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INTERNATIONAL CAMEL ORGANISATION AT SAUDI ARABIA AND CAMELID ANTIBODY FACILITY UNIT AT DUBAI, UAE

Camel based activities are strengthened in Arabia at global level by establishment of the International Camel Organisation (ICO), a non-profit entity to be headquartered in Riyadh, Saudi Arabia. It aims to expand the base of practice, equipment and sites for camel activities all over the world, ensure fair competition and raise awareness about the harmful effects of stimulants on camels. It would also envisage to encourage scientific research on camels and enriching world literature with books and translations by supporting studies, authors and translators. The organisation will hold conferences, courses, seminars and will issue regulations aimed at enhancing safety training for all its members. It will also establish research centres and provide technical expertise as well as research grants in various fields relating to camels in order to enrich cultural and scientific knowledge in the field on a global scale. The ICO will also publish magazines and newspapers and will launch TV, video and radio channels in order to contribute to the dissemination of knowledge through educational, cultural, scientific, historic, sports and media programmes.

Advances in camel research is bringing good dividends to the readers and researchers. Scientists from the Camel Research Centre at King Faisal University, in cooperation with the University of Nottingham, have discovered the most important genes responsible for the mechanism for determining the colours of 10 kinds of Arabian camels. Many kudos to Dr Nisar Ahmad Wani, scientific director of the centre for informing that first successful cloned camel of their centre- Injaz is expecting her third calf.

The Central Veterinary Research Institute, Dubai, UAE, under the supervision of Dr. U. Wernery, Scientific Director, has brought out a unique product containing Cl. perfringens A α toxin camel hyperimmune globulin (see on website www.cvrl.ae) which is highly purified and consists of pure immunoglobulins. The purified IgGs are then further concentrated to 5g/L and filled in 100ml sterile transfer bags. It helps boosting the animal's immunity. After 30 years of devoted service to the CVRL, Mrs. Renate Wernery who played a vital role in the growth and success of CVRL, has retired now after giving 30 years of service and was given an affectionate farewell by the staff of the laboratory.

The April 2019 issue contains important papers on dromedary camels which include research on Brucella melitensis caused abortion, one-humped camel in Bangladesh, pancreatic hormones-immunohistochemical localisation, ultrastructure and morphometry of prostate gland, respiratory mycoplasmosis, sequence analysis of toll like receptor 1 gene, sevoflurane anaesthesia, balantidiasis, dermatophytosis, histology and histomorphometry of the second stomach chamber, rectal prolapse, modulation of phenotype and function of neutrophils and D- and L-lactate in milk. However, the Bactrian camel research includes a special paper on Bactrian camel caravan culture in stamps, vaginal myiasis,slaughter performance and skeletal muscle fibre type and forestomach bacterial microbiota.

You will be happy to note that JCPR has started receiving more manuscripts eversince it has become triannual. A greater enthusiasm of camel scientists and researchers is sincerely acknowledged with thanks. I am sure that the future issues of JCPR would bring more new information for the readers.

(Dr. T.K. Gahlot)
Editor

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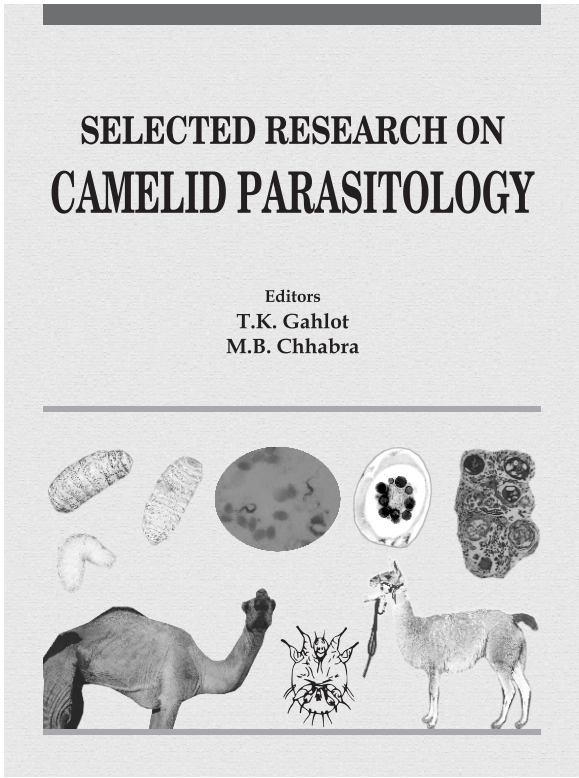

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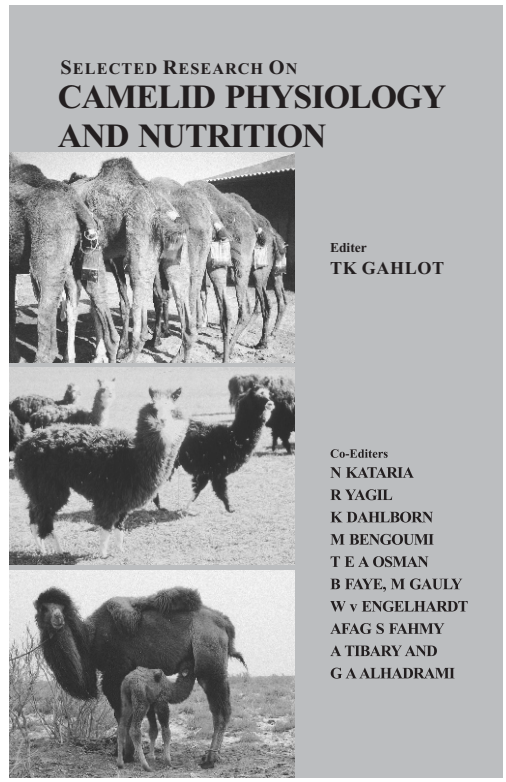

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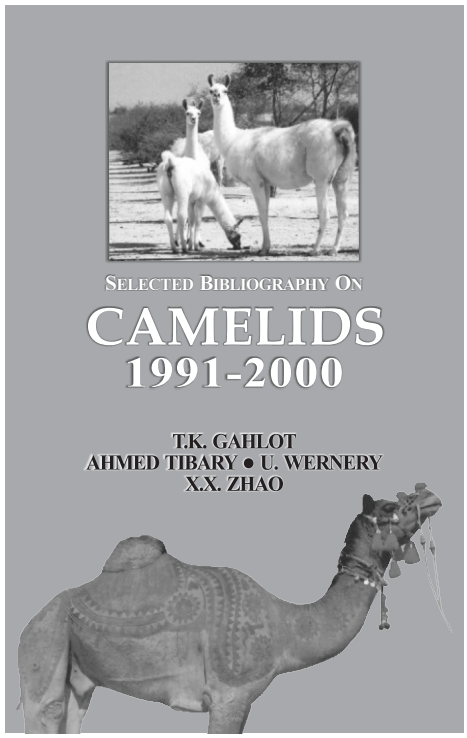

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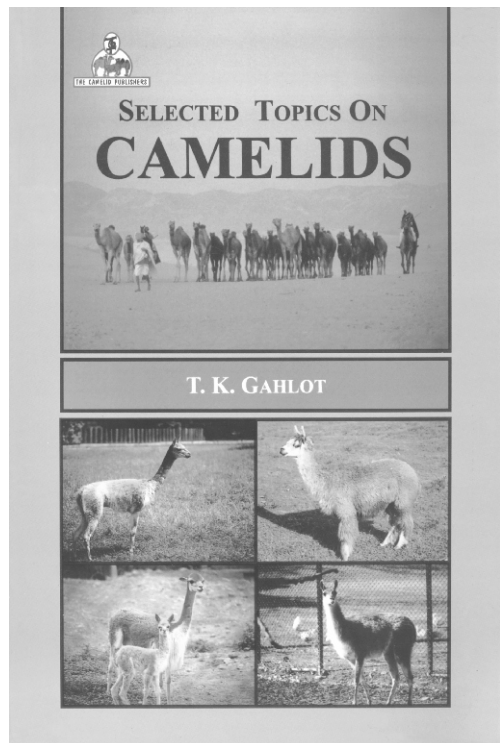

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Brucella melitensis CAUSED ABORTION IN A SEROLOGICALLY POSITIVE DROMEDARY CAMEL

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ABSTRACT

An abortion case in a dromedary camel caused by a *B. melitensis* strain belonging to the East-Mediterranean genetic group (GT42 by multiple-locus variable-number tandem-repeat analysis (MLVA8) and GT108 by MLVA11) is reported. Earlier, the dromedary was seronegative for Brucellosis for 6 years. The animal got infected between 112 and 204 days of gestation from an unknown source, developed bacteriaemia and the acute infection resulted in the abortion at 249 days of gestation. The pathological findings in the foetus and the placenta were not pathognomonic for Brucellosis. In the foetus, both pleural and abdominal cavities were filled with bloody fluid and pleurisy was observed. In the placenta, oedema, diffuse mineralisation and focal detachment of the trophoblast were detected without inflammatory changes. The *Brucella* pathogens were excreted to the environment with the aborted foetus and the placenta. The bacteria disappeared from the blood and the uterus of the dam within a short time after abortion and were isolated only from some of the lymph nodes. The *Brucella* seropositive camel in its acute phase of the disease did not infect other contact animals. In addition, the transmission of the disease to other dromedaries at the time of abortion was also prevented with appropriate biosecurity measures.

Key words: Abortion, brucellosis, *B. melitensis*, dromedary camel

Brucellosis in breeding camels occurs in all of the known forms described in ruminants and abortion is its most obvious manifestation (Acosta *et al,* 1972; Agab *et al,* 1996; Fazil and Hofmann, 1981; Radwan *et al,* 1995; Wilson *et al,* 1982). Infections may also result in stillborn calves, retained placenta and reduced milk yield, as it is common in bovine and ovine. However, retained placentas have not been reported in *Camelidae* (Wernery *et al,* 2014). Literature is scarce on the pathological changes caused by *Brucella* organisms in camelids. In serological brucellosis–positive male camels, orchitis and epididymitis have been described (Ahmed and Nada, 1993). Pathological lesions in foetuses of ten *B. abortus* naturally infected dromedary camels have also been reported (Narnaware *et al,* 2016). They included subcutaneous oedema, interstitial pneumonia, liver degeneration and mononuclear infiltration in the kidney. The placentas were oedematous, showing necrosis and mononuclear infiltration. Pathological changes were also reported (Gidlewski *et al,* 2000; Gilsdorf *et al,* 2001) in a pregnant llama which was experimentally infected with *B. abortus* through the conjunctival sac. They included lymphocytic and histiocytic placentitis with marked loss of trophoblastic epithelial cells.

B. melitensis and *B. abortus* organisms have been isolated from different tissues from Old World camels (OWCs) and New World camels (NWCs) including milk, lymph nodes, foetal stomach, placentas, vaginal swabs, hygromas and testis (Wernery *et al,* 2014).

This paper highlights the pathological alterations caused by *B. melitensis* infection in the aborted foetus and placenta as well as in its serologically positive dam. Culture and PCR results of specimens collected from the aborted foetus, placenta and dam as well as the load of *Brucella* in the specimens of the aborted foetus and placenta are described.

Materials and Methods

An aborted foetus and placenta from a *Brucella* seropositive dam from a camel farm in the UAE were submitted for necropsy to the Pathology Department of the Central Veterinary Research Laboratory (CVRL)

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and evaluated for macroscopic lesions. At necropsy, the carcass was sexed, weighed and measured. Samples from lung, liver, umbilical cord, pleural fluid, abdominal fluid and compartment one (C1) content were collected from the aborted foetus. Samples from placenta were also collected and included for further testing.

Three weeks after the abortion, the *Brucella* seropositive dam was humanely euthanised using Xylazin (Ilium Xylazil, Troy Laboratories, Australia) and T61 containing Embutramide, Mebezonium and Tetracaine (MSD Animal Health, The Netherland). Then, the carcass was submitted to CVRL where a full necropsy was performed including sampling from liver, udder, uterus and body lymph nodes. The 11 years old dromedary dam was serologically tested positive for *Brucella* antibodies, 45 days prior to abortion with Rose Bengal Test (RBT), then Brucellosis was confirmed with Complement Fixation Test (CFT), Serum Agglutination Test (SAT), Rose Bengal Test (RBT) and competitive Enzyme-linked Immunosorbent Assay (cELISA) 41 days prior to the abortion. *B. melitensis* was isolated from sodium citrate blood collected on the same day. This animal was repeatedly tested negative for brucellosis during the previous 6 years, from 2012 until 2018 (Table 3) and aborted after 249 days of pregnancy.

Formalin fixed, paraffin embedded samples of both aborted foetus and dam as well as placenta were processed for routine histopathological investigation.

Culture Methodology

All investigations were carried out in a level 2 biosafety cabinet in CVRL's high security Level 3 facility. Seven specimens collected at necropsy from the aborted foetus and placenta of the *Brucella* seropositive dromedary dam were cultured for the detection of *Brucella*. The specimens included organs (liver, right and left lung and umbilical cord), body fluids (pleural and abdominal fluid) and C1 content. From the dam, 20 specimens were collected for *Brucella* culture which included 11 lymph nodes, liver, 2 uterine tissues, 4 udder tissues, mammary secretion and blood in sodium citrate blood tubes. Blood was withdrawn from the jugular vein into EDTA tube and serum tube for PCR and serological testing, respectively. All 7 specimens from the foetus and placenta as well as the 20 specimens from the dam were cultured for the isolation of *Brucella* spp.

For the culture of specimens from the dam, 3 methods were used: direct, concentration and enrichment as described previously (Johnson *et al,* 2018). However, for the culture of aborted foetus specimens, only direct and concentration methods were used.

Two selective agars and an enrichment broth also described previously (Johnson *et al,* 2018) were used for culture. They were Farrell's media (*Brucella* medium base CM0169, Oxoid, supplemented with filtered horse serum SHS100, E and O Laboratories, UK and *Brucella* selective supplement SR0083A, Oxoid), Brain-Heart-Infusion agar (Brain Heart Infusion CM1135, Oxoid, with 1% bacteriological agar and supplemented with filtered horse serum SHS100, E and O Laboratories, UK and *Brucella* selective supplement, SR0083A, Oxoid) and Trypticase soy broth supplemented with *Brucella* selective supplement.

All inoculated agar plates and enrichment broths were incubated at 37°C in an atmosphere of 5% CO_2 for 6 days. After 6 days of incubation, all plates were examined for growth of typical *Brucella* colonies and the enrichment broths were well homogenised and 0.1 ml of each broth was quadrant streaked on *Brucella* selective agars. The streaked plates were incubated for another 6 days at 37°C in an atmosphere of 5% CO_2. After 6 days of incubation, the plates were examined for growth of typical *Brucella* colonies.

Culture for enumeration

Approximately 1 g/ml each of the 7 specimens collected from the foetus and placenta was also cultured to determine the load of *Brucella* in each specimen. For this, 1 gram of each organ sample was weighed, finely minced and homogenised in 9 ml PBS (1:10 dilution). The sample homogenates as well as 1 ml of the original body fluids (pleural and abdominal fluid) and C1 content were further serial diluted in PBS up to 10^7 dilutions and 0.1 ml of each dilution was spread plate cultured on two *Brucella* selective agars mentioned above. All inoculated plates were incubated for 6 days at 37°C in an atmosphere of 5% CO_2. *Brucella* colonies grown on the selective agars from each dilution plate were counted and cfu/g/ml were calculated.

DNA extraction and PCR

PCR for the detection of *Brucella* antigen was only performed on original samples and not on concentrated or enriched samples.

For DNA extraction, 20 mg tissue was incubated in ATL buffer (Qiagen, Germany) at 56°C for 10 min and the lysate was then loaded onto the MagNA Pure automated extraction platform (Roche Diagnostics

Ltd, UK). DNA was extracted using Magna Pure LV DNA extraction kit according to the manufacturer's instructions. The PCR was performed according to the method described previously (Probert *et al,* 2004), using Light Cycler® 2.0 instrument (Roche, Germany). PCR cycling conditions used were: initial denaturation at 95°C for 10 min followed by 45 cycles of 95°C for 15sec and 57°C for 1 min. Samples with a fluorescence signal observed before 40 cycles were scored as positive.

Serology

The dromedary dam was periodically monitored for brucellosis for the last 6 years with Rose Bengal Test (antigen - APHA Scientific-RAA0060, UK) according to methods described previously (OIE, 2018).

Clinical and molecular epidemiology

As part of the *Brucella* monitoring program, all adult dromedaries on the farm have been subjected to RBT 2-4 times a year. The positive dam was in contact with another 78 pregnant animals in 2 separate locations during a 3 month period between the time of the negative (20.3.2018) and the positive (20.6.2018) *Brucella* RBT tests. All these camels have been tested repeatedly with RBT (last date of testing: 22.11.2018). After testing positive for Brucellosis, the pregnant camel was immediately separated into a relatively small paddock (10x15 m) far from the pregnant group (> 400 m). Another camel had been moved to the same location approx. 2 weeks before the positive dam aborted. The abortion took place on 4th of August 2018 spontaneously without assistance. This contact animal has been under continuous monitoring by weekly blood samples for RBT (the time of the last testing more than 3.5 months after the abortion). In addition, DNA extract of the isolated *B. melitensis* strain was examined by multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) with different typing schemes (MLVA 8, 11 and 16) as described earlier (Gyuranecz *et al,* 2016). MLVA 8 (consisting of loci of panel 1) and MLVA 11 (including loci of panels 1 and 2A) was used to compare the genetic relatedness of this strain to previously isolated strains from the same herd. MLVA 8 uses only 8 allels. MLVA 8 is a very robust but less discriminative method, while the MLVA 11 uses 11 allels and is more discriminative method. The raw MLVA data were analysed and phylogenetic trees were constructed using neighbour-joining method with the MEGA 7 software.

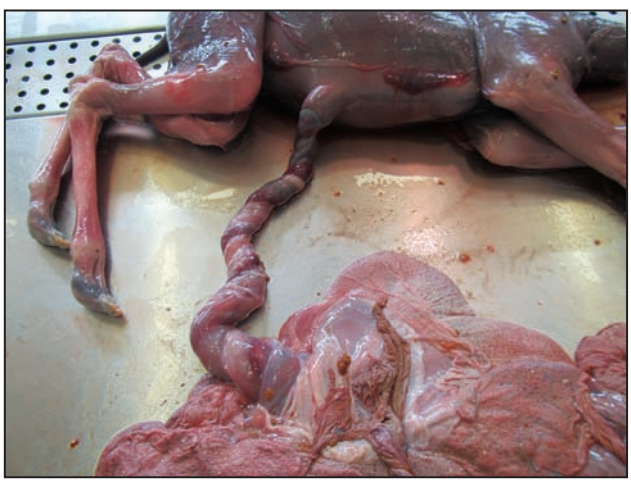

Fig 1. Female aborted foetus with greyish, oedematous placenta and twisted umbilical cord.

Results

Pathology

Aborted foetus

The aborted female foetus (13 kg, 50 cm crown–rump length) was in a fair and fresh condition at necropsy. On gross examination the placenta appeared greyish and oedematous and the umbilical cord was six times twisted (Fig 1). Both pleural and abdominal cavities were filled with bloody fluid. The lungs of the foetus were covered with a net of red fibrin which was attached to the costal pleura (Fig 2). The right lung was more affected showing marbled appearance of the surface and massive congestion of the caudal lobe. Pleurisy was observed. The liver was very soft and the C1 contained red fluid with small clusters of brownish soft material. Histopathology revealed marked oedema and diffuse severe mineralisation of the superficial chorionic stroma of the placenta with focal detachment of trophoblasts (Fig 3). However, no inflammation, no

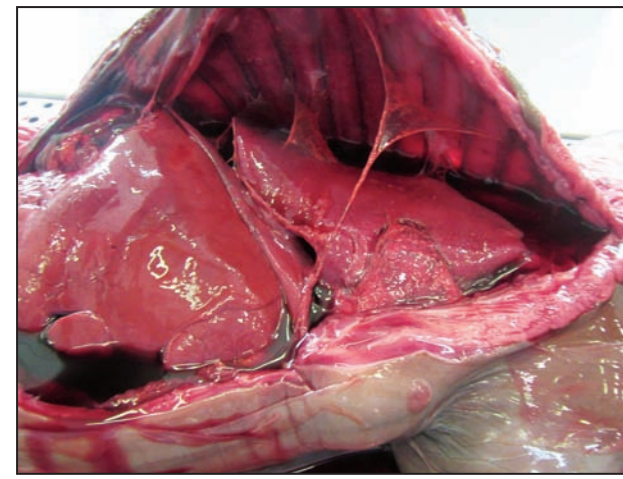

Fig 2. Pleural cavity of the aborted foetus filled with bloody fluid. The lung is covered with a net of red fibrin that is also attached to the costal pleura.

fungus, no parasites, no viral inclusions were seen. The lung showed cellular debris in subpleural alveoli and bronchioli (Fig 4).

Dromedary dam

During necropsy of the female camel (534 kg) many swollen body lymph nodes including mediastinal and intestinal lymph nodes were seen. The udder was in lactation and the lung showed massive congestion. Histopathology of the lymph nodes revealed marked proliferation of follicular and parafollicular lymphatic tissue as well as central oedema with small haemorrhages. Udder and uterus did not show any signs of inflammation.

Culture and PCR

Heavy growth of *Brucella* spp. was observed in all 7 foetal specimens and placenta when cultured by direct and concentration methods. Hence, culture from the enrichment broth tubes was not performed.

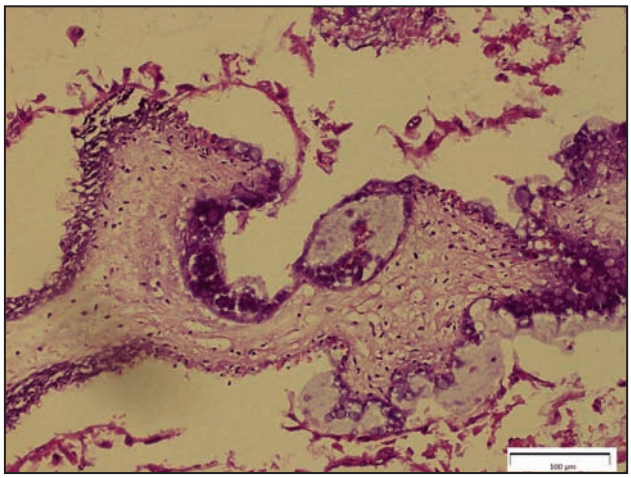

Fig 3. Light micrograph of the placenta with focal detachment of trophoblasts with a lot of cellular debris (H&E stain).

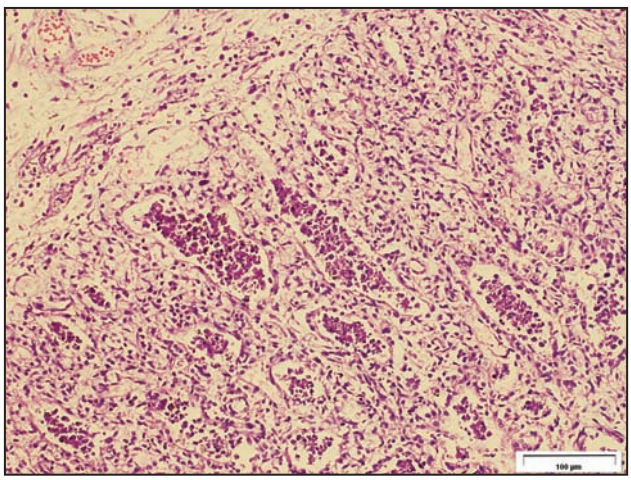

Fig 4. Light micrograph of the foetal lung with a lot of cellular debris in subpleural alveoli and bronchioli (H&E stain).

All 8 tested specimens showed 'mat growth' on both the selective agars. *B. melitensis* was also detected in all the specimens, i.e. both lungs, liver, umbilical cord, pleural and abdominal fluid, C1 content and placenta by PCR.

The culture and PCR results of specimens from dam and the enumeration results of specimens from aborted foetus and placenta are summarised in Tables 1 and 2.

Table 1 summarises the results of *B. melitensis* culture and PCR from the 20 necropsy specimens and EDTA blood from the dam. From a total of 20 specimens cultured, *B. melitensis* was isolated from 9 specimens: different body lymph nodes and the right

Table 1. *B. melitensis* culture and PCR results of 20 specimens and EDTA blood from the dam.

S. No	Specimens	Culture Results (Enrichment method)	PCR Results (Original specimens)
1	Right submandibular lymph node	Isolated	Not detected
2	Left submandibular lymph node	Not isolated	Not detected
3	Pharyngeal lymph node	Isolated	Not detected
4	Left pharyngeal lymph node	Isolated	Not detected
5	Lateral retropharyngeal lymph node	Isolated	Not detected
6	Prescapular lymph node (dorsales)	Isolated	Not detected
7	Prescapular lymph node (ventrales)	Isolated	Not detected
8	Lymph node mediastinales medii	Not isolated	Not detected
9	Lnn tracheobronchales medii	Isolated	Not detected
10	Lnn tracheobronchales sinistri	Not isolated	Not detected
11	Lung lymph node	Isolated	Not detected
12	Liver	Not isolated	Not detected
13	Right uterine horn	Not isolated	Not detected
14	Left uterine horn	Not isolated	Not detected
15	Milk	Not isolated	Not detected
16	Udder left hind quarter	Not isolated	Not detected
17	Udder right hind quarter	Isolated	Detected
18	Udder left front quarter	Not isolated	Not detected
19	Udder right front quarter	Not isolated	Not detected
20	Sodium citrate blood	Not isolated	Not detected
21	EDTA blood	Not applicable	Not detected

Lnn = lymph nodes

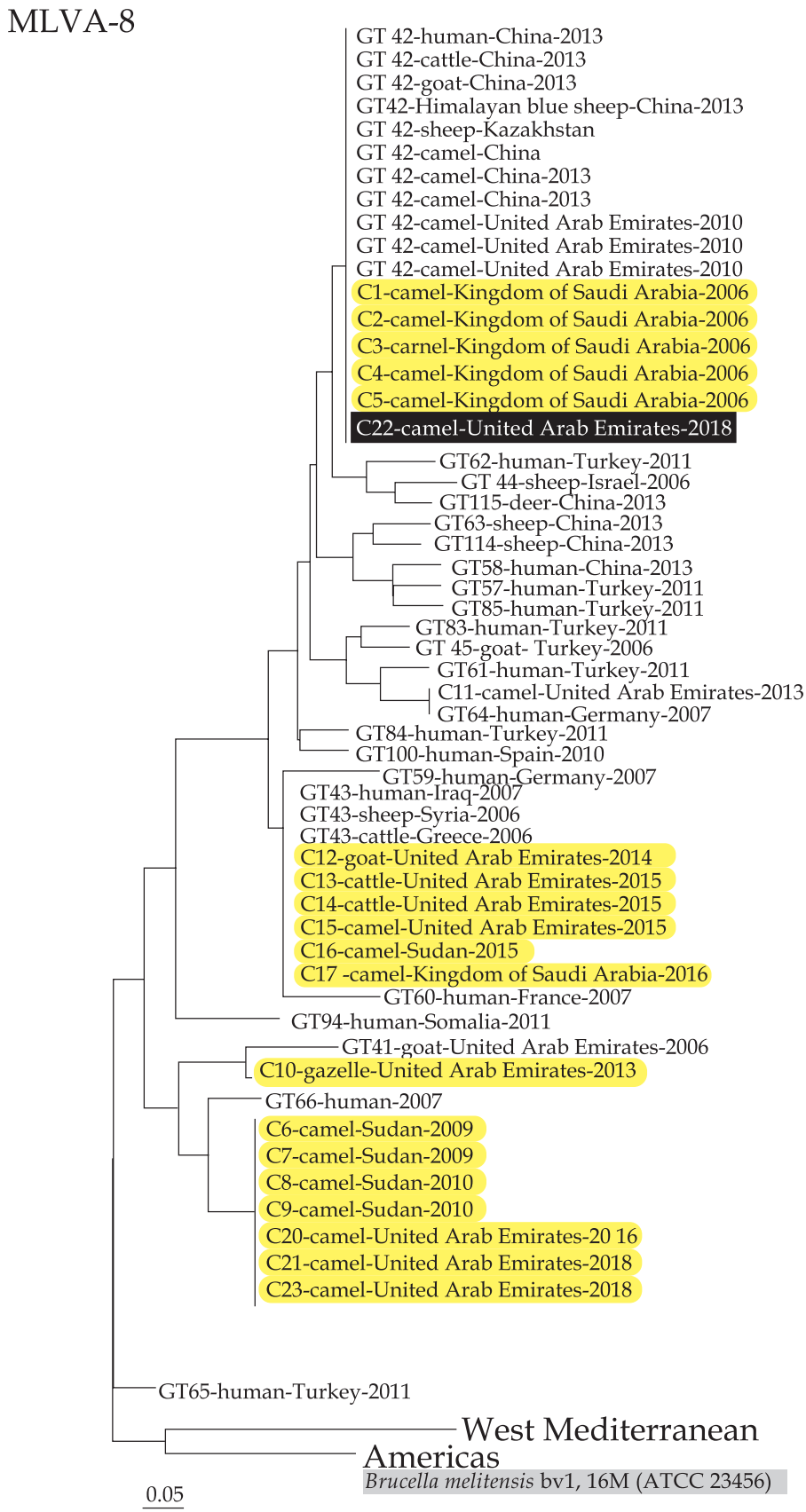

Fig 5. Neighbour joining analysis of *Brucella melitensis* isolate using multiple-locus variable-number tandem repeat analysis (MLVA) data. Isolate from the study are typed black while previous strains are highlighted in yellow (Gyuranecz *et al*, 2016).

hind udder tissue. *B. melitensis* was only detected in the right hind udder tissue by PCR.

Colony forming units of *B. melitensis* isolated per g/ml of each specimen from the aborted foetus and placenta are presented in Table 2 as mean counts obtained from 2 selective agars. In all specimens, *Brucella* was found in very high concentration ranging from 10^6 – 10^{10} cfu/g/ml. The highest concentration of bacteria was found in the placenta with 10^{10} cfu/g followed by C1 content and pleural fluid with 10^9 cfu/ml each.

Serology

Table 3 summarises the results of periodical serological screening of the dam for brucellosis throughout a period of 6 years (2012-2018). The dam was serologically negative for brucellosis by RBT over a period of 6 years. In June 2018, the dam suddenly tested positive for brucellosis and thereafter in July and August 2018, after the abortion.

Clinical and molecular epidemiology

All 78 pregnant camels that were in contact with the positive dam before or during the time of infection, remained negative for Brucellosis with RBT (last date of testing: 22.11.2018). In addition, the contact animal that was kept together with the infected dam at the time of abortion also remained negative until the time of the last testing (22.11.2018. i.e. more than 3.5 months after the abortion). The genotype of this strain was identified as MLVA8 GT42 and MLVA11 GT108. Very similar or identical strains have been described earlier in dromedary camel (Gyuranecz *et al,* 2016). The genetic relatedness of this *Bruce1lla* melitensis strain to previous isolates is shown on the neighbour-joining analysis of MLVA8 in Fig 5. This strain clustered into the main East-Mediterranean genetic group.

Discussion

Old World camels are frequently infected with brucellosis, particularly when they come into contact with infected ruminants (Wernery, 2016). Brucellosis in NWCs is rare, but outbreaks with classical signs of brucellosis have been described (Fowler, 2010). Humans are at risk through the consumption of unpasteurised milk (Wernery *et al,* 2014) but modes of transmission occur also by contact through skin with animal tissues, blood, urine, vaginal discharge and aborted foetuses, especially placentas. The pathogen is excreted in large numbers in aborted foetuses, foetal membranes and uterine discharge.

Table 2. *B. melitensis* colony forming units from aborted foetus specimens and placenta.

Specimens	Colony forming units per gram/ millilitre (cfu/g/ml)
Left Lung	1.8×10^8
Right Lung	1.4×10^8
Liver	1.2×10^6
Umbilical cord	4.6×10^7
Pleural fluid	6.0×10^9
Abdominal fluid	4.0×10^8
C1 content	3.0×10^9
Placenta	1.46×10^{10}

Table 3. Brucellosis serum RBT results obtained since 2012 from the dam.

Order	Date	Test location	Result
1	20-Nov-2012	CVRL	Negative
2	10-Jun-2013	CVRL	Negative
3	11-Dec-2013	CVRL	Negative
4	29-Apr-2014	CVRL	Negative
5	20-Nov-2014	CVRL	Negative
6	6-Jun-2015	CVRL	Negative
7	5-Dec-2015	CVRL	Negative
8	17-May-2016	CVRL	Negative
9	1-Sep-2016	DM VSS	Negative
10	18-Dec-2016	Farm in house	Negative
11	15-Feb-2017	DM VSS	Negative
12	16-Jul-2017	Farm in house	Negative
13	11-Sep-2017	DM VSS	Negative
14	14-Dec-2017	Farm in house	Negative
15	20-Mar-2018	Farm in house	Negative
16	27-Mar-2018	DM VSS	Negative
17	20-Jun-2018	Farm in house	Positive
18	24-Jun-2018	CVRL	Positive
19	25-Jun-2018	Farm in house	Positive
20	26-Jun-2018	CVRL	Positive
21	27-Jun-2018	Farm in house	Positive
22	5-Jul-2018	Farm in house	Positive
23	5-Aug-2018	CVRL	Positive
24	6-Aug-2018	Farm in house	Positive

CVRL = Central Veterinary Research Laboratory

DM VSS = Dubai Municipality Veterinary Service Section

In cattle, it is known that abortion is associated with the shedding of 10^{12} to 10^{13} *Brucella* bacteria per gram material. Survival of the organisms in the environment is enhanced by cool temperatures and high humidity, but in one case, it was proven that in the hot desert environment, 2 dromedaries in a herd

became infected with *B. melitensis* most probably through contaminated dust particles from aborted camel foetuses 500 metres away (Wernery *et al,* 2014). Air borne infections occur in animal pens, stables and laboratories (Schulze zur Wiesch *et al,* 2010). Many placental mammals, including herbivores, participate in placentophages, in which camelids are a noted exception; this fact may contribute to the spread of *Brucella* through wind.

As described in cattle and most probably in domestic small ruminants and wild ruminants, also in dromedary camels proven by this investigation, hundred million of *Brucella* pathogens per gram or millilitre are excreted with the aborted foetus. This will consequently contaminate the area where the abortion takes place which is under desert conditions mainly sand. Lochia and afterbirth material will quickly dry, but as camels are gregarious animals and very curious, they will lick and sniff at placenta and the aborted foetus. Infection occurs mainly *via* the mucous membranes of the respiratory tract and most probably also through conjunctiva. Despite this, in the present case, it seems that the contact animal did not get infected due to biosecurity measures. The staff of the farm immediately removed the foetus, placenta and the contaminated soil and also disinfected the area. This could be the reason why the other camel did not contract the disease in spite of high bacterial load of the aborted foetus.

Brucellosis is an important zoonotic disease and in humans, is characterised by recurrent fever, night sweats, joint and back pain and general weakness. People at greatest risk are those who drink unpasteurised milk and attend parturient animals. It is mainly a disease of professionals working with livestock.

As described earlier (Johnson *et al,* 2018), enrichment of tissue samples and culture on BHI agar gave the most reliable results for the isolation of the pathogen. *B. melitensis* was isolated, from 9 (45%) out of 20 specimens of the dam, mainly from lymph nodes of the respiratory tract area. It was unexpected that the left and right uterine horns were already free of the pathogen 3 weeks after the abortion. However, *B. melitensis* was cultured from the right hind quarter of the mammary gland.

According to previous studies (Johnson *et al,* 2018), PCR results were negative when specimens were tested directly without concentration or enrichment. In this study, the right hind udder tissue of the dam was positive by PCR (Table 1). However, PCR became positive when the original specimen contained high concentration of *B. melitensis*. This is evident from the positive PCR results of the aborted foetus specimens in which *Brucella* was present in high numbers (Table 2). The same was observed in the right hind udder tissue of the dam (Table 1), the only organ from which *Brucella* bacteria was isolated in high numbers. Culture remains the "gold standard" for the diagnosis of brucellosis.

The dam was from a closed camel dairy farm with no connection to any large or small ruminants. The herd is professionally managed and the entire herd is regularly tested for different infectious diseases including brucellosis using serological tests. One of the most reliable tests for camel brucellosis is the Rose Bengal Test from Vircell, Spain. Different serological tests with *B. melitensis* experimentally infected dromedaries were evaluated (Söellner, 2018). It remains obscure, why in 2018 suddenly after 6 years of being brucellosis negative the dromedary camel became positive (Table 3). Three weeks after the abortion, it was decided to euthanise the dam. *B. melitensis* was predominantly cultured from different lymph nodes where they hide as previously described (Wernery *et al,* 2007) in non-pregnant lactating camels.

The pathology of the placenta and the foetus was not pathognomonic for brucellosis. Similar findings have been described for abortions not caused by Brucellosis. Besides the greyish and oedematous placenta, the most striking pathological lesion seen on the aborted foetus was the 6 times twisted umbilical cord. Similar to most camel abortions with twisted umbilical cord seen at CVRL, both, the chest and abdominal cavities were filled with bloody fluid. Histopathology revealed marked oedema and diffuse mineralisation of the superficial chorionic stroma of the placenta with focal detachment of the trophoblast. Surprisingly, no inflammation was seen in the placenta. This is very similar to histopathological findings described in llamas and alpacas after abortion (mainly mineralisation and low incidence of placentitis) (Schaefer *et al,* 2012). The lung showed cellular debris in subpleural alveoli and bronchioli. However, similar lesions in aborted foetuses caused by natural *B. abortus* infection have been described (Narnaware *et al,* 2016), additionally with necrotising placentitis and foetal pneumonia during mid to last trimester of pregnancy.

Abortion caused by *B. abortus/melitensis* is associated with massive replication of the pathogen within the chorioallantoic trophoblasts of the placenta.

This extensive intracellular replication ruptures the infected trophoblasts releasing the bacteria and infecting the foetus. The infection of the calf and loss of placental integrity leads to the abortion or the birth of weak, infected calves (Saegerman *et al,* 2010).

The necropsy of the dam revealed no lesions besides many swollen lymph nodes, showing marked proliferation of follicular and parafollicular lymphatic tissue as well as central oedema with small haemorrhages in histopathology. Udder and uterus did not show any signs of inflammation.

Very little is known about the pathological changes caused by *Brucella* organisms in camelids. Previous studies (Abu Damir *et al,* 1984) found lesions after artificial infection of non-pregnant dromedaries with *B. abortus* in the cranial and genital lymph nodes, which showed follicular hyperplasia of cortical and paracortical areas of medullary cords and sinusoidal congestion. A mild interstitial hepatitis was also observed. The other authors (Nada and Ahmed, 1993; Wernery et al, 2007) described lesions in serologically and culture positive but non-pregnant natural infected adult dromedaries. They included inflammation with reddening of the uterus lining, fibrosis of the endometrium and atrophy of the uterine glands. Hydrobursitis was also observed, enlarging the bursa which was then filled with clear amber coloured fluid. Histopathological investigations are rare and were mainly found in lymph nodes, from which *B. melitensis* was isolated (Wernery *et al,* 2007). They showed marked sinusoidal oedema, activated follicles and histiocytosis, similar alterations as seen in this case.

In conclusion, we described an abortion case in a dromedary camel caused by a *B. melitensis* strain belonging to the East-Mediterranean genetic group (GT42 by MLVA8 and GT108 by MLVA11). The dromedary was infected during mid gestation from an unknown source, developed bacteriaemia and the acute infection resulted in abortion approx. 2 months after the infection. This specific strain of the bacteria has never been isolated on the farm but was identical or closely related to strains isolated 12 years earlier from camels originating from Kingdom of Saudi Arabia. The pathological findings in the foetus and the placenta were not pathognomonic for Brucellosis. Surprisingly, no inflammatory changes, only oedema, diffuse mineralisation and focal detachment of the trophoblast were detected in the placenta. But, hundreds of billions of *Brucella* pathogens were excreted to the environment with the aborted foetus and the placenta. Within a short time after abortion, the bacteria disappeared from the blood and the uterus of the dam and was isolated only from some of the lymph nodes and right hind udder tissue. The *Brucella* seropositive camel in its acute phase of the disease did not infect other contact animals. In addition, with appropriate biosecurity measures we could prevent the transmission of the disease to other dromedaries at the time of abortion. Successful prevention required precise laboratory diagnosis and thorough and continuous monitoring of the animals.

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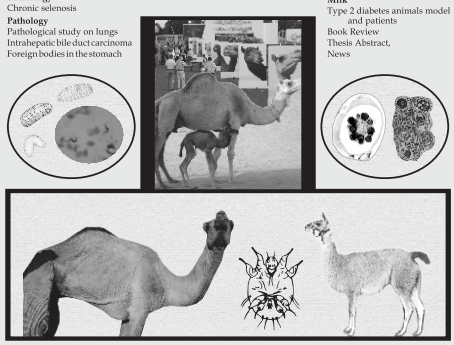

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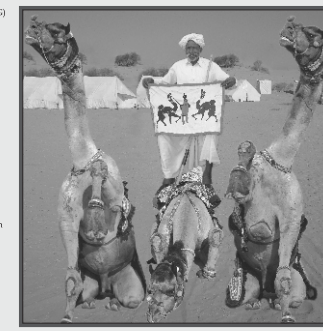

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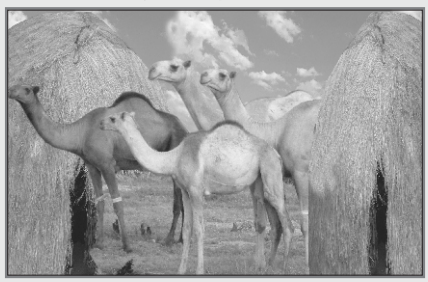

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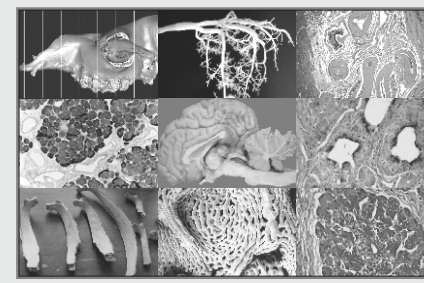

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THE ONE-HUMPED CAMEL IN BANGLADESH

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ABSTRACT

The one-humped camel is non-native to Bangladesh. A very few camels have been imported from India for production purposes and others are imported for meat purpose. In spite of intensive management on the one-humped camel farm in the country, production and productivity are low. Camels suffer from several production diseases. Imports of camels from India infected with or seropositive to Middle East respiratory syndrome-related coronavirus (MERS-COV) pose a risk to human health. Climate, physical and social environments are inimical to camel production. Development efforts should concentrate on the native and adapted species already in the country in order to improve the livelihoods and welfare of the Bangladeshi people.

Key words: Animal diseases, introductions, middle east respiratory syndrome-related coronavirus, milk production

Background

The People's Republic of Bangladesh is a developing country with a market-based mixed economy. Annual per caput income was estimated at USD 1,754 for 2018. The country is the easternmost of the South Asia region. The country is bordered on the west, north and east by India and by Burma (Myanmar) on the southeast.

The climate is tropical with a mild winter from October to March, a hot, humid summer from March to June and a monsoon season, during which most rain falls, lasting from June to October. About 80 per cent of the annual rain falls during the monsoon season usually in heavy storms. Maximum summer temperatures range between 30°C and 40°C. April is the warmest month in most parts of the country whereas January is the coldest month. Average annual relative humidity is 65.8 per cent, ranging from 45 per cent in March to 79 per cent in June (Hamid *et al*, 2016).

The share of agriculture in the national Gross Domestic Product (GDP) fell from an annual figure of around 60 per cent in 1960-1980 since when it has declined steadily to about 15 per cent in 2016. Agriculture, however, still employs about 45 per cent of the workforce and more Bangladeshis earn their living from agriculture than from any other sector (World Bank, 2018).

Bangladesh has a broad range of domestic animals that includes buffalo, cattle, goats, sheep and poultry. All species increased in number between 1961 and 2016 with the exception of buffalo (Table 1). Buffalo numbers have been decreased as the provision of energy for crop production and general transport as animal's draught power has been replaced by small tractors. The enormous increase in poultry numbers relate to industrial production of eggs and meat. The ruminant species production is almost entirely in the hands of smallholder farmers whose management is traditional and product output on a per head basis is low. Meat and milk supply is woefully below the national demand.

Camels

Camels were imported at some unknown time in the past by Dhaka Zoo but these did not survive (Dewanbag Sharif, 2018). Some camels were imported by a private entrepreneur into Bangladesh from Rajasthan in India in 2004 (Arifur Rahman Rabbi, 2016), who set up Babe Madina Camel Farm (Fig 1) (Dewanbag, 2018). By 2016, the number at the farm had increased to 36 which, given the reproductive and probable mortality rates of Bangladeshi camels that there have been further imports. In 2018, there were 45 camels at Baba Madina including 13 that had been born there (Fig 2). One calf was born at the farm on 16 September from a female that had conceived

Table 1. Livestock numbers (millions) in Bangladesh in 1961 and 2016.

Year	Livestock species				
	Buffalo	Cattle	Goat	Sheep	Poultry
1961	5.00	1.98	7.60	0.60	16.00
2016	1.47	2.38	56.10	2.17	268.92

Source: FAO, 2018

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Fig 1. Publicity poster for Babe Madina camel farm (Source: Dewanbag Sharif, 2018).

Fig 2. The camel herd at Babe Madina (Source: Arifur Rahman Rabbi, 2016).

before arrival and then calves were born from females conceived in Bangladesh on 12 February and 14 February 2006 (Dewanbag, 2018).

The presence of camels in Bangladesh has provided the opportunity for local research. The productive and reproductive performances of 28 female camels were evaluated at Babe Madina. The average milk yield of four females aged 10 years was 5.59 ± 1.11 litres whereas that of camels aged over 10 years (n = 4) was 3.28 ± 0.55 litres, the difference between the two age group being highly significant ($p < 0.01$). Lactation length in the younger group was 313.7 ± 7.41 days whereas in the older group it was 261.50 ± 56.15 ($p < 0.01$). Lactation yield in 5-10 year olds was 1839.25 ± 1021.0 but only 901.00 ± 415.05 in the over 10 years group. There were significant differences between age groups in milk composition for fat, solids not fat and ash but not for lactose and protein (Table 2). There were similar significant differences between the age groups in the number of services per conception, post-partum oestrus period, length of oestrus cycle and calving interval with the older group always performing less well than the younger one: there were no differences in the gestation period of about 369 days. The incidence of mastitis, dystocia and abortion in 18 cases was low (Fazal *et al,* 2017).

Table 2. Differences in milk composition between two age groups of camels in Bangladesh.

Component (per cent)	Age (years)	
	5-10 (n=4)	above 10 (n=4)
Fat	3.35 ± 0.37	4.37 ± 0.70**
Solids not Fat	8.34 ± 0.24	9.17 ± 0.49**
Lactose	4.75 ± 0.34	4.78 ± 0.76NS
Protein	3.88 ± 0.22	3.80 ± 0.08NS
Ash	0.73 ± 0.05	0.76 ± 0.11*

Source: Fazal *et al,* 2017

Samrat Milk Products (an Indian company) distributes processed and packed camel milk from Babe Madina Camel Farm. It is claimed that camel milk cures some incurable diseases including cancer, diabetes, tuberculosis, stomach ulcers, gastro-enteritis, psoriasis, multiple sclerosis, Cohn's diseases, severe skin condition and auto-immune diseases and that many people have been cured from chronic diseases by consuming camel milk (Dewanbag Sharif, 2018).

Data from farm records at Babe Madina were compiled in a retrospective study of diseases for the period 2012-2014 covering 43 camels in 2012, 38 camels in 2013 and 42 camels in 2014. Over the whole 3-year period disease prevalence was 33.33, 50.00 and 0.00 per cent in the age group 0-1.5 years in the three successive years, was 26.66, 20.00 and 28.57 per cent in camels 1.5 to 3 years of age and 28.00, 20.00 and 30 per cent in animals aged over three years. Disease incidence was higher in male than in females throughout the period. A higher percentage of animals suffered disease problems in the rainy season than in the winter season which was in turn higher than in the summer season. Mange was observed in 14 per cent to 18 per cent in the three years: *Sarcoptes scabiei var. cameli* was the only mite species in all skin scraping samples. Gastro-intestinal parasites were identified in 228 per cent of animals in the various years. Abscesses and allergic dermatitis were also identified as problems. Mastitis was identified in 7-15 per cent of animals depending on the year: fasciolosis was present in less than 3 per cent of animals throughout the period and ringworm and tick infestations were also minor problems. Brucellosis was seen 2.3 per cent of animals in 2012,

in 0.0 per cent in 2013 and 2.38 per cent in 2014 (Islam *et al*, 2016).

Bangladeshi traders bought "scores" of camels at the Pushkar Fair in India in 2004 for transport to Bangladesh for slaughter. These camels belonged to the Raika tribe, once celebrated for their camel culture but apparently turning to other occupations, and whose animals are an endangered breed. The Indian Congress refused a proposal to export camels to Bangladesh when it was learned they were intended for slaughter but in February 2004 Indian and Bangladeshi paramilitary forces exchanged fire as camels were being smuggled in to Bangladesh (Prakash Bandari, 2005). The trade in camels between India and Bangladesh continues.

One-humped camels are a natural host for Middle East respiratory syndrome coronavirus (MERS-CoV) which can cause fatal respiratory disease in humans. During the September–October 2015 festival of Eid ul Adha samples were collected and tested for coronaviruses from 36 camels at Babe Madina and 19 camels at a Dhaka urban market. Some 24 of the farm camels were born there whereas the remaining 12 and all the market camels were imported from India. None of the samples tested positive for MERS-CoV by Polymerase Chain Reaction (PCR) but testing with Enzyme-Linked Immuno Sorbent Assay (ELISA) showed 98.6 per cent specificity and sensitivity compared with the pseudoparticle neutralisation test. MERS-CoV antibodies were detected in 31 per cent of the samples with adults having a higher seroprevalence (36 per cent) than juveniles (9 per cent). Imported camels had a significantly higher seroprevalence (52 per cent) than domestically bred camels (4 per cent). Among the five seropositive farm camels only one was a domestically bred adult whereas the other four adults were from India. Market camels had a seroprevalence of 63 per cent whereas prevalence was only 14 per cent in farm camels (Islam *et al*, 2018).

Discussion and conclusions

The one-humped camel is not native to Bangladesh. The existing very small camel population is confined to a single farm. There has been no successful outreach to other producers and local farmers and have no tradition or experience of the animal and its management. Almost the whole of the climate and physical environments of Bangladesh are inimical to camels ad camel production. The claim that "camels play an important role to improve the livelihood in the Bangladesh" (Fazal *et al*, 2017) can not be substantiated. Were "development interventions [to] take into consideration the socio-economic characteristics of camel herders and the prevailing problems in the area" (Fazal *et al*, 2017) the prognosis could only be that there is no future for camels in Bangladesh.

The camel in Bangladesh is clearly not fit for purpose and not the right animal in the right place (Wilson, 2009). It is the wrong animal at the wrong place and development interventions for increased livestock productivity should concentrate on existing and adapted livestock resources to improve the livelihoods and welfare of the people.

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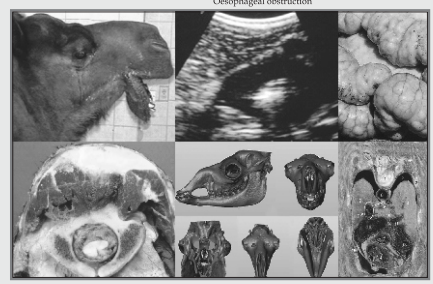

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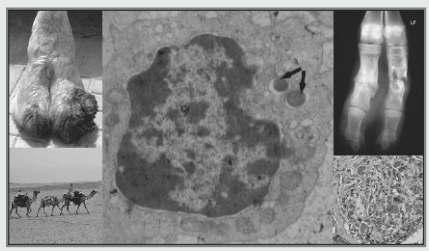

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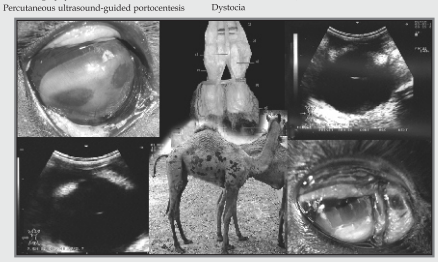

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PANCREATIC HORMONES OF DROMEDARY CAMEL: IMMUNOHISTOCHEMICAL LOCALISATION

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ABSTRACT

The persent study was aimed to investigate the endocrine cell types of the pancreas of the dromedary camels and the pattern of their distribution in the islets of Langerhans and within the different lobes, using the immunohistochemical techniques. Therefore, specimens from 5 camels (*Camelus dromedarius*) of both sexes at different ages (2-12 years) were used. The insulin, glucagon, somatostatin and pancreatic polypeptide immunoreactive cells were detected in the camel pancreas by light microscope immunhistochemistry. The current findings indicated that the insulin immunoreactive cells were located centrally in most islets. In several islets, glucagon positive cells were found in the peripheral part forming a rim or a circular zone. They were fewer in number compared to the insulin cells. The somatostatin immunoreactive cells were randomly scattered in the islets. These were characterised by long or short cytoplasmic processes. The pancreatic polypeptide reactive cells were fewer in number and peripherally located in the islet of Langerhans. These were relatively large and some have cytoplasmic processes. In conclusion, the pancreatic tissues and islets contained the insulin, glucagon, somatostatin and pancreatic polypeptide immunoreactive cells and are well distributed in the pancreas of the camel.

Key words: Camel, glucagon, immunohistochemistry, insulin, pancreas, polypeptide, somatostatin

The endocrine components of the mammalian pancreas are composed primarily of insulin (β-cells), glucagon (α-cells), somatostatin (Δ-cells) and pancreatic polypeptide (PP- cells) immunoreactive cells (Heller, 2010; Hafez *et al,* 2015). The presence of these endocrine cell types has been reported in ruminants, i.e. in bovine (Reddy and Elliott, 1985; Nakajima *et al,* 1988; Hiratsuka *et al,* 1996) sheep (Calingsan *et al,* 1984) and camel (Khatim *et al,* 1985; Adeghate, 1997).In camel, β-cells are located in the central or both central and peripheral parts of the pancreatic islets (Khatim *et al,* 1985; Adeghate, 1997; Hafez *et al,* 2015). The α-cells are fewer in number and larger in size than the β cells (Hafez *et al,* 2015). These are found in the right lobe of the pancreas of most other domestic animals (Benscosme and Liepa, 1955; Lacy, 1957; Calingsan *et al,* 1984; Nakajima *et al,* 1988; Hafez *et al,* 2015). The Δ-cells are of relatively rare occurrence and are often randomly distributed in goat, camel, dog and cat (Alumets *et al,* 1975; Khatim *et al,* 1985; Reddy and Elliott, 1985; Nakajima *et al,* 1988; Hafez *et al,* 2015). However, they are predominantly peripherally located in the islets of bovine, sheep, horse and rat (Helmstadeter *et al,* 1976; Calingsan *et al,* 1984; Nakajima *et al,* 1988; Adgehate, 1999; Hafez *et al,* 2015). The pancreatic polypeptide (PP) cells are relatively large and some of them had a long or short cytoplasmic processes (Nakajima *et al,* 1988). These are fewer in number than the insulin, glucagon and somatostatin cells (Reddy and Elliott, 1986). The PP- cells are mainly located in the peripheral parts of the islets of the pancreas of camel and rat (Khatim *et al,*1985; Adeghate, 1999; Long, 1983). Types and distribution patterns of endocrine cell types in pancreas of dromedary camels are not will studied. Therefore, the present investigation was undertaken to study the endocrine cell types of the pancreas of the dromedary camels and the pattern of their distribution in the islets of Langerhans and within the different lobes by using the immunohistochemical techniques.

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Materials and Methods

Animals and tissue preparation

Specimens of the pancreas were collected from 5 camels (*Camelus dromedarius*) of both sexes, aged 2-12 years, from Al-Hasa slaughterhouse and from the Camel Research Centre, King Faisal University, College of Veterinary Medicine, Saudi Arabia. These specimens were apparently normal and free from gross pathological changes. 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 persent study, we used 2 alternative methods for demonstration of endocrine cells; the streptavidin biotinylated horseradish peroxidase complex method and Dako labelled streptavidin biotin plus kit. 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 are 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 in the persent study was not observed.

Table 1. The primary antisera used in present study.

Antibody	Dilution	Source
Rabbit anti-Glucagon	1:40	Serotec, UK
Guinea pig anti-Insulin	1:100	Dako, USA
Rabbit anti-Pancreatic polypeptide	1:600	Dako, USA
Rabbit anti-Somatostatin-14	1:100	Serotec, UK

Results and Discussion

Insulin immunoreactive cells (β-cells)

Insulin immunoreactive cells formed the majority of the islet of Langerhans cells, however, insulin immunoreactivity was demonstrated in the pancreatic islets as evenly distributed throughout the pancreas. Single positive cells within the exocrine portion of the pancreas were observed (Fig 1A, B). They occupied the large central part of the islet leaving only a small peripheral zone or rim of unstained cells (Fig 1C, D). The β-cells were arranged in cord like clusters separated by vascular connective tissue septa that often give the pancreatic islets their multilobular appearance (Fig 1C, E). The cells were large in size, round or oval in shape with oval nuclei (Fig 1B). Few β-cells were also found in the duct epithelium (Fig 1F, G). Some cells were observed abutting or adjacent to the basal part of the duct epithelium (Fig 1H). Insulin immunoreactive cells were also observed in pancreatic islets occupying the interlobular connective tissue septa (Fig 1I).

Glucagon immunoreactive cells (α-cells)

The present investigation indicated that glucagon immunoreactive cells were fewer in number compared to insulin positive cells. These cells were polyangular in shape and had rounded nuclei (Fig 2A). They were usually located in the periphery of the islets forming a rim of single cell layer or circular zone (Fig 2B, C, D). Positive cells were sometimes encountered in the central parts of the islets (Fig 2E). Some single cells were scattered in the parenchyma (Fig2A), others abutting to the duct epithelium or in the connective tissue beside the duct (Fig 2F). It was noticed that the number of the A-cells were fewer in the right lobe compared to the rest of the pancreas.

The somatostatin immunoreactive cells (Δ-cells)

The persent findings reported that the somatostatin immunoreactive cells were fewer in number than the α-cells. These were fusiform or pyramidal in shape, with rounded nuclei and short or long cytoplasmic processes (Fig 3A). The Δ-cells were randomly distributed in the islets (Fig 3B) and also frequently distributed throughout the parenchyma (Fig 3C). Sometimes, Δ-cells were noticed within the duct epithelium and in the connective tissue around the ducts.

Pancreatic polypeptide immunoreactive cells (PP-cells)

In the persent findings, the pancreatic polypeptide immunoreactive cells were relatively large; pyramidal in outline and some of them showed long cytoplasmic processes (Fig 4A). These were scarce compared to insulin, glucagon and somatostatin immunoreactive cells. The PP-cells were mainly located in the peripheral parts of the islets of

the pancreas (Fig 4A, B). Occasionally, few PP-cells were found in the centre. Some PP-cells were also observed in the exocrine part of the pancreas, within the duct epithelium and in the connective around the ducts (Fig 4C, C).

Insulin, glucagon, somatostatin and pancreatic polypeptide immunoreactive cells were detected in the pancreas of camel by light microscopic immunohistochemistry (Hafez *et al,* 2015). These 4 endocrine cells were also demonstrated in the pancreas of camel previously (Khatim *et al,* 1985; Adeghate, 1997). Insulin immunoreactive cells (β-cells) were evenly distributed in the pancreatic islets in all parts of the pancreas. Single cells were also noticed within the exocrine portion of the pancreas. Khatim *et al* (1985) and Adeghate (1997) presented similar findings in the camel pancreas. Studies in other domestic animals have shown similar results (Reddy and Elliott, 1985; Furuzawa *et al,* 1992; Hafez *et al,* 2015). This study has also shown some β-cells in the ductus epithelium or abutting to the basal part of the duct epithelium, a finding that has not been reported in the camel before. But similar observation has been reported in the horse pancreas (Helmstaedter *et al,* 1976; Hafez *et al,* 2015). The insulin immunoreactive cells occupied a large central part of islet, confirming the previous observation in the camel pancreas by Khatim *et al* (1985). In contrast, the distribution of β-cells was different in equine, in which the β-cells occupied the peripheral part of the islet (Helmstaedter *et al,* 1976). The β-cells were arranged in cord like clusters separated by vascular connective tissue septa that often give the pancreatic islets their multi-lobular appearance. Similar

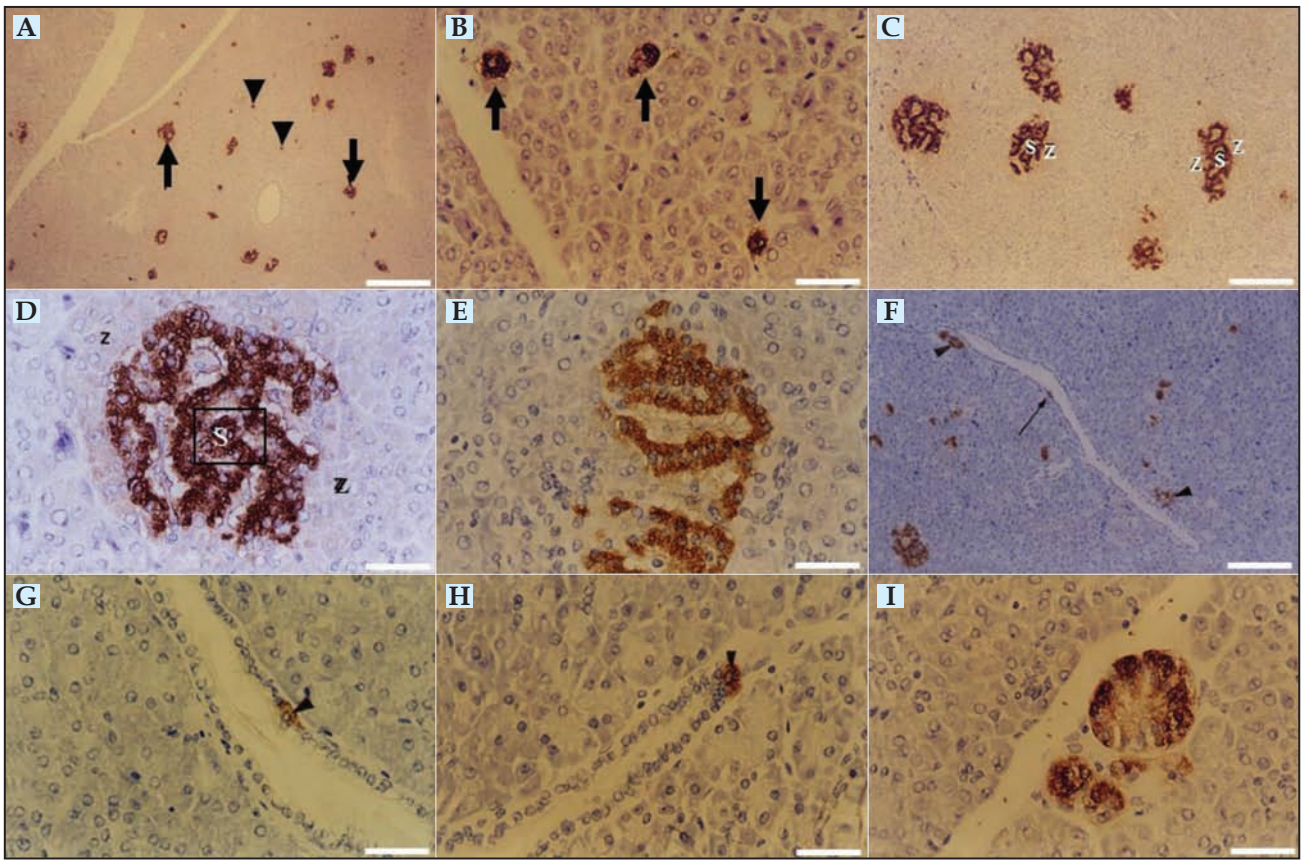

Fig 1. Localisation of insulin in camel pancreas. (A-I): Insulin immunoreactive cells. A: Insulin immunoreactive cells distributed in the pancreas islets (arrows). Single positive cells are also observed in the exocrine part (arrowheads) X40µm, B: Individual insulin immunoreactive cells in the exocrine parenchyma of the pancreas X400µm. C: Immunoreactivity for insulin is located centrally in an islet (S), which is surrounded by unstained peripheral zone (Z) X100µm., D: Immunoreactivity for insulin is located centrally in an islet (S), which is surrounded by unstained peripheral zone (Z) X400µm, E: β-cells arranged in cords giving the islet a multilobular appearance X400µm., F: β-cells both in the duct epithelium (arrow) and adjacent to the duct (arrowheads). X100µm, G: An immunoreactive insulin cell is shown in duct epithelium (arrowhead) X400µm, H: β-cells abutting to the basal part of the duct epithelium (arrowhead) X400µm and I: A pancreatic islet in the interlobular connective tissue septa of the pancreas showing insulin immunoreactive cells X400µm.

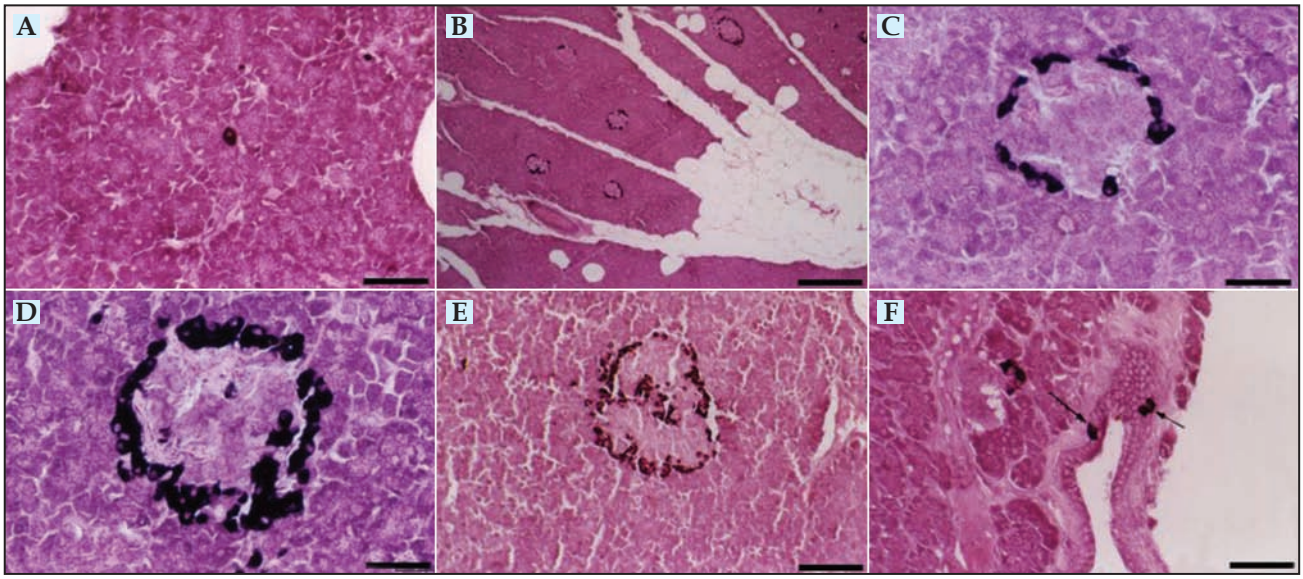

Fig 2. Localisation of glucagon in camel pancreas. (A-F): Glucagon immunoreactive cells (A-cells). A: An α-cell is shown in the exocrine portion of pancreas X200µm. The cell is oval in shape, with a rounded nucleus, B: α-cells arranged at the periphery of the islets X40µm, C: Glucagon immunoreactive cells forming a rim or a single cell layer peripherally X400µm, D: α-cells are arranged in a peripheral zone in an islet of camel pancreas X400µm, E: Majority of α-cells peripherally located and few cells are present in the centre of the islet X200µm and F: α-cells abutting to the duct epithelium (arrows) X400µm.

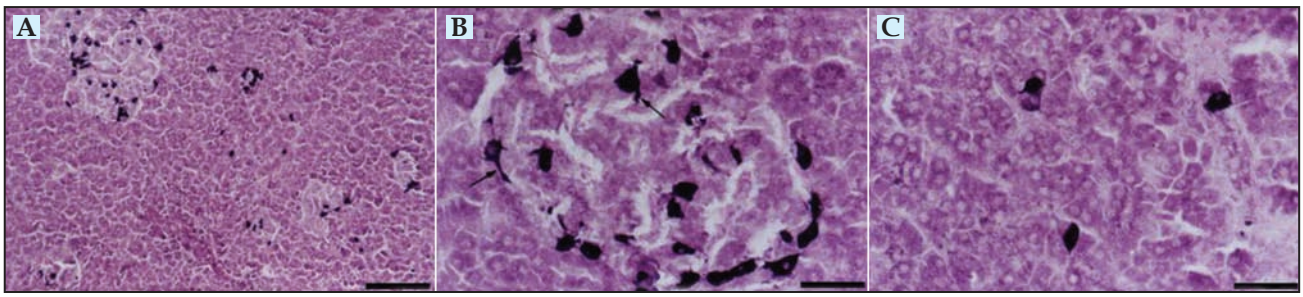

Fig 3. Localisation of somatostatin in camel pancreas. (A-C): Somatostatin immunoreactive cells (Δ-cells). A: Random distribution of the Δ-cells X100µm, B: Somatostatin immunoreactive cells distributed in the islet X400µm. Notice the cytoplasmic processes (arrows) and C: Single positive Δ-cells distributed in the exocrine parenchyma of the pancreas X400µm. Scale bar 100µm.

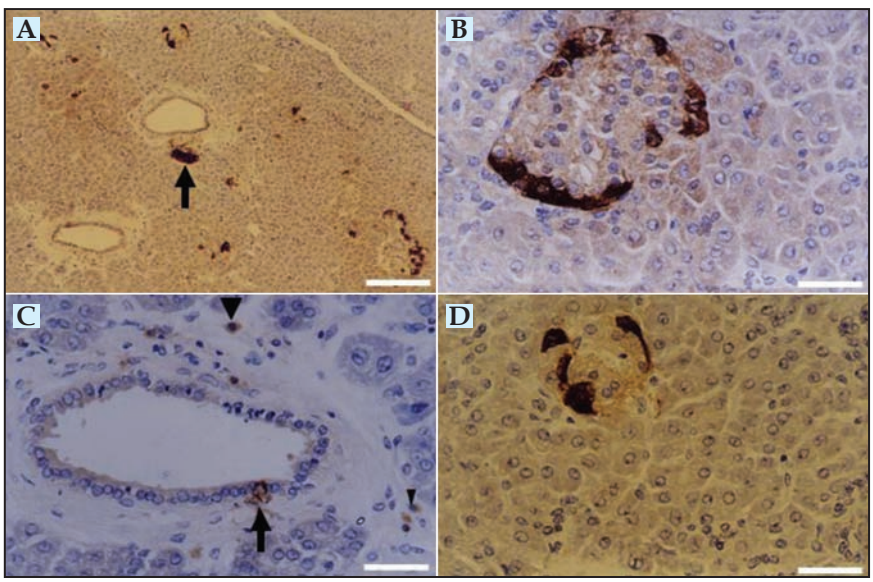

Fig 4. Localisation of pancreatic polypeptide in camel pancreas. (A-D): Pancreatic polypeptide immunoreactive cells (PP cells). A: PP-immunoreactivity. Notice a group of immunoreactive cells near a duct (arrow) X100µm. B: PP-cells located at the periphery of the islet X400µm. C: An immunoreactive PP-cell in the duct epithelium (arrow) and in the connective tissue around the duct (arrowheads) X400µm. D: PP-immunoreactive cells located at the periphery of the islet X400µm. Notice that some of the cells have long processes.

observations were reported in man (Stefan *et al,* 1983). The Glucagon immunoreactive cells (α-cells) were fewer in number compared to those of insulin. These cells were poly-angular in shape with rounded nuclei and usually located in the periphery of the islets. Similar features and distribution have been observed in the camel and other domestic animals (Aluments, *et al,* 1975; Khatim *et al,* 1985; Reddy and Elliott, 1985). But in the horse, these are often located in the core of the islets (Helmstaedter *et al,* 1976). Sometimes, the α-cells are encountered in the central part of the islets, a feature that was previously reported in camel (Khatim *et al,* 1985). This investigation has also shown some α-cells abutting to the duct epithelium or in the connective tissue beside the duct. This pattern of distribution of α-cells was similar to that observed in equine (Helmstaedter *et al,* 1976). The number of the α-cells was fewer in the right lobe compared to the rest of the pancreas. Similar observation was reported in most of the other domestic animals (Bencosme and Liepa, 1955; Lacy, 1957; Calingsan *et al,* 1984; Nakajima *et al,* 1988). On the other hand, Khatim *et al* (1985) reported that there were no obvious differences between the frequencies of the various islets cells in different pancreatic regions of camel pancreas. However, they have not ruled out their presence. They stated that the point of origin of the pancreas from the ventral anlage is small and simply have not been included in their specimens. Somatostatin immunoreactive cells (Δ-cells) have fusiform or pyramidal outline and rounded nuclei and were provided with short or long cytoplasmic processes. Similar features were reported in other animals (Larrson *et al,* 1979). The Δ-cells were randomly distributed in the islets and frequently noticed in the parenchyma. Similar pattern of distribution was also observed in camel, goat, dog and cat (Alumets *et al,* 1975; Khatim *et al,* 1985; Reddy and Elliott, 1985). A different pattern of distribution was observed in bovine, sheep, horse and rat (Helmstaedter *et al,* 1976; Calingsan *et al,* 1984; Nakajima *et al,* 1988; Adeghate, 1999), in which the Δ-cells are predominantly peripherally located. The present study has shown Δ-cells in the duct epithelium and in the connective tissue beside the duct. No similar observation was reported in camel or other animals previously. The present investigation indicates that the pancreatic polypeptide immunoreactive cells (PP-cells) were relatively large, pyramidal in outline and some showed long cytoplasmic process. Similar findings were reported in bovine (Nakajima *et al,* 1988). PP cells were less in number than insulin, glucagon and somatostatin positive cells. These findings corroborate the results obtained in the possum (Reddy *et al,* 1986). The PP-cells were mainly located in the peripheral parts of the islets of the pancreas as reported before in camel and rats (Khatim *et al,* 1985; Adeghate, 1999). Occasionally, few PP-cells were found in the centre, as it was previously observed in goat (Reddy *et al,* 1985). The PP-cells were also observed in the exocrine part of the pancreas. Similar features were also reported in camel and bovine (Khatim *et al,* 1985; Nakajima *et al,* 1988). The present study revealed the presence of PP-cells in the duct epithelium and in the connective tissue of duct. No comparative findings were reported previously. In conclusion, insulin, glucagon, somatostatin and pancreatic polypeptide immunoreactive cells were expressed in camel pancreas and these were well distributed in exocrine and endocrine portions.

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ULTRASTRUCTURAL AND MORPHOMETRIC STUDIES ON THE PROSTATE GLAND OF THE ONE-HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

The prostate glands of 40 camels were studied histologically, ultrastructurally and morphometrically. The alveolus of the gland was lined with one layer of low to high columnar cells (17.6 μm). Dark and light cells were characterised by the presence of spherical nuclei and numerous organelles. Large numbers of secretory granules, numerous vacuoles and lipid droplets were also observed. These may be 2 different cell types or stages of the development. Basal cells were characterised by large dense nuclei and they were generally poor in organelles. The mean volumes of the gland was 28.81 cm^3. The volume densities (Vv) and the total number of the different components of the prostate gland were determined by the standard morphometric methods. The glandular tissue formed 52.91% of the volume of the gland, followed by 34.68% connective tissue and muscle.

Key words: Camel, morphometry, prostate gland, ultrastructure

The prostate gland has been studied extensively in different species including boar (Aitken, 1960), bull (Schencher, 1950; Trotter, 1959; Kainer *et al,* 1969; Dyce and Wensing, 1971), dog (Bradley, 1948; Seaman, 1962; Miller *et al,* 1964), Grey Squirrel (Siwela and Tam, 1984), man (Srigley *et al,* 1990; Aumuller *et al,* 1991), ram (Aitken, 1959; May, 1964; Roy *et al,* 1985), rat (Aitken, 1959; May, 1964; Roy *et al*, 1985), stallion (Trautmann and Fiebiger, 1952; Sisson, 1969) and water buffalo (Abou-Elmagd and Wrobel, 1989).

Studies on the prostate gland of the camel (Tayeb, 1945; El-Jack, 1970; El- wishy *et al,* 1972; Ali 1975; Shahrasbi and Golbazhagh, 1975; Ali *et al,* 1977, 1978; Mosallam, 1981; Badawy and Yousef, 1982; Degan and Lee, 1982, Marroni *et al,* 1982; Aly *et al,* 1991; Yi-Wei Luo *et al,* 2010) showed that it was made of a massive dorsal corpus prostate and a small pars disseminata. According to Ali *et al* (1977), Mosallam (1981) and Yi-Wei Luo *et al* (2010) seasonal changes in the functional activity of the prostate gland were manifested by the morphology of the secretory units, as indicated by the height of the glandular cells and the contents of their secretory granules.

Preliminary observations on the ultrastructural of the prostate gland of the camel have been reported (Aly *et al,* 1991 and Yi-Wei Luo *et al,* 2010). Histochemical studies were scantly demonstrating the presence of PAS positive reactivity, acid phosphatase, alkaline phosphatase, lactate dehydrogenase and lipid (Ali *et al,* 1976; Yi-Wei Luo *et al,* 2010). Therefore, the present research was undertaken to give insight of the ultrastructure and morphometry of the prostate gland.

Materials and Methods

Prostate glands were collected from 40 apparently healthy mature camels, immediately after the animals were slaughtered (Tambul Slaughter house, Sudan)

Light Microscopy

Samples from 11 animals were used for the preparation of histological sections. Samples 5 mm thick were cut from different region of the gland fixed either in Bouin's fluid, 10% formal-saline, 10% formalin or Zenker formal, processed for routine paraffin techniques. Three to five micrometres paraffin sections were stained with Haematoxylin and Eosin (H&E) or Masson's Trichrome.

Electron Microscopy

Material for ultrastructural studies were obtained from 9 animals. Small pieces (1mm^3) of tissue were fixed in 2.3% glutaraldehyde in 2.14% sodium cacodylate buffer pH 7.4 for 2-4 hrs at 4°C.

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Samples were then washed twice for 30-60 minutes in 2.14% sodium cacodylate pH 7.4, fixed in 2% osmium tetraoxide in 2.14% sodium cacodylate buffer pH7.4, for 2 hours. Then washed in 2.14% sodium cacodylate buffer pH 7.4 twice for 30-60 minutes. Dehydration was carried out in ascending grades of acetone or ethanol 50%, 70%, 90%, 95% and 100% for 30-60 minutes each. The materials were prestained in 2% uranyl acetate and 2% phospho-tungstic acid at 4°C for 20 hrs and then embedded in resin.

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

Morphometry

Tissue samples were collected from 20 animals. The weight and volume of the glands were measured. The volume was determined by water displacement method (Scherie, 1970). Tissues were cut into 9 slices about 5 mm thick. Since the slices were more or less identical it was not necessary to analyse all of them and so every 3rd slice was taken for histological processing. The slices from each animal were used for morphometric analysis. Sections were cut at 3-5 µm thickness and then stained with Masson's Trichrome.

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

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

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

Results and Discussion

Light Microscopy

The secretory units of the gland of the camel were lined with one layer of low to high columnar cells, which extend from the basal lamina to the lumen. The cells were about 17.6 µm in height. The nuclei are spherical in shape and occupy the basal cytoplasm. Two main cell types have been identified; dark cells and light ones together with occasional basal cells seen in some units wedged between the columnar cells and basement membrane.

Electron Microscopy

The Dark Cells

The dark cell nuclei were spherical in shape, occupying the basal part of the cell and were poor in chromatin (Fig 1). The euchromatin was clearly recognisable as dense granular material distributed in the nucleoplasm, but the heterochromatin was concentrated around the periphery. One or more nucleoli were encountered and eccentrically placed. They are irregular in shape, with dense band in its outer part and lighter central portion (Fig 1).

The cells had well developed Golgi complexes occupying a juxtanuclear position. They consisted of flat saccules or cisternae piled one upon the other in close parallel arrays with curved end (Fig 2). Few granules, presumably secretory materials are seen within the lumina at the saccule.

Mitochondria, oval or rounded in shape were scattered throughout the cytoplasm. The matrix was finely granular or homogeneous and was traversed by lamellar cristae (Fig 3).

The cytoplasm was also rich in rough endoplasmic reticulum (rER) which was seen in the form of branching and anastomosing tubules and sometimes in the form of whorled lamellae (Figs 1,3 and 6). It tended to concentrate mainly near the nucleus and occasionally dilated containing moderately dense products.

A small number of membrane bounded dark particles and other dark bodies presumably lysosomes, were distributed throughout the cytoplasm (Figs 5 and 6). Numerous vacuoles were randomly distributed in the cytoplasm. Few lipid droplets were also encountered together with a large

number of dense granules. The granules however, tended to concentrate in the luminal cytoplasm (Figs 5 and 6). Some of the granules were of low density and appeared to contain a fine textured filament or flocculent material (Fig 5).

The Light Cells

Light cells were few in number (Figs 6 and 7). These were almost within the same secretory units together with the dark cells. These were characterised by light cytoplasm containing a few electron dense granules and small number of vacuoles, otherwise it is similar to dark cell (Figs 6 and 7).

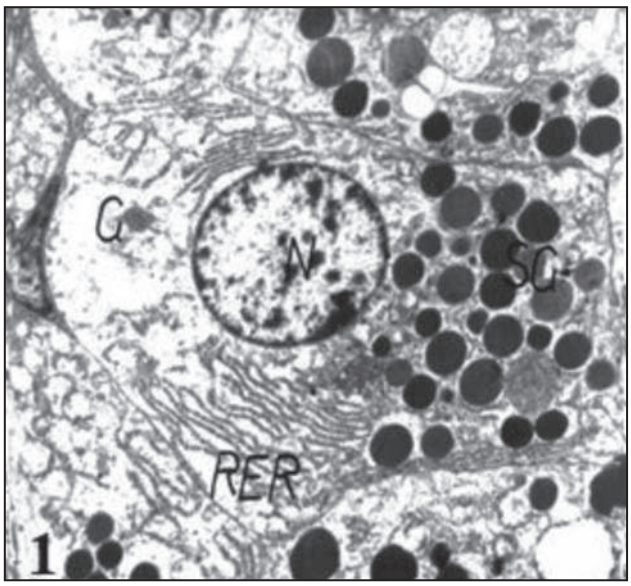

Fig 1. Electron micrograph of the prostate gland. Dark cell showing spherical nucleus (N) secretory granules (SG) in the apical cytoplasm. Note the rough endoplasmic (RER) and Golgi complex (G). 3000X.

The Basal Cells

Basal cells were small in number and they rested directly on the basal lamina. The cell membranes were straight. The lateral membranes adhered to those of the neighbouring cell by moderate junctional complex (Fig 7). These cells were somewhat pyramidal in shape with a large dense irregular nuclei that occupied most of the cytoplasm (Fig 7).

Nuclei were rich in chromatin. The euchromatin was distributed in the form of dense granules in the nucleoplasm. The heterochromatin was concentrated in the form of clumps (Fig 7).

Morphometry

The mean volume of the prostate gland of 20 camels was 28.81 cm^3, whereas measurements of the weight of the prostate gland had mean values of 24.19 gm.

Tables 1, 2 and 3 showed the mean volume of the prostate glands, total number density (Vv), volume density percentages (Vv%) and absolute volume. The greater volume of the prostate gland was occupied by glandular tissue 52.9%, followed by connective tissue and muscle 34.68%, blood vessels and nerves 6.89% and the ducts 5.52%.

In accordance to the observations of Ali *et al* (1977), Mosallam (1981) and Yi-Wei Luo *et al* (2010), the secretory units of the camel prostate gland were lined with one layer of low to high columnar cells. This is also true for the goat (Gupta and Singh,

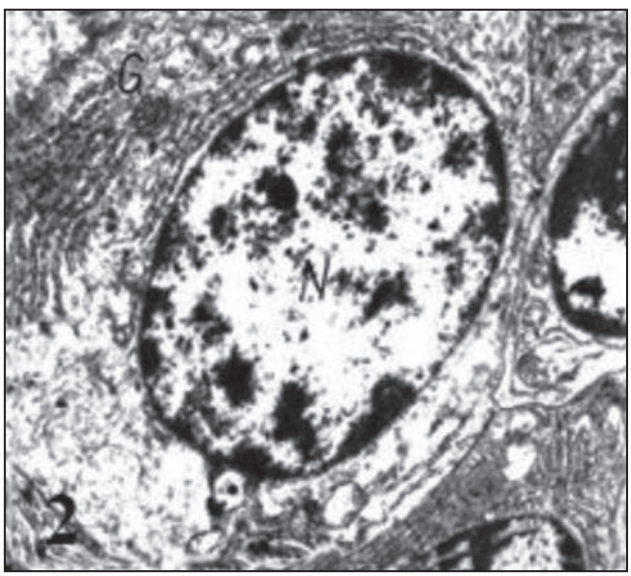

Fig 2. Electron micrograph of the prostate gland. Dark cell. A well-developed Golgi complex is seen near the nucleus (N).49000 X.

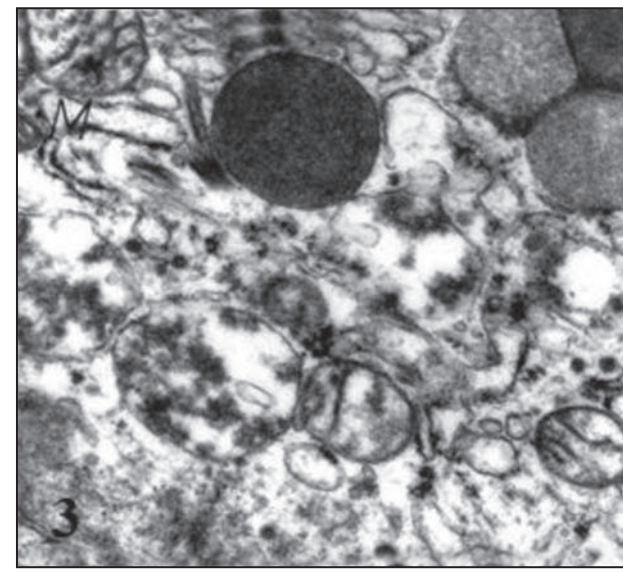

Fig 3. Electron micrograph showing oval to rounded mitochondria scattered throughout the cytoplasm.

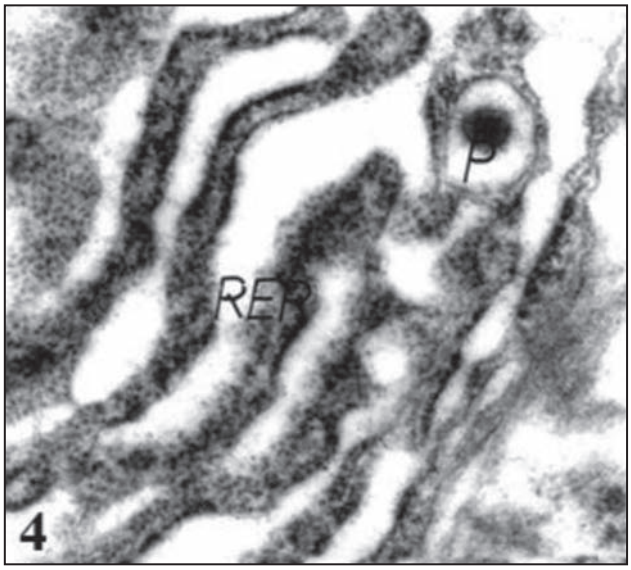

Fig 4. Electron micrograph of the prostate gland showing dark cell. Rough endoplasmic reticulum (RER) contained a moderately dense product (P). 60000 X.

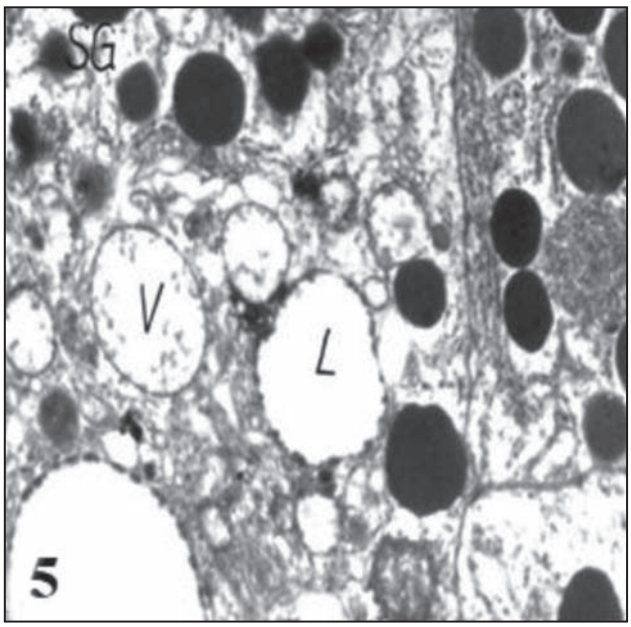

Fig 5. Electron micrograph of the prostate gland showing dark cell membrane bounded dark particles. Lipid (L), secretory granules (SG) vacuoles (V) and lysosomes. 30000 X.

1982). Similar to what has been reported by Ali *et al* (1977), the epithelium was about 17.6 µm high while in the rabbit the epithelial height amounted to be 20-30 µm (Jones *et al,* 1978). The observed difference in height between the 2 species could be attributed to differences in the volume of the secretion. In agreement with the findings of previous researchers (El-wishy *et al,* 1972; Ali, 1975; Ali *et al,* 1977; Mosallam, 1981), the nuclei of the epithelial cells are essentially basally located.

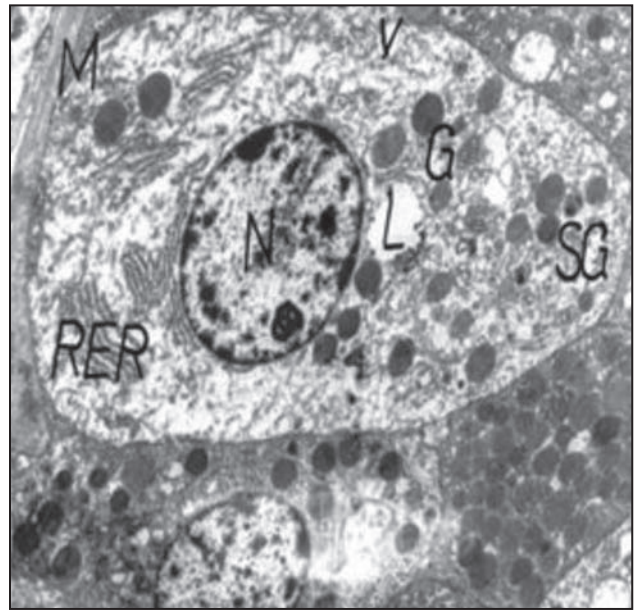

Fig 6. Electron micrograph of the prostate gland showing light cell. Nucleus (N) with nucleolus, Golgi complex(G), mitochondria (M), rough endoplasmic reticulum (RER), vacuoles (V), lipid (L) and few secretory granules (SG). 22000 X.

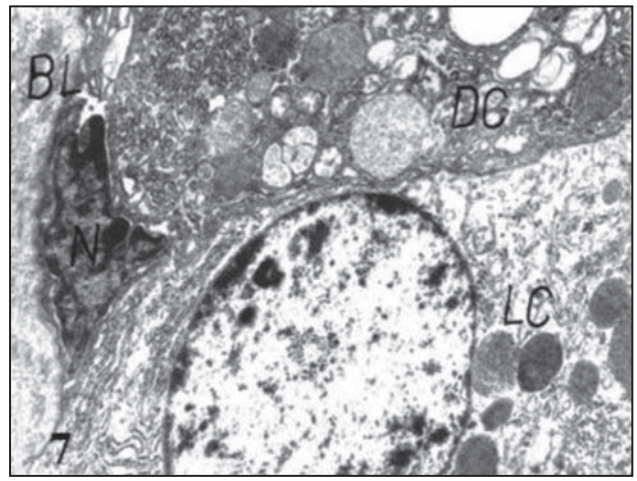

Fig 7. Electron micrograph of the prostate gland showing basal cell Located between the basal lamina (BL) and the surface epithelial cell (of dark (DC) and (LC) cells). Note the large dense irregular nucleus (N). 35200 X.

This research has established the presence of 2 cells types, dark and light in the prostate gland of the camel. Such cells types have also been identified in the prostate gland of Grey Squirrel (Siwela and Tam, 1984).

The ultrastructural observations recorded in this study confirmed earlier findings made with the light microscope (Ali *et al,* 1977; Mosallam, 1981). The dark and light cells showed enfolded basal cell membranes together with straight lateral ones. While

human prostate epithelial cells possessed interspaced junctional complexes along these lateral axes (Webber, 1975).

Table 1. Morphometric analysis of the prostate gland showing the number of points counted from section 1.

No. of F.	G. T. P	C.T+M	B.Vs+N	Ducts	Total
1	6	76	18	–	
2	–	75	25	–	
3	82	15	3	–	
4	66	11	1	22	
5	44	30	5	21	
6	78	20	2	–	
7	63	12	2	23	
8	62	35	3	–	
9	56	31	13	–	
10	8	80	12	–	
Total	465	385	84	66	1000

Number of field (N. of F), Glandular tissue of prostate (G.T.P), Connective tissue and muscle (C.T+M), Blood Vessels and nerves (BVs+N).

Table 2. Animal No.: Morphometric analysis prostate gland showing the number of points counted (P), volume density (Vv) volume density per cent (Vv%) and absolute volume (Abs.v) of the main components from three sections.

No. of F.	G. T. P	C.T+M	B.Vs+N	Ducts	Total
30	1714.0	987.0	157.0	142.0	3000
Vv	0.571	0.329	0.052	0.047	
Vv%	57.10	32.90	5.20	4.70	
Abs. v	13.19	7.60	1.20	1.09	

Table 3. Morphometric analysis of prostate gland showing the volume of gland (V) in Cm^3, volume density (Vv), volume density per cent (Vv %) and absolute volume (Abs.v) of the main components of the prostate gland of 20 camels.

	G.T.P	C.T+M	B.VS+N	Duct
Total No.	31746	20809	4134	3311
Vv ±SD	0.5291 ±.0454	0.3468 ±.0340	0.0689 ±0.1894	0.0552 ±0.0148
Vv% ±SD	52.29 ±04.02	34.68 ±03.40	6.89 ±13.40	5.52 ±01.48
Abs. v ±SD	12.06 ±2.73	07.82 ±1.91	01.25 ±0.38	01.25 ±0.38

The nucleus was oval in shape and occupied a basal position similar to that of the rat (Elisa and Fangvictor, 1974) and man (Webber, 1975). The cell possessed a highly developed Golgi complex containing secretory granules similar to those of the rat (Elisa and Fangvictor, 1974). Also, it contained oval and round mitochondria with a moderately dense matrix, randomly scattered in the cytoplasm. Similar findings were reported in the rat (Wong *et al*, 1988). The present study showed the presence of a well-developed rough endoplasmic reticulum similar to those reported in the grey squirrel (Siwela and Tam, 1984), rat (Wong *et al*, 1988) and camel (Aly *et al*, 1991; Yi-Wei Luo *et al*, 2010). Moreover, the rough endoplasmic reticulum was slightly dilated and contained moderate amounts of dense material. A similar observation was noted in the cells of the rabbit prostate (Jones *et al*, 1978). Scattered within the cytoplasm were large heterogeneous bodies with ultrastructural characteristic resembling lysosomes. This finding was previously reported in man prostate (Webber, 1975) and camel prostate (Aly *et al*, 1991). Similar ultrastructural observation coupled with histochemical localisation of hydrolytic enzymes, ascertaining their nature as lysosomes were reported in testicular excurrent duct system of the domestic fowl (Tingari, 1972; Tingari and Lake, 1972). In the view of the histochemical demonstration of hydrolytic enzymes in the lining cells of the camel prostate gland (Ali *et al*, 1976), it would be reasonable to assume that these heterogeneous bodies are different form of lysosomes.

The apical part of the dark cell camel prostate was packed with many secretory granules and vacuoles containing material of different densities, varying from finely granular to flocculent one. These granules might belong to the alpha granules type formerly described by Fawcett (1966) at the ultrastructural level. According to Fawcett (1966) there were 2 types of secretory granules; alpha and beta. Alpha granules were uniform in size and density and possessed an enveloping membrane, whereas the beta granules, which were also membrane limited, contained dense, rod-like or tubular crystals due to progressive concentration of secretory product.

The epithelial cells of the camel prostate gland contained lipid droplets similar to those of the rat prostate (Wong *et al*, 1988). Such droplets have not been reported in Bactrian camel (Yi-Wei Luo *et al*, 2010) or goat (Gupta and Singh, 1982).

The ultrastructural features of the light cell were not unlike those of the dark cell except for the smaller number of vacuoles and that of the electron dense granules. The two morphological forms may either represent two functional phases of a single cell type or 2 distinct populations of secretory cells.

Ultrastructural studies on the prostate gland of a seasonally breeding mammal, the Grey Squirrel (Siwela and Tam, 1984) has demonstrated the

presence of 2 types of secretory cells in the secretory epithelium. During the secretory period type-1 cells were characterised by a large nucleus and abundant rough endoplasmic reticulum, ribosomes and secretory granules, whereas type-2 cells were more abundant and possessed a smaller nucleus, more substantial Golgi complex and numerous secretory vesicles. In the typical atrophic gland, almost all organelles associated with secretory activity disappeared, but both types of cells could still be distinguished by their peculiar nuclei and even by their characteristic light and dark cytoplasm. In spite of the fact that the camel is not a typical seasonal breeder as it does not go through sexual quiescence (Tingari *et al,* 1984), the suggestion of Siwela and Tam (1984) that those are 2 cells types might also hold true of the camel. It is noteworthy; however, that other observation on the prostate gland of man (Webber, 1975) and rat (Wong *et al,* 1988) did not establish the presence of 2 cells types.

The basal cells were observed to be wedged among the bases of columnar cells. They were in the form of small triangular cells with a few cytoplasmic organelles, in the form of scattered profiles of rough endoplasmic reticulum, some mitochondria and a few vacuoles. A hypothesis that such cells in the human prostate were not of myoepithelial origin was investigated and confirmed employing immunohistochemical and ultrastructural methodologies (Srigley *et al,* 1990; Aumuller *et al,* 1991). Furthermore, it was suggested that the exact role of the basal cells of the prostate was not known, Nevertheless, they may serve endocrine, paracrine, or other regulatory functions and may be involved in modulating signals between prostatic stroma and epithelium.

Morphometric data obtained from the prostate gland of 20 camels showed that mean value of a volume of the gland is 28.81cm^3 and a weight of 2419 gm. The glandular tissue occupied the greater part of the prostate volume 52.91% followed by connective tissue and muscle 34.68%, blood vessels and nerves 6.89% and lastly the duct 5.52%.

It is assumed that the cellular components of the gland underwent shrinkage to the same degree since there were subjected to the same kind of treatment during processing. The relative reduction in volume densities of the components would therefore be the same. These volume densities were used to calculate the absolute volume of the components. The latter should therefore be closer to the real values since they were based on the volume of the fresh glands. The absolute volume of the glandular tissue was 12.06 cm^3, the connective tissue and muscle 7.82 cm^3, the blood vessels and nerves 1.57 cm^3 and the duct was 1.25 cm^3.

The morphometric data analysed in this study showed significant differences amongst the components. For example, the glandular tissue occupied about 52.91%, the connective tissue and muscle occupied about 34.68%, the blood vessels and nerves amounted to 6.89% and the duct system occupied 5.52%.

According to this study, the weight of prostate gland was 24.19 gm. El-wishy *et al* (1972) however, reported a weight of 19.6 gm, while Abou-Ahmed *et al* (1988) recorded a weight as low as 16.7 gm. They were of the opinion that the weight of the prostate was significantly affected by the age of the animal.

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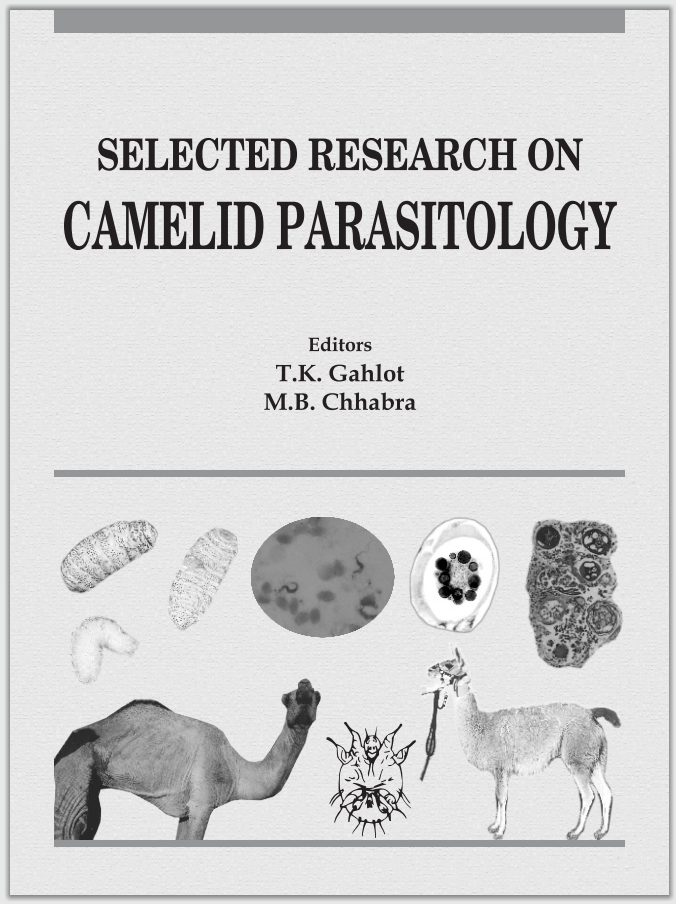

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SPECIES SPECIFICITY AND HOST AFFINITY RATHER THAN TISSUE TROPISM CONTROLS CODON USAGE PATTERN IN RESPIRATORY MYCOPLASMOSIS

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ABSTRACT

Respiratory Mycoplasmosis (rMyc) is a devastating respiratory disease causing serious pneumonia in camels, cattle and human causing fatalities and high economic loss. Codon usage pattern is an important measure of host-parasite relationship, parasites adaptation and evolution. In this work, two important species of rMyc affecting human (*Mycoplasma pneumoniae*) and camels (*Mycoplasma mycoides*) were analysed for finding their codon usage bias, factors affecting their genomes composition and their relation to their host including adaptation and disease pathogenesis. In spite of their reduced genome size, rMyc showed comprehensive and host-independent codon usage machinery with wide variability and incompatibility with their host pattern. Although, rMyc are common infectious agents of respiratory tract, they showed completely diverse codon usage, adaptation and pathogenesis profiles. *M. mycoides* showed strict highly biased A/T selection in their genomic composition, preferred codons and at the 3rd position of codons as well as highly divergent codon usage pattern compared with its host. *M. pneumoniae* showed more attempts in adaptation to mammalian host environment by showing codon usage pattern almost similar to their host. High GC content in genome, low number of overbiased and underbiased codons, high ENc values and balanced use of GC and AT in preferred codons N3s were among the factors of adaptation to its habitat. There was immune selection among rMyc. *M. mycoides* is more immune resistant by showing lower CpG dinucleotide frequency compared with *M. pneumoniae*. The higher and faster gene expression in *M. mycoides*, and to a lesser extent *M. pneumoniae*, devoted from low ENc values accounts for high pathogenicity and acute disease. Common evolutionary origin and variable codon usage indices account for variable attempts among *Mycoplasma* species to adapt their habitat regardless their tissue tropism or host specificity.

Key words: Camel, codon, mycoplasmosis, pneumonia

Several respiratory *mycoplasma*s were serologically recovered in camels (Egwu and Aliyu, 1997; Mederos-Iriarte *et al,* 2014). *Mycoplasma* is one of the most common causes of respiratory diseases. It is either primary invader or a predisposing factor for complicated respiratory diseases (Chiu *et al,* 2015; Waites *et al,* 2017). *Mycoplasma* contains the smallest essential genome capable of self-replication (Pettersson *et al,* 1996). *Mycoplasma* typically show rigorous host tissue specificities, may be due to their nutritional requirements (Razin, 1992), a direct result of the genome reduction that likely happened as a result of the metabolic complementarity of their hosts (Andersson and Kurland, 1998).

No specific virulence factor observed by *mycoplasma* as observed in other bacteria but *mycoplasma* use toxic metabolic intermediates, which they secrete and translocate to the cells of the host as virulence factors (Pilo *et al,* 2005). They contend with the cells of the host for biosynthetic precursors and can modify RNA, DNA and protein synthesis, reduce ATP and amino acid levels, introduce chromosomal alterations and alter host cell plasma membrane antigens (Olarerin-George and Hogenesch, 2015). Moreover, as they do not have cell wall, they are resistant to some antibiotics, which target synthesis of cell wall such as beta-lactam antibiotics render these microorganisms especially motivating in medicine (Waxman and Strominger, 1983).

Mycoplasma mycoides subsp. *mycoides*, the causative pathogen of contagious pleuropneumonia (CBPP) has been isolated from camels (Egwu and

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Aliyu, 1997) with very high antibody titres (Paling *et al,* 1988).

Synonymous codons in a wide variety of organisms is elected with various frequencies rather than random and equal frequencies; this phenomenon is called synonymous codon usage bias (Hershberg and Petrov, 2008). Many genetic studies denoted that the 3rd position (synonymous site) of a synonymous codon is subject to weak election and that; synonymous codon usage bias is sustained by an equilibration between translation selection, genetic drift and mutation pressure (Xia *et al,* 2017). Synonymous codon usage differs between both organisms and among genes within a genome and it arises from variations in gene expression, replication strand or GC content (Suzuki *et al,* 2008). The interaction of these factors may differ among various species depending on their evolutionary process.

With the increasing number of genes and gene annotation for the rMyc, new resources have become helpful to explore the factors that affect the codon usage tendency of gene populations and to analyse the evolutionary processes that are reflected by the overall codon usage trends (Medini *et al,* 2005). Information about the gene populations of rMyc may supply some significant evidences to understand the evolutional dynamic sustaining the unique genetic features of the mycoplasmas. In this work, the genomic content of rMyc is analysed to declare the codon usage pattern of 2 common respiratory pathogens from the same genus. Adaptation of respiratory pathogens in two different hosts is investigated. The genomic aspects of mycoplasmas were compared to that of camel as well as cattle and human genomes.

Materials and Methods

Genome Sequences, Database Collection and Software Settings:

A database of mycoplasma genomes was constructed by screening the Genbank website. A dataset of genes from *M. mycoides* subsp. *mycoides* T1-44 and *M. pneumoniae* M129 was constructed. Geneious software package was used to the extract the genes. Nucleotides composition, AT percentage and GC percentage were estimated by CLC genomics workbench (Fig 1).

Table 1. The nucleotides contents of *M. mycoides* and *M. pneumoniae*.

Mycoplasma spp.	A%	C%	G%	T%
M. mycoides subsp. *mycoides* T1-44	42	11	14	33
M. pneumoniae M129	32	20	23	28

Codon usage profile

To analyse the codon bias of all genomes, the following parameters were determined. The frequencies of the appearance of the 4 nucleotides (A%, T%, C% and G%), the presence of GC at the 1st and 2nd (GC) or 3rd position (GC3) of codons; and the rate of appearance of each nucleotide at the 3rd location of synonymous codon (A3s, T3s, G3s and C3s).

Relative Synonymous Codons Usage (RSCU):

RSCU stands for the ratio of codons' noticed rate to their expected frequency assuming that all codons for a particular amino acid are used equally. Codons revealing RSCU value of 0.6 -1.6 signifies that the codon usage frequency is equal to that expected value or that there was no bias, while codons with RSCU values >0.6 or < 1.6 indicating positive or negative codon bias, respectively. The following equation was used for RSCU estimation:

$$RSCUij = \frac{xij}{\frac{i}{ni}\sum_{j=1}^{ni} xji} \qquad (1)$$

Where **ni** is the total number of synonymous codons of the amino acid and **Xij** is the number of the noticed codons for a specific amino acid.

Relative Dinucleotide Frequencies

The existence of dinucleotides in a genome can be used as a scale of codon bias by comparing the estimates of actual to predictable dinucleotide rate. Relative dinucleotides frequency was estimated using equation no. 2.

$$(O / E)\ XpY = [f(XY) / f(X)\ f(Y)] \qquad (2)$$

Where f (XY) is the recorded nucleotides frequency and f (Y) f (X) are the single nucleotides frequencies.

Values <0.6 and >1.6 representing under-representation and overrepresentation of nucleotides, respectively. These values designate a relative plenty of dinucleotides to their random presentation.

Effective Number of Codons (ENc)

ENc is an estimate of codon bias in a gene and rates from 20 to 61. ENc lower values denote cruel codon bias in which a lower number of codons is utilised for every amino acids. In contrary, higher ENc estimates denote lack or low codon bias. Consequently, ENc estimate lower than 40 points out strong codon bias.

Codon usage Bias Mediated by Mutational pressure

ENc plot was used to know the impact of mutational pressure on codon bias. In ENc plot, ENc estimates are plotted against GC3. Mutation pressure is correlated with ENc values disseminated around standard curve of ENc-GC3 relationship. Deviation from the standard curve denotes that other factors are leading the codon bias, e.g. by natural selection. The standard curve was calculated as follows.

$$Nc = 2 + s + \left\{\frac{29}{[s^2 + (1-s)^2]}\right\} \quad (3)$$

Where, s = fGC3 and Nc ranges from 20-61.

Codon Usage Bias Mediated by Natural Selection

The codon usage bias interposed by natural selection was inspected by using codon adaptation index; neutrality plot; general average of hydropathicity (GRAVY) and aromaticity (AROMO) (21).

Multivariate Correspondence Analysis (COA)

The codon usage of *mycoplasma* genomes is statistically analysed by multivariate correspondence methods. The values of RSCU were analysed by CodonW to match the intragenomic differences. In COA analysis, every gene is geometrically given representing in a 59- dimensional vector out of 59 orthogonal axes.

Correlation analysis

Pairwise correlation statistics for codon usage indices were carried out by Graph Pad prism software (Graph Pad Inc., USA).

Results and Discussion

Mycoplasma Genomes Nucleotides Content

There were differences in nucleotides composition between rMycs. *M. mycoides* showed higher AT% (75%) and lower GC% (25%) (Fig 1, Table 1). In contrast, *M. pneumoniae* strains showed lower AT% (60%) and higher GC% (40%).

Codon usage indices

The frequencies of nucleotides at the 3rd position of codons is represented in Fig 2. The frequency of A3s/T3s records higher values in *M. mycoides* strains compared with *M. pneumoniae*.

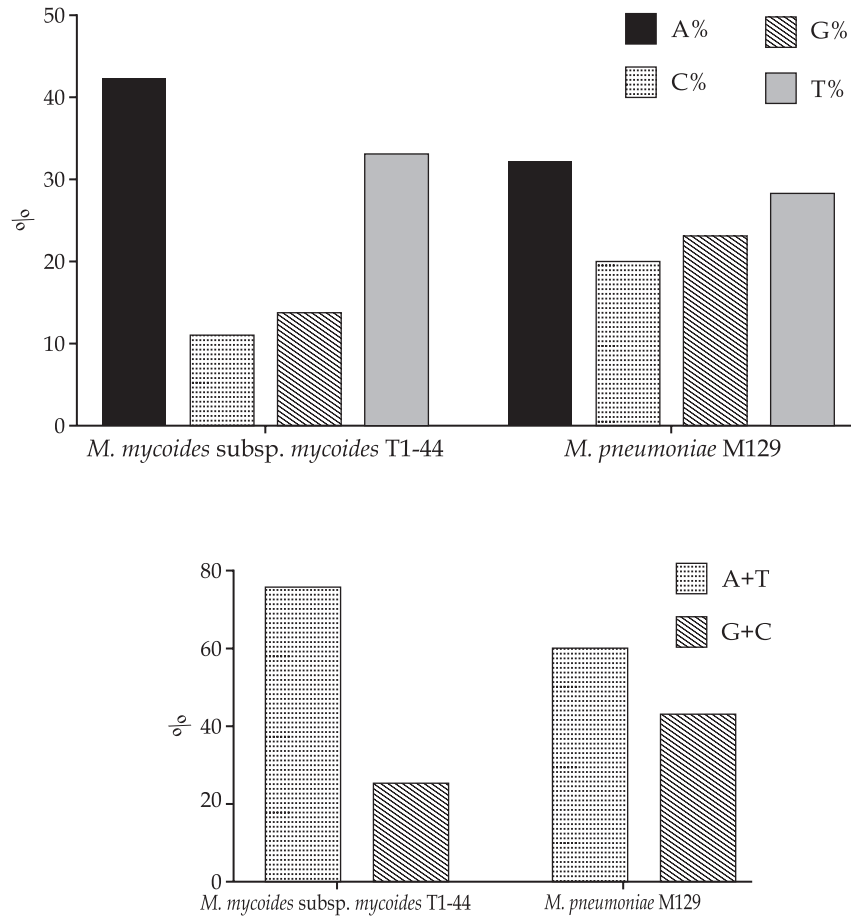

Fig 1. The AT and GC% of *M. mycoides* subsp. *mycoides* T1-44 and *M. pneumoniae* M129.

M. pneumoniae and *M. mycoides* possess higher frequency of pyrimidine nucleotides compared with the purines (Fig 2). Interestingly, *M. pneumoniae* showed 1-3 folds higher G3s/C3s frequency, compared with *M. mycoides*. NT3s frequencies rank was in the order of T3s > A3s > C3s > G3s in *M. pneumoniae* and T3s = A3s > C3s = G3s in *M. mycoides*.

M. pneumoniae showed higher ENc value (ENc = 50). While, *M. mycoides* showed a lower value (ENc = 31). Therefore, there is a large difference in codon usage bias between rMyc.

Relative Synonymous Codon Usage (RSCU) Analysis

Most of organisms encodes 61 codons for production of 20 amino acids. In the standard genetic code, methionine and tryptophan, encodes only one codon. The remaining part of amino acids have multiple codes up to 6 different codons. Therefore, synonymous codons are the alternate codons present within one amino acid (Nakamura *et al,* 2000). *Mycoplasma* uses non standard code with tryptophan has 2 codons UGG and UGA, which is a stop codon in mammals.

The detected common over-represented codons in rMyc include UUA, AUU, AGU and GGU (Table 2). Therefore, all over-represented codons are rich in A/T and ending with A3s or T3s. Only one observed C ending codon in *M. pneumoniae* M129 is for Arg, CGC. The under-represented (negatively biased) codons detected in rMyc includes UUC, CUG, ACG, CAG and GAG. Therefore, AT nucleotides are the most common in over-represented codons and G/C are common in under-represented codons. In this context, there was high variability among rMyc regarding to the total number of over-biased and under-biased codons (Table 2). *M. mycoides* contains 18 overbiased codons, compared to five codons only in *M. pneumoniae*. Similarly, *M. mycoides* showed the highest number of under-biased codons by showing 31, compared to 9 codons in *M. pneumoniae*. Within 27 under-represented codons in *M. mycoides* strains only 3 codons are ending with U or A and 24 codons are ending with G or C. 33% (3 codons) of rare codons in *M. pneumoniae* contains C3s or G3s.

In terms of host RSCU, the mammalian hosts for *M. mycoides* (camel and cattle) and *M. pneumoniae* (human) showed similar profile of low number of overbiased codons (3 codons) and balanced use of the 4 nucleotides A, G, T and C in the 3rd position of codons (Table 2). In this context, *M. mycoides* showed severe bias against GC3s (Table 2), intensive bias against G/C in nucleotide composition as well as in overbiased and optimal codons. In contrast, *M. pneumoniae* has robust similarity to their host. It has balanced use of A3s/T3s/G3s and C3s in optimal codons and lower AT and higher GC in nucleotides composition.

Dinucleotide frequencies

AA, TT, TA and AT were the most abundant dinucleotides in the *M. mycoides* (Fig 3). Additionally, CpG and GpC dinucleotides are under-represented. In contrast, *M. pneumoniae* showed lower frequencies of A/T dinucleotides and higher frequencies of C/G dinucleotide combinations. With regard to CpG frequencies, *M. pneumoniae* seems to be less virulent than *M. mycoides* due to higher interaction with host immune response due to higher CpG frequencies. *M. mycoides* are more immunological competent and escapes immune response by lowered CpG dinucleotides in their genome.

ENC- Plot

When GC3s is plotted against ENc estimates, we can sketch inferences as the role of compositional chains or mutational stress on the codon bias and RSCU. If all GC3s values locates on or below the standard ENc-GC3s relation, then codon biases is affected by mutational biases.

In both of rMyc, most of the plotted points lie under the standard curve (Fig 4). This indicates that several forces are sharing in shaping of their genomes including mutational bias, natural selection or other influences. The estimated Pearson`s correlation coefficient indicates either low or strong positive correlation between GC3s and ENc. Low r value was evident with *M. pneumoniae* M129 ($r= 0.19$, $p<0.05$). High correlation ($r = 0.6$, $p<0.01$) was evident with *M. mycoides*.

Neutrality Plot

Neutrality plot is used to assess the evolution neutrality and contribution of mutation and selection to shaping of the genome and codon usage bias. Neutral mutation by random selection is concluded when the slope of GC12 with GC3 is close to the unity. The degree of contribution of selection forces can be concluded from the slope of correlation line.

There was a moderate to high correlation of GC12 and GC3. The estimated r was 0.62 and 0.65 for *M. mycoides* and *M. pneumoniae*, respectively. The regression slopes were 0.55 and 0.53 for *M. mycoides* and *M. pneumoniae*, respectively. Subsequently, there is relative neutrality and directional mutational

pressure of 55 and 53% for *M. mycoides* and *M. pneumoniae*, respectively.

Natural Selection and Codon Usage Bias

For estimation of the contribution of natural selection to codon usage bias in rMyc, the correlation between Aromo, Gravy, ENc and GCs were investigated.

In *M. mycoides*, there was significant correlations of Aromo and Gravy with both of ENc and GC3s. This indicates the important role of translation control in codon usage. For *M. pneumoniae*, there was significant correlation between Aromo with GC3s but not and ENc. This result indicates the potential high influence of natural selection on *M. mycoides* and to a lesser extent influencing *M. pneumoniae*.

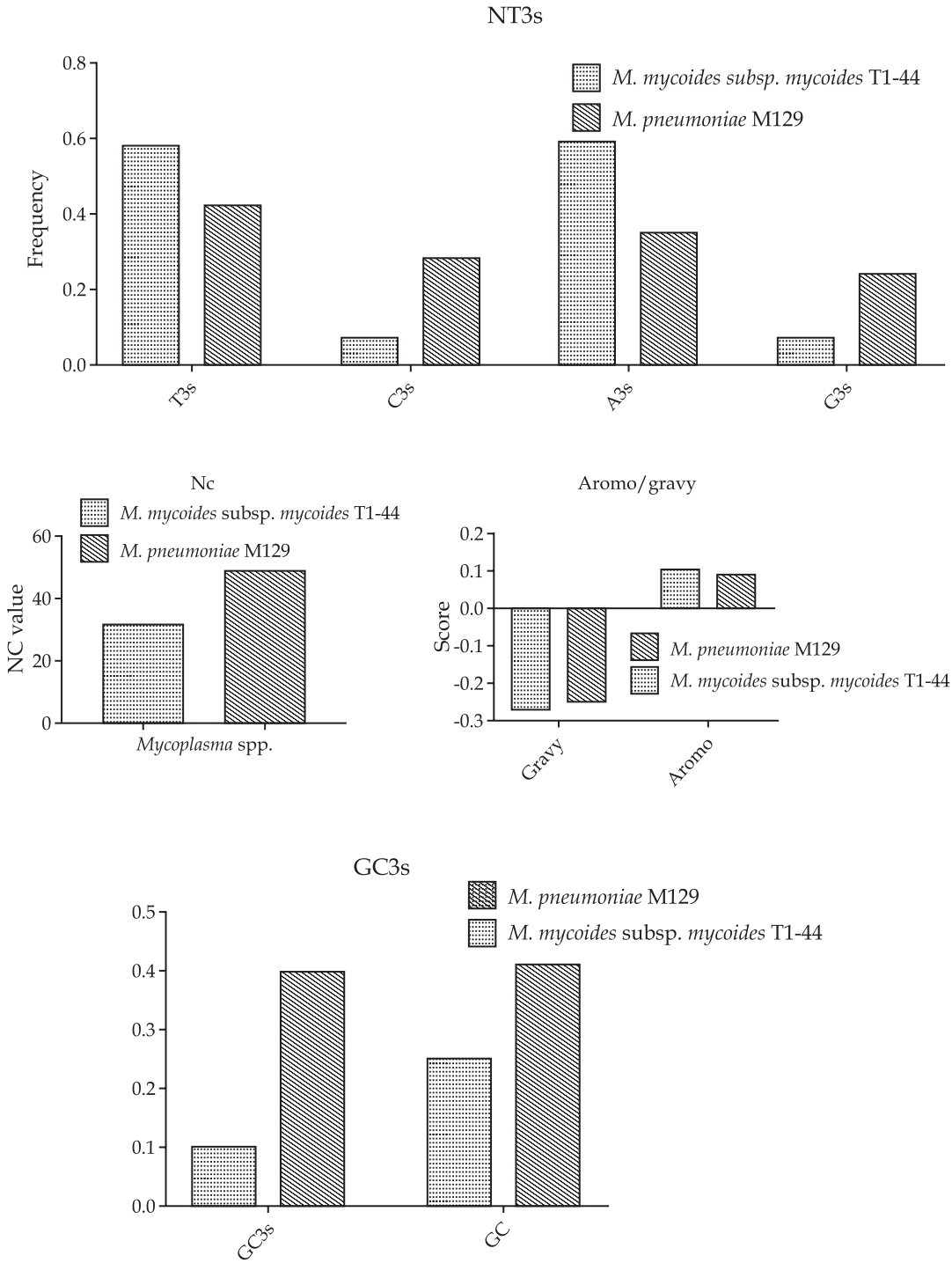

Fig 2. The codon usage indices of *mycoplasma* genomes.

Table 2. Average RSCU values from rMyc. The preferred codons for every amino acid is displayed in bold. Bold Italic indicate negative codon bias. Highlighted cells are over biased codon.

Amino acid	Codon	M. *mycoides* subsp. *mycoides* T1-44	*M. pneumoniae* M129	Camel	Cattle	Human
Phe	UUU	**1.77**	**1.49**	**1.19**	0.85	0.87
	UUC	***0.18***	0.47	0.81	**1.15**	**1.13**
Leu	UUA	**4.14**	**2.17**	1.18	***0.38***	***0.39***
	UUG	***0.42***	1.2	**1.33**	0.71	0.73
	CUU	***0.55***	0.7	0.88	0.7	0.73
	CUC	***0.06***	0.71	0.60	1.26	1.21
	CUA	0.72	0.65	0.98	***0.36***	***0.4***
	CUG	***0.09***	***0.53***	1.04	**2.59**	**2.53**
Ile	AUU	**1.98**	**1.93**	**1.34**	0.98	1.03
	AUC	***0.22***	0.64	0.65	**1.57**	**1.52**
	AUA	0.75	***0.36***	1.01	***0.45***	***0.44***
Val	GUU	**2.47**	1.43	1.00	0.64	0.69
	GUC	***0.13***	0.64	***0.53***	1.01	1
	GUA	1.16	0.86	1.00	***0.4***	***0.42***
	GUG	***0.22***	1.06	**1.46**	**1.95**	**1.9**
Ser	UCU	1.48	0.8	1.34	1.04	1.11
	UCC	***0.1***	0.89	0.80	1.37	1.39
	UCA	**2.12**	0.86	**1.30**	0.79	0.84
	UCG	***0.13***	0.6	***0.54***	***0.39***	***0.33***
	AGU	**1.89**	**1.86**	1.21	0.87	0.84
	AGC	***0.6***	0.98	0.81	**1.53**	**1.5**
Pro	CCU	1.47	0.97	**1.37**	1.08	1.12
	CCC	***0.13***	**0.99**	0.74	**1.39**	**1.35**
	CCA	**1.99**	0.68	1.33	1	1.07
	CCG	***0.12***	0.69	***0.56***	***0.53***	***0.46***
Thr	ACU	**2.21**	1.27	1.14	0.89	0.94
	ACC	***0.18***	**1.34**	0.80	**1.55**	**1.52**
	ACA	1.47	0.76	**1.41**	1.01	1.07
	ACG	***0.06***	***0.53***	0.66	***0.56***	***0.46***
Ala	GCU	**2.2**	**1.53**	1.09	1	1.09
	GCC	***0.13***	0.91	0.72	**1.71**	**1.64**
	GCA	1.45	0.85	**1.46**	0.8	0.85
	GCG	***0.11***	0.65	0.73	***0.48***	***0.42***
Tyr	UAU	**1.66**	0.86	**1.03**	0.79	0.84
	UAC	***0.23***	**1.04**	0.97	**1.21**	**1.16**
His	CAU	1.3	0.68	1.05	0.75	0.81
	CAC	***0.45***	**1.15**	0.95	**1.25**	**1.19**
Gln	CAA	**1.77**	**1.38**	1.06	***0.46***	***0.51***
	CAG	***0.17***	***0.57***	0.94	**1.54**	**1.49**
Asn	AAU	**1.6**	0.84	0.96	0.81	0.89
	AAC	***0.37***	**1.1**	**1.04**	**1.19**	**1.11**
Lys	AAA	**1.75**	**1.08**	0.96	0.78	0.82
	AAG	***0.23***	0.87	**1.04**	**1.22**	**1.18**

Asp	GAU	**1.71**	**1.18**	**1.00**	0.84	0.89
	GAC	***0.22***	0.74	**1.00**	**1.16**	**1.11**
Glu	GAA	**1.76**	**1.41**	**1.10**	0.78	0.81
	GAG	***0.19***	***0.52***	0.90	**1.22**	**1.19**
Cys	UGU	1.22	1.08	**1.18**	0.85	0.86
	UGC	***0.2***	***0.43***	0.82	**1.15**	**1.14**
Arg	CGU	***0.55***	1.53	0.84	***0.49***	0.51
	CGC	***0.1***	**1.66**	***0.54***	1.17	1.2
	CGA	***0.15***	***0.47***	***0.47***	0.68	0.63
	CGG	***0.05***	0.73	***0.49***	**1.32**	1.2
	AGA	**4.59**	0.95	**1.88**	1.14	1.2
	AGG	***0.28***	0.61	1.77	1.2	**1.26**
Gly	GGU	**1.72**	**1.87**	**1.18**	0.64	0.64
	GGC	***0.15***	0.87	1.10	**1.43**	**1.4**
	GGA	**1.75**	***0.59***	1.03	0.95	0.98
	GGG	***0.23***	0.63	0.69	0.99	0.98
Total no. over-represented codons		16	5	2	3	3
Total no. under-represented codons		17	9	6	9	8
Number of optimal codons with A3/T3		27	27	27	24	24
Number of optimal codons with G3/C3		1	23	23	25	24

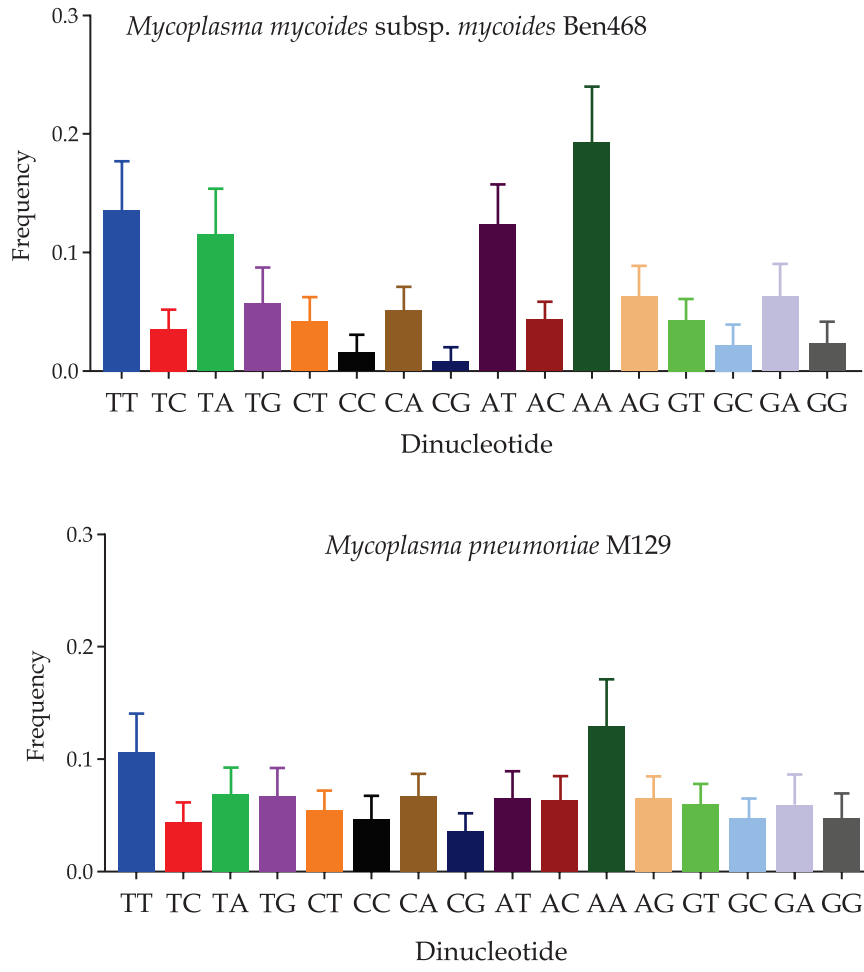

Fig 3. Dinucleotides frequency.

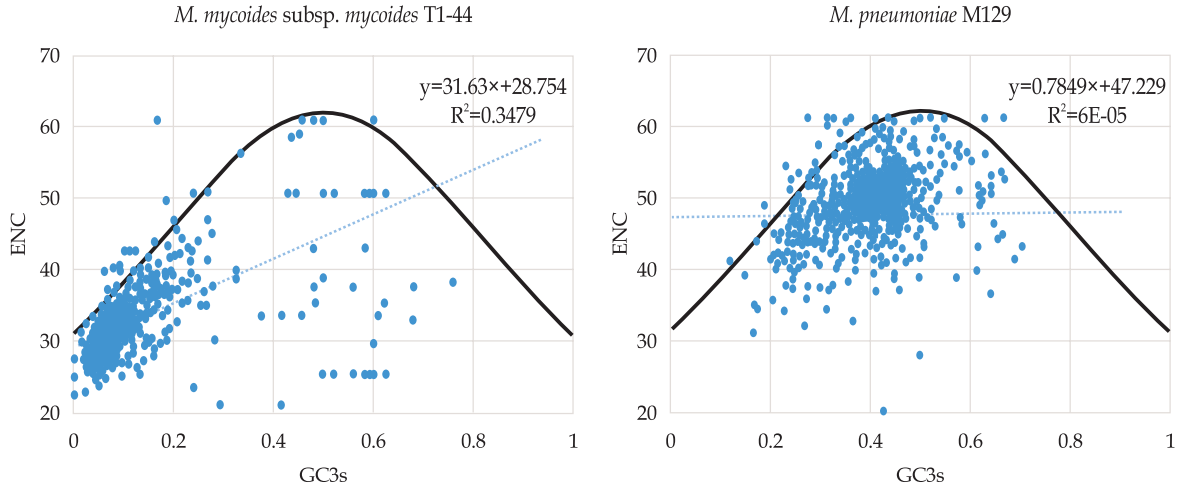

Fig 4. ENc plot. ENc is the effective number of codons plotted against G or C nucleotides at the 3rd positions in codons (GC3s). The solid black curve denotes the frequency of ENc if codon bias is due to GC3s only.

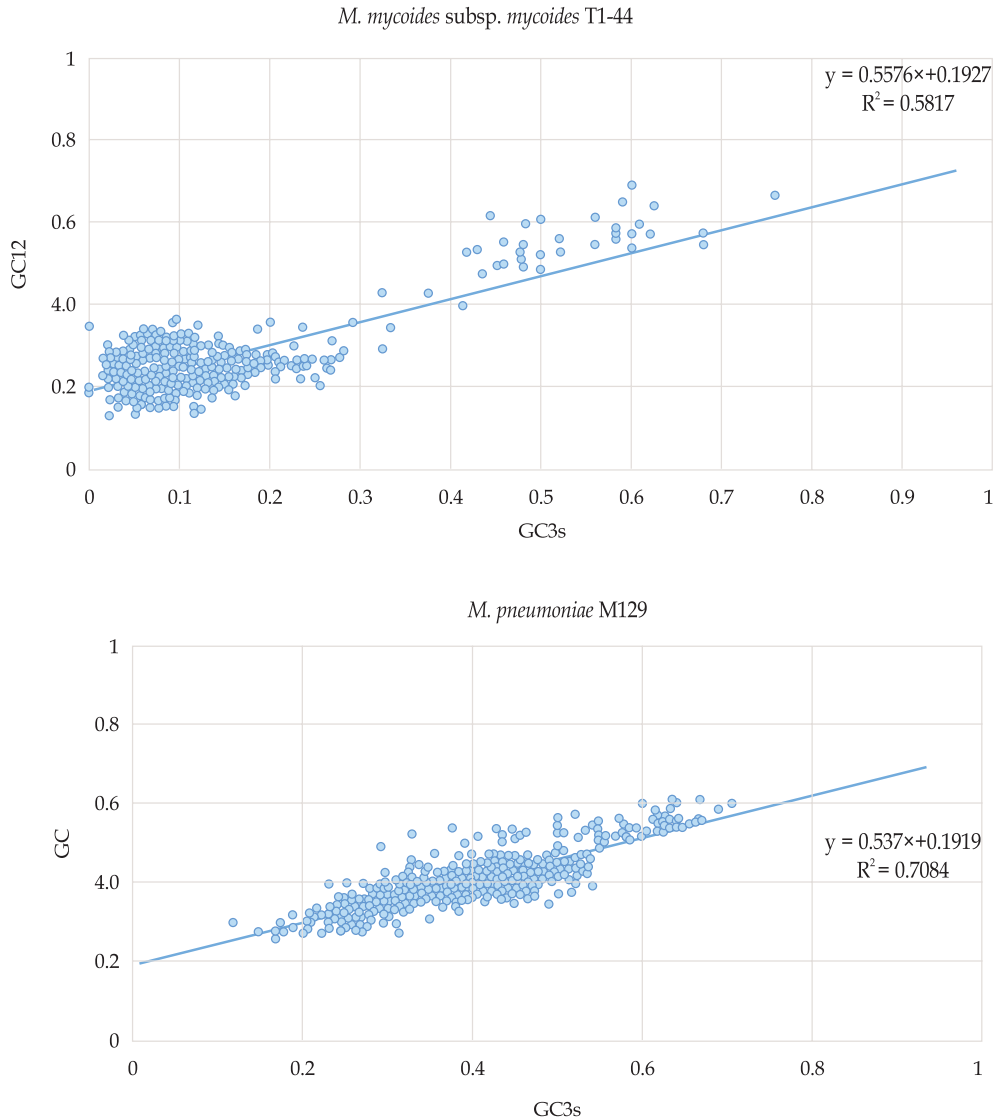

Fig 5. Neutrality plot. G/C nucleotides frequencies at the 3rd position of codons is plotted against their frequencies at the first and second positions (GC12).

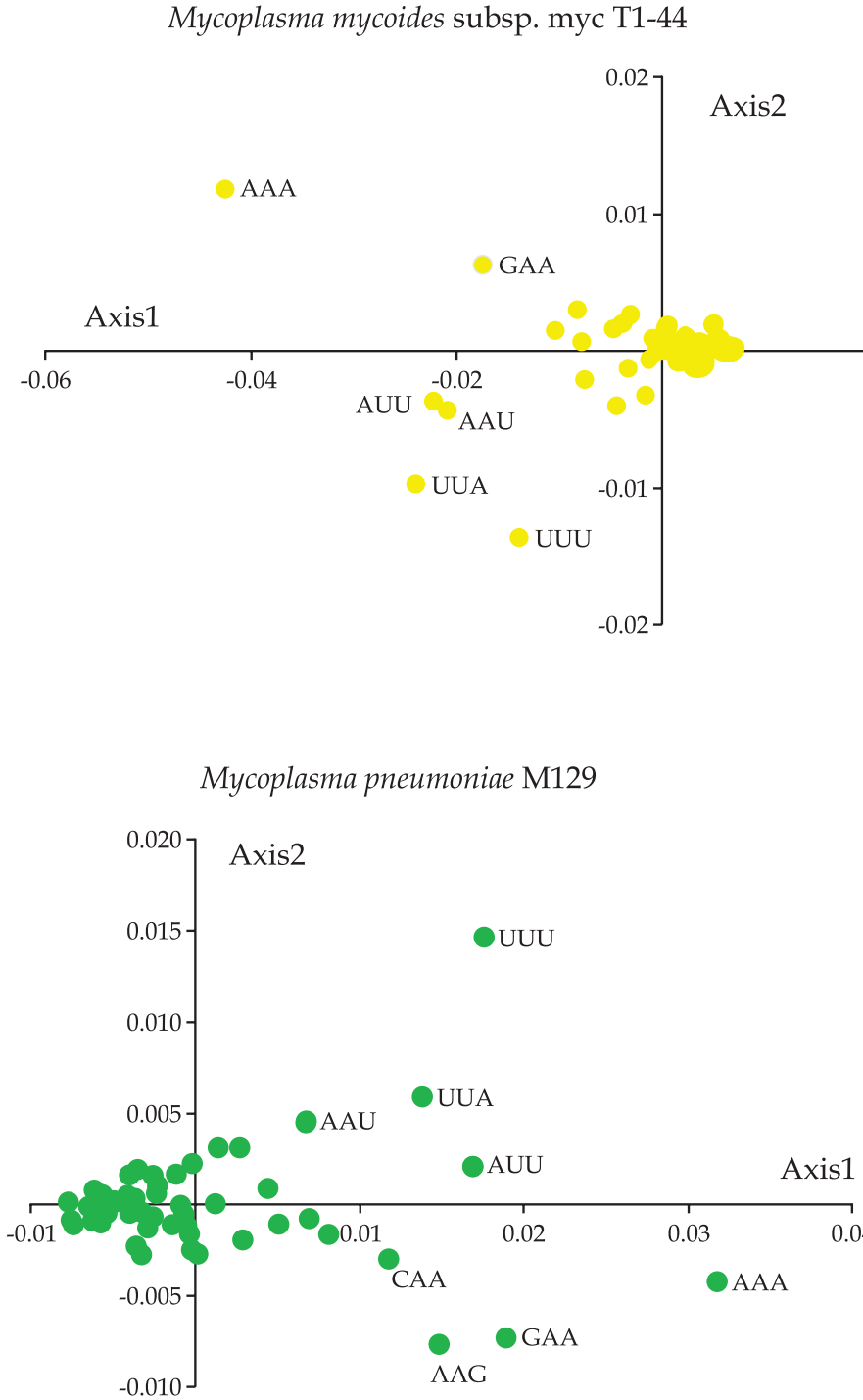

Fig 6. Correspondence analysis of codon usage in different *Mycoplasma* spp.

Table 3. Results of regression analysis of Aromo and Gravy with ENc and GC3s in different *Mycoplasma* spp.

Species	ENc		GC3s	
	Gravy	Aromo	Gravy	Aromo
M. mycoides subsp. *mycoides* T1-44	0.47	0.04***	0.4***	0.037***
M. pneumoniae M129	0.5	0.048	0.5	0.04***

Correspondence analysis

COA analysis revealed that the first 3 axes constitutes the major proportion of influences on codon usage (Table 4). The first 2 axes performed the most important influences on codon usage. The first axis accounts for more than 61.8% and 43.1 in *M. mycoides* and *M. pneumoniae*, respectively. This indicates diverse influences on codon usage in *M.*

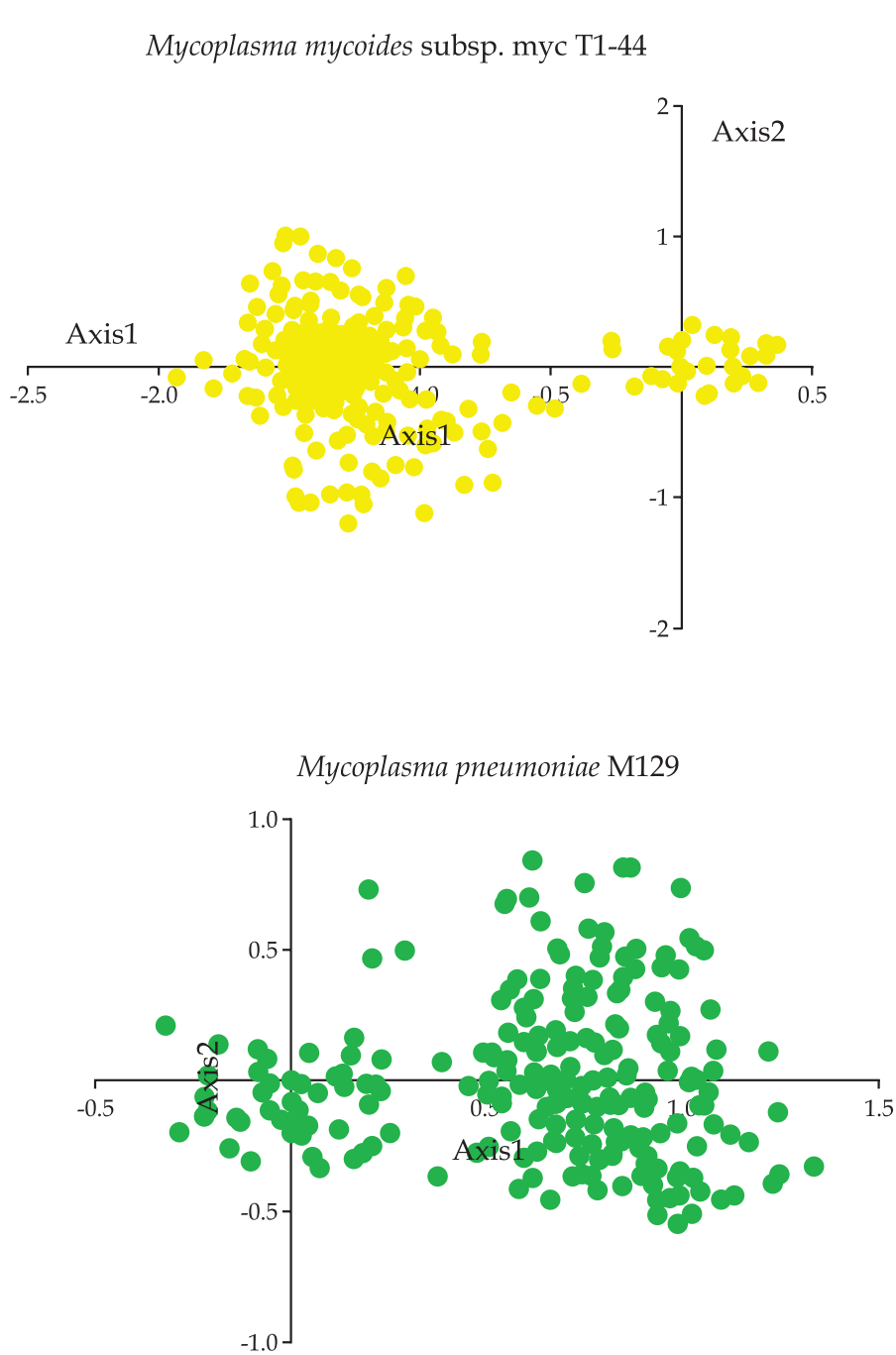

Fig 7. Correspondence analysis of RSCU in different *Mycoplasma* spp.

mycoides and *M. pneumoniae*. The distribution of codons on the first 2 axes (Fig 6) revealed moderate codon bias. The major points clustered around the origin indicates codons without bias, deviations from the origin of the scattered codons indicates the degree of bias against codons. COA of RSCU value showed different profile (Fig 7). Fewer numbers of genes were centered around the origin, while most genes cluster along the first axis indicating variable degrees of codon bias among *Mycoplasma* genes.

Table 4. The contribution of the top three COA axes on codon usage in different *Mycoplasma* spp.

Species	1st Axis	2nd Axis	3rd Axis
M. mycoides	61.8	3.9	2.2
M. pneumoniae	43.1	5.6	3.2

In this study, the trends of codon usage and codon usage bias in 2 *Mycoplasma* species affecting the respiratory system in 2 different mammalian hosts were analysed from the sequences of complete

genomes. The study of codon bias is important in understanding the microbial genetic aspects by clarifying their genome composition and its associated compositional constraints, translational pressure, frequency of codons usage, rare and preferred codons frequency and factors sharing in formation and shaping of genomes.

Codon usage is important determinant and control on gene expression efficiency. Changes in codon usage among microbes come from 2 main forces, the mutational bias and translation selection. The former model proposes the influence of nucleotide composition on codon selection. The latter assumes that adaptation in codon usage is preferred to correlate with gene expression and optimisation of translation efficiency (Vidyavijayan *et al,* 2017).

At first instance, codon bias is expected in rMyc since there was high AT compositional bias. *Mycoplasma* is considered as AT rich bacteria with an accepted low GC% not exceeding 30% (Winkler and Wood, 1988). In this work, considerable variation in genomes AT content among rMyc by showing 15% changes between two closely related species was obscured. The compositional bias is reflected on amino acids usage. *M. mycoides* showed higher frequency for valine, asparagine and tryptophan.

Most of the preferred codons in rMyc were ending with A or U indicating that mutational bias is strongly shaping their different genomes. The frequencies of NT3s showed marked differences among rMyc. *M. pneumoniae* showed 1-3 folds higher G3s/C3s and lower A3s/Ts3 values. In contrast, *M. mycoides* showed the highest G3s/C3s and lowest A3s/Ts3 values.

Analysis of RSCU denotes that rMyc are utilising more AT in both genome and preferred codon (Table 3). Codon RSCU can be divided into 3 groups: 1) codons having RSCU estimates over 1.6 represent positively biased or over-represented codons. 2) biased codons having values less than 0.6 represent under-represented or negatively biased codons. 3) codons having RSCU estimates between 0.6 to1.6 represent represented or unbiased. The codon usage bias seems to be not conserved among rMyc. It is widely accepted that ENc values above 40 indicates lack of codon usage bias. Therefore, we can conclude species differences implying strong and weak or no codon usage bias. *M. pneumoniae* showed lack of codon usage bias by showing high ENc value of 48. *M. mycoides* showed strong codon usage bias as ENc value = 31.

The relative abundance of dinucleotides has been associated with modulation of host immune response especially, the CpG frequencies. The bacterial unmethylated CpG is considered as immune stimulant by interaction with Toll-like receptor 9. As a role in escaping from immune response, *M. mycoides* seem to be more efficient immune resistant than *M. pneumoniae* by showing low CpG frequency.

ENc plot is used to assess the effect of base composition on codon usage pattern. There was positive correlation between ENc and GC3s. In rMyc, small number of genes fall on the standard solid line. The null hypothesis is that GC3s bias is only due to mutation and there is a minor role of selection pressure. In this condition, all genes should fall on the null hypothesis curve. However, most of genes in both rMyc species were located under the standard hypothesis curve suggesting a strong effect of selection on shaping of their genomes.

There were 2 patterns of distribution of genes in ENc plots. The first is strict A/T bias low ENc by *M. mycoides*: most genes are clustered toward A/T region (left side, Fig 3) with low ENc or high codon bias (ENc values below 40). In the 2nd pattern, slight or no A/T bias and high Nc by *M. pneumoniae*: most of genes were clustered at the middle part of the plot showing higher number of genes with G/C bias (right side under the curve, Fig 3) and most genes have ENc values exceeding 40. The remarkable correlation between the hydropathic and hydrophobic constituents with GCs and ENs in *M. mycoides* indicates larger selection force affecting its genome composition.

The observed low ENc values for *M. mycoides* spp. indicates higher number of highly expressed genes. This might be associated with the virulence of this strain and high pathogenicity.

Owing to noticeable dispersion of genes along the axes of COA, it was speculated that there were changes in codon usage pattern over time. Comparison between rMyc RSCU and their host values revealed interesting differences. Presumably, *M. pneumoniae* showed a profile of efficient adaptation to human codon usage environment. In contrast, *M. mycoides* RSCU was far different from camel and cattle values. The mammalian hosts in this study; camel, cattle and human, showed similar codon usage profile including 3 over-represented codons. 8-9 under-represented codons, 24 optimal codons with A3s/T3s and 24-25 optimal codons with G3s/C3s. Additionally, the preferred codons were rich in GC3s. *M. pneumoniae* showed more or less

complementarity with human codon usage. About 40% of the preferred codons contained GC3s. It shows almost similar profile of over-represented, under-represented and optimal codons compositions (Table 2). Surprisingly, *M. mycoides* has completely different profile of extreme deviation from their camel host as well as, from its human respiratory equivalent *M. pneumoniae*. From extreme AT bias to high number of over-represented and under-represented codons, *M. mycoides* has little attempts to adapt to mammalian hosts. This extreme bias might be a contributing factor to escape human immune response (He *et al,* 2017).

In conclusion, this study addressed the differences between 2 *Mycoplasma* species affecting respiratory tract with respect to codon usage, codon usage bias, genomic constituents and factors influencing its composition, encoded pathogenesis and adaptation to their host. Despite of their common evolutionary origin, they have distinct profiles of codon usage indices, adaptive paths and pathogenesis. This highlights the wide adaptability and variability in *Mycoplasma* to live in different environments. In addition, tissue tropism was not a predeterminant for the estimated indices. In contrast, some species are more adapted to live in mammals e.g. *M. pneumoniae*, while others as *M. mycoides* are highly diverged from their hosts.

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SEQUENCE ANALYSIS OF TOLL LIKE RECEPTOR 1 GENE OF CAMEL (*Camelus dromedarius*)

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ABSTRACT

In the present study complete mRNA sequence of camel TLR1 was analysed using various tools and softwares. The analysed sequence was made up of 2430 bp long contig with 2391 long open reading frame which translated into 796 aa. The sequence has been submitted in NCBI (Accession. No: MG655186). The Camel TLR1 sequence had 99% identity with the predicted TLR-1 protein of dromedary camel with two amino acid variation. The sequence alignment showed the highest similarity of camel TLR1 with pig, whereas lowest similarity was found with rabbit. The analysis of protein sequence revealed seven leucine rich repeats (LRR) domain in the extracellular region similar to that of goat. The 3D protein model showed a TLR 1 similar to that of other vertebrates.

Key words: Camel, mRNA, phylogenetic analysis, sequence analysis, toll like receptor 1

Toll like receptors (TLRs) are part of the Type I transmembrane protein family. Different TLRs are classified on the basis of their cellular location and the ligands they bind. All TLRs have the same structure which consists of three domains; extracellular domain which binds to ligand, a transmembrane domain and a TIR (Toll/IL-1R-like) cytoplasmic domain (Botos *et al,* 2011; Muzio *et al,* 2000; Zarember and Godowski, 2002; Böttcher *et al,* 2003). Although TLRs have been found to be evolutionarily conserved within and between lineages, characterising TLRs according to their domains indicates role of natural selection on TLRs (Mukherjee *et al,* 2009; Quach *et al,* 2013; Mukherjee *et al,* 2014). Studies have shown selection in TLR regions for oligomerisation and considerable degree of variation have been observed in TLR regions coding for PAMP binding (Werling *et al,* 2009). Further, taking individual species and location of receptor into account, adaptive substitutions in TLRs have been observed (Jann *et al,* 2008; Nakajima *et al,* 2008).

Amongst different TLRs, TLR1, 2, 6 and 10 form a unique subfamily within the Toll-like receptors (Matsushima *et al,* 2007; Roach *et al,* 2005). They not only do share high levels of homology and recognise related agonists, they are also the only TLRs to heterodimerise upon ligand recognition. TLR1 recognises the PAMPs with specificity for Gram-positive bacteria. It has been designated as CD281 (Rock *et al,* 1998; Elefil *et al,* 2016) and forms functional pairs with TLR6 and TLR2 to diversify its ligands recognition to bacterial lipoprotein, lipoteichoic acid and peptidoglycan (Kang *et al,* 2009; Jin *et al,* 2007; Zähringer *et al,* 2008) but can also function alone (Ozinsky *et al,* 2000).

Sequencing of TLR genes has provided the opportunity to examine the adaptive evolution of TLRs in broad range of terrestrial ungulates and cetaceans during their complex habitat diversification (Ishengoma and Agaba, 2017). *Camelus dromedarius* has acquired many special abilities and attributes to survive in harsh environments and is comparatively resilient to infections. The TLRs of dromedary camels have not been sequenced except for TLR 2 (Dahiya *et al,* 2014). Present study is aimed to identify and characterise the coding sequence of camel TLR-1 gene and provide resources to the scientific community for further investigation.

Materials and methods

Camel blood samples

Blood samples (5 ml) of dromedary camels were collected from clinics of RAJUVAS, Bikaner, India. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation using HiSepTM LSM1084 (HIMEDIA) and stored at -80°C.

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Isolation of RNA and cDNA synthesis

Total RNA was isolated from PBMCs using TRIzol reagents (Sigma) according to manufacturer's protocol; both the quantity and quality of RNA were assessed at OD260 and OD280 using Nanodrop (Implen NanophotometerTM Pearl device). An aliquot of total RNA was reverse transcribed using the RevertAid First strand c-DNA synthesis kit (Thermo scientific) as per the manufacturers protocol.

Amplification cDNA of camel TLR-1

TLR-1 cDNA was amplified by conventional PCR using specific primers (Table 1) designed by Primer3 software on the basis of predicted sequence of camel TLR-1 (XM_010980393.1). A total of 10 primer pairs were used, covering the entire coding sequence. Cycling conditions were denaturation at 94°C for 40 second, annealing at 44-51°C for 25 second, extension of 72°C for 40 second. Annealing temperatures for different primer pairs are given in table 1. Amplified PCR products were analysed on 1.5% agarose gel and were sequenced.

Sequence analysis

Contigs were prepared from resultant nucleotide sequences. These contigs were assembled into a complete coding sequence and analysed using BioEdit software version 7.0 with TLR1 of twelve animal species published in the Gene Bank. The sequence similarity was obtained using BLAST tool. The phylogenetic tree was constructed by MEGA 7.0 software and protein domain features in the translated amino acids were predicted by simple modular architecture research tool (SMART). Sequences were translated into amino acid through online utility using Expert Protein Analysis System (EXPASY) software.

Protein 3D structure prediction

The 3D protein structure of TLR-1 was constructed using SWISS-MODEL server. The best matched template was selected for 3D structure of camel TLR 1. Further, the validation of 3D model was done by Ramachandran plot (RAMPAGE).

Result and Discussion

Sequence characterisation

The different overlapping fragments of camel TLR1 cDNA obtained by PCR were sequenced and assembled into a 2430 bp long contig. Through ORF finder this contig was found to have 2391 nucleotide long open reading frame (ORF). The sequence was submitted to NCBI with the help of BankIt tool (GenBank accession no. MG655186). The predicted

Table 1. Primers used for PCR amplification of TLR-1 gene of camel (*Camelus dromedarius*).

S.No	Name	Primer	Amplicon size	Annealing temperature
1	CSTLR-1 (A)	F- ATGACTAAAATGAAGTCTGGCATC	150 bp	46°C
		R – TTTCAGGGGTAGGTCTTTGG		
2	CSTLR-1 (B)	F – CCAAAGACCTACCCCTGAAA	325 bp	47°C
		R - GCACTCAACCCCAGAAACTG		
3	CSTLR-1 (C)	F – TGGACGTGTCACTCAGCACT	333 bp	45°C
		R – TTTGTGGAAACCCAAACACA		
4	CSTLR-1 (D)	F - TGTGGAAATCTGATTAATTTGGAG	358 bp	44°C
		R - CACATCCAGGAAGGTGGACT		
5	CSTLR-1 (E)	F - GCCAAGTACCAAGTGAAGTGG	325 bp	48°C
		R – GAATCGTGCCCACTGTATGA		
6	CSTLR-1 (F)	F – TTTGTTCAGAGCGAGTGGTG	334 bp	46°C
		R – TGTTGGAACTTGCAAAAGCA		
7	CSTLR-1 (G)	F – AGTTTCTGGGGTTGAGTGCC	210 bp	50°C
		R – TGCTGAGTGACACGTCCAAA		
8	CSTLR-1 (H)	F – TACCTCCCAGGGTCAAGGTT	326 bp	51°C
		R – AGGCCAGTCCTCTACCACTT		
9	CSTLR-1 (H)	F – GTTACCGTGACCGTCCTCTG	369 bp	51°C
		R – GTTGTGGTGGGCGAAGTAGA		
10	CSTLR-1 (I)	F – TTTGGCATACGAGCATTGAG	463 bp	48°C
		R – GTCCAAGAGCAATTTCCTTCA		

open reading frame (ORF) of camel TLR1 was translated into 796 amino acid long protein sequence. The same number of amino acids was reported in pig and rabbit (Shinkai *et al,* 2006; Elefil *et al,* 2016).

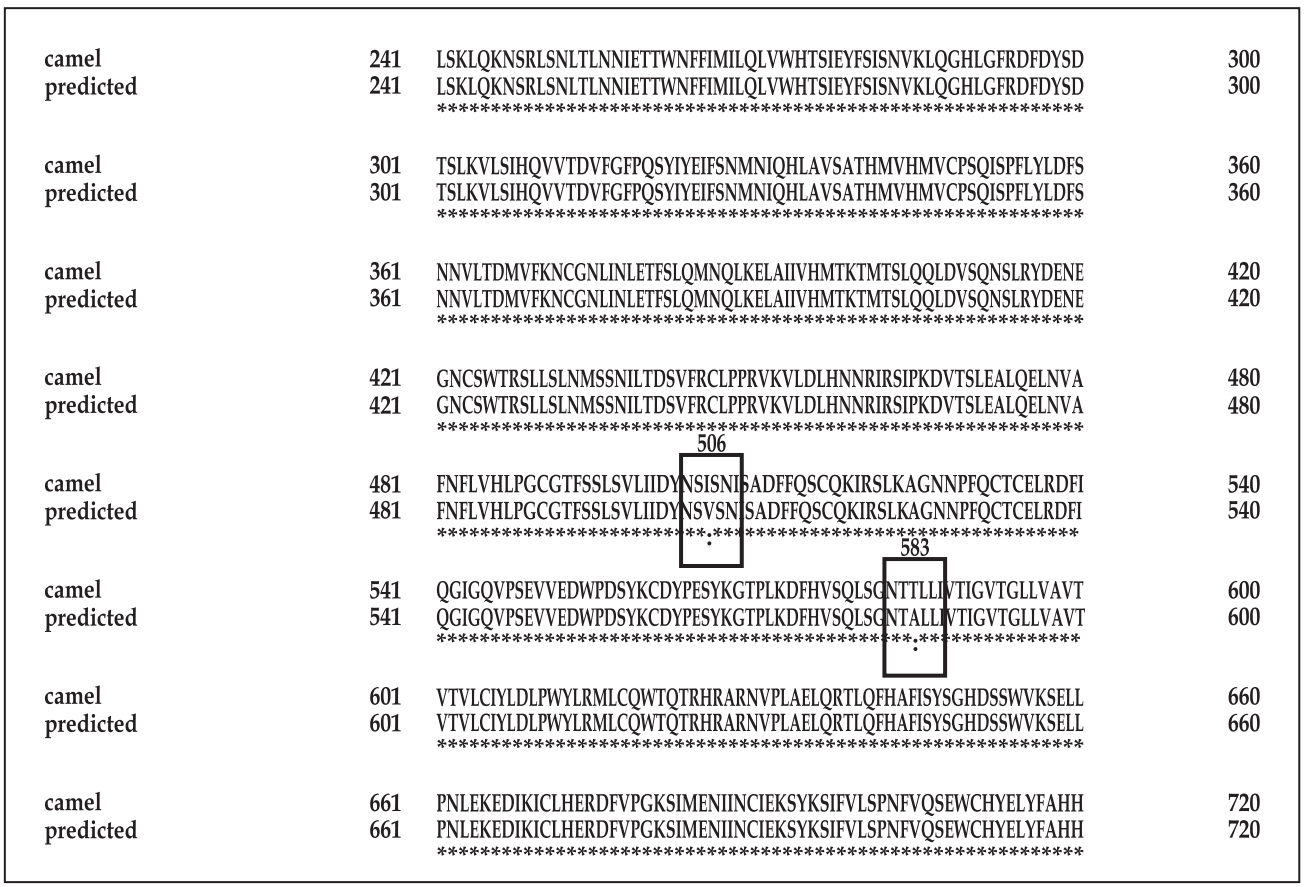
The TLR-1 sequence was aligned against the predicted TLR-1 protein sequence of dromedary camel (accession number XM_010980393.1), 99% identity was obtained. The camel TLR-1 amino acid sequence was also compared with TLR-1 of other species pig (*Sus scorfa*), buffalo (*Bubalus bubalis*), deer (*Cervus nippon*), cattle (*Bos taurus*), sheep (*Ovis aries*), dog (*Canius lupus familiaris*), goat (*Capra hircus*), horse (*Equus caballus*), gorilla (*Gorilla gorilla*), human (*Homo sapiens*), orangutan (*Pongo pymaeus*) and rabbit (*Oryctolagus cuniculus*) with similarity of 85%, 85%, 85%, 84%, 84%, 82%, 82%, 82%, 79%, 79%, 78% and 72%, respectively (Table 2).

The highest similarity of camel TLR1 was noticed with TLR1 of pig, whereas lowest similarity was found with that of rabbit. The similarity of camel TLR-1 was found to be more than 70% with other species included in this study which indicates that TLR-1 mRNA sequence is conserved among these species (Pearson, 2013; Medzhitov *et al,* 1997).

Further, variation of two amino acids was recorded between encoded polypeptide sequence of camel TLR1 and predicted polypeptide sequence of camel TLR-1 at position 506 and 583. The variation of isoleucine in place of valine at 506th and threonine in place of alanine at 583rd place was observed (Fig 1).

Analysis of Protein domain of camel TLR-1

The amino acid sequence of camel TLR1 protein was further analysed using online tool Simple Modular Architecture Research Tool (SMART). The camel TLR-1 protein consisted of signal peptides (28 amino acids from 1-28) followed by seven leucine rich repeat (LRR) domain (at position 72-95, 97-116, 117-138, 375-396, 401-424, 449-470, 471-495) and one C – terminal LRR domain of around 54 amino acids (LRR-CT, residues 528-582) in the extracellular region. The transmembrane region was composed of 22 amino acids (from position 586-608) and TIR domain of 143 amino acids (residues 640-783) in the cytoplasmic region (Fig 2) (Table 3). The TIR domain of camel was found to be similar to that of pig, horse, human and orangutan (Shinkai *et al,* 2006). This domain is responsible for signal transduction and activating innate immune inflammatory responses (Akira and



camel	241	LSKLQKNSRLSNLTLNNIETTWNFFIMILQLVWHTSIEYFSISNVKLQGHLGFRDFDYSD	300
predicted	241	LSKLQKNSRLSNLTLNNIETTWNFFIMILQLVWHTSIEYFSISNVKLQGHLGFRDFDYSD	300
camel	301	TSLKVLSIHQVVTDVFGFPQSYIYEIFSNMNIQHLAVSATHMVHMVCPSQISPFLYLDFS	360
predicted	301	TSLKVLSIHQVVTDVFGFPQSYIYEIFSNMNIQHLAVSATHMVHMVCPSQISPFLYLDFS	360
camel	361	NNVLTDMVFKNCGNLINLETFSLQMNQLKELAIIVHMTKTMTSLQQLDVSQNSLRYDENE	420
predicted	361	NNVLTDMVFKNCGNLINLETFSLQMNQLKELAIIVHMTKTMTSLQQLDVSQNSLRYDENE	420
camel	421	GNCSWTRSLLSLNMSSNILTDSVFRCLPPRVKVLDLHNNRIRSIPKDVTSLEALQELNVA	480
predicted	421	GNCSWTRSLLSLNMSSNILTDSVFRCLPPRVKVLDLHNNRIRSIPKDVTSLEALQELNVA	480
camel	481	FNFLVHLPGCGTFSSLSVLIIDYNSISNISADFFQSCQKIRSLKAGNNPFQCTCELRDFI	540
predicted	481	FNFLVHLPGCGTFSSLSVLIIDYNSVSNISADFFQSCQKIRSLKAGNNPFQCTCELRDFI	540
camel	541	QGIGQVPSEVVEDWPDSYKCDYPESYKGTPLKDFHVSQLSGNTTLLIVTIGVTGLLVAVT	600
predicted	541	QGIGQVPSEVVEDWPDSYKCDYPESYKGTPLKDFHVSQLSGNTALLIVTIGVTGLLVAVT	600
camel	601	VTVLCIYLDLPWYLRMLCQWTQTRHRARNVPLAELQRTLQFHAFISYSGHDSSWVKSELL	660
predicted	601	VTVLCIYLDLPWYLRMLCQWTQTRHRARNVPLAELQRTLQFHAFISYSGHDSSWVKSELL	660
camel	661	PNLEKEDIKICLHERDFVPGKSIMENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHH	720
predicted	661	PNLEKEDIKICLHERDFVPGKSIMENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHH	720

Fig 1. Variation of amino acid sequence between camel TLR1 and predicted camel TLR1

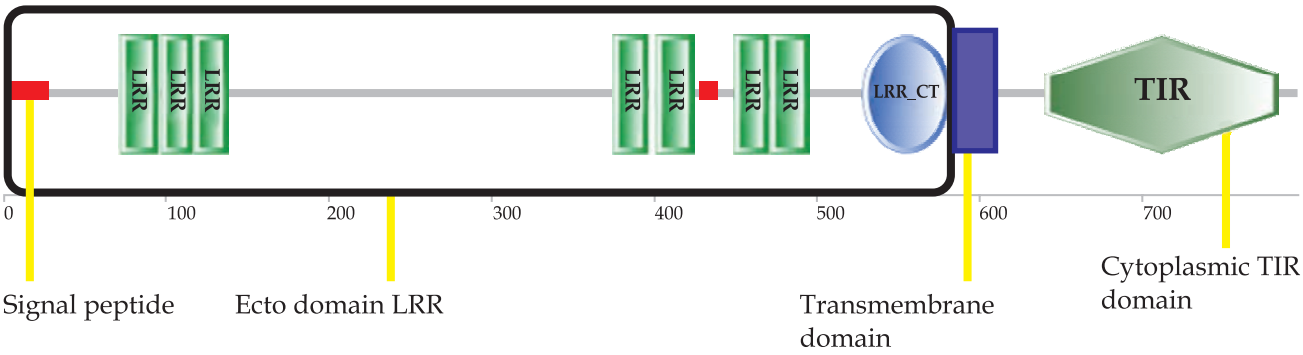


Fig 2. Diagrammatic representation of protein domain of the camel TLR-1.

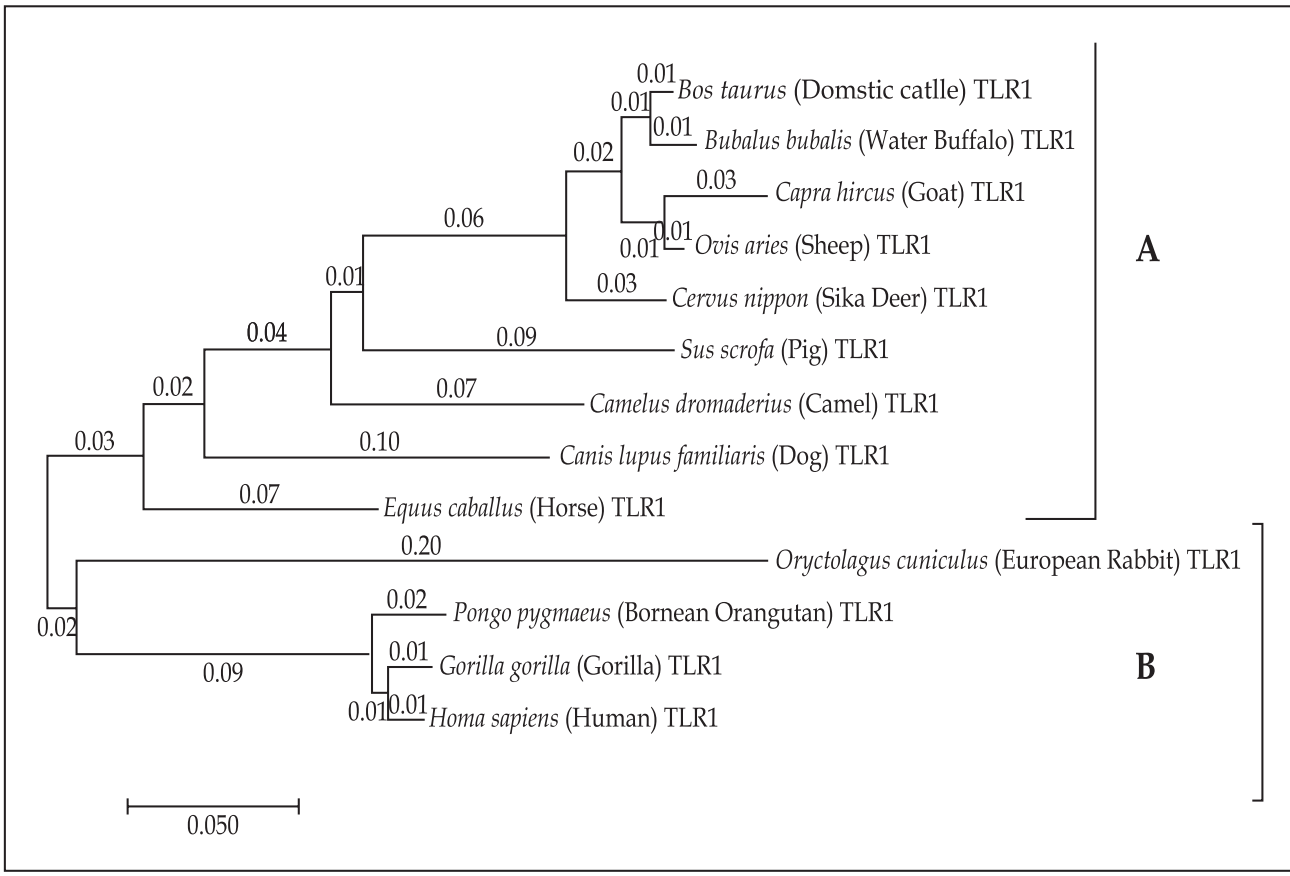


Fig 3. Phylogenetic tree of camel TLR1 on the basis of amino acid sequence

Fig 4. 3D structure model of camel TLR 1 protein.

Table 2. Percentage of amino acid sequence identity of camel TLR-1 with other species.

S.No	Species	Country and year	NCBI accession no.	Amino acid identity Percentage to camel
1	*Camelus dromedarius*	India,2017	-	-
2	*Bos taurus*	Germany ,2012	FJ147090.1	84
3	*Bubalus bubalis*	India, 2010	GU451251.1	85
4	*Canius lupus familiaris*	U.K., 2009	EU551145.1	82
5	*Capra hircus*	India, 2011	HQ263209.1	82
6	*Cervus nippon*	China, 2016	KT223118.1	85
7	*Equus caballus*	NA	NM001256899.1	82
8	*Gorilla gorilla*	Japan, 2008	AB445620.1	79
9	*Homo sapiens*	Japan, 2008	AB445617.1	79
10	*Oryctolagus cuniculus*	China, 2013	KC349941.1	72
11	*Ovis aries*	U.K., 2009	AM981299.1	84
12	*Pongo pygmaeus*	Japan, 2008	AB445621.1	78
13	*Sus scrofa*	Japan, 2006	AB208695.2	85

Takeda, 2004; Bell *et al,* 2003). Moreover, multiple sequence alignment showed that TIR domain region was more conserved than other domains.

The position of signal peptide, C-terminal LRR domain, transmembrane region and TIR domain in camel TLR-1 was found similar to the Pig, Horse, Human and Rabbit TLR-1. However, the number of leucine rich repeats in camel (7) TLR-1 is higher than rabbit (3), sheep (6), buffalo (5), pig (5), human (5) and other studied species (Elefil *et al,* 2016; Chang *et al,* 2009; Banerjee *et al,* 2012; Shinkai *et al,* 2006). Whereas, the number of LRRs (7) was similar to that of goat (Raja *et al,* 2011). LRRs are responsible for binding to different ligands (Akira and Takeda, 2004; Zhang *et al,* 2014). More number of LRR's in camel indicate that it can recognise more varity of ligand than other studied species and might be playing crucial role in the innate immune defense against pathogenic bacteria (Gao *et al,* 2016).

Phylogenetic analysis

The Phylogenetic tree was constructed using amino acid translated from coding sequence of TLR-1. Neighbour-joining method algorithm was used to construct this tree, which is a distance based method. In this study two major branches were obtained, *i.e.* branch A and B. Branch A cluster had cattle, buffalo, sheep, goat, pig, horse, camel, dog and sika deer, whereas branch B had rabbit, human, gorilla and orangutan. These findings suggest the closest phylogenetic relationship of camel TLR1 gene with pig TLR1 gene and they share a common ancestry, whereas it is distantly related to rabbit TLR-1 (Fig 3).

Table 3. Transmembrane structures of *Camelus dromedarius* TLR-1 predicted domain, repeats and motifs done by SMART tool.

Name	Start	End	E-value
Signal peptide	1	28	N/A
LRR	72	95	0.534
LRR	97	116	284
LRR	117	138	64.1
LRR	375	396	186
LRR	401	424	233
Low complexity	428	439	N/A
LRR	449	470	57.3
LRR	471	495	16.2
LRRCT	528	582	8.11e-11
Transmembrane region	586	608	N/A
TIR	640	783	3.51e-41

3D structure of camel TLR-1

In silico translation of coding DNA sequence of camel TLR1 was subjected to the online tool SWISS MODEL for the construction of 3D structure; the distinctive horseshoe/U shaped structure was formed by the extra cellular domain residues, similar to TLR 1 of different species of animals and mariens (Gay and Gangloff, 2007). This extra cellular domain had mean inner diameter of 45.1 Å, mean outer diameter of about 72.6 Å and mean thickness was 28.6 Å as analysed by Pymol software and contained amino acid sequences from 29 - 539 aa (Fig 4).

In addition, the validity of model was checked by Ramachandran plot analysis programme.

Ramachandran plot analysis showed that most of 466 residues were in favoured region, 40 residues in allowed region and very few (4) residues presented in outlier region indicating the correctness of the predicted structure of TLR 1.

Conclusion

This is the first study for characterisation of camel TLR-1 coding sequence. The camel TLR-1 encodes 2391 long nucleotide open reading frame, translated into 796 amino acids. The 3D structure of camel TLR-1 amino acids is common to TLR 1 of other vertebrates. The camel TLR-1 has 7 LRR domains which are similar to that of goat. Higher number of LRRs indicate its crucial role in the innate immune defense system as it can recognise comparatively more number of PAMPs. Further, the TLR 1 sequence of camel is closely related to that of pig and is most distant from that of rabbit.

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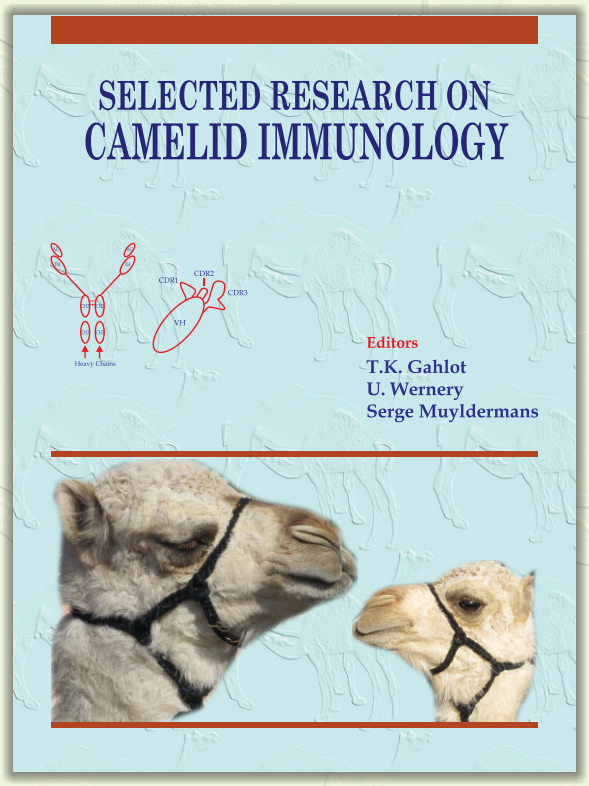
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

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

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



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EVALUATION OF SEVOFLURANE ANAESTHESIA IN DROMEDARY CAMELS (*Camelus dromedarius*) PREMEDICATED WITH XYLAZINE AND INDUCED WITH KETAMINE

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ABSTRACT

The objective of this study was to evaluate the anaesthetic effects of sevoflurane on 6 healthy adult dromedary camels which were premedicated and induced with xylazine (0.2 mg/kg, IV) and ketamine (2 mg/kg, IV), respectively. Anaesthesia was maintained with sevoflurane in 100% oxygen. The onset, duration and the depth of anaesthesia were recorded. Rectal temperature, respiratory rate, heart rate, oxygen haemoglobin saturation and mean arterial blood pressure were measured before and 20 min after the administration of xylazine and then every 10 min until recovery. Jugular blood samples were collected for haematological and blood gases evaluation. Results showed a significant reduction in mean arterial blood pressure, lymphocytes and pH during sevoflurane anaesthesia in the camels. The concentrations of oxygen haemoglobin saturation, venous carbon dioxide, oxygen and glucose were significantly increased during sevoflurane anaesthesia in dromedary camels. The quality of anaesthesia was good and recovery was excellent and relatively quick in all the camels. It was concluded that sevoflurane is an excellent inhalation anaesthetic that can be used safely for maintenance of anaesthesia in camels.

Key words: Anaesthesia, camel, ketamine, sevoflurane, xylazine

Inhalation anaesthesia has become popular for use in large animals, especially for prolonged procedures. Various inhalation anaesthetic agents have been used in large animals. In camels, the use of inhalation anaesthesia has become a routine method prior to major surgical operations. Recently, the use of halothane and isoflurane has been evaluated in dromedary camels (Alsobayil *et al,* 2016; Ahmed *et al,* 2015). Both halothane and isoflurane have been considered good inhalation anaesthetics for camels. However, hypothermia and the long duration of the recovery period were seen as problems when using halothane or isoflurane in camels (Alsobayil *et al,* 2016; Ahmed *et al,* 2015). New types of inhalation anaesthetics have been identified and used in animals. Sevoflurane is a volatile anaesthetic agent that is becoming popular in the field of large animal anaesthesia. Anaesthesia with sevoflurane has been reported in humans (Nakatsuka *et al,* 2002) and domestic animals including horses (Matthews and Hartsfield, 2004; Matthews *et al,* 1998; Aida *et al,* 1996; 1994), goats (Hikasa *et al,* 2002), sheep (Hikasa *et al,* 2000), South American camelids (Garcia Pereira *et al,* 2006; Grubb *et al,* 2003), dogs (Morgaz *et al,* 2011) and cats (Mendes *et al,* 2003; Tzannes *et al,* 2000, Hikasa *et al,* 1997). Because of its low blood solubility (Aida *et al,* 2000), sevoflurane has the advantages of rapid induction, rapid alteration of anaesthetic depth and rapid recovery compared to other inhalation anaesthetics. The use of sevoflurane in dromedary camels has not yet been investigated. Therefore, the aim of this study was to evaluate the use of sevoflurane in dromedary camels premedicated and induced with xylazine and ketamine, respectively.

Materials and Methods

The experimental protocol was approved by the Ethics Committee for Animal Research of the Scientific Research Deanship of Qassim University, Saudi Arabia.

Animals

Six apparently healthy adult female dromedary camels were used in this study. The mean body weight was 574 kg (range 540–594 kg) and mean age was 9 years (range 8–12 years). Food and water

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were withheld 36 and 12 h, respectively, before the beginning of the study. The experiment was carried out in a temperature-controlled room maintained at 25°C.

Anaesthesia

After the camels were restrained in a sitting position, 16-gauge catheters were placed aseptically in the jugular vein. The camels were first premedicated with xylazine HCl (0.2 mg/kg IV)[a]. Twenty minutes later, general anaesthesia was induced with Ketamine (2 mg/kg IV)[b]. After 10 min of ketamine injection, endotracheal intubation was performed using a 20-mm cuffed tube. The anaesthetised camel was then connected with a semiclosed circular rebreathing anaesthesia machine[c] *via* the endotracheal tube. Anaesthesia was maintained with sevoflurane[d] in 100% oxygen at a flow rate of 6 L / min. After 90 min, the vaporiser was turned off and the camel received supplemental oxygen (6 L /min) through the endotracheal tube.

The onset and duration of anaesthesia were recorded. Rectal temperature (RT), respiratory rate (RR), heart rate (HR), oxygen haemoglobin saturation (OHS) and mean arterial blood pressure (MAP) were measured before and 20 min after the administration of xylazine and then every 10 min until recovery. Rectal temperature was recorded using a digital thermometer and respiratory rate was counted by monitoring the movement of either the thoracic or abdominal wall. The concentration of OHS and HR were determined using a pulse oximeter[e] with a probe attached to the tongue. The MAP was indirectly measured using an oscillometric technique[f] with a cuff placed around the tail approximately 5 cm from where it was distally attached to the trunk. The depth of anaesthesia was determined by monitoring palpebral, jaw, tongue, digital and anal reflexes. The onset time and signs of recovery were determined. The time of standing and quality of recovery were also recorded based on a recently presented 5-point scale: 1 = poor, 2 = marginal, 3 = fair, 4 = good and 5 = excellent (Ahmed *et al*, 2015).

Immediately before and 20 min after the injection of xylazine, jugular blood samples (5 mL) were collected from each camel and transferred to vacutainer tubes containing EDTA and heparin. Collection of samples was continued every 10 min (every 20 min in the case of heparinised tubes) until full recovery. The blood samples with EDTA were used to determine the concentrations of white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), red blood corpuscles (RBC), haemoglobin (HG) and haematocrit (HCT), via an automated machine[g]. Using a blood gas analyser[h], the heparinised blood samples were analysed immediately in order to determine the concentrations of venous pH, carbon dioxide (pCO_2), oxygen (pO_2), sodium (Na), potassium (K), calcium (Ca), glucose and bicarbonate (HCO_3).

Statistical Analysis

The data were expressed as mean ± SEM and were analysed with a commercial statistical software package[i]. A repeated measures analysis of variance was used as the statistical model to evaluate the differences over time in the dependent variables including the parameters of physiological and haematological functions. The Duncan test was used to calculate multiple comparisons. Results were considered significant at $P < 0.05$.

Results and Discussion

Effects of the xylazine administration appeared after 5 ± 2 min and included increasing secretion of saliva and tears, calming, lowering of the head and neck, drooping of the lower lip and protrusion of the tongue. After 4 ± 2 min of ketamine administration, the head and neck moved suddenly toward the back of the animal, followed by lateral recumbency. Intubation was performed while the animal was in lateral recumbency.

The mean (± SEM) set value of the sevoflurane vaporizer was 3.2% (range 2.5-4.5%). Fig 1 shows the physiological parameters including RR, HR, MBP, RT and OHS of the camels during anaesthesia with xylazine/ketamine/sevoflurane and during the recovery. The MAP significantly decreased during anaesthesia with sevoflurane, while its level increased during the recovery period (Fig 1C). A significant elevation in OHS was observed during anaesthesia and recovery (Fig 1 E). The RT decreased during

a) Rompun 2%, Bayer Health Care, Monheim, Germany

b) Ketamine 10% Alfasan, Woerden, Holland

c) Surgi Vet Circuit Set, Smith Medical North America, Waukesha, WI, USA

d) Anestane®, HIKMA Pharmaceuticals, Amman, Jordan

e) 504DX Digital Oximeter, Criticare Systems Inc., Waukesha, WI, USA

f) Accutorr Plus™ Recorder, Datascope, Datascope Corp., Paramus, NJ 07652 USA

g) Vet Scan HM5, ABAXIS, Union City, CA 94587 USA

h) GEM® Premier 3000, Instrumentation Laboratory Co., Bedford, MA 01730-2443 USA

i) SAS version 8, SAS Institute Inc, Cary, NC, USA

anaesthesia and this reduction became significant during the recovery (Fig 1D). A slight elevation was seen in the concentration of HR during anaesthesia with sevoflurane in the camels; however, this increase did not reach to significant levels (Fig 1B). A non-significant reduction was observed in the levels of RR during the anaesthesia with sevoflurane (Fig 1A). The haematological parameters showed a significant reduction of total LYM during the anaesthesia with sevoflurane and recovery (Fig 2C). No marked changes in the concentration of WBC, NEU, RBC, Hct or Hb were observed throughout anaesthesia and recovery (Fig 2 A, B, D, E). Table 1 shows a significant decrease in the concentration of venous blood pH and a marked increase in the levels of pCO_2 and pO_2 during the sevoflurane anaesthesia. Glucose concentration significantly increased 10 min after ketamine administration and the elevation continued during the course of the sevoflurane anaesthesia and recovery (Table 1). Calcium levels significantly decreased after recovery from anaesthesia, while the levels of Na, K and HCO_3 did not change during anaesthesia and recovery (Table 1).

For the duration of sevoflurane anaesthesia, jaw, tongue, anal and digital reflexes were completely absent. A slight palpebral reflex was present in all camels during sevoflurane anaesthesia. At recovery, the swallowing reflex was noticed within 4.2 ± 1.6 min after sevoflurane administrative was discontinued. After the vaporiser was turned off, limb and head movement occurred 8.0 ± 2.1 and 16.4 ± 3.2 min, respectively. Sternal recumbency and standing were achieved 23.6 ± 3.5 and 36.6 ± 4.5 min, respectively, post discontinuation of sevoflurane anaesthesia. The quality of recovery was smooth and excellent in 5 animals and good in 1 camel.

This study was conducted to analyse the effects of anaesthesia with sevoflurane on the physiological and hemodynamic parameters of adult dromedary camels. The mean set value of the sevoflurane vaporizer during anaesthesia was 3.2% (range 2.5-4.5%), which was higher than the set values used for other animals as reported in previous studies (Minguet *et al,* 2013; Hikasa *et al,* 2002 and 2000). Endotracheal intubation was relatively difficult to perform in camels because of the narrow oral space, the spatula-shaped tongue with a well-developed dorsum, the wide epiglottis overlapping the soft palate and the presence of the palatine diverticulum (Singh *et al,* 1994; White *et al,* 1987). The shape of the epiglottis might be a major factor in the difficulty of endotracheal intubation in camels. It has been shown that camels have a panduriforme leaf-shaped epiglottis slightly convex on its lingual surface and slightly concave on its laryngeal surface (Eshra *et al,* 2016). However, in the present study muscle relaxation and analgesia resulting from the xylazine and ketamine injection was helpful in the intubation of the camels. A modified technique for camel intubation was used in the present study (Ahmed *et al,* 2015). Full recovery from the sevoflurane anaesthesia was relatively quick (36.6 ± 4.5 min) and smooth in the camels, which was similar to the reported findings in humans (Ebert *et al,* 1998), horses (Matthews *et al,* 1998), sheep (Hikasa *et al,* 2000) and goats (Hikasa *et al,* 2002). The blood/gas partition coefficient for sevoflurane in human blood was low, indicating a relatively rapid recovery from anaesthesia (Strum and Eger, 1987). The swallowing reflex appeared to be a good early sign for recovery in camels anaesthetised with sevoflurane. In the present study, recovery commenced with the return of the swallowing reflex 4.2 ± 1.6 min after sevoflurane

Table 1. Blood gas parameters of jugular vein samples (mean ± SEM) at times 0, after xylazine (X_{20}) and ketamine (K_{10}) administration, during anaesthesia with sevoflurane (S_{30}-S_{90}) and upon recovery (R_{30}) in six healthy adult camels. (* Values significantly different from the 0 time level).

Time / Parameter	0	X_{20}	K_{10}	S_{30}	S_{50}	S_{70}	S_{90}	R_{30}
pH	7.42 ± 0.2	7.43± 0.3	7.38± 0.3	7.32± 0.2*	7.34± 0.3*	7.33± 0.2*	7.32± 0.3*	7.33± 0.4*
pCO_2 (mmHg)	42.0± 4.2	42.8± 3.7	43.1± 3.9	54.5± 3.1*	51.5± 2.2*	56.8± 2.4*	50.8± 3.2*	50.7± 3.1*
pO_2 (mmHg)	35.0± 3.2	35.3± 3.1	43.7± 5.2	53.6± 6.4*	64.3± 4.7*	58.7±5.5*	58.2± 5.7*	43.7±4.7
Na (mmol/L)	153.1± 2.1	153.8± 2.2	152.5± 2.1	152.5± 2.2	151.3± 2.6	150.5± 3.1	150.8± 2.5	151.5± 2.1
K (mmol/L)	3.4± 0.4	2.7± 0.3	2.8± 0.5	3.0± 0.4	3.2± 0.5	3.2± 0.3	3.3± 0.5	3.3± 0.3
Ca (mmol/L)	1.29± 0.05	1.28± 0.06	1.24± 0.06	1.24± 0.05	1.24± 0.04	1.23± 0.06	1.22± 0.1*	1.21± 0.08*
Glu (mg/dL)	170.2± 10.3	181.4± 7.9	257± 14.3*	269.4± 12.7*	273.5± 8.5*	274.3± 9.7*	269.2± 12.3*	266.5± 14.3*
HCO_3 (mmol/L)	25.7± 2.1	26.4± 2.4	27.2± 2.7	27.2± 2.3	27.6± 2.2	27.4± 1.9	27.6± 1.7	27.2± 2.1

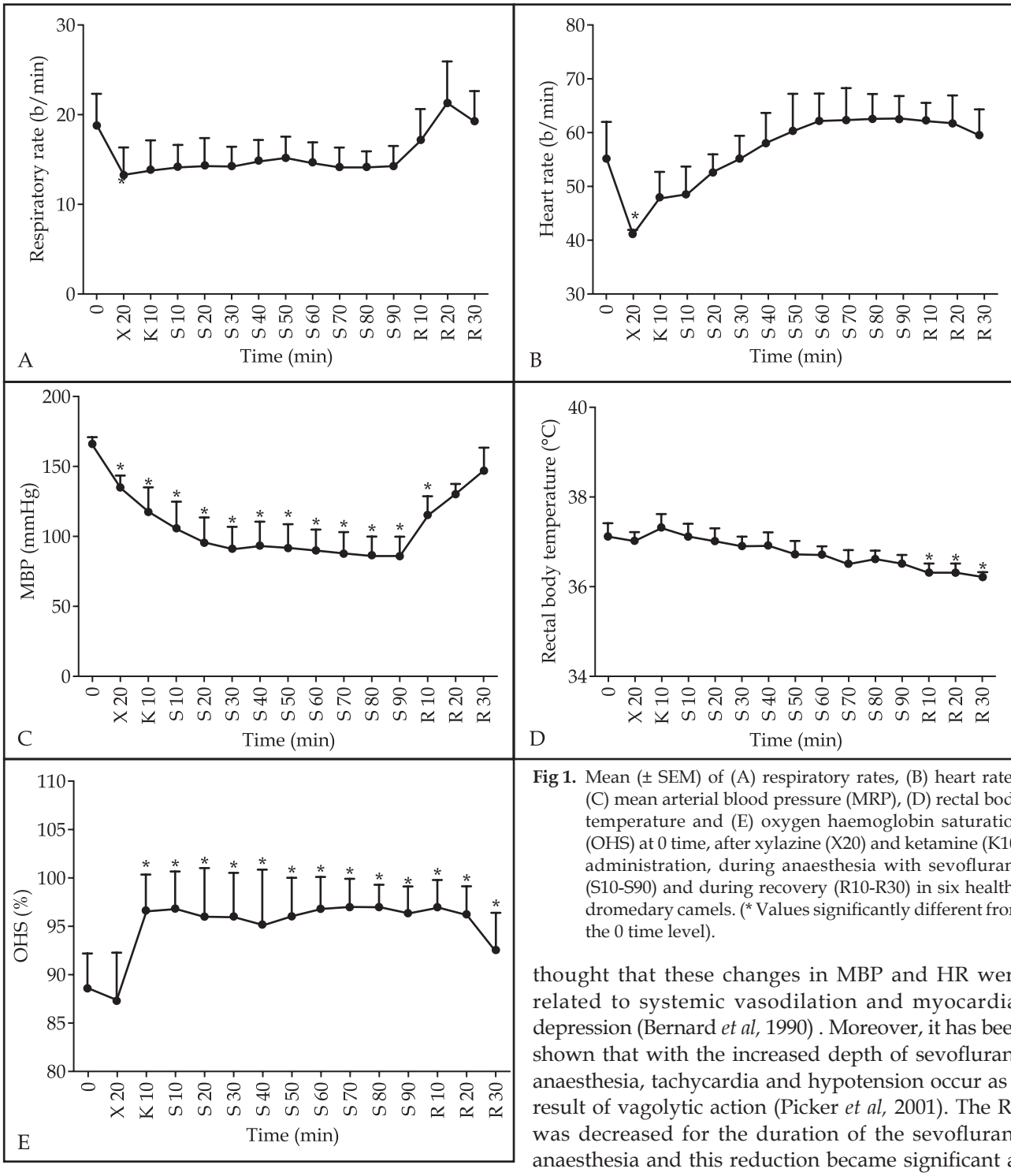

Fig 1. Mean (± SEM) of (A) respiratory rates, (B) heart rates, (C) mean arterial blood pressure (MRP), (D) rectal body temperature and (E) oxygen haemoglobin saturation (OHS) at 0 time, after xylazine (X20) and ketamine (K10) administration, during anaesthesia with sevoflurane (S10-S90) and during recovery (R10-R30) in six healthy dromedary camels. (* Values significantly different from the 0 time level).

anaesthesia was discontinued. Sternal recumbency occurred approximately 20 min after the appearance of the swallowing reflex and standing occurred about 13 min following sternal recumbency.

The MBP significantly decreased and the HR slightly increased in the camels during anaesthesia with sevoflurane. Similar results have been reported in other animals (Bernard *et al,* 1990). It has been thought that these changes in MBP and HR were related to systemic vasodilation and myocardial depression (Bernard *et al,* 1990) . Moreover, it has been shown that with the increased depth of sevoflurane anaesthesia, tachycardia and hypotension occur as a result of vagolytic action (Picker *et al,* 2001). The RT was decreased for the duration of the sevoflurane anaesthesia and this reduction became significant at the recovery period. This result was expected since inhalation anaesthetic agents reduce the metabolic activities of the body. Similar findings have been reported in humans (Kwak *et al,* 2011).

The present study demonstrated a significant reduction in the total LYM counts during anaesthesia with sevoflurane and recovery (Fig 2C). Several studies have investigated the effect of sevoflurane

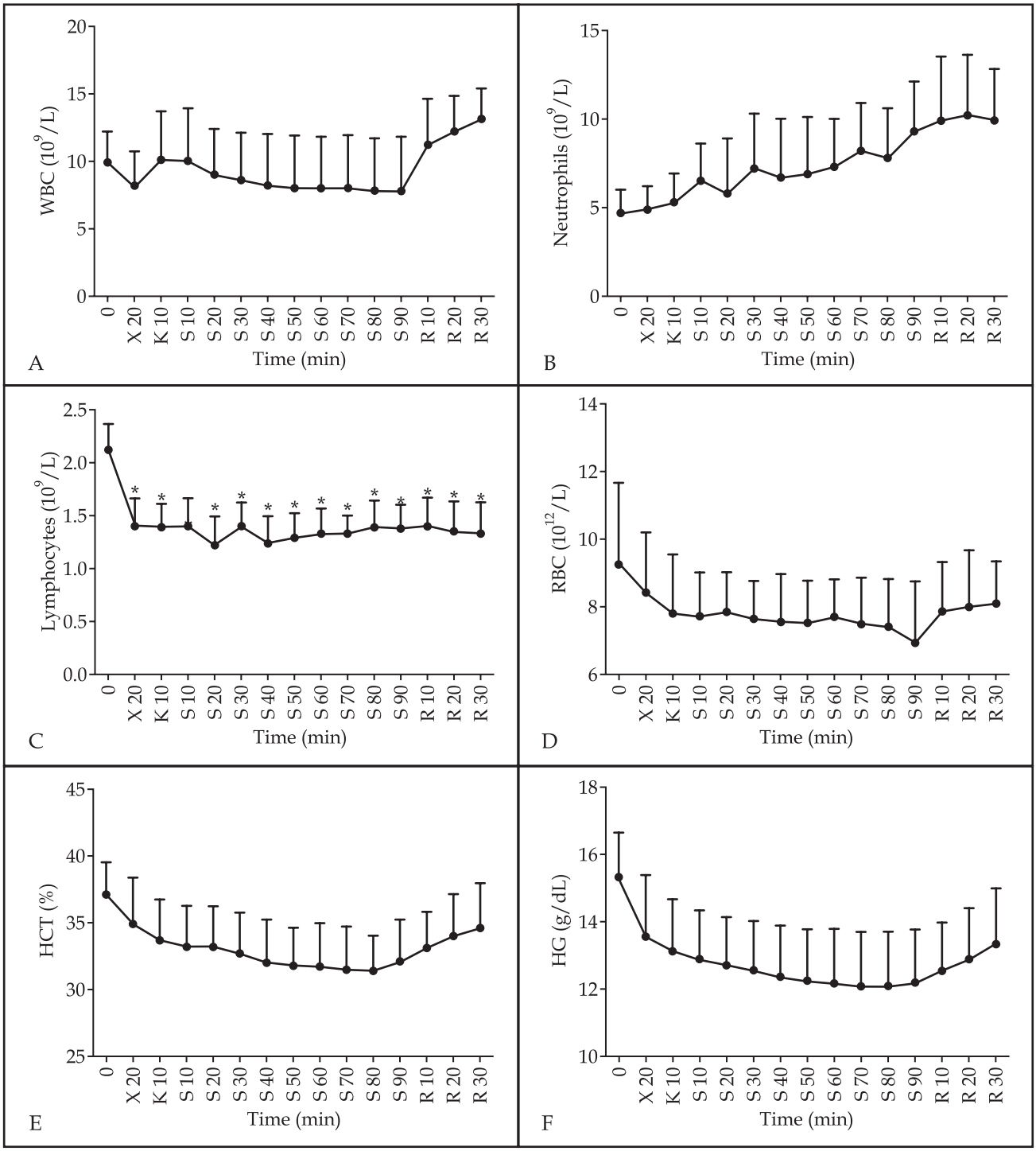

Fig 2. Mean (± SEM) of (A) total white blood cells (WBC), (B) neutrophils, (C) lymphocytes, (D) red blood cells (RBC), (E) haematocrit (HCT) and (F) haemoglobin (HG) at 0 time, after xylazine (X20) and ketamine (K10) administration, during anaesthesia with sevoflurane (S10-S90) and during recovery (R10-R30) in 6 healthy dromedary camels (* Values significantly different from the 0 time level).

administration on several components of the immune response including the lymphocytes (Adam *et al,* 2018; Cocelli *et al,* 2012; Elena *et al,* 2003). It has been reported that anaesthesia influences lymphocyte numbers, the relative composition of lymphocyte subpopulations and their proliferative profile (Elena *et al,* 2003). These changes of lymphocyte counts during anaesthesia have been attributed to different mechanisms including endocrine stress and sympathetic innervation. Release of adrenal hormones

as a result of stress from anaesthesia may signal specific leukocytes to leave the peripheral blood and enter other compartments which serve as 'battle' or 'communication stations' such as lymph nodes, Peyer's patches, bone marrow and lung, skin and mucosal tissues (Dhabhar *et al,* 1995). Moreover, some evidence suggests that the sympathetic innervation associated with lymph node and Peyer's patch vasculature might modulate lymphocyte migration, leading to rapid changes in lymphocyte sub-populations in these organs (Ottaway and Husband, 1994).

A highly significant hyperglycemia was observed in all camels starting 10 min after the administration of ketamine and continuing for the duration of the sevoflurane anaesthesia and recovery. Both the injection of xylazine as the premedication agent and the stress from anaesthesia might have caused the hyperglycemia. Xylazine-induced hyperglycemia has been reported in camels (Peshin *et al,* 1980) and other animals including dogs (Ambrisko and Hikasa, 2002), cats (Kanda and Hikasa, 2008), horses (Tranquilli *et al,* 1984) and cattle (Eichner *et al,* 1979). It has been reported that the acute hyperglycemic effect of xylazine administration was associated with decreased plasma levels of insulin, adrenocorticotropic hormone (ACTH) and corticosterone and increased levels of glucagon and growth hormone (Saha *et al,* 2006). Recent studies in non-human primates have shown that xylazine can cause hyperglycemia with no significant alterations of blood insulin and glucagon (Xiao *et al,* 2013). It was explained that the stimulation of α_2-adrenoceptors and the consequent reduction of tissue sensitivity to insulin led to the reduction of tissue glucose uptake and utilisation (Xiao *et al,* 2013). Hyperglycemia can also occur as a result of endocrine stress during anaesthesia. It has been reported that the stress of anaesthesia alters the finely regulated balance between hepatic glucose production and glucose utilisation in the peripheral tissues (Chan, 1984). With stress, the secretion of counter-regulatory hormones (catecholamines, cortisol, glucagon and growth hormone) increases and this stimulates hepatic glucose production and promotes gluconeogenesis, resulting in elevated blood glucose levels (Chan, 1984).

A significant decrease in the venous blood pH and increase in pO_2 and pCO_2 were seen in the camels for the duration of the sevoflurane anaesthesia. This study revealed that sevoflurane anaesthesia in camels produced respiratory acidosis. Hikasa *et al* (1997) reported similar results in the arterial blood of cats anaesthetised with sevoflurane. In present study, although the levels of RR decreased, this reduction did not reach significant levels. This may be due to the elevation of pO_2 and to the small sample size used in this study. In addition, the anaesthesia may have depressed the respiratory center response to the elevated CO_2 (Nakatsuka *et al,* 2002). In other species, RR significantly decreased during anaesthesia with sevoflurane (Hikasa *et al,* 2002; 1997). It has also been reported that sevoflurane produces a dose-dependent respiratory depression (Doi *et al,* 1987). The present study showed that serum ionised Ca significantly decreased post anaesthesia. Similar results have been reported in animals, with the cause being related to acidosis induced by anaesthetic agents (Hikasa *et al,* 2002; Wang *et al,* 2002). In fact, with increased blood acidity, the protein-binding of Ca increases. It was concluded from the study that, even though some physical and chemical changes occurred, sevoflurane is an inhalation anaesthetic that can be used safely in camels, with rapid and good post anaesthesia recovery.

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I, hereby declare that the particulars given above are true to the best of my knowledge and belief.

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MORPHOLOGICAL OBSERVATION OF THE LARVA OF THE ALXA BACTRIAN CAMEL VAGINAL MYIASIS

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ABSTRACT

The Alxa bactrian camel vaginal myiasis is caused by the larvae of *Wohlfahrtia Magnifica* parasitised in its vagina. In this study, the morphological structure of the larvae of vaginal myiasis in Alxa bactrian camel is observed to understand about their habits and lifestyle. The 1st instar larvae were obtained from the female *Wohlfahrtia Magnifica* captured in the field, or from the vulva of Alxa bactrian camel oviposited by the female *Wohlfahrtia Magnifica*. The 2nd and the 3rd instar larvae were taken from the vaginal lesion in the diseased Alxa bactrian camel. We observed the structure and gave a description by scanning with electron microscope, integrated anatomical microscope and ultra depth imager. Under integrated anatomical microscope, we could observe that the larva had 12 segments, in which 1 bilobed pseudocephalon segment, 3 thoracic segments, 7 abdominal segments and 1 anal segment, respectively. Under electron microscope, we could see many sensilla and a pair of mouthhooks at bilobed pseudocephalon on the 1st instar larva and mouthhooks attached to the cephaloskeleton. The surface of larva has a lot of hard spines and its number and arrangement were different on each segment. The anterior respiratory spiracles of the 2nd and the 3rd instar larva showed a fan-like structure carrying 5 branches. The anal division was the last segment, hemispherical. The posterior spiracles are hidden in the spiracular cavity.

Key words: Alxa bactrian camel, morphology, vaginal myiasis, *Wohlfahrtia magnifica* larva

Wohlfahrtia Magnifica larva is one of the important pathogens of human and animal myiasis. *Wohlfahrtia Magnifica* belongs to order Diptera, family Sarcophagidae, Adult *Wohlfahrtia Magnifica* feeds on plant juice and mate after sexual maturity. Female *Wohlfahrtia Magnifica* finds the host when the larva has matured and only parasitise in the living tissue (Robbins and Khachemoune, 2010). Under appropriate conditions, female *Wohlfahrtia Magnifica* oviposit their larvae in or near the vulva of the Alxa bactrian camel and the larvae enter the parasitic site by crawling. The Bactrian camel in Alxa has been suffering from vaginal myiasis and until now there are no effective treatments (Pirali Kheirabadi e*t al,* 2014; Schumann e*t al,* 1976). Alxa bactrian camel is basically in a semi wild state, has a big body and hard to capture, cause inconvenience to the treatment of vaginal myiasis and the healthy development of the camel industry has been seriously hindered. The larvae usually parasitise in the vagina of the Bactrian camel for 7-9 days. Because of the larvae are being kept producing in the wound, the repeating infection makes the lesion expand and difficult to heal, causing deep tissue necrosis. In milder cases, the Bactrian camel feels pain, irritability, miscarriage and in serious cases, even death (Fig 1) (Demtu, 2017). There are few studies on the Alxa bactrian camel vaginal myiasis (Schumann *et al*, 1976). In this paper, the morphological structure of *Wohlfahrtia Magnifica* larva observed, which would provide information for the prevention and treatment of vaginal myiasis.

Materials and Methods

Sample collection

Wohlfahrtia Magnifica larvae were found at the vulva of the Alxa bactrian camel. At this time, the larvae had not yet bitten the host, so the larvae are the 1st instar larvae. The 2nd and 3rd instar larvae were caught in the camel vagina, then put in hot water about 80-95°C for 30s and to avoid the subsequent deformation, stored in 70% alcohol.

Electron microscope sample preparation

The different stage larvae were removed from 70% alcohol to immobilise them for 48h with 2.5% glutaraldehyde solution. The fixative solution was replaced once during this period, then rinsed them

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twice with the phosphoric acid buffer and dehydration was carried out step by step with 80%, 90% and 99.5% alcohol. Then dried them on the critical point dryer, sprayed dry the worm's body with platinum coating on the conductive layer and observed and photographed them under the electron microscope.

Ultra depth imager sample preparation

The different stage larvae were taken out from 70% alcohol and boiled them in 5% lactic acid for 2-3 min to decompose the external soft tissue, then put larvae in glycerin to observe and photograph them under the ultra depth imager.

Integrated anatomical microscope sample preparation

The different stages of larvae were taken out from 70% alcohol. These were cut through the ventral side of the body with a surgical knife, discarding the contents, cutting off the head and the rear valve and finally fully displaying the body on a flat surface. The cleaned and flattened worm body was put in two slides and bound them with a string, so that the worm body were pressed tightly between the two slides and fixed in the 70% alcohol. Then took them out after 24h and put them into 70%, 75%, 80%, 85%, 90%, 95% and 100% alcohol each for 30 min to dehydrate. Then put these into 1:1 absolute alcohol and clove oil for 30 min and then into pure lilac oil for 30 min. Finally, the optical resin adhesive was used to seal the slides to observe and photograph them under the integrated anatomical microscope.

Results and Discussion

Adaptive structure of the 1st instar larvae

Pseudocephalo: The antennal complex had a thin conical tip, its base ring height was longer than the length of the antennae dome (Fig 2a, b). The maxillary palpus were surrounded by a few folded cuticles. There were 3 sensilla coeloconica (sc1-sc3) and 3 sensilla basiconica (sb1-sb3) in the centre, in which sb3 was not distinct, there were some small sensillum around sb1 and two additional sensillum coeloconicum (ns1-ns2) on the surface of the dorsal side of the maxillary palpus were found (Fig 2c).

Mouthhook: Mouthhooks were large and hard, the anterior part of each mouthhook strongly curves downward, with a single pointed tip and the basal part had visible lateral arm. Labrum was

Fig 1. Diseased part in vagina of the Alxa bactrian camel.

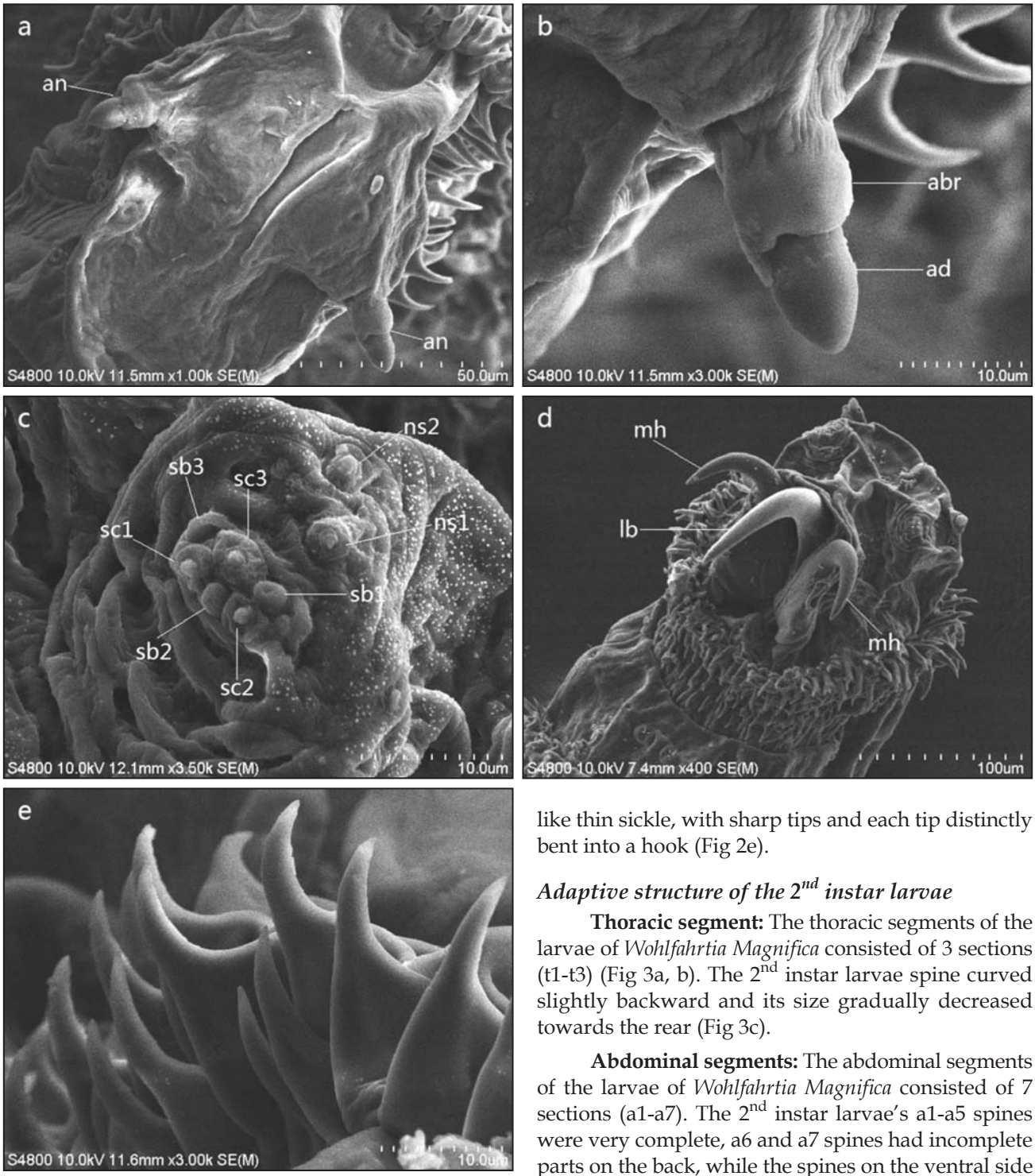


Fig 2. Electron microscope observation of the 1st instar larva of *Wohlfahrtia Magnifica*. **a:** an antennal complex; **b:** ad antennal dome, abr antennal basal ring; **c:** sc1-sc3 sensilla coeloconica, sb1-sb3 sensilla basiconica, ns1 1st additional sensillum coeloconicum, ns2 2nd additional sensillum coeloconicum; **d:** mh mouthhooks, lb labrum; **e:** spines.

very large and long and the anterior part of labrum curved downward, representing the 3rd and middle mouthhooks (Fig 2d). The 1st instar larvae spines were like thin sickle, with sharp tips and each tip distinctly bent into a hook (Fig 2e).

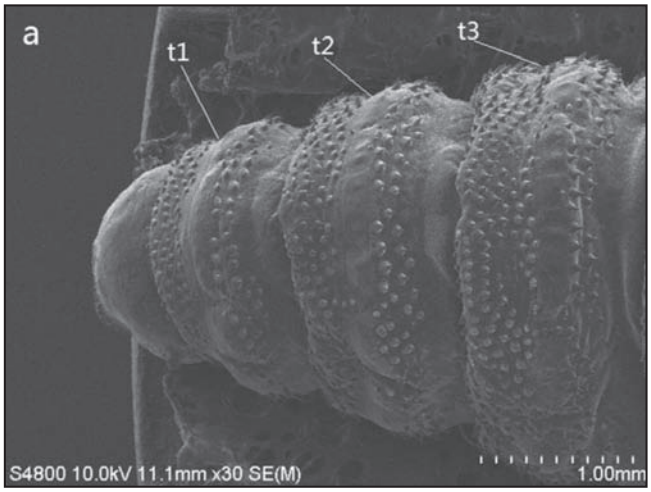
Adaptive structure of the 2nd instar larvae

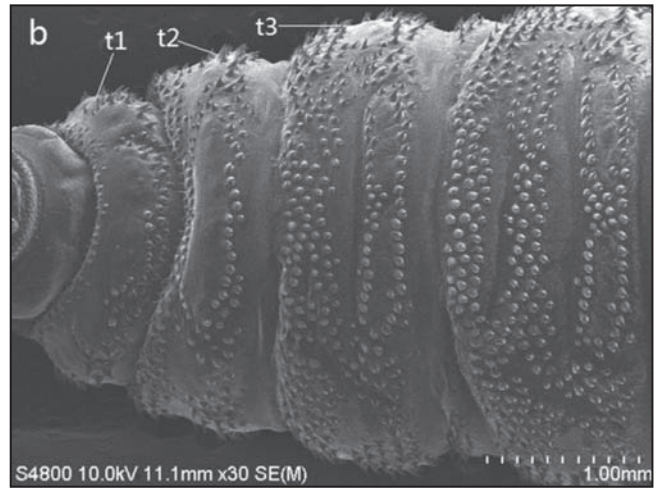
Thoracic segment: The thoracic segments of the larvae of *Wohlfahrtia Magnifica* consisted of 3 sections (t1-t3) (Fig 3a, b). The 2nd instar larvae spine curved slightly backward and its size gradually decreased towards the rear (Fig 3c).

Abdominal segments: The abdominal segments of the larvae of *Wohlfahrtia Magnifica* consisted of 7 sections (a1-a7). The 2nd instar larvae's a1-a5 spines were very complete, a6 and a7 spines had incomplete parts on the back, while the spines on the ventral side were limited (Fig 4).

Anal division: The Anal was the last section of *Wohlfahrtia Magnifica* larvae (Fig 5).

Anterior respiratory spiracles: The anterior respiratory spiracles of the 2nd instar larva showed a fan-like structures carrying 5 branches (Fig 6a). Tiny respiratory spiracles were located at the top of each branch (Fig 6b).





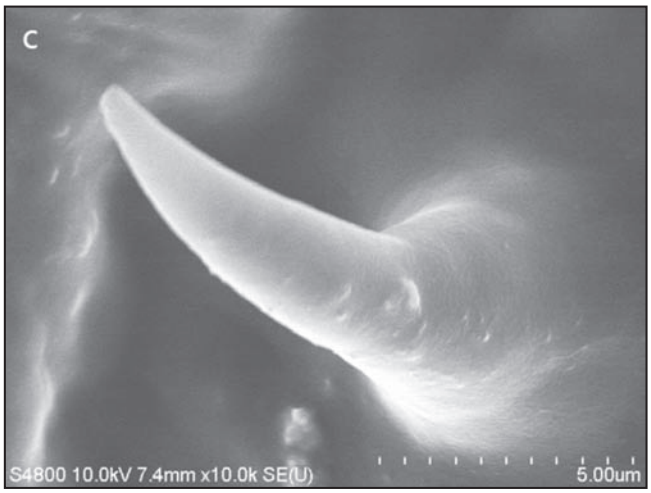
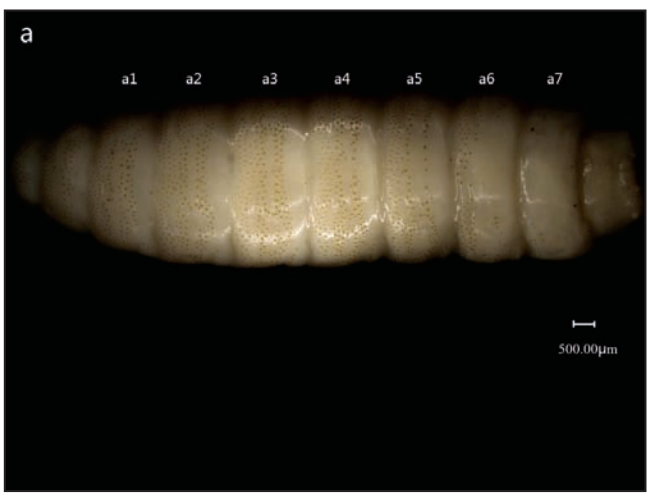


Fig 3. Electron microscope observation of the 2nd instar larva thoracic segments of *Wohlfahrtia Magnifica.* **a:** back view t1-t3 thoracic segments 1-3, **b:** ventral view **c:** spines.

Adaptive structure of the 3rd instar larvae

Cephaloskeleton: The mouth hooks of the 3rd instar larvae were the same as the 1st instar larvae, the intermediate sclerite was very short and the vertical plate was very wide and 3 times larger than the ventral cornua. The dorsal cornua was longer than the ventral cornua, but these 2 cornuas were almost the same width (Fig 7).

Wohlfahrtia Magnifica is widely distributed on the Mongolian plateau and can cause vaginal myiasis in a variety of livestock, which could cause a great obstacle to the livestock breeding industry (Valentin e*t al,*1997). Some morphological characteristics of *Wohlfahrtia Magnifica*'s larvae which were observed by the experimental group are helpful to understand their parasitic life (Ruiz-Martinez *et al,* 1990, Ruiz-Martinez e*t al,*1989). The description of the 1st instar and cephaloskeleton of *Wohlfahrtia Magnifica* obtained from gravid females is largely congruent with the present morphological characters (Szpila e*t al,* 2014).



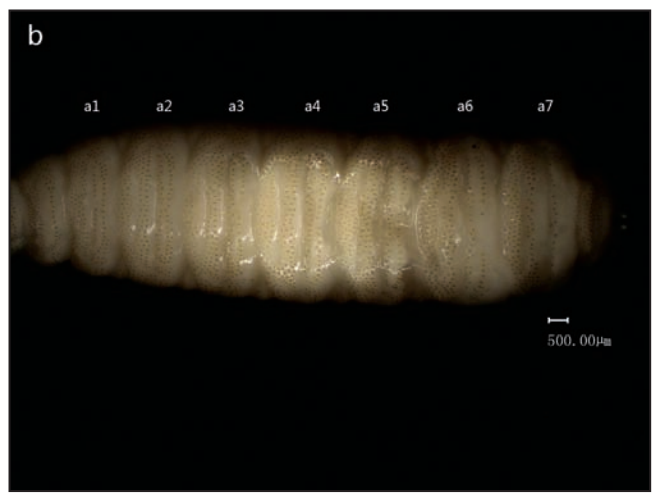


Fig 4. Anatomical microscope observation of the second instar larva abdominal segments of *Wohlfahrtia Magnifica.* **a:** back view a1—a7 abdominal segments 1—7; **b:** ventral view.

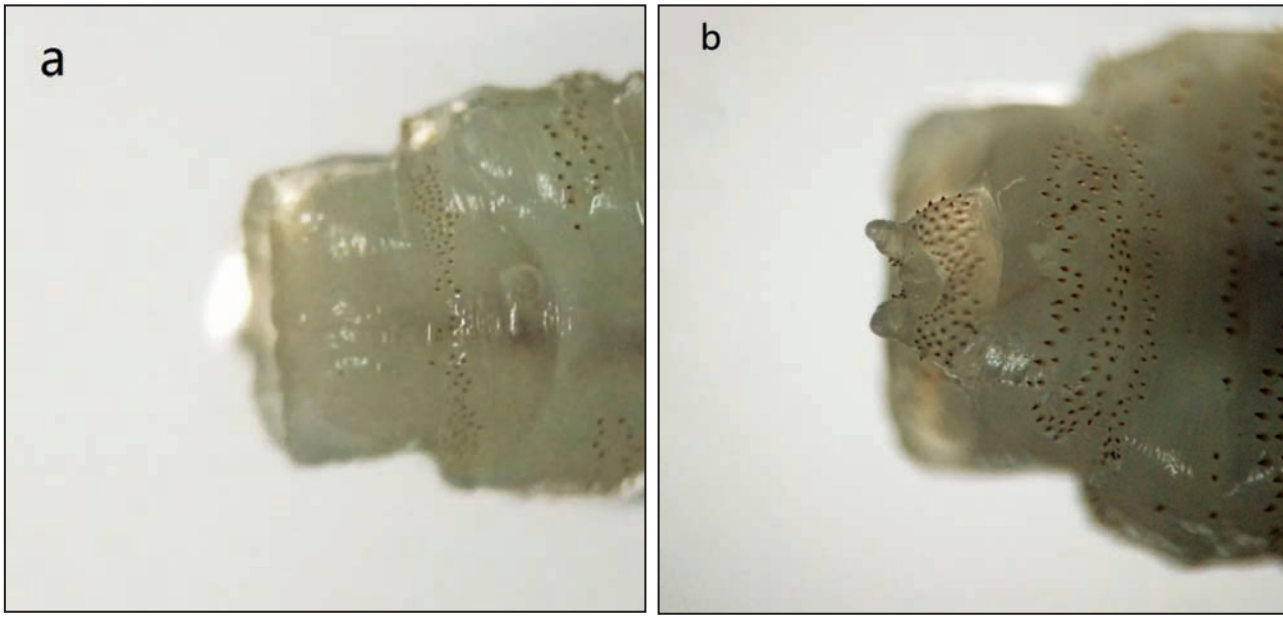


Fig 5. Anatomical microscope observation of the 2nd instar larva anal of *Wohlfahrtia Magnifica.* **a:** back view **b:** ventral view.

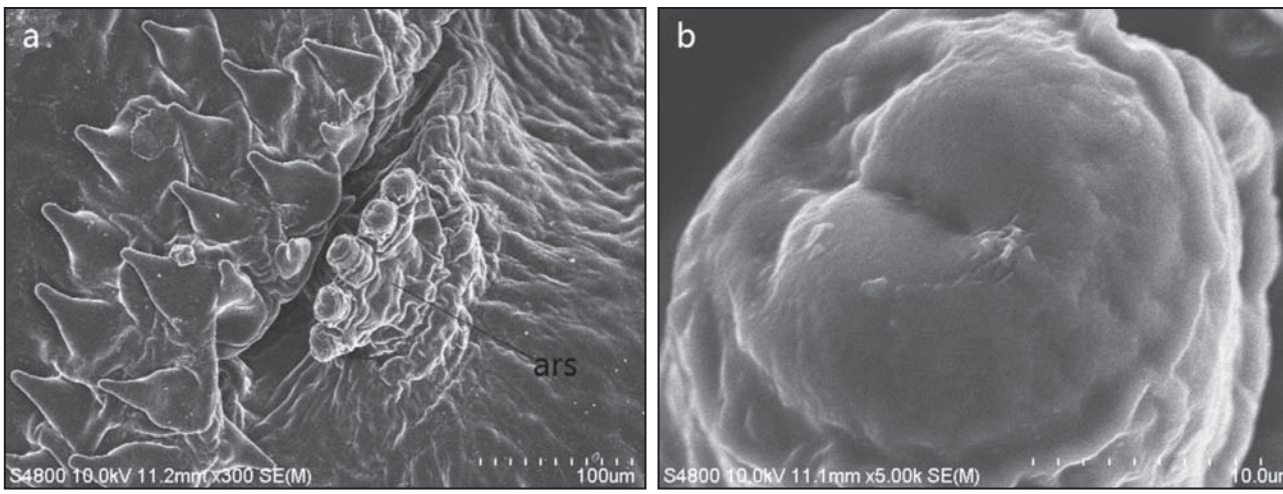


Fig 6. Electron microscope observation of the 2nd instar larva anterior respiratory spiracles of *Wohlfahrtia Magnifica.* **a:** ars anterior respiratory spiracles; **b:** spiracle branches.

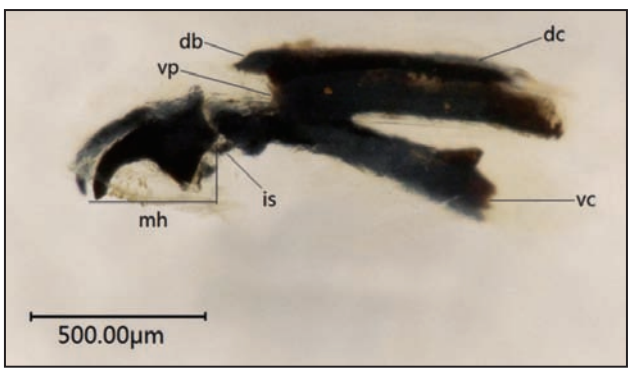


Fig 7. Ultra depth image of the 3rd instar larva cephalic framework of *Wohlfahrtia Magnifica. (*mh mouthhooks, db dorsal bridge, vp vertical plate, dc dorsal cornua, vc ventral cornua, is intermediate sclerite).

The female *Wohlfahrtia Magnifica* produces the 1st instar larvae near the vulva of bactrian camel, then the larvae crawl into the vagina. It might be the receptor on its pseudocephalo related to larvae's finding its parasitic site. The mouthhooks on the pseudocephalo of larvae and the hard spines on the surface can help the larvae crawling into the parasitic site and keeping them from falling down or washing down by the urine. Once the 1st instar larvae enter the vagina, they damage the vaginal mucosa and the mouthhooks are firmly fixed in the vagina. Since the 1st instar larvae have been fixed in the vagina, the 2nd instar larvae do not need to crawl, after the 1st molt to develop into the 2nd instar larvae, the hook-like labrum disappears

and the spines on the body surface are not as sharp as the 1st instar larvae (Xiwen *et al*, 2017). The 2nd instar larvae are fan-like and have branched anterior respiratory spiracles which is an important sign to distinguish the developmental stages of the larva. The morphological character of the anterior respiratory spiracles is largely congruent with the shape of the *W. magnifica* in camel herds in southwest of Iran and the *W. magnifica* in canine in Northeastern Iran (Moshaverinia and Kazemi Mehrjerdi, 2016; Pirali Kheirabadi *et al*, 2014). After the 2nd molt, the 3rd instar larvae are not very different from the 2nd instar larvae, but with one more shedding, there is one more posterior peritreme. The 1, 2, 3 instar larvae each has 1, 2 and 3 posterior peritremes, respectively. The arrangement and number of the spines are the marks for identifying species and posterior peritreme could also be used to identify species relationship. A thorough understanding of the morphological characteristics of *Wohlfahrtia Magnifica* would help to further understand the causes of the Alxa bactrian camel vaginal myiasis and provide new ideas for the prevention and treatment of vaginal myiasis.

Acknowledgements

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COMPARATIVE STUDIES ON SLAUGHTER PERFORMANCE AND SKELETAL MUSCLE FIBRE TYPE OF ALXA GOBI CAMEL AND DESERT CAMEL

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ABSTRACT

Alxa Gobi camel has higher meat production than desert camel. In order to explore the difference of meat production between Gobi camel and desert the present study, their slaughter performance, skeletal muscle fibre type and histological characteristics were studied. In this paper, out of 36 camels, each 6 camels were randomly taken from 3 age groups, 6, 8 and 10-tooth-age group from the Alxa Gobi camel and desert camel to measure the slaughter performance. The triceps muscle of arm, musculus longissimus dorsi and biceps femoris were taken and the tissue slice was stained with ATP enzyme. LAS 4.0 softwwere was used to analyse the image and determine the percentage of MyHC I, MyHC II a and MyHC II b muscle fibres, and SAS softwwere was used to do variance analysis. The results showed that the live weight, carcass weight and net meat weight of Gobi camel were about 60 kg, 80 kg and 90 kg, respectively. There were significant differences ($p < 0.01$; $p < 0.05$). MyHC II b muscle fibre was the main component of two camels' muscle fibres, the percentage of MyHC II b muscle fibre of the Gobi camel (39.6%) was significantly lower than that of the desert camel (48.7%) ($p < 0.05$); the fibre diameter and the cross sectional area of MyHC II b of the Gobi camel (115 μm, 8704 μm²) were significant lower than that of the desert camel (162 μm, 16743 μm²) ($p < 0.01$), and the fibre density (124 n/μm²) was significant higher than that of the desert camel (48 n/μm²) ($p < 0.01$). The meat production of Gobi camel was higher than that of the desert camel, and the difference in fibre diameter and density was related to the difference of muscle fibre composition. MyHC II b muscle fibre has the greatest effect on meat production. The content of muscle fibre in Gobi camel was about 40% and its diameter was the smallest and the density was the largest. MyHC II b muscle fibre in skeletal muscle fibre of desert camel accounts for about 50% and this muscle fibre has the larger diameter and its density was the smallest. Therefore, the meat production of desert camel was significantly lower than that of Gobi camel, which was related to its the highest MyHC II b muscle fibre content and the lowest density.

Key words: Alxa gobi camel, desert camel, skeletal muscle fibre type, slaughter performance

There are two subtypes of Alxa Bactrian camel, Gobi camel living in the vast Gobi and Desert camel living in the Desert hinterland (Tengger Desert, Badain Jaran Desert) (Ai-hua, 2016; He, 2002; Sarentuya *et al,* 2015). During the long period of evolution, there are significant differences in body features and production performance between Gobi camel and Desert camel. Generally, Gobi camel is large in size, muscular, has higher meat production, with brown red colour (Fig 1); Desert camel is small in size, with apricot yellow colour, grows high quality fine fluff, favoured in the textile industry (Fig 2).

At present, the studies on Alxa Bactrian camel are mainly focused on phenotypic traits, such as biological characteristics and economic traits (meat, milk and hair) (Hui Yang *et al,* 2017b; Liu Huiyan *et al,* 2015; Wenfang *et al,* 2017; Zhaorigetu *et al,* 2014). No variation or deletion of MSTN gene was found in Gobi camel and Desert camel in comparative studies (Grobet *et al,* 1997; Hui Yang *et al*, 2017a; Kambadur *et al,* 1997; McPherron and Lee, 1997).

According to the speed of muscle fibre contraction, the muscle fibre is divided into MyHC I and MyHC II muscle fibres to obtain energy by oxidation. MyHC II muscle fibre has poor contractility and can be divided into MyHC II a and MyHC II b according to their oxidation and glycolysis ability (Chang *et al,* 2003; Lefaucheur *et al,* 2002; Röhrle *et al,* 2011; Weisleder *et al,* 2012; Zhang *et al,* 2014b). MyHC II a muscle fibre obtains energy by

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both glycolysis and aerobic oxidation, while MyHC II b muscle fibre obtains energy only by glycolysis and its contraction is fast and short (Pasut *et al,* 2013; Zhang *et al,* 2014a).

There are no reports on the correlation of slaughter performance of Alxa Gobi camel and Desert camel with their muscle fibre types and tissue characteristics, and histological characteristics of skeletal muscle fibres, such as muscle fibre diameter, cross-sectional area and density of Gobi camel and Desert camel. In this study, the differences of slaughter performance and skeletal muscle fibre types and histological characteristics of Alxa Gobi camel and Desert camel are compared.

Materials and Methods

Total 36 camels, in which each 6 camels were randomly taken from 3 age groups, 6, 8 and 10-tooth-age group of Alxa Gobi camel and Desert camel under natural grazing conditions and the live weight, carcass weight and net meat weight were weighted before and after slaughter, respectively. The triceps muscle of arm, musculus longissimus dorsi and biceps femoris were taken and cut into 0.5cm × 0.5cm × 0.5cm, dehydrated in isopentane liquid nitrogen cooling for 30 seconds, put into the 2ml cryopreservation tube to freeze in liquid nitrogen and put in the -80 centigrade refrigerator for permanent preservation. In experiment, the samples were taken out from the refrigerator and placed in the constant temperature freeze slicer (MEV German) at -25°C for slicing. Each slice was cut into 10 micron m thick and pasted on the slide glass. After drying at room temperature, the tissue slice was stained with ATP enzyme.

Experimental reagents

Laboratory self matching: Isoamyl Yong sheng Fine Chemical Co. Ltd.; Barbiturate Sodium Technology Co. Ltd.; ATP Biotech Company; Anhydrous ethanol; $CaCl_2$; $(NH_4)S$ and so on, all are analytically pure; Tianjin chemical reagent three plant.

Instrument and equipment

MEV Freezing microtome	SLEE company
PB-10 type pH meter	Sartorius company
Electronic balance	Sardo instruments Systems Co., Ltd.
World safety fan	Shi An Ke Xing Development Co., Ltd
CX31 optical microscope	OLYMPUS

Results and analysis

Comparison of slaughter performance between Alxa Gobi camel and Desert camel

In order to understand the growing trend and slaughter performance of skeletal muscle of Alxa Gobi camel and Desert camel, the three indicators of the slaughter performance, live weight, carcass weight and net meat weight in 3 age groups were compared (Table 1).

The slaughter performance of 3 age groups of Gobi camel (live weight, carcass weight, net meat weight) was higher than that of Desert camel and the difference was significant. For both Gobi camel and Desert camel, the growth trends of live weight and carcass weight tended to increase with age; however, the growth trend of net meat weight tended to slow down after 8-tooth-age, indicating that the peak of skeletal muscle growth of Alxa Bactrian camel was at 8-tooth-age.

Comparison of skeletal muscle fibre characteristics of skeletal muscle between Alxa Gobi camel and Desert camel

In order to further understand the skeletal muscle fibre characteristics of Alxa Gobi camel and Desert camel, the fibre triceps muscle of arm, musculus longissimus dorsi and biceps femoris were studied and ATPase staining was used for staining (Fig 3).

(MyCH I type: black, MyCH II a type: dark gray, MyCH II b type: grayish)

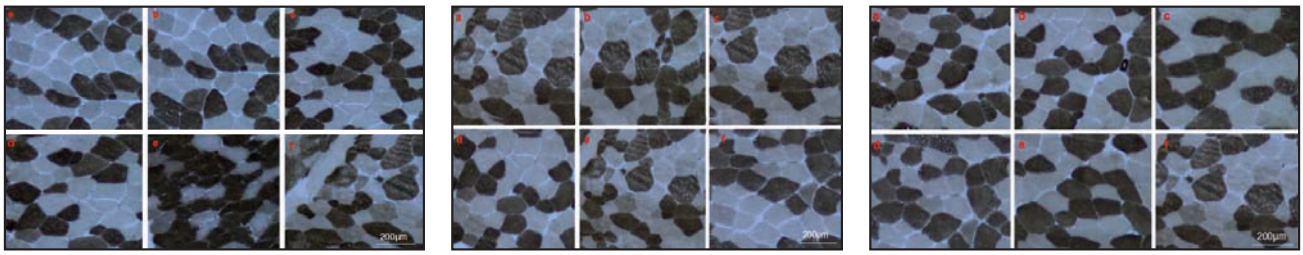
Table1. Comparison of the slaughter performance between Alxa Gobi camel and Desert camel (unit: kg; n=6).

Slaughter performance	Live weight		Carcass weight		Net meat weight	
Age group	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel
6-tooth-age	503.33^{a}±75.06	444.30^{b}±70.90	288.83^{A}±21.11	215.17^{B}±13.20	215.43^{A}±19.99	124.87^{B}±12.84
8-tooth-age	516.67^{a}±50.33	451.40^{b}±26.88	293.33^{a}±17.13	223.30^{b}±59.45	229.70^{A}±50.55	135.03^{B}±20.98
10-tooth-age	533.33^{A}±25.17	475.02^{B}±20.42	329.10^{a}±33.50	239.70^{b}±9.56	233.07^{A}±25.65	135.17^{B}±30.12

Notes: the above value is X±S; the difference between the successful bidder's shoulder mark and the lower-case letter is significant ($P < 0.05$), indicating that the difference was very significant ($P < 0.01$), and N is the sample size.

Fig 1. Alxa Gobi camel.

Fig 2. Alxa Desert camel.



at 6-tooth-age at 8-tooth-age at 10-tooth-age

Fig 3. The skeletal muscle fibre's ATPase staining in the (20×) Alxa Gobi camel and Desert camel in 3 age groups.

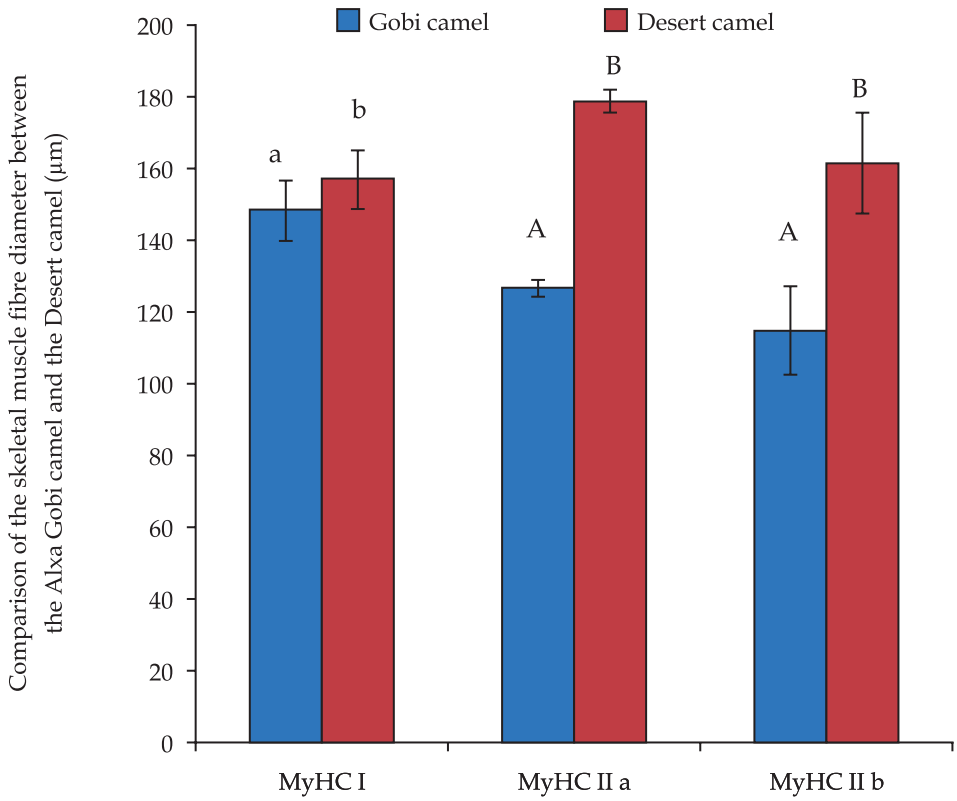


Fig 4. Comparison of the skeletal muscle fibre's diameter between Gobi camel and Desert camel (unit: μm; n=3).

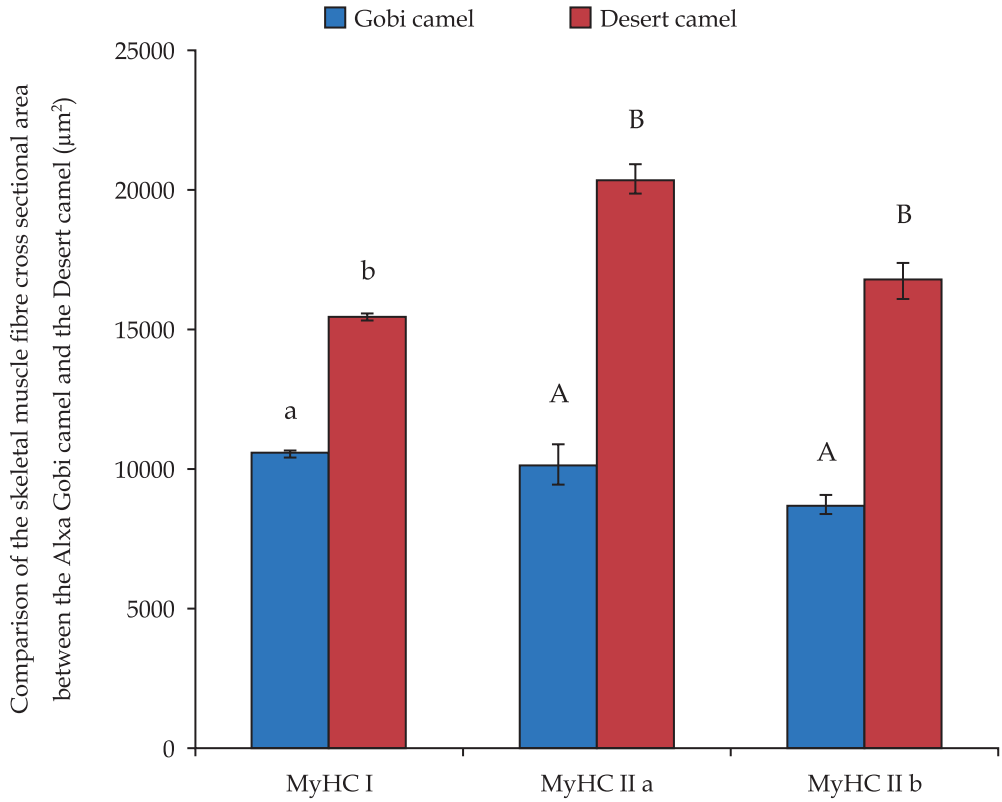


Fig 5. Comparison of the skeletal muscle fibre's cross-sectional area between Gobi camel and Desert camel (unit: μm^2; n=3).

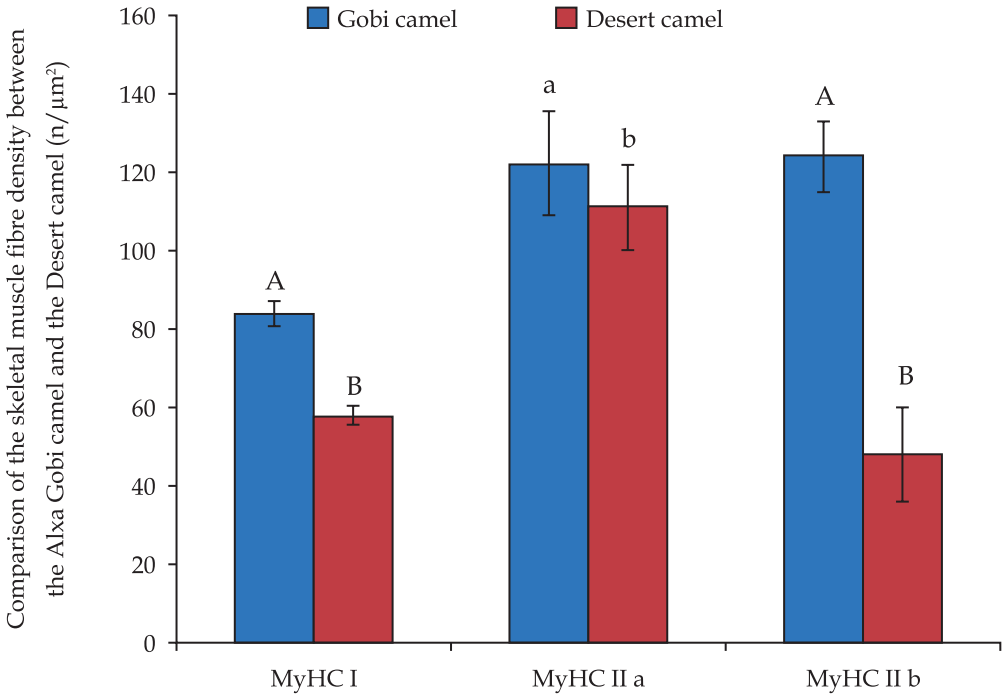


Fig 6. Comparison of the skeletal muscle fibre's density between Gobi camel and the Desert camel (unit: n/μm^2; n=3)

Table 2. Comparison of the percentage of muscle fibre between Gobi camel and Desert camel at 6-tooth-age (unit: %; n=3).

Group	Triceps muscle of arm		Musculus longissimus dorsi		Biceps femoris	
Muscle fibre type	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel
MyHC I type	30.0^{a}±4.8	33.1^{b}±4.5	19.2^{a}±4.6	24.2^{b}±3.5	22.2^{a}±2.9	19.0^{b}±3.2
MyHC II a type	47.2^{A}±2.9	26.7^{B}±3.8	45.0^{A}±5.7	36.1^{B}±3.2	57.4^{A}±3.7	37.3^{B}±4.2
MyHC II b type	22.4^{A}±3.0	39.6^{B}±3.6	35.3^{a}±6.2	39.2^{b}±4.2	20.1^{A}±3.9	43.5^{B}±4.9

Note: The above value is X±S, and the difference of the lower-case letters in the shoulder mark is significant ($P < 0.05$), and different of the capitals are very significant ($P < 0.01$).

Table 3. Comparison of the percentage of muscle fibre between Gobi camel and Desert camel at 8-tooth-age (unit: %; n=3).

Group	Triceps muscle of arm		Musculus longissimus dorsi		Biceps femoris	
Muscle fibre type	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel
MyHC I type	13.1^{a}±3.1	15.2^{b}±2.4	41.1^{A}±3.2	12.1^{B}±2.1	7.4^{a}±2.5	18.2^{b}±2.7
MyHC II a type	21.8^{a}±3.7	20.0^{b}±3.4	13.1^{A}±5	25.2^{B}±4.2	73.2^{A}±4.3	39.2^{B}±4.9
MyHC II b type	64.5^{A}±3.5	59.1^{B}±3.6	45.2^{A}±4.3	61.9^{B}±2.9	19.1^{A}±3.9	40.6^{B}±4.2

Note: The above value is X±S, and the difference of the lower-case letters in the shoulder mark is significant ($P < 0.05$), and different of the capitals are very significant ($P < 0.01$).

Table 4. Comparison of the percentage of muscle fibre between Gobi camel and Desert camel at 10-tooth-age (unit: %; n=3).

Group	Triceps muscle of arm		Musculus longissimus dorsi		Biceps femoris	
Muscle fibre type	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel
MyHC I type	22.3^{a}±2.4	16.3^{b}±1.2	38.2^{A}±1.4	21.3^{B}±2.5	20.4^{A}±3.2	10.3B±1.6
MyHC II a type	17.1^{A}±1.6	34.1^{B}±1.2	18.3^{A}±2.8	30.1^{B}±3.2	32.1^{a}±2.7	33.1b±2.3
MyHC II b type	60.0^{A}±4.1	49.4^{B}±4.5	42.5^{a}±5.7	48.3^{b}±5.3	47.2^{a}±4.3	56.2b±4.5

Note: The above value is X±S, and the difference of the lower-case letters in the shoulder mark is significant ($P < 0.05$), and different of the capitals are very significant ($P < 0.01$).

Notes: a. Triceps muscle of arm of Gobi camel; **b.** Musculus longissimus dorsi of Gobi camel; **c.** Biceps femoris of Gobi camel; **d.** Triceps muscle of arm of Desert camel; **e.** Musculus longissimus dorsi of Desert camel; **f.** Biceps femoris of Desert camel

For the changes of skeletal muscle fibre content with age, before adulthood (6-tooth-age), skeletal muscle grew rapidly and at 6-tooth-age, the muscle fibre content of the Gobi camel was mainly composed by MyHC II a, while the Desert camel was mainly by MyHC II b (Table 2); In adulthood (after 8-tooth-age, 10-tooth-age), except in the biceps femoris muscle, MyHC II b was the main type of muscle fibre of Gobi camel and Desert camel (Table 2-4).

Judging from the histological characteristics of muscle fibre of Alxa Gobi camel and Desert camel, the triceps muscle of arm, musculus longissimus dorsi and biceps femoris of Gobi camel was significantly lower than that of Desert camel, but its density is significantly higher than that of Desert camel (Fig 4-6).

The diameter and cross-sectional area of MyHC I muscle fibre of Gobi camel were significant smaller than that of Desert camel ($p < 0.05$) (Fig 4-6). The density of MyHC I muscle fibre of Gobi camel was significant lower than that of Desert camel ($p < 0.01$); the diameter and cross-sectional area of MyHC II muscle fibre of Gobi camel were all significant lower than that of Desert camel ($p < 0.05$); the diameter and cross-sectional area of MyHC II b muscle fibre of Gobi camel were significant smaller than that of Desert camel ($p < 0.01$). The density of MyHC II b muscle fibre of Gobi camel was significant higher than that of Desert camel ($p < 0.01$), the diameter and cross-sectional area of MyHC II a muscle fibre of Gobi camel were extremely significant smaller than that of the Desert camel ($p < 0.01$), the density of MyHC II type muscle fibre of Gobi camel was significant higher than that of Desert camel.

In order to further understand the skeletal muscle fibre characteristics of Alxa Gobi camel and Desert camel, the skeletal muscle fibre diameter, cross-sectional area, fibre density and percentage of muscle fibre were compared (Table 5).

The diameter of MyHC II b muscle fibre was the smallest (115 μm), and the fibre density was the highest (124 n/μm²); for Desert camel, the diameter of MyHC II b muscle fibre was largest (162 μm)

Table 5. Comparison of histological and histological types of camel fibre between Gobi camel and Desert camel (unit: μm, μm², n/μm², %).

Muscle fibre character	Muscle fibre diameter		Muscle fibre cross sectional area		Single fibre density		Average density of muscle fibre		Muscle fibre total density		Muscle fibre content	
Type	Gobi Camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel	Gobi camel	Desert camel
MyHC I type	149^{a}± 9.3	157^{b}± 8.3	10558^{a}± 121	15459^{b}± 132	84^{A}± 3.2	58^{B}± 2.4					23^{a}± 1.2	18.1^{b}± 1.4
MyHC IIa type	127^{A}± 2.4	179^{B}± 3.26	10156^{A}± 735	20410^{B}± 543	122^{a}± 13.2	111^{b}± 11	110^{A}± 9.7	72.33^{B}± 6.3	330^{A}± 7.4	217^{B}± 8.3	37^{a}± 2.2	30.5^{b}± 3.1
MyHC IIIb type	115^{A}± 12	162^{B}± 14	8704^{A}± 324	16743^{B}± 643	124^{A}± 9.0	48^{B}± 12					39.5^{a}± 2.0	48.7^{b}± 3.1

Notes: The above values are X±S, and there are significant differences ($P < 0.05$) with different lower-case letters in the shoulder mark ($P < 0.01$), and the difference between the different capitals is very significant ($P < 0.01$).

and the density was the smallest (48 n/μm²), and the difference is very significant ($P < 0.01$). MyHC I muscle fibre content is the smallest in Desert camel (18.1%), and its diameter (157 μm) was larger than that of Gobi camel (149 μm) and density (58 n/μm²) was lower than that of Gobi camel (84 n/μm²); the muscle fibre content of MyHC II a of Gobi camel was the highest (37%) and its diameter (127 μm) was smaller than that of Desert camel (179 μm), but its density (122 n/μm²) was higher than that of Desert camel (111 n/μm²). In a word, the diameter and cross-sectional area of muscle fibre of Gobi camel were smaller than that of Desert camel and the density of muscle fibre was larger than that of Desert camel, and the differences were significant.

Discussion

Skeletal muscle growth is a complex biological process (Kjobsted *et al,* 2015; Li *et al,* 2015; Reid *et al,* 2014; Toplu *et al,* 2013). Live weight, carcass weight and net meat weight are important indicators of slaughter performance (Sabuncuoglu *et al,* 2011). The results showed that the live weight, carcass weight and net meat weight of Alxa Gobi camel were higher than those of Desert camel. There was no significant difference in net meat weight between 8-tooth-age and 10-tooth-age, and the growth of net meat weight of Alxa Gobi camel and Desert camel tended to slow down after 8-tooth-age. With the increase of age, the live weight and carcass weight of Alxa Gobi camel and Desert camel increased gradually, which was related to the increase of fat deposition (DeVol *et al,* 1988; Gardan *et al,* 2006; Lee and Kauffman, 1974).

Skeletal muscle traits were determined by muscle fibre types and their propertises (Lee *et al,* 2010). From the analysis of muscle fibre content, the content of MyHC II b, MyHC II a and MyHC I in Gobi camel and Desert camel was MyHC II b > MyHC II a > MyHC I, and MyHC II b was the most dominant type of muscle fibre content in both Gobi camel (about 40%) and Desert camel (about 50%), but the density was larger (124 n/μm²) in Gobi camel, and smaller (48 n/μm²) in Desert camel. Before adulthood (6-tooth-age), MyHC II a and MyHC II b content were dominant in Gobi camel and Desert camel, while in Adulthood (8-tooth-age, 10-tooth-age), MyHC II b content is dominant in Gobi camel and Desert camel. In general, MyHC II muscle fibre has more advantage than MyHC I muscle fibre. In the analysis of muscle fibre characteristics, the diameter of muscle fibre of Gobi camel was smaller than that of Desert camel, and the density of muscle fibre was higher than that of Desert camel. The results show that the effect of MyHC II muscle fibre on meat production of Gobi camel and Desert camel was larger than that of MyHC I muscle fibre, thus MyHC II b muscle fibre plays a key role in meat production. Therefore, the meat production of Desert camel is significant lower than that of Gobi camel, which is related to its the highest MyHC II b muscle fibre content and the lowest density.

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SELECTED RESEARCH ON
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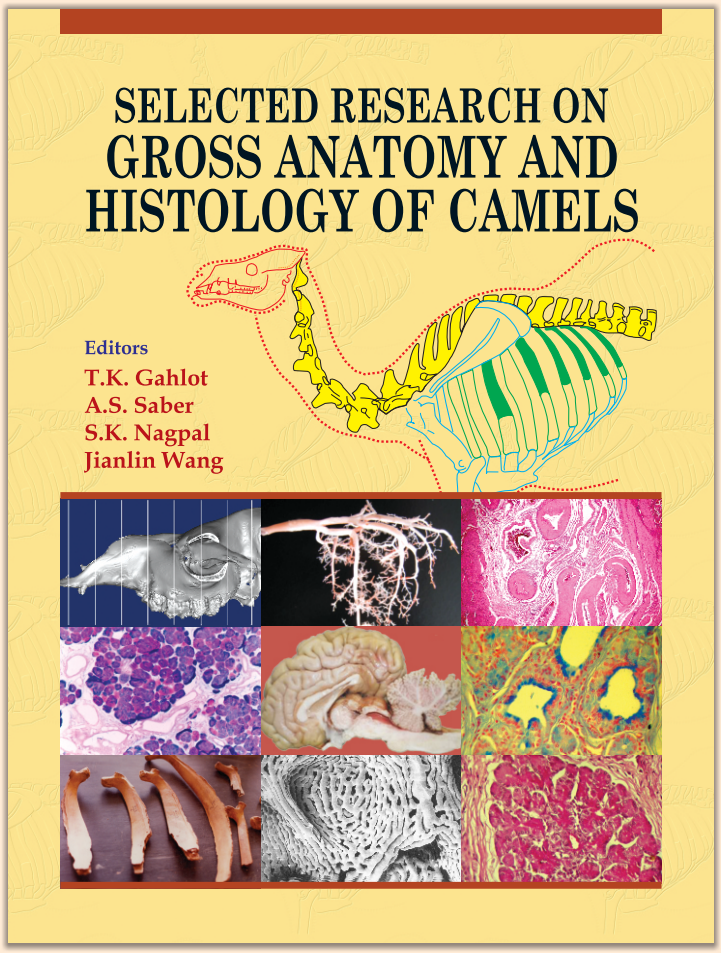

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AN ANALYSIS OF THE FORESTOMACH BACTERIAL MICROBIOTA IN THE BACTRIAN CAMEL

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ABSTRACT

The digestive systems of ruminant mammals harbour a complex gut microbiome composed of bacteria and archaea, as well as other microorganisms. These microbes influence the health of the animal and provide the host with nutrients. The digestive system of bactrian camels is unique because omasum is absent and it has three compartments. We utilised 16S rRNA gene sequencing to characterise the temporal variations in the forestomach microbiome of Bactrian camels (N=8). Different forestomach compartments had different communities and Firmicutes (34%-43%), Bacteroidetes (26%-30%) and Verrucomicrobia (7%-11%) were the most abundant taxonomic groups. Study also revealed that the forestomach microbiota may be subject to seasonal variations.

Key words: 16S rRNA, bactrian camel, forestomach microbiota, seasonal changes

The Bactrian camel is well adapted for life in extremely arid conditions, including hot summers and cold winters (Wu *et al*, 2014). The forestomach of Bactrian camel includes three chambers: the rumen, reticulum and the abomasum (Ming *et al*, 2017). Compared with other herbivores, the rumen of camels has a longer retention time (Lechnerdoll *et al*, 1995). Camelids also have a lower metabolic rate and a lower level of food intake than true ruminants (Dittmann *et al*, 2014). Camels forage for their food, which include scarce resources such as eat salt-tolerant vegetation and poisonous plants, as well as dry, prickly and bitter plants (Samsudin *et al*, 2011; Bhatt *et al*, 2013).

Previous findings have shown that like true ruminants, the degradation of plant cell wall carbohydrates in camels is entirely relient on the rumen microbiome (Samsudin *et al*, 2011). Microbial composition is similar in the forestomachs of cows in dromedary camels and is dominated by Bacteroidetes (55.5%), Firmicutes (22.7%) and Proteobacteria (9.2%) (Bhatt *et al*, 2013). Recent work has shown that the faecal microbiotas of Bactrian camel and cattle are different (Ming *et al*, 2017). This study also showed that the microbial community in Bactrian camels varied with habitat (Ming *et al*, 2017). Previous studies have investigated the bacterial ruminal microbiota in cattle (Noel *et al*, 2017), Hu sheep (Wang *et al*, 2015) and sheep (Kamke *et al*, 2016). Moreover, recent research has shown that mammalian dietary patterns can change dramatically with season and habitat (Óscar *et al*, 2012; Nakagawa, 1997; Amato *et al*, 2015). However, only a few studies have examined the ruminal microbiota of Bactrian camels. To better understand the microbial community composition in the forestomach, as well as seasonal dietary patterns in Bactrian camels, we investigated the microbial community composition of the rumen, reticulum and abomasum of eight domestic Bactrian camels over time using 16S rRNA gene sequencing.

Materials and Methods

Animal and sample collection

Eight healthy adult Bactrian camels were obtained from the Inner Mongolia XilinGol League, China and euthanised. Three fresh samples (summer - Group A) were collected from the rumen, reticulum and abomasum at the Inner Mongolia XilinGol League in August 2016. Five fresh samples (winter - Group B) were collected at the Inner Mongolia XilinGol in League in December 2016. All animals were euthanised in the morning. All samples were immediately stored at -80°C. The experimental protocol was approved by the Inner Mongolia Agricultural University Animal Care and Use Committee.

DNA extraction, 16S rRNA gene PCR and sequencing

Total DNA was extracted using the MOBio UltraClean Soil Kit (MoBio Laboratories Inc.,

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Carlsbad, CA, USA) and the DNA quality and quantity was determined via NanoDrop spectrophotometry and 0.8% agarose gel electrophoresis. The PCR conditions were: initial denaturation at 98°C for 2 min; 25–30 cycles of 15 s at 98°C for denaturation, 30 s at 55°C for annealing, extension at 72°C for 30 s and; a final extension at 72°C for 5 min. The V4 hypervariable region of the 16S rRNA gene was sequenced on an Illumina MiSeq system at Personal Biotechnology Co., Ltd., Shanghai, China.

Bioinformatics analysis

The QIIME pipeline was used to analyse the forward sequence reads (Caporaso *et al*, 2010). The sequences were clustered (97% similarity) and a representative sequence from each operational taxonomix unit (OTU) was classified using SILVA (Quast *et al*, 2013). Alpha and beta diversities were analysed in QIIME and non-parametric Mantel tests were used to compare forestomach bacterial community compositions. All p-values were corrected using false discovery rate (FDR, 0.05) (Benjamini and Hochberg, 2013).

Results

Bacterial diversity in forestomach samples

The sequences clustered into 4,251 OTUs and the rarefaction curves suggest that the sampling depth was sufficient for each sample (Fig S1). Alpha diversity was determined using a variety of metrics, including the ACE, Shannon and Chao1 indices (Table S1). There were significant differences in alpha diversity between the winter and summ2er groups (Table 1). However, the Shannon and Chao1 indices showed that overall community diversity was not significantly different between the rumen, reticulum and abomasum (Fig 1). There was a substantial amount of inter-subject variability observed between animals.

Bacterial abundance and distribution in the different segments

A total of 27 phyla s1were identified from samples; 26 were found in the rumen, 25 in the reticulum and 26 in the abomasum. The forestomach community was mainly composed of three phyla: Fimicutes (34%-43%), Bacteroidetes (26%-30%)

Table S1. Number of sequences, estimated sample coverage, diversity and operational taxonomic unit (OTU) richness in each sample.

Region	Sample ID	OTUs	Shannon	Simpson	Chao1	ACE
Rumen	LW1	943	7.55	0.98	948.00	948.00
	LW2	1134	8.12	0.99	1157.88	1175.10
	LW3	960	7.88	0.99	1020.82	1033.85
	LW7	1294	8.44	0.99	1440.61	1419.76
	LW8	1273	8.41	0.99	1401.95	1389.08
	LW9	1214	8.40	0.99	1214.00	1214.00
	LW11	1187	7.64	0.98	1266.75	1289.43
	LW12	1181	8.02	0.98	1300.94	1286.30
Reticulum	WW1	1096	8.55	0.99	1207.26	1181.52
	WW2	1061	7.88	0.98	1158.85	1150.04
	WW3	976	7.75	0.99	1016.77	1031.88
	WW7	1372	8.29	0.99	1471.11	1506.61
	WW8	1294	8.64	0.99	1300.55	1319.45
	WW9	1225	8.32	0.99	1226.00	1226.00
	WW11	1093	7.75	0.98	1093.76	1101.31
	WW12	1167	8.13	0.99	1207.90	1231.57
Abomasum	ZW1	1076	8.06	0.99	1161.36	1152.39
	ZW2	1061	8.09	0.99	1061.03	1062.65
	ZW3	865	7.28	0.98	910.19	934.11
	ZW7	1120	8.12	0.99	1120.00	1120.00
	ZW8	773	4.14	0.72	834.12	868.01
	ZW9	1309	8.50	0.99	1312.07	1325.54
	ZW11	1142	8.04	0.99	1181.84	1210.00
	ZW12	1203	7.83	0.98	1358.42	1341.51

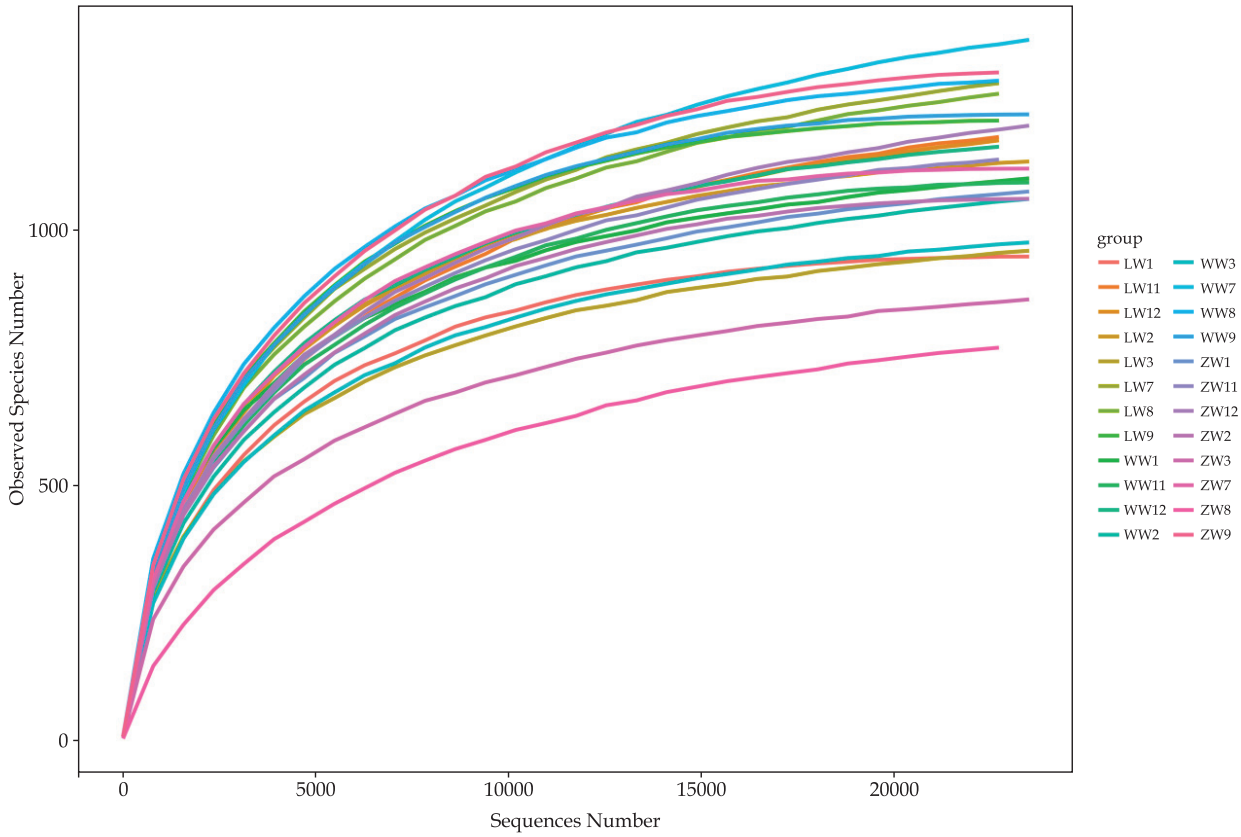

Fig S1. Rarefaction curve analysis of eight Bactrian camels.

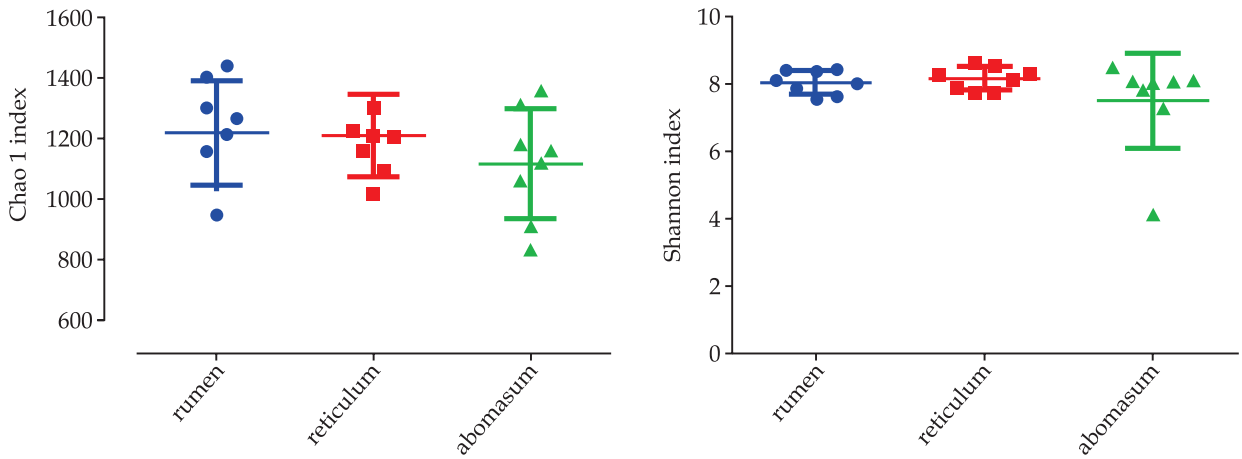

Fig 1. Alpha diversity of the rumen, reticulum and abomasum segments.

and Verrucomicrobia (7%-11%). There were 267 genera detected, with 11 genera having relative abundances greater than 1%. Among these, Christensenellaceae_R-7_group was the most prevalent, followed by Rikenellaceae_RC9_gut_group, Fibrobacter and Prevotella_1. (Fig 2). The microbiota composition in the rumen and reticulum was highly similar (Fig S2), with the exception of Aeriscardovia, which was more abundant in the abomasum than in the other ($P<0.05$) (Table S2).

Microbiota compositions in the two groups

There were 25 bacterial phyla in group A and 26 phyla in group B. The dominant phylas in the both groups were Firmicutes, Bacteroidetes and Verrucomicrobia. In group A there were three genera that each accounted for more than 5% of relative abundance in the forestomach: Christensenellaceae_R-7_group (8.62%), Rikenellaceae_RC9_gut_group (8.16%) and uncultured Bacteroidales_BS11_gut_group (6.13%). Two of these were

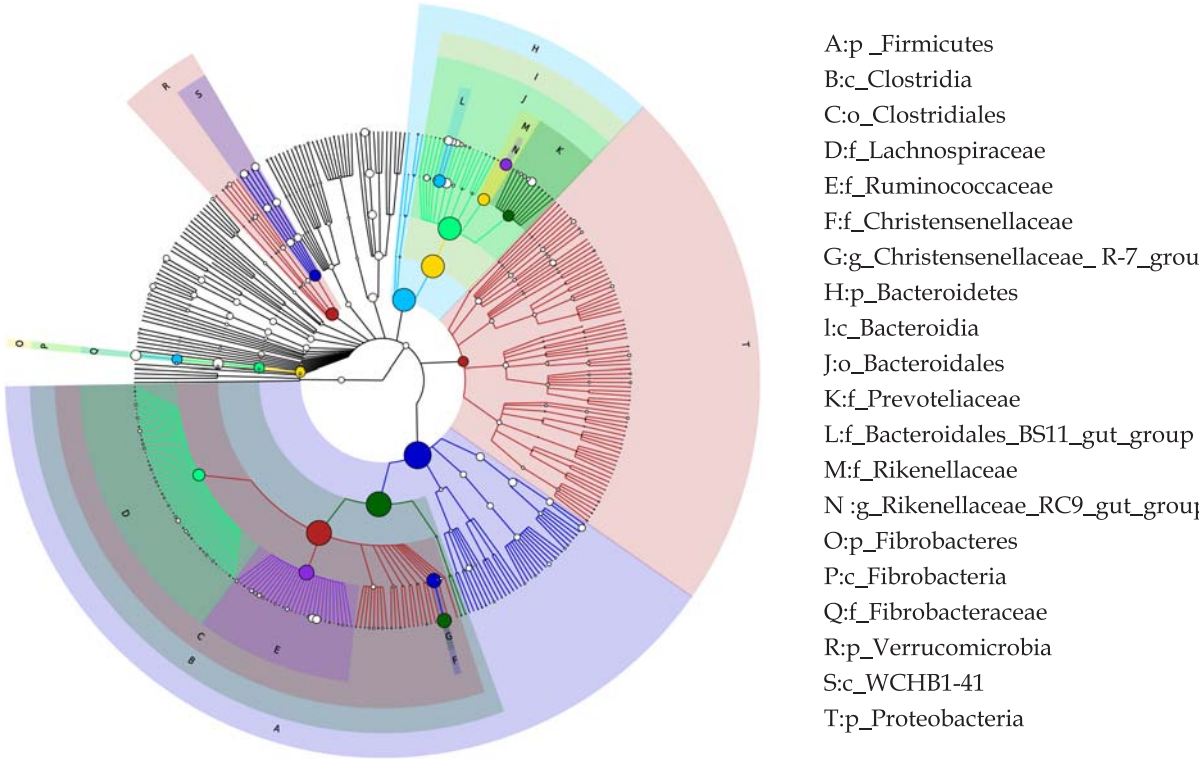

Fig 2. GraPhlAn visualisation of annotated phylogenies and taxonomies.

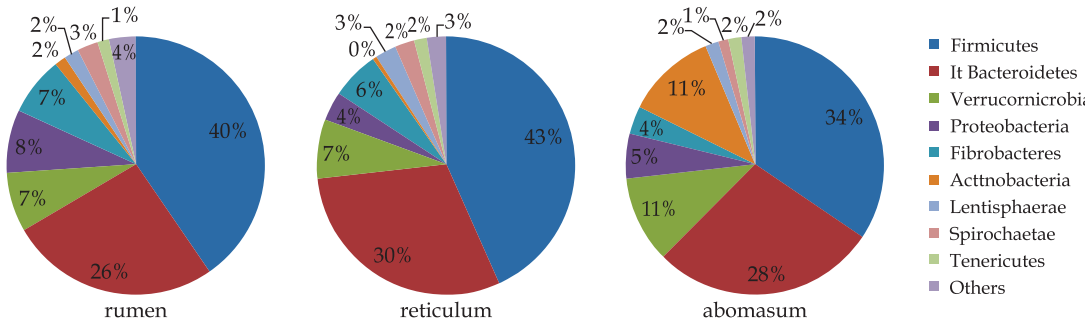

Fig S2. Forestomach bacterial composition at the phylum level per segment.

Table 1. Microbial alpha diversity indices across the two groups

sample	ACE	Chao1	Shannon	Simpson
Rumen				
Group A	1052.32	1020.82	8.00	0.99
Group B	1319.71	1300.94	8.18	0.99
Reticulum				
Group A	1121.15	1016.77	8.06	0.99
Group B	1276.99	1207.90	8.23	0.99
Abomasum				
Group A	1049.72	910.19	7.81	0.99
Group B	1173.01	1358.42	7.33	0.93

the same in group B, although one genera was different: Christensenellaceae_R-7_group (12.17%), Rikenellaceae_RC9_gut_group (6.85%) and Fibrobacter (6.88%) (Fig S2). The differences in forestomach microbiota composition between the two groups was statistically significant. Mann-Whitney U tests were used to determine differentially abundant taxa ($P<0.05$). Nine of the top 30 most abundant genera were significantly different in the rumen, seven were different in the reticulum and three were different in the abomasum. Of the nine genera identified in the rumen, three ((Anaerorhabdus)_furcosa_group, Moryella and Ochrobactrum) were more abundant in group A. Interestingly, seven of the nine genera in the rumen were Firmicutes (Table 2).

Relationships among microbiota of the two groups

Non-metric multidimensional scaling (NMDS) analysis was used to assess differences in the

forestomach microbiota between the two groups. The results of NMDS analysis were consistent the cluster analysis, as the samples clearly clustered into two groups (Fig S3). In addition, a clustered heatmap also showed the differences between the two groups.

Discussion

In ruminants, the stomachs are important for digestion and nutrient absorption. Manipulation of these processes has potential application in the areas of food fermentation and biomass utilisation (Tan

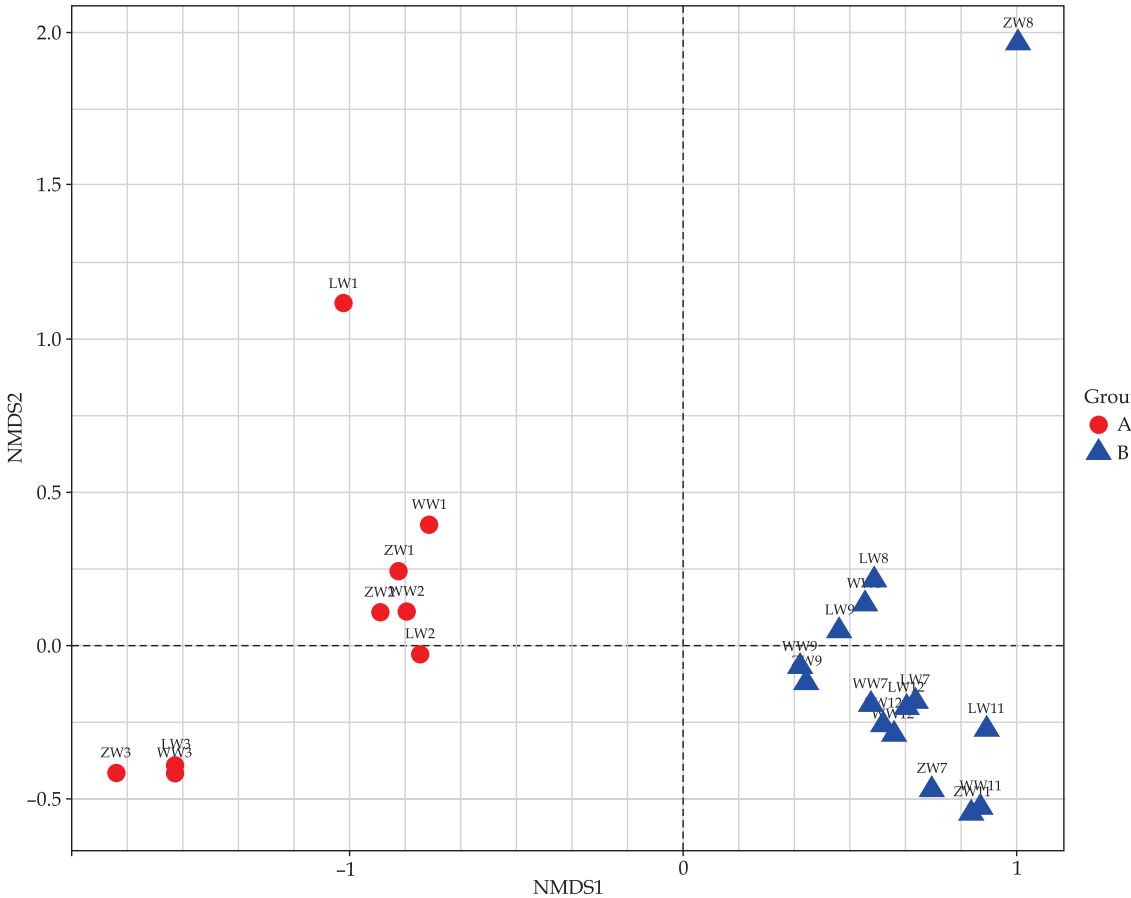

Fig S3. Multiple samples NMDS analysis of eight Bactrian camels.

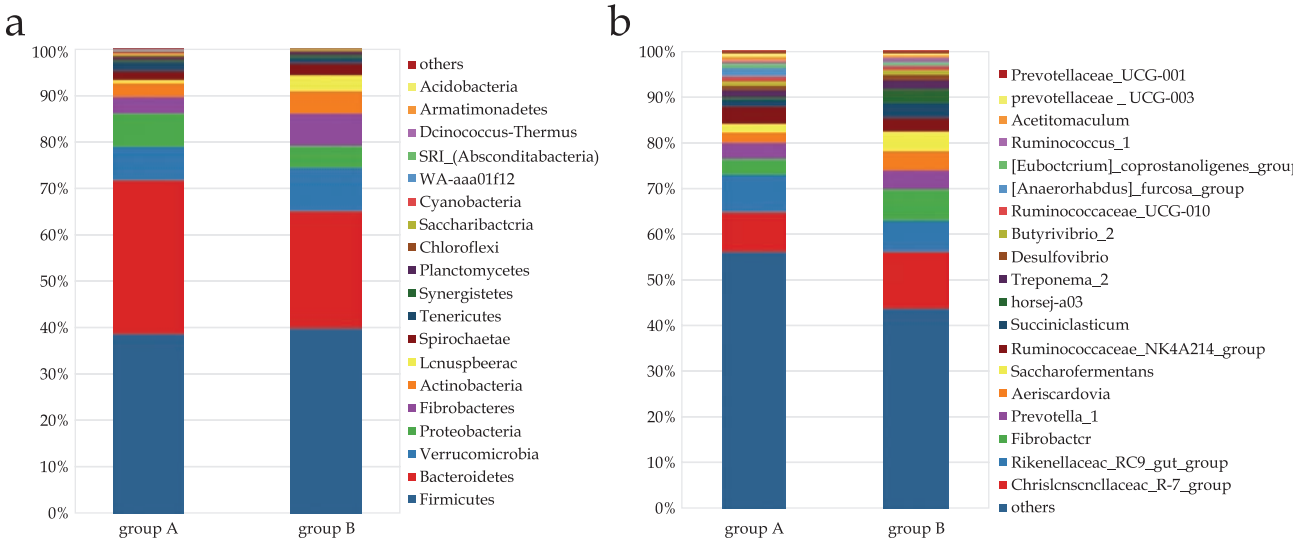

Fig 3. Median relative abundance of the main bacterial phyla and genera in the stomachs of the two groups.

Table S2. Comparisons for bacterial abundance > 1% at the genus levels.

Taxa	rumen	reticulum	abomasum	P value
Christensenellaceae_R-7_group	11.77	11.99	8.76	0.5945
Rikenellaceae_RC9_gut_group	6.63	8.03	7.35	0.6925
Fibrobacter	7.37[a]	6.01[b]	3.45[c]	0.0017
Prevotella_1	3.62	3.87	4.08	0.8332
Aeriscardovia	0.002[a]	0.001[a]	10.47[b]	0.0002
Saccharofermentans	4.13	3.60	2.53	0.1641
Ruminococcaceae_NK4A214_group	2.89	3.45	3.35	0.6126
Succiniclasticum	2.22	3.24	2.36	0.4025
horsej-a03	2.62	2.33	1.34	0.4712
Treponema_2	2.55	2.00	0.98	0.0779
Desulfovibrio	0.90	1.18	1.04	0.7065

Table S3. Seasonal comparisons for species top 30 abundance at the genus level.

Phylum	Genus	Avg (A)	Avg (B)	P value
Rumen				
Firmicutes	Christensenellaceae_R-7_group	7.03%	14.62%	0.025
Firmicutes	Saccharofermentans	2.14%	5.33%	0.025
Firmicutes	Succiniclasticum	1.13%	2.88%	0.025
Firmicutes	(Anaerorhabdus)_furcosa_group	1.44%	0.23%	0.025
Firmicutes	Ruminococcaceae_UCG-005	0.12%	0.61%	0.025
Firmicutes	Moryella	0.74%	0.12%	0.025
Firmicutes	Ruminococcaceae_UCG-011	0.09%	0.47%	0.025
Synergistete	Pyramidobacter	0.26%	0.53%	0.025
Proteobacteria	Halomonas	0.04%	0.93%	0.024
Reticulum				
Firmicutes	Saccharofermentans	2.26%	4.41%	0.025
Firmicutes	(Anaerorhabdus)_furcosa_group	2.99%	0.43%	0.025
Firmicutes	Acetitomaculum	2.71%	0.20%	0.025
Firmicutes	Moryella	1.02%	0.15%	0.025
Firmicutes	Ruminococcaceae_UCG-011	0.13%	0.46%	0.022
Proteobacteria	Burkholderia-Paraburkholderia	0.04%	0.14%	0.025
Proteobacteria	Halomonas	<0.00%	0.07%	0.025
Abomasum				
Firmicutes	(Anaerorhabdus)_furcosa_group	1.51%	0.16%	0.025
Firmicutes	Moryella	0.50%	0.11%	0.025
Proteobacteria	Halomonas	<0.00%	1.78%	0.022

et al, 2015). Although there have been some studies investigating the role of microorganisms in the different segments of the gastrointestinal tract of the Bactrian camel, there is little information on seasonal variations in the gastrointestinal microbiota. We investigated it and found that the dominant phyla in the forestomach were Firmicutes, Bacteroidetes and Verrucomicrobia; this observation is consistent with previous studies (Ming *et al*, 2017; Bhatt *et al*, 2013). However, we found that microbial community composition varied between segments of the forestomach. It is possible that these differences could be a result of multiple factors, including inter-subject variability and dietary effects (Gu *et al*, 2013). We observed variations in microbial community structure throughout the sections of the forestomach (Fig S3); this could be due to the distinct biological and physiological functions of the rumen, reticulum and abomasum.

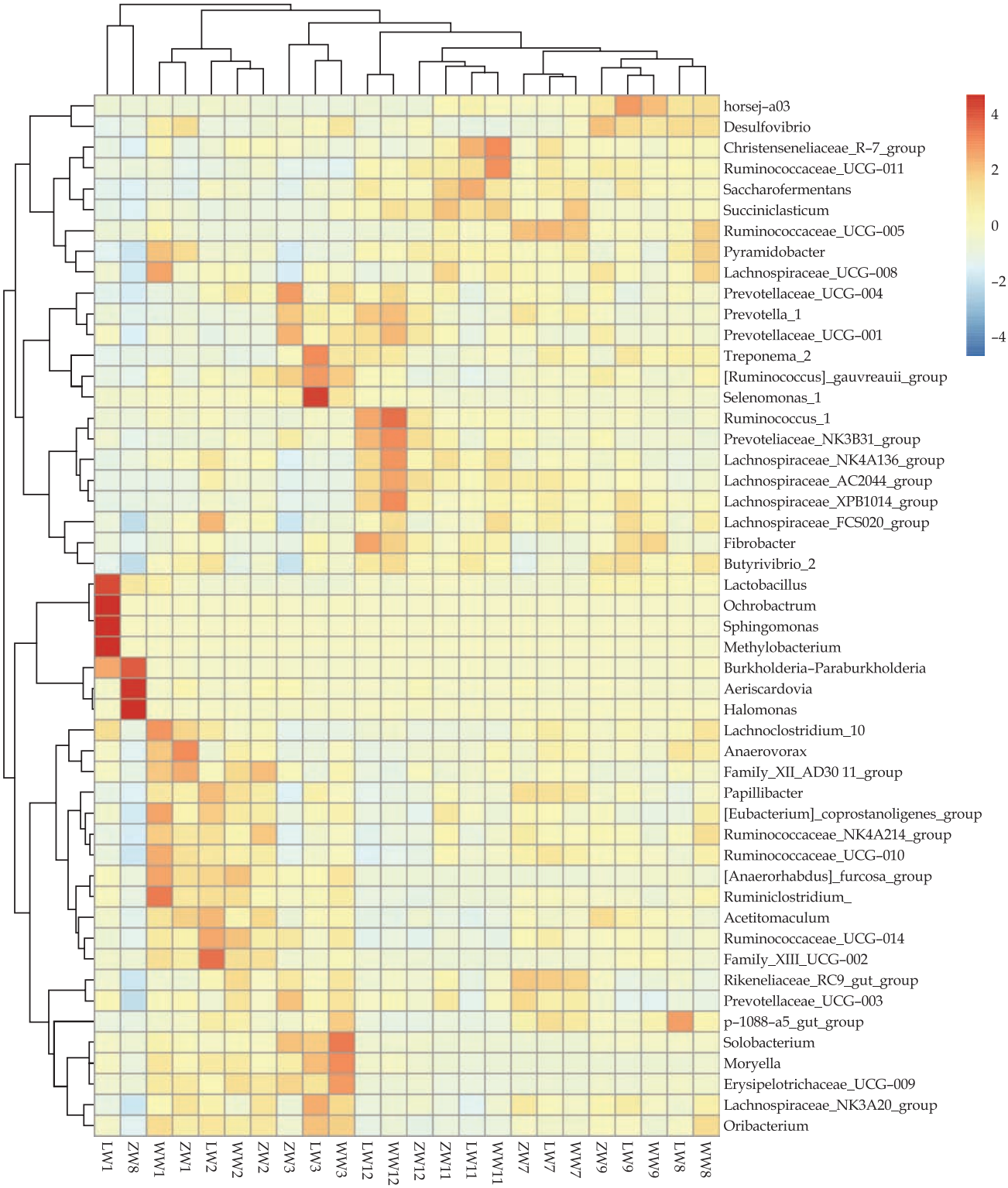

Fig 4. Heatmap analysis of the microbial communities.

Many Firmicutes and Bacteroidetes are carbohydrate-degraders; thus, the diversity and abundance of these phyla can impact the ability of the host to effectively digest fibre (Chen *et al*, 2017; Omoniyi *et al*, 2014; Noel *et al*, 2017). Firmicutes, Bacteroidetes and Spirochaetes were the dominant phyla in our study, accounting for 36, 18 and 12% of reads, respectively. The most abundant phylum in the rumen was Firmicutes, which is consistent with the results of several other studies (Noel *et al*, 2017; Carey

et al, 2013; Ley *et al*, 2008). The most abundant genera were Christensenellaceae_R-7_group, Rikenellaceae_RC9_gut_group and Fibrobacter. We hypothesise that these genera are active plant degraders in the Bactrian camel forestomach. Actinobacteria levels in the abomasum were higher than in other sites. Previous studies have shown that Actinobacteria are important secondary metabolite producers that produce potent antibiotics (Bull *et al*, 2005). Thus, it is possible that an increased level of Actinobacteria can lead to improved resistance against harmful microorganisms.

By improving our understanding of the seasonal variations in plant consumption we can better understand the dietary requirements of Bactrian camels. Previous studies have shown that many ruminants exhibit seasonal variations in plant consumption (Amato *et al*, 2015; Noel *et al*, 2017; Springer *et al*, 2017). We observed a distinct seasonal variation in microbiota composition, including differences in phylogenetic diversity and distinct groups in NMDS and cluster analyses. This suggests that the forestomach microbiota in Bactrian camels is not static and adapts to modest changes in diet. Interestingly, nine genera were differentially abundant ($P < 0.05$) between spring and winter. Seven of these genera were Firmicutes and of these seven Firmicutes, three were more abundant in winter and four were more abundant in summer. This suggests that Firmicutes may be responding to seasonal variations in diet (Carey *et al*, 2013; Ley *et al*, 2008; Davenport *et al*, 2014).

We observed seasonal variations in the composition of forestomach bacterial communities of Bactrian camel. However, the impact of these variations on host health is still unclear. Our study had a small sample size (n=8); however, our results are consistent with other studies. Another potential limitation is the significant intersubject variability. Nevertheless, this study provides a foundation for further studies of the gut microbiome in Bactrian camels.

Declarations

Abbreviations. DNA: deoxyribonucleic acid; PCR: polymerase chain reaction; NMDS: Non-metric multidimensional scaling; OTUs: operational taxonomic unit;

Availability of date and materials. The sequencing data from three samples (Group A) were deposited in the NCBI Sequence Read Archive (SRA) under accession no. SRP149038. The sequencing data from other five samples were deposited in the NCBI Sequence Read Archive (SRA) under accession no. SRP114499.

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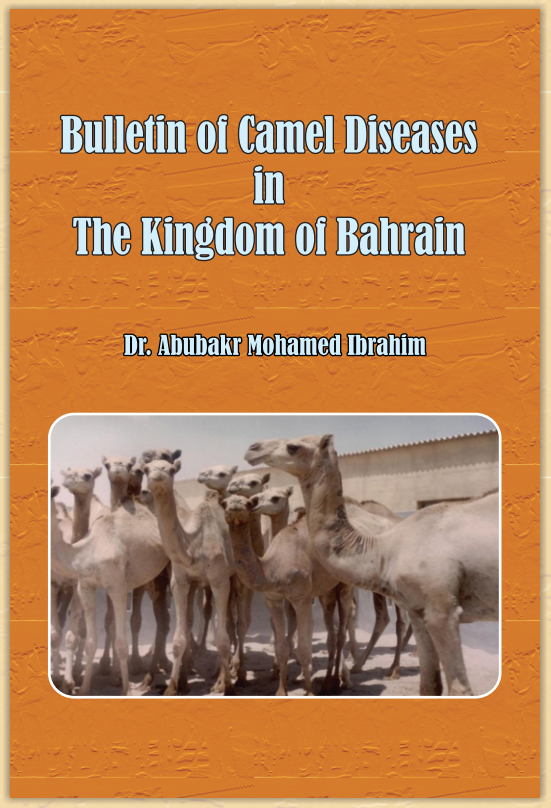
Bulletin of Camel Diseases in The Kingdom of Bahrain

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

About the Author

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



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BALANTIDIASIS IN DROMEDARY CAMELS: PREVALENCE, HAEMATOLOGY AND TREATMENT OUTCOMES

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ABSTRACT

This study was carried out in the Qassim region in central Saudi Arabia to determine the prevalence, haematological changes and treatment outcomes associated with *Balantidium coli* (*B. coli*) infection in 500 dromedary camels (45 males and 455 females) of different ages. The results showed a 21.00% prevalence of *Balantidium coli* in the camels. The prevalence of the disease was significantly different ($p < 0.01$) among different age groups and between male and female camels ($p < 0.05$). Depression, decreased appetite accompanied by normal body temperature (36-37°C) in addition to soft faeces coated with mucous were the most common clinical signs in the balantidiasis-infected camels. Haematological examination revealed no significant difference between infected and healthy camels. Treatment of infected camels using oxytetracycline yielded a 91.42% cure rate. The study concluded that *B. coli* is widespread among camels in the Qassim region and thus, treatment of both asymptomatic and clinically diseased camels is important in order to reduce and prevent environmental contamination and human infection. Furthermore, oxytetracycline is indicated in the treatment of balantidiasis in camels.

Key words: Balantidiasis, *Balantidium coli*, dromedary camels, haematology, prevalence

Balantidium coli is a common commensal parasite found in the large intestine of pigs, both domesticated and wild, indicating them as a main source of this pathogen (Tajik *et al,* 2013). Pigs are acknowledged as being the principal natural reservoir hosts and the main source in the transmission of the disease to humans *via* cyst-contaminated drinking water (Karanis *et al,* 1993; Garcia, 1999; Schuster and Visvesvara, 2004; Schuster and Ramirez-Avila, 2008). *B. coli* has been detected in camels (Ali and Abdelaziz, 1982), nonhuman primates, rodents and man (Souslby, 1982; Nakauchi, 1999; Taylor *et al,* 2007). Diagnosis of balantidiasis in camels is mainly based on the presence of chronic diarrhoea which cannot be ascribed to any other pathogenic organism, while confirmation is mainly accomplished *via* microscopic examination and detection of a large numbers of *B. coli* trophozoites and/or cysts in the faeces (Higgins, 1985). In addition, identification of trophozoites or cysts of the parasite in biopsy specimens taken through a sigmoidoscope or detection in ulcers upon autopsy are used as confirmatory tools in humans (Sampurna, 2007).

There is scarce literature on prevalence of balantidiasis in animals, especially in dromedary camels in Saudi Arabia. Therefore, the aim of this study was to determine the prevalence, haematological changes and treatment outcomes associated with *Balantidium coli* infection in dromedary camels.

Materials and Methods

In the Qassim region of central Saudi Arabia, a total number of 500 camels (45 males and 455 females) of different ages were examined for detection of *Balantidium coli* infection. Individual faecal samples (about 5 g) were collected from the rectum of each camel and stored in a clean, labeled container. These samples were examined macroscopically for any abnormalities (consistency and presence of blood and/or mucous). Direct faecal smears, sedimentation and concentration floatation techniques were done at the time of collection in order to confirm the presence of *B. coli* trophozoites and cysts according to Kaufmann (1996). Counting was done using McMaster slides.

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Epidemiological data were estimated according to Martin *et al* (1987). All camels under study were subjected to careful clinical examination according to Rosenberger (1979).

Blood samples were collected from 10 parasitologically *B. coli* infected camels in addition to 10 samples from parasitologically negative camels. These samples were collected before administration of any drugs. Erythrocyte, total and differential leucocyte counts and haemoglobin concentration were measured using VetScan HM5 haematology analyser (Abaxis, Inc.).

Infected camels were treated using long-acting oxytetracycline (Terramycin /LA®, Pfizer) in 3 doses 3 days apart *via* intramuscular injection (10 mg/kg B.W. or 1 mL/10 kg B.W. with a maximum dose of 30 mL/animal), in addition to administration of flunixin meglumine (Finadyne™ MSD Animal Health) daily for 5 days *via* intramuscular injection (2.2 mg/kg B.W. or 2 mL/45 kg B.W.). Treatment success was based on improvement of animal health and disappearance of the parasite from the faecal matter. Treated animals were examined parasitologically, 15 days post treatment.

The obtained data were analysed by T test and Chi-Square using the SPSS for Windows (Version 15.0, USA) statistical software program.

Results and Discussion

Among the 500 camels examined parasitologically for detection of *B. coli*, 105 camels proved to be infected (21% prevalence rate) (Figs 1 and 2). Regarding predisposition according to age, the disease prevalence was 27.27% among camels younger than 3 years and 17.91% in camels older than 3 years (Table 1).

Table 1. Prevalence of balantidiasis among examined camels.

Camels / Age	Examined camels	Infected camels	%
Under 3 years	165	45	27.27*
Over 3 years	335	60	17.91
Total	500	105	21

p-value is 0.01. *The results are significant ($p < 0.05$)

Concerning sex predisposition, the prevalence of the disease was 33.33% in males compared to 19.78% in female camels (Table 2).

Infected camels with a large number of *Balantidium coli* parasites showed signs in the form of depression and decreased appetite, while having a normal body temperature (36°C). Macroscopic examination of the faecal matter revealed diarrhoea or soft faeces coated with mucous having normal colour and odour. In contrast, camels infected by low numbers of parasites exhibited no clinical signs. The haematological findings of the infected camels compared to those of the parasitologically healthy camels (mean ± SD) are shown in table 3.

Table 2. Prevalence of balantidiasis among examined male and female camels.

Camels / Sex	Examined camels	Infected camels	%
Male	45	15	33.33*
Female	455	90	19.78
Total	500	105	21

*The results are significant ($p < 0.05$)

Concerning the treatment outcomes, 96 out of 105 balantidiasis-infected camels treated using oxytetracycline at 3 doses which were 3 days apart *via* intramuscular injection in addition to administration of flunixin meglumine daily for 5 doses were effectively cured, representing a 91.42% cure rate.

Table 3. Haemogram in healthy and *Balantidium coli* infected camels (Mean + SD).

Variable	Healthy camels (n=10)	Diseased camels (n=10)
RBCs (10^6/µl)	9.33 + 0.79	8.85 + 1.36
Hb (g/dl)	14.48 + 1.35	13.87 + 1.56
PCV (%)	27.64 + 2.46	26.24 + 1.46
WBCs (10^3/µl)	13.84 + 2.11	13.4 + 1.53
Neutrophils (10^3/µl)	9.61 + 1.76	8.67 + 3.05
Lymphocytes (10^3/µl)	3.20 + 1.42	2.97 + 1.12
Monocytes (10^3/µl)	0.11+ 0.09	0.10 + 0.04
Eosinophils (10^3/µl)	2.78 + 1.14	2.44 + 0.83

RBC, red blood cells; WBC, white blood cells; Hb, haemoglobin concentration; PCV, packed cell volume.

In Islamic countries where the consumption swine meat is prohibited and there are no pig farms, the reservoir host for *B. coli* is unknown (Tajik *et al*, 2013). However, Cox (2005) mentioned that camels can be considered as a reservoir host for *B. coli* in Islamic countries.

In the current study, the prevalence of balantidiasis among the camels examined was 21%. A lower rate (11.9%) was found previously by Tekle and Abebe (2001) in camels from Ethiopia, while higher prevalence rates were reported by Hamza (2007) in camels from Iraq (43.53%), by Ghoke *et al* (2010) in camels from India (53%) and by Hussein *et al* (2016) in camels from Iraq (50%). The difference

between the results of the current study and those of the other studies may be attributed to the differences in localities and climatic conditions. The *B. coli* oocyst cannot survive and is easily destroyed by direct sunlight and high temperatures in some localities and the oocyst loses its viability and infectivity (Schuster and Ramirez-Avila, 2008; Bilal *et al,* 2009).

The prevalence of the disease significantly differed among the different age groups ($p < 0.05$). The prevalence was 27.27% among camels younger than 3 years, whereas it was 17.91% in camels older than 3 years. Similar results were recorded previously by Biu and Dauda (2008) and Al-Hasan *et al* (2015) who reported a significant difference among different age groups. In addition, Hamza (2007) recorded the lowest infection rate in animals aged less than 2 years (26.82%) and the highest in the ≥10 year age group (59.37%), while Hussein *et al* (2016) found the highest infection rate in camels in the 3-<6 year age group and the lowest in the ≥9 year age group.

Regarding the predisposition depending on sex, significant ($p < 0.05$) difference was found between the prevalence in males and females. Hamza (2007), Hussin (2015) and Hussein *et al* (2016) in camels and Hussin and Al-Samarai (2016) in cattle found no significant difference in the prevalence of the disease between males and females. This may be attributed to the type of rearing and management systems used in each localities.

Depression and decreased appetite accompanied by normal body temperature (36-37°C) in addition to diarrhoea or soft faeces coated with mucous were the most common clinical signs in the balantidiasis-infected camels. Similar findings were reported previously by Abubakr *et al* (2007), who detected *B. coli* in camels suffering from diarrhoea in Bahrain and by Tajik *et al* (2013), who identified the parasite in camels exhibiting anorexia and diarrhoea in Iran.

Ali and Abdelaziz (1982) investigated the pathogenic effects of *B. coli* and its ability to cause enteric disease in camels alone without any concomitant infection. On other hand, concurrent infections of *B. coli* with other agents such as *Eimeria* spp., *Trichuris* spp. and *Strongyloides* spp. in sheep and *Trichuris vulips* in dogs were reported to intensify the disease, resulting in severe enteritis (Cho *et al,* 2006). Furthermore, Headley *et al* (2008) believed that *B. coli* acts as an opportunistic pathogen and displays its pathogenic effect after the destruction of the intestinal epithelium by other microbes.

The parasite normally inhabits the lumen of the bowel or in between the villi without harmful effect (Souslby, 1982; Jones *et al,* 1997). In some unusual situations, the parasite may invade the mucosa and submucosa of the intestine in addition to the lymphoid tissues of affected animals (Souslby, 1982; Jones *et al,* 1997). Brown *et al* (2007) indicated that *B. coli* may become an opportunist pathogen in association with disease induced by other infectious agents.

Balantidiasis is associated with enteritis and colic in man and nonhuman primates, with rare manifestations of the disease in swine and other mammalian species. However, a case of balantidiasis recorded in horses was linked to a sudden onset of colic and severe haemorrhagic and eosinophilic colitis (Headley *et al,* 2008).

The majority of human infections are asymptomatic and carriers passing cysts exhibited no significant clinical symptoms (Esteban *et al,* 1998) or may suffer only mild diarrhoea. In invasive cases,

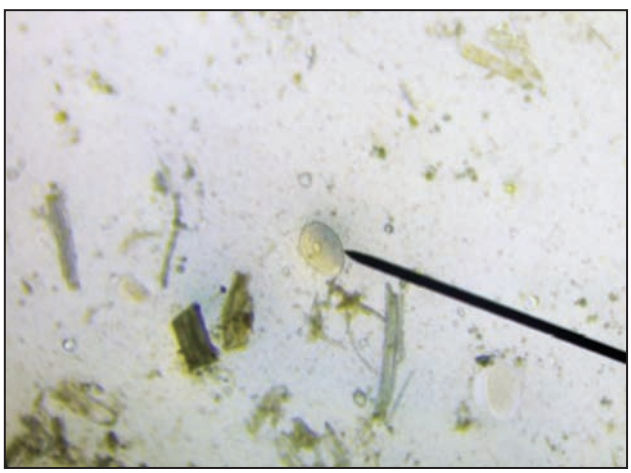

Fig 1. Faecal smear showing trophozoites of *B. coli* (Mild infection) (100 X).

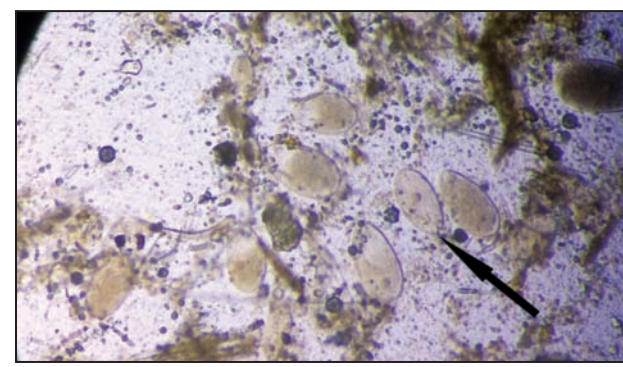

Fig 2. Faecal smear showing trophozoites of *B. coli* (severe infection) (100 X).

the trophozoites penetrate the intestinal epithelium, mainly the intestinal crypts (Zaman, 1993). Invasion of the intestinal mucosa leads to colonic ulceration resulting from the proteolytic enzymes produced by the parasites as hyaluronidase (Solaymani-Mohammadi and Petri, 2006).

Haematological examination revealed no significant differences between infected and healthy camels (mean ± SD). Similar results were reported previously by Ali and Abdelaziz (1982) and Tajik *et al* (2013), who detected no haemogram abnormality in cases of camel balantidiasis. On the other hand, Bauri *et al* (2012) found that *B. coli* in pigs caused significant decrease in the haemoglobin and packed cell volume (PCV) values whereas, the differential leukocyte count showed decreased neutrophils and lymphocytes and increased eosinophils.

The efficacy of oxytetracycline in the treatment of the infected camels was 91.42%. Similar results were observed previously by Bilal *et al* (2009), who found a final oxytetracycline treatment efficacy of 62.5% for *B. coli* in cattle in Pakistan. Moreover, Bauri *et al* (2012) observed that in the treatment of balantidiasis in pigs, oxytetracycline drugs showed higher efficacy than a combination of metronidazole + furazolidone.

Finally, it was concluded that *Balantidium coli* is widespread among camels in the Qassim region and thus, treatment of both asymptomatic and clinically diseased camels is important in order to reduce and prevent environmental contamination and human infection. Furthermore, oxytetracycline is indicated in the treatment of balantidiasis in camels.

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A Workshop Organised by Prof. Amir Niasari-Naslaji at Inner Mongolia Institute of Camel Research on Advanced Reproductive Technologies

During February 11-14, 2019, a workshop was organised by Inner Mongolia Institute of Camel Research on Advanced Reproductive Technologies in Camel Production with particular emphasis on Embryo Transfer in Camel in Alxa, Inner Mongolia, China. Prof. Amir Niasari-Naslaji, the sole invited speaker, from Faculty of Veterinary Medicine, University of Tehran, presented fifteen theoretical and three practical sessions at the workshop. More than 20 participants from the Inner Mongolia agricultural universities, veterinarians and camel breeders from East and West Banner of Alxa League attended the workshop. It was the first workshop on this subject in China and was well covered by different media such as China Daily and National TV in China. Inner Mongolia Institute of Camel Research of China wishes to apply embryo transfer to enhance camel milk production in the region.

Dr T.K. Gahlot as a Keynote Speaker at 1st International Conference at Matrouh University; Donated Camel Books and Journal

The Organizing Committee of 1st International Conference of Faculty of Veterinary Medicine–Matrouh University/ Egypt invited Dr. T.K. Gahlot, Editor, Journal of Camel Practice and Research as a keynote speaker for the lead paper "Current and Futuristic Trends of Camel Surgery of Dromedary

Camels (*Camelus dromedarius*)" in the conference which held during April 07th -08th, 2019, at Matrouh Library, Matrouh Governorate, Egypt. The said conference had theme "The Challenges and Opportunities of Veterinary Medicine in Mediterranean Countries". It was attended by more than 300 delegates. Dr. Gahlot donated many books and latest volumes of Journal of Camel Practice and Research to the library of Matrouh University which was appreciated by Dr Eman Khalifa, Acting Dean of Faculty of Veterinary Medicine – Matrouh University/ Egypt. The faculty and students felicitated Dr Gahlot in the valedictory session of the conference with a special floral garland. The President of the faculty of Veterinary Science expressed his views to seek future collaboration with Rajasthan University of Veterinary and Animal Sciences, Bikaner, India to develop clinical and health facilities for camels.

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A ZOONOTIC CASE OF CAMEL DERMATOPHYTOSIS CAUSED BY *Trichophyton violaceum*

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ABSTRACT

A case of dermal mycoses in a camel (*Camelus dromedarius*) due to *Trichophyton violaceum* with round patchy, dull whitish lesions on neck, chest and axillary regions was recorded. These lesions measured up to 5-10 cm in diameter. These alterations induced intense itching and irritation of skin in the camel. The camel was being used for riding by the camel owner. Within one week of observing the lesions, intense irritation and itching of the skin occurred in the camel owner, with the onset of tinea rashes. These rashes were circumscribed, scaly, had an inflammatory advancing margins. In the owner rashes measured up to 5-10 cm in diameter within one month of the onset of infection and these rashes were present on the arms, legs (*Tinea corporis*), cheeks (*Tinea barbae*) and inguinal region (*Tinea cruris*). Isolation and identification of *T. violaceum* from camel owner's infected skin scrapings, confirmed the zoonotic nature of this fungi.

Key words: Camel, dermatophytes, zoonoses

Zoonoses are diseases of animal origin, caused by pathogens which can be naturally transmitted to humans (WHO, 2006). Many zoonotic diseases have significant impact on human and livestock health, thereby undermining livelihoods both by causing illness in the household and threatening its livestock and their production (Ehizibolo *et al*, 2011).

Dermatophytosis is an integumentary mycotic disease prevalent in both sporadic and epidemic forms throughout the world. It is an important occupational mycozoonoses of dairymen, animal handlers, livestock farmers, pet owners, veterinarians and others (Ruben, 2010) caused by a group of pathogenic fungi collectively known as "dermatophytes" which are the most common agents of superficial mycoses in animals and humans, and are recognised as a public health menace worldwide (Mignon and Monod, 2011; Ndako *et al,* 2012). The dermatophytic fungi include various species of the three well established fungal genera that include Microsporum, *Trichophyton* and *Epidermophyton* (Nweze, 2010). Among the deramtophytes, majority of the zoonotic infections are caused by *Microsporum canis* (usually derived from pets like cats and dogs), *Trichophyton verrucosum* (usually derived from cattle), *Arthroderma vanbreuseghemii* (usually derived from cats and dogs) and *Arthroderma benhamiae* (usually derived from guinea-pigs) (Mignon and Monod, 2011). In this study, a zoonotic case of camel dermatophytosis caused by *T. violaceum* is recorded.

Materials and Methods

A riding female camel aged 6-7 years was found infected with lesions typical of dermal mycoses. Within one week these lesions showed intense irritation and itching of the skin simultaneously in the camel owner riding the camel also, with the onset of the tinea rashes. For mycological examination, skin scrapings from both camel and its owner were collected. A direct KOH mount revealed thin hyaline septate hyphae. For cultural examination samples were first mixed with Sabouraud's dextrose chloramphenicol broth and were incubated at 28°C for up to 24 hours. Then these samples were inoculated onto Sabouraud's dextrose chloramphenicol agar plates and later incubated at 28°C for 15 days. These plates were examined daily for growth of the fungi. The resultant growth was examined for colony morphology. Microscopic examination was carried out using Calcofluor-white stains using wet mount method (Halley and Standard, 1973).

Results and Discussion

Dromedary camel was infected with dermal mycoses with round patchy, dull whitish lesions on neck, chest and axillariy regions. These lesions measured up to 5-10 cm in diameter and were hard to press with finger compared to adjoining healthy skin (Fig 1). These alterations induced itching and irritation in the camel leading to scratching of the skin

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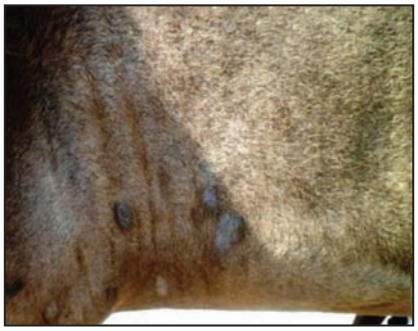

Fig 1. Lesions on chest and axillary region of the infected camel.

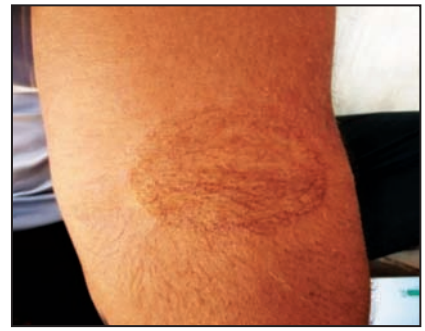

Fig 2. Rash on camel owner's arm (*Tinea corposis*).

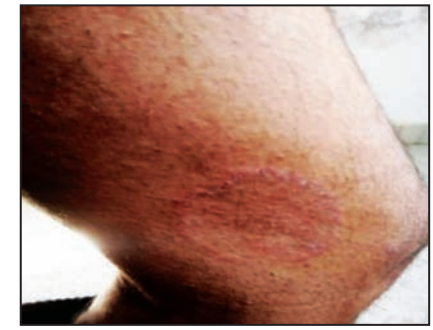

Fig 3. Rash on camel owner's leg (*Tinea corposis*).

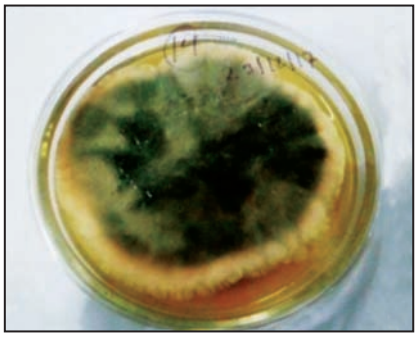

Fig 4. Colony morphology 12 days front on Sabouraud's dextrose chloramphenicol agar.

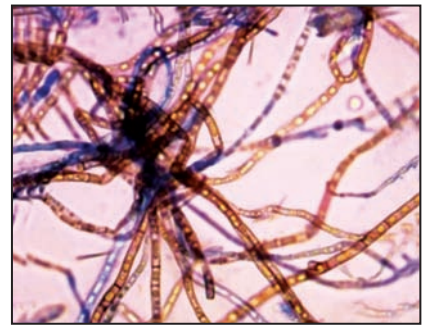

Fig 5. Hyphae showing central fat globules and granules.

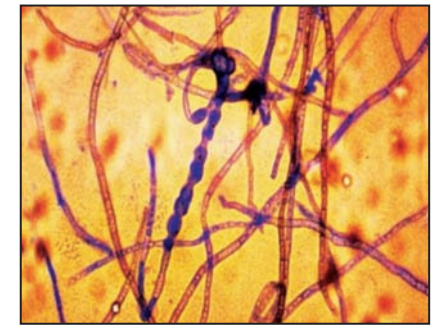

Fig 6. Microscopy showing chlamydospores.

with inanimate objects. Within one week of observing the lesions intense irritation and itching of the skin also occurred in the camel owner, with the onset of tinea rashes. These rashes were circumscribed, scaly and had an inflammatory advancing margins. The owner's rashes measured up to 5-10 cm in diameter within one month. These rashes were present on the arms (Fig 2), legs (Fig3), cheeks and inguinal region. *T. violaceum* has previously been isolated from camel skin lesions (Tuteja *et al,* 2013a). Similar rashes on scalp and cheek have been reported due to *T. violaceum* (Smriti *et al,* 2015).

The mycological culture revealed slow growing, waxy, heaped folded and deep violet colonies. Later, these became pleomorphic, forming white sectors (Fig 4). Hyphae were relatively broad, tortuous, much branched and distorted. Hyphae showed small central fat globules and granules (Fig 5). Numerous chlamydospores were present (Fig 6). These characteristics confirmed the culture of *T. violaceum* from camel and its owner.

Zoonotic fungi can be transmitted between animals and humans. Abu-Samra and Ibrahim (1988) found that horses were successfully infected with human isolates of *T. violaceum*. Pal and ChangWoo (2000) similarly reported isolation of *T. verrucosum* from skin scrapings collected from cutaneous lesions on the face of a 6-month-old male camel and on the hand of a 29-year-old camel handler from South Korea.

Zoonotic case of camel dermal mycoses

T. violaceum is an anthropophilic dermatophyte causing mainly *Tinea capitis* (Patel and Schwartz, 2011; Valari *et al,* 2012; Zaraa *et al*, 2013), *Tinea corporis* (Smriti *et al,* 2015), *Tinea pedis* (Lee and Mosser, 2015) in humans. The fungus is endemic to the arid climate zones of northern Africa, eastern Europe (Ginter-Hanselmayer *et al,* 2007) and large parts of Asia (Deng *et al,* 2008; Grover *et al,* 2010). Lamb and Rademaker (2001) reported 68 isolates of *T. violaceum* and *T. soudanense* from 60 patients in Hamilton, New Zealand. Most of these patients had migrated from East Africa. Eight of them had *Tinea corporis* due to *T. violaceum*. Magill *et al* (2007) reported an increase in recovery of *T. violaceum* and *T. soudanense* from skin, hair and nail specimens from Baltimore, Maryland, USA. They attributed this rise to increased immigration of African born individuals changing population demographic affecting epidemiology of

this organism. Ellabib and Khalifa (2001) from Libya found *T. violaceum* as a most common etiological agent in *Tinea corporis* with 44% of the total dermatophyte isolates. In another study, Singh and Beena (2003) found isolation in human of *T. rubrum* (73.27%), *T. mentagrophytes* (17.20%), *E. floccosum* (7.75%) and *T. violaceum* (1.72%). *T. violaceum* was the most common agent causing *Tinea capitis* in Turkey (Akpolat *et al,* 2005).

Zoonotic fungi can be naturally transmitted between animals and humans. Human infection results either from direct contact with an infected animal and may also be acquired indirectly through contact with a contaminated environment, such as infected hair and scales from infected animals (Mignon and Monod, 2011). The prevalence of superficial mycoses caused by zoophilic dermatophytes was found to be significant in different parts of the world especially, in the tropical countries with warm and humid climate, crowded living and poor sanitary canditions (Weese and Fulford, 2010).

Domestic livestock kept by rural Indian farmers pose increased risk of transmission of zoonotic infections to humans. In India, fungal diseases with zoonotic potential have attracted inadequate interest, probably due to the fact that fungi are yet to be recognised as a major cause of morbidity in animals and humans, leading to insufficient interest in the development of integrated platform of veterinary and medical researchers for their prevention and control. Camels can suffer from different fungal diseases (Wernery *et al,* 2014). Most of the fungi isolated and identified from camel infections (Tuteja *et al,* 2013b and Refai *et al,* 2016) are pathogenic to human beings (Mignon and Monod, 2011; Ndako *et al,* 2012; Nweze, 2010). Thus camel can be considered as a potential threat for zoonoses of fungal origin.

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A HISTOLOGIC AND HISTOMORPHOMETRIC STUDY OF THE SECOND STOMACH CHAMBER OF THE DROMEDARY (*Camelus dromedarius*)

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ABSTRACT

While a number of studies on the camel stomach have been reported, major nomenclatural inconsistencies still remain in the gross and microscopic anatomical descriptions and naming of the second compartment of dromedary stomach (C2). Additionally, there is paucity of authoritative studies that have evaluated the histomorphometric and temporal variations in the thickness of the histological layers of C2. The present study was therefore done to assess the topographical relationship of C2 with the rest of the dromedary stomach, described the microanatomical characteristics of the different histological layers and further evaluated age-related histomorphometric changes in C2. Tissue samples were taken from the initial and final portions of C2 from forty-eight healthy dromedary camels of both sexes aged between 1 and 16-years-old. The tissue specimens were routinely processed and stained with haematoxylin and eosin (H&E) and the slides microscopically evaluated for histological characteristics. The thickness of the different histological layers including the mucosa, submucosa, muscularis and serosa were also assessed histomorphometrically. On the basis of the present findings, remarkable macroscopic and histologic similarities are documented between C2 and C1 of the dromedary stomach. The different anatomical layers of the initial and final portion of the C2 showed significant intergroup thickness variation (p=0.001) that progressively increased in an age-dependent manner.

Key words: Camel, dromedary, forestomach, histology, histomorphometry, second compartment

The microbial fermentation in the large forestomachs is common to both camels and the true ruminants; however, the camelids are believed to have independently evolved much earlier than ruminants (Lechner-Doll *et al,* 1995). It is probable that the nutritional niche of the camel has influenced the development of unique histological structures in the digestive system that serve specialised functions. In addition, compared to neutral mucous produced in the glandular portions of the bovine and equine stomachs, the camel stomach secretes acidic mucus (Bello *et al,* 2012; Eerdunchaolu *et al,* 1999; Langer, 1984). Such functional and anatomical variations also suggest that the camel relies more on chemical digestion than fermentation (Raji, 2011). The first compartment (C1) of the camel stomach has a predominant non-glandular region alongside the glandular sacs, while the 2nd (C2) and 3rd compartments (C3) are glandular in nature (Abdel-Magied and Taha, 2003; Al Aiyan *et al,* 2018; Eerdunchaolu *et al,* 1999; Hansen and Schnidt-Nielsen, 1957; Lechner-Doll *et al,* 1995). These features underscore the justification of the term "pseudo-ruminant" as has been coined with reference to the camel (Bello *et al,* 2012).

Despite many camel studies focusing on the gross and histological anatomy of C2 of the stomach, major inconsistencies still remain in the nomenclature with some authors calling it a 'reticulum' (Dougbag and Berg, 1981; Hansen and Schnidt-Nielsen, 1957), while others refer to it as the "second ventricle" (Wang *et al,* 2000). Similar to the new world camelids, veterinary anatomists have popularised the terminologies C1, C2 and C3 to describe the different compartments of the camel stomach (Abdel-Magied and Taha, 2003; Al Aiyan *et al,* 2018; Eerdunchaolu *et al,* 1999; Lechner-Doll *et al,* 1995). Besides, very limited histomorphometric studies have comprehensively evaluated the temporal variations in the histological layers of the second compartment of the camel stomach. To reconcile these ambiguities, the present study assessed the topographical anatomy of the dromedary stomach, further described the histological characteristics of the mucosa, submucosa, muscularis and serosa of the 2nd stomach compartment of the dromedary and

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evaluated age-related histomorphometric changes in the study animals.

Materials and Methods

The tissue samples for this study were taken from 48 healthy dromedary camels of both sexes aged between 1 and 16 years old. The same animals were basis for a recent study of C1 recently reported by Al Aiyan *et al* (2018). In accordance with the research ethics code of the United Arab Emirates University Animal Ethics Committee, tissue samples were collected from camels slaughtered for food at the municipal slaughterhouse, Al Ain, Emirate of Abu Dhabi, UAE. Group 1 (1 - 4 years), Group 2 (5 - 7 years), Group 3 (8 - 11 years) and Group 4 (12 - 16 years). Immediately after evisceration of the camel carcasses, 3×3 cm size stomach tissue specimens were collected from the initial and final portions of C2 (Fig1) and immediately placed in 10% buffered formalin. After holding the tissue samples in 10% buffered formalin at room temperature for at least 2 weeks, the properly fixed tissues were routinely processed for histological evaluation.

The formalin fixed tissues samples were processed and stained with haematoxylin and eosin (H&E) as recently described (Al Aiyan *et al,* 2018). After drying, the stained sections were mounted with DPX (distyrene, tricresyl phosphate and xylene).

A detailed histological evaluation of the examined tissue sections was done using a light microscope (Olympus light microscope BX53, Japan). Each section was examined using 10x, 20x, 40x and 60x objectives, starting from the lumen and then scanning across the *tunica mucosa, tela submucosa, tunica muscularis,* to the *tunica adventitia*. All histological features including the type of epithelium, connective tissue, blood and lymphatic vessels, autonomic nerve ganglia, smooth muscle and adipose tissue were examined. With the aid of an Olympus light microscope (BX53, Japan), the thickness of the different histological layers were measured using the microscope's arbitrary line function to measure the linear distance between the selected initial and final points of the measured object. An analysis of variance using Statistical Packages for Social Sciences software (SPSS Inc. Version 20, Chicago IL, USA) was conducted on all thickness data. The mean of the intergroup variation in the thicknesses of the different histological layers were calculated using Duncan's multiple range test and the differences in the dimensions of the different histological layers were considered significant at $p<0.05$.

Results and Discussion

Macroscopic assessment of the second compartment of the camel stomach (C2)

A comprehensive gross (Fig 1) and microscopic assessment of the camel stomach demonstrated 3 compartments namely C1, C2 and C3. As recently reported (Al Aiyan *et al,* 2018), C1 is the largest of the 3 and occupies most of the left side of the abdominal cavity. On the other hand, C2 is ovoid in shape and situated to the right and cranioventral side of the gastric cardia directly cranial to the caudodorsal glandular sac (CDGS) of C1 and externally separated from C1 by a small deep groove (Fig1A). The orifice between C1 and the C2 is located cranial to the caudodorsal glandular sac (CDGS) of C1 and is evidently larger than the small constricted orifice between C2 and C3 (Fig 1A and C). Internally, C2 is characterised by a retiform appearance consisting of robust radiating pillars that extend across the mucosal surface (Fig 1B). These pillars are connected by numerous cross-struts forming a series of deep, mostly rectangular sub-compartments or cells that are lined by a glandular mucosa that is characterised by rugae lined by simple columnar epithelial cells. The elongate and tubular C3 originates from C2, lies cranially on the dorsal surface of the cranioventral glandular sac (CVGS) and caudally traverse across C1 on the right side after which it merges with a small 'true' gastric gland region immediately located just before the pyloric junction with the duodenum (Fig1A and C).

Histology of the initial and final portions of the second stomach compartment (C2)

Histological evaluation of a full thickness section taken from the initial portion of C2 revealed a mucosa that was lined by stratified squamous keratinised epithelium that abruptly gave way to a glandular epithelium (Fig 2A). The stratified squamous epithelium was thrown into broad-based mucosal folds of multiple cell thickness with the basal area characterised by short downward-pointing fronds of rete-peg-like structures. The epithelium was further characterised by a thin parakeratotic keratinised layer in which very few to barely visible flattened nuclei were present (Fig 2A). The propria submucosa was rich in loose to semi-dense connective tissue interspersed with variably sized blood vessels, occasional lymphatics, rare numbers of lymphocytes, plasma cells and scattered eosinophils (Fig 2A and B; Fig 3A, B and C). Multiple bundles of inner circular and outer longitudinal smooth

muscle layers separated by extensive areas of fibrovascular tissue were present within the tunica muscularis propria (Fig 2B; Fig 3A and C). In the areas of initial and terminal portions of C2 lined by a glandular epithelium composed of a simple columnar epithelium that was rich in mucous neck cells (Fig 2B and C). The mucosa was organised into rugae-like folds that bear multiple shallow gastric pits that end into simple straight tubular glands (Fig 2C and 3B). Occasionally attached to the epithelium or located within the gastric lumen were rare *Balantidium coli*-like protozoan parasites (Fig 2D) morphologically similar to the ones recently reported in C1 (Al Aiyan *et al*, 2018). The stomach glands were simple and straight in character and lined by a simple columnar epithelium while the lamina propria was infiltrated by moderate numbers of lymphocytes and plasma cells along with very low numbers of eosinophils (Fig 2C and 3B). A few follicles of lymphoid tissues were occasionally present in the lamina propria (Fig 3A) of both the initial and terminal portions of the C2. Demarcating the mucosa from the submucosa was a thin layer of smooth muscle morphologically consistent with muscularis mucosae and within the submucosa was abundant connective tissue in which many small blood and occasional lymphatic vessels were present (Fig 2C and 3C). Multiple bundles of inner circular and outer longitudinal smooth muscle layers separated by extensive areas of connective tissue were also present within the tunica muscularis.

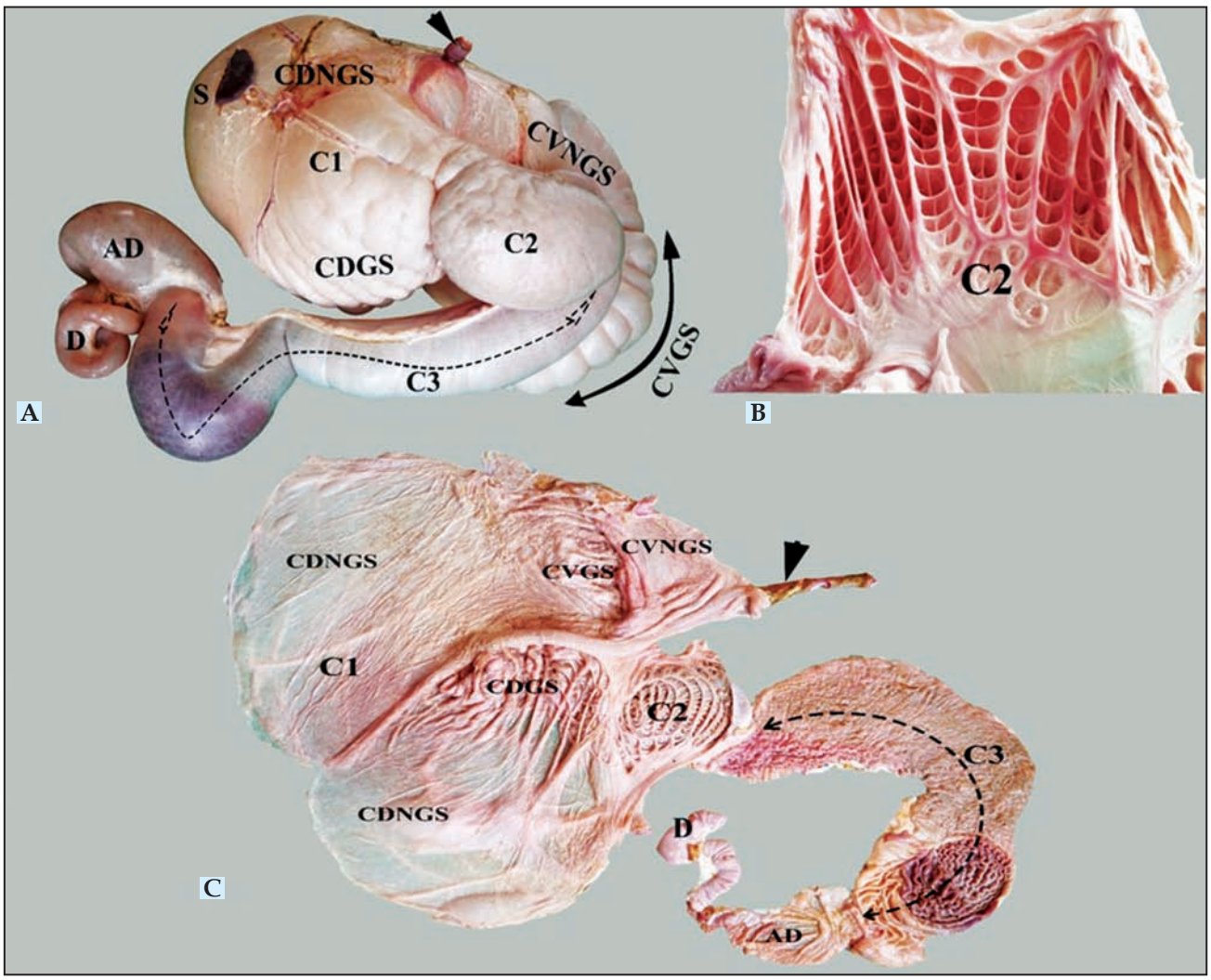

Fig 1. Composite photograph of the gross anatomy of the dromedary stomach complex where (A) right visceral aspect placed in its correct topographical position in the abdominal cavity, (B) internal view of the 2nd compartment (C2) and (C) luminal view of a dissected stomach complex. C1 represents the 1st compartment, C2 2nd compartment, C3 (interrupted black arrows) the 3rd compartment of the stomach. CDGS is the caudodorsal glandular sac, CDNGS the caudodorsal nonglandular sac, CVGS the cranioventral glandular sac, CVNGS the cranioventral nonglandular sac, AD the ampulla duodeni, D the duodenum, S the spleen and black arrowheads the oesophagus.

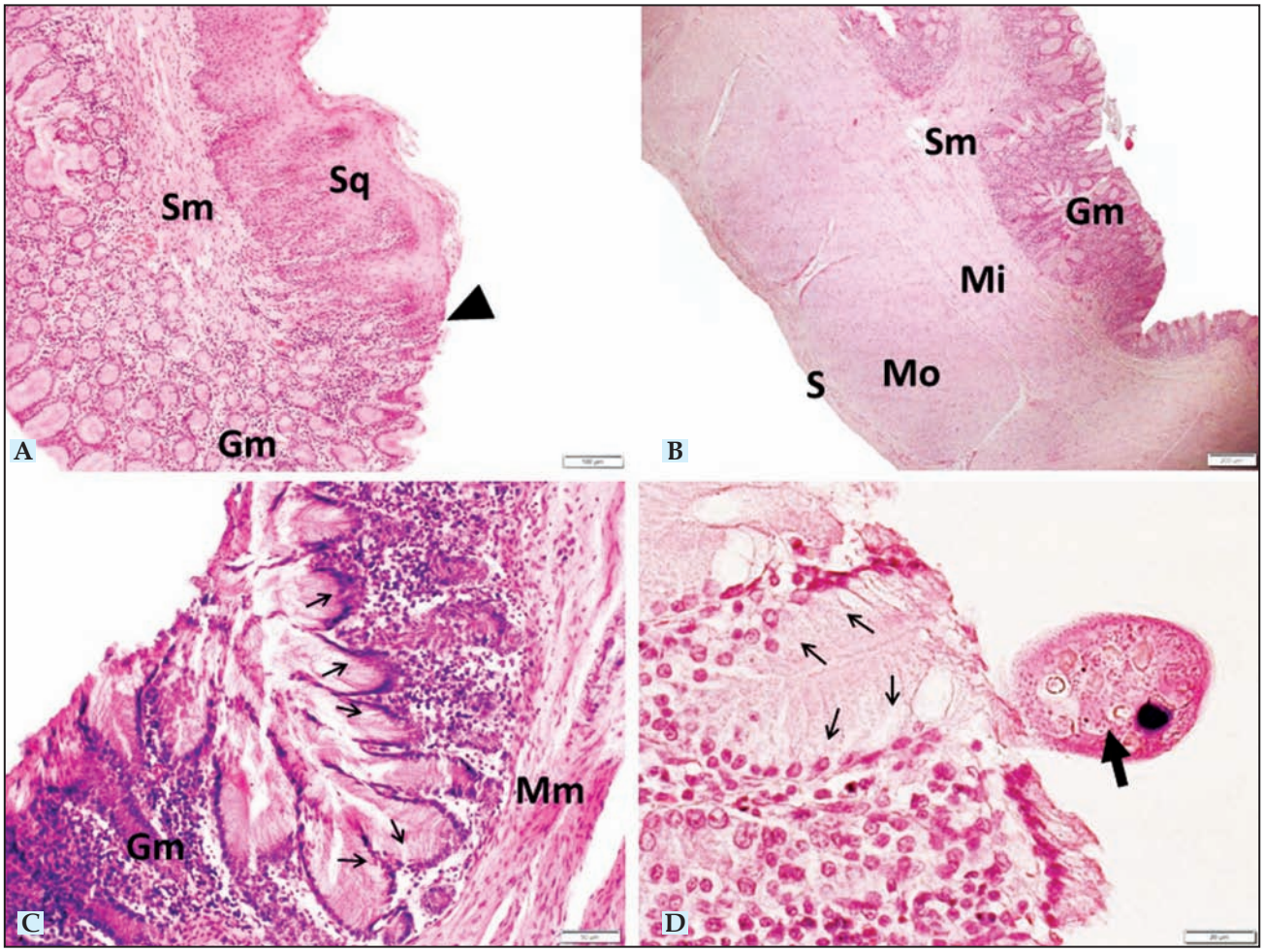

Fig 2. A photomicrograph of the initial portion of the second compartment (C2) of the dromedary stomach (A, B, C and D). The initial part of the mucosa is lined by stratified squamous keratinised epithelium and abruptly gives way to a glandular epithelium (black arrow head; Fig 2A). Note that the stratified squamous epithelium is thrown into broad-based mucosal folds of multiple cell thickness whose basal area is characterised by short downward-pointing fronds of rete-peg-like structures. On the other hand, the glandular mucosa (Gm) is composed of a simple columnar epithelium and numerous mucous neck cells (multiple small arrows) and lamina propria. Below the glandular mucosa is the submucosa (Sm), followed by the inner smooth muscle layer (Mi), outer longitudinal smooth muscle layer (Mo) and the tunica adventitia (S). The latter is characterised by abundant connective tissue and lined by a simple squamous epithelium. Note that the mucosa and submucosa are separated by muscularis mucosa (Mm) (Haematoxylin and Eosin stain).

The myenteric plexuses were randomly present within the connective tissue areas intervening between the smooth muscle layers (Fig 3C). The tunica serosa was expanded by large amounts of connective tissue in which abundant amounts of collagen, occasional fibroblasts and large numbers of lymphatic vessels and blood vessels were present, while the outer layer was lined by a mesothelium characteristically composed of simple squamous epithelial cells (Fig 3D).

Histomorphometric and statistical analysis

The histomorphometric evaluation of the initial portion of C2 revealed significant intergroup variation in the wall thickness of all the histological layers including the mucosa, submucosa, inner smooth muscle layer, outer longitudinal smooth muscle layer and tunica serosa. The greatest wall thickness was observed in animals from group 4 (12 - 16 years old), followed by groups 3, 2 and 1, respectively (Table 1). In respect to the final part of C2, the thickness of the tunica mucosa, submucosa, inner and outer smooth muscle layers showed significant intergroup variation the highest measurements being recorded for group 4 animals followed by groups 3, 2 and 1, respectively (Table 2). Similarly, the thickness of the tunica serosa also showed significant intergroup variation with highest measurements observed in group 4 followed by groups 2, 3 and 1, respectively (Table 2).

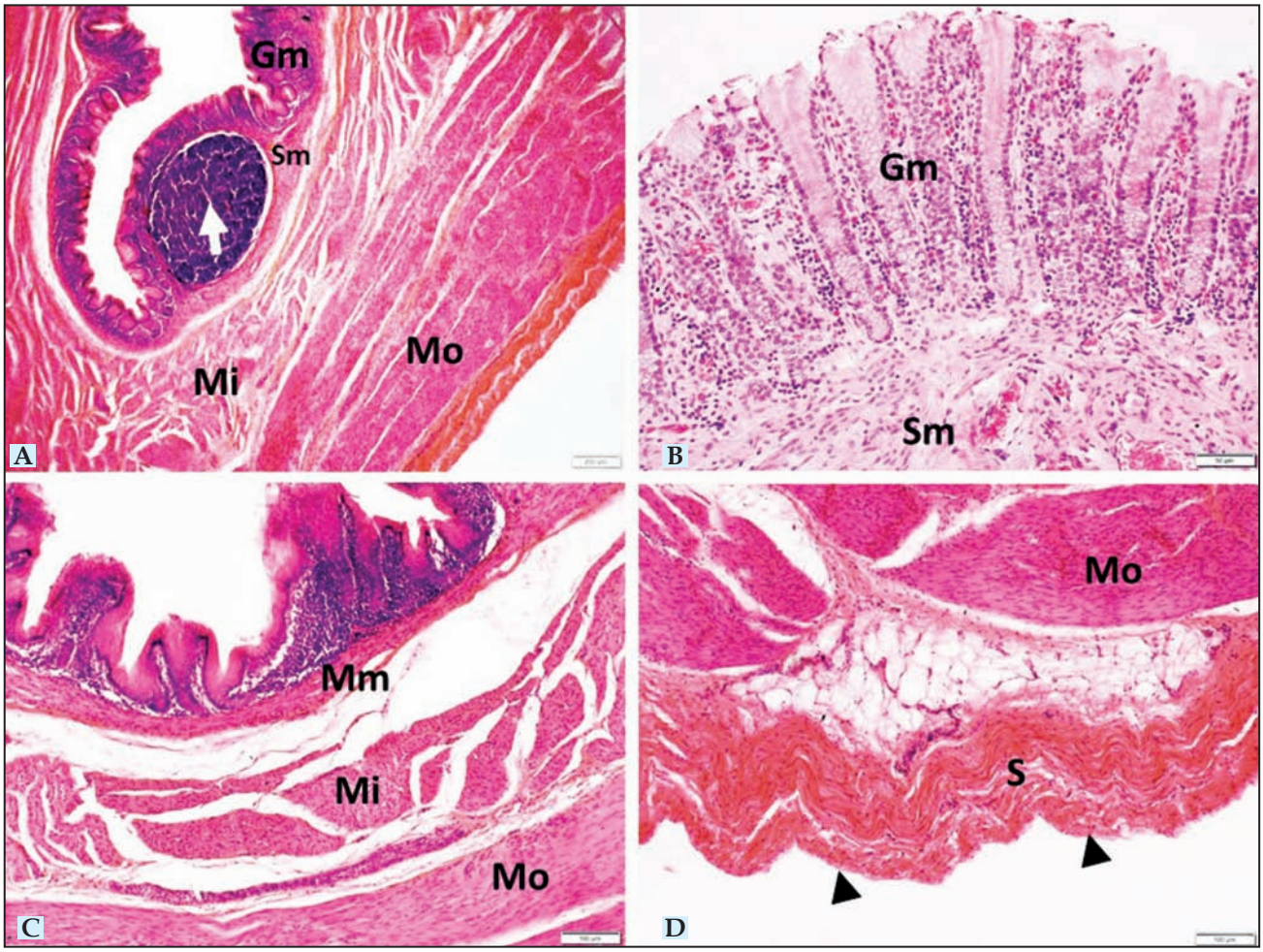

Fig 3. A photomicrograph of the final portion of the second compartment (C2) of the dromedary stomach (A, B, C and D). As demonstrated in the photomicrograph, C2 is typically composed of a glandular mucosa (Gm; composed of a simple columnar epithelium and lamina propria), submucosa (Sm), inner smooth muscle layer (Mi), outer longitudinal smooth muscle layer (Mo) and a tunica adventitia (S) that is characterised by abundant connective tissue and lined by a simple squamous epithelium (black arrowheads). The mucosa and submucosa are separated by muscularis mucosa (Mm) and rare lymphoid follicles expand the lamina propria (white asterisk) (Haematoxylin and Eosin stain).

The present study demonstrated a number of macroscopic and microscopic anatomical similarities between C1 and C2. The retiform mucosal appearance of C2 was macroscopically similar to those reported previously in the cranioventral and caudodorsal sacs of C1 (Abdel-Magied and Taha, 2003; Hansen and Schinidt-Nielsen, 1957; Hegazi, 1950; Wang *et al,* 2000). In comparison with C1, the glandular sacs in C2 were shallower and subdivided by many small mucosal folds into numerous small glandular sacs. However, the rugged mucosal folds in the initial part of C2 were divided into primary, secondary, tertiary and quaternary folds. In this portion, C2 showed histological congruence with the glandular sacs of C1 (Al Aiyan *et al,* 2018), where both structures exhibited simple columnar epithelium, gastric pits and tubular glands.

The second major similarity between C1 and C2 was the large mucosal areas lined by a stratified squamous keratinised epithelium. Lechner-Doll *et al* (1995) previously reported that the dorsal parts of C1 and C2 are lined by a stratified squamous keratinised epithelium and the ventral part by a glandular mucosa. Abuagla *et al* (2015) and Hansen and Schnidt-Nielsen (1957) have also reported that the mucosa adjacent to the junction of C1 and C2, edges of the mucosal folds as well as the oesophageal groove exhibits stratified squamous epithelium.

Similar to the findings of Lechner-Doll *et al* (1995) and Wang *et al* (2000), the present study demonstrated a wide opening between C1 and C2. However, this opening did not appear to have the gatekeeping or restrictive function as the one

between C2 and C3. As such, this makes C2 function more like a diverticulum of C1. To underscore the inconclusive interpretation of the nature and physiological relevance of the wide opening between the 2 compartments, some authors have named C2 "the third glandular sac" or "the second ventricle" of the forestomach (Lechner-Doll *et al,* 1995; Wang *et al,* 2000). As a matter of fact, Lechner-Doll *et al* (1995) clumped C1 and C2 together rather than identifying them as separate organs. Subsequently many authors also named these on the basis of histomorphological similarities. On the other hand and unlike C2 which has a comparatively smaller non-glandular region, C1 is dominated by a non-glandular epithelium. Another similarity between the 2 compartments was seen in the tunica muscularis where the inner and outer smooth muscle layers were histologically similar. Such a similarity has previously been observed in sheep (Franco *et al,* 1993). However, the current study revealed that the inner circular muscular layer in C2 is significantly thinner than in C1, ranging between 236.95±9.03 µm in the initial portion and 426.73±29.58 µm in the final portion of C2 (Tables 1 and 2). Similarly, the average thickness of the inner circular muscular layer in the non-glandular portions of C1 was between 994.62 µm to 1040.93 µm in the glandular portions and 1068.91 µm to 1309.20 µm (Al Aiyan *et al,* 2018). This difference is less pronounced when comparing the outer longitudinal layer of tunica muscularis. On the other hand, when compared to other ruminants the tunica serosa of C2 in the dromedary camel shows histological features similar to that of C1 without significant differences (Al Aiyan *et al,* 2018). Current study has also demonstrated existence of abundant smooth muscle within the core of the gastric rugae-like mucosal folds. Reminiscent of the reticulum in the true ruminants, the bands of smooth muscle fibres in question are continuous with the inner circular muscle layer. This was also previously reported by Wang *et al* (2000).

The histomorphometric evaluation of the different histological layers of the initial and final portion of the C2 showed significant intergroup variation in the wall thickness of all histological layers which progressively increased with the animal's age (Table 1 and 2). Abdel-Magied and Taha (2003) stated that the mucosal thickness of the camel reticulum (C2) was 260 µm among 1-2 year old camels, which is consistent with the result of the current study. This comparative age-dependent histomorphometry has been attempted in camel foetuses (Bello *et al,* 2012) and for other anatomical structures other than C2 (Zhang *et al,* 2012). As observed in the present study, there appears to be a gradual and continuous age-dependent maturation process of the camel stomach that continues up to the age of 16 years. Neubauer *et al* (2018) used linear ultrasound probes to measure and assess the changes of the thickness of the histological layers of the stomach as a result of certain diseases. There are remarkable macroscopic and histologic similarities between C2 and C1 of the dromedary camel stomach. Also, C2 of the dromedary

Table 1. Thickness (µm) of the layers of the initial part of the second compartment of the camel stomach in different age groups.

Group	1	2	3	4	P-value
	(1-4 year)	(5-7 year)	(8-11 year)	(12-16 year)	
Mucosa	170.66±19.12^{a}	185.13±17.71^{a}	198.06±21.43^{a}	258.98±16.39^{b}	0.010
Submucosa	134.35±12.15^{a}	180.55±12.89^{b}	215.90±13.49^{c}	283.80±10.06^{d}	0.000
Inner circular	236.95±9.03^{a}	351.81±19.55^{b}	552.98±9.46^{c}	579.64±15.14^{c}	0.000
Outer longitudinal	628.91±12.39^{a}	701.87±13.65^{b}	1008.55±23.07^{c}	1247.95±20.21^{d}	0.000
Serosa	139.39±8.23^{a}	236.39±10.10^{b}	239.26±10.56^{b}	257.61±7.38^{b}	0.000

Values with different superscripts in a row differ significantly ($p<0.05$).

Table 2. Thickness (µm) of the layers of the final part of the second compartment of the camel stomach in different age.

Group	1	2	3	4	P-value
	(1-4 year)	(5-7 year)	(8-11 year)	(12-16 year)	
Mucosa	146.95±9.55^{a}	153.68±13.40^{a}	167.25±9.67^{a}	170.73±12.30^{a}	0.041
Submucosa	169.74±12.13^{a}	176.08±15.24^{a}	214.82±15.12^{ab}	238.70±17.30^{b}	0.006
Inner circular	426.73±29.58^{a}	458.84±29.03^{a}	577.25±31.21^{b}	595.60±22.77^{b}	0.000
Outer longitudinal	1080.67±30.48^{a}	1113.83±19.77^{ab}	1185.11±29.57^{bc}	1238.62±20.26^{c}	0.000
Serosa	191.85±7.35^{a}	246.96±7.17^{b}	204.58±11.60^{a}	309.28±10.39^{c}	0.000

Values with different superscripts in a row differ significantly ($p<0.05$).

camel stomach appears to gradually and continuously grow and mature in an age-dependent manner. The results obtained from this study can be used as a basic knowledge for the diagnosis of some diseases associated with increasing or decreasing of the thickness of the histological layers of the camel stomach.

Acknowledgements

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High MERS Seroprevalence in Some Camel Workers in UAE

Among all workers, MERS-CoV seroprevalence was especially high for certain occupations, especially camel salesmen (49%) and animal or waste transporters (22%). A new MERS-CoV seroprevalence study of camel workers in Abu Dhabi found high levels for people in certain jobs, as Saudi Arabia—the country hit hardest by the virus—reported three more cases, all in people who contracted the virus from other patients. A research team from the United Arab Emirates (UAE) and the US Centres for Disease Control and Prevention (CDC) examined blood samples of camel workers from 2014 to 2017 for the seroprevalence study. Few workers were from an open-air camel market in Abu Dhabi linked to a 2015 human case, and others were at two of the local camel slaughterhouses. Self-reported diabetes was another factor linked to being seropositive, which fits with earlier reports of underlying health conditions as a risk factor for MERS-CoV infection.

International Camel Organisation Set Up

The establishment of the International Camel Organisation (ICO), a non-profit entity to be headquartered in Riyadh was announced in Saudi Arabia. It aims to expand the base of practice, equipment and sites for camel activities all over the world, ensure fair competition and raise awareness about the harmful effects of stimulants on camels. Encouraging scientific research on camels and enriching world literature with books and translations by supporting studies, authors and translators were also listed as goals.

The organisation was established by its constituent assembly made up of representatives from 96 countries who met in Saudi Arabia at the end of the six-week long third King Abdul Aziz Camel Festival held south of Riyadh. Saudi Camel Club supremo Shaikh Fahad Bin Falah Bin Hethlen was unanimously elected to a five-year term as chairman of the body. Delegates pointed to the Riyadh Charter as the basis for the organisation's profile and activities. The charter highlights the role of camels in world history and in the cultural heritage of many countries and the importance of nurturing camels and supporting those who dedicate themselves to taking care of them.

The ICO's list of objectives includes ensuring that camels and camel-related activities nurture mutual understanding, friendship and community-building, promoting and developing the moral, scientific, technical and basic skills needed to study and nurture camels. The activities are also aimed at enhancing relations with Arab, regional, continental and international organisations, institutions, committees and associations, and non-governmental organisations specialised in camels.

The organisation will hold conferences, courses, seminars and will issue regulations aimed at enhancing safety training for all its members. It will also establish research centres and provide technical expertise as well as research grants in various fields relating to camels in order to enrich cultural and scientific knowledge in the field on a global scale. The new entity will publish magazines and newspapers and will launch TV, video and radio channels in order to contribute to the dissemination of knowledge through educational, cultural, scientific, historic, sports and media programmes.

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RECTAL PROLAPSE IN CAMELS (*Camelus dromedarius*): CLINICAL FINDINGS AND TREATMENT OUTCOMES

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ABSTRACT

The present study described the clinical presentation of rectal prolapse and evaluated the treatment outcomes in 20 dromedary camels. Factors associated with incidence and clinical findings of rectal prolapse were recorded and rectal reduction, mucosal resection and amputation were evaluated as a surgical intervention. Out of 20 studied camels, incidence of rectal prolapse in Mejhem camels (55%) was higher than in Wadeh (20%), Asfar (15%) and Ashaal camels (10%). Camels of 4-10 years of age exhibited more rectal prolapse (80%) than the other age group (0-3 years) (16 *vs* 4). Moreover, male camels showed a higher incidence (65%) (13 vs 7) of rectal prolapse compared to females (35%, n=7). Rectal prolapse occurs more due to mating (50%) than diarrhoea (25%) or chronic cough (15%) or pregnancy (10%). At 3 weeks postoperatively, the rectal prolapse was reduced in comparison with pretreated (18 *vs* 2). However, only 2 cases had recurrence of the rectal prolapse. In conclusion, our results recommend reduction, mucosal resection as well as amputation as a viable and effective treatment for rectal prolapse in camels.

Key words: Camels, clinical findings, prolapse, rectum

Rectal prolapse is a common occurrence in camel and has been categorised into 3 types; partial (mucosal or type 1), complete (full-thickness or type 2) and internal (type 3). Prolapses involving only the rectal mucosa and submucosa project through the anus, are known as type 1, complete prolapse of the full thickness of all or part of the rectum is type 2, and a variable amount of small colon intussuscepts into the rectum without protrusion out of the anus is called type 3 internal (Ramadan, 2016). It occurs due to several causes such as straining, dysuria, neuropathy, chronic coughing, pregnancy, chronic constipation, diarrhoea, back pressure during the act of covering in male breeding camels, neoplasia and genetics (Jhirwal *et al,* 2004; Fowler, 2010; Steiner, 2016; Saber, 2018). A prolapse of the mucosa can often be simply reduced, but if the prolapsed tissue becomes oedematous, desiccated and traumatised, simple reduction may be impossible; so submucosal resection or amputation of the exposed tract is necessary (Anderson and Miesner, 2008; Ramadan, 2016). Rectal prolapse has been rarely reported in camels. The present study was therefore planned to classify the clinical cases of rectal prolapse and to evaluate the efficacy of diverse surgical treatments used in dromedary camels.

Materials and Methods

Study material included total of 20 dromedary camels (13 males and 7 females) aged between 6 and 120 months (mean ± SD: 96 ± 12 months), weighing between 150 and 700 kg (450 ± 100, mean ± SD) and of different breeds (11 Mejhem, 4 Wadeh, 3 Asfar and 2 Ashaal), which were admitted to Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Qassim University, Saudi Arabia. Rectal prolapse were classified according to their types as well as, in addition to presence of vaginal prolapse. The study protocol was approved by the Ethics committee (No. 367) of animal welfare and ethics, Laboratory Animal Control Guidelines of Qassim University.

Clinical examination

Camels were clinically evaluated for subjective assessment of the rectal prolapse to record the cause, duration of prolapse occurrence, shape, colour and condition of mucosa according to prolapse type. Additionally, breed, age and sex of these camels were recorded to be evaluate, compare and analyse (Table 1).

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Table 1. Incidence of rectal prolapse in camels (N= 20).

Breed	No. of camels	Incidence (%)	Age (Months/ Year)	No. of camels	Incidence (%)	Sex	No. of camels	Incidence (%)	Duration	No. of camels	Incidence (%)
Mejhem	11	55	6 months	1	5	Male	13	65	One day	7	35
Wadeh	4	20	2Yrs	2	10	Female	7	35	2 days	10	50
Asfar	3	15	3Yrs	1	5				3 days	2	10
Ashaal	2	10	4Yrs	2	10				5 days	1	5
			5 Yrs	3	15						
			6 Yrs	2	10						
			7 Yrs	2	10						
			8 Yrs	4	20						
			9 Yrs	1	5						
			10 Yrs	2	10						
			Total	20	100						
			Group 1 0-3 Yrs	4	20						
			Group 2 4-10 Yrs	16	80						
Total	**20**	**100%**		**20**	**100%**		**20**			**20**	**100%**
Cause	No. of camels	Incidence (%)	Type	No. of camels	Incidence (%)	Interference	No. of camels	Incidence (%)	Outcome	No. of camels	Incidence (%)
Mating	10	50	Type I	5	25	Reduction and Retention	15	75	Good	18	90
Diarrhoea	5	25	Type II	15	75	Mucosal resection	3	15	reccurrence	2	10
Chronic cough	3	15	Type III	0	0	Amputation	2	10			
Pregnancy	2	10									
Total	**20**	**100%**		**20**	**100%**		**20**	**100%**		**20**	**100%**

Surgical treatment

The appropriate surgical intervention of the prolapsed rectum was performed for each case. For camels who are undergoing for surgery, routine clinical and haematological evaluations were carried out depending on extent of pathology of prolapsed part. Food and water were withheld for 12 hours before surgery. Penicillin-streptomycin (Pen & Strep, Norbrook Laboratories, UK) and flunixine meglumine (Finadyin, Schering-Plough, UK) were administered intravenously (IV), preoperatively. The camel was secured in sitting position by ropes and deeply sedated by xylazine (0.3 mg/kg intravenously). A caudal epidural analgesia using lidocaine HCl 2% was achieved. The prolapsed rectum was treated by manual reduction and retention, mucosal resection and anastomosis and amputation. Normal saline (3 litres) and Ringer's Lactate (3 litres) were administered intravenously perioperatively.

Reduction and retention of the prolapsed rectum: In this technique, rectal mucosa was viable and no laceration was found on close inspection; a proper cleaning with warm 2% potassium permanganate solution was done and bathing of the prolapsed rectum with tannic acid to reduce the oedema was performed. Manual reduction by gentle massage and retention by application of a purse-string suture pattern using silk was performed. The suture (purse string) was passed in and out of the skin around the anal orifice at a distance of 2 to 4 cm from the mucocutaneous junction. A suitable opening was left after tying the purse string to enable defaecation. The suture was left in place for 5 to 10 days.

Mucosal resection and anastomosis: When only the mucosa was damaged; two circumferential incisions were performed, one on the proximal part of the prolapsed rectum (about 2 to 4 cm from the mucocutaneous junction) and the other one on the

distal part. A longitudinal incision was connecting both incisions together and the mucosa was dissected free from the submucosa. The rectal edges were sutured back together using braided coated polyglactin 910 No. 2 (United medical industries Co. Ltd. Riyadh, Kingdom of Saudi Arabia).

Amputation: The prolapsed rectum was amputated when the rectal wall was congested, oedematous and necrosed. The rectum was pulled posteriorly and away from the anus as much as possible followed by insertion of plastic syringe casing or flexible tube (with the nozzle removed) into lumen of rectum. Vertical mattress sutures were placed about 2-4 cm from the mucocutaneous junction through healthy rectal mucosa, in order to reduce haemorrhage during amputation of the prolapsed rectum, in addition to its role as the main holding suture pattern. Interrupted sutures were then added using braided coated polyglactin 910 No. 2 around the circumference to ensure good apposition and to reduce fibrosis.

Data Analysis

Camels were categorised by age 0–3 years and 4-10 years. History of the prolapse (time elapsed from prolapse to admission) was categorised as 0–2 days, 3–5 days. Prolapse type was described according to clinical findings and was classified as Type I, Type II and Type III (Table 2).

Postoperative care and follow-up

Following surgery, the preoperative anti-inflammatory and antibiotic were continued for 5 and 10 successive days, respectively, in addition to intramuscular (IM) injection of 10 ml vitamin AD_3E (ADVIT-DE, Morvel Laboratories P. Ltd.). The camel was confined in a stall rest for 3 weeks and monitored daily for healing progress. Camels were discharged approximately 3 weeks postoperatively. To evaluate the long-term results (6 months) of rectal prolapse repair, telephonic conversations were carried out. The owners were asked about recurrence of the prolapse, discharge from the surgical site and final functional results of the surgery.

Results and Discussion

Clinical findings

Out of 20 studied camels, rectal prolapse incidence was higher in Mejhem (brown- to black-coloured camels, n = 11; 55%), than Wadeh (white-coloured camels, n = 4; 20%), Asfar (light brown-coloured camels, n = 3; 15%) and Ashaal (reddish

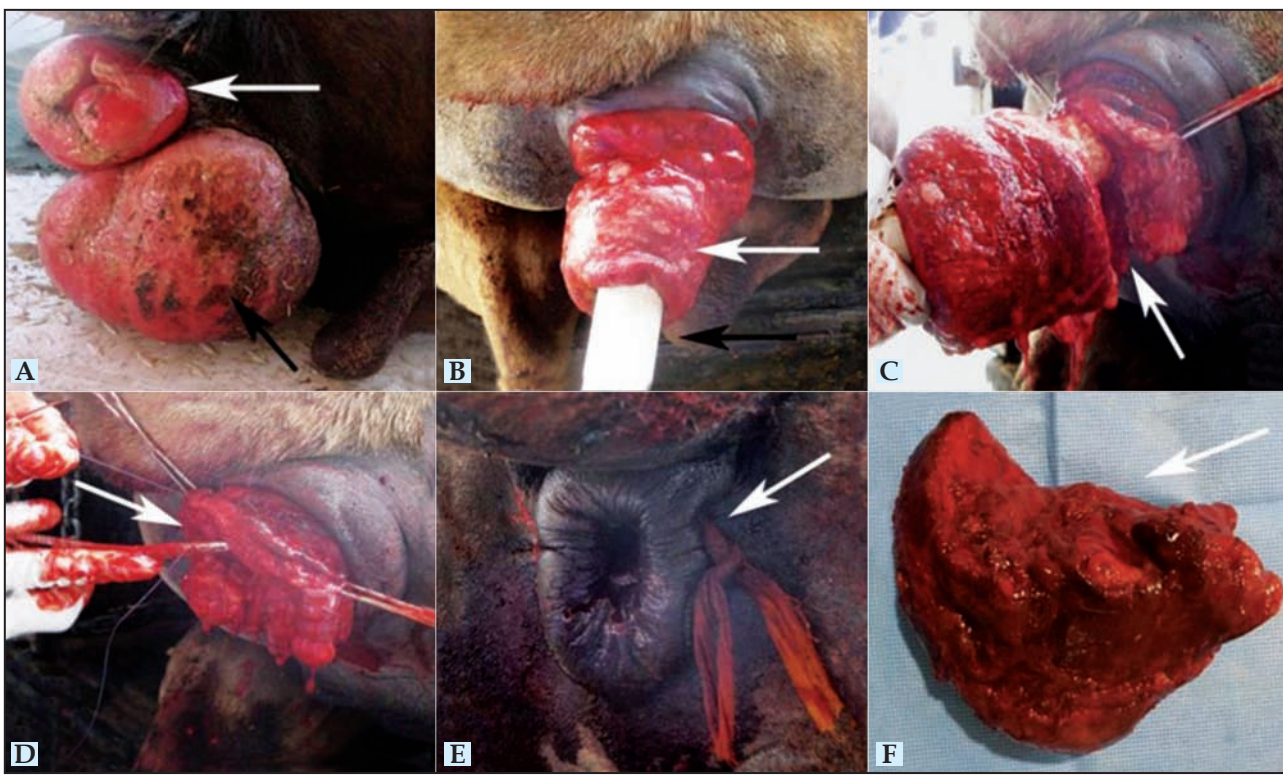

Fig 1. Rectal (Type II) (white arrow) and vaginal prolapse (black arrow) in 9 years she camel (A). Amputation of damaged and necrosed prolapsed rectum (white arrows) using flexible tube (black arrow) in 10 years male camel (B, C and D). Post operative image of prolapsed rectum retention after amputation (E) (arrow). Resected prolapsed rectum (F) (arrow).

Table 2. Clinical finding and surgical interference of rectal prolapse (N=20) in camel.

Case No.	Breed	Age	Sex	Duration	Cause	Type	State of prolapsed rectum	Interference	Outcome
1.	Mejhem	5 Yrs	Male	2 Days	Mating	Type I	Healthy mucosa	Reduction and Retention	Good
2.	Wadeh	6 M	Female	2 Days	Chronic cough	Type I	Healthy mucosa	Reduction and Retention	Good
3.	Asfar	10 Yrs	Male	3 Days	Mating	Type II	Damaged and necrosed	Amputation	Good
4.	Ashaal	4 Yrs	Male	3 Days	Mating	Type II	Unhealthy mucosa	Mucosal resection	Recurrence
5.	Mejhem	8 Yrs	Female	5 Days	Chronic cough	Type II	Damaged and necrosed	Amputation	Recurrence
6.	Mejhem	6 Yrs	Male	2 Days	Mating	Type II	Healthy mucosa	Reduction and Retention	Good
7.	Mejhem	2 Yrs	Female	One day	Diarrhoea	Type II	Healthy mucosa	Reduction and Retention	Good
8.	Wadeh	8 Yrs	Male	2 Days	Mating	Type II	Unhealthy mucosa	Mucosal resection	Good
9.	Mejhem	6 Yrs	Female	2 Days	Pregnancy	Type II	Healthy mucosa	Reduction and Retention	Good
10.	Asfar	4 Yrs	Male	One Day	Mating	Type I	Healthy mucosa)	Reduction and Retention	Good
11.	Mejhem	7 Yrs	Male	One Day	Mating	Type II	Healthy mucosa	Reduction and Retention	Good
12.	Mejhem	2 Yrs	Female	2 Days	Diarrhoea	Type I	Unhealthy mucosa	Mucosal resection	Good
13.	Mejhem	8 Yrs	Male	One Day	Chronic cough	Type II	Healthy mucosa	Reduction and Retention	Good
14.	Mejhem	5 Yrs	Male	2 Days	Mating	Type II	Healthy mucosa	Reduction and Retention	Good
15.	Mejhem	8 Yrs	Male	2 Days	Diarrhoea	Type II	Healthy mucosa	Reduction and Retention	Good
16.	Ashaal	10 Yrs	Male	One Day	Mating	Type II	Healthy mucosa	Reduction and Retention	Good
17.	Wadeh	3 Yrs	Female	One Day	Diarrhoea	Type II	Healthy mucosa	Reduction and Retention	Good
18.	Wadeh	5 Yrs	Male	One Day	Mating	Type II	Healthy mucosa	Reduction and Retention	Good
19.	Asfar	7 Yrs	Male	2 Days	Diarrhoea	Type II	Healthy mucosa	Reduction and Retention	Good
20.	Mejhem	9 Yrs	Female	2 Days	Pregnancy	Type I	Healthy mucosa	Reduction and Retention	Good

brown-coloured camels, n = 2; 10%). Camels 4-10 years of age exhibited more rectal prolapse than other age group (16 vs 4). In addition, male camels showed higher incidence (13 *vs* 7) (65%) of rectal prolapse compared to female camels (35%) (Table 1).

According to the cause, rectal prolapse due to mating were more than other causes such as diarrhoea, chronic cough as well as pregnancy (10/20 *vs*, 5/20, 3/20, 2/20, respectively). Regarding to the type of the prolapse; type II was more in occurrence than type I (15/20 *vs* 5/20) while type III was not recorded in the studied camels (0/20). History of the prolapsed rectum of 1-2 days had higher incidence than 3-5 days (17/20 vs, 3/20). The shape of prolapsed rectum was either rounded or cylindrical. The rectal wall was light red in colour, smooth and soft in recent cases and dark red with blackish areas, necrosed and indurated in old cases. Rectal prolapse was accompanied by vaginal prolapse in 2 Mejhem pregnant female camels (Fig 1A). Furthermore, manual reduction and retention by purse string sutures was performed in 15 camels, while mucosal resection and amputation were performed in 3 and 2 camels, respectively (Fig 1B – 1F).

Early diagnosed and treated cases of rectal prolapse resulted in rapid recovery, where as 18 cases investigated early were recovered rapidly. However, in 2 cases; one of them with duration of 3 days and another one admitted after 5 days were treated surgically with amputation of prolapsed part.

The treatment outcome of rectal prolapse was affected by cause, type and time elapsed after prolapse. Rectal prolapse following mating recovered easily and rapidly than other causes. Camels with rectal prolapse caused by chronic cough (n = 3) as well as diarrhoea (n=5) required medical therapies according to the cause and state of each case, in addition to surgical interference of the prolapsed rectum. There was an association between the time elapsed after prolapse and the health state of the prolapsed rectum; early

diagnoses and treatment as much as possible as well as thorough cleaning and post operative care revealed good treatment outcomes.

Treatment outcomes

At 3 weeks postoperatively, the rectal prolapse was significantly reduced. However, only 2 cases had recurrence of the prolapse out of 20 camels. By the 6th month post treatment, all treated camels recovered.

Rectal prolapse is a common occurrence in dromedary camels (Ramadan *et al,* 1986; Al-Juboori and Bake, 2011; Gahlot, 2000; Ramadan, 2016). However, in present study, 20 camels of both sexes with different types of rectal prolapse were recorded.

There are several causes of rectal mucosa prolapse in camels such as straining, dysuria due to (urolithiasis, dystocia and neoplasia), neuropathy (as a complication of spinal abscess), chronic coughing (as a complication of respiratory diseases), diarrhoea, chronic constipation and pregnancy. In the present study, rectal prolapse following mating was more common than other causes such as diarrhoea, chronic coughing and pregnancy; this could be attributed to increase of the intra-abdominal pressure results from heavy weight and position of the male camels during coitus. Similar findings were reported by Gahlot (2000), Jhirwal *et al* (2004), Fowler (2010), Steiner (2016) and Saber (2018).

Breed difference in the occurrence of rectal prolapse in camels was evident in our study and the highest incidence was observed in Mejhem camels than other studied breeds. This may be due to the presence of high number of the Mejhem breed among other camel breeds in Saudi Arabia in relation to its productive and reproductive values (Zabady *et al,* 2011). Gender had an effect on the incidence. Result of the present study demonstrated that male camels had higher incidence (65%, n=13) of rectal prolapse compared to females (35%, n=7) which could be attributed to association of prolapse with mating. This finding was similar to those observed by Zabady *et al,* (2011) and Saber (2018).

Rectal prolapse has been categorised into 3 types (Gahlot, 2000; Zabady *et al,* 2011; Ramadan, 2016). Only type I and type II were diagnosed in the 20 studied camels. The prolapsed rectum was appeared light red in colour, smooth and soft in recent cases and dark red with blackish areas, necrosed and indurated in old cases. In this study, camels 4-10 years of age exhibited more rectal prolapse than other age group (80%, n=16). This result is consistent with the data reported in the previous studies (Zabady *et al,* 2011).

Our results revealed that early diagnosed and treated cases of rectal prolapse resulted in rapid recovery and good treatment outcomes. Eighteen cases were investigated early and treated without need for amputation of the prolapsed part. However, two cases underwent amputation of prolapsed necrosed part with a duration of 3 and 5 days. This finding was in accordance with Gahlot (2000). It is construed that state of the prolapsed rectum and time elapsed after prolapse up to the presentation to the clinic used in this study became a basis for selecting a surgical procedure to treat diverse rectal prolapses in camels.

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SAUDI UNIVERSITY DISCOVERS GENETIC CHARACTERISTICS OF ARABIAN CAMELS

Researchers from the Camel Research Centre at King Faisal University, in cooperation with the University of Nottingham, have discovered the most important genes responsible for the mechanism for determining the colours of 10 kinds of Arabian camels. Results were published in an international British scientific journal, and reflected the history of the camels biological and genetic diversity in Arabia. The study reveals Arabian camels' mechanism of transmission of genetic traits, and will play a major role in the genetic classification of camels in the future. (Courtesy: Arab News)

WORLD'S FIRST CLONED FEMALE CAMEL CELEBRATES 10th BIRTHDAY IN DUBAI

Injaz, the world's first cloned female camel was created from the cells harvested from the ovary of an adult she-camel from an abattoir in Abu Dhabi, celebrated her 10th birthday in Dubai on Monday. Injaz, meaning achievement in Arabic, came to life at the Reproductive Biotechnology Centre in Dubai on April 8, 2009, following five years of dedicated research and development in in-vitro fertilisation (IVF) and somatic cell nuclear transfer technology (cloning).

Dr Nisar Ahmad Wani, scientific director of the centre said that Injaz is expecting her third calf and was our first successful cloned camel. She is completely healthy and has borne two calves so far and is pregnant again in a normal biological way of mating and conceiving.

Injaz paved the way for a well-structured and highly satisfying cloning programme at the centre, other than the normal embryo transfer and IVF methods. Other than cloning the centre also follows other *In vitro* fertilisation techniques such as Multiple Ovulation and Embryo transfer into surrogate wombs and the Intra Cytoplasmic Sperm Injection method.

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WHOLE BLOOD STIMULATION WITH LIPOPOLYSACCHARIDE MODULATES PHENOTYPE AND FUNCTION OF DROMEDARY CAMEL NEUTROPHILS

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ABSTRACT

Neutrophils play a key role in innate immunity mainly by phagocytosis and subsequent killing of bacteria. Different factors including pathogen-associated molecular patterns have modulating effects on the phenotype and function of neutrophils. The objective of the current study was to evaluate the impact of whole blood stimulation with bacterial lipopolysaccharide (LPS), the main molecular pattern associated with gram-negative bacteria, on phenotype and function of neutrophils in dromedary camel. Neutrophil shape change, the expression of adhesion molecules, phagocytosis and production of reactive oxygen species (ROS) were analysed by flow cytometry. In LPS-stimulated blood, neutrophils changed their FSC and SSC characteristics and showed modulated expression pattern of different cell adhesion molecules. In addition, LPS stimulation reduced the percentage of phagocytosis positive neutrophils as well as the number of bacteria phagocytosed by each neutrophil. However, neither the ROS production activity of unstimulated neutrophils nor the bacteria-induced ROS production were affected by LPS stimulation. Together, these results imply that phenotype and function of camel blood neutrophils are modulated by LPS stimulation.

Key words: Adhesion molecules, camel, innate immunity, lipopolysaccharide, neutrophils, phagocytosis, ROS

Polymorphonuclear neutrophils (PMNs) are innate immune cells with key role in host protection from bacterial infections (Hussen *et al,* 2016; Adrover *et al,* 2019). Activation of neutrophils and the upregulation of their adhesion molecules are key events in initiating subsequent adhesion to vascular endothelium and extravasation into inflammed tissue (Soehnlein and Lindbom, 2010; Amulic *et al,* 2012; Eger *et al,* 2016; Hussen *et al,* 2016). L -selectin, which is constitutively expressed on non-activated leukocytes, is responsible for leukocyte rolling and margination and is rapidly shed on chemotactic stimulation (Amulic *et al,* 2012). Subsequent firm adhesion to activated endothelium is mediated by the upregulation of the β2-integrin complex, particularly CD11b/CD18 (Mac-1). Thus downregulation (loss) of L -selectin and upregulation of CD11b are indicators of neutrophil activation and likely vascular transmigration (Ilton *et al,* 1999).

Neutrophil's anti-microbial activity is mainly mediated by phagocytosing bacteria and killing the ingested microbes by means of oxygen-dependent and independent mechanisms (Mantovani *et al,* 2011). Neutrophils phagocytosis activity and their capacity to produce reactive oxygen species (ROS) have been shown to be influenced by different cytokines, chemokines and pathogen-associated molecular patterns (PAMPs).

Lipopolysaccharide, an outer membrane component of gram-negative bacteria, is a potent activator of the innate immune system. The impact of LPS stimulation on phenotype and function of camel neutrophils has not been studied yet. The objective of the current study was to examine the effect of whole blood stimulation with LPS on phenotype and function of camel neutrophils *in vitro*.

Materials and Methods

Blood sampling

Blood samples were collected from seven apparently healthy dromedary camels (*Camelus dromedarius*) aged between 6 and 9 years at Camel Research Centre, King Faisal University, Al-Ahsa, Saudi Arabia. Blood was obtained by venipuncture of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany).

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LPS whole blood stimulation

Whole blood stimulation was performed according to an earlier method (Hussen *et al,* 2013). Blood from healthy camels was stimulated with Lipopolysaccharide (LPS; 1 µg/ml; Sigma-Aldrich, Germany) from *E. coli* (37°C, 5% CO_2) or left without stimulation. After incubation for 4 h blood samples were diluted with phosphate buffer saline (1:1) and centrifuged at 4°C for 10 min at 1000xg. After removing the supernatant, the cell pellet was resuspended in PBS.

Leukocytes separation

Separation of whole camel leukocytes was done after hypotonic lysis of blood erythrocytes (Hussen *et al,* 2017). Briefly, blood was suspended in distilled water for 20 sec and double concentrated PBS was added to restore tonicity. This was repeated (usually twice) until complete erythrolysis. Separated cells were finally suspended in MIF buffer (PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)) at 5 x 10^6 cells/ml. The mean viability of separated cells was evaluated by dye exclusion (propidium iodide; 2 µg/ml, Calbiochem, Germany) and it was above 95%.

Flow cytometric analysis of adhesion molecules on camel neutrophils

Adhesion molecules expression was analysed using membrane immunofluorescence test (Eger *et al,* 2015). Separated camel blood leukocytes (4 x 10^5) were incubated with monoclonal antibodies (mAbs) specific for the adhesion molecules CD18, CD11a, CD11b and CD62L cross-reactive with homologous camel molecules (Hussen *et al,* 2017). After incubation (15 min; 4°C), cells were washed twice and were incubated with mouse secondary antibodies (IgG1, IgG2a; Invitrogen) labelled with different fluorochromes. After washing, cells were analysed on a Becton Dickinson FACSCalibur flow cytometer equipped with Cell Quest software (FACSCalibur; Becton Dickinson Biosciences, San Jose, California, USA). Data of at least 100 000 cells were collected and analysed with the flow cytometric software FCS Express software Version 3 (De Novo Software, Thornton, Ontario).

Generation of ROS

ROS generation was performed in 96-well round-bottom microtitre plates (Corning, NY, USA) (Hussen *et al,* 2016). LPS-stimulated and non-stimulated camel leukocytes (1×10^6/well) were incubated without or with heat killed non-opsonised (50 bacteria/cell) *Staphylococcus aureus* (*S. aureus*) (Pansorbin, Calbiochem, Merck, Nottingham, UK) for 20 min (37°C, 5% CO_2). For the detection of ROS, dihydrorhodamine (DHR 123, Mobitec, Goettingen, Germany) was added to the cells (750 ng/ml final). After incubation cells were washed with MIF buffer and relative amount of generated ROS was determined by the median green fluorescence intensity of gated neutrophils after acquisition of 100 000 events.

Phagocytosis Assay

Heat killed *S. aureus* bacteria (Pansorbin, Calbiochem, Merck, Nottingham, UK) were labelled with fluoresceinisothiocyanate (FITC, Sigma-Aldrich, St. Louis, Missouri, USA). LPS-stimulated and non-stimulated camel leukocytes were plated in 96 well plates (1×10^6/well) and incubated with FITC-conjugated *S. aureus* (50 bacteria/cell) for 30 minutes (37°C, 5% CO_2). Control samples were incubated without bacteria. After incubation, samples were analysed by flow cytometry after addition of propidium iodide (PI, 2 µg/ml final) to exclude dead cells. Phagocytic activity of neutrophils was defined as the percentage of green fluorescing cells among viable neutrophils. Mean green fluorescence intensity (MFI) of phagocytosis-positive neutrophils was measured as indicator for the number of bacteria phagocytosed by each neutrophil.

Statistical Analysis

Statistical analysis was performed with Prism (GraphPad). Results are presented as means ± S.E. of the mean (SEM). Differences between means were tested with t test (two groups) or with one-factorial analysis of variance (ANOVA) and Bonferroni's correction for normally distributed data (more than two groups). Results were considered significant at a p-value of less than 0.05.

Results and Discussion

The present study analysed the impact of whole blood stimulation with bacterial lipopolysaccharide (LPS) on shape change, expression of adhesion molecules and anti-microbial functions (phagocytosis and ROS production) of dromedary camel neutrophils. The advantage of this type of stimulation in whole blood rely in the preservation of the microenvironment of LPS and cell interaction as it occurs *in vivo* (Gomes *et al,* 2010).

In the present study, cellular activation of neutrophils was analysed by flow cytometric assessment of shape change (change in sideward scatter (SSC) and forward scatter (FSC) characteristics) (Nicholson *et al,* 2007). Neutrophils SSC and FSC were reversely affected by LPS stimulation so that

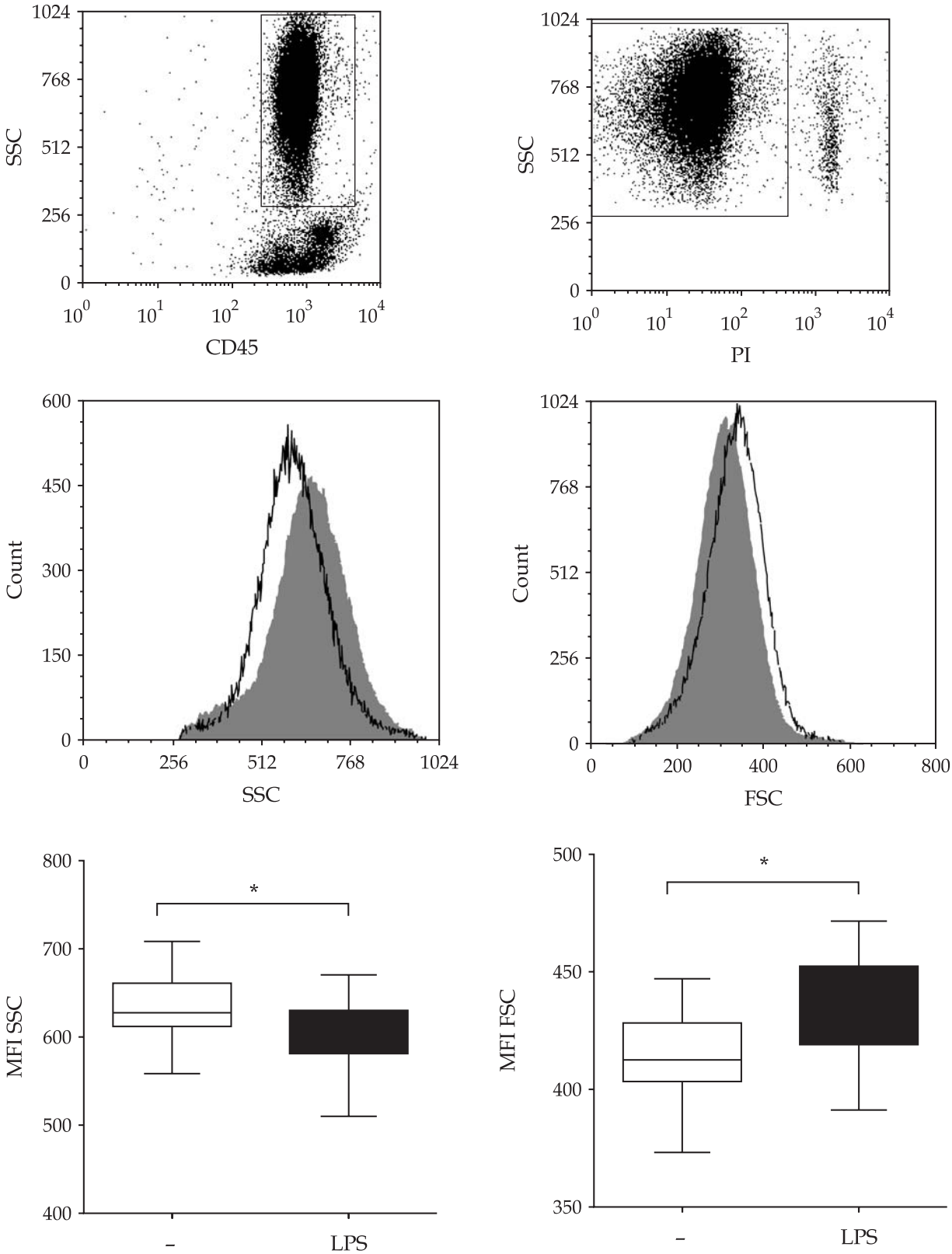

Fig 1. Gating strategy for the analysis of LPS-induced shape change in camel blood neutrophils. Camel whole blood was stimulated with LPS for 4 h. After red blood cell lysis, separated leukocytes were analysed by flow cytometry. In a SSC / CD45 dot plot, camel granulocytes were defined based on their higher side scatter characteristics and positive staining for CD45. After excluding dead cells, based on their staining with the DNA-sensitive dye propidium iodide (PI), mean side scatter (SSC) and forward scatter (FSC) values of vital neutrophils were calculated (means ± SEM). (* = $p<0.05$).

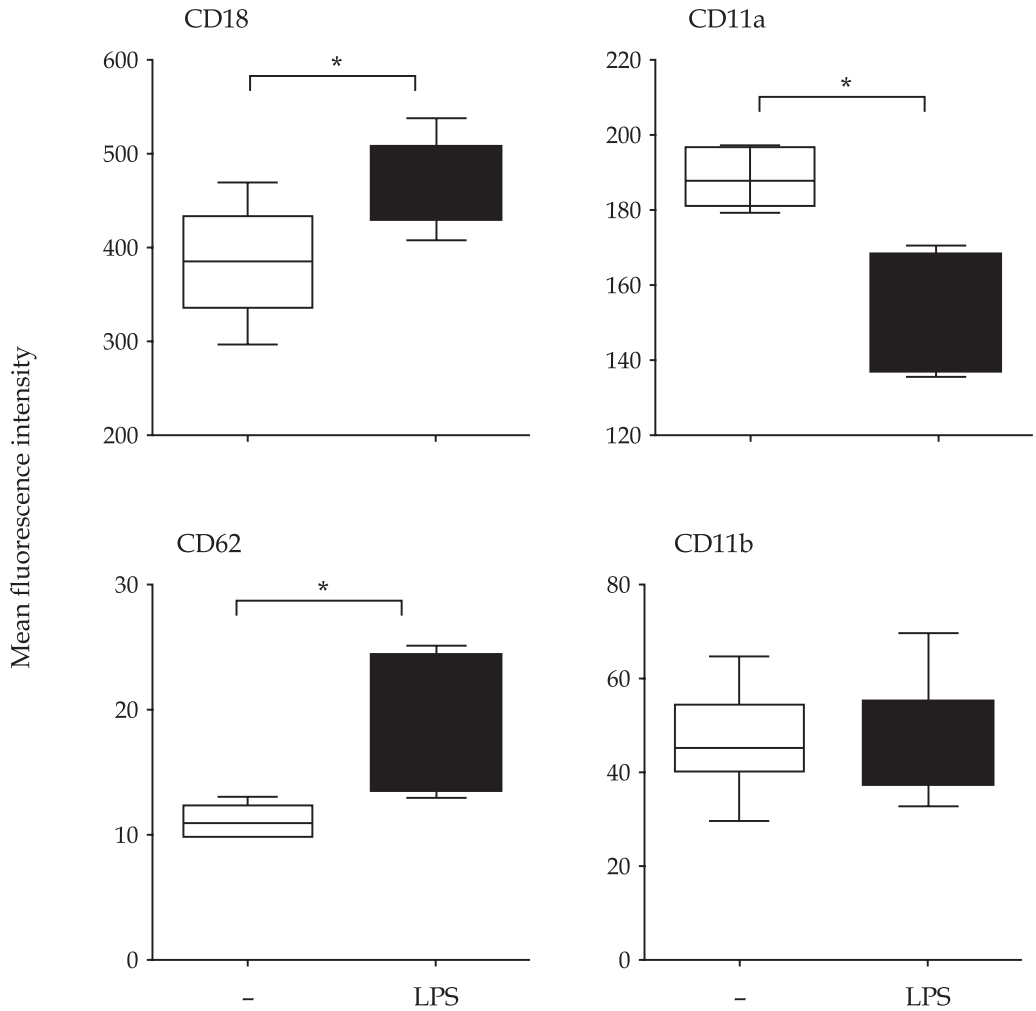

Fig 2. Influence of LPS-stimulation on neutrophil surface molecule expression. Camel whole blood was stimulated with LPS for 4 h or left without simulation. After red blood cell lysis, separated leukocytes were labeled with monoclonal antibodies to CD18, CD11a, CD11b and CD62L. Labeled cells were analysed by flow cytometry. After setting agate on vital (PI-negative) granulocytes, main fluorescence intensities of labeled cells for CD18, CD11a, CD11b and CD62L were calculated (means ± SEM). (* = $p<0.05$).

stimulated neutrophils showed increased FSC but reduced SSC in comparison to unstimulated cells. Together, the increased FSC, which is indicative for increased neutrophil cell size and the reduced SSC, which is indicative for cell degranulation indicates either a direct effect of LPS on neutrophils or an indirect effect of mediators released by other activated blood cells, like monocytes (Fig 1).

Neutrophils are the first cells that migrate to the site of infection to eliminate microorganisms (Amulic *et al,* 2012). The adhesion of neutrophils to endothelial cells and their subsequent extravasation depends on the expression of different cell surface adhesion molecules (Kolaczkowska and Kubes, 2013). Neutrophils express constitutively high levels of the integrins LFA1 (also known as α1β2; β2 integrin; CD11a/ CD18) and MAC1 (also known as αMβ2; CD11b/CD18), which mediate different phases of neutrophil adhesion and extravasation through binding to common endothelial cell surface molecules, such as ICAM1 and ICAM2 (Amulic *et al,* 2012). In the current study, LPS stimulation induced the upregulation of CD62L (L-selectin) and CD18 but downregulation of CD11a. No change in neutrophil CD11b expression was observed after LPS stimulation. In the bovine system, intramammary infusion of LPS induced the downregulation of surface CD62L with increased expression of CD11b and CD18 (Diez-Fraile *et al,* 2003). In the current study, although LPS stimulation increased the expression of CD18, the upregulated CD62L expression together with downregulated CD11a and unchanged CD11b expression argues against an adhesion stimulating effect of LPS on camel neutrophils (Fig 2).

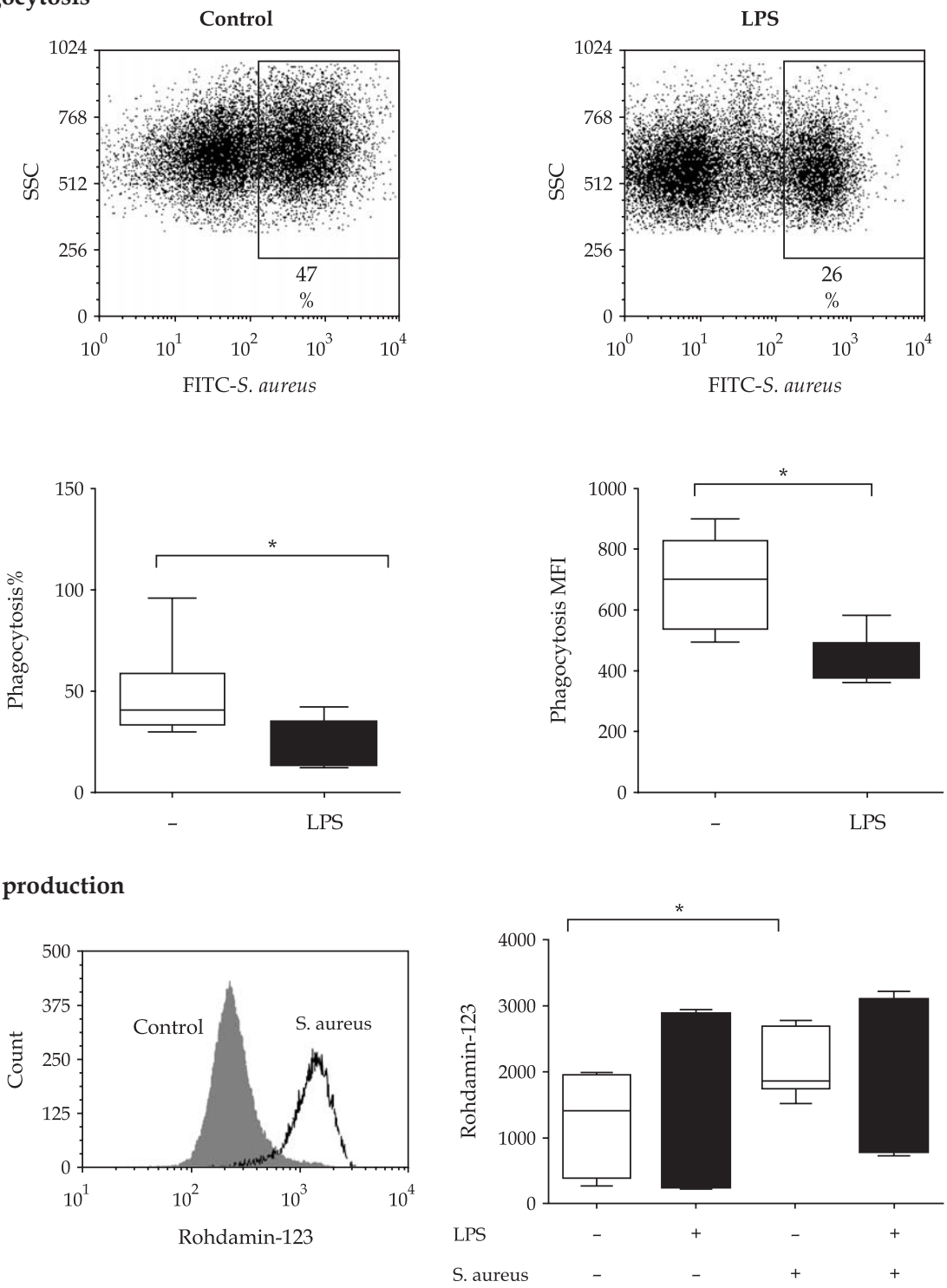

Fig 3. Influence of LPS stimulation on phagocytosis and ROS activity of camel neutrophils. Camel whole blood was stimulated with LPS for 4 h or were left without stimulation (control). A) After red blood cell lysis, LPS-stimulated and non-stimulated leukocytes were incubated with FITC-labeled heat inactivated *S. aureus* and analysed by flow cytometry. After setting agate on vital granulocytes, phagocytosis-positive cells were defined based on their higher green fluorescence (representative results are shown in A). The percentage of phagocytosis and the mean fluorescence intensities of green fluorescence-positive neutrophils were calculated (means ±SEM). (* = $p<0.05$). B) LPS-stimulated and non-stimulated leukocytes were incubated without (control) or with heat inactivated *S. aureus* in the presence of the ROS-sensitive dye dihydrorhodamin 123 and labeled cells were analysed by flow cytometry. After setting a gate on vital granulocytes, ROS production was calculated as the mean green fluorescence intensity of gated cells (means ± SEM). (* = $p<0.05$).

Phagocytosis and production of reactive oxygen species (ROS) are key antimicrobial mechanisms used by neutrophils to ingest and kill bacteria (Soehnlein and Lindbom, 2010). To see whether LPS stimulation may affect antimicrobial functions of camel neutrophils, the capacity of neutrophils to phagocytose *S. aureus* and to produce ROS upon stimulation with *S. aureus* were analysed by flow cytometry. LPS-stimulated neutrophils showed lower percentages of phagocytosis positive cells as well as reduced mean fluorescence intensity (MIF) of phagocytosis positive cells as indicator of number of bacteria ingested by each neutrophil (Fig 3). Only incubation of non-stimulated neutrophils with *S. aureus* enhanced their ROS level as measured by the MFI of the ROS-sensitive dye dihydrorhodamine 123. In contrast to this ROS stimulating effect of *S. aureus*, LPS stimulation did not induce any significant change in neutrophils ROS level. In addition, LPS stimulation did not modulate the ROS response in *S. aureus*-stimulated neutrophils (Fig 3). The reduced phagocytosis capacity and the unchanged ROS production in LPS-stimulated neutrophils indicate a negative effect of LPS on the anti-microbial functions of camel neutrophils.

In summary, the current study shows that whole blood stimulation with LPS modulates the shape, adhesion molecules expression pattern and antimicrobial functions of dromedary camel neutrophils. Further studies are needed for the analysis of molecular mechanisms mediating these effects of LPS on camel neutrophils.

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CONCENTRATIONS IN D- AND L-LACTATE IN RAW COW AND CAMEL MILK

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ABSTRACT

The present study gives some preliminary result regarding the content of the D- and L-lactate in cow and camel milk. Twenty dromedary camel milk samples and 3 cow milk samples from Kazakhstan were analysed to determine the lactate forms in order to compare these two types of milk collected in similar conditions. The content of total lactate in camel milk was comparable to cow milk (1.82 – 2.49 g/l), but the quantity of L-Lactate was 100 times more in camel milk compared to cow milk - 2.21% of the total lactate vs 0.02% in camel and cow milk, respectively. Further analyses are necessary to understand the role of the microflora present in each specific milk.

Key words: Camel milk, cow milk, lactate, stereoisomer

Camel milk contains on average the same quantity of lactose than cow milk (Al-Haj and Kanhal, 2010), *i.e.* 4.46 ±1.03 g/100ml according to the compilation of published references on camel milk composition (Konuspayeva *et al*, 2009) whereas it was 4.5 to 5.0 g/100ml in cow milk (Gaucheron, 2011). Yet, camel milk is reputed to have less effect on lactose intolerance than cow milk, and seems more easily metabolised by consumers, even those having no lactase (Cardoso *et al*, 2010). An explanation advanced by these authors is that camel milk produces less casomorphine, which provokes less intestinal motility; this would cause lactose to be more exposed to the action of lactase, the specific enzyme able to metabolise lactose into intestine gut.

Elsewhere, lactate is the product of fermentation of lactose in the digestive tract of milk consumers. The lactate has beneficial effect on health for the regulation of the milk protein digestion and mineral absorption like calcium, phosphorous and iron. Lactate is naturally present in fermented dairy product as yoghourt or fermented beverages like *koumiss* (fermented mare's milk) or *shubat* (fermented camel's milk).

Two stereoisomers of lactate are described according to the position of hydroxyl radical on the molecule: D-Lactate (for dextrogyre) and L-Lactate (for levogyre). Usually, D-lactate is regarded as having negative effects on the health of the new-born up to one year old because his metabolism is not yet mature. The enzyme responsible of the degradation of D-Lactate is lacking in new-born and the consecutive accumulation of lactate could provoke acidosis (Racinet *et al*, 2013).

In Kazakhstan, the consumption of fermented dairy products from non-conventional species is popular (Konuspayeva and Faye, 2011). So, it could be useful to compare the proportion of D-lactate and L-lactate in cow and camel milk. No data is available on the literature. Some preliminary results are presented in this short communication.

Materials and Methods

Animals

Twenty dromedary camels from the private camel farm «Daulet-Beket», Almaty region, were involved for collecting milk samples after full milking of each animal. In order to compare with cow milk, 3 cows from small private backyards of Abay village, Karasay district, Almaty region were sampled. All milk samples were transported in cooling box immediately to the laboratory ANTIGEN Co. (Abay, Kazakhstan) for analysis and achieved in less than one hour after milking for the two species.

Analytical methods used

The gross composition of milk involving total proteins, solid not fat (SNF), fat, and density was determined by using ultrasonic milk analyser (Eon Trading, Bulgaria) directly after sampling.

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Titratable acidity was determined by titration of 10 mL sample with 0.1N NaOH using phenolphthalein as an indicator. It was expressed in Turner degree. The titratable acidity expressed the total lactate. The Turner value was multiplied by the coefficient 90 (molecular weight of lactic acid) to express the lactate concentration in mg/l.

L-lactate content was determined by enzymatic Bioanalysis kit, R-Biofarm AG, Roche, Germany. The principle of the determination was as follows: L-lactate was oxidised to pyruvate by NAD (nicotinamide-adenine dinucleotide) in the presence of L-lactate dehydrogenase (L-LDH). The equilibrium of the reaction lied on the side of L-lactate. Pyruvate was trapped in a subsequent reaction catalysed by GPT (glutamate-pyruvate transaminase) in the presence of L-glutamate, leading to the displacement of the equilibrium in favour of pyruvate and NADH. The amount of NADH formed is stoichiometric to the amount of L-lactate. The increase in NADH was determined by light absorbance at 365 nm. D-lactate concentration was calculated by difference.

All the analyses of milk composition were performed in triplicate and the mean was retained in the final statistical analysis.

Statistical analysis

The proportion of D and L-Lactate between camel and cow milk we compared by applying the non-parametric test of Mann-Whitney (Lehmann, 1975). The software used for analysis was XLSTAT (Addinsoft ©).

Results and Discussion

The content of total lactate in camel milk was comparable to cow milk (1.82 – 2.49 g/l), but the quantity of L-Lactate was 100 times more in camel milk or compared to cow milk : 2.21% of the total lactate vs 0.02% in camel and cow milk, respectively.

The presence of lactate in raw milk is linked to the natural microflora responsible for the metabolisation of lactose during the acidification process by lactic bacteria. The microflora in camel milk is complex and partly unknown. In a study achieved in raw and fermented camel milk from Kazakhstan, 138 strains of microflora were isolated and among them only 37 lactic acid strains and 12 yeasts strains were identified (Akhmetsadykova *et al*, 2015). The highest proportion of L-lactate in camel milk could be explained by some unidentified specific strains present naturally just after collecting. Normally, lactate is lacking in sterilised milk and appeared with the acidification process, then increased in fermented dairy products. The L-lactate was predominant in fermented products, but D-lactate was found generally in yogurt where its proportion could reach 46% of the lactic acid (Alm, 1982). Some lactic bacteria like *Lactobacillus acidophilus* (not identified in natural camel milk) for example produced mainly D-lactate (Mullan, 2001). The presence of L-lactate being linked to the acidification process, it could be used as indicator of the milk quality (Kumar *et al*, 2016). However, in our study, the conditions of sampling, storage and the delay before analysis were identical for the two types of milk and could not explain totally the high difference between camel and cow milk. The presence of pathogenic bacteria as *Staphylococcus aureus* in milk conducted to an impressive D-lactate increase, but such increase appeared 4 hours after inoculation of the bacteria in sterilised milk (Marrazza *et al*, 1994). Thus, our analyses being achieved within one hour after sampling, it was unlikely that the difference between the two types of milk samples could be linked to an eventual contamination by pathogen bacteria. Moreover, the total quantity of lactate was similar in the two types of milk, only the ratio D/L-lactate was different.

In human gut, D-lactate is metabolised into pyruvate by D-hydroxy- acid-dehydrogenase (EC 1.1.99.6), but the efficiency of this metabolisation is low, one fifth the rate of L-lactate by L-lactate dehydrogenase (Ewaschuk *et al*, 2005). In consequence, the risk of acidosis by accumulation of D-lactate in bloodstream increased considerably with D form both in human and ruminants (Zhang *et al*, 2003). Thus, D-lactate was in higher concentrations in rumen and faeces of diarrhoeic calves, contrary to L-lactate (Ewaschuk *et al*, 2004). Moreover, according to some authors, the patients affected by autism could be more sensitive to an excess of D-lactate in the diet because its effect on mitochondrial function. The prevalence of mitochondrial disorders increases in cases of autism (Rossignol and Frye, 2012). Elsewhere, the links between alleviation of autism's symptoms and camel milk consumption are discussed but need substantiation through more clinical trials (Shabo and Yagil, 2005). The question of the role of L-lactate in higher quantity in camel milk could be investigated to explain partly this beneficial effect observed empirically.

Conclusion

The raw camel milk appeared to contain 100 times more L-lactate than raw cow milk in similar

sampling conditions. The reasons of such difference could not be explained with the present study. However, it encourages further studies by including the assessment of hygienic status of the milk and the monitoring of the ratio D/L-lactate throughout the fermentation process with or without identified starters.

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A NEW UNDERSTANDING OF BACTRIAN CAMEL CARAVAN CULTURE IN STAMPS

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ABSTRACT

The Bactrian camel and the ancient Silk road formed a special camel caravan culture that played a vital role in the development of human civilisation. The stamp has a wide range of international propaganda values and records a period of history, geography, politics, economy, natural humanities and art in the country. This study systematically collated and examined the design content, design style, artistic value, cultural connotation and themes of Bactrian camel caravan stamps. Camel caravan stamps have immeasurable status and value in art design, team spirit, global economic prosperity, collection and cultural inheritance.

Key words: Bactrian camel, caravan culture, collection, inheritance

Overview of stamps

Postage stamps are a sanctioned product of official government agencies (Li, 2002). The earliest stamp was the "Penny Black" issued by the British in May 1840 (Wang, 2013) and since then stamps began to circulate worldwide. The stamp has a wide range of international propaganda values. It records a period of history, geography, politics, economy, nature, humanities and art in a country (Yang, 2007; Cai *et al,* 2008).

Overview of Bactrian camel caravan

According to fossil data from palaeontologists, about two million years ago, Bactrian camels migrated from North America's Bering Strait into Eurasia, where they distributed widely (Jirimutu *et al,* 2009). Bactrian camels belong to the camel family, including domestic and wild species, but there is no direct relationship between them; they have different maternal origins (Ming *et al,* 2017).

Bactrian camels are a transportation method for nomadic people in central Asia and are also an important source of livestock trade between neighbouring countries (Gadari and Dachagan, 2012). The Bactrian camel is resistant to hunger and thirst, making it suitable for long-distance transport (Zhong, 1982).

According to historical records, as early as 2000 years ago, on the "Silk Road" of ancient China, people used bactrian camels to cross dangerous areas, such as the Taklimakan Desert, the Tianshan Mountains and the Pamir Plateau to transport goods over long distances (Peter, 2016).

Long-distance camel caravan were used to transport fur, silk, candy, salt, ceramics and tea, generally for several months to complete a round trip. A mid-range camel caravan usually used to takes thirty days to travel between regions. The structure of the mid and long distance camel caravan was very strict, was organised by camel merchants and comprised a team of about 10 people. The person responsible for the camel was called a Camel Man. Each man led 12–16 camels and was not allowed to ride the camels, only to walk with them. Short-distance transportation took two or three days, mainly to exchange goods between neighbouring area. The camel caravan's work schedule was strict and regular. It usually started at 12 noon, stopped at 1 or 2 o'clock at night, grazed the camels during the day and traveled at night, with a daily trip of about thirty or forty kilometres (Gadari and Dachagan, 2012; Zhong, 1991).

Collection of bactrian camel caravan stamps

Tannu Tuva issued the first bactrian camel stamp in September 1927 (Fig 1 A), which was also the first camel caravan stamp in the world and since then the bactrian camel has occupied a place in the history of stamps (Colnect, 2018).

We searched (Colnect, 2018; China post, 2018; Mongolia post, 2018; Jingdong, 2018; Taobao, 2018; Tuva, 2018) and used Colnect, e-bay, Jingdong and Taobao to collect 199 bactrian camel stamps released

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A. Tannu Tuva (1927). B. Tannu Tuva (1934). C. Mongolia (1932).

D. Mongolia (1943). E. Mongolia (1969). F. Mongolia (1975). G. Mongolia (2001).

Fig 1. Camel caravan as theme in stamps.

worldwide from 1927–2018, including 23 camel caravan stamps (Fig 1-3).

The cultural connotations of camel caravan stamps

A. Aesthetic connotation

The camel caravan stamps have three types: The camel caravan as the theme (Fig 1), as the background (Fig 2) and as a decoration (Fig 3).

The first camel caravan stamp collected, called bactrian Camel Caravan, was released by Tannu Tuva in September 1927 (Fig 1, A). The nominal value of this stamp was 14 Russian Kopeks. The national name "Tuva" was marked in Uighur Mongolian and English. The edge shows Mongolian traditional patterns, the main pattern is a camel man walking with the camel caravan in a boundless desert, with steep mountains. It depicts the camel caravan linking the East-West trade with the ringing of the camel bell and blood and sweat expended on the vast Gobi.

Colour is the main factor that determines the visual effect of stamps (Zhong, 2013). Before 1943, camel caravan stamps were mainly made using pure classical colours (Fig 1, A–D). The stamps produced after 1969 are more colourful (Fig1, E–G; Fig2, A–E; Fig 3).

Camel caravan stamps have rectangular (Fig 1, A, B, D–G; Fig 2, Fig 3) and diamond (Fig 1, B) shapes. There are definitive stamps (Fig 1, A–D), commemorative stamps (Fig 1, E–G; Fig 2, A–E) and a souvenir sheet (Fig 3). Most camel caravan stamps have a comb on the edge; only one lacks a comb (Fig 2, C).

B. Collaborative Cultural Connotations in Camel Caravan Stamps

There are many difficulties in the process of camel caravan transportation, such as shortage of food and water, loss of goods, sickness, theft and natural disasters. Therefore, collaboration is an important prerequisite for a camel caravan to reach its destination safely (Zhong, 1991; Jibuhuleng, 2017).

Collaboration between Camel Man and camel: Camels are the soul of the camel caravan. They are resistant to hunger, thirst, heat and cold. Camels never get lost in the desert and have a very sensitive sense of smell, usually smelling water thousands of meters away and have more accurate sense of fatal desert storms (Jibuhuleng, 2017). Therefore, a Camel Man relied on the camels to complete their long-distance transportation tasks and get paid for their life. On the other hand, the camel also relied on the camel man for grazing, so that it has sufficient physical strength and health to complete its daily shipping task (Zhong, 1991; Zhang *et al,* 2008).

Collaboration between camel men: Camel men were generally made up of young and strong men. An experienced person was usually the head of the camel caravan and was mainly responsible for navigation, observing the terrain and dictating the rest time. Meanwhile, a higher prestige man was placed at the end of caravan, mainly to control the camel caravan's speed, ensuring that no goods were lost. All camel men obeyed their command and cooperated with each other. During the break, the camel men quickly unloaded the goods, set up tents, grazed the

A. Mongolia (2002). B. Korea (2015).

C. Mongolia (2001). D. China (2012).

E. China (2018).

Fig 2. Camel caravan as background in stamps.

camels and prepared the food. The fate and success of the camel caravan was everyone's responsibility. Therefore, camel tourists strictly observed the rules, extended their wisdom and labour to the camel team and formed a collaborative culture with the Camel Men.

Collaboration between camel men and nature: Camel caravan life must dealt with the hard conditions of the wild, thus mastering the laws of nature, understanding the terrain, analysed the climate and adapted to different field environments was the key to survival and success (Feng, 2007). In summer, temperatures were very high and it was hard to walk through swamps on grasslands and store food and goods. Therefore, camel caravans usually started in the autumn and end in summer

Fig 3. Camel caravan as decoration in stamps (Mongolia, 2017).

(Zhong, 1991; Jibuhuleng, 2017). In addition, camels had a strong sense of smell and a premonition of sandstorms, so experienced camel men often find water sources, judge the climate change and predicted natural damage by observing the behaviour of the camels (Zhang, 2001).

Collaboration between the camel caravan and dogs: The camel caravan had an inseparable relationship with dogs (Sven, 2017) (Fig 1, G; Fig 2 A, C). Dogs were accompanied the camel caravan, explored the way and prevented threats from wild animals.

C. Silk Road on the Camel Caravan Stamp

In 138 BC, Zhang Qian set out from the Chang'an with camel caravan and travelled westward to Rome (Zhong, 1991). Chinese silk is the most representative trade goods on this long road. At the end of the 19th century, German geologist Ferdinand Freiherr von Richthofen named this East-West Road as the "Silk Road" (Peter, 2016). The ancient Silk Road was developed by camel caravan, so where there is no camel caravan, there is no Silk Road (Zhong, 1991; Jibuhuleng, 2017). Actually, camel caravan not only caused commercial activities to flourish, but also made important contributions to the spread of human beliefs, economic exchange, cultural exchange, political change, wealth expansion and ideological openness. Of course, they could also spread of greed, war and disease (Peter, 2016).

New Understanding of Camel Caravan Stamps

A. Innovation in Stamp Design

Early camel caravan stamps used narrative traditional art forms, such as figurative painting and

realism (Xiang and Tang, 1997). The pattern is mostly based on the camel caravan. The camel man leads the long camel caravan, moving towards the distant destinations, no matter how difficult it is, they would never stop (Fig 1, A–F).

Since 2001, the pattern of camel caravan stamps has changed significantly. Fig 1-G shows a bright colour with a blue sky and white clouds, implying that the camel caravan has become a leisurely peaceful culture, not a means to make a living. In Fig 2-A, the main character is the fierce Tibetan mastiff, while the camel caravan becomes a background, showing that the camel team is as precious as the Tibetan mastiff (Li, 2007). The dreamer from Gobi Dream (Fig 2-C) is more creative, with non-realistic features and surrealist descriptions (Xiang and Tang, 1997). Fig 2-D and E showing the camel caravan as a background or decoration and the theme is cultural relics, indicating that, although the camel caravan has become an ancient story, they are as precious a legacy in human history as these relics. The brightly collared camel with the camel caravan and the ancient city in the background (Fig 2-B) suggest that bactrian camels are dazzling stars from ancient times to modern times. The theme of the mini-sheet is coins (Fig 3) and the background is map of the Silk Road, on which the camel caravan is a decoration, representing the grandeur of the silk roads.

B. The teamwork spirit of the camel caravan

The core of the camel teamwork spirit is the natural human-camel collaboration, which is a spiritual force and a belief (Zhong, 1991; Jibuhuleng, 2017).

In today's world, families, departments, countries, international alliances and even global industries have engaged with a model of teamwork. A team should not only have excellent talents, but also have a team spirit to boost morale (Wang, 2013). Teamwork spirit is the soul of a high-performing team and is also a source to maximise the potential energy of the team. This is the inheritance of the valuable spiritual wealth that the camel caravan built during difficult historical periods.

C. Revival of the Silk Road

The Silk Road was created more than 2,000 years ago and camel caravan transport is the real link in this historical feat. Today, the Silk Road is prosperous again. Along this road, new cities, railways, airports, networks, resorts, commercial centres, luxury hotels and landmarks are springing up. Institutions and organisations that regulate regional relations on this road have also been established (Xi, 2013). People living in the land that connects the East and the West, regardless of their race, beliefs and cultural backgrounds, are striving to co-exist, develop together, strengthen economic ties, road links, trade links and currency flows and are forging a modern Silk Road.

D. New value of camel stamps

Nowadays, modern transportation such as railway and motor vehicles have replaced camels and the Internet has shrunk postal services; therefore, the main functions of camel stamps have been transformed into cultural heritage and appreciation. Like other cultural relics and intangible cultural heritage, camel stamps now occupied a new position in the field of art collection. Collection is not just about money, but also more importantly, culture and the spread of culture, is the essence of collection (Li, 2002).

Conclusion

Camel caravan stamp research combines stamp culture with bactrian camel caravan culture, which not only enriches the connotations of bactrian camel caravan stamps, but also explores their significance in this new epoch.

(1) In the 21st century, camel caravan stamps have made great innovations in composition, colour, content and meaning, making them collectable again increasing their value.

(2) The spirit of teamwork in modern times is an inheritance, continuing the spiritual wealth of the ancient camel caravan.

(3) The ancient Silk Road was developed by camel caravans. When there is no camel caravan, there is no Silk Road. Today, a new Silk Road is rising along this ancient way that is driving the prosperity of the global economy.

(4) With the development of science and technology, both camel caravans and stamps have withdrawn from their important historical stage. However, we cannot ignore their contributions to human civilisation. Camel caravan stamps now occupy a new position in the arts, in collections and in cultural endeavours.

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