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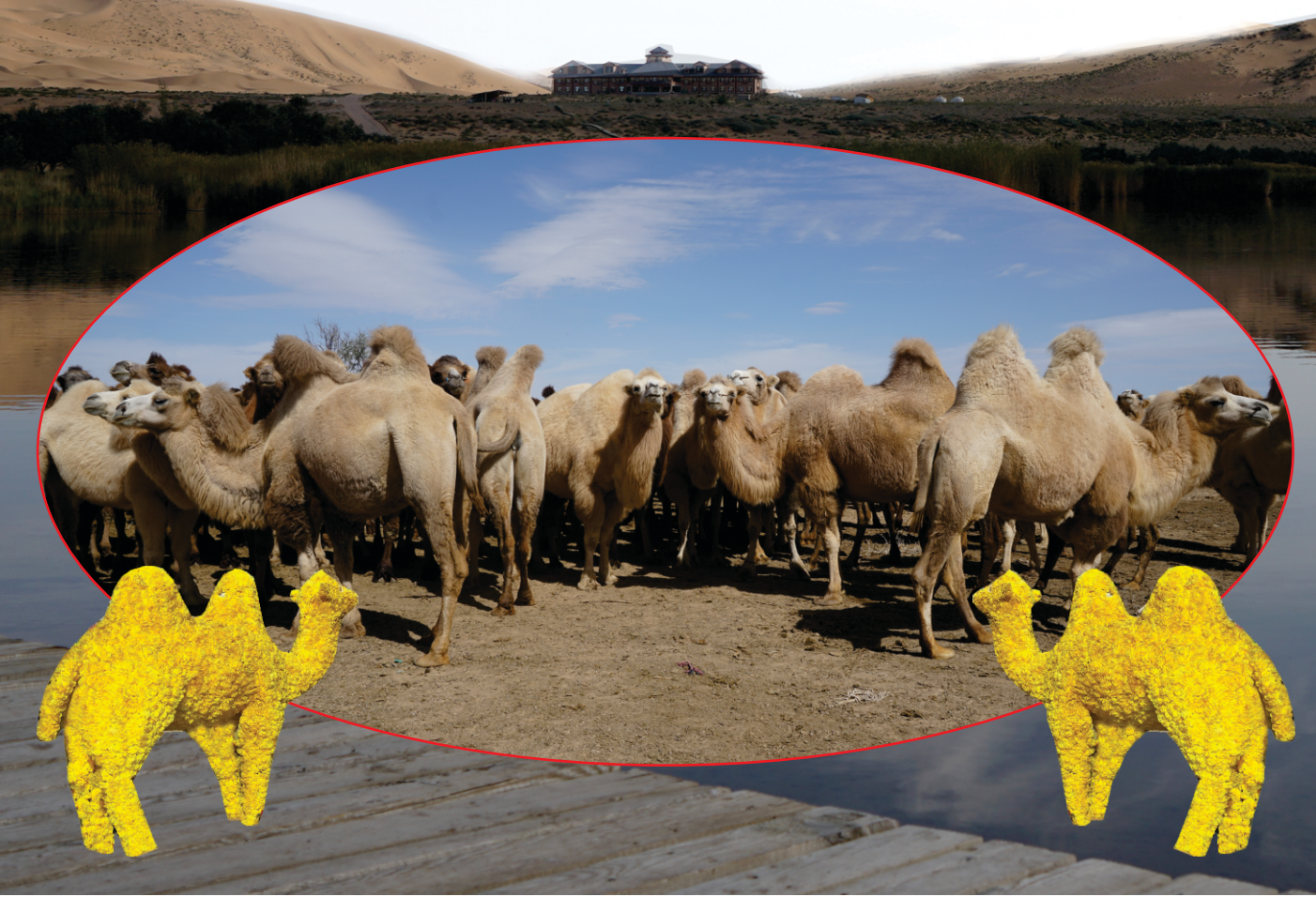

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BACTRIAN CAMEL RESEARCH MARCHING AHEAD IN CHINA

Bactrian camel research of China has not remained in main focus hitherto due to many reasons. A recently organised international conference "The Belt and Road: Camel Science, Industry and Culture" on 22-26th September 2017 at Alxa League, Inner Mongolia, China was an eye opener to scientists of other countries who were amazed to see many facets of production and research of Bactrian camels in China.

A very interesting aspect of controlling a decline in camel population in Inner Mongolia region by the Government was worth taking a note by those countries where camel population is on sharp decline, e.g. India. The measures taken by the government included formation of cooperative societies of camel herders by providing them a good platform of keeping productive camels at one place and augmenting the facilities of machine milking and reproduction (see camel city in the News section). A very nice marketing of camel products, such as milk, meat, wool or hairs with export quality finish proved Bactrian camel as a supermarket or industry, thus became a profit yielding animal to its herders. A wide range of cosmetics including soap, body lotions and creams were available for sale in the market. I noticed first time the wine produced from camel milk apart from other products including flavoured milk, sour milk, cheese etc. Establishment of Inner Mongolia Camel Research Institute was another big platform for research on Bactrian camels to the researchers and students. An exclusive museum on Bactrian camels elaborated a spectrum of these camels from history, culture and science. This conference was an important step for exchanging and sharing the camel research and production of various countries. The brain storming sessions brought lot of inputs of ongoing and future camel research. There is a greater need of translation of camelid research from Chinese to English and vice versa to benefit the researchers.

The December issue of JCPR is rich in manuscripts based on latest camel research across the globe. The topics covered are related to peste des petits ruminants virus (PPR), physocephalosis, Middle east respiratory syndrome coronavirus, genetic characterisation of camel using microsatellite markers, coccidiosis and haemonchosis, milk production, passive immunisation against *Brucella melitensis*, pharmacokinetics of cefquinome, protective effects of camel milk on inflammatory and antioxidant biomarkers, pathological disorders of the ovaries and uterine tubes, uterus, cervix and vagina, mastitis, antioxidant enriched flavoured camel milk, multidrug resistance pattern of *Escherichia coli* isolates, influence of bokhi on kidney-yang-deficiency syndrome in rats, amputation of fore limb and seroprevalence of bluetongue in dromedaries.

I am happy to note that JCPR has increased the output of publishing manuscripts because it has become triannual from 2017.

Wishing you all a happy new year 2018.

(Dr. T.K. Gahlot)
Editor

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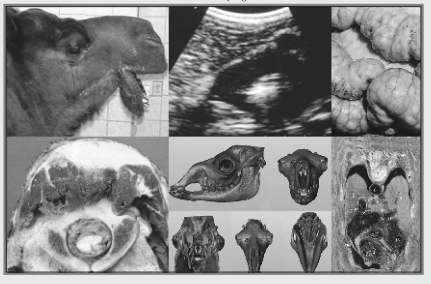

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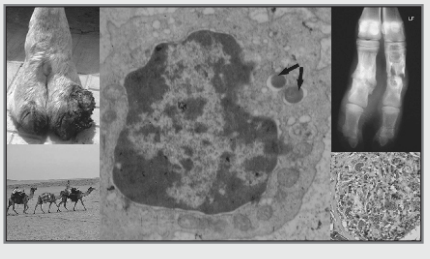

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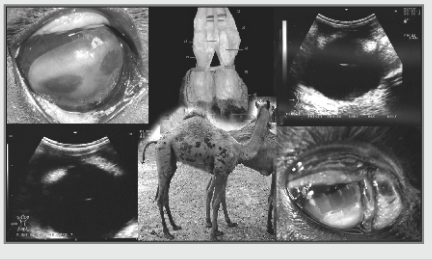

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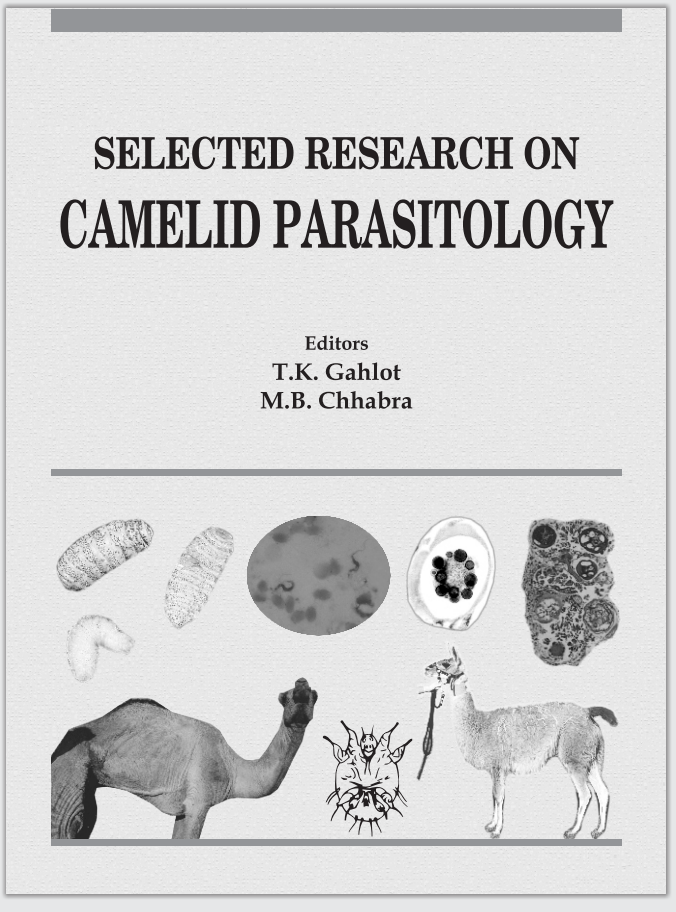
SELECTED RESEARCH ON
CAMELID PARASITOLOGY

Hard bound, 291 pages, few figures coloured

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



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Short communication

ARE DROMEDARIES A POSSIBLE HOST OF PESTE DES PETITS RUMINANTS VIRUS (PPR)

Wernery U, J Kinne, S Joseph, NAG Patteril and G Syriac
Central Veterinary Research Laboratory, P. O. Box 597, Dubai, United Arab Emirates

The recent spread of the Peste des petits ruminants virus (PPRV) to northern Algeria and Morocco approaching Gibraltar, together with continuous and increased circulation of PPRV in other parts of Africa and Asia have increased the chances of the virus entering Europe, particularly through Spain, Italy, Portugal and France. PPR is a highly contagious viral disease of small ruminants, caused by a morbillivirus with severe morbidity and mortality. Each year it affects 30 million animals and causes an estimated animal loss of between 1.2 and 1.7 billion US (Baazizi *et al*, 2017). The World Organisation for Animal Health (OIE) and the Food and Agricultural Organisation of the United Nation (FAO) have set a target to eradicate PPR from the globe by 2030 (Anonymous, 2017). PPR pathogenesis in goats that are highly susceptible to PPRV infection has been thoroughly investigated over the last decades, but whether dromedaries sharing often the same premises with goats contribute to the PPRV spread is scarcely understood.

This was the reason, why researchers from the Central Veterinary Research Laboratory (CVRL) in Dubai conducted an infection trial with the pathogenic strain Kurdistan/2011.

Material and Methods

Six dromedaries of different gender and age were intranasally infected (Fig 1) with the goat PPRV Kurdistan/2011.

For virus isolation we used 2 permanent cell lines which were Verodog SLAM tag and CHS-20.

Two days after infection (dpi), 2 dromedaries and 2 goats were housed together with the nasally infected 6 dromedaries to study the PPRV transmission. Serum, whole blood, oronasal, conjunctival and faecal swabs were collected in regular intervals and analysed for PPRV, PPRV-RNA and antibodies.

Results

Experimentally infected dromedaries did not exhibit any obvious clinical signs and no increase of rectal temperature was observed. PPRV-RNA was detected in one of the 6 infected dromedaries, but none of the contact goats and dromedary were infected with PPRV. Also no virus was re-isolated from none of the swabs taken.

Sero conversion was formed in 4 of the 6 dromedaries for a short period of time.

Discussion

For Old World camelids, PPR has recently been considered a novel disease, while the susceptibility of South American camelids has never been investigated so far. A comprehensive summary of the present situation of PPR in camelids is given by Wernery *et al* (2014). The results of our transmission experiment indicate that dromedaries are most likely dead-end hosts for PPRV. A similar observation was found by Schulz *et al* (2016 a and b) for South American camelids. Therefore, camelids (OWCs and NWCs) probably do not play a role in the spread of PPRV and do not have to be vaccinated.

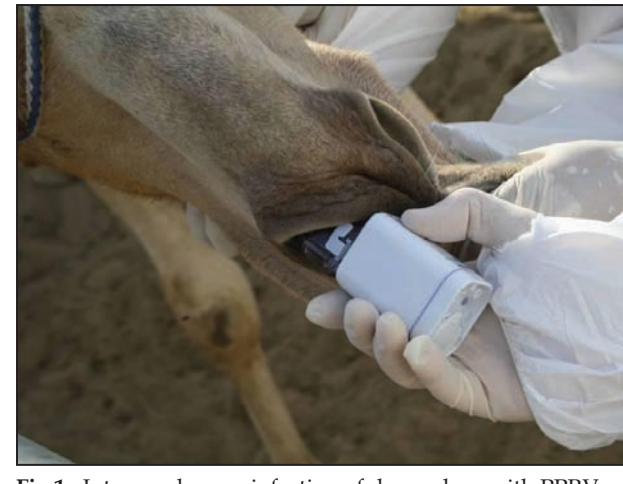

Fig 1. Intranasal spray infection of dromedary with PPRV.

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Recently, reports have emerged from Saudi Arabia by Abd El-Hakim (2006) and Sudan by Khalafalla *et al* (2010) who reported outbreaks of PPR in dromedaries. This is in contrary to our findings.

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THE EPIDEMIOLOGY OF PHYSOCEPHALOSIS IN CAMEL IN THE UNITED ARAB EMIRATES

Schuster R.K.[1], Kinne J.[1], Sivakumar S.[1], Nagy P.[2], Juhasz J.[2], Ismail A.A.[2] and Baumann M.P.O.[3]
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ABSTRACT

During a 4 year observation period (2013-2016), a total of 622 stomach of adult and young camels were examined for the presence of the stomach worm, *Physocephalus dromedarii*. The highest percentage of positive cases (26.7%) with burdens between 1 and more than 3,000 nematodes were found in dromedaries of a Dubai camel dairy farm that previously had imported camels from Sudan, Pakistan and Saudi Arabia. The overall prevalence of the parasite in camels from other farms in Dubai and Abu Dhabi added up to only 4.6% and 2.0%, respectively. Only one of the camels examined from other emirates were found infected. It is believed that *P. dromedarii* was introduced to the UAE with dromedaries from abroad. However, all positive animals were long term residents on the dairy farm or were born there. Scarab beetles serve as intermediate hosts and small vertebrates can be included in the life cycle as paratenic hosts.

Key words: Dromedary camel, *Physocephalus dromedarii*, United Arab Emirates

Nematodes of the genus *Physocephalus* in dromedaries were described for the 1st time by Seurat (1912) in Algeria and Baskakov (1924) in Turkmenistan. Badanin (1939) in his thesis, drew attention to the fact that *Physocephalus* specimens found in the abomasi of dromedaries in Turkmenistan differ from *Physocephalus sexalatus* in pigs in larger lengths and marked swelling in the mid-body of females. These features in connection with different intermediate hosts and larger measured 3rd stage larvae found in scarab beetles motivated Mushkambarova (1967) to divide *P. sexalatus* into two subspecies, *P. sexalatus sexalatus* found in pigs and *P. sexalatus dromedarii* occurring in dromedaries. Detailed microscopical and ultrastructural examination of these nematodes from dromedaries revealed morphological differences that allowed clear distinction from *P. sexalatus* from pigs and to upgrade the former subspecies to species level under the name of *P. dromedarii* (Schuster *et al,* 2013). Since eggs of the parasite cannot be found with routine coproscopic procedures, examination of the abomasum for adult nematodes at necropsy is the only diagnostic procedure at present. Despite findings of other abomasal nematodes in camels in previous years (*Haemonchus longistipes, H. contortus, Camelostrongylus mentulatus* and *Parabronema skrjabini*) 1st incidence of *Physocephalus* infection of dromedaries in Dubai were diagnosed only in July 2011 and October 2012. Repeated further findings of *P. dromedarii* in dromedaries gave point to an epidemiological study of this situation.

Materials and Methods

Between January 2013 and December 2016 a total of 165 young (>100 kg b.w.)[1] and 457 adult camels (>400 kg b.w.) sent for necropsy were specifically examined for the presence of abomasal nematodes. Of these animals, 412 were sent by veterinarians of EICMP farm and 152 originated from breeding farms in Dubai. Further camels were submitted from farms in Abu Dhabi (n=49) and the northern Emirates (n=9) (Table 1). The majority of these animals were adult females. Only EICMP farm sent also carcasses of sub-adult camels. Newborn and suckling calves were not enrolled in this study.

The diagnostic procedure included the opening of the abomasum by scissors, scratching the abomasal folds and washing the content in body-warm (40°C) normal saline in a bucket. After a sedimentation time of 20 min the supernatant was discharged and the sediment was re-suspended with warm normal saline. The process was repeated until the supernatant remained clear. The sediment of

1. Our experience showed that calves with a body weight below 100 kg were not infected with *P. dromedarii* and for this reason they were not considered.

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the abdominal content was then transferred into petri dishes and examined under a stereoscopic microscope. All nematodes in the sediment were taken out by forceps and transferred into a petri dish containing cold normal saline. Species determination and sexing was performed under a stereoscopic microscope. *P. dromedarii* could be easily recognised by the mid-body swelling in female (Fig 1) and the corkscrew twisted posterior end of the male specimens (Fig 2). In case of *Haemonchus* spp. the lengths of spicules were measured microscopically in lactic acid brightened males and taken as criterion for species differentiation.

For data analysis, the proportion of infected animals was related to the group with the respective host characteristic like age, place of origin, length of stay and examination year; the 95% confidence interval was calculated using the software package Quantitative Parasitology 3.0 (QP WEB) (Rozsa *et al,* 2000).

Results and Discussion

Overall, the examination of 622 camels revealed the presence of *P. dromedarii* in 118 (=18.9%) animals. In positive samples there was always a surplus of female nematodes. Juvenile specimens (4th larval stages) in a mixture with adults were present in 17% of the samples. Two stomachs contained in addition, a small number of *Haemonchus longistipes* as mixed infection.

Out of 412 dromedaries originating from EICMP farm, *P. dromedarii* was detected in 110 (=26.7%) carcasses with an average intensity of 108.5 (range: 1 – 3688). Adult camels were significantly more often infected than sub-adult animals (Table 2). Only 7 (4.6%) out of 152 camels from other farms in Dubai were positive for *P. dromedarii* with an intensity ranging from 1 to 30 specimens. The only positive camel that was sent for necropsy from a breeding farm in Abu Dhabi originated originally from Dubai and participated in camel races on a racecourse that is situated at a distance of only 8 km from EICMP farm. None of the 9 camels from northern Emirates were infected with *P. dromedarii*.

Documentation at the EICMP farm allowed a deeper analysis of positive cases, not only to trace back the origin of *P. dromedarii* infected camels but also to count the exact duration of stay at the farm. During the first 3 years of observation (2013–2015), the proportion of positive cases in adult and young camels varied insignificantly between 24.1 and 47.5% and 2.9 and 14.3%, respectively whereas, in 2016 there was a distinct increase in both age groups to 53.4 and 38.9%, respectively (Table 2).

Infected camels were long-term residents on the farm. The shortest stay of an infected camel was 110 days but the average sojourn time ranged from 1787 to 2265 days (149–189 months) (Table 3). For

Table 1. Origin of adult and young dromedaries examined for *Physocephalus dromedarii* at the Central Veterinary Research Laboratory in Dubai 2013-2016.

Year	EICMD farm	Other farms in Dubai	Farms from Abu Dhabi	Farms from northern Emirates	Total
2013	66	34	13	6	**119**
2014	139	53	20	1	**213**
2015	131	48	10	2	**191**
2016	76	17	6	0	**99**
Total	**412**	**152**	**49**	**9**	**622**

Table 2. Occurrence of *Physocephalus dromedarii* in adult and young camels from EICMP farm, Dubai, UAE.

Year	Adult camels			Young camels		
	Examined (N)	Positive (n)	in % (95% Confidence Interval)	examined (N)	positive (n)	in % (95% Confidence Interval)
2013	40	19	47.5 (31.5;63.9)	34	1	2.9 (0.1;15.3)
2014	72	18	25.0 (15.5;36.6)	42	6	14.3 (5.4;28.5)
2015	83	20	24.1 (15.4;34.7)	65	8	12.3 (5.5;22.8)
2016	58	31	53.4 (39.9;66.7)	18	7	38.9 (17.3;64.3)
Total:	**253**	**88**	**34.8 (28.9;41.0)**	**159**	**22**	**13.8 (8.9;20.2)**

Fig 1. *Physocephalus dromedarii* males; posterior end is corkscrew- like twisted.

Fig 2. *Physocephalus dromedarii* females; there is a swelling in the mid-body caused by loops of the uterus.

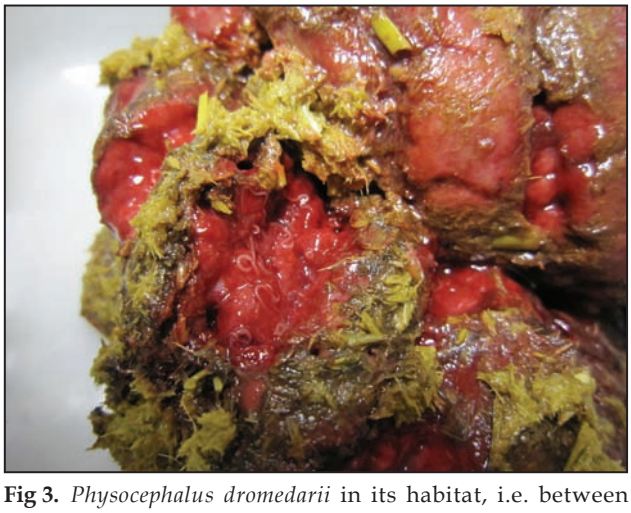

Fig 3. *Physocephalus dromedarii* in its habitat, i.e. between abomasal folds.

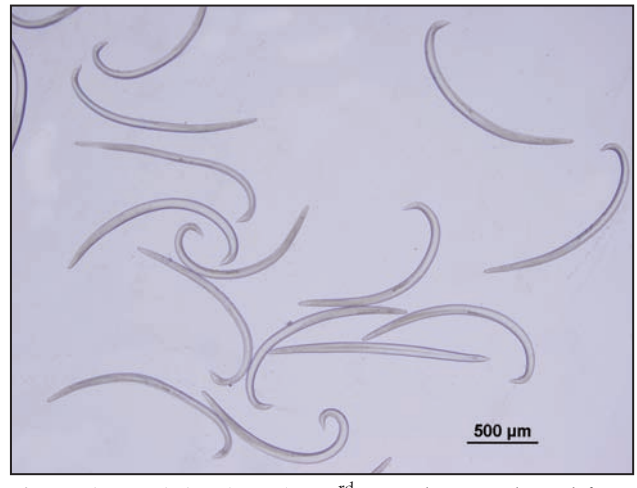

Fig 4. *Physocephalus dromedarii* 3rd stage larvae released from body cavity of *Scarabaeus cristatus*.

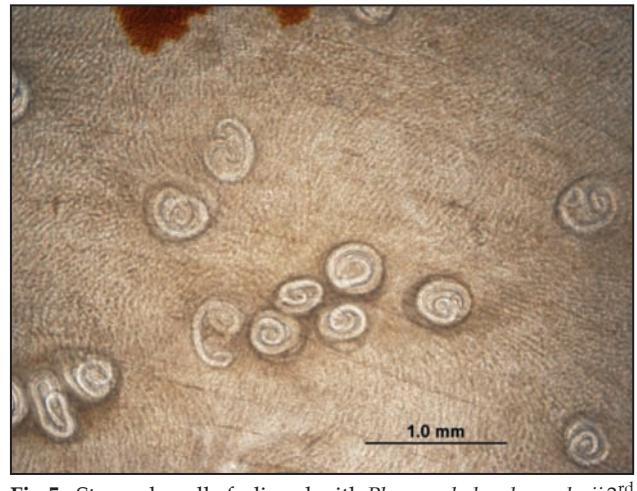

Fig 5. Stomach wall of a lizard with *Physocephalus dromedarii* 3rd stage larvae in a compressorium.

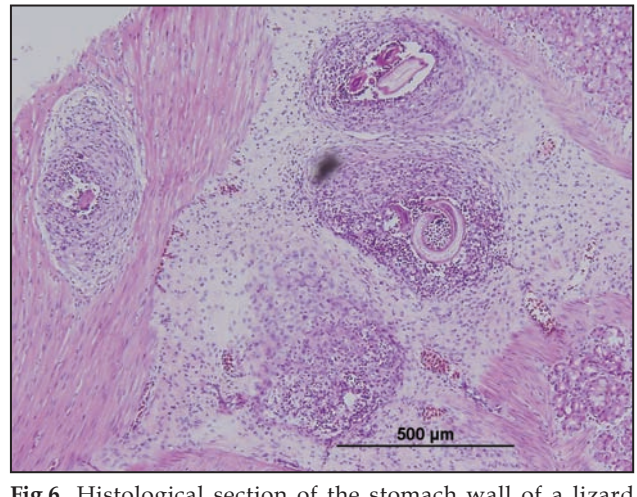

Fig 6. Histological section of the stomach wall of a lizard infected with *Physocephalus dromedarii* 3rd stage larvae surrounded by a wall of connective tissue.

this reason, the analysis of the origin of animals gave inconclusive results. The highest percentage (40.7%) of *Physocephalus* infected adult camels originally were purchased from farms within the UAE but this percentage does not significantly differ from camels bought from abroad when comparing the confidence intervals (Table 4). The fact that *Physocephalus* positive animals were found among camels that were born on the farm indicates that the parasite is well established and reproduces there.

Table 3. Dwelling time of camels infected with *Physocephalus dromedarii* on the EICMP farm.

Year	Number of positive cases	Duration of stay on the farm (in days)	
		Average	Range
2013	20	1787	110-2713
2014	24	1783	120-3242
2015	28	1933	480-3050
2016	38	2265	135-3492

Nematodes of the genus *Physocephalus* have been reported from dromedaries in few countries only. These were Algeria (Seurat, 1912; Chauve *et al,* 1990), Turkmenistan (Baskakov, 1924; Badanin, 1939; Mushkambarova, 1967; Mushkambarova and Dobrynin, 1972; Mushkambarova *et al,* 1989), Kuwait (Abdul-Salam and Farah, 1988) and Iran (Mirzayans and Halim, 1980; Anvari-Tafti *et al,* 2013; Majidi-Rad *et al,* 2015). There is no information on findings of these nematodes in East Africa, Saudi Arabia and Pakistan. In the UAE, first cases of *P. dromedarii* were detected in 2011 and 2012 only in camels of the EICMP farm. Although, *P. dromedarii* reaches a size comparable to *H. contortus* and *H. longistipes*, however, in the case of low burdens this parasite can be missed during adspection of the abomasal mucosa because of its pale pink colour and its location deep in the abomasal folds (Fig 3). For this reason, washing of the abomasal mucosa and several steps of sedimentation are necessary to increase the diagnostic sensitivity.

The relatively high number of positive cases on the EICMP farm compared to other farms in Dubai, only one infected camel from Abu Dhabi[2] and negative results in camels from the northern Emirates makes the EICMP farm a hotspot for *P. dromedarii* and suggests that this parasite might have been introduced with imported camels from abroad in an early stage of development of this farm. Unfortunately, the origin of the parasite could not be traced back since all infected animals stayed longer on the farm than the prepatent period of *P. dromedarii* lasted. Experimental infection trials showed that it takes up to 10 weeks for the parasite to reach sexual maturity. Only at 12 weeks after infection, the terminal part of the uterus is filled which eggs what makes this time span equivalent to the prepatent period (Schuster *et al,* 2016b).

Like other spirocercid nematodes *P. dromedarii* is a biohelminth and dung eating beetles serve as intermediate hosts. Both larval stages and imagines of beetles can become infected and harbor 3rd stage larvae of the parasite (Fig 4) in their body cavity.

Large concentrations of camels produce large amount of camel dung that attracts dung eating insects. *Scarabaeus cristatus* is a large dung rolling scarab beetle that has been found frequently in early morning hours and in late afternoons on the territory of the EICMP farm. The examination of 366 *S. cristatus* showed an extensity of infection with *Physocephalus* 3rd stage larvae of 93.3 % with an average burden of 1,538 reaching from one to more than 17,000 larvae (Schuster *et al,* 2016a). A part of the beetle population left the farm and was also found in a camel free equestrian area 15 km distant to the EICMP farm. These beetles showed a comparable *Physocephalus* prevalence of more than

2. The only positive camel from a farm in Abu Dhabi most probably got infected in Dubai.

Table 4. Occurrence of *Physocephalus dromedarii* in adult and young camels from EICMP farm according to country of origin.

Age group		Origin					
		Sudan	KSA	Pakistan	UAE	borne on farm	total
Adults	examined	24	70	62	86	11	253
	positives	9	26	14	35	4	88
	in % (95% Confidence Interval)	37.5 (18.8;59.4)	37.1 (25.9;49.5)	22.6 (12.9;35)	40.7 (30.2;51.8)	36.4 (10.9;69.2)	34.8 (28.9;41.0)
Young	examined	1	25	23	17	93	159
	positives	0	5	4	1	12	22
	in % (95% Confidence Interval)	0.0	20.0 (6.8;40.7)	17.4 (14.4;50.0)	5.9 (0.1;28.7)	12.9 (6.8;21.8)	13.8 (8.9;20.2)

90% but due to the absence of infected final hosts had a strikingly lower average larval burden of 605.

Apart from large dung rolling beetles there is a number of smaller scarabeids known as tunnelers and dwellers of the family Aphodiidae. These smaller insects can be eaten by reptiles, birds and rodents then functioning as paratenic hosts. In these hosts, the parasite larvae settle in the submucosa and muscular layer of stomach and intestines causing inflammation and encapsulation (Figs 5, 6) and leading to an interruption of the motoric function of the digestive tract. Heavily infected hosts eventually die and can be ingested by camels.

So far, findings of *P. dromedarii* are concentrated in one camel farm in Dubai only. However, the occurrence of the parasite in camels of other farms in Dubai indicate that *P. dromedarii* is spreading.

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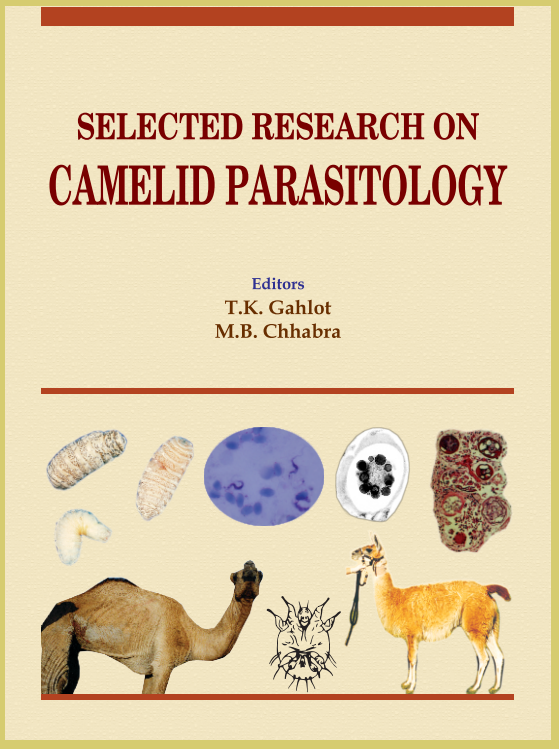

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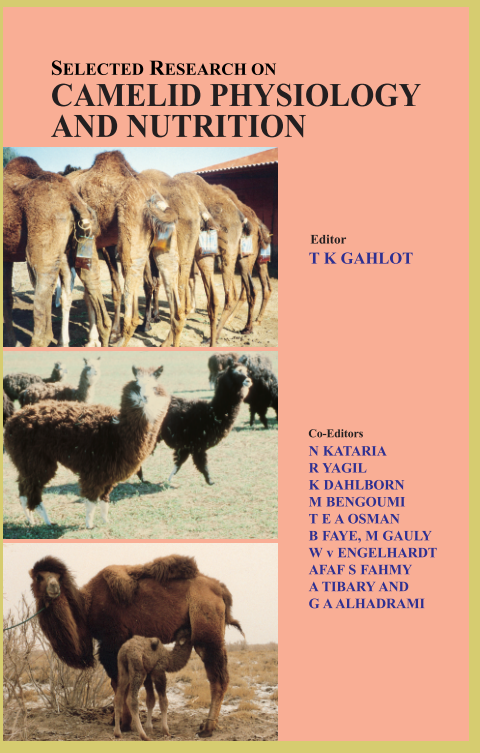

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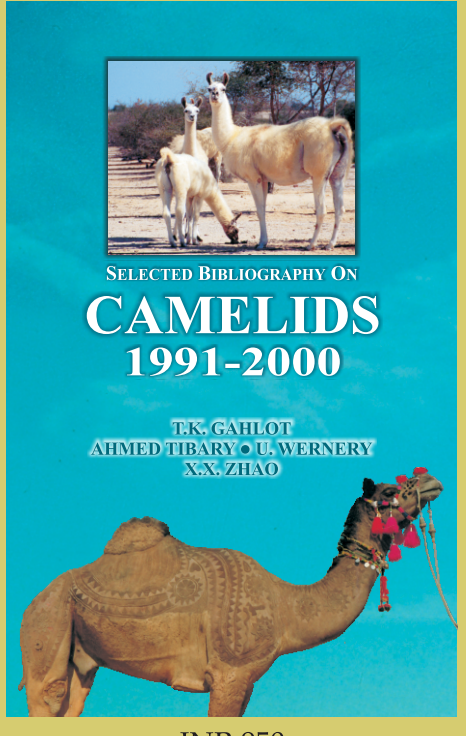

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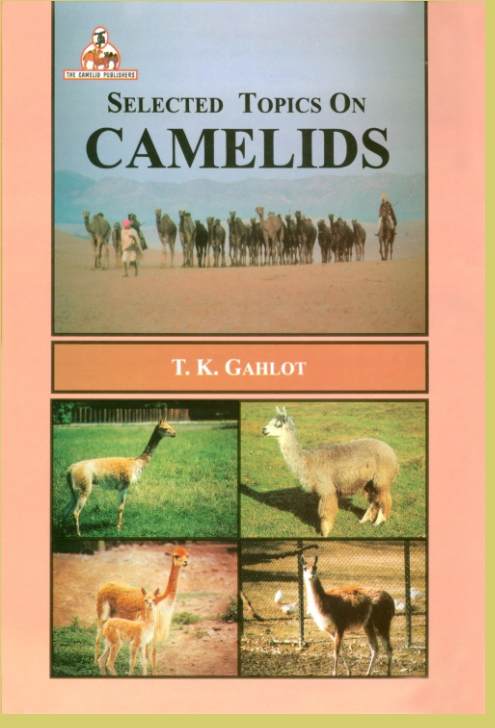

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Short Communication

MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS IN HEALTHY AND DISEASED DROMEDARIES

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ABSTRACT

In this study, we investigated the distribution of MERS CoV in Al-Ahsa region in Saudi Arabia by analysis of MERS CoV genomes isolated from Al-Ahsa as well as, tracking the virus in healthy and diseased camels. Serum and nasal swab samples were collected from different camels including sick camels submitted to an animal clinic, slaughtered camels at an abattoir in Al-Ahsa and samples from free ranging camel herds in the desert around Al-Ahsa oasis. Viral RNA was not detected in serum of all samples either healthy or sick camels. Nasal swabs were collected from two camel herds. The first herd showed 23% positive samples, while the other showed negative reaction to real time PCR. Bioinformatics analysis of 12 full genomes of MERS CoV isolated from Al-Ahsa region showed conserved sequence with no gaps in 9 genomes, while 3 genomes showed 1-3 differences. These data implies that MERS CoV is not horizontally widespread in dromedaries, and its highest occurrence was within isolated herds.

Key words: Dromedaries, MERS CoV

MERS CoV is a newly emerged virus in the Arabian Peninsula causing serious human health hazard problem (Alfuwaires *et al,* 2017; Assiri *et al,* 2013; Zaki *et al,* 2012). Soon after its discovery, several cases were recorded worldwide (Devi *et al,* 2014; Reusken *et al,* 2014). The Arabian Peninsula is considered an endemic area of MERS CoV due to circulation of the virus and identification of new infected cases. Sporadic cases were diagnosed in several countries, mainly in Middle East. More recently, MERS-CoV outbreaks were recorded in South Korea and China (Cowling *et al,* 2015, Su *et al,* 2015). Recently, we reported that camel MERS CoV genomes and enzymes bear unique evolution features that distinguish them from the human viruses (Alfuwaires *et al,* 2017; Kandeel and Altaher, 2017). The infection can be transmitted from camels to human. Additionally, camels below 1 year are the main host, while 100% of older camels show positive serum reaction (Wernery *et al,* 2017). Camel calves below 4 years show acute, epidemic and time-limited infection. Younger calves can transmit the infection to human due to separation from their mothers at a stage of high susceptibility to infection with MERS CoV (Wernery *et al,* 2015).

MERS CoV infection in both humans and camels were recorded in Al-Ahsa region. The virus was detected in camels and human beings (Khalafalla *et al,* 2015). Furthermore, the epidemiologic analysis of MERS CoV infection pattern in Al-Ahsa revealed several unexplained and unexpected results. A recent outbreak of MERS CoV infection in Al-Ahsa hospital was associated with 3 different virus strains in one event (MacIntyre, 2014). This indicates a rather complex and diverse hosts and methods of transmission in Al-Ahsa region. This may be due to the presence of several free ranging camel populations, camel barn, large market for camel trading and international camel racing tracks in the vicinity.

In this study, samples were collected from 3 different target camel populations, i.e. sick, slaughtered and free ranging healthy camels.

Materials and Methods

Collection and transport of samples

Samples were collected from 3 different groups. The 1st group comprised sick camels of different age

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and either sex (Table 1) brought to the Veterinary Clinic, King Faisal University, Al-Ahsa. Samples were collected during the period from May to July 2015 in 11 different sample collection visits. The collected samples were from camels with dystocia, respiratory distress, mastitis, fracture, fever and general health check or loss of appetite. The 2nd group was comprised of healthy camels slaughtered at Alomran abattoir, Al-Ahsa city during the period from August to September 2015 (Table 1). The source of camels were either from Al-Ahsa market, small camel raising barns or herds roaming around. The 3rd group included free ranging camels in Al-Ahsa region and small camel barns. About 10 serum samples and 26 nasal swabs were collected aseptically. Samples were stored on ice and immediately transferred to the laboratory for analysis of viral RNA. Samples from a 2nd camel herd were collected in December 2016 (18 nasal swabs). All serum samples were collected under aseptic conditions. All nasal swab samples were collected in virus transport medium (Copan diagnostics Inc, Italy).

Serum was separated by centrifugation and stored at -20°C until further investigations. Nasal swabs were clarified by centrifugation at 5000 rpm for 10min. The clarified supernatant was stored at -80°C until extraction of RNA.

RNA extraction and RTPCR

Viral RNA was extracted by either RNA extraction kit (Qiagen, Germany) or MagNA Pure 96 kits (Roche, USA) in MagNa pure 96 automated nucleic acids purification instrument. Real time PCR was performed by LightMix MERS CoV RT-PCR kit in Roche Light Cycler 480II according to the manufacturer instructions. After RNA extraction, two RTPCR assays were done, targeting upE and orf1a. The assay is combined with control reaction for tracking extraction and reverse transcription. According to WHO instructions, all upE/N gene positive results were confirmed by running Orf1a assay. Positive samples were considered after comparison with control positive and control negative standards.

Retrieval and comparisons of genomic data of complete genomes from Al-Ahsa region

Genomic data of 12 complete genomes from Al-Ahsa region were obtained from the gene bank. The files were kept in FASTA format and analysed for pairwise comparisons. The bioinformatics tools at the European bioinformatics institute were used to fully analyse the genome. Phylogenetic trees were constructed after serial amino acid or nucleotide alignments.

Results and Discussion

The present study indicated lack of MERS CoV RNA in camels serum in all collected samples. In the first herd, 6 positive nasal swab samples were confirmed, representing 23% of samples. From the second herd 18 nasal swabs were collected. All of the tested samples showed negative reaction to RTPCR test. The lack of viral RNA in the tested sera does not exclude an infection of camels with MERS CoV and may indicate a short viremia in camels. As an explanation of the results, it might be concluded that the time of sampling in summer months might be associated with decreased virus circulation among camels. The harsh desert environment in summer months might adversely contribute to MERS CoV replication. In this context, the peak of MERS CoV positive camels is expected during late November to March due to the stress of parturition and lactation. The presence of MERS CoV in nasal swabs indicates the circulation of the virus among free ranging camels. In recent human cases, severe MERS CoV disease was associated with a previous prominent viremia or detection of viral

Table 1. The number and source of the collected samples for MERS conora virus in camels.

Collection sites	Total number of samples	Sex		Age (years)			Number of MERS CoV positive samples	Ratio*
		male	female	>2	2-6	>6	(%)	
Veterinary clinic	36 serum samples**	23	13	3	11	22		
Abattoir	78 serum samples	58	20	0	18	60	zero	zero
First camel herd	10 serum samples	6	20	10	1	16	zero	zero
	26 nasal swabs						6	23
Second camel herd	18 nasal swab	16	2	7	3	8	zero	zero

* the number of MERS CoV positive samples to the number of total samples.

** the collected samples include 2 dystocia cases, 3 respiratory distress cases, 3 mastitis cases, 1 fracture case, 4 fever cases and general health check or loss of appetite in 23 cases.

Accession no.	AGV08478	AGV08490	AGV08544	AGV08571	AHI48515	AGN70927	AGN70960	AGN70971	AGV08442	AGV08556	AGV08533	AGN70949	
AGV08478		0	0	0	0	0	0	0	0	0	0	0	
AGV08490	2		0	0	0	0	0	0	0	0	0	0	
AGV08544	2	0		0	0	0	0	0	0	0	0	0	
AGV08571	2	0	0		0	0	0	0	0	0	0	0	
AHI48515	2	0	0	0		0	0	0	0	0	0	0	Gaps in genomcs alignment
AGN70927	2	0	0	0	0		0	0	0	0	0	0	
AGN70960	2	0	0	0	0	0		0	0	0	0	0	
AGN70971	2	0	0	0	0	0	0		0	0	0	0	
AGV08442	2	0	0	0	0	0	0	0		0	0	0	
AGV08556	2	2	2	2	2	2	2	2	2		0	0	
AGV08533	3	1	1	1	1	1	1	1	1	2		0	
AGN70949	2	0	0	0	0	0	0	0	0	2	1		
	Number of differences in amino acids sequences												

Fig 1. Pairwise comparison matrix of genomes of MERS CoV isolated from Al-Ahsa based on the number of gaps and differences in amino acids composition. The upper-right triangle represent the number of gaps produced during multiple genomes alignment. The lower-left triangle represent the number of differences in amino acids composition among genomes.

RNA in serum. In addition, a worse clinical course is predicted for those showing viral RNA in their blood (Kim *et al*, 2016). Therefore, the lack of RNA in camel blood in this study might indicate the course of mild disease in camels. In this context, clinical evidence and pathogenesis of MERS CoV in camels is not clearly documented and requires more investigations. Despite of the wide diversity of samples collected in this study, all positive samples were from one herd. This might imply the confinement of MERS CoV in sporadic or isolated focuses of infection but not widely distributed among camel herds.

In order to get access to the genetic aspects of MERS CoVs isolated from Al-Ahsa region, we analysed the sequence of 12 full genomes of viruses isolated from this region. Pairwise comparison matrix of 12 full genomes of MERS CoV isolated from Al-Ahsa region revealed conservation of amino acids with no differences in 9 of these genomes (Fig 1). Three of the retrieved genomes (AGV08556, AGV08533 and AGV08478) showed 1-3 differences in amino acid compositions with no gaps in sequence alignment. These genomes were sequenced within a short time frame indicating that several virus strains are circulating in Al-Ahsa region. As these genomes were sequenced in 2013, more variable composition of MERS CoV populations in Al-Ahsa is expected. Future studies for identifying the degree of changes in virus sequence will be necessary to monitor evolution and mutations.

Dromedaries are potential hosts of MERS-CoV. However, the role of camels is not well understood and needs further studies. More studies are required toward understanding the nature of this virus disease in camels, especially its pathological and clinical aspects.

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GENETIC CHARACTERISATION OF BIKANERI CAMEL USING MICROSATELLITE MARKERS

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ABSTRACT

Eleven New World Camelids microsatellite primer pairs were used to investigate the genetic polymorphism in Bikaneri camel. Genomic DNA was extracted from blood and polymerase chain reactions were carried out for 30 unrelated camels of Bikaneri breed. Microsatellite technique was used for analysis of DNA. The amplification products were resolved in 6% (denaturing) urea PAGE and stained with silver nitrate. Six microsatellite primer pairs could amplify the polymorphic microsatellite loci in Bikaneri camel. The number of alleles ranged from 2 to 5. The expected heterozygosity ranged from 0.289 to 0.686 and the polymorphic information content ranged from 0.267 to 0.639. The results indicated the utility of these microsatellite loci for studying genetic polymorphism in Indian dromedary and the potential use of microsatellite markers for further genetic investigations including individual identification, parentage testing and production enhancement.

Key words: Bikaneri, camel, genetic diversity, microsatellite markers, polymorphism

Genetic variability is the most suitable criterion that can be used when breeds are identified for conservation. Breed characterisation requires knowledge of genetic variation that can be effectively measured within and between populations (Hatzel and Drinkwater, 1992). Breed characterisation at the phenotypic and molecular genetic level has become essential to know the present status of different species and their breeds available in different agro-climatic zones of the country.

Several researchers have analysed the growth (Mehta, 2008; Mehta *et al,* 2010) and production potential (Mehta *et al,* 2014) of different Indian camel breeds. Microsatellite markers contribute most for molecular genetic characterisation of livestock breeds because of their highly informative polymorphic nature (Lubieniecka *et al,* 2000). Owing to their high heterozygosity, Mendelian co-dominant inheritance, ubiquity throughout the genome and ease of scoring by PCR, microsatellites are now considered the most powerful genetic marker (Goldstein and Pollock, 1997).

Animal genetic resources of the world, especially those of the developing countries are in the state of decline where many breeds might be lost without ever having been adequately characterised. Further, even within the same eco-system the species like camel (Mehta, 2014) have already lost much of the within breed genetic variation as compared to the more numerous species like cattle (Sodhi *et al,* 2008). This depletion of resources would result in loss of important genes responsible for remarkable adaptive traits of thriving on harsh and stressful environment. All this may lead to loss in genetic variability for selection and hence conservation of this genetic group is of top most priority, especially in the conditions where future requirements are unknown. So there is a need to study the diversity within and among breeds and also to characterise them at the molecular level using microsatellite markers. Thus the present study was conducted to screen the microsatellite markers for DNA profiling in Bikaneri breed of camel and to study the DNA polymorphism as revealed by microsatellite primers.

Materials and Methods

Location, animals and experimental setup

Present study was conducted on Bikaneri breed of camel. The animals were clinically healthy and were maintained at ICAR-National Research Centre on Camel, Bikaner, India and from the villages located in the breeding tract of the breed.

Blood sampling and DNA isolation

Blood samples (10-15 ml) were collected by jugular vein puncture under sterile conditions

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and immediately transported to laboratory under refrigeration. EDTA was used as anticoagulant. DNA was isolated using phenol- chloroform method with minor modifications (Sambrook *et al,* 1989). The concentration of DNA was assessed by standard method of ultraviolet spectrophotometry (OD 260/280 = 1.6 to 1.8) and integrity was checked by gel electrophoresis on 0.8% agarose gel.

Amplification and analysis of microsatellite loci

Eleven microsatellite primer pairs (Lang *et al,* 1996 and Obreque *et al,* 1998) were selected for present study (Table 1). PCR amplifications were carried out in 25 µl reactions containing 50 ng DNA, 5 pmol each primer, 1.5 U Taq DNA polymerase, 0.2 mM each dNTP, 2.5 µl 10X Taq DNA polymerase buffer containing 10 mM Tris – HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl and 0.01% gelatin. The PCR amplification programme consisted of an initial denaturation temperature of 95°C for 5 min, then 30 cycles at 94°C for 45s, 50-60°C for 1 min, depending on the primer pair used and 72°C for 1 min. Final extension was carried out at 72°C for 15 min. The microsatellite bands were resolved by 6% Urea Polyacryl Amide Gel Electrophoresis, stained with silver nitrate (Bassam *et al,* 1991) and sized using Gene Tools (Syngene) software.

The allele frequency was calculated by using the equation $p_i = K_i/N$, where p_i = frequency of i^{th} allele = number of observation of i^{th} allele and N = total number of alleles scored. Observed heterozygosity (H_o) was calculated by taking the ratio of number of heterozygous animals to the total number of animals scored. The expected heterozygosity (H_e) was calculated by using the equation $H_e = 1-\sum_{i=1}^{n} p_i^2$, where H_e = expected heterozygosity, p_i = frequency of i^{th} allele. The polymorphism information content (PIC) is another important measure of DNA polymorphism. Besides, being a measure of genetic variation, it is also used in the context of gene mapping. The values of PIC are lower than heterozygosity for the corresponding marker because in PIC, a quantity is subtracted from heterozygosity that corresponds to the probability of offspring being uninformative. Thus, PIC also indicates the potential of a marker to differentiate the closely related individuals or populations. The polymorphic information content were calculated (Botstein *et al,* 1980) by using $PIC = 1-(\sum_{i=1}^{n} p_i^2)-\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} 2p_i^2 p_j$, where p_i is the frequency of i^{th} allele and is the frequency of j^{th} allele.

Results and Discussion

Microsatellite markers have been the primary option for characterising genetic diversity studies in dromedary camels since protein polymorphism has shown very little genetic variation. In the present investigation, 6 primers VOLP08, VOLP10, YWLL09, YWLL44, YWLL58 and YWLL59 were successfully used in amplifying the polymorphic loci in Bikaneri camel. The other 5 primers VOLP03, VOLP67, YWLL08, YWLL19 and YWLL43 were either monomorphic or required further investigation. However, successful amplification of polymorphic microsatellite loci in Bikaneri camel (*Camelus dromedarius*) using New World Camelidae microsatellite primer pairs (Lang *et al,* 1996 and Obreque *et al,* 1998) suggests the use of a single panel of microsatellite primers to study genetic diversity in closely related individuals, breeds and species of family Camelidae.

The number of alleles, allele size and allele frequency are presented in table 1. The number of alleles ranged from 2 to 5 at different polymorphic microsatellite loci in Bikaneri camel. Five alleles in the size range of 250-264 bp were observed at VOLP10 locus whereas 3 alleles each were observed at VOLP08, YWLL44 and YWLL58, respectively in the size range of 142-146 bp, 104-108 bp and 173-177 bp. Two alleles each were observed at YWLL09 and YWLL59, respectively in the size range of 160-162 bp and 115-117 bp. The number of alleles at different marker loci and their frequencies are simple indicators of the genetic variability. The number of alleles and allele size range observed in the present investigation is in close agreement with that of Jianlin *et al* (2000), who reported 3 (146-150 bp), 7 (250-268 bp), 3 (158-162 bp), 3 (105 –109 bp) and 2 (109-111 bp) alleles (size range), respectively for VOLP08, VOLP10, YWLL09, YWLL44 and YWLL-59 loci in dromedary. Almost similar results in Jaisalmeri, Kachchhi, Bikaneri and Mewari camels have been reported by (Gautam *et al,* 2004; Mehta and Sahani, 2007; Mehta *et al,* 2007; Mehta, 2013; 2014). Obreque *et al* (1998) also reported 3 alleles each at VOLP08 (148-152 bp) and VOLP10 (231-235 bp) locus in Alpacas. However, Lang *et al* (1996) reported 13, 9, 11, 6 and 10 alleles in Alpacas and llamas at YWLL-08 (135-177bp), YWLL-09 (154-180bp), YWLL-44 (86-120bp), YWLL-58(175-194bp) and YWLL-59 (96-136 bp) locus, respectively. It is evident from the results and literature that the amplification at each locus was highly specific as the allele size range for respective locus is quite similar in all the species of family camelidae. More number of alleles in new world camelids as compared to the dromedary clearly indicates existence of relatively less genetic variation in later species as compared to the former. However, the results suggest existence of

Table 1. Allele number, size and frequency at six microsatellite loci in Bikaneri camel.

Locus	No. of Alleles	Allele Size (bp) /Allele Frequency				
		1	2	3	4	5
VOLP08	3	142/0.050	144/0.833	146/0.116		
VOLP10	5	250/0.233	252/0.033	260/0.466	262/0.183	264/0.0833
YWLL09	2	160/0.216	162/0.783			
YWLL44	3	104/0.133	106/0.366	108/0.433		
YWLL58	3	173/0.330	175/0.500	177/0.166		
YWLL59	2	115/0.283	117/0.716			

enough genetic variation in dromedary for further genetic analysis.

Heterozygosity and PIC

The observed and expected heterozygosity along with the polymorphic information content (PIC) are presented in table 2. The observed heterozygosity is based on the number of heterozygous individuals in the population under investigation. The observed heterozygosity (Ho) ranged from 0.35 (VOLP08) to 0.615(VOLP10) except at the YWLL-58 locus where all samples were observed to be heterozygous, requiring further investigation. The expected heterozygosity depends on the number of alleles and their frequency in a population at a particular locus. The expected heterozygosity (He) ranged from 0.289 (VOLP08) to 0.686 (VOLP10) with 0.50 or more value in 5 out of 6 polymorphic loci. The observed and expected heterozygosity reported in Kenyan dromedary (Jianlin *et al,* 2000) and in Indian dromedary breeds (Gautam *et al,* 2004; Mehta and Sahani, 2007; Mehta *et al,* 2007; Mehta, 2013; 2014) are quite similar to the present findings. Little higher has been reported by Lang *et al* (1996) and Obreque *et al* (1998) in new world camelids due to more number of alleles at most of the loci. The results indicate that the above 6 loci can very well be utilised for further genetic studies which may include characterisation, conservation, individual identification, parentage testing and production enhancement.

The values of PIC are lower than heterozygosity for the corresponding marker because in PIC, a quantity is subtracted from heterozygosity that corresponds to the probability of offspring being uninformative. The polymorphic information content ranged from 0.267 (VOLP08) to 0.639 (VOLP10). The PIC values reported in African dromedary (Jianlin *et al,* 2000; Hashim *et al,* 2014) and in Indian dromedary breeds (Gautam *et al,* 2004; Mehta and Sahani, 2007; Mehta *et al,* 2007; Mehta, 2013; 2014) are quite similar to the present findings. However, the PIC values reported by Lang *et al* (1996) and Obreque *et al* (1998) in New World Camelids are relatively higher due to more number of alleles at these loci. The present results and the literature suggest the probable existence of relatively less genetic variation in dromedary as compared to alpacas and llamas at these loci. Nevertheless, the above markers, due to good number of alleles and enough heterozygosity can be used to study the genetic diversity of the family camelidae.

Table 2. Heterozygosity and polymorphic information content (PIC) at 6 microsatellite loci in Bikaneri camel.

Locus	Observed heterozygosity	Expected heterozygosity	PIC
VOLP08	0.350	0.289	0.267
VOLP10	0.615	0.686	0.639
YWLL09	0.450	0.339	0.281
YWLL44	0.400	0.599	0.515
YWLL58	1.000	0.611	0.535
YWLL59	0.583	0.406	0.323

The present study suggests that characterisation of individual breed using the microsatellite primers may be of tremendous use to the planners and scientists in formulating the projects for increasing the production and utilise of specific breed. Screening by microsatellite markers may help in identification of sires of high genetic merit which are true to their breed. Thus, the information derived from microsatellite marker study for individual breed may help the conservation scientists in deciding the purity of the breed and may prove helpful in taking necessary steps for *in vivo* and *in vitro* conservation of the breed.

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Short Communication

CONCURRENT INFECTION OF COCCIDIOSIS AND HAEMONCHOSIS IN A DROMEDARY CAMEL CALF FROM RAJASTHAN, INDIA

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Haemonchosis is considered as the most severe gastrointestinal helminthosis of camels which may be associated with clinical disease and can be fatal. This nematode occurs in the abomasum of the infected camels sucking blood from the mucosal vessels leading to haemorrhagic anaemia, a characteristic feature of the disease (EL Hassan *et al*, 2011). Likewise, coccidiosis may be seen in camel calves with symptoms like diarrhoea, dysentery, dehydration, rough hair coat and anaemia (Parsani *et al*, 2008). Among gastrointestinal protozoan parasites, infection of *Eimeria* spp. is a major problem in camels. The present study is the first description of a case where in the pathology of concurrent coccidiosis and hemonchosis infection has been studied in a dromedary camel calf.

Case History and Examination

A one year old dead dromedary camel calf of an organised camel herd with a past history of prolonged emaciation, progressive anaemia, anorexia, debility and intermittent diarrhoea during winter was presented for postmortem examination. The calf was under treatment with supportive drugs and intravenous fluid but its condition did not improve and it succumbed. The necropsy was performed immediately after its death and gross lesions were observed. Tissue samples from major organs were collected and fixed in 10% formal saline for histopathology. The small and large intestinal contents were also collected in sterile vials for parasitological examination using floatation technique and direct microscopy.

Results

The gross examination of the camel calf revealed severe gelatinisation and atrophy of subcutaneous and visceral fat and pale mucous membranes. The thoracic and abdominal cavity contained moderate amount of clear watery fluid. The heart has litchi like appearance due to severe atrophy and gelatinisation of epicardial fat (Fig 1A). The other important gross changes were observed in gastrointestinal tract. The abomasum was found empty and the mucosa showed thickening, congestion and multiple petechial haemorrhages (Fig 1B). The small intestinal mucosa also showed thickening with marked corrugation and presence of yellowish watery mucous mixed contents (Fig 1C). The mesenteric fat was atrophied, gelatinised and showed prominent blood and lymphatic vessels (Fig 1D). The mesenteric lymph nodes were pale, enlarged and oedematous (Fig 1D). The lung was pale pink in colour and showed mild emphysema. No significant gross changes were observed in other organs.

The small intestinal contents on microscopic examination revealed presence of numerous oocysts of *Eimeria* spp whereas, the large intestinal contents revealed the eggs of *Haemonchus* spp. The eggs were thin-shelled, oval shape with equal poles and morula not fully filled the shells of the eggs (Fig 2A). The oocysts of *Eimeria cameli* were oval shape, distinctly brown coloured, had a single large sporont with the presence of micropyle on the 'pointed' end (Fig 2B).

The histopathology of abomasal mucosa showed moderate infiltration of eosinophils and mononuclear cells. In addition, haemorrhages, congestion of blood vessels in lamina propria and desquamation in the apical border of the villi was observed in the abomasum. The histopathological examination of small intestine showed presence of large number of different developmental stages of *Eimeria cameli* inside the mucosal layer (Fig 3) which caused severe degeneration and desquamation of the intestinal epithelium. The affected villi and crypts were also distended and disorganised due to presence of developmental stages such as giant schizonts, microgamonts, macrogametocytes and oocysts of *E. cameli*. The intestinal mucosa showed mild to

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moderate mononuclear and oeosinophilic infiltration in the lamina propria (Fig 4). The cortical area of mesenteric lymph node also showed occasional presence of developmental stages of *E. cameli* along with oeosinophilic infiltration (Fig 5). The important histopathological lesions observed in other organs were mild necrotic and degenerative changes in heart and mild emphysema in lung.

Discussion

The gastrointestinal parasites play a significant role in camel husbandry because parasites not only reduce the productivity and performance of camels but also predispose them to other infectious diseases (Radfar and Gowhari, 2013). Due to the common clinical signs and acute nature of gastro-intestinal helminths, it is practically impossible to distinguish these diseases. The general clinical signs shown by camel calf of the present case are frequently reported in most of the gastrointestinal parasitic infections including haemonchosis (Arzoun *et al,* 1984; Saminathan *et al,* 2015) and coccidiosis (Kinne and Wernery, 1998; Kumar *et al,* 2015).

The gross pathological changes of fluid in the body cavities due to hypoproteinemia, atrophied and gelatinised subcutaneous and visceral fat and petechial haemorrhages in abomasal mucosa as recorded in the present case, corresponded with the haemonchosis infection in sheep (Saminathan *et al,* 2015). The gross changes in small intestine were also more or less similar to *E. cameli* infection in camels reported in a previous study (Kumar *et al,* 2015). The histopathological changes of oeosinophilic and mononuclear cell infiltration observed in the abomasum of affected camel due to haemonchosis is similar to that reported in sheep (Saminathan *et al,* 2015). Likewise, the histopathological changes in intestinal mucosa due to presence of different

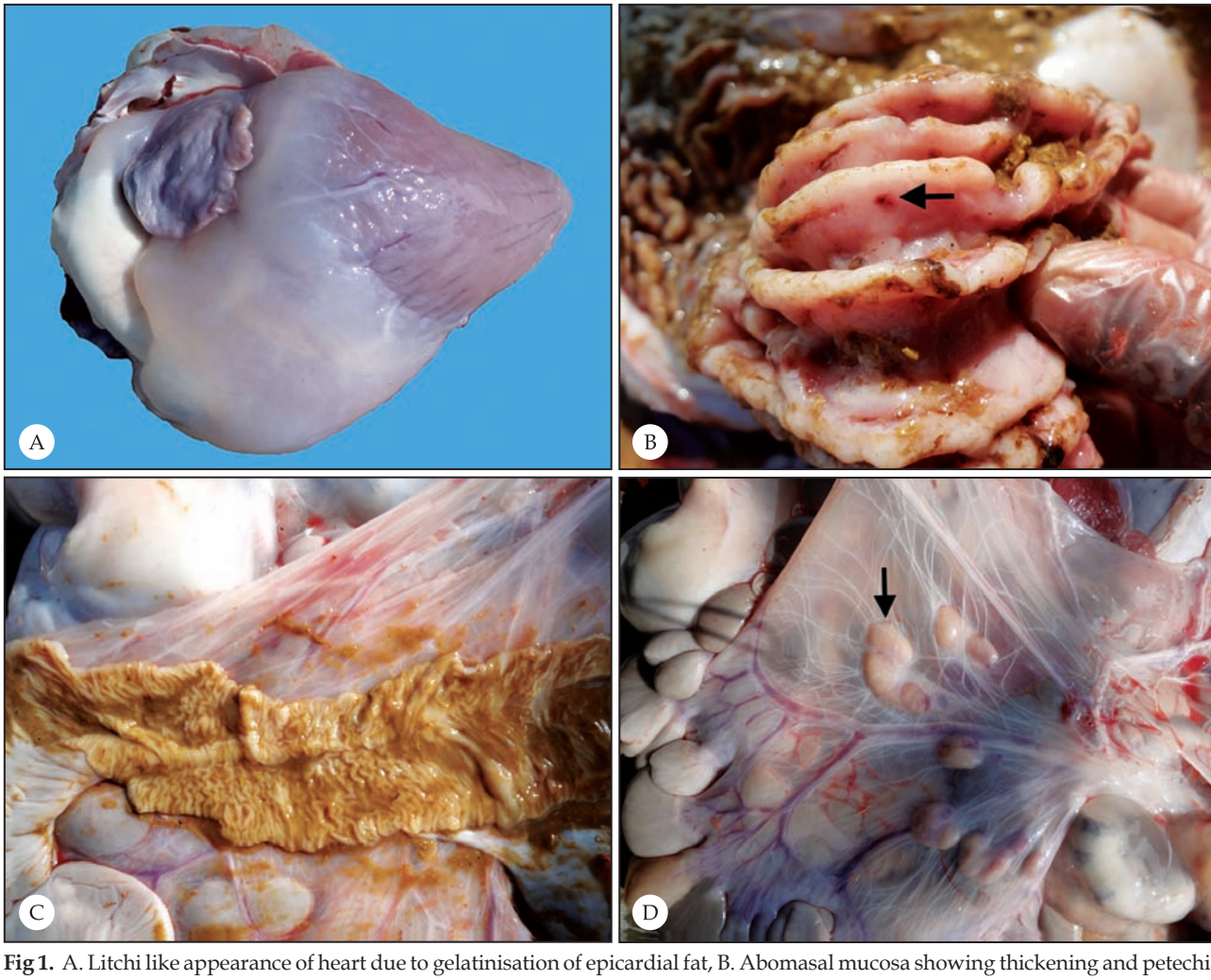

Fig 1. A. Litchi like appearance of heart due to gelatinisation of epicardial fat, B. Abomasal mucosa showing thickening and petechial haemorrhages (arrow), C. Mucosa of small intestine showing thickening, corrugation and presence of watery mucous mixed contents, D. Enlarged mesenteric lymph nodes (arrow) with prominent blood and lymphatic vessels along the mesentery.

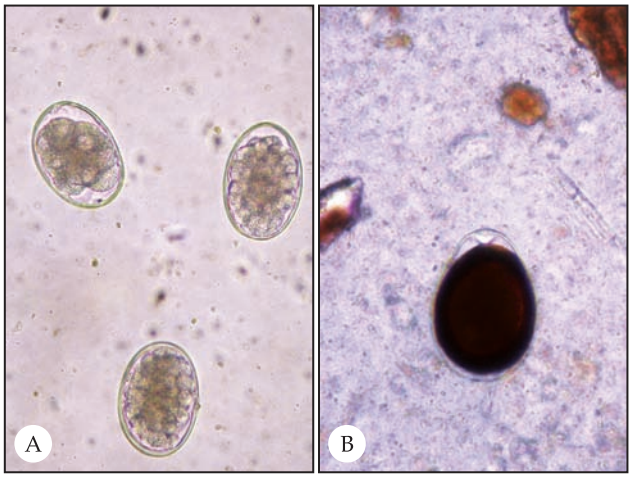

Fig 2. A. Eggs of *Haemonchus* from intestinal contents, B. An oocyst of *Eimeria cameli* from intestinal contents.

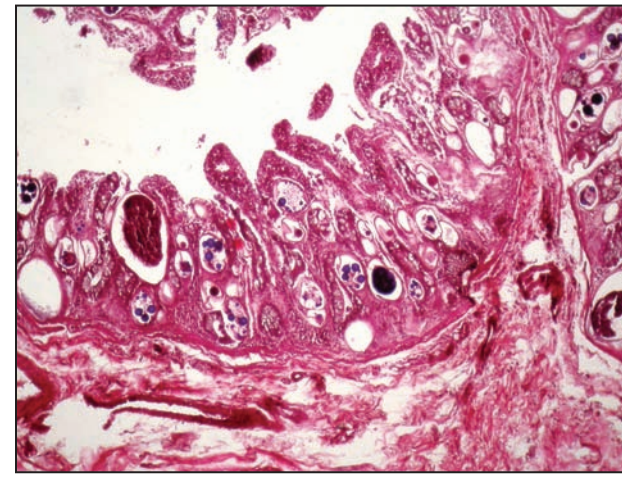

Fig 3. Various developmental stages of *Eimeria cameli* in the mucosa of small intestine (H & E X100).

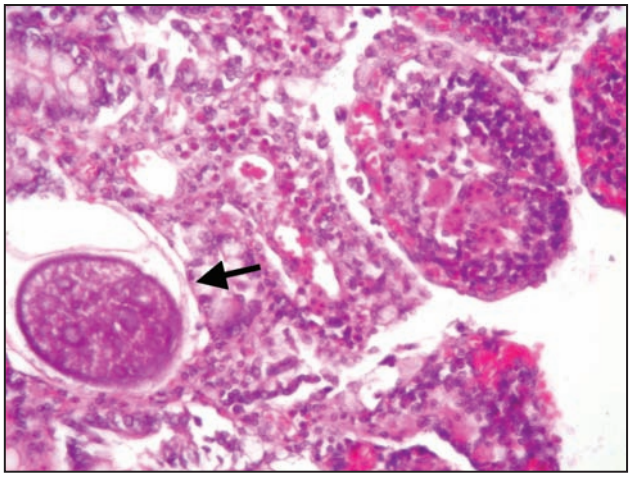

Fig 4. A large schizont of *Eimeria cameli* (arrow) containing merozoites surrounded by infiltration of eosinophils and mononuclear cells in the mucosa of small intestine (H & E X400).

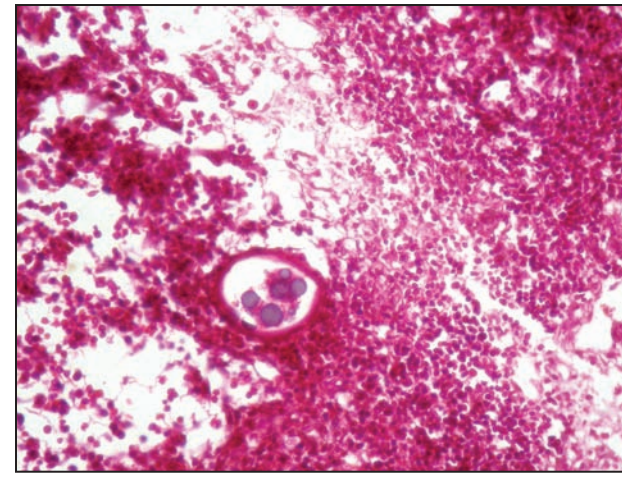

Fig 5. Mesenteric lymph node showing presence of oocyst of *E. cameli* along with eosinophilic infiltration (H & E X400).

developmental stages (oocysts, large schizonts, meronts and macrogamonts) of *E. cameli* infection were comparable with those described in previous studies in camels (Kinne and Wernery, 1998; Kumar *et al,* 2015). The coccidian parasites were also observed in the mesenteric lymph nodes of the present case which is contrary to the finding in sheep and goats (Tafti and Mansourian, 2008).

The differences between *Eimeria* spp. and their prevalence depend on some factors such as environment, animal factors, farm management and other factors (illness and stress) but clinical manifestations appear mostly under stressful conditions (Yakhchali and Cheraghi, 2007). The camel calf of the present study was also thought to be under stress due to concurrent *H. longistipes* infection and this may be the one of the reasons for more severe clinical signs and pathological lesions. In agreement with the finding of the present study, a significant correlation between age and severity of coccidiosis was observed in previous studies. The camel calves were found to be more infected with the *Eimeria* spp. than older ones, while adult camels were found to be chronic shedders of oocysts without manifesting clinical signs (Radfar and Gowhari, 2013). Contrary to this, camels only older than one year and adults revealed *Eimeria* coccidiosis in some studies (Kinne and Wernery, 1998; Sazmand *et al,* 2012). This might be due to the contamination of grazing pasture with coccidia oocysts by other camels. Another possibility of coccidial invasion is the habit of camels to ingest their own faeces (Kinne and Wernery, 1998). The winter season may also enhance the survival of coccidian parasite and the rate of infection was found higher in the winter season (Kinne and Wernery, 1998; Sazmand *et al,* 2012). The infected camel calf of

the present case also died during peak winter season. Besides this, one of the important factors was the immunosuppression due to *Haemonchus* infection. The fatal enteritis in camels caused by synergism between coccidiosis and haemonchosis has been reported in a previous study (Iyer *et al*, 1968). *H. longistipes* has been predominantly detected from the camels of Rajasthan region (Gahlot and Chhabra, 2009). The morphology of eggs and cultured larva of *Haemonchus* in the present study also corresponded with the *H. longistipes*.

On the basis of observations of the present study, it was concluded that the possible pathogenic mechanism responsible for cause of death in the camel calf of the present case having concurrent haemonchosis and coccidiosis infection could be haemorrhagic anaemia, hypoproteinemia and diarrhoea causing fluid loss and dehydration leading to hypovolemic shock and severe pathological consequences.

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INFLUENCE OF SEASON AND BREED ON MONTHLY MILK PRODUCTION OF DROMEDARY CAMELS REARED UNDER SEMI-INTENSIVE SYSTEM

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ABSTRACT

The objective of the study was to evaluate the influence of season (summer, autumn and winter) and breed on monthly milk production of dromedary camels reared under semi-intensive system. Eighteen multiparous female lactating camels in their early lactation were used. Milk production data (n=2184 milk yield samples) were collected from daily milk production records of 3 local breeds (Arabi, Kenani and Deali). The results revealed that female camels exposed to marked seasonal changes in the ambient temperature (Ta ^{o}C), relative humidity (RH %) and rainfall (mm). The higher rainfall recorded during autumn season was accompanied by an average of milk production of 3.2±1 litre/day in comparison to summer and winter values 3.0±1.8 and 2.6±1.2 litre/day, respectively. Season had a significant ($p<0.05$) effect on milk production; a significantly ($p<0.05$) higher mean value of milk production was recorded during autumn compared to winter value. Camel breed also had a significant ($p<0.05$) effect on milk production; a significantly ($p<0.05$) higher mean value of milk production was recorded by Arabi breed (3.3±1.6 litre/day) compared to Kenani and Deali breeds 2.9±1.1 and 2.6±1.1 litre/day, respectively. Season had a significant ($p\leq0.05$) effect on monthly milk production; significantly ($p<0.05$) higher mean values of milk production was observed during September and October compared to other months.

The present study confirms that both season and camel breed have significant impact on monthly milk production. Natural browsing, concentrate supplementation and water supply during summer could improve and maintain milk production in camels, regardless of environmental changes. Arabi breed can be considered as main breed for milk production in camel reared semi-intensive farms in Sudan. The effect of interaction of breed and season on milk production should be considered in the management programme of camel production system.

Key words: Breed, camels, milk production, season, semi-intensive system

Camels are recognised as suitable species for sustainable livestock production in arid and semi-arid areas (Eisa and Mustafa, 2011; Faye, 2013). The camels are not only capable of surviving under harsh and arid environmental conditions (Schwartz, 1992) but they can continue to produce milk during prolonged hot and dry periods (Guliye *et al,* 2000; Bekele *et al,* 2002). Many studies were conducted on milk production under semi intensive camel system as a current trend towards commercialisation of camel milk (Eisa and Mustafa, 2011; Shuiep and El Zubeir, 2012; Babiker and El Zubeir, 2014). However, other investigators have concluded that milk production under traditional pastoral system can provide milk with better nutritional contents compared to the farming system (Mustafa *et al,* 2014).

The significant contribution of camel milk to human nutrition in arid and semi-arid regions has been reported previously (Yagil, 1986). Faye (2005) noted that camel's milk production potential appears to be higher than that of cows reared under the same climatic and management conditions. Therefore, the vital importance of camel milk is due to its availability under semi-arid conditions when the milk from other livestock is inadequate. The camel milk is considered to be one of the most valuable food sources for people in semi-arid regions.

Milk production of camels varied depending on the region (Kamoun and Jemmali, 2012), breed (Wernery *et al,* 2004; Dowelmadina and El Zubeir, 2014; Elobied *et al,* 2015), stage of lactation (Musa *et al,* 2006; Raziq *et al,* 2008; Al-Saiady *et al,* 2012; Bakheit *et al,* 2015) and production system (Musa *et al,* 2006; Bakheit *et al,* 2008; Bakheit *et al,* 2015). Moreover, many researchers have concluded that the season has significant effect on milk production (Zeleke and Bekele, 2001; Bekele *et al,* 2002). In Sudan, milk production of the camels has been considered to be 5-10kg/day (Agab, 1993). However, few data is available regarding the interaction between

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season and breed on monthly milk production of lactating camels reared under semi- intensive system. Therefore, the objective of the present study was to evaluate the influence of season and breed on monthly milk production of dromedary camels reared under semi-intensive system.

Materials and Methods

Area of Study: The study was carried out at Camel Research Centre Farm, Faculty of Veterinary Medicine, University of Khartoum during 3 seasons: summer, autumn and winter (July 2012-June 2013). The farm was located at Shambat area, Khartoum state, Sudan (Latitude 15° North, 32° Longitude East). Shambat was described by many researchers as part of *Acacia* desert scrub (Andrews 1948; Smith 1949; Harrison and Jackson, 1958). Vegetation cover of Shambat, which was selected by lactating female camels has been reported in our previous study (Ahmed *et al,* 2014).

Climatic Data: The mean values of maximum and minimum ambient temperature (Ta max and Ta min), relative humidity (RH) and rainfall during the experimental period have been obtained from Shambat Meteorological Unit, Khartoum State.

Experimental animals and Management: Eighteen multiparous female lactating camels in their early lactation were used. The animals were reared under semi-intensive system. The animals grazed at open areas surrounding the farm until mid-day and then they were kept inside the farm for milking and supplement feeding with *Sorghum lactabiocolor* (Abu 70) and commercial concentrates. The animals had free access to fresh water.

Milk Production Data: A total number of 2184 milk yield samples were collected from daily milk production records of 3 local breeds: Arabi, Kenani and Deali. The milk yield was determined by milking front teats 2 times per day while the other 2 teats were left for suckling calves (University farm milking protocol). The total milk yield for whole udder was estimated by multiplying milk production from the two teats by two.

Statistical analysis: Statistical analysis was performed using SPSS for Windows version 20. The statistical measurements were estimated using General Linear Model (GLM), procedures (ANOVA) with reported measurements. Levine's Test and Post Hoc Test were used to assess the possible significant differences between monthly milk production, season and breed. The mean difference was considered significant at $p \leq 0.05$.

Results

Fig 1 shows that during summer (March-June) the highest Ta and RH were recorded in June (34.1°C and 27%), whereas the lowest were recorded in March (28.2°C and 17%). During autumn (July-October), the highest were recorded in July and August, respectively (32.5°C and 55%) while the lowest were recorded in October (24.3°C and 32%). During winter (November-February), the highest were recorded in November and December, respectively (35.5°C and 30%) while the lowest were recorded in December and February (16.4°C and 24%). During autumn, an average rainfall of 33.1, 40.5 and 69.2 mm was recorded in July, October and August, respectively. No rainfall was recorded during summer and winter seasons.

The season had a significant ($p<0.05$) effect on milk production (Table 1). Significantly ($p<0.05$) higher mean value of milk production was recorded during autumn as compared to winter values. Arabi and Kenani breeds recorded significantly ($p<0.05$) higher mean values of milk production of 3.22±2.7 and 3.21±1.2 litre/day during summer season compared to a value of 2.64±1.0 litre/day for Deali breed. Camel breed had a significant ($p<0.05$) effect on milk production (Table. 1); a significantly ($p<0.05$) higher mean value of milk production was recorded by Arabi breed (3.3±1.6 litre/day) compared to Kenani and Deali breeds (2.9±1.1 and 2.6±1.1 litre/day, respectively). Milk production during autumn season fluctuated significantly ($p<0.05$) among breeds; a significantly ($p<0.05$) higher mean value of milk production was recorded by Arabi breed (Table 1). During winter season, Arabi breed recorded a significantly ($p<0.05$) higher mean value of milk

Table 1. Effect of season and breed on milk production of camel reared under semi-intensive system (n=18).

Season	Number of milk samples	Breed			Mean
		Arabi	Kenani	Deali	
Summer	918	$3.22^{a\,A}$ ±2.7	$3.21^{a\,A}$ ±1.2	$2.64^{b\,A}$ ±1.0	3^{a} ±1.8
Autumn	726	$3.64^{a\,AB}$ ±0.8	$2.64^{b\,B}$ ±1.0	$3.21^{c\,B}$ ±1.0	3.2^{a} ±1
Winter	540	$3.01^{a\,A}$ ±1.3	$2.75^{a\,B}$ ±1.0	$2.04^{b\,C}$ ±1.2	2.6^{b} ±1.2
		3.3^{a} ±1.6	2.9^{b} ±1.1	2.6^{b} ±1.1	

Means within the same row (small letters) bearing different superscripts are significantly different ($p<0.05$) for breeds during 1 season.

Means within the same column (Capital letters) bearing different superscripts are significantly different ($p<0.05$) for breeds during 3 seasons.

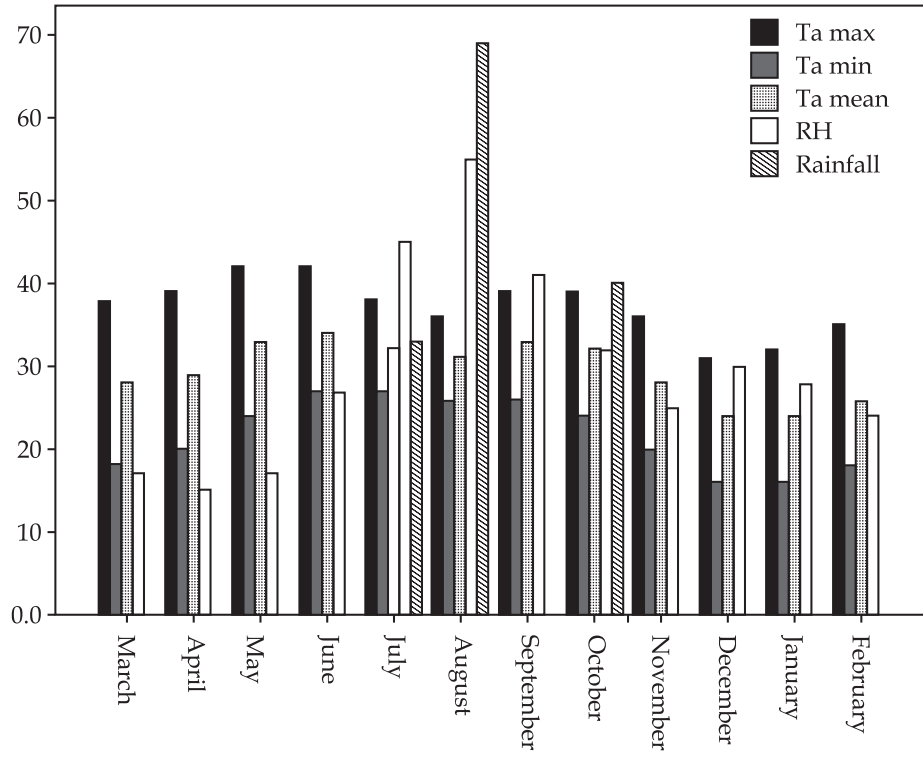

Fig 1. The ambient temperature (Ta ^{o}C), relative humidity (RH %) and rain fall (mm) during the experimental period (July 2012 - June 2013).

production (3.01±1.3 litre/day) compared to a value of 2.04±1.2 litre/day for Deali breed. Arabi breed recorded a significantly ($p<0.05$) higher mean value of milk production during autumn season compared to summer and winter values. For Kenani breed, the significantly ($p<0.05$) higher mean value of milk production was recorded during summer compared to autumn and winter values. Milk production for Deali breed showed significant ($p<0.05$) variation among seasons; the significant ($p<0.05$) higher mean value of milk production was recorded during autumn.

Season had a significant ($p\leq0.05$) effect on monthly milk production. There was a significant ($p<0.05$) gradual increase in monthly milk production during summer season (from April to June, Fig 2). Significantly ($p<0.05$) higher mean value of milk production (the peak of lactation) was observed in June compared to the other months. A gradual significant ($p\leq0.05$) increase in monthly milk production observed during autumn season (from July to September). Significantly ($p<0.05$) higher mean values of milk production was observed during September and October compared to the other months. Milk production during winter season showed a significant ($p<0.05$) higher mean value in November and December compared to the other months.

Camel breed had significant ($p\leq0.05$) effect on monthly milk production (Fig 3) except during June. Significantly ($p<0.001$-0.000) higher mean values of milk production were observed for Arabi during the whole lactation period. Monthly milk production for Kenani and Deali fluctuated significantly ($p<0.05$) among the lactation period except during the period from October to December (late autumn and early winter).

Discussion

The mean value of milk production reported in the present study for female camels reared under semi-intensive system with an average of 3 litre/day is similar to values reported by Bakheit *et al* (2008) for camels reared under semi-intensive system. However, the mean value was lower than that reported by Dowelmadina and El Zubeir (2014) and Bakheit *et al* (2015) and higher than that reported by Babiker and El-Zubeir (2014). The variation could be attributed to the variations in the stage of lactation, parity number and breed.

The results obtained in the present study showed that female camels exposed to marked seasonal changes in the ambient temperature (Ta), relative humidity (RH) and rainfall. Autumn, which is known in Sudan as rainy season was characterised by

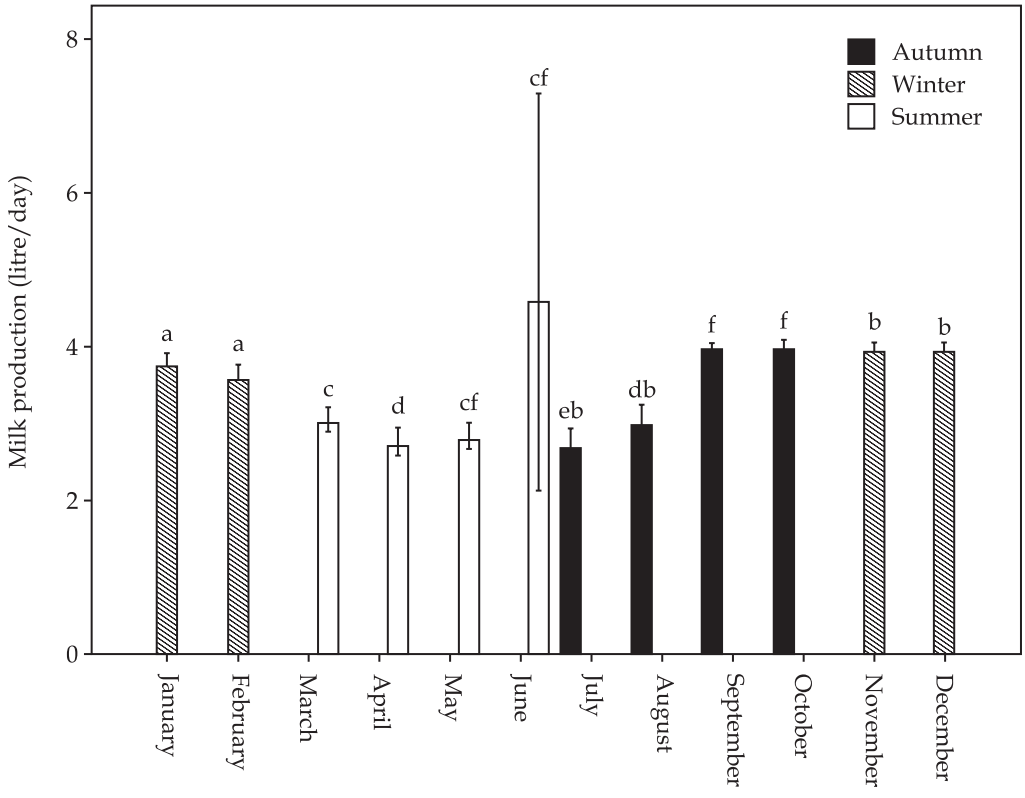

Fig 2. Effect of season on monthly milk production (litre/day) of camel (*Camelus dromedarius*) reared under semi-intensive system (n=18) (P≤0.05).

higher rainfall compared to summer and winter. The higher rainfall recorded during autumn season was accompanied by higher milk production of 3.2±1.0 litre/day in comparison to winter value (2.6±1.2 litre/day). The variation in milk production values could be attributed to the forage and water availability for lactating female camels used. The results clearly indicated that the season had significant impact on monthly milk production. Seasonal variations in milk production of camels have been observed by Salman (2002) who reported that milk production of lactating camels reached 8.0 litres/day in the rainy season and it declined to 1.38 litres/day by the end of summer season. Similar results have been reported previously by many researchers (El Amin *et al,* 2006; Zeleke, 2007; Bakheit *et al,* 2008; Haddadin *et al,* 2008; Shuiep *et al,* 2008).

The present results reported for the first time data for monthly milk production for 3 local camel breeds reared under semi-intensive system in Sudan. However, Bakheit *et al* (2015) noted that the season had significant impact on monthly milk production in camels under nomadic system. The higher mean values of milk production obtained in September and October compared to the other months could be attributed to the availability of natural browsing and water supply. The higher rainfall during July and August could give a chance for suitable and favourable environment for forage growth in the following 2 months, which in turn improved the nutritional values of the plants selected by the female camels. Mustafa *et al* (2014) indicated that natural pasture is more variable in plants and vegetations and thus provides many varieties preferred by the camels.

Significant differences were observed among the 3 studied breeds in the mean values of milk production (Table 1). The results agreed with those of other researchers (Al haj and Al Kanhal, 2010; Riyadh *et al,* 2012; Babiker and El Zubeir, 2014; Dowelmadina and El Zubeir, 2014; Dowelmadina *et al,* 2014; Elobied *et al,* 2015) who reported that milk production was significantly affected by breed of lactating camels. The results obtained in the present study indicated that Arabi breed had higher milk production value (3.3±1.6 litre/day) compared to the two breeds (2.9±1.1 litre/day and 2.6± 1.1 litre/day, respectively (Table 1). However, Elobied *et al* (2015) reported that Kenana breed showed the higher milk yield compared to Arabi. This may be attributed to variations in genetic potential for milk production between the 3 breeds, in addition to the significant impact of season as depicted in Table 1. Therefore, the present study recommends

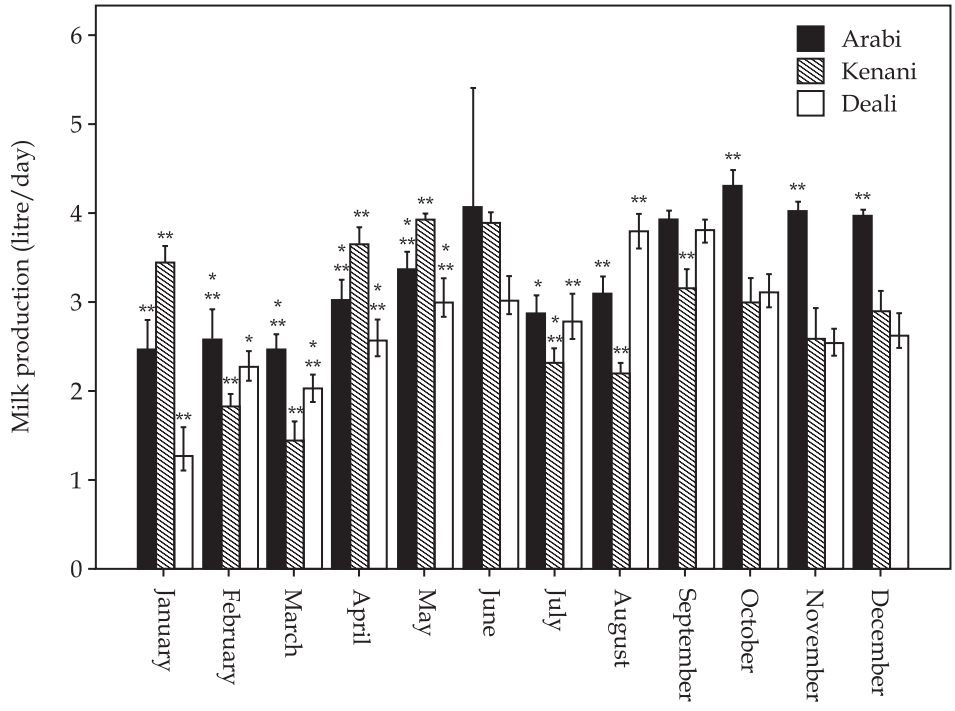

Fig 3. Effect of breed on monthly milk production (litre/day) of camel (*Camelus dromedarius*) reared under semi-intensive system (n=18) (P<0.001* and P<0.0001**).

that Arabi breed to be considered as main breed for milk production in camels reared under semi-intensive system in Sudan. The results also indicated that the interaction of breed and season on monthly milk production particularly during summer should be considered in the management programme of camel production system.

The present study confirms that both season and camel breed have significant impact on monthly milk production. Natural browsing, concentrate supplementation and water supply during summer season could improve and maintain milk production in camels, regardless of environmental changes. Arabi breed can be considered as main breed for camel milk production in semi-intensive farms in Sudan.

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PASSIVE IMMUNISATION AGAINST *Brucella melitensis* IN DROMEDARIES

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ABSTRACT

Four female and 1 male dromedaries serologically positive for *Brucellosis,* were treated 3 times with enrofloxacin and Brucella IgGs, intravenously over a time period of 3 months. Despite this treatment, the brucellosis positive dromedaries remained serologically positive.

Key words: *B. melitensis*, dromedary, hyperimmune serum, passive immunisation, zoonosis

Passive immunisation involves the production of antibodies in an animal by active immunisation. These antibodies can be stored as immunoglobulins (IgGs) and then administered to the diseased or susceptible animals to achieve immediate but short-lived protection which may last between 4 weeks to 3-4 months. IgGs are generally produced in young horses after a series of immunising inoculations. Their most important role is the protection against toxigenic organisms like *Clostridium tetani* or *C. perfringens* (Toxoid vaccines).

Brucellosis is a contagious disease caused by bacteria of the genus *Brucella*. Taxonomically, the genus *Brucella* is divided into 10 classified species and subdivided into *biovars*. Human brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 new cases annually (WHO and FAO, 1986). Infection prevalence in the animal reservoirs determines the incidence of human cases (Von Hieber, 2011). Brucellosis in camelids has a wide distribution and is mainly caused by *B. melitensis* on the Arabian Peninsula (Wernery, 2016). Antibiotic treatment has been tried in dromedaries with different results (Radwan *et al*, 1995; Wernery, 2016). However, treatment is forbidden in many countries and unlikely to be cost efficient or therapeutically effective because of the intracellular sequestration of the organisms, mainly in lymph nodes (Wernery *et al*, 2007).

We, therefore, tried another approach by giving 5 serologically positive *Brucella* dromedaries, a *B. melitensis* hyperimmune serum, the results of which are presented here.

Materials and Methods

Five dromedaries were selected for this experiment, of which 4 were non-pregnant females between 4 to 6 years of age and a 12 year-old non-castrated bull. All 5 dromedaries were serologically positive for *Brucellosis* which was confirmed by Rose Bengal Test (RBT) and Complement Fixation Test (CFT) several times over a period of 2 months before passive immunisation. Their blood was also regularly tested for the presence of *Brucella* using a culture method described by Wernery *et al* (2015).

Production of the *B. melitensis* hyperimmune serum

For the production of the hyperimmune serum, a 23 year-old female dromedary was chosen which tested negative for *Brucella* antibodies on different occasions using RBT and CFT tests. A *B. melitensis* strain named EM2 (East Mediterranean strain 2, Soellner, 2018 in press) was selected for the active immunisation of the 23 year-old camel. EM2 was isolated from the placenta of a dairy dromedary. The strain was grown on Farrell's media and an inoculum of 4.8×10^8 cfu/ml was prepared. The bacteria was later killed by adding concentrated formalin (37%) at a ratio of 50% of the inoculum. Before immunisation, 0.5 ml Advax adjuvant (ADVAX^{TM} CxL vaccine adjuvant, vaccine Tty Ltd., Australia) was added to 1.0 ml of the killed *B. melitensis* antigen and thoroughly mixed. This mixture was given subcutaneously at the base of the neck 6 times, one each week.

When RBT and CFT results showed high levels of antibodies, the camel was bled. The production of IgG followed the procedure described by Wernery *et*

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al (2009) and Joseph *et al* (2012) for the production of a *Clostridium perfringens* A hyperimmune serum, which is described herewith briefly.

Approximately 6 litres of jugular blood was collected in blood bags and after centrifugation the plasma was frozen at -20°C. When 30 litres of plasma was gained, IgG was extracted. The dromedary plasma was subjected to a series of processes which included solvent-detergent extraction that effectively inactivates possible lipid-enveloped viruses. Serum protein of the product was then precipitated by caprylic acid (octanoic acid). Subsequent filtration and chromographic separations resulted in highly purified IgGs with a content of 14g/l. The hyperimmune serum was filled in 100 ml sterile transfer bags (Compoflex, Fresenius Kabi AG, Germany) and stored at 4°C before being used.

Passive immunisation of dromedaries against *B. melitensis* infection

Five *Brucella* serologically positive dromedaries were given Baytril™ (Enrofloxacin, Bayer Healthcare, USA) and anti-*Brucella* hyperimmune serum. The 4 female dromedaries weighing approximately 280 to 300 kg each received for 7 days 20 ml Baytril i. v. and on the 7th day additionally, 500 ml hyperimmune serum i.v. The 420 kg bull received 25 ml of Baytril for 7 days and 1,000 ml hyperimmune serum i. v. The entire procedure was repeated 3 times within a time frame of 3 months. After this procedure, every week, their sera were tested for *Brucella* antibodies using RBT and CFT.

Results and Discussion

Four female dromedaries and one bull were chosen for this experiment. They were tested *Brucella* positive 5 times using RBT and CFT before the treatment with Enrofloxacin and hyperimmune serum. No *Brucella* organisms were isolated from their blood on all 5 occasions.

No side effects were observed in any of the 5 dromedaries after the 3 intravenous administrations of the hyperimmune serum. However, all 5 dromedaries remained *Brucella* serologically positive 6 months after start of the treatment.

Passive immunisation and immunotherapy products are commercially available against tetanus, rabies, botulism, hepatitis A and B, diphtheria and Eebola. During the 1995 Ebola outbreak in the Democratic Republic of Congo, 8 infected persons were given antibodies from Ebola survivors and 7 survived.

It seems that antibiotherapy of camelid *Brucellosis* is not effective due to the intracellular sequestration of the pathogen. Therefore, with this study we chose a different approach of passive immunisation with anti-*Brucella* IgGs produced in a homologous system and not in equines or ovines which is the usual procedure. We also chose this therapy, because of the unique properties of camelid IgGs which lack light chains. They are therefore, smaller, had shown to migrate across blood/tissue barriers and penetrate deeper into tissues (Harrison and Wernery, 2016).

Meddeb-Monetli *et al* (2003), Harrision *et al* (2006) and Wernery *et al* (2012) used camel IgG to neutralise scorpion toxins, snake venoms and *Clostridium perfringens* A toxins.

Despite, the 3 times treatment with enrofloxacin and *Brucella* IgGs, the serological *Brucella* positive dromedaries remained positive. The passive immunisation had no effect and the *Brucella* positive dromedaries remained serologically positive 6 months after treatment.

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PHARMACOKINETICS OF CEFQUINOME FOLLOWING INTRAVENOUS AND INTRAMUSCULAR INJECTION IN CAMELS

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ABSTRACT

The pharmacokinetics of cefquinome was determined in camels following single intravenous and intramuscular injection at a dose of 1 mg/kg into 5 healthy she-camels. A crossover study was carried out in 2 periods separated by 30 days clearance period. Cefquinome concentrations in plasma were determined by LC-MS/MS assay. Cefquinome concentration *vs* time data after IV and IM was best fitted to a two-compartment open model. Cefquinome mean values of area under concentration–time curve (AUC) were 15.37 ± 1.06 and 12.85 ± 2.15 µg/ml/h after IV and IM injection, respectively. Distribution and elimination half-lives were 0.14 ± 0.04 h and 3.15 ± 0.22 h after IV dose and 1.42 ± 0.11 h and 6.68 ± 0.87 h after IM administration. The value of total body clearance (Cl_{tot}) was 0.07 ± 0.001 L/kg/h and volume of distribution at steady state (V_{ss}) was 0.27 ± 0.02 L/kg. In conclusion, cefquinome persisted in plasma for 12 hours at concentration that exceeds the MIC for many microorganisms such as *Streptococcus* spp., *Staphylococci*, *Klebsiella* spp., *Pasteurella* spp., *Salmonella* spp. and enteric and systemic *Escherichia coli*. Therefore, it is suggested using cefquinome twice daily intravenously or intramuscularly at a dose of 1mg/kg in camels.

Key words: Camels, cefquinome, LC-MS/MS assay, pharmacokinetics

Cefquinome is an injectable aminothiazolyl cephalosporin derivative. In veterinary medicine, cefquinome is approved and used for several animal species in many countries worldwide (Aarestrup and Skov, 2010).

The pharmacokinetics of cefquinome has been studied in various animal species including, sheep (Uney *et al,* 2011), goats (Dumka *et al,* 2013), buffalo calves (Dinakaran *et al,* 2013), cattle (Shan *et al,* 2014; Ahmad *et al,* 2015), piglets (Li *et al,* 2008), horses (Winther *et al,* 2011), dogs (Zhou *et al,* 2015), boars (Liu *et al,* 2012), ducks (Yuan *et al,* 2011) and chickens (Xie *et al,* 2013). Camels have peculiar physiological and biochemical features, which may be revealed in their response to xenobiotics and in the disposition of drugs given to them (Kadir *et al,* 1997 and Oukessou *et al,* 1999). Camels have comparatively low glomerular filtration rate and renal plasma flow (Etzion and Yagil, 1986). The pharmacokinetics of cefquinome in camels following IM injection was studied. Data concerning the pharmacokinetic profile of cefquinome after IV injection and bioavailability after IM injection in camels are lacking. The aim of this study was to investigate pharmacokinetic profile of cefquinome in healthy camels following single IV and IM administration and to recommend a rational dosage schedule for potential use of cefquinome in camel diseases caused by susceptible microorganisms.

Materials and Methods

Drugs

Cefquinome sulfate analytical standard was obtained from Intervet International (Mechelen, Belgium). Cefquinome (Cobactan® IV 4.5%) was procured from Intervet International Company, Netherlands for present study.

Animals

The present experiment was accomplished at the Centre for the Studies and Development of Camels in Matrouh Governorate (Animal Production Research Institute), Egypt. The study was conducted on 5 she-camels with 440-570 kg body wt. Animals were kept under the best hygiene condition, fed on green fodder, concentrated mixture, hay and water was provided *ad-libitum*. None of the animals

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were treated with chemical agents for one month before the trial. Apparently healthy animals were clinically inspected and blood and faecal samples were examined to assure that animals are free from blood and intestinal parasites. The she-camels were injected into the left jugular vein with cefquinome 1 mg/kg. b.wt. The animals were then marked and blood samples (10 ml) were collected before and at 5, 15 and 30 min, 1, 2, 3, 4, 6, 8, 12 and 24 h after cefquinome injection from the right jugular vein. The samples were drawn into heparinised tubes and the plasma was separated immediately by centrifugation at 3000 rpm for 20 min and stored at -20°C until analysis. Animals were then left for 30 days after the intravenous injection to ensure complete elimination of cefquinome from their bodies. Then, each she-camel was injected intramuscularly into the deep gluteal muscle of the hindquarter with cefquinome in the same dose. Blood samples were withdrawn after intravenous injection and plasma was collected for determination of cefquinome concentration.

Analysis of cefquinome

Preparation of standard solution

Stock standard solutions of cefquinome were prepared at concentrations of approximately 1000 µg /ml in MeOH, divided into small portions and stored in amber glass vials at −20°C. Working solutions for plasma (0.005, 0.01, 0.05, 0.1, 0.5,1, 2, 5, 10 µg/ml) were obtained by further diluting the stock solution with MeOH. Standard curve in plasma solution was drawn by plotting the peak area against the corresponding concentration of cefquinome. All chemicals utilised in this study were of analytical grade or HPLC grade quality.

LC/MS analysis:

LC/MS/MS 4000 QTRAP (Applied Bioscience): Advanced Linear ion trap liquid chromatography was utilised for quantitative analysis of cefquinome. The mass conditions were adopted according to Shi-juan *et al* (2012). The extraction procedure was carried out according to the method described by Li *et al* (2014). The European Commission guidelines and criteria were utilised to assess the method validation. Selectivity was determined from retention time, ion ratios and identification points (IP) for cefquinome (EC, 2006).

Pharmacokinetic analysis

The pharmacokinetic parameters were calculated by PK Solver: An add-in program for Microsoft Excel, version 2 (Zhang *et al*, 2010). The mean pharmacokinetic variables were obtained by averaging the variables calculated for drug disposition after IV or IM administration to each camel. The proper pharmacokinetic model was determined by visual examination of individual concentration–time curves and by application of Akaike's Information Criterion (AIC).

Statistical Analysis

Differences between means of data obtained from intravenous and intramuscular routes were tested for significance by the Student 't' test using SPSS 14.

Results and Discussion

No clinical signs of adverse effects or intolerance were observed to cefquinome after IV injection in camels. The used analytical method proved linear and reproducible for the detection of cefquinome in plasma samples at concentration ranged from 0.005 to 10 µg/ml. The limit of detection (LOD) and the limit of quantification (LOQ) of the assay were 0.001 and 0.005 µg/ml, for plasma the recovery of cefquinome in plasma was 91.16 ± 0.036%. The intraday and the interday variation coefficients were less than 10 and 15% in all cases, respectively.

Following a single intravenous or intramuscular injection of cefquinome in camels, the drug plasma concentration *vs* time followed the 1st order 2 compartments open model (Fig 1). Cefquinome was detected in plasma after 24 h of IV and IM administration at a concentration of 0.023 ± 0.003 and 0.017 ± 0.002 µg/ml, respectively (Fig 1 and 2). The pharmacokinetic parameters of cefquinome following IV and IM injection are recorded in table 1. Cefquinome after an intravenous dose revealed a rapid distribution half-life ($t_{½\alpha}$) of 0.14 ± 0.04 h). The apparent volume of distribution at steady state (V_{ss}) was 0.27 ± 0.02 l/kg. The half-life ($t_{½\beta}$) of elimination was 3.15 ± 0.22 h. Cefquinome was cleared by all clearance processes in the body at a rate of 0.07 ± 0.001 l/kg/h. The mean residence time (MRT) was 4.21 ± 0.29 h.

Following a single IM injection, cefquinome achieved maximum serum concentration (C_{max}) of 3.2 ± 0.39 µg/ml after a maximum time (T_{max}) of 0.82 ± 0.06 h. The absorption half-life ($t_{½ab}$) was 0.26 ± 0.03. The elimination half-life $t_{½\beta}$ was 6.68 ± 0.87 hours. The mean systemic bioavailability of cefquinome following a single IM injection was 85.52 ± 11% (Table 1).

In Comparison of pharmacokinetics parameters of cefquinome following a single intravenous and intramuscular injection, the results revealed that A, α, B, β, k_{10}, k_{12}, k_{21} were significantly lower after intramuscular than those after intravenous administration of the same dose. On the other hand, the $t_{1/2\alpha}$, $t_{1/2\beta}$ and MRT were significantly longer although, the AUC was lower after intramuscular injection.

Cefquinome plasma concentration following a single intravenous (IV) injection in healthy camels indicated that the disposition of cefquinome obeyed the 1st order 2 compartments open model, as the decline in the drug concentrations is curvilinear on the semilogarithmic scale. In this study, the difference between the distribution rate constant (α, 5.28 ± 1.59 h^{-1}) and the slow post-distribution rate constant (β, 0.22 ± 0.02 h^{-1}) is vast. This indicates the existence of a two-compartment model (Jambhekar and Breen, 2009) and reflecting a very short distribution half-life in comparison to the long elimination half-life, the fact that was obvious in the present study. The obtained result was consistent with those reported for cefquinome in sheep (Uney *et al,* 2011), goats (Dumka *et al,* 2013), buffalo calves (Dinakaran *et al,* 2013), cattle (Shan *et al,* 2014; Ahmad *et al,* 2015), piglets (Li *et al,* 2008), horses (Winther *et al,* 2011), dogs (Zhou *et al,* 2015), boars (Liu *et al,* 2012), chickens (Xie *et al,* 2013) and ducks (Yuan *et al,* 2011). Plasma cefquinome concentration decreased gradually until reaching to 0.02 ± 0.003 µg/ml 24 hours post intravenous injection.

In this study, the 1st-order elimination rate constant of cefquinome from the central compartment (K_{10}) following a single IV injection (0.59 ± 0.05 h^{-1}) indicates the faster elimination rate. This observation is about similar to that reported after IV administration of cefquinome in buffalo calves (Dinakaran *et al,* 2013) and in cattle (Shan *et al,* 2014). However, higher values were previously recorded for cefquinome in goats (Dumka *et al,* 2013) and in porcine (Zhang *et al,* 2014) but lower values were registered in horse (Winther *et al,* 2011) and cattle (Ahmad *et al,* 2015).

Cefquinome was transferred from the central to the peripheral compartment at higher rate (K_{12} = 2.96 ± 1.09 h^{-1}) than its passage from the peripheral to the central compartment (K_{21} = 1.95 ± 0.47 h^{-1}). This pattern coincided with that reported for cefquinome in cattle (Shan *et al,* 2014). The value of K_{12} was about like to that reported for cefquinome in the porcine (Zhang *et al,* 2014). The value of K_{21} of cefquinome in camel was higher than the value in goat (Dumka *et al,* 2013), in horse (Winther *et al,* 2011), in cattle (Ahmad *et al,* 2015) but it was lower than the value in porcine (Zhang *et al,* 2014).

The elimination rate constant [β] of cefquinome following a single IV injection was 0.22 ± 0.02 h^{-1}. This

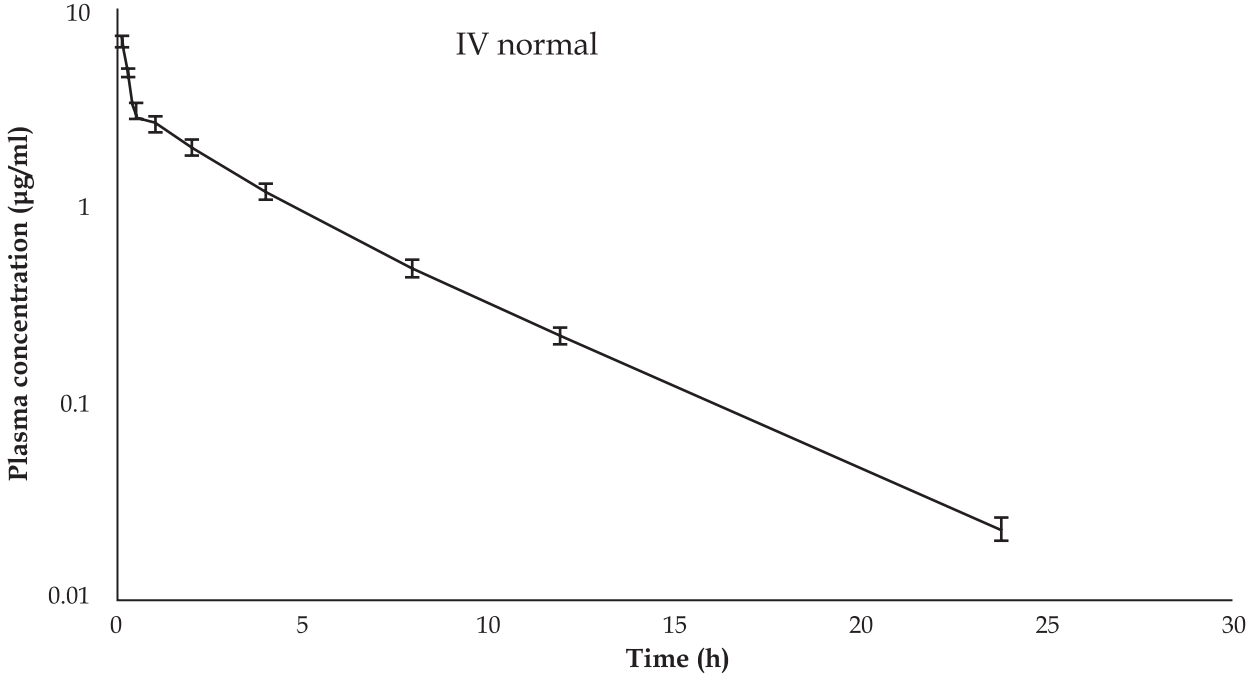

Fig 1. Semilogarithmic graph depicting the time course of cefquinome in plasma of camels (n=5) following a single intravenous injection of 1 mg/kg b.wt.

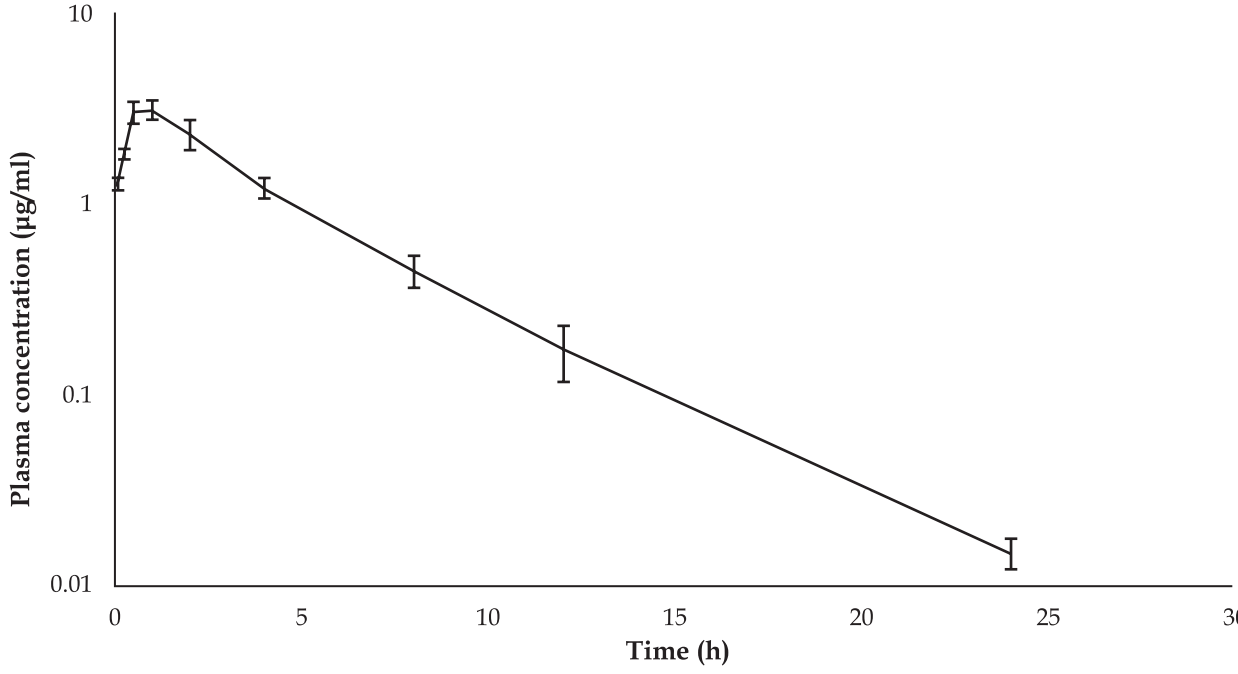

Fig 2. Semilogarithmic graph depicting the time course of cefquinome in plasma of camels (n=5) following a single intramuscular injection of 1 mg/kg b.wt.

is nearly similar to that reported in the horse (Winther *et al,* 2011). However, the obtained value was shorter than that recorded in other species such as dogs (Zhou *et al,* 2015) and piglets (Li *et al,* 2008). The elimination half-life ($t_{½\beta}$; 3.15 ± 0.22 h) of cefquinome following a single IV injection was nearly similar to that reported in the horse (Winther *et al,* 2011) and buffalo calves (Dinakaran *et al,* 2013). On the contrary, this obtained value was longer than that recorded in cattle (Ahmad *et al,* 2015), dog (Zhou *et al,* 2015) and piglet (Li *et al,* 2008; Zhang *et al,* 2014) but it was shorter than that reported in goat (5.76 ± 0.19 h) (Dumka *et al,* 2013).

The value of AUC obtained in the present study was 15.44 ± 1.07 µg/ml.h. This value is consistent with the values reported in the horse following IV administration of similar dosage rate (Winther *et al,* 2011) and nearly similar to that reported in crossbred wild boars (13.85 ± 2.57 µg/ml.h) following IV administration of cefquinome at double doses (Liu *et al,* 2012). Although it is much lower than that reported in goats (33.83 ± 2.53 µg/ml.h) (Dumka *et al,* 2013) and buffalo calves (32.9 ± 0.56 µg/ml.h) (Dinakaran *et al,* 2013) following IV of double the dose. On the contrary, this obtained value was higher than that reported in other species such as sheep (5.83 ± 0.45 µg/ml.h)(Uney *et al,* 2011), piglets (8.07 ± 1.91 µg/ml.h) (Li *et al,* 2008) following IV administration of 2 mg/ kg b.wt. It appears that species of the animal rather than dose is the more important factor for these discrepancies.

The rate of total body clearance of cefquinome (CL_{tot}; 0.07 L/kg/h) was similar to that reported in buffalo calves (0.06 L/kg/h) (Dinakaran *et al,* 2013) after IV administration of 2 mg/kg. On the contrary, the reported value, was lower than that reported after IV administration of cefquinome in the horse (0.12 ± 0.02 L/kg/h) (Winther *et al,* 2011) and cattle (CL 0.12 ± 0.00 L/kg/h) (Ahmad *et al,* 2015) and (0.11 ± 0.02 L/kg/h) (Shan *et al,* 2014) after IV administration of cefquinome at a similar dosage rate. The slower clearance rate (CL_{tot}) of cefquinome in camel in comparison to other species could be related to the comparatively low glomerular filtration rate and renal plasma flow in camels (Etzion and Yagil, 1986) and to their specific physiological and biochemical features, which may be reflected to their response to xenobiotics and in the disposition of drugs given to them (Kadir *et al,* 1997; Oukessou *et al,* 1999). Dissimilarities in the kinetic parameters of drugs are relatively common and might be attributed to assay methods used, age, animal species, breed, health status of the animal and formulation of the used drug (El-Sayed *et al,* 1989).

In the present study, cefquinome was rapidly absorbed from the site of injection after a single IM administration with a short absorption half-life ($t_{½ab}$; 0.26 ± 0.03 hours). This value was about like

Table 1. Pharmacokinetic parameters of cefquinome following a single IV and IM injection of 1 mg/kg b.wt. in camels (mean ± SD, n=5).

Parameters	Unit	Intravenous	Intramuscular
B.wt	Kg	519.4 ± 46.57	519.4 ± 46.57
A	µg/ml	6.02 ± 1.02	4.98 ± 0.77***
α	h^{-1}	5.28 ± 1.59	0.49 ± 0.04***
B	µg/ml	3.15 ± 0.33	0.57 ± 0.24***
β	h^{-1}	0.22 ± 0.02	0.11 ± 0.01***
k_a	h^{-1}	-	2.7 ± 0.35
k_{10}	h^{-1}	0.59 ± 0.05	0.35 ± 0.01***
k_{12}	h^{-1}	2.96 ± 1.09	0.1 ± 0.02***
k_{21}	h^{-1}	1.95 ± 0.47	0.15 ± 0.03***
$t_{1/2\alpha}$	h	0.14 ± 0.04	1.42 ± 0.11***
$t_{1/2\beta}$	h	3.15 ± 0.22	6.68 ± 0.87***
$t_{1/2ab}$	h	-	0.26 ± 0.03
C^0	µg/ml	9.18 ± 1.26	-
T_{max}	h	-	0.82 ± 0.06
C_{max}	µg/ml	-	3.2 ± 0.39
V	L/kg	0.11 ± 0.01	-
V/F	L/kg	-	0.22 ± 0.05
Cl_{tot}	L/kg/h	0.07 ± .001	-
V_2	L/kg	0.16 ± 0.02	-
V_2/F	L/kg	-	0.15 ± 0.02
CL_2	L/kg/h	0.32 ± 0.07	-
CL/F	L/kg/h	-	0.08 ± 0.02
CL_2/F	L/kg/h	-	0.02 ± 0.001
AUC_{0-24}	µg/ml.h	15.37 ± 1.06	12.85 ± 2.15*
$AUC_{0-\infty}$	µg/ml.h	15.44 ± 1.07	13.25 ± 2.23
AUMC	$\mu g/ml.h^2$	65.06 ± 7.04	68.64 ± 14.58
MRT	h	4.21 ± 0.29	5.14 ± 0.27***
V_{ss}	L/kg	0.27 ± 0.02	-
F	(%)	-	85.52 ± 11.0

C^0 plasma concentration, α and β, distribution and elimination rate constants; k_{10}, k_{12}, k_{21} and k_a; the first-order rate constants, $t_{1/2\alpha}$, $t_{1/2\beta}$ and $T_{1/2ab}$ distribution, elimination and absorption half-life, V and V_2 apparent volume of central and peripheral compartment, Cl_{tot} and CL_2; total body and intercompartmental clearances; T_{max}, the time point of maximum plasma concentration C_{max}, AUC_{0-24} and $AUC_{0-\infty}$, area under plasma drug concentration vs time curve to 24h and to infinity, AUMC, area under the first moment curve; MRT, mean residence time; V_{ss}, volume of distribution at steady state; F%, Bioavailability, V/F, volume of central compartment corrected for bioavailability; CL/F, body clearance corrected for bioavailability; V_2/F, volume of peripheral compartment corrected for bioavailability; CL_2/F, intercompartmental clearance corrected for bioavailability.

to that reported for cefquinome in cattle (0.29 ± 0.07 h) (Shan *et al,* 2014), sheep (0.31 ± 0.05 h) (Uney *et al,* 2011), goats (0.64 ± 0.23 h) (Dumka *et al,* 2013) and piglets (0.41 ± 0.36 h) (Li *et al,* 2008), although higher value was reported previously in camel (4.35 ± 27 h) (Al-Taher, 2010) who used the microbiological assay method for estimation of cefquinome concentration. This assay method measure the activity of the drug in serum rather than estimation of the drug itself. However, the reported value was higher than that reported for cefquinome in dogs (0.14 ± 0.05h) (Zhou *et al,* 2015).

The reported maximum serum concentration (C_{max}; 3.2 ± 0.39 µg/ml) was higher than (C_{max}; 1.23 ± 0.08 µg/ml) that reported in camel (Al-Taher, 2010) and was achieved at short time (T_{max}; 0.82 ± 0.06 hours) than that reported previously (T_{max}; 4.25 ± 0.1 h). This is probably due to the use of different assay method. The reported Cmax and time to maximum concentration in this study was nearly similar to

those reported in crossbred wild boars (C_{max} 3.89 ± 0.51 µg/mL and Tmax 0.66 ± 0.07 h) (Liu *et al,* 2012). However, the reported C_{max} was higher than that reported in cattle (C max 2.34 ± 0.12 µg/ml) receiving cefquinome at similar dosage rate (Shan *et al,* 2014), although, it was attained after similar T_{max} (0.78 ± 0.32 h). On the contrary, the obtained results were lower than that reported in goat (C_{max} 4.84 ± 0.23 µg/ml, Tmax 1.50 ± 0) (Dumka *et al,* 2013) and dogs (C_{max} 4.83 ± 0.79 µg/ml, Tmax 0.43 ± 0.11 h) (Zhou *et al,* 2015) following IM administration of cefquinome at double doses. The differences could be attributed to the differences in doses in addition to species difference. The bioavailability of cefquinome in normal camels, which assesses the per cent of the dose, entered the systemic circulation after IM injection was 85.52 ± 11%. This indicates proper absorption of cefquinome after IM injection. This value was similar to those recorded in pigs (85.13 ± 9.93%) (Lu *et al,* 2007) and in sheep (89.31 ± 6.06 %) (Uney *et al,* 2011) but it was higher than that reported in goat (Dumka *et al,* 2013). However, higher values were reported in dogs (Zhou *et al,* 2015).

Since cefquinome is a β-lactam antimicrobial and acts as a time-dependent bactericidal drug (Thomas *et al,* 2006), the most suitable PK–PD parameter to describe drug efficacy is the time during which the drug's concentration exceeds the minimum inhibitory concentration (T > MIC) (Zonca *et al,* 2011).

The lower values of A, α, B, β, k_{10}, k_{12}, k_{21} after intramuscular injection were expected because when drug is absorbed from outside the systemic circulation, as with intramuscular doses, the peak plasma drug concentration occurs sometime after time zero rather than at time zero, as with an IV drug injection. The peak plasma concentration occurs at the point where the amount eliminated and the amount absorbed is equal. When a drug is absorbed more slowly, such as after an intramuscular injection, it will have a smaller peak concentration and a slightly longer duration of action than the IV administration of the same drug. The slow intramuscular absorption allows significant drug elimination to occur before absorption is complete. This could explain the relatively lower value of AUC after an intramuscular injection. Both physicochemical and physiologic factors influence the rate of drug absorption from the site of an intramuscular injection that explains the delayed distribution half-life and explain the longer elimination half-life. One potential determinant is the drug's partition between aqueous and lipid phases. Lipophilic drugs can diffuse directly through the membranes in contrast to cefquinome, which is characterised by low fat solubility. The concentration of the injected solution can also affect the rate of absorption. Another factor that influences the rate of absorption is the total surface area available for diffusion with which the injected solution is in contact (Koch-Weser and Greenblatt, 1976).

The results of this study indicate that a dosage regimen of 1 mg /kg body weight at 12 h intervals following IV or IM injection of cefquinome would maintain the plasma levels between 0.28 and 0.18 µg/ml which is ≤ MIC for susceptible bacterial pathogens particularly *S. agalactiae, S. dysgalactiae, P. multocida, E. coli* and Enterobacteriaceae. The IM route exhibited longer elimination half-life.

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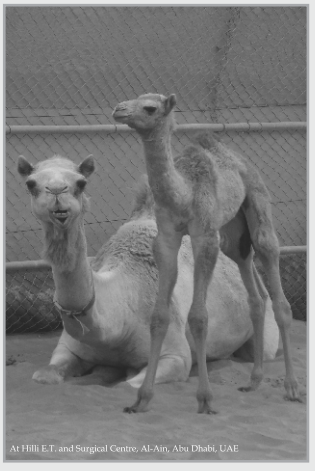

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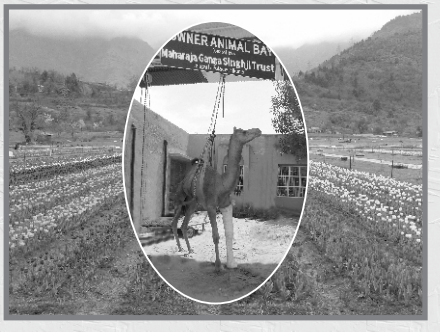

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PROTECTIVE EFFECTS OF CAMEL MILK ON INFLAMMATORY AND ANTIOXIDANT BIOMARKERS IN THE OFFSPRING WITH EXPERIMENTAL STREPTOZOTOCIN-INDUCED DIABETES

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ABSTRACT

Camel milk possess rich content of antioxidants help in ameliorating some chronic disease. The purpose of this study was to investigate the effects of supplemental camel milk on apoliproteins, leptin, hyperhomocyteinemia as well antioxidant status in rats subjected to induced-streptozotocin (STZ) diabetes. Pregnant rats were assigned to 3 groups of 12 rats in each group; Group 1: Control; Group 2: Diabetic with citrate and Group 3: Diabetic with camel milk. Diabetes was induced by STZ at a dose of 49 mg/kg dissolved in citrate buffer. Blood glucose concentrations was measured on a weekly basis after inducing diabetes and the concentrations exceeded 200 mg/dL is a confirmed diabetic rats. The study indicated that camel milk improved blood profile of the measured parameters and these effects are related to antioxidant properties. Current findings confirmed an increase in homocysteine (Hcy), cathepsin G and apolipoprotein B (ApoB) levels and decreases in apolipoprotein A (ApoA) and leptin levels in the group with no supplemental camel milk. Restoration of the elevated levels of biomarkers were found in the camel milk treated group. An improved in Total Antioxidant Status (TAS) and reduced Malondildehyde (MDA) is clearly seen in camel milk-treated offspring compared with non-treated group. Camel milk is suggested to have cardio protective effects as it improved these markers of inflammation associated with heart changes.

Key words: Antioxidant, camel milk, diabetes, inflammation, rats

In recent years, particularly in the Arabian countries there is a change in life style, with increasing consumption in refined sugar. Diabetes Mellitus (DM) is a global health issue with macro and micro vascular complications posing a threat to health sectors and productivity (Majid *et al,* 2016). There is greater consensus about the relationship between food and health and studies on functional food increased dramatically in recent years. The diabetic complications include not only hyperglycaemia, but also cardiomyopathy due to oxidative stress. There is an urgent need for non-conventional therapy for diabetes with less adverse effects. Therefore, the World Health Organisation recommended more research on herbal and medicinal plants (Ameh *et al,* 2010). The aim of this study was to examine the protective effects of camel milk in diabetic animal model of rats. It also tested whether treatment of camel milk can alter oxidative and inflammatory stress agents.

Materials and Methods

Pregnant rats were matched for body weight and assigned to 3 groups of 12 rats in each group (diabetic with citrate and diabetic with camel milk and control), of 15 each. Diabetes was induced by streptozotocin at a dose of 49 mg/kg dissolved in citrate buffer (STZ Sigma, USA) injection. Control rats received (i.v.) citrate buffer. Blood glucose concentrations was measured on a weekly basis after inducing diabetes and the concentrations exceeded 200 mg/dL is a confirmed diabetic rats (Volpato *et al,* 2009). Total antioxidant status was measured in the plasma as the ability of the plasma to prevent ABTS oxidation in comparison to Trolox (Cayman Chemical Co, USA). Malondialdehyde concentration was estimated in the plasma according to the method of Fernandez *et al* (1997). Apolipoprotein (ApoA) and B amounts were measured using the nephelometric method of Randox, UK. The amount of plasma leptin was measured using a commercial kit (Randox, UK). Plasma cathepsin and Hcy were measured using Randox, UK kits. The assays of plasma hormones (LH, FSH and T_3) were done in accordance with the manufacturer's instruction (EIA-5179; DRG Diagnostic, Marburg, Germany).

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The experimental plan followed the requirement of the National Committee of Bio and Medical Ethics, Saudi Arabia. Data were tested for normality and homogeneity of variance and the differences were tested by one way ANOVA, followed by a Turkey's post hoc test. P value < 0.05 was considered statistically significant.

Table 1. Nutritional composition of the experimental diets during pregnancy and lactation in rats.

Nutrients	
Fibre	48
Carbohydrate (% of total kcal)	61
Protein (% of total Kcal)	18
Fat (% of total Kcal)	16
Energy (Kcal/Kg)	3750

Table 2. Supplemental camel milk on antioxidant status and hormonal profile in rat offspring.

Variable	Control	Diabetes	Diabetes+ camel milk
TAS Trolox Equiv./L	0.53±0.08	0.34±0.071[a]	0.51±0.01[b]
MDA (Nmoles of MDA/mg protein)	1.63±0.39	4.12±0.13[a]	1.71±0.42[b]
LH (ng/mL)	6.34+1.00	5.11+1.01[a]	6.00+0.77[b]
FSH (ng/mL)	5.34+0.44	3.55+0.44[a]	4.85+0.65[b]
T_3 (ng/dL)	0.80+0.06	0.49+0.03[a]	0.74+0.03[b]

Values are presented as means±standard error for 10 rats per group

[a]Significant differences at P < 0.05 compared to the control group

[b]Significant differences at P < 0.05 compared to the diabetic group

Results and Discussion

The main components of diets given to experimental rats are shown in table 1. Malondialdehyde is one of the major products of oxidation of polyunsaturated fatty acids, causing damage to tissue and this is manifested in pathological conditions, including DM (Nguyen *et al,* 2016). As shown in table 2, a reduction in TAS as well as an increase in TBARS is indicated in diabetic rats, with camel milk ameliorated such effects. Similar trends were observed by medicinal plant extracts, as reported by Naseem *et al* (2016). The authors confirmed that Panax ginseng is effective in ameliorating diabetes via increasing antioxidant profile, catalase and lowering lipid peroxidation biomarker.

The influence of camel milk on the plasma concentrations of LH, FSH and T_3 as well as antioxidant status in rat offspring is presented in table 2. Diabetes resulted in a significantly decreased hormonal status compared with the control ones. However, supplemental camel significantly (P < 0.05) reversed the suppressive effects of diabetes, by normalising hormonal levels. Similar trend of adjusting the hormone in diabetic rats were observed by the work done by Adedara *et al* (2105), in which the authors identified *Garcinia kola* seed extract as anti-diabetic agent. In another study, *Ficus pumila Linn* extract altered serum hormonal levels in rats, reducing the negative effects of hyperprolactinemia in rats (He *et al,* 2016).

Table 3. Effect of camel milk on diabetes-induced changes in plasma apolipoproteins, leptin and Hcy of rat offspring.

	Control	Diabetes	Diabetes+ camel milk
ApoA (g/L)	138.2±1.9	103±2.9[a]	129±4.3[a,b]
ApoB (g/L)	109±4.3	168±4.3[a]	115±2.9[b]
Leptin (ng/mL)	4.2±0.13	2.9±0.2[a]	3.35±0.8[a,b]
Cathepsin	4.33±0.9	8.5±0.28[a]	5.4±0.36[b]
Hcy (µmol/L)	3.12±0.18	4.55±0.11[a]	2.81±0.23[b]

Values are presented as means±standard error for 10 rats per group

[a]Significant differences at P < 0.05 compared to the control group

[b]Significant differences at P < 0.05 compared to the diabetic group

As indicated in table 3, camel milk proved effective in normalising inflammatory biomarkers in diabetic rat offspring. Leptin adjust the energy balance and it has specific effects of cardiac function and in reported studies lack of its receptor is linked with diabetes (Friedman, 2016). The plasma leptin of the control group was lower than the rest of the groups and camel milk significantly increased its level.

Homocysteine is a non-protein alpha amino acid. Its high levels (hyperhomocysteinemia) leads to inflammation. It can alter innate immunity and can lead to disturbance in diabetes mellitus (Joshi *et al,* 2016). The apolipoprtien A and B are used to assess the risk for cardiovascular diseases (Sharma *et al,* 2017). Due to its antioxidant properties, 50 mg of daily dose of ginger extract significantly improved the status of apo, leptic, cathepsin and Hcy in wistar rats (Likhanizadeh *et al,* 2016). Similar protective effects against inflammatory biomarkers were observed in the study in which cloudy apple juice and apple peel extract were used (Fathy and Drees, 2016).

Inflammatory biomarker are differently expressed between control and diabetic rats (La

Fontaine *et al,* 2014). In another study, lycopene proved effective in enhancing the antioxidant status, by increasing SOD and also reducing the inflammatory response in diabetic rats (Li *et al,* 2016). The studies are comparable to that of camel milk and this could give extra support about the role of antioxidant in either the milk or medicinal plants in alleviating some markers.

This study outline similar effects in both camel milk and ginger extract in normalising some inflammatory biomarkers. Such biomarkers may produce cardiac abnormalities and thus future studies on camel milk and its cardio-protective properties may be recommended. In conclusion, the supplementation of rat offspring with camel milk restored the levels of inflammatory ApoA, ApoB, Hcy and leptin in the experimentally-induced diabetes in rats. Further studies are required to understand the mechanism behind these effects.

Acknowledgement

The author would like to extend his sincere gratitude to the Deanship of Scientific Research at Al Baha University for its funding this research through the project No. 21-1436.

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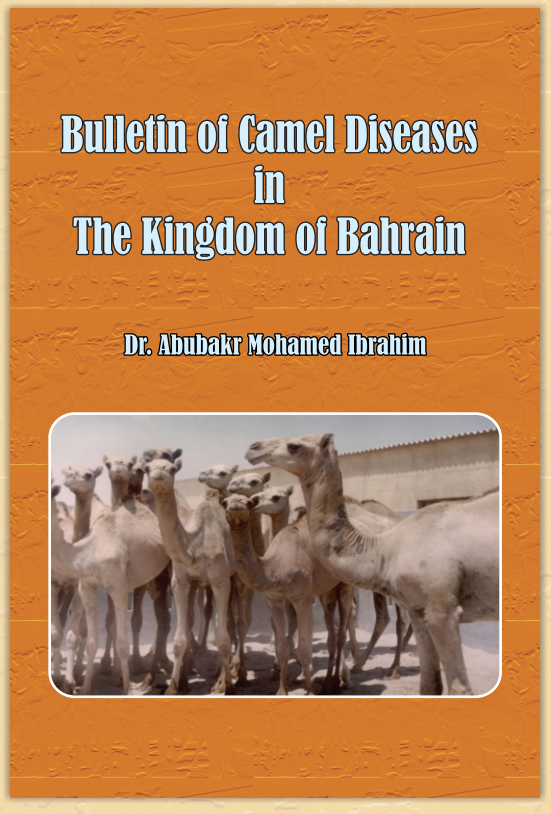
Bulletin of Camel Diseases in The Kingdom of Bahrain

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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PATHOLOGICAL DISORDERS OF THE OVARIES AND UTERINE TUBES IN CAMELS (*Camelus dromedarius*) SLAUGHTERED AT TAMBOUL ABATTOIR, SUDAN

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ABSTRACT

This study was carried out to investigate the different pathological lesions, reproductive diseases and pathological conditions in the ovaries and uterine tubes of camels slaughtered at Tamboul abattoir, Sudan. Samples collected from genitalia of 2158 female camels at different ages of *Arabi* breed were examined. Gross lesions of affected organs were observed and recorded. Representative samples from the gross lesions were fixed in 10% neutral formal saline, processed, sectioned and stained with Haematoxylin and Eosin (H & E) for histopathological examinations. Tissue samples and swabs from affected organs (ovaries and uterine tubes) were taken for bacteriological examination. The results showed that 43 organs were affected representing 1.99% of the total she-camels investigated (2158). The various pathological lesions and conditions included; ovarian cysts (41.86%), ovarian hypoplasia (23.26%), ovarian oedema and enlargement (13.95%), ovarian fibrosis (9.30%), oophoritis (4.65%), hair in ovaries (dermoid cyst) (2.33%) and thickening and corrugation in uterine tubes containing pus (4.65%). It should be highlighted that fibrotic and oedematous ovaries were reported for the first time in dromedaries. Dermoid cyst (hair in ovaries) was reported for the first time in Sudanese dromedary camels. Various microorganisms were isolated from affected cases including; *Staphylococcus aureus, Streptococcus* spp. and *Corynebacterium* spp.

Key words: Camel, ovaries, pathological disorders, uterine tubes

Diseases and infections of the reproductive system of camels may cause complications resulting, infertility or poor reproductive performance and consequent loss of productivity (Yagoub, 2005; Tibary *et al* 2006; Al-Afaleq *et al,* 2012). These are usually associated with repeat breeding, early embryonic death, foetal loss and abortion. Repeat breeding is one of the major reproductive problems among she-camels which is mainly due to ovulation failure (Tibary and Anouassi, 1998).

The purpose of the present study was to investigate the different pathological changes, reproductive diseases, pathological conditions and their bacteriological causes in the ovaries and uterine tubes in camels (*Camelus dromedarius*) slaughtered at Tamboul abattoir, Sudan.

Materials and Methods

This study was carried out to investigate the different pathological lesions and pathological conditions in the ovaries and uterine tubes of she-camels slaughtered at Tamboul slaughterhouse.

Samples collected from 2158 female camels at different ages of *Arabi* breed were examined and those from affected female genitalia were collected. Gross examinations of affected ovaries and uterine tubes were observed and recorded. Representative samples from the gross lesions were fixed in 10% neutral formal saline, processed, sectioned and stained with Haematoxylin and Eosin (H & E) for histopathological examination according to Bancroft *et al* (1996). Tissue samples and swabs from ovaries and uterine tubes were taken for microbiological examination according to the method of Barrow and Feltham (1993).

Results

Gross and histopathological examination

The parts of 2158 female reproductive systems were examined carefully during the post-mortem inspection at Tamboul slaughterhouse and different pathological changes were observed and described. The results showed that 43 organs were affected representing 1.99% of the total camels investigated.

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Table 1 shows the prevalence % of the encountered lesions as calculated from the affected organs. These were ovarian cysts (41.86%), ovarian hypoplasia (23.26%), ovarian oedema and enlargement (13.95%), ovarian fibrosis (9.30%), oophoritis (4.65%), hair in ovaries (teratoma) (2.33%) and thickening and corrugation in uterine tubes containing pus (4.65%).

Ovaries

Types and distribution of pathological changes encountered in the ovaries and uterine tubes are summarised in Table (1). These included.

1. Ovarian cysts

Cysts were found to be the most prevalent pathological disturbances in ovaries. They represented (41.86%) of the pathological lesions of affected ovaries and uterine tubes. Four different types of ovarian cysts were described (Table 1).

Table 1. Types and distribution of pathological lesions encountered in the ovaries and uterine tubes in slaughtered females.

Organ	Pathological lesion	Number of lesions	% to number of organs affected
Ovary	Ovarian cysts	18	41.86
	Hypoplasia	6	13.95
	Atrophy	4	9.30
	Oedema	6	13.95
	Fibrosis	4	9.30
	Oophoritis	2	4.65
	Teratoma (hair in ovary) and caseous necrosis	1	2.33
Uterine tube	Thickening , corrugation and pus accumulation	2	4.65
Total affected organs		**43**	**100%**
Total examined animals		**2158**	
Per cent of affected organs to total examined animals		**1.99**	

1.1. Follicular cysts

Follicular cysts were either solitary (Fig 1) or multiple; with average diameter 3-5 cm, larger cysts exceeding 5 cm in diameter were also seen. The cysts were thin-walled and the wall was either semitransparent and well vascularised or slightly opaque with little vascularisation. The thin-walled cysts were filled with a straw coloured serous fluid, When multiple cysts were seen, they took a grape punch like appearance (Fig 2). Histopathologically, the ovum and the surrounding cells were completely degenerated or absent. The basement membrane was not found in most of the cases and it was difficult to differentiate the theca interna from the theca externa.

1.2. Luteal cysts

Luteal cysts appeared as dark red masses protruding from the ovarian surface and filled with a colourless, semi-coagulated fluid (Fig 3). The cysts were surrounded by a thick opaque and tense fibrous connective tissue capsule containing greyish yellow colour semi coagulated fluid. Histopathologically, the granulosa cells were changed into granulosa leutein cells due to leutilisation that appeared polyhedral with large vacuolated cytoplasm. No para-ovarian cysts were found.

1.3. Haemorrhagic cysts

The cysts had a thick and highly vascular wall (Fig 4). In cut section, the internal cavity was filled by brownish fluid. Histopathologically, this fluid was mixed with a large amount of R.B.C. giving it a brownish colouration. The cyst wall was vascularised and covered by a thick connective tissue capsule (Fig 5).

1.4. Infundibular cysts

These were lined by stratified squamous epithelium and are distinguished from cystic teratomas of the ovary by the absence of skin adnexae. It was filled with thin slightly yellowish serous fluid (Fig 6).

2. Teratoma (Dermoid cyst)

It is a bizarre tumor, usually benign, in the ovary. Usually it contains a diversity of tissues including hair, teeth, bone etc. In this study it contained hair and caseous material and surrounded by a fibrous wall (Fig 7).

3. Oophoritis

Two cases of Oophoritis were recorded. The ovary was enlarged congested and haemorrhagic. It was distended with thick, caseous pus surrounded by a thick fibrous capsule (Figs 8 and 9). Histopathological examination revealed haemorrhage, oedema, caseous necrosis and infiltration of inflammatory cells predominantly neutrophils.

4. Ovarian Atrophy

This condition was found in 4 cases, the affected ovaries were much smaller than normal (Fig 10) and showed no evidence of ovulation. Histopathological examination revealed atrophy of the ovarian cortex, which consisted almost exclusively of fibrovascular tissue, with no evidence of follicular activity.

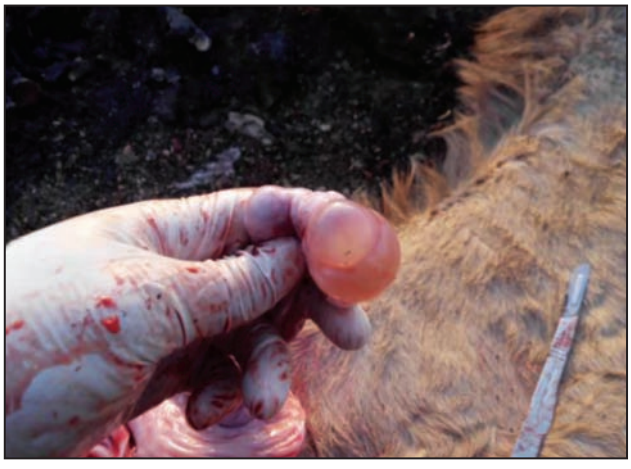

Fig 1. Solitary follicular ovarian cyst filled with straw coloured fluid.

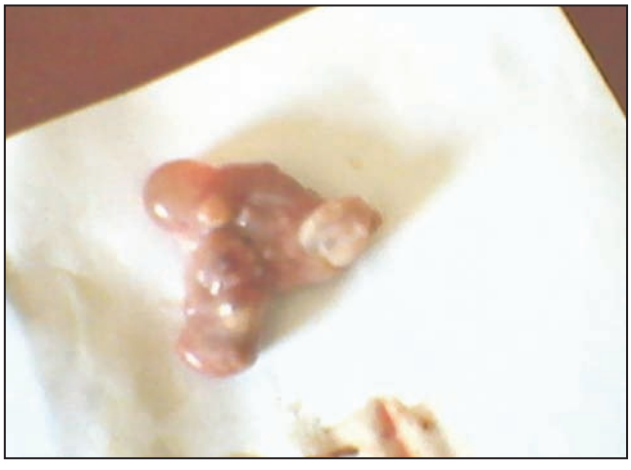

Fig 2. Grape punch appearance of multiple ovarian cysts.

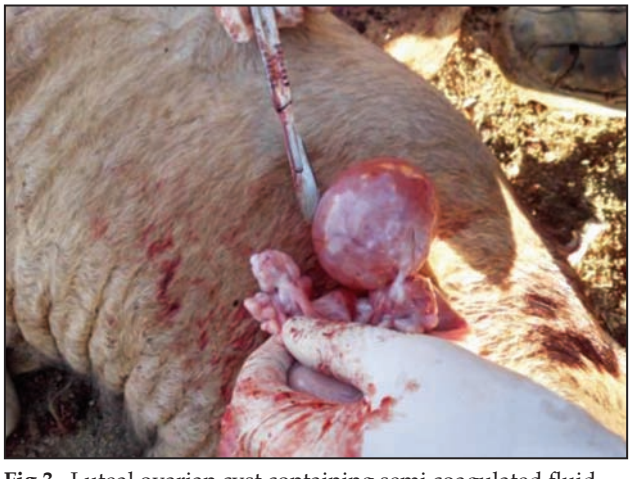

Fig 3. Luteal ovarian cyst containing semi coagulated fluid.

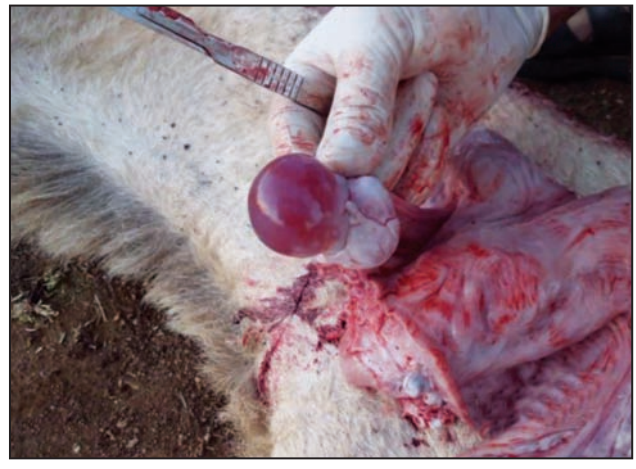

Fig 4. Haemorrhagic ovarian cyst showing a thick and highly vascular wall.

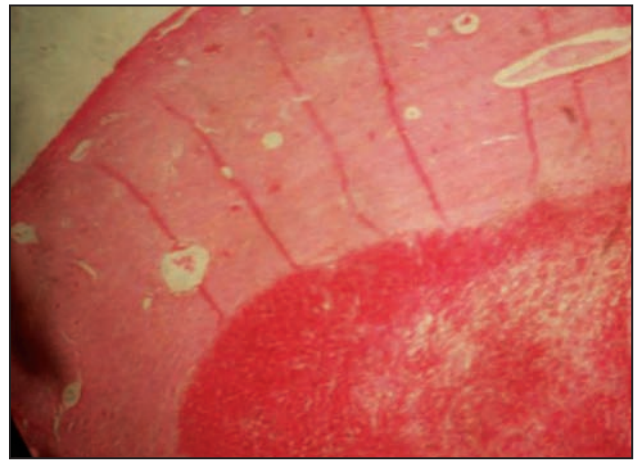

Fig 5. Haemorrhagic ovarian cyst with vascularised haemorrhagic wall.

Fig 6. Infundibular ovarian cyst containing yellowish fluid.

5. Ovarian hypoplasia

This congenital condition was found in 6 cases where the affected ovary showed complete lack of follicles. In this study uni or bilateral hypoplasia (Fig 11) were encountered, characterised by small firm smooth ovaries, complete absence of follicular or luteal development and fibrous connective tissues proliferation.

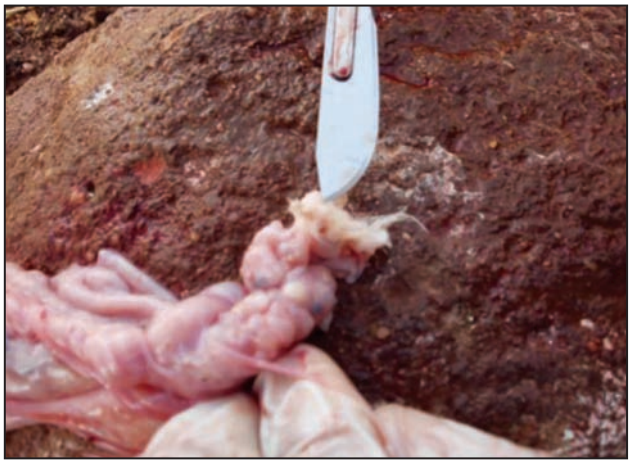

Fig 7. Camel's ovary showing teratoma (hair in ovary) and caseous necrosis.

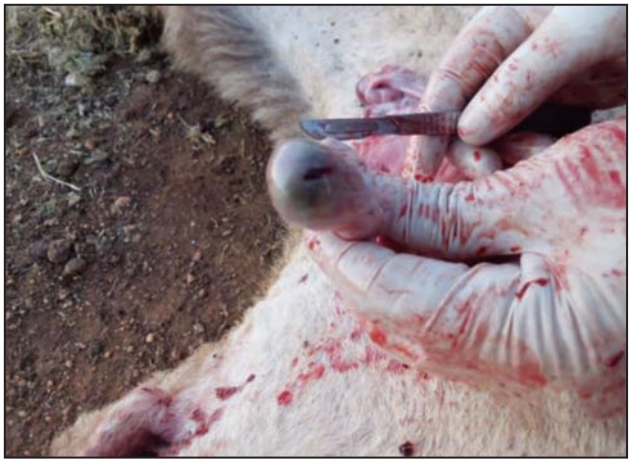

Fig 8. Oophoritis showing enlarged ovary with pinkish material.

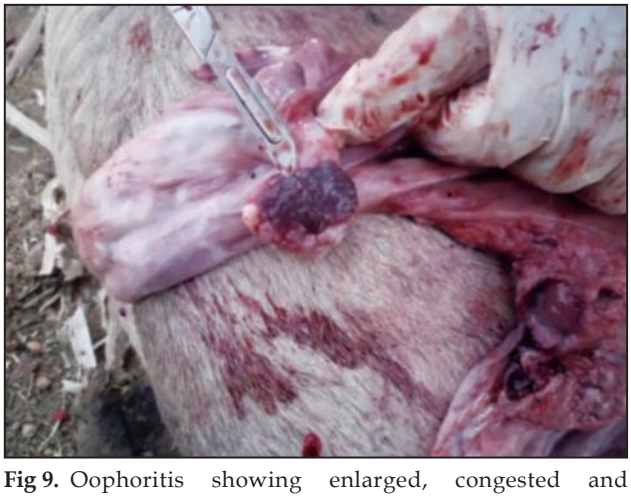

Fig 9. Oophoritis showing enlarged, congested and haemorrhagic ovary.

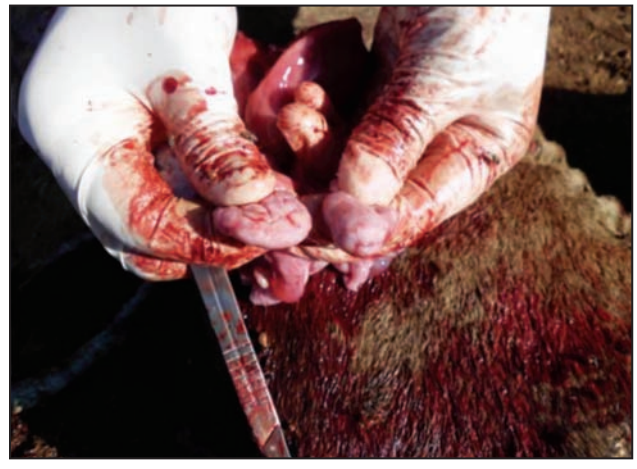

Fig 10. Atrophied left ovary.

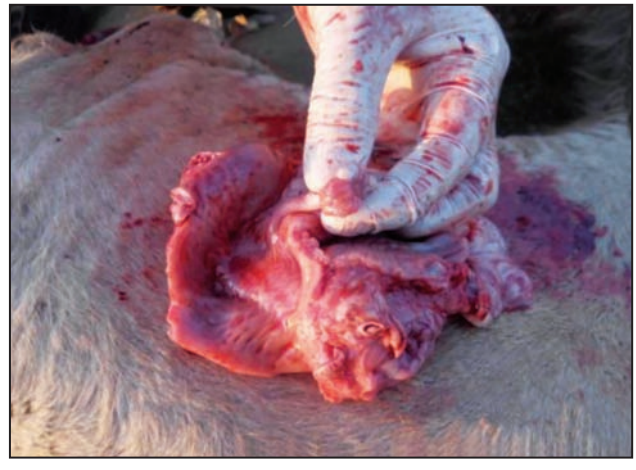

Fig 11. Bilateral hypoplasia.

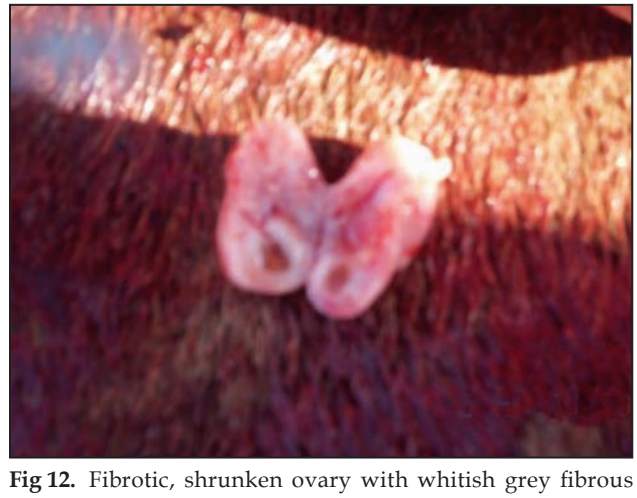

Fig 12. Fibrotic, shrunken ovary with whitish grey fibrous tissue.

6. Ovarian fibrosis

This lesion was found in 6 cases. It was characterised by excessive proliferation of fibroblasts. The primary pathological features of ovarian fibrosis are a thick capsule (Fig 12) increased mesenchymal connective tissue and decreased or absent follicles

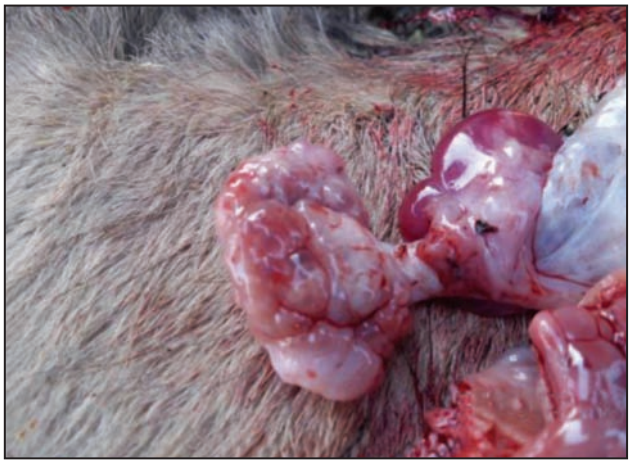

Fig 13. Oedematous ovary and oviduct.

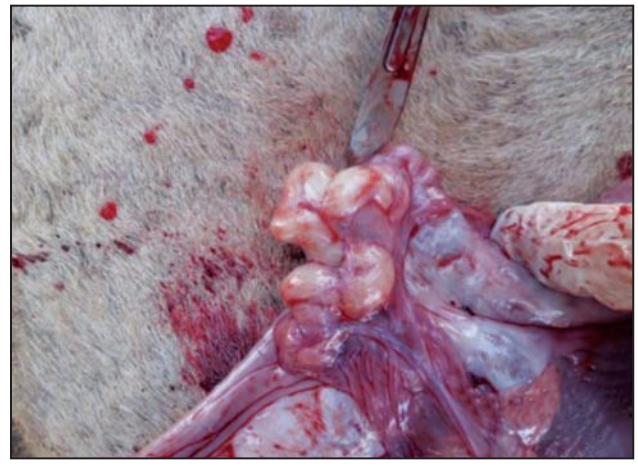

Fig 15. Pyosalpinx, with tortuosity and thickening of the oviduct.

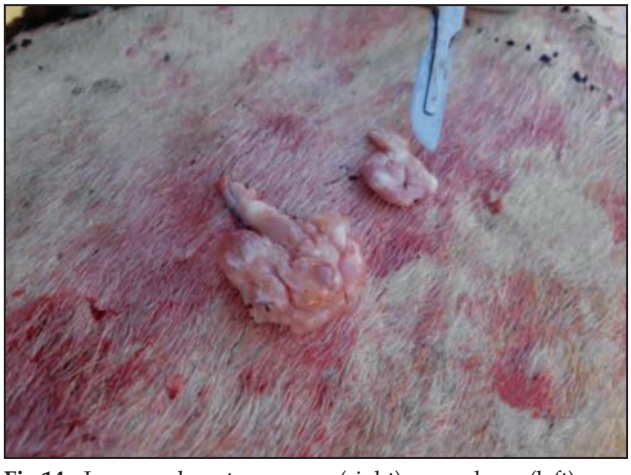

Fig 14. Large oedematous ovary (right) normal one (left).

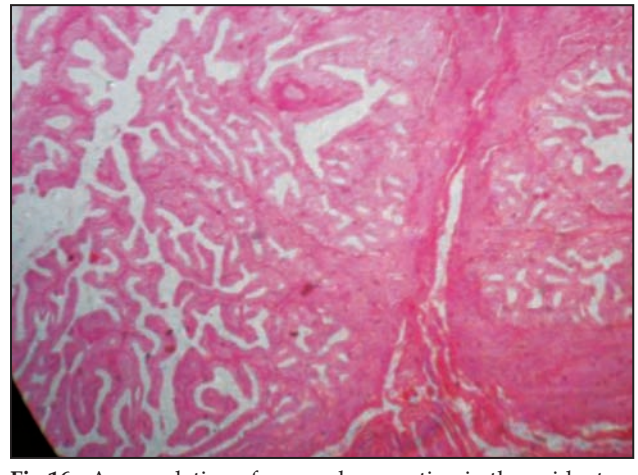

Fig 16. Accumulation of pus and congestion in the oviduct.

7. Ovarian oedema

This was found in 6 cases representing 13.95% of total affected cases. The ovary was enlarged. Clear serous fluid oozed out when the ovary was cut (Figs 13 and 14).

Uterine tube

1. Pyosalpinx

Pyoalpinx was observed in two cases representing 4.65%. of the total affected cases. The lesion was characterised by bilateral salpingeal distension due to accumulation of pus. The uterine tubes wall was irregularly thickened and tortuous (Fig 15 and 16).

Organisms isolated from affected cases

The organisms that were isolated from affected cases included; *Staphylococcus aureus, Streptococcus* spp. and *Corynebacterium* spp.

Discussion

The results showed a prevalence that 43 ovaries and uterine tubes were affected representing 1.99% of all examined she-camels. These included ovarian cysts (18), ovarian hypoplasia (6), ovarian atrophy (4), ovarian oedema (6), ovarian fibrosis (4), oophoritis (2), teratoma with caseous necrosis (1) and thickening and corrugation in uterine tubes containing pus (2).

Incidence of ovarian cysts in she-camels in this study was 41.86% of the lesions in the ovary. This finding was higher than that reported by Hamouda *et al* (2011) and Al-Afaleq *et al* (2012) in Saudi Arabia who reported 7.3% and 6.9%, respectively. Shawky *et al* (2004) in Egypt (7.6%) and Nourani and Khodakaram (2004) in Iran (18.75%). This high incidence could be attributed to heat stress in Butana region and hence increased activity of the pituitary and thyroid gland. It should be stressed that many non-bred female dromedaries normally tend to

develop follicular ovarian cysts since ovulation in these animals is induced during coitus (mating). Haemorrhagic cysts may be due to some pathological changes during growth of follicular cyst resulting in quick bleeding with accumulation of the blood within the cyst.

Ovarian hypoplasia is a congenital condition which is defined as incomplete ovarian development due to germ cell deficiency, where the affected ovary or part of the ovary shows complete lack of follicles. In this study 6 cases (13.95%) of both unilateral and bilateral ovarian hypoplasia were seen. The occurrence was higher than that reported by Shawky *et al* (2004) in camels in Egypt (0.4%) and Al-Afaleq *et al* (2012) in Saudi Arabia (3.19.%).

Dermoid cyst (hair in ovary) was found in one ovary (2.33%). Several authors reported dermoid cysts in the camel's ovary, i.e. Al-Afaleq *et al,* 2012; (1.06%); Shawky *et al,* 2004 (0.4%); Hamouda *et al,* 2011 (0.17%) and Nourani and Khodakaram, 2004 (1.04%). These lesions are sometimes classified as benign cystic teratomas and are believed to originate as congenital developmental anomalies.

Fibrosis of the ovary was found in 4 cases (9.34%). This finding appears to be the first report for ovary fibrosis in dromedaries.

The pathological conditions of uterine tubes was seen in 2 cases (4.65%). The lesion was characterised by tortuous and thickened tubes contain pus. Pathological lesions in the uterine tube have rarely been reported in female dromedaries. The present results revealed that pyosalpinx, corrugation, thickening and cystic changes could occur in dromedaries and could be one of the causes of infertility in this species.

Different species of microorganisms isolated from the affected cases of the camels indicated that camel environment is contaminated with the isolated organisms or that such organisms flourish under stressful conditions.

In conclusions, this study indicated that the pathology and infections are more common in the ovaries of dromedary camels. Reproductive tract pathology and infections in Sudanese female dromedary camels are more common than originally thought. Fibrotic and oedematous ovaries and dermoid cyst (hair in ovary) are reported for the first time in Sudanese camels.

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PATHOLOGICAL STUDY OF CAMEL MASTITIS IN TAMBOUL AREA, SUDAN

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ABSTRACT

This study was carried out to determine different types of mastitis and their incidence in camels in Tamboul area. A questionnaire was designed to collect data on mastitis in 10 herds in 4 different localities with total population 1649 camels during 3 seasons (summer, winter and autumn) for 1 year. In addition a slaughter house study in which, udders of 2158 female camels at different ages of Arabi breed were examined. Gross lesions of affected udders were observed and recorded. Representative samples from the gross lesions were fixed in 10% neutral formal saline, processed, sectioned and stained with haematoxylin and eosin (H & E) for histopathological examinations. The prevalence of mastitis as calculated from the questionnaire results was 18.98%.

The results showed that prevalence of clinical mastitis was 18.98% (acute 6.9%, chronic 12% and gangrenous 0,061%). The results revealed that 47.92% of mastitis cases were found to use (surar), 19.17% were found to use gourab and 32.91% didn't use any device. Mastitis cases observed in the initial stage of lactation was 19%, middle stage of lactation 30.1% and the highest prevalence of mastitis was found in late stage of lactation (50.9%). Slaughter house results showed that 353 udders were affected representing 16.36% of the total she-camels investigated (2158). The various pathological lesions and conditions included chronic mastitis (66.29%), acute mastitis (31.73%), abscess (1.70%) and gangrenous mastitis (0.28%) of the total affected udders.

Key words: Camel, mastitis, pathological study, Sudan

Mastitis is a complex condition, which occurs worldwide among dairy animals, with heavy economic losses. Incidence of mastitis may increase in dairy camels due to hand milking and teat malformation (Almaw and Molla, 2000). Clinical mastitis (chronic, acute and gangrenous) causes abnormalities in udder and/or milk and these can be detected during physical examination and systemic signs. The clinical mastitis in camel is diagnosed by palpation and examination of udder or milk. Acute mastitis has been reported to occur during the first few days following parturition and with signs including anorexia, fever, general depression, swelling, severe inflammation and pain of the udder (Quandil and Oudar, 1984; Obeid and Bagadi, 1996; Tibary and Anouassi, 2000). Chronic mastitis can be observed by peresence of firm, swollen fibrous udder often with nodular indurations and abscess formation, atrophy of one or more quarters and presence of pustules on the surface (Barbour *et al,* 1984; Saad and Thabet, 1993). Gangerenous mastitis is characterised by bluish, oedematous and cold to the touch udder. The teat secretion is usually purplish violet in colour mixed with gas.

This study was carried out to determine the incidence of different types of mastitis in different herds in Tamboul area (Butana) using a questionnaire coupled by gross and histopathological examination of the pathological lesions in udders in female camels slaughtered at Tamboul slaughterhouse.

Materials and Methods

The Survey

Ten camel herds comprising of 1649 females were surveyed for 1 year to determine the incidence and types of mastitis. A questionnaire was designed for data collection. Clinical examination was made to diagnose mastitis and identify the type.

Slaughterhouse study

These studies were carried out to find out the pathological changes of udders of 2158 females (Arabi breed) slaughtered at Tamboul slaughterhouse. Gross examination of affected udders was made and the findings were recorded. Representative samples from the gross lesions were fixed in 10% neutral formal saline for histopathological examination. Sections were prepared and stained with haematoxylin and eosin (H & E) for histopathological examination according to the method of Bancroft *et al* (1996).

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Results and Discussion

Survey results

Ten camel herds (1649 females) were examined during summer, winter and autumn for 1 year to investigate prevalence of clinical mastitis and their types in Tamboul area, Sudan. The most important sign was enlarged, hard, hot and painful quarter which were apparent before changes appeared in the milk. In some cases, serous secretion was also seen. There was prominent subcutaneous oedema with teat congestion enlargement and severe inflammation of supra-mammary lymph nodes. Chronic mastitis was characterised by firm red, swollen and fibrosed udder often with nodular induration and abscess formation. Some cases showed hypertrophy of mammary gland tissue and watery secretion or pus. Other cases of chronic mastitis showed obstruction of the teat canal and enlargement of the teat due to the anti-suckling devices (surar or gourab). The gangrenous mastitis showed bluish discolouration of udder; this was found associated with injury by anti-suckling devices (surar).

The effect of anti-suckling devices (surar and gourab)

The owners of the camels used 2 kinds of local anti-suckling devices (surar and gourab). Surar is a piece of wood and cloth which ties two pairs of teats together. It is injurious and traumatic to the udder and neck of the teat leading to its fibrosis.

Gourab is a plastic sac or cloth covering the udder and flank which are considered as source of contamination to the udder. Out of 313 cases of mastitis cases, 150 (about 48%) were found to be associated with the use of surar, while 60 cases (19.17%) were associated with the use of gourab. In remaining 103 cases (32.91%) of mastitis she-camels were free from these two devices.

Seasonal effects on mastitis

The highest prevalence (23.16%) (107 cases from 462 cases) of clinical mastitis were reported in summer season, followed by winter season (19.54%) (120 out 614 cases) and the lowest prevalence was reported in autumn season (15.1%) (86 out of 373 cases) (Table 1).The survey revealed that the highest prevalence of acute mastitis was seen in summer (9.09%), followed by autumn (8.03%) and winter (4.23%) (Table 1).

The survey revealed that the highest occurrence of chronic mastitis was recorded in winter (15.31%), followed by summer (13.85%) and autumn season (6.98%) (Table 2). Only one case of gangrenous mastitis was seen in summer (0.22%) (Table 1).

Prevalence of mastitis among age groups

The age of camels affected with clinical mastitis (acute, chronic or gangrenous) varied between 4 and 18 years.

Acute mastitis was highest (8.54%) in the age group of 4-8 years, followed by 9-13years (7.06%) and 14-18 years (5.55%) (Table 2). The survey also revealed the highest prevalence of chronic mastitis in the age group 14-18 years (12.94%), followed by age groups of 9-13 years (11.66%) and 4-8 years (12.94%) (Table 2). Only one case of gangrenous mastitis was reported in she-camels age 9-13 years (prevalence 0.22%).

Effect of stage of lactation on mastitis

Few mastitis cases were observed during the first stage of lactation (19%), the cases increased at the middle stage of lactation (30.1%) and the highest prevalence of mastitis was diagnosed in the late stage of lactation (50.9%) (Table 3).

Slaughterhouse investigation

The organs of 2158 female udders were examined carefully during the post-mortem inspection at Tamboul slaughterhouse and different pathological changes were observed and described. The main pathological lesions diagnosed were 353, representing 16.36% of the total udders examined. According to the lesions chronic mastitis constituted (66.29%), acute (31.73%), suppurative (1.70%) and gangrenous (0.28%) Table (4).

Table 1. Prevalence of clinical mastitis among she-camels according to season in Tamboul area.

Season	Number tested	Acute mastitis		Chronic mastitis		Gangrenous mastitis		Overall	
		+ve	Prevalence	+ve	Prevalence	+ve	Prevalence	+ve	Prevalence
Summer	462	42	9.09%	64	13.85%	1	0.22%	107	23.16%
Winter	614	26	4.23%	94	15.31%	-	-	120	19.54%
Autumn	573	46	8.03%	40	6.98%	-	-	86	15.1%
Overall	1649	114	6.91%	198	12%	1	0.061%	313	18.98%

Table 2. Prevalence of clinical mastitis among she-camels according to the age group in Tamboul area.

Age group	Number tested	Acute mastitis		Chronic mastitis		Gangrenous mastitis		Overall	
		+ve	Prevalence	+ve	Prevalence	+ve	Prevalence	+ve	Prevalence
4-8 years	445	38	8.54%	50	11.23%	1	0.22%	89	20%
9-13 years	609	43	7.06%	71	11.66%	-	-	114	18.72%
14-18 years	595	33	5.55%	77	12.94%	-	-	110	18.49%
Overall	1649	114	6.91%	198	12.00%	1	0.06%	313	18.98%

Table 3. Effect of stage of lactation on clinical mastitis in Tamboul area.

Stage of lactation	Number Tested	+ve	Prevalence
First	200	38	19%
Middle	295	89	30.1%
Late	318	162	50.9%
Overall	814	139	17.07%

Table 4. Type of mastitis observed in the udders of camels slaughtered at Tamboul abattoir.

Type of mastitis	Number affected	% affected
Acute mastitis	112	31.73
Suppurative mastitis	6	1.70
Gangrenous mastitis	1	0.28
Chronic mastitis	234	66.29
Total	353	100%

Acute mastitis

In this type of mastitis, the affected udders were often swollen, hard, reddened and hot to touch (Fig 1). Abscessation was observed with discharge of white, yellow or green pus according to the causative bacteria (Fig 2). Mammary secretions were watery, yellowish or bloody. Mammary lymph nodes were increased in size. Microscopic examination revealed congestion, haemorrhage and oedema. Mature and immature abscesses were also seen. The mature ones were surrounded by a thick fibrous capsule and infiltration of neutrophils and macrophages.

Gangrenous mastitis

It was observed in one case representing 0.28%. The affected quarters were necrotic, oedematous bluish in colour (Fig 3), and sloughing was seen (Fig 4). Mammary lymph nodes were enlarged. Microscopic examination revealed necrotic area surrounded by inflammatory cells, mainly neutrophils and giant cells.

Chronic mastitis

In chronic mastitis, the affected udders were often hard with fibrosis. Abscessation were observed with evidence of white, yellow or green pus (Fig 6). Lactiferous ducts were blocked by accumulations of keratin. Microscopic examination revealed necrosis of alveolar epithelium, hyperplasia of epithelial lining, proliferation of fibrous tissue, and thickening of alveolar septa that led to shrunken udder. In some cases abscesses surrounded by a thick fibrous capsule and infiltration of neutrophils and lymphocytes.

The occurrence of clinical mastitis in the herds surveyed was 18.98%. This is in agreement with reports by Alamin *et al* (2013) in Kordofan state, Sudan and Al-Juboori *et al* (2013) in Abu Dhabi, United Arab Emirates. These authors found an incidence of 25% and 24.7%, respectively. Such incidence was higher than that reported by Abdella, (2015) in Butana area, Sudan (9.09%).

The local anti-suckling devices used for lactating females proved to be a risk factor for mastitis as 67.09% of animals used these showed mastitis. Tick infestation which causes teat lesions was also found to predispose to mastitis. These lesions or wounds together with poor udder hygiene facilitated bacterial entry and hence infection of the udder. In this connection Alamin *et al* (2013) in western Sudan and Abdella (2015) in Butana area, Sudan reported that mastitis spread between she-camels due to the bad milking practises and/or the use of local anti-suckling devices which caused wounds facilitating invasion by *Staphylococcus* spp. into mammary gland tissue. Hussein *et al* (2013) reported that tick infestation causes lesions and is one of the potential risk factor for occurrence of mastitis by creating a suitable condition for infection by the majority of mastitis causing microorganisms.

The highest prevalence of clinical mastitis in this study was that of chronic mastitis (12%) followed by acute (6.91%) and least by gangrenous mastitis (0.061%). These results were different from that reported by Abdella (2015) in Butana area, Sudan who reported acute, chronic and gangrenous mastitis and these constituted prevalence 24.14%, 72.41% and 3.45%, respectively. Al-Tofaily and Alrodhan (2011) in Iraq reported a very low mastitis prevalence. The results of the present survey were in agree ment with the finding of Yagoub (2005) who reported that acute and chronic mastitis were among important

Fig 1. Acute mastitis showing swelling and redness of udder.

Fig 2. Camel's udder showing abscess and escape of pus.

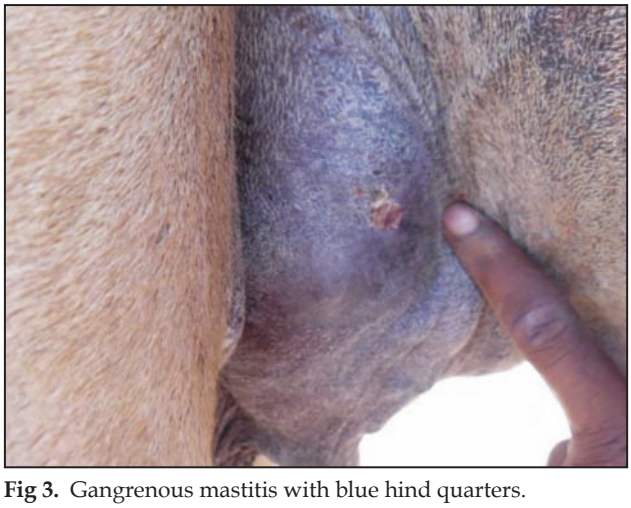

Fig 3. Gangrenous mastitis with blue hind quarters.

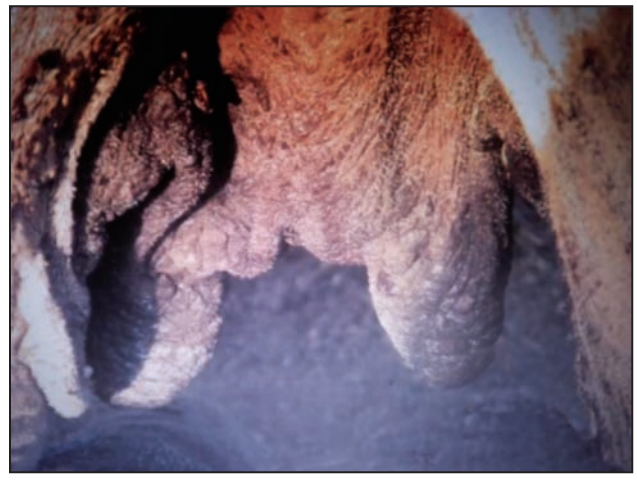

Fig 4. Sloughed quarter and black bluish skin in the teat in case of gangrenous mastitis.

Fig 5. The shrunken quarters in a case of chronic mastitis.

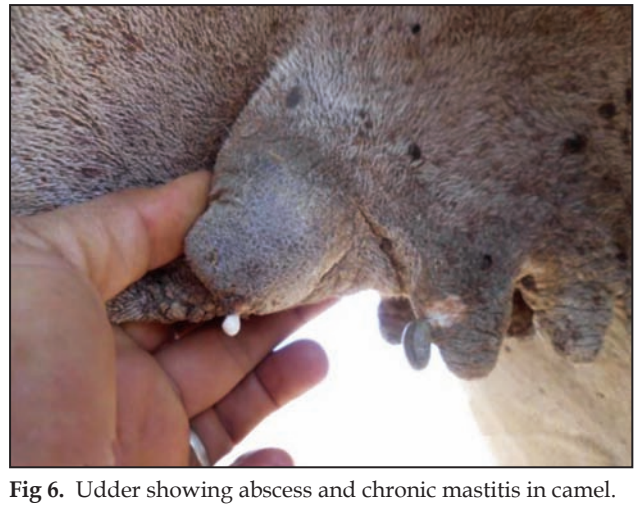

Fig 6. Udder showing abscess and chronic mastitis in camel.

pathological conditions in she-camels in eastern Sudan.

The results indicated that the age of she-camels had no significant effect on the occurrence of clinical mastitis as 6.90% of mastitis cases were found at the age between 9-13 years, 6.39% at the age 14-18 years and 6.07% for the age group 4-8 years. Abdella, (2015), on the other hand, demonstrated that she-camels above 9 years old were the most susceptible to clinical mastitis. Hussein *et al* (2013) reported

that the incidence of mastitis was influenced by age, where the lowest prevalence of mastitis in she-camels was between 5-7 years in Jijiga town, Ethiopia. The present findings indicated that there was a correlation between the stage of lactation and mastitis; this agrees with the studies of Abdella (2015) and Suheir (2004) who reported that the percentage of mastitis increased with the progress of lactation.

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Continued on page 268

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PRODUCTION AND EVALUATION OF ANTIOXIDANT ENRICHED FLAVOURED CAMEL MILK

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ABSTRACT

The objective of the study was to incorporate freeze dried Sapota (*Manilkara zapota*) fruit powder in skimmed camel milk to develop a naturally flavoured milk product with enhanced antioxidant activity. Freeze dried sapota fruit powder was incorporated at 3 levels (w/v) *viz.* 3% (T1), 5% (T2) and 7% (T3) in skimmed camel milk (milk fat: <0.5%) and compared with control (C: flavoured camel milk-pineapple). The developed products were subjected for sensory evaluation and highest overall acceptability scores were recorded for flavoured camel milk with 5% freeze dried sapota fruit powder (T2). The optimised product (T2) and control samples were stored at refrigerated temperature (4±1°C) and samples were drawn at 0, 2nd, 4th, 6th and 8th days for physico-chemical, sensory quality, antioxidant properties and storage stability analysis in aerobic packaging condition. The pH and TA of C and treatments did not differ significantly (P<0.05) among groups as well as during storage. However, with the advancement of storage period, the pH decreased while TA increased in all groups. Colour and appearance, flavour, apparent viscosity and overall acceptability scores were recorded significantly (P<0.05) higher for fruit flavoured products as compared to control. These scores were also better maintained during storage but were comparable to that of control at the end of storage. The ABTS radical scavenging activity of treated products were significantly (P<0.05) higher than control and were better maintained during refrigerated storage. Standard plate counts were comparable among all groups throughout the storage period. The findings suggested that antioxidant enriched flavoured camel milk with good quality and storage stability could be prepared by adding 5% (w/v) freeze dried sapota fruit powder.

Key words: Antioxidant, camel flavoured camel milk, sapota

The farmers of arid region have still inclination towards Camel because of its survival capacity and unique utility in tourism and for milk production. Now-a-days, more emphasis is being given to use this animal for milk production to sustain the camel farming system.

The compositional differences in different components of dromedary camel (*Camelus dromedarius*) milk differentiate it from other milk in its functional and biological properties (Kumar *et al,* 2016c).

The research evidences from the last 2 decades on cause and consequences of oxidative stress as a result of excessive production of free radicals, which redefined the disease condition as "imbalance in the equilibrium status of oxidants and antioxidants in biological system" (Aruoma, 1994). The use of natural dietary antioxidant sources to supplement the defective or insufficient endogenous antioxidant system is gaining priority (Kumar *et al,* 2015). Camel milk itself has good antioxidant properties and get enhanced when proteins are hydrolysed by proteolytic enzymes (Kumar *et al,* 2016a; 2016b; 2016c; 2016d) or through fermentation process. However, enrichment of whole/skim milk with antioxidant rich fruit products might be another way to develop functional milk products. Fruits are identified as rich sources of antioxidants and many a times used to overcome oxidative stress. The presence of large number of nutraceutical phytochemicals *viz.*, polyphenols, carotenoids, sterols, saponins, terpenes and vitamins are responsible for health-beneficial property of fruits (McCarty, 2004). Some of the phytochemical like phenolics, ascorbic acid and carotenoids may have direct influence over the radical-scavenging potential (Frei *et al,* 1989; Byers and Perry, 1992; Rice-Evans *et al,* 1996; Zhang and Hamauzu, 2004). Sapota (*Manilkara zapota*) belongs to family Sapotaceae and is one of the major fruit crops in India, Mexico, Guatemala and Venezuela. The ripened fruit contain sugars, acids, protein, amino acids, phenolics, *viz.*, gallic acid, catechin, chlorogenic acid, leucodelphinidin, leucocyanidin and leucopelargonidin, carotenoids, ascorbic acid and minerals like potassium, calcium and iron. Shui *et al* (2004) have reported the change of antioxidant levels

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during storage of *Manilkara zapota* L. Kulkarni *et al* (2007) also reported the multiple radical scavenging activities of sapota fruits. Therefore, the aim of this study was to develop and evaluate antioxidant enriched flavoured camel milk utilising sapota fruit powder.

Materials and Methods

Chemical and reagents

Fine chemical such as 2, 2-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), dehydrated microbiological media used in the study were obtained from Standard firms (Sigma-aldrich, HiMed, Merck). Other chemicals were of analytical grade from reputed companies and used without further purification. All solutions, prepared with double-distilled water, were kept at 4°C before further use.

Production of freeze dried sapota fruit powder (FDSFP)

Fresh ripened sapota fruits were purchased from local market, cleaned, seeds were removed manually and frozen in -20°C deep freezer. The frozen pieces of fruit pulp were dehydrated using freeze drier (Christ Alpha 2-4 LD Freeze Dryer, SciQuip Ltd, UK) and the resultant dried product was pulverised using mixer grinder and packed in LDPE bags and stores under refrigeration till further use.

Production of Sapota powder added flavoured camel milk

Fresh camel milk was collected from Camel Dairy, ICAR-National Research Centre on Camel, Bikaner, pre-heated and skimmed to fat less than 0.5% using cream separator and boiled for 5 minutes. While heating the skim milk, sugar (4%) and FDSFP were added at 3 different levels *viz*., 3% (T1), 5% (T2) and 7% (T3) and pine apple flavored camel milk was used as control (C). All the treatment groups were prepared separately, cooled to below 5°C using crushed ice containing box, packed in LDPE pouches and subjected to sensory evaluation. One selected product among 3 treatments and control flavoured milk samples were stored in refrigerator (4±1°C) and samples were drawn on 0, 2nd, 4th, 6th and 8th days and evaluated for sensory, physico-chemical and microbiological quality.

Measurement of pH and Titratable acidity

The pH was determined by inserting a pH electrode (HANNA) directly into the product. The titratable acidity was determined by titrating 10 g of flavoured camel milk with 0.1 N NaOH to the phenolphthalein end point and calculated as follows:

$$\text{Titratable acidity (\% lactic acid)} = \frac{\text{ml of 0.1 N NaOH} \times 0.1 \times \text{meq wt. of lactic acid}}{\text{Weight of sample in g}} \times 100$$

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

The spectrophotometric analysis of ABTS+ (Sigma–Aldrich Chemical Co. India) radical scavenging activity was determined according to method described by described by Salami *et al* (2009) with slight modification. Briefly, 20µl supernatant was mixed with 1 ml of ABTS+ working standard solution (OD 0.70) and absorbance was measured after 20 min (t20) at 734 nm in UV spectrophotometer. The ABTS+ activity was calculated by using formula:

$$\text{ABTS activity (\% inhibition)} = \frac{0.7 - At20}{0.7} \times 100$$

Microbiological quality analysis

Standard Plate Counts, Coliforms count and Yeasts and Mold counts of the samples were enumerated following the methods as described by American Public Health Association (APHA, 1984).

Sensory evaluation

A seven member trained panel comprising of scientists and technical staffs of ICAR-NRC on Camel, Bikaner evaluated the samples for the attributes *viz.* appearance and colour, flavour, texture/consistency and overall acceptability using 8 point descriptive scale (Keeton, 1983), where 8=extremely desirable and 1=extremely undesirable. The panelists were seated in a room free of noise and odours and suitably illuminated with natural light. The potable water was provided in between samples to cleanse the mouth palate.

Statistical analysis

All the experiments were repeated 3 times and parameters were analysed in duplicate (n=6). Data were expressed as means with standard error. Two-way analysis of variance (ANOVA) was done for comparing the means by using Duncan's multiple range test (DMRT), at 95% confidence level using a SPSS package (SPSS 20.0 for Windows, SPSS Inc., USA).

Results and Discussion

Optimisation of level of incorporation of FDSFP in Skimmed Camel milk FDSFP were incorporated

at 3 different levels T1: 3%, T2: 5% and T3: 7%) and compared with control for sensory quality attributes (Table 1). The colour/appearance scores were significantly (P<0.05) higher for T2 and T3 than C. However, these scores were comparable among treatments. The flavour score of T2 was significantly (P<0.05) higher than T3, but were comparable with C and T1. Comparatively, lower flavour score in T3 might be due to more sweetness resulted from higher level of incorporation of FDSFP. The texture/ consistency of C, T1 and T2 were comparable and lower than T3 as this product was more viscous than other groups. The overall acceptability score of T2 was higher followed by T1, C and T3, might be because the flavour is probably the most important attributes that influence the overall acceptability of flavoured milk products. On the basis of results of sensory evaluation tests, product containing 5% FDSFP (T2) was selected for further storage studies.

Physico-chemical, microbiological and antioxidant properties of flavoured camel milk during refrigerated storage

The pH of treatment (T) as well as control (C) decreased significantly during refrigerated storage and at the end of storage; it was significantly higher in C than T which might be due to higher sugar content in optimised product (Table 2). During initial days of storage, pH of C and T were comparable. Decrease in the pH might be due to microbial growth and/or due to production of organic acids and amino acids due to the action of ascorbic acid on sugar and protein content of developed product (Kumar *et al,* 2017). Similar results were reported by Sampedro *et al* (2009) in orange juice and milk based beverage after PEF and thermal processing and Baljeet *et al* (2013) in whey based pine apple and bottle gourd mixed beverages. Similar to pH, but opposite trends were observed for titratable acidity (TA) of treatment (T) as well as control (C) wherein it increased significantly at the end of storage. The results were in accordance with the findings of Samaddar *et al* (2015) in essential oil enriched flavoured milk. Increase in titratable acidity might be due to the growth of micro-organisms in flavoured milk during storage or due to the conversion of lactose into lactic acid by ascorbic acid present in the juice. The conversion of proteins into amino acids could also be the reason for increased acidity during storage. The higher acidity in T during storage might be due to higher sugar content in finished product. Similar results were also reported by Hassan *et al* (2015) in fruit flavoured milk based beverages and Sakhale *et al* (2012) in mango flavoured whey beverage during refrigerated storage.

The Standard plate count (log_{10} cfu/g) for C as well as T increased significantly (P<0.05) with the advancement of storage period. However, between 2 groups the C had significantly lower counts from 4th day of storage. As per BIS specifications (1981), the standard plate count and coliform count should not exceed 50,000 cfu/ml and 10 cfu/ml, respectively. The prepared flavoured milk showed the total viable count/Standard plate count in normal range and the coliform and yeast and mould counts were not detected in developed products. The results obtained are in agreement with the findings of Sakhale *et al* (2012) and Sampedro *et al* (2009) in whey based RTS beverage from mango and orange juice and milk based beverage, respectively.

The cationic radical scavenging activity of ABTS+ is most frequently utilised to measure antioxidant activity of food ingredients and processed food products. Since, the reagents dissolve well in both aqueous hydrophilic and organic solvent hydrophobic groups, this assay measures both the hydrophilic and lipophilic antioxidants (Kwang *et al,* 2012). Its efficiency depends upon the number of aromatic rings, nature of hydroxyl groups and molecular weight (Hangerman *et al,* 1998). The ABTS (% inhibition) activity of developed flavoured milk (T) was significantly (P<0.05) higher than C throughout the storage period, however, it decreased significantly from day of production to the end of storage study (Table 2). Higher ABTS (% inhibition) activity in developed flavoured milk (T) might be due to higher antioxidant activity of test ingredient. The Shui *et al*

Table 1. Sensory quality scores (Mean±SE) of flavoured camel milk incorporated with freeze dried sapota powder (n=21).

Groups	Sensory Parameters			
	Colour/ appearance	Flavour	Texture/ Consistency	Overall acceptability
C	6.88±0.08^{A}	7.07±0.12^{AB}	7.05±0.12^{A}	7.07±0.11^{AB}
T1	7.19±0.10^{AB}	7.12±0.10^{AB}	7.33±0.09^{AB}	7.24±0.09^{B}
T2	7.26±0.08^{B}	7.36±0.09^{B}	7.40±0.07^{AB}	7.38±0.08^{B}
T3	7.36±0.09^{B}	6.88±0.08^{A}	7.45±0.10^{B}	6.79±0.10^{A}

Mean±SE values bearing same or no superscript (row-wise) do not differ significantly (P<0.05).

Table 2. Physico-chemical, microbiological and antioxidant properties of flavoured camel milk (Mean±SE) during refrigerated storage.

Groups	Storage Period (days)				
	0	2	4	6	8
	pH				
C	6.40±0.01^{b}	6.37±0.01^{b}	6.35±0.01^{b}	6.32±0.01^{ab}	6.25±0.05^{a}
T	6.39±.0.01^{b}	6.37±0.01^{b}	6.36±0.01^{b}	6.32±0.02^{ab}	6.22±0.07^{b}
Titratable Acidity (% lactic acid)					
C	0.164±0.004^{a}	0.176±0.002^{ab}	0.187±0.004^{bc}	0.195±0.004^{cd}	0.209±0.004^{d}
T	0.162±0.005^{a}	0.173±0.003^{ab}	0.186±0.003^{bc}	0.194±0.003^{c}	0.210±0.002^{d}
Standard Plate Count (log10 cfu/g)					
C	3.65±0.04^{a}	3.77±0.02^{b}	4.07±0.01^{Bc}	5.11±0.01B^{d}	5.24±0.01^{Be}
T	3.66±0.03^{a}	3.71±0.03^{a}	3.99±0.02^{Ab}	5.06±0.02^{Ac}	5.11±0.02^{Ac}
ABTS (% inhibition)					
C	33.14±0.97^{Ac}	31.78±1.00^{Abc}	30.40±0.81^{Aabc}	29.19±0.84^{Aab}	26.73±0.77^{Aa}
T	50.80±1.26^{Bb}	49.11±1.14^{Bb}	47.85±1.14B^{b}	43.54±0.63^{Ba}	41.78±0.83^{Ba}

Mean±SE values bearing same or no superscript (row-wise: capital and column-wise: small alphabets) do not differ significantly (P<0.05). (n=6) ; C=control (with pineapple flavour), T= Flavoured camel milk with 5% sapota fruit powder

Table 3. Sensory quality score of flavoured camel milk (Mean±SE) during refrigerated storage.

Groups	Storage Period (days)				
	0	2	4	6	8
Colour/ Appearance					
C	7.33±0.21^{b}	6.83±0.17^{ab}	6.67±0.21^{ab}	6.33±0.21^{a}	6.17±0.17^{a}
T	7.67±0.21^{c}	7.33±0.21^{bc}	6.83±0.17^{abc}	6.50±0.22^{ab}	6.33±0.21^{a}
Flavour					
C	7.00±0.26	6.83±0.17	6.67±0.21	6.17±0.17	6.00±0.37
T	7.50±0.22^{b}	7.33±0.21^{ab}	7.00±0.26^{ab}	6.67±0.21^{ab}	6.50±0.22^{a}
Texture/ Consistency					
C	7.33±0.21	7.17±0.31	7.00±0.26	6.83±0.17	6.67±0.21
T	7.50±0.22	7.50±0.22	7.50±0.22	7.33±0.21	7.17±0.17
Overall Acceptability					
C	7.00±0.26	6.83±0.17	6.50±0.22^{A}	6.33±0.21	6.17±0.31
T	7.83±0.17^{c}	7.50±0.22^{bc}	7.33±0.21^{Babc}	6.83±0.17^{ab}	6.67±0.21^{a}

Mean±SE values bearing same or no superscript (row-wise: capital and column-wise: small alphabets) do not differ significantly (P<0.05). (n=21) ; C=control (with pineapple flavour), T= Flavoured camel milk with 5% sapota fruit powder

(2004) and Kulkarni *et al* (2007) have reported the multiple radical scavenging activities of sapota fruits.

Changes in sensory quality of sapota flavoured camel milk during refrigerated storage

Mean sensory scores for the C and T are shown in table 3. The colour/appearance scores were comparable for 2 groups during storage. However, it decreased significantly (P<0.05) with the advancement of storage period. The flavour and texture/consistency scores were almost comparable between groups and also during storage period. The overall acceptability score of T was higher throughout the storage period than control.

Popularisation and effective utilisation of camel milk is one of the challenges for the scientists/ researchers for sustaining the decreasing camel population in India. The sapota fruit powder could be incorporated at 5% (w/v) in camel milk for development of good quality flavoured camel milk product. The findings of this experiment could also be helpful in developing other value-added milk and other food products.

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Periodicals: Sharma SD, Gahlot TK, Purohit NR, Sharma CK, Chouhan DS and Choudhary RJ (1994). Haematological and biochemical alterations following epidural administration of xylazine in camels (*Camelus dromedarius*). Journal of Camel Practice and Research 1(1):26-29.

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PATHOLOGICAL AND BACTERIOLOGICAL STUDIES IN UTERUS, CERVIX AND VAGINA OF THE FEMALE CAMELS (*Camelus dromedarius*) SLAUGHTERED AT TAMBOUL ABATTOIR, SUDAN

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ABSTRACT

This study was carried out to investigate the different pathological lesions of the uterus, cervix and vagina of 2158 female camels at different ages of *Arabi* breed slaughtered at Tamboul abattoir, Sudan. Gross lesions of affected organs were observed and recorded. Representative samples from the gross lesions were obtained, fixed in 10% neutral formal saline, processed, sectioned and stained with haematoxylin and eosin (H & E) for histopathological examinations. Tissue samples and swabs from these organs were taken for bacteriological examination. The results showed that 214 organs were affected representing 9.92% of the total she-camels investigated. The various pathological lesions and conditions as calculated from total affected organs included uterine haemorrhage (23.37%), uterine congestion (20.56%), uterine white purulent spots (16.82%), uterine thickening and corrugation (9.35%), pyometra (5.60%), vaginal prolapse (4.67%), uterine abscess (3.73%), vaginal necrosis (2.80%), cervical abscess (2.80%), uterine necrosis (2.80%), uterine prolapse (1.87%), vaginal abscess (1.87%), uterine diminution (0.94%), uterine oedema (0.94%), uterine fibrosis (0.94%) and cervical oedema (0.94%). Tissues for bacteriological examination were taken from 37 samples representing all the pathological lesions encountered in this study revealed 37 isolates. The isolates consisted of 32 Gram-positive isolates (86.49%) and 5 Gram-negative ones (13.51%). The percentage of isolates in order of frequency as calculated from the total isolates were; *Staphylococcus* spp. 22(59.45%), *Streptococcus* spp. 2 (5.41%), *Bacillus* spp. 6 (16.22%) and *Corynebacterium* spp. 2 (5.41%). The Gram-negative bacteria isolates were *Escherichia coli* 4 (10.81%) and *Pseudomonas* spp. 1 (2.70%). It should be stressed that *Staphylococcus aureus* and *Pseudomonas* spp. were isolated for the first time from white purulent spots lesions in the uterus of dromedary camel.

Key words: Bacteriological study, camels, reproductive system, Sudan

Diseases and infections of the reproductive system of camels may cause complications resulting in infertility or poor reproductive performance and consequent loss of productivity (Tibary and Anoussi, 2001; Yagoub, 2005; Tibary *et al,* 2006; Shawky *et al,* 2004 and Al-Afaleq *et al,* 2012).

Generally, infection of the reproductive tract during the prepartum period leads to metritis and endometritis with consequent lowering of the reproductive efficiency and repeat-breeding (Gani *et al,* 2008; Mshelia *et al,* 2012). Some bacterial diseases of camel's reproductive system have been reported. Fayed (1992) and Refai (1992) classified the main genital tract infections in Egyptian camels as acute catarrhal, suppurative endometritis, chronic endometritis, pyometra and abscesses of the uterine wall. Literature on camel diseases and in particular those related to reproductive system is very scanty (Wernery and Kaaden, 2002).

The purpose of the present study was to investigate the different pathological lesions and bacteriological causes in the uterus, cervix and vagina in camels slaughtered at Tamboul abattoir, Sudan.

Materials and Methods

The genital tract of 2158 female Arabi camels of different ages slaughtered at Tamboul slaughterhouse were examined and affected genital organs were collected. Gross examination was done and observations were recorded. Representative specimens from the gross lesions were obtained, fixed in 10% neutral formal saline. Sections were processed, prepared and stained with haematoxylin and eosin (H & E) for histopathological examination (Bancroft *et al,* 1996). Tissue samples and swabs from affected organs were taken for bacteriological examination according to the method of Barrow and Feltham (1993).

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Results

Gross and histopathological examination:

The results showed that 214 organs were affected representing 9.92% of the total camels investigated (Table 1). The pathological lesions per cent as calculated from total affected organs comprised uterine haemorrhage (23.37%), uterine congestion (20.56%), uterine white purulent spots (micro abscesses) (16.82%), uterine thickening and corrugation (9.35%), pyometra (5.60%), vaginal prolapse (4.67%), uterine abscesses (3.73%), vaginal necrosis (2.80%), cervical abscesses (2.80%), uterine necrosis (2.80%), uterine prolapse (1.87%), vaginal abscesses (1.87%), uterine atrophy (decrease in size) (0.94%), uterine oedema (0.94%), uterine fibrosis (0.94%) and cervical oedema (0.94%).

Uterus

The diverse pathological conditions recorded in the uterus are summarised below.

Pyometra

This condition was characterised by accumulation of a large amount of pus in the uterine horns, distension of the uterus and thinning of its walls. The pus showed different colours (white, grey, green or yellow) according to the causative pyogenic bacteria (Fig 1). Congestion, oedema, necrosis of mucosal epithelium of uterus and infiltration of neutrophils and mononuclear cells were seen microscopically. Various organisms isolated from affected cases were *Staphylococcus aureus, Streptococcus* spp., *Corynebacterium* spp. and *E. coli.*

Table 1. Pathological usionl condions of the female genital tract in camels slaughtered at Tamboul abattoir.

Organ	Pathological lesion	Number of lesions	% of lesions
Uterus	Haemorrhage	50	23.37
	Congestion	44	20.56
	White purulent spots	36	16.82
	Thickening and corrugation	20	9.35
	Pyometra	12	5.60
	Abscess	8	3.73
	Necrosis	6	2.80
	Uterine prolapse	4	1.87
	Fibrosis	2	0.94
	Oedema	2	0.94
	Decrease in size	2	0.94
Cervix	Oedema	2	0.94
	Abscess	6	2.80
Vagina	Vaginal prolapse	10	4.67
	Vaginitis	6	2.80
	Abscess	4	1.87
Total affected organs		**214**	**100%**
Total examined animals		**2158**	

*Overall percentage (%) of affected organs to total examined animals = 9.92%.

White purulent spots (Micro abscesses)

White spots (micro-abscesses) were found scattered on the endometrial surface. The endometrium was congested and oedematous. Microscopic examination revealed endometrial purulent spots containing pus surrounded by fibrous capsules, neutrophils and mononuclear cells infiltration. Various bacteria isolated from affected cases were *Staphylococcus aureus* and *Pseudomonas* spp.

Thickening and corrugation

The uterus was enlarged and increased in weight and size. The external surface of uterine wall was corrugated and thickened. The endometrium was also thickened following recent history of abortions (Fig 5).

Uterine oedema

The uterus was enlarged, with an increase in weight and size due to oedematous uterine wall. Clear and serous fluid was released when the surface of the uterus was cut or opened (Fig 6).

Uterine abscess

The abscesses were embedded in the uterine wall either internally or exteriorly with, whitish and yellowish colour pus surrounded by a thick fibrous capsule (Fig 7). Microscopic examination showed liquefactive necrosis and leucocytic infiltration predominantly neutrophils surrounded by a fibrous tissue capsule.

Uterine atrophy

A she-camel had much smaller uterus than the normal one of similar age. The weight of the affected uterus was also lower than normal (Fig 8).

Uterine congestion and haemorrhage

The endometrium was severely congested, with petechial and echymotic haemorrhages (Fig 9). Blood plaques were found scattered throughout the endometrium (Fig 10). Microscopic examination revealed red blood cells leaking out of the endometrial blood vessels.

Uterine fibrosis

Fibrosis was often seen in the uterus that was affected with chronic metritis at angles of adhesion. The collagenous fibres were red in colour when stained with H&E (Fig 11).

Uterine prolapse

This condition was recorded in 4 cases and occurred after parturition. The protruding part of the uterus was oedematous, congested and haemorrhagic and in some cases the uterus was necrosed (Fig 12).

Cervix

Different pathological changes in the cervix are summarised as under:

Cervical oedema

The oedematous cervix was enlarged, with increase in size and fluid escaped when the surface was cut. The cervix contained clear serous transudate.

Abscess (Suppurative Cervicitis)

Gross examination revealed mucosal congestion of the cervix wall along with many abscesses (Fig 13). The latter contained a small or large amount of pus surrounded by a thick fibrous capsule.

Vagina

Different pathological changes in the vagina are summarised as under:

Vaginal prolapse

These were seen clinically in pregnant camels. The protruding part of the vagina was congested and haemorrhagic, (Fig 14). In some cases necrotic areas were also seen, while the protruding part of the vulva was pale.

Vaginitis

Gross examination revealed mucosal congestion of the vagina with accumulation of pus, hyperaemia and coagulative necrosis of the mucosa. This condition was noticed after parturition (Fig 15). Microscopic examination revealed congestion, necrotic area, accumulation of pus and infiltration of neutrophils surrounding the capsule of the abscess.

Bacteriological findings

Tissues for bacteriological examination were taken from 37 samples representing all types of pathological lesions encountered in this study. All samples were positive for bacterial growth with the exception of one tissue lesion that didn't show any growth in media.

The results showed 32 Gram-positive (86.49%) and 13 Gram-negative isolates (13.51%). The percentage of Gram-positive isolates in order of frequency were *Staphylococcus* spp. 22(59.45%), *Streptococcus* spp. 2(5.41%), *Bacillus* spp. 6(16.22%) and *Corynebacterium* spp. 2(5.41%). The Gram-negative bacterial isolates were *Escherichia coli* 4(10.81%) and *Pseudomonas* spp. 1(2.70%) (Table 2).

Table 2. Percentage of bacteria isolated from pathological lesions.

Bacterium	Total No. of isolates	%
Staphylococcus spp.	22	59.45
Bacillus spp.	6	16.22
Escherichia coli	4	10.81
Streptococcus spp.	2	5.41
Corynebacterium spp.	2	5.41
Pseudomonas spp.	1	2.70
Total	**37**	**100%**

Discussion

The incidence and pathological lesions of the genital tract affections of the she-camel could provide information that could aid in evaluating the reproductive status of these animals in the Sudan. The present study provided valuable data on the prevalence and causes of pathological changes of the uterus, cervix and vagina in camels slaughtered at Tamboul abattoir, Sudan.

The results revealed that the total incidence of female genital disturbances was 9.92%. This incidence is lower than that recorded in Saudi Arabia by Al-Afaleq *et al* (2012) who found an incidence between 15.43 and 17.14%. Incidence of genital tract affections of she-camel was 26% in Egypt (Shawky *et al* 2004).

The study also indicated that the uterine affections were the most frequently estimated affections, followed by the lesions of the vagina and cervix. These findings were in accordance with Shawky *et al* (2004) in Egypt. Concerning the gross pathological examination of she-camel uterus, the present study revealed that uterine affections constituted the highest incidence (8.62%) of all examined cases and represented 86.91%. This result is less than that reported by Shawky *et al* (2004) in Egypt (13.2%) and Al-Afaleq *et al* (2012) in Saudi Arabia (16.99%).

Pathological lesions in the camel uterus particularly haemorrhage and congestion in the present study has not been reported in female dromedary camels previously.

Fig 1. Gross examination revealed distended uterine horns and escape of pus from cut surface.

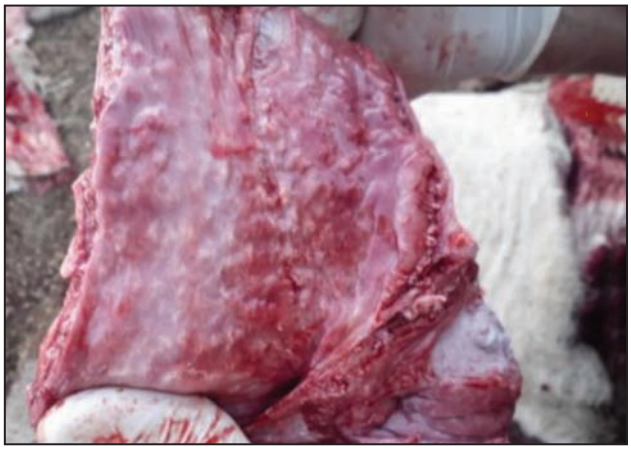

Fig 2. Gross examination revealed white spots scattered on the endometrial surface.

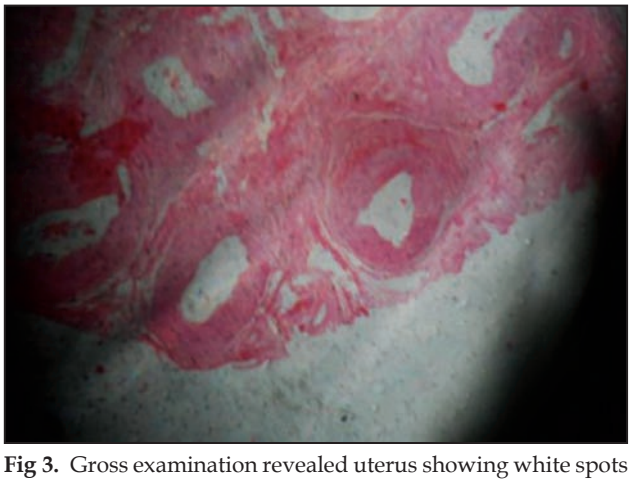

Fig 3. Gross examination revealed uterus showing white spots (micro-abscesses) surrounded by fibrous capsules.

The occurrence of small uterine abscesses scattered on the uterine endometrial surface and isolation of *Staphylococcus aureus* and *Pseudomonas* spp. from these lesions was reported previously.

Thickening and corrugation of the uterine wall was corroborated with the abortion of these animals two weeks before slaughtering.

Fig 4. Gross examination revealed corrugated and thickened external surface of the uterus.

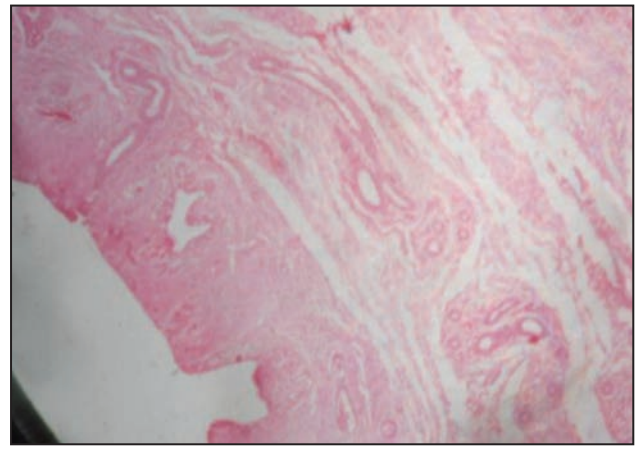

Fig 5. Photomicrograph showing thickened endometrium (H&E).

Fig 6. Gross examination revealed enlarged oedematous wall of uterus with released serous fluid.

Large uterine abscesses (2-3cm) found in this study have not been reported previously in dromedaries. The occurrences of pyometra was 5.60% in animals of present study, however, Al-Afaleq *et al* (2012) found it 3.19% and Shawky *et al* (2004) 6.4%.

The incidence of oedematous cervix in the present study was 0.94%, whereas Shawky *et al*

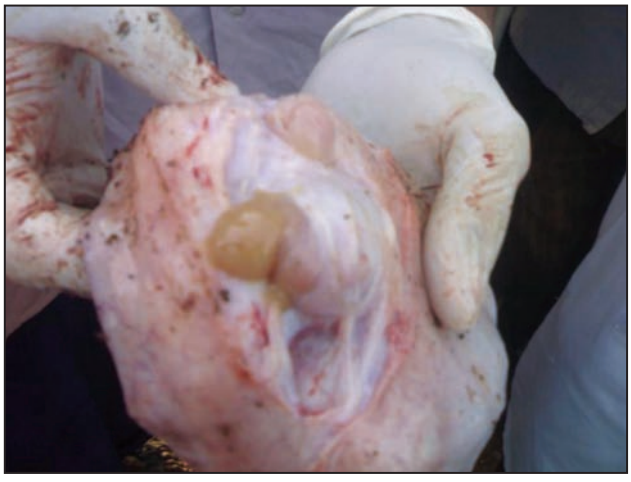

Fig 7. Gross examination of uterus revealed yellowish pus released from an abscess.

Fig 8. Gross examination of uterus revealed atrophied uterus which appeared smaller in size than normal.

Fig 9. Gross examination revealed congested and haemorrhagic endometrium.

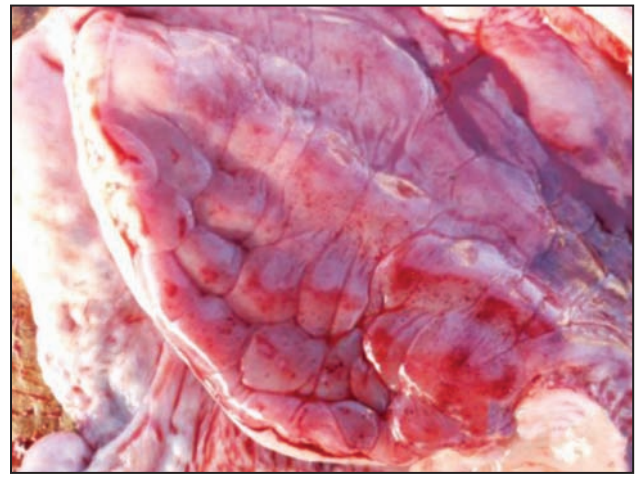

Fig 10. Gross examination revealed ecchymotic haemorrhages and blood plaques on the endometrium.

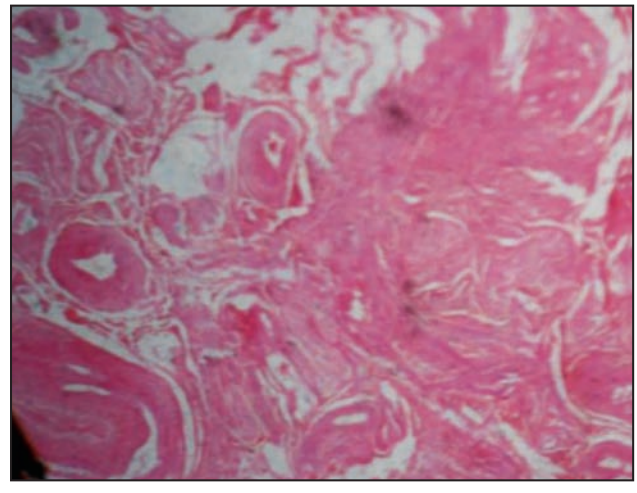

Fig 11. Histopathological examination showing collagenous fibres (H&E) indicating fibrosis of uterine wall.

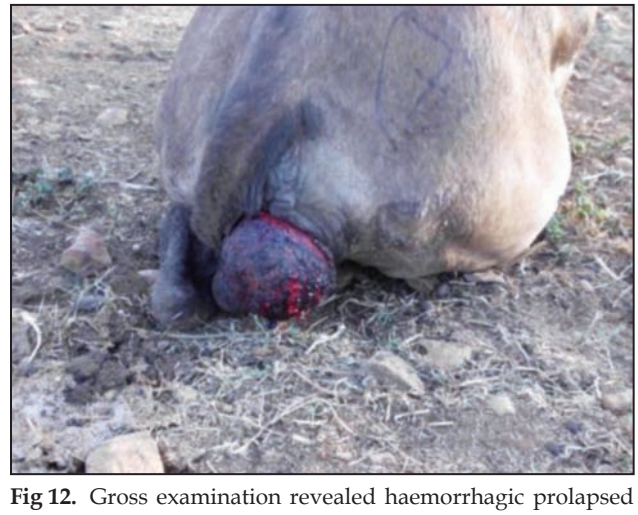

Fig 12. Gross examination revealed haemorrhagic prolapsed uterus.

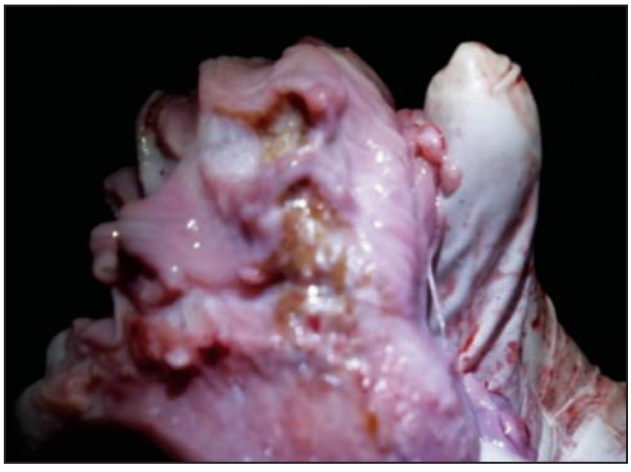

Fig 13. Gross examination revealed cervical abscesses.

Fig 14. Gross examination revealed congested, haemorrhagic prolapsed vagina.

(2004) reported it to be 0.4%. However, suppurative cervicitis and cervical abscesses have not been reported previously.

Different species of microorganisms isolated from the uterus, cervix and vagina in animals of present study which was similar to reports by other investigators (Yagoub, 2005, Tibary *et al,* 2006 and Al-Afaleq *et al,* 2012).

In the present study, *Staphylococcus aureus* and *Pseudomonas* spp. were isolated for the first time from white spots lesions in the uterus of camels.

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Fig 15. Gross examination revealed vaginal congestion and pus accumulation.

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MULTIDRUG RESISTANCE PATTERN OF *Escherichia coli* ISOLATES OBTAINED FROM HEALTHY AND DISEASED CAMEL (*Camelus dromedarius*)

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ABSTRACT

The present study was attempted to detect multidrug resistance pattern of *Escherichia coli* isolates obtained from nasal discharge of healthy and diseased camels. Out of total 112 samples of deep nasal swab (47 healthy and 65 diseased camels), 55 (20 from healthy and 35 from diseased camels) *E. coli* isolates were obtained. Further all isolates were subjected to 24 antibiotics of various groups and found marked difference in multidrug resistance profile of the isolates. All the 35 isolates from diseased camels were 100% sensitive to faropenem, gentamicin, imepenem whereas the all 20 isolates from healthy camels were 100% sensitive to ampicillin+sulbactam, cefepime, cephotaxime, ceftazidime, ciprofloxacin, faropenem, gentamicin, imepenem, norfloxacin and trimethoprim. Isolates from diseased camels showed 100% resistance to ampicillin, bacitracin, clindamycin and sulfadiazine whereas isolates from healthy camels showed 100% resistance to clindamycin and sulfadiazine. Overall resistance pattern revealed that all the 55 *E. coli* isolates were susceptible to faropenem, gentamicin and imepenem while resistant to clindamycin and sulfadiazine. Among isolates from healthy camels, 14 different resistotypes patterns were detected with 0.9579 discriminatory index and 0.13 to 0.38 multiple antibiotic resistance (MAR) index while isolates from diseased camels existed with 30 different resistotypes patterns, 0.9899 discriminatory index and 0.21-0.75 MAR index. The higher number of resistotypes, more discriminatory index and high value of MAR index indicated higher diversity and severity of MDR existed among *E. coli* isolates obtained from diseased camels.

Key words: Camel, *Escherichia coli*, MAR index, resistotype

Escherichia coli is not only associated with respiratory tract infection but also resides as commensal in domestic animals and also commonly found in the nasal cavity either through inhalation, direct or indirect contact or during drinking (Al-Sultan *et al,* 2014; Ahmed and Musa, 2015).

Escherichia coli acquires antibiotic resistance in short time with various mechanisms such as Beta-lactamase and Extended-Spectrum Beta-lactamase (ESBL) production, multidrug efflux system, low outer membrane permeability, mutations in chromosomal genes and additional acquired resistance genes via plasmids, transposons and phage makes this organism highly resistant (Sorum and Sunde, 2001; Al-Sultan *et al,* 2014). Multidrug resistance is not only public health threat but also severely affects the characteristics of pathogens and management of infection. Increased resistance to antibiotics may pose a challenge for the effective treatment, therapy options and disease containment strategies (Nontongana *et al,* 2014). It is necessary to compare and evaluate antibiotic resistance pattern of pathogens not only from diseased animal but also from healthy. It would help designing strategy and control program to combat antibiotic resistance especially in camel to control respiratory infection and population downfall also. The present study was designed to characterise the *Escherichia coli* isolates obtained from nasal discharge of healthy and diseased camels in context of antibiotic resistance pattern.

Materials and Methods

Isolation and Identification

A total of 112 specimens of deep nasal discharge (47 healthy and 65 diseased camels) were collected aseptically with sterile absorbent swabs soaked in nutrient broth. All samples has been collected from Teaching Veterinary Clinical Complex of College of

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Veterinary and Animal Science, Bikaner (Rajasthan) India on the basis of clinical symptoms of acute respiratory tract infection irrespective of age, sex and breed of camels. The samples were inoculated on nutrient agar and MacConkey agar plates and then processed for isolation and identification of *Escherichia coli* (Cowan and Steel, 1975; Quinn *et al,* 1994). All the samples underwent phenotypic and biochemical tests such as cultural characteristics, motility, lactose fermentation, carbohydrate fermentation, IMViC pattern and metallic sheen on EMB agar.

Genotypic confirmation (23S rRNA based Ribotyping)

All the 55 phenotypically confirmed isolates were found to be *Escherichia coli* by 23S rRNA ribotyping described by Khaled *et al* (2010) with some minor modification. The Primer-1F - 5′ GCT TGA CAC TGA ACA TTG AG 3′ and Primer-2R - 5′ GCA CTT ATC TCT TCC GCA TT 3′ was used. The reaction mixture for single PCR reaction was prepared by mixing 12.5µl Go Taq® Green Master Mix (2X), 1.0µl Primer-1F (25 pM/µl), 1.0µl Primer-2R (25 pM/µl), 3µl DNA template (25 ng/µl) and Nuclease free water to make 25µl total reaction volume. Amplification was carried out in a Veriti thermal cycler (Applied Biosystems) as follows: initial cycle of amplification at 94°C for 5min, 35 cycle at (denaturation at 94°C for 45 sec., primer annealing at 57°C for 60 sec and primer extension at 72°C for 45 sec.), and final extension at 72°C for 10 min. The isolates were considered positive with presence of 662bp size species specific amplicon (Fig 1).

Antibiotic Sensitivity test

Antibiotic sensitivity test was conducted by method of Bauer *et al* (1966) against 24 antibiotics (Table 1) of different groups. The isolates were inoculated in sterile 5 ml nutrient broth tubes and incubated for 18 hour at 37°C. The opacity was adjusted to 0.5 McFarland opacity standards (Quinn *et al,* 1994) and inoculums were well spread over the agar surface with the help of sterilised swab. Plates were allowed to dry for 10 minutes at 37°C and then antibiotic discs (Hi Media, Mumbai) were carefully placed on the surface with enough space around each disc for diffusion of the antibiotic. Plates were incubated for 24 hour at 37°C and the diameter of zone of inhibition of growth around each disc was measured in millimetres. After inhibition zone measurement, interpretation of resistant, sensitive and intermediates was drown as breakpoints defined by The Clinical and Laboratory Standards Institute (CLSI, 2007).

Multiple antibiotic resistance (MAR) index

All Multidrug resistant isolates were evaluated for their Multiple Antibiotic Resistance (MAR) index (Krumperman, 1983).

MAR Index of single isolate = a/b where (a -number of antibiotics to which the isolate was resistant and b- number of antibiotics to which the isolate was exposed).

Discriminatory index

The discriminatory power of the different typing system i.e. their ability to distinguish between unrelated strains was determined by the number of types defined by the test method and the relative frequency of their types. The numerical index of discrimination was calculated using the formula given by Hunter and Gaston (1988).

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} nj(nj-1)$$

Where,

D = Discriminatory index, S = Total number of type used, nj = Number of strains belonging to jth type, N = Total number of strains.

Result and Discussion

All suspected isolates were subjected to phenotypic confirmation as pink lactose fermenting colonies on mac-conkey agar, metallic seen on EMB

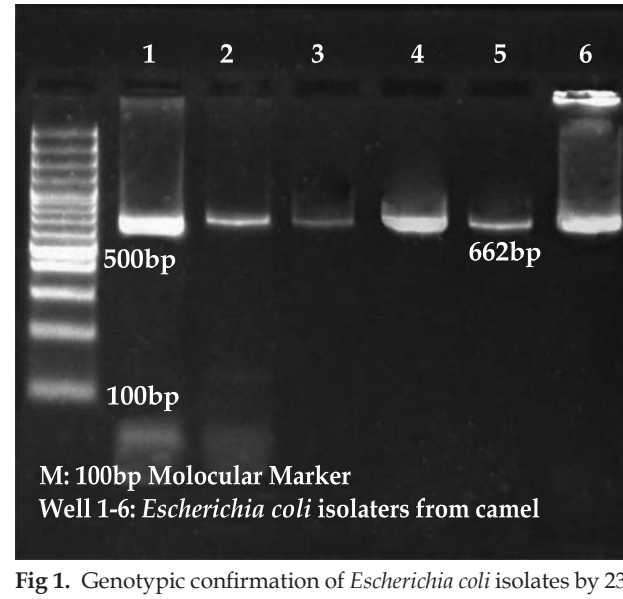

Fig 1. Genotypic confirmation of *Escherichia coli* isolates by 23S rRNA based ribotyping.

Table 1. Antibiogram of *Escherichia coli* isolates from healthy and diseased camels.

S. No.	Antibiotic	Conc. (mcg/ disc)	Diseased Camels			Healthy Camels		
			Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
1	Amoxicillin (AMX)	10	-	48.57% (17)	51.42% (18)	-	40% (8)	60% (12)
2	Amoxicillin+ Clavulanic Acid (AMC)	20/10	5.71% (2)	20% (7)	74.28% (26)	-	100% (20)	-
3	Ampicillin (AMP)	10	-	-	100% (35)	10% (2)	10% (2)	80% (16)
4	Ampicillin + Sulbactam (AMS)	10/10	94.28% (33)	2.85% (1)	2.85% (1)	100% (20)	-	-
5	Bacitracin (B)	10 Units	-	-	100% (35)	20% (4)	20% (4)	60% (12)
6	Cefepime(CPM)	30	85.71% (30)	11.42% (4)	2.85% (1)	100% (20)	-	-
7	Cephotaxime (CTX)	30	37.14% (13)	45.71% (16)	17.14% (6)	100% (20)		
8	Ceftazidime (CAZ)	30	65.71% (23)	17.14% (6)	17.14% (6)	100% (20)	-	-
9	Cephalothin (CH)	30	31.42% (11)	42.85% (15)	25.71% (9)	40% (8)	50% (10)	10% (2)
10	Ciprofloxacin (CIP)	5	65.71% (23)	17.14% (6)	17.14% (6)	100% (20)		
11	Clindamycin (CD)	2	-	-	100% (35)	-	-	100% (20)
12	Erythromycin (E)	15	-	60% (21)	40% (14)	-	80% (16)	20% (4)
13	Faropenem (FAR)	5	100% (35)	-	-	100% (20)	-	-
14	Gentamicin (G)	10	100% (35)	-	-	100% (20)	-	-
15	Imepenem (I)	10	100% (35)	-	-	100% (20)	-	-
16	Kanamycin (K)	30	45.71% (16)	51.42% (18)	2.85% (1)	50% (10)	50% (10)	-
17	Norfloxacin (NX)	10	85.71% (30)	2.85% (1)	11.42% (4)	100% (20)	-	-
18	Oxacillin (OX)	1	17.14% (6)	34.28% (12)	48.57% (17)	10% (2)	50% (10)	40% (8)
19	Polymyxin-B (PB)	300 Units	20% (7)	-	80% (28)	50% (10)	-	50% (10)
20	Rifampicin (R)	5	-	5.71% (2)	94.28% (33)	-	10% (2)	90% (18)
21	Tetracycline (T)	30	48.57% (17)	34.28% (12)	17.14% (6)	20% (4)	80% (16)	-
22	Trimethoprim (TR)		77.14% (27)	-	22.85% (8)	100% (20)	-	-
23	Sulfadiazine (SZ)	100	-	-	100% (35)	-	-	100% (20)
24	Vancomycin (VA)	30	14.28% (5)	5.71% (2)	80% (28)	-	10% (2)	90% (18)

agar and typical IMViC pattern (+ + - -) of *E. coli*. Further the isolates were also confirmed by presence of 662bp species specific amplicon during 23S rRNA based ribotyping (Fig 1).

After phenotypic and genotypic confirmation, 35 isolates (CPE1 to CPE35) were obtained from deep nasal swabs of 65 respiratory tract infected camels and 20 isolates (CHE1 to CHE20), from 47 nasal swabs of healthy camels. The overall 49.10% occurrence of *E. coli* isolates was detected among both healthy and diseased camels, which may further divide as 53.8% occurrence among diseased and 42.5% in healthy camels.

High prevalence of *E. coli* was also recorded by Ahmed and Musa (2015) who recorded 61.75% occurrence (among 247 samples) from pneumonic cases in autumn and 38.25% (153 samples) in summer, probably due to the effects of climatic changes. Sharma *et al* (2013) detected 85.71% occurrence of *E. coli* from faecal samples of enteritis in dogs in same study area and Al-Sultan *et al* (2014) also recorded 85% prevalence of *E. coli* isolates from ovarian hydrobursitis in eastern region of Saudi Arabia.

Similarly, Awol *et al* (2011) reported 17.7% occurrence from lung lesions of slaughtered camels and Al-Tarazi (2001) detected 26.6% *E. coli* from pneumonic camels. The high isolation rate of *E. coli* may be due to regular contacts of camels with the natural habitat of *E. coli*, where it can survive in faecal particles, dust and water for weeks and months (Quinn *et al,* 1994). Contrary to our study, Al-Doughyam *et al* (1999) and Alhendi (1999) reported low prevalence of *E. coli viz.* from diseased and healthy camel nasal samples as 7% and 5%, respectively.

All the 35 isolates from diseased camels were 100% sensitive to faropenem, gentamicin and imipenem followed by ampicillin + sulbactam (94.28% sensitive), cefepime and norfloxacin (85.71% sensitive) and trimethoprim (77.14% sensitive)

Table 2. Resistotypes of *Escherichia coli* isolates from healthy camels.

S.no	Pattern no	Resistance pattern	Number of resistant antibiotics	Isolates	Number of isolates	MAR index
1	R1	AMP,CD,SZ	3	CHE18,CHE19,CHE20	3	0.13
2	R2	AMX,CD,SZ	3	CHE15	1	0.13
3	R3	AMP,E,CD,SZ	4	CHE12	1	0.17
4	R4	B,AMX,CD,SZ	4	CHE6	1	0.17
5	R5	AMP,AMX,CD,SZ	4	CHE14,CHE16	2	0.17
6	R6	AMP,VA,AMX,CD,SZ	5	CHE9,CHE11,CHE17	3	0.21
7	R7	AMP,B,VA,AMX,CD,SZ	6	CHE8	1	0.25
8	R8	AMP,B,VA,AMX,CD,SZ	6	CHE5	1	0.25
9	R9	AMP,VA,AMX,CD,PB,SZ	6	CHE1,CHE13	2	0.25
10	R10	AMP,B,OX,VA,AMX,CD,SZ	7	CHE4	1	0.29
11	R11	AMP,E,VA,AMX,CD,PB,SZ	7	CHE10	1	0.29
12	R12	AMP,OX,VA,AMX,CD,PB,SZ	7	CHE3	1	0.29
13	R13	AMP,B,E,VA,AMX,CD,PB,SZ	8	CHE7	1	0.33
14	R14	AMP,B,CEP,OX,VA,AMX,CD,PB,SZ	9	CHE2	1	0.38

Number of unrelated strains:20 ; Number of types:14 ; Discriminatory power: 0.9579

while all 20 isolates from healthy camel were 100 % sensitive to ampicillin + sulbactam, cefepime, cephotaxime, ceftazidime, ciprofloxacin, faropenem, gentamicin, imepenem, norfloxacin and trimethoprim. In continuation, isolates from diseased camels showed 100% resistance to ampicillin, bacitracin, clindamycin and sulfadiazine followed by rifampicin (94.28% resistance), vancomycin and polymyxin-B (80% resistance) while isolates from healthy camel showed 100% resistance to clindamycin and sulfadiazine followed by vancomycin and rifampicin (90% resistance) and ampicillin (80% resistance) as described in Table 1.

Out of the total *E. coli* isolates, it was observed that the isolates from diseased camels showed resistance phenomena with more antibiotics while isolates obtained from healthy camels were more susceptible for similar antibiotics. It indicated acquired resistance phenomena among *E. coli* isolates during diseases conditions due to incorporation of antibiotics for treating diseased camels thus the organism developed antimicrobial resistance to the respective antibiotics. Similar to our observation, Al-Doughaym *et al* (1999) recorded that *E. coli* were 100% sensitive to gentamicin and 50% to tetracycline. Similarly, Al-Sultan *et al* (2014) also found highest susceptibility towards carbepenem, floroquinolones and aminoglycosides antibiotics while Kumar *et al* (2013) observed high level of resistance against nalidixic acid (87.3%) followed by ampicillin (85.5%), norfloxacin (74.5%), amoxicillin/clavulanic acid (74.5%), tetracycline (67.3%) and trimethoprim/ sulfamethoxazole (67.3%) among *E. coli* isolates associated with urinary tract infections.

During resistotyping, 14 different resistotypes were detected with 0.13-0.38 multiple antibiotic resistance (MAR) index and 12 isolates were found to be multidrug resistant (MDR= MAR index > 0.2) isolates among total *E. coli* isolates from healthy camels (Table 2). While 30 different resistotypes with 0.21-0.75 MAR index were detected as MDR among total *E. coli* isolates from diseased camels (Table 3).

The higher value of MAR index indicates severity of MDR property of isolates and capabilities of isolates as potential source to spread multiple antibiotic resistance among environment of other microorganisms and ability of resistant organism to hamper disease management during infections (Krumperman, 1983). Since more number of resistotypes were detected among *E. coli* isolates from diseased camels indicated more variability among detected isolates and required to determine discrimination between different isolates. Thus the discriminatory index recorded in present study was 0.9899 and 0.9579 for isolates of healthy and diseased camels, respectively. The highest discriminatory index of resistotyping among isolates from diseased camels revealed MDR variability among isolates and capabilities of resistotyping as powerful tool to discriminate isolates.

The marked difference in resistance pattern of *E. coli* isolates from diseased and healthy camel indicated indiscriminate use of antibiotics during disease management and evolving pattern of *E. coli*

Table 3. Resistotypes of *Escherichia coli* isolates from diseased camels.

S. no	Pattern no	Pattern	Number of resistant antibiotics	Isolates	Number of isolates	MAR index
1	R1	AMP,B,VA,CD,SZ	5	CPE8	1	0.21
2	R2	B,AMX,CD,PB,SZ	5	CPE33	1	0.21
3	R3	AMP,B,AMX,CD,SZ	5	CPE35	1	0.21
4	R4	AMP,B,VA,CD,PB,SZ	6	CPE14	1	0.25
5	R5	AMP,B,OX,AMX,CD,SZ	6	CPE31	1	0.25
6	R6	AMP,B,E,VA,AMX,CD,SZ	7	CPE26	1	0.29
7	R7	AMP,B,VA,AMX,CD,PB,SZ	7	CPE17,CPE18,CPE32	3	0.29
8	R8	AMP,B,OX,AMX,CD,PB,SZ	7	CPE1	1	0.29
9	R9	AMP,B, VA,AMX,CD,PB,SZ	7	CPE13	1	0.29
10	R10	AMP,B,VA, AMX,CD,PB,SZ	7	CPE16	1	0.29
11	R11	AMP,B,CIP,AMX,CD,PB,SZ	7	CPE4	1	0.29
12	R12	AMP,B,E,VA,TE,CD,PB,SZ	8	CPE11	1	0.33
13	R13	AMP,B,E,VA,AMX,CD,PB,SZ	8	CPE7	1	0.33
14	R14	AMP,B,OX,E,VA,AMX,CD,SZ	8	CPE3, CPE9	2	0.33
15	R15	AMP,B,OX,VA,AMX,CD,PB,SZ	8	CPE5,CPE12	2	0.33
16	R16	AMP,B,VA,AMC,AMX,CD,PB,SZ	8	CPE20	1	0.33
17	R17	AMP,B,CEP,AMX,CTX,CD,PB,SZ	8	CPE34	1	0.33
18	R18	AMP,B,CIP,E,VA,AMX,CD,PB,SZ	9	CPE15	1	0.38
19	R19	AMP,B,CIP,NX,VA,AMX,CD,PB,SZ	9	CPE6	1	0.38
20	R20	AMP,B,OX,VA,AMC,AMX,CD,PB,SZ	9	CPE2	1	0.38
21	R21	AMP,B,CEP,NX,E,VA,AMX,CD,PB,SZ	9	CPE23	1	0.38
22	R22	AMP,B,OX,VA,AMC,AMX,CTX,CD,SZ	9	CPE24	1	0.38
23	R23	AMP,B,CIP,CAZ,E,TE,TR,AMX,CD,PB,SZ	11	CPE27	1	0.46
24	R24	AMP,B,OX,E,VA,TE,TR,AMC,AMX,CD,PB,SZ	12	CPE10	1	0.50
25	R25	AMP,B,CEP,OX,E,VA,AMC,AMX,CTX,CD,PB,SZ	12	CPE21	1	0.50
26	R26	AMP,B,CAZ,CEP,OX,VA,TR,AMC,AMX,CD,PB,SZ	12	CPE19	1	0.50
27	R27	AMP,B,CEP,OX,E,VA,TE,TR,AMC,AMX,CD,PB,SZ	13	CPE29,CPE30	2	0.54
28	R28	AMP,B,CIP,CAZ,CEP,NX,OX,VA,TE,TR,AMX,CD, PB,SZ	14	CPE25	1	0.58
29	R29	AMP,B,CIP,CAZ,CEP,NX,OX,E,VA,TR,AMX,CPM, CTX,CD,PB,SZ	16	CPE22	1	0.67
30	R30	AMP,B,CIP,CAZ,CEP,NX,OX,E,VA,TE,TR,K,AMS, AMX,CTX,CD,PB,SZ	18	CPE28	1	0.75

Number of unrelated strains:35; Number of types:30; Discriminatory power: 0.9899

isolates from natural environment (healthy camel) to acquired resistance (diseased camel) condition.

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INFLUENCE OF BOKHI ON KIDNEY-YANG-DEFICIENCY SYNDROME IN RATS

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ABSTRACT

In Mongolian folk medicine, Bokhi, which comes from male camel occipital gland secretions, is used to treat Kidney-Yang-Deficiency Syndrome (KYDS) which has similar clinical signs as glucocorticoid withdrawal syndrome. Model KYDS rats were established by multipoint subcutaneous injection of hydrocortisone for 14 successive days and then the rats were treated by oral administration of Bokhi from two regions in China and at both a high and a low dose for a further 14 successive days. The growth rate, food intake, urine volume, vesicula seminalis, spleen, kidney, testes and the general condition of rats were recorded. The levels of serum creatinine, blood urea nitrogen, testosterone, thyroid stimulating hormone, superoxide dismutase and nitric oxide in rat serum were quantified. Results demonstrated that the symptoms of KYD were gradually alleviated by the administration of Bokhi, which also affected urine volume. Bokhi increased the growth rate and levels of testesterone, superoxide dismutase and nitric oxide. The levels of serum creatinine, blood urea nitrogen, thyroid stimulating hormone were reduced. However, Bokhi had little effect on food intake or organ indices. This experiment demonstrated that high doses of Bokhi could improve KYDS.

Key words: Camel, Bokhi, KYDS, Hydrocortisone, Occipital gland secretion

Kidney-Yang-Deficiency Syndrome (KYDS) is a term used in traditional Chinese medicine (TCM) to describe an illness characterised by paleness, chills, cold extremities, a weak and slow pulse, poor semen production and general spiritual malaise (Hao *et al,* 2008). In modern medicine it is recognised that KYDS is related to problems in the neuroendocrine immune system. Specifically, KYDS results from dysfunction of the hypothalamus-pituitary-adrenal axis, the thyroid axis, the gonadal axis (to varying degrees), but also organs involved in metabolism and the immune system resulting in hypofunction and pathological change (Lu and Wo, 2007). Experimentally, a valid method to establish KYDS in a model animal (e.g. rats), is by subcutaneous injection of high doses of an exogenous glucocorticoid such as corticosterone or hydrocortisone, which induces atrophy of the hypothalamic-pituitary glands and reduced secretion from those glands (Gou *et al,* 2009; Li *et al,* 2013; Zhao *et al,* 2013). Following injection these animal models show the same symptoms as KYDS and are widely employed in the evaluation of the mechanisms for establishment of KYDS and the therapeutic effects of curative drugs (Huang *et al,* 2013).

The Alxa League and Bayan Nur areas in the Autonomous Region of Inner Mongolia, have more bactrian camels than elsewhere in China. To adapt to the harsh desert and semi-desert conditions (extremes of heat and cold, arid conditions, poor grazing), camels have evolved many special abilities and attributes over a long period of natural selection (Jirimutu *et al,* 2012). Amongst these characteristics, camels secrete particular sex hormones during the rut. The sexual season in camels is relatively stable and sexual activity only occurs during the period of the rut. Female camels come into 'heat', or oestrus, between the end of one year and the spring of the next year. As with Asian elephants, during this time, male camels secrete a light brown foul smelling sticky liquid (Gosling, 1985) from their occipital glands (Yagil and Etzion, 1980), known locally as Bokhi (Mongolian transliteration). The volatiles produced by Bokhi are the male camels' sex hormone and they induce females into oestrus (Tingari and George, 1984). During this time the males' occipital glands increase in size secreting more Bokhi and then, during the summer, they shrink again in size. Bokhi production is a distinctive feature of male camels and the size of occipital glands and associated rate of

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Bokhi secretion is a measure of likely sexual activity and mating success.

In Mongolia Bokhi is used for the treatment of KYDS. When Bokhi from a male camel's mane is dipped in water and the resulting solution eaten over a period of a week, the positive effects on alleviation of KYDS is obvious. However, this is a folk method and the most effective dose of Bokhi has not been quantified. Here we describe an experiment to study the influence of Bokhi (from camels from two regions in China) on KYDS in a rat model in which the KYDS has been induced using hydrocortisone. This provides pharmacological evidence for the effects of Bokhi on KYDS.

Materials and Methods

Preparation of Bokhi. At the peak of the male camel rutting season, 60 Bokhi samples (each sample containing approximately 100 camel hairs) were collected from the neck of 60 mature, domesticated, bactrian camels from the western region of Inner Mongolia; 30 samples came from camels in Alxa League and 30 samples came from camels in Bayan Nur. From each sample 20 camel hairs were selected and placed in 200 mL of distilled water and soaked for 24 h. The resulting 'Bokhi solution' was filtered through filter paper and the filtrate freeze-dried to produce a black solid powder.

Chemicals. Hydrocortisone injections were purchased from Zhengzhou Lingrui Pharmaceutical Company (Henan, China). Sodium chloride (control) injections (0.9 %) were purchased from Jilin Kelun Connell Pharmaceutical Company (Jilin, China). Sildenafil citrate was purchased from Pfizer Pharmaceuticals Limited (Liaoning, China). Serum creatinine (SCR), blood urea nitrogen (BUN), testosterone (T), thyroid stimulating hormone (TSH), superoxide dismutase (SOD) and nitric oxide (NO) reagent kits were purchased from Nanjing Institute of Biological Engineering (Jiangsu, China).

Experimental animals and groups. All protocols were approved by Animal Care and Use Committee at Inner Mongolia Agricultural University. Seventy healthy adult male Spague Dawley (SD) rats (weighing 200-240 g, Specific Pathogen Free, animal licence No. SCXK (Jing) 2006-0009) were supplied by Vital River Laboratory Animal Technology Company Limited (Beijing, China). The rats were maintained under standard laboratory conditions (temperature of 21-23°C, relative humidity of 45-55 % and 12 h/12 h light/dark cycle) with food and water freely available. After one-week of acclimation to standard laboratory conditions, cardiac blood samples were taken from all animals. The blood was centrifuged (3,000 g centrifugation for 15 min) and the serum isolated. The levels of SCR, BUN, T, TSH, SOD and NO in the serum from each rat was determined using the ELISA kits; there was no significant difference ($P > 0.05$) in the levels of these compounds amongst the rats. The 70 rats were randomly divided into seven groups as follows: Control, Model, AHDB, BHDB, ALDB, BLDB and SC (Sildenafil citrate), ten rats per group.

Treatment administration. The experimental design followed the recommended methods from published work (Shen *et al,* 2007; Zhou *et al,* 2007) and is fully described in Fig 1. Rats in the Control group received multipoint subcutaneous injections of 25 mg/kg body weight of medical physiological saline daily for 14 consecutive days and the remaining rats received multipoint subcutaneous injections of 25 mg/kg body weight of hydrocortisone daily for 14 consecutive days. The rats were weighed every other day and, at the same time, their activity, hair gloss and shedding were observed and recorded. After the 14 days, the rats in the KYDS Model groups were shedding hair and appeared thin, dispirited, less dynamic and cold, which are clear symptoms of KYDS and demonstrated that the KYDS model had been established. On the 15th day the Control and Model groups were each given 2 mL sterilised tap water. In contrast the AHDB and BHDB groups were given Bokhi 50 mg/kg body weight, the ALDB and BLDB groups were given Bokhi 10 mg/kg body weight and the SC group was given SC 10 mg/kg body weight. The treatments were administered daily (9:00-11:00 a.m.) for 14 consecutive days. Every other day, at a fixed time, each rat was weighed and the figure used to adjust the dosage of the drugs. The food intake of each group rats was weighed at a fixed time every day. After the final treatment, food was withheld and all rats were immediately kept in individual metabolic cages to collect urine for 24 h. Then they were euthanised by bleeding from the femoral artery under anaesthesia. The serum was extracted from the femoral blood as described previously. The serum was used to quantify SCR, BUN, T, TSH, SOD, NO using the ELISA kits. The vesicula seminalis, spleen, kidney and testes were dissected from each rat and weighed.

Statistical Analysis. Data was expressed as mean ± standard deviation (SD). SPSS 17.0 software was used for all statistical analysis. Differences between mean values of normally distributed data

were assessed by one-way analysis of variance (ANOVA) followed by post hoc least significant difference (LSD) comparison tests. For comparisons between two groups, t-tests were used. Statistical differences were considered significant at $P < 0.05$. GraphPad Prism 5 software was used for all figures analyses. R language software was used for principal components analysis using the ggplot2 package for data visualisation.

Results

Changes in Growth Rate of rats

Before the experiment began the rats were randomly divided into groups and there was no significant difference in the growth rate of rats amongst the groups ($P > 0.05$). After the 14 days during which the KYDS model had been established, growth rate of the Model groups were significantly slower than the Control group ($P < 0.01$, Fig 2 A). There was no significant difference ($P > 0.05$) in growth rate amongst rats in each of the model groups. These differences in growth rate before and after establishing the model demonstrated that the KYDS model had established successfully.

After the 14 days during which the KYDS model groups had received treatment (Fig 2 B), growth rate in the treatment groups (AHDB, BHDB, ALDB, BLDB and SC) was significantly faster than the Control group ($P < 0.01$). The growth rates of AHDB and BHDB groups were the fastest.

Changes in food intake of rats

During the KYDS model establishment period rats in the Control group consumed significantly more food (and achieved a steady growth rate) than the other six groups ($P > 0.05$, Fig 3). This demonstrated that the rats had been stimulated by the drugs, resulting a significantly reduced food intake.

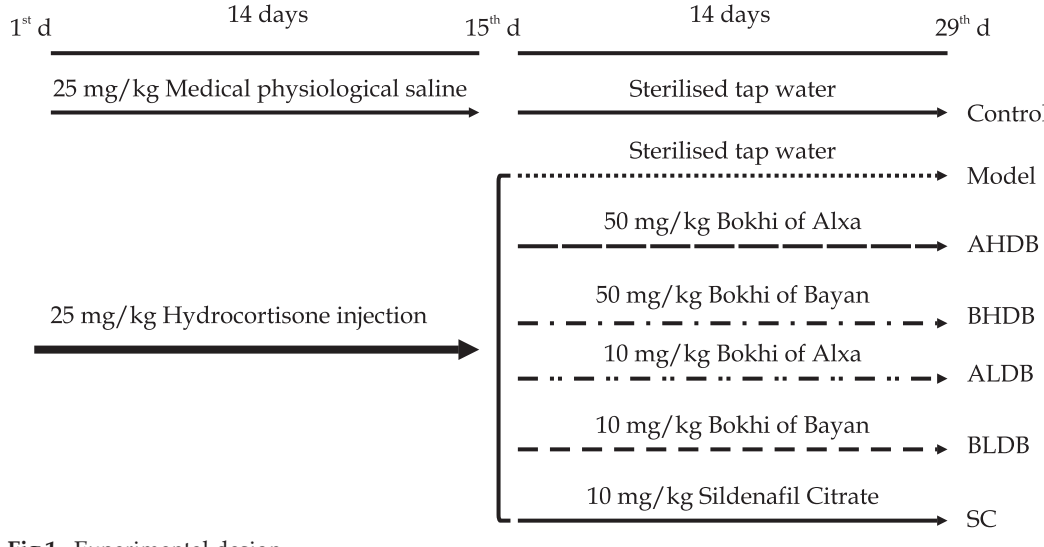

Fig 1. Experimental design.

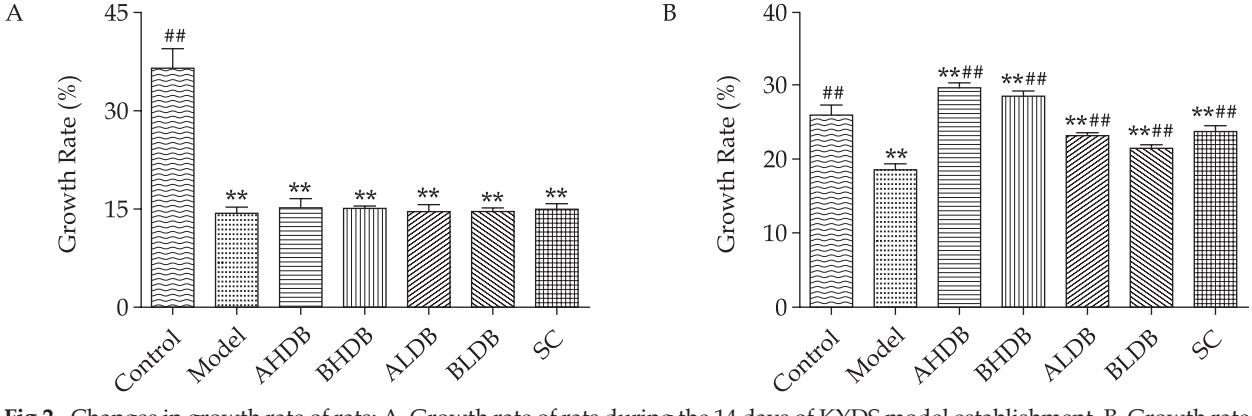

Fig 2. Changes in growth rate of rats: A. Growth rate of rats during the 14 days of KYDS model establishment. B. Growth rate of rats during the 14 days of treatment application. **P < 0.01 and *P < 0.05 compared with the Control group; ##P < 0.01 and #P < 0.05 compared with the Model group.

During the treatment period (days 15-28), rats in the Control group had the highest daily food intake and steady growth ($P < 0.05$, Fig 4 A). After hydrocortisone injections were stopped normal metabolic regulation returned to the remaining six groups of rats and there was a gradual recovery of physical condition and increased food intake (Fig 4 B-D). By the end of the experiment, the daily intake of food was the same as in the Control group of rats ($P > 0.05$). This showed that curative drug treatment resulted in no differences in food intake between the KYDS rats and the Control rats.

Effects of Bokhi on urine volume of KYDS model rats

After 14 successive days of Bokhi administration the Model group had obvious urinary dysfunction producing more urine than the other groups ($P < 0.01$, Fig 5). The urine volume level of AHDB group was close to that of the Control group ($P > 0.05$) and they regulated the urine volume better than the other four treatment groups (Fig 5).

Observations on General State and Condition of rats

Before the experiment began, the rats showed normal activity, shiny hair and there were no obvious differences between the groups. During model establishment (days 1-14), the model group rats: ate

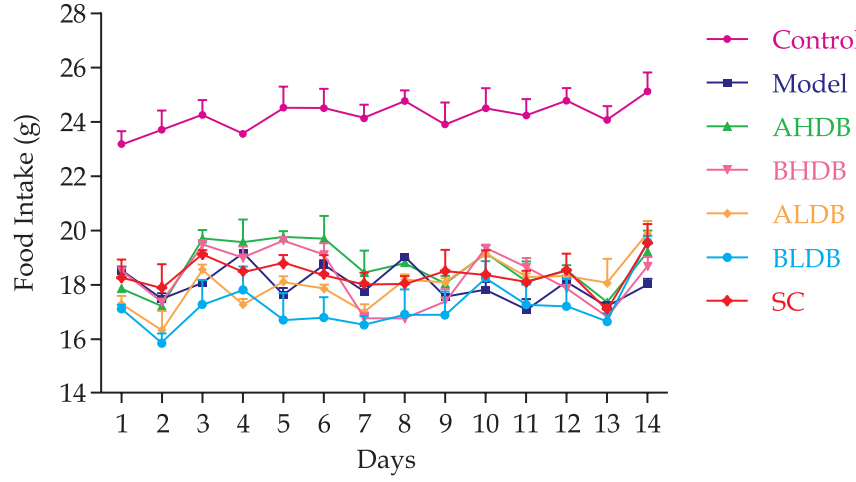

Fig 3. Changes in daily food intake during KYDS model establishment in rats.

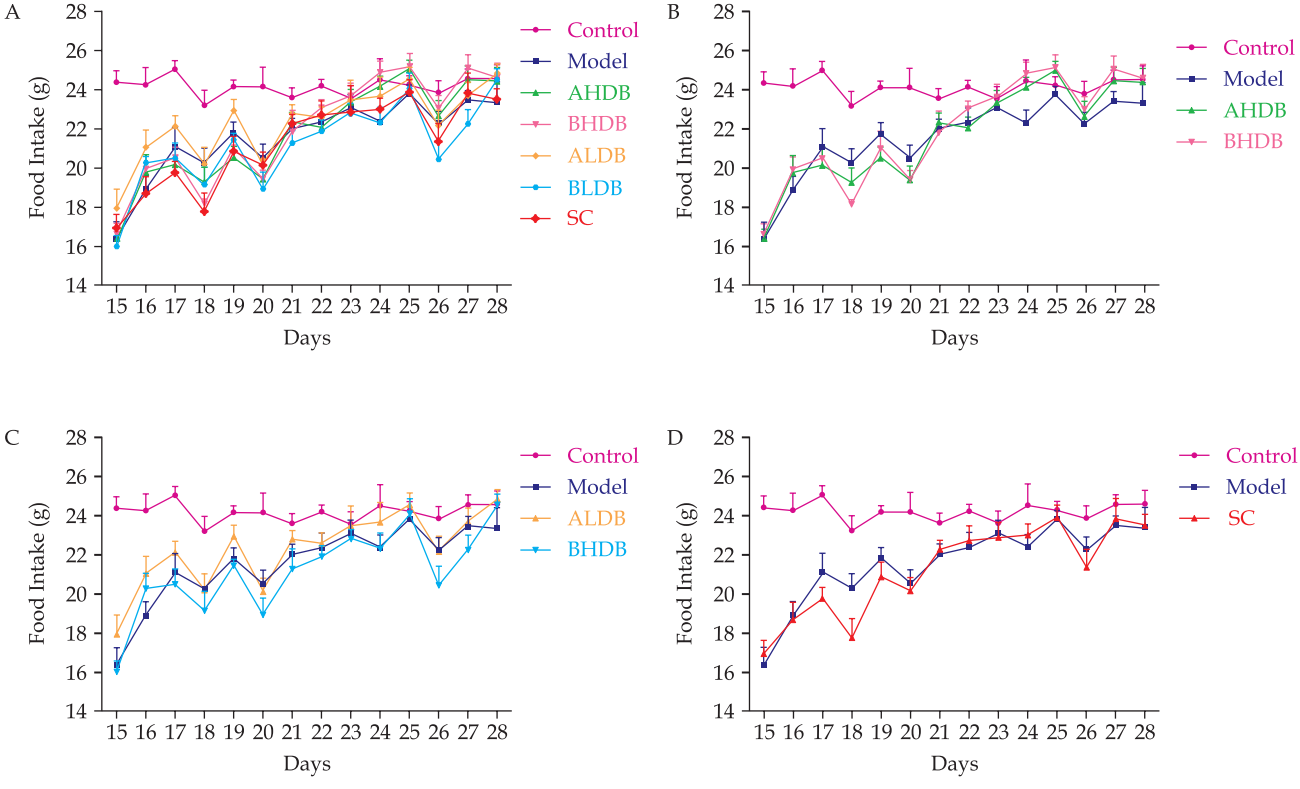

Fig 4. A. Changes in daily food intake during treatment of KYDS rats with Bokhi. B. Changes in daily food intake during treatment of KYDS rats with high doses of Bokhi (AHDB and BHDB). C. Changes in daily food intake during treatment of KYDS rats with low doses of Bokhi (ALDB and BLDB). D. Changes in daily food intake during treatment of KYDS rats with SC.

less; became unresponsive inactive and dispirited; their eyes became glazed; they appeared chilled; suffered hair loss, weight loss and anal pollution; engaged in arching their backs and twining. These symptoms caused by using large doses of hormones to induce KYDS were consistent with the research as discussed by Gou *et al* (2009) and matched the symptom diagnosis standard for an animal model of KYDS from the 'Reference standard for syndrome differentiation of traditional Chinese Medicine' (Shen and Wang, 1986).

Following the establishment of the model (days 15-28), the weight of the Model group rats did increase, but there was no improvement in the other symptoms and indeed in some cases they were even worse. Symptoms in the drug therapy groups receiving Bokhi or SC improved: urine volume normalised, their hair gradually regained luster, depilation reduced, their spirits improved and their activity increased. From the changes in appearance of the drug therapy groups it could be seen that, while the KYDS model had established successfully, that Bokhi had a positive effect on alleviating the symptoms of KYDS.

Effects of Drug therapy on SCR and BUN in KYDS model rats

After 14 successive days of administration of drug therapy, SCR levels in the AHDB, BHDB and ALDB groups were not significantly different to the Control group (P > 0.05, Table 1). However, levels of SCR in the AHDB, BHDB and ALDB groups were all significantly different to levels in the Model group (P < 0.01, Table 1). This showed that in the AHDB, BHDB and ALDB groups that Bokhi had a good effect on SCR regulation compared with KYDS rats.

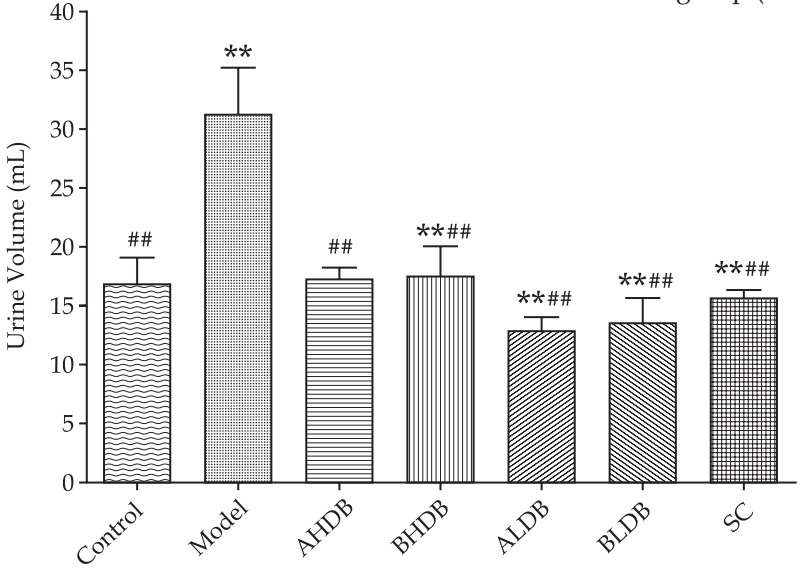

Fig 5. Effects of 14 successive days of Bokhi administration on urine volume (24 h) of KYDS model rats. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Table 1. Effects of Bokhi on SCR and BUN in KYDS model rats compared with control rats and rats receiving drug therapy.

Group	SCR (umol/L)	BUN (mmol/L)
Control	68.174±0.736$^{\#\#}$	4.592±0.158$^{\#\#}$
Moldel	76.916±0.827**	6.699±0.279**
AHDB	67.612±0.835$^{\#\#}$	4.861±0.238$^{\#\#}$
BHDB	68.324±0.921$^{\#\#}$	4.856±0.222$^{\#\#}$
ALDB	67.821±0.647$^{\#\#}$	5.256±0.079$^{*\#\#}$
BLDB	70.248±0.931$^{*\#\#}$	5.130±0.585$^{\#\#}$
SC	76.810±0.000**	5.370±0.066$^{*\#\#}$

The data shown are mean scores ± SD, n = 10. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Levels of BUN were not significantly different in the AHDB, BHDB and BLDB groups compared with the Control group (P > 0.05, Table 1). However, levels of BUN in the AHDB, BHDB and BLDB groups were all significantly different to levels in the Model group (P < 0.01, Table 1). This showed that in the AHDB, BHDB and BLDB groups that Bokhi had a good effect on BUN compared with KYDS rats.

Effects of drug therapy on hormones in KYDS model rats

Levels of the hormone, T, in the AHDB and BHDB were significantly different to the Control group (P < 0.01, Fig 6). Levels of T were significantly different in the AHDB and BHDB (P < 0.01, Fig 6) compared with the Model group. Provision of 14 successive days of drug therapy to rats after the establishment of KYDS did not restore T levels to that of the Control group. However, the greatest improvements were found in the high dose Bokhi groups (i.e. AHDB and BHDB, Fig 6).

Levels of TSH in the AHDB and BHDB groups were not significantly different to levels in the Control group (P > 0.05, Fig 7). Levels of TSH in the AHDB and BHDB groups were significantly different to the Model group (P < 0.01, Fig 7). Levels of TSH in the Model group were seriously unbalanced, while in the drug therapy groups there was a regulatory effect on

TSH. The effects of AHDB and BHDB were the best as TSH levels in KYDS rats returned to normal levels.

Effects of Bokhi on SOD and NO in KYDS model rats

Levels of SOD in the AHDB, BHDB and SC groups were not significantly different to the Control group ($P > 0.05$, Fig 8). Levels of SOD were significantly different in the AHDB, BHDB and SC groups ($P < 0.01$) compared with the Model group. The effects of the high dose Bokhi were similar to the effect of SC. Both the high dose Bokhi and SC returned the level of serum SOD in rats with KYDS to near normal values.

Levels of NO in all six groups were significantly different to the levels in the Control group ($P < 0.01$, Fig 9). Levels of NO were significantly different in the Control, AHDB, BHDB and ALDB groups ($P < 0.01$) and the BLDB and SC groups ($P < 0.05$) compared with the Model group (Fig 9). Provision of 14 successive days of drug therapy to rats after the establishment of KYDS, did not restore NO levels to that of the Control group. But the best effects were found in the AHDB, BHDB and ALDB groups.

Effects of Bokhi on the Organ Indices of KYDS model rats

The vesicula seminalis index was not significantly different in the AHDB and BHDB groups ($P > 0.05$, Table 2) compared with the Control group. The vesicula seminalis index was significantly different in the AHDB and BHDB groups ($P < 0.01$) compared with the Model group (Table 2).

All treatment groups ($P < 0.01$, Table 2) were a significant difference in the spleen index compared with the Control group and the Model group. Drug therapy to rats after the establishment of KYDS did not restore the Spleen index to the levels of the Control group.

There was no significant difference in the kidney index in the AHDB group compared with the Control group ($P > 0.05$, Table 2). There was a significant difference in the kidney index of the AHDB group ($P < 0.01$) compared with the Model group (Table 2).

All treatment groups ($P < 0.01$, Table 2) were significantly different in the testes index compared with the Control group and the Model group. Drug therapy to rats after the establishment of KYDS did not restore the testes index levels to that of the Control group.

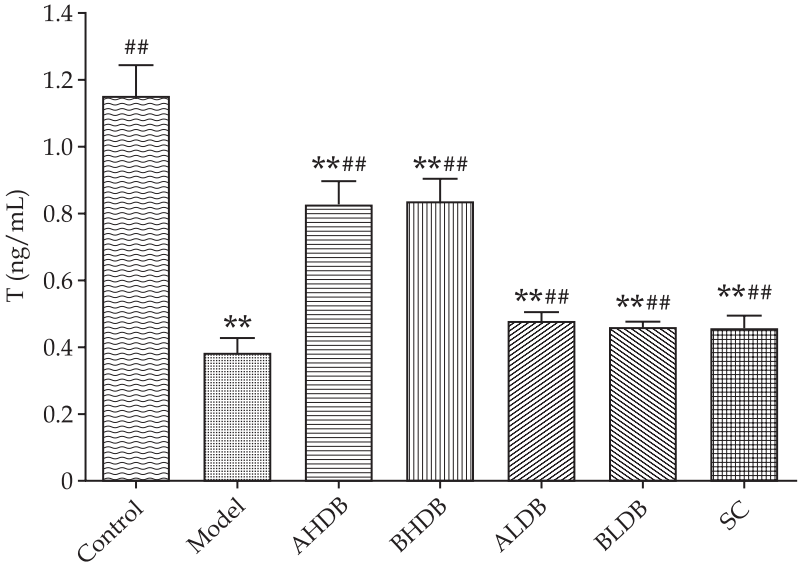

Fig 6. Effects of Bokhi on the hormone, T, in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Table 2. Effects of Bokhi on the vesicula seminalis, spleen, kidney and testes indices of KYDS model rats compared with control rats and rats receiving drug therapy.

Group	Vesicula Seminalis (g/100g)	Spleen (g/100g)	Kidney (g/100g)	Testes (g/100g)
Control	$0.382\pm0.038^{\#\#}$	$0.191\pm0.012^{\#\#}$	$0.729\pm0.010^{\#}$	$1.010\pm0.055^{\#\#}$
Moldel	$0.333\pm0.038^{**}$	$0.287\pm0.022^{**}$	$0.707\pm0.011^{*}$	$1.239\pm0.095^{**}$
AHDB	$0.390\pm0.013^{\#\#}$	$0.223\pm0.003^{**\#\#}$	$0.745\pm0.015^{\#\#}$	$1.125\pm0.070^{**\#\#}$
BHDB	$0.374\pm0.012^{\#\#}$	$0.215\pm0.005^{**\#\#}$	$0.750\pm0.026^{*\#\#}$	$1.106\pm0.079^{**\#\#}$
ALDB	$0.342\pm0.050^{**}$	$0.251\pm0.004^{**\#\#}$	$0.778\pm0.020^{**\#\#}$	$1.151\pm0.051^{**\#\#}$
BLDB	$0.351\pm0.011^{**\#\#}$	$0.246\pm0.001^{**\#\#}$	$0.767\pm0.020^{**\#\#}$	$1.177\pm0.012^{**\#\#}$
SC	$0.409\pm0.017^{**\#\#}$	$0.389\pm0.008^{**\#\#}$	$0.816\pm0.012^{**\#\#}$	$1.151\pm0.070^{**\#\#}$

The data shown are mean scores ± SD, n = 10. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Principal Component Analysis (PCA)

The data (growth rate, urine volume, SCR, BUN, T, TSH, SOD, NO, vesicula seminalis index, spleen index, kidney index and testes index) was used in PCA (Fig 10). The Model group and the Control group was very distant from each other showing that the biochemical function of the Model group had been pathological changed. It also indicated that the establishment of the KYDS model rats had been successful. The Bokhi high dose groups (AHDB and BHDB) were the closest to the Control group and also distant from the Model group. The distance between the Bokhi low dose groups (ALDB and BLDB) and the Control group was slightly closer than the distance between Bokhi low dose groups (ALDB and BLDB) and the Model group. The SC group was distant from both the Control group and the Model group. SC had a therapeutic effect on erectile dysfunction caused by KYDS (Xu *et al*, 2010) but had little effect on the other symptoms of KYDS. Therefore, the treatment effect of high doses of Bokhi on KYDS rats was the most beneficial.

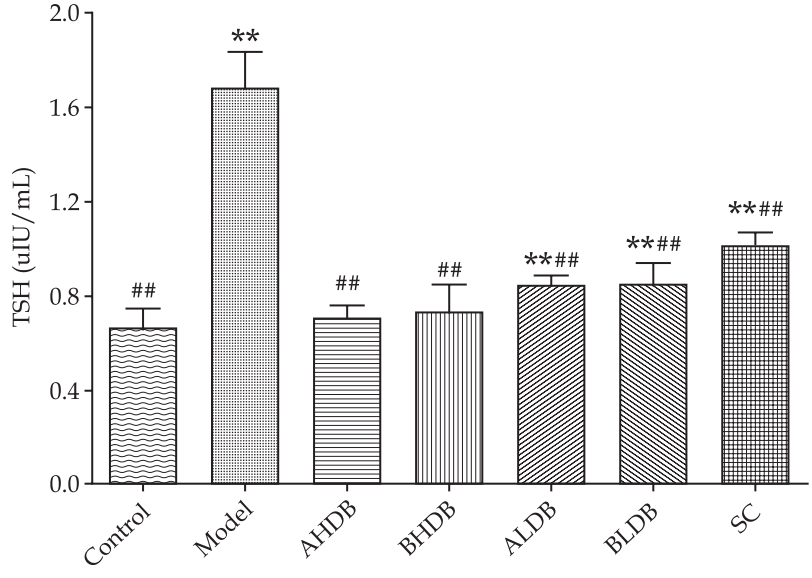

Fig 7. Effects of Bokhi on levels of the hormone, TSH, in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

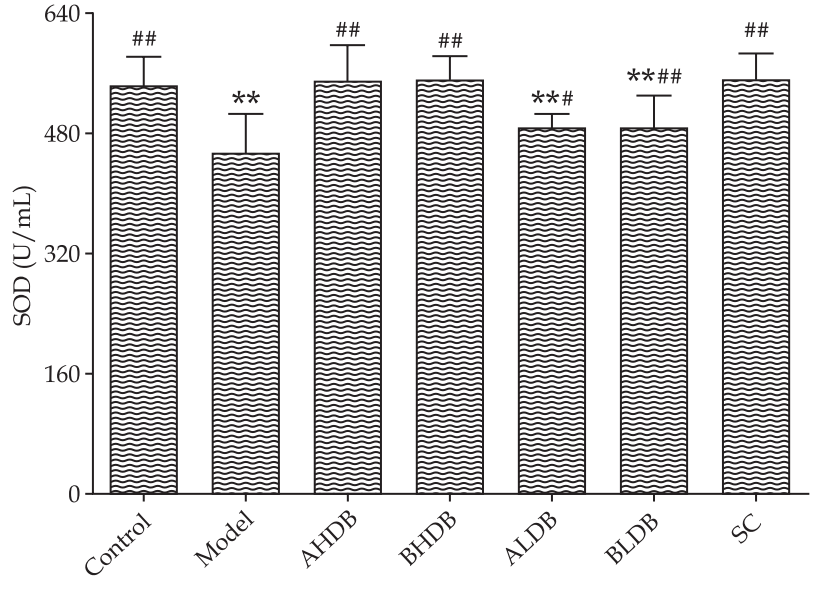

Fig 8. Effects of Bokhi on SOD in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

Discussion

Growth rate of model rats following 14 successive days of subcutaneous injection with hydrocortisone grew similarly and significantly more slowly than rats in the Control group. This phenomenon was consistent with the report as discussed by Xiao *et al* (2008) who used hydrocortisone injection over a short-term (7 days) to induce Kidney-Yin Deficiency and, over a longer period (10 days) to induce Kidney-Yang-Deficiency when weight increased slowly. The general condition of rats during the period of model establishment was the same as the condition observed as discussed by Liang *et al* (1999) who observed that long-term high doses of hormones resulted in slow increases in body weight, lethargy, dull hair, crowding together, polyuria and oliguria.

SCR and BUN, respectively, are the end products of the metabolism of nitrogen-containing organic compounds and proteins and, to a certain extent, reflect renal function. Treatment with Bokhi significantly improved the permeability of the glomerular filtration membrane. The excretion of SCR and BUN in metabolic products improved renal function and reduced the symptoms of KYDS. The high dose of Bokhi regulated SCR and BUN most effectively as they returned to close to the normal value.

KYDS does not only cause a functional disorder of the hypothalamus-pituitary-adrenal-cortex axis, but

also dysfunction to varying degrees in different target gland axes (such as the thyroid axis and the gonadal axis) and the neuroendocrine system. These can cause declines in reproductive function in patients with KYDS due to decreases in plasma androgen in males and plasma oestrogen levels in females (Si, 1994). The experimental results showed that following establishment of KYDS in rats, subsequent drug therapy was unable to return T levels to the level of the Control group. Serum T content decreased, which showed that the changes in sex hormones resulted in different levels of gonadal axis dysfunction (Qiu *et al,* 1999; Hu *et al,* 2014). The level of TSH in rats with KYDS was higher than that in control rats, which showed that there was a certain degree of functional disorder in the thyroid axis (Wang *et al,* 2015). High doses of Bokhi was the best treatment to improve levels of T in KYDS rats and reduce the levels of TSH, thereby indirectly regulating KYDS caused by rat gonad axis and thyroid axis disorders.

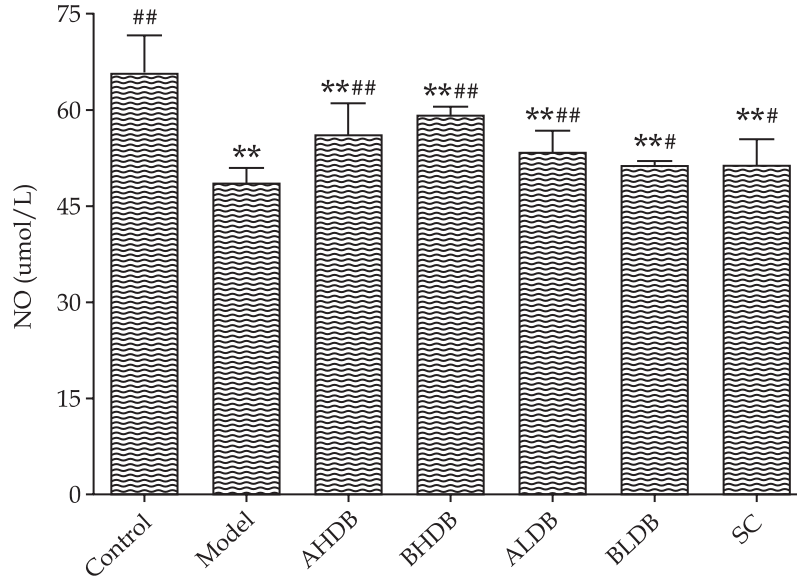

Fig 9. Effects of Bokhi on NO in KYDS model rats compared with control rats and rats receiving drug therapy. $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with the Control group; $^{\#\#}P < 0.01$ and $^{\#}P < 0.05$ compared with the Model group.

KYDS can lead to decreased SOD activity, i.e. the body's ability to eliminate free radicals is weakened (Rong *et al,* 2016). High doses of Bokhi were most effective at increasing levels of SOD, improving scavenging of free radicals and reducing symptoms of premature senility. In KYDS rats the serum NO content decreased significantly. This reduced secretion was probably caused by insufficient cell damage, leading to cell proliferation, which causes glomerular sclerosis and may be important for the symptoms of KYDS caused by hydrocortisone. High doses of Bokhi increased the content of NO. The mechanism for this may be due to increased activity of NOS, which can directly relax blood vessels, increase renal blood flow and glomerular filtration rate. This would reduce kidney damage, causing smooth muscle relaxation, increased intracavernosal blood flow and increased erectile potential (Chancellor *et al,* 2003).

Injection with high doses of an exogenous glucocorticoid, such as hydrocortisone, is a classical method of establishing KYDS in a model animal and induces adrenocortical insufficiency after abrupt withdrawal of the glucorticoid (Tan *et al,* 2014). To a certain extent this animal model mimicks the pathological state of suppression of the hypothalamic–pituitary-adrenal (HPA) in humans with KYDS and can contribute greatly to important advances in the current understanding of the underlying mechanisms of KYDS as well as treatments (Yang *et al,* 2008; Wang *et al,* 2012). In this study, Bokhi collected from different regions had similar effects as a treatment for KYDS; the only difference occurred between the high and low doses. High doses of Bokhi significantly improved symptoms of KYDS in rats and the treatment effect was better than treatment with SC, a drug commonly used for treatment of KYDS. The research as discussed by Guo *et al* (2013) has reported that acute toxicity of camel Bokhi and showed that it produced no toxic effects within 24 h in mice at a dose of 5,000 mg/(kg bw). We therefore propose that it is safe to use Bokhi in the short term without obvious side effects.

Bokhi, which has a dark brown colour, watery consistency and heavy, somewhat sweet aroma saturates the long nape hair of sexually mature animals and is used to scent mark the hump and objects in the environment. The research as discussed by Ayorinde *et al* (1982) has reported that the male Bactrian camel's occipital scent gland produces a series of steroids including 5a-androst-16-en-3-one in addition to a series of fatty acids and γ-dodecalactone. 3-Methylbutanoic acid is the most volatile of the acids which include hexanoic, a decenoic and the saturated acids from C15 to C25 with the exception of C24. Mass spectrometry results showed that their molecular ions of these compounds have high indices of hydrogen deficiency. The constitution of this secretion changes appreciably with the season.

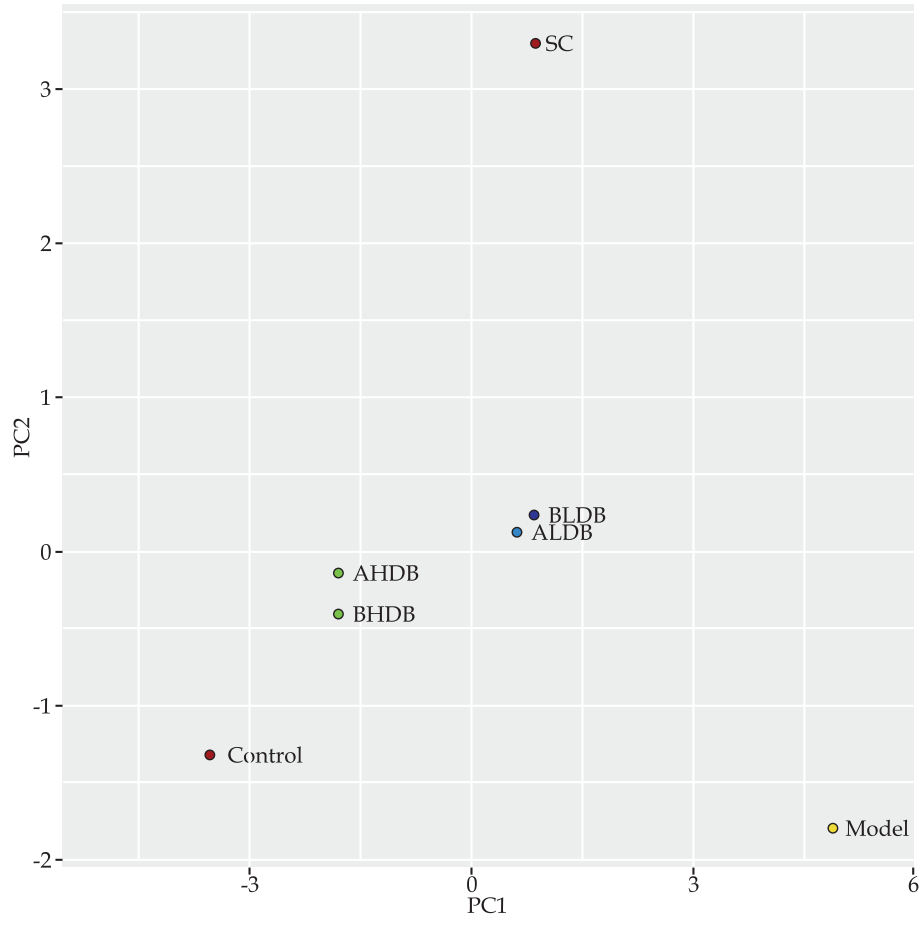

Fig 10. Effects of Bokhi on PCA in KYDS model rats compared with Control rats and rats receiving drug therapy.

To date, the underlying mechanisms of KYDS have been unclear, especially as there are no effective drug treatments for KYDS. What the components of Bokhi are that improve the symptoms of KYDS also needs further research. Bokhi has naturally high levels of medicinal ingredients and would have considerable potential as a new drug.

Acknowledgements

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Ethical Approval

All animal procedures were approved by Animal Care and Use Committee at Inner Mongolia Agricultural University.

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SEROPREVALENCE OF BLUETONGUE IN DROMEDARIES

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ABSTRACT

Bluetongue (BT) is an infectious, non-contagious, arthropod-borne viral disease, mainly of sheep but many domestic and wild animals are also affected by this disease. A serological study was aimed at the detection of BTV antibodies by Agar Gel Immunodiffusion test (BT-AGID) and competitive Enzyme Linked Immunosorbent Assay (c-ELISA) in dromedaries of North Gujarat and Kachchh regions. Out of 533 serum samples, the BT-AGID test detected antibodies against BTV in 83 cases (15.57%) while c-ELISA test was positive in 136 cases (25.51%).

Key words: Bluetongue, BT-AGID, camels, c-ELISA, seroprevalence

Bluetongue (BT) is an infectious and non-contagious arthropod borne viral disease of domestic and wild ruminants mainly sheep, goat, cattle, camels, deer and antelopes (Molalegne *et al*, 2013). It is transmitted by arthropods of the genus *Culicoides* and mostly prevalent in tropical, semitropical and temperate regions of the world (Tabachnick, 2004). It is a notifiable disease and is listed with those diseases that can spread rapidly and that have a considerable impact on the health of livestock (Mozaffari *et al*, 2013). The economic losses due to BT is around 3 billion US$ per year in the world (Sperlova and Zendulkova, 2011). Direct losses are caused by death, abortions, weight loss, reduced milk and meat productions. Indirect losses are through export restrictions of live animals, semen and foetal calf serum (Bitew *et al*, 2013). The diagnosis of a BT infection relies primarily on assays detecting antibodies to a group-specific antigen. Agar Gel Immunodiffusion (AGID) test is the most widely used assay for this purpose (Khimaniya *et al*, 2013). Although, the AGID test is simple and rapid to perform, however, it gives cross-reactions with other orbiviruses, e.g. epizootic haemorrhagic disease virus. To overcome this problem, monoclonal antibody based competitive ELISA (c-ELISA) is a highly specific as well as sensitive test for detection of BTV antibodies (OIE, 2016).

Materials and Methods

Test samples

A total of 533 sera were collected from dromedaries of North Gujarat (363) and Kachchh (170) regions of Gujarat state including 2 different breed Kachchhi (436) and Marwari (97). Agewise sera were collected from three different group of age 3 to 5 years old (55), 5 to 7 years old (153) and more then 7 years old (325) dromedaries. Approximately 10 ml of blood were collected from individual animals aseptically from the jugular vein using plain BD Vacutainer. The vacutainer tubes were kept in slanting position at room temperature for 2 hours and centrifuged at 3000 rpm for 10 minutes. The separated serum was collected in screw capped plastic vial and heat inactivated at 56°C for 30 minutes. The sera were held at –20°C temperature until tested.

All 533 dromedary sera were tested with commercially available AGID and cELISA. The test methods used followed strictly the kit manufacturer's description and the protocol of Pearson and Jochim (1979) and Afshar *et al* (1987).

The performance of c-ELISA and BT-AGID tests for the detection of BTV group specific antibodies in camel sera was compared with each other on 533 dromedary sera. Considering c-ELISA as reference test, cross tabulation of c-ELISA and BT-AGID was recorded as per the method described by Martin (1977) to determine relative sensitivity and specificity of BT versus AGID by the following formula:

$$\text{Sensitivity (\%)} = \frac{\text{(c-ELISA and BT-AGID positives)}}{\text{c-ELISA positives}} \times 100$$

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$$\text{Specificity (\%)} = \frac{\text{c-ELISA and BT-AGID negatives}}{\text{c-ELISA negatives}} \times 100$$

Results and Discussion

Seroepidemiological studies are important tool for the epidemiology of BT (Mehrotra and Shukla, 1990) and serological surveys are used to analyse the infection status of ruminants in an area (Sreenivasulu and Subba Rao, 1999). Eighty three sera (15.57%) were positive by BT-AGID, while 136 (25.51%) were positive by c-ELISA. Khimaniya *et al* (2013) reported BTV antibodies of 14.30% and 24.35% by AGID and cELISA tests, respectively. Mohamed *et al* (2012) reported a 25.70% seroprevelance of BT in camels in Iran by cELISA. In contrast to our findings, a lower rate of BTV antibodies were reported by Chandel and Kher (1999) in Gujarat and Mallik *et al* (2002) in Rajasthan by AGID test, while Chauhan *et al* (2004) reported higher rates of seroprevalence in camels by AGID and c-ELISA tests. An overview of BT seroprevalence in camelids were also given by Wernery *et al* (2014).

In North Gujarat, overall seroprevalence found was 16.25% and 27.27% by AGID and cELISA, respectively while in Kachchh region, the seroprevalence found was 14.11% by AGID and 21.76% by cELISA.

Comparing the seroprevalence of the 2 different dromedary breeds tested, the results showed that Marwari breed showed a higher prevalence over Kachchi breed (19.6% and 32% to 14.7% and 24.1%).

Sera were collected from different age group of camels. The highest seroprevalence was reported in the old age group while in case of the young age group, it was lowest both, by AGID and c-ELISA.

$$\text{Overall agreement (\%)} = \frac{\text{c-ELISA and BT-AGID positives + c-ELISA and BT-AGID negatives}}{\text{c-ELISA positives + c-ELISA negatives}} \times 100$$

Out of 533 sera tested, 136 sera were found positive and 397 sera were found negative by both the tests, while 53 samples detected positive by c-ELISA were negative in BT-AGID test. Relative to c-ELISA results, the sensitivity of BT-AGID was 61.02% and specificity was 100.00%. Overall agreement between both the tests was 90.05%.

Acknowledgement

The Bluetongue virus (BTV) antibody test kits AGID and c-ELISA were made available by Dr. M. M. Jochim, Veterinary Diagnostic Technology Incorporation, U S A.

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Special session on camels in 11th International Veterinary Congress at Berlin, Germany

Special session on camels with a theme "Camel Research: Challenges and Opportunities" will take place in 11th International Veterinary Congress at Berlin, Germany scheduled on 2-3 July 2018.

There will be limited participants in this special session and their deliberations will be of great value to the participants. Desirous participants are requested to submit their abstracts at an earliest.

Dr. T.K. Gahlot is the Organising Secretary for the special session and he can be contacted on email tkcamelvet@yahoo.com

Note: Please find Registration fees of this conference at https://veterinary.conferenceseries.com//registration.php

For queries please contact at Email: veterinary2017@veterinaryseries.com

Camel Conference held at Inner Mongolia, China

The international conference "The Belt and Road: Camel Science, Industry and Culture" was held on 22-26th September 2017 at Alxa League, Inner Mongolia, China.

The main topics discussed were Camel Genetics and Genomics, Camel Products: camel milk, meat, hair, camel blood, leather & bones, Camel Reproduction and Management, Camel Nutrition and Metabolism, Camel Health and Diseases and Camel Culture and Tourism. It was attended by more than 200 delegates from China and other countries. The conference included important visits to various places of camels and camel products. The opening ceremony of Bactrian camel festival was also witnessed by the delegates. The cooperative camel breeder society, machine milking of she camels, market of camel products and good cultural programmes were seen by the delegates in and around inner Mongolia.

Glimpses of International Camel Conference, Inner Mongolia, China

Delegates of International Camel Conference, Inner Mongolia

Delegates tasting Bactrian camel milk products

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Short Communication

AMPUTATION OF FORE LIMB FOLLOWING COMPOUND FRACTURE OF RADIUS ULNA IN A FEMALE CAMEL

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A 9 year old female camel was presented to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, Bikaner with a history of accident causing compound fracture of left radius-ulna just above the knee joint along with a wound over the fracture site. Clinical examination revealed the compound fracture of distal end of left radius-ulna (Fig 1). There was complete loss of sensation with coldness of distal region of the foot. Extensive necrosis of tissue with foul odour was observed at the fractured site. Limb amputation was done at distal end of radius-ulna bone above the fracture site under xylazine sedation (0.4 mg/kg body weight, intravenously). The skin, subcutaneous tissue and muscles were incised to the bone. The bone was amputated by a wire saw. The haemorhage was checked by ligating the vessels. The muscles were sutured in continuous suture pattern using chromic catgut no. 2 to cover the bony stump and skin was closed in horizontal mattress pattern using silk no. 2. Postoperatively (Oxytetracycline @ 10 mg/kg body weight, for 5 days and Meloxicam @ 0.4 mg/kg body weight, were given intramuscularly for 3 days and vitamin B_1, B_6 and B_{12} 20 ml intramuscularly for 7 days. Antiseptic dressing on every alternate day along with proper bandaging was done for 2 weeks. Skin sutures were removed after 2 weeks. The female camel showed remarkable ability to walk on remaining 3 limbs without any assistance and complication (Fig 1).

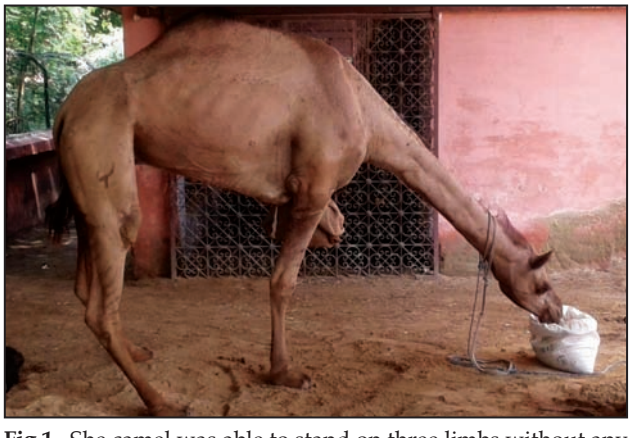

Fig 1. She camel was able to stand on three limbs without any support and assistance 5 day after leg amputation. Animal showed uneventful recovery and returned to normal feeding.

Fracture is a common surgical affection in camel mostly traumatic in origin. The most common fracture is that of horizontal rami of mandible in camels followed by fractures of metatarsus and metacarpus (Gahlot and Chauhan, 1994). The fracture of radius-ulna is not very common (Siddiqui and Telfah, 2010). The external fixation by plaster of paris cast does not provide adequate immobilisation due to inability to incorporate the elbow joint in the cast (Siddiqui and Telfah, 2010). The primary goal should always be to save the limb but if it is not possible, limb amputation can be performed (Desrochers *et al,* 2014). The limb amputation is a drastic treatment in untreatable cases of fracture. Kumar *et al* (2013) performed limb amputation in 2 camels suffering from compound fracture along with gangrene of distal part of metacarpus.

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Glimpses of International Camel Conference, Inner Mongolia, China

Delegates with Prof Jirimutu who attires traditional Mongolian dress

An artistic Bactrain camel chariot during Bactrian camel festival was important attraction

Delegates respecting National Anthem during inaugural session

Bactrian camel caravan during Camel Festival inaugural session

Dias and dignitaries during inaugural session

Depiction of Bactrian camel as a super market. Camel products were displayed through a cut out window of Bactrian camel

Glimpses of International Camel Conference, Inner Mongolia, China

Marketing of camel meat products in attractive packings

Delegates of Inner Mongolia, China with Indian delegates

Cosmetics made out of camel milk in export quality finish

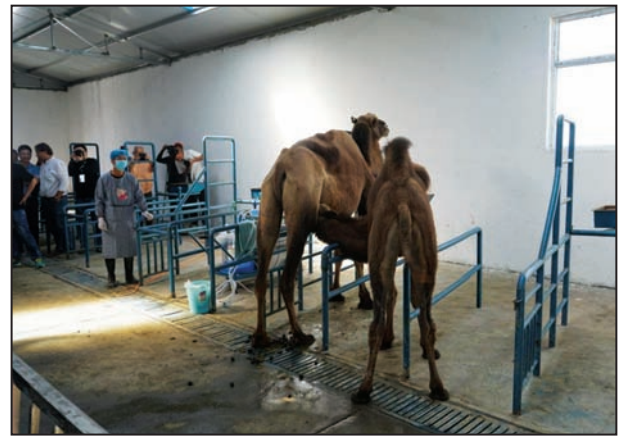
Twins born from Bactrian female attracted attention of all delegates

Various products made out of camel milk on display

Camel city was made for cooperatives of camel herdsman

Glimpses of International Camel Conference, Inner Mongolia, China

Dr. T.K. Gahlot, Editor of camel books and Journal of Camel Practice and Research presenting journals and books to Prof Jirimutu



Milking of camels by machine milking

A herd of Bactrian camels

A discussion of Indian delegation with authorities of Inner Mongolia Research Centre of Camels

Camel carvings discovered in Saudi Arabia

Archaeologists (a joint Franco-Saudi research team) have discovered 2,000-year-old life-size camel carvings, unlike any others in the region, in the northwestern Al Jawf province in Saudi Arabia. Scientists believe that the area's proximity to caravan routes suggest it could have served as a place of worship or boundary marker. Engravings and paintings are the most common techniques found in Arabian rock art, making the latest discovery unique for its use of sunken reliefs. Rock art is widespread in the kingdom. The Rock Art in the Ha'il region, which also depicts camels, is featured on UNESCO's list of World Heritage Sites.

Saudi Arabia pushes for international forum on camels

Organisers of the King Abdul Aziz Camel Festival are planning to hold an international forum that would bring together people with interests in camels to reflect on ways to generate greater attention, better care and more business about the desert animals. This platform can be used to research ways to benefit from camels whether economically, nutrionally or other ways.

Dr Fahad Bin Abdullah Al Samawi, the Secretary General of the Riyadh-based King Abdul Aziz Foundation for Research and Archives (Darah) and the general supervisor of the festival, informed about a major plan to build on the success of the current King Abdul Aziz Camel Festival by launching a forum that would promote research about camels and bring together people from across the world to exchange views about them. The forum will enable the participants to explore new ideas about how to take care of camels in a better way. It will be an opportunity for camel experts to exchange views on ways that would benefit camel owners, the economy and tourism. They can tell owners about economic and health benefits of camel milk. The main goal of the forum will be raise awareness about importance of camels like horses.

World's first camel hospital just opened in Dubai

Last week, in a world's first, a new 40 million dirhams ($10.9 million) camel hospital opened in Al Marmoum, Dubai, with state of the art treatment on par with that offered for racehorses. The hospital will be able to treat up to 20 camels at any given time and is equipped with a small racetrack to get the camels rehabilitated after their medical procedures. Its customised equipment were adapted from equestrian medical equipment to accommodate camel treatment. A surgery starts at around 3500 dirhams ($990) and an X-ray or ultrasound at 400 dirhams ($110). The hospital intends to help in the research and development of camel medicine.

Camels carry a heavy viral load

A burden of mammalian viruses makes camel a breeding ground for novel human diseases. Camels are a melting pot for mammalian viruses, according to new research, and may serve as an incubator for the production of novel viruses which would infect humans. Despite thousands of years of close proximity to humans, camels generally haven't been considered a major source for human diseases. However, the MERS coronavirus (MERS-CoV) which caused a disease outbreak in Saudi Arabia in 2012 is known to have originated in bats and incubated in camels before infecting humans. Camels are thought to serve as a major reservoir of MERS-CoV, and a 2014 study identified a range of mammalian viruses in pooled camel faecal samples. In the new study, a team led by researchers from

the Abu Dhabi Food Control Authority and the US Centres for Disease Control and Prevention (CDC) sequenced DNA from nasopharyngeal samples taken from 108 camels known to carry MERS-CoV. They found sequences related to mammalian viruses from 13 genera in 10 families, including viruses known to infect humans and other animals, as well as some potentially novel camel viruses. Many of the camels were infected with viruses from two or three different genera, and MERS-CoV was found with another coronavirus, alpha-CoV, in more than 90% of the samples. The high co-infection rates raise the risk that viruses might recombine, borrowing genetic material from each other, gaining the ability to infect a different host species. This risk is exacerbated by the fact that humans and camels often mix in live animal markets which are home to many other species such as cattle, sheep, and goats and even occasionally chickens, dogs, or cats.

Saudi Arabia establishes the first official camel club

A camel club is established for the first time in Saudi Arabia. King Salman issued a decree to launch the club, reported Al Arabiya. King Salman endorsed on April 13 the closing ceremony of King Abdulaziz Camel Festival in its new version for this year in Siahed, northeast of Riyadh. The Saudi king announced the inauguration of a specialised village for camels to boost its folkloric significance,

researches and trade in the Kingdom. The initiative is an expression of the Kingdom's attentiveness in supporting the country's heritage, culture and support for camels and its importance to Saudi Arabia's heritage and history. The club will be an important reference for health related issues and historical researches that would function as an official umbrella for camel owners in the Kingdom. It will also play social and cultural roles linking the current generation and the desert icons. It will also work for preservation of rare breeds of camels, provide accurate statistics in cooperation with sectors on the numbers and types of camels, research in camel diseases and cooperation with their owners, communicate with similar clubs in the region and learn about their experiences. It will also help supervising duties on all festivals, competitions and auctions for camels. It will provide cooperation with government sectors such as the Ministry of Education to encourage young people to attend camel competitions and activities.

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