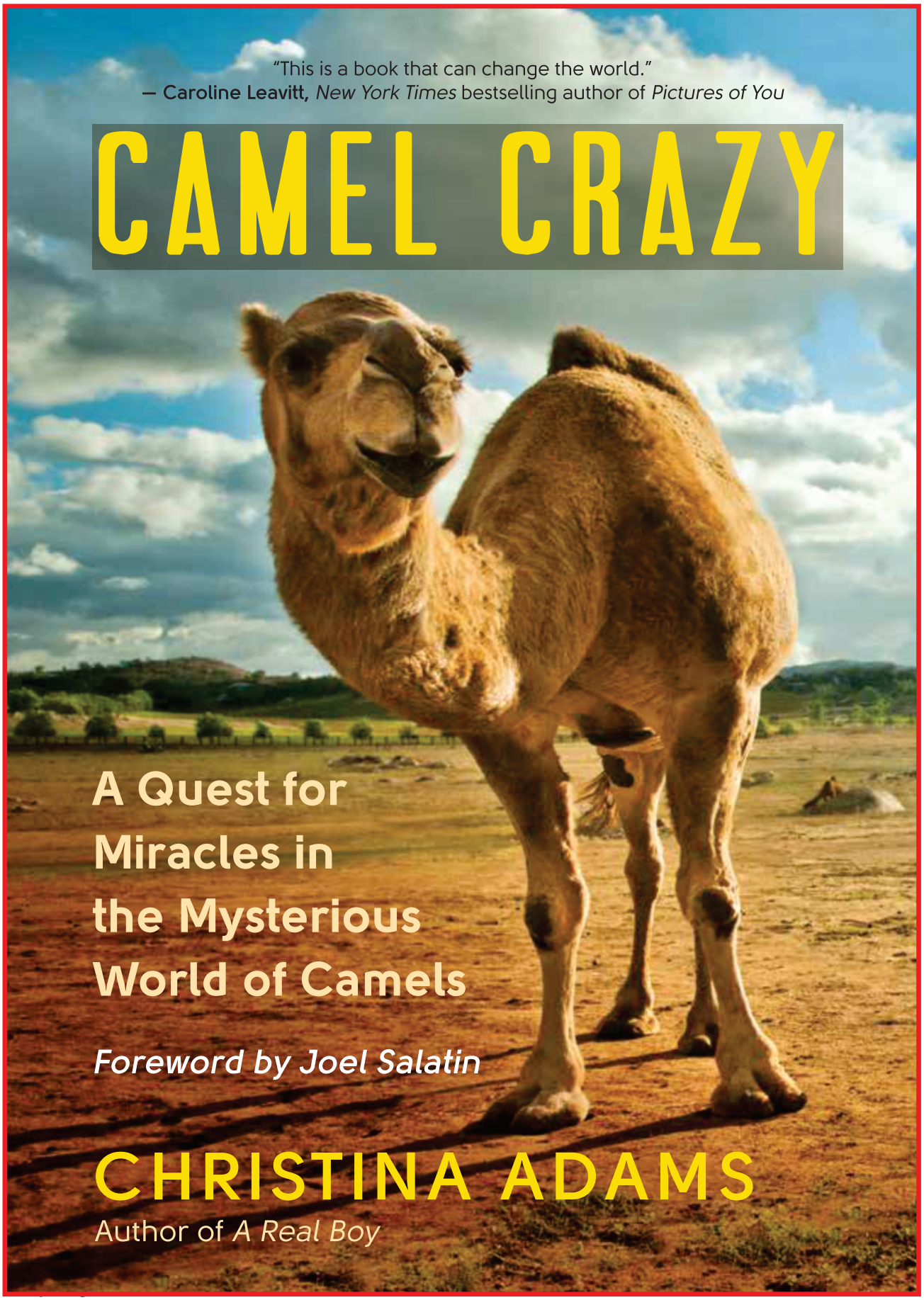
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DOI : 10.5958/2277-8934.2019.00023.7 Vol 26 No 2, p 149-156

EVALUATION OF PRESLAUGHTER STRESS RESPONSES DURING WAITING TIME AT LAIRAGE IN DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

Stress responses were evaluated during waiting time at lairage at the slaughterhouse by analysing some physiological, haematological, biochemical and hormonal parameters in the camel. Sixteen male animals belonging to the municipal slaughterhouse of Casablanca (west of Morocco) were divided into 2 groups according to their waiting periods before slaughter: short period (12hs≤time<16hs, Group I, n=8) and long period (16hs≤time≤20hs, Group II, n=8). In groups I and II, neutrophil/lymphocyte ratio, haemolysis and circulating levels of cortisol, triiodothyronine and thyroxine were measured before waiting (at the end of transport and after unloading) and these were significantly ($P<0.05$) lower than those observed after the 2 waiting periods. After waiting time, all these parameters and plasma levels of glucose remained significantly ($P<0.05$) lower in Group II than those analysed in Group I. In the same conditions, rectal temperature, heart rate, respiratory rate and circulating levels of calcium, phosphorus and magnesium, showed no significant variation either between the stages (before and after waiting), or between the 2 waiting periods. In the camel, the waiting period at the slaughterhouse could be an important preslaughter stress factor, capable of altering the animal's physiology and the post-mortem quality of its meat. After transport and unloading, a waiting period of 16h to 20h could be considered less stressful than that of 12h to 16h in the camel. The impact of waiting period on antioxidant status will be evaluated later.

Key words: Dromedary, haemolysis, hormones, lairage period, leukocyte formula, preslaughter stress

Stress is completely defined as the combination of mental and biological responses of an animal to novel and threatening physical and psychological stimuli (Broom, 2008). Several responses, *i.e.*, increased heart rate (HR), respiratory rate (RR), adrenal activity and reduced immunological response, have been considered as stress indicators (Broom, 2014). These responses involve activation of the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis, inducing release of catecholamines, corticotrophin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and cortisol. In camels, investigation of stress responses had used cortisol measurement in blood (El khasmi *et al,* 2010; 2013; 2015; Lemrhamed *et al,* 2018), urine (El khasmi *et al,* 2010), saliva (Majchrzak *et al,* 2014), hair and faeces (Sid-Ahmed *et al,* 2013; Bargaa *et al,* 2016) as an indicator for the activity of the HPA axis. Other neuroendocrine systems are also involved, such as thyroid hormones in the camel (Saeb *et al,* 2010; El khasmi *et al,* 2010; Lemrhamed *et al,* 2018) and several domestic animals (Ferlazzo *et al,* 2018).

The steps preceding the slaughter of an animal for human consumption need to maintain product quality as well as protecting animal welfare (Shimshony and Chaudry, 2005; Broom, 2014). Currently, during the pre-slaughter period, domestic animals are exposed to various potentially stress-inducing factors of psychological origin, such as social disturbances, handling, transportation and novelty, or physical origin such as food and water deprivation, pain or fatigue (Terlouw *et al,* 2008; Ferlazzo *et al,* 2018). In the dromedary camel, stressful situations induced by transport, travel distance and loading density was evaluated by physiological [rectal temperature (RT), respiratory rate (RR), heart rate (HR)], haematological [haematocrit (Hct), neutrophil/lymphocyte ratio (NLR), haemolysis (H%)], endocrine [cortisol (COR), triiodothyronine (T3), thyroxine (T4)] and biochemical [glucose, malondialdehyde,

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catalase activity] responses (El Khasmi *et al,* 2010, 2013, 2015; Saeb *et al,* 2010; Lemrhamed *et al,* 2018). Pre-loading handling, transportation and unloading are crucial steps in the production chain of camel destined for slaughter and may lead to several stress reactions. Thus, the best conditions to reduce stress and promote the maintenance of good welfare of camel must be respected. In present study, the stress responses induced by waiting period before slaughter in the camel were studied, *i.e.* stress-related physiological (RT, RR, HR) and blood variables (Hct, NLR, haemolysis, COR, T3, T4, glucose, Ca, Pi and Mg).

Materials and Methods

Animals

The study was carried out at Casablanca Municipality slaughterhouse (West of Morocco, 33.35° N, 7.36° W and 27 m altitude) during the winter season, from January to February, with temperatures ranged between 11-16°C. Sixteen adult one-humped male camels (*Camelus dromedarius*), aged 4–7 years were used. These animals were maintained under similar conditions, fed with some barley concentrate and dry hay straw and exposed to the same preslaughter conditions. All the camels were transported by truck from the market to the slaughter house within 2h and spent approximate 12h to 20h in the lairage before slaughtering. Prior to transportation, these animals were ensured as clinically healthy and were deprived of water and food for 1 to 3 hours before being loaded. These were transported in a side-facing position and squatting position holding the forelegs tight by a rope at the knees. During transportation, the camels could not feed and drink and the road was asphalted until the arrival to the slaughterhouse. On arrival at the abattoir, the animals were carefully and calmly unloaded to avoid stress and were guided into the waiting station before slaughtering. These were divided into 2 groups of 8 animals according to their waiting period at lairage: short period (12h≤time<16h, Group I) and long period (16h≤time≤20h, Group II). During waiting, the animals were kept together without mixing of different groups. Each animal had enough space to stand up, lie down and turn around. Water and feed was not available to the animals on their arrival and throughout the waiting time.

Physiological parameters

Physiological parameters (RT, HR and RR) were measured before waiting (just after transport and unloading) and just at the end of waiting before slaughtering.

Blood sampling

Blood samples were collected from each camel before waiting (just after transport and unloading) and just at the end of waiting before slaughtering. EDTA blood was used for Hct, NLR and haemolysis (H%) measure, whereas heparinised blood was used for the determination of plasma levels of all biochemical and hormonal parameters. The plasma was separated by centrifugation at 750×g for 15 min at 4°C, pipetted into aliquots and then stored at -20°C until analysis.

Haematocrit

The Hct was determined on whole blood with capillary tubes and centrifuged (Hettich Haematokrit D-7200) using a microhaematocrit reading device and was expressed as follows:

Hct = (level of pellet/overall height)x100.

Neutrophil/lymphocyte ratio

To determine the leukocytes differential distribution (%), blood smears were stained with May-Grunwald-Giemsa, *i.e.* 5 min of May-Grunwald and 5 min of Giemsa 10^{th} diluted in water. Of 100 leukocytes, the percentage of neutrophils (neutrophils, eosinophils, basophils), lymphocytes and monocytes and the neutrophil to lymphocyte ratio (NLR) were determined.

Haemolysis test

The profile of H% was analysed by using the slightly modified method of O'Dell *et al* (1987). A 100 µl aliquot of blood was added to test tubes containing 5ml of various concentrations of buffered salt solutions (BSS, pH 7.4) ranging from 0.1 to 0.9%. The contents of these tubes were gently mixed by inverting them five times and were allowed to stand at 37°C for 30 min. Thereafter, these tubes were centrifuged at 1270xg for 10min to pellet the cells. The supernatant was then transferred into a glass cuvette and the absorbance was measured at 540 nm using a spectrophotometer. The H% in each tube was expressed as a percentage, taking as 100% the maximum value of absorbance of distilled water. BSS (0.9%) was considered as a control sample. The per cent haemolysis was calculated according to Faulkner and King (1970) as follows:

H(%)= (Optical density of test/Optical density of distilled water)x100.

H(%) curve was obtained by plotting per cent haemolysis against the saline concentrations. H50 was determined as the saline concentration responsible for an haemolysis of 50% of red blood cells.

Biochemical and hormonal parameters analysis

Plasma Glu, Ca, Pi and Mg concentrations were measured using a spectrophotometric procedure from commercially available kits. Plasma levels of COR, T3 and T4 were analysed by radioimmunoassay (RIA) method in the National Centre of Science and Nuclear Technical Energy in Maâmoura, Morocco, by using commercially available coated RIA tubes. The hormones were quantified according to the manufacturer's instructions. These kits proved efficient in previous experiments in dromedary camels (El Khasmi *et al*, 2013, 2015; Lemrhamed *et al*, 2018) and was purchased from DIAsource (Immunoassays S.A., Nivelles, Belgium). The areas of validation for cortisol assays included limits of detection and precision in the standard curve following sample dilution, inter- and intra-assay coefficients of variation results were considered.

Statistical analysis

Statistical Analysis System (SAS, Version 9.0) was used. The data were classified into 2 groups according to waiting periods at lairage (short period, 12hs≤time<16hs, n=8; and long period, 16hs≤time≤20hs, n=8). Blood samples were analysed by one-way (general linear model procedure) analysis of variance. A difference of $P < 0.05$ was considered statistically significant.

Results and Discussion

In Groups I and II, NLR, H50 (mOsmol/L) (Fig 1) and plasma levels of COR (ng/mL), T3, T4 (nM) (Fig 2) measured after waiting time showed a significant ($P<0.05$) increase when compared with those observed before waiting (at the end of transport and after unloading) (1.31±0.1 *vs* 0.91±0.1; 139.6±3.5 *vs* 121.6±3.9; 54.33±5.33 *vs* 25.21±2.67; 3.56±0.25 *vs* 1.42±0.17 and 117.8±16.93 *vs* 66.50±6.86, respectively in Group I and 0.93±0.1 *vs* 0.86±0.1; 131.1±3.2 *vs* 123.1±3.1; 43.67±5.74 *vs* 30.34±5.52; 2.71±0.21 *vs* 1.34±0.19 and 81.445±8.24 *vs* 53.62±7.21, respectively in Group II). By comparison to glucose levels (mM) measured after waiting, those analysed before rest were significantly ($P<0.05$) higher in Group I and lower in Group II (8.14±0.02 *vs* 7.28±0.03 and 6.23±0.04 *vs* 7.37±0.05, respectively) (Fig 3).

In the same conditions, at the end of waiting, NLR, H50 (mOsmols/L) (Fig 1) and plasma levels of COR (ng/mL), T3, T4 (nM) (Fig 2) and glucose (mM) (Fig 3) remained significantly ($P<0.05$) higher in group I (short waiting time) than those analysed in group II (long waiting time) (1.31±0.1 *vs* 0.9±0.1; 139.6±3.5 *vs* 131.1±3.2; 54.33±5.33 *vs* 43.67±5.74; 3.56±0.25 *vs*

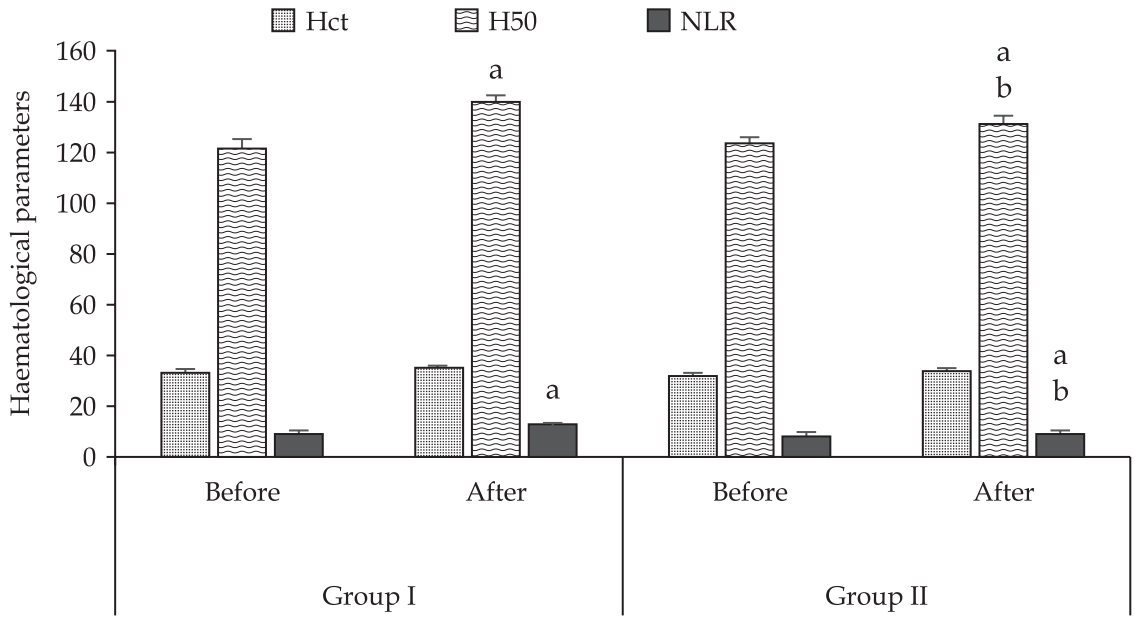

Fig 1. Haematocrit (Hct) (%), concentration of saline solution (mOsmol/L) inducing the haemolysis of 50% of erythrocytes (H50) and neutrophil/lymphocyte ratio (NLR) ($x10^{-1}$) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE, $^{a}P<0.05$, comparison before and after waiting for the same group, $^{b}P<0.05$, comparison after waiting between Groups I and II).

2.71±0.21; 117.8±16.93 *vs* 81.44±8.24 and 8.14±0.02 *vs* 6.23±0.04, respectively).

However, Hct (Fig 1), plasma levels of Ca, Pi and Mg (Fig 3), RT, HR and RR (Fig 4) showed no significant variation either between the stages (before and after waiting), or between the 2 waiting times.

The parameters analysed in this work (RT, HR, RR, H%, NLR, COR, glucose, T3, T4) were previously used by several studies as good indicators of stress responses in the dromedary camel (El khasmi *et al,* 2010, 2013), (Saeb *et al,* 2010; El khasmi *et al,* 2015; Lemrhamed *et al,* 2018). In domestic animals, the pre-slaughter stress can start in the farm, breeding site and market, continues with loading, transport, unloading, reception, conduction to the lairage area in the slaughterhouse where the animal can wait several hours and ends at the bleeding (Terlouw *et al,* 2008; Kadim *et al,* 2008; De la Fuente *et al,* 2010). In order to avoid stress at each of these stages, the International Committee of the World Organisation for Animal Health has developed recommendations for each of the pre-slaughter and slaughter processes for domestic animals, on the basis of available scientific data (Shimshony and Chaudry, 2005). So far, no recommendations concerning the dromedary welfare have been developed. However, research works had reported that this species has been more sensitive to road transport stress (El khasmi *et al,* 2010, 2013; Saeb *et al,* 2010), distance of transport (El khasmi *et al,* 2015) and loading density during transport (Lemrhamed *et al,* 2018). These stress situations were marked by a significant increase of H%, NLR, glycaemia and circulating levels of COR and thyroid hormones.

In the dromedary camel, among pre-slaughter stress factors, waiting at lairage after road transportation might be a potential source of stress, marked by significant high NLR, haemolysis and circulating levels of COR and thyroid hormones. Work shows that beyond 2 hours, increased waiting time promotes overlap in calves (Grigor *et al,* 2004). Resting animals in the lairage for before slaughter, is highly recommended for reduce transport stress and improve meat quality characteristics (Thompson, 2004). However, this requires good waiting conditions, while this period can also be a source of stress leading animals to become reactive and stressed. Furthermore, the rate energy gain by animals depend upon the amount of stress from transportation and the conditions of the lairage at the abattoir (Gregory, 2008; Grandin, 2010). In fact,

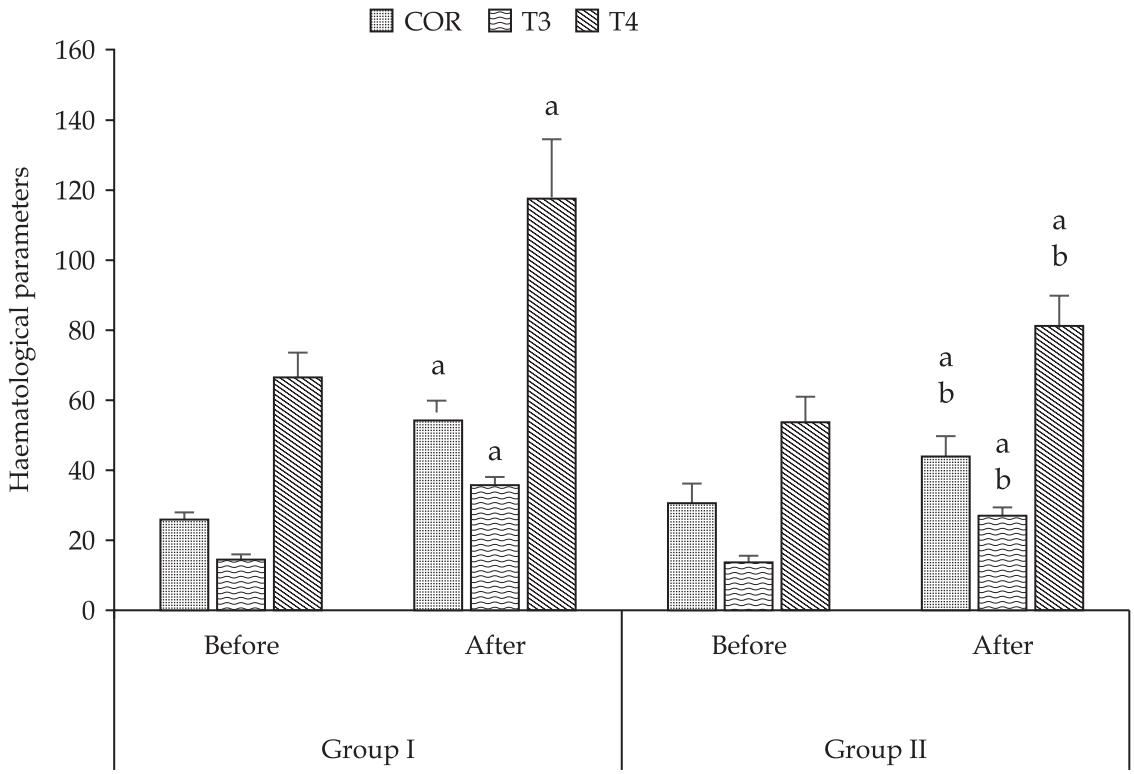

Fig 2. Plasma levels of cortisol (COR) (ng/mL), total triiodothyronine (T3) ($x10^{-1}$nM) and total thyroxine (T4) (nM) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE, $^{a}P<0.05$, comparison before and after waiting for the same group, $^{b}P<0.05$, comparison after waiting between Groups I and II).

during transfer to the lairage station, animals can be exposed to various stressors such as fasting or forced exercise, breakdown of social group and the familiar environment, background noise, handling and novelty, resulting in a physical exhaustion and a psychological stress (Terlow, 2005). For example, Tume and Shaw (1992) found cortisol concentrations in cattle slaughtered in commercial abattoirs to be higher than in animals slaughtered at research abattoirs owing to the inherent noise and movements of animals and people in the yards. In pigs, a significant increase of COR levels was found in blood after more than 4h (Rey-Salgueiro *et al,* 2018) or after 9h (Pérez *et al,* 2002) in lairage and in saliva after 20h of waiting (Jama *et al,* 2016) at the slaughterhouse. According to Hambrecht *et al* (2005) and Jama *et al* (2016), in pigs, decreasing lairage duration increased significantly plasma lactate and urine and plasma cortisol, suggesting that pre-slaughter rest can alleviate stress induced by pre-slaughter handling operations. Finally, the return to basic physiological and behavioural state prior to slaughter without feed but with access to water, could be observed after waiting 24 to 48 hours in cattle (Mounier *et al,* 2006), 12–24 hours in camel (Kadim *et al,* 2013), 24 hours in steers (Tadich *et al,* 2005) and more than 17 hours in pig (Jama *et al,* 2016).

The findings reported in this work, showed an increase of number of neutrophils and a decrease of number of lymphocytes in the camels after waiting. These variations were more pronounced after the short waiting time than the long waiting time. An excessive release of neutrophils occurs through the action of endogenous COR, causing mobilisation of the marginal neutrophils of the microvasculature, as well as the induction of increased reserve release of these cells from the bone marrow (Jain, 1993). Increased release of neutrophils at stress during short waiting time may weaken the camel's immune function (Thrall, 2006), leaving them susceptible to infections and inflammatory diseases. However, in pigs, lymphopenia with neutropenia has been reported (Chacon *et al*, 2005), as well as neutrophilia accompanied by lymphopenia (Gupta *et al,* 2007). These results may be explained by an excessive release of adrenaline (Jones and Allison, 2007) as well as by emotional changes or excess muscular effort (Jain, 1993). The increased circulating levels of COR in camels during short waiting time, might reduce mobilisation of circulating lymphocytes by inhibition of production. COR decreases the number of circulating lymphocytes, particularly T-helper cells involved in response to foreign substances resulting in a decrease of all cell-mediated immunity (Earley *et al,* 2017).

In this work, plasma glucose levels increased after the short waiting time and decreased after the long waiting time in the camel. The high levels of

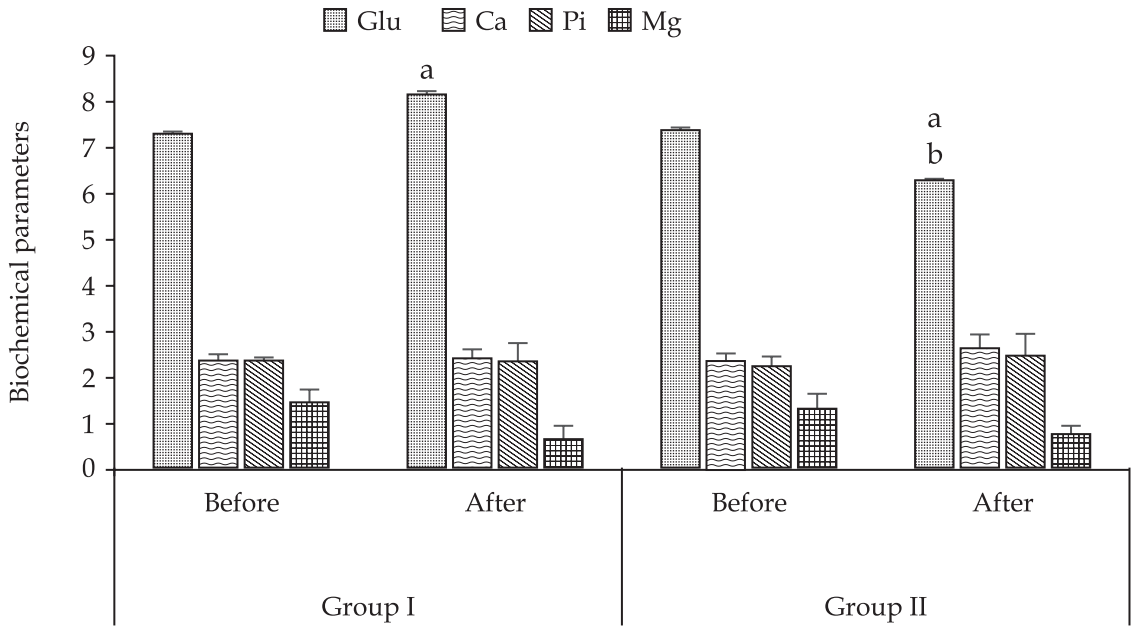

Fig 3. Plasma levels (nM) of glucose (Glu), calcium (Ca), phosphorus (Pi) and magnesium (Mg) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE, $^{a}P<0.05$, comparison before and after waiting for the same group, $^{b}P<0.05$, comparison after waiting between Groups I and II).

glucose observed just before slaughter, might impact the meat industry, since glycogen which is a precursor of glucose, is essential during the postmortem transformation of muscle into meat. The requirement of muscle glucose during waiting period at lairage by using glycogen can directly affect the blood concentration of glucose and quality of the meat, which are directly dependent on all pre-slaughter stages (Minka and Ayo, 2010; Gruber *et al,* 2010; Chulayo *et al,* 2016). According to Jama *et al* (2016) muscle glycogen content could therefore increase with waiting time, leading to a decrease of circulating levels of glucose in pigs. Tadich *et al* (2005) reported in the steers that had been transported for 3 h, glucose levels started to recover after 24 h of lairage. In comparison, a decline in glucose levels was observed in cattle that had been transported for up to 31 h even after 24 h in lairage (Mounier *et al,* 2006). Lairage duration might affect the circulating levels of glucose, however, the magnitude of waiting stress response is highly dependent on micro-ambient temperature and humidity leading to increased production of biochemical reactions and reduced blood levels of glucose (Chulayo *et al,* 2012, 2016; Romero *et al,* 2014). In addition, new environment where camels were mixed with unfamiliar animals, increase of probability of interactions between camels, waiting stressful conditions and adrenaline and COR secretion might be responsible for increased levels of circulating glucose and glycogen breakdown in the muscle (Terlouw *et al,* 2008).

The stress responses observed during waiting in the camels used in this investigation, might be explained by an activation of the hypothalamic-pituitary adrenal axis, inducing the release of catecholamines, glucocorticoids and other hormones that may alter the blood biochemical and cellular physiology, metabolism and immune function (Stanger *et al,* 2005). In the same conditions, the hypothalamus-pituitary-thyroid (HPT) axis may be involved in stress responses in animals (Joseph-Bravo *et al,* 2015), which underlines the evaluation of iodothyronines and notably of T3, as markers of their welfare and stress (Ferlazzo *et al,* 2018).

In camel, homeostatic changes due to waiting period at lairage were observed, with effects on immune function, integrity of erythrocyte membrane and corticotropic and thyrotropic axis. These pre-slaughter stress responses could alter the animal's physiology and the post-mortem quality of its meat. After transport and unloading, a waiting period of 16h to 20h could be considered less stressful than that of 12h to 16h in the camel. Nevertheless, our data do not enable us to recommend a specific waiting time, however, good waiting conditions are recommended aiming to reduce stress and improve camel welfare. The impact of waiting period on the blood and muscle antioxidant status will be evaluated later in this species.

Acknowledgements

The authors thank the President of urban municipalities of Casablanca, the Director of the abattoir of Casablanca and Dr. ABOUHAFS Rachid the responsible for prefectural veterinary service of Casablanca, to carry out this work.

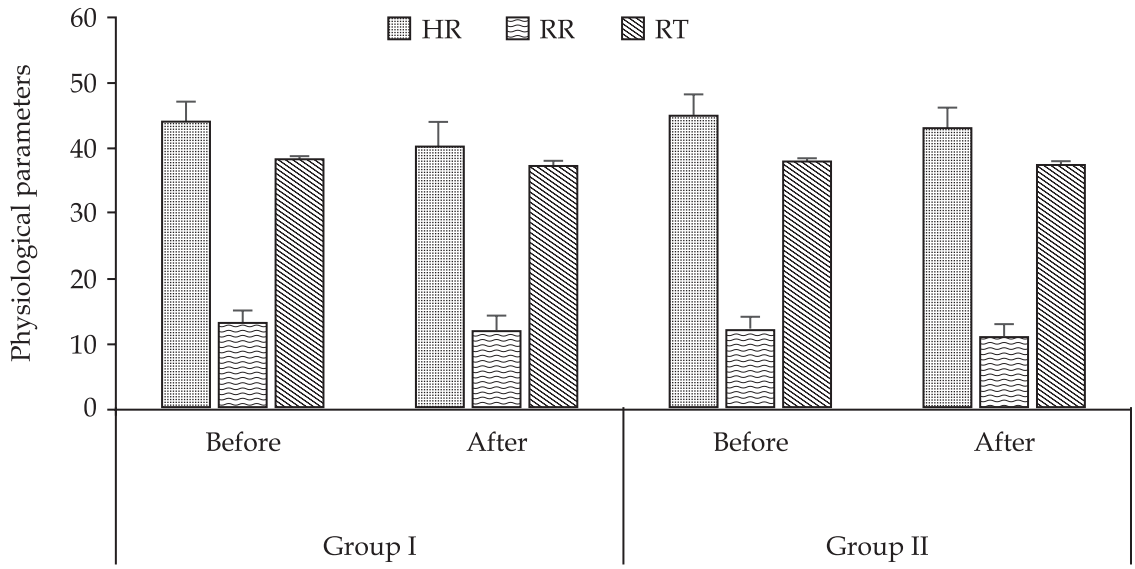

Fig 4. Heart rate (HR) (beats/mn), respiratory rate (RR) (cycles/mn) and rectal temperature (RT) (°C) before waiting (just at the end of transport after unloading) and after 2 different waiting periods: short period (12h≤time<16h, n=8, Group I) and long period (16h≤time≤20h, n=8, Group II). (Means±SE).

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DOI : 10.5958/2277-8934.2019.00024.9 Vol 26 No 2, p 157-162

TEAT CHARACTERISTICS OF INDIAN DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

The present study describes teat characteristics of Indian dromedary females (n=75). Mean±SE of teat diameters measured using Vernier caliper at Right front top, Right front middle, Right front lower or tip; Right Rear top, Right Rear middle, Right Rear lower or tip, Left front top, Left Front middle, Left front lower or tip, Left Rear top, Left Rear middle and Left Rear lower or tip, were 48.45±1.45; 31.77±1.14; 12.16 ±0.42; 52.12±1.48; 34.58±1.3; 13.25±1.01; 47.01±1.58; 33.51±1.26; 12.33±0.49; 54.07±1.62; 36.48±1.41; 12.42±0.47 mm, respectively. Mean±SE of length of Right front, Right rear, Left front, Left rear teat were 63.83±1.88; 64.88±2.1; 64.71±1.8 and 63.92±1.87 mm, respectively with a wide range of 20-110.04 mm. The effect of parity was significant on most of the teat measurements. Ultrasonographic examination revealed presence of definite gland and teat cisterns in camel. The present results give baseline data about teat dimensions with respect to parity, age in female dromedary of Bikaneri, Jaisalmeri, Kachchi and Mewari breeds of Indian camels. This will help to develop teat cups and liners and in turn milking machine for Indian dromedary camels.

Key words: Age, breeds, *Camelus dromedarius*, India, parity, teat characteristics

Camel is the fifth most important dairy animals in world after dairy cattle, buffalo, goat and sheep. According to FAO 2012, camel milk production is around 2.8 million tons which equals to 0.3% of total world milk production. However, others believe that global camel milk production is much higher, around 5.4 million tons per year (Faye, 2008; Faye and Konuspayeva, 2012). The camel milk dairies have come up as business activity in most camel possessing countries (Musaad *et al,* 2017). The market potential for camel milk could be highly developed in the future (Faye *et al,* 2014).

In order to meet out increased demand, milk production through intensive camel dairy management are increasing and resulted into development of camel mechanical milking. Besides behaviour and reaction to human presence and contact, udder and teat traits are important improvement in milking ability. Selection of best udder and teat shape or traits is an important step towards adaption to machine milking (Marnet *et al,* 2016).

Identification of factors like udder and teat characteristics is very important for milking management and machine milking development for camel (Marnet *et al,* 2016; Nagy *et al,* 2015). However, udder and teat morphology have received little attention by camel scientists (Atigui *et al,* 2016). The present study was taken to identify the teat characteristics in Indian dromedary camel breeds.

Materials and Methods

The lactating camels belonging to ICAR-National Research Centre on Camel, Bikaner situated at 28.0229° N, 73.3119° E, 242 m above sea level were studied for teat characteristics. There were 75 camels of four breeds *viz* Bikaneri (n= 23), Jaisalmeri (n= 17), Kachchi (n= 15) and Mewari (n= 20). Lactating camels were supplemented with 1 kg of concentrate pellets besides 10 kg of dry fodder.

The teat measurements were taken immediately after "let down" of milk and just before morning milking. The following measurements were taken using a Vernier caliper (VITA PQ150, Taiwan)- Teat diameter at the orifice, middle and base of the teat, teat length as distance from the teat insertion base to the teat orifice. A total of 1200 measurements (16 from each camel) were recorded as,

Diameters- Right front top (RA1), Right Front middle (RA2), Right front orifice or tip (RA3)

Right Rear top (RB1), Right Rear middle (RB2), Right Rear orifice or tip (RB3)

Left front top (LA1), Left Front middle (LA2), Left front orifice or tip (LA3)

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Left Rear top (LB1), Left Rear middle (LB2), Left Rear orifice or tip (LB3)

Length- Right front length (RAL), Right rear length (RBL), Left front length (LAL), Left rear length (LBL).

Ultrasound examination and body measurement

The ultrasound examination of udder and teat was performed using linear probe (6.5 MHz, V-5 portable ultrasound machine, Med-India) with camels in standing position immediately after "let down" of milk and just before morning milking.

Statistical analysis

The data was analysed to study the effect of breed, age and parity on various teat measurements. In order to study the effect of various factors affecting teat measurements a linear fix model was used. Data was analysed using GLM procedure of SPSS20. The linear model included fix effect of breed (4 levels- Bikaneri, Jaisalmeri, Kachchi and Mewari), parity (4 levels- first, second, third and fourth and above) and age of she camel at the time of calving (4 levels- 3-5 year, 6-8 year, 9-11 year and more than 11 year).

$$Y_{ijkl} = \mu + A_i + B_j + C_k + e_{ijkl}$$

Where, Yijkl is individual teat measurement, μ is overall population mean, Ai is fixed effect of age at calving, Bj is the fixed of breed, C_k is the fix effect of parity and e_{ijkl}= is a normally distributed random variable with mean zero and variance σ_e^2.

One way ANOVA was used to study differences in teat measurements which was recorded from different quarters and different sides.

Results

The results of the present study give baseline data about teat dimensions with respect to parity and age in female dromedary of four Indian breeds namely Bikaneri, Jaisalmeri, Kachchi and Mewari. The shape and size of the teat and udder was observed to vary greatly among the individual camels (Fig 1).

Overall least squares means for teat measurements of right quarters RA1, RA2, RA3, RAL, RB1, RB2, RB3 and RBL were 48.70 ± 1.73 cm, 33.04 ± 1.30 cm, 13.15 ± 0.51 cm, 66.10 ± 2.25 cm, 53.11 ± 1.87 cm, 36.64 ± 1.65 cm, 14.64 ± 1.41cm and 67.03 ± 2.80 cm, respectively (Table 1). Overall least squares means for teat measurements of left quarters LA1, LA2, LA3, LAL, LB1, LB2, LB3 and LBL were 48.84 ± 2.15 cm, 34.87 ± 1.65 cm, 13.24 ± 0.66 cm, 67.11 ± 2.38 cm, 53.72 ± 2.09 cm, 38.56 ± 1.66 cm, 13.07 ± 0.65 cm and 66.11 ± 2.36 cm, respectively (Table 2). The effect of age of she camels did not affect the teat measurements significantly except RA3. Effect of breed of camel was also found non-significant for majority of the teat measurements parameters except RA1, RB1 and LB1. Effect of breed was highly significant ($p \leq 0.01$) on RA1 and RB1 and significant on LB1. These measurements were higher in Bikaneri and Kachchi breeds compared to Jaisalmeri and Mewari Breeds. The parity of animals significantly affected most of the teat measurements parameters except RB1, RB3, RBL, LA3, LB1, LB3 and LBL. The parity of animals affected RA1, RA2, RA3, RAL, LA2, LAL significantly ($p \leq 0.01$) and affected RB2, LA1 and LB2 significantly ($p \leq 0.05$) (Table 1 and 2). Increasing trend for various teat measurements was seen up to third parity and thereafter decrease in teat measurements was observed for fourth parity animals. This trend was more consistent for right quarter teat measurements (Fig 2 and Fig 3).

The overall mean value of teat diameters at top/base, middle and tip/orifice and Length were 50.41±0.78, 34.08±0.65, 12.54±0.32 and 64.34±0.96 cm, respectively.

The ultrasound examinations clearly revealed milk in the teat canal and hence increase in dimensions of the teat canal just before the milking and empty teat canal after the milking. Teat's ultrasound scanning showed distinct teat canal connected to well defined teat cistern in each quarter. These were separated from each other by middle cistern wall (Fig 4 and 5).

Discussion

Investigating the teat characteristics is important step in developing machine milking in dromedary camels. Prerequisite for machine milking is the need to use a same liner/ cluster type for all the camels of one flock. During the past 10-15 years intensive camel milk production using machine milking has been introduced in some traditional camel keeping countries like United Arab Emirates, Saudi Arabia and Tunisia (Wernery *et al,* 2004; Hammadi *et al,* 2010; Ayadi *et al,* 2013; Atigui *et al,* 2015). Small scale farms have also come up in Netherlands, Australia and USA. The present study revealed that Indian dromedary camels have a well-developed udder and teats. The shape and size of the teat and udder was observed to vary greatly among the individual camels. Shehadeh and Abdelaziz (2014) has also reported big variability between camels but also intra-camels.

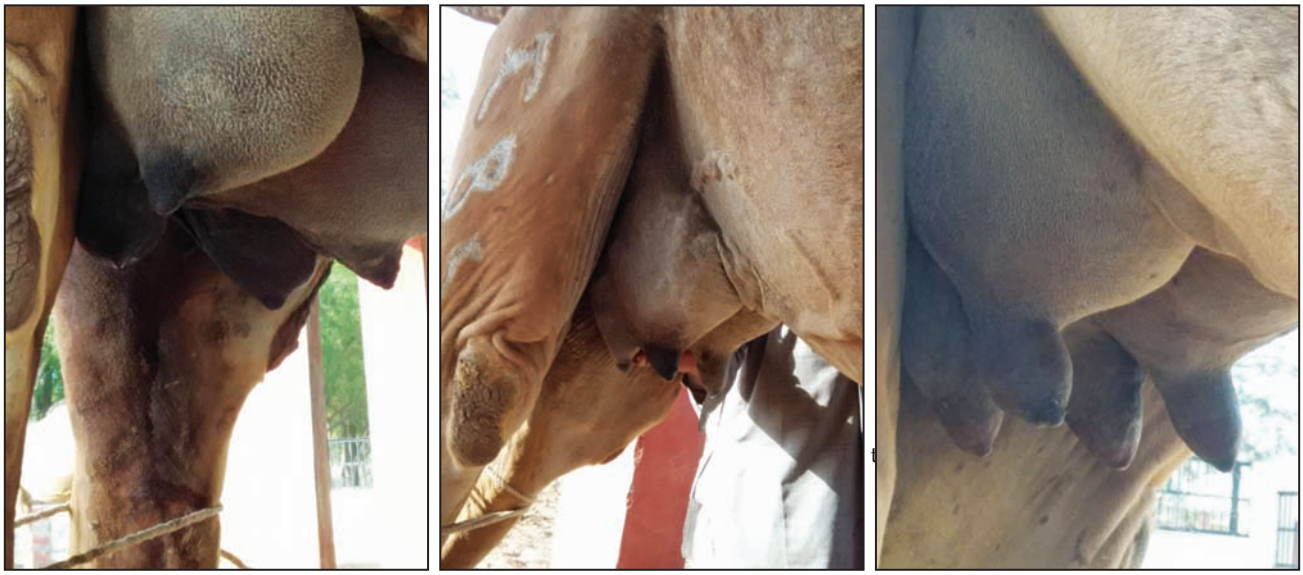

Fig 1. Udder and teats of different shapes and size in Indian *Camelus dromedarius.*

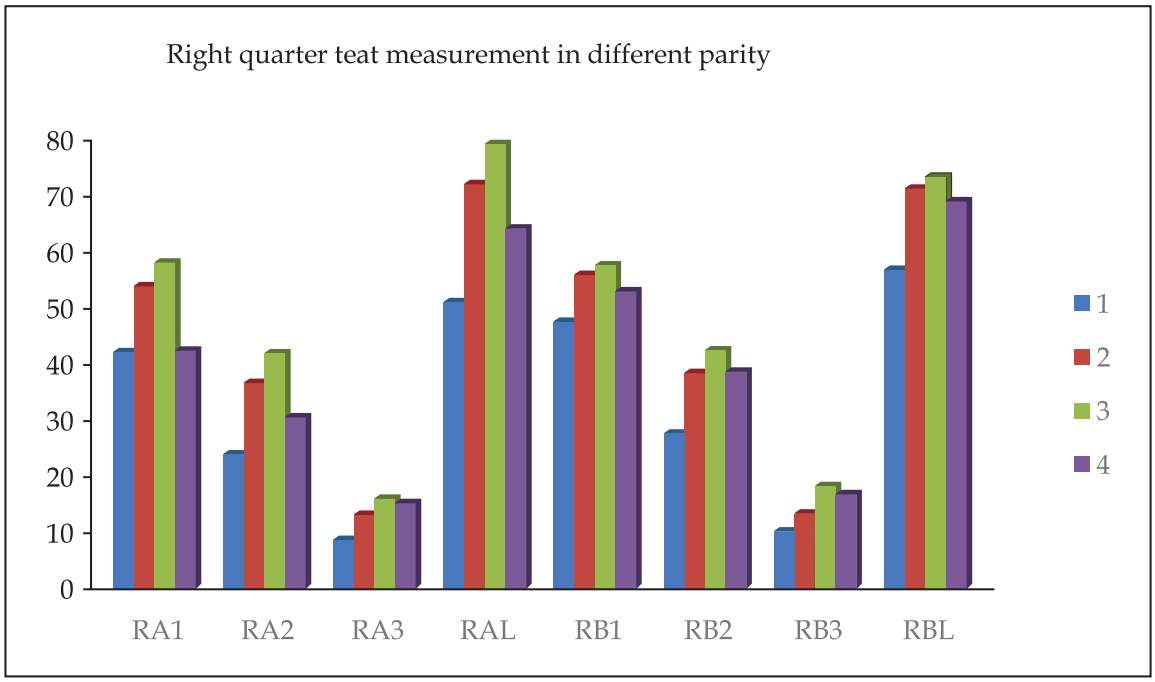

Fig 2. Right quarter teat measurements in different parity.

Teat length in NRCC camels was larger than those reported previously by Eisa *et al* (2010) (mean length 4.3 to 5.3 cm) and similar to that reported by Atigui *et al* (2016) using B-mode ultrasonography (mean length 6.16 to 6.17 cm) and Nagy *et al* (2015).

Teat characteristics are quite different in dromedary camels compared to other dairy animals. Such large teats might cause some problems during machine milking and require special settings and practice during milking. This should be taken into account when developing a milking machine for dairy camels. The teat length in Indian camels in the present study was more than the values reported for dairy cows (around 2.5 cm, Rogers and Spenser, 1991; Zwertvaegher *et al,* 2012) and buffaloes (2.76 ± 0.02 cm, Prasad *et al,* 2010). This indicates that existing milking machines used for cows and buffaloes should be modified before these are used for camels.

The current milking machines work by alternate periods of suction and massage by the liner wall pressure on the teats. The buckling point of the liner is generally situated in the middle of its barrel.

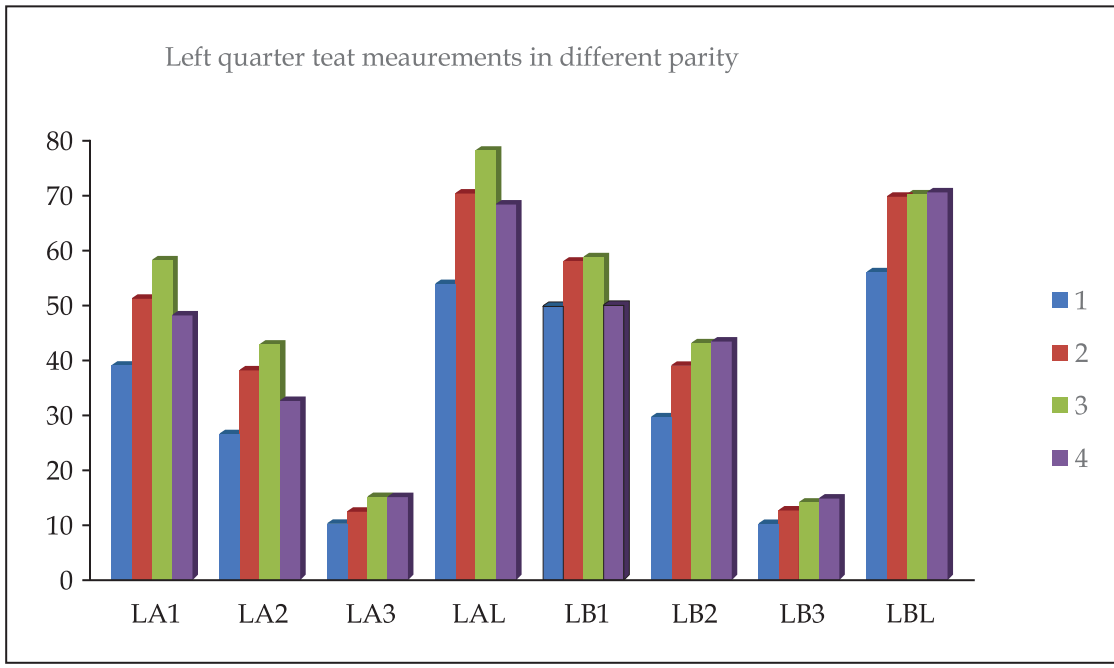

Fig 3. Left quarter teat measurements in different parity.

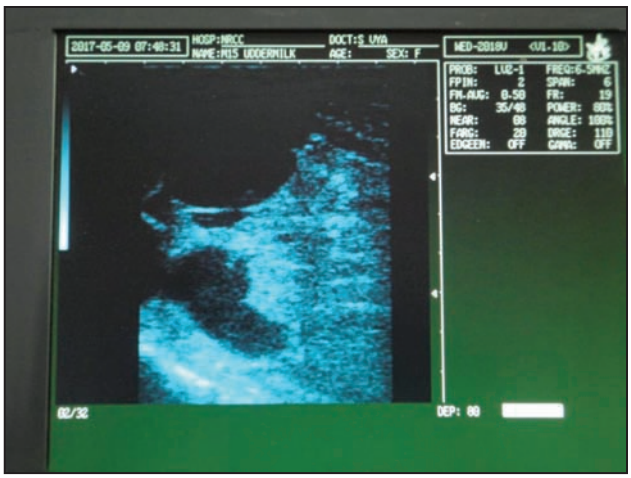

Fig 4. Distinct teat cistern and teat canal engorged with milk before milking.

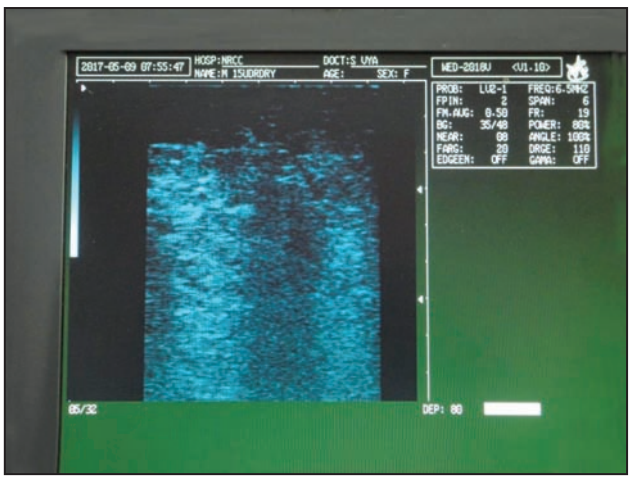

Fig 5. Ultrasonogram of udder and teat emptied after milking.

Therefore the length of liner must be adapted to the teat length to avoid massaging only at the teat apex (teat too short for the liner) or in the upper part of teat (teat too long for the liner) (Mein *et al,* 2003).

The size of dromedary teat undergoes great variation before (*i.e* after milk let down) and after milk ejection. Nagy *et al* (2015) described that change in teat size parameters are related to pre-milking milk ejection. During milk let down and ejection, teat cisterns are filled with milk causing changes in teat size and volume. This functional characteristic underlines the importance of effective pre-milking udder stimulation in this species. During milk let down or stimulation the teat length and volume is reported to increase by 40 to 50% and 130%, respectively. After milking teat length and volume returned to pre-stimulation (n=44, Nagy and Juhasz, 2016). Therefore the liners with large mouth piece (> 30 mm) tend to climb up with decreasing teat size while smaller liner (25 mm) can only accommodate the lower third of an enlarged teat (Nagy and Juhasz, 2016).

B-mode ultrasonography in the present study confirms the presence of definite gland and teat cisterns in camel as reported previously (Abshenas *et al,* 2007 and Atigui *et al,* 2016). This is in contrast to previous reports of a notion /conception that there is no mammary or teat cisterns in camels but the milk rapidly descends directly into the teats causing an enormous swelling, often requiring two hands to

Table 1. Least squares means of teat measurements of right quarters.

Traits	N	RA1	RA2	RA3	RAL	RB1	RB2	RB3	RBL
Overall Mean	75	48.70±1.73	33.04±1.30	13.15±0.51	66.10±2.25	53.11±1.87	36.64±1.65	14.64±1.41	87.03±2.80
Age at calving		NS	NS	*	NS	NS	NS	NS	NS
1(3-5 years)	13	50.74±4.44	35.39±3.34	13.61±1.31	69.75±5.77	53.16±4.80	38.61±4.23	13.80±3.62	66.82±7.18
2 (6-8 years)	26	48.63±2.99	35.28±2.25	15.26±0.89	72.25±3.89	53.23±3.24	38.27±2.86	15.14±2.44	69.79±4.84
3 (9-11years)	15	47.22±3.28	30.29±2.47	12.03±0.97	61.54±4.27	53.76±3.55	34.71±3.13	17.10±2.68	66.37±5.31
4 (>11years)	21	48.22±2.61	31.20±1.96	11.71±0.77	60.88±3.40	52.29±2.82	34.99±2.49	12.52±2.13	66.15±1.22
Breed		**	NS	NS	NS	**	NS	NS	NS
Bikaneri	23	53.87±2.65	33.92±1.99	13.41±0.78	68.11±3.44	58.46±2.86	38.45±2.52	13.46±2.16	69.68±4.28
Jaisalmeri	17	45.85±2.67	32.47±2.01	13.42±0.79	64.21±3.47	49.81±2.88	35.17±2.54	17.55±2.17	64.07±4.31
Kachchhi	15	52.37±3.01	36.03±2.27	12.90±0.89	70.74±3.92	58.03±3.26	41.07±2.87	13.03±2046	73.87±4.88
Mewari	20	42.73±3.06	29.75±2.30	12.89±0.91	61.36±3.89	45.15±3.31	31.89±2.92	14.51±2.50	60.50±4.95
Parity		**	**	**	**	NS	*	NS	NS
1	32	41.81±2.57	23.87±1.93	8.71±0.76	50.71±3.34	47.26±2.78	27.56±2.45	10.23±2.10	56.42±4.16
2	20	53.34±2.53	36.38±1.91	13.10±0.75	71.51±3.29	55.66±2.74	38.22±2.42	13.38±2.07	70.79±4.10
3	17	57.57±3.15	41.63±2.37	15.78±0.93	78.48±4.10	57.05±3.41	42.37±3.00	18.12±2.57	72.64±5.10
4 and above	6	42.10±5.28	30.29±3.97	15.03±1.56	63.71±6.86	52.48±5.75	38.43±5.03	16.83±4.30	68.27±8.54

N denotes number of observation. ** denotes (p≤0.01), * denotes (p≤0.05) and NS denotes- Non-significant at (p >0.05).

Table 2. Least squares means of teat measurements of left quarters.

Traits	N	LA1	LA2	LA3	LAL	LB1	LB2	LB3	LBL
Overall Mean	75	48.84±2.15	34.87±1.65	13.24±0.66	67.11±2.38	53.72±2.09	38.56±1.66	13.07±0.65	66.11±2.36
Age		NS	NS	NS	NS	NS	NS	NS	NS
1 (3-5 yrs)	13	54.09±5.50	37.92±4.23	13.78±1.70	69.79±6.11	51.26±5.34	37.69±4.25	12.84±1.67	62.98±6.05
2 (6-8 yrs)	26	51.75±3.71	37.02±2.85	13.63±1.15	71.52±4.12	54.25±3.61	41.97±2.87	13.83±1.12	69.13±4.09
3 (9-11yrs)	15	45.38±4.07	31.03±3.13	13.06±1.26	66.48±4.52	52.35±3.95	35.97±3.15	12.93±1.23	88.30±4.48
4 (>11yrs)	21	44.16±3.23	33.50±2.49	12.48±1.00	60.64±3.59	57.02±3.14	38.61±2.50	12.68±0.98	64.04±3.56
Breed		NS	NS	NS	NS	*	**	NS	NS
Bikaneri	23	51.98±3.28	34.77±2.52	13.60±1.01	69.32±3.64	59.18±3.19	42.51±2.53	14.59±0.99	62.98±6.05
Jaisalmeri	17	47.54±3.30	36.81±2.54	12.27±1.02	67.44±3.67	49.03±3.21	34.45±2.55	12.24±1.00	69.13±4.08
Kachchhi	15	50.98±3.73	37.25±2.87	13.84±1.15	65.07±4.15	58.82±3.63	45.07±2.89	12.99±1.13	68.30±4.48
Mewari	20	44.86±3.79	30.64±2.91	13.24±1.17	66.61±4.21	47.85±3.69	32.21±2.93	12.47±1.15	64.04±3.56
Parity		**	**	NS	**	NS	**	NS	NS
1	32	38.78±3.18	26.41±2.45	10.18±0.98	53.52±3.54	49.35±3.09	29.46±2.46	10.26±0.96	55.75±3.50
2	20	50.94±3.14	37.93±2.41	12.48±0.97	69.79±3.45	57.46±3.05	38.81±2.43	12.80±0.95	69.20±3.45
3	17	57.77±3.91	42.56±3.00	15.10±1.12	77.42±4.34	58.33±3.80	42.82±3.02	14.12±1.18	69.52±4.29
4 and above	6	47.88±6.54	32.56±5.03	15.19±2.02	67.71±7.26	49.74±6.35	43.15±5.05	15.11±1.98	69.98±7.19

N denotes number of observation. ** denotes (p≤0.01), * denotes (p≤0.05) and NS denotes- Non-significant at (p >0.05).

encompass a teat (Yagil *et al,* 1999 and Simpkin *et al,* 1997).

At present camel farmers at large in India are skeptical about possibility of machine milking for camels. But it could be possible if they overcome their traditional mindset and opt for selection of camels for udder and behavioural traits suitable for efficient milk ejection. Development or modification in existing milking machines using new material and settings is also necessary for successful machine milking in camels.

The present results offer a good base data which can be used effectively for selection camels for bringing an improvement in performance of dairy

camel. The findings of the present study will help to develop teat cups and in turn milking machine for Indian dromedary.

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DOI : 10.5958/2277-8934.2019.00025.0 Vol 26 No 2, p 163-168

COMPARATIVE TRANSCRIPTOME ANALYSIS OF LIVER TISSUES IN BACTRIAN CAMEL (*Camelus Bactrianus*)

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ABSTRACT

In the present study, comparative transcriptome sequencing was used to compare liver tissue transcriptome profiles between domestic bactrian camel (BCL), cattle (CL) and sheep (SL). The RNA-seq data from these two groups revealed that 3254 genes were differentially expressed between the BCL and CL groups, 2426 genes were found to differ significantly in expressional levels between BCL and SL. Pathway analysis revealed that the differentially expressed genes between the BCL and CL belong to non-alcoholic liver disease, bile secretion, as well as metabolic related pathway such as fatty acid metabolism and degradation, xenobiotics by cytochrome P_{450}, drug metabolism-cytochrome P_{450}, and drug metabolism-other enzymes; between the BCL and SL groups. A complete dataset of bactrian camel liver tissue using RNA-seq will facilitate the understanding of the unique biological characteristics of bactrian camels and supply essential information towards further studies on the genomes of bactrian camel and other ralated mammals.

Key words: Bactrian camel, orthologous gene, RNA-seq, transcriptome

The bactrian camel (*Camelus bactrianus*) represent an important livestock species of Gobi desert area and are a key source of animal products including meat, milk and transportation (Cardellino *et al,* 2004). The Northeast Asia and Central Asia, including Inner Mongolia, Xinjiang and Qinghai of China are the main distribution area of the domestic Bactrian camels (Ji *et al,* 2009).

In the vast grasslands, there are many kinds of plants that are toxic, such as *Peganum harmala, Cynomorium songaricum, Gelsemium elegans, Amygdalus mongolica* and *Mongolian almonds*. These plants not only have certain toxicity, but also mostly are bitter and hard to time. Generally, livestock such as cattle, sheep and horses instinctively avoid these plants. Once they are fed, they cause seriously ill and animals die. Camels have unique resistance to stress and toxic plants low rates of poisoning and mortality after eating, and some can even eat normally. According to the research, the reason of the low rates of poisoning is related to the liver tissue, and the liver has long been known as the major site of xenobiotic biotransformation in mammals (Al Katheeri *et al,* 2006; Elsheikh, 1997; Kandeel *et al,* 2016). To analyse this unique biological function of the bactrian camel, it is necessary to do transcriptomics research.

However, the complexity of the camel liver transcriptome has not yet been fully described. In recent years, with the rapid development of sequencing technology, high-throughput and deep- sequencing technologies have become new strategies to analyse the functional complexity of transcriptomes (Mortazavi *et al,* 2008). In the present study, RNA-Seq approach was used to explore the liver transcriptome of domestic bactrian camel, cattle and sheep. The main goal of this study was to identify differentially expressed genes which might be useful for the molecular breeding of domestic bactrian camel, and will extend our understanding on how genetic background may influence detoxification function.

Materials and Methods

Sample collection

A total of 5 female cattle, 5 female sheep and 5 male domestic bactrian camels were used for this experiment. All individuals were healthy and development was consistent with age. Cattle, sheep and domestic bactrian camel were slaughtered

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following the "Guidelines on Ethical Treatment of Experimental Animals" (2006) No. 398, which was formulated by the Ministry of Science and Technology, China. Liver tissues were collected and frozen in liquid nitrogen within 10 min of slaughter. Subsequently, liver tissues were stored at -80°C until RNA isolation.

Total RNA Extraction, cDNA Library Construction and Illumina Hiseq 4000 Sequencing

Total RNA (5µg) from the liver tissues of domestic bactrian camel, cattle and sheep was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. The total RNA concentration and purity of the RNA was detected by Ultra-micro spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA); the integrity of RNA was found by agarose gel electrophoresis and Biological analyser Agilent 2100 (Agilent, Santa Clara, CA, USA) was employed to calculate the RNA Integrity Number (RIN). A Truseq RNA Sample Prep Kit (Illumina, SanDiego, CA, USA) was employed in mRNA purification and cDNA library construction according to the manufacturer's instructions. The cDNA library was amplified by PCR enrichment, and was examined by 2% electrophoresis agarose gel to recover PCR fragments. Illumina sequencing was conducted on a Hiseq 4000 sequencer (Hiseq 4000 Truseq SBS Kit (300 cycles), Illumina). These experiments were completed by Shanghai Majorbio Bio-pharm Biotechnology Co. (http://www.majorbio.com, Shanghai, China).

Data Analysis of RNA-Seq

Software SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle) were used to filter noises for the original sequencing reads. The sequencing adapter sequence, low quality reads, higher N rate sequences, and too short sequences were removed to get clean data. At the same time, Q30, GC-content, and sequence duplication level of the clean data were calculated. Further, the remaining high-quality reads were submitted for mapping analysis against camel (Jirimutu *et al,* 2012), cattle (Elsik *et al,* 2009) and sheep (Jiang *et al,* 2014) reference genome (*Camelus bactrianus*: ftp://ftp.ncbi.nih.gov/genomes/*Camelus_ferus/; Bos taurus:* version UMD 3.1.1; *Ovis aries:* version GCA_000298735.2(1)) using Hisat^2 (Kim *et al,* 2015), allowing 2 base mismatches. Sequencing saturation and duplicate reads were performed by the RSeQC-2.6.3 (http://rseqc.sourceforge.net/) software (Wang *et al,* 2012). Orthologous and paralogous genes were identified by RSEM (http://deweylab.biostat.wisc.edu/rsem/) (Li and Dewey, 2011); The expression quantity of each gene (fragments per kilobase of exon model per million mapped fragments, FPKM) was estimated by edgeR (http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html) (Robinson *et al,* 2010; Tang *et al,* 2008). "FDR (False Discovery Rate) < 0.05 and log2|FC| (Fold Change) >= 1" were used as the threshold for judging the significant of gene expression difference. Gene Ontology (GO, http://www.geneontology.org/) and functional enrichment analysis were conducted on all identified differentially expressed

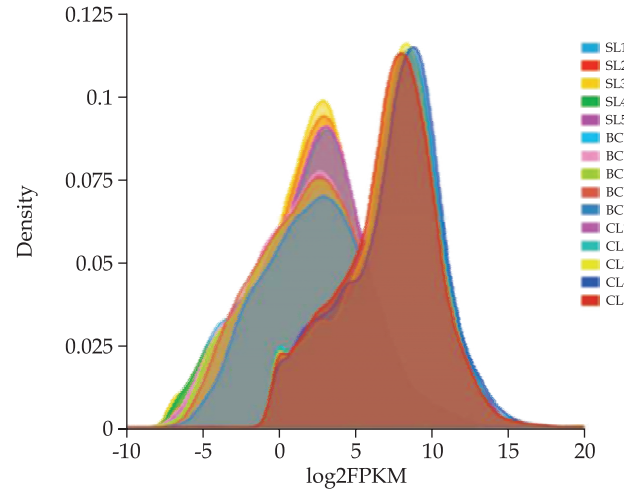


Fig 1. Expression density distribution.

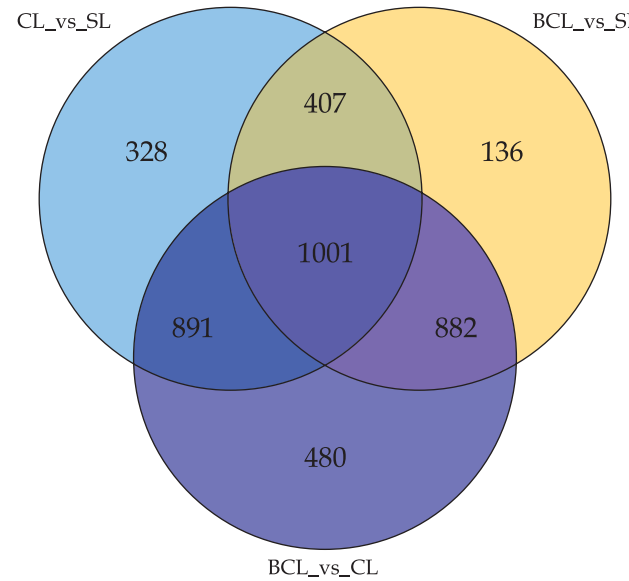


Fig 2. Distribution of differential expressed orthologous genes among the 3 species.

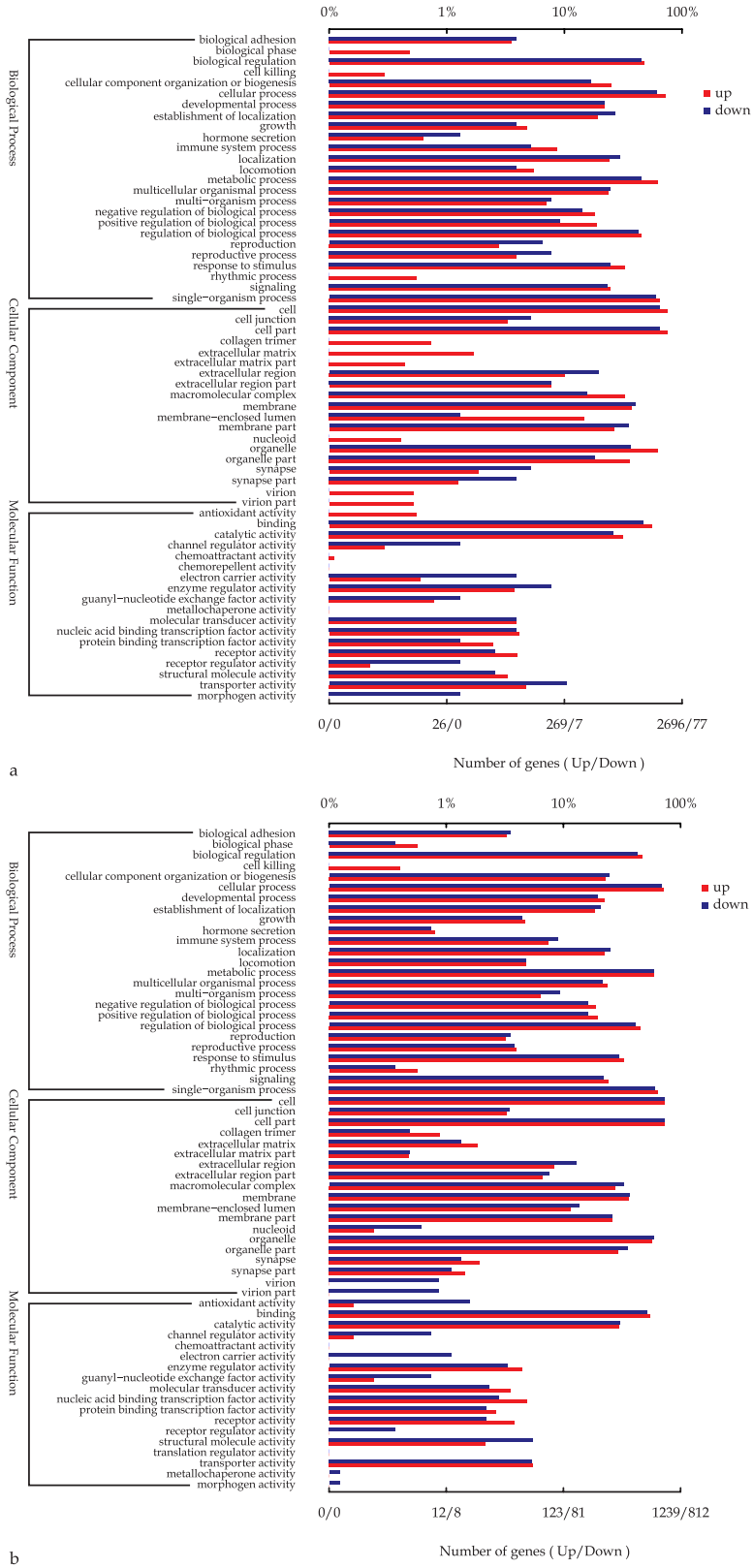


Fig 3. Histogram illustration of GO classification in all differentially expressed gene in bactrian camel and cattle (**a**), and bactrian camel and sheep (**b**). Results were summarised in 3 main categories: biological process, cellular component, and molecular function. The y-axis indicates the number of genes involved in each category.

genes (DEGs) using the Goatools software (Klopfenstein *et al,* 2018) (https://github.com/tanghaibao/goatools). Finally, metabolic pathway analysis was performed on all identified DEGs in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/genes.html) using Blastx/Blastp and KOBAS (http://kobas.cbi.pku.edu.cn/home.do) (Xie *et al,* 2011). Venn map of differential genes was made by VennDiagram software (http://en.wikipedia.org/wiki/Venn_diagram), which observed the distribution of differential genes among the samples.

Results and Discussion

Transcriptome Sequencing and Sequence Alignment

In this study, the transcriptome sequencing analysis of the domestic bactrian camel liver (n=5), cattle liver (n=5) and sheep liver (n=5) was conducted using an Illumina Hiseq 4000 platform. After removing reads containing poly-N or adaptors and low quality reads, a total of 841.01million 150-bp pair-end clean reads were obtained, the total read length was 121.93 gigabases (Gb). The Q30 scores of clean bases were more than 93% for all these 15 samples, implying the high quality of our sequencing data. We next aligned the clean reads and about 89.26-91.09% of reads were mapped, aligned the clean reads onto the *Bos taurus* reference genome (version UMD 3.1.1); about 89.43-90.96% of reads were mapped, aligned the clean reads onto the *Ovis aries* reference genome (version GCA_000298735.2(1)); about 91.14-91.59% of reads were mapped aligned the clean reads onto the *Camelus bactrianus* reference genome (Jirimutu *et al,* 2012); (Table 1).

Orthologous gene analysis

Firstly, we used OrthoMCL software (http://orthomcl.org/orthomcl/) to analyse predicted protein sequences, and classified genes from

different species; then each group of homologous genes were aligned by muscle (version 3.8). However, large number of paralogous genes were contained by some homologous gene clusters, and it was necessary to remove the paralogous genes and further analysed them with a single copy of the gene. At present, RAxML software (version: 8.0.20) (Stamatakis, 2014) was used to establish a gene tree for homologous gene clusters, and the agalma software package (Dunn *et al,* 2013) was used to trim the trees to get only one ortholog gene of each species. The result we found 6,112 ortholog genes in the 3 species. Further, orthologous gene expression was analysed (Fig 1), and the result displayed that the gene expression of bactrian camel liver sample was higher than cattle and sheep samples.

It is well known that, the expression profiles of liver tissue changed considerably in different species. A total of 2627 genes were found to differ significantly in expressional levels between CL and SL; a total of 2426 genes were found to differ significantly in expressional levels between BCL and SL; a total of 3254 genes were found to differ significantly in expressional levels between BCL and CL. When mixed together, there were 1001 differentially expressed among all the 3 species. The distribution of differential orthologous genes among the 3 species was shown in fig 2.

To validate the differential orthologous expressed genes, normal distribution analysis was performed with the results of the differential orthologous expressed genes, and all of their coefficients were <0.5. Based on the 4125 known genes that were shared as DEGs in three species, cluster analysis of all differential orthologous expressed genes was performed using the Cluster 3.0 software. The results demonstrated that expression profiles of samples at the same species were polymerised together (Supplementary Fig 1).

Gene Ontology Analysis

To further investigate the biological processes associated with the differentially expressed genes, we performed Gene Ontology (GO) analysis by running queries for each differentially expressed gene against the GO database, which provides information on the relevant molecular functions, cellular components, and biological processes. The results of analysing the GO functional annotations are presented in Fig 3.

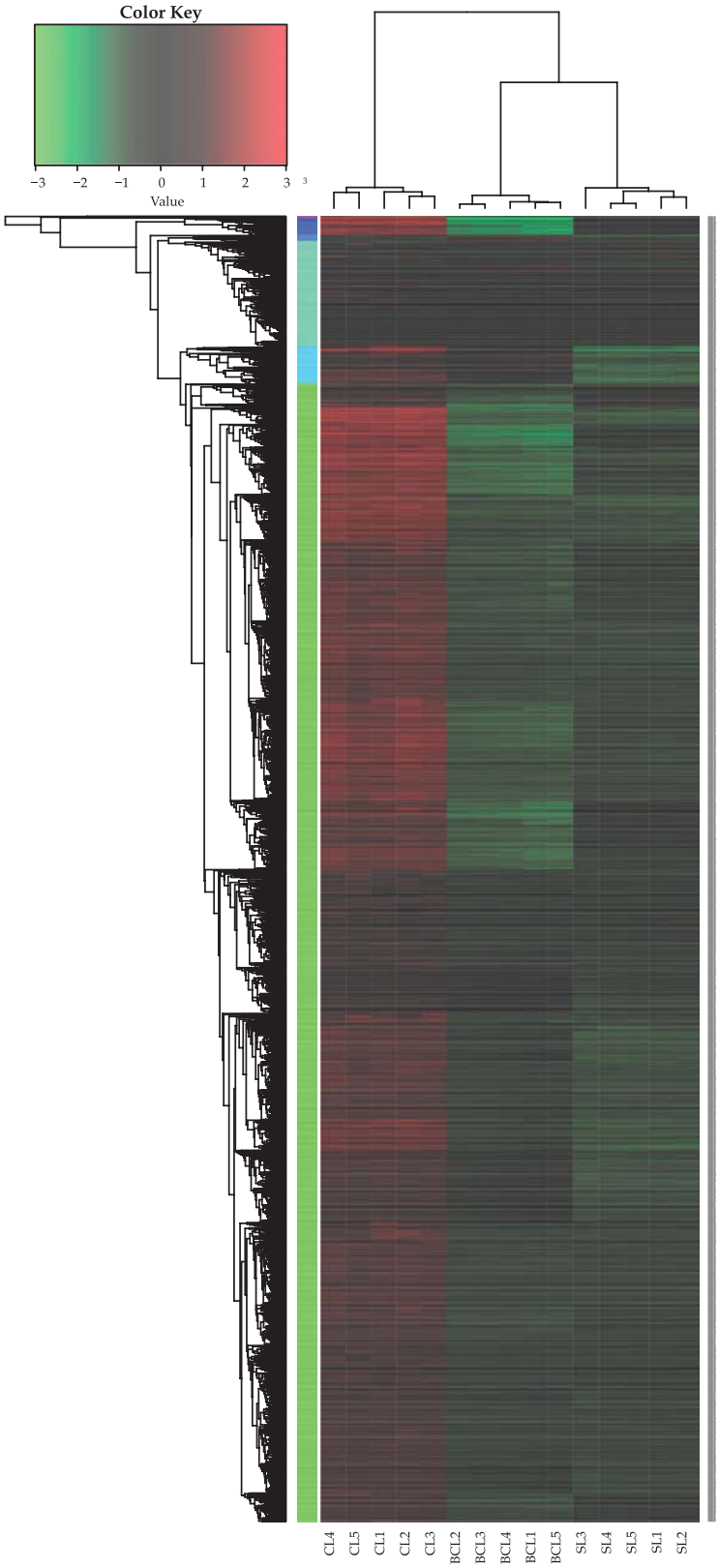
Functional enrichment of differential expressed orthologous genes in bactrian camel

KEGG pathway analysis of differential expressed orthologous genes from BCL and CL groups indentified several known pathways including non-alcoholic liver disease (61 genes) and bile secretion (13 genes), as well as, metabolic related pathway such as fatty acid metabolism and degration (18 and 14 genes), xenobiotics by cytochrome P_{450} (10 genes), drug metabolism-cytochrome P_{450} (8 genes), and drug metabolism-other enzymes (7 genes); in BCL

Table 1. Number of reads sequenced and mapped to reference genome of domestic bactrian camel, cattle and sheep, respectively.

Sample ID	Raw bases (bp)	Raw reads	Q30 %	Clean reads (bp)	Clean reads	Q30 %	Mapped reads/total reads	Proportion (%)
BCL1	8539083300	56927222	95.68	8036906798	55434508	94.26	38749336/43033084	90.05%
BCL2	8952820200	59685468	95.67	8413666600	58085812	94.40	59839114/65820148	90.91%
BCL3	8896200000	59308000	96.09	8425145944	58014126	94.80	47349828/52057218	90.96%
BCL4	8167500600	54450004	95.70	7681775401	53037902	94.38	39112157/43275402	90.38%
BCL5	8708774302	57674002	97.23	8207311698	55834858	95.44	49932685/55834858	89.43%
CL1	9196513500	61310090	95.77	8660393564	59691334	94.49	54371197/59691334	91.09%
CL2	10544123700	70294158	95.62	9905366787	68321982	94.21	62461010/68321982	91.42%
CL3	8693765400	57958436	94.96	8068750843	55825214	93.58	50780243/55825214	90.96%
CL4	9602182800	64014552	95.66	9037948384	62316468	94.26	56674147/62316468	90.95%
CL5	7943402100	52956014	95.19	7407653740	51230822	93.72	45729709/51230822	89.26%
SL1	6591963300	43946422	96.02	6240744838	43033084	94.66	53802575/59031928	91.14%
SL2	10114684200	67431228	95.85	9526217955	65820148	94.51	50771359/55434508	91.59%
SL3	8034809100	53565394	95.52	7518888495	52057218	94.12	52999896/58085812	91.24%
SL4	6721945200	44812968	95.13	6250965626	43275402	93.74	53090145/58014126	91.51%
SL5	9097595400	60650636	95.65	8546407787	59031928	94.28	48566276/53037902	91.57%

Note: BCL represents domestic bactrian camel liver sample, CL represents cattle liver sample and SL represents sheep liver sample.



Supplementary Fig 1. Heatmap showing the expression level of the differential orthologous expressed genes between domestic bactrian camel, cattle and sheep. Columns represent individual samples; rows indicate genes with significant expression differences between the three species.

and SL groups, we determined fat digestion and absorbtion (8 genes) and regulation of lipolysis in adipocytes (5 genes), as well as metabolic related pathway such as drug metabolism-cytochrome P_{450} (9 genes), xenobiotics by cytochrome P_{450} (10 genes), inositol phosphate metabolism (10 genes), fatty acid degradation (11 genes) and steroid hormone biosynthsis (5 genes), which may contribute to liver detoxification and drug metabolism.

The detoxification function in the liver of bactrian camel plays an important role in energy reserve during drought seasons and, thus, the analysis of the liver transcriptome in bactrian camel reveals the functional complexity of metabolising toxic substances. The RNA-seq identified a large number of DEGs, including new candidate genes, with respect to camel, cattle and sheep with significantly different amounts of liver tissues. These DEGs could be important for understanding the molecular basis of detoxification function in bactrian camel.

Acknowledgements

This work was supported by grants from the Inner Mongolia Natural Science Foundation Project (2018BS03017), High-level talents introduce scientific research start-up project (NDYB2017-28) and the Major Project of Inner Mongolia Autonomous Region Science and Technology.

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DOI : 10.5958/2277-8934.2019.00026.2 Vol 26 No 2, p 169-172

CHRONIC PERITONITIS IN DROMEDARY CAMELS: CLINICAL, ULTRASONOGRAPHIC AND PATHOLOGIC FINDINGS

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ABSTRACT

The aim of the present study was to emphasise the clinical, ultrasonographic and pathologic findings in dromedary camels with chronic peritonitis. For this purpose, 15 dromedary camels underwent transabdominal ultrasonographic examination of the viscera. Postmortem examination was also carried out in 5 camels. Clinical signs recorded were anorexia, scanty faeces, fits of colic that appeared in the form of rolling and moderate abdominal distension. Abdominal ultrasonography revealed echogenic fibrin threads floating in the peritoneal effusion. Fibrinous peritonitis was seen as heterogeneous deposits of echogenic and anechoic materials between the intestines, liver, kidneys rumen, spleen and abdominal wall. Peritoneal effusion appeared echogenic showing fibrinous tissue deposits interspersed with anechoic areas of fluid pockets. Ultrasound-guided aspiration of the peritoneum yielded a deeply turbid fluid. A treatment follow-up revealed that only 3 of the 15 camels made a full recovery. Postmortem examination showed massive fibrin network with adhesion of the viscera to the abdominal wall. Foul-smelling serosanguineous abdominal fluid was evacuated from the abdomen. In conclusion, ultrasonography is feasible diagnostic tool for verifying chronic peritonitis in camels. The procedure provides information about the scale and localisation of inflammatory lesions of the peritoneum. The clinical examination should therefore be supplemented by this imaging modality in camels suspected of having peritoneal lesions.

Key words: Abdomen, camel, pathology, peritonitis, ultrasound

There are numerous causes of peritonitis, among which are uterine tears, ruptured bladder, abscessation and gastrointestinal perforation. In camels, the cause of peritonitis may be associated with intestinal perforation (Tanwar *et al,* 2000). In another report, reticular foreign body penetration was the cause of peritonitis in a male dromedary camel (Suthar *et al,* 2010). The causative agent of peritonitis in a llama (Hewson and Cebra, 2001) and dromedary camel calf (Stoughton and Gold, 2015) was *Streptococcus equi* subsp. *zooepidemicus*. Haematogenous dissemination from suspected pneumonia was considered as the route of infection in this case. Similarily, *Streptococcal zooepidemicus* septic peritonitis was also recorded in a male dromedary camel (Heller *et al,* 1998). Anderson *et al* (1995) reported septicemic salmonellosis in two llamas caused by *S. cholerasius* and it was characterised by fibrinopurulent pericarditis, pleuritis and peritonitis.

Ultrasonography is an integral part of contemporary bovine medicine (Braun, 2004; Tharwat *et al,* 2012a). Ultrasonography of the peritoneum has been cited to be the best method to assess the extent of peritoneal reaction/abscessation in cattle (Braun, 2004, 2009; Tharwat *et al,* 2012a) and horses (Stewart, 2006; Radostits *et al,* 2010). Recently, ultrasonography was used extensively for the diagnosis of diverse abdominal disorders in camels (Tharwat *et al,* 2012b; Tharwat and Al-Sobayil, 2016). The aim of the present study was to describe the clinical, ultrasonographic and pathologic findings in dromedary camels affected with chronic peritonitis.

Materials and Methods

Animals, history and clinical examinations

Fifteen dromedary camels were presented at the Veterinary Teaching Hospital, Qassim University, Saudi Arabia, with a history of anorexia, abdominal distension, decreased faecal output and fits of colic that appeared in the form of rolling. Camels underwent a thorough physical examination which included general behaviour and condition,

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auscultation of the heart, lungs, rumen and intestine, recording of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion auscultation of both sides of the abdomen and rectal examination (Köhler-Rollefson *et al,* 2001).

Ultrasonography, abdominocentesis and post mortem examination

Ultrasonographic examination was carried out in sedated camels using xylazine 2% (0.3 mg/kg BW IV) while securing them in sternal recumbency using a 3.5 MHz sector transducer (SSD-500, Aloka, Tokyo, Japan). Scanning was done on both sides of the abdomen from the dorsal midline to linea alba. The abdominal viscera including the C_1, C_2, C_3, peritoneum and small and large intestines were imaged (Tharwat *et al,* 2012b,c; Tharwat and Al-Sobayil, 2016). Abdominocentesis was carried out under ultrasound guidance (Tharwat *et al,* 2013). Due to a poor prognosis and based on the owner request, 5 camels were euthanised and necropsy was done.

Treatment outcome

Animals were treated with penicillin streptomycin 40.000 IU/kg BW IM/14d (Pen & Strep, Norbrook Laboratories, UK), flunixin meglumine 2.5 mg/kg BW IV/7d (Finadyne, Intervet/Schering-Plough Animal Health, UK) and Ringer solution (5000 mL daily). In addition, in cases with decreased faecal output, animals were drenched 1L of liquid paraffin/3d. In addition, anti-inflammatory and diuretic were also administered for 4 days.

Results

Clinical findings included anorexia (n = 15), scanty faeces (n = 9), fits of colic that appeared in the form of rolling (n = 11) and moderate abdominal distension (n = 7), (Fig 1). Physical condition was moderate to poor in all animals. Rectal temperature varied from 35.6 to 39.2°C. Heart rate ranged from 35 to 74 beats/min and the respiratory rate between 7 and 15 breaths/min. Ruminal motility was either reduced or completely absent. One of the affected cases was admitted 20 days after parturition. Another camel was presented with a history of bleeding before and after mating; ultrasonographic examination revealed a highly thickened, echogenic endometrium and a perforated dorsal uterine wall.

Ultrasound-guided aspiration of a peritoneal fluid sample from post-xipoid region using a 14G and 15cm long needle yielded a deeply turbid peritoneal fluid. Sediment was formed after fluid centrifugation (Fig 2). Microscopy of the collected peritoneal fluid was not done.

Transabdominal ultrasonography revealed bilateral echogenic fibrin threads floating in a hyperechoic peritoneal effusion. Fibrinous peritonitis were seen at the sonogram as heterogeneous deposits of echogenic and anechoic materials between the intestines, liver, kidneys rumen, spleen and abdominal wall Peritoneal effusion appeared as echogenic areas of fibrinous tissue deposits interspersed with hypoechogenic areas of fluid pockets (Fig 3).

A follow-up of 15 days after being discharged from the hospital showed that only 3 of the 15 camels made a full recovery beginning from the 7th day after discharging from the clinic. Postmortem examination of 5 camels showed massive fibrin network with adhesion of the viscera to the abdominal wall. Foul-smelling serosanguineous abdominal fluid was evacuated from the abdomen (Fig 4). In one camel, the endometrium was thickened, haemorrhagic and the dorsal wall of the uterus was perforated.

Discussion

Peritonitis is a common disease in cattle, less common in horses and rarely, if ever, identified

Fig 1. Moderate abdominal distension in a female camel with chronic peritonitis.

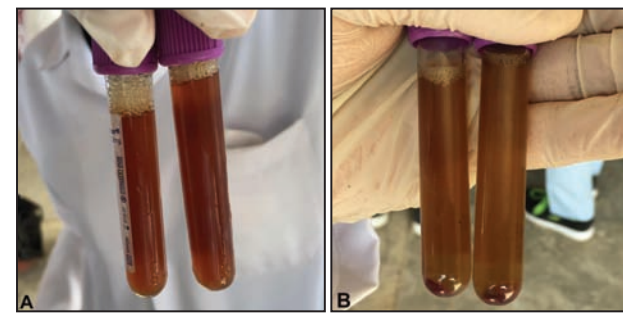

Fig 2. Abdominocentesis of a deeply turbid peritoneal fluid in a female camel with chronic peritonitis (A). Image B shows sediment that formed after centrifugation of the collected fluid.

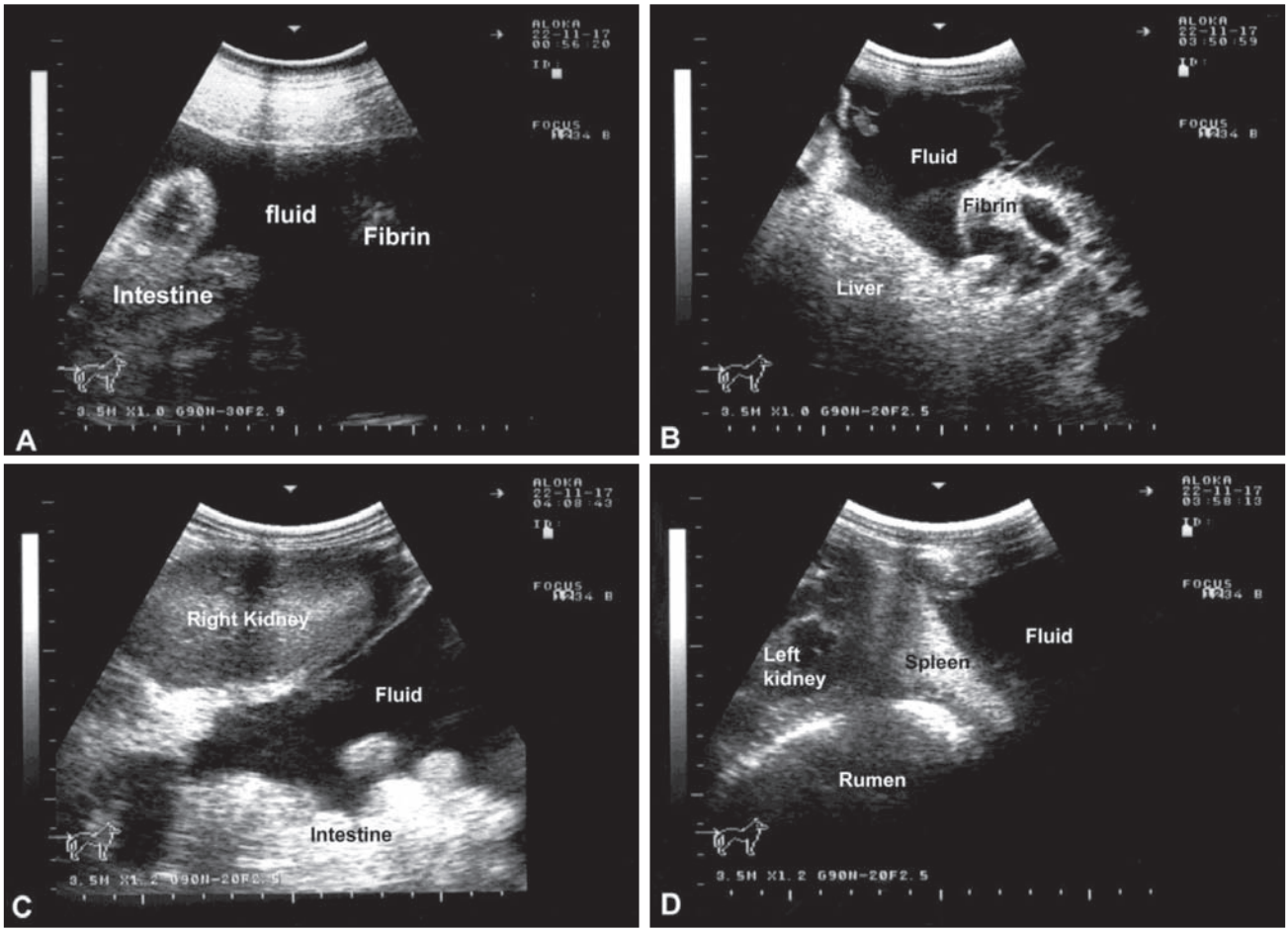

Fig 3. Ultrasonographic findings in dromedary camels with chronic peritonitis. Echogenic fibrin and anechoic fluid were imaged within the peritoneum exudation. Intestine (**A**), liver (**B**), right kidney (**C**) and left kidney and spleen (**D**) were imaged floating within the peritoneal fluid.

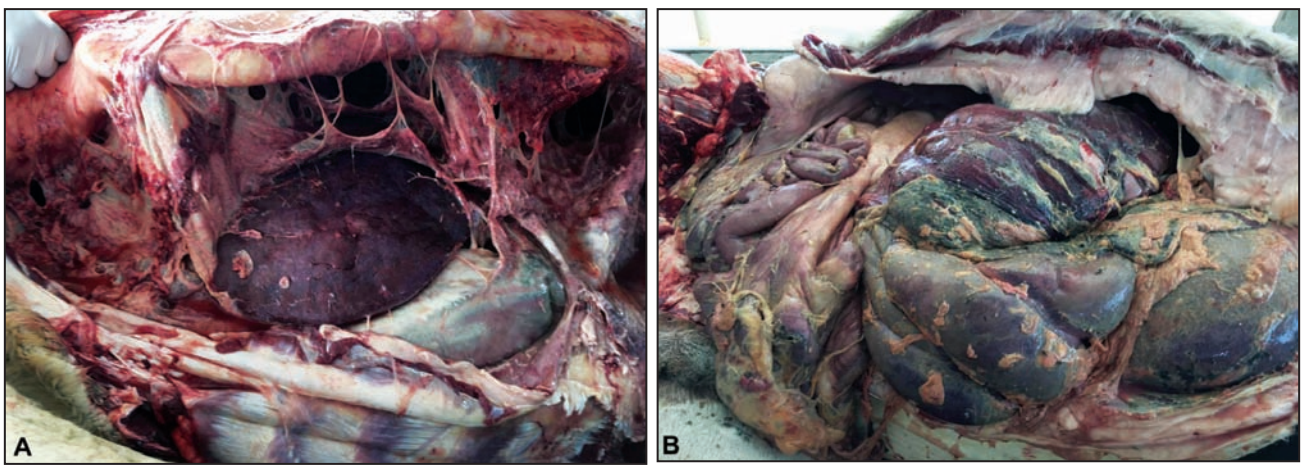

Fig 4. Necropsy findings in camels with chronic peritonitis. **A.** Massive fibrin network and adhesions of the viscera together and to the abdominal wall and serosanguineous abdominal exudate. **B.** Necropsy was done 6h after collapse revealed viscera covered with fibrin sheath and adhesions were seen with adjoning organs and abdominal wall.

clinically in sheep, pigs or goats (Radostits *et al*, 2010). There are general signs applicable to all species and most forms of the disease in a general way. In addition, there are special findings peculiar to individual species and to various forms of the disease. Unfortunately, the clinical signs are reported to be nonspecific and the disease needs further diagnostic tool to confirm the diagnosis

(Tharwat *et al,* 2012a). However, in the present study, although the case history of abdominal pain, scanty faeces and abdominal distension were not indicative of peritonitis, but it was quiet informative for an initial preliminary diagnosis of peritonitis. More profound clinical signs were reported by Fowler (2010) for peritonitis in camels. *Streptococcus equi* subsp *zooepidemicus* was the causative agent in a camel calf (Stoughton and Gold, 2015) and in a young male camel (Heller *et al,* 1998), however, the causative agent were not determined in the present study.

The ultrasonograms of the peritoneal effusion in camels were similar to the results of other studies in cattle (Braun, 2003; Tharwat *et al,* 2012a). In present study the peritoneal effusion was considerable in quantity and extended up to the caudal abdomen and appeared as anechoic or hypoechogenic upon ultrasonography. Hewson and Cebra (2001) have also detected a large volume of free peritoneal fluid with a pattern of mixed echogenicity during transabdominal ultrasonography of a llama with peritonitis.

Necropsy findings were confirmative to the ultrasonographic observations in camels of the present study, where a massive fibrin network and adhesions of the viscera to the abdominal wall was found. Similarly, Heller *et al* (1998) found a copious abdominal fluid with fibrin clots during exploratory laparotomy in a young dromedary camel with *Streptococcal* peritonitis. Diffuse suppurative peritonitis, with extensive fibrinous adhesions throughout the abdominal cavity was also found in a llama with peritonitis caused by *Streptococcus equi* subsp. *zooepidemicus* (Hewson and Cebra, 2001).

Ultrasonography proved good diagnostic tool in present study and should alway be clubbed with clinical examination and other requisite laboratory tests in case of peritonitis in camels.

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DOI : 10.5958/2277-8934.2019.00027.4 *Vol 26 No 2, p 173-177*

ASSOCIATIONS OF BACTERIAL ISOLATION WITH ENDOMETRIAL CYTOLOGY IN CAMELS (*Camelus dromedarius*) WITH ENDOMETRITIS

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ABSTRACT

The objective of the present study was to evaluate uterine cytological examination as a reliable diagnostic tool for camel endometritis in 108 repeat breeding female camels (*Camelus dromedarius*) aged 6–18 years. With the aid of rectal palpation of reproductive tract, vaginal examinations as well as transrectal ultrasonography, 46 camels were diagnosed with clinical endometritis. Bacteriological swabs and low volume uterine lavage were collected for bacteriological and cytological analysis, respectively. Bacteriological growth could be identified in 65% (30 swabs) of investigated samples. No growth was reported in 35% (16 swabs) of the investigated samples. With 9 and 8 positive swabs, *Staphylococcus aureus* and *Escherichia coli* were responsible for the majority of uterine infections within studied animals. Cytological analysis revealed that the cellular contents of studied samples were significantly different according to isolated bacterial species. In our study, five cases (11%) of studied animals were negative for both cytological and microbiological examination, whereas 29 cases (63%) were positive in bacteriology and cytology. The compatibility in the bacteriological and cytological results in the case of both positive or negative in present study appeared in 74% of animals. In 24% of studied animals there was an absence of bacterial growth on the culture, though these samples were positive for cytology. Our study confirmed the importance of combined employment of cytological and bacteriological results in the diagnosis of endometritis in dromedary camels.

Key words: Bacteria, camel, cytology, endometritis

Uterine infections are considered as main cause of reproductive failure and infertility in dromedary camels (Ali *et al,* 2010; Tibary and Anouassi, 2000; Tibary and Anouassi, 2001; Tibary *et al,* 2006; Wernery and Kumar, 1994). The high rate of reproductive failure and endometritis in camels may be explained by the persistent use of traditional systems of reproductive management (Al-Eknah, 2000; Skidmore *et al,* 2010). Local studies in Saudi Arabia revealed that endometritis and metritis are the most common cause of infertility in dromedary camels (Al-Humam, 2016; Ali *et al*, 2010; Ghoneim *et al,* 2013; Khalafalla *et al,* 2017; Tibary *et al,* 2006; Tibary and Anouassi, 2000). In addition to postpartum complications, overbreeding and unsanitary gynaecological manipulation were found as major contributing factors to camel uterine infections (Tibary, 2004). Based on bacteriological studies, *Salmonella* spp, *Proteus* spp, *Escherichia* spp *Seeatia* spp, *Klebsiella* spp, *Pseudomonas* spp, *Streptococcus* spp, *Staphylococcus* spp, and *Corynebacterium* spp were found as main pathogens responsible for reproductive diseases and abortion in dromedary camel (Ali *et al*, 1987; Arthur *et al,* 1985; Wernery, 1991; Wernery and Wernery, 1992). *Escherichia coli* was the most common bacteria isolated from camels with purulent vaginal discharge and repeat breeding (Wernery and Wernery, 1992; Ali *et al,* 2010).

Endometritis can be diagnosed by different methods including rectal palpation of the uterus, examination of the vaginal discharge, vaginoscopy, transrectal ultrasonography, endometrial cytology, histological examination of uterine biopsy and bacterial culture (LeBlanc *et al,* 2002). However, most of uterine bacteriological investigations in dromedary camels were performed with slaughterhouse animals with lack of information about reproduction history (Al-Afaleq *et al,* 2012; Enany *et al,* 1990; Hussein *et al,* 2006). Cytological examination of the uterus has been considered as a rapid and reliable

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diagnostic technique especially in case of subclinical endometritis, when clinical signs are absent (Overbeck *et al,* 2011).

The early diagnosis of uterine infections represents a key factor for choosing specific treatment to prevent disease progression and chronic uterine disorders. The present study was, therefore, aimed to evaluate uterine cytological examination as a reliable diagnostic tool for camel endometritis.

Materials and Methods

This study was donw on 108 repeat breeding female camels (*Camelus dromedarius*) aged 6–18 years admitted between October and December 2018 to the Veterinary Teaching hospital (King Faisal University, Saudi Arabia). Camels had a history of failing to conceive after more than two services.

Clinical Examination

All camels were clinically examined for any signs of abnormal vulvar discharge, rectal palpation of the reproductive tract and ovaries, vaginal examination as well as transrectal ultrasonography using UST-588U-5, SSD-500V, attached to 7.5 MHz transducer (ALOKA, Co., LTD JAPAN). Forty six animals were diagnosed endometritis and uterine swabs were collected under complete aseptic conditions from these animals. A double-guarded swab (Equi-VET® Kruus) was guided manually through the vulva to reach the right uterine horn. A swab was rolled on the endometrial surface for 20 seconds in the right uterine horn and repeated with the left uterine horn. Then, the swab was retracted into the protecting tube of the double guarded swab. Swabs were transported to the laboratory at ambient temperature in Amies modified medium and cultured within 24 hours.

Uterine fluid collection and cytological analysis.

The uterus was flushed with a small quantity of sterile saline using either a foley catheter or a mare insemination pipette. The fluid was collected, fixed in 40% ethanol and centrifuged to concentrate cells. Samples were smeared on a microscope glass slide and smears were labelled, air-dried for 10-20 minutes, fixed with methyl alcohol at five minutes, and followed by washing in distilled water and subsequent drying and processed using Diff Quick (Hemal Stain Co. Inc., Danbury, CT). The degree of inflammation is assessed by evaluation of the amount and morphology of epithelial cells (Epith) and neutrophils (PMN) (Tibary *et al,* 2006). The total and differential cell counts of epithelial cells and neutrophils were counted using oil immersion (1000X) per high power field in order to assure the specific morphologic characteristics of cell.

Bacterial Isolation and Identification

Individual swabs were cultured on Columbia agar (CM331; Oxoid, Basingstoke, UK) containing 5% citrated sheep blood and MacConkey agar (Oxoid, Basingstoke, UK). After inoculation, the plates were incubated aerobically and anaerobically for 18 to 24 h at 37°C and for a further 24 h if bacterial growth had not ensued. If > 90% of the grown colonies in an incubated agar were similar phenotypically, the result was considered as substantial growth in primary culture. Mixed cultures with dissimilar colonies were discarded as no growth. Presumptive identification was done according to descriptions in textbooks (Koneman *et al,* 2005). Confirmation of the identification of isolates was done by Vitek 2 technique (bioMerieux, Marcy L'Etoile, France).

Statistical analysis

The data obtained for number and percentage of bacteriology results and cytological results were analysed for each type of bacteria and grade of cytology and compared with others using SPSS software. Differences were considered statistically significant (P>0.05) using Duncan multiple range test for Mean seperation.

Results and Discussion

Although some studies have investigated endometritis in camelids (Wernery, 1991; Wernery and Wernery, 1992), many aspects regarding the incidence, diagnosis and treatments of endometritis in camels need further clarification (Tibary *et al,* 2006). Early detection of uterine infection using a rapid and reliable diagnostic technique is very important for treatment and disease control through the prevention of venereal transmission of infection to other animals (Tibary and Anouassi, 2001). Uterine bacteriological culture has been considered as the gold standard diagnostic method of endometritis in cows (Duvel *et al,* 2014; Mancill *et al,* 2011; McKinnon, 2011; Sheldon *et al,* 2009). In different animal species, low volume uterine lavage and cytobrush are considered as the main techniques used to collect samples for cytological examination in endometritis (Cocchia *et al,* 2012; Dini *et al,* 2015; Kasimanickam *et al,* 2004).

In the current study, 42.59% of the infertile female camels (46 out of 108) were diagnosed as suffering from endometritis. This seems to be in line with reports made by Tibary and Anouassi

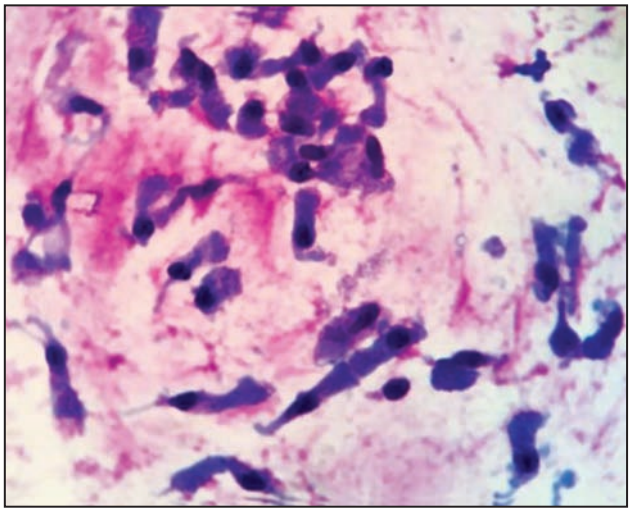

Fig 1. Microscopic evaluation of endometrial cytological samples of low lavage fluid. Note integrity of epithelial cells

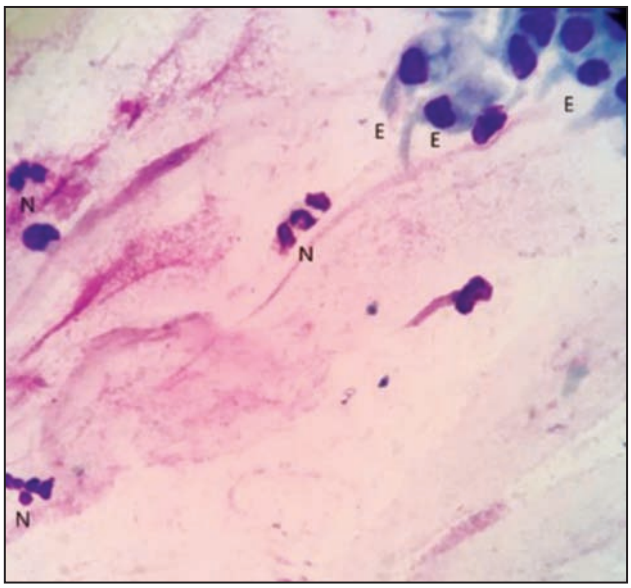

Fig 2. Microscopic evaluation of endometrial cytological samples of low lavage. Note integrity of epithelial cells (E) and neutrophils (N).

(2001), who found that uterine infections are the most common cause of reproductive failure in camelids. Bacteriological growth could be identified in 65% (30 swabs) of investigated samples, whereas no growth was reported in 35% (16 swabs) of the samples. Similar results of no growth in some examined swabs was found by Tibary *et al* (2006) in camels with chronic endometritis.

Isolated bacterial species as well as their frequency in the investigated animals are presented in Table 1. With 9 and 8 positive swabs, *Staphylococcus aureus* and *Escherichia coli* were responsible for the majority of uterine infections within studied

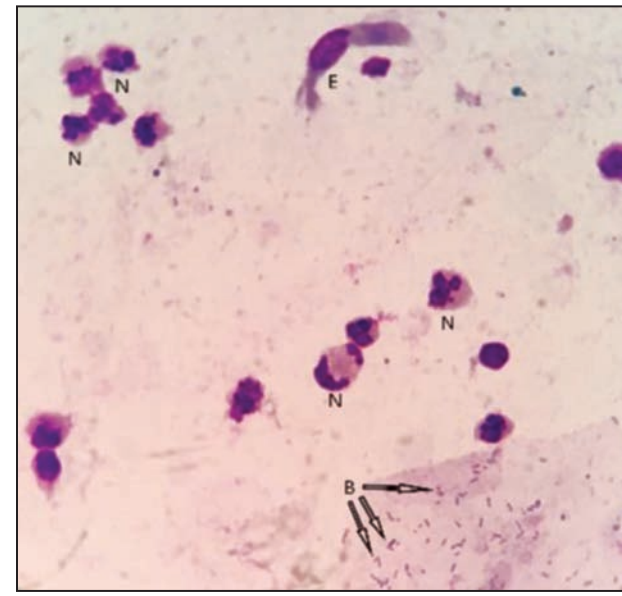

Fig 3. Microscopic evaluation of endometrial cytological samples of low lavage fluid. Note low number of epithelial cells (E) and increased number of neutrophils with bacteria (B).

animals. Other bacterial species found in the current study included *Streptococcus agalactiae* (6 swabs), *Klebsiella* sp. (4 swabs), *Proteus mirabilis* (2 swabs), and *Arcanobacterium pyogenes* (1 swab). Similar bacterial spectrum was found by Tibary *et al* (2006). In a study with Egyptian camels suffering from endometritis, Ali *et al* (1987) isolated mainly *Escherichia coli*, *Corynebacterium pyogenes, Proteus* spp. and *Pasteurella multocida*. In addition, Wernery (1991) and Wernery and Wernery (1992) found that *Escherchia coli* represents the most common bacteria isolated from camels with endometritis.

Cytological examination of endometrial smears is an easy tool for quick and easy diagnosis of uterine inflammation under practical field conditions (Knudsen, 1964; Reiswig *et al,* 1993; Riddle *et al,* 2007). In addition, the interpretation of microbiological results of uterine swabs is very difficult given the wide range of bacteria that can be isolated. Moreover, some of these germs are part of the normal vaginal flora whereas others are opportunistic and can become pathogenic if the right conditions are present (Tibary and Anouassi, 2001).

To evaluate the diagnostic value of endometrial cytological analysis for diagnosis of camel endometritis, stained smears obtained from the surface of the endometrium were evaluated for their cellular content of neutrophils and epithelial cells (Figs 1, 2 and 3). In agreement with other studies (Nielsen, 2005), the cellular contents of studied

samples was significantly different according to isolated bacterial species (Table 1). The degree of inflammation can be assessed by the evaluation of number and morphology of neutrophils. The presence of three to five neutrophils per high power field is usually considered as a significant indicator of endometritis, and the microorganism responsible may sometimes be observed in the cytological evaluation (Tibary and Anouassi, 2001).

Table 1. Relationship between uterine cytological results and uterine bacteriological analysis in 46 camels suffered from endometritis.

Bacteria	Epith+ 0-2 PMN in field	Epith + 3-5 PMN in field	Epith+ >5 PMN in field	Total N (%)
Escherichia coli	0 (0)	3 (7)	5 (11)	8 (18)[a, b]
Proteus mirabilisa	1 (2)	0 (0)	1 (2)	2 (4)[a]
Klebsiella spp.	0 (0)	1 (2)	3 (7)	4 (9)[a]
Strept. agalactiae	0 (0)	1 (2)	5 (11)	6 (13)[a, b]
Staph. aureus	0 (0)	3 (6)	6 (13)	9 (19)[a, b]
Arcanobacterium pyogenes	0 (0)	0 (0)	1 (2)	1 (2)[a]
Negative growth	5 (11)	6 (13)	5 (11)	16 (35)[b]
Total N (%)	6 (13)[x]	14 (30)[y]	26 (57)[z]	46 (100)

[a-b] Percentage within the last column with different superscripts are significantly different ($P < 0.05$).

[X-Z] Percentage within the lower row with different superscripts are significantly different ($P < 0.05$). Epith (Epithelial cells), PMN (polymorphonuclear cells).

In the current study, epithelial cells were in abundance in all studied samples. About 57% of studied animals showed more than 5 neutrophils in high power field, while only 13% of studied animals had less than two neutrophils in high power field. Results of cytological analysis were compared with results of bacteriological analysis in table 2.

Table 2. Comparison of the results of microbiological and cytological examinations from 46 camels with endometritis.

Microbiological and cytological results	Numbers	Percentage %
Microbiological (+ve) and cytological (+ve)	29	63 %
Microbiological (+ve) and cytological (-ve)	1	2 %
Microbiological (-ve) and cytological (+ve)	11	24 %
Microbiological (-ve) and cytological (-ve)	5	11 %

In our study, five cases (11%) of studied animals were negative for both cytological and microbiological examinations, whereas 29 cases (63%) were positive for bacteriology and cytology. The compatibility between the bacteriological and cytological results appeared in 74% of animals studied. However, in 24% of studied animals, which were positive for cytology, there was no bacterial growth. Similar results were reported by Ball *et al* (1988), where neutrophils were present in cytology but no bacterial growth was found.

It was concluded that the integration of the microbiological and cytological investigation is essential for precise diagnosis of endometritis in camels and cytological results of endometritis should be interpreted together with a bacteriological examination.

Acknowledgements

The authors thank the Deanship of Scientific Research, King Faisal University, Saudi Arabia for the financial support of this work (grant number:186112). The authors would also like to thank the authorities of Veterinary Teaching Hospital, College of Veterinary Medicine, King Faisal University and Prof. Ali Fadel Almawla for their kind cooperation in conducting this valuable study.

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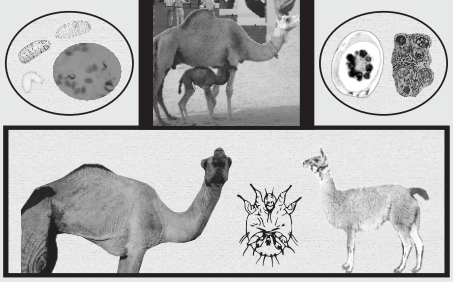

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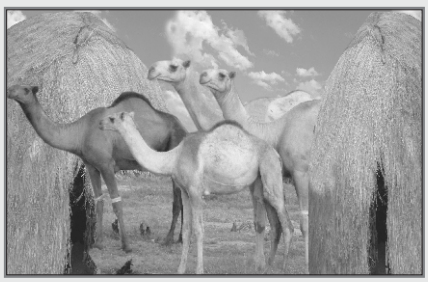

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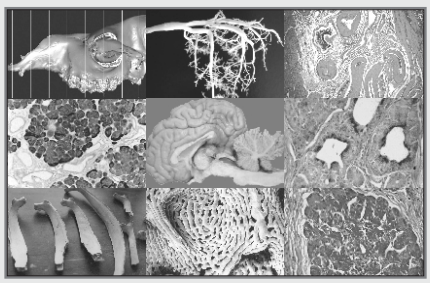

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DOI : 10.5958/2277-8934.2019.00028.6 Vol 26 No 2, p 179-187

ASSESSMENT OF TRACE ELEMENTS IN CAMEL (*Camelus dromedarius*) MEAT, HUMP AND LIVER CONSUMED IN SAUDI ARABIA BY INDUCTIVE COUPLED PLASMA MASS SPECTROMETRY

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ABSTRACT

This study focused on the determination of trace elements in camel meat consumed in Abha and Khamis Mushyt cities in the Kingdom of Saudi Arabia (KSA). Trace elements were measured by inductively coupled plasma mass spectrometry (ICP-MS). In total, 25 samples of the liver, neck, shoulder, thigh and hump of five camels were collected from local slaughterhouses in the two cities. Ten elements were measured in each sample: V, Cr, Mn, Co, Ni, Cu, As, Se, Cd and Pb. The mean concentrations (µg/g wet weight) of the trace elements were V (0.03), Cr (0.28), Mn (0.89), Co (0.04), Ni (0.47), Cu (2.21), As (0.01), Se (0.37), Cd (0.05) and Pb (0.71) in all organs. For Cu and Pb, the highest concentrations were observed in liver (7.48) and hump (1.32), respectively. Statistical analysis showed a significant difference ($p < 0.05$) for V, Ni and As in the hump and all other organs and Co, Cd and Pb in the hump and all other organs, except the liver. For Cr, no significant difference ($p > 0.05$) was observed between the hump, shoulder and neck. A significant difference ($p < 0.05$) was observed for Mn and Cu in the liver and all other organs. For Se, no significant difference ($p > 0.05$) was observed between the liver, shoulder and neck. A similar distribution of elements was observed in all organs with high concentrations of essential elements (Cu, Se, Mn, Ni, Co and V) and lower concentrations of toxic elements (As, Cd, Cr and Pb), except that Pb in the hump was high. Generally, positive correlations were observed for elements in all organs. However, no correlations were observed for As/V and Cu/Co in the liver and hump, respectively. All organs had positive correlations related to toxic elements. The statistical analysis showed a significant difference ($p < 0.05$) for correlations between all organs and the liver regarding V, Mn, Co, Cu, Se and Cd. Cu had the highest estimated dietary intake (EDI) values (µg/kg bw day, where bw is the average body weight) in the liver (20.90), shoulder (2.37), neck (2.23), thigh (3.27) and hump (2.12). The hump had the highest Pb value (3.68). Moreover, the hazard quotients (HQs) for all elements were less than 1, except the HQ > 1 for Pb in the hump. Therefore, we concluded that consumption of a camel's hump could lead to adverse health effects in humans due to exposure to high levels of Pb. A comprehensive study with larger number of samples is required to confirm the results of this study based on limited number of samples.

Key words: Camel, EDI, HQ, ICP-MS, meat, trace elements

Camel meat contains essential elements which are beneficial for human health (Faye and Bengoumi, 1994). Compared with beef, camel meat is significantly different in terms of amounts of major elements, containing K, Na and Mg and some trace elements such as Fe and Zn (Dawood and Alkanhal, 1995). This was confirmed by another study (Alamin and Ahmed, 2013), which reported that camel meat has higher amounts of major elements (Ca, Na, K and Mg) and some trace elements (Fe and P) compared with other red meat such as goat meat and beef. A previous study, conducted in a slaughterhouse in Casablanca, Morocco analysed the organs of camels and sheep, including the liver, lung, heart, kidney and meat for elements, i.e. Cu, Zn, Cd and Pb and found that copper had the highest concentration in the liver and camel meat was found to be rich in zinc compared with sheep meat. Moreover, high lead concentrations were reported in camel liver (Abdelbasset *et al,* 2014). Abdou *et al* (2015) investigated elements, i.e. Cu, Zn, Pb and Cd in camel meat and blood and revealed that the liver

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had a higher level of Pb compared with the lung and kidney. Some heavy metals were estimated in fish as well as camel meat in some regions (Tabuk, Dammam, Riyadh and Jazan) of the KSA and found that camel meat had the lowest levels of most of the studied elements (Alturiqi and lbedair, 2012). Chafik *et al* (2014) investigated the levels of Zn, Cu, Cd and Pb in camel meat and found highest concentrations in the liver. Levels of Cu, Mn, Zn and Mg in the blood, liver, kidney and meat were investigated for two camel breeds (Maghateer (white coat colour) and Majaheem (black coat colour) in Riyadh, KSA. In yet another study there were higher concentrations of Cu and Mg and lower concentrations of Zn in the liver of the black camels compared with white camels of KSA. Higher Cu and lower Mn and Zn levels were reported in kideny of Majaheem compared with Maghateer camels. In addition, a higher concentration of Mg was reported in black camel meat compared with white camels. These differences were ascribed to different metabolism of Mg, Mn, Cu and Zn between white and black camels possibly due to genetics (Abdelrahman *et al,* 2013b).

The objectives of this study were to determine the levels of essential (V, Mn, Co, Ni, Cu and Se) and toxic (As, Cd, Pb and Cr) elements in camel organ samples (liver, shoulder, neck, thigh and hump) using inductively coupled plasma mass spectrometry (ICP-MS); to assess distribution of these elements in different camel organs and to calculate the estimated dietary intake (EDI) and hazard quotient (HQ) for these elements.

Materials and Methods

In total, 25 samples were collected from 5 camels, which included 5 organs (liver, shoulder, thigh, neck and hump) from each camel during September to October 2018 in slaughterhouses of Abha and Khamis Mushyt cities, KSA. The camels were 6-7 months of age and all were apparently healthy. The samples were kept in plastic bags and were immediately transported to a laboratory at King Khalid University. All samples were oven-dried over night at 70°C; only the hump samples were kept in the refrigerator at -4°C until the digestion process. The dried samples were powdered before digestion.

For each sample, a quantity of 0.5 g was digested with 4 mL of HNO_3 (≥69%) (Sigma-Aldrich, Steinheim, Germany) and 2 mL of H_2O_2 (33%) (AppliChem Panreac, Darmstat, Germany). A microwave (ECO, Anton Paar Multiwave, Austria) digestion procedure was used, which consisted of three steps: ramping time for 5 min up to 800 W, then heating time for 40 min held at 800 W and finally cooling time for 15 min. The temperature was held at 125 °C during the digestion process. The digested samples were made up to 25 mL with deionised water in preparation for ICP-MS analysis.

Measurement of the Elements Using ICP-MS

ICP-MS (iCAP Q, Thermo Fisher Scientific, Waltham, MA, USA) was used for the elemental analysis of all samples. Ten elements (V, Cr, Mn, Co, Ni, Cu, As, Se, Cd and Pb) were measured in all samples (n = 25). The ICP-MS operating conditions used here were the same of those reported by Brima (2016).

Chemicals, Reagents and Analytical Method

A mixture of 29 elements at a concentration of 10.0 ± 0.05 µg/mL from ULTRA Scientific (North Kingstown, RI, USA) was used to prepare a daily fresh single-stock solution. The calibration standards for trace elements (including all 10 elements) were 5, 10, 20, 50 and 100 µg/L. Internal standards—Sc (100 µg/L), Rh (20 µg/L) and Ge (20 µg/L)—were prepared from a stock solution (1000 µg/mL) obtained from ULTRA Scientific (North Kingstown, RI, USA), a stock solution (1 g/L) of standard Rh was obtained from AppliChem (Panreac, Germany) and a stock solution (1g/L) of standard Ge was obtained from AppliChem (Panreac, Germany).

Quality Control (QC) and Quality Assurance (QA)

The sensitivity and background signals of the ICP-MS were checked using a tune solution (B iCAP) containing U, In, Li and Co, which contained 1 µg/L of each element in 2.0% HNO_3 and 0.5% HCl. Kinetic energy discrimination (KED) mode was used for element measurements by using helium gas. The limits of detection (LODs) and limits of quantification (LOQs) for all 10 elements were calculated. The calculation was carried out by measuring the blank (1% HNO3) 10 times, then the standard deviation (SD) was used for LODs and LOQs as follows: LOD = 3 x SD and LOQ = 10 x SD. LODs and LOQs were as follows (µg/L): V (0.2 and 0.7), Cr (0.2 and 0.74), Mn (0.24 and 0.81), Co (0.24 and 0.82), Ni (0.40 and 1.35), Cu (0.36 and 1.2), As (0.24 and 0.81), Se (0.94 and 3.14), Cd (0.14 and 0.47) and Pb (0.18 and 0.60).

For QC, continuing calibration verification (CCV) was used. In addition, QC was also performed by measuring 50 µg/L of mixed standards of 10 measured elements after each set of 10 samples. In the QC, each element was measured three times (n = 3).

Throughout the whole session, the QC analysis was repeated four times; thus, each element was measured 12 times (n = 12). The recoveries in one session were as follows: V (100.23%), Cr (100.41%), Mn (102.98%), Co (99.53%), Ni (96.83%), Cu (96.70%), As (98.13%), Se (93.57%), Cd (100.85%) and Pb (90.75%).

For QA, the accuracy of the measurement was determined by measuring certified reference material (CRM Hair, NCSDC 73347). The results of the measured essential and toxic elements were similar to those of the CRM Hair, NCSDC 73347. The concentrations (µg/g) for certified and measured were as follows: As (0.28 ± 0.05; 0.25 ± 0.03), Cd (0.11 ± 0.03; 0.11 ± 0.03), Co (0.071 ± 0.012; 0.06 ± 0.0012), Cr (0.37 ± 0.06;0.34 ± 0.01), Cu (10.6 ± 1.2; 8.91 ± 0.78), Mn (6.3 ± 0.8; 5.64 ± 0.48), Pb (8.8 ± 1.1; 7.46 ± 0.53) and Se (0.9 ± 0.04; 0.81 ± 0.13). Moreover, spiked samples were also used for the QA. A mixture of 50 µg/L was spiked in a sample before digestion and then throughout the digestion process. The recoveries were as follows: V (92.83%), Cr (89.83%), Mn (86.43%), Co (91.56%), Ni (81.64%), Cu (65.46%), As (103.62%), Se (79.77%), Cd (94.16%) and Pb (70.92%).

EDI and HQ

The EDIs were calculated for all measured elements (V, Cr, Mn, Co, Ni, Cu, As, Se, Cd and Pb) in all camel meat organs (liver, shoulder, neck, thigh and hump). The following equation was used: EDI = (D × Cc)/bw (Mohammed *et al,* 2019), where D is the average daily intake of meat (199.8 g/day), Cc is the calculated concentration (µg/g wet weight) of each element and bw is the average body weight (in kg), which is 71.53 kg in the KSA. The HQ was calculated as follows: HQ = EDI/PMTDI (Mohammed *et al,* 2019), where PMTDI is the provisional maximum tolerable daily intake (PMTDI) established by world health organisation (WHO) and European food safety agency (EFSA). The PMTDIs (µg/kg bw day) for all elements were V (0.3), Cr (300), Mn (428.57), Co (120), Ni (2.8), Cu (500), As (2.14), Se (6.67), Cd (1) and Pb (3.57). The PMTDIs values were cited/calculated from the following references: V (EFSA, 2004), Cr (EFSA, 2014), Mn (EFSA 2013), Co (EFSA, 2012), Ni (EFSA 2015), Cu (FAO/WHO, 1982), As (FAO/WHO, 1989), Se (FAO/WHO, 2004), Cd (FAO/WHO, 2003) and Pb (FAO/WHO, 2003), respectively.

Statistical analysis

A correlation was assessed for all 10 measured elements in all organs (liver, shoulder, neck, thigh and hump) from 5 camels by using Excel (Microsoft office 2010). ANOVA (SPSS version 20) was used to evaluate the differences among camel organs related to element concentrations with a 95% confidence level.

Results

Both essential and toxic elements were found in all different organs with various concentrations. The levels of the different elements in the different organs in decreasing order were for the liver: Cu > Mn > Pb > Se > Ni > Cr > Cd > Co > V > As (Table 1). This is a very promising order of an arrangement because the essential elements are at the top of the list and toxic elements are at the bottom. This is because the liver is the organ of storage for essential elements. The decreasing order of the elements in the shoulder was as follows: Cu > Pb > Mn > Ni > Se > Cr > Cd > Co ≥ V > As. Although Cu was at the top of the list and As at the bottom, the high presence of lead is surprising. The order of the elements in the neck was as follows: Cu > Pb > Ni > Mn > Se > Cr > Cd > Co > V > As. Cu was also at the top and As at the bottom but still again the high concentration of lead was remarkable. The order of the elements in the thigh was more or less comparable to the other part of meat: Cu > Pb > Mn > Se > Ni > Cr > Cd > V > Co > As. Contrary to muscle, the order of the elements in the hump was different with the higher concentrations in lead. Indeed, the list was as follows: Pb > Ni > Cu > Mn > Cr > Se > Cd > Co > V > As. In all five organs, the As level was the lowest and with the exception of the hump, the Cu level was the highest.

Copper and arsenic had the highest and lowest concentrations in all organs, respectively (Table 1). In general, the liver had the highest concentrations of most elements measured, while the shoulder had the lowest concentrations of these elements. Both essential and toxic element levels had similar trends in the liver and shoulder. V and Cr levels were low in the neck and high in the hump, while Mn, Co, Ni, As, Se and Cd were low in the shoulder and high in the liver. Cu concentration was low in the hump and high in the liver. This confirms the nature of the liver as it is the normal organ for copper storage in all animals and in camel (Bengoumi *et al,* 1998). The Pb level was low in the shoulder and high in the hump. Three elements (V, Cr and Pb) had high levels in the hump. These elements could be associated to pesticides or other toxic compounds with lipophilic characters (Rodriguez-Mercado and Altamirano-Lozano, 2006; Gorman *et al,* 2011). Seven elements (Mn, Co, Ni, As, Se, Cd and Cu) had high concentrations in the liver. In contrast, concentrations

of Mn, Co, Ni, As, Se, Cd and Pb but not Cu were low in the shoulder.

The hump was rich in V, Cr, Co, Ni and Pb compared to the other studied tissues while the liver was characterised by high concentrations of Mn, Cu, Se and Cd (Table.1). The hump was characterised by significantly increased V and As concentration compared to the liver (p- value= 0.008), neck and shoulder (p- value = 0.011, p = 0.003, respectively). The Cr concentration of the hump was significantly more than its concentration in the shoulder and neck (p- value= 0.023 and 0.016, respectively). The hump contained significantly increased concentration of Co compared to its concentration in the shoulder, neck and thigh (p- value= 0.001, 0.003 and 0.002, respectively). Regarding the concentration of the Ni in the hump, its concentration was significantly high than its amount in all the other tissues (p- value< 0.001). The Pb content of the hump was significantly more than the Pb content of the shoulder, neck and thigh (p-values were < 0.001, < 0.001 and 0.001, respectively).

The liver was highly and significantly rich in Mn and Cu compared to the other tissues (p-value<0.001). The concentration of the selenium in the liver was significantly more than its concentration in the shoulder and neck (p- value= 0.03 and 0.047, respectively). The liver Cd concentration was more than the Cd concentration in the shoulder, neck and thigh (p- value= 0.012, 0.017 and 0.035, respectively).

Copper had the highest EDI value in all organs except the hump, for which Pb had the highest value (Table 2). All HQs were less than 1 (Table 2). In the hump, Pb had an HQ of >1, which could be harmful to human health.

Positive correlations (>0.5) were as follows (Table 3): All measured elements had positive correlations in all camel organs. However, Cu/Co and As/V did not have any correlation in the hump and liver, respectively. Mn had the highest correlations and Co had the lowest. Mn had nine correlations and other element such as Pb, V, Ni and Cu had seven correlations. Cr and As had six correlations, Se and Cd had five correlations and, finally, Co had four correlations. All the highest correlations (>0.9), except As/V (0.83), were among the liver and shoulder. There were five correlations (Pb/Ni, Pb/Cd, As/V, Cu/Co and Mn/V) in the shoulder, while four correlations (Pb/Ni, Cr/Cd, Se/Co and Pb/Cd) were in the liver. Moreover, Pb/Ni, Pb/Cd and Pb/Cr had correlations in both the liver and shoulder.

The liver had four positive correlations (Table 4) for toxic elements (Cr, Pb, Cd and As) with all other organs (shoulder, hump, neck and thigh). The neck had positive correlations for essential elements (V, Mn, Cu and Se) with the thigh, while the hump showed a positive correlation with the thigh for Ni and Co. ANOVA analysis showed that V, Mn, Co, Cu, Se and Cd had a significant difference ($p < 0.05$) between the liver and all other organs.

Discussion

In our study, the concentrations of Cu, Cd and Pb in the liver and thigh (meat) were similar to those results reported by Chafik *et al* (2014). For those authors, the concentrations of Cu, Cd and Pb in the liver and meat were 14.2 and 1.1, 0.3 and 0.1, and 1.3 and 0.7, respectively. A recent study (El-Ghareeb *et al,* 2019) conducted in Saudi Arabia reported lower concentrations (µg/kg wet weight) of Pb (9.26), Cd (2.18), As (12.89) and Cu (1.66) in camel liver as compared to present study. These differences could be due to various animal feeds. A positive correlation (0.68) was observed in their study between Cd/Pb in camel liver, which was similar to our result (0.84). Similar high correlation between

Table 1. Concentrations (µg/g wet weight) of the ten elements measured in 25 samples of camel meat (n = 3; mean± SD), including; liver, shoulder, leg, neck and hump.*

	V	Cr	Mn	Co	Ni	Cu	As	Se	Cd	Pb
Liver	0.032± 0.007	0.320± 0.209	2.591± 0.809	0.059± 0.033	0.449± 0.173	7.481± 4.343	0.011± 0.003	0.534± 0.208	0.078± 0.061	0.945± 0.371
Shoulder	0.013± 0.003	0.180± 0.058	0.360± 0.117	0.013± 0.001	0.249± 0.073	0.848± 0.178	0.005± 0.001	0.226± 0.047	0.024± 0.010	0.435± 0.199
Neck	0.017± 0.004	0.160± 0.026	0.385± 0.128	0.021± 0.005	0.401± 0.289	0.797± 0.300	0.006± 0.001	0.252± 0.062	0.027± 0.004	0.515± 0.125
Thigh	0.021± 0.012	0.256± 0.186	0.454± 0.242	0.017± 0.016	0.330± 0.168	1.170± 0.823	0.007± 0.001	0.412± 0.407	0.034± 0.026	0.598± .377
Hump	0.048± 0.012	0.468± 0.298	0.656± 0.092	0.076± 0.007	0.924± 0.047	0.758± 0.318	0.017± 0.003	0.419± 0.085	0.077± 0.17	1.316± 0.255

* The mean difference is significant at the 0.05 level,with significant difference ($P < 0.05$), as analysed by one-way ANOVA.

Pb and Cd in camel milk collected in polluted areas from Kazakhstan was reported (Konuspayeva *et al,* 2009). However, other positive correlations were also observed in their study between Cd/As (0.753) and Pb/As (0.701) in the liver, which were similar to correlations observed in our study in other organs (Table 4). This indicates that positive correlations between toxic elements (As, Pb and Cd) occurred in camel meat, regardless of the specific organ. A previous study (E1-Faer *et al,* 1991) was carried out to measure elements in the shoulder, hump, neck and thigh of camel. Their results (mg/100 g) compared with our results (mg/100 g) are as follows: shoulder: Cu (0.073, 0.085), Mn (0.004, 0.04) and Cr (0.005, 0.02); thigh: Cu (0.085, 0.12), Mn (0.009, 0.05) and Cr (0.008, 0.03); hump: Cu (0.033, 0.08), Mn (0.00, 0.07) and Cr (0.00, 0.05); neck: Cu (0.094, 0.08), Mn (0.006, 0.04) and Cr (0.03, 0.02). There was agreement between their results and ours for Cu in all different organs. Moreover, after rounding the concentrations to one decimal point, all organs for their and our results were equal (0.1 mg/100 g), except in the hump. This is a surprising finding considering that these studies were done 28 years apart, which could implicate the efficient metabolism of Cu in camel which maintains a specific level throughout the entire body, except the liver which is the mean target organ for copper storage. For other elements (Mn and Cr), our results were almost 10-fold higher than their results. This could be due to the modern trend of feeding animals with various processed foods rich in elements. A study was conducted in Egypt which investigated Cd, Pb, Mn, Fe, Cu and Zn concentrations in camel blood and tissues (muscles samples, lung, kidney and liver) in relation to age. Pb and Fe showed the highest concentrations in the kidney. Mn (2.14 ppm) and Cu (54.52 ppm) were the highest in the liver, which is in agreement with our results (Table 1), whilst Cu was 7-fold of our value. The authors concluded that Cd (0.23 ppm) and Pb (0.15 ppm) concentrations are

Table 2. Estimated Dietary intake (EDI) for all measured elements (µg/kg bw day) and Hazard Quotient (HQ) for all measured elements (EDI/PMDI).

	V	Cr	Mn	Co	Ni	Cu	As	Se	Cd	Pb
Liver	0.089	0.894	7.237	0.165	1.254	20.896	0.031	1.490	0.218	2.640
Shoulder	0.036	0.503	1.006	0.036	0.696	2.369	0.014	0.631	0.067	1.215
Neck	0.047	0.447	1.075	0.059	1.120	2.226	0.017	0.705	0.075	1.439
Thigh	0.059	0.715	1.268	0.047	0.922	3.268	0.020	1.152	0.095	1.670
Hump	0.134	1.307	1.832	0.212	2.581	2.117	0.047	1.169	0.215	3.676
Hazard quotient (HQ)										
Liver	0.298	0.009	0.020	1.4x10-3	0.448	0.042	0.014	0.223	0.218	0.739
Shoulder	0.121	0.005	0.003	3.0 x10-4	0.248	0.005	0.007	0.095	0.067	0.340
Neck	0.158	0.004	0.003	4.9 x10-4	0.400	0.004	0.008	0.106	0.075	0.403
Thigh	0.196	0.007	0.004	4.0 x10-4	0.329	0.007	0.009	0.173	0.095	0.468
Hump	0.447	0.013	0.005	1.8 x10-3	0.922	0.004	0.022	0.175	0.215	1.030

Table 3. Correlations between essential and toxic elements (V, Cr, Mn, Co, Ni, Cu, As, Se, Cd and Pb) within different organs (liver, shoulder, neck, thigh and hump).

	V	Cr	Mn	Co	Ni	Cu	As	Se	Cd	Pb
Liver										
V	1									
Cr	0.65	1								
Mn	0.69	0.60	1							
Co	-0.27	-0.07	-0.77	1						
Ni	0.71	0.79*	0.21	0.30	1					
Cu	0.18	0.67	-0.04	0.62	0.64	1				
As	-0.56	0.12	-0.10	0.20	-0.29	0.49	1			
Se	-0.08	0.25	-0.57	0.94*	0.55	0.81*	0.24	1		
Cd	0.45	0.93*	0.26	0.28	0.84*	0.85	0.24	0.58	1	
Pb	0.51	0.70	-0.04	0.53	0.96*	0.73	-0.17	0.75*	0.84*	1

Shoulder										
V	1									
Cr	0.63	1								
Mn	0.95*	0.60	1							
Co	0.34	0.39	0.56	1						
Ni	0.71	0.67	0.84*	0.57	1					
Cu	0.60	0.48	0.79*	0.95*	0.74	1				
As	0.83*	0.70	0.77*	0.52	0.44	0.64	1			
Se	0.75*	0.74	0.54	-0.07	0.30	0.11	0.78*	1		
Cd	0.46	0.34	0.65	0.44	0.91*	0.59	0.06	-0.05	1	
Pb	0.66	0.57	0.79*	0.46	0.98	0.65	0.31	0.22	0.96*	1
	Neck									
V	1									
Cr	0.37	1								
Mn	0.71	0.57	1							
Co	0.12	0.79*	0.30	1						
Ni	-0.44	-0.75*	-0.31	-0.27	1					
Cu	0.62	0.22	0.88	-0.19	-0.22	1				
As	-0.87*	-0.56	-0.68	-0.07	0.79*	-0.66	1			
Se	-0.46	0.08	-0.25	0.66	0.48	-0.59	0.68	1		
Cd	-0.90	-0.02	-0.37	0.05	0.16	-0.34	0.62	0.35	1	
Pb	-0.95	-0.26	-0.81	0.06	0.33	-0.83	0.84*	0.57	0.79*	1
Thigh										
V	1									
Cr	-0.02	1								
Mn	0.39	0.74	1							
Co	-0.16	-0.52	-0.03	1						
Ni	0.46	-0.23	0.27	0.62	1					
Cu	0.57	0.63	0.72	-0.24	0.52	1				
As	-0.57	-0.72	-0.80	0.54	0.12	-0.61	1			
Se	-0.45	0.54	-0.16	-0.71	-0.54	0.13	-0.03	1		
Cd	0.13	0.31	-0.08	-0.52	0.15	0.58	-0.04	0.68	1	
Pb	-0.56	-0.43	-0.40	0.73	0.43	-0.25	0.86*	-0.07	0.02	1
Hump										
V	1									
Cr	0.05	1								
Mn	0.91*	0.22	1							
Co	-0.05	-0.38	0.23	1						
Ni	0.39	0.63	0.72	0.38	1					
Cu	0.35	-0.05	0.26	-0.37	-0.10	1				
As	0.46	0.31	0.73	0.32	0.77*	0.50	1			
Se	0.26	-0.49	-0.16	-0.48	-0.73	0.08	-0.66	1		
Cd	0.90*	0.14	0.67	-0.47	0.11	0.43	0.18	0.52	1	
Pb	0.86*	0.25	0.86*	-0.15	0.49	0.69	0.75*	-0.04	0.78	1

*Correlation is significant at the 0.05 level

related to old age (Sharkawy *et al,* 2002) because the Cd concentration was highest in the kidney and liver of the older group. Similar to our study Cd and Pb were in the highest concentrations in the liver and Pb was in the top highest concentration in the hump (Table 1). A previous study from Pakistan (Mahmud *et al,* 2011) reported levels (mg/100 g wet weight calculation based on reported moisture (72.03%)) of some elements in camel meat. A comparison with results of present study revealed that the values of Co and Ni were the same but values for Cr and Pb were higher and for Cd were found lower. Abdelrahman *et al* (2013a) investigated the selenium and iodine status in Majaheem and Maghateer breeds raised under a semi-intensive system in Saudi Arabia and found higher Se concentrations (µg/kg, wet weight) in black camel tissues (liver and meat) compared to white camels. The Se concentrations were 200.43 and 114.12 in black camel liver and meat compared with 128.12 and 81.84 in white camel liver and meat, respectively. Our study showed higher Se concentrations in the liver and meat (Table 1) than their results, with significant differences ($p < 0.05$). Concentrations of Se in camel liver (921 µg/kg) and meat (351 µg/kg) was higher than our results (Ma, 1995). The highest Se concentration was found in liver, followed by meat; a similar trend was seen in their study. They mentioned that a significant difference ($p < 0.05$) in Se level was on account of camel breed specificity, but in present study we did not focus on a specific breed. A study

Table 4. Correlations between different organs (liver, shoulder, neck, thigh and hump) within essential and toxic elements (V,Cr,Mn, Co,Ni, Cu,As, Se, Cd and Pb).

	Liver	**Shoulder**	**Neck**	**Thigh**	**Hump**	**Liver**	**Shoulder**	**Neck**	**Thigh**	**Hump**
	V					**Cr**				
Liver	1					1				
Shoulder	0.5	1				0.58	1			
Neck	0.2	0.6	1			0.10	-0.37	1		
Thigh	0.3	0.2	0.7	1		0.26	0.7	0.23	1	
Hump	0.82*	0.14	-0.33	-0.24	1	0.99*	0.53	0.13	0.22	1
	Mn					**Co**				
Liver	1					1				
Shoulder	0.91*	1				-0.39	1			
Neck	0.86*	0.97*	1			-0.53	-0.38	1		
Thigh	0.86*	0.63	0.52	1		0.44	0.61	-0.75	1	
Hump	0.16	0.18	-0.04	0.40	1	0.81*	-0.14	-0.31	0.64	1
	Ni					**Cu**				
Liver	1					1				
Shoulder	0.81*	1				-0.19	1			
Neck	-0.42	-0.29	1			0.27	0.80*	1		
Thigh	0.98*	0.78*	-0.49	1		0.36	0.65	0.97*	1	
Hump	0.97*	0.63	-0.40	0.96*	1	0.12	0.004	0.12	0.12	1
	As					**Se**				
Liver	1					1				
Shoulder	0.06	1				-0.56	1			
Neck	0.64	0.07	1			-0.52	0.37	1		
Thigh	-0.52	-0.84	-0.33	1		-0.76	0.38	0.57	1	
Hump	-0.42	0.47	0.13	-0.26	1	-0.97	0.39	0.57	0.71	1
	Cd					**Pb**				
Liver	1					1				
Shoulder	0.92*	1				0.71	1			
Neck	-0.87	-0.64	1			-0.59	-0.94	1		
Thigh	0.43	0.52	-0.46	1		0.30	-0.02	0.24	1	
Hump	-0.10	0.19	0.51	-0.08	1	0.38	0.39	-0.11	0.11	1

*Correlation is significant at the 0.05 level

from China (Zongping, 2003) found 2.11 and 0.63 mg/kg (dry weight) Se level in liver and muscles, respectively of Bactrian camel, which was similar to our results (Table 1). This may lead to conclusion that the highest Se concentration occurs in the liver, followed by meat, regardless of the camel's type (*Camelus bactrianus* or *Camelus dromedarius*). Kadim *et al* (2008) reported that vanadium levels in camel muscle were 0.072, 0.90 and 0.11 mg/100 g in the age groups 1–3, 3–5 and 5–8 years, respectively. However, in present study it was lower possibly due to nutritional or the age factor because camels in our study were 6-7 months old, while the camels in study of Kadim and associates were 1-8 years old. This led us to conclude that the vanadium concentration in camel meat increases with age, which is for most of the essential elements because of the storage.

Because the high EDI for lead, especially in hump, the consumption of camel hump is a source of exposure to Pb, which could have adverse health effects on humans. Lead (Pb) is well-known toxic element to human, especially for neurodevelopment in children. Moreover, it was reported by World Health Organisation (WHO) that Pb exposure (4.4%) to children between 0-4 years may lead to minor mental retardation in Europe (Nordberg *et al,* 2007).

The compounds containing lead are added to cars' fuel as anti-knock agents, which have lipophilic properties (CES, 2019). Therefore, we could notice an accumulation of Pb in camel hump, as we observed in our study, could be due to intensive cars traffic in Saudi Arabia (Algadhi *et al,* 2002).

Conclusion

Both essential elements (Cu, Se, Mn, Ni, Co and V) and toxic elements (As, Cd, Cr and Pb) exist in various concentrations in different camel organs (liver, shoulder, neck, thigh and hump). In this study, the distribution of these elements was similar in all investigated camel organs. All measured elements showed positive correlations in all camel organs. However, higher level of Pb was reported in hump. Thus, we conclude that consumers of camel humps may be exposed to higher Pb levels, which could lead to adverse health effects. Particularly, for children of young age, Pb was established as neurodevelopmental toxicant to humans by WHO. The source of Pb pollution could be originated from feed or car traffic for camels reared in rangelands near the highways or oil areas. More samples are needed to confirm our findings.

Acknowledgements

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through research groups program under grant number R.G.P.1/7/40.

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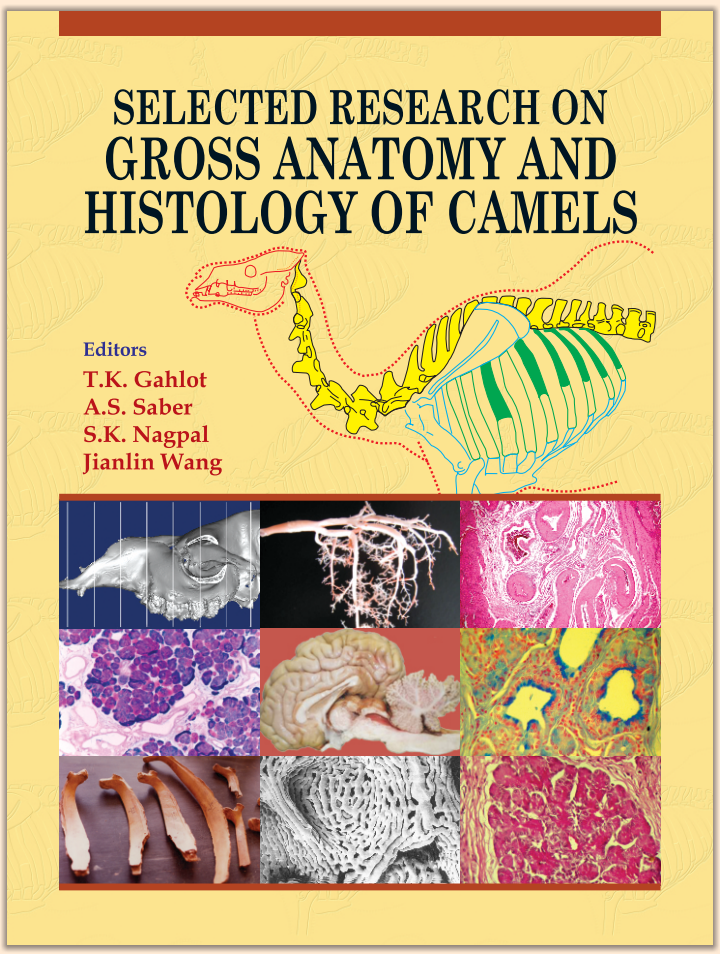

Editors:
T.K. Gahlot, A.S. Saber, S.K. Nagpal and Jianlin Wang

Edition: 2011

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

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

ISBN: 81-903140-1-7

DOI : 10.5958/2277-8934.2019.00029.8 Vol 26 No 2, p 189-190

Short Communication

MANAGEMENT OF MANDIBLE FRACTURES USING INTERDENTAL WIRING (IDW) IN CAMELS

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Fractures of the mandible are very common in male camels and these occur during rut season due to fighting with other male camels and grasping of the hard objects. These are generally compound in nature and become contaminated later. The presence of mental canal, alveoli of tusks and comparative long length of the body of lower mandible makes this area more weak and susceptible to fractures (Gahlot and Chouhan, 1994). A variety of fixation methods have been utilised to manage mandible fractures in camel including using transfixation pins along with plaster of Paris bandaging (Gahlot and Chouhan, 1994), bone plating (Kumar and Singh, 1979), plaster of Paris bandage and a wooden plate as a splint (Lavania, 1998), interdental wiring (Gahlot *et al,* 1989) and application of a U-shaped aluminium bar (Ahmed and Al-Sobayil, 2012). Successful management of mandible fractures using interdental wiring in camels is documented in this report.

History and Clinical Examination

Present study was conducted on 3 adult male camels presented with history of the trauma and hanging of the lower jaw with salivation (Fig 1). The feed and water intake were completely suspended. Clinical examination revealed bilateral fracture of the mandible which were further confirmed by a lateral radiograph. Radiographs revealed transverse bilateral mandibular fracture usually rostral to canine teeth (Fig 2). The interdental wiring (IDW) technique using copper wire was used to immobilise the bilateral mandible fracture in these cases.

Treatment

The camels were restrained in sternal recumbency and sedated with xylazine @ 0.3 mg/kg body weight, intravenously. The oral cavity was irrigated with light potassium permanganate solution to remove the feed straws, clots and debris. Interdental wiring (IDW) was done as per the method of Gahlot *et al* (1989). The twisted ends of the wires were trimmed about 1 cm from the base and directed towards the central incisors to prevent injury to the mucosa of lower lip. The fractured fragments of the mandible were reduced adequately by manual palpation, however, a slight ventral deviation of the lower jaw was noticeable in one case.

Post operatively, broad spectrum antibiotic and analgesics were administered parenterally for 5 days. Three doses of Injection containing vitamin A, D_3, E; 20 ml was also administered intramuscularly on alternate days. Mineral mixture supplementation was followed for 4 weeks. The irrigation of oral cavity with light potassium permanganate solution was done daily till healing of the oral cavity wound. Postoperative radiographic evaluation revealed satisfactory anatomical alignment of the fracture (Fig 3). Clinical and radiographic evaluation on 7 weeks also revealed good anatomical alignment and callus formation (Fig 3). The animals were allowed free access to water and semisolid foods for two weeks. Later, leafy feed was offered for next 4 weeks. Follow-up of the animals up to three months after surgery revealer a satisfactory restoration of prehension and mastication of roughage. No complication was observed and wires were removed after 8 weeks.

Discussion

The main objective of mandibular fracture treatment was to provide an adequate anatomical reduction, satisfactory fixation, restoration of normal occlusion and prehensile functions. Mandibular fractures close to the alveoli of tushes are often. The fracture fragment's sharp edges easily disrupt the gingiva. Standard interdental wiring technique using 1.0 mm diameter stainless steel, silver or copper wire has been reported to repair such fractures in camels (Gahlot *et al,* 1989). Purohit *et al* (2013) also documented the successful management of lower

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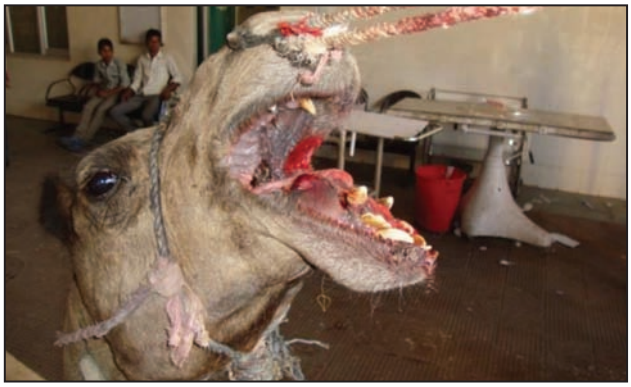

Fig 1. Dropping of lower jaw, blood tinged salivation and pointing out of the bone indicating the compound mandibular fracture.

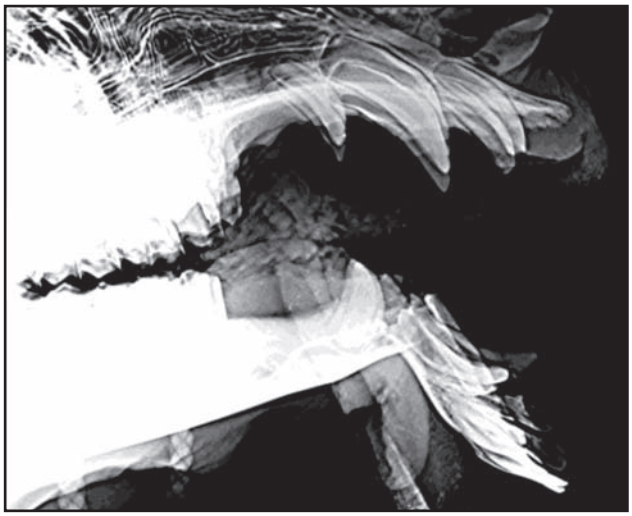

Fig 2. Lateral radiograph showing bilateral fracture of lower mandible just rostral to tushes of camel.

jaw fracture using IDW technique in adult bovine. IDW method is relatively simple, provide adequate fracture stability and does not require expensive or specialised equipment. The development of submandibular abscesses and osteomyelitis were not observed clinically as well as radiographically in these cases as documented previously (Gahlot *et al*, 1989; Al-Dughaym *et al*, 2003). It is not recommended to remove teeth at the fracture line unless these are loose or damaged or disturb interdental wiring. In present study, tooth was not required to be removed in all the cases. Present report documents the successful management of bilateral mandibular fracture by IDW technique using copper wire.

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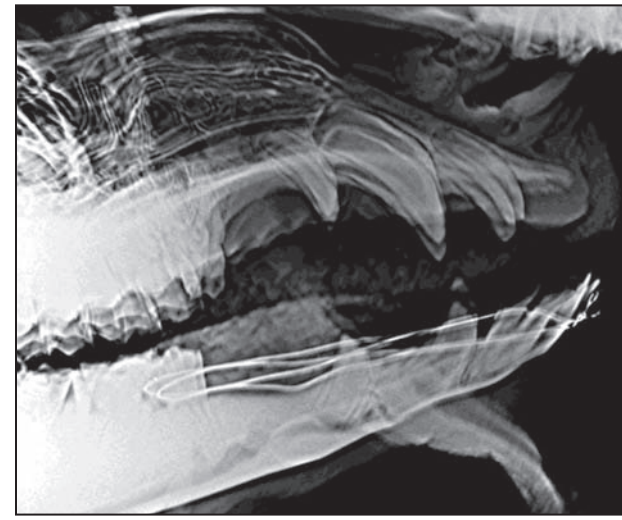

Fig 3. Lateral radiograph at 7th post-operative day showing adequate immobilisation, occlusion of dental table and good callus in case no. 3.

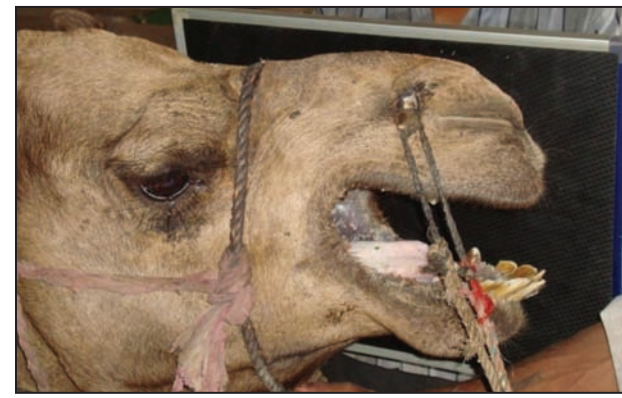

Fig 4. Same camel on 7th postoperative day showing good immobilisation in case no. 3.

DOI : 10.5958/2277-8934.2019.00030.4 Vol 26 No 2, p 191-193

STUDY ON PHYSICAL PROPERTIES OF CAMEL CASHMERE

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ABSTRACT

Camel cashmere is safe and pure natural product which is loved by consumers. The anti ultraviolet property, heat transfer, thermal contact property and air permeability of camel cashmere were qualitatively measured in this study. Experimental results show that camel cashmere has strong anti ultraviolet property, good thermal insulation, nice warm feeling and high air permeability.

Key words: Air permeability, anti ultraviolet property, camel cashmere, heat transfer, warm-cool feeling

Bactrian camel is a national class II protected animal in China and mostly scattered in the sunny areas of Gobi and desert (Zhang *et al,* 2008; Feng, 2007). Camel cashmere is safe and pure natural product which is loved by consumers because of its comfort and warmth properties (Zhang, 2001). The degree of customer's affection for fabric is determined by its hand values, comfortability, warm-cool feeling, or other special characteristics such as anti ultraviolet property etc (Tu and Sachiko, 2017). These subjective characteristics correspond to the physical properties of fabric. Camel cashmere physical properties are easily affected by the thickness, texture, colour and surface conditions of the fabric. The heat transfer, thermal contact property and air permeability of camel cashmere were qualitatively measured in this study.

Materials and Methods

The samples used in this experiment are four type fabrics, such as camel cashmere (plain weave, known as camel1), camel cashmere (twill weave, known as camel2), goat cashmere (twill weave, known as goat2) and cotton (twill weave, known as cotton2). Those are provided by Inner Mongolia Zhaojun camel cashmere Industry company Limited. Fabric characterisation parameters are shown in Table 1.

Experimental conditions: the test area 20 cm^2, the diameter of sample ≥=45mm and under 100 Pa pressure.

YG(B)912E UV Tester, KES-F7 Contact Cooling and Warming Sensor Tester, YG461E Digital Permeability Meter were used in this experiment.

Table 1. Fabric characterisation parameters.

Fabric	Camel1	Camel2	Goat2	Cotton2
weave	plain	twill	twill	twill
Thickness (cm)	0.1644	0.2266	0.2426	0.2275
Weight (g/m^2)	450	834	798	762

Results and Discussion

Anti ultraviolet (UV) property of camel cashmeres

The standard of evaluation on anti UV properties of fabrics issued by the State Quality Inspection Bureau stipulates that as long as the UPF value of the sample is greater than 30 and the transmittancy (TR)of UVA is less than 5%, it can be called "anti ultraviolet product" and these two conditions are indispensable. (Jiang and Chen, 2012).

Table 2. UV protection factor (UPF) of samples.

Parameters	Camel1 (plain)	Camel2 (twill)	Goat2 (twill)	Cotton2 (twill)
UPF	30.16	100+	28.99	27.23
UVA	3.57	1.01	5.01	4.05
UVB	2.82	0.67	3.38	3.05

From Table 2, it can be seen that UPF of samples are: camel2 (twill) >camel1 (plain) >goat2 (twill) >cotton2 (twill) ; The transmittancy of UVA and UVB are: goat2 (twill) >cotton2 (twill) >camel1 (plain) >camel2 (twill). Twill weave Camel cashmere has the strongest anti UV performance with UPF =100 > 30, TR(UVA) = 1.01% < 5%. Plain weave Camel cashmere ranks second with UPF =30.16 > 30, TR(UVA) = 3.57% < 5%.

Preliminary qualitative experiments using YG(B)912E UV tester, the samples were tested every 5nm in the wavelength range of about 280~400nm.

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The experimental results are shown in Fig 1-2 and Table 2.

Fig 1-2 shows that the UV transmittancy of twill camel fabric is less than 2 % in a large range, so camel cashmere has the strongest UV protection property.

We can infer that camel cashmere has strong anti ultraviolet property than others.

Heat transfer property

Thermal conductivity K is an intrinsic property of material that indicates its ability to conduct the heat. It is the flux of heat (energy per unit area per unit time) divided by temperature gradient (temperature difference per unit length) (Yamini *et al*, 2014; Tu and Sachiko, 2017). In general, the lower the thermal conductivity K of the object, the weaker the thermal transfer performance of the object and its thermal insulation is better (Cui and Sun, 2013).

Using the KES-F7 contact cold and warm sensing tester, the thermal conductivity of the samples was measured 5 times for each sample. The measurement results are shown in Table 3.

Table 3. Thermal conductivity of samples.

Samples	Camel1 (plain)	Camel2 (twill)	Goat2 (twill)	Cotton2 (twill)
1	1.762	1.444	1.892	2.927
2	1.761	1.439	1.897	2.956
3	1.720	1.467	1.867	2.947
4	1.698	1.450	1.809	2.944
5	1.712	1.425	1.856	3.012
mean	1.731	1.445	1.864	2.957

From Table 3, it can be seen that the thermal conductivity K of samples are: cotton2 (twill) > goat2 (twill) >camel1 (plain) > camel2 (twill).

It is therefore opined that camel cashmere has better thermal insulation property.

Thermal contact property

Thermal contact properties determine the feeling when the human skin touches an object for a brief period of time. The more a material absorbs thermal energy, the more it acts as a thermal conductor and the cooler it seems at the very first moment of contact with a warmer body (Sinem *et al*, 2005; Vivekanadan *et al*, 2011). The characteristic value Q was proposed by Kawabata (Kawabata, 1984) and has been shown to estimate the temperature perception of the fabric. This value is the maximum rate of heat flux from a heated plate to the fabric and is convenient to measure. Q is usually measured with the KES-F7 (Kato Tech Co., Ltd., Japan). Large Q values correspond to cold sensations and small Q values correspond to thermal sensations. Hence, the larger the Q, the colder it is (Jonko *et al*, 1987).

Using the KES-F7 contact cold and warm sense tester, the heat obtained per square centimetre of the sample was measured 5 times per sample. The measurement results are shown in Table 4.

Table 4. The instant warm and cold value Q of the samples.

Samples	Camel1 (plain)	Camel2 (twill)	Goat2 (twill)	Cotton2 (twill)
1	0.063	0.053	0.069	0.087
2	0.060	0.057	0.071	0.091
3	0.066	0.055	0.072	0.089
4	0.064	0.051	0.069	0.084
5	0.067	0.054	0.070	0.086
mean	0.064	0.054	0.070	0.087

From Table 4, it can be seen that the heat Q obtained by each square centimetre are : cotton2 (twill) > goat2 (twill) >camel1 (plain) >camel2 (twill).

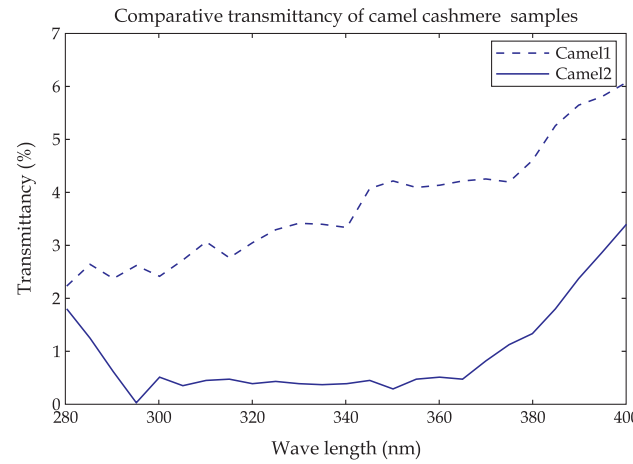

Fig 1. Comparative transmittancy of camel cashmere samples.

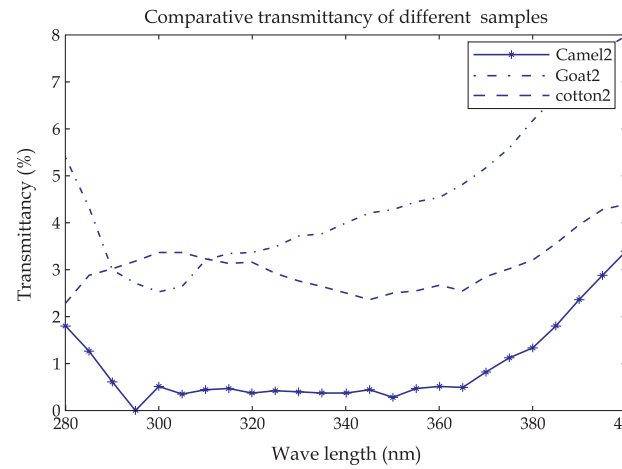

Fig 2. Comparative transmittancy of different samples.

Camel cashmere has nice warm fleeing property.

Air permeability of camel cashmere

Air permeability (AP) is described as the rate of air flow through a known area under a prescribed air pressure differential between the two surfaces of material (Yamini *et al,* 2014). Fabrics with small air permeability can prevent thermal divergence, which can also play a good thermal effect (Zhang *et al,* 2009).

This experiment used YG461E digital permeability meter to measure the air permeability of the sample and each sample was measured 5 times. The measurement results are shown in Table 5. Experimental conditions: The test area was 20cm^2, under 100Pa pressure.

Table 5. The air permeability of the samples.

Samples	Camel1 (plain)	Camel2 (twill)	Goat2 (twill)	Cotton2 (twill)
1	2050.12	488.22	1074.66	1213.65
2	2049.32	498.28	1072.45	1234.77
3	2051.03	485.67	1069.49	1209.32
4	2049.10	490.78	1071.90	1213.78
5	2052.33	484.90	1075.12	1234.10
mean	2050.38	489.57	1072.72	1221.12

From Table 5, it can be seen that the permeability of samples are: camel1 (plain) >cotton2 (twill) > goat2 (twill) > camel2 (twill).

Camel cashmere has high air permeability, so has a good warmth effect.

Conclusion

Experimental results showed that camel cashmere had strong anti ultraviolet property, good thermal insulation, nice warm feeling and high air permeability.

Another study of our team found that camel hair had small diameter, hollow structure, inlaid scale. These unique microscopic structures determined the special physical properties of camel cashmere.

Acknowledgement

This research paper was supported by a grant from Inner Mongolia Natural Science Foundation (2018MS06011) and Chinese Natural Science Foundation (31560710).

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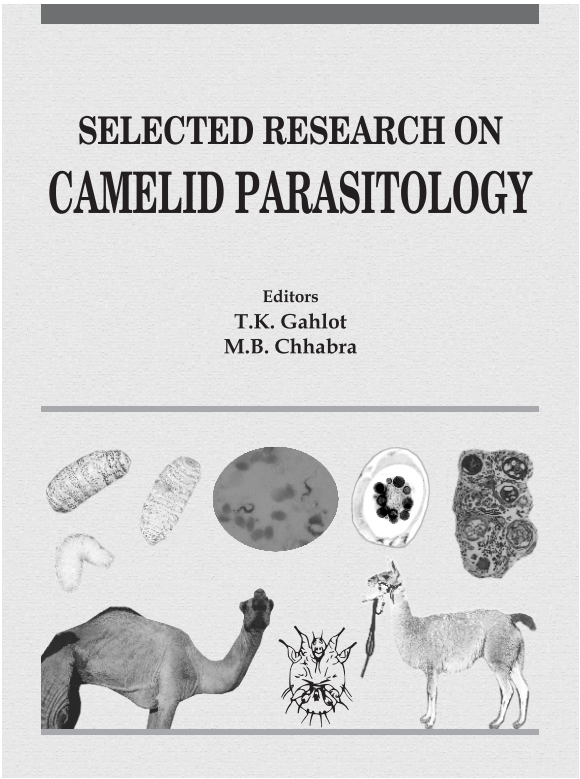

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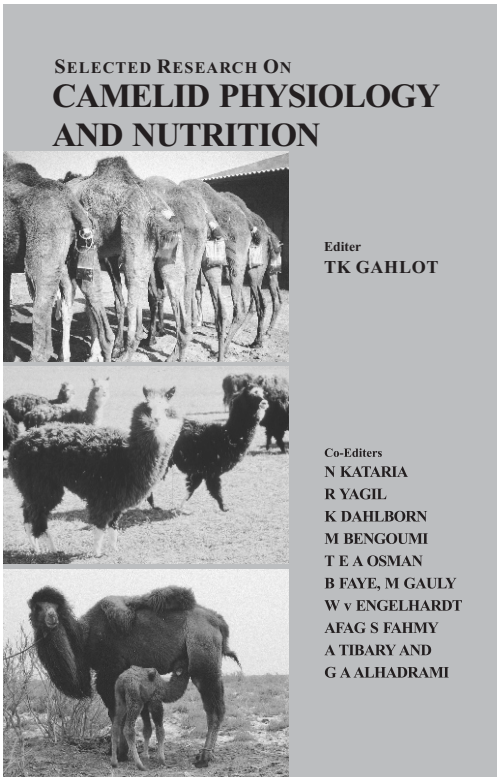
Price: US $ 200 (Abroad) INR (Rs) 3000 (India)
ISBN: 81-903140-0-9



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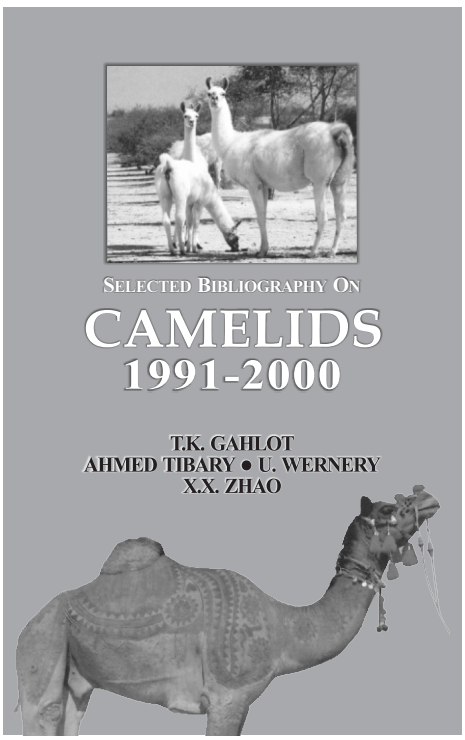

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Price: US $ 90 (Abroad) INR (Rs) 950 (India)
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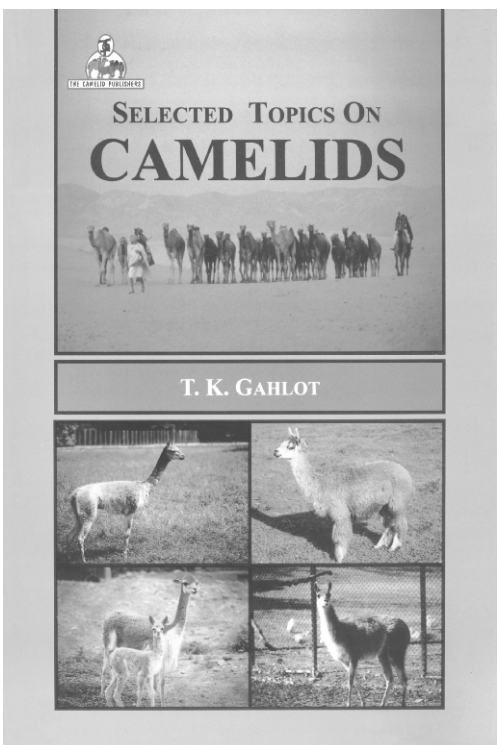

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Price: US $ 180 (Abroad) INR (Rs) 1950 (India)
ISBN: 81-901141-0-7

DOI : 10.5958/2277-8934.2019.00031.6 Vol 26 No 2, p 195-196

Short Communication

SURGICAL MANAGEMENT OF SOFT PALATE HAEMATOMA IN CAMEL

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Palatine diverticulum (dulaa or gulaa) is a distendable diverticulum on the lower part of the soft palate and well developed in male dromedary camels (*Camelus dromidarius*), balloons out in 'rut' or breeding season as a form of sexual behaviour (Arnautovic and Abdel Magid, 1974; Reece and Chawla, 2001; Al-Sobayil and Ahmed, 2011). The exact mechanism of distension of the dulaa is not well understood. It has been assumed that the dulaa distends during expiration when the camel closes its nares and air is forced from the lungs into the oro-pharynx to inflate the soft palate (Arnautovic and Abdel Magid, 1974; Al-Sobayil and Ahmed, 2011). Trauma of protruded soft palate by sharp teeth, other object like tree and manger may lead to perforation of the soft palate wall, causing submucosal haematoma, necrosis, gangrene, food impaction leading to characteristic signs of dysphagia and dyspnoea (Gahlot *et al,* 1988; Gahlot, 1993; Gahlot, 2000; Al-Sobayil and Ahmed, 2011; Tanwar *et al,* 2016). The inflammatory condition with oedematous swelling of protruded dulaa becomes more complicated by the time. Successful surgical management of soft palate haematoma in camel is reported here.

History and Clinical Examination

Adult male dromedary camel aged 6 years was presented with a history of protruding the elongated mass "dulla" and salivation sine last 2 days (Fig 1). The camel had dysphagia and dyspnoea. The feed and water intake were completely absent. However, owner drenched some water and liquid feed into oral cavity to fulfil their daily requirement. Clinical examination revealed it as a case of protrusion of soft palate. Soft palate resection under xylazine sedation was planned.

Treatment

The camel was restrained in sternal recumbency and sedated with xylazine @ 0.3 mg/kg body weight, intravenously. The oral cavity was irrigated with light potassium permanganate solution and the feed material, clots and debris were removed. The animal's mouth was opened by application of a rope each at upper and lower jaw and were pulled in opposite direction. Tongue was pulled out. The soft palate was grasped using a cotton towel and pulled rostrally. The distended pedicle of soft palate was resected close to its attachment using a long handle Mayo scissors. The head of camel was lowered down to prevent aspiration of blood. Haemostasis occurred spontaneously (Fig 2). On dissection of the resected soft palate, clotted blood was found inside the soft palate indicating haematoma (Fig 3, 4).

Post operatively, broad spectrum antibiotic and analgesic were administered parenterally for 5 days. Three doses of injection containing vitamin A, D, E, 20 ml was also administered intramuscularly on alternate days. The irrigation of oral cavity with light potassium permanganate solution was done daily till healing of the oral cavity wound. The animal was allowed free access to water but semisolid foods and leafy hay for 2 weeks. The eventless recovery was reported by the owner.

Discussion

In the present case the protruded dulaa was ulcerated, oedematous with occurrence of haematoma due to injury by sharp teeth. It has been reported that the injuries of dulaa cause laceration of mucosa and rupture of its blood vessels (Gahlot, 2000; Al-Sobayil and Ahmed, 2011; Tanwar *et al,* 2016). An increase in the size of the protruded dulaa occurs due to accumulation of inflammatory fluid, oedema, feed particles and haematoma. Camel owners are advised to rasp sharp teeth in male camels before every rut season to decrease the chance of soft palate injury and further complications. The delay in amputation of the injured dulaa may lead to

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Fig 1. Hanging out of pendulous, oedematous, elongated, soft palate indicating injury with haematoma.

Fig 2. Camel just after excision of the soft palate.

abscession and gangrene (Gahlot *et al,* 1988) which is a sequalae to the contamination of wounds and decreased blood supply in hanged out portion of soft palate (Vashishtha *et al,* 1980). Dysphagia has been reported as a characteristic sign seen in cases of soft palate injuries. Inadequately cut stump might be sucked into the laryngeal cavity to cause death by asphyxia (Gahlot *et al,* 1988). Haemorrhage was minimal which might be due to the prolonged tension on the distended palatine vessels and auto sealing of the blood vessels by coiling and elastic nature of remaining short stump of soft palate; hence no sutures were applied (Reece and Chawla, 2001; Tanwar *et al,* 2016). Present report documented the successful surgical management of soft palate injury and haematoma in camel and recommends its suitability for field condition.

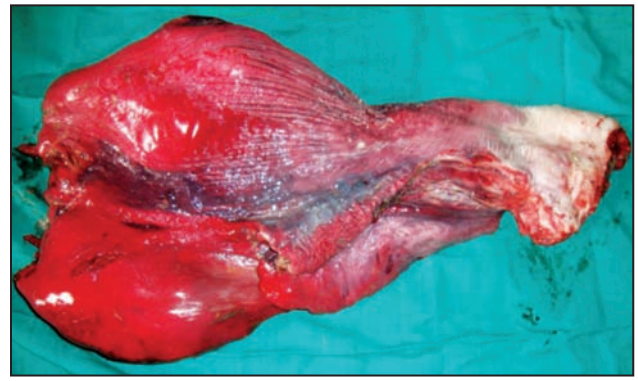

Fig 3. Photograph showing elongated resected soft palate mass with collection of haematoma on its tip.

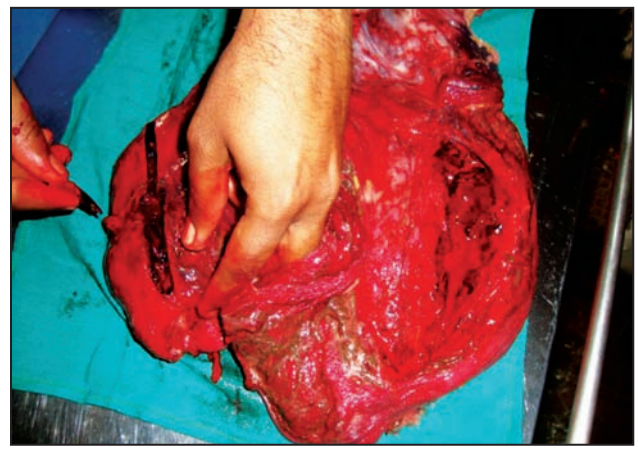

Fig 4. Blood clots were seen in the resected soft palate.

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FIRST CONFERENCE OF INTERNATIONAL CAMEL ORGANISATION HELD AT BISHKEK, KYRGYZSTAN

First Conference of International Camel Organisation (ICO) with theme- Camel Culture in Central Asia- Historical Heritage and Prospects, was held on 28-29 August 2019 at Bishkek, Kyrgyzstan. The International Camp Organisation (ICO) is an international Organisation created as per the decision of the Organisation's Constituent Assembly, with the participation of special Organisations from 36 countries of the world, on 22 March 2019 in the city of Riyadh in the Kingdom of Saudi Arabia. Sheikh Fahd bin Farah bin hitliin, the first Vice President of akimat, was elected President of the International Organisation for International Camps (ICO). The mission of the international Organisation, is to promote the cultural heritage of the world's people through the development of the culture of verblûdovodstva. The international Organisation "ICO" - is the organiser of cultural, camel festivals with the participation of more than 50 countries of the world.

CAMEL DAIRY MARKET: GLOBAL INDUSTRY TRENDS, SHARE, SIZE, GROWTH, OPPORTUNITY AND FORECAST 2019-2024

In 2018, the global market for camel dairy products reached a value of US$ 5.64 Billion growing at a CAGR of 3.89% during 2011-2018. The price of camel milk is significantly higher compared to traditional cow milk, owing to the fact that camel milk production is lower than cow milk, and camel breeding costs are also higher compared to that of cows. Its immense health benefits, however, significantly outweigh the higher prices. Camel milk has several times more iron and vitamin C compared to cow's milk. Morever, camel's milk is lower in cholesterol, fat and has a higher quantity of proteins. Medical research has also suggested that camel milk created a positive impact on children with autism to live a better life. Individuals with lactose intolerance were also found to cope with camel milk much better compared to cows milk. Driven by these nutrient rich features, camel dairy products have been slowly gaining popularity across the world. Manufacturers of camel dairy products are also diversyfing the range and flavours of products that can be made from camels milk. Popular products include fresh milk, flavoured milk, laban, cheese, ice-cream, yoghurt, milk powder, infant formulae, etc. Hence, the global camel dairy market is expected to grow at a CAGR of 8.01% during 2019-2024 reaching levels worth more than US$ 8 Billion by 2024.

PROBIOTIC CHARACTERISTICS OF LACTIC ACID BACTERIA (LAB) ISOLATED FROM CAMEL MILK

Scientists of UAE university identified LAB (*Lactococcus lactis* KX881768, *Lactobacillus plantarum* KX881772, *Lactococcus lactis* KX881782 and *Lactobacillus plantarum*) which showed auto-aggregation ability, high cholesterol removal ability, high co-aggregation, strong antimicrobial activity and EPS production. Among the isolates, *Lactococcus lactis* KX881768, *Lactobacillus plantarum* KX881772, *Lactococcus lactis* KX881782 and *Lactobacillus plantarum* KX881779 exhibited remarkable cholesterol removal abilities. Similarly, *Lactobacillus plantarum* KX881779, and I KX881782 showed very promising fermentation profiles. They investigated their probiotic characteristics such as physiological properties, cell surface properties (hydrophobicity, autoaggregation, co-aggregation), acid and bile tolerance abilities, bile salt hydrolysis, cholesterol removing property, exopolysaccharide (EPS) production ability, hemolytic and antimicrobial activities, resistance toward lysozyme and six antibiotics, and fermentation profile (growth, pH, and proteolysis) and rDNA sequencing to identify those isolates.

(Source:https://www.probiotic-conference.net)

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Books (Personal authors): Gahlot TK and Chouhan DS (1992). Camel Surgery, Ist Edn. Gyan Prakashan Mandir, Gauri Niwas, 2b5, Pawanpuri, Bikaner, India. pp 37-50.

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