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This issue is dedicated to Dr. U. Wernery

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Journal of Camel Practice and Research (JCPR) publishes only research and clinical manuscripts related to the Camelids (Old and New World camelids), hence published contents are consistent with the title and scope of the journal. Review articles on emerging research are invited and published. JCPR also publishes the news related to the New or Old World Camelids, specially those related to new products, conferences, books, trainings or workshops etc.

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CAMEL COLOSTRUM BANK- MAKING THERAPEUTIC USE OF IgG POSSIBLE

In a recent study by a team of camel scientists led by Dr. U. Wernery of CVRL, Dubai (UAE), there is a new hope of raising a colostrum bank for camel calves to protect them from many diseases. In their research, camel calves were found to acquire an adequate passive transfer of IgG through colostrum intake together with the intake of maternal antibodies against Brucellosis, MERS, and *Trypanosoma evansi*, which eventually protects them against these infections for at least two months of age. Dr. Wernery and co-researchers conducted another important research by serologically testing for seven infectious diseases of camels from different countries. All sera were found negative for FMD, PPR, and Anaplasma. However, high numbers of antibodies were found against CCHFV and WNV and, to a lesser extent, against *Trypanosoma evansi* and Brucellosis. Many salutes to dedicated camel scientist-Dr. U. Wernery, who benefitted camels not only in the middle east but at a global level as well. Fortunately, he turns octogenarian on 7th August, remains active, diligent, and indefatigable as a dynamic scientist, and will remain a legend. We all wish him good health and longevity.

World Camel Day was celebrated on 22nd June in many countries to raise awareness about camels and their importance to human societies and ecosystems and to celebrate these unique creatures.

The August issue of JCPR is an amalgamation of research on diverse aspects of camel science. It contains two review papers. The first is related to camel milk and other dietary treatments for autism. Christina Adams, the author of the famous book –Camel Crazy, made a fair attempt to co-author me in this review paper. Scientists from Mongolia and CVRL, Dubai, authored another review of bacterial and viral infections of Bactrian camels in Mongolia. A team of Indian scientists worked on the cytotoxic activity of camel milk whey protein as a nutraceutical against HeLa cells and found that camel milk whey may inhibit cell migration, induce DNA fragmentation, and activate the Caspase 3 mediated pathways. Dose specific effects of ionomycin on parthenogenetic activation of *in vitro* matured dromedary oocytes, expression of aquaporin 1 in the testis and epididymis, and glycosidase activities and steroids hormones concentrations during the follicular phase and parturition are few reproduction-based research papers which found a space in the current issue. Two camel milk-based papers are optimising processing conditions for camel's kefir milk and *in vitro* digestibility of spray-dried camel milk powder during accelerated storage. Other miscellaneous manuscripts are on the effect of roughage replacement in camel diet with tannin-containing tree leaves on digestibility and nutrient intake of lactating camel, histology of adrenal gland, and isolation and characterisation of a nitrate reductase positive *Corynebacterium pseudotuberculosis* strain from caseous lymphadenitis.

New research may come up in camel science this year as few laboratories were affected during the Covid-19 pandemic which hampered the research. I wish great times and good health to all editors, authors, and readers of the Journal of Camel Practice and Research.

(Dr. Tarun Kumar Gahlot)
Editor

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CAMEL MILK AND OTHER DIETARY TREATMENTS IN AUTISM: AN OVERVIEW

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ABSTRACT

The present study is an overview of diverse treatments adopted over the last three decades using camel milk or other complementary alternative therapies for autism. A pertinent review revealed that many hypotheses have been reported for using such treatments for autistic persons. Some researchers believe camel milk contains many essential vitamins, minerals, and immunoglobulins, which impart many health benefits for diseases and various health conditions, including autism. It is hypothesised that the consumption of camel milk by autistic persons reduces their oxidative stress by altering and increasing the levels of antioxidant enzymes and other benefits. Researchers have reported a decrease in the Childhood Autism Rating Scale (CARS) scores in autistic persons when camel milk is administered. Additionally, diverse psychopharmacological and dietary interventions such as gluten-free, casein-free diets, and ketogenic diets also are perceived to provide symptom abatement to many autistic persons but studies are mixed. The authors state that exclusive and highly focused research on camel milk and other alternative treatments is recommended to differentiate, authenticate and, if needed, expand various hypotheses pointed out by researchers over the last few decades.

Key words: Alternative treatments, autism, camel milk

Autism spectrum disorders (ASD) are classified as neurodevelopmental disorders and are categorised as developmental disabilities. In some children ASD symptoms appear within the first 12 months of life while in others they may not show up until 24 months of age or later. However, in some persons it can persist throughout life, although symptoms may improve over time. ASD-affected children often show different behaviour in addition to altered communication and social interaction. Children with ASD often show an approximately 3:1 male to female ratio (Loomes *et al,* 2017). The causes of ASD are unknown, but several genetic and non-genetic risk factors have been characterised that, alone or in combination, are implicated in the development of ASD (Sauer *et al,* 2021). Strathearn (2009) found that over the past six decades, public and scientific opinion on the etiology held it to be either a genetic abnormality, spawning a search for the "autism gene," or due to specific environmental factors. It was hypothesised that autistic children were both genetically predisposed to develop autism, but were also affected by parenting behavior (Kanner, 1949); however, the early focus on the parenting effect on autism has long since been rejected. Rossignol and Frye (2014) examined the evidence linking oxidative stress, mitochondrial dysfunction, and immune dysregulation/inflammation in the brain of ASD individuals. This pointed out that ASD has a clear biological basis with features of known medical disorders. In another study, Taylor *et al* (2020) found that genetic factors were primarily associated with autistic traits and were consistent over time, whereas environmental factors played a smaller role. Yet there is still no clarity on causation and the differing rates among types of children. Autism rates continue to climb globally, and autism is found in 1 of every 36 US children in 2020 (Maenner *et al,* 2023), with notable differences by sex and race. Additionally, mounting evidence of immune involvement in both schizophrenia and autism (Patterson, 2009) has been identified. The expression of immune-related molecules, such as cytokines in the brain and cerebral spinal fluid (CSF) show significant abnormalities. McGuinn *et al* (2020) studied a satellite-based model to assign air pollutant exposure averages during several critical periods of neurodevelopment of foetus during pregnancy till one year of age and found a positive association between early life air pollution exposure and ASD.

Psychopharmacological interventions

In recent years, research has increased on complementary alternative treatments to treat

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autism, though evidence supporting many of these is minimal. DeFilippis and Wagner (2016) mentioned that among current treatments, psychosocial therapies, such as Applied Behavioral Analysis (ABA) for the core symptoms of autism, are preferred, but their use is limited. Such professional services are expensive and labor-intensive. Lord *et al* (2018) opined that ASD children and adolescents should be treated primarily with educational and behavioral services and therapies, and medication is considered an important adjunct. Genovese and Butler (2020) pointed to diverse behavioral interventions, including high-intensity ABA, early intensive behavioral intervention (EIBI), and social skill interventions.

Currently, the most reliable medicines used to treat severely disruptive behavior and hyperactivity in ASD cases are risperidone and aripiprazole, duly approved by the US FDA. Still, the core symptoms are not cured (Alsayouf *et al,* 2021). Politte *et al* (2014) observed that children with ASD are generally more susceptible to the side effects of psychoactive medications. Children with ASD are also treated by psychopharmacological interventions, most commonly with stimulants, alpha-2 agonists, antipsychotics, anticonvulsants, and antidepressants (Madden *et al,* 2017). Therefore, alternative therapies are required due to the lack of effective and accessible options.

Dietary interventions

Dietary interventions, including the gluten-free and casein-free diet (GFCF), gluten-free diet (GFD), and ketogenic diet (KD), come into view as alternative therapies for ASD. Current reports are mixed. In a study of 62 adults and children with ASD, significant improvement in nonverbal intellectual ability was seen in the treatment group compared to the non-treatment group (+6.7 ± 11 IQ points vs. -0.6 ± 11 IQ points, $p = 0.009$) based on a blinded clinical assessment. A semi-blinded assessment showed the treatment group, in comparison to the non-treatment group, had significantly greater improvement in autism symptoms and developmental age. The treatment group also showed significantly greater increases in EPA, DHA, carnitine, and vitamins A, B2, B5, B6, B12, folic acid, and Coenzyme Q10. The results indicate that comprehensive nutritional and dietary intervention is effective at improving nutritional status, non-verbal IQ, autism symptoms, and other symptoms in most individuals with ASD (Adams *et al,* 2018). Nutritional intervention also offered neuroprotective factors against autism trait development in rat pups (Alsubaiei, 2023).

However, some available research data does not support the use of such diets as a primary treatment for individuals with ASD (Buie *et al,* 2010; Yu *et al,* 2022). This divergence between results is unexplained, partly because studies are scarce and factors such as intestinal permeability, bacterial population, enzymatic and inflammatory gastrointestinal activity should be assessed to identify possible responders (González-Domenech *et al,* 2022). For example, prevalence and risk of Functional Gastrointestinal Disorders (FGIDs) is significantly higher in ASD children and correlates with the severity of ASD, and bacterial and fungal diversity differ between ASD and NT children, indicating a difference in taxonomic abundance profiles (Lasheras *et al,* 2019) but existing studies are limited.

As it is generally believed by many parents and some medical clinicians specializing in autism that certain dietary modifications help alleviate gastrointestinal and behavioral symptoms for certain subsets of ASD children, further investigation is needed. Many researchers have found that children with ASD may not get the nutrition they need for appropriate growth and development (Adams *et al,* 2011; Arnold *et al,* 2003; Zimmer *et al,* 2012 and Herndon *et al,* 2009). Avoidant-restrictive food intake disorder (ARFID) is an eating disorder often associated with a heightened sensitivity to the sensory features of different types of food and may be more prevalent among children with ASD (Schimansky *et al,* 2023).

Camel Milk and Autism

Camel milk has been adjudged as a therapeutic agent for diabetes, autism, cancer, various infections, heavy metal toxicity, colitis, and alcohol-induced toxicity (Mihic *et al,* 2016). These researchers scanned 430 relevant studies by searching through MEDLINE (1946 to March 2016), EMBASE (1974 to March 2016), and Google Scholar; out of these 24 were included after assessment to reach a conclusion about clinical efficacy of camel milk in various diseases. Bashir and Al-Ayadhi (2014) investigated the role of the effectiveness of camel milk (CM) (raw and boiled) on thymus and activation-regulated chemokine (TARC) serum levels and Childhood Autism Rating Scale (CARS) score in subjects with autism and concluded that camel milk therapy over the course of two weeks significantly improved clinical measurements of ASD severity (per CARS scores). Furthermore, there were significantly decreased levels of serum of TARC among the study subjects.

Tahereh (2020) found that camel milk plays an important role in decreasing oxidative stress by alteration and increasing of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, myeloperoxidase and nonenzymatic antioxidants, and improved autistic behaviours.

A therapeutic advantage of camel milk in autism has been demonstrated by Al-Ayadhi and Elamin (2013). Shabo and Yagil (2005) and Yagil (2013) found that autistic children under 10 years had extremely high benefits while children over 15 years had remarkable gains by use of camel milk. Adams (2013) reported on her son, who was diagnosed with autism spectrum disorder (ASD) at nearly three years of age, and the positive results observed when he started consuming camel milk daily. Initially at age nine, he drank one half-cup (four ounces) of raw camel milk a day and experienced an overnight improvement in his symptoms, with eventual overall symptom improvement of 30%. There were sustained improvements in symptoms following his continued regular consumption of camel milk for six consecutive years (2007-2013) and it also worked as an fast-acting antidote to the negative behavioral effects and stomach pain resulting from bovine dairy and sugar consumption.

Oxidative stress has a key role in autism. Al-Ayadhi and Elamin (2013) evaluated the effect of camel milk consumption on oxidative stress in autistic children and showed that camel milk reduces the oxidative stress and improves the behavior of autistic children. Wernery *et al* (2012) reported similar results in autistic children after the use of camel milk as they displayed better social skills and had a reduction in hyperactivity and increased vigilance, plus sustained regular bowel movements. Mostafa *et al* (2021) concluded that a short course of camel milk consumption for two weeks neither significantly decreased the severity of autism nor increased serum anti-inflammatory vasoactive intestinal peptide levels. Kandeel and El-Deeb (2022) did a risk assessment and meta analysis of randomised clinical trials of application of natural camel milk products to treat autism spectrum disorders. The study of these authors found out that camel milk contains essential vitamins, minerals, and immunoglobulins, making it rich in antioxidant, antibacterial, and antiviral properties which reduces oxidative stress in consumers and improve many conditions, including ASDs. They opined that therapy with raw and boiled camel milk significantly lowers the CARS scores, leading to the conclusion that camel milk intake offers an advantage to those suffering from certain central nervous system (CNS) disorders. Liu *et al* (2022) summarised recent studies about the mechanisms and biomarkers of oxidative stress. These potential biomarkers could be used for early diagnosis and evaluation of ASD intervention, as well as to inform and target the pharmacological or nutritional treatment interventions for ASD.

Conclusions

An overview of published work on camel milk and other complementary alternative treatments in autism points to many hypotheses which may offer advantages to autistic persons to a variable extent. It is believed that camel milk reduces oxidative stress by altering and increasing the levels of antioxidant enzymes. Camel milk consumption is also noted to decrease the CARS scores in autistic persons. Diverse psychopharmacological and dietary interventions have also provided good relief to autistic persons although research is mixed and limited. The authors state that exclusive and highly focused research on the topic of camel milk and other alternative treatments is recommended to differentiate, authenticate and, if needed, expand various hypotheses pointed out by researchers over the last few decades.

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SEROEPIDEMIOLOGICAL STUDIES FOR THE DETECTION OF ANTIBODIES AGAINST SEVEN INFECTIOUS DISEASES IN OLD WORLD CAMELS FROM DIFFERENT COUNTRIES

U. Wernery, R Raghavan, NM Paily, S Thomas, S Raja and S Joseph

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ABSTRACT

One hundred fifty Old World Camel sera from different countries were serologically tested for seven infectious diseases. All sera were negative for FMD, PPR and *Anaplasma*. However, high numbers of antibodies were found against CCHFV and WNV and to a lesser extent against *Trypanosoma evansi* and Brucellosis.

Key words: Infectious diseases, Old World Camels, serology

Infectious diseases can play an important role in the economic viability of camel dairy enterprises and in camel races on the Arabian Peninsula. In many western countries, cattle health schemes for infectious diseases like IBR, BVD, Johne's disease and leptospirosis are licensed and regulated by 'Cattle Health Certification Standards', which is a self-regulatory body aiming to help control and eradicate non-statutory diseases (Winden and Pfeiffer, 2008). Such schemes have led to the eradication of certain diseases and for certified disease-free animals, a premium is paid when traded. Before these animal schemes can be applied to the camel industries, serological test methods, which are generally used for cattle, must first be evaluated for camels.

The evaluation has been started at Central Veterinary Research Laboratory (CVRL) some years ago and several scientific papers have been published on this subject (Wernery *et al,* 2007a; Wernery *et al,* 2008; Soellner *et al,* 2018), which was supported by WAHO (OIE) for brucellosis.

This work has now been extended to seven infectious diseases on Old World Camels (OWCs) including dromedaries, Bactrian camels and OWC hybrids from different countries. It is a serological epidemiological survey of 150 sera, the results of which are presented herewith.

Materials and Methods

A total of 150 sera from OWCs were collected or sent to CVRL for different investigations over a span of 2019-2022. At CVRL these were tested for seven different diseases. The serological test methods used are described here in more detail.

Foot-and-mouth disease (FMD)— PrioCHECK® FMDV-NS was used, which detects antibodies directed against non-structural 3ABC proteins of FMD independent of the serotype that causes the infection. It is a blocking ELISA, which discriminates between infected and vaccinated animals (Sorensen *et al,* 1998). The conjugate is directed against the non-structural 3ABC proteins and will block the binding of the antibody, if present, in the test sera. The conjugate is not directed against the animal species tested and therefore, the test can be used for different animal species.

West Nile Fever (WNF)— The ID screen, West Nile competitive ELISA was used, which detects antibodies directed against the pr-E envelope WNV protein. The test uses a conjugate against the IgG of the WNV. The conjugate is not directed against the animal species tested and can therefore be used for different animal species including camelids.

Peste des Petits Ruminants (PPR)— ID Screen PPR Competition ELISA was used which detects antibodies against nucleoprotein of PPR virus. An anti-NP peroxidase (FIRP) conjugate is used as the conjugate. The conjugate is not directed against the animal species tested and can therefore be used for different animal species including camelids.

Camel Trypanosoma antibody ELISA is an in-house developed ELISA based on the indirect enzyme

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linked immunosorbent assay (iELISA) technique. In this procedure, pre-diluted camel-sera are added to ELISA plate wells coated with non-infectious *T. evansi* antigen. If antibodies directed to the *T. evansi* antigen are present in the serum sample, they will bind to the parasitic antigen in the wells. An optimum dilution of Protein A horseradish peroxidase (HRP) conjugate is added to the wells, which will bind to the antigen antibody complex, which is further detected by the addition of substrate solution. Subsequently, colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by the development of blue colour. Upon addition of the stop solution the colour turns to yellow, the plates are read in a microplate absorbance reader, in which the optical density (OD) is measured at 450nm. Corrected OD_{450} of all test samples and controls is obtained by subtracting the mean OD_{450} of the blocking buffer control. The interpretation is based on the in-house determined cut-off criteria.

***Brucella* ELISA** multispecies enzymatic assay based on the blocking ELISA technique purchased from Gold Standard Diagnostics, Spain. The plates are coated with purified LPS of *Brucella abortus*. After adding the serum sample to the well containing specific antibodies against *Brucella abortus*, they will bind to the antigen, while if the sample does not contain any specific antibodies they will not bind to the antigen. When mAb (conjugated with peroxidase) specific for LPS is added, it will compete with the antibodies of the serum. If the serum samples contain specific antibodies, they will not permit the binding of the labelled mAb to the antigen, whereas if it does not contain specific antibodies, mAb will bind to the antigen on the plate. The binding of mAb is detected by a colorimetric reaction after the addition of the substrate. After the addition of the stop solution, the plates are read in a microplate absorbance reader, where the OD is measured at 450nm. The percentage of Inhibition (PI) of each sample and controls are calculated as follows, PI =100x {1- (OD sample/ OD negative control)}. There are innumerable *Brucella* antibody ELISAs commercially available, but according to our previous investigations, this ELISA gave the most reliable results (Soellner *et al,* 2018).

CCHF ELISA detection of antibodies against **CCHF** in serum was performed with the help of a double antigen multi-species commercial ELISA kit IDScreen®, CCHF Double Antigen Multispecies purchased from IDVet, France. This ELISA kit is based on an indirect technique where plates are coated with non-infectious recombinant N-protein of the lbAr10200 CCHFV strain (expressed in *E. coli*). Anti-NP antibodies, if present in the serum, form an antigen-antibody complex. A recombinant purified CCHF nucleoprotein antigen-HRP conjugate is added to the plate after the washing step. It fixes to free sites of the bound serum anti-NP antibodies. In the presence of antibodies, a blue colouration appears which becomes yellow after the addition of the stop solution. The plates are then read at 450nm and S/P % is calculated as follows: S/P%=100x (OD sample/ OD positive control).

Antibodies against **Anaplasma spp.** were investigated by using a commercial competitive ELISA (cELISA) from VMRD Inc, USA which detects antibodies against the major surface protein 5 (MSP5) of *A. marginale, A. centralei and A. ovis*. In principle, antibodies in the serum samples to *Anaplasma* inhibit the binding of a HRP labelled monoclonal antibody to the *Anaplasma* antigen coated in the plate wells. Binding or lack of binding HRP-labelled monoclonal antibody conjugate is detected by the addition of enzyme-substrate and is quantified after colour development. The presence of *Anaplasma* antibodies in the serum samples is indicated by weak or no colour development due to the inhibition of monoclonal antibody binding to the antigen of the solid phase. The plates are read at 630nm and the percentage of inhibition (PI) of each sample and controls are calculated as follows, PI= 100 x {1- (OD sample/OD negative control)}.

Results

One hundred fifty serum samples collected from OWCs from different countries were serologically tested. The summarised results, including origin of sera, are shown in Table 1.

As summarised in Table 1, all 150 sera tested were negative for FMD, PPR and *Anaplasma* antibodies. However, high numbers of antibodies were detected against CCHFV and WNV and to a lower extent against *Trypanosoma evansi* and *Brucella*.

Discussion

Old World Camels (OWCs) inhabit countries in North and East Africa, the Middle East and Far East Mongolia and China where FMD is endemic. Infection experiments with FMD serotypes O and A carried out at the CVRL in Dubai have shown, that dromedaries are resistant to FMD (Wernery *et al,* 2006; Wernery, 2007a), whereas Bactrian camels become diseased after experimental infection (Larska *et al,* 2008) as well from field infection (Kouba, 2005; Hohoo *et al,*

Table 1. Results of a serological survey of 150 camel sera from different countries investigated for 7 infectious diseases.

Disease	Test method	Racing dromedaries UAE (n=44)			Breeding dromedaries UAE (n=50)			Hybrid camels UAE (n=9)			Bactrian camels Mongolia (n=9)			Pakistan dromedaries (n=38)		
		Neg	Pos	Pos %	Neg	Pos	Pos %	Neg	Pos	Pos %	Neg	Pos	Pos %	Neg	Pos	Pos %
FMD	3ABC indirect ELISA (for field infection)	44	0	0	50	0	0	9	0	0	9	0	0	38	0	0
PPR	Ab competitive ELISA	44	0	0	50	0	0	9	0	0	9	0	0	38	0	0
Anaplasma	Ab competitive ELISA	44	0	0	50	0	0	9	0	0	9	0	0	38	0	0
CCHF	Double Antigen indirect ELISA	35	9	20	22	28	56	2	7	78	1	8	89	0	38	100
WNV	IgG Ab competitive ELISA	43	1	2	42	8	16	8	1	11	9	0	0	2	36	95
Brucellosis	Ab competitive ELISA	43	1	2	48	2	4	7	2	22	5	4	44	38	0	0
Trypanosomosis	Ab indirect ELISA	44	0	0	34	16	32	9	0	0	9	0	0	25	13	34

(Abbreviations: Neg=Negative, Pos=Positive)

2001). Dromedaries do not transmit the FMD virus to susceptible animal species even by very close direct contact.

The current results again show that dromedaries are resistant to FMD as no antibodies were detected in their blood. On the contrary, the two-humped or Bactrian camel can contract FMD as it has been shown in experimental infection as well as in the field. It is worthwhile to note, that two closely related camel species possess noticeably different susceptibilities to FMD. Our investigation, however, of 9 sera from Mongolian Bactrian camels was negative for FMD antibodies, but the number of sera received was very less for any conclusion.

Infection experiments have shown that dromedary camels including New World Camelids (NWCs) infected intra nasally with the virulent PPRV lineage IV (LIV)-strain Kurdistan/2011 developed no clinical signs, no viraemia and did not transmit any PPR virus to contact animals (Schulz *et al,* 2019). They are considered dead-end hosts for the PPR virus, that do not contribute to the spread of the PPR virus. After the intranasal infection of the dromedaries, no seroconversion took place. Our investigation of 150 OWC sera from different countries confirmed these results as no antibodies were detected against the PPR virus.

Several different *Anaplasma* species have been detected in dromedary and Bactrian camels either in their blood or in ectoparasites attached to their skin by molecular biological techniques by several researchers. Only very few serological investigations were performed and no bacterial culture methods were performed. A review of *Rickettsiales* infections in Camelids has recently been published (Wernery, 2022) and shows these micro-organisms were detected in healthy camels indicating the presence of asymptomatic carrier states. The current investigation of 150 OWC sera showed a negative antibody result, but it should be mentioned, that the cELISA used, detects only antibodies against *A. marginale, A. centrale* and *A. ovis*. Many other *Anaplasma* taceae species have been found in OWCs, for which there are no serological tests available. To overcome the uncertainty of Rickettsial infection in camelids, experimental infections are necessary to investigate, if this bacteria group is pathogenic to camels or not. This is, however, a challenge as most of these microorganisms are not available. A recent experiment conducted at CVRL, in which 2 dromedaries were intravenously injected with 2 different strains of *A. marginale* did not produce any antibodies or disease (not published).

High seroprevalences of Crimean Congo Haemorrhagic Fever (CCHF) in dromedaries have been reported by Wernery *et al* (2021) and Camp *et al* (2020) in dromedaries of the UAE. Our investigation shows that the virus is not only endemic in the UAE, but also in Pakistan as all 38 dromedary camels from this country had antibodies to the CCHF virus. Additionally, a seroprevalence of 89% was also found in Bactrian camels from Mongolia confirming previous investigations (Wernery *et al,* 2021). CCHF is enzootic in many countries, but asymptomatic in many animal species such as cattle, sheep, goats, camels and hares (Schwarz *et al,* 1996). In experimental inoculations with the CCHF virus, sheep

and cattle become infected but do not produce disease (The Merck, 2016). The asymptomatic infection in camels poses a severe risk to people working with camels. Therefore, camels harbouring ticks must be treated especially before slaughtering or grooming animals.

West Nile virus encephalitis (WNV) is an infectious disease caused by a mosquito-borne virus that belongs to the *Flaviviridae* family. The genotyping of WNV has demonstrated two main lineages with several subtypes. Lineage II includes strains from sub-Saharan Africa and Madagascar, as lineage I is an American strain. The virus is transmitted by mosquitoes which become infected when taking a blood meal from a bird carrying WNV. Many mammalian species including human beings as well as reptiles can be infected by the insect. However, they are dead-end hosts, because they do not produce enough virus particles in their blood to infect biting mosquitoes. It is highly likely that camelids, like other mammals, are dead-end hosts as well. WNV infections have only been reported in New World Camels (NWCs) (Wernery *et al,* 2014) and antibodies were found in 38% of dromedaries with the cELISA in a dairy farm (Wernery *et al,* 2007a) without any clinical signs for WNF.

Joseph *et al* (2016) recently isolated a WN virus from a dromedary calf showing no clinical signs. This virus belonged to lineage Ia. High prevalences were found in the present study in camels from Pakistan with 95% positivity, in breeding dromedaries with 16% and in hybrids in the UAE with 11%. No antibodies to the WN viruses were found in Bactrian camels from Mongolia, but the sample size was too small for any conclusion. More research is necessary to elucidate the role of WNF in camels. The pathogenic role of the WN virus in camels remains to be determined. However, it is important to examine camels properly showing central nervous signs for WNF.

Brucellosis in camelids is common and has been reported in many different camel-rearing countries. It is mainly caused by *Brucella melitensis* (Wernery *et al,* 2014). The disease poses a severe health risk to people, who do not heat-inactivate, milk before consumption. Brucellosis in breeding camelids is found in all known forms, whereby abortion is its most obvious manifestation (Wernery *et al,* 2007b). In this investigation ELISA antibodies had been detected in UAE racing camels and breeding camels, in UAE hybrid camels and in Mongolian Bactrian camels, but not in Pakistani dromedary camels, which were transported to the UAE for milk production and tested in their homeland by RBT before sent to the UAE.

Trypanosoma evansi casues trypanosomosis known also as surra, which affects a large number of wild and domesticated animal species in Africa, Asia Central and South America. Surra is an arthropod-borne disease and the principal host species are camels, horses, buffalos and cattle. Several species of haematophagous flies, including Tabanids and Stomoxys mechanically transmit the parasite from host to host. Our investigation shows the UAE-breeding camels and Pakistani dromedaries have a high seroprevalence of 32% and 34%. Recent studies by Schuster *et al* (2022) revealed a drug resistancy of one *T. evansi* strain.

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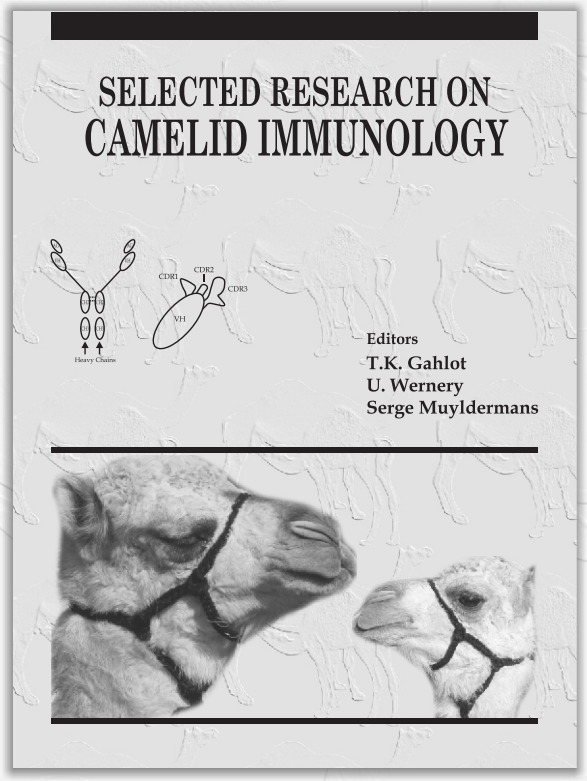
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

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

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



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STUDIES ON CYTOTOXIC ACTIVITY OF CAMEL MILK WHEY PROTEIN AS A NUTRACEUTICAL AGAINST HeLa CELLS

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ABSTRACT

The present study was based on cytotoxic properties of camel milk and its whey against the cervical cancer-derived cell line, HeLa. High concentrations of camel milk and whey were cytotoxic against these cells. Camel milk whey inhibited the migration of these transformed cells, increased the activity of caspase 3, fragmented DNA by PCD, and regulated the CYP1A1 oncogene at the transcriptional level. Thus, camel milk whey can be considered as a potent nutraceutical with anti-cancer property.

Key words: Camel milk, casein, caspase, cytotoxicity, HeLa cells, nutraceutical, protein, whey

Camel milk is an adapted dietary supplement with multiple antimicrobial and immuno-stimulatory properties. Diabetes, infant diarrhoea, hepatitis, allergy, lactose intolerance, and alcohol- induced liver damage have been treated with it (Galil *et al,* 2016). Numerous immunologically essential molecules, such as lysozymes, lactoferrin, lactoperoxidase, serum albumin, acidic whey protein, peptidoglycan recognition protein, and small peptides, contribute to its health benefits (Dubey *et al,* 2016). Recently, the anti-microbial and antioxidant properties of camel milk and its role as an anti-cancer and anti-hepatitis agent has been demonstrated (Khan *et al*, 2021). Camel milk also has the normal isotypes of antibodies shared with other mammalian species. Actually not only camel's milk but even its urine is among such natural products enriched with molecules that are safe to humans and endowed with profound anti-cancer properties (Alebie *et al*, 2017).

Aryl hydrocarbon receptor (AhR) is an endogenous transcription factor with known preventative and therapeutic benefits for patients with cancer associated with organs like the liver, breast, prostate, etc. (Xie *et al,* 2012; Richmond *et al,* 2014). Aberrant AhR expression is involved in carcinogenesis (Korzeniewski *et al,* 2010). Upon activation by its ligands, it regulates tumor suppressor genes and oncogenes. Besides endogenous ligands, components of camel milk also serve as ligands for the aryl hydrocarbon receptor. Some antitumour drugs like amino flavone and benzothiazoles induce DNA damage *via* AhR-mediated signaling.

Camel milk has been traditionally used for cancer prophylaxis and treatment in middle-eastern countries. Its proteins are extremely thermostable and acid resistant (Atri *et al,* 2010). Recent studies have shown that camel milk inhibits the malignant transformation of colon carcinoma, hepatocellular carcinoma, lung cancer cells, human glioma cells, and leukemia *in vitro* (Badawy *et al,* 2018; Dubey *et al,* 2016). Furthermore, multiple caspases, including caspase 3, are involved in the cytotoxicity mediated by programmed cell death. Many anticancer drugs act via the caspase group of enzymes.

We have conducted cytotoxic mechanism-related studies previously by analysing the DNA fragmentation pattern and the caspase 3 activity of cells treated with camel milk whey (Mahala *et al,* 2023). The present study explores the cytotoxicity induced by camel milk whey on cell migration. The aryl hydrocarbon receptor expression on camel milk whey-treated Hela cells has been studied at a transcriptional level in the CYP1A1 gene.

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Materials and Methods

Camel milk sample collection

Aseptic samples of camel milk were collected directly into sterile containers by trained personnel. To prevent bacterial growth, sodium azide (0.02 % w/v) was added, and the solution was immediately transported in a freezing chamber and stored at -20°C for future use.

Separation of whey and casein from camel milk

Camel milk sample was processed to separate whey and casein as per the standard by acidification protocol (Mahala *et al,* 2022a). The supernatant containing whey proteins was saturated with ammonium sulphate (273 g/L) by stirring at 800 rpm. Subsequently, the sample was kept at 4°C overnight and centrifuged at 10,000 rpm for 15 mins at 15°C to give the whey pellet. Whey was further desalted using dialysis membrane-60 with PBS buffer. The final pellet was obtained by 15 minutes of centrifugation at 10,000 rpm.

Quantitation of milk proteins

Aliquots of skimmed milk and its whey and casein protein were diluted 1:20 in PBS, and Lowry's method was used to calculate protein content taking bovine serum albumin (BSA) as a standard (Lowry *et al,* 1951). In brief, varying concentrations of the BSA standard and the test solution were made, and the volume was adjusted to 1 ml using Milli-Q water. Five ml of alkaline $CuSO_4$ reagent was added and incubated at room temperature. After 10 minutes, 0.50 ml of Folin's reagent was added to each test tube and the reading was measured at 640 nm after 20 minutes of incubation. The amount of protein in the given unknown solution was calculated using the standard graph.

SDS PAGE for protein profile

A 12% gel was used to separate proteins by SDS-PAGE. Before electrophoresis, the proteins were diluted to 2 µg/µl with 5X sample buffer and denatured for 10 minutes at 100°C. Twenty microlitres of protein were added to each well, and the electrophoresis was performed at 80 V in a Bio-rad vertical gel electrophoresis apparatus. Separated proteins were identified using the wide-range molecular weight marker. The protein bands on the gel were stained with Coomassie Brilliant Blue R-250 for one hour, followed by overnight destaining in a solution of methanol (15%), acetic acid (10%), and water (75%). BioRad Gel DocTM XR imaging system was used to observe the gel.

Culture of Hela cells

Certified HeLa cells were obtained from National Centre for Cell Science (NCCS) in Pune, India. Minimal essential medium (MEM; Gibco, Thermo Fisher Scientific) supplemented with 10% heat-inactivated foetal bovine serum (FBS; Gibco, Thermo Fisher Scientific), 0.2% sodium bicarbonate (HiMedia India, Ltd. Mumbai, India), and 1% antibiotic solution (Gibco, Thermo Fisher Scientific) containing 100 U/ml penicillin and 100 g/ml streptomycin was used to culture cells in 5% CO_2 at 37°C. Before treatment, cells were grown to a confluence of about 60–70% in a tissue culture flask (Tarsons). Trypsin EDTA solution containing 0.05% Trypsin (Gibco, Thermo Fisher Scientific) was used for cell disaggregation. The detached cells were rinsed in phosphate-buffered saline and transferred to a fresh medium.

Cytotoxicity assay

The MTT assay was conducted to analyse the cytotoxicity of samples against Hela cells. Cells were incubated in a 96-well plate at a concentration of 5.0 x 10^4 cells/well with an increasing concentration of camel milk and whey samples. After 24 or 48 hours, the media was aspirated, and 5 mg/mL of MTT solution in 100 µl of the medium was added to each well. The plate was then incubated at 37°C for 4 hours. Subsequently, the media was discarded to stop the reaction, and 150 µl of DMSO was added to each well to dissolve the formed crystals. A microplate reader was used to measure the colour intensity at 630 nm wavelength. The percentage cell viability was calculated using the following formula.

% Cell Viability = OD of treated cells/OD of Control (without treatment) *100

Cell migration assay

This assay was conducted to evaluate the ability of camel milk whey samples to inhibit HeLa cell migration under optimal growth conditions (Krishnankutty *et al,* 2018). Briefly, 3-4×10^6 cells were seeded in 6 well plates to produce a confluent monolayer. Using a sterile 200 µl pipette tip, a scratch was made in the middle of the plate. PBS was used to remove cellular debris from the monolayer. Fresh media containing various IC50 of whey was used for treatments and further incubated for 24 hours. Plates were photographed initially and after 24 hours of treatment using a Primovert Compact Inverted

Microscope (ZEISS). Cells treated with IC50 of cisplatin were used as a positive control whereas untreated cells served as a negative control.

Caspase Assay

Caspase-3 colourimetric protease assay kit (Invitrogen, Thermo Fisher Scientific) was used to measure the enzyme activity of caspase 3. 4×10^5 HeLa cells were seeded in a 60 mm dish and treated with IC50 of whey and Cisplatin for 24 hours. Following this treatment, cells were washed with PBS, lysed, and protein concentration was determined using the Bradford method. For caspase assay, 50 µl of 2x reaction buffer (100 µg/ml) with 10mM DTT was added to the required protein concentration, and 5 µl of 4mM DEVD-PNA substrate was added to the plate before incubation for 2 hours at 37°C in the dark. The microplate reader was used to obtain a reading at 405 nm, and fold change was calculated relative to the untreated control.

RNA isolation, cDNA synthesis and real-time PCR (RT-PCR) for studying expression of CYP1A1 gene at transcriptional level

To extract total cellular RNA using TRIzolTM reagent, 5×10^5 HeLa cells were seeded in a 60 mm dish and treated with (i) 2.5 nM of TCDD (molecule binding AhR), (ii) A combination of 2.5 nM of TCDD and 25 µM of resveratrol (positive control) (iii) A combination of 2.5 nM of TCDD and 10 mg/mL of whey (IC50 dose). After 24 hours incubation, the total cellular RNA was extracted from treated and untreated cells using TRIzol® reagent. Briefly, TRIzolTM reagent were added to the cells and mixed by gentle pipetting and incubated on ice for 45 minutes. The cell suspension was transferred to a new tube, and 200 µl of chloroform was added before incubation at room temperature for 5 minutes. The suspension was centrifuged for 15 minutes at 12000 rpm at 4°C, and the upper aqueous layer was transferred to a new tube. An equal volume of isopropanol was added, and the mixture was incubated for 30 minutes at -20°C. Subsequently, it was centrifuged at 12000 rpm for 15 minutes at 4°C. The pellet was washed with 70% ethanol, air-dried, and then resuspended in RNase-free water. RNA was pretreated with DNase to eliminate any contaminating DNA. The concentration of this pure RNA was determined by measuring the absorbance at 260nm, and the quality of the RNA was determined by measuring the 260/280 ratio using a Nanodrop analyser.

cDNA was synthesised using the high-capacity cDNA reverse transcription kit, as per the manufacturer's instructions, using 1µg of extracted RNA. A Real-time PCR system amplifying cDNA templates was used for evaluating CYP1A1 gene expression. The β- actin gene was used as an internal control gene. In a 20µl PCR reaction, 4 µl of diluted cDNA, 1 µl of forward and reverse primer, and 10 µl of power-up SYBR Green master mix were used. Pre-denaturation for 5 min at 95°C followed by 35 cycles at 94°C, denaturation for 30 seconds and annealing temperature of gene-specific primers for 30 seconds was conducted. Extension at 72°C for 1 minute and a final extension at 72°C for 3 minutes was conducted. The gene expression results were analysed using the $2^{-\Delta\Delta Ct}$ method with untreated cells used as the control (Livak *et al,* 2001). The data were represented as the mean fold change ± standard error (SE) for three experimental replicates.

DNA fragmentation assay

For this assay, cells were seeded at a density of $3 \text{ X } 10^5$ cells/ well in a 6-well plate and treated for 24 hours with varying sample concentrations. Both, adherent as well as floating cells were harvested, pelleted, and washed in PBS for DNA isolation. 100 µl of DMSO was added to the cell pellet and thoroughly mixed. This was then followed by the addition of an equal volume of Tris-EDTA buffer (pH 7.4 containing 2% SDS), mixing and centrifugation for 15 minutes at 12000 rpm at 4°C. The DNA- containing supernatant was run on a 1.5% agarose gel electrophoresis at 60V and visualised under a UV scanner (Suman *et al,* 2012). DNA extracted from cells treated with 5-fluorouracil was used as a positive control, and untreated cells were used as a negative control.

Statistical analysis

The data were analysed statistically by Graphpad prism5 using one-way ANOVA, followed by Bonferroni multiple comparison test. Statistically, significant differences are indicated as given ahead: $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$ and ns indicated non- significant difference.

Results and Discussion

Our study showed that the cytotoxic property of camel milk was associated with its whey fraction. Upon SDS-PAGE, the whey fraction showed proteins with anticancer potentials, such as lactoferrin and soluble TRAIL. The camel milk whey treatment of the cervical cancer cell line (HeLa) showed its ability

to induce nuclear fragmentation and a DNA ladder pattern characteristic of apoptosis. The whey elicited an ability to inhibit HeLa cell migration. It also activated caspase 3, a vital enzyme in programmed cell death. Furthermore, the ability of camel milk whey to down-regulate cancer-promoting genes like CYP1A1 at a transcriptional level was suggested by real-time PCR.

Camel milk, whey, and casein protein profile by SDS PAGE

The protein components of camel milk, casein and whey components have been depicted in Fig 1. The proteins present in skimmed camel milk (CSM) have been depicted in lane 4, and the identification of proteins has been done based on molecular weight. This lane shows the presence of bands of proteins like α-Lactalbumin visible at 14.2 kDa, a very faint band of TRAIL at a molecular weight of about 20 kDa (Mariani *et al,* 1998; Melendez *et al,* 2018), faint bands of different casein proteins from 24 to 36 kDa. The light chain (25 kDa) and heavy chain (50 kDa) of immunoglobulin are also visible (Omar *et al,* 2016). Protein bands are visible from 66 kDa to 76 kDa, with bands of CSA and Lactoferrin in lane 4. Lane 2 depicts the caseins extracted from camel milk to get whey. Prominent bands of the casein components i.e., β-casein (24 kDa), k- casein (29 kDa), αS1-casein (29 kDa), αS2-casein (36 kDa), respectively, are shown in here. The components of camel whey proteins (CWP) are depicted in lane 3. A band of α-Lactalbumin, a very faint band of TRAIL, and then faint bands of different casein proteins (depicting traces of casein protein in the purified whey sample). Protein bands of light and heavy-chain immunoglobulins, CSA and lactoferrin are also visible. The molecular weight marker has been depicted in lane 1.

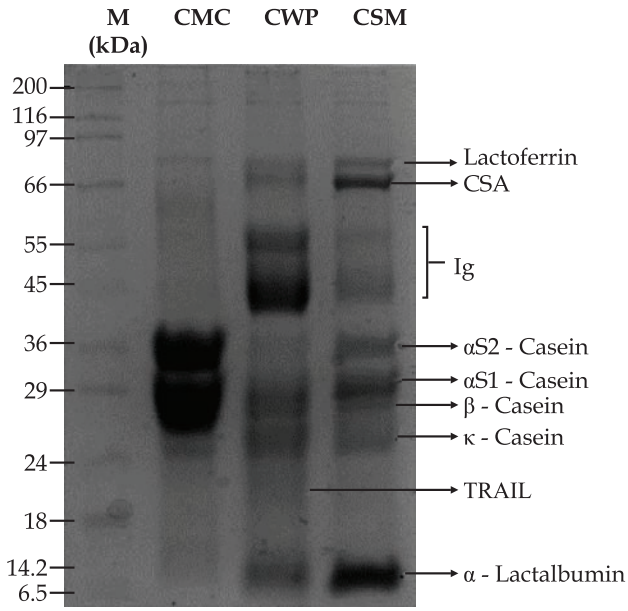

Fig 1. Protein profile of defatted camel milk, camel milk whey, and camel milk casein by SDS-PAGE.
Abbreviations: M (Molecular weight marker); CMC (Camel milk casein); CWP (Camel whey protein); and CSM (Camel skimmed milk); CSA (Camel serum albumin); Ig (Immunoglobulin).

Cytotoxic potential of camel milk and whey fraction

Fig 2A and 2B illustrate the cytotoxicity of camel milk and whey against HeLa cells, respectively. All concentrations of camel milk were observed to be cytotoxic to HeLa cells after 48 hours of treatment, whereas only high concentrations of camel milk were able to effectively induce cytotoxicity after 24 hours of treatment (Fig 2a). The whey fraction of camel milk was cytotoxic to Hela cells after 24 and 48 hours of treatment at all concentrations in a dose-dependent manner (Fig 2b). This study indicated that the cytotoxicity of camel milk is associated with its

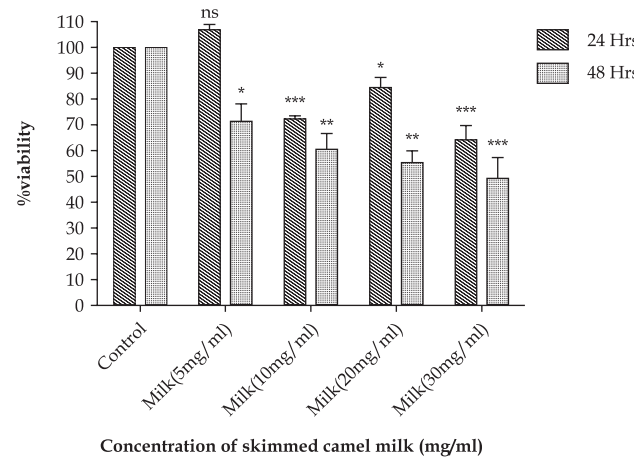

Fig 2a. Percentage viability of defatted camel milk treated HeLa cells compared with control (untreated HeLa cells).

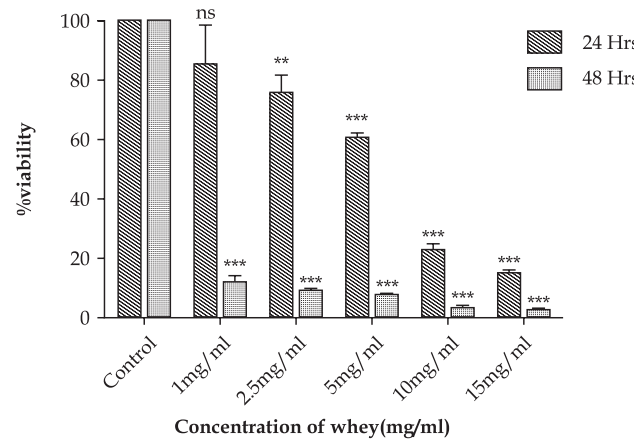

Fig 2b. Percentage viability of camel milk whey fraction treated HeLa cells.

whey fraction. Whey induced cytotoxicity is even effective at 24 hours, unlike defatted milk, which only has cytotoxicity after 48 hours. Therefore, only the whey fraction has been used for further studies.

Effect of camel milk whey on cell migration

The cell migration assay tested the ability of whey to inhibit the migration of HeLa cells (Fig 3). Cisplatin-treated and untreated cells were used as the positive and negative controls, respectively. It was observed that HeLa cells incubated with whey at its IC50 (7.5 mg/ml) showed more migration inhibition than the control untreated cells after 24 hours of treatment. Cisplatin-treated cells under similar conditions showed maximum migration inhibition. This suggests that camel milk whey has the potential to inhibit the migration of the transformed HeLa cells, although to a lesser extent than cisplatin.

Caspase-3 activity of camel milk whey-treated cells

The ability of camel milk whey proteins to induce apoptosis by caspase- 3 activation was also studied (Fig 4). The caspase-3 assay was performed on HeLa cells treated with the IC50 camel milk whey for 24 hours. Cells treated with the IC50 of cisplatin were treated as a positive control, whereas untreated cells were used as negative controls, respectively. Compared to untreated control cells, there is a 1.4-fold increase in caspase-3 activity in whey-treated cells. This was slightly lesser than caspase-3 activity induction by cisplatin, which showed a 1.6-fold increase.

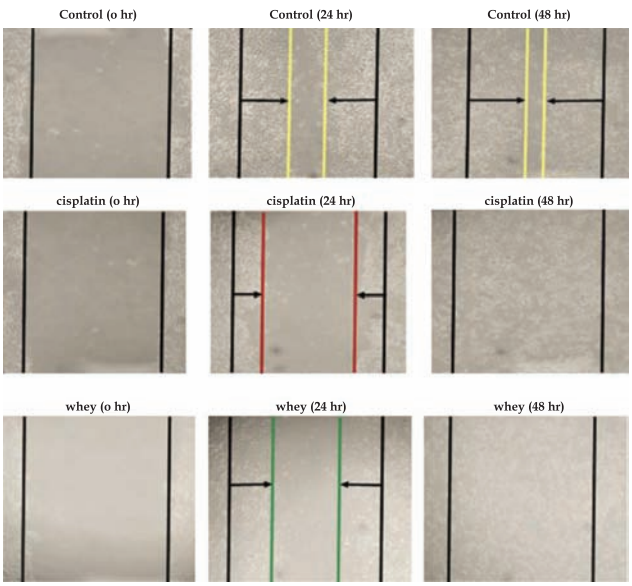

Fig 3. Effect of camel milk whey and cisplatin treatment on cell migration. Migration of untreated HeLa cells (uppermost row), cisplatin-treated (middle row) and camel milk whey-treated cells (lowermost row) after 24 hrs. Treatment of HeLa cells was given with the lC50 of Cisplatin (4µg/ml) and whey (7.5mg/ml) for 24 hrs.

Induction of DNA fragmentation by camel milk whey

DNA from the camel milk whey-treated cells were isolated and used for the DNA fragmentation assay (Fig 5). Cells treated with 5-fluorouracil served as a positive control and separated into 180-300 bp DNA fragments characteristic of apoptotic (lane 1). Further, the DNA extracted from the cells treated in increasing concentrations of camel milk whey also demonstrated the DNA ladder pattern, characteristic of cell death by apoptosis (lanes 2-5). DNA from

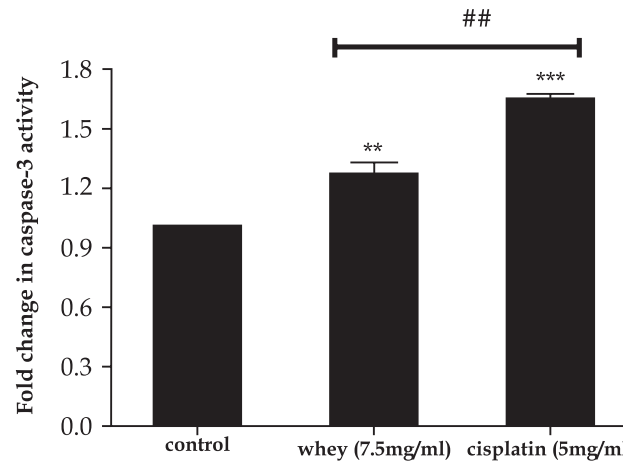

Fig 4. Induction of Caspase-3 activity by whey protein at IC50 compared with Cisplatin and Control after 24 hrs of treatment.

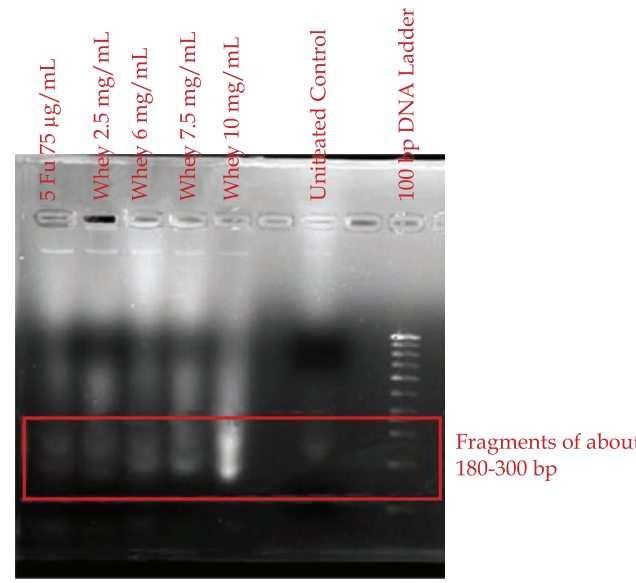

Fig 5. Induction in DNA fragmentation by camel milk whey and the positive control drug 5- Fluorouracil. Untreated cells serve as the negative control.

the untreated control cells showed a single high molecular weight band, as expected (lane 7). Lane 10 depicts the standard DNA ladder pattern.

Induction of CYP1A1 gene expression by camel milk whey

Further, the effect of camel milk whey on transcription of the pro-apoptotic gene, CYP1A1 was studied here (Fig 6). TCDD, a molecule binding AhR and activating the CYP1A1 gene, was used to study the inhibitory effect of camel milk whey proteins. HeLa cells treated for 24 hours with TCDD showed a 7-fold induction of CYP1A1 mRNA, as measured by RT PCR. In the presence of resveratrol, the positive control, the expression of this gene was significantly reduced. Similarly, camel milk whey-treated cells could reduce CYP1A1 mRNA levels in HeLa cells. Untreated cells served as the negative control. This indicates the possible role of whey proteins in suppressing the expression of the cancer-causing gene, CYP1A1 at a transcriptional level.

Few authors have recently studied the ability of mammalian milk and its fractions to kill cancers. Maliheh *et al (*2017) have studied the effect of milk, whey, and casein protein derived from many different mammals on MCF7 and demonstrated that the whey and casein proteins of mare, cow, donkey, and camel milk have dose-dependent cytotoxic activity against MCF7 cells. In contrast, goat and sheep milk proteins did not show cytotoxic activity. Our study also exemplifies the cytotoxic potential of camel milk and whey against the HeLa cervical cancer cell line. Our earlier work does not show casein to have cytotoxic potential (Mahala *et al*, 2023). Similarly, in another study the *in vitro* cytotoxic effect of camel milk and whey has been observed. Here too it has been noted that they inhibited the growth of HeLa cells (Abdallah *et al*, 2019). Although, not cytotoxic against transformed cells, casein from camel milk has been used as a carrier for synthetic and natural drugs (Esmaili *et al,* 2011; Mittal *et al,* 2021).

We have further investigated the milk components that may be associated with cytotoxicity. According to our study, the biomolecules present in camel milk that is associated with an inhibitory potential against cancer are likely to be lactoferrin, TRAIL (a soluble form of the apoptosis-inducing ligand related to TNF) and α-lactoglobulin, the latter exhibiting its potential upon the combination with oleic acid forming HAMLET in humans (Lal *et al,* 2020). It has been shown that the OA-α-cLA complex from camel milk does not affect normal cells but has noticeable anti-cancer activity especially, against MCF-7 cells (Uversky *et al*, 2017). Interestingly, the structure and stability analysis of cytotoxic complex of camel α-lactalbumin and unsaturated fatty acids produced at high temperature has also been studied (Maliheh *et al*, 2017). Lactoferrin is involved in various physiological and immunomodulatory functions and has antimicrobial, antioxidant, and anticancer activities (Mahala *et al,* 2022b). In an earlier study, it has been suggested that nano-formulation of camel milk lactoferrin and OA potentiates their activity and selectivity toward cancer cells, (EL-Baky *et al*, 2021). The present study points in this direction with reference to camel milk, but the validation would require further studies. In our earlier study we have indicated the presence of Lactoferrin (~77kDa)

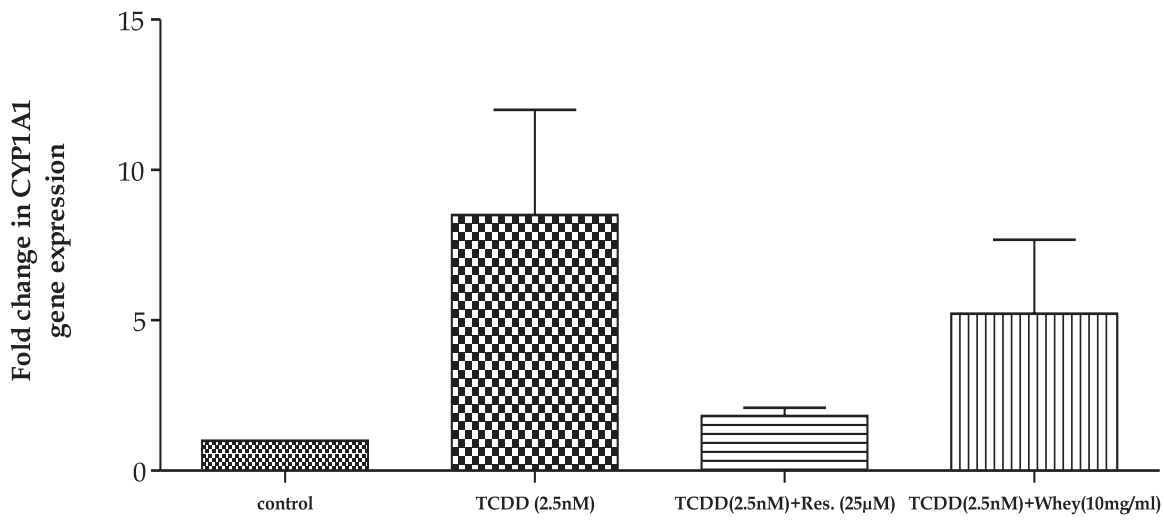

Fig 6. Gene expression of CYP1A1 after 24hrs of treatment.

in the cytotoxic fractions purified by gel filtration chromatography from camel milk whey (Mahala *et al,* 2023).

Similarly, Korashy *et al* (2012a) found camel milk to be cytotoxic against human breast cancer (MCF7) and hepatoma (HepG2) cell lines. They investigated the impact of camel milk on apoptotic signaling pathways in both cell lines. HepG2 and MCF7 cells treated with camel milk exhibited decreased cell proliferation and increased expression of caspase-3 mRNA activity levels and induction of death receptors. The transcriptional inhibitor, actinomycin D, completely blocked the increase in caspase-3 mRNA levels by camel milk, indicating that camel milk increased *de novo* RNA synthesis. Inhibition of the mitogen protein kinases differentially modulated the camel milk-induced caspase-3 mRNA levels. According to their study, camel milk inhibited cell survival and proliferation through the activation of both the extrinsic and intrinsic apoptotic pathways. Camel milk enhanced the expression of oxidative stress markers, heme oxygenase-1 and reactive oxygen species production in both these cells.

Scientists have also investigated the physiological properties of camel whey protein hydrolysates and found an associated enhanced anti-proliferative, anti-diabetic, and anti-inflammatory activity (Kamal *et al,* 2018). Animal models of colon and mammary tumorigenesis have demonstrated that whey proteins are superior to other dietary proteins in inhibiting the development of tumors (Brandelli *et al,* 2015). These studies indicate the potential of using camel milk whey and its hydrolysates as a functional food ingredient.

DNA fragmentation was induced in transformed cells upon treatment with camel milk whey. These fragments represent chromatin cleaved at the linker sites of the nucleosome, thus leading to the formation of nucleotide oligomers (Schliephacke *et al,* 2004). The cytotoxicity induced by camel milk and the underlying mechanism in breast and colorectal cancer cell lines was investigated and found autophagy as a mechanism for inducing cytotoxicity (Krishnankutty *et al,* 2018). Furthermore, they also observed a reduced migration induced in cancer cells.

Similarly, in our study, we also observed that whey treatment reduced the migration of HeLa cells. Earlier researchers have also done the examination of the activity of camel milk casein against hepatitis C virus and its apoptotic potential in hepatoma and HeLa cell lines has been studied (Almahdy *et al,* 2011).

CYP1A1 induction is regarded as a valuable biomarker of exposure to cancer- causing agents (Williams *et al,* 2000). Attenuation of carcinogen-activating genes and CYP1A1 signalling pathways is one of the strategies for protecting cells from the toxic effects of carcinogens and their cytotoxic metabolites. Multiple lines of evidence showed a strong correlation between the induction of CYP1A1 and an increased incidence of colon, rectal, human, and lung cancers (Slattery *et al,* 2004; Shah *et al,* 2009). TCDD, an environmental toxicant, also acts *via* the aryl hydrocarbon receptor. It generally acts more as a cancer promotor than an initiator. It acts by inducing oxidative stress- mediated DNA damage. A transcriptional mechanism is responsible for the inhibitory effect of whey protein on TCDD-mediated CYP1A1 activity induction. Research by Korashy *et al* (2012b) demonstrated that camel milk has the ability to modulate the expression of a well-known cancer-activating gene, Cytochrome P450 1a1 (*CYP1A1*), and cancer-protective genes, NAD(P)H:quinone oxidoreductase 1 (Nqo1) and glutathione S-transferase a1 (*Gsta1*), in murine hepatoma Hepa-1c1c7 cell line at the transcriptional and post-transcriptional levels. Their results showed that camel milk significantly inhibited induction of CYP1A1 gene expression by 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD), most potent CYP1A1 inducer and known carcinogenic chemical, at mRNA, protein, and activity levels in a concentration- dependent manner. Additionally, camel milk significantly decreased the xenobiotic responsive element (XRE)-dependent luciferase activity, suggesting a transcriptional mechanism is involved. This inhibitory effect of camel milk was associated with a proportional increase in heme oxygenase 1. Also, camel milk significantly induced Nqo1 and Gsta1 mRNA expression levels in a concentration-dependent fashion. The RNA synthesis inhibitor, actinomycin D, completely blocked the induction of Nqo1 mRNA by camel milk suggesting the requirement of de novo RNA synthesis through a transcriptional mechanism. Our study has demonstrated an inhibitory effect of camel milk whey on lactoferrin on TCDD-mediated CYP1A1 activity induction at a transcriptional level in HeLa cells.

AhR-mediated signalling pathways are a novel approach to treat cancers. New antitumor drugs such as benzothiazole and aminoflavone have been shown to act *via* this pathway to induce DNA damage and have been used to treat breast cancers. Similarly, components of camel milk also act on the

aryl hydrocarbon receptors (Mariani *et al,* 1998). Consequently, it is hypothesised that camel milk can protect against or reduce the harmful effects of numerous environmental carcinogens and toxicants by modulating AhR-regulated genes.

Interestingly, scientists have also reported the *in vivo* and *in vitro* anti-cancer effects of camel milk and its exosomes. They observed that the oral administration of camel milk, local injection and oral administration of camel milk-derived exosomes inhibited the progression of breast tumors through increased apoptosis, DNA fragmentation, caspase-3 activity, and Bax gene expression. In addition, they observed suppression of oxidative stress-induced apoptosis and suppression of oxidative stress, angiogenesis, inflammation, and metastasis in the tumor microenvironment by treatment with camel milk (Badawy *et al,* 2018).

This study has investigated camel milk whey-related cytotoxicity in multiple dimensions (Fig 7). Our study suggests the association of the cytotoxicity of camel milk with its whey fraction. It also suggests that camel milk whey may inhibit cell migration, induce DNA fragmentation, and activate the Caspase 3 mediated pathways. It can further induce the expression of cancer activation genes such as CYP1A1 at a transcriptional level. Thus, our study suggests the potential of camel milk whey as a nutraceutical with cytotoxic ability against transformed cells. In future, the specific protein responsible for this effect can be further studied.

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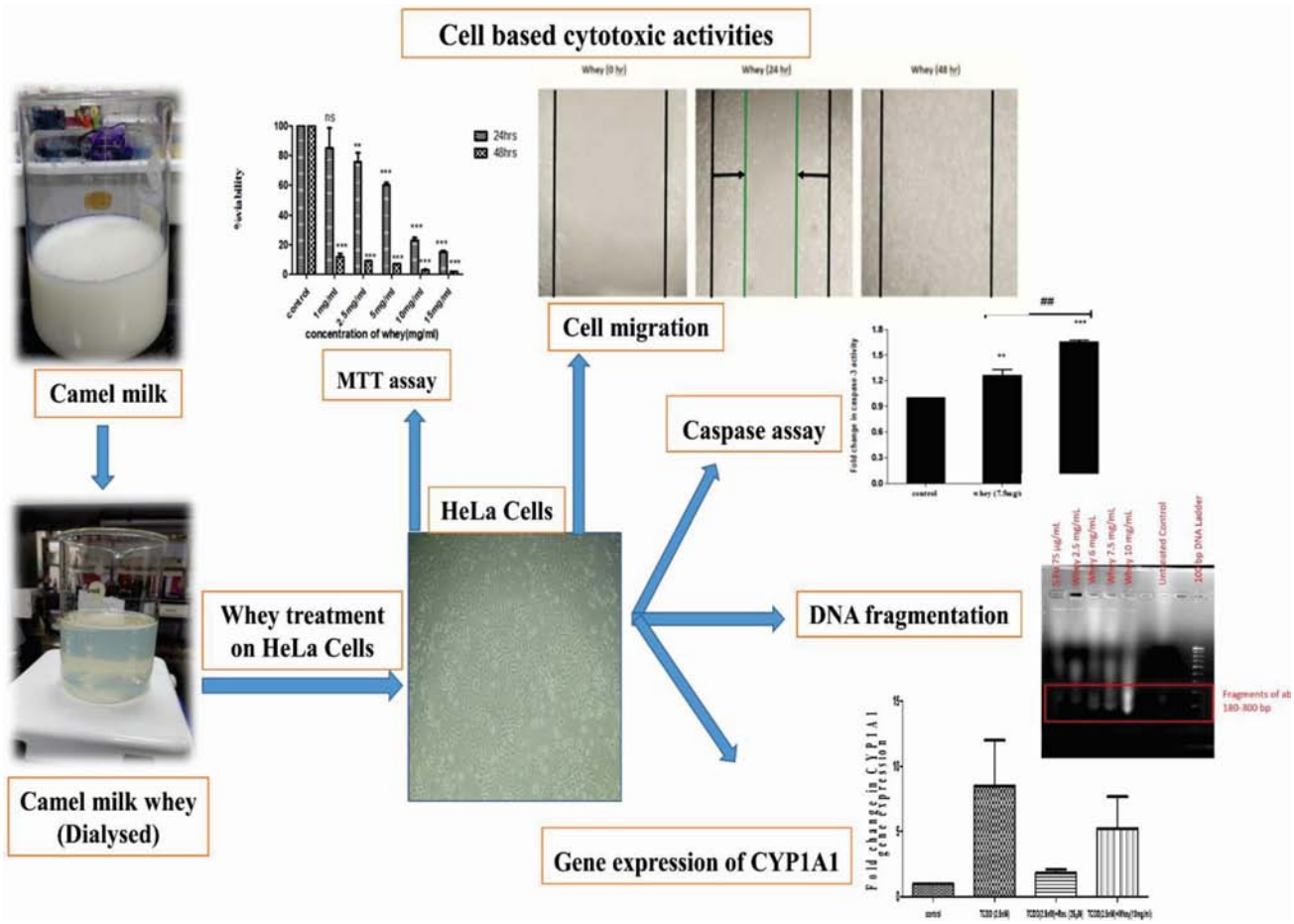

Fig 7. A graphical representation of cell based cytotoxic activities of camel milk whey.

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SEROLOGICAL AND PASSIVE TRANSFER INVESTIGATIONS OF TWO DROMEDARY CALVES AFTER DRINKING COLOSTRUM FROM THEIR INFECTED DAMS

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ABSTRACT

ELISA antibodies were tested in two dromedary camel dams for Brucellosis, MERS and *Trypanosoma (T.) evansi*. The same tests were applied to their calves before suckling and several times after intake of colostrum. One mother possessed high antibodies to Brucellosis and MERS. The second mother to *T. evansi* and MERS, whereas the two offsprings were negative at post-partum (pp) to the dams infection. After suckling colostrum from their mothers, both calves acquired antibodies to all three infections which were, however, lower than in their dams. These antibodies disappeared between 30 to 60 days' pp; whereas the dams antibodies remained high. Additionally the sera of both mothers and calves were also tested for total protein (TP) and immunoglobulins (IgG). TP and IgGs in the dams were high due to their mature immune system. In the calves, both proteins increased after colostrum intake, indicating an adequate intake of colostrum.

Key words: Brucellosis, camel calves, MERS, passive transfer, *Trypanosoma evansi* antibodies

Passive acquisition of antibodies is an important survival mechanism for the new born. Immunoglobulins, principally IgGs, are transferred from the dam to its off spring by colostrum intake after birth. The intake of colostrum has to be fast, as the rate of decay of antibodies is also fast. For IgGs, the half-life is 9 to 20 days and for IgM 3 to 5 days. Failure of passive transfer (FPT) of maternal immunoglobulins is the most important immunological deficit in veterinary medicine, as it is significantly correlated to numerous infections in postnatal life. The passive transfer (PT) of maternal antibodies from serum to colostrum, to the intestinal tract and finally to the neonatal vascular system, is a complex process with many sites of disruption (Wernery *et al,* 2014).

Camelids have a thick-layered epitheliochorial placenta that prevents transplantal transfer of IgG directly to the camelid foetus. Camels, therefore must obtain passive immunity by intestinal absorption of IgG from colostrum. Investigations have shown that for example new born dromedary calves have very little or none demonstrable serum IgG prior to ingesting colostrum, named agammaglobinanaemia (Ungar-Warom *et al,* 1987).

We report here the passive transfer (PT) of antibodies of 3 infectious diseases from two dromedary dams to their neonates and their duration in them.

Materials and Methods

Despite the infection with *Brucella, MERS* and *Trypanosoma evansi*, two dromedary camels delivered healthy calves. Before drinking colostrum, blood were withdrawn from their jugular veins, centrifuged and stored at -20°C until testing. At the same time, also from their mothers blood were collected and stored at -20°C. All four animals were bled on different days.

Antibodies were tested for Brucellosis, MERS and *T. evansi*, the details of which had been previously reported (Wernery *et al,* 2023). The Brucellosis ELISA was from Gold Standard Diagnostics, Spain; the MERS ELISA from Euro Immune, Germany and the *T. evansi* ELISA is an indirect ELISA developed at CVRL.

Additionally, to the ELISA antibody investigation, the camel sera were tested in the Cobbas C311 for total protein (TP) and immunoglobulins (IgG).

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Table 1. Serological results of 2 dromedary dams infected with Brucellosis, MERS and Trypanosomosis and of their calves.

Sample details	Serological results															
	Brucellosis (values as Percentage Inhibition)					*T. evansi* (values as Optical Density at 450nm)					MERS (values as ratio of Optical Density of sample to calibrator at 450nm)					
	At postpartum	9 days postpartum	12 days postpartum	30 days postpartum	60 days postpartum	At postpartum	9 days postpartum	12 days postpartum	30 days postpartum	60 days postpartum	At postpartum	9 days postpartum	12 days postpartum	30 days postpartum	60 days postpartum
Dam 1	Positive 97%	Positive 95%	Positive 94%	Positive 96%	Positive 93%	Neg	Neg	Neg	Neg	Neg	Positive 4.2	Positive 4.0	Positive 4.0	Positive 4.0	Positive 4.0
Calf 1	Neg	Positive 54%	Positive 56%	Positive 41%	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Positive 1.4	Positive 1.4	Doubtful 0.9	Neg
Dam 2	Neg	Neg	Neg	Neg	Neg	Positive 2.4	Positive 2.3	Positive 2.3	Positive 2.2	Positive 2.1	Positive 2.3	Positive 3.6	Positive 3.0	Positive 2.7	Positive 2.6
Calf 2	Neg	Neg	Neg	Neg	Neg	Neg	Positive 2	Positive 1.6	Positive 1.1	Positive 0.6	Neg	Positive 3.2	Positive 1.3	Doubtful 0.9	Negative

Results

Before delivery both dromedary dams possessed high antibodies against two diseases, whereas their neonates had no antibodies before colostrum intake. However, ELISA antibodies were found in their bloods after drinking colostrum and they remained in their circulatory system between 30 and 60 days after delivery. Details of these investigations are shown in Table 1.

The assessment of the immune status of all four dromedaries, two dams and their calves, was measured by TP and IgG estimation. The results are found in Table 2.

The mean values for the dams were 71g/l and 76 g/l for TP; 32g/l and 33g/l for IgG and for the calves 51 g/l and 55 g/l for TP and 13 g/l and 18 g/l for IgG.

Table 2. Total Protein (TP) and Immunoglubulin G (IgG) status of 2 camel dams and their offsprings.

Sample details	Immune status						Mean Immune status	
	TP (g/l)	IgG (g/l)	TP (g/l)	IgG (g/l)	TP (g/l)	IgG (g/l)	TP (g/l)	IgG (g/l)
	At Postpartum		60 days postpartum		180 days postpartum			
Dam 1	66	31	75	35	71	29	71	32
Calf 1	51	13	50	13	51	13	51	13
Dam 2	77	28	75	35	75	35	76	33
Calf 2	51	20	57	17	56	17	55	18

Discussion

Although the neonatal camelid is immunocompetent at birth, it is immunologically naïve and therefore dependent on passively acquired humoral antibodies. The immunity will steadily mature until it reaches its peak within 4 to 6 months, which has been shown by testing IgGs or TP concentrations (Barrington *et al,* 1997). New borns that fail to acquire adequate passive immunity are at much greater risk of developing diseases such as enteritis, septicaemia, arthritis, omphalitis and pneumonia. Successful PT is achieved when neonates possess TP levels between 50-60 g/l or above (Wernery *et al,* 2001) at 48h of age (Whitehead and Anderson, 2006). Both investigated camel calves of this study possessed at the age between 60 and 180 days post-partum a mean TP value of 53 g/l and an IgG value of 16 g/l which is in the range of protection. To achieve the goal of PT, it is important that new born camelids should be standing within 30 to 60 mins and nursing should start after 2 to 4 hours, whereas the placenta should be passed within 24 to 36 hours. If PT is not guaranteed, therapeutic administration of IgG has nowadays an important place in veterinary medicine, for example by establishing a colostrum bank.

In the present study, both camel calves acquired an adequate PT of IgG through colostrum intake. With this

intake they also received maternal antibodies against Brucellosis, MERS and *Trypanosoma evansi* which eventually protects them against these infections for at least 60 days of their young lives. This was shown in previous investigations by von Hieber *et al* (2010) for brucellosis.

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SELECTED RESEARCH ON
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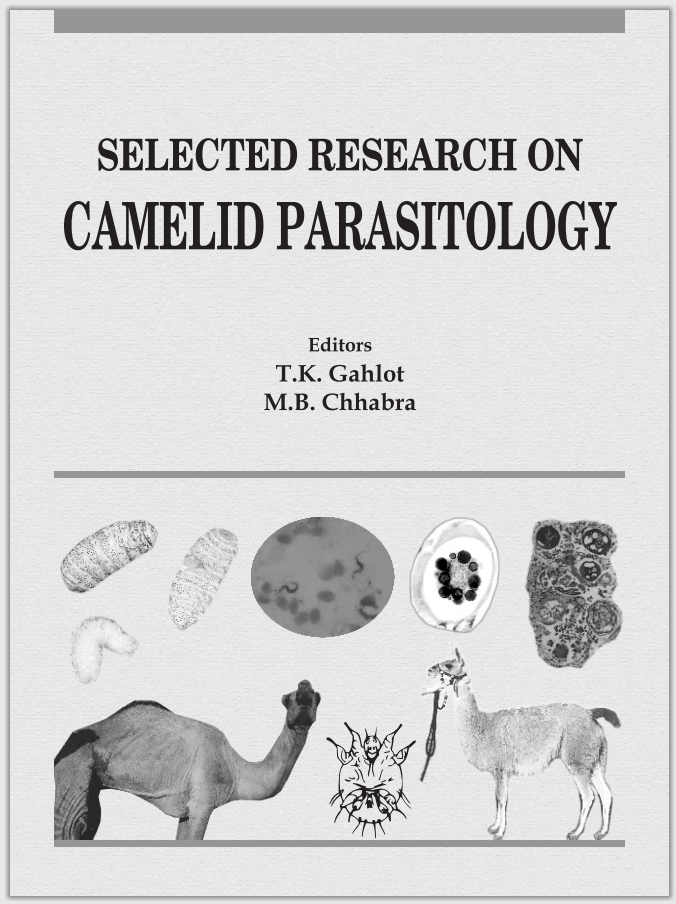
New research and experience always broaden our knowledge, and help us adopting new diagnostic methods and treatments. Camel Publishing House has taken a step forward to compile this knowledge in form of a book and this Herculian task was accomplished with the help of dedicated editors. viz. Drs. T.K. 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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REVIEW OF BACTERIAL AND VIRAL INFECTIONS OF BACTRIAN CAMELS IN MONGOLIA

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ABSTRACT

This second part of infectious diseases of Bactrian camels of Mongolia deals with bacterial and viral infections: anthrax, pasteurellosis, glanders, brucellosis, tuberculosis, plague, salmonellosis, infectious enterotoxaemia, necrobacillosis, abscess, anaplasmosis, ehrlichiosis, rickettsiosis, food and mouth disease, camel pox, orf, rabies, influenza, MERS and Crimean Congo Haemorrhagic fever.

Key words: Bacterial infection, bactrian camels, Mongolia, viral infection

Details of Mongolia, its territory, camel husbandry, population numbers and more have been published in our manuscript on parasitic infections of Bactrian camels in Mongolia (Munkhjargal *et al,* 2023). It is expected that both informations on parasites, bacteria and viruses can be used by relevant authorities in the field of both human and veterinary medicine. These important scientific pages may encourage camel scientists and others to continue and deepen camel research in Mongolia and adjacent countries with scientists outside Mongolia.

Bacterial diseases

2.1. Anthrax

Anthrax is a worldwide zoonotic disease caused by *Bacillus anthracis*, a gram positive bacteria which forms highly resistant and long-lasting spores in the environment. The spores are highly resistant to extreme temperatures, radiation and chemical substances and can persist in soil for several decades (Mock and Fouet, 2001). In Mongolia, anthrax is an endemic. From 1964 to 2008, 212 human anthrax cases were reported from 17 provinces and from the Ulaanbataar, the capital city of Mongolia. Most of the patients had acquired the infection through contact with diseased livestock; however, in recent years, transmission from soil and livestock barns has increased (Okutani *et al,* 2011). Between 1976 and 1995, 84.8% of all anthrax cases were from cattle, 9.2% from small ruminants and each 3 % from horses and camels. Of the total animals that died of anthrax, 4.3% were camels. Camels were vaccinated only when there was an outbreak in the region (Odontsetseg *et al,* 2007).

2.2. Pasteurellosis

Pasteurella multocida is a small gram-negative coccobacillus. High morbidity and mortality rates are associated with significant economic losses to livestock. A first case of *P. multocida* in Mongolia was recorded in 1950. Pasteurellosis epizootics occurs in winter and spring and mostly affected younger farm animals (83.6%) with high mortality rates in the country. Recently, Erdenechimeg and Ankhbayar (2020) isolated and identified *P. multocida* for the first time from tissue samples from Bactrian camels in Ömnögovi province by bacteriological and molecular examinations. However, isolating *Pasteurella* bacteria from nasal swabs or tissues of camels does not mean that these organisms disease them as they are normal commensals. Wernery *et al* (2014) stated that pasteurellosis as a disease does not exist in dromedaries. They are resistant as infection and experiments did not produce clinical signs or disease.

2.3. Glanders

Glanders is a zoonotic bacterial infection caused by *Burkholderia mallei*, a gram negative, non-motile, non-encapsulated and non-spore-forming bacillus of the family Burkholderiaceae. Typical clinical signs of glanders in horses are nasal discharge, ulcerations of the nasal mucosa and multiple skin nodules, especially in hind limbs and abdomen.

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Transmission of *B. mallei* occurs mainly through ingestion of contaminated feed or water, or otherwise through direct skin or mucous membrane contact with excretions from infected animal tissues (World Organisation for Animal Health, 2023).

Up to 1940s, glanders was one of the most prevalent infectious diseases in Mongolia, which was mostly reported in horses from almost all the provinces of the country. Starting in 1960, diagnostic, therapeutic and preventive projects were carried out for the control of glanders in horses and the prevalence of the disease decreased significantly (Odontseteg *et al,* 2005). However, glanders is still present in Mongolia (Erdemsurakh *et al,* 2020).

There is little information about the epidemiology of glanders in camels. From 1966 to 1968, a project named "Veterinary expeditions of Central and Eastern European countries against brucellosis, tuberculosis and glanders in Mongolia" in the frames of the Council for Mutual Economic Assistance successfully eliminated glanders in Mongolia. Within this project, 332,684 camels were tested using the mallein test. Of these, 380 (=0.1%) were seropositive for glanders (Kouba, 2010). Dromedaries can contract glanders as recently shown by Wernery *et al* (2011) and in Russa Bactrian camels were experimentally infected with *B. mallei* and developed a severe disease (Curasson, 1947).

2.4. Brucellosis

Brucellosis is a zoonosis caused by the intracellular, gram negative bacteria of the genus *Brucella*. Brucellosis is thought to be the most economically important zoonosis worldwide because it is endemic in many countries and impacts both human and livestock health. In 2001, approximately 8000 cases of human brucellosis were registered in Mongolia (Ebright *et al,* 2003). Camels are susceptible to both *B. abortus* and *B. melitensis*; however, camels are considered to be secondary hosts of *Brucella* spp. (Gwida *et al,* 2012).

Clinical signs of brucellosis in camels are less obvious compared to other livestock species (Wernery *et al,* 2014). This may be a reason why little information is available on epidemiology of brucellosis in camels and its impact on human health, notably in Mongolia. A serological investigation carried out in two provinces of Mongolia (Bulgan and Övörkhangai) between 1966 to 1968 by Czechoslovak veterinary expedition showed that 3.41% of examined camels were *Brucella* positive (Kouba, 2010). In a more recent examination, 1822 camels, together with other farm animals in Dornod and Sükhbaatar and in the Southern & Western provinces of Dornogovi, Ömnögovi and Khovd between 2013 and 2015 were tested with the Rose Bengal test and a seroprevalence of 2.3 % was found. *Brucella abortus* was found in vaginal swabs and milk samples from camels and cows Bayasgalan *et al* (2018).

2.5. Tuberculosis

Tuberculosis is one of the major zoonotic diseases in animals and humans caused by members of the bacterial genus *Mycobacterium* and Mongolia is one of the countries with the highest tuberculosis prevalence with around 4000 human tuberculosis cases reported every year, out of which 10% is pediatric (WHO, 2019). There are several reports published on tuberculosis in dromedaries from African countries, India and Bactrian camels from European zoos (Wernery *et al,* 2014). The situation of tuberculosis in Bactrian camels in Mongolia is unclear and should be investigated in future.

2.6. Leptospirosis

Leptospirosis, caused by pathogenic members of the genus *Leptospira*, is one of the most widespread zoonoses and the infection is an important cause of reproductive failure and production losses in big animals throughout the world (Odontsetseg *et al,* 2005). Serovars of leptospirosis having antigenic similarities are formed into serogroups and over 250 pathogenic serovars classified into 25 serogroups have been described (WHO, 2023). Serological examination of leptospirosis of cattle, sheep, goats, camels and rodents were carried out in seven provinces of Eastern, Central and Southern Mongolia in 2009-2010. The results of the study indicated the epidemiological significance of the Tarassovi serogroup in cattle and the Sejroe serogroup (probably hardjo serovar) in goats, sheep and camels as well as in desert and steppe dwelling rodents of Mongolia, indicating a circulation of these bacteria in natural foci (Anan'ina *et al,* 2011). A so far unknown *Leptospira* serovar circulating in ground squirrels and marmots is suspected.

2.7. Plague

Mongolia is a key area of plague genesis and marmots are the main reservoir for *Yersinia pestis*. Epizootics and consequent human plague cases were favoured by sufficient populations of fleas and susceptible rodents as well climatic conditions (Riehm *et al,* 2011). About 40 human cases were reported every year (Ebright *et al,* 2003). In Mongolia the

human plague is mainly contracted by skinning or preparing marmots for cooking, when an individual cuts its hand, or through eating raw infected marmot organs, meat or fat (Fijn and Terbisch, 2021). Enzootic plague also may be maintained in the Mongolian gerbil, *Meriones unguiculatus* and its flea, *Nosopsyllus laeviceps*, (Jun *et al,* 1993). Cases of plague in Bactrian camels in Mongolia, China and former USSR were reported by Sotnikov (1973). But there is no evidence for camels as direct source for human plague infection in Mongolia.

2.8. Salmonellosis

Salmonellosis is an infectious disease in camels and young camels that manifests as fever and bowel disturbances in the acute phase and pneumonia in the chronic phase.

Yondondorj *et al* (1986) isolated *Salmonella Dublin* and *S. Thyphimurium* from Bactrian camels with diarrhoea and from a dead juveline camel. Furthermore, the author indicated that some camel herds contained *Salmonella* carriers. Carrier camels can spread the disease to other non-infected camel herds, which will then develop diarrhoea (Erdenebileg, 2001).

2.9. Infectious enterotoxaemia

Camel calf diarrhoea is among the common illness in suckling dromedary calves, resulting in high mortality rate among this age group particularly in intensively kept camel herds. *Clostridium perfringens* type C can be one reason of diarrhoea in Bactrian camel calves. Baatar (1970) reported that one of the major reasons for camel deaths from enterotoxaemia lies in immune-incompetence and disorders of the gastro-intestinal track due to a lack of rangeland nutrients and harsh environmental factors.

2.10. Necrobacillosis

The Mongolian Bactrian camel suffers from necrobacillosis mainly in the steppe region of Mongolia (Davaa, 1987). Any form of abrasion of the camel's feet are main sources of infection. When camels stay for a long time in water and damp environments, such as lakes, bogs and slush, the food pads become soft and easily can be damaged by hard thorn bushes. The bacteria, *Fusobacterium necrophorum*, enters through the wound, causing the disease. At the early stage of the disease, Davaa (1987) recommended to wash the damaged part of the camel's feet in warm water with soap. After washing, the lesions on the feet should be powdered with manganese and boric powders.

2.11. Abscess

Some researchers (Yondondorj *et al,* 1986; Luvsannyam and Lkhagva, 1990) have studied the etiology of camel abscesses in Mongolia. They mentioned that the disease is caused by pathogenic bacteria such as *Staphylococcus aureus*. Abscess occurs more often in young and female Bactrian camels. In Mongolia, the disease starts in early spring and continues to late autumn. Sources of infection are sick animals with draining abscesses, purulent discharges from wounds. Also infected water points and animal handling equipment such as halters, ropes and fences may play a role. The incubation period of abscesses in camels is from 2-3 days to 1-2 weeks depending on the virulence of the causative agents and the resistance of the camels to the pathogenic organism. The clinical course of camel's abscess can be acute and chronic. Acute cases are characterised by abrupt fever, rapidly increased pulse and respiration rate, foaming from the mouth, red mucosa due to sepsis and generalised intoxication. This acute form usually occurs in spring. "Haemotherapy" is recommended for abscesses of camels (Luvsannaym and Lkhagva, 1990).

2.12. Rickettsiales

The *Rickettsia*les are an order of small Alphaproteobacteria. They are obligate intracellular parasites and some are notable pathogens, including *Rickettsia*, which causes a variety of diseases in humans and *Ehrlichia* and *Anaplasma* which causes diseases in livestock. *Rickettsia*, *Ehrlichia* and *Anaplasma* species are transmitted by numerous types of arthropods, including chigger, ticks, fleas and lice.

2.12.1. Anaplasmosis

Anaplasmosis is an infectious disease caused by a Gram-negative obligate intracellular bacterium of the *Anaplasmataceae* family (order *Rickettsia*les). It is a non-contagious, insect bite or tick borne disease of domesticated and wild ruminants including camels. *Anaplasma* spp. have been identified in tick species (*Dermacentor nuttalli* and *Ixodes persulcatus*) across Mongolia (Narankhajid *et al,* 2018; von Fricken *et al,* 2020). A study detected *Anaplasma* antibodies in 53.8% of Bactrian camels in Mongolia (von Fricken *et al,* 2018). Recently, *Anaplasma* spp. were detected in 18.2% of Bactrian camels in Töv and Dornogovi provinces of Mongolia by 16S metagenomics and confirmed by PCR assays (Chaorattanakawee *et al,* 2022). There is a need to investigate, if *Anaplasma*

bacteria may induce a disease in Mongolian Bactrian camels.

An interesting molecular study was carried out in Inner Mongolia of China that borders the southern Gobi district (Li *et al,* 2015). This study found *Anaplasma platys* DNA in 20 (=7.2%) out of 279 tested Bactrian camels but failed to find *Anaplasma* in blood smears. Six (=9.5%) out of 69 *Hyalomma sanguineus* ticks collected from these camels harboured *A. platys* DNA. *A. platys* DNA was also found in *H. sanguineus* collected from vegetation while examination of *Hyalomma asiaticum*, *Hy. dromedarii* and *Dermacentor niveus* gave negative results.

2.12.2. Ehrlichiosis

Ehrlichiosis is an important emerging tick-borne diseases, which, however may present itself asymptomatically or with a range of signs like elevated liver enzymes, fever, fatigue, lymphadenopathy, leukopaenia and weight loss (Doudier *et al,* 2010). Chaorattanakawee *et al* (2022) detected an *Ehrlichia* DNA in a Bactrian camel in Dornogobi province of Mongolia and characterised it as as *Candidatus E. regneryi*. Further studies are needed to clarify the epidemiology and risk factors of camel ehrlichiosis in Mongolia.

2.3.12. Rickettsiosis

Rickettsiosis are a group of diseases generally caused by species of Rickettsia, a genus of obligate intracellular bacteria. Most of the Rickettsioses are transmitted by ticks, but they can also be transmitted by fleas, lice and mites. There are not enough studies about the frequency of camel ricketsiosis in Mongolia. *Rickettsia* spp. exposure as measured by IgG was detected in 1.9% of Bactrian camels in Mongolia (von Fricken *et al,* 2018). According to reports on camel rickettsiosis, there is a necessity to further study this disease to figure out the clinical signs and its importance in transmitting diseases to humans in Mongolia.

3.2. Viral diseases

3.2.1. Foot-and-mouth disease

Foot-and-mouth disease (FMD) is an endemic viral disease of cloven-hooved livestock present throughout large parts of the African and Asian continents. Evidence of infection and/or disease has been reported in wildlife and Bactrian camels of Mongolia (Nyamsuren *et al,* 2006). During the outbreaks of FMD between 1963 and 1966 in Mongolia, mortality of affected camels reached 1.2-2.5 % (Erdenebileg, 2001). Khukhuu *et al* (2000) described clinical findings of FMD in camels during the outbreak in 2000 in the Western Gobi region. A large upsurge of FMD cases was observed in 2017/2018. For the first time, Ulziibat *et al* (2020) isolated the virus from camels in Mongolia. These were multiple lineages of serotype O (O/SEA/Mya-98; O/ME-SA/PanAsia; O/ME-SA/Ind-2001) as well as a single lineage of serotype A (A/ASIA/Sea-97). After vaccination, titres in camels were significantly lower than in cattle or sheep.

3.2.2. Camelpox

The camelpox virus infection causes a severe generalised disease in Bactrian camels and dromedaries that is characterised by extensive skin lesions. It is an important disease, especially in countries of Africa, the Middle East and southwestern Asia, where the camel is used as a beast of burden and for milk and it is zoonotic. In 1979, a pustular dermatitis caused by a virus belonging to the family Poxviridae, genus *Orthopoxvirus*, was observed among camels in some areas of Mongolia. The morbidity in adult camels ranged from 10 to 80%; in camel calves aged two to three months it was between 50-70% and reached 100% in 1-year-old animals. At the beginning, pustules developed around the mouth followed by papular elevations and scab formation. The virus wrongly designated as camel contagious ecthyma grew on the chorionallantoic membrane of 11-day-old chick embryos. Vaccination with the material containing contagious pustular dermatitis virus was promising. In contrast, camels were not protected after immunisation with vaccinia virus and with a vaccine against sheep and goat contagious ecthyma (Dashtseren *et al,* 1984).

3.2.3. Contagious ecthyma/orf

The disease is caused by the camel contagious ecthyma virus. Some researchers (Khukhuu, 1988; Dashtseren *et al,* 1984) reported outbreaks of contagious ecthyma in Mongolian Bactrians, especially in young camels. Contagious ecthyma in camels is usually characterised by local pox-like lesions on the face. Outbreaks of the disease occur from March to mid-July and sometimes from mid-September to November. The virus is resistant to cold weather conditions. The virus gains entry through injured mouth mucosa of camel calves due to their inexperience in eating bushy pasture. Vaccinated camels vaccinated with *Parapoxvirus ovis* vaccine were protected from the diseases for at least six months (Dashtseren *et al,* 1984).

3.2.4. Rabies

Rabies is a viral disease, characterised by fatal encephalitis in virtually all mammals, including camels. The first official report of rabies in Mongolia appeared in 1968 and since that time, both human and animal rabies cases occurred every year in Mongolia. Nearly 6000 animal rabies cases were reported between 1972 and 2006. Using molecular methods, Botvinkin *et al* (2008) for the first time characterised a rabies virus isolated from a Bactrian camel from western part of Mongolia and classified it within the steppe-type virus clade. Rabies virus of the same clade were determined from camels that died in the Govi Altai and Ömnögovi districts (Boldbaatar *et al,* 2010, Tuvshintulga *et al,* 2015). So far, the source of transmission is unknown.

3.2.5. Hepatitis E

Hepatitis E virus (HEV) infects humans and a wide variety of other mammalian hosts. Recently, HEV strains belonging to genotypes 7 and 8 within the **Orthohepevirus** A species of the Hepeviridae family, were identified in Bactrian camels in China (Sridhar *et al,* 2017). In Mongolia, 71 (35.5 %) out of 200 domestic Bactrian camels in three provinces were positive for anti-HEV IgG, with prevalence ranging between 4.2-75.0 %. From two camels in the Bayankhongor province the RNA of the virus was isolated. The two HEV strains (BcHEV-MNG140 and BcHEV-MNG146) obtained from the viremic camels in the present study shared 97.7 % nucleotide identity. They were closest to the reported G8 Chinese camel HEV strains but differed from them by 13.9-14.3% over the entire genome, with a nucleotide difference of 24.0-26.5 % from the reported G1-G7 HEV strains. A phylogenetic tree indicated that the BcHEV-MNG140 and BcHEV-MNG146 strains were located upstream of a clade consisting of the Chinese camel HEV strains and formed a cluster with them, with a bootstrap value of 100 %, suggesting that they may represent a novel subtype within G8. These results indicate a high prevalence of HEV infection in Mongolian camels and suggest that the variability of camel HEV genomes is markedly high (Nishizawa *et al*, 2021).

3.2.6. Influenza

Several outbreaks of severe respiratory diseases in Bactrian camels have occurred between 1978 and 1988 in Mongolia and were suspected to be caused by influenza viruses. During the epizootic of 1979-1980, approximately 4000 camels exhibited severe clinical signs, 375 died, 148 aborted and 270 became extremely exhausted. Between 1980 and 1983 thirteen isolates of H1N1 viruses were obtained from diseased camels (Yamnikova *et al,* 1993). Genetic and antigenic analyses of these isolates confirmed their relatedness to influenza A/USSR/90/77 (H1N1) which is a reassortant vaccine strain obtained by reassortment between the two H1N1 strains (A/ PR/8/34 and A/khabarovsk/77), possibly through transmission from vaccinated humans to camels. Interestingly, experimental infection of Bactrian camels with these isolates resulted in productive infections and antibody responses, indicating that camels are a potentially unexplored permissive host for influenza A virus (Yamnikova *et al,* 1993). Further active and enhanced surveillance of Bactrian camels in Mongolia also resulted in the isolation of influenza A H3N8 viruses that are phylogenetically related to equine influenza A virus, suggesting possible interspecies transmission (Yondon *et al,* 2014).

3.2.7. Middle East Respiratory Syndrome Coronavirus

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was identified in humans for the first time in 2012 in Saudi Arabia. MERS-CoV is a zoonotic disease and acute and severe respiratory disease in humans. The source of MERS-CoV are dromedary camels. They are a natural host of the MERS-CoV.

For Mongolia, there are two published studies on MERS-CoV in Bactrian camels. Chan *et al* (2015) sampled a total of 200 Bactrian camels (nasal swabs for real time PCR; serum samples for ELISA) from Ömnögovi and Dundgovi provinces in southern Mongolia and all MERS-CoV test results were negative. In a second study, out of 180 serum samples from Bactrian camels collected in 2016 and 2017, 21 were positive in an ELISA. However, none of the positives samples were positive in virus neutralization test (Bold *et al,* 2021).

3.2.8. Crimean-Congo Haemorrhagic Fever

Many different animal species including small ruminants and camels are infected with Crimean-Congo Haemorrhagic Fever virus (CCHFV) without showing any clinical signs (Wernery *et al,* 2022). Dromedaries in the United Arab Emirates for example have a sero-prevalence of more than 90%. The virus has a high fatality in humans.

The CCHFV is usually transmitted by tick bites and has an enzootic cycle including vertebrates. A serological survey based on human serum samples revealed an overall sero-prevalence of 1.4%. The

highest seroprevalence of 2.63% was found in the Bayankhongor district (Voorhees *et al,* 2018). Of the two examined ixodid tick species, *Hyalomma asiaticum* and *Dermacentor nutralli*, only one pool of *H. asiaticum* from Ömnögovi was positive for the virus DNA. It is likely that also Bactrian camels in the Gobi Desert are infected with CCHFV since they are the main host for *H. asiaticum*.

Conclusions

A comprehensive enumeration of infectious diseases in Bactrian camels of Mongolia is presented in two articles (includes Munkhjargal *et al,* 2023 also) which includes 15 parasitic, 12 bacterial and 8 viral infections, occurning in Mongolian Bactrian camels. So far, nothing is known about fungal diseases including dermatomycoses. The content of these articles may encourage scientists and researchers to carryout further research on these diseases. This may also lead to the development of prevention methods and to the development of vaccines.

Conflict of interests

The authors declare that they have no conflict of interest.

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ANALYSIS OF GLYCOSIDASE ACTIVITIES, STEROIDS HORMONES CONCENTRATIONS AND SELECTED BIOCHEMICAL PARAMETERS IN SERUM OF DROMEDARY CAMELS (*Camelus dromedarius*) DURING FOLLICULAR PHASE AND PARTURITION

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ABSTRACT

In the current study, blood samples were collected from 18 camels during the follicular phase and 9 camels at parturition. The obtained sera were used for estimation of glycosidase activities, steroids hormones concentrations and selected biochemical parameters in dromedary camels during follicular phase and parturition. The current findings indicated that the activities of β-N-acetylglucosaminidase and α-N-acetylgalactosaminidase increased significantly during parturition compared to that during follicular phase. α-L-fucosidase activities remained comparable in both phases. The serum concentration of progesterone, cortisol and prostaglandin F2α increased significantly during parturition compared to that during the follicular phase. However, oestradiol 17-β decreased significantly during parturition compared to that during the follicular phase. The serum concentration of nitric oxide, alkaline phosphatase, cholesterol, creatine kinase, glucose and magnesium decreased significantly during parturition compared to that during the follicular phase. The activities of AST and the concentrations of calcium and phosphorus remained comparable in follicular phase and parturition. In conclusion, the current study indicated that there is a difference in glycosidase activities, steroids hormones concentrations and some biochemical parameters in serum of dromedary camels (*Camelus dromedarius*) during follicular phase and parturition.

Key words: Camels, follicles and steroids, glycosidase, hormones, parturition

The dromedary camel (*Camelus dromedarius*) is a seasonal breeder with a relatively short breeding season (Wilson, 1984 and Tibary and Anouassi, 1997). The oestrous cycle in camel has no luteal phase (Musa and Abusineina, 1978) because the ovulation is a response to the stimulus of mating (El wishy, 1987). However, in induced ovulators such as the camel, there are three phases of the follicular wave which could be categorised as the growth phase (10.9±3.0 days), the mature phase (7.6±4.2 days) and the regressing phase (11.9±4.2 days) (Skidmore *et al*, 1995). Follicles mature in 6 days, maintained their size for 13 days and regressed in eight days (Musa and Abusineina, 1978). The oocyte grows and matures in a biochemical environment (follicular fluid) that changes from small to large follicles (Leroy *et al,* 2004).

Glucosidases are believed to control early embryonic development by altering membrane permeability. Tsiligianni *et al* (2003) studied the activity of -N-acetyloglucosaminidase (bNAGASE) and α-manosidase in the luminal fluid from the uterine horns of ewes after superovulation and found a relationship between b-NAGASE and α-manosidase activity and superovulatory response.

The non-mated animals has low concentrations of progesterone in but it increases in mated camels 3-4 days after ovulation (day of ovulation=Day 0) to reach maximum concentrations on days 8-9 before decreasing rapidly on Days 10-11 in the non-pregnant animal (Skidmore, 2011).

Skidmore *et al* (1998) found that, as in other large domestic animal species, release of PGF2

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alpha from, presumably, the endometrium controls luteolysis in the dromedary camel and the release of endometrial PGF2 alpha in the non-pregnant camel may not be controlled by the release of oxytocin.

Mohamed *et al* (2021) revealed significant correlations between steroid hormones and the oxidant indicators, antioxidant biomarkers, lipid profile indices and renal functions biomarkers that provided better understanding for physiological stress during pregnancy in camels.

Tharwat *et al* (2015) investigated the hematological, biochemical, acid-base and blood gas parameters in 12 healthy pregnant dromedary camels, their calves and umbilical vein blood at spontaneous parturition. A complete blood count (CBC) and serum concentrations of calcium, phosphorus, magnesium, blood urea nitrogen (BUN) and glucose were measured. The serum activity of cardiac troponin I (cTnI), γ-glutamyl transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK) and alkaline phosphatase (ALP) were also measured. None of the female camels showed any evidence of clinical disease at parturition.

In view of importance of above parameters, we collected the sera from camels during the follicular phase and at parturition for estimation of glycosidase activities, steroids hormones concentrations and selected biochemical parameters in dromedary camels.

Materials and Methods

The Scientific Research Deanship Ethical Committee of King Faisal University approved the protocol and conduct of this study.

Animals and sample collection

This study was conducted during the breeding season (November – March) at the Camel Research Centre, King Faisal University (located between 25° 05′ and 25° 40′ northern latitude and 49° 55′ eastern longitude), Saudi Arabia. The animals involved in this study were cycling with follicular activity (n= 18) and pleuripara pregnant (n= 9) dromedary female camels ranging from 6 to 15 years of age and weighing 500 – 800 kg. During the study, the camels were maintained under standard conditions of feeding and management. The cycling females were examined routinely with ultrasonography using linear-array 7.5 MHz transducer (MyLab Five VET, Esaote, Italy; Tibary and Anouassi, 1996) to determine their follicular activity. Based on the breeding records of the Camel Research Centre, King Faisal University and the average length of gestation archived for the Centre, the signs of approaching parturition (Musa, 1983) were carefully examined 10 days before the due dates for parturition. Briefly, the labour was graded as follows: Score 1 (no assistance; n= 366), score 2 (farm-staff assistance provided with calf in normal or mal posture; n=20). The pregnant animals of this study included 7 female camels with scores of 1 and 2 animals with scores of 2. Blood samples (10 ml/ each animal) were collected in plain vacutainer tubes from all cycling animals and within the first 15 min after delivery in pleuripara females. Blood serum was separated by centrifugation at 3000rpm for 10 min and stored at -80^{o}C with pending analysis.

Enzymes' analysis

Three enzymes were estimated in the serum of dromedary camels during follicular phase and at parturition using ELISA reader (Absorbance Microplate Reader ELx 800TM BioTek®, USA; Microplate Strip Washer ELx 50TM BioTek®, USA) and commercial ELISA diagnostic kits (My Biosource®). These enzymes were camel β-N-acetylglucosaminidase (Catalog # MBS094638), camel α-N-acetylgalactosaminidase (Catalog # MBS053019) and camel α-L-fucosidase (Catalog # MBS092780). The procedures for analysis and calculation were adopted according to the manufacturer instructions.

Steroid hormone analysis

The concentrations of oestradiol 17-β (E17-β; pg/ml; catalog No. 60155), progesterone (P4; ng/ml; catalog No. 582601) and prostaglandin $F_2\alpha$ (pg/ml; catalog No. 516011) in serum and follicular fluids were analysed using enzyme immune assay (EIA) kits (Cayman Chemical Company, Ann Arbor, MI, USA). Cortisol analysis (ng/ml) was determined by EIA kits (Oxford Biomedical Research Inc., Oxford, MI, USA). All assays were performed according to the manufacturer's directions and the optical densities were measured using an ELISA reader (Absorbance Microplate Reader ELx800TM, BioTek®, Highland Park, VT, USA and Microplate Strip Washer ELx800 TM, BioTek®, Highland Park, VT, USA).

Biochemical parameters analysis

Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of glucose (mg/dL; Catalog No. EP37L-660), cholesterol (mg/dL; Catalog No. EP24-660), ALP (IU/L; Catalog No. EP04L-660), AST (IU/L; Cataloge No. EP15-500), CK (IU/L; Catalog No. EP28-310), Calcium (mg/dL; Catalog No. EP22-660),

phosphorus (mg/dL; Catalog No. EL46-1200) and magnesium (mg/dL; Catalog No. EL50-1000). The analysis was conducted by using a full-automated chemistry analyser (ELIPSE, Rome, Italy). Nitric oxide was assayed using commercially available kits (Cayman, Kit number (µM; Catalog No. 780001).

Statistical analysis

The data analysis of biochemical constituents and hormones in serum was carried out using a General Linear Model (GLM) procedure and means were compared by Least Significant Difference (LSD) using SPSS 22.0 statistical software (SPSS, 2013).

Results and Discussion

The persent findings indicated that the activities of β-N-acetylglucosaminidase and α-N-acetylgalactosaminidase increased significantly during parturition compared to that during follicular phase (Table 1). Alpha-L-fucosidase activities remained comparable in both phases (Table 1). The serum concentration of progesterone, cortisol and prostaglandin F2α increased significantly during parturition compared to follicular phase (Table 2). However, oestradiol 17-β decreased significantly during parturition compared to follicular phase (Table 2). The serum concentration of nitric oxide, alkaline phosphatase, cholesterol, creatine kinase, glucose and magnesium decreased significantly during parturition compared to that during the follicular phase (Table 3). The activities of AST and the concentrations of calcium and phosphorus remained comparable in follicular phase and parturition (Table 3).

Table 1. Comparison of β-N-acetylglucosaminidase, α-N-acetylgalactosaminidase and α-L-fucosidase concentrations (mean ± SEM) in serum of the dromedary camel (*Camelus dromedarius*) during the follicular phase and parturition.

Enzymes	Follicular phase (n=18)	Parturition (n=9)
β-N-acetylglucosaminidase (ng/ml)	2.52^{a} ± 0.16	4.16^{b} ± 0.61
α-N-acetylgalactosaminidase (ng/ml)	2.57^{a} ± 0.23	4.22^{b} ± 0.62
α-L-fucosidase (µmol/ml)	383.33 ± 34.65	379.17 ± 13.23

Means with different superscripts in the same row are different (P<0.05)

The present study revealed high serum concentrations of glycosidases (β-N-acetylglucosaminidase and α-N-acetylgalacto-saminidase) at parturition in comparison to these concentrations during the follicular phase in dromedary camels. Glycosidases have a pivotal role in many reproductive aspects like cumulus cells expansion (Takada *et al,* 1994), sperm capacitation, zona pellucida binding, and prevention of polyspermy (Miller *et al,* 1993; Miranda *et al,* 2000; Zitta *et al,* 2006; Taitzoglou *et al,* 2007), and early embryos development (Tsiligianni, 2018). Moreover, glycosidases are considered as markers of embryo quality, embryo recovery rate, and super-ovulatory response (Reilas *et al,* 2000; Tsiligianni *et al,* 2007; Tsiligianni, 2018).

Table 2. Concentrations (mean ± SEM) of different hormones in the serum of camels during follicular phase and parturition.

Hormones	Follicular phase (n=18)	Parturition (n=9)
Progesterone (pg/mL)	412.26^{a} ± 28.32	768.44^{b} ± 81.50
Oestradiol 17-β (pg/mL)	216.60^{a} ± 31.33	137.00^{b} ± 19.45
Cortisol (pg/mL)	1374.91^{a} ± 275.23	3404.80^{b} ± 486.80
Prostaglandin F2α (pg/mL)	68.44^{a} ± 21.16	296.89^{b} ± 116.09

Means with different superscripts in the same row are significantly different (P < 0.05)

Table 3. Concentrations (mean ± SEM) of different biochemical constituents in the serum of camels during follicular phase and parturition.

Biochemical constituents	Follicular phase (n=18)	Parturition (n=9)
Nitric oxide (µM)	2.37^{a} ± 0.10	1.82^{b} ± 0.04
Alkaline phosphatase (IU/L)	55.50^{a} ± 5.00	38.04^{b} ± 2.34
Aspartate aminotransferase (IU/L)	73.08 ± 4.91	69.57 ± 3.51
Calcium (mg/dL)	8.62 ± 0.19	6.70 ± 0.23
Cholesterol (mg/dL)	52.17^{a} ± 4.25	31.00^{b} ± 2.34
Creatine kinase (IU/L)	180.28^{a} ± 33.64	92.40^{b} ± 8.32
Glucose (mg/dL)	260.10^{a} ± 30.00	103.31^{b} ± 12.42
Inorganic phosphorus (mg/dL)	4.48 ± 0.58	3.81 ± 0.22
Magnesium (mg/dL)	2.00^{a} ± 0.39	1.21^{b} ± 0.14

Means with different superscripts in the same row are significantly different (P < 0.05)

Glycosidases activities change during different reproductive events such as oestrous cycle in ewes (Roberts *et al,* 1976) and mares (Reilas and Katila, 2002), and pregnancy in cows (Roberts and Parker, 1974). Fluctuations in glycosidase activity during the oestrous cycle are hormonally controlled (Hansen *et al,* 1985). The activity of glycosidases is regulated by progesterone and oestrogens (Gladson *et al,* 1998; Buhi *et al,* 2000; Reilas and Katila, 2002).

In the present study, a significant increase in serum progesterone, cortisol and $PGF_2\alpha$ concentrations at parturition compared to during the follicular phase was observed. Progesterone, which is crucial for pregnancy in mammals was released in the corpus luteum and the placental trophoblast (Wooding *et al,* 2003). In dromedaries, corpus luteum was necessary for maintaining pregnancy all over the gestation period (Musa and Abu Sineina, 1976; Skidmore *et al,* 1996; Al-Eknah *et al,* 2001). Progesterone levels remained elevated all over pregnancy and started to decrease towards the parturition day in both dromedary camels and llamas (León *et al,* 1990; Bravo *et al,* 1996; Skidmore, 2011). Moreover, the source of the significant high peripheral progesterone concentrations reported in camels may be the adrenal cortex (Asher *et al,* 1989). This study reported a high significant peripheral cortisone concentration at parturition compared to the follicular phase. Similarly, in previous studies, glucocorticoid concentration was markedly increased during parturition in camels (El-Belely, 1994) and cattle (Smith *et al,* 1973; Hudson *et al,* 1975; Heuwieser *et al,* 1987; Nakao and Grunert, 1990). As in other farm animals (Silvia *et al,* 1991), uterine $PGF_2\alpha$ was the essential luteolytic factor in dromedaries (Skidmore *et al,* 1998). In dromedary camels (El-Belely, 1994) and llamas (Aba *et al,* 1998), an excessive production of $PGF_2\alpha$ occured on the day of parturition.

In this study, serum oestradiol level was significantly higher during the follicular phase than at parturition. Oestradiol in pregnant camels was feto-placental in origin (Ayoub *et al,* 2003). However, unlike other ruminants (Wood, 1999), a raised level of oestrogens is found at parturition in dromedaries (El-Belely, 1994). The parturition process was ruled by maternal and foetal hormones (Thorburn *et al,* 1977; Brooks and Challis, 1988; Wood and Keller-Wood, 1991; El-Belely, 1994; McMillen *et al,* 1995).

In the present study, serum concentrations of nitric oxide, alkaline phosphatase, cholesterol, creatine kinase, glucose and magnesium decreased significantly during parturition compared to that during the follicular phase. Nitric oxide has a regulatory role within the reproductive systems (Rosselli *et al,* 1998), it is released in the ovary (Van Voorhis *et al,* 1994; Yamauchi *et al,* 1997) and controlled uterine contractions during pregnancy and parturition (Rosselli *et al,* 1998). On the contrary, alkaline phosphatase, released from the uterus or placenta, peaked during parturition in cows (Peter *et al,* 1987; Wallach, 2000).

Most steroid hormones like progesterone were synthesised from cholesterol (Pineda and Dooley, 2003). In women, cholesterol tended to be highest during the follicular phase and declined during the luteal phase (Mumford *et al,* 2011). However, Civelek *et al* (2008) recorded marked increase in cholesterol levels in animals during normal birth.

In this study, the serum creatine kinase, glucose and magnesium levels were significantly lower at parturition than during follicular phase. Similarly, low serum creatine kinase activity was recorded in women with early labour pains (Chemnitz *et al,* 1979). Creatine engaged in energy metabolism all over female reproduction and played a role in successful fertilisation, pregnancy and parturition (Muccini *et al,* 2021).

Glucose was the primary source of energy that maintained ovaries (Rabiee *et al,* 1997; Leroy *et al,* 2004; Krasnow and Steiner, 2006). Glucocorticoids stimulate the liver to secrete great quantities of glucose (Lange *et al,* 1994; Schmoll *et al,* 1996; Cassuto *et al,* 2005). Furthermore, heifers with normal ovarian function had significantly higher plasma glucose concentrations at days 2 – 4 of oestrus (Butler *et al,* 2012).

In the postpartum period, serum glucose was insignificantly lowered than in the pregnancy (Debski *et al,* 2017). Similarly, low blood glucose concentrations occurred in early postpartum dairy cows (Garverick *et al,* 2013; Lucy *et al,* 2013). On the contrary of the present study, blood serum magnesium was not different in cyclic and pre-pubertal water buffaloes (Shoushtari *et al,* 2014). However, similar to the present study, low serum magnesium levels have been found in post-partum cows (Van de Braak *et al,* 1987).

Conclusion

There was a difference in glycosidase activities, steroids hormones concentrations and some biochemical parameters in serum of dromedary camels (*Camelus dromedarius*) during follicular phase and parturition.

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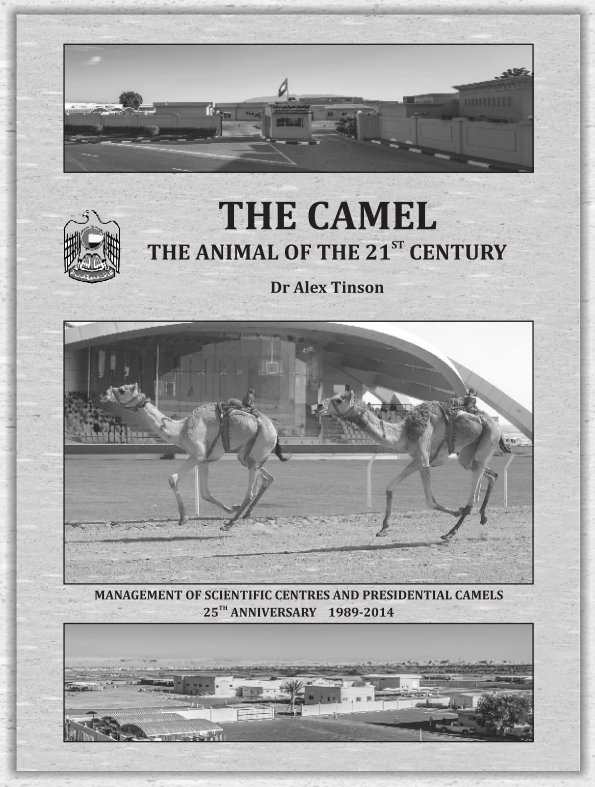
THE CAMEL

THE ANIMAL OF THE 21ST CENTURY

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



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DOSE SPECIFIC EFFECTS OF IONOMYCIN ON PARTHENOGENETIC ACTIVATION OF *in vitro* MATURED DROMEDARY OOCYTES

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ABSTRACT

Evaluation of the dose-specific effects of ionomycin on the parthenogenetic activation of *Camelus dromedarius* oocytes was done in the present study. Ovaries were collected from a local slaughterhouse. Cumulus-oocyte-complexes (COCs) were aspirated and cultured in a commercial IVM medium (BO-IVM) for 42 hrs in a humidified atmosphere containing 5% CO_2 at 38°C. All metaphase II oocytes were activated either 2.5 µM, 5.0 µM or 10.0 µM of ionomycin for 4 min. Activated oocytes were immediately incubated in 2.0 mM 6-dimethylaminopurine (6-DMAP) in a commercial embryo culture medium (BO-IVC) for 4 hrs at 39°C in a humidified incubator with 5% CO_2. Oocytes were cultured in BO-IVC for 7 days and developmental stages were monitored and recorded. After 42 hrs of *in vitro* maturation, 71.28% of oocytes were found with the extruded first polar body (metaphase II oocytes). The oocytes in the 5.0 µM of ionomycin group showed the highest blastocyst formation rate (56.99%) compared to the 2.5 µM (6.58%) and 10.0 µM (1.81%) groups. We recommended 5.0 µM of ionomycin for 4 min followed by incubation in 2.0 mM 6-DMAP for 4 hrs to activate camel oocytes.

Key words: Camel, *in vitro* maturation, ionomycin, oocytes, parthenogenetic

Parthenogenesis provides valuable insights into genomic imprinting during early embryonic development and it is helpful to understand the effectiveness for activation of oocytes. Among those, general principles of cell signaling systems (Solter, 1998; Ma *et al,* 2005). Furthermore, artificial activation of oocytes has a functional role in somatic cell nuclear transfer process as reconstructed oocyte needs to be activated for further embryonic development (Campbell, 1999; Kishikawa *et al,* 1999).

Following fertilisation, the intracellular calcium oscillation leads to cortical reaction and resumption of meiosis and formation of the second polar body to the effectiveness for activation of oocytes. Among those, perivitelline space (Küpker *et al,* 1998). Parthenogenesis mimics the functions of spermatozoa during fertilisation (Nakada and Mizuno, 1998). Different methods, such as chemical, mechanical or physical have been used for the creation of parthenogenetic embryos (Machaty, 2006; Brevini and Gandolfi, 2008). Some chemicals such as strontium (Cuthbertson *et al,* 1981) and ionomycin release calcium from cytoplasmic stores to increase intracellular free calcium, while electrical stimulus causes the influx of calcium from the extracellular medium and some chemicals such as ethanol promote both effects (Loi *et al,* 1998).

Various chemical agents have been tested for the resumption of meiotic II arrest and proved effective for activation of oocytes. Among those, calcium ionophores, such as ionomycin and A23187, together with protein synthesis or kinase inhibitors, such as 6-dimethylaminopurine (6-DMAP), is the most popular method for oocytes parthenogenesis in several different species (Heindryckx *et al,* 2009). Although, ionomycin has been previously used to derive parthenogenetic camel embryos, even SCNT-reconstructed oocytes were also activated with ionomycin (Wani, 2008; Hossein *et al,* 2021; Son *et al,* 2022), the optimum treatment conditions of ionomycin have not been studied systematically in camels. Accordingly, the objective of the present study was to evaluate the dose-specific effects of ionomycin treatment on the parthenogenetic activation of dromedary oocytes.

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Materials and Methods

The present study did not utilise any live animals, only slaughterhouse samples at post-mortem were collected and used for experimentation; hence had no ethical consideration.

Oocyte collection from abattoir ovaries

The experiments were conducted from November 2021 to January 2022. Ovary samples were collected from a local public slaughterhouse and transported to the laboratory within 3 hrs of collection in lukewarm 0.9% saline solution. Cumulus oocyte complexes (COCs) were aspirated from 2–6 mm diameter antral follicles using an 18-gauge hypodermic needle attached to a 10 ml disposable syringe. The COCs were selected based on cumulus cell layers and cytoplasm homogeneity; oocytes with two or fewer layers of compact cumulus cells or without a homogenous cytoplasm were excluded for *in vitro* maturation (IVM). The COCs were washed 3 times in Dulbecco's phosphate-buffered saline (DPBS; Welgene, Gyeongsan, Korea) supplemented with 5 mg/ml bovine serum albumin (BSA; Thermo Fisher Scientific, Waltham, MA, USA) and 1% antibiotic–antimycotic (Thermo Fisher Scientific, Waltham, MA, USA). We used a commercial IVM medium (IVF Bioscience, Falmouth, UK) for culturing COCs for 42 hrs at 38°C in a humidified atmosphere containing 5% CO_2.

Parthenogenetic activation

After 42 hrs of IVM, cumulus layers were removed from the oocytes by gentle pipetting with 0.1% (v/v) hyaluronidase in the washing medium (HEPES buffered TCM-199 supplemented with 10% (v/v) FBS). The denuded oocytes were examined under a stereomicroscope for the presence of the first polar body. Oocytes with no polar body, abnormal size or shape, or without homogenous cytoplasm were excluded from this study. All metaphase II oocytes (oocytes with an extruded polar body) were activated with ionomycin according to the experimental design. Three different concentrations of ionomycin, such as 2.5 µM, 5.0 µM 10.0 µM were evaluated in the present study. In each case, ionomycin was treated for 4 min. The activated oocytes were washed several times in the washing medium. After that, oocytes were incubated in 2.0 mM 6-DMAP in a commercial embryo culture medium BO-IVC (IVF Bioscience, Falmouth, UK) for 4 hrs at 39°C in a humidified incubator with 5% CO_2.

In vitro culture of parthenogenetic embryos

Following activation, oocytes were washed three times and cultured in a commercial embryo culture media (BO-IVC) for 7 days. Six to 8 oocytes were cultured in a 30 µl oil-covered droplet at 38°C in a humidified atmosphere with 5% CO_2 and 5% O_2. Developmental stages of embryos were evaluated on days 2, 4 and 7 of IVC and the number of two-cell stage, four-cell stage, morula and blastocyst were recorded.

Statistical analysis

All data were analysed using statistical analysis software, SPSS (version 15; SPSS Inc., Chicago, IL, USA). The two-cell rate was calculated from the number of activated oocytes and the four-cell, morula and blastocyst rates were calculated from the number of two-cells. Pre-implantation developmental competence of activated oocytes was analysed using a one-way analysis of variance (ANOVA) and a Duncan post hoc test. Statistical significance was set at $p < 0.05$.

Results and Discussion

In vitro maturation of camel oocytes

A total of 1271 cumulus-oocyte-complexes were collected from 124 ovaries. After 42 hrs of culture, 906 (71.28%) oocytes were found where the first polar body was extruded. Oocytes without a first polar body (n=256; 20.14%) or with abnormal morphology (n=109; 8.58%) were excluded from the experiment. Representative photographs of COCs at collection and after 42 hrs of culture and mature oocyte with the first polar body are shown in Fig 1.

Embryonic development of activated oocytes

Dose specific effects of ionomycin treatment on embryonic development of camel oocytes is shown in table 1. The average cleavage (two-cell) rate was 20.22 ± 1.04, 73.87 ± 4.76 and 19.91 ± 1.89 for 2.5 µM, 5.0 µM and 10.0 µM of ionomycin, respectively ($p<0.05$). The rate of morula was 12.88 ± 1.10, 64.11 ± 1.68 and 3.15 ± 1.97 for 2.5 µM, 5.0 µM and 10.0 µM of ionomycin, respectively ($p<0.05$). The oocytes in 5.0 µM group showed the highest blastocyst formation rate (56.99 ± 2.33) compared to 2.5 µM (6.58 ± 2.39) and 10.0 µM groups (1.81 ± 1.81). Representative photograph of various developmental stage is shown in Fig 2.

Ionomycin has been widely used in the parthenogenetic activation of camel oocytes, including in the SCNT program for the production of cloned embryos (Wani, 2008; Hossein *et al,* 2021; Son *et al,* 2022). However, little information regarding the dose-

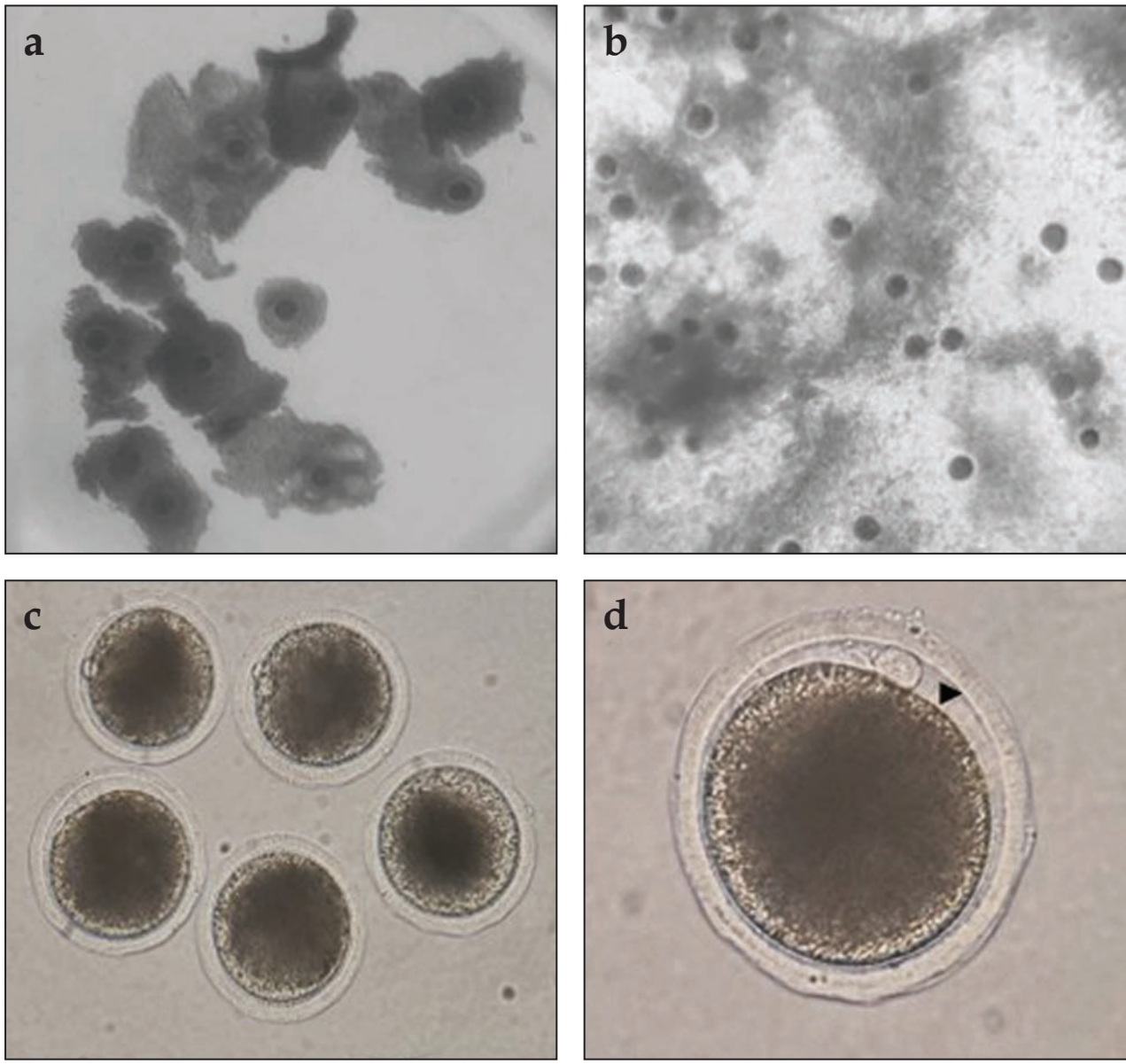

Fig 1. Representative photograph of oocytes (a) cumulus oocytes complex (COCs), (b) COCs after 42 hrs of *in vitro* maturation (c) Oocytes after denuding (d) Metaphase II oocytes (arrow head indicates the first polar body).

specific effects of ionomycin in camel oocytes has been reported. In the present study, three different concentrations of the ionomycin such as 2.5 µM, 5.0 µM and 10.0 µM were evaluated for activation of *in vitro* matured camel oocytes. This protocol used immediate incubation of activated oocytes in 2.0 mM of 6- DMAP, a kinase enzyme inhibitor, for 4 hr. The oocytes in the 5.0 µM of ionomycin group showed the highest developmental competence compared to the 2.5 µM and 10.0 µM groups.

Variable results were reported from different laboratories regarding IVM of camel oocytes. Some studies reported only 25% to 40% of the oocytes with the extruded first polar body after IVM (Gabr *et al,* 2014; Mesbah *et al,* 2016), while others reported more than 50% maturation rate (Kafi *et al,* 2005; Wani and Nowshari, 2005; Wani and Wernery, 2010; Saadeldin *et al,* 2017). It was observed 71% maturation rate in the present study; even in one earlier studies, approximately 70% maturation rate was reported (Hossein *et al,* 2021; Son *et al,* 2022). The source of oocytes, oocyte culture condition and duration of the culture period may influence the differences in maturation rate. However, in most laboratories at least 50% of oocytes reached to the metaphase II stage. Therefore, slaughterhouse ovaries, in conjunction with IVM of COCs, could be the potential source of mature oocytes for embryo production in camels.

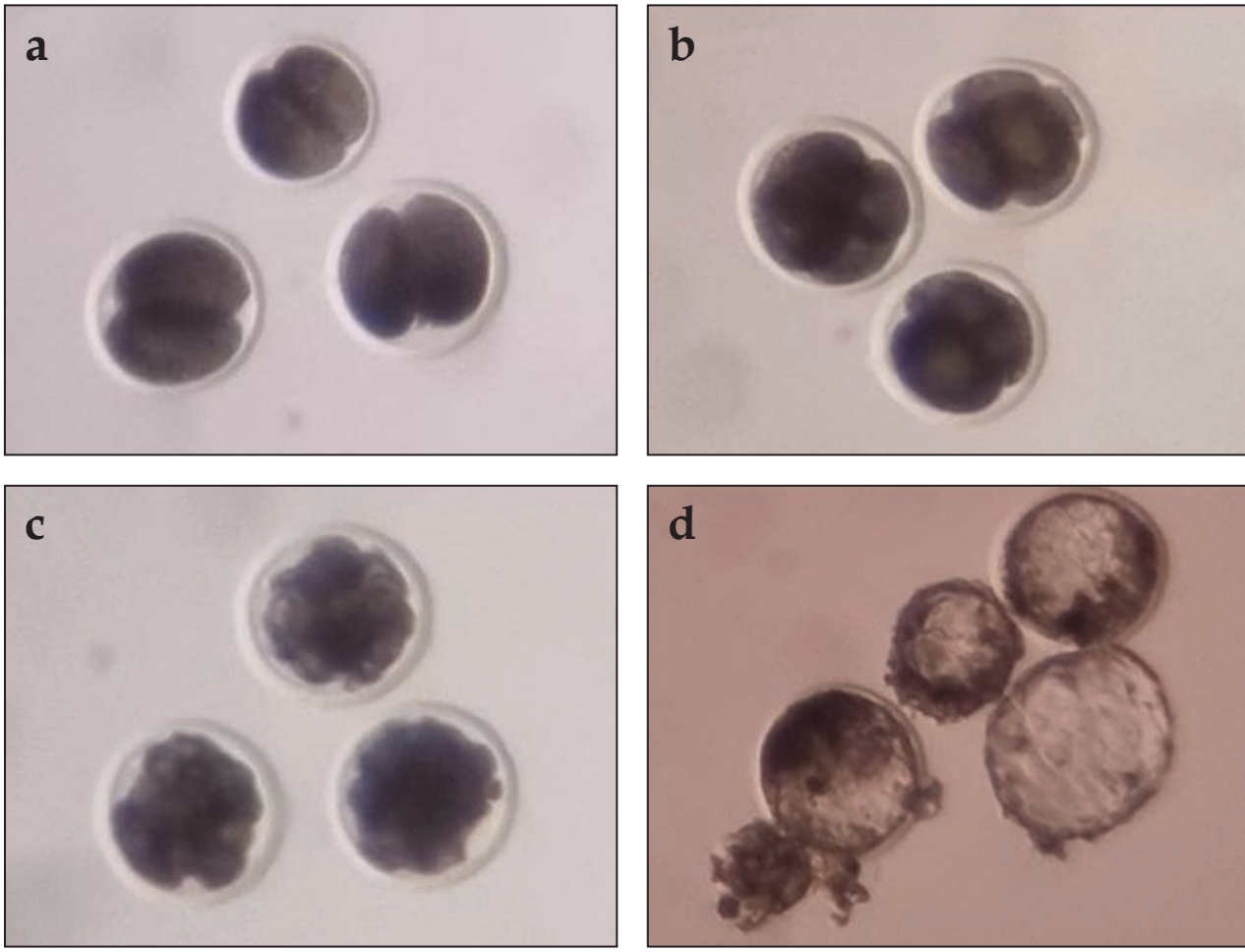

Fig 2. Developmental stages of parthenogenetic embryos (a) two-cell stage embryo, (b) four-cell stage embryos, (c) morula, (d) blastocysts.

Table 1. *In vitro* development of camel embryos derived by parthenogenetic activation of *in vitro* matured oocytes

Treatment groups	No. of oocytes	Cleaved† (%)	4 cells‡ (%)	Morula‡ (%)	Blastocyst‡ (%)
2.5 µM (n=5)	299	20.22 ± 1.04^{a}	26.44 ± 2.46^{a}	12.88 ± 1.10^{a}	6.58 ± 2.39^{a}
5.0 µM (n=5)	293	73.87 ± 4.76^{b}	83.19 ± 1.83^{b}	64.11 ± 1.68^{b}	56.99 ± 2.33^{b}
10 µM (n=5)	307	19.91 ± 1.89^{a}	20.20 ± 2.30^{a}	3.15 ± 1.97^{a}	1.81 ± 1.81^{a}

† The percentage of cleaved oocytes was calculated based on the number of oocytes.

‡ The percentage of embryos developed in each stage was calculated relative to the number of cleaved embryos.
Different superscript in a column is significantly different ($p<0.05$).

Two forms of calcium ionophore (ionomycin and A23187) are widely used for the artificial activation or parthenogenesis of camelid oocytes. Ionomycin mainly utilised the internal storage of oocytes' own calcium to increase calcium concentration in oocytes (Loi *et al*, 1998), but it can also use external sources of calcium from the media (Cuthbertson *et al*, 1981). These increased calcium concentrations inactivate MPF and CSF and thus oocytes are released from the second meiotic arrest (Collas *et al*, 1993; Lorca *et al*, 1993) and commence embryonic development. A combined treatment of ionomycin and 6-DMAP is shown to induce higher embryonic development in bovine and rabbits (Susko-Parrish *et al*, 1994; Liu *et al*, 1998; Mitalipov *et al*, 1999). We also used 6-DMAP treatment for parthenogenesis in camel oocytes. Wani (2008) compared ionomycin and ethanol combined with 6-DMAP or roscovitine and reported that ionomycine/6-DMAP is the best combination to activate camel oocytes. The author further reported that the ionomycin-activated group produced a higher blastocyst rate compared to *in vitro* fertilised group. Similar results were observed in bovines

(Rho *et al*, 1998), buffalo (Saikhun *et al,* 2004) or goat oocytes (Onger *et al,* 2001).

The present study showed that all the studied concentrations of ionomycin have the ability to activate camel oocytes and support their development upto the blastocyst stage. However, a higher proportion of oocytes activated by 5.0 µM ionomycin reached the blastocyst stage compared with 2.5 µM or 10.0 µM of ionomycin. Che *et al* (2007) used 5.0 to 20.0 µM of ionomycin to activate porcine oocytes and reported that 15.0 and 20.0 µM of ionomycin developed the highest blastocyst rate. The study revealed that concentration more than 5.0 µM of ionomycin significantly reduce the blastocyst formation rate. The species-specific variation of oocyte morphology and intrinsic quality of cytoplasm might be responsible for these differences.

In conclusion, the majority of immature camel oocytes collected from slaughterhouse ovaries reached to the metaphase II stage after 42 hrs of *in vitro* culture. Ionomycin treatment at a concentration of 5.0 µM for 4 min followed by culture in 2.0 mM 6-DMAP for 4 hrs resulted in the best parthenogenetic activation of camel oocytes.

Acknowledgements

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EXPRESSION OF AQUAPORIN 1 IN THE TESTIS AND EPIDIDYMIS OF THE DROMEDARY CAMEL (*Camelus dromedarius*) IN RUTTING AND NON-RUTTING SEASONS

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ABSTRACT

The present study clarified the expression of AQP1 in the testis and epididymis of dromedary camels. The immunohistochemistry showed that AQP1 was moderately expressed in the cranial and caudal parts of the testis and the rete testis during the first half of the rutting season in October and increased rapidly until December. The expression decreased and remained strong until March of the following year. The testis recovered to being highly immunoreactive to AQP1 during the whole non-rutting season. The same pattern expression of AQ1 was present in the testicular spermatozoa. The testis had distinct histological changes where the seminiferous tubules' size and the interstitial tissue's density varied by season. In the initial part of the breeding period, the epididymal head's epithelium exhibited moderate reactivity to AQ1 and increased strongly towards the tail. Through the remainder of the rutting season, a moderate immunoreactive to AQ1 was recognised in the epididymal epithelium and sperms. The non-breeding time showed very strong immunoreactive to AQP1 in the epididymal epithelium and spermatozoa. The epididymis' histology revealed a noticeable seasonal variation. These findings could suggest that AQ1 is essential for the dromedary camels' spermiogenesis.

Key words: Aquaporin1, dromedary camel, expression, epididymis, testis

Aquaporins (AQPs) are biologically active proteins affecting mammalian species' reproductive activity. AQPs are a class of small, hydrophobic, integral membrane proteins that frequently facilitate the passive movement of water, by which they regulate the transportation of fluids in cells and tissues such as the male reproductive organs (Verkman and Mitra, 2000; Agre *et al,* 2002; Kannan *et al,* 2020). In mammals, thirteen distinct isoforms of AQPs have been detected; AQP0 to AQP12 (Carrageta *et al,* 2020). Among them, AQP1, AQP2, AQP7, AQP8, and AQP9 have been seen in the male reproductive tract (Stevens *et al,* 2000), where AQP1 function is the absorption of water and regulation of sperm concentration in the male genital organs (Brown *et al,* 1993; Nicotina *et al,* 2004; Nicotina *et al,* 2005; Lu *et al,* 2008; Ishibashi *et al,* 2009; Arrighi *et al,* 2010).

AQP1 was expressed in the organs of the male reproductive tract of many mammals, including the Bactrian camel, human, horse, buffalo-bull, dog, cat, mouses and fruit-eating bat (Danyu *et al,* 2008; Ito *et al,* 2008; Lu *et al,* 2008; Domeniconi *et al,* 2008; Skowronski *et al,* 2009; Yeung *et al,* 2010; Oliveira *et al,* 2013; Klein *et al,* 2013; Arrighi and Aralla, 2014; Arrigh *et al,* 2016; An and Wang, 2016). Al-Thnaian (2023) found that the highest level of AQP-7 mRNA expression was in the testis of rutting and non-rutting dromedary males compared to the ductus deferens, epididymis and prostate. AQP1 in the dromedary's reproductive system are least studied.

Thus, the present study was conducted to detect AQP1 and histological changes in the testis and epididymis of the dromedary camel during rutting and non-rutting season throughout the year, applying the immunohistochemistry techniques.

Materials and Methods

Samples were obtained from 36 healthy adult local bread dromedary camels (age 4-8 years) from Al Omran slaughterhouse, Al-Ahsa, Saudi Arabia, at regular monthly intervals for 12 months. All animal sampling methods were carried out according to the animal slaughter regulations and ethical guidelines of the Ministry of Municipality, Rural Affairs, and Housing, Saudi Arabia. The King Faisal University's ethics committee approved the animal protocol (Ref.

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No. KFU-REC-2023-MAY-ETHICS886). Samples were taken from the testes (cranial and caudal parts of testis and rete testis) and epididymis (head, body and tail) for immunohistochemical (IHC) analysis. Tissue samples were fixed in 10% buffered formalin for 36 hours, then were thoroughly washed in phosphate-buffered saline (PBS) dehydrated in graded ethanol and embedded in paraffin wax.

Five micrometre- thick sections were cut from each tissue by a rotary microtome. After that, tissue sections were deparaffinised in xylene, washed in ethanol alcohol and rehydrated in PBS. Antigen retrieval was performed in a microwave oven in 0.01M PBS (pH 7.4) for 15 min. Thereafter, the sections were cooled at 25°C and rewashed in PBS. Endogenous peroxidase was blocked by using 3% hydrogen peroxide for 30 min. After washing in PBS three times, the goat serum (10%) was used for 20 min to avoid non-specific reactions. Then, the primary antibody, polyclonal rabbit anti-AQP1, was applied (Abcam, dilution 1:200, Cambridge, Cambridgeshire, UK) and incubated overnight in a wet chamber. Sections were incubated with biotin-labelled secondary antibodies and avidin-horseradish peroxidase (HRP) third antibodies, Dibutylphthalate polystyrene xylene (DAB) was used to detect the positive staining. Hematoxylin stain was used for section counter-staining. Negative control sections have the same procedure except for skipping the primary antibody. Slides were examined under light microscopy for histological and immunohistological studies, and photo-micrographs were photographed.

Results

AQP1 immunoreactive protein was expressed using immunohistochemistry in the testis and epididymis of the dromedary camel during rutting and non-rutting season for 12 consecutive months. Localisation of AQP1 in different parts of the testis and epididymis during these periods was recognised and recorded in Tables 1 and 2, respectively.

Table 1. Localisation of AQP1 in different parts of the testis during the year.

Part Month	January-March	April-September	October	November-December
Cra	+++	++++	++	++++
Cau	+++	++++	++	++++
Ret	+++	++++	++	++++

Cra, cranial; Cau, caudal; Ret, rete testis; ++, moderate reaction; +++, strong reaction; ++++, very strong reaction

Table 2. Localisation of AQP1 in different parts of the epididymis through the year.

Part Month	January-March	April-May	June	July-September	October-December
Head	++	++++	++++	+++	++
Body	++	+++	++++	+++	+++
Tail	++	++++	+++	+++	+++

++, moderate reaction; +++, strong reaction; ++++, very strong reaction

Testis

During the rutting season from October to March, the cranial and caudal parts of the testicles of the dromedary camel consisted of numerous seminiferous tubules of different shapes and sizes (oval, ovoid and circular). In samples collected during October, the seminiferous tubules were found lined by a thin layer of spermatogenic cells (spermatogonium, spermatocytes, spermatid and spermatozoa) and Sertoli cells. The connective tissue stroma of the testis surrounded the seminiferous tubules and comprised Leydig cells together with some fibroblasts, collagenous fibres, lymphatic vessels and blood capillaries. AQP1 was localised moderately in all parts of the testis, including the capsule, seminiferous tubules, and interstitial tissue (Figs 1a & b). The lining epithelium of the rete testis also showed a moderate reaction to AQ1 (Figs 1c & d).

In samples collected during November and December months, the seminiferous tubules' lining epithelium was found thickened, and the spermatozoa filled the tubules' lumen. AQP1 was detected as very strong immunostaining in a variety of cell types in the cranial and caudal parts of the testis, the spermatogonia and sertoli cells of the seminiferous tubule epithelium, the interstitial cells, the blood vessels in the capsule and parenchyma, as well as in the lining epithelium at the rete testis (Figs 1e & f). For the rest of the season until March, the seminiferous tubules became adjacent and the interstitial connective tissue of the testis was reduced in amount, whereas AQ1 was decreased slightly and expressed strongly in the testicular structures (Figs 1g & h). The testicular sperms reacted positively to AQ1 antibodies during this season (Figs 1e & g).

Outside of the rutting season, from April onwards, the histological anatomy of the testis revealed seminiferous tubules with medium and large diameters with occasional lower density in the interstitial connective tissue between them. There was high to medium lining epithelium in the seminiferous tubules. The AQP1 immunoreactive

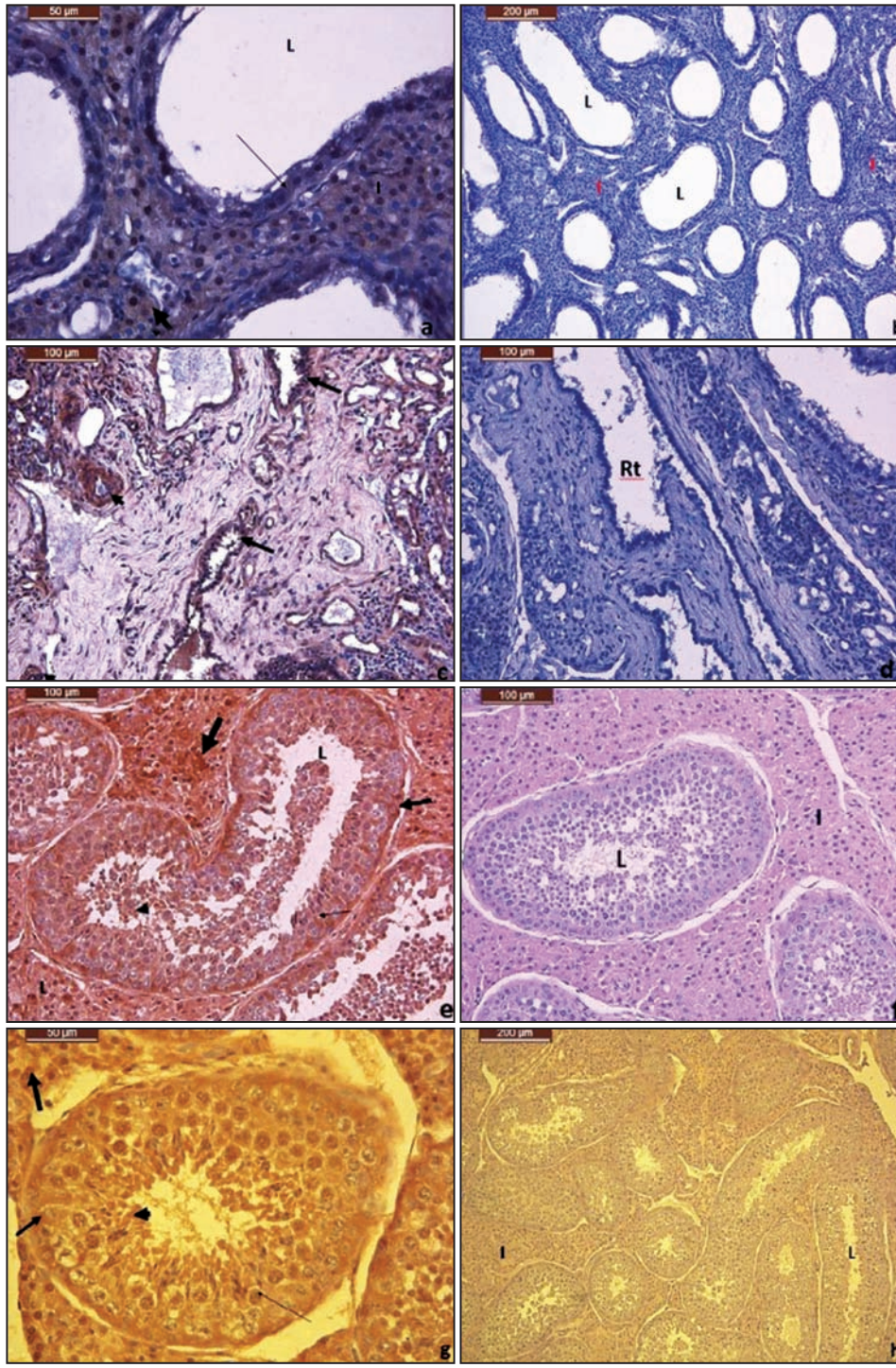

Fig 1. Photomicrograph of the dromedary camel's testis in the rutting season showing: (a) a moderate immunoreactive of AQP1 in the lining epithelium (arrow) of the seminiferous tubules (L) and interstitial cells (head arrow) of the cranial part of the dromedary camel's testis in October, whereas, (b) showing a negative AQ1 the seminiferous tubules of different shapes and sizes lined by a thin layer of spermatogenic cells (arrow) and connective tissue stroma (I). In (c), a moderate immunoreactive of AQP1 in the lining epithelium of the rete testis (arrow) and blood vessel (arrowhead) in October, while, (d) showing negative AQ1 in the rete testis (Rt) in October. In (e), very strong immunostaining of AQP1 was recognised in the spermatogenic cells (thin arrow), Sertoli cells (arrow), sperms (arrowhead) and Interstitial cells (thick arrow) of the cranial part in December, (f) showing a negative AQ1 staining of the lining epithelium of the seminiferous tubules and interstitial cells in the cranial part in December. In (g) strong immunostaining of AQP1 in the spermatogenic cells (thin arrow), Sertoli cells (arrow), sperms (arrowhead) and Interstitial cells (thick arrow) of the caudal part in March, while, (h) shows a negative AQ1 staining of the lining epithelium of the seminiferous tubules and interstitial cells in the caudal part in March.

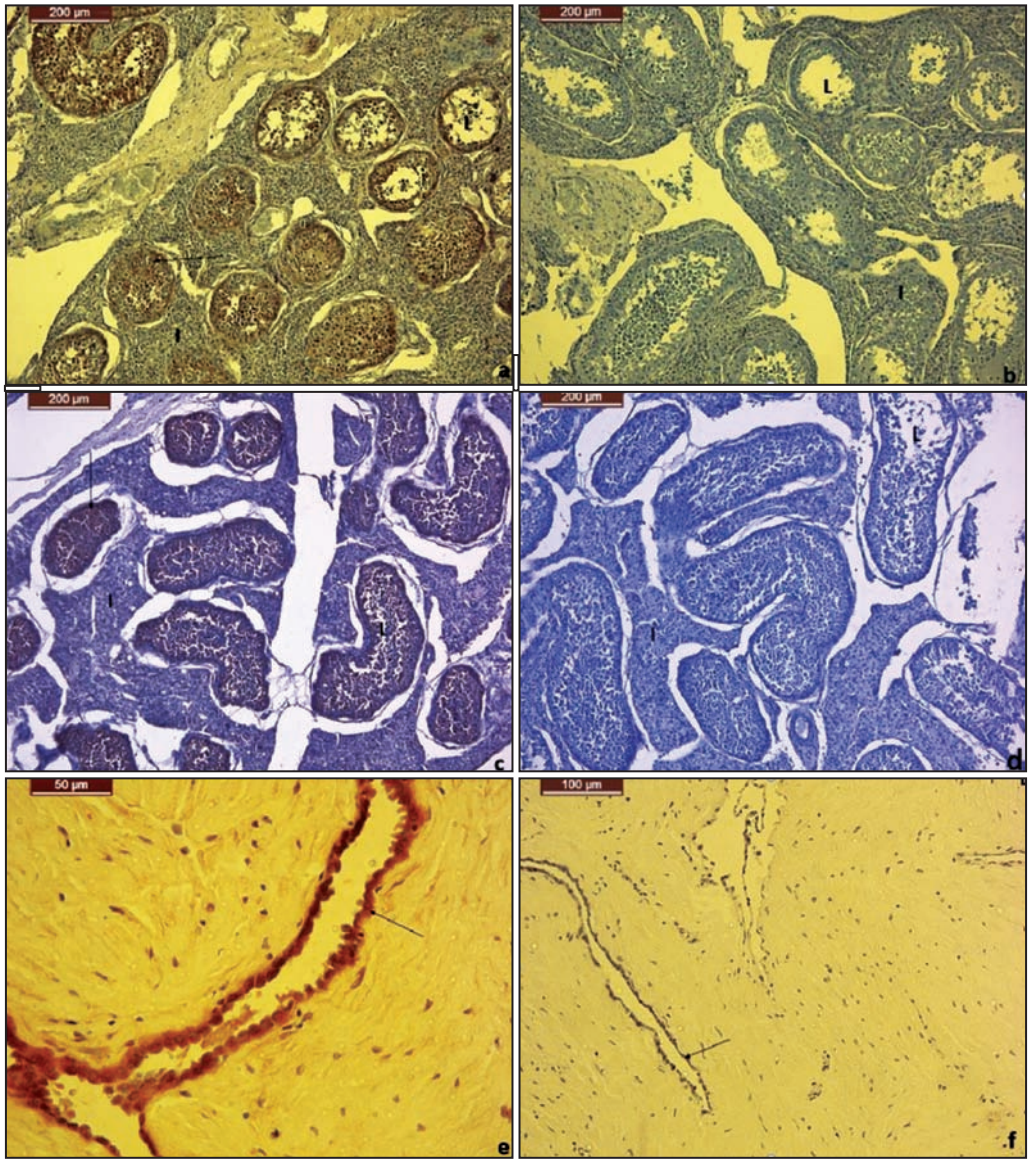

Fig 2. Photomicrograph of the dromedary camel's testis in the non-rutting season showing: (a) very strong immunostaining of AQP1 in the lining epithelium of the seminiferous tubules (thin arrow) in the cranial part in June, whereas, (b) showing seminiferous tubules (L) with medium and large diameters with occasional lower density in the interstitial connective tissue (I) in the cranial part in June. The lining epithelium of the tubules has a negative staining for AQ1. In (c), very strong immunostaining of AQP1 appeared in the lining epithelium of the seminiferous tubules (thin arrow) in the cranial part in August, while in (d), the seminiferous tubules (L) have medium and large diameters with occasional lower density in the interstitial connective tissue (I) in the cranial part in August. The lining epithelium of the tubules has a negative staining for AQ1. In (e), the lining epithelium of the rete testis (arrow) shows a strong immunoreactive of AQP1 in April, while (f) shows negative AQ1 in the rete testis (Rt) in April.

in the spermatogonia, Sertoli cells, Leydig cells, the capsular and interstitial blood vessels and the rete testis epithelial cells revealed very strong reaction (Figs 2 a, b, c & d). The simple cuboidal epithelium lined the rete testis in this period showed strong immunostaining to AQ1 antibodies (Figs 2e & f).

In both seasons, the caudal part of the testis usually showed more immunoreactivity than the cranial part (Figs 3a, 4a).

Epididymis

AQP1 protein was expressed differently on the head, body and tail of the epididymis of dromedary camels in rutting and non-rutting seasons throughout the year (Table 2).

During the rutting season, the convoluted tubules of the epididymal head were large, lined with a stereociliated pseudostratified columnar epithelium and their lumen was filled with a medium amount

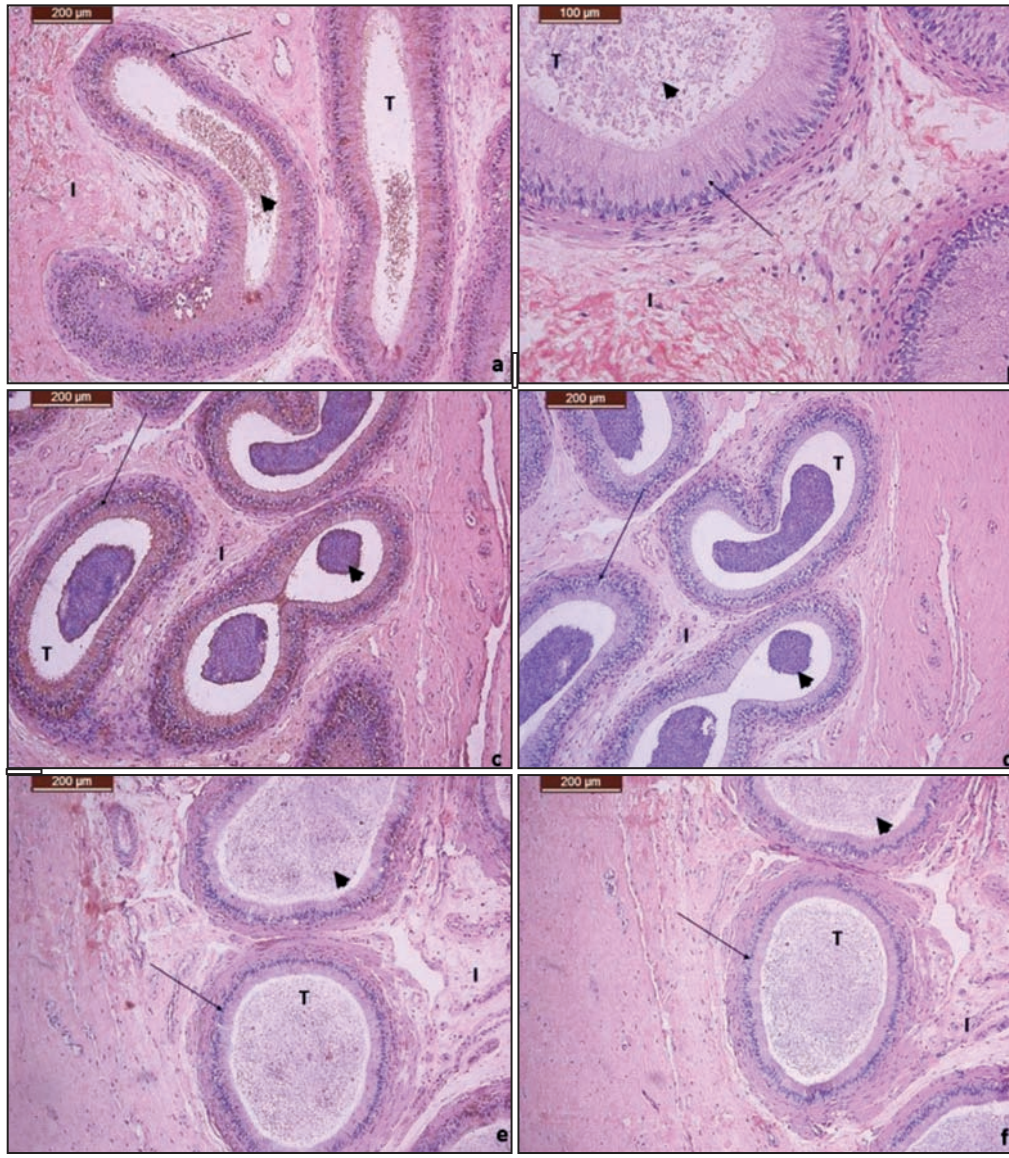

Fig 3. Photomicrograph of the dromedary camel's epididymis in the rutting season showing: (a) a moderate intensity immunoreactive of AQP1 in the stereociliated pseudostratified columnar lining epithelium of the convoluted tubule (T) of the epididymal head (arrow) and epididymal sperm (arrowhead) in November, while in (b), the lining epithelium (thin arrow) of the convoluted tubules of the epididymal head and their sperm (arrowhead) in November have negative staining for AQ1. The interstitial tissue (I) appears between the tubules. In (c), a strong intensity immunoreactive of AQP1 was clarified in the lining epithelium of the epididymal body (arrow) and epididymal sperm (arrowhead) in December, whereas, (d) showing the lining epithelium (thin arrow) of the large convoluted tubules (T) of the epididymal body and their sperm (arrowhead) in December have a negative reaction for AQ1 antibodies. In (e), a moderate intensity immunoreactive of AQP1 showed in the lining epithelium of the epididymal tail (arrow) and epididymal sperm (arrowhead) in February, while (f) showed the lining epithelium (thin arrow) of the large convoluted tubules (T) of the epididymal tail and their sperm (arrowhead) in February have a negative for AQ1 antibodies.

of sperm. There was connective tissue separated between these tubules. Moderate intensity of the immunoreaction of AQP1 was observed in the basilar region of the lining epithelium and the epididymal sperms (Fig 3a & b). At this time of the year, the body and tail displayed only large convoluted tubules, and their lumens were occupied by sperms (Fig 3d & f). AQ1 was strongly expressed in both the lining epithelium and the sperm of these parts between October and December (Fig 3c). While, in the rest period of the season, the immunoreaction became moderate in the epithelium of the three parts and the spermatozoa (Fig 3g).

Over the non- rutting season, the histological structure of all parts of the epididymis revealed that the convoluted tubules became small to medium size,

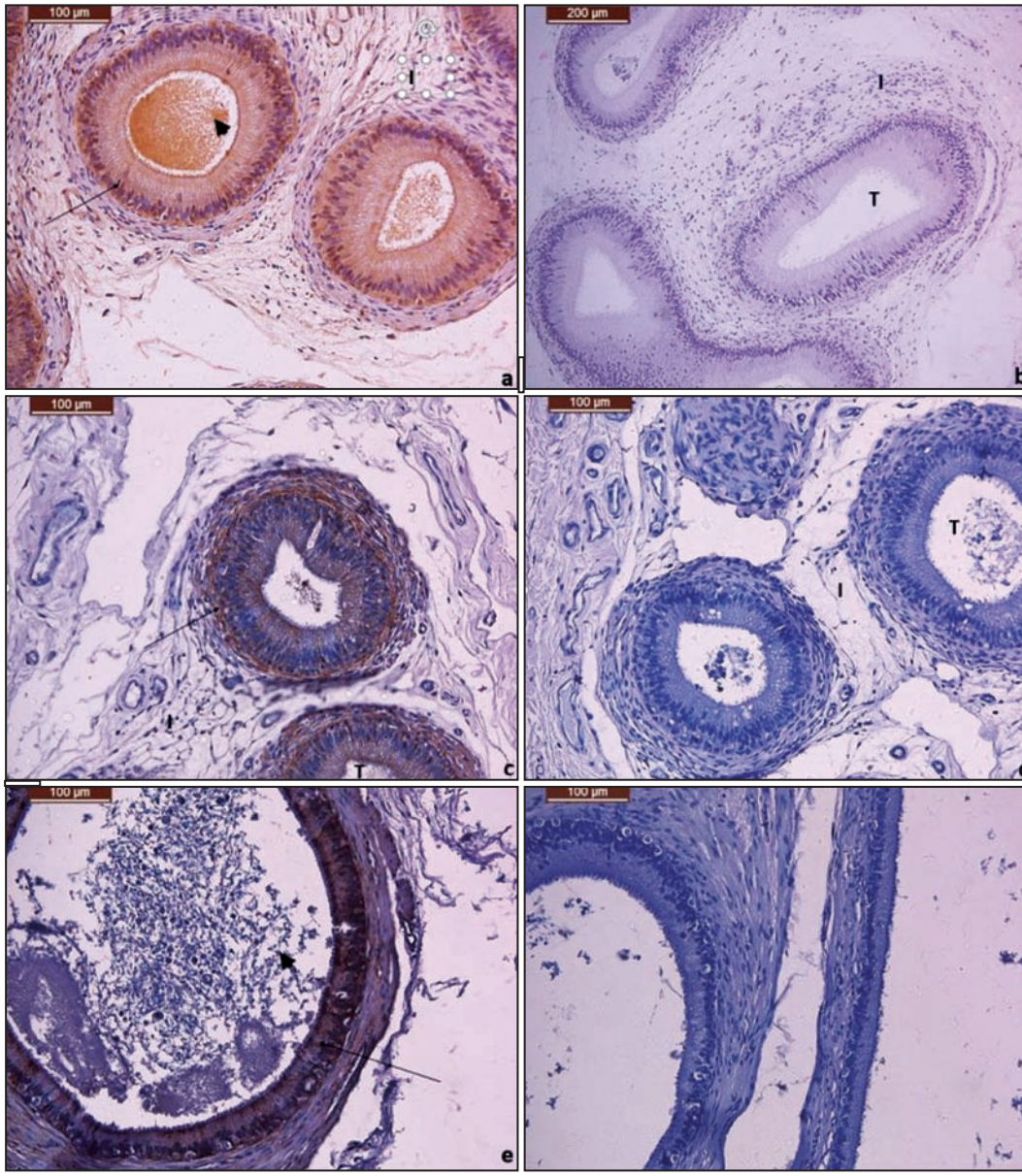

Fig 4. Photomicrograph of the dromedary camel's epididymis in the non-rutting season showing: (a) AQP1 reaction is very strong in the lining epithelial cells of the tubules of the epididymal head (arrow) and epididymal sperm (arrowhead) in April, while, (b) showing the lining epithelium (thin arrow) of the medium convoluted tubules (T) of the epididymal head empty of sperms in April. The epithelium is negative for AQ1 antibodies. In (c) and (e), the body and tail, expressed strong reactions to the protein in the epithelial cells in June and July, respectively. Whereas, the histological structure in (d) and (f) displayed that the convoluted tubules were small to medium size and a spermatozoon occupied some of their lumens.

and their number steadily increased towards the tail. A spermatozoon occupied some of their lumens. The connective tissues between tubules were not dense (Fig 4 b, d&f). The AQP1 reaction was expressed very strongly in the lining epithelial cells of the tubules and the epididymal sperms of the head (Fig 4a) whereas, in the body and tail, the expression of the protein in the epithelial cells was strong (Figs 4c & e). The sperms in the lumen of both previous parts, body and tail, showed a weak affinity to AQP1 (Figs 4c & e).

Discussion

The histological structures of the dromedary's testis in the present study during the rutting season showed variety in the size of the seminiferous tubules and interstitial tissue, which started dense and decreased throughout the season. While during the non-rutting season, the dromedary camel's testes were characterised by many medium to large seminiferous tubules and a decrease in the intensity of the interstitial connective tissue. These

findings throughout the year were confirmed with that mentioned by Tingari *et al* (1984); Singh and Bharadwaj (1978) who reported that the diameter of the seminiferous tubules was smaller with an increase in the volume of the interstitial tissue between winter months than between the summer months.

The immunohistochemical results of this study during the first half of the rutting season, from October to December, AQP1 was found moderately expressed during October. It was increased rapidly to be very strong in various cell types in the cranial and caudal parts of the testis, emphasising the caudal parts and also in the epithelia of the rete testis. The expression became strong in these structures for the rest of the season from January until March. AQ1 was also expressed in the testicular spermatozoa. While throughout the entire period of the non-rutting season, the testicular cells returned to be highly immunoreactive to AQP1.

The presence of AQP1 in the testes of the dromedary camel in this study was comparable to that reported in the Bactrian camel by An and Wang (2016), and other mammals like a dog, fruit-eating bat, horse, cat, buffalo-bull, ram, rat and agouti by Domeniconi *et al* (2008), Oliveira *et al* (2013), Klein *et al* (2013), Arrighi and Aralla (2014), Schimming *et al* (2015), Arrigh *et al* (2016), Ablimit *et al* (2021) and Schimming *et al* (2021), respectively. The pattern of AQP1 distribution in the testis during rutting and non-rutting seasons also reflects the need for water in the testis for sperm production.

In the present study, the histological structure of the convoluted tubules in the caput epididymidis was medium size; the numbers and sizes of the tubules steadily increased towards the cauda epididymidis and filled with spermatozoa at the onset of the rutting season in October onward end of the season. In non-rutting season, the seminiferous tubules become small to medium size with an increase in the number towards the tail and decreased in spermatozoa in their lumens, distributed connective tissue present between these tubules. These observations of regional differences in the epididymis throughout the year were consistent with those of numerous mammalian species (Parillo *et al,* 2009 in alpaca; Hafez *et al,* 2011 and Ibrahim and Singh, 2014 in dromedary). These regional variations are assumed to be related to the functional differentiation along the duct necessary for spermatozoa maturation.

AQP1 is expressed differently in all parts of the epididymis of the dromedary camels. The epithelial cells of the head had a moderate reactivity in the first half of the breeding time, which subsequently developed to become strong towards the tail. In contrast, for the remainder of the rutting season, the lining epithelium and epididymal sperms in all parts of the epididymis displayed a moderate immunoreactive to AQ1. Whereas, throughout the non-breeding time, the epithelial cells and spermatozoa in various regions of the epididymis showed very strong immunoreactive to AQP1.

Our results validated previous studies' findings that AQP1 was present in the epididymis of animals at various levels (Arrighi *et al,* 2010; Hermo and Smith 2011; Klein *et al,* 2013; Arrighi and Aralla 2014; Schimming *et al,* 2015; Lee, 2021).

According to the present findings, AQP1 cellular and sperm distribution varied throughout the year in the testis and epididymis of the dromedary camel, which may be related to diversity in the androgen and oestrogen components over the entire year, as reported in camel by Mohamed *et al* (2018). The seasonal variance investigated by Oliveira *et al* (2013) on a large fruit-eating bat supported the current study distribution. Thus, the present results provide evidence that AQP1 may be significant for spermatozoa differentiation and possibly allow the efflux of water required for cytoplasmic condensation and cell size reduction during the spermiogenic process and which is consistent with AQP1's primary function as a selective water channel. Therefore, AQP1 could one day serve as a distinctive biomarker of male fertility and infertility and would also be a useful indicator of the quality of sperm both before and after semen cryopreservation.

Conflict of interest

None declared

Acknowledgements

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A LEGENDARY CAMEL SCIENTIST- PRIV. DOC. DR. MED VET ULRICH WERNERY – AN APPRAISAL ON HIS 80th BIRTHDAY

Ulrich Wernery was born on the 7th of August 1943 in Marienwerder in western Prussia (today: Kwidzyn in Poland), who later migrated to Schleswig-Holsteinin northern Germany. U. Wernery obtained his doctoral degree in veterinary bacteriology from the Free University of Berlin in 1970. After graduation, he worked in a veterinary diagnostic institution in Neumuenster (Germany) post which he joined as a veterinarian in German Agency for Technical Cooperation in Somalia (from 1974 to 1976). After staying abroad in Papua New Guinea (from 1980 to 1983), in 1987, he became staff member of a small haematological laboratory in Dubai at the age of 45 years. Having gained remarkable practical experience in diagnosing infectious diseases in animals, he convinced the ruler of Dubai to create a diagnostic laboratory for research on infectious diseases of dromedaries, and thus the Central Veterinary Research Laboratory (CVRL) was founded. With his habilitation thesis on bacterial and viral diseases of dromedaries, he became the status of Private-docent at the University of Munich in 1994. A great support to the Journal of Camel Practice and Research (JCPR), since it's inception (1994), was extended by Dr. Wernery. He usually authored one or two research or review manuscripts or short communications in every issue of JCPR. His views and recommendations for every new issue of JCPR as an active member of editorial board, consistently helped us in raising the academic standards of this exclusive scientific journal on camels over the years.

Dr. Wernery, the longstanding director of CVRL is the author and coauthor of more than 500 scientific publications and numerous research presentations at national and international conferences. He is acclaimed for writing several textbooks, including Infectious Diseases of Camelids, Camelid Haematology, Falcon Medicine, and Camelid Infectious Disorders. His research activities are dominated by bacterial and viral diseases, such as brucellosis, glanders, salmonellosis, clostridium, pox and herpesvirus infections in different host species, African horse sickness (AHS), PPR, FMD, influenza, MERS, and others.

Among his other pursuits, he actively engages in research about camel milk, camel nanobodies, and equine grass sickness. He and his team developed veterinary vaccines against a number of diseases, such as camelpox, falcon pox, houbara pox, AHS, Caseous Lymphadenitis (CLA), and Newcastle disease.

The idea for cloning camels was a brainchild of Dr. Wernery at CVRL. It was his vision to establish a modern camel dairy farm in Dubai which became a reality in 2007. At present, this farm is home to more than 8000 dromedaries and produces camel milk and milk products, like milk powder, ice cream, and others.

In 2007/ 08 CVRL was refurnished and a Biosafety Level 3 Laboratory, the first high-security lab in the country was established and a new state-of-the-art necropsy hall was added in 2016. Today the institute consists of nine scientific departments and employs 77 staff members. CVRL is the only accredited laboratory in the country and the region to perform tests for the import and export of horses and pets.

Apart from the other positions Dr. Wernery holds, he is also the WOAH Reference Laboratory expert for glanders, camelpox, brucellosis, and MERS.

As a naturalist, ornithologist and animal lover, Dr. Wernery raised his voice against plastic pollution which led to death of several camels and wildlife by ingesting the littered natural biotopes with plastic waste. 'Plastic is not fantastic' is his motto.

We gratefully acknowledge his exceptional achievements and his friendship and wish him many more years of good health and creative energy. Congratulations on your 80th birthday. You are no longer just a vintage, now you are a classic.

(Compiled by Rolf K. Schuster and Sunitha Joseph, Central Veterinary Research Laboratory, Dubai, United Arab Emirates and Dr T.K. Gahlot, Editor, JCPR)

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ISOLATION AND CHARACTERISATION OF A NITRATE REDUCTASE POSITIVE *Corynebacterium pseudotuberculosis* STRAIN FROM CASEOUS LYMPHADENITIS IN A DROMEDARY CAMEL (*Camelus dromedarius*) FROM INDIA

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ABSTRACT

The present study reports isolation of a nitrate reductase positive strain of *Corynebacterium pseudotuberculosis* from caseous lymphadenitis in a dromedary camel housed in an organised dromedary camel farm located at Bikaner, Rajasthan, India. The bacteria were identified on the basis of biochemical properties as well as PCR based amplification of 16s rDNA, rpoB, plD and narG genes. Phylogenetic analysis using partial sequences of 16s rDNA and rpoB genes revealed its resemblance to equine strains. It was hypothesised that the organism might have originated from the equine herd located in the vicinity of the camel farm. The isolated strain was resistant to penicillin, oxacillin, cloxacillin, amoxicillin/ sulbactam and methicillin but sensitive to amikacin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline hydrochloride, erythromycin, kanamycin, lincomycin, rifampicin, streptomycin, teicoplanin, tetracycline, trimethoprim and vancomycin. The present report suggests that nitrate reductase positive *Corynebacterium pseudotuberculosis* originating from equine or other animal species may cause caseous lymphadenitis in dromedary camel.

Key words: Antimicrobial-resistance, camel, caseous-lymphadenitis, *Corynebacterium pseudotuberculosis,* nitrate reductase

Corynebacterium pseudotuberculosis (CPs) is a Gram-positive, pleomorphic, non-capsulated, non-motile, non-sporulating, facultative anaerobic and intracellular bacterium (Zidan *et al,* 2013; Algammal, 2016). It is reported to cause suppurative lesions in superficial lymph nodes and sub-cutaneous tissues in sheep, goat, horses, cattle and camel (Dorella *et al,* 2006 and Terab *et al,* 2021). CPs has been classified into two biovars or serovars based on their nitrate-reducing ability. Strains isolated from sheep and goat show negative nitrate reduction and are termed biovar Ovis or biovar I; while strains from horse and cattle exhibit positive nitrate reduction and are called biovar Equi or biovar II (Oliveira *et al,* 2016).

In large dromedary herds, enlargement and suppuration of lymph nodes may occur as an outbreak (Afzal *et al,* 1996; Tejedor-Junco *et al,* 2004; Wernery, 2012; Wernery and Kinne, 2016; Ranjan *et al,* 2018). Although, biovar I or biovar Ovis is reported as the most common cause of caseous lymphadenitis in camel, occasional involvement of biovar Equi or biovar II is also reported (Tejedor-Junco *et al,* 2004; Tejedor-Junco *et al,* 2008). Moreover, *Corynebacterium ulcerans* can also cause identical clinical lesions in dromedary camel (Tejedor-Junco *et al,* 2000). Whole-genome sequence of CP strain Cp162, isolated from camel has been done previously (Hassan *et al,* 2012). Though, identification and classification of genus *Corynebacterium* are largely based upon their biochemical characteristics, large variation in biochemical properties has been recorded in different *Corynebacterium* strains isolated from the same host species (Cetinkaya *et al,* 2002). Differentiation between *C. pseudotuberculosis* and *C. ulcerans* on the basis of their biochemical properties is difficult, but can be done accurately on the basis of their genomic analysis and polymerase chain reaction based tools (Heggelund *et al,* 2015). The present study reports isolation of a CPs biovar Equi or biovar II from

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suppurative lymph node of a dromedary camel, identified on the basis of multiplex PCR and its phylogenetic analysis using partial sequences of 16s rDNA and rpoB genes. Antimicrobial sensitivity pattern of the isolate is also reported.

Materials and Methods

Isolation and biochemical characterisation

Creamy to caseated, odourless pus samples were collected from enlarged, suppurated peripheral lymph nodes from a dromedary camel housed at ICAR-National Research Centre on Camel, Bikaner, Rajasthan, India. The samples were inoculated on cystine tellurite blood agar base (HiMedia Laboratories Pvt. Ltd., Nasik, India) supplemented with 5% v/v of 1.0% potassium tellurite and 5% defibrinated sheep blood at 37°C for 48-72 hours. Black coloured colonies appearing on the culture plate was further processed for Gram's staining and biochemical tests to exclude other bacteria like *Staphylococcus* Spp.

After repeated sub-culture, pure colonies were isolated which were further grown in BHI broth (HiMedia Laboratories Pvt. Ltd., Nasik, India) supplemented with 0.4% potassium tellurite. Black coloured colonies appeared in BHI broth as flakes due to clumping of bacterial colonies. Biochemical tests were conducted using Enterobacteriaceae Identification kit and HiStrep identification kit (KB003 and KB005A HiMedia Laboratories Private Ltd., Mumbai, India).

Bacterial DNA isolation

Bacterial genomic DNA was extracted using commercially available kit (Maricon DNA bacteria plus Kit; Cat. No. 69534, Qiagen India Pvt. Ltd., New Delhi). In a 2 ml sterile microcentrifuge tube, 400 µl Fast Lysis Buffer was taken and few isolated bacterial colonies were added to it. The mixture was vortexed for 5 minutes for proper mixing and then transferred to a pathogen lysis tube supplied with the kit. The PLT was placed vertically in a vortex adapter after tightly closing the lid and vortexed at maximum speed for 10 min. The PLT was centrifuged at 4°C at 13,000 X g for 5 min. Thereafter, 100 µl of the supernatant was transferred to a fresh 1.5 ml microcentrifuge tube and directly used as DNA sample for PCR reaction. The DNA purity was checked by electrophoresis on 0.8% agarose gel and stored at -20°C till PCR reaction or maximum up to 3 weeks.

Molecular identification

The genomic identification of *Corynebacterium pseudotuberculosis* was carried out on the basis of amplification of 16S rDNA, rpoB, plD and narG genes using multiplex PCR using primers (synthesised by Europhin Genomics India Pvt. Ltd., Bangalore, India) as listed in Table 1 (Almeida *et al,* 2017).

PCR was carried out in a final volume of 50 µl, containing 5 µl (~20 ng of genomic DNA), 1 µM of each primer (diluted to 10 pM/µl), 12 µl of MilliQ water and 25 µl of Master Mix (Go Taq Hot Start Green Master Mix; Promega Biotech India Private Limited, New Delhi). The Master Mixt-1x PCR assay buffer consisted of $MgCl_2$ (2 mM), dNTP (0.25 mM), 1unit Taq DNA polymerase (0.05 µl) and DNA loading dye. Amplification was performed using the thermal cycler (Prima- 96 Thermal Cycler, HiMedia Laboratories Pvt. Limited, Mumbai) as follows: the first denaturation at 95°C for 4 min; followed by 30 cycles of denaturation at 95°C for 30s, annealing at 58°C for the 30s, and extension at 72°C for 1.5 min. The amplified products were subjected to electrophoresis in 1.0% agarose gel (w/v) in Tris-borate-EDTA (TBE) buffer (89 mM Tris Base, 89 mM Boric Acid and 2 mM EDTA pH 8.0) and stained with 0.5 mg/ mL ethidium bromide. The amplified PCR products were visualised and photographed under UV light (Gel Pro CCD516, Episome Lab Solutions (P) Ltd., Ghaziabad, India). The PCR bands were

Table 1. The sequence of the primer pair used in the multiplex PCR test.

Target gene	Primers	Sequence (5′-3′)	Amplicon size (bp)
16S rDNA	Forward	ACCGCACTTTAGTGTGTGTG	816
	Reverse	TCTCTACGCCGATCTTGTAT	
rpoB	Forward	CGTATGAACATCGGCCAGGT	446
	Reverse	TCCATTTCGCCGAAGCGCTG	
plD	Forward	ATAAGCGTAAGCAGGGAGCA	203
	Reverse	ATCAGCGGTGATTGTCTTCCAGG	
narG	Forward	ACCCGTACTTGCACTCTTTC	612
	Reverse	AGTCAGTACTTCCGCAGGTC	

identified on the basis of their molecular size by comparing with molecular weight marker loaded simultaneously in the gel.

The rpoB gene was amplified separately with slight change in PCR protocol as, initial denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for the 1 min, and extension at 72°C for 2 min (Ilhan, 2013). The PCR product was purified using PCR Clean-Up System (Wizard, SV Gel and PCR Clean-Up System, Promega, USA) and submitted to Biologia Research India Pvt. Ltd., Karnal, Haryana, India for sequencing in both forward and reverse directions using Sanger sequencing technique. The aligned sequence consisting 458 base pairs was submitted to GenBank (Accession No. OK562597).

The partial sequence of 16S rDNA was amplified separately using universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R (5'-GGTTACCTTGTTACGACTT-3'). For PCR, 50µl reaction mixture was subjected to initial denaturation for 5 min at 95°C. Thereafter, 30 cycles each of 30s denaturation step at 94°C, a 45s annealing step at 54°C and 2 min elongation step at 72°C was performed. At last, the final extension step was performed for 5 min at 72°C (Lane, 1991). The PCR product obtained was purified and sequenced. The aligned sequence consisting of 1347 bp was submitted to GenBank (Accession No. MT649220.1).

Phylogenetic analysis

The partial sequences of 16s rDNA and rpoB of the present isolate and other sequences obtained

Table 2. Details of 16s rDNA sequences (downloaded from GenBank) used for phylogenetic analysis.

S. No.	Bacteria	Strain	Host	Country	Accession No.
1	*Coryne pseudotuberculosis*	CD070	Domestic cat	USA	NR_117210.1
2	*Coryne pseudotuberculosis*	NCTC 3450	-	UK	NR_119175.1
3	*Coryne pseudotuberculosis*	E23	-	France	NR_037070.1
4	*Coryne pseudotuberculosis*	DSM7177	Equine (Pectoral muscle)	Germany	GU818733.1
5	*Coryne pseudotuberculosis*	ATCC19410	Sheep	Japan	NR_115562.1
6	*Coryne pseudotuberculosis*	CIP52.97	Equine	USA	X81907.1
7	*Coryne pseudotuberculosis*	CRY1	Dromedary (blood)	UAE	MW276079
8	*Coryne pseudotuberculosis*	879	Sheep (CLA)	Russia	MK965655.1
9	*Coryne ulcerans*	CD 361	Domestic cat	USA	NR_117211.1
10	*Coryne ulcerans*	BRAD 41	Domestic dog	Brazil	KF924749.1
11	*Coryne ulcerans*	MIT07-3331	Ferret (Faeces)	USA	KF564646.1
12	*Coryne ulcerans*	CVUAS 4292	Wild boar	Germany	GU818734.1
13	*Coryne ulcerans*	CD02-59	Domestic cat	UK	AY987817.1

Table 3. Details of rpoB gene sequences (downloaded from GenBank) used for phylogenetic analysis.

S.No.	Bacteria	Strain	Host	Country	Accession No.
1	*Coryne pseudotuberculosis*	CD070	-	UK	GQ409651.1
2	*Coryne pseudotuberculosis*	Elmaani 2	Sheep (lymph node)	Sudan	MG692442.1
3	*Coryne pseudotuberculosis*	CPS-H1	Deer (Pectoral muscle)	Chile	KY560450.1
4	*Coryne pseudotuberculosis*	RA1	Goat (CLA)	Iraq	MT974521.1
5	*Coryne pseudotuberculosis*	DSM7177	Equine (Pectoral abscess)	Germany	GU818740.1
6	*Coryne pseudotuberculosis*	CP162	Camel	UK	CP003652.3
7	*Coryne pseudotuberculosis*	CIP52.97	Horse	Kenya	CP003061.3
8	*Coryne pseudotuberculosis*	258	Horse	Belgium	CP003540.3
9	*Coryne pseudotuberculosis*	31	Buffalo	Egypt	CP003421.4
10	*Coryne pseudotuberculosis*	VD57	Goat (CLA)	Brazil	CP009927.1
11	*Coryne ulcerans*	469-YAMAGATA	Human (Abscess)	Japan	AB751261.1
12	*Coryn eulcerans*	1310100012-1	Water rats	Germany	KM595079.1
13	*Coryne ulcerans*	MIT07-3331	Ferret	USA	KF539859.1
14	*Coryne ulcerans*	BR-AD22	Dog	Brazil	FJ545133.3

from GenBank (Table 2 and 3) were aligned using CLUSTAL W program (Thompson *et al,* 1994) and phylogenetic trees were constructed through Neighbour-joining method with a bootstrap value of 1000 repetitions using Mega X software (Tamura *et al,* 2004). Partial sequences of16s rDNA and rpoB of *Corynebacterium ulcerans* obtained from GenBank was included as the out-group.

Antimicrobial sensitivity profile

Antimicrobial sensitivity profile of the CPs against 20 antibiotics was determined using the discs diffusion method (Doughari *et al,* 2007). Few isolated pure colonies of the organism were transferred into Brain Heart Infusion Broth (HiMedia Laboratories Pvt. Ltd., Nasik, India) added with 0.1% Tween 20 and incubated at 37°C for 24 to 72 hours (Norman *et al,* 2014). The optical density of the organism in the broth at 500 nm was adjusted to 0.5 McFarland (0.08 to 0.1). With the help of a micropipette fitted with sterile tip, 80 µl of broth was spread evenly on the surface of BHI agar plate (supplemented with defibrinated 5% sheep blood). The antibiotic discs were placed and plates were incubated for 24-48 hours at 37°C. The inhibition zone obtained was measured using a vernier caliper.

Results and Discussion

Isolation and biochemical characterisation

Small, black coloured, round colonies appeared on cystine tellurite agar plate. Gram's staining of small colonies revealed presence of Gram positive cocco-bacilli with Chinese letter like appearance. Metachromatic granules were also evident after Albert's staining. Pure culture of CPs was submitted to ICAR-National Centre for Veterinary Type Cultures, Hisar, India (Accession number VTCCBAA1496). The bacterial isolate was catalase and nitrate reductase positive, but oxidase negative. urease and methyl red were positive but negative for esculin hydrolysis. It fermented glucose, but was unable to ferment sucrose, lactose, arabinose, sorbitol and mannitol.

Molecular identification

The extracted bacterial DNA was subjected to PCR assays that targets 16S rDNA, rpoB, plD and narG genes. The product of multiplex PCR after agarose gel (1.0%) electrophoresis and staining with ethidium bromide revealed characteristic bands at 446 bp, 612 bp and 816 bp. However, amplification of plD gene was very low in multiplex PCR, as evident from faint band at 203 bp (Fig 1). Using the same genomic DNA with slightly altered PCR protocol, plD gene amplified well showing clear band at 203 bp (Fig 2).

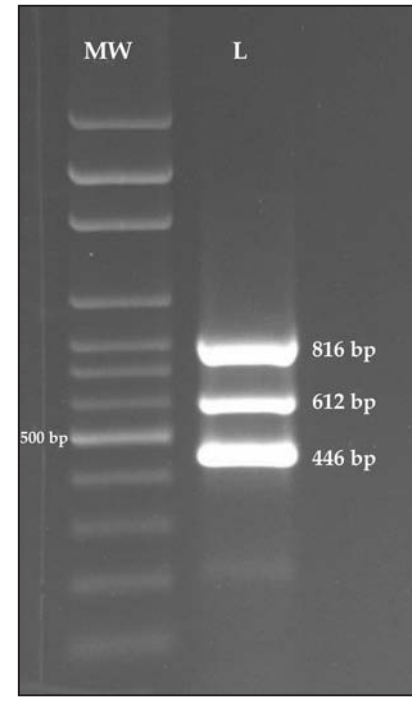

Fig 1. Electrophoretic pattern of multiplex PCR product in 1% agarose gel showing the amplified product at 446 (rpoB gene), 612 (narG gene) and 816 (16s rDNA gene) bp (Lane MW: 100 bp DNA ladder; L: PCR product).

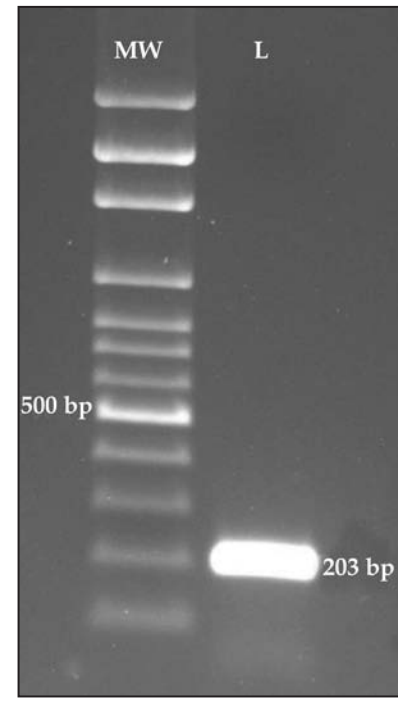

Fig 2. Electrophoretic pattern of PCR product in 1% agarose gel show amplified product at 203 bp (plD gene) (Lane MW: 100 bp DNA ladder; L: PCR product).

Phylogenetic analysis

Phylogenetic analysis of 16s rDNA revealed that the present isolate was closely related to CPs isolated from domestic cat (USA), equine (Germany) and sheep (Japan), while it was distinctly related to CPs isolated from dromedary camel in UAE, sheep in Russia and equine in USA (Fig 3). CPs from dog (Brazil), ferret (USA) and wild boar (Germany) formed separate cluster in the phylogenetic tree.

Phylogenetic analysis of rpoB gene revealed that the present strain was closely related to CPs isolated from equine (Germany and Belgium) and buffalo (Egypt) than CPs isolated from camel in UK and equine in Kenya (Fig 4). CPs from goat (Brazil and Iraq) and sheep (Sudan) and deer (Chile) were also distinctly related to the present isolate. However, *Corynebacterium ulcerans* from water rats (Germany), ferret (USA), human (Japan) and dog (Brazil) formed a different cluster from CPs.

Antimicrobial sensitivity profile

The bacterial isolate was resistant to penicillin, oxacillin, cloxacillin, amoxicillin/ sulbactam and methicillin but sensitive to amikacin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline hydrochloride, erythromycin, kanamycin, lincomycin, rifampicin, streptomycin, teicoplanin, tetracycline, trimethoprim and vancomycin.

CPs have been reported to cause enlargement and suppuration of superficial lymph nodes in camel from various countries across the globe (Tejedor-Junco *et al,* 2004; Wernery, 2012; Wernery and Kinne, 2016; Ranjan *et al,* 2018). It is a demanding organism from a nutritional standpoint, growing well on enriched media such as blood agar, brain heart infusion (BHI) agar or broth or enriched medium with animal blood, serum, yeast extract, tryptone or lactalbumin producing yellowish-white, opaque, flat colonies with a matt surface at 24 to 48 hr incubation (Cameron and Swart 1965; Holt *et al,* 1994; Bastos *et al,*

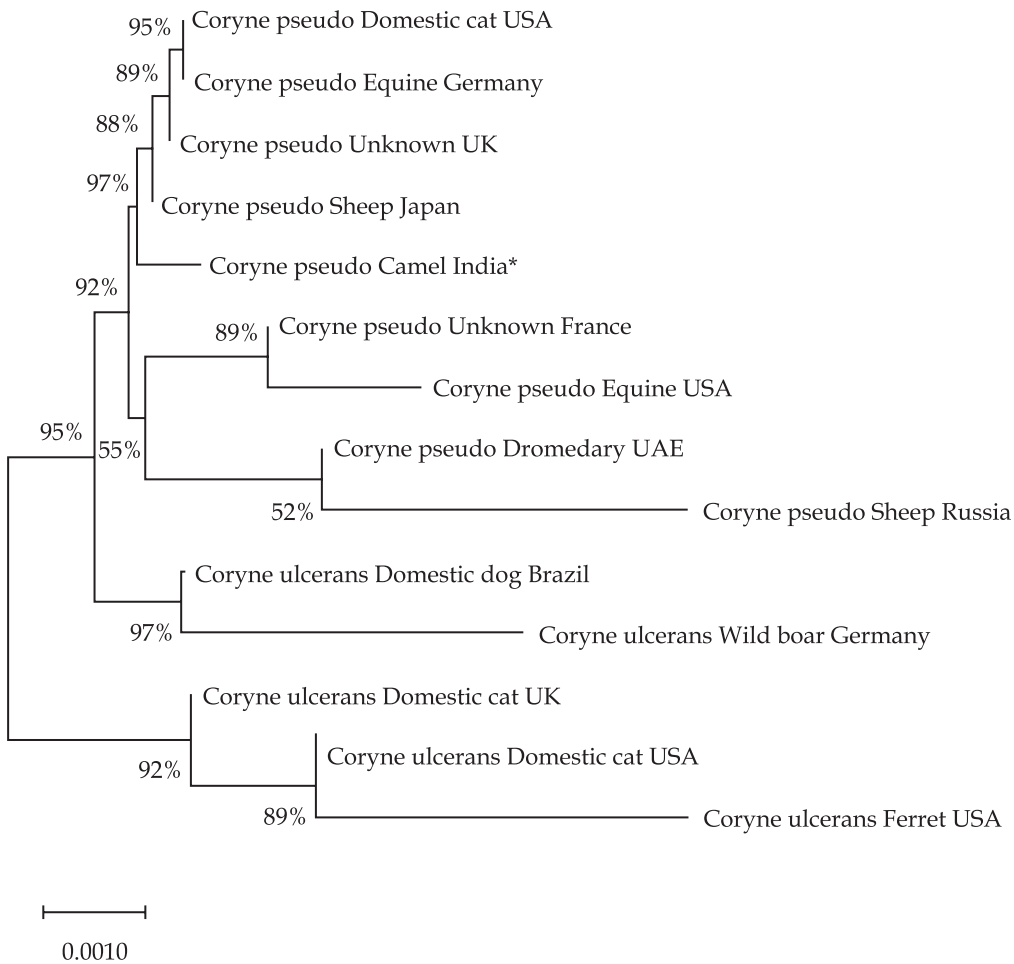

Fig 3. Phylogenetic tree based on partial sequences of 16s rDNA gene of *Corynebacterium pseudotuberculosis* isolated from different livestock species using Neighbour-joining method with a bootstrap value of 1000 repetition. *Corynebacterium ulcerans* was included as the out-group. Horizontal distances are proportional to the genetic distances while vertical distances are arbitrary. Strength of each branch is indicated in the respective node.

Fig 4. Phylogenetic tree based on partial sequences of rpoB gene of *Corynebacterium pseudotuberculosis* isolated from different livestock species using Neighbour-joining method with a bootstrap value of 1000 repetition. *Corynebacterium ulcerans* was included as the out-group. Horizontal distances are proportional to the genetic distances while vertical distances are arbitrary. Strength of each branch is indicated in the respective node.

2012). In the present study, bacterial colonies on blood tellurite agar appeared small, uniformly blackish, low convex with matt surface, which matched to the previously reported colony characteristics of CPs (Holt *et al,* 1994). The growth in BHI broth was also luxurious, which indicated that it is a suitable media for seed culture preparation for antibiotic sensitivity testing.

Nitrate reductase negative CPs are more frequent cause of lymph nodes abscesses in dromedary camel (Radwan *et al,* 1989; Tejedor-Junco *et al,* 2004), though sporadic reports suggest that nitrate reductase positive CPs may also be involved (Tejedor-Junco *et al,*2008; Wernery and Kinne, 2016). The present isolate was nitrate reductase positive that was further confirmed by polymerisation of narG gene in the multiplex PCR. Considering involvement of both strains, it was suggested that CLA-vaccines for camelids should include both nitrate reductase negative and positive strains (Wernery and Kinne, 2016).

Corynebacterium ulcerans can also cause enlargement and suppuration of peripheral lymph nodes in dromedary camel, mimicking those caused by CPs (Tejedor-Junco *et al,* 2000). *Corynebacterium ulcerans* can infect humans and a variety of animal species like cattle, pigs, dogs and cats (Lartigue *et al,* 2005; Wagner *et al,* 2011). Differentiation between *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* using conventional biochemical tests requires due care due to high similarity in their biochemical properties (Tejedor-Junco *et al,* 2000).

Molecular diagnostic techniques like PCR assay has been reported as a reliable test for accurate identification of CPs biovar and its differentiation from *Corynebacterium ulcerans* (Ilhan, 2013; Almeida *et al,* 2017). A multiplex PCR test involving simultaneous amplification of phospholipase D (plD) gene, rpoB gene, narG gene and 16S rDNA gene was tested and found quite effective in accurate identification of *Corynebacterium pseudotuberculosis* by several workers in the recent past (Almeida *et al,* 2017; El-Sebay *et al,* 2021). Genomic analysis of rpoB helped in accurate identification of *C. diptheriae* that were wrongly identified by automated testing system VITEK 2 ANC based on biochemical properties of the organism (Yasmon *et al,* 2020). In the present study, amplification of 16S rDNA, rpoB, plD and narG genes clearly established that the isolated bacteria were nitrate reductase positive *Corynebacterium pseudotuberculosis*. The presence of plD gene indicated that the strain was virulent, as phospholipase D (plD) is the major factor that increases vascular permeability, inactivates complement, inhibit chemotaxis and facilitates dissemination of bacteria inside lymph nodes (Markey *et al,* 2013).

The 16s rDNA gene sequences are the most widely used molecular marker for the bacterial identification and phylogenetic analysis. However, the rpoB gene is more polymorphic than 16s rDNA, hence considered as a better candidate for identification as well as phylogenetic analysis of the species *Corynebacterium* (Khamis *et al,* 2004; Dorella *et al,* 2006). Gene rpoB is involved in many important cellular processes, such as maintenance of cellular integrity, cell survival and several metabolic reactions and is a strong candidate for phylogenetic analysis because it is evolutionarily conserved. The complete rpoB sequence comprises approximately 3500 bp. However, amplification and sequencing of area (with 434 to 452 bp) with a high degree of polymorphism, bordered by conserved sequences may serve as useful tool for identification of *Corynebacterium* species (Khamis *et al,* 2004). In a study on phylogenetic analysis of hyper-variable sequences of rpoB gene, three distinct ovine, caprine and equine strains were found to exist among CPs isolated from domestic animals, though a close genetic relationship was observed between ovine and caprine strains (Retamal *et al,* 2011).

In the present study, phylogenetic analysis using both 16s r DNA and plD genes affirmed that CPs isolate was more closely related to equine strain than CPs isolated from camel in other countries. This may be due to proximity of the equine production centre of ICAR-National Research Centre on Equine, Bikaner with approximate herd strength of 120 equines. The CPs isolated from different animal species is reported to show genetic variability (Oliveira *et al,* 2016). However, limited studies have been conducted to evaluate the genetic variability and presence or absence of virulence genes in *Corynebacterium pseudotuberculosis* isolated from dromedary camel. In a study on camel isolates of CPs applying Pulsed-Field Electrophoresis (PFGE), it was concluded that camel isolates not only differ from isolates of small ruminants, but also from each other thus making herd specific vaccines necessary (Wernery and Kinne, 2016). Therefore, a large-scale study using more camel isolates are required to reach at any conclusion regarding their genetic relationship with ovine and equine strains.

Antimicrobial sensitivity pattern of CPs isolated from dromedary camel is also reported to vary with geographical location of the host (Abubakr *et al,* 1999; Ali *et al,* 2001; Tejedor-Junco *et al,* 2004). The present bacterial isolate was sensitive to most of the common antibiotics including amikacin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline hydrochloride, erythromycin, kanamycin, lincomycin, rifampicin, streptomycin, teicoplanin, tetracycline, trimethoprim and vancomycin. Most of the CPs isolated from different animal species have been reported to be sensitive to penicillins, macrolides, tetracyclines, cephalosporines, lincomycin, chloramphenicol and rifampicin (Judson and Songer, 1991; Algammal, 2016). In a study, 66 isolates of CPs from sheep and goats were tested for their sensitivity to 16 antimicrobial agents. The isolates were highly sensitive to nitrofurantion, chloramphenicol, rifampicin, cotrimoxazole, erythromycin, and ampicillin (Abdel Wahab and Shigidi, 2013). In a study, Abubakr *et al* (1999) reported that all 13 CPs isolates from dromedary camel were sensitive to ampicillin, amoxicillin/clavulanic acid, ceftazidime, clindamycin, erythromycin, tetracycline, gentamicin, ciprofloxacin, enrofloxacin, trimethoprim/suphamethoxazole, vancomycin and rifampicin but resistant to streptomycin. Nevertheless, few reports suggest that under natural infective environment, the bacteria may show résistance to those antibiotics that appears effective under *in vitro* conditions. For example Olson *et al* (2002) grew *C. pseudotuberculosis* as a biofilm, and observed that this bacterial species was highly resistant to all antimicrobials tested under such growth conditions. Multidrug resistant

Corynebacterium spp have been isolated from blood cultures of human patients in China (Qin *et al,* 2017). Thus, regular monitoring of drug sensitivity profile is essential to minimise the emergence of drug resistance problem in CPs.

In conclusion, nitrate reductase positive strains of *Corynebacterium pseudotuberculosis* (CPs) may play an important role in development of caseous lymphadenitis in camel; the risk of transmission increases with presence of equine herds in the vicinity. Cephalosporines, aminoglycosides, tetracyclines, chloramphenicol and rifampicin seems to be more useful in management of CPs infection than penicillins.

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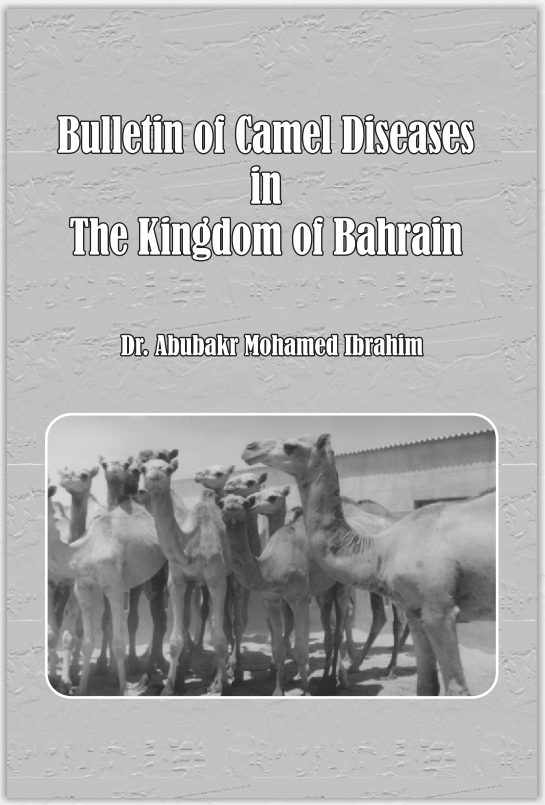
Bulletin of Camel Diseases in The Kingdom of Bahrain

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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HISTOLOGICAL STUDY OF ADRENAL GLAND OF ONE HUMPED CAMEL (*Camelus dromedarius*)

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ABSTRACT

The investigation was carried out on the adrenal glands of 6 recently dead adult camels. Histologically, the adrenals were divided into stroma and parenchyma. Stroma consisted of capsule and trabeculae. Collagen and reticular fibres were present at the capsule and trabeculae. Parenchyma was composed of the cortex and medulla. The cortex was divided into three parts according to their cell arrangement: zona glomerulosa, zona fasciculata, and zona reticularis. The trabeculae entered into the cortex at various distances. The medulla was divided into the inner and outer parts. The outer zone was lined by columnar-shaped cells, and the inner area had polyhedral cells. Patches of the medulla were seen in some cortical areas. The adrenal gland of the camel was surrounded by a thick layer of dense connective tissue fibres, specially collagen and reticular predominance over the elastic and muscle fibres.

Key words: Adrenal gland, fibres, histology, one humped camel

The biological structure of the dromedary camel's adrenal gland is vital in adapting to extreme environments. The adrenal gland is one of the most important organs because it plays a significant role in the body's activities and is essential for maintaining whole life. It regulates many physiological functions both in foetal and postnatal life (Hill, 2007). The adrenal gland is an indispensable organ that forms the hypothalamic pituitary adrenal axis (HPA), the body's stress system. Moreover, the HPA mainly controls cortisol levels and other critical stress-related hormones (Hu and Funder, 2006; Pippal and Fuller, 2008).

The cortex mainly produces aldosterone, cortisol, and androgens responsible for regulating blood pressure, electrolyte balance, glycogen and lipid metabolism, and oestrogen biosynthesis, respectively. In contrast to the direct innervation in the medulla, the cortex is regulated by neuroendocrine hormones secreted from the pituitary gland, which are under the control of the hypothalamus as well as by the renin-angiotensin system.

The study was aimed to investigate the structure of the camel's adrenal glands to justify the body's importance and essentiality. A better knowledge of the morphological norms and the causes of their variations is essential for a better understanding of the physiology and a correct diagnosis and prognosis of the diseases. The detailed description of the adrenal gland of the camel needs to be more explicit. However, the lack of available literature on the mature adrenal gland of the camel has prompted this research work.

Materials and Methods

The adrenal glands were collected from 6 recently died adult camels from VCC, CVAS, Bikaner, Rajasthan. The recommendations of the ethical committee were followed for the present research, and an investigation of the organs was carried out in the Department of Veterinary Anatomy, CVAS, Bikaner. For light microscopic studies, the samples were fixed in 10% formalin for routine staining and in bouin's fluid for special staining for 48 to 72 hours. The tissues were proceeded by the Alcohol-xylene method using cedarwood oil (Thamiselvan *et al,* 2021), paraffin blocks were prepared, numbered, and stored at 4°C in the refrigerator. Sections of 5-6 µm thickness were obtained, placed on albumenised slides, kept overnight in a hot air oven at 36°C, and finally stained for general histomorphological observations.

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Results and Discussion

The adrenal gland of the one-humped camel consisted of two distinguished parts: stroma and parenchyma. The stroma was composed of a capsule and trabeculae. The parenchyma consisted of the outer adrenal cortex and inner medulla (Figs 1 and 2), which was corroborated by the observation of Hullinger (1978) in the dog and Kour *et al* (2017) in Bakerwali goat.

Capsule

The adrenal gland of the camel was enclosed by a thick layer of dense connective tissue fibres, specially collagen and reticular predominance over the elastic and muscle fibres (Figs 3 and 5). The findings were in agreement with the report of Prasad and Yadava (1972) on Indian buffalo, Panchal *et al* (1998) on sheep, Nagpal *et al* (1991) on camel, Baishya *et al* (1998b) on mithun and yak reported similar findings as a predominance of the collagenous fibres over reticular and elastic fibres and Nabipour *et al* (2008) in camel. The finding was in partial harmony with the findings of Olukole *et al* (2016) who observed that collagen fibres were found in the capsule and zona glomerulosa in African giant rats. In disagreement with the present study, Stokoe (1959) found an abundance of elastic fibres in the capsule of sheep adrenal, and Paul *et al* (2016) in the black Bengal goat also found connective tissues in the capsule.

The trabeculae were found extended from the capsule (Figs 4 and 7). The findings of the present study conformed with the reports of Al-Bagdadi (1968) on camel and Nabipour *et al* (2008) on camel, Teixeira *et al* (1993) on African buffalo, Alsadek (2012) on Baladi goats, Paul *et al* (2016) in black Bengal goat and Abass (2017) in the guinea pig. The finding was in partial harmony with the results of Balash and Al-Saffar (1999) who described that the capsule trabeculae entered in zona glomerulosa and zona fasciculata in ox and Sohal and Chaturvedi (1962) found that strong trabeculae entered the deeper zones of the cortex in buffalo.

The adrenal glands were highly vascularised in camel (Nabipour *et al,* 2008). The blood vessels (Figs 8, 9, and 11) were present within the capsule and outside in present study. The finding was in partial harmony with the results of Al-Bagdadi (1968), who stated that the inner layer of the capsule was more richly supplied with blood vessels than the outer layer in camel. Alsadek (2012), found that a supra capsular network of blood vessels was detectable in Baladi goat and Balash and Al-Saffar (1999) found that the capsule of the adrenal gland was richly supplied with blood vessels in ox.

The elastic fibres were present on the walls of blood vessels (Fig 9 and 11). Capsule penetrated the parenchyma forming trabeculae, which constituted the part of mesenchyme, while the trabeculae extended into the cortex to various distances (Fig 1 and 5) and mainly consisted of collagen fibres and reticular fibres. These findings resembled the studies of Sohal and Chaturvedi (1962) and Prasad and Sinha (1981) in buffalo.

The trabeculae reached the zona fasciculata and medulla in some area (Fig 1 and 5). This was in uniformity with the findings of Nama *et al* (2009) in the sheep and Kour *et al* (2017) in Bakerwali goat.

The trabeculae reached up to zona fasciculata, which simulated Al-Bagdadi (1968) finding in camel and Balash and Al-Saffar (1999) in ox. The finding was in partial harmony with the results of Teixeira *et al* (1993) who found that connective tissue trabeculae from the capsule penetrate into the cortex in African buffalo. Nabipour *et al* (2008) stated that the capsule formed the trabeculae and penetrated the cortex in camel. Paul *et al* (2016) revealed that trabeculae originating from the capsule penetrated the cortex and sometimes, the medulla in black Bengal goats and Abass (2017) noticed that this capsule had projections trabeculae into the cortex and medulla as supported by the adrenal gland matrix in guinea pig.

There were clusters of polyhedral cells at the inner side of the capsule area, which formed accessory nodules found in the capsule as well as in the cortical part in zona glomerulosa, which were surrounded by the fibres and cells were similar to zona glomerulosa area (Fig 8). Iskander and Mikhail (1966) in camel, Prasad and Sinha (1980) in goat and sheep, Ahmadpanahi (2007) in Caspian miniature horses, Sanyal *et al* (2005) in goat, Nama *et al* (2009) in sheep and Kour *et al* (2017) in Bakerwali goat, Prasad and Sinha (1980) in dog and Badaway *et al* (1982) also observed the zona glomerulosa cells in the accessory cortical nodule, which was in disagreement with the findings of Nagpal *et al* (1991) who found that there was an arrangement of glomerulosa, fasciculata and reticularis in the cells of a cortical nodule in camel and Jamdar and Ema (1983) stated that there were melanin-containing cells in the capsule of the adrenal gland of goat. These observations were not consistent with the findings of our study.

Cortex

The cortex was the parenchyma's outer part, consisting of three zones, namely zona glomerulosa, zona fasciculata and zona reticularis (Fig 1 and 5). This was in conformity with the observations of Sohal and Chaturvedi (1962) in buffalo, Prasad and Yadava (1972) in buffalo, Ganguly and Ahsan (1978) in goat, Dellman (1993) in ruminants, Svendson *et al* (1998) in mini pig, Yilmaz and Girgin (2005) in porcupine, Sanyal *et al* (2005) in goat, Eroschenko (2008) in ruminants, Nama *et al* (2009) in sheep, Qiu *et al* (2013) in Beagle dog and Nwaogu and Francis (2009) in Kano brown goats. In contrast, Fujioka (1956) in pig, Bacha and Bacha (2000) in ruminants, Ahmadpanahi (2007) in Caspian miniature horses, and Ye *et al* (2017) in the Bactrian camel reported the intermediate zone between zona glomerulosa and zona fasciculata.

Zona glomerulosa

The zona glomerulosa was the outer most part of the cortex and was smallest in size, lying just below the adrenal capsule (Figs 1 and 5). It was similar to the findings of Bacha and Bacha (2000) in ruminants, Sanyal *et al* (2005) in goat and Yilmaz and Girgin (2005) in porcupine.

The cells were mainly cuboidal, but some were low columnar and large and irregular in shape (Fig 13). Similar finding was also reported by Al-Bagdadi (1968) in camel, Sanyal *et al* (2005) in goat, Ganguly and Ahsan (1978) in goat and Bacha and Bacha (2000) in ruminants.

The outermost zone of zona glomerulosa was formed of cell clusters or tufts as an inverted U-shaped formation by the trabeculae (Fig 7), which was in partial harmony with the findings of Alsadek (2012), who found that cuboidal epithelial cells collected in clusters (glomerules) surrounded by delicate connective tissue fibres in Baladi goat. Ye *et al* (2017) revealed that the ovoid cells of zona glomerulosa were arranged in the nest. The cell clusters in this region were almost uniform in Bactrian camel.

Some zona glomerulosa cells were surrounded in the trabeculae (Fig 7). This finding resembled that of Panchal *et al* (1998) in Marwari sheep. The result was in partial harmony with the findings of Sohal and Chaturvedi (1962), who recorded that the strong trabeculae enter the deeper zones of the cortex in buffalo.

Zona fasciculata

Zona fasciculata was the widest zone formed by the radiating, anastomosing arranged cords of polyhedral and columnar cells (Fig 13). The findings were in agreement with the report of Al-Bagdadi (1968) in camel and Baishya *et al* (1998a) in mithun (Bosgrunnies). The finding was in partial harmony with the findings of Dellman (1993) described that the zona fasciculata consisted of radially arranged cords of cuboidal or columnar cells in ruminants. Bacha and Bacha (2000) observed that zona fasciculata was the widest zone of the cortex in ruminants. Sanyal *et al* (2005) observed that the zona fasciculata was the widest zone present in 65% of the area of the cortex in goat. Yilmaz and Girgin (2005) studied that the zona fasciculata was 990µm thick and occupied about half (48.5%) of the adrenal gland in porcupine. Eroschenko (2008) also observed that the intermediate and widest zone was the zona fasciculata where the cells were arranged into narrow cords in ruminants. Nwaogu and Francis (2009) noticed zona fasciculata as the largest zone and cells of the zona fasciculata were arranged in a cord-like pattern in Kano brown goats. Alsadek (2012) found that the zona fasciculata was built of radially orientated cords of cells in Baladi goats. Ye *et al* (2017) revealed that the zona fasciculata was the thickest part of the cortex of the adrenal gland in the Bactrian camel.

The area was arranged in a cord-like arrangement towards the medulla (Fig 13). This conformed with the findings of Nwaogu and Francis (2009) in Kano brown goats. The finding was in partial harmony with the findings of Ganguly and Ahsan (1978) who stated that zona fasciculata cuboidal cells were arranged in parallel cords in goat.

The zona fasciculata layer possessed the capsular fibres from zona glomerulosa. These findings resembled to those recorded by Al-Bagdadi (1968) in camel.

Few fibres also invaded the zone as trabeculae. Reticular fibres were present throughout the layer (Fig 5). The findings agreed with the report of Sohal and Chaturvedi (1962) on buffalo. The result was in partial harmony with the findings of Balash and Al-Saffar (1999), who described that the capsule trabeculae entered in zona fasciculata in ox.

The cells of the upper part of the zona fasciculata were larger and foamier than the cells of the lower part. The area appeared foamy and vesiculated due to the presence of lipid droplets (Figs 13). The result was in partial harmony with the findings of Svendsen *et al* (1998) elucidated that the larger middle zone was the zona fasciculata, which was composed of large rectangular cells, arranged

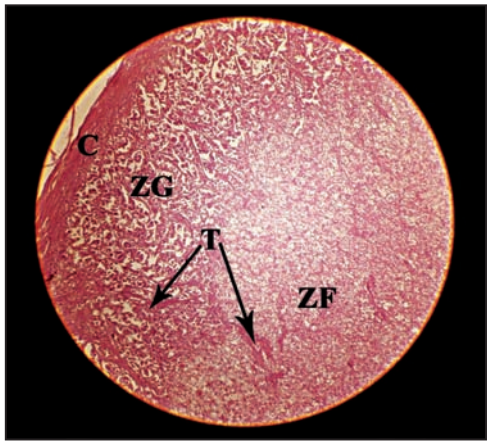

Fig 1. Photomicrograph of adrenal gland of camel showing C – Capsule, ZG – Zona Glomerulosa, ZF – Zona Fasciculata and T- Trabeculae. (H & E Stain, 100X).

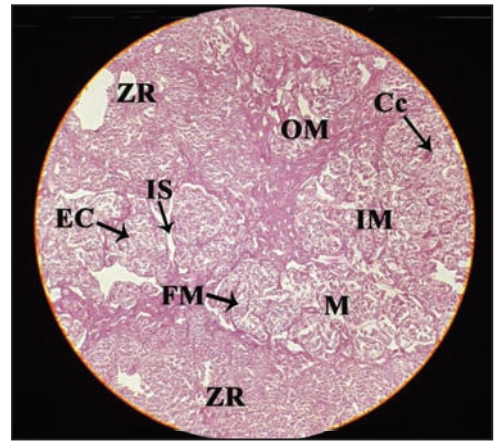

Fig 2. Photomicrograph of adrenal gland of camel showing of the cortex and medulla zones. ZR – Zona Reticularis, EC – Epitheloid cells, IS – Irregular Sinusoids, FM – Follicular Manner, M – Medulla, OM – Outer Medulla, Cc – Columnar shaped cells, IM – Inner Medulla. (H & E Stain, 100X).

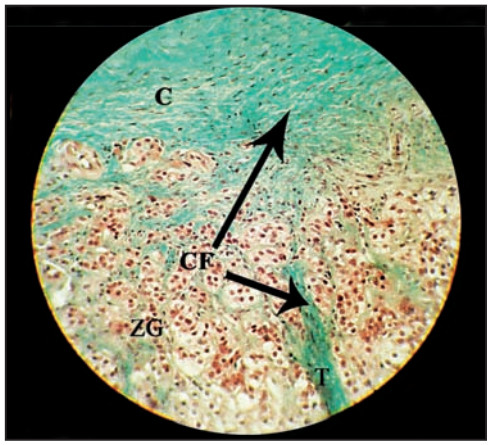

Fig 3. Photomicrograph of adrenal gland of camel showing the collagen fibres in the capsule and cortical zones. C – Capsule, CF – Collagen Fibres, ZG – Zona Glomerulosa, T – Trabeculae. (Masson's trichrome stain, 400X).

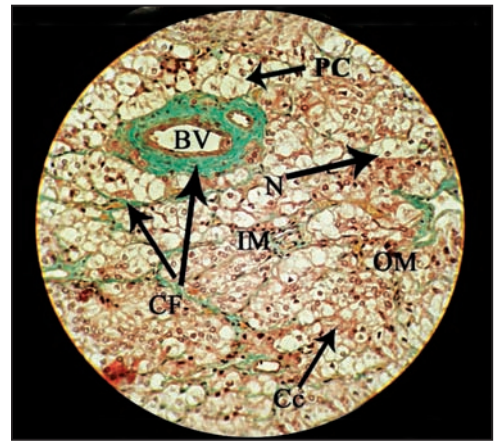

Fig 4. Photomicrograph of adrenal gland of camel showing the collagen fibres in medullary zones, CF – Collagen Fibres, N – Nucleus, Cc – Columnar Cells, BV – Blood vessels, OM – Outer Medulla, IN – Inner Medulla, PC – Polyhedral Cells. (Masson's trichrome stain, 400X)

in vertical columns 2 to 3 cells wide separated by capillaries. These cells had extensive lipid vacuoles in mini pig, and Yilmaz and Girgin (2005) studied that the zona fasciculata appeared foamy because of numerous lipid vacuoles in porcupine.

Zona reticularis

The zona reticularis was the innermost and deepest part of the cortex (Fig 2). This was in uniformity with the findings of Bacha and Bacha (2000) in pig and horse, Eroschenko (2008) in ruminants, Alsadek (2012) in Baladi goats and Ye *et al* (2017) in Bactrian camel.

The cells of reticularis were irregularly arranged network as anastomosing cords (Fig 8). These observations were in close harmony with the findings of Ganguly and Ahsan (1978) in goat, Dellman (1993) in ruminants, Ye *et al* (2017) in Bactrian camel and Baishya *et al* (1998a) in mithun (Bosgrunnies). The finding was in partial harmony with the results of Al-Bagdadi (1968) who stated that the zona reticularis consisted of cuboidal to polyhedral cells anastomosing with each other to form cell cords in bovine, Yilmaz and Girgin (2005) studied that the zona reticularis appeared as irregular cords in porcupine. Eroschenko (2008) evaluated zona reticularis, in which the cells were arranged into groups of branching cords and clumps in ruminants. Nwaogu and Francis (2009) noticed that the cells of the zona reticularis were arranged in oval clusters and

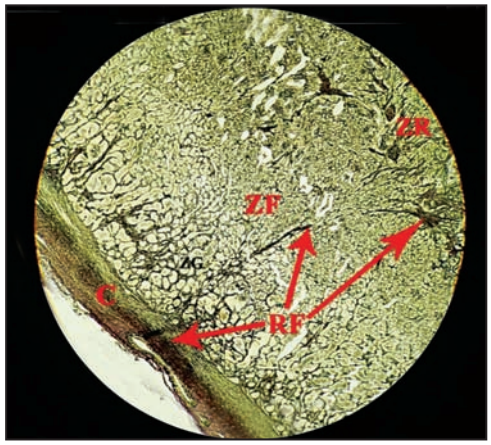

Fig 5. Photomicrograph of adrenal gland of camel showing the reticular fibres in the zones of the capsule and cortex. C – Capsule, ZF – Zona Fasciculata, ZG – Zona Glomerulosa, ZR – Zona Reticularis, RF – Reticularis fibres. (Gomori's stain for reticular fibres, 100X).

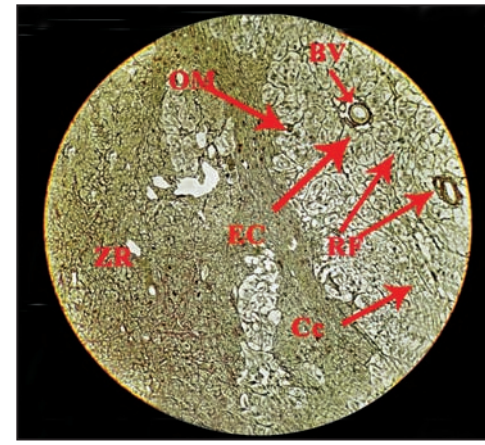

Fig 6. Photomicrograph of adrenal gland of camel showing the reticular fibres in the zona reticularis and medulla. BV – Blood Vessels, RF – Reticular Fibres, ZR – Zona Reticularis, Cc – Columnar cells, OM – Outer Medulla, EC – Epitheloid cells. (Gomori's stain for reticular fibres, 400X).

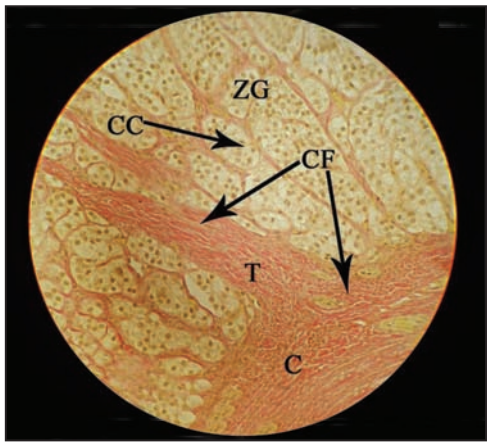

Fig 7. Photomicrograph of adrenal gland of the camel showing the collagen fibres in the capsule and the zona glomerulosa. C – Capsule, T – Tufts, CF – Collagen Fibres, CC – Cuboidal Cells, ZG – Zona Glomerulosa. (Van Gieson's stain, 400X).

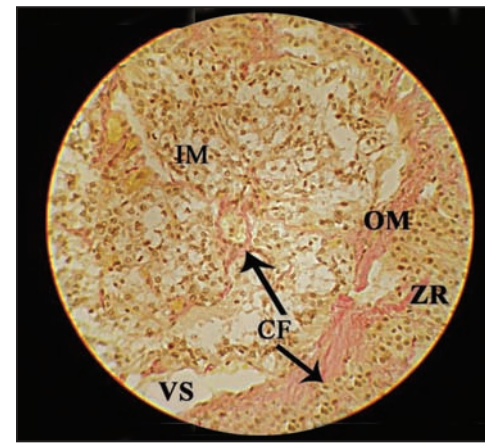

Fig 8. Photomicrograph of adrenal gland of the camel showing the collagen fibres in the zona reticularis and the medulla. CF – Collagen Fibres, OM – Outer Medulla, IM – Inner Medulla, ZR – Zona Reticularis, VS – Vascular Space. (Van Gieson's stain, 400X).

also as an irregular network of cords in Kano brown goats, and Alsadek (2012) found that zona reticularis consisted of interconnecting, irregular cords of small Baladi goats.

There was no clear distinct division between zona fasciculata and the zona reticularis (Fig 1 and 5). The zona fasciculata cells were changed in the network-like arrangement and became reticularis zone (Fig 8). The finding was in partial harmony with the findings of Hullinger (1978) stated that the zona reticularis could be delineated by its tissue organisation in dog.

The cells of the zona reticularis were invaded in the medulla. Some cells of the medulla were also found in this zone (Fig 2). The cells were cuboidal and polyhedral in shape. The finding was in partial harmony with the findings of Al-Bagdadi (1968) stated that the zona reticularis consisted of cuboidal to polyhedral cells anastomosing with each other to form cell cords in bovine. Dellman (1993) described that the cells were polyhedral in ruminants. Sanyal *et al* (2005) observed that the zona reticularis comprised cuboidal cells in goat, and Ye *et al* (2017) revealed that the cells of zona reticularis were polyhedral in Bactrian camel.

The zona reticularis was the second largest zone of the cortex. The cords and plates were divided by irregular large sinusoids at some parts (Figs 1, 2, and 5). Similar findings were reported by Al-Bagdadi (1968) in bovine and Alsadek (2012) in Baladi goats.

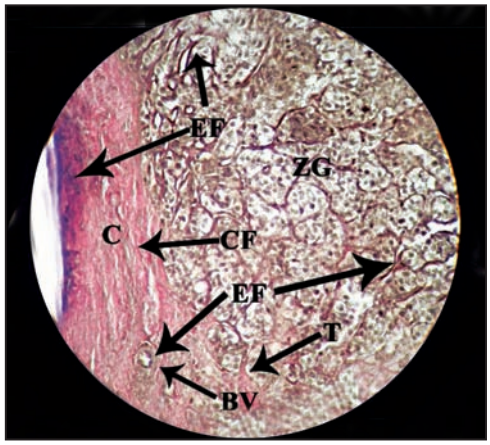

Fig 9. Photomicrograph of adrenal gland of the camel showing the fibres in thecapsule and the cortex zones. C – Capsule, ZG – Zona Glomerulosa, EF – ElasticFibres, CF – Collagen Fibres, BV – Blood Vessels, T – Trabeculae. (Weigert's stain, 400X).

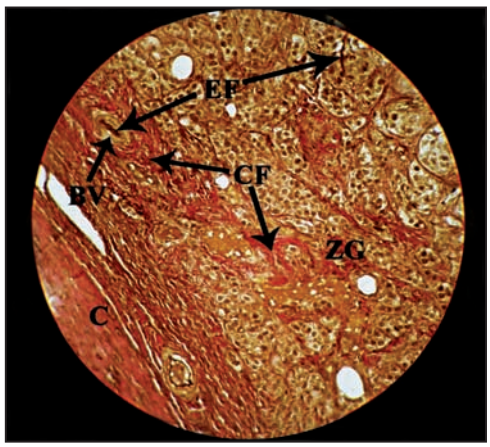

Fig 11. Photomicrograph of adrenal gland of thecamel showing the collagen & elastic fibres in thecapsule and cortex zones. C – Capsule, CF – Collagen Fibres, EF – Elastic Fibres, BV – Blood Vessels, ZG – Zona Glomerulosa. (Verhoeff's stain, 400X).

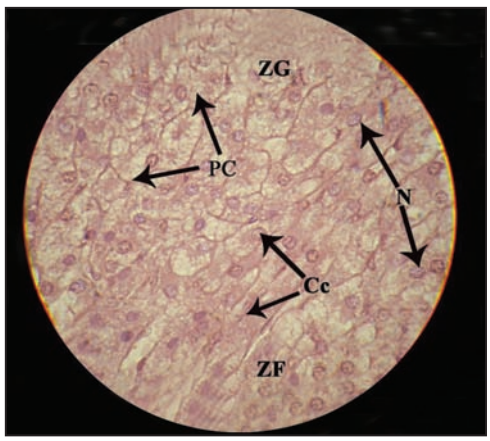

Fig 13. Photomicrograph of adrenal gland of thecamel showing the cells of zona glomerulosa and fasciculata zones. PC – Polyhedral Cells, N – Nucleus, Cc – Columnar cells, ZG – Zona Glomerulosa, ZF – Zona Fasciculata. (H & E stain, 1000X).

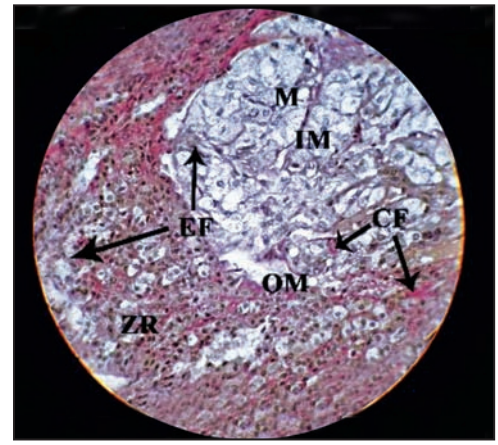

Fig 10. Photomicrograph of adrenal gland of thecamel showing the fibres in thecortex and medulla zones. ZR – Zona Reticularis, M – Medulla, OM – Outer Medulla, IM – Inner Medulla, CF – Collagen fibres, EF – Elastic Fibres. (Weigert's stain, 400X).

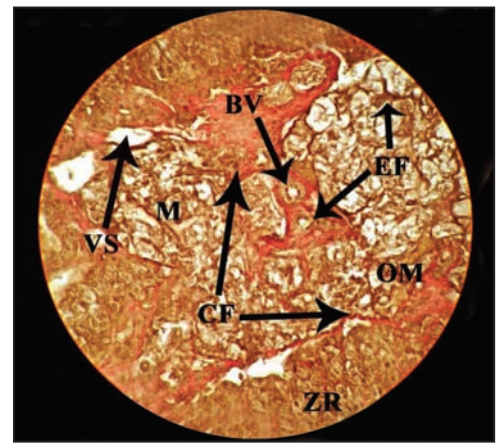

Fig 12. Photomicrograph of adrenal gland of thecamel showing the collagen & elastic fibres in thezona reticularis and medulla zones. M – Medulla, BV – Blood Vessels, CF – Collagen Fibres,EF – Elastic Fibres, OM – Outer Medulla, VS – Vascular Space. (Verhoeff's stain, 400X).

Reticular and collagen fibres were seen in this zone (Fig 5, 6, 8, 10 and 12). The finding was in partial harmony with the findings of Al-Bagdadi (1968) who found that fibrous connective tissue runs into the cortex between the individual glomerulosa and the columns of the fasciculata, and these were also distributed in the zona reticularis in camel. Fujioka (1956) observed that the zona reticularis contains the most plentiful connective tissue fibres in pig. Yilmaz and Girgin (2005) studied that collagen fibres appeared in intercellular spaces in porcupine. This was in disagreement with the findings of Olukole *et al* (2016) who noticed that collagen fibres were absent in zona reticularis in African giant rat.

Medulla

The medulla cells were polyhedral (cuboidal to columnar), arranged in nests or short cords (Fig

2), which simulated the finding of Al-Bagdadi (1968) in camel, Esther (1978) in swine and Ye *et al* (2017) in camel. The result was in partial harmony with the findings of Krumery and Buss (1969) who found irregular groups of columnar epithelial cells in the medulla in African elephants. Gelberg *et al* (1979) examined that packs of these round to polygonal cells occasionally were seen in the medulla of horse. Qiu *et al* (2013) stated that chromaffin cells in the medulla were loosely arranged in clusters and cords in Beagle dog, and Abass (2017) noticed that chromaffin cells were columnar in guinea pig.

Abundant blood vessels, sinusoids, and a small amount of connective tissue are distributed in the medulla (Fig 4 and 6). The findings of the present study were in conformity with the reports of Al-Bagdadi (1968) on camel and Ye *et al* (2017) on camel.

There was the irregular boundary between the zona reticularis and medulla (Fig 2) which was in close agreement with the reports of Al-Bagdadi (1968) in camel and Ye *et al* (2017) in camel. The present study contradicted the observation of Prasad and Yadava (1972), who opined that there was a cortico medullary junction distinguished sharply by the presence of reticular fibres in Indian buffalo. Prasad and Yadava (1973) found a distinct boundary between cortex and medulla in Indian buffalo and Fazekaz (1996) found that medulla was clearly demarcated from the cortex with a distinct border in antelope.

The medulla was a follicular manner arranged area present at the centre of the adrenal gland (Figs 2, 6, and 12). This was in conformity with the findings of Smollich (1967) in cattle and Prasad and Yadava (1973) in Indian buffalo.

The medulla comprised epitheloid cells called chromaffin cells, which were irregular and pale staining medullary granules. Chromaffin cells formed anastomosing strands, separated by sinusoids. A single layer of endothelial cells lined these sinusoids. The findings agreed with the report of Al-Bagdadi (1968) on camel and Kour *et al* (2017) on Bakerwali goat. The finding was in partial harmony with the findings of Holmes (1961) who reported that the cells of the medulla appear large and pale-staining, with granular cytoplasm in ferret, and Shehan *et al* (2017) observed that the adrenal medulla contained a large amount of blood sinusoidal among the cells in local Iraqi goat.

The reticular fibres bound the chromaffin cells (Fig 6). These cells were in the medullary outer and inner zones. These were columnar-shaped cells with granular cytoplasm (Fig 2). The findings agreed with the report of Al-Bagdadi (1968) on camel. The result was in partial harmony with the findings of Krumery and Buss (1969), who described irregular groups of columnar epithelial cells in the adrenal medulla in African elephants.

The medulla was separated from the cortex by connective tissue, and the medulla was extended into the cortex in some areas (Figs 2, 6, 8, 10 and 12). Patches of the medulla were present in some areas of the cortex. These findings of the present study were in conformity with the reports of Al-Bagdadi (1968) in camel and Abdalla and Ali (1989) in camel. The finding was in partial harmony with the findings of Prasad and Yadava (1972) who investigated that the cortico medullary junction is distinguished sharply by the presence of reticular fibres in Indian buffalo.

There were 2 or 3 central veins present in the medulla (Fig 6). The lumen of central veins was lined by endothelial cells (Fig 6). The finding was in partial harmony with the findings of Al-Bagdadi (1968) in camel and Prasad and Yadava (1973) referred to the presence of central veins in the medulla in Indian buffalo.

Outer medulla

The outer zone was lined by columnar-shaped cells, and inner area had polyhedral cells with apical nuclei (Fig 13). which was in close agreement with the reports Al-Bagdadi (1968) on camel, Gelberg *et al* (1979) in horse, Panchal *et al* (1998) on Marwari sheep and Shehan *et al* (2017) on local Iraqi goat. The finding was in partial harmony with the findings of Esther (1978), who mentioned that columnar cells predominantly situated at a cortico-medullary junction in swine, and Jelinek and Konecny (2010) noticed that columnar cells were located in the superficial zone of the adrenal medulla in cattle.

There were collagen and reticular fibres in the medulla (Fig 4, 6, 8 and 12). The outer medulla was in the peripheral part of the medulla (Fig 6) with sinusoids clearly demarcated (Fig 2). There were numerous large vascular spaces (Fig 8 and 12). The findings of the present study were in conformity with the reports of Al-Bagdadi (1968) in camel. The finding was in partial harmony with the findings of Prasad and Yadava (1972), who investigated that the zona reticularis, along with the collagenous fibres bundles, surrounded each group of medullary cells in Indian buffalo. Gelberg *et al* (1979) reported that there were also numerous large vascular spaces in horse, which was in disagreement with the findings of Olukole *et*

al (2016), who noticed that collagen fibres were absent in zona reticularis in African giant rat.

Inner medulla

The central medullary region contained smaller, round to polygonal cells with less densely stained, finely granular cytoplasm. These cells had central nuclei (Fig 2 and 4). The findings were in agreement with the report of Al-Bagdadi (1968) on camel, Gelberg *et al* (1979) on horse, Baishya *et al* (1998b) on mithun and yak, Panchal *et al* (1998) on Marwari sheep and Shehan *et al* (2017) in local Iraqi goat. The finding was in partial harmony with the findings of Jelinek and Konecny (2010), who noticed that small cuboidal cells containing light cytoplasm were found in the inner medulla of cattle.

The chromaffin cells were more prominently seen in this area. There were collagen fibres in the inner medulla (Fig 4 and 12). The polyhedral cells were arranged around the central vein (Fig 4). The findings were in agreement with the report of Qiu *et al* (2013) on Beagle dog. The finding was in partial harmony with the findings of Al-Bagdadi (1968), who found that mainly collagenous fibres in the inner medulla of the camel, Krumery and Buss (1969), who described irregular groups of columnar epithelial cells in the adrenal medulla in the African elephant, Prasad and Yadava (1972) who reported on the collagenous fibres bundles surrounded each group of medullary cells in the Indian buffalo. Esther (1978) studied that compact clusters of polygonal norepinephrine cells were present in between the large medullary veins in swine and Baishya *et al* (1998b) found that the cells of the inner medulla were located around the central vein, made up of clusters in mithun and yak, which disagreed with the findings of Olukole *et al* (2016) who noticed that collagen fibres were absent in zona reticularis in African giant rat.

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REVIEW

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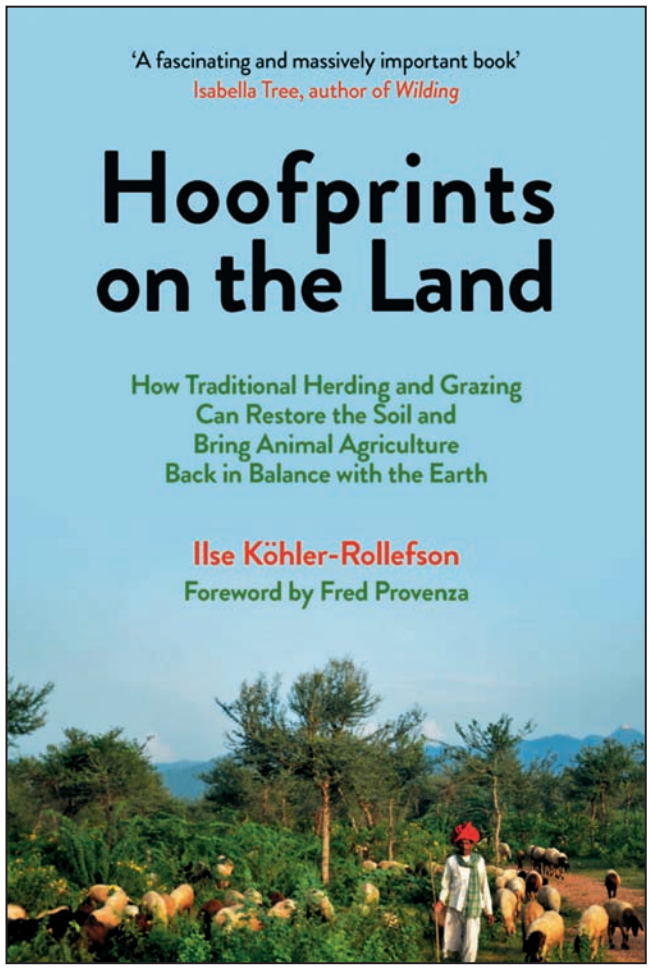

A unique book authored by Ilse Kohler-Rollefson is all about traditional herding and grazing, which can restore the soil and bring animal agriculture back in balance with the Earth. This book shows that herding cultures are not a thing of the past but a regenerative model for our future.

The book contains three parts, i.e., part one- The Herding Universe, part two- Herding Therapies, and part three- Why Herding is Future. It is a fascinating and lyrical book exploring the deep and ancient working partnerships between people and animals. The author writes a passionate rallying cry for those invisible and forgotten herding cultures that exist worldwide and how we can help restore and regenerate the Earth by embracing these traditional nomadic practices. The author has spent over three decades living with and studying the Raika camel herders in Rajasthan, India. She shows how pastoralists can address many of the problems humanity faces. The author indicates that free-range animals and animals in pastoral cultures are a solution to climate change. The book explains the vital role of pastoralists concerning the health of the planet and its grazing animals. This book highlights the critical role of nomadic herding in securing the future of people in drylands, which helps in environmental management in a big way. This is a passionate, important book recommended for those interested in ecology, food, or the coexistence of man with domestic animals, especially ruminant or pseudo-ruminant species.

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CAMEL'S KEFIR MILK: OPTIMISATION OF PROCESSING CONDITIONS

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ABSTRACT

This research was aimed to optimise an experimental process to transform camel milk from semi-intensive rearing system to a fermented milk named "kefir". Three doses (2%, 5% and 10%) of kefir grains, three incubation stages (18h, 20h and 24h) were tested at a fixed temperature 25°C. The physiochemical and microbiological quality of raw camel milk, Kefir grains and their fermented product were determined. In parallel, the effect inoculation dose and incubation time were studied to determine the necessary conditions for the manufacture of camel's kefir. Results showed that camel milk from semi-intensive rearing system had 104.76 g/l total solids, 24.2 g/l proteins, 32.71 g/l fat, 8.63 g/l ash, 41.23 g/l lactose, 15.77 °D acidity and a pH of 6.52. The microbiological analysis (cfu / ml) showed a low load in aerobic bacteria [7.19(10^3)], Coliforms [1.61(10^3)], lactic acid bacteria [3.98(10^4)] and Yeast and mold [3.9(10^2)]. A significant effect of the grain dose on the pH as well as the acidity of the obtained Kefirs was revealed. It is noted that the camel kefir with a dose of 10% had the highest acidity (97. 35±2.9^a °D, pH 4. 03±0.16^c) and the highest viscosity (58. 67±2. 4). However, the incubation time had no significant effect on the physicochemical quality of the obtained kefirs. According to the *Codex Alimentarius* (Codex Stan 243-2003) and sensorial tests, a typical camel's kefir milk from semi-intensive rearing system required 2% kefir grains and t18h of incubation time.

Key words: camel milk, kefir, optimisation

Fermented milks have been developed around the world to preserve, enhance shelf life and improve the flavour of milk (Konuspayeva *et al,* 2023). They became an important part of the diet in many cultures and over time fermentation has been associated with many health benefits (Maoloni *et al,* 2020). Many researchers claimed their nutritional benefits (Nevin *et al,* 2019). Among these products, kefir, a fermented milk of Caucasian origin, owes its specific taste. It differs from other fermented products because it is produced from kefir grains that comprise a specific and complex mixture of lactic acid- and acetic acid-producing bacteria and lactose-fermenting and non-fermenting yeast, which live in a symbiotic association (Lopitz-Otsoa *et al,* 2006). Currently, an increase in kefir consumption in many countries has been reported, due to its unique sensory properties associated with beneficial effects on human health (Otles and Cagindi, 2003; Farnworth, 2005; Tamime, 2006; Farag *et al,* 2020). The benefits of consuming kefir in the diet are numerous, as it is reported to possess an antibacterial, immunological, antitumoural and hypocholesterolemic effects (Irigoyen *et al,* 2005).

In Tunisia, the knowledge of this beverage, its benefits, as well as its manufacturing process are not very widespread. It can be made from any type of milk, such as goat, sheep and cow. The use of camel milk is more limited than milk from other domestic animal species; because it is less suitable for processing, despite its nutritional and health effect (Muthukumaran *et al,* 2022; Marete *et al,* 2023). The nutritional composition of kefir varies widely and is influenced by milk type and composition, the origin and composition of the used grains the time/temperature of fermentation, size of inoculating starters and their heat treatment influencing their metabolic activities. Therefore, the present study was focused on the valorisation of camel milk and its dairy products such as Kefir. It was also aimed to optimise its manufacturing diagram according to the *Codex Alimentarius* (Codex Stan 243-2003) and the sensorial evaluation.

Materials and Methods

Camel milk was collected from a semi-intensive farm belonging to the Livestock and Wildlife

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laboratory of the Arid Land Institute (IRA), Medenine, Tunisia. The camel diet was based on natural feedstuff from rangelands.

Plants grazed by camels rearing in the semi-intensive system had total crude protein (from 4.84 to 19.83%) and parietal compounds i.e; NDF (from 33.89 to 81.11%) (Arroum *et al,* 2022).

Camel milk was collected under hygienic conditions, in order to analyse its physicochemical and microbiological properties before and after the addition of the kefir grains, as quickly as possible in Livestock and Wildlife laboratory.

Kefir grains were obtained from local kefir users and stored in the laboratory. Before each use, the kefir grains were sieved and washed with distilled water and subsequently inoculated into camel milk the various fermentation media. For accuracy, each fermentation was repeated 5 times.

Kefir Manufacture

In this study, the kefir was prepared according to the traditional protocol of Otles and Cagindi (2003). Camel milk was homogenised and heated to 72°C for 20 seconds. The pasteurised milk was cooled down to 35°C. To obtain kefir, cooled milk was inoculated with 2%, 5% and 10% (wt/vol) kefir grains rate and incubated at 25°C for 18 h, 20h and 24h. At the end of the incubation, kefir grains were separated via a sieve. Kefir grains were transferred to fresh milk allowing further fermentation. In other words, kefir is the drink, however, kefir grains are the starter culture that used to produce the beverage.

The physicochemical composition and microbiological status

The study of physicochemical parameters included pH, Acidity, Viscosity, dry matter DM (g/l), mineral matter MM (g/l), fat (g/l), total protein TP (g/l), lactose (g/l). Microbiological status included the determination of total aerobic mesophilic flora (TAMF), total coliforms (TC), lactic acid bacteria (LAB) and yeast and mold (YM).

The pH was measured using a Thermo Orion pH meter (Cumming Centre Beverly, USA). The Dornic acidity was determined by sodium hydroxide titration N/ 9 in the presence of phenolphthalein (AFNOR, 1993). The viscosity (in cP) was determined by a Brookfield type viscometer (model DV-E, MA, USA). DM expressed in g/l was calculated after weighing the sample at 105°C for 24 h of its dry residue. MM (g/l) of milk was determined after drying at 550°C (AFNOR, 1993). Fat content (g/l)was measured using a "neusol solution" cited by Wangoh *et al* (2004). This method is a direct reading on a butyrometer measuring the amount of fat contained in 12ml of sample after centrifugation in the presence of butyric alcohol.

TP were determined by quantifying nitrogen using Kjeldahl method (N×6.38) (AFNOR, 1993) after distillation unit NITRO PRO-I and titration with 0.1 N hydrochloric acid. Lactose was determined by HPLC technic (Rajan *et al,* 2009). All analyses were repeated 5 times.

We used conventional methods recommended by the French law or official French method (AFNOR, 1996) which gives details of the followed techniques. All samples studied have undergone a preliminary treatment to obtain the dilutions according to standard NF V08-0IO (AFNOR, 1996).

Raw camel milk, kefir (1 ml) and Kefir grains samples (1g) were dispersed with 9 mL of sterile sodium chloride solution (0.1% w/v) and homogenised. The number of aerobic bacteria was carried out on plate count agar (PCA, Sharlau Chemie S.A), incubated at 30°C for 72h (ELZiney and AL-Turki, 2007). TC was grown in Violet Red Bile Agar (VRBA) (AppliChem. Biochemical. Chemical services) in double layer. After solidifying of the agar, the plates were incubated at 30°C for 24 h (Federal Register, 1992). LAB counts were determined on MRS solid (Man Rogosa Sharpe agar, Charlau Chemie S.A.) (De Man *et al,* 1960) after incubation at 30°C on surface for 48 h. YM were enumerated on Sabouraud Chloramphenicol (Pronadisa Micro and Molecular Biology) culture medium and incubated at 25°C for 3 to 5 days.

Description of the composition of Kefir (FAO & OMS, 2003)

According to the *Codex Alimentarius* (Codex Stan 243-2003), a typical kefir (fermented milk obtained from kefir grains) should contain at least 2.7% protein, 6% lactic acid and less than 10% fat. The percentage of alcohol is not established. The total number of micro-organisms in the fermented milk produced should be at least 10^7 colony-forming units (CFU)/ml and the yeast number not less than 10^4 CFU/ml (Codex Stan 243-2003).

Sensory evaluation

The final camel kefir was evaluated in sensory test, according to the Hedonic scale (Moraes, 1993; Puerari *et al,* 2012; Jrad *et al,* 2019) by 65 untrained tasters (males and females, aged between 21–55

years). Randomised, 30 ml of camel kefir samples with different inoculations dose and incubation time were served in glasses with a volume of 50 ml; these were marked with three digit random numbers.

The considered attributes were salinity, viscosity, odour, acidity and colour of the beverages. Each attribute was scored on an increasing scale from 0 (not present) to 9 (very intense). Finally, the tasters indicated whether they liked or disliked camel kefir based on their acceptability.

Statistical analysis

The statistical analysis were carried out by the SPSS software (11.5), the chemical composition were treated by an ANOVA analysis of variance with 2 factors (effect of dose and incubation time) using the Duncan test ($P < 0.05$). The sensory quality were treated by an ANOVA analysis to evaluate the statistical significance ($P < 0.05$) of differences between the beverages and to compare the means among the samples.

Results and Discussion

Physico-chemical and microbiological composition of milk and kefir grains

Camel milk pH was 6.52±0.09 (Table 1), slightly higher than the pH value found by Fguiri *et al* (2018) in milk from semi-intensive camel farms (6.46±0.16), but lower than 6.77±0.07 reported by Khaskheli *et al* (2005). According to El-Hatmi *et al* (2004), pH is influenced by the stage of lactation. The density of camel milk was (1.029±0.001) similar to value reported by Fguiri *et al* (2017) (1,027). Fat content and TP were 32.71±4.4 g/l and 24.2±2.07g/l, respectively. Lactose was 41.23±0.76 g/l and similar value was reported by Sboui *et al* (2016) and Baig *et al* (2022).

The microbiological quality of camel milk (Table 1) revealed a very low load in aerobic bacteria (TAMF) of $7.19\pm0.1*10^3$ cfu/ml, remained below the limit announced by the Tunisian standard which is equal to $5*10^5$ cfu/ml (Kamoun, 2012). These results are due to the good health of the animal and the precautions taken to avoid any contamination of samples. The number of TC in the camel milk samples was $1.61\pm0.3* 10^3$ cfu/ml .This result was higher than the average noted by Fguiri *et al* (2018) which was $3.38*10^2$ cfu/ml. The presence of less than 100 coliforms per ml of milk is indicating a good hygiene status due to proper management during production, handling and distribution of milk (Jrad *et al,* 2013). The average level of LAB was $3.98 \pm1*10^4$ cfu/ml. These bacteria are reported to be the origin of some bacteriocins which contain antibacterial activities in milk (Singh, 2018). The yeast microbial load was $3.9 \pm1.1*10^2$ cfu/ml. These values were higher than those reported by Jrad *et al* (2013). The presence high level of YM in camel milk can affect the quality of the milk because of their lipolytic activity which modifies the flavour of the milk (Jrad *et al,* 2013).

The pH of kefir grains was 3.9±0.2. All grains produced acid products with pH between 3.5 and 4.0 (Garrote *et al,* 2001). The fat content of kefir grains was negligible (0.02 g/l). DM, TP and MM were 122.8±0.02g/l, 44.5 ± 0.1 g/l and 9±0.81g/l, respectively. Liutkevičius and Šarkinas (2004) found that the biomass of kefir grains contained 13.7% DM which comprised 4.5% protein, 1,2% ash and 0,03% fat. Quantitatively, kefir grains contained a variable number of LAB ($3\pm1.2*10^8$ cfu/g) and yeasts ($4.3\pm2.3*10^6$ cfu/g). According to Dong *et al* (2018) LAB and yeasts are the major population in kefir grains. The complex microbiota is an example of a symbiotic community where LAB (10^8–10^9 cfu per gram of grain), yeasts (10^7–10^8 cfu per gram of grain) share their bioproducts as energy sources and microbial growth factors (Garrote *et al,* 2010; Nielsen *et al,* 2014; Plessas *et al,* 2016; Tamang *et al,* 2016). The ratio of LAB to yeast was found 10^9:10^6 (Guzel-Seydim *et al,* 2005).

Table 1. Composition of milk and Kefir Grains.

Parameters	Camel milk n=30	Kefir Grains n=15
pH	6.52±0.09	3.9±0.2
Acidity (°D)	15.77±1.96	135 ±0.1
Viscosity (cP)	2,94±1,09	-
Density (g/l)	1.029±0.001	-
Dry Matter DM (g/l)	104.76±1.26	122.8±0.02
Mineral Matter MM (g/l)	8.63±0.73	7.9±0.81
Fat (g/l)	32.71±4.4	0.02±0.01
Total Protein TP (g/l)	24.2±2.07	44.5 ± 0.1 g / l
Lactose g/l	41.23±0.76	-
Total Aerobic Mesophilic Flora TAMF ufc/ml	7.19 103 ±0.1	$8\pm3.4*10^9$
Total Coliforms TC ufc/ml	$1.61 \pm0.3*10^3$	-
Lactic Acid Bacteria LAB ufc/ml	$3 .98\pm1* 10^4$	$3\pm1.2*10^8$
Yeast and Mold YM ufc/ml	$3.9 \pm1.1*10^2$	$4.3\pm2.3*10^6$

Effect of inoculated grain dose on kefir characteristics

Inoculation dose showed a significant effect on pH, acidity as well as ash content of the obtained kefirs (Table 2). With increasing dose pH decreased

while acidity and viscosity increased. Camel's kefir with a dose of 10% of grain was highly acidic (97.35±2.9a, pH=4.03±0.16^{c}) and viscous (58.67±2.4). The kefir pH is usually reported between 4.2 and 4.6 (Botelho *et al,* 2014). Other studies reported lower values of pH that reached 3.98 (Pop *et al,* 2015; Zajsek *et al,* 2011) which was possibly due to the presence of some components, such as carbon-dioxide, acids, lactose, ethanol, proteins and fat content (Kesenkas *et al,* 2011). The increase of acidity is due to the production of lactic acid according to LAB growth during fermentation (Guzel-Seydim *et al,* 2000).

Fat decreased with increasing dose from 32.4±1.54 g/l at 2%, to 31.17±0.54 g/l at 5% and 30.55±3.3 g/l at 10%). According to Zourari and Anifantakis (1988), the fat content depends on the origin of the milk used (cow, sheep, goat etc) and the choice of the manufacturer of whole, skimmed or partially skimmed milk.

In our study, the ash content of camel Kefir made by 2% of kefir grain was 8.2±0.22 g/l. This value was higher than obtained by Kök-Taş *et al* (2014), who found a range of values from 0.55% to 0.66%.

Lactose content, which is the predominant sugar in milk, is reduced in the camel kefir. Such a decrease is due to fermentation (Otles and Cagindi, 2003) and LAB activity. Such camel kefir was a good option for lactose-intolerant individuals. The load of LAB and LM increase was mainly due to the production of microbiota within the grains matrix, which can be transferred to the fermented milk. No significant difference in lactose content was detected depending on inoculation dose. LAB were sufficiently active with all tested doses.

Effect of incubation time on the quality of camel kefir

The incubation time had no significant effect on the physico-chemical quality of the obtained camel kefir (Table 3) while a significant effect was observed on microbiological quality. Production of kefir was based on symbiotic relation between LAB and yeasts. pH decreased from 6.52±0.09 to 4.36±0.23 after 18 hours of incubation. With longer incubation time (24 h), kefir pH decreased to 4.14±0.21. These results agreed with those reported by Magalhães *et al* (2011) who showed a decrease in pH value from 6.61 to 4.42 after incubation of Brazilian kefir grains for 24 h in pasteurised whole milk while acidity and viscosity increased.

The content of lactose decreased according to the incubation time due to the grains' LAB which turn the milk lactose into lactic acid. Over approximately 18 hours, the microorganisms in the kefir grains multiplied and fermented the sugars (lactose) of the milk, turning the liquid milk into viscous and fermented beverage "kefir".

The microbial composition of kefir is subjected to variations (Londero *et al,* 2012; Rosa *et al,* 2017). These variations may be due to the factors such as the origin and storage of the kefir grains, the type of milk used as well as the processing conditions of the product, especially the grain/milk ratio and the fermentation temperature (Garrote *et al,* 1998; Nielsen *et al,* 2014).

Sensory evaluation of kefir from camel milk

Statistical analysis revealed that all 9 produced kefir with different inoculation dose and incubation time had similar colour (p=0.38). Kefir from pasteurised camel milk tasted most acidic after 20 h of incubation with 5% grain dose (7.07) compared to other types of kefirs. The camel kefir at a dose of 10% and an incubation time of 20 hours (CK, 20h, 10%) was characterised by a saltiest taste (6.32±2.53). Kefir from pasteurised camel milk after 18 hours of incubation with 2% grain dose was more viscous. According to Garrote *et al* (2001), kefir is viscous and slightly effervescent fermented milk with an acidic flavour. Camel kefir can be made with different dose and incubation time and had the following characteristics: taste was acid, prickly and slightly yeasty. The sharp acid and yeasty flavour, together with the prickly sensation produced by the yeast flora can be considered as the typical kefir flavour (Irigoyen *et al,* 2005). Camel milk, due to its chemical composition and physicochemical properties is an excellent raw material to produce this type of dairy products.

Table 2. Effect of the dose on the quality of kefir.

Dose	pH	Acidity	Viscosity	Fat (g/l)	MS (g/l)	Ash (g/l)	Lac (g/l)	LAB	LM
2%	4.56±0.3^{a}	72.45±1.6^{b}	44±1.72	32.4±1.54	105.34±1.2	8.2±0.22^{a}	12.66±1	1±0.2*10^{5}	3±0.2*10^{4}
5%	4.26±0.2^{b}	80.8±1.7^{b}	53.33±1.4	31.17±0.54	103.31±1	7.92±0.26^{b}	12.2±0.3	3±0.2*10^{5}	4.5±0.6*10^{4}
10%	4.03±0.1^{c}	97.35±2.9^{a}	58.67±2.4	30.55±3.3	98.78±1.9	7.75±0.3^{b}	11.3±1.3	5±0.6*10^{5}	4,6±0,8*10^{4}

a, b, c: the means on the same row with different letters are statistically different (P <0, 05);Total solid: MS (g/l); Lactose: Lac lactic acid bacteria: BL; Yeast and mold: LM

Table 3. Effect of incubation time on the quality of kefir.

Times	pH	Acidity°D	Viscosity	Fat (g/l)	MS(g/l)	Ash (g/l)	Lac (g/l)	LAB	LM
18h	4.36±0.23	76±0.1	46±5.3	29.28±0.22	105±0.55	7.79±0.3	13±2.4	4.9 ± 0.5^{ab} *10^5	4.9 ± 0.6^{a}*10^5
20h	4.35±0.43	84.5±3.1	46.22±2.7	35.61±6.8	103.86±7.4	8.06±0.3	12±1.6	5.3 ± 0.2^{b} *10^4	4 ± 0.2^{ab}*10^4
24h	4.14±0.21	90.1±3.7	63.56±4.5	29.22±4.04	98.55±7.7	8.04±0.2	11±2.8	5.4 ± 0.2^{a} *10^5	3.7 ± 0.3^{b}*10^4

a, b, c: the means on the same row with different letters are statically different (P <0, 05);Total solid: MS (g/l); Lactose: Lac lactic acid bacteria: BL; Yeast and mold: LM.

Table 4. Sensory analysis of camel kefir.

Camel Kefir	Salinity	Viscosity	Odour	Acidity	Colour
CK (18h) 2%	4.41 ± 3.35^{bc}	4.79 ± 2.77^{a}	3.73 ± 3.09^{bcd}	5.37 ± 2.77^{bc}	3.7 ± 2.84^{a}
CK (20h) 2%	2.75 ± 2.48^{de}	2.82 ± 2.73^{cd}	3.44 ± 3.46^{cd}	3.48 ± 2.55^{d}	3.39 ± 3.01^{a}
CK (24h) 2%	1.86 ± 2.21^{e}	2.19 ± 2.54^{d}	3.13 ± 3.62^{d}	3.06 ± 3.17^{d}	3.99 ± 3.49^{a}
CK (18h) 5%	4.19 ± 2.95^{cd}	4.26 ± 2.55^{abc}	3.61 ± 2.74^{bcd}	5.27 ± 2.45^{bc}	4.11 ± 3.63^{a}
CK (20h) 5%	5.99 ± 3.73^{ab}	4.55 ± 2.87^{ab}	5.15 ± 3.35^{abc}	7.07 ± 3.4^{a}	3.86 ± 3.45^{a}
C K (24h) 5%	4.84 ± 3.55^{abc}	3.83 ± 2.66^{abc}	5.74 ± 3.41^{a}	5.91 ± 3.47^{ab}	4.69 ± 3.72^{a}
CK (18h) 10%	$5.51\pm2,54^{abc}$	3.26 ± 2.4^{bcd}	$5.04\pm2,78^{abc}$	3.87 ± 2.5^{cd}	2.6±2.17
CK (20h) 10%	6.32 ± 2.53^{a}	4 ± 2.45^{abc}	5.23 ± 2.63^{ab}	$6.43\pm2,66^{ab}$	3.58±2.49
CK (24h) 10%	5.93 ± 3.21^{ab}	$4.22\pm2,67^{abc}$	$5.88\pm3,3^{a}$	$5.03\pm2,64^{bc}$	3.5 ± 2.35^{a}
P	0.0001	0.002	0.001	0.0001	0.38

CK : Camel Kefir.

By using the acceptability test in the sensory evaluation, camel kefir coming from semi-intensive rearing system at a dose of 2% and an incubation time of 18 hours was accepted by 60% of tasters. This selected camel kefir was thicker and smoother than the other kefirs. It had a creamier and smoother texture, smelled lighter and was less acidic (5.37±2.77). In general, due to the higher microbial population (4.9±0.6a*105 especially yeast), kefir taste was sour. The results for certain attributes agreed with the results reported by Irigoyen *et al* (2005), who studied the correlations of the different sensory attributes with acceptability indicated that the panel was positively influenced by milky taste, milky odour and smooth viscosity. The changes in the organoleptic characters of camel kefirs showed that acidity, taste and odour were due to the dose and time applied of the culture. They exerted a significant impact on the results of the sensory evaluation of its acceptability.

The necessary process conditions of making camel kefir

After studying the nutritive quality of the obtained camel kefirs, the dose of kefir grains necessary to obtain a camel kefir conforming to the Codex Standard for fermented milks CODEX STAN

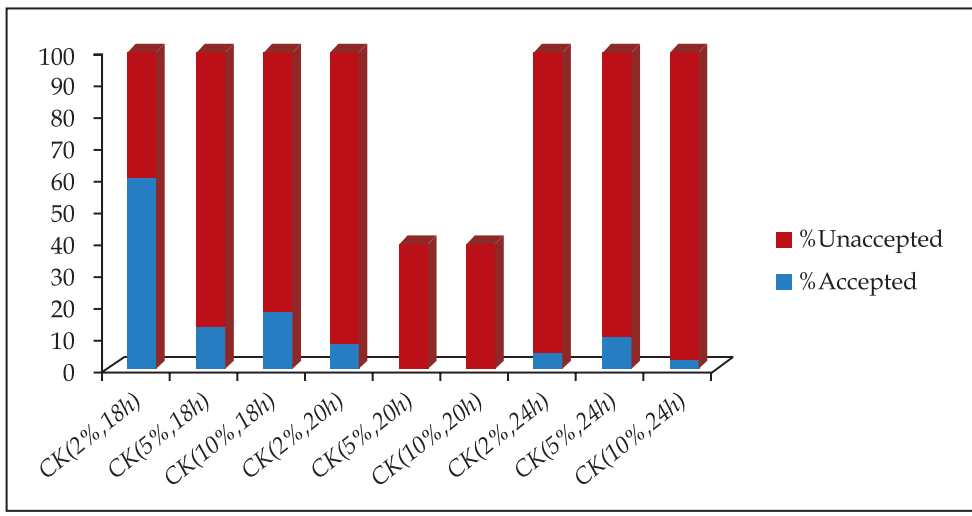

Fig 1. Acceptability Test (CK : Camel Kefir).

243-2003 and sensorial test was 2% and the incubation time to be applied for camel milk was 18 hours. To obtain a kefir that complies with the standard (Farmworth *et al,* 2005), considering the acidity (72.45 ± 9.6), the kefir of camel milk from semi-intensive rearing system obtained with 2% dose and incubated for 18 hours was the most valuable. It had the highest acceptability rate by tasters (60%). This fermented beverage, because of its nutritive interest as well as taste and smell values, was occupying a high position for the tasters. It raised interest due to its suggested beneficial and organoleptic properties.

The present study contributed for a better knowledge of the microbiological and chemical constitution of the camel kefir beverage prepared in Tunisia. Future works involving nutritional and therapeutic benefits would improve the characterisation of Tunisian camel kefir, packaging, storage and post-production treatment as well as methods of preservation of kefir grains.

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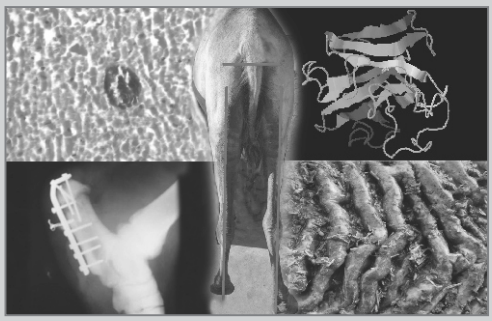

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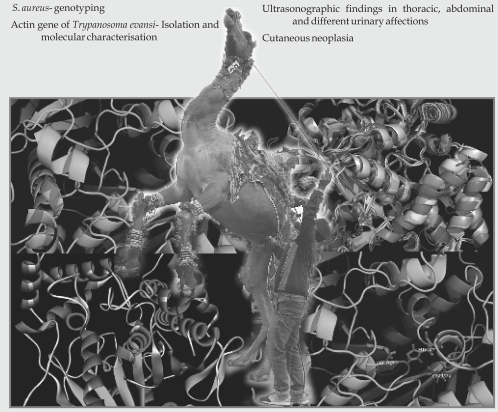

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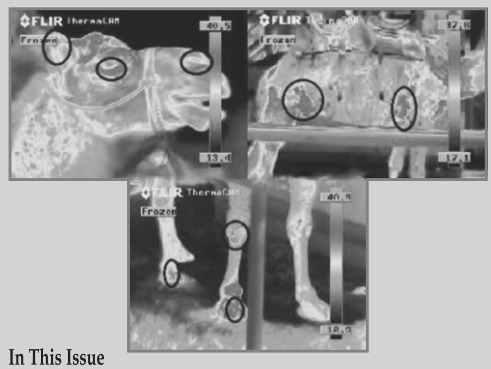

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COMPARATIVE STUDY ON ANTIOXIDANT CAPACITY, OXIDATIVE CHANGES AND *in vitro* DIGESTIBILITY OF SPRAY DRIED CAMEL, GOAT AND COW MILK POWDER DURING ACCELERATED STORAGE

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ABSTRACT

The antioxidant capacity, oxidative changes and rate of digestibility of camel milk powder were compared with that of goat and cow milk powder. The average antioxidant capacity of all milk powder varied range from 14.27 to 33.13 per cent inhibition. Among all powder samples, camel milk powder was found to have higher antioxidant capacity while the lowest antioxidant capacity was observed in cow milk powder. The initial values of free fatty acids varied from 1.93 to 2.60, 1.80 to 3.22 and 2.17 to 2.60 per cent oleic acid for camel, goat and cow milk powder, respectively. The peroxide and TBA value of all the samples increased at the end of the storage. Camel milk powder had higher rate of digestion as compared to goat and cow milk powder.

Key words: Antioxidant capacity, camel, cow, goat, *in vitro* digestibility, milk powder, oxidative changes

Camel milk is popular in many countries because of its reputed health benefits. It contains all the essential nutrients found in bovine milk. Camel milk includes higher levels of immune-active proteins, vitamin C, minerals, antioxidant properties and insulin like protein which increase the therapeutic values of camel milk. Goat milk differs from cow milk in its composition, nutritional and therapeutic attributes. Goat milk is considered to be superior in terms of numerous health benefits and low risk of allergy. Additionally, goat milk has low levels of orotic acid than cow milk, which significantly reduces the risk of developing fatty liver syndrome (Haenlein and Caccese, 1984). Goat milk has recently been found to provide health benefits for anaemia recovery, reduced plasma cholesterol induction and greater levels of insulin-like growth factor I, which boosts metabolism and promotes tissue regeneration and repair in adults (Kalyan *et al,* 2018).

The antioxidative power of spray-dried camel milk powders increases with the inlet temperature of drying (Marija *et al,* 2021). Physico-chemical properties and the effect of operating parameters on the surface of spray-dried camel milk powders have been studied (Haileeyesus *et al,* 2018). Ho *et al* (2019) studied alterations in surface chemical composition relating to rehydration properties of spray-dried camel milk powders during accelerated storage (11–33% RH, 37°C) over 18 weeks. Researchers found that even though fresh camel milk powder had very poor wettability, it displayed very high dispersibility and solubility (99%) but it declined with increasing storage time and increasing RH levels, which is correlated with an increase in surface lipid content. Notably, camel milk powder retained very high solubility at the end of the storage period (>93%).

The camel and goat milk powder are now used for the creation of a variety of food products, including chocolate and confections. The Food Safety and Standards Authority of India has issued a legal requirement of a minimum 2 and 3 to 3.5% milk fat and a minimum 6 and 9% solids not fat emphasising its commercialisation potential for camel and goat milk, respectively (FSSR, 2011).

Drying of milk helps in preserving its physio-chemical and nutritional qualities, lower transportation costs and extend its shelf life. Although milk powder can be stored for a long time, the storage

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conditions, such as time, temperature and relative humidity have a significant impact on these changes. The oxidative changes may produce rancidity in the milk products and some of the oxidised components may adversely affect the health leading to heart attack, cancer and mutation of the gene (Thomas *et al,* 2004). The digestion of milk fat is also important from nutritional point of view. Milk is regarded as a highly digestible food product. The dietary lipids are the most energy-dense component. The digestibility of fat depends on various factors like types of animals, fatty acid composition and its positional distribution, interaction of fat with other milk components during processing/storage. Based on the lipolysis of fat in the digestive system, this study aims to understand the digestibility of milk fat in various types of spray dried milk powders.

Materials and Methods

Chemicals

The chemicals were procured from reputed companies. DPPH (HiMedia Laboratories Pvt.Ltd., Mumbai, India), methanol (Loba Chemie Pvt. Ltd., Mumbai, India), glacial acetic acid (Loba Chemie Pvt. Ltd., Mumbai, India), potassium iodide (Thermo Fisher Scientific India Pvt. Ltd., Mumbai), chloroform (Merck Life Science Pvt. Ltd., Mumbai, India), sodium thiosulphate (Loba Chemie Pvt. Ltd., Mumbai, India), starch indicator (Thermo Fisher Scientific India Pvt. Ltd., Mumbai), TCA (Trichloroacetic acid (Loba Chemie Pvt. Ltd., Mumbai, India), 2-Thiobarbituric acid (TBA) (Himedia Laboratory Pvt. Ltd., Mumbai) and ethanol ((Loba Chemie Pvt. Ltd., Mumbai, India).

Samples of milk powder

The most commonly available two different brands of spray dried camel milk powder (COMP1 and COMP2), goat milk powder (GMP1 and GMP2) and cow milk powder (COM1 and COM2) used for this study were purchased from the market. One of the commercially available cow milk powder (COM2) was A2 types that was manufactured from breed of Gir cow. The total five different batches of each powder were procured at different interval of times from the suppliers. All fresh powders were subjected to antioxidant capacity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. All samples of powder were stored in BOD incubator at 40±2°C in LDPE bags (50 µ) and subjected for accelerated storage study upto 90 days. The oxidative changes were monitored using free fatty acids (FFA), peroxide value and 2-Thiobarbituric acid (TBA) values at the 15 days of interval during storage study. Moreover, the samples of powder (CMP1, GMP1, COM1 and COM2) were also studied for *in vitro* digestibility at the fresh (0 day), 45 day and 90 days of interval during the storage.

Determination of antioxidant capacity of milk powder

Antioxidant capacity of milk powder was determined using procedure given by Brand-Williams *et al* (1995) and modified by Song *et al* (2010). Sample 0.5 g powder was treated with 10 mL of methanol–water (8:2, v/v) in a shaking water bath at 35°C for 24 h. The mixture was then centrifuged at 3000 rpm for 10 min and filtered through Whatman no. 42 filter paper. Filtrate (0.5 ml) and methanol (0.5 ml) were taken in test tube and mixed properly and 3 ml of DPPH (0.05 mM) was added. The tube was incubated for 35 min at room temperature. Methanol was used (as blank) for the baseline correction. Absorbance of the control and samples were measured at 517 nm using a spectrophotometer against blank (methanol). Radical-scavenging capacity was expressed as the inhibition percentage and was calculated using the following formula.

$$\text{Radical Scavenging capacity (\%inhibition)} = \frac{(\text{Absorbance of control-Absorbance of sample})/}{(\text{Absorbance of control})}$$

Determination of free fatty acids content in milk powder

Free fatty acids content was determined by volumetric method given by Deeth *et al* (1975). In this volumetric method, 5 g of powder sample was weighed into a 60 ml test tube then 10 ml of extraction mixture which contained iso-propanol: petroleum ether: 4 N sulfuric acid (ratio 40:10:1) was added and shaken thoroughly, followed by the addition of 6 ml petroleum ether and 4 ml distilled water. The test tube was stoppered and tempered at 40°C for 10 minutes. The contents were vigorously shaken for 20 seconds. The two layers were allowed to separate for 10-15 minutes and an aliquot of the upper layer (5-8 ml) was withdrawn and titrated against 0.02 N methanolic KOH solution using 1 per cent methanolic phenolphthalein indicator.

$$FFA\ (\%\ Oleic\ acid) = \frac{(T \times N) \times 10^3)}{P \times W}$$

Where, T = ml of 0.02 N methanolic KOH ; N = Normality of methanolic KOH solution; P =

Proportion of upper layer of aliquot and W = Weight of sample taken in g

Determination of peroxide value in milk powder

The peroxide value of powder was determined by method described in AOAC (2006). Five grams of milk powder sample was soaked in 30 ml of chloroform for the period of 12 h in an airtight flask. After that the content was filtered through Whatman filter paper No. 42 to get the clear chloroform extract. The obtained filtrate was then transferred to 50 ml test tube; to which 1 g potassium iodide powder and 10 ml glacial acetic acid was added. The tubes were kept in boiling water bath until the content of tubes started to boil. The tubes were then immediately cooled by dipping in tap water. In a conical flask, 20 ml potassium iodide solution (5% w/v) was taken, to which the content of the above tube was transferred by washing with 20 ml distilled water. The content of the conical flask was then titrated against a 0.002 N sodium thiosulfate solution using starch as an indicator. The peroxide value was expressed in mEq/kg fat.

$$Peroxide\ Value = \frac{(A\text{-}B)\times N\times 1000}{M}$$

A = volume of $Na_2S_2O_3$ required for the sample; B = volume of $Na_2S_2O_3$ required for the blank

N = Normality of $Na_2S_2O_3$ solution; M = mass in g of the sample taken

Determination of TBA value in milk powder

The TBA value was determined according to the method described by Sun (2013). The milk powder was reconstituted by 12%, w/v and the 35.2 ml of reconstituted milk was poured in sugar tube and kept in a water bath at 30°C. Then, 2 ml trichloroacetic acid solution of 40% (w/v) and 4 ml of ethanol solution of 95%, were added to sugar tube. After shaking, the mixture was allowed to rest for 15 min. The milk fat and proteins were removed by filtration. One ml of TBA solution of 0.1 mol/L was added to the clear filtrate and the mixture was incubated in a water bath at 60°C for 60 min. The absorbance was measured at 538 nm at room temperature and expressed in optical density (OD).

In vitro fat digestion of milk powder in terms of free fatty acid

The rate of fat digestion was evaluated in terms of free fatty acids released after digestion as per the method of Meena *et al* (2014). Free fatty acids released during intestinal digestion (0, 30, 60, 90, 120,150, 180 and 210 min) were estimated by pH titration against 0.01 M NaOH and expressed in terms of oleic acid from the standard curve (250 to 1500 µmol oleic acid mL^{-1}). The digestion of milk powder was performed in intestinal phase. The pancreatic-lipase catalysed digestion of milk fat was studied using a pH-stat titration approach, where the release of free fatty acids was titrated with NaOH (Meena *et al,* 2014). To simulate *in vivo* intestinal conditions for fat breakdown, simulated intestinal fluid (SIF) was created. It contained 39 mM K_2HPO_4, 190 mL 0.2 M NaOH and 150 mM NaCl (Ye *et al,* 2010).

The 13 grams of sample of powder was reconstituted to 100 ml distilled water. The pH of the reconstituted milk was then raised to 7.5 using 1 N NaOH and 10 ml was used. Milk samples were immediately incubated for 10 minutes at 37°C following pH adjustment. The 10 mL of SIF (250 mg of bile extract and 40 mg of pancreatin for 50 mL SIF buffer) was added to a reaction mixture of 10 mL of milk that had already been pH-adjusted to 7.5. In control samples, 10 mL of SIF was added to 10 mL of milk (without pancreatin or bile extract). Samples were then incubated in a water bath at 37°C. The 10 mL of digested milk sample was transferred into a conical flask which was withdrawn after 30, 60, 90, 120,150, 180 and 210 min and released free fatty acids were estimated by pH titration against 0.01 M NaOH. The amount of free fatty acids was expressed in µmol.

Preparation of standard curve

A standard curve of oleic acid was created with concentrations ranging from 200 to 1600 µmol in order to determine the quantity of free fatty acids generated after the fat digestion by pancreatic lipase (Table 1). Oleic acid standard solution was made in skimmed milk. The standard curve of oleic acid is mentioned in Fig 1.

Table 1. Preparation of oleic acid standard solution.

Concentration of oleic acid (µmol)	ml of 0.01 N NaOH used for titration
0	3.50
200	13.4
400	22.6
600	39.6
800	46.2
1000	59.9
1200	66.6
1400	88.0
1600	99.0

Statistical analysis

The Statistical analysis was done by using the simple and factorial completely randomised design (CRD) (Steel and Torrie, 1980). Five samples of each milk powder were procured at different interval of time in the study.

Results and Discussion

Antioxidant capacity of camel, goat and cow milk powder

The antioxidant capacity of milk powder is mainly due to sulphur containing amino acids, Vitamin E, Vitamin C and various enzymes. Antioxidant capacity of the samples is shown in Table 2.

Table 2. Antioxidant capacity of camel, goat and cow milk powder.

Types of milk powder	DPPH (% inhibition)	
	Range	Average
CMP1	11.21-31.49	18.83±9.91[a]
CMP2	32.13-32.86	33.13±1.37[b]
GMP1	24.48-34.26	30.18±3.61[b]
GMP2	24.55-30.66	27.72±2.02[b]
COM1	11.30-17.21	14.27±8.26[a]
COM2	15.30-20.53	17.38±1.80[a]
SEm	2.81	
CD	8.20	
CV%	26.07	

[a-b]: values with different letters between a row were significantly different at 5 % (i.e., $p<0.05$).
SEm: standard error of means, CD: Critical difference, CV: Coefficient of variance
CMP: Camel milk powder; GMP: Goat milk powder; COM: Cow milk powder

Our result shows that a significant difference was observed in the antioxidant capacity of various milk powders obtained from various brands. The average antioxidant capacity of milk powder varied range from 14.27 to 33.13 per cent inhibition. Among all powder samples, CMP2 was found to posses higher antioxidant capacity as compared to other samples of milk powder while the lowest antioxidant capacity was observed in COM1. The antioxidant capacity of COM1 was found significantly ($p<0.05$) lower in comparison to GMP1 and GMP2. The non-significant ($p>0.05$) difference was observed for samples GMP1 and GMP2. The antioxidant capacity of GMP1 varied from 24.48 to 34.26 per cent inhibition, whereas 24.55 to 30.66 per cent inhibition in GMP2. The antioxidant capacity was found 15.30 to 20.53 per cent inhibition in COM2.

The variation in the antioxidant capacity among different brands of samples might be due to losses of vitamins and sulfur-containing amino acids during heat treatments and drying process. Vitamin C content also influences the antioxidant capacity of the powder. Vitamin C content in goat milk powder was significantly higher than the cow milk powder might be the reason for this variation (Somnath, 2023). Antioxidant capacity milk powder is affected by milk of different species and breeds, hot air temperature, design of drying chamber, contact time of hot air with the milk particles, storage conditions, etc. There are limited literatures available on the antioxidant capacity of various milk powders. Al-Saleh *et al* (2014) studied the antioxidative capacity of camel milk casein hydrolysate. They reported a range of 27.05 to 36.82 per cent inhibition of camel casein samples and 27.05 and 11.95 per cent inhibition for unhydrolysed camel casein and cow casein, respectively. The addition of soybean extract in camel and cow's milk revealed 15.4 to 61.70 per cent inhibition of DPPH antioxidant capacity (Shori *et al,* 2021). Lugonja *et al* (2021) investigated the antioxidant capacity and quality of human milk and infant formula by direct current polarography. They reported 35.51% inhibition of cow milk powder. The DPPH capacity of the CMP2 falls in the range reported by Al-Saleh *et al* (2014). Kalyan *et al* (2018) reported antioxidant capacities of 54.86 and 56.55 per cent in pasteurised and unpasteurised goat milk, respectively. The antioxidant capacity of milk powder was found less than milk could be due to variations in processing conditions such as preheating, evaporation and spray drying as well as different physicochemical interactions between ingredients.

Changes in Free fatty acids (FFA) content in camel, goat and cow milk powder

The changes in FFA content during accelerated storage in camel, goat and cow milk powder are shown in Table 3.

All the powder samples were found significantly ($p<0.05$) different from each other. The initial values of free fatty acids were 2.60, 1.93, 1.80, 3.22, 2.60 and 2.17 per cent oleic acid for CMP1, CMP2, GMP1, GMP2, COM1 and COM2, respectively. The FFA content of CMP1, CMP2, GMP1, GMP2, COM1 and COM2 were increased to 2.61, 3.94, 3.87, 5.75, 2.97 and 4.91 per cent oleic acid, respectively at the end of storage. The FFA values steadily increased in CMP2 throughout the storage period except was decreased on 30th day.

The significantly ($p<0.05$) increase in FFAs value on 0^{th} day to 45^{th} day was found in GMP2. The FFAs value in GMP1 was observed continuously increased from 1.69 per cent oleic acid on 30^{th} day to 5.10 per cent oleic acid on 75^{th} day. The significantly more FFAs value was observed in 75^{th} day of both sample GMP1 as well as GMP2 with FFAs value of 5.10 and 6.65 per cent oleic acid, respectively. The highest FFA value was observed in GMP2 on 90^{th} days of storage. There was no definite trend observed in FFA values of the milk powder samples throughout the storage periods.

There is no literature available on the FFA value of camel milk powder. However, few researchers observed FFA value changes in various milk powders during storage. Celestino *et al* (1997) evaluated the influence of refrigerated storage of raw cow milk on the quality of whole milk powder. They mentioned FFA values ranged from 2.63 to 3.95 mEq/100gm of powder at 8 months of storage in WMP. The FFA values obtained in our study were closer to the range reported by Celestino *et al* (1997). The increase in FFA value in our study might be due to the occurrence of lipolysis and oxidation in the whole camel and cow milk powder. However, during storage, the FFA content slightly decreased and this might be due to the formation of a complex between FFA and unstable protein (Andersson, 1980). According to Queiroz *et al* (2021) lactose hydrolysis of goat milk results in greater darkening and increased free fat content. Celestino *et al* (1997) reported the free fatty acid content in cow milk powder as 7.55, 9.10, 10.19, 10.38 and 10.96 mEq/kg powder on 0^{th}, 2^{nd}, 4^{th}, 6^{th} and 8^{th} months, respectively. Fonseca *et al* (2013) reported that the FFAs content in whole goat milk powder were 5.9 to 10.1 mEq/kg, 5.1 to 9.5 mEq/kg, 5.6 to 10.0 mEq/kg and 6.3 to 15.4 mEq/kg on 0^{th}, 60^{th}, 120^{th} and 180^{th} days. Definite trends were not found by Fonseca *et al* (2013) but overall, the increase in FFAs content during storage was observed. The free fatty acid contents of present study were comparatively less than the reported in literature and the changes that occurred during storage were in general agreement with changes observed by Fonseca *et al* (2013) and Celestino *et al* (1997).

Changes in peroxide value of camel, goat and cow milk powder

The changes in peroxide value during accelerated storage in camel, goat and cow milk powder are shown in Table 4 which indicated that the sample (T) and storage period (P) had a significant effect ($p<0.05$) on the peroxide value of milk powder. The initial peroxide value for samples CMP1, CMP2, GMP1, GMP2, COM1 and COM2 were 2.60,0.67, 0.23, 0.39, 0.74 and 1.01 mEq/kg, respectively. In all the samples, changes in peroxide value were found indefinite throughout the storage periods. However, the peroxide values increased at the end of storage to 8.31, 7.76, 1.28, 1.11, 1.03 and 1.60 mEq/kg in CMP1, CMP2, GMP1, GMP2, COM1 and COM2 samples, respectively. There was no significant difference observed in the peroxide value of COM1 from the day 15^{th} to the 75^{th} of the storage period. It was observed that the peroxide value increased significantly ($p<0.05$) in CMP1 and GMP1 till 30^{th} days with average value 5.99 and 0.80 mEq/kg fat, respectively. The increase in the peroxide value indicated an accumulation of intermediates formed from lipid oxidation in the dried milk whereas a decrease in the peroxide value can be attributed to further oxidation with the formation of small molecules, such as aldehydes, ketones and acids. This might be the reason for the variation found in TBA value at storage. The oxidation can also occur at very low water capacity. Some authors opined that the long storage duration of raw milk and dried milk may lead to higher peroxides in powders (Nursten, 1997; Thomas *et al,* 2004; Fonseca *et al,* 2013).

A scarce literature is available on the peroxide value of camel, goat and cow milk powder. Few researchers observed peroxide value changes in milk powder during storage. Baldwin *et al* (1991) found peroxide values ranged from 0.03 to 3 mEq/kg fat in milk powder stored at room temperature for 18^{th} months. The peroxide value of fresh WMP was 0.55 mEq /kg fat which increased to 2.40 mEq oxygen/kg fat at 8 months of storage at 25°C (Celestino *et al,* 1997). Romeu-Nadal *et al* (2007) evaluated on oxidation stability of milk powder formulas and found peroxide value ranged from 0.52 to 0.98 mEq/kg fat for different infant formulas at the beginning of storage. The peroxide value was increased to 12.9 and 20.3 mEq/kg fat at 25°C and 37°C, respectively at 8 months of storage and declined thereafter in supplemented infant formula. Li *et al* (2012) reported that the initial peroxide value of whole milk powder was 0.14 mEq/kg of fat which increased to 2 mEq/kg fat and 9 mEq/kg fat at 3 and 6 months of storage, respectively. Similarly, the peroxide value reached 11.23 mEq/kg fat after storage for 6 months and then decreased to 8.74 mEq/kg fat at 9 months of storage in infant formula. It follows varied trend during storage which has similarities with our findings.

Baldwin *et al* (1991) found peroxide values 0.03 mEq/kg fat on 0^{th} day and 3 mEq/kg fat on 18^{th} months in milk powder stored at ambient temperature. Celestino *et al* (1997) worked on the effects of refrigerated storage of raw milk on the quality of whole milk powder stored for different periods. The peroxide values recorded on 0^{th}, 2^{nd}, 4^{th}, 6^{th} and 8^{th} months were 0.55, 0.93, 1.09, 1.51 and 2.40 mEq/kg fat, respectively. The researchers observed that the release of sulfhydryl groups and packaging material which allow permeation of oxygen could affect the peroxide values. Fonseca *et al* (2013) worked on the storage of refrigerated raw goat milk affecting the quality of whole milk powder for 180 days at 25°C. They reported the peroxide value 0.1 to 0.2 mEq/kg fat on 0^{th} day, 0.1 to 0.5 mEq/kg fat on 60^{th} day, 0.3 to 0.7 mEq/kg fat on 120^{th} day and 0.4 to 0.7 mEq/kg fat on 180^{th} day in goat milk powder. Llyod *et al* (2009) studied on effect of nitrogen flushing and storage temperature on flavour and shelf-life of whole milk powder at 23°C. The peroxide value observed in fresh whole milk powder was less than 0.05 mEq/kg of fat and 1 mEq/kg fat on 6^{th} months in air-packaged sample. The air-packaged sample reached 2.7 mEq/kg of fat on 10^{th} months and then decreased by 12^{th} months. They also observed that peroxide value in

Table 3. Changes in FFA content of camel, goat and cow milk powder during storage at 40±2°C.

Storage period (days) (P)	FFA content (% oleic acid)					
	CMP1	CMP2	GMP1	GMP2	COM1	COM2
0	2.60 ± 0.07^{cC}	1.93 ± 0.09^{aA}	1.80 ± 0.17^{aA}	3.22 ± 0.56^{aD}	2.60 ± 0.16^{cC}	2.17 ± 0.22^{aB}
15	2.21 ± 0.45^{bA}	2.66 ± 0.10^{cB}	4.57 ± 0.52^{dD}	4.24 ± 0.03^{bC}	2.27 ± 0.09^{bA}	2.72 ± 0.11^{bB}
30	1.33 ± 0.14^{aA}	1.73 ± 0.19^{aB}	1.69 ± 0.08^{aB}	5.05 ± 0.27^{cD}	1.47 ± 0.09^{aAB}	2.49 ± 0.34^{bC}
45	2.03 ± 0.33^{bA}	2.30 ± 0.22^{bA}	3.40 ± 0.16^{bB}	6.49 ± 1.07^{eD}	3.19 ± 0.14^{eB}	3.99 ± 0.12^{cC}
60	3.13 ± 0.09^{dA}	3.27 ± 0.25^{dA}	3.89 ± 0.25^{cB}	5.13 ± 0.71^{cC}	3.67 ± 0.09^{fB}	5.27 ± 0.10^{eC}
75	2.33 ± 0.34^{bA}	3.84 ± 0.53^{eC}	5.10 ± 0.19^{eD}	6.65 ± 0.32^{eE}	2.87 ± 0.09^{cdB}	5.03 ± 0.42^{dD}
90	2.61 ± 0.29^{cA}	3.94 ± 0.17^{eC}	3.87 ± 0.72^{cC}	5.74 ± 1.09^{dE}	2.97 ± 0.09^{deB}	4.91 ± 0.35^{dBD}
Source of variation			Sample (T)	Days (P)	T x P	
SEm			0.10	0.11	0.27	
CD (0.05)			0.28	0.30	0.75	
CV %			13.55			

The values are mean of three replications.
$^{a-e}$: values with different letters between a row are significantly different at 5 % (i.e., $p<0.05$).
$^{A-E}$: values with different letters between a column are significantly different at 5 % (i.e., $p<0.05$).
CMP: Camel milk powder, GMP: Goat milk powder; COM: Cow milk powder

Table 4. Changes in peroxide value of camel, goat and cow milk powder during storage at 40±2°C.

Storage period (days) (P)	Peroxide value (milliequivalent/kg fat)					
	CMP1	CMP2	GMP1	GMP2	COM1	COM2
0	2.60 ± 0.34^{bD}	0.67 ± 0.27^{bB}	0.23 ± 0.02^{aA}	0.39 ± 0.02^{abA}	0.74 ± 0.23^{bB}	1.01 ± 0.03^{bcC}
15	3.35 ± 1.29^{cD}	0.83 ± 0.04^{bC}	0.46 ± 0.05^{abB}	0.75 ± 0.05^{cC}	0.13 ± 0.02^{aA}	0.94 ± 0.04^{bC}
30	5.99 ± 0.79^{eD}	0.36 ± 0.03^{aAB}	0.80 ± 0.07^{cC}	0.46 ± 0.05^{abB}	0.17 ± 0.02^{aA}	0.79 ± 0.05^{bC}
45	4.30 ± 0.70^{dE}	0.47 ± 0.05^{aB}	0.72 ± 0.03^{cBC}	$0.57\pm0.04b^{cBC}$	0.20 ± 0.03^{aA}	1.24 ± 0.03^{cD}
60	2.67 ± 0.16^{bC}	0.6 ± 0.16^{bB}	0.61 ± 0.05^{bcB}	0.47 ± 0.12^{abB}	0.21 ± 0.03^{aA}	0.52 ± 0.03^{aB}
75	1.54 ± 7.37^{aC}	1.27 ± 0.18^{cB}	1.64 ± 0.03^{eC}	0.31 ± 0.05^{aA}	0.28 ± 0.07^{aA}	2.30 ± 0.05^{eD}
90	8.31 ± 0.22^{fE}	7.76 ± 0.20^{dD}	1.28 ± 0.15^{dB}	1.11 ± 0.12^{dA}	1.03 ± 0.01^{cA}	1.60 ± 0.15^{dC}
Source of variation			Sample (T)	Days (P)	T x P	
SEm			0.08	0.08	0.20	
CD (0.05)			0.22	0.23	0.57	
CV %			23.88			

The values are mean of three replications.
$^{a-e}$: values with different letters between a row are significantly different at 5% (i.e., $p<0.05$).
$^{A-E}$: values with different letters between a column are significantly different at 5% (i.e., $p<0.05$).
CMP: Camel milk powder, GMP: Goat milk powder; COM: Cow milk powder

nitrogen-flushed sample was less than 1 mEq/kg of fat after 12th months. The peroxide value of all the sample in the present study was in general agreement with the reported value in literature.

Changes in TBA value of camel, goat and cow milk powder

The changes in TBA value during accelerated storage in camel, goat and cow milk powder are shown in Table 5.

The TBA values of all the powder sample were found significantly ($p<0.05$) different throughout the storage. The initial peroxide value of CMP1, CMP2, GMP1, GMP2, COM1 and COM2 were 0.112, 0.051, 0.039, 0.027 and 0.012, respectively. The TBA value of all the samples were increased at the end of the storage. Among all the samples, CMP1 had a significant higher TBA value and least value was observed in GMP1 during end of storage. The significantly ($p<0.05$) increased TBA value of COM2 was found throughout the storage period. The significantly ($p<0.05$) increased TBA value of GMP1 was observed from 0th day to 60th day. The non-significant ($p>0.05$) difference was observed between sample GMP1 and COM2 on 90th day with average TBA value of 1.06 and 1.07. In GMP2, the TBA value was significantly ($p<0.05$) increased from 0th day to 45th days with a TBA value of 0.027 to 0.122 which decreased to 0.105 on 60th days.

There is no literature available on the TBA value of camel, goat and cow milk powder. However, few researchers observed TBA value changes in whole milk powder during storage. Stapelfeldt *et al* (1997) studied on influence of heat treatment, water capacity and storage temperature on the oxidative stability of whole milk powder. They mentioned that TBA value of whole milk powder ranged from 0.059 to 0.063 and 0.133 to 0.233 at 25°C and 45°C, respectively. It follows varied trend during storage which has similarities with our finding. Our tabulated value shows a similarity with the results obtained by Li *et al* (2012) who reported that the TBA value of whole milk powder varied from 0.044 to 0.221 during 9 months of storage. Another sample showed a 0.477 to 0.880 range at the end of the storage. An increase in TBA value seen in present study, may be due to major changes in the powder structure, leaving the lipids more accessible to oxygen and water capacity changes through the storage period. Chudy *et al* (2015) reported the TBA value in whole milk powder ranged from 0.02 on 0th day and 0.28 on 24th month during study of effect on physicochemical properties of spray-dried milk due to storage. Sorensen *et al* (2017) worked on storage stability of whole milk powder and found an increase in the secondary lipid oxidation products such as hexanal, heptanal and nonanal. According to Li *et al* (2019), the aldehydes and ketones are closely related to the oxidation of milk powder. They found the TBA value in whole milk powder which 0.044 on 0th day and 0.221 on 9th months of storage. The TBA value was found highest in GMP2 with mean value 0.086 followed by GMP1 with mean value of 0.076 and lowest in COM2 with mean value 0.067. Thus, the results of TBA value in present study of goat and cow milk powders are similar to the previously reported values.

In vitro fat digestion of milk powder during storage

In terms of released free fatty acid, the rate of milk fat digestion for various powders of camel, goat and cow was estimated. Table 6 shows the quantity of free fatty acid (µmol oleic acid/ mL) liberated from milk fat of various milk powder after *in-vitro* digestion.

The fresh camel milk powder (CMP1) had a larger release of fatty acid (21.44 µmol oleic acid/ mL) within 30 mins of intestinal digestion followed by COM1 (23.14 µmol oleic acid/ mL), COM2 (18.90 µmol oleic acid/mL) and GMP1 (18.73 µmol oleic acid/ mL) when compared it at 0 mins. The rate of fatty acid release increased continuously during intestinal digestion in all the samples of powder. However, the rate of fatty acid release was slower with increase in time. After completion of intestinal digestion, the free fatty acids in the fresh samples of COM1, CMP1, GMP and COM2 were 66.70, 62.80, 60.60 and 37.04 µmol oleic acid/mL, respectively. Tunick *et al* (2016) found that the release of FFA was faster during first 15 min of intestinal digestion. The rate of FFA slows down with further digestion of milk fat and this may be attributed to the accumulation of released fatty acids (higher surface capacity) on the interface of the lipid droplet. This leads to the inhibition of lipolysis as the accessibility of triglyceride core reduces for action of pancreatin lipase until the solubilisation of these accumulated lipolytic particles into micelles.

It is cleared from the Fig 2 that among all the samples of milk powder, the rate of fatty acid release higher in COM1 samples and least in COM2 samples throughout storage period. The rate of fatty acid release was relatively higher in CMP1 samples as compared to that of GMP1 samples. Camel milk

Table 5. Changes in TBA value of camel, goat and cow milk powder during storage at 40±2°C.

Storage period (days) (P)	Peroxide value (milliequivalent/kg fat)					
	CMP1	CMP2	GMP1	GMP2	COM1	COM2
0	0.112±0.001^{aC}	0.051±0.016^{aB}	0.039±0.003^{aA}	0.027±0.003^{aA}	0.012±0.002^{aA}	0.018±0.002^{aA}
15	0.142±0.030^{abC}	0.057±0.019^{aA}	0.045±0.003^{aA}	0.068±0.002^{bB}	0.059±0.008^{bA}	0.037±0.002^{aA}
30	0.117±0.005^{aB}	0.108±0.010^{bB}	0.063±0.002^{aA}	0.081±0.002^{bA}	0.066±0.001^{bA}	0.069±0.002^{aA}
45	0.235±0.038^{cD}	0.155±0.004^{cC}	0.086±0.002^{bA}	0.122±0.002^{cB}	0.094±0.008^{bA}	0.073±0.001^{aA}
60	0.243±0.036^{cC}	0.284±0.137^{dD}	0.099±0.003^{cA}	0.105±0.003^{cA}	0.128±0.033^{dB}	0.079±0.002^{bA}
75	0.433±0.026^{dC}	0.107±0.008^{bB}	0.076±0.002^{cA}	0.106±0.006^{cAB}	0.111±0.00^{cB}	0.088±0.002^{bA}
90	0.687±0.172^{eC}	0.256±0.068^{dB}	0.106±0.006^{cA}	0.111±0.004^{cA}	0.106±0.009^{cA}	0.107±0.003^{cA}
Source of variation			Sample (T)	Days (P)	T x P	
SEm			0.01	0.01	0.03	
CD (0.05)			0.03	0.03	0.08	
CV %			37.5			

The values are mean of three replications.
$^{a-e}$: values with different letters between a row are significantly different at 5% (i.e., $p<0.05$).
$^{A-E}$: values with different letters between a column are significantly different at 5% (i.e., $p<0.05$).
CMP: Camel milk powder, GMP: Goat milk powder; COM: Cow milk powder

Table 6. Amount of FFAs released during storage in different milk powders.

Time (min)	Amount of FFAs released (µmol/ml)											
	Camel (CMP1)			Goat (GMP1)			Cow (COM1)			Cow (COM2)		
	0 days	45 days	90 days	0 days	45 days	90 days	0 days	45 days	90 days	0 days	45 days	90 days
0	12.80	13.99	15.17	13.31	12.97	14.16	15.51	16.19	16.36	14.33	14.16	14.66
30	21.44	24.33	26.87	18.73	20.60	21.61	23.14	27.72	29.24	18.90	19.75	20.60
60	33.48	37.38	38.90	26.53	25.51	26.36	43.14	44.16	49.58	22.46	24.83	24.83
90	37.38	40.60	45.00	33.48	32.80	35.34	46.53	52.97	54.66	27.72	27.55	27.55
120	45.68	49.07	51.78	47.38	46.53	50.09	58.73	61.11	61.11	31.61	34.49	34.66
150	48.39	50.60	53.82	52.46	54.33	56.53	59.92	61.78	62.97	34.16	34.83	35.68
180	52.12	55.34	60.60	56.70	61.11	62.29	63.48	65.17	66.53	35.34	36.53	36.19
210	62.80	63.14	64.16	60.60	63.14	63.82	66.70	67.38	67.55	37.04	37.72	37.38

powder has the higher rate of digestion as compared to goat milk powder and cow milk powder (COM2). The camel milk has relatively lower fat globule size

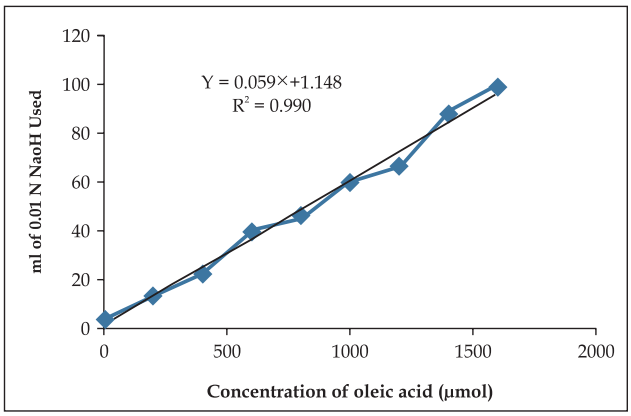

Fig 1. Standard curve for oleic acid with concentration ranging (200-1600 µmol) for free fatty acid release during digestion.

(3.0- 3.2 µm) as compared to that of goat milk (3.5-3.7 µm) and cow milk (4-6 µm). Berton *et al* (2012) indicated that surface-to-volume ratio of small-size fat globules is higher in smaller the globule leads to the faster digestion. They investigated the effects of size and interface composition on the ability of human pancreatin enzyme to digest native and homogenised milk fat. They discovered that smaller native fat globules, measuring around 1.6 mm, were more responsive to pancreatic lipase than were bigger native globules, measuring 3.9 mm. This implied that smaller fat globules would digest more quickly since they had a bigger surface area for the action of digestive enzymes than do larger fat globules (Singh *et al,* 2009, Berton *et al,* 2012, Meena *et al,* 2014). Homogenised and UHT milk is digested more quickly than raw milk because the MFGM is dislodged and casein micelles accumulate as a result of mechanical

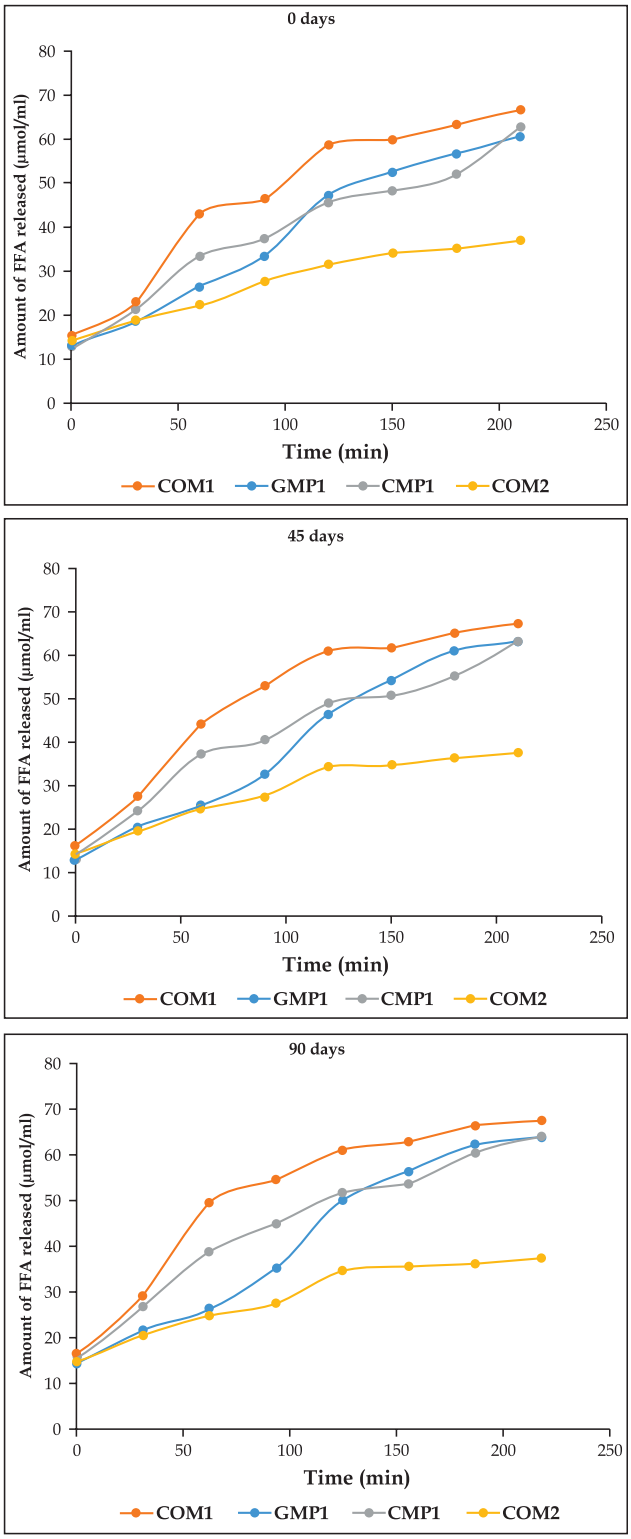

Fig 2. *In vitro* fat digestion of milk powder in terms of release of FFAs during storage.

shearing and heating, respectively. This is because the TAG core is more accessible to the digestive enzyme, resulting in quicker digestion. According to Meena *et al* (2014), camel, goat, cow and buffalo had the lowest rates of milk fat digestion, with 146.22, 136.83.3, 118.5 and 102.5.31 mol/ml, respectively. This showed that camel and goats released free fatty acids more quickly than cows and buffaloes. However, cow milk fat was released more free fatty acids than buffalo milk fat. This variation in free fatty acid concentration and release between species was related to differences in fat globule size. The other parameters such as milk fat globule size, composition of milk fat globule membrane and amount of adsorbed proteins on the milk fat globule membrane will also affect the effectiveness of digestive lipases (Garcia *et al*, 2014).

Conclusions

The monitoring the oxidative deterioration, antioxidant capacity as well as *in vitro* digestibility on camel, goat and cow milk powder during storage are important aspect to understand the nature of the dried products. Camel milk powder showed higher antioxidant capacity which ultimately affect the oxidative changes in the stored product. The rate of fatty acid release was relatively higher in camel milk powder as compared to that of goat milk powder due to smaller size of fat globule in camel milk. However, there is need of further study on the effect of various drying conditions as well as interaction of fat with other milk components during processing and storage on oxidative stability and digestibility of the camel milk powder.

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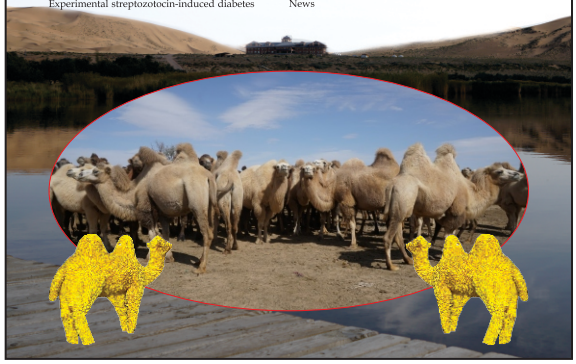

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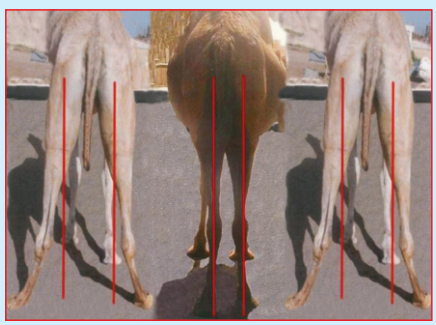

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EFFECT OF ROUGHAGE REPLACEMENT IN CAMEL DIET WITH TANNIN CONTAINING TREE LEAVES ON DIGESTIBILITY AND NUTRIENT INTAKE OF LACTATING CAMEL

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ABSTRACT

This study investigated the effect of roughage replacement in camel diet with tannin containing tree leaves on digestibility, nutrient intake and water intake. Fifteen lactating camels (average BW 554 kg) in mid lactation stage were used to study the impact of tree leaves. Animals were blocked by weight and milk production into 3 groups, where these were offered roughage to concentrate ratio of 70:30. Roughage components included crop residues viz. groundnut straw (GS), guar phalgati (GP) and khejri leaves (KL)/pala leaves(PL) in different ratio. Group T_0 fed with GS and GP ratio of 50:50, Group T_1 fed with GS, GP and KL ratio of 40:40:20 and Group T_2 were offered GS, GP and PL ratio of 40:40:20. Results revealed that DM and OM consumption was lower ($P<0.5$) in camels fed 20% tree leaves as compared to other groups, but an enhanced CP digestibility referred to economic usage of forage biomass in arid and semi-arid ecology.

Key words: Arid zone, roughage, digestibility, nutrient intake, tree leaves

Livestock production in many tropical environments is negatively affected by low feed availability and quality (Sahoo and Karim, 2011). Shortage of feed is evidenced during the prolonged dry season in arid and semi-arid areas. Camel, a unique animal species of desert ecosystem, is adapted to sustain on a variety of feeds and fodders like grasses, tree leaves, crop residues and agro-industrial byproducts (Nagpal *et al,* 2003; Sahoo and Sawal, 2021). In general, an increase in the productivity of ruminants can be achieved by improving environmental factors like management, nutrition and health care. Several indigenous browse species growing in fallow lands are commonly used by smallholder farmers as a roughage source for ruminants. Quantitative information on biomass production and chemical composition of forages from tree/browse species of arid and semi-arid regions of India has been documented (Sharma and Sahoo, 2017). Pala (*Zizyphus mauritiana*) and Khejri (*Prosopis cineraria*) trees are native to desert ecosystem and form natural feeds of camel but feed intake is often limited due to presence of some anti-nutritional factors such as tannins, saponins, alkaloids, oxalates and flavonoids (Abo-Donia and Nagpal, 2015). The Pala leaves are readily eaten by camels (Poonia *et al,* 2022) and contain 12.5-16.9% CP, 13.9-17.1% CF, 1.5-2.7% EE, 10.2-11.7% ash and 55.3-56.7% NFE (Abdu *et al,* 2007). Similarly, Khejri leaves are also nutrient rich and preferred by livestock of arid and semi-arid climatic regions (Sharma and Sahoo, 2017). However, little information exists on livestock performance on combination of roughages involving crop residues and available tree/browse plants of the region. There is deficit water availability in the arid climatic region, which may have variable adverse effect on livestock performance. But, camel seems to sustain production even during prolonged period of water scarcity (Sahoo, 2020). It is thus hypothesised that replacement of conventional dry roughages with tannin containing tree leaves in the diet of lactating camel may have a modulatory effect on nutrient digestibility feed and water intake.

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Materials and Methods

Animals

Fifteen lactating camels in mid lactation were divided into 3 groups of 5 each, based on comparable milk yield, body weight, number of lactations completed and days in lactation. These were housed in individual stalls and provided with uniform management practices except feeding. Three dietary combinations were prepared by substituting conventional roughage groundnut (*Arachis hypogaea* L.) straw (GS) and guar (*Cyamopsis tetragonoloba* L.) phalgati (GP) with khejri (*Prosopis cineraria*) leaves (KL) and pala (*Zizyphus mauritiana*) leaves (PL). The control (Group T_0) received a standard diet of roughage (GS:GP at 50:50 ratio) and concentrate in the ratio of 70:30 to meet the nutrient requirements as per ICAR (2013). The treatment group T_1 received a similar ration with a varied roughage combination involving KL to feed GS, GP and KL at 40:40:20 ratios and T_2 involving PL to feed GS, GP and PL at 40:40:20 ratios. The animals were offered with measured quantity of feed and *ad libitum* water. A digestibility trial was conducted at the end of 180 days experimental feeding to assess intake and digestibility of nutrients. Daily water intake was also recorded during the trial.

Crop fodder, khejri leaves, pala leaves and different concentrates were analysed for proximate principles (AOAC, 2005), fibre fractions (Van Soest *et al,* 1991) and total tannins (Makkar, 2003).

Statistical analysis

Statistical analysis was performed using SPSS version 24 (SPSS Inc., Chicago IL). The effects of tree leaves feeding on parameters were analysed by ANOVA with the Tukey post hoc test.

Results and Discussion

Chemical composition of crop fodder, khejri leaves, pala leaves and concentrates has been given in table 1. Observations on the chemical composition revealed that values of OM CP and EE of tree leaves obtained in the present study were also reported by Nagpal *et al* (2017), Abo-Donia *et al* (2015) and Santra and Karim (2019). The total tannins content in khejri leaves was higher compared to pala leaves, while it is negligible in the conventional crop residue,s groundnut straw and guar phalgati used in the feeding of camel. Similar concentration of tannins in these tree leaves was also observed earlier (Raghuvansi *et al,* 2007; Pal *et al,* 2015; Aderao *et al,* 2019). The possible reason of variability in chemical composition of tree leaves might be due to environmental conditions like soil nutrients, water availability, temperature, leaves maturation stages and season.

Intake of DM and OM was lower ($P<0.5$) in camels fed khejri leaves compared to control, which might be attributed to the effect of tannins on voluntary feed intake. On the contrary, a less tannin content in pala leaves had less adverse effect on its intake. Similar findings were reported by Olafadehan *et al* (2014), Aderao *et al* (2020) and Bhatt *et al* (2020). A decrease in consumption of DM in T_1 indicated that the palatability of khejri leaves was lower than the crop fodder alone, while the pala leaves exhibited moderate palatability. Fernandez *et al* (2012) also observed that intake of tannin-containing supplements were lower than intake of supplements without tannin ($P<0.01$) in sheep. The level of condensed tannins higher than 50 g/kg DM significantly ($P<0.05$) reduced voluntary feed intake in ruminants while medium or low levels seemed to have a minor effect (Frutos *et al,* 2004; Singh and Sahoo, 2004). Frutos *et al* (2004) found that when sheep were fed 8 g of tannic acid per kg live weight, their voluntary feed intake fell drastically after 24 h (from 18 to 2.5 g DM/kg LW). Three mechanisms have been suggested to explain the negative effects of high tannin concentrations on voluntary feed intake viz. i) a reduction in feed palatability; ii) slowing of digestion; and iii) the development of conditioned

Table 1. Chemical composition (% DM basis) of crop fodder, khejri leaves, pala leaves and concentrate.

Particulars	OM	CP	EE	TA	NDF	ADF	Tannins
Groundnut straw	89.30	8.36	1.24	10.70	44.03	28.67	0.42
Guar phalgati	88.05	7.50	0.97	11.95	60.41	40.96	0.23
Khejri leaves	87.04	15.60	3.10	12.96	37.76	30.62	8.56
Pala leaves	89.60	11.80	2.90	11.40	34.23	29.57	2.69
Concentrate 1	89.79	12.12	1.33	10.21	40.22	20.34	-
Concentrate 2	88.74	10.47	1.93	11.26	40.02	20.56	-
Concentrate 3	89.12	11.89	1.62	10.88	40.67	20.33	-

aversions (Olafadehan *et al,* 2014). A similar intake of CP in camels fed 20% khejri leaves (KL) or pala leaves (PL) mix diet compare to control group on conventional roughage alone was in line with the treatment design. A lower (P<0.05) intake of ADF and NDF in T1 can be linked to lower DM intake and a reduced level of ADF and NDF in khejri leaves compared to groundnut straw and guar phalgati. But, between T_1 and T_2, the difference in DM and nutrient intake was non-significant, while the values of DM, OM, ADF and NDF intake in T_2 were closer to that in the control group. The observed differences in nutrient intake other than CP were in line with altered feed intake.

Table 2. Average nutrient intake in different treatment groups.

Attributes	T_0	SEM	T_1	SEM	T_2	SEM
DM (kg/d)	10.67^b	0.167	9.43^a	0.177	10.06^{ab}	0.151
OM (kg/d)	9.58^b	0.133	8.37^a	0.119	8.97^{ab}	0.134
CP (g/d)	394	10.4	407	11.2	400	9.4
EE (g/d)	17.2	0.18	17.3	0.10	17.2	0.19
ADF (kg/d)	5.84^b	0.272	4.62^a	0.137	5.19^{ab}	0.131
NDF (kg/d)	7.15^b	0.107	6.57^a	0.122	6.84^{ab}	0.111

Values with different superscript in a row are significantly different.

The digestibility of DM, OM, EE, NDF and ADF was similar (P>0.05) in all the 3 groups (Table 3). But, there was significant increase in digestibility of CP in both the tree leaves fed groups compared to control. It might be due to higher tannin contents of tree leaves that contributed to improved protein utilisation. Aderao *et al* (2020) observed a higher total tannins and condensed tannins (CT) content in khejri leaves and they also observed improved nitrogen (NDF) utilisation due probably attributed to ruminal protection of protein against degradation and an increased microbial protein synthesis. Bhatt *et al* (2020) also observed increased utilisation efficiency with a reduced excretion through urine and higher rumen microbial protein synthesis. The apparent digestibility of fiber showed a different pattern, NDF and ADF showed a decline in T_1, but not significantly different from T_0 and T_2, whereas hemicellulose and cellulose showed a reverse trend (Table 3). The cellulose digestibility was higher in tree leaves fed groups compared to control, which may be linked to possible increase in microbial protein synthesis as highlighted by Aderao *et al* (2020) and Bhatt *et al* (2020). On the contrary, T_1 had lower (P<0.05) hemicellulose digestibility compared to T_0, but between T_1 and T_2, it was non-significantly different. Raghuvansi *et al* (2007) observed higher hemicellulose digestibility and no effect on NDF and cellulose digestibility due to inclusion of tree leaves in the diet. Yang *et al* (2009) also reported non-significant effect of tree leaves on DM, OM and NDF digestibility. The results of ADF digestibility get support from the findings of Dida *et al* (2019) who also found non-significant effect of tree leaves on ADF digestibility in goat. Earlier, Bhatta *et al* (2005) reported that tannins do not have any significant effect on ADF digestibility.

Table 3. Digestibility of nutrients in different groups.

Attributes	Treatments			SEM
	T_0	T_1	T_2	
DM	69.20	66.88	65.21	2.22
OM	72.29	70.03	68.12	2.27
CP	72.16^a	81.91^b	79.58^b	1.14
EE	68.47	73.81	70.74	3.54
ADF	55.76	51.24	55.13	2.48
NDF	56.75	52.75	55.79	2.97
Hemicellulose	80.60^a	67.96^b	75.81^{ab}	2.04
Cellulose	71.17^a	78.87^b	79.50^c	1.65

Replacement of conventional roughage (crop residue fodder) with tannin containing tree leaves reduced the voluntary feed intake, but significant improvement in CP digestibility without any adverse effect on digestibility of DM, OM and fibre components can be inferred as positive outcome as it enhances the usage of locally available tree forages for sustaining production. A reduced feed intake may be an added advantage to sustain productivity with efficient use of scarce forage biomass in a desert environment.

Acknowledgement

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DIFFERENT TREATMENT PROTOCOLS FOR ENDOMETRITIS IN CAMELS

A total of 112 dromedary she-camels with uterine infection were subjected to uterine swabbing for bacterial culture and received one of the following treatments, i.e., Uterine douching with lotagen every other day for three doses or Single parenteral oxytetracycline injection, or Subcutaneous injection with ceftiofur for five days, or Combined oxytetracycline-ceftiofur injection.

The results showed that *Escherichia coli* was the most isolated bacteria, followed by Streptococcus species. Treatment efficacy was ($P < 0.05$) higher in ceftiofur and oxytetracycline-ceftiofur protocols compared with lotagen and oxytetracycline protocols. The fertility indexes, services per conception, and pregnancy rate were improved in ceftiofur and mixed oxytetracycline plus ceftiofur protocols as the pregnancy rate was ($P < 0.05$) higher in those protocols compared with lotagen and oxytetracycline protocols (71.4 and 67.9% vs. 39.3 and 42.9%, respectively). On the other hand, the number of services per conception was significantly lower in ceftiofur and oxytetracycline-ceftiofur protocols (1.2 for each protocol) than in lotagen and oxytetracycline protocols (1.8 and 1.7, respectively). In conclusion, subcutaneous injection of 1 ml ceftiofur per 50 kg body mass for five days can efficiently treat uterine infection in female dromedary camels caused by *E. coli* and Streptococcus species for improving their fertility indexes.

(Source: Zaher, Hany Ahmed and Al-Fares, Abdullah F. and Mesalam, Ayman, Efficacy of different treatment protocols for endometritis in *Camelus dromedarius,* Frontiers in Veterinary Science,2023; Vol 10. DOI 10.3389/fvets.2023.1136823)

ISOLATED BACTERIA FROM THE UTERI OF CAMELS

The uterine samples from 856 dromedary camels yielded a total of 17 different bacterial species, with a higher proportion of sub-fertile camel uteri being colonised by bacteria (66.6%) as compared to nulliparous, recently calved, and those with unknown reproductive history combined (44.2%; $p < 0.05$). Camels with body condition scoring < 3 and those with a consistently echogenic appearance of the uterine lumen by sonography were more likely to be positive on uterine culture. In contrast, the presence of pus in uterine discharge was not associated with the odds of bacterial isolation ($p > 0.05$). While certain strains were more likely to be obtained from the uteri of the sub-fertile group ($p < 0.05$), embryo transfer to camels with a positive uterine culture in the absence of other gross reproductive pathologies did not necessarily affect the overall pregnancy rate compared to recipients with a negative uterine culture ($p > 0.05$). In conclusion, a relatively high bacterial load can be identified from the uteri of sub-fertile and normal dromedary camels, with a higher frequency among the former.

(Source: Asadi B, Seyedasgari F, Ashrafi Tamai I, Yarmohammadi M, Ebadi R, Kim E, Barin A. Isolated Bacteria from the Uteri of Camels with Different Reproductive Backgrounds: A Study on Sampling Methodology, Prevalence, and Clinical Significance. Vet Sci. 2023 Jan 5;10(1):39. doi: 10.3390/vetsci10010039..)

SEASONAL VARIATIONS OF VITAMIN A, D AND E LEVELS IN SERUM OF FEMALE CAMELS AND THEIR CALVES IN SAUDI ARABIA

Sixty sera samples were collected and tested for vitamins A, D, and E levels, and the results were statistically analysed. The statistical mean value of vitamin A was within the reported range, but for D and E, there were minor variations. The effect of season was insignificant ($p > 0.05$) for vitamins A and E in the combined results of the dam and newborn. This seasonal effect was highly significant in dam serum ($p < 0.05$). Region effect was significant for vitamin A in the northern area ($p < 0.05$) and for vitamin E in the southern region ($p < 0.05$). Correlations analysis revealed significant results in the season vs. vitamin A and E $p < 0.05$. Mean values of vitamins A, D, and E in dam and newborn did not observe significant variations; however, in the season and regions, there were significant variations which can be attributed to the climate difference, availability of balanced rations, and camel management in each location of the five main regions of Saudi Arabia.

(Source: Abdullah HM, Almathen F, Sheikh A, Alfattah M, Khalid AM, Bakhiet AO, Abdelrahman MM. Seasonal variations of vitamin A, D and E levels in serum of female camels (*Camelus dromedarius*) and their calves raised in five geographic regions of Saudi Arabia. Saudi J Biol Sci. 2023 Jun;30(6):103675. doi: 10.1016/j.sjbs.2023.103675)

COMPARISON BETWEEN TRANS-VAGINAL AND RECTO-VAGINAL TECHNIQUES FOR TRANSFERRING EMBRYOS IN THE DROMEDARY CAMELS

The present study was conducted in two breeding seasons to compare the effect of two non-surgical techniques for embryo deposition intrauterine, trans-vaginal (TV) versus recto-vaginal (RV) techniques, on the pregnancy rate and early pregnancy loss (EPL) in dromedary camels. Embryos were collected from 70 donors and transferred to 210 recipients by TV (n = 256 transfers) or RV technique (n = 186 transfers). Pregnancy diagnosis was conducted on Day 10 after embryo transfer (ET) by using the progesterone-ELISA test and by trans-rectal ultrasonography at Day 60 of gestation. EPL was calculated as the recipients that were diagnosed pregnant on Day 10 post-ET and lost their pregnancy between Days 20–60 of their gestation. Using the RV technique in ET of a single embryo showed higher pregnancy rates at Day 19, especially with the embryos of folded, semi-transparent shapes, or those collected after superovulation with the recovery of >4 embryos per flush. While, the pregnancy rates at 60 days showed increases after ET with the RV technique of single, folded, transparent, and semi-transparent, medium-size embryos and/or those collected after superovulation with any number of the recovered embryos than those transferred by the TV technique. The rate of EPL was increased when the TV technique was used for ET of single, spherical, folded, semi-transparent, medium-sized embryos and those collected without or with the superovulation and recovery of >4 embryos per flush. In conclusion, using the RV technique to deposit the embryos intrauterine improves the pregnancy rate and reduces EPL compared to the TV technique.

(Source: Aly Karen, Nabil Mansour, Comparison between trans-vaginal and recto-vaginal techniques for transferring embryos in the dromedary camels, Theriogenology, 205, 2023, Pages 130-136, ISSN 0093-691X, https://doi.org/10.1016/j.theriogenology.2023.04.020.)

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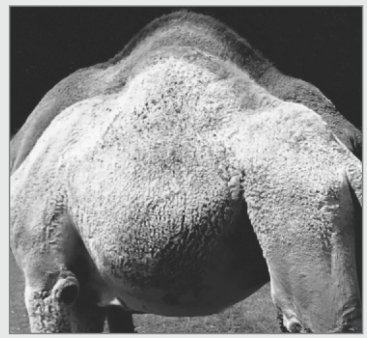

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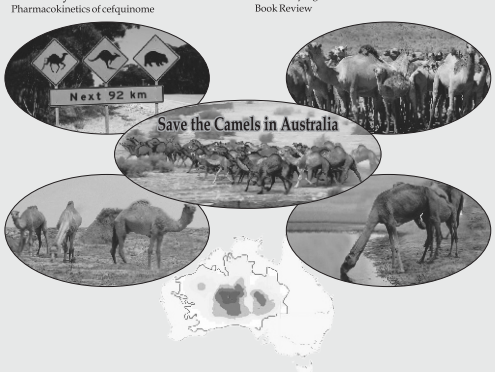

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