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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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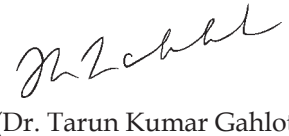
ROLE OF CAMELS IN FOOD SECURITY IN ARID AND SEMIARID REGIONS

The growing population of the world is projected to reach 9.7 billion by the year 2050. The priorities of the 21st century are food and water security which cannot be achieved without sustainable and intensive agricultural production. There is a continuously rising trend of number of camels in the last few decades and the population is estimated to be approximately 40 million head of Bactrian and dromedary camels and this may go above 60 million in 25-years' time (Faye, 2020). In parallel with the number of animals, the world's annual camel milk production has also increased from 0.63 million tonnes in 1961 to 3.15 million tonnes in 2020 (FAO, 2020; https://www.fao.org/faostat/en/#data/QCL); this is a 5-fold increase over the 60-yr period. With this quantity, camels are the fifth most important dairy animals. Although most camels are kept in developing countries under pastoral, extensive, or semiintensive systems, well-planned intensification might potentially help the further development of the species and its integration into the food production chain. In order to make the milk residue free, the controlled use of veterinary drugs under the supervision by a qualified veterinarian and regular monitoring is required (Nagy *et al,* 2022).

(Faye, B. 2020. How many large camelids in the world? A synthetic analysis of the world camel demographic changes. Pastoralism. 10:25. doi: 10.1186/s13570-020-00176-z and Nagy PP, Skidmore JA, Juhasz J. Intensification of camel farming and milk production with special emphasis on animal health, welfare, and the biotechnology of reproduction. Anim Front. 2022 Aug 12;12(4):35-45. doi: 10.1093/af/vfac043)

The current issue of the Journal of Camel Practice and Research has brought 4 review papers, i.e. MVA-based vaccine efficacies on the immune response and seroprevalence of MERS-CoV, methanogenesis and superovulation in dromedary camels, and parasitic infections of Bactrian camels in Mongolia. These review papers would not only help researchers in continuing research but would also enrich the literature and citations on the diverse topics and research being discussed in those papers. Another interesting research has come up on use of camel milk whey proteins with cytotoxic potential against cervical cancer cell line; this antineoplastic attribute of camel milk protein will be greatly appreciated, if positive results are achieved. Good research on pathology involving pulmonary lesions and cutaneous histoplasmosis in dromedary camels and immunological study in Bactrian camels on serological diagnosis of *Parabronema skrjabini* infection using a recombinant antigen also finds space in this issue. The pharmacokinetics and pharmacodynamics of betamethasone (phosphate and dipropionate), triamcinolone acetonide and hydrocortisone; molecular characterisation of diacylglycerol o-acyltransferase (dgat1) gene in camels marks important study of this issue. Cheese made from mixed milk of camel and goat, constituents of follicular and oviductal fluid, ultrasonographic assessment of follicular size, ultrastructure of pelvic urethral gland, prosthodontic application of acrylic polymer in buccal fistula and dexmedetomidine as sedative are other notable research published in the April 2023 issue of JCPR.

I am sure that all camel researchers and practitioners would continue their support for JCPR in future as well.



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REVIEW OF PARASITIC INFECTIONS OF BACTRIAN CAMELS IN MONGOLIA

Tserendorj Munkhjargal[1], Boldbaatar Chinchuluun[1], Sukhbaatar Lkhagvatseren[1], Sambuu Gantuya[1], Gonchigoo Battsetseg[1], Zayat Batsukh[1] and Rolf K. Schuster[2]

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ABSTRACT

Mongolia is home to 454,000 domesticated two-humped camels (*Camelus bactrianus*) and several hundred wild Bactrian camels (*Camelus ferus*) but knowledge about the diversity of camel infectious pathogens in this country is still limited. In this article, all studies carried out on camel infectious diseases in Mongolia are reviewed. More than 100 documents published between 1936 and 2022 were systematically analysed to get information on this topic. In this communication we review articles that dealt with parasitic infections of Bactrian camels in Mongolia such as trypanosomosis, coccidiosis, toxoplasmosis, sarcocystosis, piroplasmosis, hydatidosis, trichostrongylidosis, parabronemosis, dipetalonemosis, tick infestation, sarcoptic mange, myiasis and vermipsillosis. The results showed that parasititoses are an issue in Mongolia in terms of economics and public health.

Key words: *Camelus bactrianus,* infectious diseases, prevalence, Mongolia

Mongolia is a landlocked country in East Asia covering a territory of 1.5 million km^2 and a population of just three million people. According to the Food and Agriculture Organisation of the United Nations, there were 57.5 million small ruminants, 5 million cattle, 4 million horses and more than 450 thousand Bactrian camels in Mongolia (Fig 1) (FAOSTAT, 2021). Camel husbandry in Mongolia is practiced primarily by pastoralists in the Gobi Desert. Camels produce milk, wool and meat and are also used for racing and less commonly now, as a beast of burden.On the other hand, camels are carriers of zoonotic pathogens and sources of infection for humans, livestock and wildlife in Mongolia.

Outbreaks of camel diseases between 1947 and 1966 occurred in the Gobi Desert and steppe regions of Mongolia with clinical findings such as ataxia and intractable diarrhoea but the aetiology was not determined. Hundred-thousands of camels were affected in these disease outbreaks and more than 90,000 camels died (Erdenebileg, 2001).

In this paper, the parasitic diseases of Bactrian camels in Mongolia are outlined by focusing on the aetiology of diseases and infections and determining their distribution pattern in order to show camel's public health importance in Mongolia.

Materials and Methods

Relevant studies were reviewed through Medline (PubMed), ISI Web of Scopus and Google Scholar. These were systematically searched to find all publications from Mongolia using the keywords of "Bactrian camel", "Infectious disease" and "Mongolia". The retrieved papers and books that reported camel diseases with major public health importance were included in the present study. More than 100 documents published between 1936 until late 2022 were analysed. The included papers were written in English, Russian and Mongolian languages.

Results

Protozoal infections

Surra

Camel trypanosomosis (surra) is caused by *Trypanosoma evansi*. This extracellular blood parasite is mechanically transmitted by haematophagous dipterea such as tabanids, stomoxes and hippobosces. Bactrian camels were suspected of having surra in the western part of Mongolia, but little information is available. Several serological studies on non-tsetse transmitted trypanosomoses were carried out in horses from all districts in Mongolia. Since serological methods do not allow to differentiate between infections with *Trypanosoma equiperdum* (dourine)

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and *T. evansi*, the presence of surra in Mongolia cannot be ignored. Thus, Clausen *et al* (2003) examined 1122 horse sera and detected CFT and ELISA seroprevalence of 7.6 and 6.7%, respectively with only one stallion showing typical clinical signs of dourine. The isolation of *T. equiperdum* from the urethral tract of a stallion in the Töv province by Suganuma *et al* (2016) was the first prove for the presence of dourine in Mongolia. Davaasuren *et al* (2017) reported an outbreak of a horse disease on a farm in Ulaanbaatar with one stallion showing typical signs of dourine and a total of 46% of the 50 horses gave positive serological (ELISA) results. Molecular examination revealed that beside typical *T. equiperdum* another closely related trypanozoon was present on the farm. In a nationwide survey on horse trypanosomosis with an rTeGM6-4r-based immunochromatographic test, Mizushima *et al* (2018) tested 1701 equine sera and detected 42 positives. Later, Mizushima *et al* (2020) used a rTeGM6-4r-based ELISA, which was applied for surra against cattle and water buffalo and dourine against horse. This test revealed that the overall sero-prevalence of non-tsetse transmitted trypanosomoses in Mongolia was 4.8%. It is interesting that the sero-prevalence in horses in two Gobi provinces (Ömnögovi, Govi-Altai) with high Bactrian camel populations was 11.0 and 10.4%. In order to clarify the role of Bactrian camels in the epidemiology of surra, specific investigations have to be carried out.

Coccidiosis

Coccidiosis of camels is an intestinal protozoan infection caused by apicomplexan parasites of the genus *Eimeria*. *Eimeria* spp. are gut-dwelling intracellular parasites, transmitted by faecal-oral route; oocysts are shed from infected animals and sporulated oocysts are then ingested via contaminated feed or water. Examination of faecal samples of camels from three Mongolian provinces, namely Bayanhongor, Övörkhangai and Ömnögovi revealed the presence of *E. rajasthani*, *E. dromedarii* and *E. cameli* in a prevalence of 40.8, 33.5 and 19.7 %, respectively (Khatanbaatar *et al,* 2017). Mixed-*Eimeria* spp. infections were more frequently (31.6 %) observed than mono infections.

Toxoplasmosis

Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii*, which infects warm-blooded animals including human and livestock as an intermediate host. The cat and other felids are the only final host for this parasite.

Little is known about Toxoplasma in Bactrian camels and there is only one serological study from Qinghai Province, in northwestern China where seven out of 234 serum samples from Bactrian camels were positive for *Toxoplasma* antibodies in a commercial indirect haemagglutination test at a cut-off of 1:64. A study of *T. gondii* DNA in Bactrian camel milk revealed 5 out of 9 examined camels from the

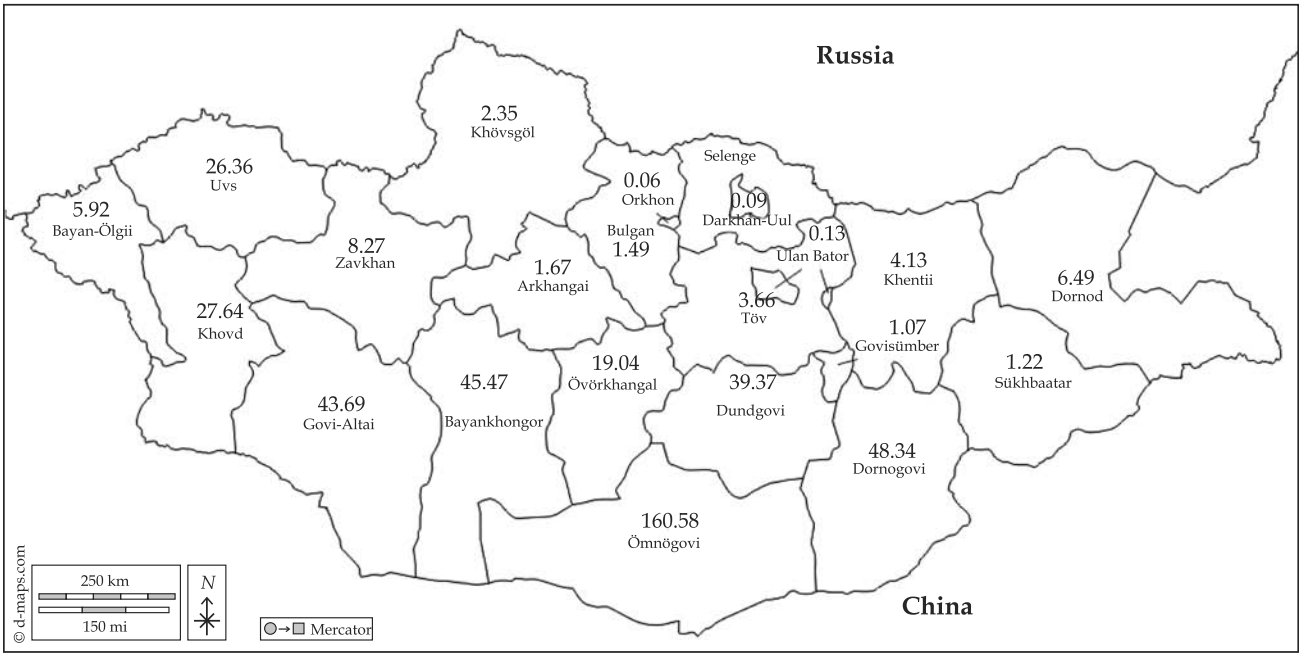

Fig 1. The number of Bactrian camels (in thousands) categorised by provinces according to the National Statistics Office of Mongolia. https://en.nso.mn/ 2022.

Ömnögovi province positive (Iacobucci, 2019). The presence of Toxoplasma types I, II and III linages was suggested.

Sarcocystiosis

Sarcocystosis is a diheterooxenic protozoan infection that is usually asymptomatic for the herbivorous intermediate host. A survey of *Sarcocystis* infection in farm animals (cattle, yak, hainag, sheep, horse and camel) was conducted in Mongolia between June 1998 and July 1999. Compressed muscle samples from the diaphragm, heart, tongue, esophagus and intercostal region were examined under the microsope. In 5 camels from Zawkhan Province, muscle micro cysts of *Sarcocystis* sp. were detected (Fukuyo *et al,* 2002). The species was not determined. Three species of *Sarcocystis* that form microcysts in the muscles of dromedaries are mentioned in the literature (Wernery *et al,* 2014). One species of muscle macrocysts was found in camels in Kazakhstan (Kuraev, 1981).

Piroplasmosis

Babesiosis and Theileriosis are caused by intraerythrocytic protozoan parasites that are transmitted by ticks. Although, Bactrian camels are not described as hosts of *Babesia* and/or *Theileria* species, no confirmed information is available about camel babesiosis due to the lack of experimental infections. Svoboda *et al* (2011) examined horses, camels and dogs in Khentii province for the presence of *Babesia* and *Theileria* and found 67% of horse blood smears are positive for *Theileria equi*. *T. equi* DNA was detected in 93% of these horses but camels and dogs were negative, both in blood smears and DNA detection.

Molecular examination of 305 blood samples of Bactrian camels from 6 Mongolian provinces showed the presence of DNA of 3 different *Babesia* species, *Babesia bovis*, *B. bigemina*, and *Babesia* sp. Mymensingh, with prevalences of 32.1%, 21.6%, and 24.3%, respectively and 52.5% of the surveyed animals harboured DNA of at least one of these species (Otgonsuren *et al,* 2022). Despite this high prevalence, so far, *Babesia* development stages have not been demonstrated in blood smears of Bactrian camels and no disease was reported. The DNA of these *Babesia* species had previously been reported from bovines in Mongolia (Otgonsuren *et al,* 2020). In Mongolia, cattle and Bactrian camels usually share common pasture lands for grazing and tick species infesting cattle use also Bactrian camels as hosts and inoculate *Babesia* sporozoites DNA which were then detected in the camel blood. In a molecular study on the role of ticks as vectors for tick borne diseases, Narankhajid *et al* (2018) examined ticks from camels in the Gobi Altai province and detected *Babesia* DNA in 17 out of 64 specimens. *Babesia* positive ticks belonged to *Dermacentor nuttalli, De. marginatus* and *Rhipicephalus asiaticum*. The role of Bactrian camels in the epidemiology of babesiosis remain unclear. Finding of DNA alone is not an indicator that the host is involved in the life cycle.

Helminth infection

Scientists of the Soviet Union started to investigate the helminth fauna of Mongolia in the first half of the last century and the results obtained by the Mongol Agricultural Expedition of the Academy of Science of the USSR from 1947 to 1952 were published by Ivashkin (1955). Later Sharhuu (1986) continued research in this field and proposed control programmes. In Mongolia a total of 21 helminth species were listed for Bactrian camels. *Dipetalonema evansi*, *Dictyocaulus cameli* and *Nematodirella cameli* were camel specific and were reported for Bactrian camels in Mongolia for the first time (Sharhuu and Sharkhuu, 2004).

Orientobilharziosis

The schistosome *Orientobilharzia tukestanica* was described by Skrjabin (1913) from a cow in Kazakhstan. *O. turcestanica* inhabits blood vessels of intestines and livers of ruminants as main final hosts, and pulmonate snails (*Radix auricularia*) act as intermediate host. In Mongolia, this trematode species was first mentioned by Shumakovic (1936). Apart from Bactrian camels Sharhuu and Sharkhuu (2004) named cattle, sheep and goat as hosts. Final hosts become infected when wading in shallow waters or at drinking when water is infested with cercariae. In humans, *O. turcestanica* cercariae can cause cercarial dermatitis (Wang *et al,* 2009).

Echinococcosis/ Hydatidosis

Echinococcosis is a zoonotic disease where canids act as final hosts and herbivores and omnivores including humans are the intermediate hosts. Already Shumakovic (1936) and Ivashkin (1955) mentioned the presence of *Echinococcus granulosus* Bactrian camels in Mongolia without giving data on the distribution of the parasite. A more recent study carried out in the Ömnögovi district found 22 (= 30.5%) out of 72 slaughtered camels infected with hydatid cysts. A low number of cysts (1–3) was recovered mainly from the lungs (n=12) and less frequent from livers (n=6). Mixed infections of livers

and lungs were seen in four animals (Chinchuluun *et al,* 2018). Bold *et al* (2019) concluded that human cystic echinococcosis is significantly related to camel density. Nineteen (19.8%) out of 96 slaughtered camels in the Ömnögovi district had hydatid cysts. On the other hand, the Ömnögovi district had the highest incidence of cystic echinococcosis in humans in Mongolia (8 cases per 100,000).

Based on molecular peculiarities, the former species *E. granulosus* was split into different strains that had become five independent species of which *E. granulosus* sensu stricto (G1-3) and *E. canadensis* (G6-10) were found in human cases from Mongolia (Jabbar *et al,* 2011; Ito *et al,* 2014). Based on recent findings, Ito and Budke (2015) drew a map on the distribution of 85 human cystic *Echinococcus* cases in the country. Of these, 27 cases, mainly *E. canadensis* (G6/7) but also *E. granulosus* sensu stricto (G1) were diagnosed in Gobi districts where most of the Bactrian camels are kept.

Little is known about echinococcosis in final hosts in Mongolia. In nomadic husbandry systems, dogs play a major role as a direct source for human infections. Thus, Zoljargal *et al* (2001) found 17 (25.4%) out of 67 dogs in the Govi Altai positive in a copro-antigen test and in a second study, 5 out of 14 necropsied dogs had a patent *Echinococcus* infection in the Bulgan province (Wang *et al,* 2009). With regards to possible final hosts amongst wild canids, 118 wolfs were examined in northern Mongolia. Of these, two harboured the G6/7 strain and three were infected with the G10 strain of *E. canadensis* (Ito *et al,* 2014).

Trichostrongylidosis

According to Sharhuu and Sharkhuu (2004), the following trichostrongylid species were found in Bactrian camels in Mongolia: *Marshallagia mongolica, M. skrjabini, Ostertagia dahurica, O. orloffi, Teladorsagia kasachstanica, Trichostrongylus colubriformis, T. probuluris, Cooperia surnabada, C. oncophora, Nematodirus oiratianus and N. cameli.* Most of these species were found at necropsies carried out by Sharhuu (1986).

Dipetalonemosis

Dipetalonemosis in camels caused by *Dipetalonema evansi*, a filarial nematode of the family of Onchocercidae, is considered an economically significant mosquito-borne disease. The life cycle of *D. evansi* was studied by Katajceva (1969) in Turkmenistan and *Aedes caspius* was found as intermediate host and vector. It is quite possible that also other mosquitos are involved in the life cycle. The adult filarial worms have predilection sites in the blood vessels of lungs, hearts and tecticles of dromedaries and Bactrian camels. For the first time in camels of Mongolia, Ivashkina (1953) observed and described *D. evansi*.

During an examination of blood in connection with a surra survey, microfilaria as an additional finding were diagnosed and DNA of *D. evansi* and another *Dipetalonema* species was detected in 4.5% of 400 blood samples (Bilegjargal *et al,* 2021).

Parabronemosis

Parabronemosis is caused by the spirurid *Parabronema skrjabini* that inhabits the abomasum of domestic and wild ruminants and Old World Camelids. The horn fly, *Haematobia irritans*, acts as intermediate host (Liu *et al,* 2020, 2021). Apart from Bactrian and wild Bactrian camels in Mongolia, Sharhuu and Sharkhuu (2004) listed the following hosts for *P. skrjabini*: cattle, yak, sheep, goat, maral, roe deer, argali ibex Mongolian and goitered gazelles and Mongolian saiga.

Arthropod infections

In unpublished documents of an ectoparasite control programme (1970 – 1989) based on bilateral governmental treaty between Mongolia and the German Democratic Republic, three parasitoses of Bactrians caused by arthropods were mentioned, namely sarcoptic mange, tick infestation and cephalopinosis.

Tick infestation

Hard ticks are frequent ectoparasites of Bactrian camels in Mongolia. *Hyalomma asiaticum*, *Dermacentor nuttalli, D. dagestanicus, Rhipicephalus pumilio* ticks are reported in Mongolian Bactrian camels. *Hyalomma asiaticum* is more widespread in the Gobi Desert and is often found in the armpits and groins of camels. *Dermacentor nuttalli* and *D. dagestanicus* ticks are distributed among camels in Gobi Gurwansaikhan mountain steppe regions: Altai, Mongolian Altai and Gobi. Rh. pumilio spread in camels around Bor Tsonji Gobi in the East Gobi region (Dash, 1986; Erdenebileg, 2001). In a more recent study, Narankhajid *et al* (2018) collected to *De. nuttalli, De. marginatus* and *Hy. asiaticum* from camels in the Gobi Altai district. A survey of ticks in Bactrian camels in the Gobi of Inner Mongolia of China revealed the presence of *Rhipicephalus sanguineus, Hyalomma dromedarii, Hy. asiaticum* and *Dermacentor niveus* (Li *et al,* 1997).

Camel mange

Camel mange, an extremely contagious parasitic disease caused by the *Sarcoptes scabiei* and

is transmitted by direct or indirect contact. Although it is one of the most important parasitic diseases affecting camel (Erdenebileg, 2001) little is known about the disease in Bactrian camels in Mongolia.

Wohlfahrtiosis

Wohlfahrtiosis is a parasitic disease caused by the larval stages of flesh flies of the genus *Wohlfahrtia*. While *Wohlfahrtia magnifica* causes obligate myiasis in in animals and men, *W. nuba* mainly colonise carcasses and only few cases of (facultative) myiasis are known. Schuman *et al* (1976) found *W. magnifica* as the cause of genital myiasis in female camels in Mongolia. Genital myiasis occurs 4-6 weeks after calving. A spontaneous recovery was seen at the beginning of October when adult flies disappear due to unfavourable cold temperatures.

Examination of 1676 Bactrian camels from 45 selected herds in six different areas of the Chatanbulag Sumon in the Eastern Gobi district, Mongolia, led to an estimate of *W. magnifica* infestation rates between 8-10%. Most myiasis cases were found in older females (> 4 years); younger animals were infested at a lower rate. Highest prevalence rates (15%) were seen during June and July in the Aman Us Chudak region (Valentin *et al,* 1997).

Cephalopinosis

Cephalopinosis or nasopharyngeal myiasis in dromedaries and Bactrian camels is caused by the larval stages of the camel nasal bot fly *Cephalopina titillator*. Compared to dromedaries, little research has been done on cephalopinosis in Bactrian camels. The inconspicuous female fly deposits its eggs around the nostrils and first stage larvae migrate through the labyrinth of the ethmoid bone of the nasal cavity. Second and third stage larvae are found in the nasopharynx. Under conditions of continental climate in east Asia, only one generation is produced per year and the whole larval development lasts 9-11 months. Fully grown third stage larvae are sneezed out and pupate in surface layers of the ground. The presence of *C. titillator* in Mongolia was mentioned by Erdenebileg (2001). Li *et al* (1997) and Yao *et al* (2022) studied the distribution of camel nasal bots in Bactrian camels in the neighbouring province of Inner Mongolia in China and found a prevalence of 96.2 and 54.2%, respectively. Under similar climatic conditions as in Mongolia, fly imagines are present around camel herds between June and September.

Vermipsillosis

Fleas of the family Vermispsyllidae occur on farm and wild animals in countries of central and east Asia. Three species, *Vemipsylla alacurt*, *Docardia ioffi* and *D. docardia* prevail in Mongolia (Ioff *et al,* 1965; Hiepe and Ribbeck, 1982). The life cycles of Vermispsyllidae spp. and their host spectrum under conditions of Mongolia was studied by Zedev (1976). Camels and other farm animals become infected in late autumn and the fleas hibernate on the host and suck blood. Thereby, the body of the females become worm like stretched and resembled a striped worm. Deposition of eggs takes place during the whole cold time of the year and the parasites die in spring. Preimaginal development takes place on the ground during the summer months. According to Erdenebileg (2001), heavily infested camels lose weight and condition over the winter.

Discussion

Camel husbandry is the main source of living for pastoralists in the semi-arid and arid zones of Mongolia, including the Gobi regions. Undoubtedly, camels represent a vital contribution to food security and human welfare in vulnerable households of dry areas. However, infectious diseases of livestock are major cause of production losses and may lead to even death.

Remote distances, poor infrastructure and lack of adequate diagnostic centres were the main reason for poor investigation of infectious diseases in Bactrian camels in Mongolia. As a matter of fact, linear distance from Ulaanbataar to places of the Gobi Desert where the majority of camels in Mongolia live is more than 500 km, mostly on unpaved roads. Also, sampling of materials under conditions of nomadic pastoralist systems was a challenge. Although, the situation has changed in the past 20 years, not all aspects of parasitic diseases are fully understood.

According to Wernery *et al* (2014) one of the most important camel parasitic diseases is surra but so far, there are only speculations on the presence of surra in Mongolia since serological results cannot differentiate between surra and dourine. There is no information about the presence of biting flies as the main vectors for surra. Findings of *O. trurkestanica* in camels in previous reports (Shumakovic, 1936, Sharhuu and Sharkhuu, 2004) suggested that muddy biotopes, such as watering places for animals are present in areas were camels are reared. Such places are the habitats of mud snails and offer good conditions for the larval development of horseflies,

the main vectors of surra. In other papers, the presence of the stomach worm *P. skrjabini*, in camels of Mongolia was mentioned (Sharhuu and Sharkhuu, 2004). The intermediate host for this parasite are hornflies, that can also act as a vector for surra.

Camels are prone to the faecal-oral way of transmission. Examples for this are the *Eimeria* coccideans, *T. gondii*, *Sarcocystis*, *Neospora* and *Echinococcus* spp. Toxoplasmosis in herbivores is always linked to the presence of cats but cats do not play a role in nomadic husbandry systems. More interesting would be a survey of neosporosis since it is linked to canids. Confirmed cases of hydatidosis in camels (Chinchuluun *et al,* 2018, Bold *et al,* 2019) do not present a direct danger for humans but feeding slaughtering offal to herding dogs is a jeopardy for both humans and livestock.

Some research work was done in Mongolia with regards to piroplasms and DNA of bovine and equine piroplasms were detected in camel blood. The researchers did not detect the parasites in examined blood smears. DNA of bovine and equine piroplasms was also detected in apparently healthy dromedaries from Jordan, Saudi Arabia and Egypt but blood smears were not examined (Quablan *et al,* 2012; Omer *et al,* 2021; Salman *et al,* 2022).

Hard ticks are important vectors for a number of viral, bacterial and parasitic diseases in animals and humans and camels may serve as a reservoir for these pathogens and also as a nutritional source for these vectors under the harsh climatic conditions of Mongolia. Thus, *Anaplasma* antibodies and their DNA were detected in Bactrian camels in Mongolia (von Fricken *et al,* 2018, Altantogtokh *et al,* 2022, Chaorattanakawee *et al,* 2022). A very interesting observation was made by Tyron (1983) who found that adult *Dermacentor nuttalli* sitting on shrubs in the Mongolian steppe and waiting for suitable hosts in early spring when temperatures were still below freezing point. This is important for control strategies.

Conflict of interests

The authors declare that they have no conflict of interest.

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MVA-BASED VACCINE EFFICACIES ON THE IMMUNE RESPONSE AND SEROPREVALENCE OF MERS-COV: A SYSTEMATIC REVIEW

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ABSTRACT

Modified vaccinia virus Ankara (MVA) is an attenuated type of poxvirus vaccine. Many vaccines against infectious diseases, which include influenza, HIV/AIDS, tuberculosis, cancer and lately, Ebola virus and MERS-CoV, have been developed using MVA as a viral vector. MERS-CoV encodes 4 structural proteins, nucleocapsid (N) or spike (S), also the membrane (M), in addition to (envelope) (E) (proteins), are all encoded by the MERS-CoV genome. Most viral vector-based MERS vaccines exhibit immunogenicity in vaccinated animals and use the full-length S or S1 protein of MERS-CoV as the coding antigen. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines were utilised. The research strategy used keywords, keyword combinations, MeSH terms, field tags, Boolean operators "AND" and "OR," and truncations. Search strings were built from these elements to ensure an accurate acquisition of the best output. The population, exposure, control, outcome, and studies (PECOS) criteria were used in this study. In a homologous vaccination regimen, MVA-MERS-S generated potent antibodies as well as specific B-cells, although T-cell responses showed a heterogeneous design among cohorts. A booster or third immunisation is significant because it increases the longevity and levels of antibodies and B-cells specific to MERS-CoV-S. Furthermore, the antibodies' levels and capacity to neutralise is improved after the late booster immunisation. Follow-up studies and large-scale clinical trials are required to confirm the circulation and immunity status against MERS-CoV in camels.

Key words: MERS-CoV, meta-analysis, MVA, spike protein, seroprevalence, vaccine

Modified vaccinia virus Ankara (MVA) is an attenuated type of poxvirus vaccine (Altenburg *et al*, 2014). Many vaccines against infectious diseases, including influenza, Middle East respiratory syndrome corona virus(MERS-CoV), HIV/AIDS, tuberculosis, cancer and lately, Ebola virus infection, are developed using MVA as a viral vector (Alharbi, 2019). The MVA strain of the vaccinia virus has 6 significant genomic deletions compared to the parental virus, which attenuated it by repetitive passage in chick embryo fibroblasts (CEFs). However, replication of MVA is low in most mammalian cells but robust in CEFs (Blanchard *et al*, 1998). MVA is a virus deficient in replication that has been tested in several experiments and has superior immunogenicity and profile of safety (Sutter and Staib, 2003). Primarily, this MVA vaccine has been used as a booster in heterologous prime-boost vaccination regimens, enhancing recombinant antigen-specific T-cells that have already been primed. Oftentimes, adenoviral vectors are employed in these schedules to accomplish the priming immunisation (Gilbert *et al*, 2006; Reyes-Sandoval *et al*, 2010).

Since its initial detection in 2012, the Middle East respiratory syndrome (MERS) virus has spread to many countries (Masood *et al*, 2020). There is evidence that human dipeptidyl peptidase 4 (hDPP4) is a functional receptor for MERS-CoV(Wang *et al*, 2013). There is still widespread concern about MERS-CoV infections in both humans and camels. Proof of human-to-human transmission of MERS-CoV has been found (De Wit *et al*, 2016). Humans who have any kind of contact with MERS-CoV-infected camels may be at a higher risk of contracting the virus. MERS-CoV remains a persistent threat to global health security, with new cases and outbreaks occurring regularly in the Middle East. MERS-CoV was shown to have a moderate to high prevalence, but a high seroprevalence. Despite the virus's extensive presence in camel herds, zoonotic

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transmissions were less common (Kandeel, 2022). Effective interventions, such as vaccinations and medications are urgently needed to halt the spread of MERS-CoV among camels and prevent transmissions from camels to humans, as well as to treat and prevent infections among people.

MERS-CoV encodes four structural proteins nucleocapsid (N), spike (S), membrane (M)and envelope (E) proteins (Malik, 2020). Vector-based MERS vaccines exhibit immunogenicity in vaccinated animals and have their coding antigen as proteins S1 or the full-length spike protein (Zhou *et al*, 2018). Immunised mice produced MERS-CoV S-specific antibody responses in response to recombinant Ad5 vectors that encode the full-length or S1 extracellular domain of the MERS-CoV S protein, neutralising MERS-CoV infection *in vitro* (Kim *et al*, 2014). Infected mice have produced MERS-CoV-specific antibody responses, neutralising antibodies, and T-cell responses in response to Ad5 or Ad41 vectors expressing full-length S protein of MERS-CoV. MVA-MERS-S, an MVA-based viral vaccine that expresses viral S protein, showed effectiveness against MERS-CoV infection in Ad5/DPP4-transduced mice. MVA-MERS-S is a promising vaccination candidate due to its high rate of genetic stability and favourable development properties. Serum antibodies against MERS-CoV were abundant in vaccinated mice. Therefore, MVA-MERS-S could be used to advance research toward an urgently needed vaccine against MERS-CoV(Song *et al*, 2013).Vaccines with viral carriers like the measles virus, adenovirus and MVA can trigger strong humoral and cellular immune reactions (Rollier *et al*, 2011). In dromedary camels exposed to MERS-CoV, an MVA-based, full-length S MERS-CoV vaccine candidate (MVA-S) elicited mucosal immunity and decreased viral shedding (Haagmans *et al*, 2016; Volz *et al*, 2015). In addition, less infectious MERS-CoV particles and little to no viral RNA were found in the nasal cavities of immunised camels.

Developing MERS vaccine candidates has been challenging due to a lack of understanding of the mechanisms that determine protective immunity and the difficulties of conducting effective trials. Although, optimal animal models for discovering protective immunological correlates for MERS are still missing, current mice models show that antibodies and T-cells are crucial for building protective immunity. This study looks into how well MVA-based vaccines protect against MERS-CoV.

Materials and Methods

Study Design

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used. The preparation for this work also included the use of PRISMA extensions published in the Cochrane Handbook for Systematic Reviews and Extensions – Chapter 4 (Page *et al*, 2021).

Search Strategy

This study identified Embase, Cochrane Central, PubMed, and Google Scholar as the electronic databases for research. The search strategy used keywords, keyword combinations, MeSH terms, field tags, Boolean operators "AND" and "OR," and truncations. Search strings were built from these elements to ensure an accurate acquisition of the best articles. Search strings used in the study included "MVA-Based"[All Fields] AND ("vaccin"[Supplementary Concept] OR "vaccin"[All Fields] OR "vaccination"[MeSH Terms] OR "vaccination"[All Fields] OR "vaccinable"[All Fields] OR "vaccinal"[All Fields] OR "vaccinate"[All Fields] OR "vaccinated"[All Fields] OR "vaccinates"[All Fields] OR "vaccinating"[All Fields] OR "vaccinations"[All Fields] OR "vaccination's"[All Fields] OR "vaccinator"[All Fields] OR "vaccinators"[All Fields] OR "vaccine s"[All Fields] OR "vaccined"[All Fields] OR "vaccines"[MeSH Terms] OR "vaccines"[All Fields] OR "vaccine"[All Fields] OR "vaccins"[All Fields]) AND "vaccin"[Supplementary Concept] OR "vaccin"[All Fields] OR "vaccination"[MeSH Terms] OR "vaccination"[All Fields] OR "vaccinable"[All Fields] OR "vaccinal"[All Fields] OR "vaccinate"[All Fields] OR "vaccinated"[All Fields] OR "vaccinates"[All Fields] OR "vaccinating"[All Fields] OR "vaccinations"[All Fields] OR "vaccination's"[All Fields] OR "vaccinator"[All Fields] OR "vaccinators"[All Fields] OR "vaccine's"[All Fields] OR "vaccined"[All Fields] OR "vaccines"[MeSH Terms] OR "vaccines"[All Fields] OR "vaccine"[All Fields] OR "vaccins"[All Fields]. Identified articles were sought to get the most relevant for this study.

Eligibility Criteria

The researchers selected eligibility guidelines for the selection of studies to be included in this study. The population, exposure, control, outcome, and studies (PECOS) criteria were used in this study. The population in the included studies had a high risk of getting MERS-CoV. Exposure considered for

inclusion was to be vaccination with an MVA-based vaccine. This study had no specific comparator for the control. The efficacy of the MVA-based vaccine was the outcome prioritised for this study. Study designs considered for this study were any studies that indicated the vaccination of MVA-based vaccines for MERS-CoV. Consideration was also done for articles with different study designs but had very relevant material. The studies had to have clinical or experimental trials for the MVA-based vaccines. Only English-published articles or those translated were considered for inclusion.

Data Extraction

Two researchers conducted the extraction of data. A pre-designed excel worksheet was used in the recording of extracted data. Information on the authors, year of publication, outcomes, and the results of the included studies was extracted. Engagement between the two researchers was constant to ensure the results' congruence. A third party quelled disputes that arose.

Quality Assessment

Utilising the Critical Appraisal Skills Program (CASP) standard checklist, the quality of the included studies was evaluated. Four sections were used to conduct the quality assessment. Three questions were used in Section I to verify the study designs of the included studies. Three questions were used to evaluate the listed studies' methodological soundness. Three questions were used to evaluate the results' validity, and two were used to evaluate their applicability. The answers "YES," "NO," and "CAN'T TELL" were used for evaluation. "Y," "N," and "CT" were used as the abbreviations for these answers. The studies were ranked for quality assessment, with 11 being the highest score possible, using the responses from the checklist. Studies of excellent quality received scores between 8 and 10. Scores of 6 and 7 were considered to be of moderate quality, while scores of 5 or less were considered to be of low quality. The questions used in the checklist are displayed in the table 1.

Results Analysis

This study made use of only one form of investigative analysis. The method used was a qualitative assessment and a systematic review. Literal analysis was also conducted from the included studies.

Table 1. CASP standard checklist used in this systematic review.

Validity of the Study Design
C1. Was the research question from the study focused?
C2. Was randomisation of the participants towards the interventions done?
C3. In conclusion, was there accountability of the participants?
Methodological Soundness of the Study
C4. Was blinding done for the following:
• The participants?
• The investigators?
• Results analysers?
C5. Were the study groups similar at the start of the trial?
C6. Was there a similarity in the level of care among the study groups and the participants?
Validity of the Results
C7. Was a comprehensive report on the effects of the interventions done?
C8. Was there a report on the precision of the effect of treatment or the estimate of the intervention?
C9. Were the efficacies of MVA-based vaccines identified?
Applicability of the Results
C10. Was there compatibility between the population and the results?
C11. Was there a benefit in the application of implantable cardioverter defibrillators compared to those without?

Results

Study Selection

One hundred and six articles were identified from the electronic databases used in the study strategy. Of these, 19 articles were excluded as duplicates. The remaining 87 studies were then screened using titles and abstracts to determine their suitability. Sixty-nine studies were excluded thus remaining only 18 studies. Further screening was conducted, which led to exclusion of 9 studies. Nine research/studies in total have been used in this systematic review. The PRISMA flow chart (Fig 1) shows the study selection process.

Study Characteristics

Information obtained from incorporated research/study was as shown in Table 2.

Quality Analysis

The study below shows the quality assessment conducted using the CASP checklist.

Table 1. Study characteristics including subjects, vaccine, test groups, outcomes and results summary.

Studies	Year	Subjects	Vaccine development	Test groups	Outcomes	Results summary
Alharbi *et al*	2022	Mice and Camels	(Encoding) (Full)-(length) (spike)of the proteins for(MERS)-(CoV) and(M.V.A)-(MERS)	Three mice groups (Group 1: (MVA)-wt)/(MVA)-(MERS); and the second (Group): (MVA)-(MERS)/(MVA)-(wt); including third Group: (MVA)-(MERS)/(MVA)-(MERS). 3 (camel)-(groups) (Group 1: (MVA)-(wt)/(MVA)-(MERS); second Group: (MVA)-(MERS)/(MVA)-(wt); including the third group: (MVA)-(MERS)/(MVA)-(MERS)	Humoral immune responses (Antibody (Ab))	A high dosage of (MVA-MERS) was included to induce stronger (Ab-responses) for the mice as well as camels and included(neutralising-antibodies). (MVA-MERS)–(vaccine), that was administered via (homologous) for (prime–boosting) regimen, inducing (high-levels) (neutralising) anti-(MERS-CoV) antibodies into mice as well as in the camels. (ELISA) in every(time-point): (Log10) end-point (titers)
Alharbi *et al*	2017	Mice	(Full-length) spike-for(MERS-CoV)	(Heterologous)(prime-boost) regimen – vaccines were given to the mice with (ChAdOx1) (MERS) together with boosting them with an (MVA-MERS) that was 28d.p.i. (Homologous-regimens) – vaccines were administered to mice which had (MVA-MERS) including boosting them with an (MVA-MERS) that was (21d.p.i).	Humoral immunogenicity	Both vaccine candidates with a sample of (14) including(28d.p.i) which induced higher levels for(S1), (specific) for (antibodies) with a mean-(endpoint)-(titre) of (Log10)=(4.8) and a (t.P.A) of (4.7) and without (tPA), besides controlled vaccines, (ChAdOx1)- encoding enhancing greener (fluorescent) (protein) of (ChAdOx1)-(e-GFP), with a mean-endpoint-titre of (Log10)=(1). (ELISA) with a mean-endpoint-titre (Log10)= (3.2)
Koch *et al*	2020	Humans	(Full-length) (MERS-CoV) with a (spike-glycoprotein)	Prime immunisation: Low dose group got; doses of (1×107) (plaque), (foming) units (P.F.U), High dose group; (1×10^8) (P.F.U). Booster shot: similar dose, which was 28-days later.	Frequency as well as the severity of adverse events, immunogenicity	Adverse events: (67, 10) out of (14) persons who participated in (the low-dose-group), (The high-dose-group) were (10) out of (12). Pain, fatigue, swelling, and indurations. (Sero-conversion) after booster: (9/12) of (low-dose-group) together with (111/11) of (high-dose-group). (Biding-antibody-titers) matched up (ME, RS-CoV) specific (neutralising) antibody). ELISA: (Spearman) matched up with(r=086) and (95%) CI, of (0·6960–0·9427), (p=0·0001)
Fathi *et al*	2022	Humans	(Fulpike)(glycol-protein) (MERS CoV-S)	(Homologous) primary immunisation: (Low) (dose) group); (1×10^7) (PFU), (High-dose-(group); (1×10^8) (PFU) Booster; after one year (±4months). Re-enrolment of ten persons to participate; three from(LD) and the other seven from (HD) groups: a dosage of (1×10^8)(PFU)	Safety and immunogenicity	Adverse events: (51) in (9/10) Participants. (40/51) were related to the vaccine. (Reactogenicity) and fatigue/malaise. (32/40) were mild cases. (37/40) were solicited. Occurrence of adverse events (AE): median 1 day. ELISA-optical-density (OD) (0.08) of (95%) CI (0.03–0.13). Elevated frequency and persistence for spike-specific (B-cells) were observed from a (late-booster-immunisation), which binds (immunoglobulin-G1) - (IgG1) as well as (neutralising-antibodies) and not (T-cell) response.

Table 1. Continued

Song *et al*	2013	Mice	Recombinant MVA, mature full-length S glycoprotein	Immunisation: (10^8) (PFU) (MVA)-(MERS) – (S) at zero and three weeks.	Immunogenicity	Every animal is given a (booster- immunisation) shortly after producing (circulating-antibodies) elevated levels, neutralising (MERS-CoV). (Serum-samples) did not detect (neutralising-antibodies) in a controlled animal that was inoculated via (non-recombinant) (MVA) and either (saline).
Volz *et al*	2015	Mice	(Full-length) (MERS-CoV) spikes (S) (protein) (MVA)-MERS) (S)	(Single-subcutaneous) that is (s.c.) immunisation via dosage with (10^7) and either (10^8) (PFU)	Immunogenicity	(MVA-MERS) – (S) brought out noticeable MERS-CoV) – (neutralising-antibodies). Immunisation used to boost (s.c.) gives results with elevated titers for (MERS-CoV) – (neutralising-antibodies). (Serum), (antibody) (titers) (log2).
Weskamm *et al*	2022	Humans	(MVA)-(MERS) – (S) that encodes (MERCoV) – (spike), (protein)	Administering of 3(homologous immunisations) during 0 to 28 days. (Booster-vaccination) were given at months (12) ± (4).		ELISA:r = 0.9383, p < 0.0001, 50% plaque-reduction neutralisation test (PRNT50),
Veit *et al*	2018	Mice	MERS CoV spike (S) protein	Female sets of (BALB/c) mices with (n=2) and (5) got immunised two times within intervals (21-days) for (10^8) (plaque forming units) (P.F.U) with recombinant (MVAMERS) – (N) and either (non-recombinant) (MVA) besides (P.B.S) used for (mock-vaccine).	Immunogenicity	Formation of (MVA-MERS) – (N) for the (recombinant-virus) using(CEF) infected with (MVA) together with (trans-infected) with (MVA) (vector-plasmid) (pIIIH5red) – (MERS) – (N).
Langenmayer *et al*	2018	Mice	(MERS-CoV) spikes (S) (protein)	Vaccination: (1×10^7) (PFU) together with(1×10^8)(PFU)	(MVA-MERS) – (S) distribution	(Real-time) (P.C.R) analysed via(> 240) (tissue-samples) detection(MVA-DNA)(pre-dominant) during injecting-site as well as draining (lymph nodes). The considerations of Level for (parenteral-site-inflammation) together with (hyperplasia) for (draining-lymph-nodes) incorporation with (immunological-response) into (vaccine-inoculation).

Discussion

MERS-CoV vaccines were created in addition to being tested *via* various animal models, including non-human primates, camels, and human clinical trials (Alharbi *et al*, 2017; Langenmayer *et al*, 2018). MVA-based vaccination that included full-length spike antigen testing was done in a mouse model and in dromedary camels (Alharbi *et al*, 2022). The vaccination elicited stronger T-cell mediated immune responses in mice, and increased levels for binding together with neutralising antibodies were obtained from camels. Homologous prime-boosters immunisation strategy in mice demonstrates the effectiveness of MVA viral vectors in priming and boosting. When compared to a heterologous prime-boost regimen, where for instance, primed with ChAdOx1based-vaccine together with boosting it with MVA encoding similar vaccine prompted elevated neutralising Abs of mouse models. It has been demonstrated that the use of MVA-based vaccine in prime and boost immunisation is suboptimal. Therefore, vaccines were established based on two alternative vectors, avoiding immune reactions unique to the priming vector. Additionally, it has been demonstrated that the vector-based vaccine is hindered by earlier immune reactions to a vaccine vector. Although, the MVA-based vaccine may not be influenced by pre-existing immunity

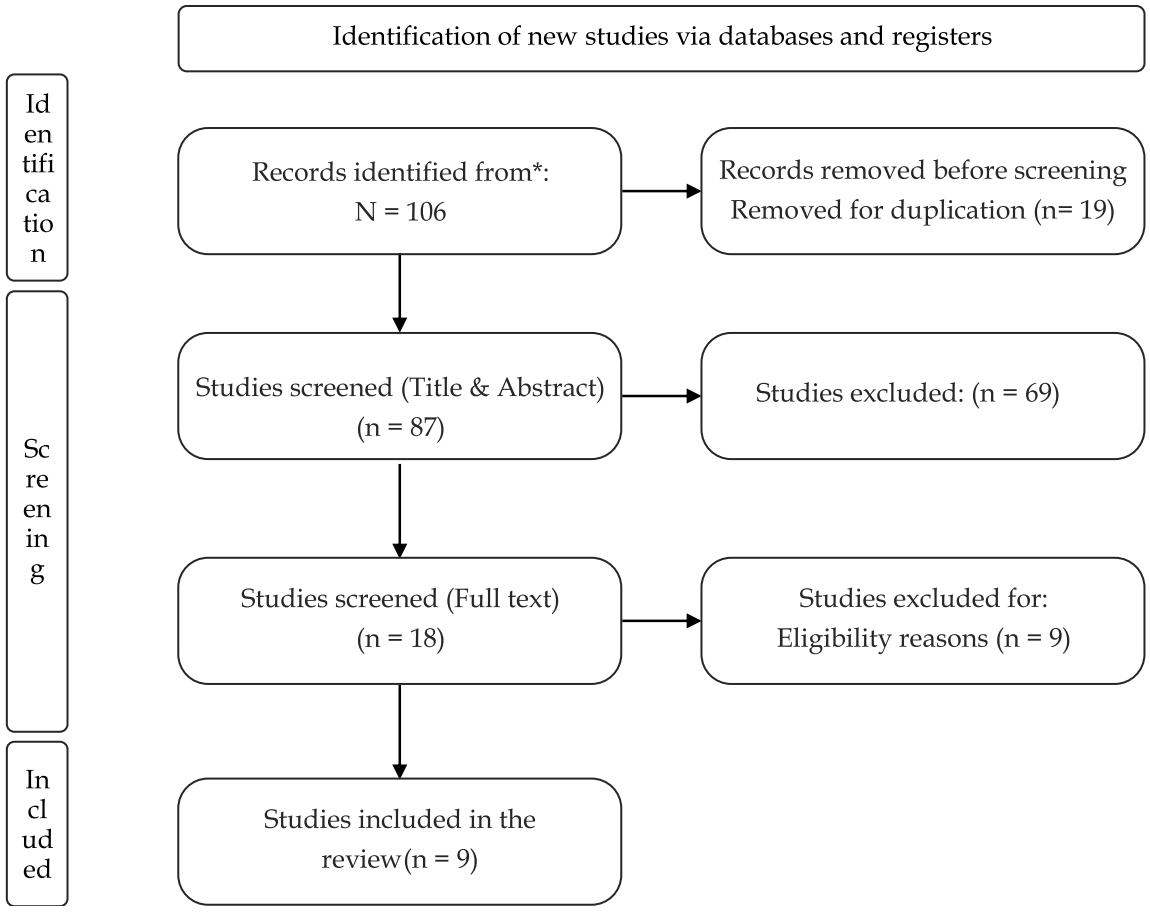

Fig 1. PRISMA flow diagram of studies in the systematic review.

Table 2. Quality Analysis of the included studies.

STUDY	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
Alharbi *et al*, 2022	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Alharbi *et al*, 2017	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Koch *et al*, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Fathi *et al*, 2020	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y
Song *et al*,	Y	CT	Y	Y	CT	Y	Y	Y	Y	Y	Y
Volz *et al*, 2015	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y
Weskamm *et al*, 2022	Y	CT	Y	Y	CT	Y	Y	Y	Y	Y	Y
Veit *et al*, 2018	Y	CT	Y	Y	CT	Y	Y	Y	Y	Y	Y
Langenmayer *et al*, 2018	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y

given that smallpox has been eradicated, there may still be problems with using MVA as a vector for numerous antigens or multiple vaccines, should any be approved in the future.

The anti-MERS-CoV spike Ab level was enhanced and unaffected by the anti-MVA-induced immune responses at prime. Anti-spike Ab levels that were at a high level in response to MVA-MERS were produced in mice with MVA-wt. This indicated that MVA has a potent effect on priming immunological reactions in animals with anti-MVA antibodies. The increase in anti-antigen Ab levels in homologous MVA vaccination regimens are often lower after the boost vaccination when compared to after prime immunisation. Most dromedary camels in Africa, the Middle East, and the Arabic Peninsula are MERS-CoV-seropositive. The prime vaccination with MVA did not appear to cause any elevation of spike Abs that were anti-MERS-CoV (Alharbi *et al*, 2022). After the boost vaccine, the immunisation displayed high neutralising and binding Abs levels.

The use of the tPA leader sequence being used by the F11 promoter and ChAdOx1 in MVA caused immunogenicity to increase slightly as compared to the use of mH5 promoter or no leader sequence, respectively (Alharbi, 2019). MVA vectored vaccine is the only MERS vaccine investigated in camels,

which also protected hDPP4 transgenic mice. Those were vaccinated with a requirement of two doses of a homologous prime-boost regimen administered both intramuscularly and intranasally to achieve protection partially and reduce viral shedding in camels.

According to Fathi *et al* (2022), in terms of the dynamics, kind, and severity of adverse events, the reactogenicity of the prime immunisation regimen and the booster vaccination appeared to be similar. Vaccination with two doses of MVA-MERS-S resulted in transient changes, specifically in haematologic parameters, which can be seen as a biological reaction to vaccination. A late MVA-MERS-S homologous booster was found to boost insert-specific immunity significantly. All participants in the study experienced an increase in anti-MERS-CoV-specific neutralising and binding antibody titers, regardless of whether they initially received low-dosage or high-dosage primary vaccinations, which was observed even if they had been unable to produce neutralising antibodies previously after the initial two-dose regimen.

In a study by Song *et al* (2013), all immunised animals developed significant circulating antibodies that neutralised MERS-CoV following booster immunisation. In contrast, control animals' blood samples given phosphate-buffered saline [PBS] or non-recombinant MVA injections did not show the presence of neutralising antibodies. According to Volz *et al* (2015) investigation, neutralising MERS-CoV antibodies were detectable following a single subcutaneous vaccination with 10^7 or 10^8 PFU of MVA-MERS-S. Increased titres of MERS-CoV-neutralising antibodies were the consequence of booster immunisations. Even a small dose of 10^6 PFU of MVA-MERS-S caused detectable neutralising antibodies. Similar antibodies were produced by MVA-MERS-S vaccination dosages of 10^7 and 10^8 PFU.

In Volz *et al* (2015) study, booster subcutaneous immunisations enhanced the quantity of IFN-γ-secreting MERS-S291-specific CD8+ T cells even more, especially with the lower dosage of 10^6 or 10^7 PFU of MVA-MERS-S. After single and prime-boost immunisations, intramuscular immunisations produced equal levels of CD8+ T-cell responses for all doses of the MVA-MERS-S vaccine. The intramuscular booster roughly tripled the amount of T-cell responses specific to MERS-S291. Histology revealed that the candidate vaccine in the investigation by Langenmayer *et al* (2018) did not cause organ lesions peripheral to the parenteral site or generalised lesions.

According to Weskamm *et al* (2022), the high-dose cohort experienced a seroconversion of 100% after receiving the first two doses of the MVA-MERS-S vaccine. These responses included T cell and antibody production. Immune reactions brought on by vaccination are frequently multilayered and can vary depending on the vaccine candidate. For instance, the strength and quality of immune response boosters can be affected by vector immunity, innate immunological reactions, and the stage of memory B cells (MBCs) maturation. It has been demonstrated that allowing more time between the prime and boost immunisations can significantly improve immunogenicity. It has been demonstrated that vaccines work better and produce stronger immune responses when the prime-boost period is increased from 28 to 84 days. Studies have shown that antigen-specific T-cell responses vary significantly between different cohorts. Antibody-secreting cells are effectively generated after the late boost.

According to Koch *et al* (2020), MVA-MERS-S vaccination generated both humoural and cellular immune responses to the MERS-CoV spike, mainly apparent after boost immunisation rather than prime immunisation. Most study participants reached baseline antibody levels by the end of the study, 6 months after vaccination, according to the humoral immune responses, which were assessed by ELISA and two different viral neutralisation assays. The humoral immune responses peaked at 42 and 56 days, were maintained through the 84^{th} day, and then declined to baseline levels.

In the Veit *et al* (2018) study, the MVA-MERS-N recombinant virus produced stable levels of antigen MERS-CoV N upon *in vitro* infection of cells from people, which indicated the unimpaired expression of the target gene at the level of late viral transcription using the synthetic, vaccinia virus-specific promoter PmH5. Additionally, antibodies from experimentally infected laboratory animals strongly recognised the MERS-CoV-N antigen generated in MVA-MERS-N infected cells, demonstrating that N-specific immune responses were potently activated upon MERS-CoV infection. According to descriptions of other viruses, the presence of N in these respiratory epithelial cells may lead to effective identification by innate and adaptive immune cells, causing strong protective immunity. As a result, the activation of N-specific immune responses in these animals highlights the MERS-CoV-N protein's potential use as a vaccine antigen. Veit *et al* (2018) claim that the MERS-CoV N protein can potentially effectively trigger CD8+

T cell responses specific to the virus. The MVA-MERS-N vector virus created by Veit *et al* (2018) for the study showed to be a stable recombinant virus that can be multiplied easily to produce vaccine formulations technically meeting all standards for further experimental or even medical development.

The study sought to investigate the efficacies of MVA-based vaccines on the seroprevalence of MERS-CoV. In a homologous vaccination regimen, MVA-MERS-S generated potent antibodies as well as specific B cells although, T-cell responses showed a heterogeneous design among cohorts. The late third immunisation is significant in that it increases the longevity and number of antibodies and B cells specific to MERS-CoV-S. After the late boost, the antibodies' levels and capacity to neutralise are reported to have stabilised. This supports a growing body of data that suggests late boosting may be a useful strategy for enhancing the immune response to CoVs generated by vaccination. This study showed that MVA-based vaccines on the seroprevalence of MERS-CoV were efficient.

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Data availability statement

"All data are within manuscript. Further details can be requested from the corresponding author".

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Conflict of interest disclosure

None

Ethics approval statement

Not apply

Patient consent statement

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Permission to reproduce material from other sources

Not apply

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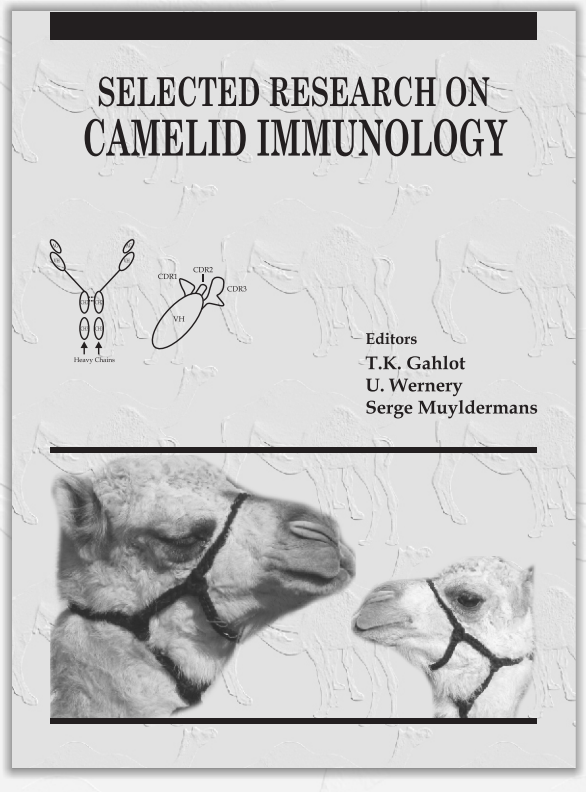
SELECTED RESEARCH ON CAMELID IMMUNOLOGY

(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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SUPEROVULATION IN CAMEL: STATE OF THE ART

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ABSTRACT

Superovulation (multiple ovulation) and embryo transfer is relatively an old, cheap and efficient reproductive technique for taking advantage of an elite she-camel's genome. It starts with the selection and preparation of donors followed by the stimulation of ovaries (superovulation) to produce more ovulatory follicles. The rest of the procedure including mating and/or AI, fertilisation, and construction of zygote and embryo follow the same natural sequence of embryo production *in vivo*. The quality of embryo produced by this technique has nearly similar competence to the one produced normally during reproductive cycle in camel resulting in the birth of healthy and genetically selected calf. This review article summarises the majority of references used for superovulation in camel in order to sum up the approaches for synchronisation of elite donors, varying types of gonadotropins and the way of their administration in camel.

Key words: Follicular wave synchronisation, gondadotropins, multiple ovulation, superovulatory response

Camel has low reproductive efficiency resulting in low genetic progress (Skidmore, 2003). She-camel could deliver the first calf around 5 to 6 years of age (Merkt *et al,* 1990). Considering the calving rates of 40% (Wilson, 1984), she-camel may produce 7 to 8 calves throughout the life span of 15 years (Merkt *et al,* 1990). Therefore, embryo production technology could boost reproductive efficiency of she-camel.

Three main approaches were used to produce camel embryos. The first approach relied on *in-vivo* production of embryos and introduced since early nineties in camel (Anouassi and Ali, 1990; Cooper *et al,* 1990; McKinnon and Tinnson, 1992; Skidmore *et al,* 1992). This is the most accepted approach to produce camel embryos well known as MOET (Multiple Ovulation and Embryo Transfer). In this particular method, the elite donors receive FSH and/or FSH like factors during the procedure named superovulation to induce more follicles to grow. Then, the donor is mated and following fertilisation and construction of embryo *in vivo*, the uterus is flushed and the embryos are recovered, graded and transferred into synchronised recipients (Niasari-Naslaji *et al,* 2009, 2014; Ararooti *et al,* 2018a,b). Due to the relatively simple, unexpensive and good embryo quantality and quality, MOET is well established in several labs and became the main source of embryo production in camel (Anouassi and Tibary, 2013).

The second approach, introduced in 2006 in camel (Khatir and Anouassi, 2006), relied on the aspiration of oocyte from ovary either collected from abattoir or through ultrasound-guided transvaginal ovum pickup (OPU). Good quality oocytes go through maturation and fertilisation procedures to produce presumptive zygotes from which after several cleavage cycles, the embryos are produced *in vitro* (IVEP; Wani, 2021). Unfortunately, due to the special configuration of follicle in camel ovary (El-wishy, 1992) and high incidence of bleeding following OPU in camel, the *in vitro* production of embryos in camel did not become a popular approach for embryo production like in other species. Moreover, as a result of several abnormalities of embryos and health issues in newborns following *in vitro* production of embryos in cattle (Ealy *et al,* 2019), there could be a chance of such abnormalities in camel as well which requires further investigation.

The third approach, introduced in 2010 in camel (Wani *et al,* 2010), relied on somatic cell nuclear transfer (SCNT/cloning). In this particular technique, the somatic cell was transferred into enucleated oocyte. Following reprogramming of the somatic cell, the totipotent cells started to grow toward embryo production. Apart from low success rates and being relatively expensive technique in camel (100000 USD per calf), there are great concerns for the quality of embryos and the health of offspring produced by SCNT (Malin *et al,* 2022). Recently, there are great investment on producing an expensive cloned embryo in camel (Olsson *et al,* 2021); however, like other

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species, there might be several future health problems in cloned camels as well, mainly due to epigenetic modifications (Malin *et al,* 2022), which is neglected in camel at the present time.

In this review, superovulation was described in detail as the most important part of MOET programme in camel. Synchronisation of donors prior to superovulation, different types of gonadotropins, their advantages and disadvantages and the way of gonadotropin administration were elaborated.

Follicular growth in camel

Camel follicular growth occurs in a wave like pattern so called as follicular wave cycle (Nawito *et al,* 1967; Skidmore *et al,* 1995; Nikjou *et al,* 2009). There is no luteal phase in camel, therefore using the term "oestrous cycle" may not be appropriate in this species. Camel is an induced ovulator (Marie and Anouassi, 1987), seasonal breeder, displaying complete follicular wave cycles from November to March in most countries (Al Eknah, 2000). Each follicular wave cycle includes recruitment, growing, mature and regressing phases (Skidmore *et al,* 1995, 1996; Nikjou *et al,* 2009). During non-breeding season, the follicle may not reach to the diameter suitable for mating or AI (Nawito *et al,* 1967). It is possible to enhance follicle growth to the final stage of development or superovulate during non-breeding season using gonadotropins (Nowshari and Ali, 2005). However, due to the lack of suitable male with proper libido and sperm production during non-breeding season (Ali *et al,* 2021) breeding of female during non-breeding season could be a challenging subject until the availability of fertile frozen semen to achieve successful fertilisation and pregnancy.

Preparing donors for superovulation

The main concept for successful superovulation is to initiate superovulation between the emergence of follicular wave (Ararooti *et al,* 2018a, b) and before follicle deviation (Skidmore *et al,* 2005; Nikjou *et al,* 2008; Anouassi and Tibary, 2013; Manjunatha *et al,* 2019). Using this strategy, the follicles that initially emerged by endogenous FSH surge grow simultaneously with the support of exogenous FSH. Moreover, there is no mature follicle to suppress the growth of subordinate follicles. To achieve the emergence of new follicular wave, nearly all synchronising treatments relied on eliminating the existing follicle either by inducing ovulation in mature follicle using GnRH (Moghiseh *et al,* 2008), or by regressing existing follicles using progestogens (McKinnon and Tinson, 1992; McKinnon *et al,* 1994). Another non-practical way to eliminate mature follicles in camel is to ablate mature follicle by ultrasound-guided transvaginal follicle aspiration (Skidmore *et al,* 2009).

Camel is induced ovulator with follicular wave cycles and corpus luteum is present only in camels that have previously had sterile mating or have been ovulated by ovulating agents (Nawito *et al,* 1967; Skidmore *et al,* 1996; Moghiseh *et al,* 2008). In non-mated camel, the interval between formations of two consecutive mature follicles (inter-wave interval) is about 18 days for dromedary (Skidmore *et al,* 1996) and 19 days for Bactrian camels (Nikjou *et al,* 2009). Induction of ovulation in the mature follicle reduces the inter-wave interval to about 14 days. When GnRH is injected at a random stage of follicular wave cycle, emergence of new follicular emergence occurs variably within 2 to 6 days after GnRH injection, depending on the size of follicle at the time of treatment (Nikjou *et al,* 2008). However, the tightness of synchrony for follicular wave emergence is increased when the second GnRH is administered 14 days later (Nikjou *et al,* 2008; Skidmore *et al,* 2009). Similar results were found using the combination of GnRH and prostaglandin $F_2\alpha$ (Manjunatha *et al,* 2014, 2015). Accordingly, she-camel receives three GnRH injections on day -22, -12 and 0 (Day of the last GnRH injection) and two prostaglandin $F_2\alpha$ on Days -15 and -5. Superovulation could be started at any time between emergence of new follicular wave (2 days after the last GnRH injection; Ararooti *et al,* 2018a, b), and prior to the follicle deviation (4 days after the last GnRH injection; Skidmore *et al,* 2005; Nikjou *et al,* 2008; Anouassi and Tibary, 2013; Manjunatha *et al,* 2019).

The second approach for synchronising donors is to use follicle regressing agents such as progesterone (McKinnon and Tinson, 1992; McKinnon *et al,* 1994). In this particular approach used in dromedary camel, superovulation is initiated when there is limited follicular activity in the ovaries (i.e. no follicle > 7 mm in diameter) which can be achieved by daily intramuscular injection of progesterone (100 mg) for 8–15 days (McKinnon and Tinson, 1992, 1994). However, we could not find any benefit of using progestogens in controlling follicular dynamics in Bactrian camel (Nikjou *et al,* 2008).

Evaluation of superovulatory response

Superovulatory response could be evaluated by the number and diameter of follicles induced

to grow prior to ovulation, the number of corpora lutea (CL), the number of total ova/embryos, the number of transferable embryos, and the number of un-ovulated follicles at the time of embryo recovery. The best superovulatory response occurs when there is high number of CL and transferable embryos (≥ 5; Table 1) with low number of un-ovulated follicles and no unfertilised ova. This could be achieved when good type, right amount and right procedure for gonadotropin injections is used. Having good and safe gonadotropin such as highly purifies FSH (Folltropin-V) and/or hMG, still it is necessary to use right total amount and correct procedure of daily gonadotropin administration (Ararooti *et al,* 2017, 2018a). Small proportion of camels (10–20%) do not respond at all; whereas, some donors (15–20%) could be over-stimulated and produce ≥ 30 follicles in each ovary (McKinnon *et al,* 1994; Tibary and Anouassi, 1997; Skidmore *et al,* 2002; Anouassi and Tibary, 2013). The great number of un-ovulated follicles at the time of embryo recovery, in association with the low number of CLs and recovered embryos, could imply that the type, the amount and/or the way of gonadotropin administration may not be suitable. The presence of great number of un-fertilised ova could imply over-response to gonadotropin and/or using immature bull or using the bull at the beginning of breeding season.

Prediction of superovulatory response could be an essential part of superovulation program in order to estimate the right number of recipients necessary to receive embryo. We have demonstrated positive correlations between the ratio of follicles >6 mm/follicles ≤6 mm, detected on Day 4 of 5.5 days program, and the number of CLs and embryos in FSH treated camel (r=0.9; P<0.05; Ararooti *et al,* 2018a). As a result, a great percentage of follicles >6 mm, detected on Day 4 of superovulation, ovulated and established corpora lutea (90%), and embryos (70 %). This protocol allows the estimation of superovulatory response and the number of recipients that has to be prepared prior to embryo recovery in dromedary camel.

Table 1. Summary of the number of transferable embryos found following different superovulation protocols in dromedary camel since 1990.

Investigators	Year	No. Donors	No. Responders (%)	Superovulation	Transferable Embryo (mean)
Anouassi & Ali	1990	9	3 (33)	eCG (1500 IU)	2.1
Anouassi & Ali	1990	21	7 (33)	eCG (2000 IU)	4.9
Skidmore *et al*	1992	30	5 (17)	eCG (2000-4000 IU)	1.3
Skidmore *et al*	1992	11	3 (27)	Ovagen (20—30 IU)	2.5
Mckinnon and Tinson	1992	14	9 (62)	eCG (4500 IU)	2.7
Mckinnon and Tinson	1992	13	9 (69)	Ovine FSH (?)	4.5
Mckinnon *et al*	1994	68	N/A	Folltropin-V (?)	3.8
Mckinnon *et al*	1994	84	N/A	eCG (3000-6000IU)	2.3
Skidmore *et al*	2002	42	36 (86)	eCG (2500IU) + Folltropin-V (400 mg)	6.7
Skidmore *et al*	2005	12	10 (83)	eCG (2500IU) + Folltropin-V (400 mg)	4.0
Nowshari *et al*	2005	15	15 (100)	eCG (2000IU) + Folltropin-V (400 mg)	5.1
Nowshari *et al*	2005	11	10 (91)	Folltropin-V (400 mg)	1.8
Anouassi & Tibary	2013	153	106 (69)	eCG (3000 IU)	7.1
Anouassi & Tibary	2013	176	123 (70)	Folltropin-V (400 mg)	8.2
Ararooti *et al*	2017	6	6 (100)	hMG (16.5 ampules)	5.8
Ararooti *et al*	2018	5	5 (100)	Folltropin-V (390 mg)	16.2
Ararooti *et al*	2018	5	5 (100)	eCG (1000IU) + Folltropin-V (330 mg)	7.2
Manjunatha *et al*	2019	13	N/A	Pluset (2000 IU)	6.1
Manjunatha *et al*	2019	45	N/A	Folltropin-V (200 mg) dissolved in hyaluronan	5
Manjunatha *et al*	2019	42	N/A	Pluset (1000 mg) dissolved in hyaluronan	5.2
Manjunatha *et al*	2020	11	N/A	Folltropin-V (400 mg)	5.6
Manjunatha *et al*	2020	14	12 (86)	Recombinant eCG (3000 IU)	4.6
Average number of transferable embryos					5.1

Superovulation in camel

Three main gonadotropins used for superovulation in camel are equine Chorionic Gonadotropin (eCG), Follicle Stimulating Hormone (FSH) and human Menopausal Gonadotropin (hMG). Early efforts to superovulate camel in the early 1990s used eCG at various doses (Anouassi and Ali, 1990; McKinnon Tinson, 1992; Skidmore *et al,* 1992; Table 1). Anouassi and Tibary (2013) summarised the result of single injection of eCG (3000 IU) on 153 dromedary camels. They found that 31% of donors did not respond to eCG and the number of transferable embryos were 7.1±4.3 with great variation (0-19; Table 1). More recently, Manjunatha *et al* (2020) used newly released recombinant eCG (reCG) to superovulate camel. Accordingly, single injection of 3000 IU reCG could produce between 5-25 ovulation and 4.6±1.3 (0-7) transferable embryos. Most studies using eCG suffers the variation in response due to the inherent impact of eCG of any kind on superovulatory response. Two main advantages of using eCG for superovulation are easy administration (single injection) and low cost compared to other gonadotropins. However, several negative impacts of eCG on superovulatory response need to be considered including long half-life, the possibility of producing different generation of follicles, having both FSH and LH like effects, the possibility of luteinisation of follicles prior to ovulation which delays or entirely inhibits ovulation, and antigenic properties resulting in ovarian refractoriness following repeated administration of eCG, which ultimately results in low or lack of desired superovulatory response (Skidmore, 2003; Anouassi and Tibary, 2013).

The main negative impact of gonadotropins with prolonged half-life or at the doses beyond the requirement for suitable superovulation is continuous daily recruitment of different generation of follicles resulting in the formation of follicles with different diameters that could interfere in the process of ovulation followed by the production of un-ovulated follicles at the time of embryo recovery, which in turn, interrupt with the process of uterine flushing and embryo recovery (Ararooti *et al,* 2018a).

Ovine FSH (20 or 30 IU; Ovagen), twice daily, over 3 days were used in camel (Cooper *et al,* 1992; Skidmore *et al,* 1992; Table 1). However, follicular response and embryo recovery using these protocols were poor. From more than two decades ago, highly purified porcine FSH (Folltropin-V; LH to FSH ratio:0.12; Mikkola and Taponen, 2017) was used for superovulation in camel. Because of very short half-life of FSH, about 5 hours, typically, donor animals received FSH twice daily in decreasing doses manner, over 4 days (Anouassi and Tibary, 2013; Ararooti *et al,* 2017, 2018a). Anouassi and Tibary (2013) summarised the result of Folltropin-V injections (400 mg, twice daily over 4 days) on 176 dromedary camels. They found that 30% of donors did not respond to FSH and the number of transferable embryos were 8.2±6.1 with great variation (0-36; Table 1).

Three gonadotropins for superovulation in dromedary camel have been investigated (Ararooti *et al,* 2018a). Donors received porcine FSH alone (Folltropin-V; 390 mg), or combination of eCG (1000 IU im) and FSH (330 mg), and hMG (17.5 ampules. Donors in the first group, received twice daily FSH in decreasing doses (80, 50, 30, 20, 10 mg) for 5 days and single dose of FSH on Day 6 (10 mg). Donors in eCG-FSH group, received a single dose of eCG (1000 IU) on Day 0, followed by twice daily FSH in decreasing doses (60, 40, 30, 20, 10 mg), beginning on Day 0, followed by a single dose of 10 mg FSH on Day 5. In hMG group, donors received hMG (3, 2, 1.5, 1, 1 ampules) twice daily between Days 0 to 4, and 0.5 ampule on Day 5. The number of transferable embryos were 16.2, 7,2 and 1.6 in FSH alone, eCG-FSH and hMG, respectively (Table 1). We hypothesised that low superovulatory response in hMG group could be due to high total dose of hMG and/or the way of hMG administration. In another study, we have reduced the total dose of hMG to 16.5 ampules and we changed the injection protocol to twice daily injections over 5 days of 4, 2, 1, 0.5, 0.5 ampules and 0.5 ampule on Day 6. These modifications resulted in 5.8 transferable embryos which was comparable to FSH treatment (Ararooti *et al,* 2017; Table 1).

In earlier study porcine FSH (Folltropin-V; 400 mg) in decreasing doses (80, 60, 40, 20 mg) over 4 days was associated with low response (1.8 transferable embryos; Nowshari and Ali, 2005). However, in more recent study using the same amount of porcine FSH, twice daily, in decreasing dosages (60, 50, 40, 30, 20 mg), over 5 days was associated with good superovulatory response (5.6 transferable embryos; Manjunatha *et al,* 2020).The later authors used 2000 IU Pluset (LH to FSH ratio: 1; Mikkola and Taponen, 2017) in twice daily, in decreasing dosages (300, 250, 200, 150, 150 mg), over 5 days, which was associated with good superovulatory response (6.1 transferable embryos; Manjunatha *et al,* 2019). In order to simplify the administration of FSH, two intramuscular injections of FSH dissolved into 5 mg/

ml hyaluronan solution, 48 hrs apart, were examined to superovulate dromedary camel (Manjunatha *et al,* 2019). Using 200 mg Folltropin-V (120 and 80 mg at 48 hrs interval) or 1000 IU Pluset (600 and 400 IU at 48 hrs interval) were associated with 5 and 5.2 transferable embryos, respectively (Manjunatha *et al,* 2019; Table 1). Although this method of FSH delivery seems to be very simple, it needs to be repeated in large scale and by different groups to become routine procedure for superovulation in camel. The combination of eCG and FSH were also used in camel. Donors received a single injection of eCG (2000-2500 IU), in association with porcine FSH (400 mg) administered in decreasing doses (80, 60, 40, 20 mg twice daily) over 4 d. The average numbers of 4, 5.1 and 6.7 embryos were recovered using the latter protocol (Skidmore *et al,* 2002, 2005; Nowshari and Ali, 2005).

In conclusion, different gonadotropins could be used for superovulation in camel with great variation in superovulatory response. Although eCG seems to be a simple and cheap gonadotropin for superovulation, great variation and several drawbacks of eCG, might remove this gonadotropin as the first choice for superovulation in camel. Highly purified FSH seems to be the first choice for superovulation in camel. Although it is more expensive than eCG, it provides better response. The number of FSH injections could be reduced by dissolving FSH in hyaluronan. It could be possible to predict superovulatory response by using the right amount and the way of gonadotropin administration.

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REVISITING METHANOGENESIS IN CAMEL- A REVIEW

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ABSTRACT

Camel is often blamed for emitting methane, one of the potent greenhouse gas (GHG), to the environment. On the contrary, camel survives in the most vulnerable arid environment of the desert and may thus be considered environmentally friendly that resists the ensuing climate-change effect on the globe. This review discusses rumen ecological differences with respect to its structure, microbiome environment, substrate availability, fermentation behaviour to elucidate methanogenesis and its meagre emission contribution to GHG compared to other ruminant livestock. Besides, the feeding habits, nutrient metabolism and efficiency in energy utilisation are highlighted to corroborate 'Camel' as the environmentally friendly animal. Amongst the group camelids, the methane emissions from camel is principally discussed because of its predominant population, growth and structure in various parts of the world. In the hindsight, an environmental-friendly animal camel should not be blamed as GHG contributor due to relatively less feed intake and substantial reduced methanogenesis.

Key words: Camel, greenhouse gas, methane

The world camel population stands at ~35.03 million (FAOSTAT, 2021), mostly in the desert ecology of Asian and African countries (viz. Arabian and Sahara deserts) like Pakistan, Arab countries, Chad, Somalia, Sudan, Niger, Kenya, Ethiopia, Mali and Mauritania. Camel is considered as the most adapted domestic animal to desert conditions due to its anatomical features, thirst resistance, novel feeding behaviour and digestive physiology (Sahoo, 2020). Methane is a product of normal fermentation of feedstuffs which is produced in the rumen. Researchers found that 89% of methane emitted from ruminants is produced in the rumen and exhaled through the mouth and nose (Murray *et al,* 1976). The methane emissions at global level are from the agricultural sector (50-60%), specifically from livestock production operations, however, ruminants remain the principal source of methane emission (Ellis *et al,* 2007 and NRC, 2002). As per an estimate the domesticated ruminants produce about 86 million metric tonnes (Tg) of methane per year (McMichael *et al,* 2007). According to Johnson and Ward (1996), estimates the global yearly methane contribution of buffalo to be 6.2–8.1 Tg, 0.9–1.1 Tg from camels, and methane production within the hindgut of pigs and horses to be approximately 0.9–1.0 Tg and 1.7 Tg, respectively. A loss of diet energy for buffaloes and camels in the form of methane ranges from 7.5–9.0% and 7.0–9.0%, respectively (Johnson and Ward, 1996). Ciliate protozoa produce abundant H2, the main substrate for methanogenesis in the rumen, and their removal (defaunation) results in an average 11% lower methane emissions *in vivo*, but the results are not consistent (Tapio *et al,* 2017). Haque (2018) reviewed biology of methane emission from ruminants and its mitigation through dietary manipulation. The identification of methanogens in the rumens of cattle and sheep and methane mitigation strategies which were effective *in vivo*, were described by Hook *et al* (2010).

Methane emissions from ruminant livestock have been intensively studied and the enteric methane emission is found to be one of the major contributors to GHG. As camels share some features of their digestive anatomy and physiology with ruminants, it has been proposed that they produce similar amounts of methane per unit of body mass. Unfortunately, this unique eco-friendly animal of the desert is blamed for competing biomass reserve and contributing to greenhouse gases (GHG). The paper analyses unique anatomical, physiological, rumen ecological, feeding habit and digestive metabolism of camel in relation to its contribution to socio-economic livelihood besides maintain a greener environment.

Pseudo-rumen and its ecology

It is understood that unlike other ruminants, camel has no omasum and thus called as pseudo-

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ruminant. The digestive tract differs anatomically from that of true ruminants (cattle, buffaloes, sheep, goats), which have a 4 chambered forestomach, while that of camel is differentiated into three compartments and are referred as C1 (equivalent to the rumen), C2 (reticulum), and the gastric secreting compartment C3 (abomasum) (Sahoo, 2020). The C1 compartment or the camel rumen is an enlarged anaerobic fermentation chamber similar to other ruminants and it houses a similar complex microbial community consisting of bacteria, archaea, protozoa and fungi. The rumen microbial population as a whole shared a symbiotic relationship to decompose plant polysaccharides into their oligomers and monomers, and ferments further to short-chain volatile fatty acids (SCFA), which are subsequently absorbed from the rumen and utilised as a source of energy by the host animal (Flint, 1997; Jouany, 2000; von Engelhardt *et al,* 2007). Similarly, the microbial community inhabiting the rumen has an obligate symbiotic association with the host animal benefitting each other, maintenance of a dynamic complex microbial community that multiplies in the host and provides microbial cells and fermentation products as a source of protein and energy to the host by degrading plant lignocellulosic compounds that are indigestible for the host digestive system (Kamra, 2005). The rumen microbiota thus makes a large contribution to promotion of health, productivity, and immunity of the host animal. It is often difficult to quantify the role played by any particular microbial types or groups due to diversity and complicated synergism and antagonism among them. These microbes survive in the rumen under different constraints, be it natural or climate driven scarcity in feed resources to thrive on available shrubs, browses/trees, salt-tolerant vegetation, thorny plants, poor grazing conditions and scarcity of water (Sahoo *et al,* 2022).

The absence of omasum in camel may have also significant bearing on gastric emptying and retention of digesta. The proximal parts of the C3 (also referred as the 'gastric tube') are functionally similar to the ruminant omasum, but different anatomically (Langer, 1988). The omasum has been interpreted as the organ that reabsorbs fluid and hence facilitates an efficient sorting mechanism (digesta washing) and a great fluid throughput linked to high feed intake (Clauss and Hummel, 2017).

But, camel without omasum is not handicapped but showed adequate efficiency due to the suction-pressure pump mechanism exerted by motility of the canal at the proximal part of C3 comparable to reticulo-omasal canal in ruminants (von Engelhardt *et al,* 1988). Further, there are systematic differences in fermentative behaviour that corresponds to adaptations of the respective herbivores to forage-type and mean retention time (MRT) of feed in the fermentation chamber (Hummel *et al,* 2006). Dittmann *et al* (2015) observed a potentially slower particle sorting in camelids than in ruminants, with larger particles being retained longer in relation to small particles. The camel digestive system has adapted to harsh environmental ecology that are not favourable to other herbivore species (Sahoo, 2020) and it has a large foregut, leading to a much longer MRT for the ingested feed substrates. Camel has entirely a different basic pattern of motility and certain separation of feed particles from fluid seems to occur throughout the motility cycle, thereby achieving a longer MRT of fibrous feed particles (Lechner-Doll and Engelhardt, 1989). Thus, camel can utilise low-quality lignocellulolytic feeds, which are not preferred by other livestock species.

Rumen ecology: Camels live in deserts and dry lands, and usually graze on low quality natural forages and woody shrubs, which are highly fibrous and have a greater content of antinutritional compounds, such as tannins, saponins and lignins. They can survive in harsh and inhospitable environmental conditions, such as high salinity and drought with the minimal use of water, and their digestive system has evolved in order to adapt to these unfavourable conditions. A longer MRT of feed particles in the camel forestomach prolongs the exposure of plant biomasses to the symbiotic microorganisms and helps in efficient digestion of low-quality fibrous diets. In addition, the camel ruminal pH is largely constant and close to neutral, which further provides a suitable condition for growth and colonisation of cellulolytic microorganisms (Russell and Wilson, 1996; Samsudin *et al,* 2011). These unique characteristics of the camel digestive system, along with the distinct microbial community inhabiting its rumen, allow the animal to digest, ferment and extract the nutrients efficiently from plant lignocellulosic material.

Rumen fermentative behaviour

Rumen fermentative behaviour is dependent on type of substrates, maintenance of pH, retention time, passage rate and above all the anatomy and physiology of the forestomach and the inhabiting microbial population. Hummel *et al* (2006) observed highest maximal gas production in grass followed by herbs and legumes, and the lowest in browse

leaves and twigs. A longer passage time of feed particles seems to be adaptive for grazing ruminants, as over a wide range of fermentation times, absolute gas production rate is higher in grass compared with dicots and therefore, a higher intake level is expected to balance energy requirements of animals relying especially for browse leaves. But, camel has lesser metabolic requirement due to its unique thermoregulation mechanism (Sahoo, 2020) and it could meet its daily nutritional requirement even at a lower intake level. According to Dittman *et al* (2014a), camelids in general are characterised by relatively low metabolism and food intake, which might explain why these animal species are currently limited to arid environments with low food resources where a reduced metabolism represents an advantage. Upon comparative evaluation of food intake of herbivores, Meyer *et al* (2010) observed that camelids generally had lower food intakes per kg of metabolic body mass than ruminants at comparable forage fibre contents. It is hypothesised that this may be at least partly due to a different set of morphophysiological adaptations in the forestomach required to achieve 'rumination' compared to the Ruminantia, which might limit the food intake capacity of camelids.

Bhatt *et al* (2014) evaluated eleven different crop residues of the arid and semi-arid regions, viz. cereal crop residues- pearl millet (*Pennisetum typhhoids*), barley (*Hordeum vulgare*), sorghum (*Sorghum bicolour*), wheat (*Triticum aestivum*); leguminous crop residues- chick pea (*Cicer arietinum*), groundnut (Arachis hypogaea), clusterbean (*Cyamopsis tetragonoloba*); oilseed crop residues- sesamum (*Sesamum indicum*), mustard (*Brassica compestris*); and spices crop residues- cumin (*Cuminum cyminum*), fennel (*Foeniculum vulgare*) and found crop residues from pearl millet, cumin and groundnut as the promising ones with less CH_4 without compromising substrate degradability. Similarly, Pal *et al* (2015) screened eighteen tree leaves for methane production potential, degradability and rumen fermentation characteristics *in vitro* and found that the leaves of *Syzygium cumini*, Azadirachta indica, Ficus religiosa and Acacia nilotica have low methane production potential with greater OM degradability and microbial biomass production. In an explorative investigation, Bhatt *et al* (2020) assessed methane emitting potential of different feeds and fodders and found lower values in the tree forages and shrubs of arid climatic regions. Further, Bhatt *et al* (2021) assessed the effect of incorporating nonconventional roughages at 40% level in the diet of sheep and found significant reduction in enteric methane emission for Blepharis scindica and Trigonella foenum-graecum by 49.3% and 26.8%, respectively. This is evident from these reports that the browsing species and other available biomass of the arid and semi-arid regions of India are rich in phytochemicals and they exert rumen modulatory response in livestock (camel and other ruminants) to produce lower methane and other positive attributes to yield fermentation byproducts for use in maintenance and productivity.

Rumen microbiome: an update

The rumen ecosystems are typified by strong metabolic interactions between microbes that facilitate the fermentation of plant material to products useful for both the host and other rumen microbes. Therefore, the rumen microbiome of camel is structurally similar but compositionally different in comparison to other ruminants. Taxonomic analysis of metagenomic reads indicated Bacteroidetes (55.5 %), Firmicutes (22.7 %) and Proteobacteria (9.2 %) phyla as predominant camel rumen taxa (Bhatt *et al,* 2013). Unclassified Bacteroidales and Ruminococcaceae were more abundant in all animals fed forages, while Prevotella and unclassified Succinivibrionaceae were more abundant in animals fed diets containing concentrate and animals fed mixed diets were intermediate between these (Henderson *et al,* 2015). The unclassified Veillonellaceae were proportionally more abundant in sheep, deer, and camelids, which may be related to differences in rumen and camelid foregut sizes, anatomy, and feeding frequencies compared to bovines (Hofmann, 1989). Samsudin *et al* (2011) sequenced 267 bacterial 16S rRNA gene clones and clustered the sequences into 151 operational taxonomic units (OTUs) at a 99% sequence identity level. The majority of OTUs were annotated to the phyla Firmicutes (67%) and Bacteroidetes (25%), and were dominated by members of the families Eubacteriaceae, Clostridiaceae, Prevotellaceae, Lachnospiraceae, and Rikenellaceae. In another study the same group (Samsudin *et al,* 2012) inspected the camel rumen microbiota by using 283 16S rRNA gene clones for the presence of cellulolytic bacteria by inoculating the rumen digesta in three different enrichment media and identified, 33 OTUs affiliated to the genera Butyrivibrio, *Clostridium, Eubacterium*, Pseudobutyrivibrio, Schwartzia, Selenomonas, Anaerobiospirillum, Shigella, and Succinivibrio. Mishra *et al* (2020) applied 18S rRNA amplicon sequencing to explore eukaryotic diversity in the rumen of Indian camel (*C. dromedarius*; Bikaneri and

Kachchhi breeds) and found the presence of 92 genera belonging to 16 different divisions representing dominant Ciliophora (73%), followed by Fungi (13%) and Streptophyta (9%).

Henderson *et al* (2015) analysed rumen microbiota from 32 animal species and inferred that the composition of the rumen microbiota is largely determined by diet and it is less likely influenced by the host. But, rumen and camelid foregut microbial community structure could be expected to be shaped by morphological, physiological, and even behavioural characteristics that evolved along with the varied feeding strategies in the various ruminant lineages. Therefore, besides feed composition effects, host adaptations might also play a role in regulating rumen microbial community structure. In general, camel diet is dominated by a variety of woody shrubs and tree biomasses, along with various halophytes, species which are not favored by most ruminants (Sahoo and Sawal, 2021; Sahoo *et al,* 2022). Consequently, the rumen microbes must, therefore, have the capacity to degrade such recalcitrant feedstocks which are rich in lignocellulosic materials. We need to focus research on the digestive physiology, feeding types and nutrition and ruminal microbial community of camels to establish its climate-resilient production system and dairy potential with preservation of all nutraceutical properties for human health.

Rumen microbiome and methanogenesis

Characterisation of the ruminal microbial community provides opportunities to improve animal food digestion efficiency, mitigate methane emission, and develop efficient fermentation systems to convert plant biomasses into biofuels. Camel rumen has very close taxonomic and functional resemblance with that of cattle as both the animal species are herbivores with similar fermentative digestion process. There is dominant Bacteroides species (55.5%) followed by Firmicutes 22.7% and Proteobacteria 9.2% (Bhatt *et al,* 2013). Gharechahi *et al* (2015) applied 16S rRNA gene amplicon pyrosequencing to explore the structure of the bacterial community inhabiting the camel rumen and reported significant enrichment for cellulolytic bacteria different from those of other ruminants. There is relatively strong representation of Fibrobacteres species, known to target plant fibre and pectin, which may reflect the uniqueness of the camel's diet that includes material having a high content of lignocellulose. Faridi *et al* (2017) characterised the methanogenic archaeal community in the C1 compartment of the dromedary camel and found the most prevalent (76.82%) genus Methanobrevibacter (order Methanobacteriales) followed by archaea from the orders Methanomassiliicoccales (17.21%) and Methanomicrobiales (5.96%). An apparent shift of abundance from genus Methanomicrobium to Methanobrevibacter in camels can be attributed to their different digestive anatomy from true ruminants and high feed efficiency on low resources. Judging by their reported substrate utilisation, these species might be classed as hydrogenotrophic methanogens, i.e., using hydrogen or formate (Mbb. millerae, Mbb. smithii, Mbb. ruminantium, Methanocorpusculum bavaricum) and methylotrophic methanogens, i.e., using methanol or other methyl compounds ("Candidatus" *Methanoplasma termitum*). Rabee *et al* (2020a) is also of opinion that the camel rumen has differences in proportional dominance of bacterial (Firmicutes and Bacteroidetes) and archaeal (Candidatus, Methanomethylophilus) community and the abundant bacterial genera are Prevotella, Fibrobacteres, Ruminococcus and Butyrivibrio. Further, by applying total rRNA sequencing to identify active microbial communities in 22 solid and liquid rumen samples from 11 camels, Rabee *et al* (2020b) found dominance in order of bacterial, protozoal, archaeal and fungal communities represented by Firmicutes, Diplodinium, Thermoplasmatales and Neocallimastix, respectively.

Functional and substrate-specific microbes

Rumen microbiota facilitates nutrition through digestion of recalcitrant lignocellulosic substrates into energy-accessible nutrients and essential metabolites. Although there is high similarity in rumen microbiome structure, there might be distinct functional capabilities that enable camel to thrive on some unique plant biomass of the arid hot and cold regions with various lignocellulosic and phytochemical-rich substrates as feed. Functional analysis revealed clustering-based subsystem and carbohydrate metabolism as the most abundant SEED subsystem representing 17 and 13 % of camel metagenome, respectively (Bhatt *et al,* 2013). Metagenomic analysis of the camel rumen's microbiome identifies major functional group microbes responsible for lignocellulose degradation and fermentation (Gharechahi and Salekdeh, 2018). The camel rumen is host to a number of Spirochaetes species, which are also found in other lignocellulose degrading environments, such as the moose rumen (Svartström *et al,* 2017) and the termite hindgut (Warnecke *et al,* 2007). The camel

rumen's microbiome harbored a higher number of genes encoding glycoside hydrolases (GH) and carbohydrate-active enzymes (CAZyme) profile and an array of sequence features unique to the cellulosome complex, an elaborate multi-enzyme assembly that allows efficient decomposition of plant lignocellulosic materials different from those of the bovine rumen (Gharechahi and Salekdeh, 2018). Analysis of microbial composition through the solid and liquid fractions of rumen digesta revealed differential enrichment of members of Fibrobacter, Clostridium, Ruminococcus, and Treponema in the solid fraction, as well as members of Prevotella, Verrucomicrobia, Cyanobacteria, and Succinivibrio in the liquid fraction (Gharechahi *et al,* 2015). Compared to the bovine rumen's microbiome, the contribution of Bacteroidetes species to the production of CAZymes in the camel rumen was relatively high (56% vs 40%) and this unique carbohydrate degrading capability of the camel's rumen microbiome, as members of the phyla Bacteroidetes and Firmicutes are known as potent lignocellulose degraders. In addition to a high level of functional redundancy with respect to their lignocellulose degrading capability and VFA fermentation ability, the camel rumen's microbiome also displayed a degree of diversity with respect to types of lignocellulosic substrate. Members of the Bacteroidetes phylum showed broad substrate specificity as they contained a varied combination of CAZymes in the form of polysaccharide-utilisation loci (PUL), which are clusters of genes encoding catalytic CAZymes, sugar transporters and regulatory proteins, required for the sequestration, break-down and transport of glycan substrates. This may be inferred that the camel rumen exploits Bacteroidetes (PUL) enzymes to assimilate complex dietary carbohydrates. The Bacteroidetes and Firmicutes species have an equal capability for acetate, propionate and butyrate production in the camel rumen (Gharechahi and Salekdeh, 2018). The microbiota and CAZyme profile of Indian dromedary camel was studied by using 16S rRNA amplicon and shotgun metagenomics (Hinsu *et al,* 2021), which revealed abundant glycoside hydrolases (54.5%) followed by Glycosyl transferases (25.3%), Carbohydrate esterases (11.9%). They also observed similar abundance of Bacteroidota (60%) phylum followed by Firmicutes (14%) and Fibrobacteres (4%) with 13.3% remaining unclassified, and the most classified genera were Prevotella (23%), Bacteroides (4%), Fibrobacter (4%), Ruminococcus (1%) and Butyrivibrio (1%). Gharechahi *et al* (2022) applied genome-centric metagenomics for diversity analysis to explore phylogenetic diversity, lignocellulose-degrading potential and fermentation metabolism of biofilm-forming microbiota colonising 11 different plant substrates in the camel rumen and found significant variations in the community of rumen microbiota colonising different substrates in accordance with their varied physicochemical properties. Analysis of the genomic potential of the reconstructed genomes for CAZymes and the associated pathways for fermentation and utilisation of the degraded products revealed a significant diversity among different members of the rumen community and their widespread interdependencies for intermediate metabolites.

Earlier, Brooker *et al* (1994) isolated and identified tannin-tolerant and/or degrading bacteria such as *Streptococcus bovis*, Coprococcus sp., and Streptococcus caprinus, *Selenomonas ruminantium* and *Streptococcus gallolyticus* from the rumen fluids of camels. Ephraim *et al* (2005) found all five isolates from the faecal samples of camel hydrolysed tannic acid to pyrogallol and gallic acid to phloroglucinol. Camel are known to browse on tannin-rich leaves of the arid-climatic regions (Sahoo *et al,* 2022) and therefore substrate dependent adaptation of these isolated rumen microbes could play a potential role in the utilisation of tannin-containing feed. It is suggested that rumen microbial communities have been evolutionarily developed to complement each other and to support the host animal with energy-rich fermentation products, intermediate metabolites and vitamins. The familiarity of camel to a wide range of plant secondary metabolites highlights the key role of these small metabolites in rumen microbial interaction and function besides enriching host milk with nutraceutical and pharmaceutical effects to support human health. Further, existence of a wide range of secondary metabolite gene clusters with potentially diverse antimicrobial properties highlights the key role of these small metabolites in rumen microbial interaction and function most likely contributing to shaping the structure of the microbial community in the gut environment by targeting other microbes (Gharechahi *et al,* 2022) and therefore, a possible lower methanogenesis in camel contributing to greener environment cannot be ruled out. From the culture collection of different ruminal microbes at National Research Centre on Camel (NRCC), Bikaner, India having one or multiple functional properties e.g. fibre degrading, lipid-metabolising, tannin degrading, phytochemical-resistant, CLA-producing, etc. we envisaged that the peculiarity and superior health-

advantage in camel milk and meat can be modulated for human-health attributes. Application of NGS-based metagenomics surveys to characterise the constituents of the rumen microbiome can be applied to understand the nature of the carbohydrate or fibre degradation processes in the rumen with a reduced methanogenesis to achieve energy-efficient production.

Methanogenesis

Camels are known to produce methane similar to other ruminants, which is a potent GHG. When they eat, they regurgitate their food and chew it again, allowing microbes in their stomachs to break down the food and produce methane as a byproduct. The exact amount of methane produced by camels can vary depending on their diet, age, and other factors. While camel methane emissions are a concern for the environment, they are not a major contributor to global methane emissions compared to other sources such as cattle, rice cultivation, and fossil fuel extraction. However, efforts to reduce methane emissions from all sources are important to mitigate the impact of climate change. As there was insufficient information on camels' nutrition and digestion processes the IPCC Tier 1 method was used to arrive at approximate enteric methane emissions by extrapolation from main livestock categories that are considered to have a similar digestive system (IPCC, 2006). Accordingly, the estimate of enteric methane emission was 46 kg of CH_4/head/year for a camel weighing approximately 570 kg, which corresponds to 0.3942 kg CH_4/kg0.75/year or 1.08 mg/kg0.75/day. This figure was derived from an earlier report by Gibbs and Johnson (1993), who extrapolated methane emission figures of camels from cattle measurements.

Equation for predicting methane production

Blaxter and Clapperton prediction model: The earliest equation on predicting methane emission from ruminants is derived from a series of *in vivo* methane measurements experiments on sheep and cattle fed different diets based on digestible energy and feed intake as variables relative to maintenance (Blaxter and Clapperton, 1965).

$CH_4 = 1.30+0.122\ D+L\ (2.37-0.05\ D)$,

where, D Digestibility at maintenance level of feeding; L level of feeding

Later, different application and evaluation trials of Blaxter and Clapperton equation showed an overestimation and is not found suitable to apply on a wide range of feedstuffs varying in chemical constituents (CF, crude fibre; NfE, Nitrogen free extract; CP, crude protein; EE, ether extract). Therefore, nutrient data were used to predict the expected amount of CH_4 produced by domestic cattle on the corresponding diet using the equation of Kirchgessner *et al* (1991):

CH_4 (g/d) =63 + 806CF (kg/d) + 116NfE (kg/d) + 196CP (kg/d) – 1956EE (kg/d).

Similarly, an equation was developed to predict methane production potential of feedstuffs based on chemical composition (CP; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin) and *in vitro* dry matter degradability (IVDMD) (NIANP, 2021):

Methane (ml/100 mg digested substrate) = 14.346 + 0.010 × CP × NDF -0.069 × ADF - 0.001 × ADL - 0.136 × IVDMD

According to Bell *et al* (2016), increasing digestible organic matter (DOM) concentration in feed increases CH_4 yield, but increasing dietary ether extract (EE) and the feeding level above maintenance intake reduces CH_4 yield.

CH_4 (g/kg DM intake) = 0.046 × DOM - 0.113 × EE (g/kgDM) - 2.47 × (feeding level - 1)

Another equation is derived from theoretical fermentation balance based on the stoichiometry of the main rumen fermentative pathways, H2 transfer reactions and methanogenesis (Alemu *et al,* 2011).

CH_4 (mol/d) = (2Ac − Pr + 2Bu − Bc)/4 and CH_4 (MJ/d) = CH_4 (mol/d) × 0.882

Where, Ac- acetate, Pr- propionate, Bu-butyrate, Bc-branched chain fatty acids and 0.882 is heat combustion of CH_4 in MJ/mol.

But all these prediction equations need further studies to increase the accuracy of the prediction equation under different production conditions and variables, particularly animal species with unique feeding habbits such as camel that presents a different substrate fermentation and degradability pattern.

Camel versus other ruminants

The transhumance feeding system, foraging habit, ecology (environmental as well as host forestomach) and difference in metabolic adaptation between camel and other ruminants might have significant bearing on fermentative digestion process and emission of gases to the atmosphere. Although the report has acknowledged lack of information on camels, the default figure is not categorically evaluated by accounting for the differences in intake,

feeding behaviour, fermentation processes and production between camels and cattle. Guerouali and Wardeh (1998) has assessed the calorimetric estimates of methane emission from camels fed different levels of a diet at 0.999, 0.285, and 0.642 mg/kg0.75/day, during the periods fasting, feeding (consisting of barley grain and wheat straw) and refeeding, respectively. These values correspond to a total 26.3, 32.6 and 38.6 kg CH_4/year during feeding; 7.5, 9.3 and 11.0 kg CH_4/year during fasting and 16.9, 21.0 and 27.3 kg CH_4/year during re-feeding periods in 300, 400 and 500 kg live weight camels, respectively. It is to note that the feeding practice followed in this study is not a normal phenomenon in the field as camels are generally reared by the pastoralist communities that feed on ranges. Guerouali and Laabouri (2018) has compared dromedary camel with Holstein dairy cattle under the same diets (lucerne hay 2 kg/d and barley grain 3 kg/d) and housing conditions (housed individually) and measured CH_4 emission using face mask open circuit system. They found CH_4 emission from camels 1/3rd of that from cattle (47.7 vs 138.7 g/d or 17.4 vs 50.6 kg/year) and these estimates are similar to those reported earlier by Guerouali and Wardeh (1998). An Australian experiment on quantitative methane emission measurements in Bactrian camel revealed less methane emission (0.23 L/d/kg) compared to literature data on domestic ruminants, viz. sheep and goats (0.55 L/kg/d), cattle (0.66 L/kg/d) fed on similar roughage diets (Dittmann *et al,* 2014b). It may thus be inferred that the differences between camel and other ruminant livestock is attributed to substrate type (feeding habit), level of intake, microbial fermentation process, particulate fractional outflow rate, energy metabolism and underlying physiological mechanism responsible for methanogenesis and emission. The information compiled in NRC (2007) and ICAR (2013) inferred that camels consume less feed than other ruminants, due to their lower energy requirements. This differences in intake level between camel and sheep have also been confirmed in earlier and later studies (Kayouli *et al,* 1993; Khattab *et al,* 2021).

Ruminant livestock have been found to produce more enteric methane than other mammalian herbivores. But, camel is grouped as pseudo-ruminant and it shares some features of their digestive anatomy and physiology with that of true ruminants, the principal difference being absence of omasum. It has been proposed that they produce similar amounts of methane per unit of body mass. The compiled information in Table 1 has inferred differently. The CH_4 emission measured from the Bactrian camels on average amounted only

Table 1. Methane emission in camel versus other ruminants.

Animal species	Body weight	DMI%BW	Methane production				References
			L/d	L/d/kgBW	L/kgDMI	%DEI	
Cattle	525±42.2	2.06±0.168	346±27.6	0.66±0.030	32.0±2.09	12.0±1.71	Dittmann *et al,* 2014b
	350	-	194±17.8	0.55±0.193	42.2±4.09	-	Guerouli and Laabouri, 2018
Beef cattle	499±57	1.40±0.002	274±44.9	0.55±0.090	39.5±3.89	-	Bell *et al,* 2016
	400		119.0	0.297			Zeng, 2015
Dairy cow	571±61	2.99±0.006	567±100	0.99±0.175	33.8±5.09	-	Bell *et al,* 2016
	560±63	2.80±0.112	548±95.8	0.98±0.171	35.0±4.19		
	500		170.1	0.340			Zeng, 2015
Buffalo	450		82.0	0.182			Zeng, 2015
Sheep & Goat	53±3.62	2.09±0.116	29.2±1.96	0.55±0.028	26.4±1.04	11.6±0.58	Dittmann *et al,* 2014b
Sheep	50		38.5±4.79	0.77±0.096	43.8±5.39	-	Bell *et al,* 2016
	50		10.08	0.201			Zeng, 2015
Goat	40		7.49	0.187			Zeng, 2015
Camel	658±32.0	1.32±0.116	149±11.6	0.23±0.022	17.4±1.12	7.3±0.48	Dittmann *et al,* 2014b
	570	-	126	0.221	-	-	Al-Jassim and Hogan, 2013
	330	-	66.6±3.46	0.202±0.018	15.2±0.49	-	Guerouli and Laabouri, 2018
	400		64.8	0.162			Zeng, 2015
Camelids	106±12.8	1.75±0.098	38.0±5.18	0.35±0.028	21.2±1.28	8.3±0.75	Dittmann *et al,* 2014b

to 46% of the CH_4 production estimated from the equation derived from ruminant data (Kirchgessner *et al,* 1991) and adopted by IPCC (2006). Dittmann *et al* (2014b) demonstrated 45-66% less methane emission in Bactrian camel compared to the estimated values based on equations. This is important, when calculating GHG budgets for countries that harbour large populations of camels. The methane emissions expressed in per cent digestible energy intake is found to be lower in camels (7.3%) than in other ruminant livestock (11.7%). This lower methane emission of camelids can be explained by their generally lower relative food intake (NRC, 2007), but with higher utilisation efficiency. It is to note that the statement, 'Camel emits large amounts of methane', is untrue and thus, camel can be considered as the 'The Green Livestock' and most environmental-friendly animal of the planet.

Taking into account the lower ruminal short-chain fatty acid production by camel compared to other ruminants fed on same diet (Khattab *et al,* 2021; Torbati *et al,* 2023), the estimated methane by applying the stoichiometric equation (Alemu *et al,* 2011) also gave a lower value in camel. This phenomenon can be correlated to lower rumen fluid and substrate volume in camel due to scarcity of both in the arid region, longer retention time (Lechner *et al,* 1995; Dittmann *et al,* 2015) and larger difference between fluid and particle passage rate (Heller *et al,* 1986). It is interesting to note that this peculiarities in camel helps in enhanced energy gain due to increase in digestive efficiency (Clauss *et al,* 2009) accompanied by relatively lower feed intake. To correlate this fermentative digestion process with methane emission, a longer retention time is generally correlated with longer exposition to microbial fermentation that may lead to higher CH_4 production per unit food ingested. On the contrary, a lower feed intake, increased digestibility of dry matter (DM) and NDF (Kayouli *et al,* 1993; Khattab *et al,* 2021), lower protozoa population (Rouissi and Guesmi, 1996; Poonia *et al,* 2022) and difference in rumen microbiome (Bhatt *et al,* 2013; Gharechahi *et al,* 2015; Gharechahi and Salekdeh, 2018; Faridi *et al,* 2017; Hinsu *et al,* 2021) are in line with the concurrent findings that demonstrates a lower methane production (Table 1). Therefore, equations based on estimates following the IPCC guidelines for national GHG inventories developed for ruminant livestock to estimate CH_4 emissions have to be applied carefully and assessments should rather rely on specific measurements.

Camel and the environment

Camel is often blamed for emitting methane to the environment, one of the potent GHG. But, as per the findings (Table 1), ruminal methanogenesis seems to be different and the camel actually produces ~50% of methane compared to other ruminant livestock. Due to environmental concern, Australian Government run a series of projects to assess distribution and abundance of the feral camel (*C. dromedarius*), impacts of feral camel on rangeland in competition with other animal species, impacts on the environment and social/cultural values and economics of camel control in camel dense areas of central Australia (Edwards *et al,* 2010; Drucker *et al,* 2010; Saalfeld and Edwards, 2010). The dromedary camels have flexible adaptations and thrive where other species perish and, in many ways, they are the natural habitat in this harsh environment. But, numerous Australians, particularly ranchers, conservation managers, and increasingly local and national governments, perceived camel as pests and unwelcome invaders.

Reducing enteric methane emission

Since, emission of methane is a loss of fermentation energy above and beyond a concern for the environment, strategies to modulate methanogenesis is aimed at increasing the animals' energetic efficiency. Amongst the dietary manipulation, optimal use of phytochemicals in the ration of ruminant herbivores seems to be quite promising that modulates principally by i) decreasing methanogenesis, ii) diverting hydrogen sink with alteration in acetate: propionate ratio, iii) reducing ciliate protozoa population, iv) increasing microbial protein synthesis, v) improving nutrient intake and utilisation (Jakhmola *et al,* 2012). In an *in vitro* fermentation study with camel rumen inoculum, different doses of natural essential oils from semi-arid medicinal plants (Cotula cinereal, Juniperus phoenicea, Artemisia campestiris) showed significant reduction in methane production (Medila *et al,* 2017). Some plants or their extracts with high concentrations of bioactive plant secondary metabolites (PSM) such as saponins, tannins, essential oils, organosulphur compounds, flavonoids and many other metabolites appear to have potential to inhibit methane production in the rumen (Patra, 2010; Patra and Saxena, 2010; Jakhmola *et al,* 2012; Cieslak *et al,* 2013). The possible role of PSM and phytochemical-rich forages in modulating rumen fermentation and methanogenesis has been outlined as i) saponins mitigate methanogenesis mainly by

reducing the number of protozoa and the commensal microbes, ii) condensed tannins both by reducing the number of protozoa and by a direct toxic effect on methanogens, iv) essential oils act mostly by a direct toxic effect on methanogens and concurrently, v) combinations of phytochemicals modulating the process with additive or corroborate effects. Modulating rumen fermentation through additives and use of probiotics seems promising in enhancing intake, digestibility and utilisation of nutrients. A mixture of yeast (Saccharomyces cerevisiae) and microalgae (*Spirulina platensis* and *Chlorella vulgaris*) modulated the rumen microbial community (Phylum Bacteroidetes and Firmicutes) towards increasing degradation of dietary fibre and crude protein with improved feed intake and rumen fermentation (Rabee *et al,* 2022). Most often the resultant *in vivo* effect of efficient nutrient utilisation or energy utilisation with reduced methanogenesis seems to be the primary objective of any feeding strategy. On similar concept, differences between high and low residual feed intake (RFI) animals have been reported in terms of methane production (Nkrumah *et al,* 2006), as well as of some differences in microbial composition (Hernandez-Sanabria *et al,* 2012). It is also understood that dietary regime in early life might be another potential way to select for low methane emission during animal development (Sahoo *et al,* 2005; Furman *et al,* 2020).

The dependence of *Methanobrevibacter ruminantium* on simple substrates (H_2 +CO_2, formate) and its interaction with the rumen environment via surface proteins and exopolysaccharides are potential targets for inhibition. Attwood *et al* (2008) analysed the M. ruminantium draft genome to understand methanogen biology and speculated that uncovering approximately half of the identified genes within the genome that have no known function will help in exploring methane emissions control mechanism from ruminant animals. Understanding the metabolism of this organism and how it interacts with other microbes will identify conserved features among methanogens that may be inactivated to prevent or reduce methane formation in the rumen.

Advances in bioinformatics now allows us to get insight into the microbiome's metabolic networks, functional capabilities and species interactions. Moreover, future breeding strategies would benefit from fundamental research of isolated heritable microorganisms that show strong associations with the methane production trait and the mechanism underlying these associations. It is to keep in mind that mitigation strategies should only interfere to a minimal extent, not to affect food to feed ratio and to meet the food/protein necessity of humanity.

Conclusion

Methane emission from camel needs to be assessed separately as it inhabits in an arid and feed-scarce environment that is often not suitable to other animal species and in the process, has evolved as the most adaptive animal. The anatomy and physiology of the gastro-intestinal tract and the feeding habits separate out camel from other ruminants that possess a not-so-similar rumen ecology, anaerobic fermentation process and methanogenesis leading to less GHG emission. Efforts should go into characterising the metabolism and roles of bacteria that are responsible for the majority of feed fermentation, with the aim of enhancing animal productivity and reducing methane emissions. Combining metagenomic data with metatranscriptomic, metaproteomic and metabolomic data would help us understand various contributions of the camel rumen's microbiome in quality milk production having nutraceutical properties. The scope for future research also includes the identification of genes encoding enzymes involved in methanogenesis, e.g., methyl-coenzyme M reductase and formylmethanofuran transferase, to facilitate the understanding of mechanisms that mediate digestion in the ruminants. More studies are needed to characterise functional metabolic groups as well as rumen manipulation strategies to mitigate methane emission while optimising digestibility and utilisation of nutrients from the scarce and phytochemical-rich tough-to-be-digestible feed resources of the arid regions.

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LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION, PHARMACOKINETICS AND PHARMACODYNAMICS OF TRIAMCINOLONE ACETONIDE AND HYDROCORTISONE IN CAMEL PLASMA

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ABSTRACT

A sensitive, specific and rapid liquid chromatographic–tandem mass spectrometric (LC–MS/MS) method was developed and validated to quantify triamcinolone acetonide and hydrocortisone concentrations in camel plasma. Samples were prepared by solid-phase extraction using Oasis HLB cartridges. Separation was achieved with an Phenomenex Kintex C18 column (2.6 µm × 2.1 mm × 50 mm). The mobile phase was 0.1% formic acid (solvent A) and methanol with 0.1% formic acid (solvent B). A linear gradient was used at a flow rate of 0.3 ml/min with a run time of 7.5 minutes. The ionisation was optimised with positive electrospray source using multiple reaction monitoring transitions. The method was successfully applied to study the pharmacokinetic triamcinolone acetonide in camels (n = 5) following intramuscular administration of a dose of 50 µg/kg body weight. The data was analysed by non-compartmental analysis. The results obtained (median and ranges) were as follows: the terminal elimination half-life (t1/2) was 77.7 (42.9-120.2) h, AUC0-∞ was 747.7 + 383.4 (h*ng ml^{-1}), Cmax was 14.2 (11.8-26.1) ng/ml and Tmax was 3.0 (0.5-4.0) h. The plasma cortisol concentration was significantly lower than basal levels at time 1.5 h and remained significantly depressed until the last sampling day.

Key words: Camels, chromatography, hydrocortisone, pharmacodynamics, pharmacokinetics, spectrometry, triamcinolone acetonide

Triamcinolone acetonide (TRA), like other synthetic corticosteroids, possess anti-inflammatory effects. Synthetic corticosteroids are widely used in veterinary practice (Pugh, 1991; Minamijima *et al,* 2018). The rationale behind their use in race animals by practicing veterinarians and trainers is their ability to decrease the severity of inflammation particularly in the early phases of muscle strain injury and its modulation may be critical to rehabilitation and early return to racing activity following race injuries ((Drezner, 2003; Toumi and Best, 2003).

The pharmacokinetics (PK) of synthetic corticosteroids has been studied in various species including man, with fewer reports in camels. However, there are scant reports on the pharmacodynamics (PD) of synthetic corticosteroids in farm animals. We have previously reported on the PD of dexamethasone (DXM) in camels using integrated PK/PD modeling (Al Katheeri *et al,* 2004). Characterisation of these parameters in camels, together with controlled clinical efficacy trials, is one important factor that would allow development of appropriate dosage regimens. TRA is classified as a controlled drug in the 2021 Fédération Equestre Internationale prohibited substances list. Several analytical methods for TRA have been published previously. Most of them have employed high-performance liquid chromatography with ultraviolet detection (HPLC-UV), which suffers from low sensitivity (Beer *et al*, 2003; Yi *et al*, 2008) or used a laborious gas chromatography–mass spectrometry method (Chu *et al*, 2007). Few LC–MS/MS methods to quantify TRA in serum, plasma, lymph, urine, muscle and aqueous humor of human, equine or porcine have also been published in recent years (Kaklamanos *et al*, 2009; Cheng *et al*, 2009; Qu *et al,* 2007; Taylor *et al*,

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2004; Andersen *et al*, 2008; Zhang, 2011; Ho *et al,* 2019; Honeder *et al,* 2014; Dahm *et al,* 2021). However, these methods were either insensitive or involved time-consuming multiple liquid–liquid extraction steps, used expensive solid-phase cartridges, or had long run times. We have recently seen several positive cases of TRA in race camels in Saudi Arabia. The objectives of the present study were therefore, to develop a highly sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of TRA in camel plasma, apply the method to establish PK parameters for TRA in camels after intramuscular (i.m.) administration, study its effect on plasma cortisol, glucose and lymphocyte count and also to advise on preliminary withdrawal time based on analytical screening limit before racing.

Materials and Methods

All solvents and chemicals were of analytical grade or HPLC grade and TRA, hydrocortisone (HCOR) and the internal standards DXM and cortisol-D3 (COR-D3) were purchased from Sigma Aldrich with ≥ 98% purity (St. Louis, MO, USA). Oasis HLB cartridges, 3 CC, 60 mg were purchased from Gulf Scientific Corporation, Dubai, U.A.E.

Animals

Five clinically healthy male camels (*Camelus dromedarius*), 4-6 years old and ranging in body weight from 300 to 400 kg were used in this study. The camels were out of training and were kept in open pens. These were fed good quality hay and lucerne (alfalfa) once daily, with water allowed *ad libitum*. The Ministry of Agriculture, Veterinary Department, UAE approved the protocol.

Treatment

TRA (Vetalog Parenteral, 6 mg per mL, Boehringer Ingelheim Vetmedica, Inc.) was administered as i.m. injection at a dose of 50µg/kg body weight (manufacturer recommended dose for horses). Venous blood samples were drawn from the jugular vein in heparinised blood tubes at 0, 5, 10, 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 12, 24 h and at 2, 3, 4, 5, 6, 7, 8, 9 and 10 days timed from the start of injection of TRA. Blood samples were immediately placed on ice; plasma was separated by centrifugation at 4500 x g at room temperature for 10 min. The harvested plasma was frozen at -20°C until analysis for TRA, HCOR and glucose.

Extraction of plasma TRA and HCOR

Plasma samples (1 ml) were pipetted in duplicates into glass test tubes, and 3 mL phosphate buffer pH 6.0 was added. DXM (10 ng) and COR-D3 (50 ng) were added as internal standards for TRA and HCOR, respectively. Samples were vortexed and then centrifuged at 3000 rpm for 10 min, and the supernatant was decanted for solid-phase extraction. Oasis HLB cartridges were conditioned with 2 mL methanol and 2 mL water. Then samples were loaded onto the cartridges. Cartridges were washed with 2 ml 5% (v/v) methanol in water and dried for 5 min at 20 mm Hg. The analytes were eluted with 2 ml methanol. The eluent was evaporated under a nitrogen stream at 40°C, reconstituted in 100 µl of mobile phase and was analysed by a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

LC-MS/MS conditions

LC–MS/MS analysis for TRA, HCOR, COR-D3 and DXM was performed using a 5500QTrap mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an Agilent 1200 series HPLC system consisting of a binary gradient pump (Agilent Technologies, Palo Alto, CA, USA) and autosampler. A Phenomenex Kintex C18 column (2.6 µm × 2.1 mm × 50 mm) linked to a Phenomenex pre-column filter (4 mm × 2.0 µm, Torrance, CA) operating in gradient mode at 35°C was used. The mobile phase was 0.1% formic acid (solvent A) and methanol with 0.1% formic acid (solvent B). A linear gradient was used at 0.3 ml/min, with 40% solvent B at the start (t = 0 min), increasing to 90% solvent B at t = 3 min. The gradient was then returned to 40% solvent B at t = 4.0 min, and stabilised until t = 7.5 min before starting the next injection. The source was operated in positive ESI mode at 500°C with the nebuliser gas and heater gas set to 45 and 55 psi, respectively. Ion spray voltage was set to 5500V, curtain gas was set to 22 psi, and collision gas was set to medium. The resolution for the selection of the precursor ions in Q1 and the product ions in Q3 was set to unit mass. Detection of the analytes and internal standards were performed in the MRM mode with a single time segment and the scan time was 50 ms per transition. The mass transitions: m/z 435.3 → 415.2, and m/z 435.3 → 397 for TRA; m/z 393 → 373.1 and 393 → 355 for DXM; m/z 363.2→327.1 and m/z 363.2 → 309.1 for HCOR and m/z 366.2 → 121.1 for COR-D3 were used to selectively monitor precursor ions and corresponding product ions. The SRM transitions, declustering potentials, collision energies and the collision cell exit potentials for TRA, DXM, HCOR and COR-D3 were optimised by infusion of reference material. Data processing was performed using Analyst software (Version 1.5.1).

Analysis of plasma glucose

Plasma glucose concentration was determined by reflectance photometry using a clinical chemistry analyser (Hitachi 704, Hitachi Naka Works, Japan). Reagents, calibrators, and QC samples were supplied by the manufacturer. The calibration curves were linear from 0 to 500 mg/dl (r2= 0.9999).

Estimation of lymphocyte count

Two millilitres of blood were collected in blood tubes containing EDTA for the estimation of lymphocytes count by a Coulter instrument (Coulter model T 890, Northwell Drive, Luton, Beds, England).

LC-MS/MS method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, matrix effects limit of detection (LOD), limit of quantification (LOQ) and stability. Drug-free camel plasma was used for TRA validation. For plasma COR, being an endogenous compound and a small molecule, water was used instead as a surrogate matrix for the validation (Wakamatsu *et al,* 2018). Validation runs were conducted on five consecutive days. Each validation run consisted of one set of calibration standards and two replicates of QC samples (n = 30 total values in 5 days). For intra-assay coefficients of variation, 10 replica were used in the same day.

The selectivity of the method was evaluated by analysing 15 blank camel plasma samples. Calibration curves were constructed by analysing spiked calibration samples on five separate days. Peak area ratios of TRA and HCOR to DXM and COR-D3 were plotted against TRA and HCOR concentrations, respectively. Standard curves were fitted to the equations by linear regression. The calibration levels for TRA were: 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 15.0 and 30.0 ng/mL and the QC levels were 0.1, 1.0 and 10 ng/ml. The calibration levels for plasma HCOR were: 1.0, 5.0, 10.0, 15.0, 25, 50 and 100.0 ng/ml. QC levels were 5, 25 and 50 ng/ml. The lower limit of quantification (LOQ) was defined as the lowest concentration on the calibration curve, which can be quantified reliably, with acceptable accuracy (80–120%) and precision (<20%).

The LOD was determined as the lowest concentration detectable with a signal-to-noise ratio (S/N) > 3.

Accuracy and precision were assessed by the determination of QC samples in five validation days. The precision was expressed by the coefficient of variation (CV). Accuracy was expressed as relative error (RE %) to the nominal concentration.

The recovery of TRA and HCOR (A/B 100%) was evaluated by comparing the peak areas of QC samples (A) with those of reference QC solutions reconstituted in blank plasma (for TRA) and water (for HCOR) after extraction (B, n = 6). Recovery of the internal standards was determined in the same way. To evaluate the matrix effects (B/C × 100%), the extract of blank camel plasma samples were spiked after extraction with QC levels (B). The corresponding peak areas were then compared to those of neat standard solutions at equivalent concentrations dissolved in the mobile phase (C), and this peak area ratio was defined as the matrix effect. In the same way, the matrix effect of the internal standards was assessed at the working concentration (10 ng/mL for DXM and 50 ng/ml for COR-D3). For matrix effects investigations, 15 pooled samples of camel plasma were employed. Carry-over was assessed following injection of a blank plasma sample immediately after three repeats of the upper limit of quantification (ULOQ) and the response was checked. Carryover giving no greater than 5% of the response of the ULOQ in the blank samples was accepted. The stability of TRA in plasma and HCOR in water was examined for various storage or handling conditions. These included short-term tests and assessments were performed with the three QC levels. Short-term stability testing included one freeze–thaw cycle following storage at −20°C for 14 days, and concentrations were quantified before and after this process. Stability was also assessed by reinjection of processed QC samples after 24 and 72 h kept in the auto-sampler (10°C).

Pharmacokinetic calculations

Non-compartmental analysis for plasma TRA vs time for each animal was performed using least-squares nonlinear regression analysis program (WinNonLin Standard edition, version 1.5, USA). The PK parameters determined by the software were the elimination rate constant, plasma elimination half-life, MRT0-∞, AUCoberved, AUC0-∞, Cl/F, Volume/F, Cmax and Tmax.

Statistical calculations

Pharmacokinetic parameters are reported as medians and ranges. Plasma HCOR and glucose concentrations and lymphocyte counts were expressed as percentages relative to time zero values, which were regarded as 100%. Statistical analysis involved repeated measures analysis of variance and posthoc Dunnet testing where groups were compared to group zero (before TRA administration). A p-value less than 0.05 was considered statistically significant.

Results

LC-MS/MS method validation

The method was efficient and sensitive with acceptable validation criteria and there was more than 15 data points across the chromatographic peaks which is necessary for reproducible quantitation. Tables 1 and 2 summarise the method validation findings. The LOQ and LOD for TRA were 0.05 ng/mL and 0.01 ng/mL, respectively. The intra-assay coefficients of variation (n = 10) for TRA was less than 12% for all QC samples. The inter-assay coefficients of variation (n = 10) for TRA was less than 17% for all QC samples. The LOQ and LOD for HCOR were 0.025 ng/mL and 0.01 ng/mL, respectively. The intra-assay coefficients of variation (n = 10) for HCOR was less than 8% for all QC samples. The inter-assay coefficients of variation (n = 10) for HCOR was less than 11% for all QC samples. Accuracy (RE) was less than 15% for both TRA and HCOR. Extraction recovery for TRA and HCOR and their corresponding internal standards ranged from 79-88% and 90-102%, respectively. Specificity for TRA was confirmed by the absence of significant chromatographic peaks interfering with TRA and DXM in fifteen different camel plasmas. No interference was observed in water spiked QC samples. TRA and HCOR calibration curves were linear (r2= >0.995) and slopes of the standard curves varied by <13% over five different runs for TRA and <10% with five different runs for HCOR. Matrix effect was insignificant for both analytes and internal standards. Stability for all analytes was acceptable (+ 11% change from initial concentration) at 10°C for 24 and 72 h (in the autosampler) and after one freeze–thaw cycle following storage at −20°C for 14 days (Table 2).

Table 1. Precision, accuracy, extraction recovery and matrix effects for the determination of triamcinolone acetonide (TRA) in camel plasma and hydrocortisone (HCOR) in water.

Nominal concentration (ng/mL)	Intra-day RSD (%)	Inter-day RSD (%)	RE (%)	Extraction recovery (%)	Matrix Effect (% response)	LOQ	LOD
TRA							
0.1 (n =10)	11.8	16.2	14.1	71.3 ± 2.75	82.1	0.05	0.01
1.0 (n =10)	9.9 1	1.9	9.2	68.9 ± 0.39	84.8		
10 (n =10)	7.6	9.9	7.3	76.8 ± 1.97	87.5		
HCOR							
5.0 (n =10)	7.8	10.8	10.2	90.3 ± 2.46	95.2	0.025	0.01
25.0 (n =10)	5.9	8.8	7.8	100.9 ± 1.39	97.3		
50.0 (n =10)	6.8	8.3	8.1	101.2 ± 1.97	89.4		

Pharmacokinetic Analysis

The estimated PK parameters are presented in Table 3. TRA plasma concentration time profile

Table 2. Calculated concentration of triamcinolone acetonide (TRA) in camel plasma and hydrocortisone (HCOR) in water, demonstrating acceptable stability at 10°C for 24 h and 72 h (in the autosampler) and after one freeze-thaw cycle following storage at −20°C for 14 days.

QC level (ng/ml)	Initial run (ng/ml)	After 24 h in Autosampler (ng/ml)	After 72 h in Autosampler (ng/ml)	After one freeze-thaw cycle (ng/ml)
TRA				
0.1 (n =5)	0.11	0.093	0.089	0.102
1.0 (n =5)	1.05	0.93	0.91	0.93
10 (n =5)	9.73	9.23	9.45	11.4
HCOR				
5.0 (n =5)	4.95	4.27	4.56	4.86
25.0 (n =5)	25.1	26.1	24.81	23.98
50.0 (n =5)	50.9	48.9	47.95	49.52

Table 3. Pharmacokinetic parameters of TRA following intramuscular administration to 5 healthy camels @ 50 µg/kg BW. Values are presented as median and range.

Variable	Value Median and (range)
λz (h^{-1})	0.008 (0.006-0.016)
$T_{1/2\,\lambda z}$ (h)	77.7 (42.9-120.2)
$MRT_{0-\infty}$ (h)	118.1 (81.7-162.7)
$AUC_{0\rightarrow t10}$ (h*ng ml^{-1})	701.3 (229.6-1047.7)
$AUC_{0-\infty}$ (h*ng ml^{-1})	747.7±383.4
C_{max} (ng ml^{-1})	14.2 (11.8-26.1)
T_{max} (h)	3.0 (0.5-4.0)

λz = terminal slope; $T_{1/2\,\lambda z}$ = terminal elimination half-life; $MRT_{0\rightarrow\infty}$ = Mean residual time from moment 0 to infinity; T_{max} = time to maximal plasma concentration; C_{max} = maximal plasma concentration; $AUC_{0\rightarrow t}$ = area under the plasma concentration time curve to last measured concentration; $AUC_{0-\infty}$ = area under the plasma concentration time curve to infinity.

is presented in Fig 1. The plasma drug profiles were characterised by a very long terminal elimination half-life. The maximum plasma TRA concentration attained was 26.1 ng/ml and was observed at 3.0 h. At 240 h, the average TRA plasma concentration was 0.99 ng/ml.

The effect of TRA on plasma HCOR, Glucose and Lymphocyte count

In all animals there was a marked suppression of plasma HCOR concentration and lymphocyte number as well as a marked elevation on of plasma glucose concentration. The plasma HCOR concentration was significantly lower than basal levels at time 1.5 h and remained significantly depressed until the last sampling day (day 10) (Fig 2). Lymphocytes count followed a similar pattern and statistical significance was observed at 5 h post TRA administration, while return to basal levels was observed at time 72 h (Fig 2). Statistical significance of the increase of plasma glucose was observed between 4 to 120 h after TRA administration (Fig 2).

Discussion

The method proved to be efficient and sensitive with acceptable validation criteria. The method, with a run time of 7.5 minutes, was also quick; such that in one day, more than 80 samples could easily be processed and analysed. Our method for TRA with a LOQ of 0.05 ng/ml was more sensitive than methods reported previously by other authors (Ho *et al,* 2019; Honeder *et al,* 2014; Dahm *et al,* 2021). Ho *et al* (2019) made a ten time dilution of rat plasma with methanol and reported a LOQ of 0.2 ng/ml. Honeder *et al* (2014) reported a LOQ of 1.0 ng/ml with a run time of 15 minutes, while Dahm *et al* (2021) used a 4 times precipitation of plasma with methanol and obtained a LOQ of 0.5 ng/ml with a run time of 10 minutes.

Initially, we attempted to fit the data by compartmental analysis, looking for a best fit based on Akaike (1976) and Schwarz's (1978) criteria, analysis of residual plots, and correlation matrix, but that was not successful. Accordingly, a noncompartmental analysis was used because of the erratic absorption of TRA from the i.m. site into the bloodstream. Although TRA is not labelled for use in camels, it is widely used in race camels by camel owners and trainers. Following detection of TRA in plasma of race camels by our laboratory, camel owners usually claim that they have used it for more than three to four weeks earlier which is suggestive of a long terminal plasma half-life. This study has shown that TRA has a very wide range (42.9-120.2 h) of terminal elimination half-life in plasma in camels, with an average half-life of about three days. Indeed, intra- and inter-subject variability, which can be a significant source of inaccuracy, is one of the main issues for all PK *in vivo* research (Královicová *et al,* 2022). This can be explained by differences in genetic make-up, susceptibility to stress, feeding habits that can vary even among animals that are fed similarly, physical activity levels, and a variety of physiological factors. Many of these variables are impossible to measure or regulate throughout the studies. The slow absorption from the injection site, being an ester formulation, must have also resulted in a long plasma half-life of TRA. To the best of our knowledge, there are no previous studies on the PK of TRA in camels, thus we have no data to compare our findings with. However, in horses Knych *et al* (2013) reported a half-life of 11.4 days following an i.m. dose of 0.1 mg/kg body weight. On the other hand, Soma *et al* (2011) after an i.m. dose of 40 µg/kg body weight in horses reported an elimination half-life of 150.2 h and an elimination half-life of 23.8 h after intra-articular administration of the same dose into the carpal joint. Thus, in camels, the elimination half-life appears to be shorter than in horses. However, it should be noted that we stopped sampling at day 10 while our analytical method was capable of quantifying TRA concentration for more days. Had we extended our sampling to more days, the terminal slope might have been better characterised and a longer elimination half-life might have been seen in camels. On the other hand, French *et al* (2000) fitted their data to a three-compartment PK model following an intravenous dose of TRA of 0.2 mg/kg body weight to horses. A terminal elimination half-life of 12 hours was reported.

One of the objectives of the present study was to advise on TRA withdrawal times before camel racing. The international screening limit for controlling equine therapeutics for TRA is 0.5 ng/ml in urine. However, no limits are mentioned for equine plasma. Our laboratory receives camel blood samples for dope testing. The camel racing club adopts a zero medication rule (that is, it does not permit the presence of any drug and/or its metabolite at the time of racing).

PD parameters, like half-maximal inhibitory concentration (IC50), may be used to advise on withdrawal times of drugs. However, glucocorticoids possess a complex array of PD effects (Knych *et al,* 2020). We have used the suppression of plasma HCOR and blood lymphocyte numbers as well as

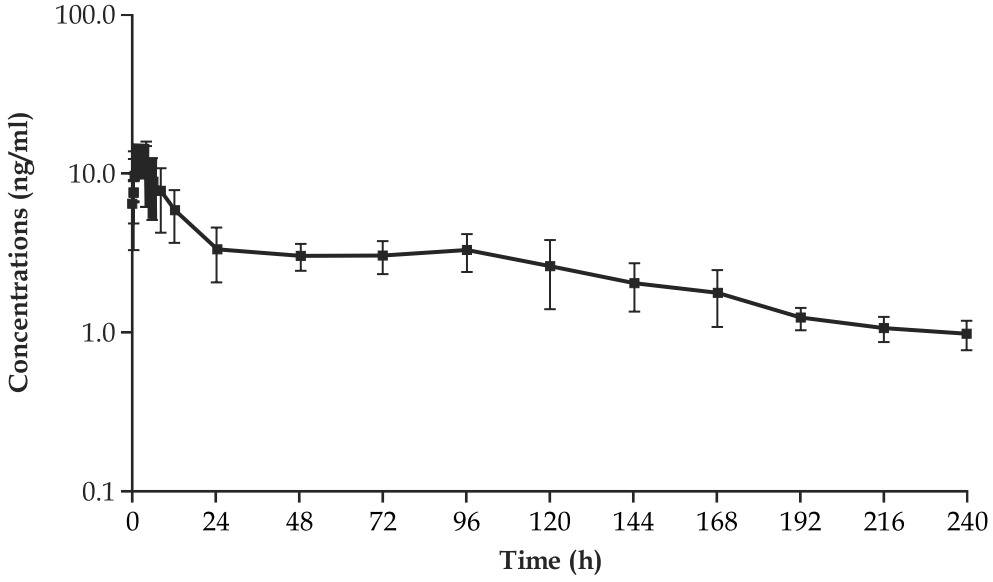

Fig 1. Triamcinolone acetonide plasma concentrations -time profile of five camels after an i.m. dose of 50 ug/kg BW. Values are presented as means ± standard errors of the mean.

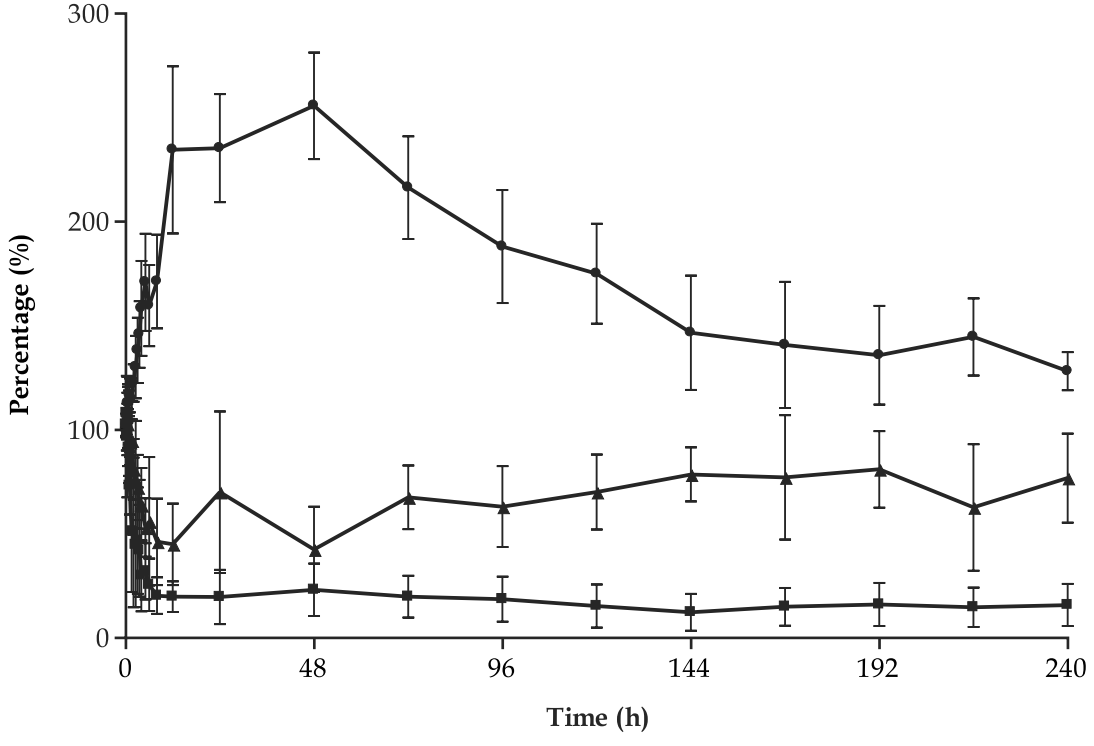

Fig 2. Effect of triamcinolone acetonide on plasma hydrocortisone (squares), glucose (circles) and lymphocyte count (triangles) after an i.m. dose of 50 µg/kg BW. Values are presented as mean + S.D.

the elevation of plasma glucose as PD effects of TRA administration. Of these, the suppression of plasma HCOR was mostly used in several studies previously in camels, horses, and dogs (Al Katheeri *et al*, 2004; Wasfi *et al,* 2018; Ekstrand *et al,* 2015; Knych *et al,* 2013; Soma *et al,* 2011; Morris *et al,* 2021). A significant reduction of plasma HCOR occurred at 1.5 h post TRA administration. There was a large variation in plasma HCOR but this is common in camels from different locations. Camel owners regularly buy and sell race camels, thus the origin of the camels used in the current experiment is not known. Moreover, plasma HCOR values are known to be affected by other factors like stress, transport, exercise, and

diurnal rhythm which would vary from animal to animal (El Khasmi *et al*, 2015; Krumrych *et al,* 2018). The maximum suppression effect occurred at around 144 h and persisted up to the last sampling time. A similar finding was reported in camels following i.v. DXM administration (Al Katheeri *et al,* 2004), as well as in horses (Soma *et al,* 2011). Glucocorticoids have both rapid and slower PD effects. The latter effect ocurs when a glucocorticoid binds to its receptor and stimulates target gene expression followed by protein synthesis, and hence a delay in some effects is expected. It is possible that plasma HCOR suppression would persist when the plasma TRA concentration is below detectable levels and below its IC50. In fact, we frequently observe extremely low plasma HCOR levels in samples from races where there are no measurable TRA levels in the blood or other glucocorticoids. Therefore, based only on PD values, it would be difficult to suggest TRA withdrawal durations prior to racing in camels, especially considering the significant inter-animal variation of established PD parameters.

Instead, recommendations on a withdrawal time-based analytical screening limit before racing might be made utilising PK values and the analytical detection capacity. A plasma concentration of TRA of 0.01 ng/ml is anticipated to be present 20 days after TRA injection. This is based on the plasma half-life reported in this study, the last plasma concentration seen at time 240 h (0.98 ng/ml), and the current LOD of the analytical method, which is 0.01 ng/ml. However, given to the substantial variability of the plasma half-life of TRA observed in this investigation (42.9–120.2 h), a preliminary withdrawal time of 40 days is indicated. It should be noted, however, that this withdrawal duration is based on a small number of animals, and additional research is required on various doses and routes of TRA administration in camels.

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None

Conflicts of interest

The authors declare no conflicts of interest

Animal welfare and ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have adhered to US standards for the protection of animals used for scientific purposes. Drug administration and blood sampling was conducted by qualified veterinarians.

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THE PHARMACOKINETICS AND PHARMACODYNAMICS OF BETAMETHASONE (PHOSPHATE AND DIPROPIONATE) IN CAMELS

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ABSTRACT

The pharmacokinetics and pharmacodynamics of a betamethasone (formulation dipropionate and sodium phosphate) were evaluated in 4 healthy camels after a single intramuscular dose of 35 µg/kg body weight. A sensitive, validated LC-MS/MS method for the quantification of plasma betamethasone and hydrocortisone was developed. Plasma betamethasone versus time concentration was best described by a two-compartment open model. The pharmacokinetic parameters median and range were as follows: terminal elimination half-life was 7.17 (6.93-7.58) h, Cmax was 15.9 (10.8-20.85) ng/ml, and Tmax was 0.5 (0.25-0.75) h. The estimated IC50 for hydrocortisone (mean ± SD) was 0.09 ± 0.08 ng/ml. Based on the analytical method and plasma terminal elimination half-life, a 4-day withdrawal period of the betamethasone formulation is advised prior to racing.

Key words: Betamethasone, camels, pharmacokinetics, pharmacodynamics

Betamethasone (BMT) is a synthetic corticosteroid and is a stereoisomer of dexamethasone (DEX). Synthetic corticosteroids depress formation, release and activity of endogenous mediators of inflammation, including prostaglandins, kinins, histamine, liposomal enzymes, and the complement system (Barnes, 1998). Synthetic corticosteroids are potent drugs widely used in veterinary practice. These are prohibited by most racing authorities and the Fédération Equestre Internationale. These can be administered either by a systemic or topical route (Popot *et al,* 2003). These are usually used for the treatment of joint disorders, such as osteoarthritis and muscle injuries (Sokolowski, 1982; French *et al,* 2000; Murray *et al,* 2002). Treatment during the early stages of inflammation is critical for early rehabilitation and return to racing activity following race injuries (Drezner, 2003; Toumi and Best, 2003). The use of corticosteroids administered by intra-articular injection has become commonplace recently. Direct injection into the site of action allows the use of low doses of corticosteroids, which results in relatively low urine and plasma concentrations compared with systemic administrations (Popot *et al,* 2003). There are reports describing the detection times of BMT in horses after intra-articular administration (Popot *et al,* 2003; Knych *et al,* 2017; Menendez *et al,* 2016). However, the use of corticosteroids administered by intra-articular injection in camels is not a common practice. We are not aware of any report describing intra-articular administration of a corticosteroid in camels. An injectable formulation of BMT for intramuscular (i.m.) administration is available on the UAE market and is frequently used by veterinarians on race camels. Our laboratory receives plasma samples following camel races. The authorities and camel owners both request information on the BMT withdrawal period following a therapeutic dose of BMT.

The goal of the current investigation was, therefore, to create a sensitive and validated LC-MS/MS method for the quantification of BMT in camel plasma and use it to describe the pharmacokinetics (PK) of BMT in camels following a single i.m. injection. Using plasma hydrocortisone (HCOR) suppression, we also wanted to assess the pharmacodynamics (PD) of BMT in camels. Another goal of the study was to provide advice to the camel racing authority regarding the timing of BMT's withdrawal based on the analytical method and PK values.

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Materials and Methods

BMT and triamcinolone acetonide (TRA), used as internal standard, were purchased from Sigma Aldrich with 98% purity (St. Louis, MO, USA). All solvents and chemicals were of analytical grade or HPLC grade and were obtained from Sigma Aldrich (St. Louis, MO, USA). Oasis HLB cartridges, 3 CC, 60 mg, were purchased from Gulf Scientific Corporation, Dubai, U.A.E.

Animals

Four clinically healthy male camels (*Camelus dromedarius*), 5-7 years old and ranging in body weight from 350 to 500 kg, were used in this study. The camels were out of training and were kept in open pens. They were fed good quality hay and Lucerne (alfalfa) once daily, with water allowed *ad libitum*. The Ministry of Agriculture, Veterinary Department, UAE, approved the study protocol.

Treatment

Combine BMT (Diprospan injection contains betamethasone dipropionate equivalent to 5 mg betamethasone and betamethasone sodium phosphate equivalent to 2 mg betamethasone in a sterile buffered and preserved vehicle, Schering-PlowLabo NV, Belgium) was administered as an i.m. injection at a dose of 35 µg/kg body weight. Venous blood samples were drawn from the jugular vein in heparinised blood tubes at 0, 5, 10, 15, 30, 45 min and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 12, 24, 48. 72, 96 and 120 h. Blood samples were immediately placed on ice; plasma was separated by centrifugation at 4500 x g at room temperature for 10 min. The harvested plasma was frozen at -20°C until analysis for BMT and HCOR.

Extraction of plasma for BMT and HCOR

Plasma samples (1 ml) were pipetted in duplicates into glass test tubes, and 3 ml of phosphate buffer pH 6.0 was added. TRA (5 ng) was added as an internal standard. Samples were vortexed and then centrifuged at 3000 rpm for 10 min, and the supernatant was decanted for solid-phase extraction. Oasis HLB cartridges were conditioned with 2 ml of methanol and 2 ml of water. Then samples were loaded onto the cartridges. Cartridges were washed with 2 ml of 5% (v/v) methanol in water and dried for 5 min at 20 mm Hg. The analytes were eluted with 2 ml of methanol. The eluent was evaporated under a nitrogen stream at 40°C, reconstituted in 100 µl of mobile phase, and analysed by a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

LC-MS/MS conditions

LC–MS/MS analysis for BMT and TRA was performed using a 5500QTrap mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an Agilent 1200 series HPLC system consisting of a binary gradient pump (Agilent Technologies, Palo Alto, CA, USA) and an autosampler. A PhenomenexKintex C18 column (2.6 µm × 2.1 mm × 50 mm) linked to a Phenomenex pre-column filter (KrudKatcher ULTRA, 2.0µm Depth Filter x 0.004 in ID) operating in gradient mode at 35°C was used. The mobile phase was 0.1% formic acid in water (solvent A) and methanol with 0.1% formic acid (solvent B). A linear gradient was used at 0.3 ml/min with 40% solvent B at the start (t = 0 min), increasing to 90% solvent B at t = 3 min. The gradient was then returned to 40% solvent B at t = 4.0 min and stabilised until t = 7.5 min before starting the next injection. The source was operated in positive ESI mode at 500°C with the nebuliser gas and heater gas set to 45 and 55 psi, respectively. Ion spray voltage was set to 5500V, curtain gas was set to 22 psi, and collision gas was set to medium. The resolution for the selection of the precursor ions in Q1 and the product ions in Q3 was set to unit mass. Detection of the analyte and internal standard was performed in the MRM mode with a single time segment and the scan time was 50 ms per transition. To selectively monitor precursor ions and corresponding product ions, the mass transitions m/z 393→355 and m/z 393→147 for BMT; m/z 435.3→415.2 and m/z 435.3→397 for TRA were used. The SRM transitions, declustering potentials, collision energies, and the collision cell exit potentials for BMT and TRA were optimised by infusion of reference material. Data processing was performed using Analyst software (Version 1.5.1). Plasma HCOR was analysed as previously reported (Wasfi *et al*, 2018).

LC-MS/MS method validation

The method was validated for selectivity, linearity, accuracy, precision, recovery, matrix effect, limit of detection (LOD), limit of quantification (LOQ), and stability. Pooled drug-free camel plasma from 15 camels was used for BMT validation. Validation runs were conducted on four consecutive days. Each validation run consisted of one set of calibration standards and two replicates of QC samples (n = 24 total values in 4 days). For intra-assay coefficients of variation, 10 replicas were used on the same day.

The selectivity of the method was evaluated by analysing 15 blank camel plasma samples. Calibration curves were constructed by analysing

spiked calibration samples on four separate days. Peak area ratios of BMT and TRA were plotted against BMT concentrations. Standard curves were fitted to the equations by linear regression. The calibration levels for BMT were: 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 15.0, 30.0 ng/ml, and the QC levels were 0.1, 1.0, and 10 ng/ml. The lower limit of quantification (LOQ) was defined as the lowest concentration on the calibration curve that can be quantified reliably with acceptable accuracy (80–120%) and precision (± 20%). The LOD was determined as the lowest concentration detectable with a signal-to-noise ratio (S/N) > 3.

Accuracy and precision were assessed by the determination of QC samples in four validation days. The precision was expressed by the coefficient of variation (CV). The accuracy was expressed as a percentage of the nominal concentration (RE%).The recovery of BMT (A/B × 100%) was evaluated by comparing peak area of QC samples (A) with those of reference QC solutions reconstituted in blank plasma after extraction (B, n = 6). Recovery of the internal standard was determined in the same way.

To evaluate the matrix effect (B/C × 100%), the extract of blank camel plasma samples were spiked after extraction with QC levels (B). The corresponding peak areas were then compared to those of neat standard solutions at equivalent concentrations dissolved in the mobile phase (C), and this peak area ratio was defined as the matrix effect. The matrix effect of the internal standard was evaluated at the working concentration (5 ng/mL) in the same manner. Fifteen pooled camel plasma samples were used for matrix effects experiments. Carry-over was assessed following injection of a blank plasma sample immediately after three repeats of the upper limit of quantification (ULOQ) and the response was checked. Carryover giving no greater than 5% of the response of the ULOQ in the blank samples was accepted. The stability of BMT and TRA in plasma was examined for various storage or handling conditions. This included short-term tests and assessments were performed with the three QC levels. Short-term stability testing included one freeze–thaw cycle following storage at −20°C for 14 days and concentrations were quantified before and after this process. Stability was also assessed by reinjection of processed QC samples after 24 and 48h kept in the auto-sampler (10°C).

Pharmacokinetic and pharmacodynamics analysis

PK analysis of plasma BMT concentrations for each animal was performed using the least-square nonlinear regression analysis programme (WinNonLin Standard edition, version 4.0.1, Pharsight, Sunnyvale, CA, USA). One and two-compartment models were tested for the best fit to the i.m. administration data. The best fit was based on Akaike (1976) and Schwarz (1978) criteria, analysis of residual plots, and the correlation matrix. The PK-PD surrogate, the IC50 for the reduction of plasma HCOR concentration, was calculated. Calculations were performed on individual data using least-square nonlinear regression analysis (WinNonLin Standard edition, version 1.5, USA). An indirect response model describing the PD effect of the drug with the mechanism producing the effect was used (Dayneka *et al,* 1993; Al Katheeri *et al,* 2004). Good modelling could be obtained if corticosteroids were assumed to modify the influx of HCOR by decreasing Kin, causing a reduction of HCOR concentration in blood (Al Katheeri *et al,* 2004; Wasfi *et al,* 2018). As corticosteroids exert their effects via receptors, the Emax model was reported as appropriate (Möllmann *et al,* 1998) which is described by the equation:

$$\frac{dC_{BMT}}{dt} = K_{in} \quad \frac{(1- C_{BMT})}{IC_{50} + C_{BMT}} \; - K_{out} \cdot C_{BMT}$$

where C_{BMT} is the plasma free BMT concentration, and IC_{50} is the concentration of free BMT that will result in 50% of maximum inhibition. IC_{50} is unique to each corticosteroid and reflects its potency.

Statistical calculations

PK parameters are reported as medians and ranges and PD parameters are reported as mean ± SD.

Results

LC-MS/MS method validation

The LOQ and LOD for BMT were 0.05 ng/ml and 0.025 ng/mL, respectively. The intra-assay coefficient of variation (n = 10) for BMT was less than 9% for all QC samples. The inter-assay coefficient of variation (n = 10) for BMT was less than 15% for all QC samples. The accuracy (RE) was less than 12%. Extraction recovery for BMT and TRA ranged from 75-88%. Specificity was confirmed by the absence of significant chromatographic peaks interfering with BMT and TRA in fifteen different camel plasmas. The BMT calibration curves were linear (r^2 = > 0.995) and the slopes of the standard curves varied by 13% over four different runs. The matrix effect was insignificant for both BMT and TRA. Stability for BMT was acceptable (± 11% change from initial concentration) at 10°C for 24 and 72 h (in the autosampler) and after

one freeze–thaw cycle following storage at -20°C for 14 days.

PK-PD Analysis

BMT was well tolerated in camels, and no side effects were observed during the experimental period. Pharmacokinetic parameter estimates of BMT (median and range) following i.m. administration are shown in Table 1. Fig 1 depicts the BMT plasma concentrations–time profile. The plasma drug profiles were characterised by a fast distribution phase and a terminal elimination half-life of 7.17 (6.93-7.58) h. The plasma HCOR concentration was significantly lower than basal levels at time 2.0 h and remained significantly depressed until day 4 (Fig 2). The estimated IC50 of BMT for COR (mean ± SD) was 0.09 ± 0.08 ng/ml.

Table 1. Pharmacokinetic parameters of betamethasone following intramuscular administration to 4 healthy camels at a dose of 35 µg/kg body weight. Data are expressed as median and ranges.

Variable	value
$T_{½\alpha}$ (hour)	1.00 (0.47-1.99)
$T_{½\beta}$ (hour)	7.17 (6.93-7.58)
$AUC_{0-\infty}$ (ng $hour^{-1}$ per mL)	67.91 (50.23-78.42)
Tmax (h)	0.5 (0.25-0.75)
Cmax (ng/ml)	15.92 (10.8-20.85)
T ½ absorption (hour)	0.05 (0.02-0.20)

$T_{½\alpha}$ = half–life of distribution phase; $T_{½\beta}$ = half–life of elimination phase; AUC= area under the curve to infinity; Tmax= time of maximum concentration; Cmax= maximum concentration; $T_{½}$ absorption = absorption half-life.

Discussion

The validated method developed in this report proved to be sensitive and robust with a LOQ and LOD of 0.05 ng/mL and 0.025 ng/mL, respectively. The method will allow the control of low doses of BMT in camel races, which results in relatively low plasma concentrations. In contrast, BMT phosphate ester was not detected in camel plasma in the current study. However, BMT phosphate ester is shown in humans to have an exceedingly short half-life of 4.7 min (Petersen *et al,* 1983) and that is rapidly and completely converted to BMT *in vivo* so that BMT phosphate ester were rarely determined for the quantification in human plasma *in vitro* and *in vivo* (Salem *et al,* 2012; Ahmed and Atia, 2013). Additionally, neither the BMT dipropionate ester nor its monopropionate esters were found in camel plasma, which may also indicate that they have a very short half-life or that they were degraded by esterases at the injection site or in the plasma. However, Chen *et al* (2016) used a similar formulation in humans with an i.m. dose that was 2.6 times larger than the dose we used in camels. These authors measured BMT, betamethasone dipropionate, and betamethasone 17 propionate in plasma, the latter at low concentration (Cmax, 2.2 ng/ml). Betamethasone 21 propionate, however, could not be quantified by the authors.

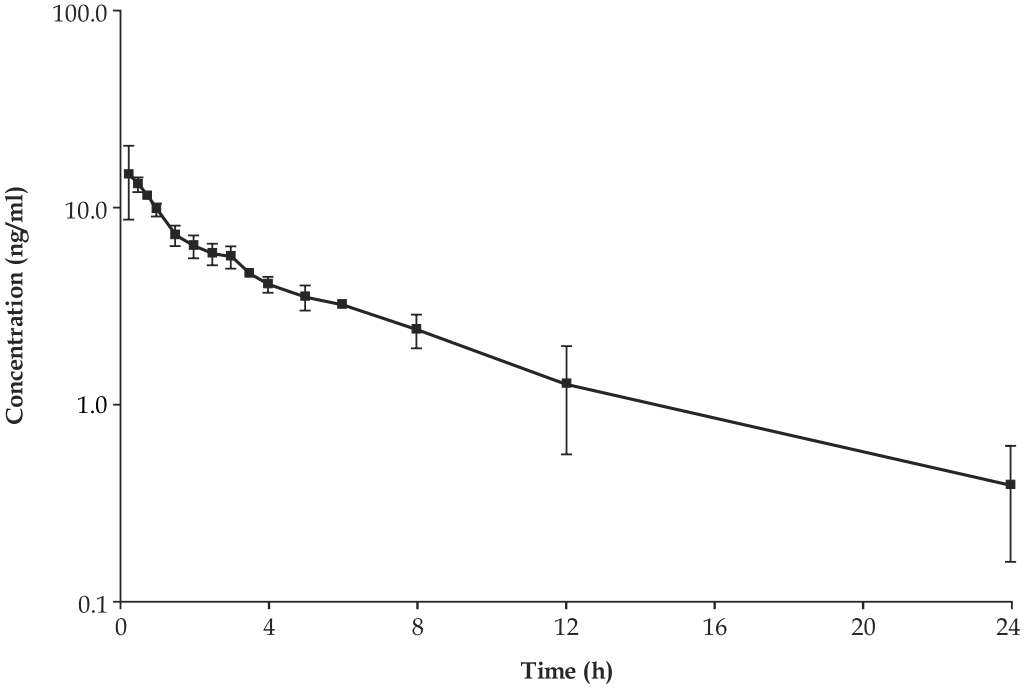

Fig 1. Betamethasone plasma concentrations -time profile of four camels after an i.m. dose of 35 µg/kg BW. Values are presented as means ± standard deviations of the mean.

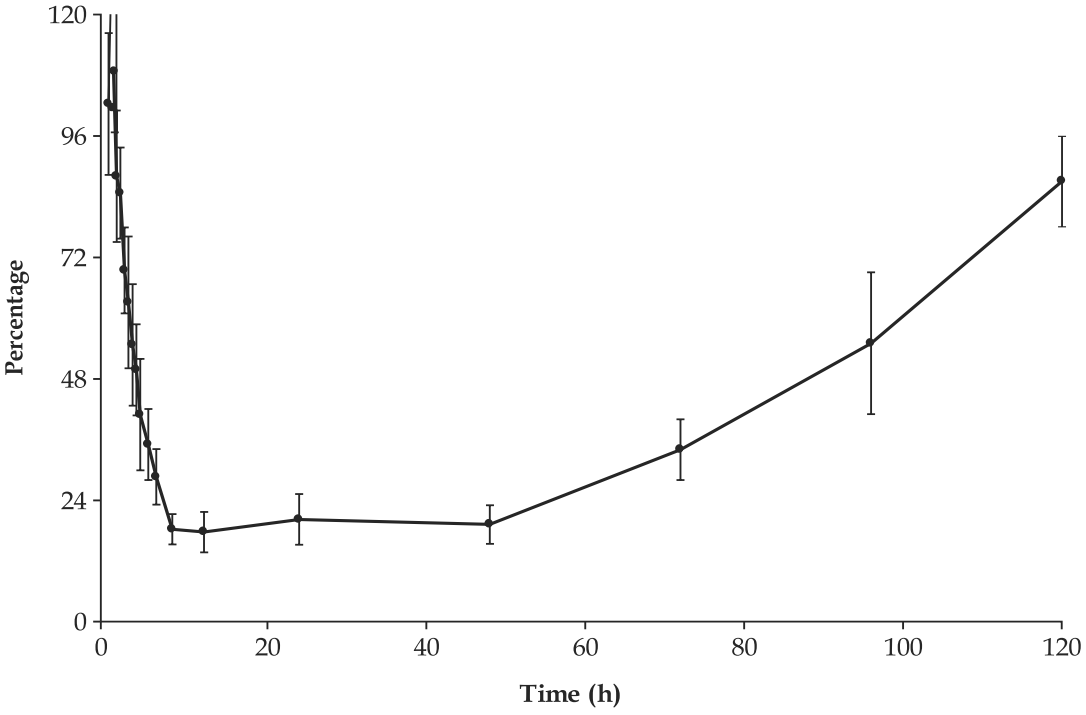

Fig 2. Hydrocortisone plasma concentrations -time profile of four camels after an i.m. dose of betamethasone of 35 µg / kg BW. Values are presented as means ± standard deviations of the mean. The plasma hydrocortisone concentration was significantly lower than basal levels at time 2.0 h and remained significantly depressed until 96 h.

The research on PK/PD of BMT in camels is not traceable in available literature hence our results could not be compared with other studies. However, DXM, a stereoisomer of BMT, was reported to have a half-life of 7-8 h in camels after an i.v. of 50 µg/kg body weight (Al Katheeri *et al,* 2004), which is similar to the half-life of BMT in camels reported in the current study. There are two reports which describe the PK of BMT in horses after intra-articular administration (Knych *et al,* 2017; Menendez *et al,* 2016). In one study (Knych *et al,* 2017), the authors administered a single intra-articular administration of 9 mg of betamethasone sodium phosphate and betamethasone acetate injectable suspension in the right antebrachiocarpal joint of exercised thoroughbred horses. The reported terminal elimination half-life in plasma was 7.48 ± 0.39 h (mean ± SE), which was close to the plasma terminal elimination half-life in camels reported in this study (7.17, 6.93-7.58 h, median and range). According to those authors, BMT could be found in plasma at a maximum concentration of 3.97 ng/ml, compared to the value of 15.92 (10.8-20.85) ng/ml reported in this study (median and range). In the other study (Menendez *et al,* 2016), the authors administered a similar preparation of the BMT formulation used by the authors in the first study. However, the dose used was 30 mg divided into two different joints, which resulted in a maximum plasma concentration of BMT of 26.64 ± 4.79 ng/ml (mean ± SE) and a slightly longer half-life of 9.22 ± 0.86 h. Samtani *et al* (2005) studied the PK of two formulations of BMT in sheep. Following i.m. administration of BMT phosphate (0.25 mg/kg) and betamethasone phosphate/acetate (0.5 mg/kg), they reported a plasma terminal half-life of 4h and 14 h, respectively. The formulation used in the current study produced a peak concentration of about 10.8–20.85 ng/ml at 0.25-0.75 h, which reflects the fast input from the prodrug (Ka half-life of 0.05 h).

It has been reported that administering BMT preparations results in a significant decrease in endogenous HCOR concentrations (Petersen *et al,*1983; Popot *et al,* 2003). Exogenous synthetic corticosteroids have been shown to suppress HCOR in horses (Bayer *et al,* 2001) and camels (Al Katheeri *et al,* 2004; Wasfi *et al,* 2018). In the current study, the IC_{50} of BMT for the suppression of endogenous HCOR was 0.09 ng/ml, indicating that it is a very potent corticosteroid when compared to values reported by us previously for dexamethasone (3.74 ng/ml) (Al Katheeri *et al,* 2004) and flumethasone (4.52 ng/ml) in camels (Wasfi *et al,* 2018).

The maximum suppression effect occurred at around 6 h and persisted up to day 4 after BMT

administration. A similar finding was reported in camels following i.v. DXM administration (Al Katheeri *et al,* 2004). Corticosteroids have both rapid and slower PD effects. It is possible that plasma HCOR suppression would persist when the plasma BMT concentration is below detectable levels and below its IC_{50}. In fact, we frequently observe extremely low plasma HCOR levels in samples from races where there are no measurable BMT levels in the blood or any other corticosteroid.

The Asian Racing Federation and the International Federation of Horse racing Authorities describe a screening limit for BMT in horse urine of 0.2 ng /ml but no plasma values are described (Wong and Wan, 2014). The Camel Racing Association in Saudi Arabia adopts a zero medication rule in plasma samples taken post-racing. The LOQ reported in this study was 50 pg/ml and the average plasma BMT concentration at time 24 h was 390 pg/ml. Accordingly, based on the analytical results and plasma terminal elimination half-life, it will take 2-3 additional half-lives, or 2 days after BMT treatment, for the plasma BMT concentration to fall below the LOQ. In fact, BMT could be quantified in one camel at day 2 but not in all camels at days 3, 4, or 5 after injection. However, a 4-day withdrawal period is advised before racing due to animal variability and as a safety measure. It should be noted, however, that this withdrawal period is based on a limited number of animals and further work is required on various doses and routes of BMT administration in camels.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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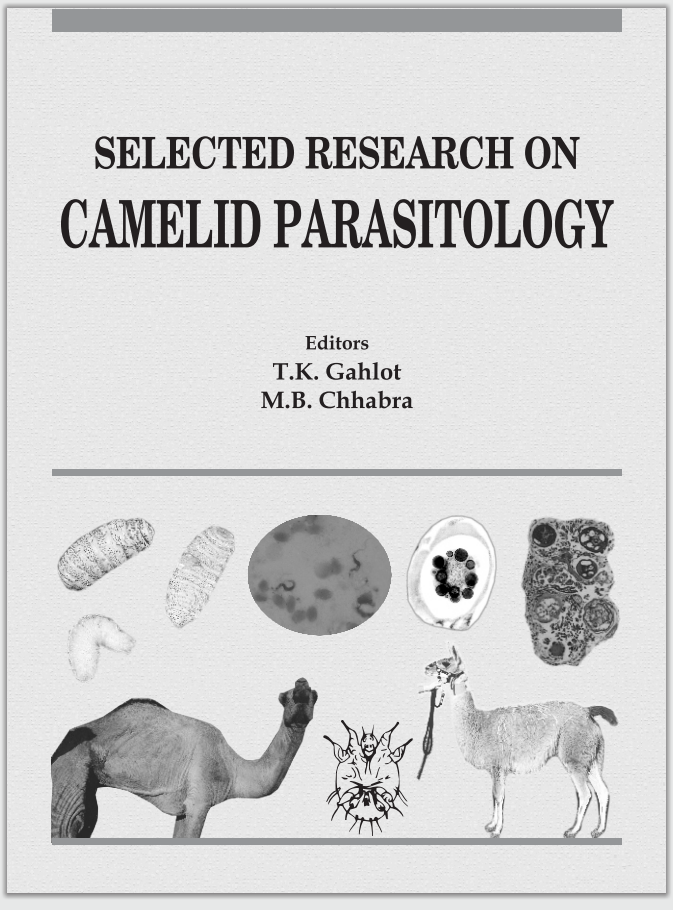

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CHROMATOGRAPHIC PURIFICATION OF PROTEINS WITH CYTOTOXIC POTENTIAL FROM CAMEL MILK WHEY AGAINST CERVICAL CANCER CELL LINE

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ABSTRACT

The objective of the present work was to purify bioactive components from camel milk and test them for antineoplastic properties. Camel milk was separated into casein and whey fractions and their cytotoxicity was assessed. Only camel milk whey and not casein were found to be cytotoxic to Hela cells. Further, camel milk whey was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE), liquid chromatography-mass spectrometric (LC-MS) analysis and subsequent size exclusion chromatography. The cytotoxic potential of fractions obtained from chromatography were tested against the cervical cancer cell line, Hela. The present study indicated the presence of Lactoferrin (~77kDa) in the cytotoxic fractions purified from camel milk whey. This study indicates that camel milk whey and its components can be used as a nutraceutical with health benefis. It also suggests the potential of using camel milk lactoferrin as an important molecule with cyotoxic potential.

Key words: Camel milk, cervical cancer, chromatography, cytotoxicity, Hela, lactoferrin, LCMS, whey

Camel milk has therapeutic potential against many diseases including insulin-dependent diabetes mellitus (IDDM), infant diarrhea, hepatitis allergy, lactose intolerance, and alcohol-induced liver damage, and has been traditionally used for cancer prevention and treatment in Middle Eastern countries (Wernery, 2006). It has been traditionally believed that camel milk has both preventive and therapeutic potential against cancer. There is presence of numerous molecules with proven potential of antineoplastic activity, in other species (Dubey *et al,* 2016).

Studies have shown that camel milk works to combat and eradicate cancer hepatoma (HepG2) and human breast (MCF7) cancer cells, because camel milk contains high levels of lactoferrins, immunoglobulins, and iron-binding glycoprotein. Camel milk contains lactoperoxidase which possesses anti-tumour activity and peptidoglycan recognition protein which helps in fighting against cancer (Korashy *et al,* 2012). Lyophilised camel milk has been shown to inhibit the growth and proliferation of human breast cancer BT-474, laryngeal HE-p2 (Hasson *et al,* 2015). In addition, camel milk protein lactoferrin has been shown to inhibit the proliferation of human colorectal cancer HCT 116 cells by exerting antioxidant and DNA damage activities (Habib *et al,* 2013). Some of the *in vivo* studies have also shown the potent inhibitory effects of camel milk on pro-inflammatory, pro-angiogenic, and pro-fibrogenic cytokines (Alhaider *et al,* 2014).

Camel whey protein supplementation is shown to maintain a high concentration of cellular antioxidants and boost immune defenses that promote carcinogen detoxification (Ajarem *et al,* 2015). Camel whey proteins promote the production of IL-1β, IL-8, IL-6, and tumour necrosis factor (TNF-α) which enhances lymphocyte functions, chemotaxis, phagocytic activity, granulocyte, and NK cell activity (Gauthier *et al,* 2006; Rusu *et al,* 2010). Owing to these properties, camel whey protein supplementation is viewed as a non-pharmaceutical adjunct therapy in the treatment of cancer. In fact, camel whey protein is a new dietary supplementation to the management of free radicals and for the treatment of different health disorders (Badr *et al,* 2017).

The present study explores the cytotoxicity induced by the whey and casein fraction of camel milk. The composition of the bioactive whey fraction

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is examined by LC-MS, and the impact of camel milk whey treatment on Hela cell migration is studied. Thereafter, we studied the cytotoxicity of camel milk whey fractions obtained by size exclusion chromatography, against Hela cells. The cytotoxic fractions were further subjected to SDS-PAGE analysis for the identification of protein components.

Materials and Methods

Milk processing

Camel milk samples were collected from healthy camels at NRCC, Jorbeer, Bikaner, Rajasthan, India. The experiments were conducted from 2017-19 at BITS Pilani, Pilani Campus. Fat components from the milk sample were removed by centrifugation at 8000 rpm for 30 minutes at 4°C to obtain skim milk. Whey was obtained from the skimmed milk by acid precipitation of caseins with 1 N HCl till pH 4.6 and centrifugation at 12000 rpm for 30 minutes at 4°C. The supernatant was saturated with ammonium sulphate (273 g/L of whey supernatant) by constant stirring at 800 rpm and incubated at 4°C overnight. It was subsequently spun at 10,000 rpm, 15°C for 15 minutes to obtain the whey pellet. Whey was extensively dialysed using dialysis membrane-60 (HiMedia) with PBS buffer (Saliha *et al,* 2013).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

GE SimpliNano Spectrophotometer was used to quantitate the protein concentration of camel skim milk and camel milk whey was analysed by SDS PAGE on 12% gel using a Bio-Rad mini gel electrophoresis unit run. The proteins were diluted to 2 µg/µl with sample buffer and denatured for 10 min, at 100°C. Then the samples were added to each well, and electrophoresis was performed at 80 V. Proteins in the gel were stained by Coomassie Brilliant Blue R-250. The gel image was viewed using a gel documentation system (BioRad). Separated proteins were validated for identification using the stained wide range 6.5-200 kDa molecular weight marker (SIGMA #S8445) (Laemmli, 1970).

Measurement of cytotoxicity of fractions against Hela cells

The MTT assay determined the fractions efficacy to induce cytotoxicity against the Human cervical cancer cell line, HeLa (van Meerloo *et al,* 2011). These cells procured from NCCS, Pune, India were cultured at 37°C, 5% CO_2 in RPMI supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin mixture. Trypsin- EDTA solution was used for the detachment of cells. 5.0 x 10^4 cells/ well were incubated with fraction 1 to Fraction 10 in a 96-well cell culture plate under the conditions described above. After 24h and 48h, the media was aspirated. 20µl of MTT solution (5mg/ml in PBS) and 100 µL of media were added to each well. The plate was then re-incubated for 4 hours. To stop the reaction, the media were removed, and 150µL of DMSO was added to each well. The absorbance was measured at 630 nm using a Multiskan™ Thermo Scientific™ microplate reader. Cell viability was calculated as the percentage of the control wells using the following formula:

% Cell Viability = {O.D of treated cells / O. D of Control (without treatment)} X 100

Statistical analysis of the data was performed by GraphPad prism5 using one-way and two-way ANOVA followed by Bonferroni and Dunnett's multiple comparison test. Statistically significant differences with respect to control were represented by *P < 0.05, **P<0.001, ***P<0.0001, and NS = non-significant, respectively. Each experiment was done in triplicate and performed atleast thrice.

LC-MS Analysis of Proteins

The camel milk whey sample for its LC-MS analysis was outsourced to Vproteomics, New Delhi for identifying and determining the main features of proteins. The sample was reduced with 5 mM TCEP and then alkylated with 50mM iodoacetamide. This was followed by digestion with trypsin, and the digests were cleaned using a C18 silica cartridge to remove the salt and dried using a speed vac. The dried pellet was suspended in buffer A (5% acetonitrile, 0.1% formic acid). Peptide mixture analyses were performed using the EASY-nLC 1000 system (Thermo Fisher Scientific), coupled with Thermo Fisher-Q Exactive equipped with a nanoelectrospray ion source. RAW files generated were analysed with Proteome Discoverer (v2.2) against the Uniprot *Camelus* reference proteome database. This was used to identify and determine the proteins' main characteristic features in camel milk whey samples. Trypsin digestion of the sample was followed by nano-ESI LC-MS/MS analysis.

Size exclusion column chromatography

For size exclusion column chromatography, a column of diameter 1.5 cm and length 75 cm was used to separate camel milk whey proteins. Initially, 5g of Sephadex G 100 gel beads were soaked in phosphate buffer saline (pH 7.4) containing 0.02%

sodium azide then heated at 90°C for 5 hrs. Gel beads were degassed, loaded, and allowed to settle in the column. Then the column was washed with sodium phosphate buffer (pH 7.4), 17mg whey in a 5 ml buffer was loaded, 60 fractions were collected, and the absorbance was measured at 280 nm to quantitate the amount of protein content (Garcia Rojas *et al,* 2004). SDS PAGE was performed on 12% gel to profile protein present in fractions.

Silver staining of SDS PAGE gel

The gel was stained using silver stain kit (Himedia # ML123). Briefly, after running the gel, its fixation for 10 min, sensitisation for 1 min, and washing 3 times for 20 min was done. For staining, the gel was incubated in a silver staining solution for 20 min and rinsed for 1 min. A developing solution was added to the gel and incubated until the colour developed. In the end, a stopping solution was added, and the gel was washed before visualisation.

Results and Discussion

The protein profile of camel skim milk (CSM) obtained by SDS PAGE electrophoresis has been shown in lane 3 of Fig 1. It exemplifies the presence of all casein proteins and whey proteins. The camel milk whey (CMW) proteins are also depicted separately in lane 3. The major whey proteins are lactoferrin (Lf), camel serum albumin (CSA), multiple immunoglobulins (Ig), soluble TRAIL, and α-lactalbumin (α-LA) in CMW as well as CSM of these, lactoferrin and TRAIL are associated with an antineoplastic property. Additionally, CSM exemplifies the presence of the 4 casein proteins, namely, αs1-Casein (αs1-CN), αs2 - Casein (αs1-CN), beta Casein (β-CN), and kappa Casein (κ-casein), having molecular weight range of 23 kDa, 25 kDa, 24 kDa, 19 kDa, respectively. The molecular weight marker is depicted in lane 2.

MTT-based cell viability assay was performed against the HeLa cell line after 24h as well as after 48h treatment of Hela cells with whey and casein (Fig 2A and Fig 2B). A dose-dependent decline was observed in the viability of cells after whey treatment. The cytotoxicity after 48h was always more than after 24h, as to be expected. At 7.5 mg/mL concentration of whey, approximately 50% cell cytotoxicity was observed after 24h, indicating the IC50 of whey to be 7.5 mg/mL. Unlike whey, the casein treatment did not induce any cytotoxicity to the cells at the different concentrations after 24h and 48h as shown in Fig 2B. Therefore, only the whey fraction has been used for further studies.

In order to facilitate the identification of components present in whey fraction, it was subjected to analysis by LC-MS. The results of proteins having high protein scores (> 19) have been shown in table 1. The proteins corresponding to bands obtained upon fractionation have also been depicted in the table. It also facilitated the determination of the relative abundance of individual proteins. Numerous proteins such as alpha S1 casein, beta-casein, and lactoferrin could be identified. It also showed fragmented forms of kappa casein, milk fat globule EGF factor 8, α-lactalbumin, and alpha S1 casein. Amongst the molecules present, lactoferrin and α-lactalbumin are two molecules associated with anti-cancer properties, the latter only in combination with oleic acid. The SDS-PAGE gels also validate the presence of the proteins mentioned above.

Subsequently, gel filtration chromatography was conducted and the number of proteins present in fractions 1-60 has been depicted in Fig 3 which depicts the presence of proteins only in the initial fractions. Therefore, subsequently, further studies pertaining to SDS PAGE were conducted only on the initial 10 fractions.

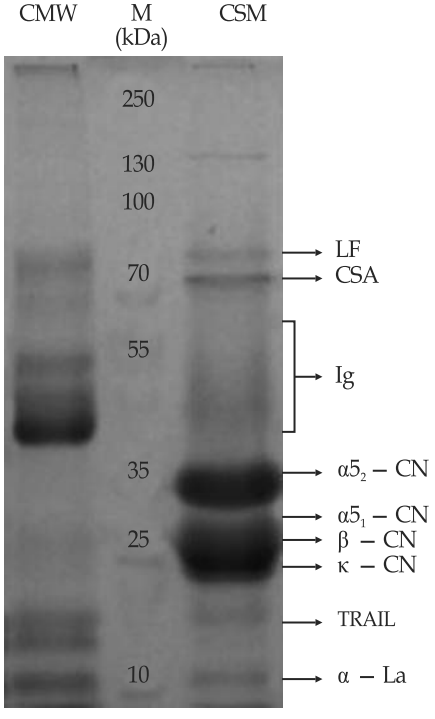

Fig 1. Protein profile of camel skim milk and camel milk whey by SDS-PAGE (L to R). Abbreviations: M (Molecular weight marker); CMW (Camel Milk Whey); CSM (Camel skimmed milk); CSA (Camel serum albumin); Ig (Immunoglobulin); αs1-Casein (αs1-CN); αs2 - Casein (αs1-CN); Beta Casein (β-CN); Kappa Casein (κ-casein); TRAIL (TNF-related apoptosis-inducing ligand), α-LA (α-Lactalbumin).

Fig 4 depicts the protein profile of the fractions obtained by gel filtration chromatography. The profile of molecular weight marker, whole milk (WM), whey (WH), and bovine lactoferrin (LF) are shown in the first 4 lanes sequentially. The other lanes were loaded with fractions 1-10. SDS- PAGE of only the fractions showing cytotoxicity (first 10) was performed. The functional significance of the proteins obtained has been shown in Table 2.

The fractions showed the presence of some biologically essential proteins, identified by their molecular weight. Lactoferrin and soluble TNF-related apoptosis-inducing ligand (TRAIL) are 2 proteins with antineoplastic properties were present out of which only the presence of lactoferrin could be confirmed by LCMS. Besides, these bands corresponding to camel serum albumin and heavy chain antibodies were also observed. The details of the fractions, along with the functions of proteins present, are shown in table 2.

The cytotoxic activity of these whey fractions was checked against HeLa cells to determine their potency against transformed cells. A decrease in per cent viability of the transformed cells was observed only in the initial 10 fractions and not the later fractions; therefore, keeping the objective in focus, further experiments were carried out using the initial 10 fractions only (data not shown).

Fig 5 depicts the cytotoxicity induced by whey fractions after a treatment of 24 hrs and 48 hrs. Fraction 1-3 did not induce any significant cytotoxicity after 24 hrs, although they could do so after 48 hrs. Fraction numbers 4-10 were able to induce statistically significant cytotoxicity at both time points. They further observed that more cytotoxicity is induced by all the fractions after 48 hrs compared to 24 hrs, as expected.

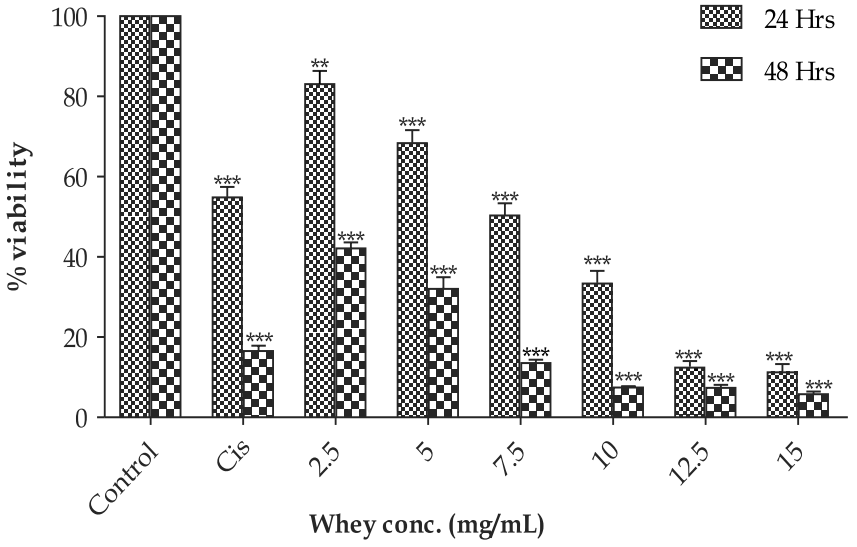

Fig 2A. Viability of HeLa cells after the treatment of camel milk whey by MTT assay after 24 and 48 hrs. The untreated cells (negative control) and cisplatin IC50 (14.9 μM) treated cells (positive control) are also depicted.

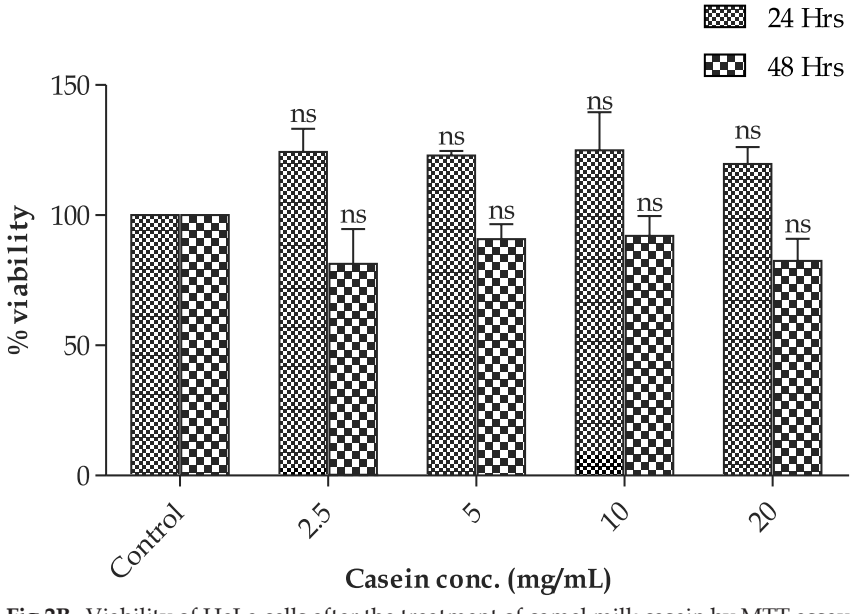

Fig 2B. Viability of HeLa cells after the treatment of camel milk casein by MTT assay after 24 and 48 hrs. The untreated cells are used as a control.

This study suggested that the cytotoxicity of camel milk is largely associated with its whey fraction but not its casein fraction. The whey fractions were purified by size exclusion chromatography and further subjected to SDS-PAGE analysis for the identification of proteins. Proteins with molecular weights 20kDa and 77kDa were found in the cytotoxic fractions purified by size exclusion chromatography. Literature suggests the possibility of soluble TNF-related apoptosis-inducing ligands (TRAIL) of molecular weight 20kDa and Lactoferrin of molecular weight 77kDa as the cytotoxic molecules in human

Table 1. Analysis of camel (*Camelus dromedarius*) milk whey protein by LC-MS.

Accession	Description	Score	Coverage	Peptide spectrum match	Amino Acid	Mol. wt [kDa]	Calc. pI
W0K8B9	κ-casein	118.52	60.13	45	153	17.1	8.60
K7DXB9	α-s1-casein	71.97	40.99	33	222	25.8	5.08
F5BZ34	Milk fat globule EGF factor 8	67.39	37.07	30	294	32.8	8.03
M1E4K4	β-casein	43.36	39.22	18	232	26.2	5.58
W6GH05	Lactoferrin	28.67	12.01	13	708	77.3	8.24
A0A2H4WWA5	A-lactalbumin	24.36	42.31	12	52	6.1	4.81
A0A0U2KTX5	Single variable on heavy chain (VHH)	1.73	7.38	1	122	13.0	10.8

Table 2. Protein profile of fractions.

Protein	Molecular Weight	Function	Fraction number
Lactoferrin	77	Immunomodulatory, antimicrobial and anticancer	7, 8, 9 & 10
Camel serum albumin	68	Transport of biomolecules	6, 7 & 8
Heavy chain antibody	50	Immune response	7, 8 & 9
TRAIL	20	Anticancer property	4, 6 & 7

milk (Davanzo, 2013). Besides these, the presence of Ig heavy chain and camel serum albumin was also obtained in the fractions, but these are not associated with any anticancer activity. Camel milk casein, although not cytotoxic against transformed cells but has been used as a carrier for natural and synthetic drugs (Mittal *et al,* 2021). Camel milk whey was found to be cytotoxic to these cells at 24 and 48 hrs in a dose-dependent manner. This indicates that the cytotoxicity of camel milk is primarily associated with the whey fraction. Besides whole milk, some scientists have also studied the anti-cancer property of camel whey protein hydrolysates. Animal models for colon and mammary tumourigenesis have shown that whey proteins are better than other dietary proteins in suppressing tumor development (Parodi, 2007). These studies indicate that camel milk whey can be potentially utilised as a functional ingredient in nutraceuticals. In our study camel milk casein was not toxic to cervical cancer cell line Hela.

LC-MS was used to identify the other proteins in the whey fraction. Although, it showed the presence of many proteins like lactoferrin, α-lactalbumin, fragmented forms of kappa casein, milk fat globule EGF factor 8, and alpha S1 casein. Amongst the molecules present, lactoferrin is the only molecule associated with anti-cancer properties. Although the molecule of soluble TRAIL could be identified by SDS PAGE the same could not be verified by LCMS. One of the possible reasons for missing TRAIL in LCMS could be the nonavailability of camel milk TRAIL sequence in the database used for identifying proteins. We know that camel milk TRAIL has not been studied sufficiently.

It is well known that camel milk contains numerous bioactive molecules with immunomodulatory, antimicrobial, and anticancer abilities. Camel milk proteins are highly thermostable besides being resistant to acid hydrolysis. The SDS-

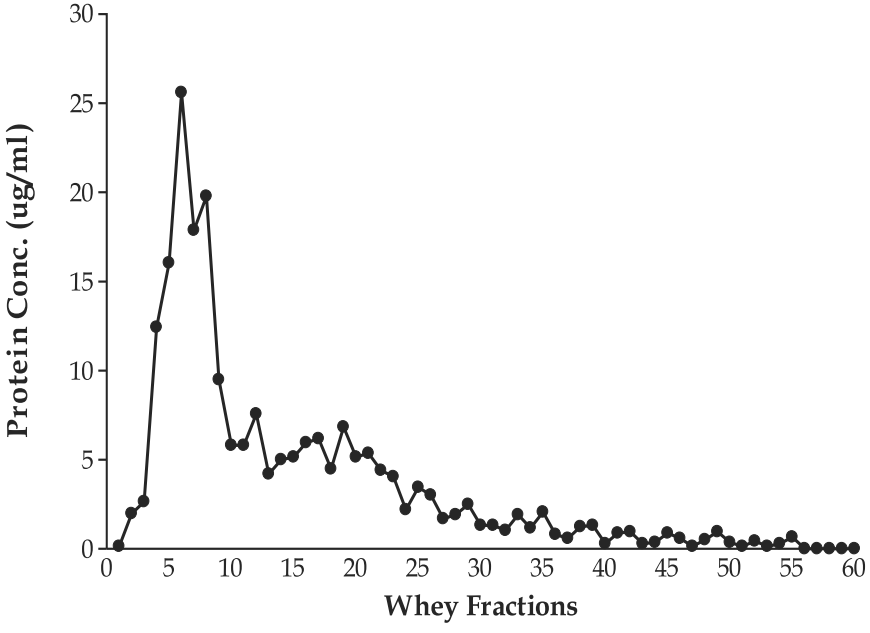

Fig 3. The protein content of fractions separated by Gel Filtration Chromatography.

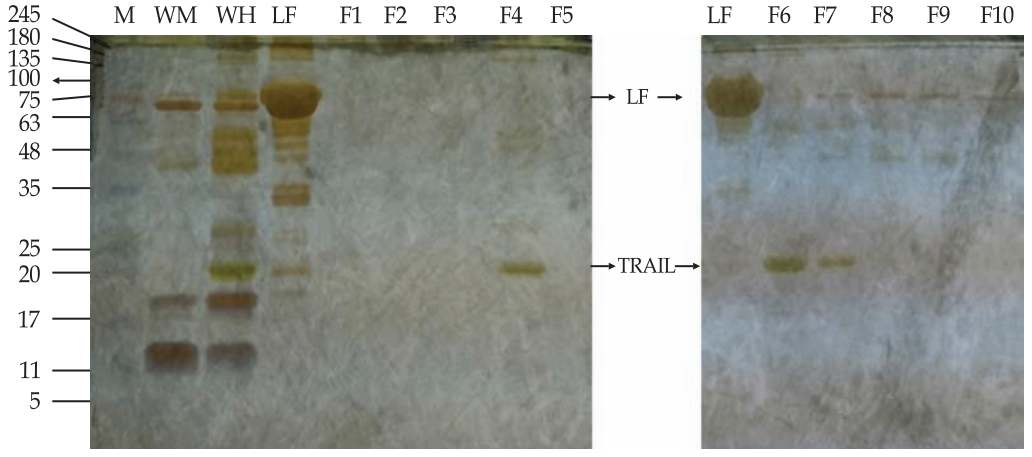

Fig 4. Protein profile of fractions obtained from whey using gel filtration column chromatography by SDS-PAGE (L to R). Abbreviations: M (Molecular weight marker); WM (Whole Milk); WH (Whey), LF (Bovine Lactoferrin) F1 – F10 (Fraction 1 to Fraction 10).

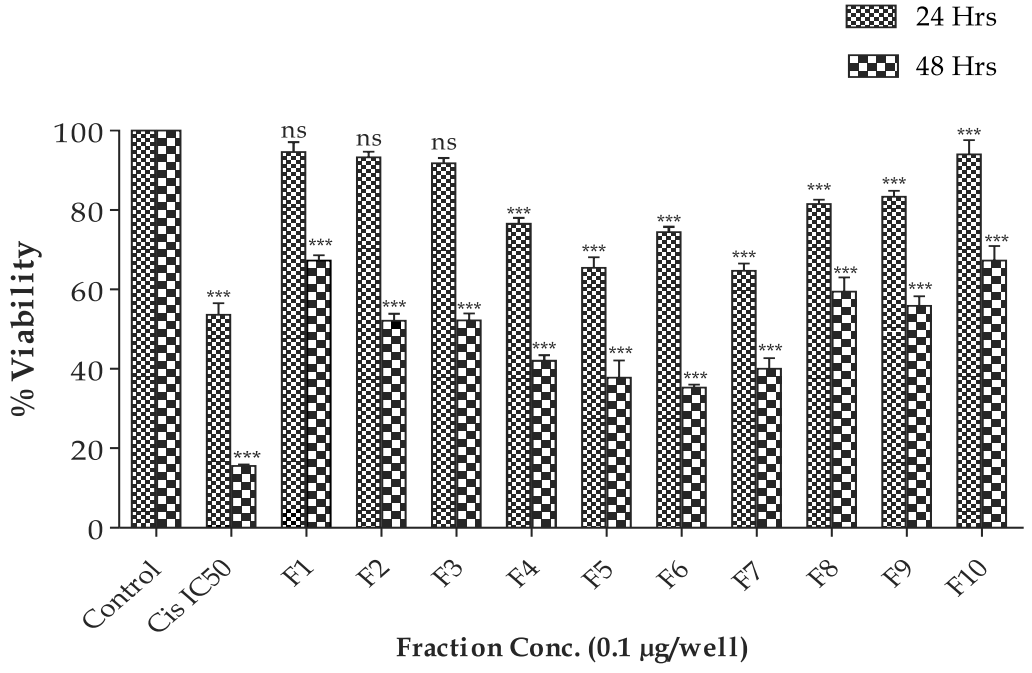

Fig 5. Viability of HeLa cells after the treatment of 0.1 µg/well of indicated protein fraction by MTT assay after 24 and 48 hrs. The untreated cells (negative control) and cisplatin IC50 (14.9 µM) treated cells (positive control) are also depicted.

PAGE analysis of this study of skimmed camel milk and whey showed additional proteins such as different caseins, serum albumin, multiple immunoglobulins, and α-lactalbumin, respectively (Lal *et al,* 2020; Mittal *et al,* 2021).

Camel milk's whey significant proteins contain the entire array of normal antibodies besides having the unique featured camelid antibodies, single-domain antibodies (SdAb) containing only the heavy chain and not the light chain; they retain their specificity and stability i.e., the only heavy chain immunoglobulins antibodies (VHH antibodies) (Desmyter *et al,* 2001). They are often referred to as nanobodies due to their small size. Therefore, the unique features of these antibodies and the presence of other biologically important molecules and their derivatives confer camel milk with unique medicinal properties (Abdel Gader and Alhaider, 2016).

Few authors have recently studied the ability of mammalian milk and its fractions to kill cancers (Shariatikia *et al,* 2017). They have studied the effect of milk, casein, and whey proteins derived from many different mammals on MCF7. Their results showed that mare, donkey, cow, and camel milk, casein,

and whey proteins have potent cytotoxic activity against MCF7 cells in a dose-dependent manner. In contrast, sheep and goat milk, whey and casien did not reveal any cytotoxic activity. Korashy *et al* (2012) have reported that camel milk has the ability to significantly inhibit the induction of the Cyp1A1, a cancer-activating gene. Further, it also induces NQO1, a cancer chemo-preventive gene in murine hepatoma Hepa 1c1c7 cells. Both these functions were studied at the transcriptional and post-transcriptional levels. It was observed that the survival of HepG2 cells was significantly reduced upon incubation with camel milk. Further, they observed that camel milk significantly induced caspase-3 and DR4 mRNA expression levels. The induction of Caspase-3 was blocked by the action of Act-D. This indicates that camel milk increased the caspase-3 mRNA level by the de novo synthesis of RNA. It was further observed that pretreatment of cells with MAPK inhibitors alone, slightly, but not significantly decreased the basal expression level of caspase-3 mRNA. Furthermore, it was reported that the induction of caspase-3 mRNA by camel milk in HepG2 cells was significantly decreased by both the JNK and p38 MAPKs inhibitors and was potentiated by an ERK inhibitor (Korashy *et al,* 2012).

Our study also exemplifies the ability of camel milk whey to be cytotoxic to the cervical cancer cell line, HeLa. Earlier, we also purified the whey proteins to get lactoferrin by a novel pH-dependent procedure (Dubey *et al,* 2022). In this work, SDS-PAGE and LC-MS analysis of the lactoferrin fraction was done for its identification and characterisation. Furthermore to evaluate of the bioactivity of the isolated lactoferrin MTT-based cytotoxicity against the Hela cells was also conducted (Mahala *et al,* 2022).

Similarly, numerous studies point towards the protective character of lactoferrin against malignancies. Bovine lactoferrin has been reported to inhibit the development of experimental metastases in mice with chemically induced tumours (Iigo *et al,* 2009). Bovine lactoferrin-mediated inhibition of tumour growth might be related to apoptosis of these cells, induced by the activation of the Fas signaling pathway (Fujita *et al,* 2004). Treatment of lactoferrin knockout mice with lactoferrin post-chemotherapy accelerated the reconstitution of the immune system, reducing the chances for infection following chemotherapy treatment (Ye *et al,* 2014).

Lactoferrin can scavenge free iron in fluids and inflammed and infected sites, suppressing free radical-mediated damage and decreasing the availability of the metal to pathogens and cancer cells. Also, lactoferrin hinders migration in a model of human glioblastoma by reverting an epithelial-to-mesenchymal transition like process (Adlerova *et al,* 2008; Cutone *et al,* 2020).

Furthermore, camel milk lactoferrin reduces the proliferation of colorectal cancer cells and exerts antioxidant and DNA damage inhibitory activities (Habib *et al,* 2013). The administration of bovine Lactoferrin in a randomised placebo-controlled clinical trial setting has also been reported to elicit beneficial effects for blocking the growth of polyps that are often thought to lead to colon cancer (Kozu *et al,* 2013). Additionally, lactoferrin knockout mice demonstrated a significant susceptibility to inflammation-induced colorectal dysplasia. Lactoferrin carries out different functions owing to its ability to activate specific signaling pathways. It is therefore, of utmost importance to consider the iron saturation rate when carrying out *in vitro* and *in vivo* experiments (Cutone *et al,* 2020).

Contrary to the notion of effectiveness of oral administration of camel lactoferrin, many other therapeutic proteins typically require other invasive routes of administration (Leader *et al,* 2008). Oral administration of bovine Lactoferrin inhibits carcinogenesis in the colon and other organs in rats and lung metastasis in mice. A likely mechanism by which bovine lactoferrin mediates its anti-carcinogenesis effects is by enhanced expression of cytokines and subsequent activation of immune cells (Iigo *et al,* 2009). Although the level of lactoferrin is comparable to cow's milk, even then its bioactivity is slightly higher (Conesa *et al,* 2008; Narmuratova *et al,* 2006).

Studies have shown high levels of TRAIL in human milk and colostrum. Its presence has been implicated as one of the many molecules for anticancer properties in human milk, but camel milk soluble TRAIL has not been studied sufficiently. TRAIL is a cytokine that is produced and secreted by most normal tissue cells. It causes apoptosis primarily in tumour cells by binding to specific death receptors. TRAIL and its receptors have been used as the targets of several anticancer therapeutic modalities. TNF family members, the ligand TRAIL, is primarily expressed as a type 2 transmembrane protein, which proteases can process to release the soluble form (Shepard and Badley, 2009). The protein profile of whole camel milk resulting from commercial thermal treatment was examined by liquid Chromatography combined with Tandem Mass Spectrometry (LC-

MS/MS) to identify proteins. In this study, total 807 proteins were identified which were mainly involved in biological processes like metabolic process and cellular processes and mostly related to signaling pathways including RNA transport, PPAR signaling pathway, and ribosome (Li *et al,* 2020).

In our study, camel milk whey has been fractionated by size exclusion chromatography to yield proteins with potential cytotoxicity toward Hela cells. The present fraction study indicated lactoferrin to be the most vital anticarcinogenic molecule in camel milk whey. sTRAIL, the other potential molecule, needs to be further studied.

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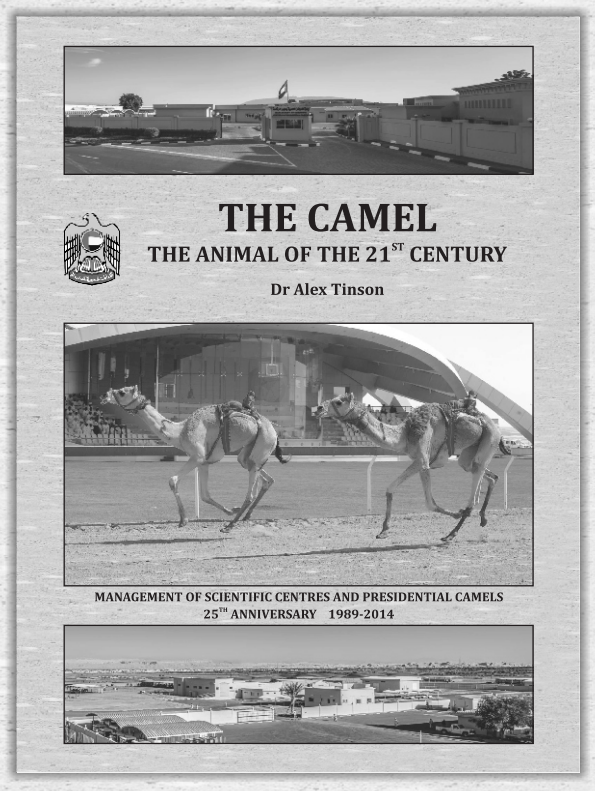
THE CAMEL

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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AN ABATTOIR STUDY OF PULMONARY LESIONS IN ADULT CAMELS (*Camelus dromedarius*) FROM EASTERN REGION OF SAUDI ARABIA

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ABSTRACT

This study describes the gross and microscopic findings in the lungs of 450 adult camels at the AL-Ahsa abattoir (352 male and 98 female). The study showed that 14.7% of the examined lungs had one or more lesions. The most common lesions were pneumoconiosis with dilation of the alveoli 12 (17.91%), pulmonary fibrosis 9 (13.43%) and hydatid cysts 33 (49.25%). In addition various inflammatory lesions including acute mucous bronchopneumonia 2, (2.98%), chronic bronchial pneumonia 1 (1.49%), lung nodules 1 (1.49%), and lung oedema 3 (4.47%) were observed. Moreover secondary cancers, represented by malignant lymphoma 3 (4.47%) , malignant epithelial cell tumour 2 (2.98) %, and Malignant fibroblastoma 1 (1.49%) were seen. Possible explanations for the occurrence of the pulmonary lesions are discussed and the necessary recommendations are made.

Key words: Camel, fibroblastoma, pneumoconiosis, pneumonia, Saudi Arabia

Studies on the pathogenesis of camel respiratory diseases are limited. Although early reports were recorded on the incidence of tuberculosis, in Egypt (Refai, 1992), United Arab Emirates (Wernery and Kaaden, 2002; Kinne *et al,* 2006), Pakistan (Zubair *et al,* 2004), Australia (Manefield and Tinson, 1997), Ethiopia (Bekele, 2008; Mamo *et al,* 2011). Influence of camel pulmonary lesions on some hematological and clinicopathological parameters were previously reported by Nourani *et al* (2009), Abubakar *et al* (2011), Jenberie *et al* (2012), Bani (2017) and Hamid *et al* (2021). Many authors have observed the association of bacterial pneumonia with the high levels of condemnation of lungs of camels in abattoirs, AL-Tarazi (2001), Tigani *et al* (2007), Nasar Eldien (2010), Awol *et al* (2011) and Muna *et al* (2017). Isolation and characterisation of Mycobacterium species from camels with pneumonia were reported, (Elfaki *et al*, 2002; Mederos-Iriarte *et al,* 2014). However, recent studies on MERS Coronavirus in dromedary camel were carried out in Jordon (Reusken *et al,* 2013), Saudi Arabia, (Alagaili *et al,* 2014; Hemida *et al,* 2013), and Egypt (Chu *et al,* 2014). The respiratory diseases of camels have received little attention, even though they are emerging diseases in several countries causing considerable loss of production and deaths (Bekele, 1999; Rufael, 1996; Tafesse, 1996). In Ethiopia, the relevant clinical, etio-epidemiological and pathological data regarding gross and microscopic pulmonary lesions of dromedary camels and their causative agents were reviewed and summarised (Tolossa, 2022). The aim of the present study was to determine the incidence of gross and microscopic pulmonary lesions in adult camels brought for slaughter to several abattoirs in the Eastern Region of Saudi Arabia.

Materials and Methods

A total of 450 apparently healthy adult camels (*Camelus dromedarius*) of both sexes (352 males and 98 females) brought for slaughter at Al Ahsa Central Abattoir and Alomran Abattoir were included in this study. The lungs of camels were collected and grossly examined immediately after the slaughter and lesions were recorded. Samples were collected from the affected parts of each lung in 10% neutral formalin solution, which were prepared for histopathological examination using the wax paraffin method. Sections were cut 4-5 µm and stained with H&E according to the method of Bancroft and Gamble (2008).

Results and Discussion

The prevalence rate of hydatidosis between infected cases in camels under this study was 7.33% (Fig 1). This percentage was lower than previous abattoir survey reports from Egypt (Haridy *et al,* 1998), and from Ethiopia (Bekeke, 2008; Muskin, 2011 and Etana *et al,* 2015). This could be attributed to the

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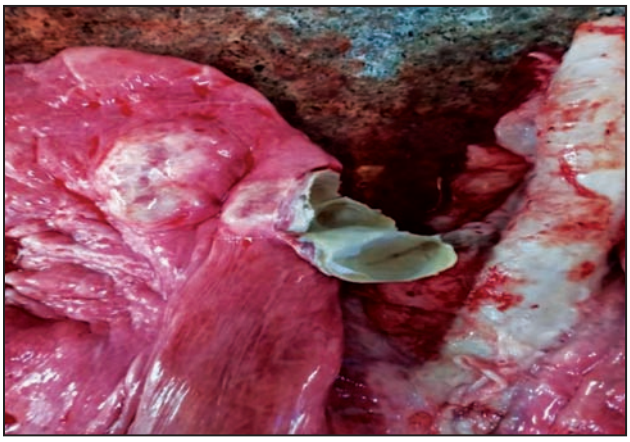

Fig 1. Hydatid cyst. The vesicles are protruding on the surface or within the parenchyma of the lung.

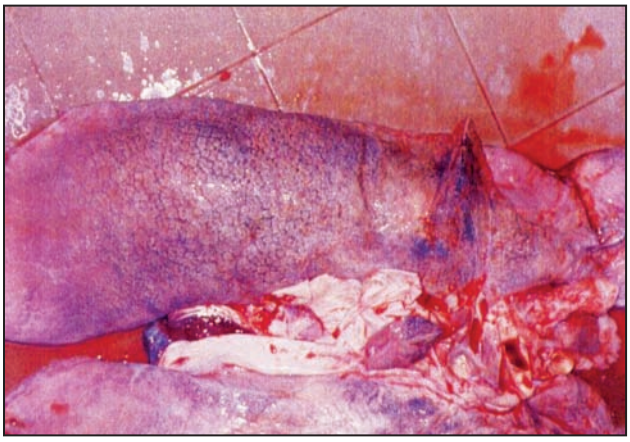

Fig 2. Pneumoconiosis. Dark deposits in the lung tissue, accompanied by the appearance of prominent lung lobules, and dilation of the alveoli.

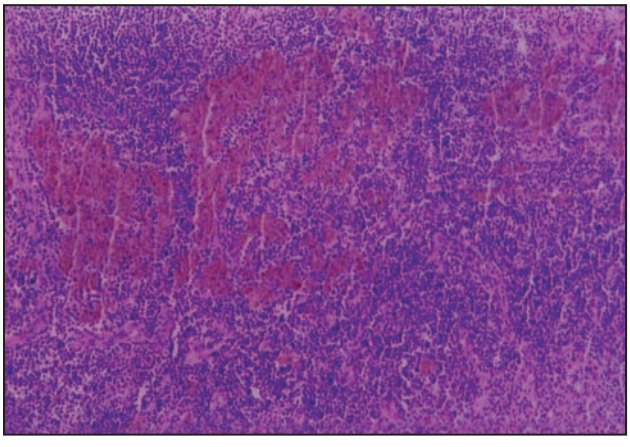

Fig 3. The bronchial lymph nodes were obliterated with aggregations of macrophages containing carbon or silicon granules. H&E × 100.

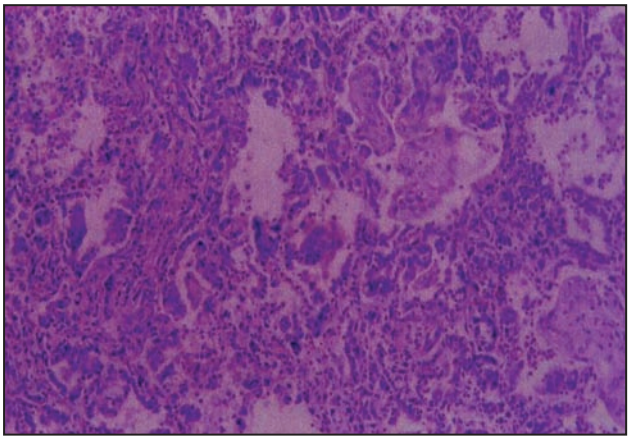

Fig 4. Chronic bronchial pneumonia-destruction of the bronchioles with fibrosis and proliferation of inflammatory cells. H&E × 100.

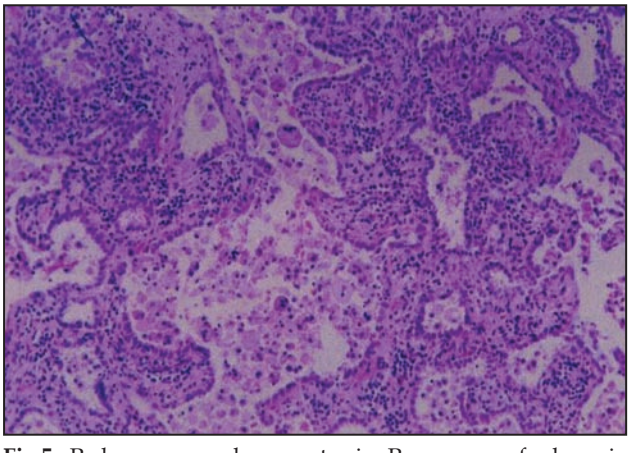

Fig 5. Pulmonary adenomatosis Presence of chronic pneumonia with hyperplasia of type 2 pneumocytes and multinucleated cells and alveolar adenomatosis. H&E × 100.

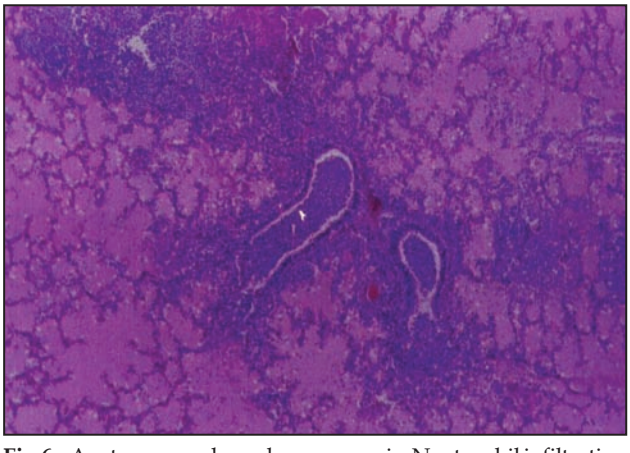

Fig 6. Acute mucous bronchopneumonia. Neutrophil infiltration inside and between the respiratory bronchioles (thin arrow) and presence of pink staining serous exudate and fibrin in the alveoli. H&E × 100.

proper condemnation procedures of carcasses after slaughter adopted in all abattoirs of the eastern region of Saudi Arabia. In this study lung infections, were few and comparable with that previously reported by Farah *et al* (1984) in Egypt and Al Darraji and Wajid (1990) in Iraq. The recorded pulmonary lesions

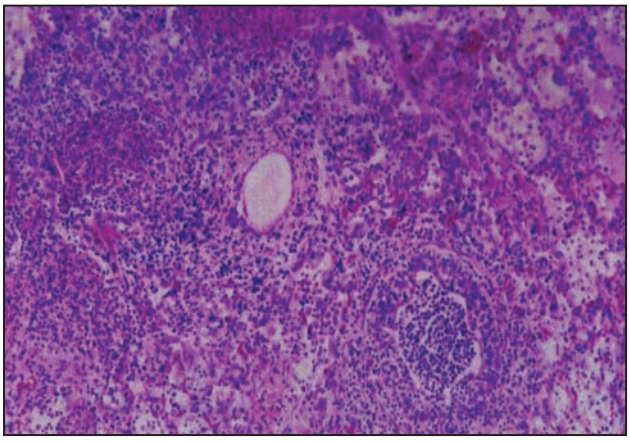

Fig 7. Chronic bronchial pneumonia obliteration of bronchioles and alveoli with inflammatory cells mainly lymphocytes and presence of serofibrinous exudate in some alveoli. H&E × 100

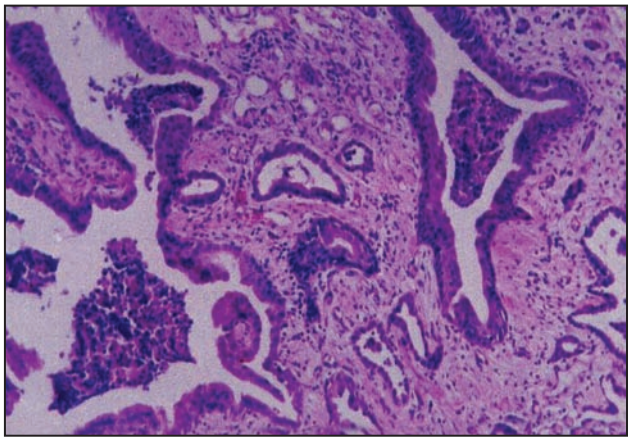

Fig 8. Pulmonary Fibrosis : Alveoli were replaced by spreading fibrous tissue, with the transformation of the mucous membrane lining the bronchi to layered epithelial cells. H&E ×200.

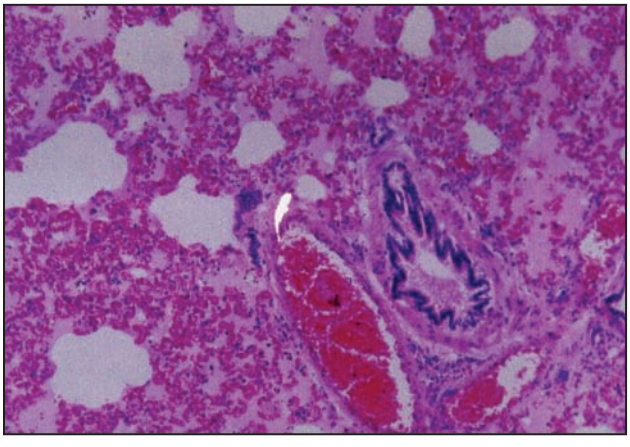

Fig 9. Pulmonary oedema: Presence of clear fluid in the alveoli with capillary congestion. H&E × 100.

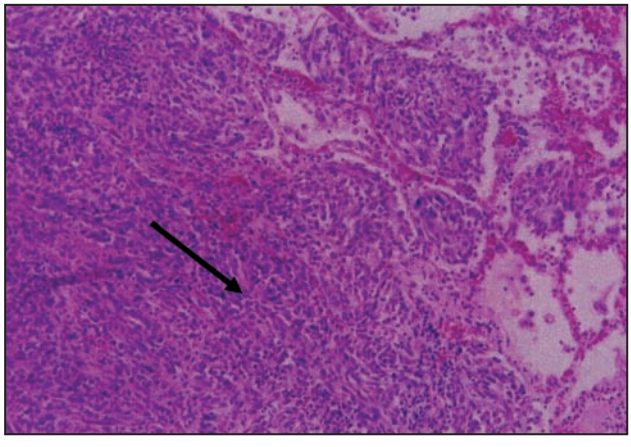

Fig 10. Malignant lung fibroblastic carcinoma. Note the presence of groups of malignant fibroblasts in the lung tissue. H&E × 100.

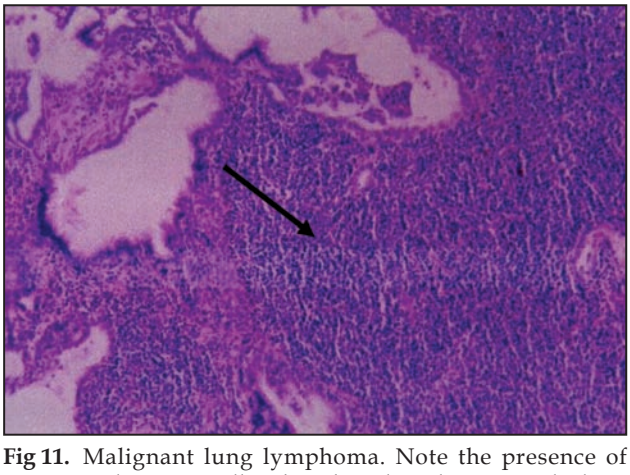

Fig 11. Malignant lung lymphoma. Note the presence of malignant small and medium lymphocytes in the lung tissue. H&E × 100.

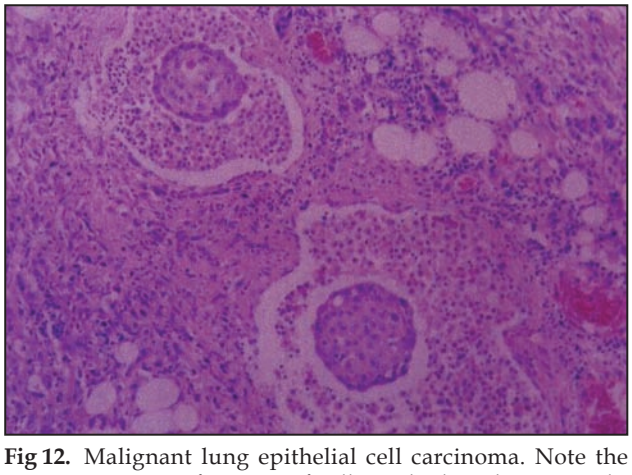

Fig 12. Malignant lung epithelial cell carcinoma. Note the presence of groups of cells in the lymphatic vessels. H&E × 200.

of acute mucous bronchopneumonia (1.49%) and chronic bronchial pneumonia (2.98%) (Figs 6, 7) may indicate previous exposure to bacterial or viral infections (Carlton and McGavin, 1995; Dungworth, 1993; Jones *et al*, 1997). However, the occurrence of respiratory infections in animals could be due

Table 1. Prevalence of naturally occurring pulmonary lesions in camel lungs (n=450).

Lesion Type	Number 450	Percentage between infected cases	Percentage between the total number of cases
Hydatid cyst	33	49.25	0.22
Pneumoconiosis	12	17.91	2.66
Pulmonary Fibrosis	9	13.43	2.0
Acute mucous bronchopneumonia	2	2.98	0.44
Chronic bronchial pneumonia	1	1.49	0.22
Pulmonary adenomatosis	1	1.49	0.22
Lung oedema	3	4.47	0.67
Malignant lymphoma	3	4.47	0.67
Malignant epithelial cell tumour	2	2.98	0.44
Malignant Fibroblastoma	1	1.49	0.22
Total	67	%	14.87

to stress and compromised immunity created by adverse environmental conditions (Mohamed, 2002; Shewen *et al,* 1993). The percentage of pneumoconiosis between infected cases in present study was 17.91%. Pneumoconiosis was demonstrated grossly as dark deposits in the lung tissue, accompanied by the appearance of prominent lung lobules, and dilation of the alveoli (Fig 2). Microscopically focal aggregates of dust laden macrophages were observed (Fig 3). This could be attributed to the dusty environment where camels are reared in the eastern region of Saudi Arabia. The diagnosed cancers, malignant epithelial cell carcinoma (Fig 12), malignant fibroblastoma (Fig 10) and malignant lymphoma (Fig 11) demonstrated in present study were possibly secondary cancers and were not reported previously. The rare occurrence of neoplasms in camelids could be due to a low prevalence of neoplasia within the population or a lack of presentation for clinical and histopathological examination (Al-Ani *et al,* 2004; Singh *et al,* 1991). In conclusion, the present study has demonstrated the common naturally occurring pulmonary lesions in camels, but the detailed epidemiological information and identification of the primary causative agents of respiratory diseases in camels need further investigation.

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SELECTED RESEARCH ON
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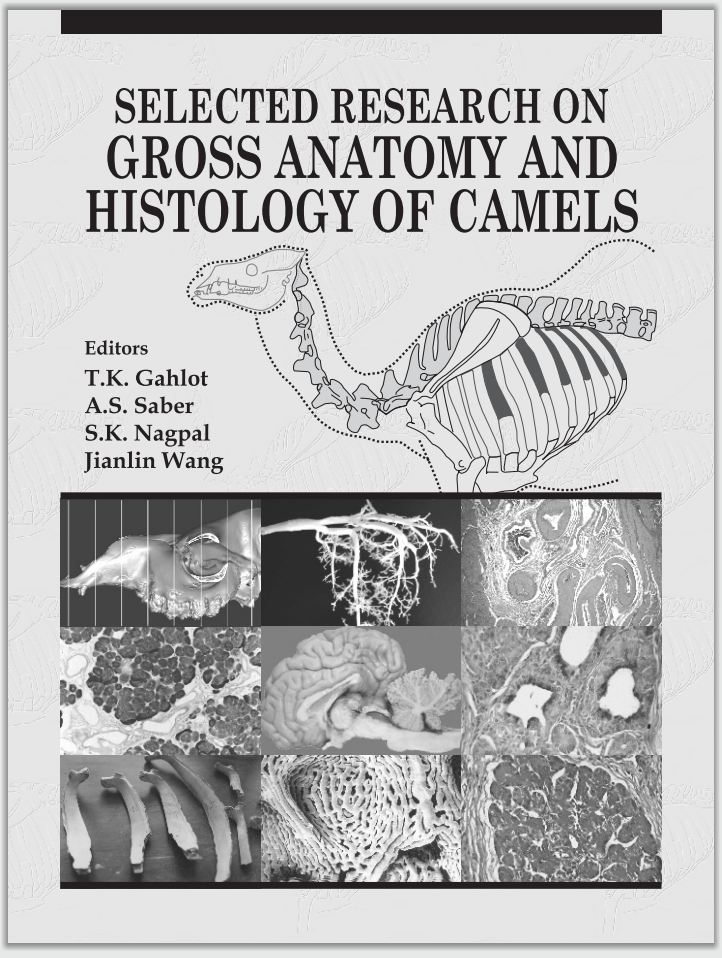
Selected Research on Gross Anatomy and Histology of Camels is a unique reference book on anatomy of dromedary and bactrian camels. This book contains a first ever wide spectrum of histological description of various organs of camels which is depicted by special stains and scanning electronmicroscopy in addition to the gross anatomy, histochemical and immunohistochemical studies. The book has 92 manuscripts in 9 sections, e.g. radiographic anatomy, anatomy of various systems (skeletal, digestive, respiratory, circulatory, urogenital and nervous), common integument and miscellaneous. These manuscripts were published by 158 authors working in 37 laboratories or colleges or institutions from 14 countries in the Journal of Camel Practice and Research between June 1994 to June 2010. Bactrian camel anatomy research was exclusively contributed by the researchers of China. The countries involved in camel anatomy research were China, Egypt, India, Iran, Saudi Arabia, Iraq, Jordan, Japan, Pakistan, Sweden, United Arab Emirates, United States of America, France and Germany. Camel Publishing House has taken a step forward to compile this knowledge in form of a book and this herculian task was accomplished by its dedicated editors, viz. T.K. Gahlot (India), S.K. Nagpal (India), A.S. Saber (Egypt) and Jianlin Wang (China). This classic reference book will serve as a one stop resource for scientific information on gross anatomy and histology of camels.



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Short Communication

DIAGNOSIS OF RARE CASES OF CUTANEOUS HISTOPLASMOSIS IN DROMEDARY CAMEL IN INDIA

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ABSTRACT

The present investigation was aimed to diagnose the mycoflora associated with dermal mycoses infected camels in the Bikaner city and the nearby villages, Rajasthan. In this investigation 16 camels infected with dermal mycoses irrespective of sex and age were included. Out of 16 infected cases, two cases were found infected with cutaneous histoplasmosis caused by *Histoplasma capsulatum*. Cutaneous histoplasmosis appeared as small multiple nodular lesions, whitish to gray mainly found over the neck, axillaries and legs. In addition, alopecia, itching and emaciation were also noticed. The cutaneous histoplasmosis in both camel calves was confirmed by cultural and microscopic examination.

Key words: Histoplasmosis, mycosis, camel, skin

Camel is likely to be more prone to get fungal skin infections in comparison to bacterial skin infections. Among other fungi the *Histoplasma capsulatum* has been reported to cause various diseases in camelids (Al-Ani and Roberson, 2005). *Histoplasma capsulatum* is an environmental dimorphic fungus (Wheat *et al,* 2007). Gitao *et al* (1998) found high infection of dermatophilosis in the moist season in comparison to dry season in camels of Kenya, Sudan and Saudi Arabia. Mammals including wild, domestic animals and humans are the host of *Histoplasma capsulatum* which is a dimorphic fungus widely dispersed in the tropical or subtropical areas of the globe (Chermette and Guillot, 2010; Guillot *et al,* 2018). The dimorphism presented by these pathogens is considered one of the most relevant virulence factors in the pathogenesis of these fungi. The severity of the diseases relies on factors such as the immunity of the host and antigenicity of the pathogen. The single species *H. capsulatum* is supposed to include 3 distinct subspecies which do not share exactly the geographical distribution and which are responsible for variable clinical signs in different animal species and humans (Guillot *et al,* 2018; Brilhante *et al,* 2016).

Histoplasma capsulatum remains in a mycelial form at ambient temperatures and grows as yeast at body temperature in mammals. Although, the fungus that causes histoplasmosis can be found in temperate climates throughout the world. These fungi can cause disease even in immune-competent individuals. The establishment of these pathogens in the host directly depends on the conversion of the filamentous phase to the parasitic yeast phase (Nemecek *et al,* 2006). The case of cutaneous histoplasmosis in Indian dromedary camel is reported here.

Materials and Methods

The present investigation was carried out to diagnose dermal mycoses in camels in the post rainy season. Skin scrapings were collected from the affected camels as per the standard practices without using anaesthesia.

Animals

Sixteen camels (9 males and 7 females) showing any dermatological disorder were included in this study. These were screened at Veterinary Clinical Complex, CVAS, Rajasthan (University of Veterinary

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and Animal Sciences, Bikaner, Rajasthan, India and National Centre on Camel, Bikaner. These camels showed typical skin lesions and clinical signs like scab formation, pruritus and hair fall. The cases of dermal mycoses were confirmed by microscopic examination and culture examination of skin scrapings. The camels which were found positive for ectoparasite infestation were excluded from this study.

Collection of samples: Debris and/or faecal materials present over the clear skin lesions of camels were first removed with an alcohol wipe and then lesions were firmly scraped by using blunt scalpel, particularly at the advancing border and were collected in sterile vials.

Direct microscopic examination: The scrapings were placed on grease free glass slide with 2-3 drops of 20 per cent KOH and cover slips were placed. The samples were kept for 5 minutes over flame and examined microscopically after attaining normal temperature for the presence of sporing heads and conidia.

Culture examination: Samples were mixed with sabouraud's dextrose chloramphenicol broth and incubated for 24 hours. Thereafter, the samples were inoculated onto sabouraud's dextrose chloramphenicol agar (SDCA) plate and incubated at 25°C for 15 days. These plates were examined daily for the growth of the fungi. The resultant growth was examined for the colony morphology. Microscopic examination was carried out using lacto phenol cotton blue and/or calcoflour white stains using wet mount method (Halley and Standard, 1973). Fungal species were identified on the basis of colony morphology and microscopic characteristics (Colin *et al,* 2013).

Haemato-biochemical examinations: Haemato-logical examination was performed as per the method described by Jain (1986) and biochemical analysis was carried out by the Vet Test Chemistry Analyser using standard kits supplied by Idexx laboratories.

Results and Discussion

Cutaneous histoplasmosis was found in 2 male camel calves (Age < 1 year) out of 16 dermal mycoses (12.5%) infected camels.

The skin lesions appeared as small multiple nodular lesions, whitish to gray in colour associated with alopecia, itching, thickening of skin and emaciation. The lesions were most commonly found on nose, mouth, legs, neck, axilla, chest and abdomen (Fig 1). Similar findings due to cutaneous histoplasmosis in two years old domestic cat was seen with no clinical signs of systemic involvements of gastrointestinal or respiratory system and no abnormality was noticed in complete blood count and serum biochemical profiles (Larsuprom *et al,* 2017).

Colonies of *Histoplasma capsulatum* on SDCA were flat, spreading, irregular white or buff-brown, suede-like to cottony in the front view of the plate (Fig 2) and appeared pale yellow-brown in reverse view. Morphological characters under the microscopic observed were single celled, typically large, tuberculated macroconidia on short branches, rounded, hyaline and undifferentiated conidiophores (Fig 3). Small microconidia, round to pear-shaped, were present either on short branches or on the sides of the hyphae. The morphological character of colony and microscopic findings were similar to *Histoplasma capsulatum* as described by Colin *et al* (2013). *Histoplasma capsulatum* shows thermal dimorphism which grows on living tissue and/or in the culture as a yeast-like fungus on body temperature and in environment as a mould at temperatures under 30°C.

Haemato-biochemical analysis revealed low haemoglobin concentration, PCV and TEC and high neutrophil count in affected camel calves (Table 1). Foutah *et al* (2012) also reported reduction of erythrocytic count, haemoglobin content, neutrophilia and lymphocytosis in camels infected with ringworm whereas Mathur *et al* (2011) found variations in most of the haematological parameters in dermatomycoses infected camels, but these were within the normal physiological range. Biochemical values of both infected camel calves were also found within the normal ranges.

Histoplasma is generally found in the soil of temperate and subtropical regions but some parts of the world are considered to be highly enzootic. Infection generally remains limited to the skin and subcutaneous lymph nodes but may also cause lesions in the lungs and gastrointestinal tract. The possible mode of transmission is inoculation, ingestion and inhalation are frequently reported in humid and hot altitude regions (Ameni, 2006). However, some cases of direct contact transmission have been reported between infected and healthy animals having wounds during mating (Al-Ani and Al Delaimi, 1986).

Cutaneous histoplasmosis was reported in some animal species and humans. *H. capsulatum var. farciminosum* causes epizootic lymphangitis in horses (Mahajan *et al,* 2017). Guillot *et al* (2018) reported *Histoplasma capsulatum* var. *duboisii* in Western and

Fig 1. Skin lesions of *Histoplasma capsulatum* on neck and mouth of affected camel calf.

Fig 2. Photograph showing colony of *Histoplasma capsulatum* in front view of plate.

Central Africa which develops as large-sized yeasts with lymphadenopathy and dissemination to the skin and bones in primates. Macedo *et al* (2021) reported feline cutaneous histoplasmosis in the north-central region of Paul. Ameni (2006) recorded 18.8% prevalence of equine histoplasmosis (EH) in Ethiopia. *Histoplasma capsulatum* has been reported from dromedary camel to cause miliary necrosis of the lungs (Chandel and Kher, 1994). Young camels are supposed to be more susceptible to the fungal infections in comparison to the adults, that could be attributed to stronger immunity development in adult animals due to the diverse contacts with the fungus (Descamps *et al*, 2003; Moriello *et al*, 2003).

Conflict of interest

The authors declare no competing interests.

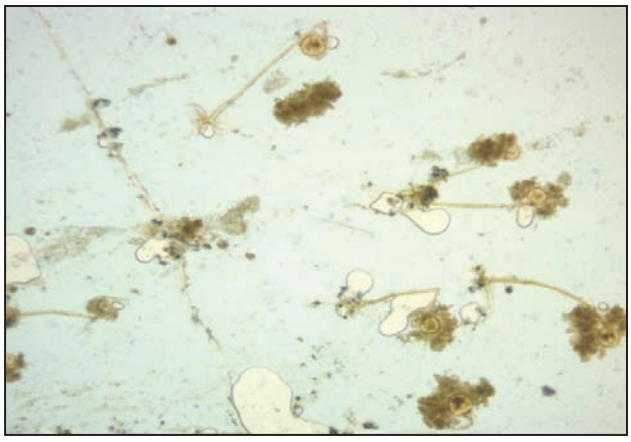

Fig 3. Microphotograph showing large, rounded, single-celled, capsulated macroconidia and small microconidia of *H. capsulatum* (Lactophenol cotton blue stainX100).

Table 1. Haemato-biochemical parameters of *Histoplasma capsulatum* infected camel calves.

Parameters		Mean value of infected camels (n=2)	Reference value of healthy camel Hozifa *et al* (2016); Islam *et al* (2019); Kant *et al* (2019)
Hb%		8.5	11-15
PCV%		22	25-38
TLC(10^3/ µl)		11.5	8 - 13
TEC (10^6/ µl)		6.6	7.6-9.2
Differential leukocyte count (DLC) %	N%	61.5	24-50
	M%	2.5	2-7
	L%	38.5	30-45
	E%	2.5	0-6
	B%	0	0-2
Platelet count ($\times10^3$/µl)		338	230-360
Total protein (g/dl)		6.3	5.8- 10.5
Albumin (g/dl)		3.8	3.50-7.8
Globulin (g/dl)		2.5	2.8-2.2
ALKP (IU/l)		37.5	30-122
AST (IU/l)		34	20-80
ALT (IU/l)		25	9-30

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EFFECT OF TANNIFEROUS TREE LEAVES IN CAMEL'S DIET ON MILK YIELD AND QUALITY

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ABSTRACT

Effect of tannin containing tree leaves in the diet on camel milk production and its quality was studied in 15 lactating camels (average body weight 554 kg) in mid-lactation stage. The animals were distributed following randomised-block design into 3 groups on the basis of similar live weight and milk production. The control group (GG) was fed with a standard basal diet of roughage and supplemental concentrate in the ratio of 70:30, where the roughage component included equal proportions (1:1) of groundnut (*Arachis hypogaea* L.) straw (GS), guar (*Cyamopsis tetragonoloba* L.) phalgati (GP). The treatment groups GGK received a similar ration with a varied roughage combinations, one having tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios and the other GGP received pala leaves (PL) at GS40:GP40:PL20 ratios. Milk yield was recorded daily and the milk samples were collected fortnightly for assessing the quality parameters. The milk yield (kg/d) of camel increased to 7.11±0.14 in GGK and 6.65±0.21 in GGP compared to 6.15±0.24 in GG. The physical parameters of camel milk like electrical conductivity and freezing point decreased in the tree-forage fed groups. Amongst the chemical constituents, milk protein content increased and fat:SNF ratio decreased in GGK and GGP compared to control. An improvement in antioxidant properties of camel milk was observed in the test groups due to consumption of tanniferous tree leaves. It may thus be concluded that incorporation of tanniferous tree forages of hot-arid climatic regions at 20% level by replacing the basal crop-residues in the diet of lactating camel had positive influence on milk quality and its antioxidant properties, which will have far-reaching economic impact in the trade of camel milk and milk products and thus uplifting the socio-economic status of camel rearers.

Key words: Arid zone roughage, leaves, milk production, tanniferous leaves

Camel milk is considered to be an important nutrition source for the inhabitants in arid and semiarid areas (Singh *et al,* 2017; Sahoo, 2021a). Unlike other milk-producing animals, camels can thrive under extreme hostile conditions of temperature, drought and lack of pasture and still produce milk (Sahoo, 2020).

The general composition of camel milk varies in various parts of the world, i.e. protein (3.5-4.5%), lactose (3.4-5.6%), fat (3.07-5.50%), ash (0.7-0.95%) and TS (12.1-15%) (Konuspayeva *et al,* 2009; Singh *et al,* 2017). This variation may be attributed to factors such as breed, age, the number of calving, nutrition, management, the stage of lactation and the sampling technique used. Thus, production of animals is directly affected by availability of feeds and feeding strategy adopted during the course of seasonal cycle, available in plenty after rain to continue end of winter and then a long summer scarcity period ranging from March to July (Sahoo, 2021b). An economic feeding schedule adheres to maximum and optimal utilisation of the locally available feed resources. The camel population of India is concentrated mostly in the 'Thar Desert' of India (AHSD, 2019) and the available forage biomass in this western arid Rajasthan comprises of seasonally grown pasture, salt bushes, browses and tree forages (Sharma and Sahoo, 2017). Therefore, suitable inclusion of alternate tree forages with conventional crop-residue based ration could be an economical way of sustaining lactation in camel. But, the tree leaves of this region are mostly tanniferous Khejri (*Prosopis cineraria*) and Pala (*Ziziphus nummularia*) leaves, which may exert antinutritional effect if not fed judiciously. Various approaches involved in the use of these unconventional feed resources have delineated newer concepts that implies inclusion at low levels to explore pro-nutritional effect for improving animal production (Singh and Sahoo, 2004). The phytochemical-rich tree leaves have bioactive properties (Makkar *et al,* 2007; Durmic

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and Blache, 2012; Xiao and Bai, 2019) that often alters the quality milk, meat and their products (Vasta and Bessa, 2012; Morales and Ungerfeld, 2015; Jerónimo *et al,* 2016; Sahoo, 2021a). There is limited information on modulation of antioxidant properties of camel milk. The present investigation was thus aimed at incorporating 20% of tree leaves in the basal roughage diet of lactating camel on milk yield and its composition and possible alteration in antioxidant constituents.

Materials and Methods

Fifteen lactating she camels in mid lactation were randomly divided into 3 groups of 5 camels each based on comparable milk yield, body weight, number of lactations completed and days in lactation. The control group was fed with a standard basal diet of roughage and supplemental concentrate in the ratio of 70:30 (ICAR, 2013), where in the roughage component included equal proportions (1:1) of Groundnut (*Arachis hypogaea* L.) straw (GS), Guar (*Cyamopsis tetragonoloba* L.) *phalgati* (GP) and designated as GG. One treatment group received a similar ration with a varied roughage combinations having tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios and referred as GGK. Similarly, the other group (GGP) received Pala leaves (PL) at GS40:GP40:PL20 ratios. The experimental animals were housed in individual stalls and provided with uniform management practices during the course of this study, i.e. 12 fortnights.

Data recording and sample collection

Samples of feed and forage samples were collected at fortnight intervals and pooled after drying for phytochemical analysis. The milk yield of individual camel was monitored daily and fortnightly average yield per animal was recorded during the whole period. Milk samples were collected at fortnight intervals in clean plastic sampling bottles and kept in an icebox during transportation and subjected to analysis directly.

Phytochemical analysis

Representative samples of feed offered during the study were collected, dried in drafted hot-air oven at 55-60°C, ground to pass through 1 mm screen and stored in poly propylene air-tight containers for further analysis. Polyphenols were extracted in 70% acetone solution after treating with 2% acetic acid and petroleum ether with the help of sonicator (Sonirep 150, Ultrasonic disintegrator and process timer, MSE, UK), total phenols (TP) and total tannins (TT) were assessed by a modified Folin-Ciocalteu method using polyvinyl polypyrrolidone (Makkar, 2003) for separating non-tannin phenols (NTP) from tannin phenols. Condensed tannins (CT) were analysed by the butanol-HCl-iron method (Porter *et al,* 1986). Hydrolysable tannins (HT) were estimated as the difference between total tannins and condensed tannins. TP and TT were expressed as tannic acid equivalents, while CT as leucocyanidin equivalents.

Chemical assay of milk

The physicochemical parameters including fat, protein, lactose, electrical conductivity (EC), density and freezing point determined by the infrared milk analyser (Lactoscan, MCC) previously standardised for camel's milk (Aple Industries services–LaRoche/ Foron, France), as per the to manufacturer's instructions. The total solid was determined from its dry matter (DM) content by drying in a drafted hot-air oven and subsequently its mineral content i.e. inorganic matter (or total ash) was analysed as per AOAC (2000).

Antioxidant assay

Total antioxidant capacity (TAC) was determined by ferric reducing antioxidant potential (FRAP) based on a redox reaction in which ferric tripyridal triazine complex (Sigma-Aldrich) reduced to its coloured ferrous form in the presence of antioxidant, which is measured after 40 min of incubation with an absorption maximum at 593 nm (Benzie and Strain, 1996) in a UV-VIS spectrophotometer (Uvikon XL; Bio-Tek Instruments, Winooski, VT, USA). The potential of the antioxidants in the milk serum to reduce Fe^{3+} to Fe^{2+} was expressed in µmol Fe^{2+} in 1L milk serum, using a calibration curve of standard $FeSO_4.7H_2O$ (0–1000 mM; Sigma-Aldrich).

Free radical scavenging activity (FRSA) was measured by employing 2,2-diphenyl-1-picrylhydrazyl (DPPH; Fluka Chemicals) method that is based on the reduction of the stable free radical by antioxidants present in a methanol solution (Brand-Williams *et al,* 1995). The reduction of DPPH (105 mM) was followed by monitoring a decrease in its absorbance after 40 min (Uvikon XL). For each sample, six different volumes of milk serum were measured in a ratio of DPPH solution to milk serum varying between 3:1 and 1.5:2.5 (v/v), depending on the number of antioxidants present in the milk serum. Before measuring the absorption, the reaction mixture is centrifuged (5 min 1300 g; Sorvall RC 26 Plus) to obtain clear medium. For each volume of milk

serum, the percentage of DPPH remaining at 40 min was calculated based on different standard curves of DPPH in methanol: water (ratio varying between 3:1 and 1.5:2.5, v/v). The antioxidative capacity is calculated as the amount of milk serum necessary to decrease the initial DPPH concentration by 50% (EC_{50} value) and expressed as mL milk serum per 1mg DPPH and thus a lower EC_{50} value is indicative of less milk serum needed to reduce the concentration of DPPH to 50%.

Statistical analysis

Statistical analysis was performed using SPSS version 24 (SPSS Inc., Chicago IL). The effects of tree leaves feeding on milk quality parameters were analysed by ANOVA with the Tukey post hoc test. The milk yield data recorded fortnightly was subjected to repeated measure analysis. Any significant differences between the dietary groups was declared at $P<0.05$.

Results and Discussion

Tanniferous constituents

The conventional basal forages had least and/or negligible phenolic constituents, GS accumulated more than GP (Table 1). On the other hand, all the phenolic and tannin constituents were high in Khejri leaves compared to Pala leaves, the most significant being TP (10.22 *vs* 5.41) and CT (4.47 *vs* 1.42). The phenolic composition of the test forages was in line with the values reported earlier (Sharma and Sahoo, 2017). The tree forages accumulate more phenolic constituents as a defence mechanism against browsing by herbivores (Salminen and Karonen, 2011) compared to cultivated crops (e.g. groundnut, guar) which are harvested for seed production. A similar difference in polyphenolics composition between Khejri and Pala leaves was also reported by Kanika *et al* (2022).

Table 1. Tanniferous constituents (% DM) of forages used in camel feeding.

Attributes	Khejri leaves	Pala leaves	Groundnut straw	Guar phalgati
Total phenols	10.22	5.41	1.21	0.55
Non-tannin phenols	1.51	1.20	0.39	0.28
Total tannins	8.71	4.21	0.82	0.27
Hydrolysable tannins	4.24	2.79	0.54	0.23
Condensed tannins	4.47	1.42	0.28	0.04

Milk yield and composition

The milk yield of camels recorded highest in GGK followed by GGP and GG during the 12 fortnights observation (Table 2). The lactation pattern was more consistent in GGK compared to GGP and GG, where in the fall was sharp during 5 to 8 fortnights (Fig 1). Repeated measure analysis showed significantly higher milk yield in GGK compared to GGP and GG during 6^{th} and 7^{th} fortnights that ultimately influenced the average milk yield of GGK over that of the other two groups. The average milk yield of camel was in line with the observations made earlier (NRCC, 2020; Nagpal *et al,* 2003; Nagpal and Jabbar, 2005). An increase in milk yield in GGP and GGK compared to GG may be attributed to difference in nutrient uptake and utilisation of the polyphenolic-rich diet. More specifically, GGK recorded higher milk yield than GGP and this could be ascribed to difference in polyphenolic composition between Khejri and Pala leaves (Table 1). Bhatt *et al* (2020) reported a lower growth performance of lambs on a complete diet at higher levels of inclusion (50%) of tanniferous tree leaves. On the country, Aderao *et al* (2020) observed comparable lamb growth performance when the conventional cowpea hay was replaced with Pala leaves. Nagpal *et al* (2002a) observed higher feed intake and growth in camel calves fed on guar phalgati and Khejri leaves at 76:24 ratio. It may be inferred that the present levels of incorporation of Khejri and Pala leaves in the diet of camel could support higher milk yield in camels during the mid-lactation stage.

With regard to milk composition (Table 2), the physical attributes *vis* EC and freezing point were higher ($P<0.05$) in control compared to groups fed on tanniferous leaves. This was a typical observation and a possible role of plant polyphenols or tannins on electrolyte redistribution that affected these two attributes cannot be ruled out. Invariably, tree leaves have wider calcium: phosphorous ratio than the conventional crop residues (Nagpal *et al,* 2002b). Moreover, soil-plant-animal inter-relationship is one of the principal determinants in regulating the difference in mineral acquisition process in different agroecological regions (Kumar *et al,* 2016). According to Henno *et al* (2008), possible effects of season and diet and their combined effect should be considered for interpreting the milk freezing point data. The milk nutrient components except protein are relatively similar in all the dietary groups. The milk protein content in the tree leaves fed groups (GGK and GGP) was higher ($P<0.05$) than the control. This is most

Table 2. Milk yield and composition of lactating camels under different dietary groups.

Item	GG	GGK	GGP
Milk yield/day	6.15 ± 0.24[a]	7.11 ± 0.14[c]	6.65 ± 0.21[b]
Physical parameters			
pH	6.48 ± 0.009	6.49 ± 0.010	6.50 ± 0.008
Electrical conductivity (EC; mS/cm)	6.48 ± 0.072[a]	6.34 ± 0.089[ab]	6.17 ± 0.078[b]
Density (kg/m^3)	26.7 ± 0.216	26.08 ± 0.223	26.5 ± 0.243
Freezing point (^{0}C)	0.48 ± 0.006[a]	0.45 ± 0.004[b]	0.46 ± 0.005[b]
Chemical constituents			
Total solids	10.50 + 0.105	10.64 + 0.105	10.56 + 0.091
Fat (%)	2.66 ± 0.119	2.51 ± 0.111	2.47 ± 0.104
Protein (%)	3.06 ± 0.059[b]	3.29 ± 0.044[a]	3.28 ± 0.034[b]
Lactose (%)	4.00 ± 0.050	4.04 ± 0.062	4.02 ± 0.058
Solid not fat (SNF; %)	7.84 ± 0.084	8.13 ± 0.098	8.09 ± 0.076
Minerals (%)	0.78 ± 0.010	0.80 ± 0.011	0.79 ± 0.010
Fat: SNF ratio	0.339 ± 0.0042[a]	0.309 ± 0.0035[b]	0.305 ± 0.0034[b]

GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)
GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios
GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio
abc Values within a row without a common superscript letter are significantly different ($P < 0.05$)

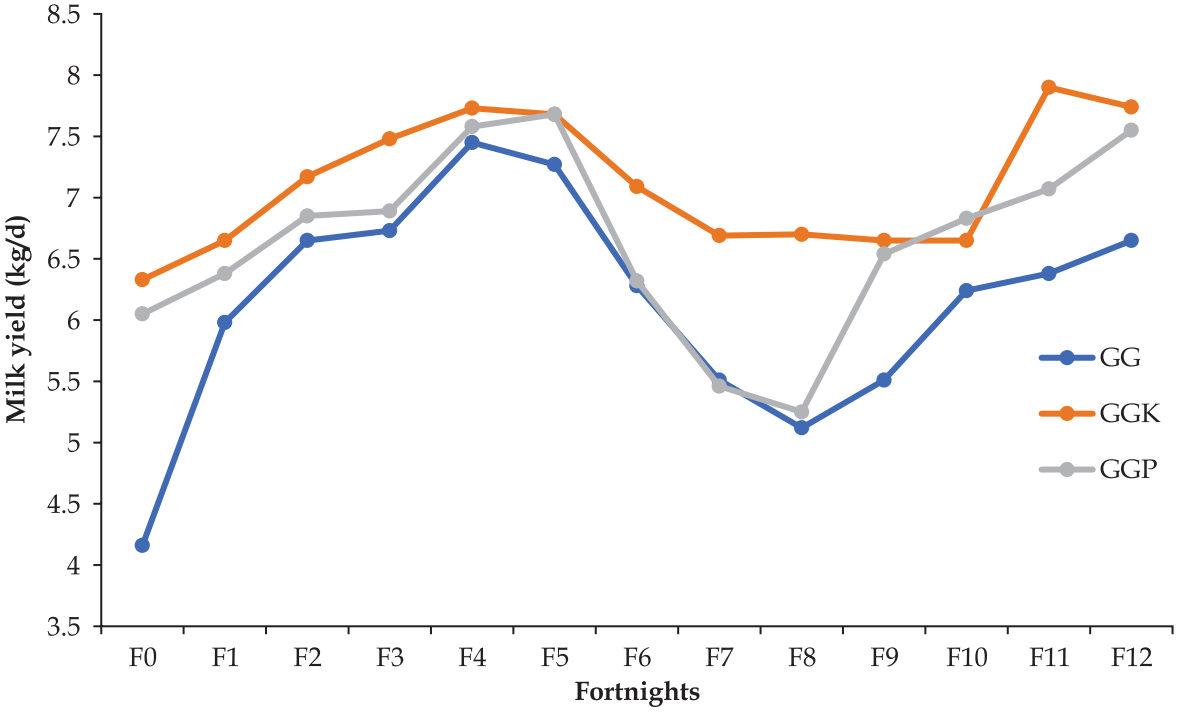

Fig 1. Fortnightly average milk yield of camels in different dietary groups.
GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)
GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios
GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

likely attributable to tanniferous forage intake in GGK and GGP that might have modulatory effect on N or protein utilisation. Plant tannins in moderate amounts reduces the degradability of proteins in the rumen, which induces greater absorption of amino acids by the gut and synergistic actions of

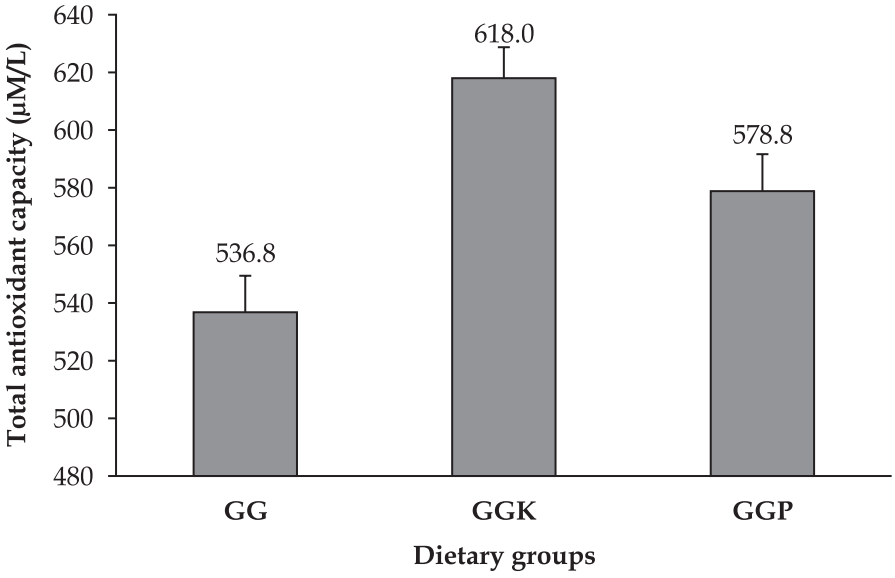

Fig 2. Total antioxidant capacity of milk serum of lactating camels in different groups
GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)
GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios
GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

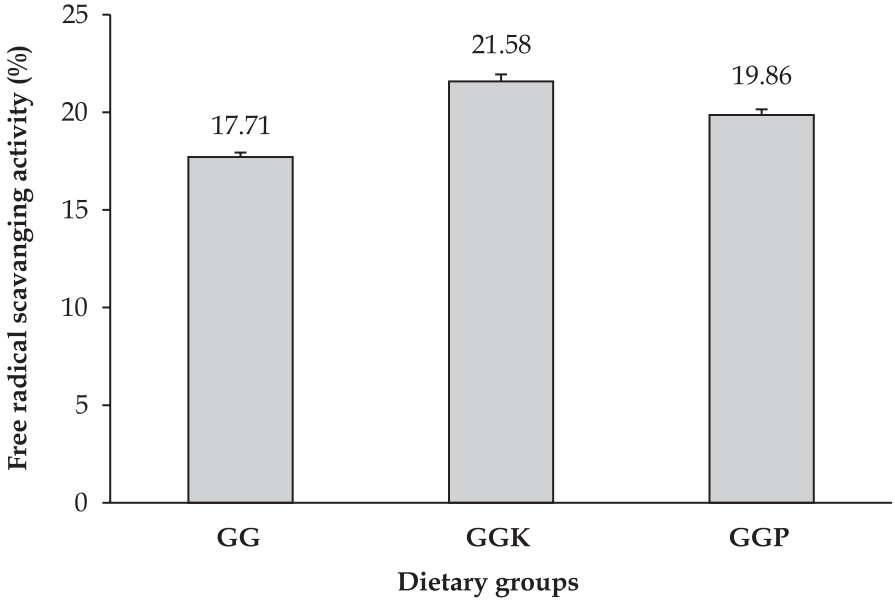

Fig 3. Free radical scavenging activity of milk serum of lactating camels in different groups.
GG: Control, fed with roughage (70%)-Groundnut straw (GS) and Guar phalgati (GP) at 1:1 ratio with supplemental concentrate (30%)
GGK: treatment group having similar control diet with tanniferous Khejri leaves (KL) at GS40:GP40:KL20 ratios
GGP: treatment group having similar control diet with tanniferous Pala leaves (PL) at GS40:GP40:PL20 ratio.

its role in host-microbe metabolism improve the efficiency of utilisation of dietary protein for milk protein synthesis (Jeronimo *et al,* 2016). The other nutritional constituents like total solids, fat, lactose, SNF and minerals were similar between the groups. Nagpal and Jabbar (2005) reported enhancement in total solids, protein and lactose content of milk when the nutritional profile of lactating camel was improved through complete feed block. But, a similar milk nutrient composition in all the three dietary groups would thus be indicative of adequate nutritional status of camels, while additional nutrient input supported increment in milk yield. Similar nutrient composition of milk in lactating camels has been reported earlier (NRCC, 2020).

Upon critical analysis, a decrease in fat: SNF ratio in tanniferous forage fed groups can be interpreted differently. This decline was due to non-significant decrease in fat content with a concomitant increase in SNF content of milk in response to phytochemical-rich diet in GGK and GGP. Milk fat, SNF:fat ratio and, to a lesser extent, total solids contents were strong individual predictors of cheese yield (Caro *et al,* 2011). It is suggested that standardisation of fat: SNF ratio is essential for production of uniform quality and economic aspects of manufacturing any dairy product (Chaudhary *et al,* 2015).

Antioxidant activity

Regarding the effects of feeding regimen, the results revealed that the milk from camels fed on Khejri and Pala leaves exhibited significantly higher ($P < 0.05$) TAC and FRSA values than the milk from the control (Figs 2 and 3). It was obvious that the

uptake of phytochemicals from these tree forages are metabolised by the camels that eventually secreted through the milk to exert higher antioxidant activities in these groups. De Feo *et al* (2006) reported positive relationships between forage intake and antioxidant compounds. Similarly, some other studies in small ruminants have reported similar findings with regards to milk quality (De Feo *et al,* 2006; Jordán *et al,* 2010). Between the two tree-forage fed groups, GGK exhibited higher FRSA activity than GGP and this difference was due to higher TP, TTP and CT content in Khejri leaves than that in the Pala leaves. An increase in milk protein content in tree-forage fed groups may be ascribed to a positive influence on availability of bioactive peptides to exert antioxidant properties to camel milk (Khan *et al,* 2021). Thus, our results showed that the phenolic constituents of tree forages can affect the antioxidant activities of milk through its secondary compounds or degraded metabolites that entered into milk and may provide additional value in terms of oxidative status and thus, incorporation of alternate tanniferous tree forage seems to be a promising strategy for improving milk quality.

Incorporation of tanniferous tree forages at 20% level by replacing the conventional crop residues in the basal diet of lactating camel could be a promising strategy to support or enhance milk yield besides improving the milk quality parameters and providing an impetus to milk processing for commercial cheese manufacturing. Additionally, an increase in antioxidant properties in the milk due to possible influence of polyphenolic metabolites may have a role in increasing the economic value of camel milk and its processed products. This will ultimately have far-reaching socio-economic impact in the life of camel rearers. The top-feed biomass of hot-arid deserts could be a cost-effective solution for nourish lactating camel during scarcity of crop fodder in extreme climatic conditions.

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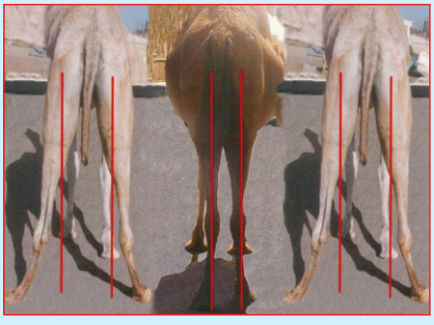

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MANUFACTURE AND CHARACTERISATION OF FRESH CHEESE MADE FROM MIXED MILK OF CAMEL AND GOAT

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ABSTRACT

The aim of this research is to diversify and improve the processing of camel milk. The response surface methodology was used to optimise the coagulation parameters (pH (X_1) and temperature (X_2)) of the camel and goat milk mixture. The coagulation of the milk mixture was performed with camel rennet and was compared to the milk mixture coagulated with microbial rennet. The physicochemical composition and microstructural of optimised fresh cheese was determined and the rheological and sensory properties were studied. Results deduced to optimum points of camel rennet (CR) [X_1=6.16, X_2=37.75°C] and microbial rennet (MR) [X_1=6.31, X_2=38.84°C], which were used in adopted cheese-making process. The physicochemical characteristics of cheeses revealed a significant difference in pH, dry matter, protein and fat contents. Whereas rheological and microstructural analyses, revealed a dilatent coagulum with compact texture characterised with numerous and small pores. Sensory, camel rennet cheese (C_{CR}) was more accepted and had particular profile with creamier texture than microbial rennet cheese (C_{MR}).

Key words: Camel-goat milk, cheese, coagulation, microstructure, rheological properties, sensorial proprieties

Among the processed dairy products, cheese occupy a very important place. In addition to their undeniable nutritional values, they are appreciated for their specific sensory characteristics. The most processed milks are cow, goat and sheep milk. However, camel milk is the least used. Only a few studies have been conducted on the processing of camel milk into cheese. It is therefore not surprising that many scientists have become involved in the study of cheese making from camel milk (Fox *et al,* 2017). In addition, cheese from camel milk remains a challenge under existing conditions (Konuspayva *et al,* 2021; Baig *et al,* 2022).

Haileeyesus and Shimelis (2016) indicated that camel milk is more difficult for processing into cheese, owing to the smaller size of its caseins micelles, fat globules, and its low content of kappa caseins and consequently, limit ability for enzymatic coagulation. Addition of calcium chloride and rennet to camel milk causes a clotting reaction and the formation of a fresh light coagulum (Konuspayva *et al,* 2017; Derar and El Zubeir, 2016). Karoui and Kamal (2017) noted that limited studies are available regarding rennet-induced coagulation of camel milk. Indeed, it exhibits a rennet-induced coagulation time two to three-fold longer compared with bovine milk (Farah and Bachmann, 1987). Siboukeur *et al* (2005), Mahboub (2009) and Boudjenah-Haroun *et al* (2014) studied the properties of camel rennet with their action on camel milk. According to Leksir *et al* (2019) coagulating enzymes of animal origin have been used in Algeria on a traditional scale in the manufacture of cheese.

In Algeria, camel occupies a preponderant place in the socio-economic activity since it represents an indisputable asset in the food security of desert and arid regions (Senoussi *et al,* 2017). In arid areas, goat farming is often conducted in association with camel farming, which makes it possible to have both camel milk and goat milk. In addition, goat milk is the basis

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of excellent cheese widely valued as local product (Boumendjel *et al,* 2017).

In terms of mixed milk, camel milk is used with sheep milk for production of white soft cheese (Derar and El Zubeir, 2013; Derar and El Zubeir, 2016), with buffalo milk for the production of soft cheese (Shahein *et al,* 2014) and with cow milk for the production of Mozarella cheese (Abdallah *et al,* 2022). In addition, the exploitation of goat milk mixed with camel milk remains an untapped task.

Considering the excellent cheese-making ability of goat milk, and in order to improve the camel milk coagulation, this present work targets the optimisation of the coagulation of camel-goat mix milk using camel rennet and microbial rennet, and the characterisation of the manufactured fresh cheese with physicochemical, rheological, sensory and microstructure properties.

Materials and Methods

Milk samples

Camel milk was collected from herds of *Sahrawi* population dromedaries (*Camelus dromedarius*) living in semi-extensive breeding in natural ranges. Goat milk was collected from herds of Arbia goats. The two types of milk were from El Oued "South East of Algeria". All samples were collected cleanly and sent to laboratory with a cold chain using ice packs, then frozen at -18°C until further use. The mixed milk had an equal volume of camel milk and goat milk (50%/50%) (V/V).

Camel abomasum (Last part of Compartment 3-C3)

C3 used in this study from *Camelus dromedarius* (less than a year) was obtained from a slaughterhouse from the same region and was washed with tap water, degreased and covered in a sterile bag and frozen at -18°C.

Microbial rennet (MR)

It's a commercial rennet used in this study as standard rennet. It's a fungal enzyme obtained from *Rhizomucor miehei* (Marzyme R 150MG) with a coagulating force of 1/5000.

Extraction of camel rennet (CR)

The C3 obtained was sliced ($1cm^2$), macerated in a 6% NaCl solution (1:10 w/v) containing 2% boric acid continuously for 4 days at 5°C. Then, the mixture was filtered and centrifuged at 1500 rpm for 15 minutes. The pH of the supernatant was decreased from 5.5 to 4.7 with HCl (1N) and the extracts were kept at 25°C for 24 h to activate the zymogens. The pH was then raised to 5.5 with NaOH (1N). The final rennet extract was obtained by centrifugation at the same speed (Wangoh *et al,* 1993).

Optimisation of the coagulation time of a mixture of camel and goat milk using camel rennet and microbial rennet

Experimental design

The response surface method was used for optimisation of the coagulation time of a mixed volume of camel-goat milk.

The central composite plan (CCD) with two factors (X_1 and X_2) and five levels (-α, -1, 0, +1, + α) was constructed. This plan allowed us to know the effect of pH (X_1) and temperature (X_2) on the coagulation time of milk in a mixture of camel milk and goat milk (linear, quadratic and interactive effects). The pH and temperature varied from 5 to 6.7 and from 30°C to 42°C, respectively (Table 1). The formula (1) of the second degree equation was determined from the experiments to predict the different responses as a function of the parameters studied (pH and temperature).

Table 1. Factors codes and levels of the experimental design for parameters of pH [from 5 to 6.7] and temperature (T) [from 30°C to 42°C].

	Factors (not coded)	
Levels (coded)	pH	T (°C)
Min Point (-α)	5	30
Point (-1)	5.25	31.76
Central point (0)	5.85	36
Point (+1)	6.45	40.24
Max Point (+α)	6.7	42

$$Y=b_0 + b_1 X_1+ b_2 X_2+ b_{11} X^2_1 +b_{22} X^2_2 +b_{12} X_1 X_2 \quad (1)$$

With:

Y: predicted response;

b_0, b_1, b_2, b_{11}, and b_{22}: coefficients of the equation with

b_0: constant;

b_1 and b_2: coefficients of the linear terms;

b_{11} and b_{22}: coefficients of the quadratic terms;

b_{12}: interaction coefficients;

X_1 and X_2: uncoded values of the independent variables (pH and T°).

The experiment matrix of orthogonal composite plane centered with two factors for the 13 tests is shown in table 2.

Table 2. Experimental matrix of orthogonal composite plane centered with two factors.

	Coded values		Uncoded values	
Test	**A**	**B**	**pH**	**T**
1	**0**	**0**	5.85	36
2	**1.414**	**0**	6.7	36
3	**0**	**1.414**	5.85	42
4	**0**	**-1.414**	5.85	30
5	**-1.414**	**0**	5	36
6	**0**	**0**	5.85	36
7	**0**	**0**	5.85	36
8	**-1**	**-1**	5.25	31.76
9	**0**	**0**	5.85	36
10	**1**	**1**	6.45	40.24
11	**0**	**0**	5.85	36
12	**-1**	**1**	5.25	40.24
13	**1**	**-1**	6.45	31.76

The coagulation time was obtained by adding 1mL of camel rennet and /or microbial rennet to 10 mL of mixed milk (50%/50%) (V/V). For all tests, pH adjustment was made by lactic acid solution (10%) (V/V). The coagulation time was completed when a rigid coagulum appeared in the tube (Alais, 1974).

Statistical analysis of data and graphical representation

The regression coefficients of the responses (clotting time) were determined by Minitab software (Minitab Inc., State College, PA). The value of P determined the degree of significance (α=0.05). Plots for camel and microbial rennets are shown using Statistica software (version 10, Statsoft Poland).

Camel and goat milk cheese-making process

The experimental diagram that summarises the procedure for making fresh cheese from camel milk and goat milk, coagulated with camel rennet (CR) or microbial rennet (MR) is presented in Fig 1.

The freshness of milk was ensured by measuring the pH of camel milk and goat milk. Then, a mixture of milk was made (50%/50%) (V/V) and filtered. Then $CaCl_2$ (0.01%-0.015%) was added and the milk mixture was allowed to stabilise for 30 minutes. The mixed milk was heated to the optimal temperature for coagulation. Afterwards, salting (5g/L) was carried out and followed by adjustment of the optimal pH with lactic acid. At these optimal parameters (temperature and pH), and based on the coagulant strength of each rennet, the camel rennet (CR) was added at a concentration of 0.24ml/l and that of the microbial rennet (MR) at 0.2 ml/l. After coagulation, which took a maximum 45 minutes, the cheese was drained spontaneously and then molded. The cheese made with camel rennet (C_{CR}) and with microbial rennet (C_{MR}) were placed in clean food trays and kept in a refrigerator at 4°C.

Analysis of the crude composition and yield of the cheese

The physicochemical composition (protein, dry matter, fat and ash) and pH were determined from cheese stored at 4°C for a maximum of 24h. The methodological standards for analysis were followed as described by Bradley *et al* (1993). The pH of the cheese was measured by a digital pH meter (inolab, Germany). Total nitrogen was determined by the Kjeldahl method. Dry matter was calculated by drying 2g of fresh cheese at 103°C±2°C for 3h (dry oven, Memmert, Spain), fat was measured by the Van Gulik method (FUNKE GERBER centrifuge, Germany) and ash was determined by total incineration (muffle oven, Memmert, Spain).

Cheese yield is the percentage of total cheese weight (kg) in relation to the initial milk weight (kg) (Mahaut *et al,* 2000).

Cheese yield (%) = (cheese weight/milk weight) × 100.

Rheological analysis (flow test)

The flow test of cheese produced with camel rennet (C_{CR}) and/or with microbial rennet (C_{MR}) was measured by a rheoviscosimeter HAAKE 550 (HAAKE MessTechnik Gmbh Co, Karlsruhe, Germany). The cheese samples were placed on a parallel plate geometry. The plate diameter was 50 mm (Haake, PK 5, 0.5 grads), and the gaps between plates were 4.5 mm. The viscosity of cheese samples was measured for 180s with an initial shear rate of 1.2 s^{-1} and 200 s^{-1} as the final shear rate. Rheological data were analysed with HAAKE Rheo Win software version 2.09. The temperature was maintained at 20°C by a thermostat bath. The number of measuring points were 100. Each test was performed in triplicate. The rheological analysis method was cited by Boughellout (2007) and Djeghim *et al* (2021).

The apparent viscosity was determined using the low power law model (Ostwald de Waele):

$\eta_{ap} = K.\gamma^{n-1}$

where

ηap: apparent viscosity (Pa.s);

γ: shear rate (s^{-1});

n: flow behaviour index;

K: consistency index ($Pa.s^n$) represents the stress required to obtain a shear rate of $1s^{-1}$.

Microstructural analysis by environmental scanning electron microscopy (ESEM)

The purpose of this analysis was to visualise the microstructure of the enzymatic gel based on the mixture of camel and goat milk by observation under an environmental scanning electron microscope (ESEM – FEI QUANTA 250) operating under a large file detector (LFD) and a low vacuum with an accelerating voltage of 10.00 KV.

First, a small piece of each fresh cheese, newly clotted, was finely cut (0.5 cm in length, 0.5 cm in width) and air-dried for 4 to 5 hours. Then, drying in an atmosphere saturated with glutaraldehyde overnight (12h). Then, each piece of cheese was fixed by a series of ethyl alcohol from 10° to 100° for 5 minutes by solution. Thereafter, the pieces of cheese were dried in open air for a few hours before proceeding to observation (Attia *et al,* 1991).

Sensorial profile analysis

The objective of this analysis was to give the sensory profile of the cheese and check its quality. It involved giving a subject of cheese sample and the sensory characteristics were assessed through visual observations and tastings. The characterisation relates to the appearance and texture; smell and taste with aroma. It was made with 20 'student and teacher' tasters. Cheese samples were cut into small squares at about 10g and placed in a closed box for one hour at room temperature before testing. The taster answers the questions on the evaluation grid and assesses the sensory characteristics (Berodier *et al,* 2003).

The comparison between means of crude composition, rheological analysis and the sensory profile values of the studied cheese was done.

Results and Discussion

Effect of pH and temperature on clotting time of mixed milk of camel and goat with camel and microbial rennet

The optimisation gave us the optimum points of pH and temperature for obtaining a suitable coagulation time of mixed milk camel and goat. Table 3 shows the performance of camel and microbial rennets to coagulation of mixture of camel and goat milk.

Results showed that camel rennet coagulated the milk mixture at a minimum time of 15±0.00s at pH:5.85/T:36°C pair and a maximum time of 180±0.00s at pH:5.25/T:31.76°C pair. According to Bouras *et al* (2022), the camel milk coagulation with camel rennet gives a minimum and maximum time of 60±0.01s and 248±0.04s, respectively. In addition, Hailu *et al* (2016) reported that time gelation of camel milk with camel chymosin is mainly affected with temperature and enzyme concentration. Whereas, microbial rennet takes a minimum time of 7.24±0.00s, and a maximum time of 106±0.03s for pH:6.45/ T:40.24°C and for pH:5/T:36°C pairs in mixed milk of camel and goat.

Table 3. Clotting time responses of mixed milk camel and goat with CR and MR.

Test	Coded values		CR	MR
1	0	0	43±0.00	12.31 ± 0.01
2	1,414	0	59 ± 0.00	13.85 ± 0.00
3	0	1,414	50 ± 0.03	20.36 ± 0.02
4	0	-1,414	97 ± 0.04	9.69 ± 0.00
5	-1,414	0	120 ± 0.04	106 ± 0.03
6	0	0	19 ± 0.01	9.39 ± 0.01
7	0	0	15 ± 0.00	10.5 ± 0.01
8	-1	-1	180 ± 0.00	14.14 ± 0.02
9	0	0	57 ± 0.02	7.78 ± 0.00
10	1	1	42 ± 0.02	7.24 ± 0.00
11	0	0	49 ± 0.01	8.79 ± 0.02
12	-1	1	159 ± 0.00	8.29 ± 0.02
13	1	-1	105 ± 0.00	18.5 ± 0.04

CR : Camel Rennet, **MR** : Microbial Rennet.

For camel rennet, plot in fig 2 showed that the pH had a significant effect (linear and quadratic) on the coagulation of milk mixture (b1=0.012, b11=0.014) with a significant effect (quadratic) on temperature (b22=0.038). Based on surface plot analysis and relationship between response and variables, a milk mixture clotting time is optimal assuming these pairs of pH and temperature: [X_1=6.16, X_2=37.75°C]. Concerning microbial rennet, the coagulation of milk mixture is sensitive to the variation of pH (linear and quadratic effects: b1=0.000, b11=0.000) with the interaction of pH and temperature (interactive effect) (b_{12}=0.000). Based on surface plot analysis and relationship between response and variables, a milk mixture clotting time is optimal assuming these pairs of pH and temperature: [X_1=6.31, X_2=38.84°C].

Results showed that the addition of goat milk to camel milk considerably increases the performance of camel milk to coagulate with camel rennet. Subsequently, the reducing of coagulation time increases the cheese-making ability of camel

milk. So, the optimum pH and temperature pairs are subsequently used in fresh cheese processing using camel rennet or microbial rennet.

Crude composition and yield of fresh cheeses

Table 4 is the summary of the main physicochemical properties of C_{CR} and C_{MR} fresh cheese.

Table 4. Physicochemical properties of C_{CR} cheese and C_{MR} cheese (mean ± standard deviation for 100g).

Parameters	C_{CR}	C_{MR}	P-value
pH	6.48±0.01	6.28±0.01	**
Dry matter (%)	26.7±2.1	45.2 ±0.9	**
Proteins (%)	11.1±1.5	23±0.7	**
Fat (%)	9.7±0.4	18.7±1.25	**
Ash (%)	6.81±0.5	9.29±1.09	NS
Yield (%)	17±1.8	18.3±0.4	NS

C_{CR}: Camel Rennet Cheese, C_{MR}: Microbial Rennet Cheese. Presented values are the means of three replicate trials. NS: Non-Significant, **P <0.01 (Student's t-test).

For pH, it appears to be a significant difference between cheese coagulated with camel rennet and cheese coagulated with microbial rennet (P<0.05). In addition, both types of cheese were characterised by the absence of acidity. These results are explained by the fact that C_{CR} and C_{MR} cheese are initially acidified only by the addition of lactic acid in order to achieve the optimum pH of coagulation.

For the main components, the dry matter of C_{CR} and C_{MR} were significantly different (P<0.05). C_{CR} cheese produced by camel rennet had a dry matter content of 26.7±2.1% compared with C_{MR} cheese, which has a dry matter content of 45.2±0.9%. According to Hailu *et al* (2014), several factors were considered into the cheese, variation in dry matter, such as the nature of the ingredients added during the cheese making, the dry matter content of the raw material and the addition of salt.

For proteins content, there was a significant difference between the two cheese types (P <0.05) and these values for C_{CR} and C_{MR} cheese were 11.1±1.5% and 23±0.7%, respectively. Concerning lipids, the fat content of C_{MR} cheese was 18.7±1.25% and higher than that of C_{CR} cheese 9.7±0.4%.

Cheese yield showed no significant difference between C_{CR} and C_{MR}. It was therefore construed that the enzymatic extract of camel rennet can be a strong competitor of microbial rennet in terms of cheese yield in camel-goat cheese. There appeared a clear impact of the type of enzyme on the composition of the fresh cheese.

Shahein *et al* (2014) produced fresh cheese from camel milk and buffalo milk. These cheese were characterised by a dry matter of 13.58% to 15.15%, proteins of 3.62% to 3.9%, fats of 3.6% to 5.3%, ash of 0.81% to 0.84% and a cheese yield of 14.7% to 20.1%.

Fresh cheese were also made from camel milk only using different proteolytic enzymes. Saima *et al* (2003) reported 97.04% total solids not fat and 78.38% total proteins using commercial rennet and skimmed camel milk. El Zubeir and Jabreel (2008) reported 7.5% dry matter, 4.2% protein, 1.9% fat and 0.2% ash using Camifloc as a coagulant. Bekele *et al* (2018) reported a dry matter content of 34.76±0.26 % to 43.44±2.8%, a protein content of 11.12±0.02% to 17.49±1.73%, a lipid content of 17.99±0.45% to 20.91±0.82% and a yield of 9.5±0.34% to 13.44±0.09% using camel chymosin. The latter was also valorised in the study of Mbye *et al* (2020), who reported a yield of 12.3±1.2%.

The fresh cheese coagulated with recombinant camel chymosin had pH 5.27±0.05%, proteins content 15.62±0.71%, fat content 20.21±0.67%, ash content 2.47±0.53% and a cheese yield 8.75±1.68% (Al-Zoreky and Almathen, 2021).

Rheological analysis (flow test)

Rheological parameters of C_{CR} fresh cheese made with camel rennet and C_{MR} fresh cheese made with microbial rennet are represented in table 5.

Table 5. Rheological parameters of C_{CR} cheese and C_{MR} cheese.

Parameters	K (Pa.sn)	n	$\chi 2$	R
C_{CR}	2174±226.9*	-1.4±0.48 ^{NS}	5.58±3.79	0.82±0.03
C_{MR}	5694±850*	-0.92±0.27 ^{NS}	1.48±0.50	0.77±0.14

C_{CR}: Camel Rennet Cheese, C_{MR}: Microbial Rennet Cheese. Presented values are the means of three replicate trials. K: consistency coefficient; n: flow behaviour index; r: statistical correlation coefficient; $\chi 2$: Chi-square, NS: Non-Significant, *P <0.05 (Student's t-test).

According to Ostwald model, the statistical analysis showed low values of chi-square ($\chi 2$) and high values of regression coefficient (r) for both C_{CR} and C_{MR} cheese formulations. The consistency index (K) is an indicator of the viscous nature of the system (Koocheki *et al,* 2009).The C_{CR} cheese produced with camel rennet had a coefficient of consistency K value, which was 2174±226.9 (Pa.sn); whereas C_{MR} cheese produced with microbial rennet had 5694±850 (Pa.sn). Statistical analysis revealed a significant difference between the two values of K (P <0.05).

The flow index (n) may vary from n=1 (leading to the Newtonian law) to n<1 or n>1 to describe shear

thinning or shear-thickening flow behaviour (Steffe, 1996; Fischer *et al,* 2009).

The C_{CR} cheese n value was greater than 1 indicating a dilatent coagulum (shear thickening) and this value of C_{MR} cheese was less than 1 indicating a pseudoplastic coagulum (shear-thinning).

Attia *et al* (2001) recorded a plastic gel behaviour for fermented camel milk; where as Ayyash *et al* (2022) reported a pseudoplastic gel for a heat treated and fermented camel milk.

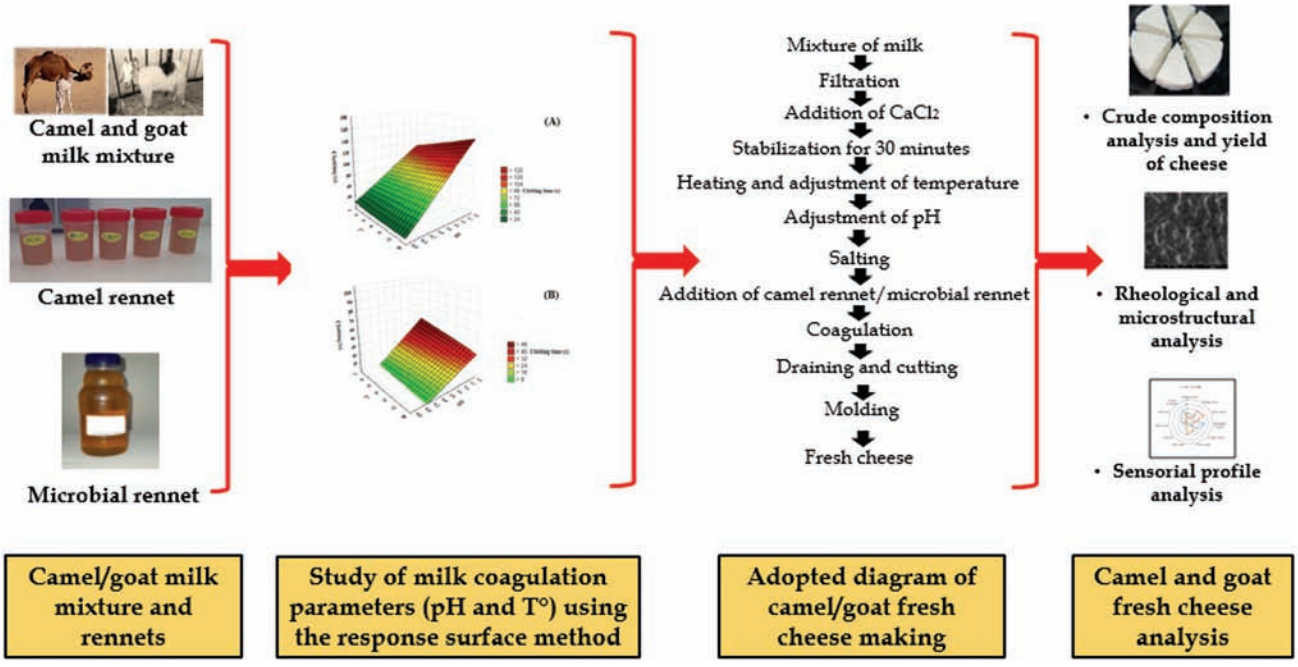


Fig 1. Experimental diagram of cheese.

The behaviour of the shear thinning coagulum has been molecularly interpreted as the molecules or structural units of the substance gradually align in the direction of flow as the shear rate increases. In the case of the shear thickening coagulum, this behaviour was less frequent than the thinning coagulum. It was molecularly interpreted by a redistribution of the solvent (lubricant) at the level of the particles, after swelling of the liquid phase (Couarraze and Grossiord, 1991).

Coefficient of consistency K and flow index depend on the temperature and the formulation (Cheremisinoff, 1990; Djeghim *et al,* 2021). Negative values of flow index n can be justified by molecular degradation of the sample, viscosity dispersion or presence of slippage in the fluid (Padmanabhan *et al,* 1991; Drozdek and Faller, 2002; Djeghim *et al,* 2021).

In rheological characterisation of cheese it is generally classified as a viscoelastic material because its stress–strain ratio exhibits both solid and liquid behaviour. In addition, cheese rheology depends on its internal environment, such as its pH and temperature (Giha *et al,* 2021).

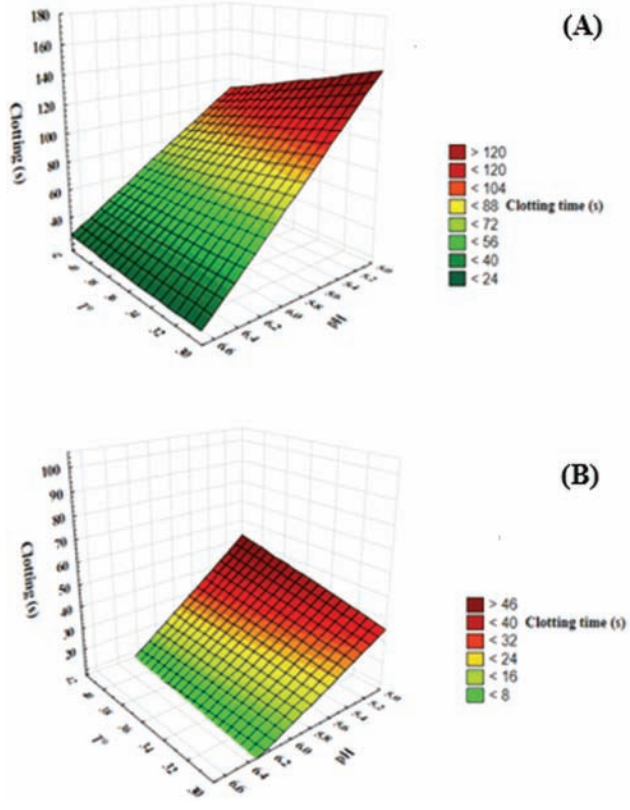
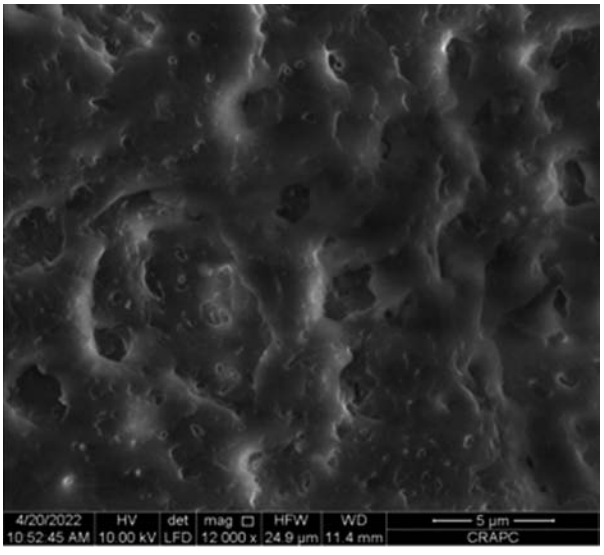
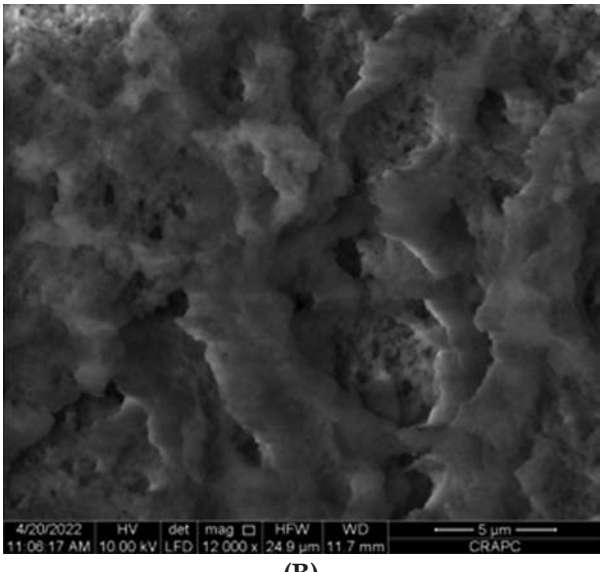


Fig 2. Surface response plot of camel-goat milk mixture with CR (A) and MR (B).



(A)



(B)

Fig 3. Microstructure of C_{CR} cheese (A) and C_{MR} cheese (B).

The difference in the coagulum behaviour of C_{CR} cheese and C_{MR} cheese could be due to specific affinity of each rennet towards the caseins of camel milk, and by the effect of the addition of goat milk.

Microstructure

The ESEM images of cheese produced with camel rennet and commercial rennet are shown in Fig 3.

Results of coagulated C_{CR} cheese and C_{MR} cheese microstructure showed that the type of coagulant had an effect on the casein network formation. It appears that the network of C_{CR} and C_{MR} cheese are continuous. In addition, the pores of C_{CR} cheese are more numerous and smaller compared

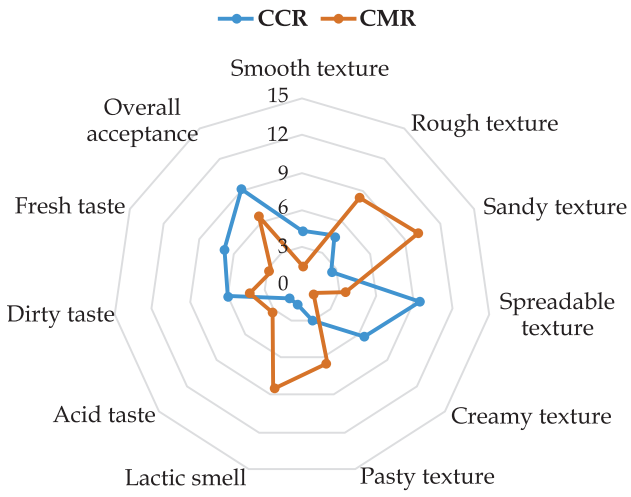


Fig 4. Sensory profile of C_{CR} cheese and C_{MR} cheese.

to C_{MR} cheese. The protein network brins of C_{MR} cheese are thicker than C_{CR} cheese. The presence of evident spaces in cheese is related to the specificity of the coagulant (Fox *et al,* 2000). The ESEM image showed that the protein network of C_{CR} cheese is more compact than that of C_{MR} cheese.

Boudjenah-Haroun (2012) found that the microstructure of cheese made from camel milk and produced with coagulant enzyme extracts isolated from C3 (abomasum) was a compact and uniform structure.

The presence of the small pores may be justified by the reduction in protein activity of the camel chymosin, which was leading to a more compact network, in comparison with the proteolytic activity of the microbial rennet of *Rhizomucor miehei* (Soltani *et al,* 2016).

Sensorial profile analysis

The sensory profile of C_{CR} and C_{MR} fresh cheese are shown in Fig 4. All cheese sensory characteristics were evaluated from 0 to 15 scale.

For the texture, C_{CR} cheese is weakly smooth, rough and pasty with a score of 4.25±1.64, 4.6±0.72 and 2.85±0.99, respectively. In addition, it is characterised by a moderately spreadable texture of 9.2±2.66. Whereas, C_{MR} cheese is weakly smooth, spreadable and creamy with a score of 1.6±0.34, 3.35±0.79 and 1±0.58, respectively. On the other hand, it had a moderately rough texture with 8.3±2.72 and pasty with 6.35±1.76.

For taste and aroma, C_{CR} and C_{MR} cheese are characterised by a weakly acidic taste, which is consistent with the 2 pH cheeses results. In addition, both types of cheese have an average salinity of 6.1±1.19 for C_{CR} cheese and 4.3±1.88 for C_{MR} cheese.

There is a significant difference ($P<0.05$) between C_{CR} and C_{MR} cheese in terms of texture, smell and taste. For the texture, the C_{CR} cheese was less sandy and creamier than the C_{MR} cheese. For smell, C_{CR} cheese had less lactic smell. For taste, C_{CR} cheese was more fresh than C_{MR} cheese.

On the scale of general acceptance, C_{CR} cheese more accepted by tasters compared to C_{MR} cheese with a score of 9.1±2.53 and 6.55±3.39, respectively.

In the literature, fresh cheese made from camel milk only was characterised by a slightly acidic taste, a moderately smooth texture, slightly rough with a salty taste and an acceptance of fresh cheese as reported by El Zubeir and Jabreel (2008). The appreciation of a certain roughness in the texture of fresh cheese made from camel milk was still observed in the study by Ramet (2001) which was justified by the reduction in the fat content of camel milk. At this level, our results are compatible with that of the fat composition of C_{CR} and C_{MR} cheese even with the addition of goat milk. In addition, Mbye *et al* (2020) reported a weakly hard and moderately spreadable and crumbly texture.

For salinity, it was appreciated in C_{CR} and C_{MR} fresh cheese. This assessment was compatible with the considerable ash content for both types of cheese. Besides, the salinity of fresh cheeses was further justified by the high content of camel milk in the region of El Oued in the study by Bouras *et al* (2022).

According to Mehaia (1993), the acceptability score of fresh cheese made from camel milk was between 4.10 and 7.80. In another study by Benkerroum *et al* (2011) found the acceptability score to be 2.90 and 4.70. In this study, we can say that the addition of goat milk to camel milk increased the acceptability score of C_{CR} and C_{MR} cheese.

Conclusion

The coagulation of camel milk is a key step in cheese making process. In this study, the type of rennet used had a significant effect on the overall quality of the cheese. The coagulation of camel milk was improved when mixed with goat milk, regardless of the type of enzymes used (camel or microbial rennet). The fresh cheese produced had very interesting physicochemical and textural characteristics. Microscopic analysis showed that the coagulum obtained was quite firm and on the other hand it presented specific sensory characteristics. These results seem therefore encouraging to exploit the camel rennet in the manufacture of other cheeses, mainly ripened.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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MOLECULAR CHARACTERISATION OF DIACYLGLYCEROL O-ACYLTRANSFERASE (DGAT1) GENE IN DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

Present study was undertaken to characterise Diacylglycerol O-Acyltransferase (DGAT1) gene of dromedary camels (*Camelus dromedarius*). Molecular characterisation of 773 bp long DGAT1 gene fragment was performed in Indian dromedary camel through PCR amplification, sequencing and bioinformatics analysis. The characterised DGAT1 gene fragment covered partial exon-6, exon-7, 8, 9 and partial exon-10 and complete intron-6, 7, 8, 9 regions. A single nucleotide variation was seen at position 463 C>T of exon-8 in both Bikaneri and Kachchhi breeds. Animals with single and double peaks in the sequence chromatograms were observed at this particular locus. Accordingly, two alleles (C, T) and only two genotypes (CC and CT) were identified in the Bikaneri and Kachchhi camel breeds. Bikaneri and Kachchhi camel (*Camelus dromedarius*) sequence showed highest similarity (99.71%) with predicted dromedary camel, Ferus (wild) camel, Bactrian camel and Vicugna DGAT1 sequences. With other species like cattle, buffalo, pig, sheep, goat, yak, dog, cat, killer whale, naked mole rat and human DGAT1 sequence similarity ranging from 73.55 % to 84.93 % was observed. Pig was closest species (84.93%), whereas American Shorthair breed of cat (78.08%) was least similar to dromedary camel. Similar relationship was observed between species on the phylogenetic analysis of DGAT1 gene nucleotide sequences.

Key words: Camel, DGAT1 gene, dromedary, phylogeny

The vital role of DGAT1 in fat metabolism makes them a best choice as a candidate marker in animal production (Khan *et al,* 2021). DGAT1 was documented to have a significant influence on milk production in cattle in Germany (Molee *et al,* 2012). Diacylglycerol O-acyltransferase 1 (DGAT1) was identified as one underlying QTL for milk production traits located on the centromeric region of the bovine chromosome 14 having 17 exons with 14,117 base pair (bp) (Grisart *et al,* 2002 and Winter *et al,* 2002) and was located on chromosome 15 has a size of 10,733 bp distributed in 19 exons in buffaloes (Amaral *et al,* 2008).

The marker genes for camel milk traits can give the pathway to improving camel milk yield and quality which can result in increased economic importance of camel. The polymorphism in candidate genes like CSN1S1, CSN1S2, CSN2, CSN3, ACACA, DGAT1, DGAT2,ME1, SCD, LPL, LIPE, BTN1A, MFGE, GH, PRLR, PITX2, POUF1, and STAT5 has been linked with milk yield and composition traits (Tesema and Alemayehu, 2018). So far only some information on polymorphism in Casein gene (Gahlot *et al,* 2019; Jadhav *et al,* 2019; Jadhav *et al,* 2020), Growth hormone gene (Prakash *et al,* 2021; Jyotsana *et al,* 2021) and Leptin gene (Choudhary *et al,* 2022) have been reported in the case of Indian dromedary camel. DGAT1 gene have one of most important QTL associated with variation in milk composition and parameters (Fernir *et al,* 2002; Kuhn *et al,*2004). Structure and role of DGAT1 gene has been widely studied in cattle, buffalo and to some extent in sheep, goat. Allelic variants of DGAT1 are associated with Fat %, Protein %, Lactose %, and SNF % and milk yield (Thaller *et al,* 2003; Hanusova *et al,* 2014; Faraj *et al,* 2020).

Samuel *et al* (2022) included fifteen haplotypes/ sequences of milk producing farm animals from Genbank including Bos taurus (AJ318490, EU077528, MF069174 and MF445056), *Bubalus bubalis* (MZ230553, MZ230553, MF069172 and KX965992), *Camelus dromedarius* (MF069170 and MF069171), *Capra hircus* (LT221856 and FJ415876) and *Ovis aries* (KJ918741, FJ415875 and EU178818) from Germany, Turkey, India, Iran and Benin in the analysis.

The whole genome Illumina assemblies or shotgun sequence (CamDro3) of chromosome 25 on

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North African dromedary camels reported camel DGAT1 gene extends from 41215260 to 41224586 bases (GenBank: NC_044535.1) with a size of 9327 bp (Elbers *et al,* 2019). It splits into 16 exons and 15 introns (Gen Bank: KAB1258102.1). The molecular structure of DGAT1 gene in Indian dromedary and bactrian camels are not available. Hence, the present investigation was undertaken for molecular characterisation of DGAT1 gene in Indian dromedary camels.

Materials and Methods

Blood samples were randomly collected from 10 camels each from Bikaneri and Kachchhi breeds maintained at ICAR-National Research Centre on Camel (NRCC) farm at Bikaner, Rajasthan (India). The DNA was extracted from blood cells using standard phenol-chloroform extraction protocol (Sambrook *et al,* 2001). PCR amplification of 773 bp DGAT1 gene fragment was performed utilising primers designed with the help of Primer-BLAST system of NCBI (Stephen *et al,* 1997) and were synthesised from Eurofins genomics. Primer sequence (5′-3′) in Forward direction was CCTTCCTGCTTGAGTCCAT, whereas reverse primer was ACTTGGAGCTGGGTAAGG. The gradient PCR programme was used to find out the appropriate annealing temperature. The PCR reaction was carried out with Green master mix-13 µl (Promega, USA), 1µl of each primer with concentration of 10 pM, 1µl of 80-100 ng camel genomic DNA and nuclease free water (Promega, USA) to make total volume up to 25µl. Amplification was performed in thermal cycler (Applied biosystem Veriti™ Thermal cycler) programmed for initial denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 94°C for 60 s, annealing at variable temperatures for 45 s, extension at 72°C for 45 s and final extension at 72°C for 10 min. The PCR products were checked for amplification by electrophoresis on 1.0% agarose gel (Himedia) in parallel with 1kb plus DNA marker (Thermo-scientific). Standardised temperature was further used for the amplification of all the samples. This bidirectional sequencing using forward and reverse primers was performed using Sanger Dideoxy Chain termination method (GeneOmBio Technologies Pvt. Ltd.). The forward, reverse sequences obtained for each animal were edited using Codon Code Aligner software (USA) and different sequences patterns were generated. The pair wise and multiple alignment of the different sequence pattern were analysed to find out the differences and relationship between Indian camels DGAT1 gene sequences. Obtained sequences were compared with camelid and other domesticated species DGAT1 gene sequences available in National Centre for Biotechnology Information (NCBI) database using BLAST software program (http://www. ncbi. nlm.nih.gov/) to study the sequence variation and relationship. The estimation of evolutionary relationship between different species was inferred by neighbour joining method by using the Blast Tree View system of online BLAST® software of NCBI and phylogenetic tree was also studied.

Results and Discussion

The annealing temperature of 57^{o} C was found optimal for amplification of the target DGAT1 gene fragment. Single clear bands were observed, when the PCR products were checked for amplification by electrophoresis on 1.0 % agarose gel in parallel with 1 bp plus DNA marker (Fig 1).

After bidirectional sequencing of PCR products, visualisation of sequence chromatograms and editing of sequence reads using Codon Code aligner software, 773 base pair DGAT1 gene fragment and its genetic variants were identified. The amplified DGAT1 gene fragment covered partial exon-6, complete intron-6, 7, 8, 9, complete exon-7, 8, 9 and partial exon-10.

Population genetic differentiations based on the DGAT1 genes of the breeds had been evaluated by Ne's genetic distance (GST) by DnaSP software (Librado *et al,* 2009).

Nucleotides were conserved at all the positions in 773 bp long DGAT1 gene fragment sequence, except 463rd position which was part of exon-8. At position 463 the sequence chromatogram of some animals showed one peak and some had two peaks due to presence of different allele at the same locus. This variation was due to change in nucleotide sequence 463 C>T in Bikaneri and Kachchhi breed. The nucleotide variation caused change in last codon of 8th exon from CGC to CGT. However, it was a synonymous mutation as both codons code same amino acid (Arginine). The nucleotide change resulted in 2 allelic (C, T) and 3 genotypic variations (CC, CT, and TT) in Indian dromedary camels (Fig 2).

The sequence variation and identity percentage were determined on the basis of pairwise nucleotide BLAST of Indian dromedary DGAT1 gene (C allele) with Ferus (wild), Bactrian, dromedary camel, vicugna, and other species like cattle, buffalo, pig, sheep, goat, yak, dog, cat, killer whale, naked mole rat and human. Dromedary DGAT1 gene sequences with 773 fragment size showed 99.17% identity with predicted DGAT1 cDNA sequences of *Camelus ferus*,

dromedary and Bactrian camel. Per cent identity of Indian dromedary with other species varied between 73.55 % to 84.93 % (Table 1). The differences could be due to transition, transversion, insertion and deletion type of mutations during the course of evolution. This DGAT1 gene is present on different chromosomes between DGAT1 genes (Fig 3) of different species as observed on the basis of percentage identity of the nucleotide sequences.

Multiple Sequence alignment was prepared between DGAT1 gene and DNA, cDNA sequences from different species with NCBI MSA programme

Table 1. Percentage identity between DGAT1 gene sequence of Indian dromedary camel with DGAT1 gene sequences of another species.

Accession Id.	Organism and type of Reference Sequence	Query Cover	% Identity	Acc. Len
XM_032467564.1	PREDICTED: *Camelus ferus* DGAT1, mRNA	43%	99.17%	1708
XM_006211243.3	PREDICTED: *Vicugna pacos* DGAT1, mRNA	42%	99.17%	2205
XM_031439877.1	PREDICTED: *Camelus dromedarius* DGAT1, variant X1	44%	99.17%	1559
XM_010961176.1	PREDICTED: *Camelus bactrianus* DGAT1, partial mRNA	43%	99.17%	1624
AY116586.1	Sus scrofa DGAT1 gene, complete cds	89%	84.93%	9303
OW443377.1	*Orcinus orca* genome assembly, chromo:17	99%	84.81%	87542868
AY999090.1	*Bubalus bubalis* DGAT1 gene, complete cds	91%	80.06%	12372
CP027082.1	*Bos mutus* isolate yakQH1 chromosome 14	77%	79.83%	81354091
EU178818.1	*Ovis aries* DGAT1 gene, complete cds	87%	79.66%	8676
LR962869.1	*Bos taurus* genome assembly, chromo: 14	83%	79.46%	82271483
EF636701.1	*Bos indicus* breed Butana DGAT gene, partial cds	83%	79.46%	8107
LT221856.1	*Capra hircus* dgat1 gene	87%	79.34%	37251
CP050604.1	*Canis lupus* familiaris breed Labrador retriever chromosome 13a	86%	79.23%	63905973
AP023169.1	*Felis catus* Senzu DNA, chromo: F2, American Shorthair breed	100%	78.08%	86116642
OX090950.1	*Hetero cephalus* glaber genome assembly, chromosome: 10	82%	73.95%	94825129
NG_034192.1	*Homo sapiens* DGAT1, RefSeq Gene chromosome 8	90%	73.55%	19337

in different species, as it is present on chromosome number 25 in members of Camelidae like wild camel, vicugna, dromedary and double humped Bactrian camel. Its location in human, cattle, buffalo, sheep, pig, dog, cat and whale is on chromosome number 8, 14, 15, 9, 4, 13, 2 and 17, respectively. Its nature as transposon or jumping gene (Pray, 2008) can also be investigated. The evolutionary relationship inferred using Neighbour joining method of phylogeny tree construction showed similar relationship pattern

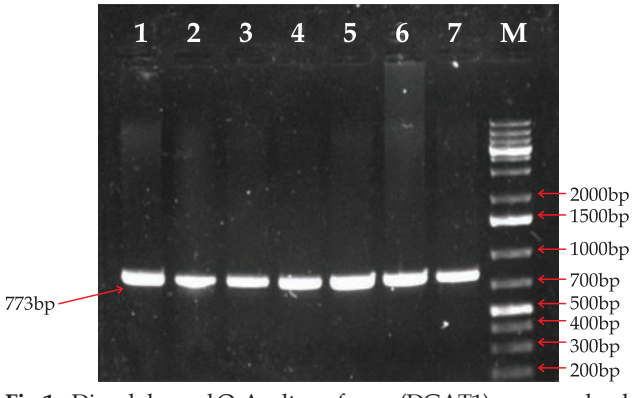

Fig 1. Diacylglycerol O-Acyltransferase (DGAT1) gene resolved on 1.0% agarose gel Lane-Marker 1k bp plus DNA ladder (M), Lane-1 to 7 are DGAT1 gene product.

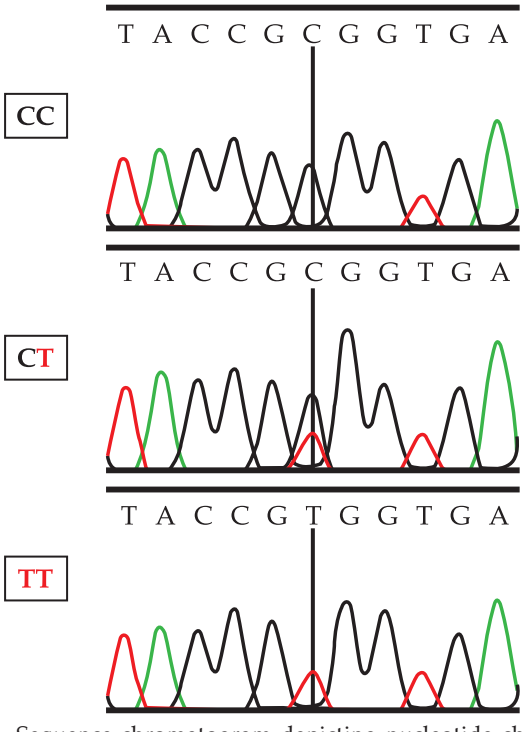

Fig 2. Sequence chromatogram depicting nucleotide change at position 463 in DGAT1 gene sequence of Indian dromedary camel. Different colour and type of peak at position 463 show genotypes pattern

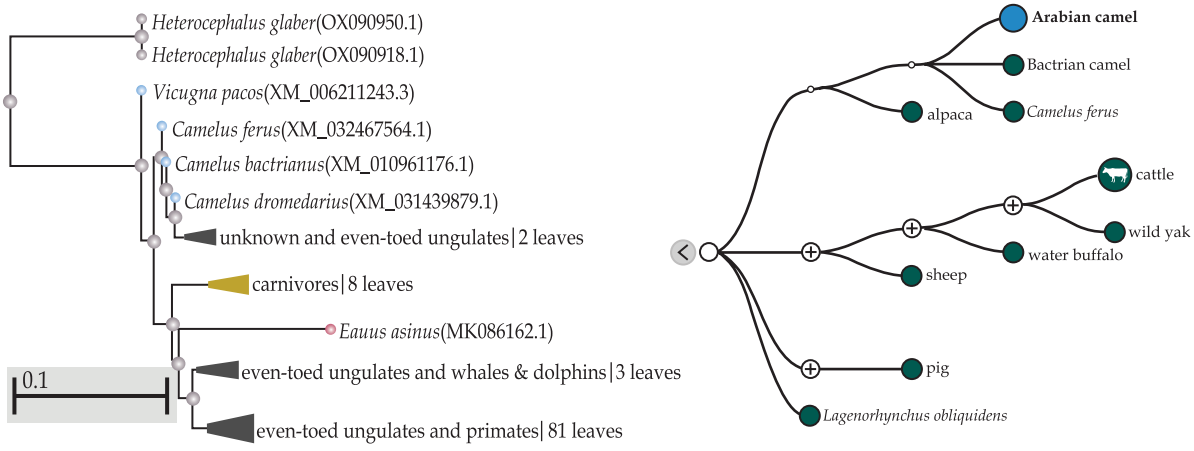

Fig 3. Phylogeny tree of DGAT1 gene based upon Neighbour joining method.

Fig 4. Multiple Sequence alignment between DGAT1 gene and cDNA/DNA sequences from different species. (At extreme left of Fig Sequence ID is denoted as accession number, at extreme right of Fig zoological names of species are mentioned).

(Ver: 1.22.0), which show all possible genetic similarities and differences between targeted 15 species (Fig 4).

A study conducted by Samuel *et al* (2022) found that the DGAT1 gene may provide baseline information for in-depth understanding, exploitation of milk linked gene variation and could be used as a marker in selection programmes to enhance the production potential and genetic gain in Ethiopian cattle populations.

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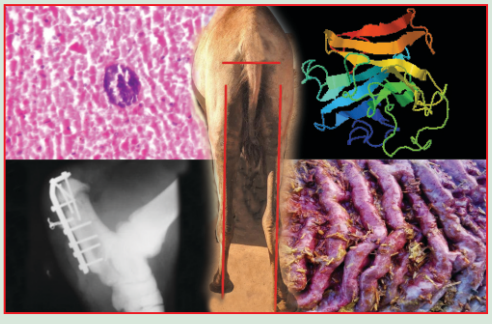

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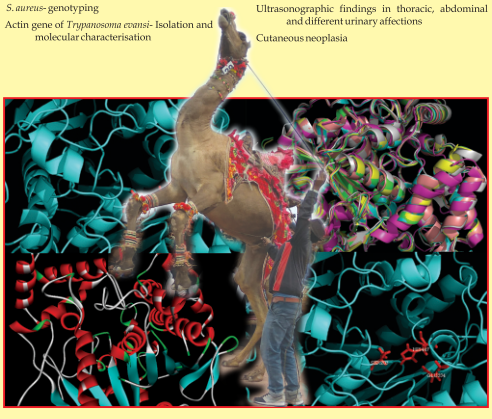

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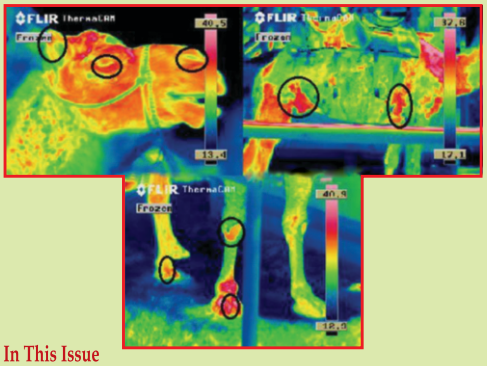

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SEROLOGICAL DIAGNOSIS OF *Parabronema skrjabini* INFECTION USING A RECOMBINANT ANTIGEN IN BACTRIAN CAMELS

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ABSTRACT

In order to find out an effective method for the *in vivo* diagnosis of parabronemosis and on the basis of results of histological analysis obtained in the laboratory, the serine threonine protein kinase (STPK) gene, an immune-related secretory gene of *P. skrjabini*, was screened. A total of 140 Bactrian camel sera samples from different regions of Inner Mongolia were screened. The results of bioinformatics analysis showed that the nucleic acid sequence of STPK gene was 579 bp, contained a complete open reading frame encoding 199 amino acids, had no signal peptide, was atypical secretory protein and had 8 antigenic epitopes. The total RNA of *P. skrjabini* was extracted and the STPK gene was obtained by RT-PCR amplification. The recombinant expression plasmid was constructed and expressed in *Escherichia coli* BL21 (DE3). A recombinant protein, rSTPK, 28 kDa in size, that was primarily expressed in inclusion bodies, was obtained. An indirect enzyme-linked immunosorbent assay (iELISA) using rSTPK as an antigen was established using recombinant protein. We observed a positive detection rate of 85.7% (120/140), indicating that rSTPK-iELISA can be used as a serological method to diagnose parabronemiasis in camels.

Key words: Camel, enzyme-linked immunosorbent assay, *Parabronema skrjabini*, recombinant antigen, serine threonine protein kinase

Parabronema skrjabini is a blood-feeding nematode that resides in the abomasum of ruminants and compartment 3 of camels. However, camels are considered the primary definitive host (Hasheminasab *et al*, 2016). *P. skrjabini* is widely distributed in Africa and Asia and it is especially common in Mongolia (Sharkhuu, 2001), Kazakhstan (Morgan *et al*, 2006), Saudi Arabia (El-Azazy, 1990), Namibia (Krecek *et al*, 1990), Turkey (Umur and Yukari, 2005) and Iran (Eslami and Nabavi, 1976). Camels infected with *P. skrjabini* experience inflammation, ulcers and bleeding of the diarrhoea, anaemia and even death. In the main camel breeding grounds of Inner Mongolia, *P. skrjabini* infections are common hence the research has primarily focused on its morphological identification, classification (Habronematidae; Hasheminasab, 2015) and transmission (Chen *et al*, 2016; Deng *et al*, 2017). Traditional nematode detection methods, such as saturated sodium chloride solution floating can be used for the diagnosis of the disease, but the detection rate is low. Polymerase chain reaction (PCR) can be used to detect a small number of parasites in the skin tissue, but this method is not suitable for the detection of *P. skrjabini* (Fischer *et al*, 1998; Morales-Hojas *et al*, 2001). In order to find out an effective diagnostic method for *P. skrjabini* the indirect ELISA detection method was established (Wang *et al*, 2022) using recombinant antigen rCPI which had good sensitivity and specificity and proved that the detection method based on recombinant antigen is feasible. The use of recombinant protein in iELISA was an effective tool for the serological diagnosis of parasitic diseases (Santos *et al*, 2019).

In this study, we developed a diagnostic method for identifying *P. skrjabini* based on the detection of serine threonine protein kinase (STPK) screened by sequencing the transcriptome of *P. skrjabini* and comparing it to common ruminant parasite genes from GenBank. The gene was cloned, expressed using a prokaryotic vector, verified antigenically and the recombinant protein was used as a diagnostic antigen to establish a serological diagnosis method.

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Materials and Methods

Parasites

Female and male *P. skrjabini* individuals were collected from the compartment 3 of Bactrian camels (*Camelus bactrianus*) in Bayannaoer, Inner Mongolia, China. The collected portions were stored in liquid nitrogen after morphological identification using microscopy, packing and marking. In total, 81 positive sera samples infected with *P. skrjabini* were collected from camels in Inner Mongolia during postmortem and nine negative serum samples not infected with *P. skrjabini* were collected from camels. Twenty four sera samples from sheep infected with *Moniezia* spp., *Haemonchus contortus, Dictyocaulus filaria, Trichuris ovis, Chabertia ovina, Nematodirus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp. and *Oestrus ovis* were collected from the sheep farm in Bayannaoer City, Inner Mongolia and preserved in the laboratory.

Bioinformatics analysis of STPK gene

Bioinformatics analysis of STPK gene of *P. skrjabini* was carried out by bioinformatics software. The open reading frame was predicted by ExPASy (http://web.expasy.org/translate/) and ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/) software and the signal peptide sequence was predicted by SignalIP4.1 server (https://www.ncbi.nlm.nih.gov/orffinder/) online software. SecretomeP.2.0 server (http://www.cbs.dtu.dk/services/SecretomeP/) software for prediction of atypical protein secretions, TMHMM server.v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/) software for transmembrane protein domain analysis, Target P 1.1 server (http://www.cbs.dtu.dk/services/TargetP/) software for prediction of subcellular localisation of eukaryotic proteins. BepiPred 1.0 Server (http://www.cbs.dtu.dk/services/BepiPred/) software was used to predict the antigenic epitopes.

Cloning and expression of STPK gene in Escherichia coli

P. skrjabini specimens were placed in a mortar filled with liquid nitrogen and ground thoroughly. Total RNA was extracted using the TRIzol method, followed by ethanol precipitation. The precipitate obtained was dissolved in RNase-free water and stored at −80°C.

The PCR primers were designed according to the nucleotide sequences of the STPK gene (Genbank with the following accession numbers: 2488713), which were 5′-GCC GAA TTC ATG GTT ATG AGG AAC GG-3′ and 5′-TTG CTC GAG CAC GCC TAT GCC CTG AG-3′ (italics in the primer sequence indicate the *EcoRI* and *XhoI* sites in the expression vector pET30a). The extracted total RNA was reverse-transcribed into cDNA. The cDNA was used as a template and mixed with PCR buffer, dNTP mixture and DNA polymerase for PCR amplification. PCR amplification conditions: 94°C pre-denaturation for 5min; 94°C denaturation for 30s; 57°C annealing for 30s; 72°C extension for 1.5min; 35 cycles; 72°C extension for 10min. The recombinant plasmid pET-STPK was confirmed by restriction digestion and sequencing and subsequently constructed and expressed in *E. coli* BL21 (DE3). Finally, the recombinant bacteria were completely lysed using ultrasonic cell fragmentation, the supernatant and precipitate were collected and the expression of the recombinant protein was detected by SDS-PAGE electrophoresis. The recombinant protein was purified using the Ni-NTA Sefinose™ Resin Kit and used as an antigen for enzyme-linked immunosorbent assay (ELISA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis of rSTPK

A recombinant protein rSTPK was separated *via* SDS-PAGE and transferred to polyvinylidene difluoride membranes (Jeong *et al*, 2018). The membranes were blocked using 5% skim milk. Positive serum of camel parabronemiasis (1:1000) was used as the primary antibody and rabbit anti-camel IgG labeled with horseradish peroxidase (IgG-HRP) was used as the secondary antibody (1: 5000). A negative serum control was established.

rSTPK indirect ELISA (iELISA)

According to the chessboard test, purified rSTPK was diluted with 50 mmol/L carbonate buffer and 1, 2, 4, 8 and 16 µg was added per well. The negative and positive sera were diluted (1:25, 1:50, 1:100, 1:200 and 1:400) and the rabbit anti-camel IgG-HRP was diluted 5000 times. The reaction was carried out by adding a substrate solution containing tetramethylbenzidine chromogenic substrate and terminating the reaction with H_2SO_4. Finally, the optical density (OD) at 450 nm was measured using a mini tablet reader (Bio-Tek, USA). The optimal reaction conditions was determined according to the calculated P/N value. Based on this condition, nine camel negative sera were detected and the average value and standard deviation were calculated (cut-off value=negative serum average + 3 standard deviation). The recombinant protein rSTPK antigen

was used to detect the specificity of 24 positive sera infected with *Moniezia* spp., *Haemonchus contortus, Dictyocaulus filaria, Trichuris ovis, Chabertia ovina, Nematodirus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp. and *Oestrus ovis*.

Field sera testing

The established iELISA was used to screen 140 camel sera collected from Inner Mongolia. According to the principle of statistics, when the OD450nm value of the sample to be tested is greater than the cut-off value, it is judged to be positive and if it is less than the cut-off value, it is negative. When the OD450nm of the sample to be detected was close to the critical value (the cut-off value ± 0.005) and the result of the second test was the same as that of the first test, it was determined as suspected infection of *P. skrjabini*.

Results

Bioinformatics analysis of STPK gene

The characteristics of STPK gene were analysed by ExPASy and ORFfinder online software. the results showed that the nucleic acid sequence of STPK gene was 579 bp and its molecular weight was 22.79 kDa. It contained a complete open reading frame encoding 199 amino acids, including 27 strong acid amino acids, 24 strong base amino acids, 66 hydrophobic amino acids and 43 polar amino acids and the isoelectric point was 6.208.

Using SignalP and SecretomeP software to predict the signal peptide sequence of gene-encoded protein and to analyse the atypical secretory protein, it was found that there was no signal peptide in STPK, but it was considered to be atypical secretory protein NN-Score=0.842 in the prediction of SecretomeP software (if NN-Score > 0.5, it was determined to be secretory protein) (Fig 1).

The antigenic epitopes of the gene-encoded protein were predicted by BepiPred software. The results showed that there were 8 antigenic epitopes of STPK protein: DS (aa 45~46), LLPGKDNYDQ (aa 66~75), S (aa 88), P (aa 98), LFT (100~102), SEKTED (aa 122~127), VERPKPTE (aa 144~151), DVHQAPPQGIGV (aa 182~193). It indicated that the secretory protein encoded by STPK had better antigenicity. The protein transmembrane region and protein subcellular localisation of the gene were predicted by TMHMM and TargetP software and the number of amino acids in extracellular region, transmembrane region and intracellular region was analysed. The results showed that STPK was not a transmembrane protein and the whole protein was extracellular (Fig 2).

Expression of STPK gene in E. coli

STPK was amplified with specific primers using the reverse transcription product of RNA from *P. skrjabini* as a template. The results of the agarose gel electrophoresis revealed a specific band of approximately 579 bp (Fig 3).

The recombinant plasmid pET-STPK was transformed into *E. coli* BL21 (DE3) cells. After induction, the molecular weight of rSTPK protein was 28 kDa (Fig 4).

Western blot

rSTPK was probed using camel serum infected with *P. skrjabini* (Fig 5). A specific band was observed at 28 kDa, but no band was observed upon probing with negative sera samples. This showed that rSTPK can specifically bind to IgG in the sera of camels infected with *P. skrjabini*.

Evaluation of P. skrjabini infection in camels using rSTPK ELISA

An iELISA was established for the serological diagnosis of *P. skrjabini* using rSTPK as a coating antigen. The chessboard experiment determined that the best coating concentration of rSTPK was 2 μg/well (Table 1).

The recombinant antigen rSTPK was used to detect 9 negative sera samples from camels uninfected with *P. skrjabini*. The average OD450nm value of negative serum screened by rSTPK was 0.229 and the standard deviation was 0.025. Therefore, the cut-off value was determined to be 0.304. The 24 sera samples used for the specificity test did not exhibit any cross-reactivity with other parasitic-infected sera samples.

Table 1. Chessboard method to determine the best dilution conditions for ELISA, based on the OD value of the positive and negative serum.

Conjugate dilution	Antigen (μg/mL)				
	16	8	4	2	1
1:25	4.38	4.45	4.79	5.11	5.28
1:50	4.74	4.91	5.43	7.23	6.60
1:100	5.24	5.85	5.72	6.41	5.91
1:200	4.92	5.45	5.70	5.97	5.87
1:400	5.03	5.81	6.10	6.03	4.88

Field serum test results

We used iELISA to test 140 camel serum samples, of which 120 were positive (85.7%). Twenty

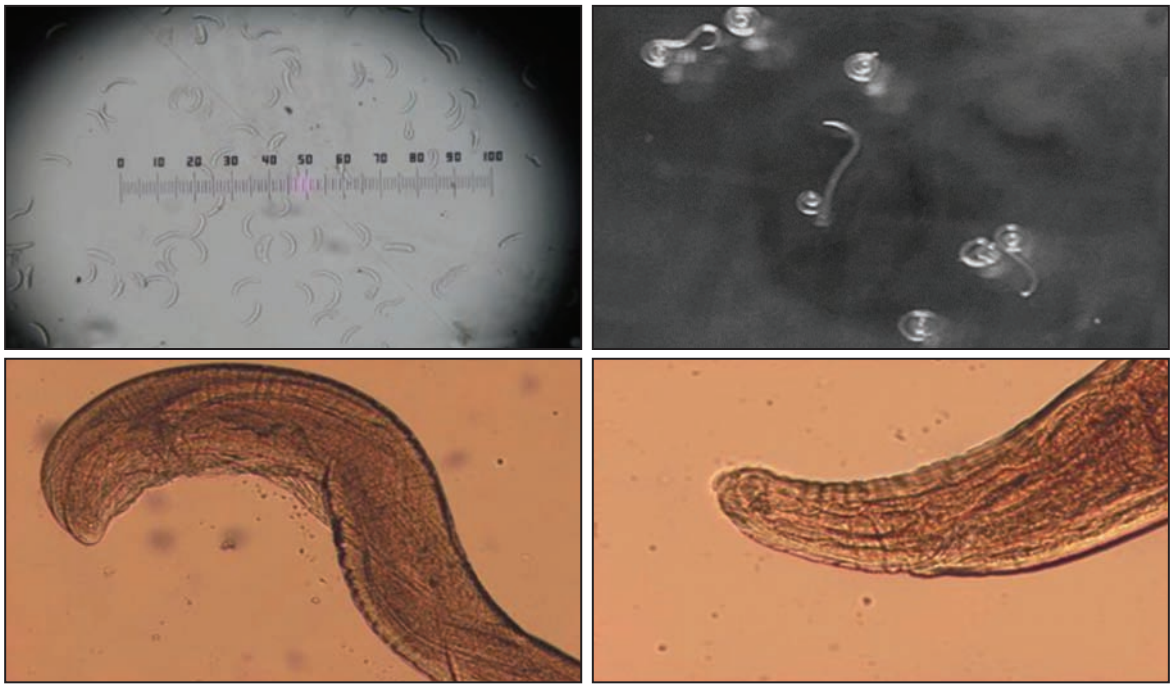
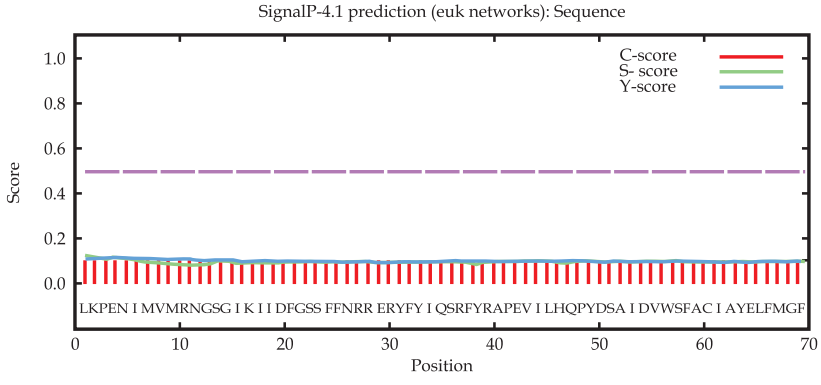


Fig 1. Morphology of *P. skrjabini*: egg, larva, adult (Left, female; Right, male).



#	Measure		Position	Value	Cutoff	signal peptide?
	max.	C	51	0.108		
	max.	Y	11	0.108		
	max.	S	1	0.125		
	mean	S	1–10	0.106		
		D	1–10	0.107	0.450	NO

Name=Sequence SP= 'NO' D=0.107 D–cutoff=0.450 Networks=SignalP–noTM

Fig 1. The analysis of signal peptide sequence of STPK gene.

Pj_STPK Length: 193
Pj_STPK Number of predicted TMHs: 0
Pj_STPK Exp number of AAs in TMHS 0.04552
Pj_STPK Exp number, first 60 AAs: 0.0339
Pj_STPK Total prob of N-in: 0.13969
Pj_STPK TMHMM2.0 outside 1 193

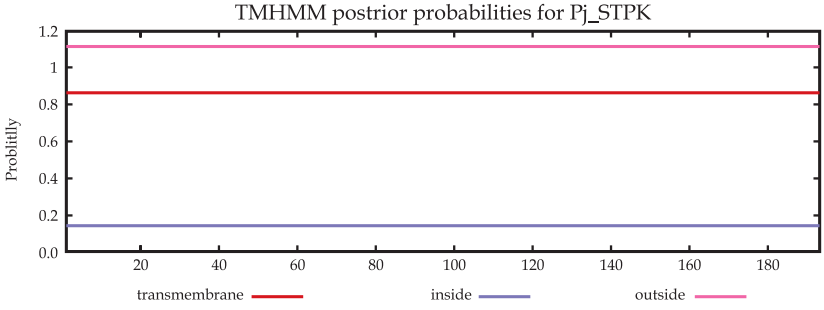


Fig 2. The analysis of STPK transmembrane domain.

samples tested negative, of which 4 were suspected of being infected with *P. skrjabini*. The results showed that the camels in Inner Mongolia were seriously infected with *P. skrjabini*.

Discussion

The diagnosis of this nematode disease primarily depends on the postmortem of diseased animals, but this method cannot provide a reference for the *in vivo* detection of the disease. The improved CTAB+SiO_2 adsorption method was used to extract DNA from artificially simulated positive faeces and established a method for *in vivo* diagnosis of the disease *via* PCR (Zheng, 2015). However, the detection rate of *P. skrjabini* eggs in infected animal faeces was low, making it difficult for practical applications. The serological method established with recombinant antigen by our research team in the early stage was highly feasible.

With the continuous application of high-throughput sequencing technology in parasite research,

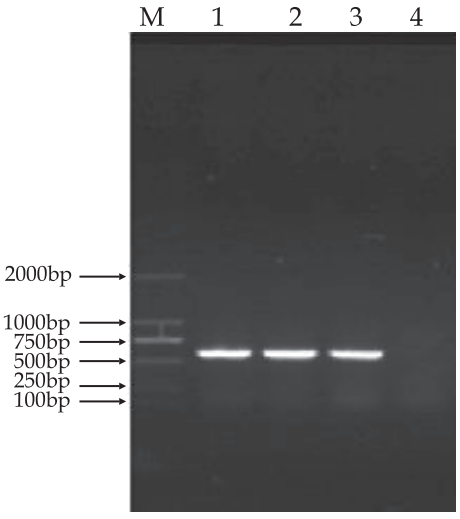


Fig 3. Electrophoresis following the reverse transcription polymerase chain reaction for STPK. M: DNA standard DL2000; line 1-3: STPK; line 4: negative control.

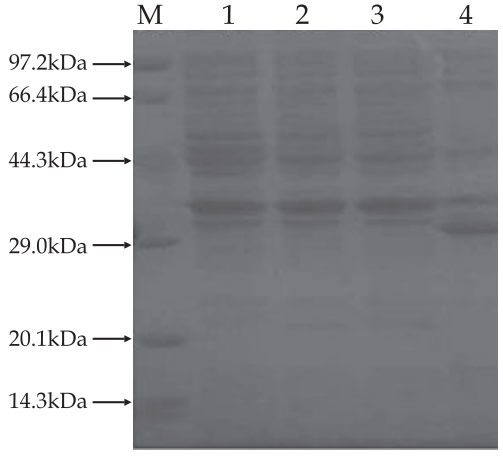


Fig 4. Electrophoresis following the reverse transcription polymerase chain reaction for STPK. M: molecular protein marker; Line 1: BL21 (DE3) empty bacteria; Line 2: Before induction of BL21 (pET30a); 3: After induction of BL21 (pET30a); 4: After induction of BL21 (pET-STPK).

many scholars began to use multi-omics sequencing analysis technology to carry out more in-depth basic research in immune regulation and immune protection of parasites and identified many specific diagnostic antigens and vaccine candidate genes (Blazie *et al*, 2015; Hewitson *et al*, 2011; Laing *et al*, 2013; Zheng *et al*, 2011). A comparative analysis of the secretory gene and protein data predicted in the transcriptome and proteome studies revealed that 76.6% of the secretory proteins were consistent with the transcriptome secretory gene data (Feng *et al*, 2017), indicating that these genes are secreted at the mRNA and protein level and can be used as candidate genes for immune-related gene screening of *P. skrjabini*. The secretory antigen expressed on the body

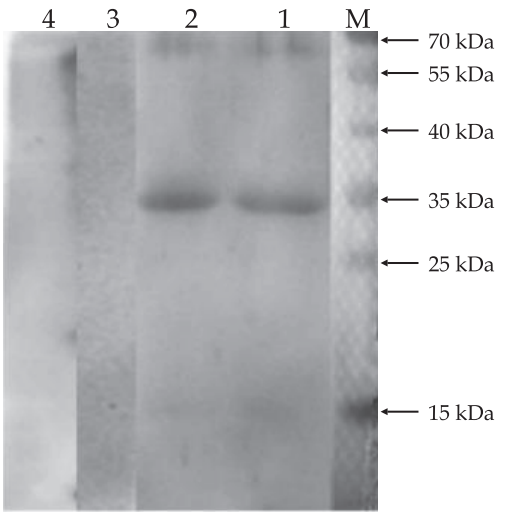


Fig 5. Western blotting analysis of recombinant rSTPK. M: Molecular protein marker; Line 1-2: Positive serum test; Line 3-4: Negative serum test.

surface of *Schistosoma mansoni* can directly interact with the host immune system and thus influence the host immune response. It was reported that serine / threonine protein kinase can be expressed in the body surface of *Schistosoma mansoni* as a TGF- β receptor (Forrester *et al*, 2004), but its biological characteristics and functions were unknown, therefore, STPK was selected as a diagnostic candidate gene to provide a reference for the diagnosis and control of *P. skrjabini*. Bioinformatic analysis showed that STPK gene was a specific gene of *P. skrjabini* and was an atypical secretory protein without a signal peptide sequence. The identification of the transmembrane region and subcellular localisation analysis showed that STPK was not a transmembrane protein; instead, the whole protein was located in the extracellular region. The protein was predicted to have eight antigenic epitopes, which indicates sound antigenicity. The selected STPK gene conforms to the characteristics of candidate genes for immunological diagnosis and immune prevention.

Toxoplasma gondii secretory protein particle antigens (GRA1 and GRA7) was used for recombinant expression and their diagnostic value by iELISA assay was assessed with a great significance (Wang *et al*, 2014). Teimoori *et al* (2015) used a recombinant antigen of the cysteine protease of *Opisthorchis viverrini* from Thailand for the serological diagnosis of *Fascioliasis hepatica*. The iELISA results showed that the sensitivity and specificity of this diagnostic antigen were 62.1% and 84.05%, respectively. In this study, STPK was cloned into the plasmid pET30a and expressed in *E. coli*. Western blotting showed

that the rSTPK protein could stimulate host-specific antibodies. Using recombinant protein rSTPK as an antigen, an iELISA detection method for the diagnosis of *P. skrjabini* infection was established. This method exhibits good specificity. A total of 140 clinical sera samples were screened and the positivity rate was 85.7%. Therefore, the detection method established in this study can provide a reference for the prevention and treatment of camel parabronemiosis and can be used for early diagnosis.

In this study, STPK was identified as a candidate antigen gene which was cloned into pET30a and recombinant rSTPK was used as an antigen for the serological diagnosis of camels infected with *P. skrjabini*. rSTPK was also used as the antigen to establish an iELISA method for diagnosing parabronemiosis. The results showed that the method had high specificity, suggesting that rSTPK is a potential serological marker for detecting *P. skrjabini* in camels.

Acknowledgements

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Ethics statement

Our research was conducted strictly in accordance with the Guide for the Care and Use of Laboratory Animals of the Ministry of Health, China and the protocol was reviewed and approved by the Research Ethics Committee of Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China. Permission was obtained from a local official slaughterhouse before specimen collection.

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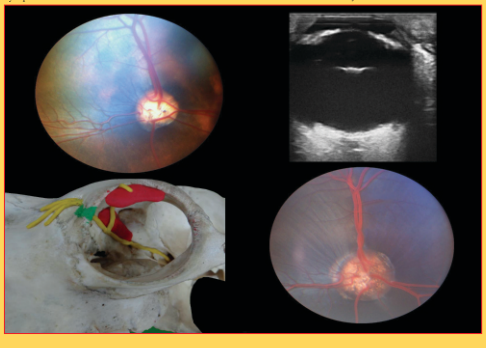

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STUDIES ON SOME BIOCHEMICAL CONSTITUENTS OF FOLLICULAR AND OVIDUCTAL FLUID IN DROMEDARY CAMEL (*Camelus dromedarius*)

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ABSTRACT

The culture media used for *in vitro* embryo production (IVEP) technology in camels have been adopted from ones used in other domestic animal species. In order to improve maturation, fertilisation, and embryo culture media and thereby IVEP, a need exists to determine the ionic composition of follicular and oviduct fluids in this species. Reproductive tracts were collected from a local abattoir immediately after slaughter and transported to the laboratory within 2 h in separate ice-chilled sterile plastic bags. Oviductal fluid was aspirated from individual oviducts using sterilised Drummond pipettes. The follicular fluid was aspirated in sterile syringes attached to 22 gauge hypodermic needles. The biochemical constituents of follicular and oviductal fluid were measured by an automatic chemistry analyser (Roche Hitachi 912) using kits, reagents and instructions provided by the manufacturer. The concentration of sodium in developing and mature follicles was lower than that of the serum and cystic follicles, which tended to be similar. The concentration of potassium, phosphate and lactate significantly ($P < 0.05$) decreased as the follicle size increased with the highest concentration in 5-10 mm follicles and lowest in >20 mm cystic follicles. No difference was observed in the concentration of calcium, magnesium and chloride between serum and follicular fluid from different size follicles. No difference was observed in the concentration of glucose in the follicular fluid from different size follicles, however, it tended to be higher when compared with serum.

The concentration of sodium was lower in the oviductal fluid when compared with serum during all the stages of follicular development. During the oestrous/receptive phase of the cycle when ovaries were with mature follicles, sodium concentration was higher ($P< 0.05$) when compared with the other stages of follicular development. Potassium and phosphate concentrations were very high in the oviductal fluid when compared with serum through all the stages, however, it was lower during the peri-ovulatory period when compared with other stages of the reproductive cycle. No difference was observed in the calcium and chloride concentrations during the different phases of oestrous/follicular cycle, however, both of them were lower than that present in the serum. Magnesium levels were higher in the oviductal fluid compared to serum but did not differ during different follicular developmental stages. High concentrations of glucose and lactate were observed during all the stages of reproductive cycle in the oviductal fluid when compared with the serum.

Key words: Camel, follicular fluid, oviductal fluid

Development of *in vitro* embryo production technology (IVP) has been slow in camelids when compared with other domestic animal species. The culture media used for this species have been adopted from ones used in other domestic animal species. Therefore, a need exists to determine the ionic composition of follicular and oviduct fluids, which may lead to improved maturation, fertilisation and embryo culture media and thereby improve *in vitro* embryo production in this species. The most important factors regulating the production of embryos *in vitro* are the culture systems used for *in vitro* oocyte maturation and culture of embryos. The components of the culture media and culture conditions can affect and even modulate the meiotic regulation of mammalian oocytes (Downs and Mastropolo, 1997; Kito and Bavister, 1997). Culture conditions for *in vitro* maturation in other domestic species have been improved such that nowadays, a large percentage of oocytes successfully complete nuclear maturation (Krisher and Bavister, 1998; Eppig, 1991). Although, there are many studies on *in vitro* oocyte maturation and *in vitro* embryo production in dromedary camels from oocytes collected from slaughterhouse ovaries or by OPU from live donors, but only a limited number of studies have reported pregnancies and offspring produced from such embryos (Wani, 2021). The embryos produced

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through IVEP techniques have been reported to have a low cell numbers and lower pregnancy rates when compared to their *in vivo* produced counterparts (Vettical *et al,* 2019), which could mean improper culture media and conditions.

During *in vivo* oocyte maturation, besides meeting nutritional requirements of the developing oocyte, follicular fluid also maintains a proper environment for the maturation of the oocyte. The follicle, which is an avascular compartment within the mammalian ovary, is separated from the peri-follicular stroma by the follicular wall that constitutes a blood–follicle barrier (Bagavandoss *et al,* 1983). Within the ovarian follicle, the developing oocyte is surrounded by the follicular fluid, which is a serum transudate modified by follicular metabolic activities. Besides a serum transudate, follicular fluid also contains locally produced substances that share the metabolic activity of follicular cells (Gerard *et al,* 2002). Similarly, after fertilisation, the zygote/embryo is dependent on the nutrients provided by the oviduct and uterine fluids for its growth and survival (Ellington, 1991; Leese, 1995; Leese *et al,* 1985; Bavister, 2000). In particular, the ionic composition of these fluids has been shown to be important for early embryo development (Bavister, 2000; Grippo *et al,* 1992; Mathews *et al,* 1998; Leese, 1988). Oviductal fluid is produced by selective transudation of blood constituents and by secretion of specific constituents. Ions, which play an essential role in the formation of oviduct fluid, move through the epithelial cells of the oviduct and uterus into the lumen of the reproductive tract causing a concentration gradient which in turn causes an osmotic gradient providing the driving force to transport water by osmosis out of the epithelial cells into the oviduct or uterine lumen (Leese and Gray, 1985). Despite the importance of ions in oviduct, zygote and early embryo development there is little published information on the ion concentrations of oviduct in camelids.

This study was, therefore, carried out to investigate the biochemical composition of follicular and oviductal fluid in dromedary camel.

Materials and Methods

Collection of Oviductal fluid

Reproductive tracts were collected from she dromedary camels of unknown reproductive history from a local abattoir immediately after slaughter and transported to the laboratory within 2 h in separate ice-chilled sterile plastic bags in a thermos flask on ice (0.0–2.0°C). Reproductive tracts were grouped as per the status of follicular development on ovaries as under:

1. Developing follicles (5 to 10 mm),
2. Mature follicles (>10 to < 20 mm),
3. Cystic follicles (≥20 mm),
4. Corpus haemorrhagicum (Fresh corpora lutea with indications of fresh ovulation).

Ovaries were trimmed off the tracts and stored in thermos flask on ice for experiment 2. Oviductal fluid was then carefully aspirated from individual oviducts within a short period of time (5 min/sample) under aseptic conditions using sterilised Drummond pipettes (Drummond Scientific, Broomall, PA). The pipette tip was inserted gently in the lumen of the oviduct at the side of the fimbriae and the fluid was aspirated along the lumen toward the uterine end as a result of the negative pressure created by the release of the Drummond plunger. The fluid recovered was pooled separately for each group, centrifuged at 2500 g for 5 min to remove cellular debris and stored at -20°C for later analysis.

Collection of follicular fluid

The ovaries from experiment 1 were trimmed off the surrounding tissues and based on their diameters, follicles were categorised as:

Developing follicles (5 to 10mm),

Mature follicles (>10 to <20 mm) and

Cystic follicles (≥20 mm)

The follicular fluid was aspirated in sterile syringes attached to 22 gauge hypodermic needles in three different tubes depending on the size of follicle. The follicular fluid from the same group collected on a day was pooled, centrifuged, aliquoted and stored at –20°C until further analysis.

Collection of Serum

Before slaughter, jugular blood was collected randomly from 5 to 6 animals each time (N = 35) by sterilised 18-gauge hypodermic needles and syringes. The serum was harvested and stored in aliquots at –20°C for further analysis.

Estimation of biochemical constituents

The biochemical constituents of follicular and oviductal fluid were measured by an automatic chemistry analyser (Roche, Hitachi 912) using kits, reagents and instructions provided by the manufacturer. The analyser uses several operational systems to perform the required function. It includes control system, sampling system, reagent system,

photometric measuring system, cell rinse system and ISE system. The test principle is a colourimetric assay. The photometric measuring system detects colour or turbidity produced by the chemical reactions between reagents and analyte of interest in the sample, while in the reaction cells. This is capable of monochromatic and bi-chromatic photometry of end point; kinetic, ultraviolet and visible light chemistry determinations.

Statistical analysis

The data is presented as mean ± SEM. The concentrations of various biochemical constituents in the follicular fluid from different size follicles and serum were analysed by ANOVA with Fisher protected least significant difference test (MINITAB statistical software, Minitab ltd, CV3 2TE, UK). The concentrations of various biochemical constituents in the oviductal fluid collected from the reproductive tracts of different ovarian status were also analysed by ANOVA with Fisher protected least significant difference test.

Results and Discussion

The concentration of various biochemical constituents in the follicular fluid and the serum are summarised in table 1. The concentration of sodium in developing and mature follicles was lower than that of the serum and cystic follicles, which tended to be similar. The concentration of potassium, phosphate and lactate significantly ($P < 0.05$) decreased as the follicle size increased with the highest concentration in 5-10 mm follicles and lowest in >20 mm cystic follicles. No difference was observed in the concentration of calcium, magnesium and chloride between serum and follicular fluid from different size follicles. No difference was observed in the concentration of glucose in the follicular fluid from different size follicles, however, it tended to be higher when compared with serum.

The concentration of various biochemical constituents in the oviductal fluid are summarised in table 2. The concentration of sodium was lower in the oviductal fluid when compared with serum during all the stages of follicular development. During the oestrous/receptive phase of the cycle when ovaries were with mature follicles, sodium concentration was higher ($P< 0.05$) when compared with the other stages follicular development. Potassium and phosphate concentration was very high in the oviductal fluid when compared with serum through all the stages, however, it was lower during the peri-ovulatory period when compared with other stages of the reproductive cycle. No difference was observed in the calcium and chloride concentrations during the different phases of oestrous/follicular cycle, however, both of them were lower than that present in the serum. Magnesium levels were higher in the oviductal fluid compared to serum but did not differ during different follicular developmental stages. High concentrations of glucose and lactate were observed during all the stages of reproductive cycle in the oviductal fluid when compared with the serum.

Despite low fertility and high embryonic losses in camels, there is very little published information on the ion concentrations of follicular and oviductal fluid in this species. To the best of our knowledge this is the first study in which the ionic and biochemical

Table 1. Concentration of different biochemical constituents in the follicular fluid and serum of camel (*Camelus dromedarius*)

Follicular size	Sodium	Potassium	Calcium	Magnesium	Chloride	Phosphate	Glucose	Lactate
5.0 to 10 mm	137.7 ± 9.41^{b}	13.2 ± 0.71^{a}	2.4 ± 0.15^{a}	1.06 ± 0.07^{a}	108.2 ± 10.11	2.5 ± 0.15^{a}	4.9 ± 0.36^{a}	11.5 ± 0.77^{a}
>10 to < 20 mm	133.8 ± 5.87^{b}	8.30 ± 0.27^{b}	2.3 ± 0.12^{a}	0.9 ± 0.05^{a}	111.8 ± 8.39	2.0 ± 0.09^{b}	5.5 ± 0.28^{a}	6.7 ± 0.30^{b}
≥20 mm	149.3 ± 2.18^{a}	6.5 ± 0.12^{c}	2.3 ± 0.05^{a}	0.9 ± 0.06^{a}	119.5 ± 3.66	1.5 ± 0.08^{c}	4.6 ± 0.39^{ab}	5.2 ± 0.34^{c}
Serum	149.7 ± 1.4^{a}	4.3 ± 0.0^{d}	2.4 ± 0.05^{a}	1.3 ± 0.03^{a}	114.0 ± 0.0	1.40 ± 0.0^{c}	4.03 ± 0.07^{b}	2.9 ± 0.03^{d}

Values in same column with different superscripts differ significantly at $P < 0.05$.

Table 2. Concentration of different biochemical constituents in the oviductal fluid and serum of camel (*Camelus dromedarius*)

Ovarian status	Sodium	Potassium	Calcium	Magnesium	Chloride	Phosphate	Glucose	Lactate
Developing follicles	81.3 ± 4.9ac	70.8 ± 2.3^{a}	1.1 ± 0.07^{a}	3.5 ± 0.13^{a}	61.0 ± 4.5^{a}	15.9 ± 0.61^{a}	20.9 ± 1.5^{a}	30.4 ± 0.75^{a}
Mature follicles	108.4 ± 5.48^{b}	60.8 ± 1.65^{b}	1.3 ± 0.05^{a}	3.6 ± 0.18^{a}	62.0 ± 4.46^{a}	13.6 ± 0.59^{b}	14.3 ± 0.83^{b}	32.2 ± 0.73^{a}
Corpus haemorrhagicum	91.0 ± 9.26^{c}	61.6 ± 1.51^{b}	1.06 ± 0.23^{a}	3.02 ± 0.24^{a}	73.4 ± 7.07^{a}	13.02 ± 0.54^{b}	13.7 ± 0.67^{b}	26.6 ± 0.97^{b}
Cystic follicles	70.9 ± 3.65^{a}	71.8 ± 4.27^{a}	1.08 ± 0.10^{a}	3.3 ± 0.17^{a}	66.3 ± 5.58^{a}	16.3 ± 1.34^{a}	16.8 ± 1.30^{b}	30.2 ± 1.89^{a}
Serum	149.7 ± 1.4^{d}	4.3 ± 0.0^{c}	2.4 ± 0.05^{b}	1.3 ± 0.03^{b}	114.0 ± 0.0^{b}	1.4 ± 0.0^{c}	4.03 ± 0.07^{c}	2.9 ± 0.03^{c}

Values in same column with different superscripts differ significantly at $P < 0.05$.

composition of oviductal fluid in camels determined. We observed a lower concentration of sodium in developing and mature follicles when compared with the cystic follicles and serum, which is similar to earlier studies (Zia-ur Rahman *et al,* 2008) in the same species. The concentrations of sodium in developing follicles in our study was similar to 137.05 ± 3.6 mEq/L observed in small follicles by these authors. Iwata *et al* (2004) also reported difference in sodium concentrations between fluids from small and large follicles in bovine. Serum sodium concentration was also higher than small follicular fluid in pigs (Chang *et al,* 1976) similar to our findings in camel.

A decrease in potassium concentration with the increase in follicle size was observed, similar to that in bovine (Iwata *et al,* 2004: Leroy *et al,* 2004) and swine (Chang *et al,* 1976). Higher potassium in small follicles compared with large follicles has been attributed to glucose utilisation, a process that leads to the transfer of potassium from extracellular fluid to intracellular sites (Chang *et al,* 1976). Perhaps the same is true for the camel as higher glucose concentration was observed in developing and mature follicles in our study.

No significant difference in the chloride concentration between different follicle groups and serum was observed in present study. These findings are in contrast to the observations of Leroy *et al* (2004) who reported a decrease in chloride concentrations with the advancement in follicular growth in dairy cattle and Zia-ur Rahman *et al* (2008) who also reported a lower chloride concentration in follicular fluid when compared with serum in camels. The chloride is known to initiate the LH-stimulated steroidogenesis in the chicken granulosa cells (Morley *et al,* 1991), human chorionic gonadotropin-stimulated steroidogenesis in oocytes of amphibians (Skoblina and Huhtaniemi, 1997) and steroidogenesis in adrenal glands by influencing the cAMP production (Cooke *et al,* 1999). Chloride ions are also responsible for enhancing the activity of angiotensin-converting enzyme, a metalloenzyme, which has been discovered in the follicular fluid of the porcine ovaries (Matsui and Takahashi, 2002).

Calcium concentration in developing and mature follicles was similar to that of the serum in our present study, which does not agree to the earlier findings (Zia-ur Rahman *et al*, 2008) in same species, where authors have reported a lower concentration in follicular fluid when compared with serum. Our observations are, however, in agreement with Iwata *et al* (2004) who reported that calcium concentration did not differ between the small and large follicles in bovine. Calcium is required for normal functioning of the granulosa cells (Leung and Steele, 1992) and steroidogenesis in granulosa cells during *in vitro* studies (Eckstein *et al,* 1986).

The concentration of phosphate was higher in the developing and mature follicles when compared with serum and cystic follicles in the present study. These observations were in total contrast to what has been reported in an earlier study in same species (Zia-ur Rahman *et al,* 2008) where authors have reported a lower concentration of phosphorus in follicular fluid when compared with the serum. We have also observed a decrease in the phosphate concentration with the increase in follicular size in contrast to earlier study (Zia-ur Rahman *et al*, 2008) where the authors reported an increase with the follicular growth. Phosphate is known to be a vital part of cAMP, as the second messenger in physiological action of steroid hormones (Hafez, 1993). The concentration of cAMP increases with the maturation of follicles in pigs (Chang *et al,* 1976). Thus, higher phosphorus concentration in developing and mature follicles can be related to a high demand of cAMP in the present study.

Higher concentration of glucose in follicular fluid when compared with serum was observed, which is in agreement with the findings of Zia-ur Rahman *et al* (2008). However, no significant difference in the glucose concentration of fluid from different size follicles was seen in the present study which is in contrast to the findings of Zia-ur Rahman *et al* (2008). Glucose being the major energy source for the ovary, might be the reason for its higher concentration in follicles. It seems that follicles have the ability to filter and reserve the high concentrations of glucose from blood for utilisation in their development. Gerard *et al* (2002) and Iwata *et al* (2004) observed a decrease in the glucose concentration in the fluid of pre-ovulatory follicles developing from a dominant follicle in mares and cows, respectively. However, in dairy cows Leroy *et al* (2004) observed an increase in the glucose concentration of follicular fluid with an increase in the follicle size. Our observations are, however, in contrast to their findings of low glucose concentration in follicular fluid when compared with the serum, which might be due to species differences. A higher concentration of lactate was observed in the fluid from follicles compared to the serum. There was a negative correlation between the lactate in follicular fluid and the follicular size. Lactate was higher

in small follicles because it might be the alternate sources of energy for the cells in follicles (Harlow *et al,* 1987; Leroy *et al,* 2004).

Sodium is the major cation present in oviductal fluid and in blood serum in camels. Sodium concentration was highest in the oviductal fluid of animals with mature follicles, which are receptive to males, but it was still lower than that of serum. The concentration in present study was lower than that reported in different phases of the oestrous cycle in cattle (Kenny *et al,* 2002; Grippo *et al,* 1992) and about 8 times lower than that reported in oviductal fluid of Alpacas (Apichela *et al,* 2015). Sodium is essential for blastocyst expansion because the formation of fluid and the resulting blastocyst expansion is dependent on the pumping of sodium into the blastocoel cavity by the Na+K+-ATPase sodium pump (Hobbs and Kaye, 1986; Brison and Leese, 1993). Oviduct potassium was affected by the ovarian follicular activity. Potassium concentrations in the oviduct in the persent study were higher during the follicular development stage while as it significantly decreases around the receptive phase when the ovaries have mature follicles or with a fresh ovulation. These concentrations were similar to those reported for human oviduct fluid (David *et al,* 1973; Lippes *et al,* 1972). Potassium concentration in the oviduct was 15 times higher than in serum. In another study on llama, oviductal fluid (Apichela *et al,* 2015) the concentration of potassium was half of what we have observed in our study. Earlier studies in cattle (Jordan *et al,* 1983) and human uterine fluid (Casslen and Nilsson, 1984) also reported to have the highest potassium concentrations during late luteal phase of the oestrous cycle. The significantly higher potassium concentrations in oviduct fluid when compared with blood serum recorded in the persent study agrees with the previous reviews (Leese, 1988 and Hunter, 1988) who suggested that the oviduct potassium concentration of most mammalian species is higher than blood values. A higher potassium concentration than in blood was found to be necessary to support the fertilising capacity of spermatozoa and the development of the murine preimplantation embryos (Roblero *et al,* 1990). Furthermore, high potassium concentrations were found to have a beneficial effect in a culture medium, based on human tubal fluid, for the development of human preimplantation embryos (Quinn *et al,* 1985). The higher potassium concentrations in oviduct fluid compared to blood suggests an active transport mechanism and/or an active secretion of potassium ions by the oviductal epithelium thus regulating the composition of the secreted fluids.

The concentration of chloride in the oviductal fluid was not affected by the ovarian activity in the present study. It was, however, lower than that of the serum and its secretion pattern was similar to what was observed for sodium. Our results are in agreement with Schultz *et al* (1971) who reported a similar concentration range in cows. Chloride is the major ion responsible for fluid secretion into the reproductive tract. For example, *in vitro* studies on rabbit oviduct tissue (Schultz *et al,* 1971; Gott *et al,* 1988) and studies on monolayers of bovine oviduct epithelial cells (Reischl *et al,* 2000) have shown that the net movement of chloride ions in the secretory direction causes an electrochemical gradient which is coupled to the transport of water into the lumen of the oviduct. An *in vivo* study on human uterine fluid composition (Casslen and Nilsson, 1984) also reported high chloride concentrations and concluded that this ion was actively involved in fluid secretion across the endometrium through absorptive and secretory activities.

Phosphate concentration in oviduct fluid was lower during the peri-ovulatory period when compared with the follicular development period, however, it was still 9-10 times more than the serum concentrations. Thompson *et al* (2000) reported an attempt to measure phosphate concentrations in bovine uterine fluid on day 7, however, they failed to do so as the concentrations were below the detection limit of their assay. There are no other published data on cattle oviduct or uterine phosphate for comparison.

Magnesium concentrations in oviduct fluid were similar in all the groups but higher than that of serum. Our observations were in contrast to another study on llama oviductal fluid where in authors mentioned a 10 times lower concentration (Apichela *et al,* 2015). The concentrations of oviduct magnesium recorded in the present study, however, reflect the concentration used in *in vitro* capacitation and acrosome reaction studies on bull spermatozoa (Parrish *et al,* 1988). Magnesium plays a major role in embryo and foetal development as foetal malformations had been reported to be linked to severe magnesium deficiencies (Jordan *et al,* 1983).

Calcium concentration in oviductal fluid was numerically lower than the serum concentrations, however, we did not observe any difference in its concentration in the fluid from tracts with developing,

mature, cystic follicles or even with fresh ovulations in contrast to an earlier report in cattle (Grippo *et al,* 1992), where authors reported maximum oviduct calcium concentrations at oestrus and ovulation. Our observed values were 5 times higher than what has been reported in a study on oviductal fluid of llama (Apichela *et al,* 2015). The oviduct calcium is considered to be essential for sperm viability as it enables the binding of oviduct proteins to spermatozoa (Lapointe and Sirard, 1996) and is essential for sperm capacitation, acrosome reaction and fertilisation processes in humans (Stock and Fraser, 1989).

Higher levels of glucose and lactate were observed in the oviductal fluid from all the groups when compared to serum in the present study. Lactate concentration was highest during the follicular development stage; however, it reduced significantly after ovulation. Our results are similar to those reported in women where Lactate (L (+) isomer) increases from 4.9 mM in the follicular phase to 10.5 mM at ovulation and then decreases to 6.2 mM in the luteal phase (Gardner *et al,* 1996). Interestingly, they also used a suction pipette to collect small volumes (0.5 µL) of luminal fluids from naturally cycling patients who were being investigated for infertility. Glucose levels decreased from about 21 mM during the follicular development to around 14 mM peri-ovulatory stage in the present study. Dickens *et al* (1995) also reported a decrease in glucose concentration from follicular phase to midcycle in the human tubal fluid. The trend is similar in our observations as well; however, the values are higher, which might be due to species differences. The decrease in glucose in oviduct fluid peri-ovulation is consistent with it being a major energy source of the oviduct for utilisation in secretory activity, and muscular and ciliary movement (Gardner *et al,* 1996).

In conclusion, the present study has quantified the *in vivo* concentrations of anions and cations in follicular and oviductal fluid of camels and compared them to blood serum concentrations. The physiological importance of the ionic composition of oviduct and follicular fluid is in the context of its influence on spermatozoa motility and function, oocyte quality and embryo viability and development. This information increases our understanding of the *in vivo* environment of the oocyte maturation, early embryo development, and provides valuable information that may lead to improved *in vitro* culture media and thereby improved *in vitro* embryo production in camelids.

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ULTRASONOGRAPHIC ASSESSMENT OF FOLLICULAR SIZE TO REDUCE THE AGE OF FIRST SERVICE IN THE DROMEDARY HEIFERS

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ABSTRACT

In the present study we attempted to breed dromedary camel heifers (n= 68) at a younger age compared to the common practice of breeding camel heifers at 4 years of age. In the first experiment, heifers (n=15) were maintained on different nutritional groups viz. intensive system with fodder (ISF; n=5), intensive system with extra protein (ISP; n=5) and semi-intensive system with fodder (SIF; n=5) starting from 2.5 years of age. In experiment II, heifers (n=18) were maintained under SIF (n=12) and ISF (n=6) and in experiment III, heifers (n=35) were maintained under SIF. The heifers in all experiments were evaluated every 10th day during the breeding season by transrectal ultrasonography and those evidencing a follicle (diameter ≥ 9 mm) were mated with virile stud camels. Ultrasound examination revealed follicular growth diameter ≥ 9 mm in 14/15 (93.3%), 18/18 (100%) and 31/35 (88.5%) of the camel heifers in Experiments I, II, III, respectively, at 3 yr ± 2 months of age and weighed at least 360 kg; these were successfully mated and 28 out of 68 heifers conceived at approximately 3 yr and delivered calf at around 4 yr age. The age of first service was positively correlated with body weight, heart girth and body girth. It was concluded that supplementary feeding of camel heifers had positive effects on the body weight and reduced the age at first service. Camel heifers attained puberty at 3 yr of age and >360 kg body weight and were successfully bred through ultrasonographic monitoring of follicle.

Key words: Age of first service, body measurements, *Camelus dromedarius*, heifers, ultrasound for ovarian status

The reproductive performance of *Camelus dromedarius* is termed poor because of limited breeding season, lengthy gestation period and delayed puberty (Purohit and Pareek, 2000; Vyas *et al,* 2004a; Purohit *et al,* 2020). Improved reproductive performance is required in camel to provide an opportunity for selection, genetic improvement and profitable production. The reported age at puberty in the female camel varies widely; 3-4 yr (Musa *et al,* 1993), 5 yr (Rathore, 1986) of age. It is reported to vary from 4-6 yr for camels maintained at farm (Khanna *et al,* 1990) and at field level from 5-7 yr (Arthur, 1985). The age of first service at an organised farm is reported to vary between 1679.5±49.1 to 1863±44.1 days (NRCC, 2012). In India, dromedaries are not bred until 4 yr of age, resulting in an age at first calving of 5 yr or more (Khanna *et al,* 1990; Agarwal *et al,* 1996). In Saudi Arabia, majority of camel herds breed females at 4 yr (77/115 herds, 67%) and fewer at 5 yr (30/115 herds, 26%) and less than 7% at 3 yr (8/115 herds) (Ali *et al,* 2018). Tura *et al* (2010) observed that the age at first calving was 60 months in camels in Kenya implying that 47-48 months was the age of first service. Reducing the age of first service can improve the reproductive performance and quality of camel herds. The prospect of increasing the reproductive performance of camels is impeded by the persistent use of traditional systems of reproductive management in most breeding herds (Al Eknah, 2000; Almutairi *et al,* 2010; Skidmore *et al,* 2010). The lack of strategy to improve age at first calving affects reproductive efficiency and productivity of camel herds (Gherissi *et al,* 2020).

Detecting oestrus efficiently and accurately is one of the key factors for reproductive success in camels (Manjunath *et al,* 2015). The oestrus behaviour in female camel is not characteristic and is rather vague because it does not correlate with the ovarian status or presence of follicle over ovaries (Vyas *et al,* 2004b). The male can be observed following

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females, sniffing their vulva exhibiting flehmen and these females also exhibit signs like frequent urinating, straddling hind legs and even sitting beside a copulating male, but upon ovarian examination, follicular activity was not observed in many of females exhibiting such behaviour (Skidmore *et al,* 1996; Vyas *et al,* 2008). At many times, male suddenly select a female which is not receptive, running away from male and chase her and force her to sit and engage in copulation. These females, on examination, were reported to possess mature follicle. The information about ovarian status, bodyweight and body size in relation to age at puberty is lacking in camel (Mahla *et al,* 2015). Therefore, in the present study trans-rectal ultrasonography was used to detect follicular growth with the following objectives—

(i) To know the effect of intensive and semi-intensive feeding systems on body measurements and follicular growth at 3 yr of age in dromedary camel heifers.

(ii) To ascertain the presence of follicle at 3 yr of age and its relation with body weight and other body measurements.

Materials and Methods

Experimental animals

A total of 68 dromedary camel heifers belonging to ICAR- National Research Centre of Camel, Bikaner situated at 28° 01′ N and 73° 22′ E and 228 m above sea level were utilised for the experiments.

Experiment 1 (first year)

Fifteen camel heifers (2.5 yr) were divided into three groups. Group I (ISF, intensive stall-fed fodder, n=5) camel heifers were stall fed 6 kg *Cymopsis tetragonaloba* crop residue (Guar phalgati*; CP- 8%; TDN- 48%) and not allowed to graze outside the stall.

Group II (ISP, intensive stall-fed pellets, n=5) camel heifers were fed 6 kg diet containing 20% protein and energy guar phalgati 50%, moong fali chara** (*Arachis hypogea*)- 20%, khejri leaves*** (*Prosopis cineraria*)- 5%, moong churi- 2%, crushed maize- 17%, mineral mixture- 2%, common salt-1% and molasses- 3% in pellet form and were not allowed to graze out side the stall.

Group III (SIF, semi-intensive fodder n=5) camel heifers were fed 6 kg *Cymopsis tetragonaloba* crop residue and were kept under semi-intensive management, i.e. they were sent to grazing for about 6-7 hr daily.

* Vernacular name of *Cymopsis tetragonoloba*

** Vernacular name of *Arachis hypogea*

*** Vernacular name of *Prosopis cineraria*

Ultrasound examination

The ultrasound examination was performed using linear probe (6.5 MHz, V-5 portable ultrasound machine, Med-India) with camels restrained in sitting posture as described previously (Vyas and Sahani, 2000). The heifers were examined when they attained the age of 3 yr ±2 months at fortnightly intervals till a follicle was observed.

Body measurements

Body weight and body measurements like heart girth (HG), height at withers (HE), body length (BL) and tail length (TL) were measured (using a measurement tape) at the age of 3 yr as per standard protocol at the farm. HG was measured as the circumference of anterior base of hump and behind the sternum. HE was the measurement between wither or front base of hump and foot pad of fore limb. BL was the measurement between shoulder joint to pin bone (pelvic). BG was the circumference at posterior base of hump and anterior to udder. TL was the length of tail from the base. The measurements were done by same technician. The heifers were mated with virile studs when a follicle of ≥ 9 mm diameter was observed (Skidmore *et al*, 1996) and copulation time was recorded.

Experiment II (second year)

The camel heifers (age 2.5 yr) were either maintained under ISF (n=6, Group I) or SIF (n=12, Group II), examined with ultrasound at similar intervals and mated as in experiment I once they attained the age 3 yr.

Experiment III (third year)

The experiment III was planned in subsequent year for validation of findings of experiment II and camel heifers (n=35) were maintained under SIF condition. They were examined for ovarian status at fortnightly interval from the age of 3 yr±2 months. The heifers were mated with virile studs when a follicle of ≥ 9 mm diameter was observed.

The heifers in all experiments were kept separated from male camels during the period of experiment including grazing period except at mating.

All animal experiments were conducted adhering strictly to institutional ethical management practices.

Statistical analysis

The ANOVA was used to analyse the results of experiment I and unpaired t test was used to compare the results of experiment II.

Results

Experiment I

The overall mean age at first service in camel heifers was 1147±5.93 days and body weight were 422.4±12.5 kg. The body weights attained in the three different groups were significantly different (P<0.05; Table 1). The heifers in ISF group were the heaviest. The age at first service (days) i.e., when follicle was observed for the first time was lowest in heifers under SIF, but difference was not statistically significant. Ultrasound examination revealed that 13 out of 15 females had follicle at 3 yr ± 2 months. The successful calving was higher in Group III. Camel heifers maintained under ISF and ISP attained higher body weights compared to SIF but there was no difference in age of the first service.

The overall means of different body measurements like body length (BL), heart girth (HG), height at wither (HE), tail length (TL), body girth (BG) were 143.87±2.0, 203.2±1.4, 188±1.18, 55.47±0.78 and 146.1±1.39 cm, respectively. The body measurements did not differ among different groups.

The age of the first service was positively correlated with body weight, heart girth and body girth and the heart girth was positively correlated with body length (Table 2).

Experiment II

A high proportion (91.66%; 11/12) of camel heifers in SIF group revealed follicles at 3 yr±2 months and were mated with virile stud camels. Six out of these 11 heifers (54.54 %) delivered the calf. All camel heifers (100%) in ISF group revealed ovarian follicles, were mated with virile stud and two out of six heifers (33.3%) delivered the calf. The age at first service in SIF camel heifers was 1108±5.0 days and it was significantly higher (P<0.05) compared to ISF camel heifers (1062±13.6 days) (Table 3).

Table 1. Mean ± SEM of growth parameters and age at first service in camel heifers.

Feeding group	Age of first service (days)	B. wt. (kg)	CT (s)	BL (cm)	HG (cm)	HE (cm)	TL (cm)	BG (cm)	Birth wt of calves born (kg) (n)
ISF	1162 ± 11.2	500.6 ± 21.4^{c}	403.6±31.8	146.6±4.0	206±2.7	189.2±0.7	56.4±0.7	146.6±2.8	36 (1)
(ISP	1143.6±10.3	471.8±16.3^{b}	424.6±119.6	143.2±3.6	202.6±2.5	188.8±3.0	54.8±2.2	148±2.8	40 (2)
SIF	1134.8±5.9	422.4±27.0^{a}	353±99.7	141.8±3.4	201±1.9	186±1.2	55.2±0.8	143.8±1.4	32.5 (1)
Overall	1147±5.93	464.9±12.5	393.73±49.6	143.8±2.0	203.2±1.4	188±1.2	55.5±0.8	146.1±1.39	35
f	2.25	5.357	0.160	0.44	1.139	0.69	0.344	0.76	
P value	0.147	0.021	0.85	0.65	0.35	0.52	0.72	0.48	
F crit	3.88	3.88	3.88	3.88	3.88	3.88	3.88	3.88	
df between	2								
within	14								
MS	44.1								

P<0.05; s- seconds; B.wt- Body weight; CT- Copulation time; BL-Body length; HG- Heart girth; HE- Height at wither; TL- Tail length; BG- Body Girth; ISF- heifers under intensive management with fodder; ISP- Heifers under intensive management with concentrate pellets; SIF- Heifers under semi-intensive management with fodder; n- number.

Table 2. Correlation of body measurements and age at first service in camel heifers.

Correlation coefficient								
	Age of first service	Body weight	CT	BL	HG	HE	TL	BG
age of first service	1	0.3058	-0.0182	0.1092	0.4595	0.262	0.3161	0.3062
Body weight		1	0.1417	0.1649	0.562	0.1906	-0.028	0.3605
CT			1	-0.0045	-0.2088	-0.0432	0.2242	0.0535
BL				1	0.5649	0.1099	0.4195	0.7453
HG					1	0.4358	0.3704	0.6648
HE						1	0.4696	0.4025
TL							1	0.2331
BG								1

CT- Copulation time; BL-Body length; HG- Heart girth; HE- Height at wither; TL- Tail length; BG- Body Girth.

The difference in mean bodyweight at first service in SIF (402.17±8.7 kg) and ISF camel heifers (421.1±9.5 kg) was not significant.

Experiment III

Ovarian follicle (>0.9 cm) was observed in a high proportion of camel heifers (88.57%; 31/35) managed under SIF at the mean age of 1089±6.51 days. The mean body weight at the time of first service was 407.48±8.58 kg (range- 360-506 kg). They were mated with virile stud. Eighteen heifers (58.06%; 18/31) became pregnant and sixteen heifers delivered healthy calf at around four years of age.

Discussion

In the present study positive effects of intensive feeding on the body weight and body dimensions of camel heifers were recorded. ISF camel heifers had significantly (P<0.05) higher body weight and non-significantly higher body dimensions compared to ISP and SIF camel heifers, yet SIF camel heifers had the lowest age at first service. However, in subsequent trials (Expt. II) ISF camel heifers had non-significantly higher body weight and significantly (P<0.05) lower age at first service compared to SIF camel heifers. This reflects that supplementary feeding has positive effects on the body weight of camel heifers, yet the appearance of an ovulatory sized follicle was less closely synchronised with feeding in camel heifers. A previous study (Saini *et al,* 2014) found that supplementary stall feeding of young camels (330 kg, n=20) resulted in significantly higher body weight compared to camels allowed grazing only. Similarly, Bakheit *et al* (2017) noticed significant higher growth of male and female camel calves maintained under semi-intensive management from 3 to 18 months of age compared to those maintained under traditional management system. Another study observed no appreciable difference in weight gain in male camels receiving supplementary diet (13% crude protein) although supplemented camels had higher testosterone concentrations (Al-Saiady *et al,* 2013). Under pastoral management conditions in Algeria a recent study recorded that the age at first oestrus in camels was 31.07±8.97 months and the age at first mating was 35.52±8.55 months (Gherissi *et al,* 2020). The body dimensions at puberty recorded in the present study are similar to previous report in camel (Kamoun and Wilson, 1994). Ultrasound examination of camel heifers (n= 68) at the age of 3 yr±2 months with a body weight of at least 360 kg revealed presence of follicle in 63 out of 68 heifers. They were mated and 28 out of 63 heifers got conceived at 3 yr±2 months of age and delivered healthy calf at around 4 yr of age. This finding is like a previous study (Abdel-Rahim, 1997) that recorded puberty in stall fed two camel breeds at 336 to 360 kg body weight attained at 173 weeks of age.

In most management systems dromedaries are not bred until the female has almost reached her mature physical size at 4 years of age, resulting in an age at first calving of 5 yr or more (Khanna *et al*, 1990). Growth and weight of young females also seem to be important factors in the onset of ovarian activity and influence the chances of conceiving. Low reproductive performance in camels is mainly ascribed to higher age at first calving, long calving interval and limited breeding season (ElWishy, 1987). The age of puberty and first service is reported to be influenced by complex interactions among genetic, nutritional, environmental and economic factors (Perry, 2016).

Studies on puberty in the female dromedary are based on a few clinical and field observations (Agarwal *et al,* 1996). Inadequate body weight caused by lack of sufficient food appears to be the cause of delayed puberty in the camel (Chatty, 1972). Nutrition seems to play a vital role in the various physiological

Table 3. Comparison of semi-intensive and intensive management systems on age at first service in camel in camel heifers (Experiment II).

	Age at first service		Body weight		Calves born; mean Calf birth weight (kg)	
	SIF	ISF	SIF	ISF	SIF	ISF
Mean±SE	1108.1±5.04[a]	1062.5±33.5[b]	402.2±8.7	421.2±9.5	6; 31.5±0.8	2; 32.5±1.5
N	12	6	12	6	12	6
P value	0.0014		0.1963		0.56	
t	3.8494		0.3485		0.6124	
df	16		16		16	
SE of difference	11.84		14.09		1.633	

ISF- heifers under intensive management with fodder; SIF- Heifers under semi-intensive management with fodder Values with different superscripted letters in a row are significantly (P<0.05) different.

events for the attainment of sexual maturity and in the reproductive process (Maynard, 1979). The overall pregnancy rate did not differ significantly between the herds where camel heifers were bred by the age of 3 yr or age > 3 yr (Ali *et al,* 2018).

Another report suggests that first service is allowed when the female reaches about 70 % of their adult body weight (Tibary and Anouassi, 1997). The average adult body weight in females of different Indian camel breed is reported to be 577.95±0.79, 537.0±11.66, 563.74±14.73 kg for Bikaneri, Jaisalmeri and Kachchi breeds, respectively (Khanna *et al,* 2004). The bodyweight of heifers considered for mating in the present study is 380 kg which comes to be 65.8, 70.7 and 67.5 per cent of adult body weight for Bikaneri, Jaisalmeri and Kachchi breeds, respectively. Breeding in camels based on visual observations appears to be difficult and sonographic presence of a follicle can be a better option for mating. In one previous study parturient camels were monitored ultrasonographically and when a follicle was observed these were mated resulting in pregnancies in 52.7% camels (Vyas *et al*, 2000). A recent study (Faraz *et al*, 2022) reported that the age at first service was 1091, 1109 and 1162 days in camel heifers that were forcefully mated with male camels. This reflects the complexity of camel breeding and probably the forcefully mated camel heifers had an ovulatory sized follicle yet did not evidence overt signs of oestrus or receptivity.

Conclusion

It was concluded that supplementary feeding of camel heifers has positive effects on the body weight and age at first service. Camel heifers attain puberty at 3 yr of age and >360 kg .and can be successfully bred if the follicle is monitored by ultrasonography.

Declaration of competing interest

Authors of the manuscript declare no conflict of interest for this research work.

Contributions of authors

SV - conceptualisation, methodology, investigation, execution, writing-original draft preparation; RKS- investigation, execution; MMA- investigation, execution; KN- investigation, execution; BDK- investigation, execution, SV- Statistical analysis, GNP-Writing-reviewing and editing.

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

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ABSTRACT

The urethral glands from 40 dromedary camels were investigated using histological, morphometric and electron microscopic techniques. The secretory units of the gland were characterised by columnar or pyramidal cells rich in rough endoplasmic reticulum, Golgi complex and various forms of mitochondria. Basal cells were observed located between the cells of the secretory units. Morphometric data revealed that the connective tissue occupied most of the gland (81.66%) while the glandular tissue was reduced to only 8.94%. Morphometric data was indicative of the relatively minor contribution of the urethral gland to the seminal plasma.

Key words: Camel, dromedary, morphometry, ultrastructure, urethral gland

The male urogenital tract was confirmed to contain urethral glands in many rodents (Hall, 1936; Hebel and Stromber, 1986). The pelvic urethral glands have been studied in the male cat (Cullen *et al,* 1983), dog and stallion (Trautman and Fiebiger, 1952; Bharadwaj and Calhoun, 1959) and man (Sirigu *et al,* 1991).

Various studies described the histology or histochemistry of the camel urethral glands (El Jack, 1970; El-Wishy *et al,* 1972; Ali, 1975; Ali *et al,* 1976, 1978; Mosallam, 1981; Badawy and Yousef, 1982; Degan and Lee, 1982; Marroni *et al,* 1982; Mobarak *et al,* 1990; Hafez and Hafez, 2001; Yousefi *et al,* 2010; Masood *et al,* 2022).

The urethral glands are located just behind the body of the prostate and extend to the level of the urethral bulb before opening into the urethral lumen *via* numerous ducts. These glands and the pelvic urethra are responsible for the contraction of the muscle and expulsion of glandular secretion (Ali *et al*, 1978). The seminal colliculus is fibroglandular in the males of camel, ox, buffalo and pig are found on the medial aspect of the dorsal wall of the urethra, little caudal to the internal opening of the urethra from the bladder (Shehata, 1980).

The ultrastructure and morphometry of urethral glands of camels are least studied. The present study was, therefore, undertaken to reveal the ultrastructure and morphometry of this gland.

Materials and Methods

The pelvic urethral glands were collected from 40 adult one-humped camels (*Camelus dromedarius*) immediately after slaughter at Tambul Slaughterhouse, Sudan. All collected samples were apparently normal without any pathological lesions.

Light Microscopy

Samples from 11 animals were used for the preparation of histological sections. Tissue pieces from the urethral glands were fixed either in Bouin's fluid, 10% formal-saline, 10% formalin or Zenker formal. Sections, 5 mm^3 thick were cut from the different levels of the gland, then processed for normal paraffin techniques. Paraffin sections at 3-5µm were cut and stained with Haematoxylin and Eosin (H&E) or Masson's Trichrome.

Electron Microscopy

Materials for ultrastructural studies were obtained from 9 animals. Small pieces (1mm^3) of tissues were fixed in 2.3% glutaraldehyde in 2.14% sodium cacodylate buffer pH 7.4 for 2 hours. These were then washed in 2.14% sodium cacodylate pH 7.4, postfixed in 2% osmium tetraoxide in 2.14% sodium cacodylate buffer pH 7.4 for 2 hours. These were then

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

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

Morphometry

Tissue samples were collected from 20 animals. The weight and volume of the organs were measured. The volume was determined by water displacement method (Scherie, 1970).

Each gland was cut into 4 slices about 5 mm thick. Since the slices were more or less identical it was necessary to analyse all of them and therefore, every second slice was taken for histological processing. The slices from each animal were used for morphometric analysis. Sections were cut at 2-5 µm thick and stained with Masson's Trichrome.

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

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

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

Results and Discussion

Light Microscopy

The secretory units were lined with one layer of pyramidal or columnar cells about 17 µm in height. Their nuclei were spherical in shape and usually occupied a basal position. Some units presented basal cells wedged between the lining cells and the basement membrane.

The camel pelvic urethra was described by El Jack (1970) and Degan and Lee (1982). Camel urethra was different from other species, as it was glandular along its entire length. Such an observation was reported by Ali *et al* (1978) and confirmed in the present study.

Current findings on pelvic urethral gland of the camel confirmed those of Ali (1975), Ali *et al* (1978) and Mosallam (1981). The secretory units were lined with a single layer of pyramidal or columnar cells (13µm) with spherical basal nuclei. Basal cells were observed in some secretory units, an observation which were not reported elsewhere.

Electron Microscopy

Columnar Cells

The luminal border of the columnar cells carried microvilli which were long, numerous and pleomorphic; some were short (Fig 1). Pinocytotic vesicles were observed between the bases of the microvilli. The lateral plasma membrane showed extensive junctional complexes towards the luminal tips (Fig 3). There was also an extensive infolding of the basal part of the lateral plasma membranes whereas the basal plasma membrane was moderately folded. Nuclei of the columnar cells were spherical in shape and frequently occupied basal positions (Fig 1) and rich in chromatin. Heterochromatin, is the form of irregular, dense granular masses disposed around the periphery of the nucleoplasm, while euchromatin occupied the central areas of the nuclei (Figs 1-4). Columnar cells contained sizeable Golgi complex, usually seen in the supranuclear cytoplasm, consisting of several arrays of cisternae and vesicles of various sizes (Fig 2). Numerous mitochondria were scattered throughout the cytoplasm in a form of oval, rounded and elongated bodies (Fig 3). Rough endoplasmic reticulum (RER) consisting of branching and anastomosing tubules were distributed throughout the cytoplasm. Some profiles were observed in the form of whorled lamellae (Fig 4). Large electron-lucent bodies, numerous vacuoles and secretory granules were often seen in the cytoplasm (Fig 5).

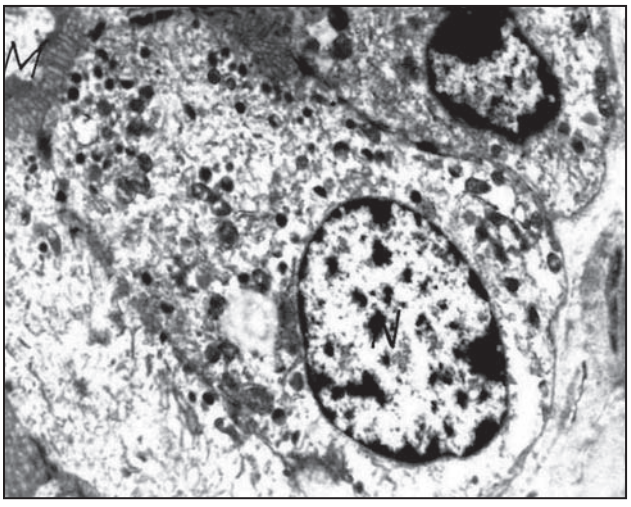

Fig 1. TEM micrograph of the urethral gland. The columnar cell carries microvilli (M) with spherical nucleus (N) in the basal cytoplasm. X30800.

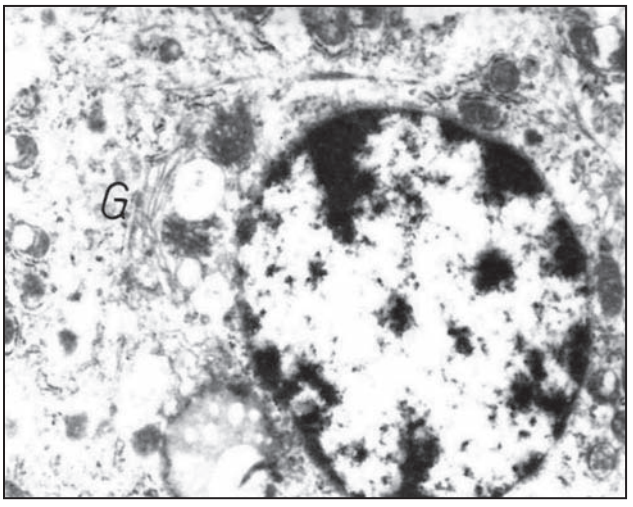

Fig 2. TEM micrograph of the urethral gland. The cell showing a sizeable Golgi complex (G). X32700.

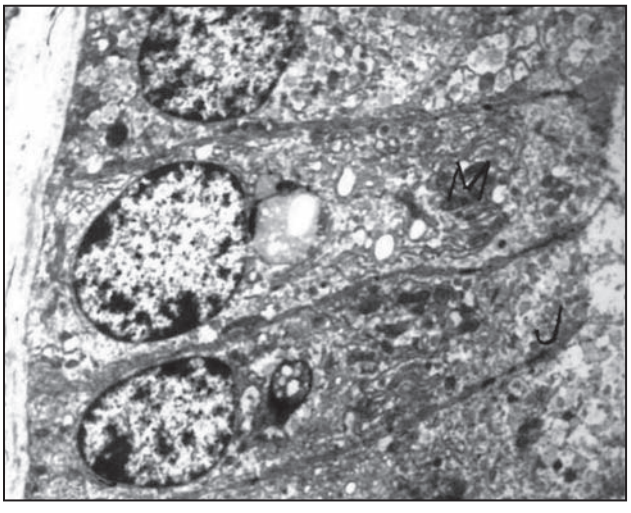

Fig 3. TEM micrograph of the urethral gland. Two cells showing many mitochondria. Note the junctional complex (J) towards the luminal tips. X19500.

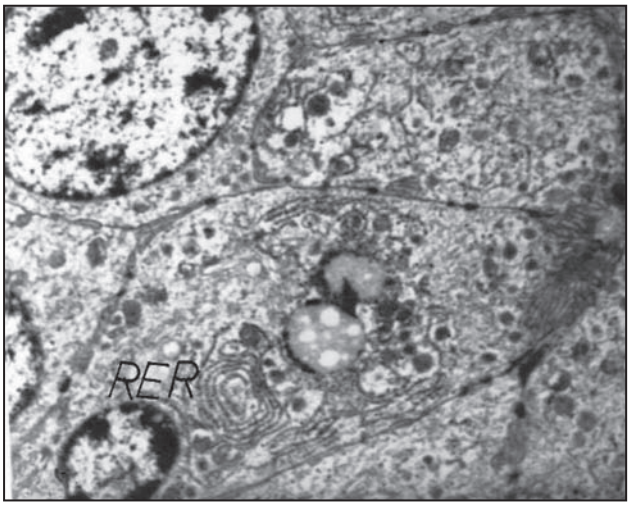

Fig 4. TEM micrograph of the urethral gland. A cell showing Rough Endoplasmic Reticulum, some profiles observed in the form of whorled lamellae (RER). X44000.

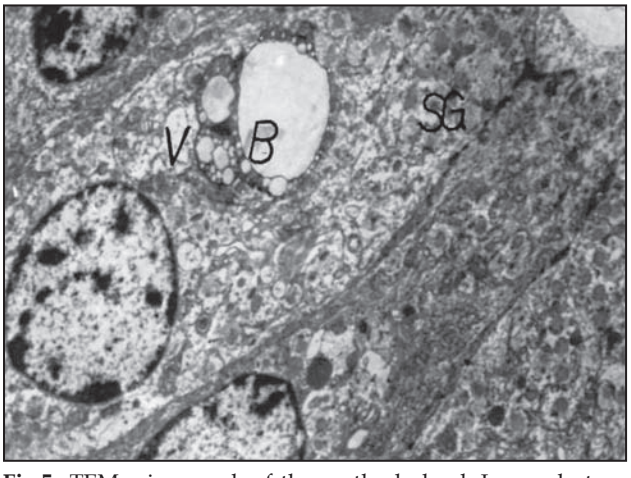

Fig 5. TEM micrograph of the urethral gland. Large electron lucent bodies (B) are observed, secretory granules (SG),and vacuoles (V). X21900.

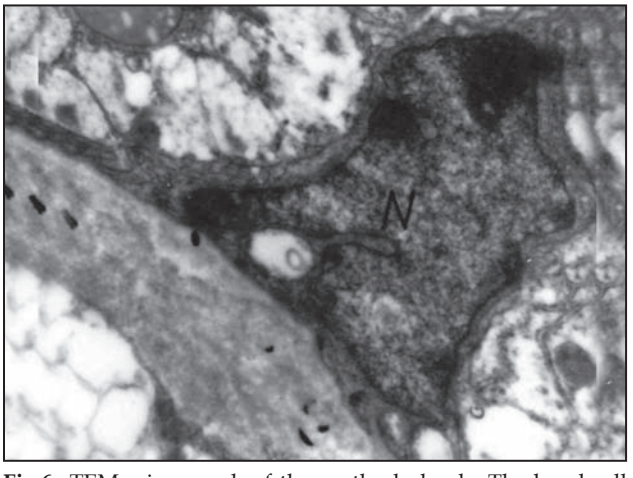

Fig 6. TEM micrograph of the urethral glands. The basal cell is irregularly pyramidal in shape with dense nucleus occupies most of the cell. X23000.

Basal Cells

The basal cells were irregularly pyramidal in shape and rested directly on the basal lamina. Their cell membrane were somewhat irregular. These cell possessed large dense irregularly pyramidal nuclei that filled most of the cell and had heterochromatin concentrated peripherally (Fig 6). These cells had small Golgi complex, few mitochondria, rounded or elongated and some profiles of rough endoplasmic reticulum (RER). The cytoplasm contained also a small number of vacuolar spaces.

As revealed by electron microscope the luminal surface of the lining cell was provided with tiny microvilli together with interspaced pinocytosis vesicles. The lateral plasma membrane was extensively folded in it was basal part and showed large junctional complex towards the luminal tip. The basal plasma membrane was only moderately folded.

The nucleus was spherical and basally located, similar results was reported by Ali *et al* (1978) and Mosallam (1981). The heterochromatin was located at the periphery. A large Golgi complex indicative of secretory activity, appeared in the supranuclear cytoplasm. There was an abundance of mitochondria which was either oval, round or elongated with lamellar cristae. The cytoplasm also contained elements of rough endoplasmic reticulum (RER). Some were observed in the form of whorled lamellae as in prostate gland.

The cells were characterised by the presence of a large electron-lucent bodies located above the nucleus and a large number of secretory granules were concentrated in the luminal cytoplasm.

Morphometry

Tables 1, 2 and 3 present data and results obtained by using the point-counting technique. The mean volume of the urethral gland, the total points falling on each component, the volume density of each component (Vv), the volume density percentage (Vv%) and the absolute volumes were recorded in these tables. Measurement of the volumes of the urethral glands of the 20 camels gave mean values of 64.7 cm^3; and those of weight of the urethral glands gave mean values of 69.97 gm.

The components of the urethral gland studied were glandular tissue, connective tissue and muscles, blood vessels and nerves and ducts. The greater volume of the urethral glands was occupied by connective tissue and muscle (81.66%) followed by glandular tissue 8.94%). The blood vessels and nerves occupied 6.04% and ducts occupied the least volume 3.36% (Tables 1-3). The absolute volume of connective tissue and muscle was 53.20 cm^3 while that of glandular tissue was 5.10 cm^3. The ducts showed an absolute volume of 2.01 cm^3 and that of blood vessels and nerves was 3.50 cm^3 (Tables 1-3).

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

No. of fields	G. T. P	C.T+M	B.VS+N	Ducts	Total
1	-	85	15	-	100
2	65	25	03	07	100
3	-	94	06	-	100
4	75	12	06	07	100
5	81	09	03	07	100
6	-	95	05	-	100
7	67	16	04	13	100
8	87	12	01	-	100
9	-	100	-	-	100
10	35	40	05	20	100
11	-	90	10	-	100
12	25	48	05	22	100
13	17	72	08	03	100
14	-	89	11	-	100
15	07	86	03	04	100
16	-	96	04	-	100
17	-	97	03	-	100
18	-	100	-	-	100
19	-	96	04	-	100
20	-	88	12	-	100
21	-	94	06	-	100
22	-	94	02	-	100
23	-	100	-	-	100
24	-	95	05	-	100
25	-	94	06	-	100
26	-	100	-	-	100
27	-	97	03	-	100
28	-	100	-	-	100
29	-	98	02	-	100
30	-	90	10	-	100
Total	459	2316	142	83	3000

Glandular tissue of urethral gland (G.T.P), Connective tissue and muscles (C.T+M), Blood vessels and nerves (B.Vs+N).

Search in the literature has shown that quantitative data on the urethral gland was virtually lacking. In the camel the mean values of the volume was 64.7 cm^3, and that of weight was 69.97 gm. The connective tissue and muscle occupied about 81.66%

of the gland, followed by glandular tissue 8.94%, blood vessels and nerves 6.04% and lastly the duct 3.36%.

Table 2. Morphometric analysis of urethral gland showing the number of points counted (P), volume density (Vv), volume density percentage (Vv%), and absolute volume (V) of the main components from four sections.

No. of fields	G. T. P	C.T+M	B.VS+N	Ducts	Total
120	1070	9775	736	419	1200
Vv	0.089	0815	0.061	0.035	
Vv%	8.90	81.50	6.10	3.50	
Abs. v	5.10	46.70	3.50	2.01	

Glandular tissue of urethral gland (G.T.P), Connective tissue and muscle (C.T+M), Blood vessels and nerves (B.Vs+N).

Table 3. Morphometric analysis of urethral glands showing the volume of gland (V) in cm^3, volume density (Vv), volume density percentage (Vv%), and absolute volume (V), of the main components of 20 camels.

No. of fields	G. T. P	C.T+M	B.VS+N	Ducts
Total No.	21459	195984	14488	8069
Vv	0.0894	0.8166	0.0604	0.0336
±SD	±.0.0165	±0.0111	±0.1032	±0.0071
Vv%	8.94	81.66	6.04	3.36
±SD	±1.65	±1.12	±1.02	±0.71
Abs. v	5.79	53.20	3.92	2.22
±SD	±1.37	±9.59	±0.97	±0.70

Glandular tissue of urethral gland (G.T.P), Connective tissue and muscle (C.T+M), Blood vessels and nerves (B.Vs+N).

The absolute volume of connective tissue and muscle was 53.20 cm^3, glandular tissue 5.79 cm^3, blood vessels and nerves was 3.93 cm^3 and duct was 2.22 cm^3.

The analysis of the quantitative data of the pelvic urethra showed that the glandular tissue occupied about 8.94% being much lower than those of prostate gland (52.91%) and bulbourethral glands (54.02%). The connective tissue and muscle occupied 81.66%, a ratio which exceeds other glands of the dromedary camel (Shaaeldin and Tingari, 2019; Shaaeldin *et al*, 2020). Blood vessels and nerves amounted to 6.4%, this ratio was lower than that of prostate gland and exceeded that of the bulbourethral glands. The quantitative data of the pelvic urethra, especially that of the glandular tissue was indicative of relatively minor contribution of the pelvic urethral glands to the seminal plasma.

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PROSTHODONTIC APPLICATION OF ACRYLIC POLYMER AS DIASTEMA FILLER FOR MANAGEMENT OF BUCCAL FISTULA IN CAMELS

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ABSTRACT

Buccal and buccal cum salivary fistula were diagnosed in four male adult dromedary camels of age between 12-18 years. In all cases fistulous wound was surgically repaired by debridement and suturing of wound edges with the interior and exterior application of rubber sheath as a wound protectant. In cases with buccal cum salivary fistula (2 cases), along with fistulous wound repair, Stenson's duct was ligated and parotid gland cauterised with retrograde infusion of Lugol's iodine. Surgery was performed in all cases under xylazine (0.4 mg/kg bwt.) and ketamine (2 mg/kg bwt.) administered intravenously. The fistula recurred in three cases after 6-8 weeks. Abnormal interdental gap between maxillary molar teeth (cheek teeth diastemata) against the buccal fistulous wound was present in these cases as evident by lateral radiograph of skull region. Coarse feed straw was found trapped in these gaps causing injury to the buccal mucosa. The interdental space in these 3 animals with recurring fistula and in a new case was filled with acrylic material under general anaesthesia after complete cleaning and drying the space. In a long term follow up of 2-3 months, the filling of interdental space with acrylic material remained in place and no recurrence of buccal fistula was seen in all the cases.

Key words: Buccal fistula, cheek teeth diastemata, polymethyl methacrylate (PMMA), salivary fistula

Salivary fistula is an abnormal tract that connects between the lumen of the Stenson's duct of the parotid salivary gland and the skin surface where as buccal fistula is an abnormal tract that connects between the oral cavity and the skin surface of the face mostly at the level of cheek tooth. In camels, penetrating wounds of buccal cavity due to trauma caused by accumulation of feed straw between cheeks and molars have been reported (Gharu and Gahlot, 2015; Gahlot, 2000 and Singh *et al,* 2020). The oral cavity fistulae are more common in horses due to accidental injury, following periodontal disease in which exfoliation of the affected teeth leads to oral fistula, cheek teeth diastemata, fractured cheek teeth and a central defect in a worn cheek tooth (Hawkes *et al,* 2008 and Misk and Misk, 2020).

Polymethyl methacrylate (PMMA) is commonly used for prosthetic dental applications, including the fabrication of artificial teeth, denture bases, dentures, obturators, orthodontic retainers, temporary or provisional crowns and for the repair of dental prostheses. Additional dental applications of PMMA include occlusal splints, printed or milled casts, for treatment planning and the embedding of tooth specimens for research purposes (Zafar, 2020). The unique properties of PMMA, such as its low density, aesthetics, cost-effectiveness, ease of manipulation and tailorable physical and mechanical properties, make it a suitable and popular biomaterial for the dental applications.

Dental defect can be treated successfully by filling the diastema with PMMA after removing food and exudate from the sinuses or fistula. In case of fractured tooth PMMA alveolar packing can be done following dental extraction (Hawkes *et al,* 2008). In present clinical study the use of PMMA as filler of abnormal cheek teeth diastema in the treatment of buccal and buccal cum salivary fistula in dromedary camels is reported.

Materials and Methods

In camels, buccal fistula (n=2) (bilateral in one) and buccal cum salivary fistula (n=2) were treated by using PMMA as cheek tooth diastemata filler along with surgical repair of fistulous wounds. DPI RR Cold Cure Acrylic (Fig 1) repair material (Dental Product of

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India) was used as a prosthodontic which contained powder (polymethyl acrylate) and liquid (methyl methacrylate monomer, cross linking agent- Ethylene glycol dimethcrylate).

Camel I (C_I)– It was a male camel (aged 14 years) which had a fistulous opening on cheek just below the lower eyelid of left side and masticated feed straw was escaping out from this opening since last 2 months. An abscess was also present close to the fistulous opening (Fig 2) which was first drained by a criss-cross incision and treated with antiseptic fluid flushing and dressing.

Camel II (C_{II})– It was a male camel (aged 18 years) which had a bilateral fistulous opening on cheek below lower eyelid on either side with escape of feed straw and exudate.

In both C_I and C_{II} cases the fistulous tract was cleaned and flushed with light potassium permanganate (P.P.) solution. The feed straw in the interdental space close to the external fistulous opening was removed.

Camel III (C_{III})– It was a male camel (aged 16 years) which was presented with a history of wound on cheek below lower left eyelid alongwith painful swelling near to the wound since 4 weeks.

Camel IV (C_{IV})– It was a male camel (aged 14 years) which was presented with a history of wound on cheek below lower right eyelid alongwith engorged parotid salivary duct (Stenson's duct) (Fig 3).

In these cases (C_{III} & C_{IV}) the feed mixed with saliva exuded from the external fistulous opening and wetting of hairs below the skin wound was evident. These were diagnosed as buccal cum salivary fistula.

In all cases primary antiseptic dressing of fistulous wound alongwith parenteral administration of antibiotic* and anti-inflammatory** drugs was done for 3 days. The surgery was planned under general anaesthesia using xylazine (0.4 mg/kg body weight) and ketamine (2 mg/kg body weight) intravenously. The surgery was performed in lateral recumbency with affected side up in 3 camels (C_I, C_{III} and C_{IV}) and sternal recumbency in 1 camel (C_I). The buccal fistula and oral cavity were flushed with light P.P. solution in head down position, after that fistulous wound was cleaned and debrided. Two pieces of smooth edged rubber sheath (3 mm thickness) were cut in a circular manner of diameter slightly greater than that of fistulous wound for applying on oral and dermal side of wound as protectant. The rubber pieces were secured on either side of surgically repaired buccal fistula using non-absorbable (silk or polyester) suture material passing through the centre of both rubber sheets and the suture knots were kept on external side of skin side rubber sheet (Fig 4). Buccal mucosa and muscles of fistulous wound were apposed by polyglactin 910 suture (vicryl no.1) and skin edges were sutured using silk or nylon no.1 and pattern used in both the layers was simple interrupted. After complete surgical repair of wound, the preplaced internal and external rubber sheets were apposed against the buccal mucosa and skin by pulling the ends of suture material and applying the knot externally.

In C_{III} and C_{IV} camels with buccal cum salivary fistula, an oblique skin incision was placed on masseter muscle region rostral to angle of jaw and subcutaneous tissue was dissected out to exteriorise the stenson's duct which was engorged in C_{IV} (Fig 5A). Exploratory aspiration of saliva was done to ensure the presence of Stenson's duct. Lugol's iodine (10 ml) was injected into the duct in retrograde manner to destroy the secretory activity of parotid gland (Fig 5B) and the duct was immediately ligated with a preplaced non-absorbable silk suture material. The skin wound was closed in a routine manner. The buccal fistula wound was surgically repaired as described previously.

Postoperatively, camels were offered soft feed material for at least 2 weeks. Antibiotic and anti-inflammatory drug administration was done for 7 and 3 days, respectively. Surgically repaired wounds got healed at 2 week postoperative period. At 6-8 week postoperative period, recurrence of buccal fistula was seen in 3 animals which was evidenced by a small wound exuding saliva/feed material. Clinical and radiographic examination revealed presence of coarse feed material trapped in the interdental space between the maxillary cheek teeth opposite to the fistulous wound and cheek teeth diastema (Fig 6). The continuous trauma caused by this trapped feed material resulted in recurrence of the fistula.

It was planned to fill that space with PMMA. After initial treatment of cleansing the wounds and fistulous tract with light PP the interdental filling was performed under general anesthesia as described earlier. The accumulated feed material in the cheek teeth diastema was manually removed, flushed with light P.P. solution and dried (Fig 7). For prosthodontic applications of acrylic polymer (Polymethyl

* Oxytetracycline @ 10mg/kg, intramuscularly.

** Meloxicam@ 1mg/kg, intramuscularly

Fig 1. DPI RR cold cure acrylic material.

Fig 2. Buccal fistula and abscess in C_I.

Fig 3. Buccal cum salivary fistula and engorged Stenson's duct.

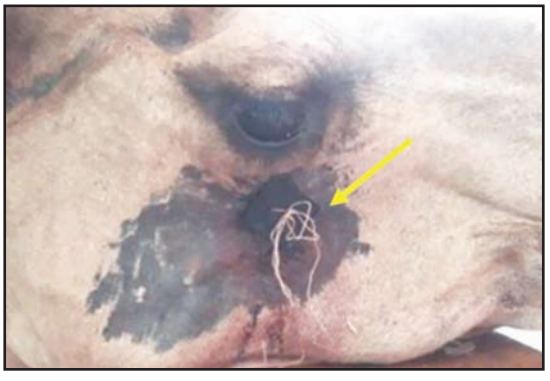

Fig 4. Repair of buccal fistula with rubber sheet application.

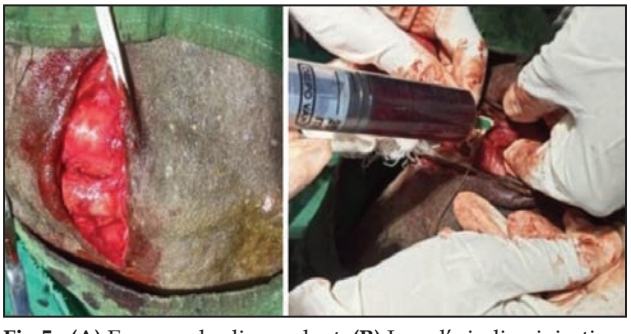

Fig 5. **(A)** Engorged salivary duct. **(B)** Lugol's iodine injection.

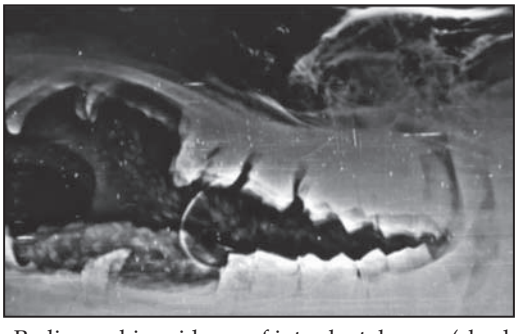

Fig 6. Radiographic evidence of interdental space (cheek teeth diastemata).

Methacrylate) its powder was mixed with provided liquid till it took semisolid form (Fig 8) and it was applied immediately in the interdental space (Fig 9) up to the alveolar border alongwith bridging between rostral and caudal teeth (Fig 10). The acrylic material was ensured not to protrude below the occlusal surface. *In situ* chemical exothermic heat generation was felt during the acrylic polymer application and the material got hardened within few minutes. The extra hardened acrylic polymer was removed to prevent soft tissue trauma to the buccal mucosa and tongue. Postoperatively, soft feed diet was offered for at least 2 week period.

Results and Discussion

In present study buccal fistula and buccal cum salivary fistula in adult dromedary camels were recorded and treated (Table 1). The history provided by animal owners could not ascertain the etiology of this condition. However, on the basis of clinical and radiographic examination the common etiological factor noticed in all cases was presence of abnormal interdental space between maxillary cheek teeth against the fistulous wound. Similarly, Hawkes *et al* (2008) also reported cheek teeth diastema as a common etiology leading to oromaxillary fistula in

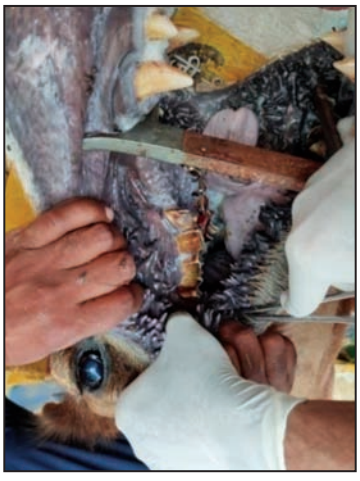

Fig 7. Manually evacuation of accumulated feed material at cheek teeth diastemata, with mouth gag in position.

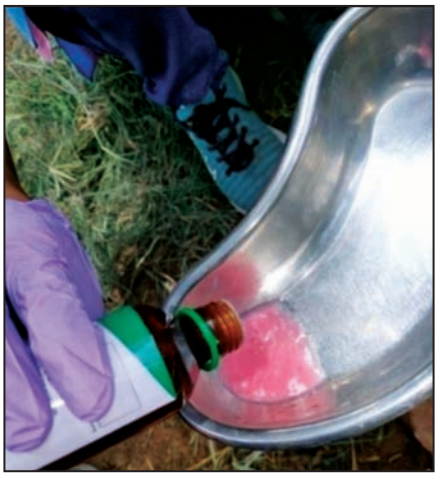

Fig 8. Acrylic powder mixing with liquid.

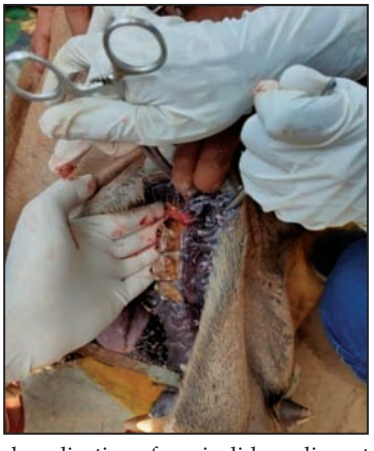

Fig 9. Manual application of semisolid acrylic material at cheek teeth diastemata.

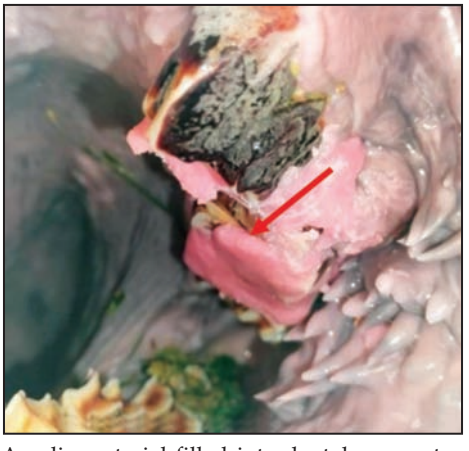

Fig 10. Acrylic material filled interdental space at occlusion level surface.

horses. However, in previous reported cases causes of buccal fistula in camels were traumatic injury (Purohit *et al,* 2011), accumulation of feed between cheek and molars (Gharu and Gahlot, 2015) and feed straw lodgement at interdental space between maxillary cheek teeth and entry of feed into opening of parotid salivary duct leading to concurrence of buccal and salivary fistula (Gahlot, 2000).

Table 1. Details of camels affected with buccal and buccal cum salivary fistula.

Case	Age (Years)	Surgical disorder	Affected side	Chronicity of fistula	Site of cheek teeth diastemata
C_I	14	Buccal fistula	Unilateral (Left)	2 months	P3-M1
C_{II}	18	Buccal fistula	Bilateral	4 months	M1-M2 (Rt.) P3-M1 & M1-M2 (Lt.)
C_{III}	16	Buccal cum-salivary fistula	Unilateral (Left)	4 weeks	P3-M1
C_{IV}	14	Buccal cum-salivary fistu la	Unilateral (Right)	2 weeks	P3-M1

C- Camel; Rt.- Right; Lt.- Left; P- Premolar; M- Molar

In all cases feed straw protruded out from the opening of fistula exteriorly at skin wound and was mixed with exudate. In a previously reported clinical case, chronic wound (3 cm diameter) was found about 2 cm below the lower eye lid on right side and partially masticated feed and saliva exuded through that opening (Kaswan *et al,* 2016). In C_{III} and C_{IV} cases of present study the hairy area below the fistula opening was moist with drooling saliva. In the clinical case study of Gharu and Gahlot (2015), the buccal fistulous wounds were 1-3 cm in diameter and found 1-2 inches below the lower eyelid on the cheek. Concurrence of salivary and buccal fistula was observed, in cases where the Stenson's duct was also involved; the saliva leaked through the

wound during mastication. In salivary fistula cases, saliva exuded in large amounts which may lead to dehydration and indigestion, if treatment is delayed (Singh *et al,* 2020).

In C_{III} camel, the fibrosis was observed at surgical site and Stenson's duct was not engorged whereas in C_{IV} camel the duct was engorged and filled with cloudy coloured salivary fluid alongwith flakes. The parotid duct arises on the ventral, rostral border of parotid gland and then traverses towards the side of the face medial to platysmas muscle, approximately 1-1.5 cm dorsal to the facial vein. It empties into the oral cavity through an orifice on a flattened papilla that is located 1 cm dorsal to the gingival border opposite the 4th maxillary cheek tooth (Fowler, 2000).

In the initially treated 3 cases, surgical repair of buccal fistula was done with application of internal and external rubber sheaths as a wound protectant. Gharu and Gahlot (2015) and Kaswan *et al* (2016) also used rubber sheets for the management of buccal fistula in camels. Rubber sheets got dislodged within 1 week postoperative period in C_I and C_{III} camels and after two weeks in C_{II} camel. In C_{IV} camel rubber sheets were not applied because there was a simultaneous cheek teeth diastema filling and repair of fistula was performed. In C_I, C_{II} and C_{III} camels recurrence of fistula was recorded following straw feeding. Presence of unusual interdental space due to absence of maxillary tooth at this location was root cause of fistula and filling of that space with PMMA helped healing the fistula.

In the presence of a healthy alveolus, an acrylic plug could firmly retain in place for 4–6 weeks which prevents alveolar food contamination and thus allows granulation tissue to close the apical aspect of the alveoli and thus allows buccal fistula to heal. The prosthodontic application of acrylic material as an interdental space filler has its inability to stay firmly in position for this length of time. After the initial period, the normal rostral and caudal dental drift of adjacent teeth into the tooth extraction space compresses the sides of the acrylic plug and usually keeps it firmly in place, sometimes for many years (Dixon, 2019).

In present clinical study, long term follow up was done in all cases and found that acrylic plug was retained between adjoining teeth till a follow up of 4 to 6 weeks post-application. The fistulae got completely healed and no recurrence was observed after prosthodontic application. Hawkes *et al* (2008) observed a similar case of oro-nasal and oro-maxillary fistula in equine treated with application of acrylic material, where PMMA got dislodged when the horse masticated as it was protruding below the occlusal surface. Any loose pieces of PMMA protruding bucally or palatally should be removed as they later cause soft tissue trauma when hardened. If the PMMA becomes too hard and does not attach firmly, it should immediately be replaced (Dixon, 2019). Prostheses should be regularly examined up to 2 or 3 months following their initial application and then at least annually.

Presence of cheek teeth diastemata facilitates the entry of hard fibres of feed straw which can repeatedly cause trauma to the duct and buccal wall hence, diastema space should be filled at an earliest by prosthodontic application of acrylic polymer PMMA, as done in the present study. It was inferred that prosthodontic application of Acrylic polymer (PMMA) and its retaining at diastemata for longer duration can promote the granulation tissue formation and subsequent healing of buccal fistula wounds.

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USE OF DEXMEDETOMIDINE AS SEDATIVE IN MALE DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The study was conducted to evaluate the sedative, physio-haemodynamic and haemato-biochemical effects of two doses of dexmedetomidine in camels. The experiment was conducted on 6 adult male camels in a randomised cross over design. The camels were randomly divided into two treatment groups, Dex_1 (dexmedetomidine 2.5 µg/kg b.wt) and Dex_2 (dexmedetomidine 4 µg/kg b.wt). Sedation, analgesia, physio-haemodynamic parameters and haemato-biochemical parameters were recorded at T- 0 (pre-administration) and at T -15, 30, 60, 90 and T-120 minutes post-administration. The onset of sedation was quicker (6.10 ±0.44 min) in Dex_2 than (6.85± 0.45 min) Dex_1 group. The duration of sedation and complete recovery time in Dex_2 group (40.05 ± 1.47 min and 64.32 ± 1.72 min, respectively) was significantly ($P<0.01$) longer than Dex_1 group (25.85 ±0.97 min and 43.14 ± 1.13 min, respectively). The extent of sedation and analgesia was significantly ($P<0.05$) more prominent in Dex_2 than Dex_1 group. Heart rate and pulse rate decreased significantly ($P<0.05$) in Dex_2 group at 15, 30 and 45 min. interval but no significant difference was observed between the groups. Systolic and diastolic blood pressure increased initially in both groups and later followed a decreasing trend. Haematological studies revealed non-significant changes in haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count at various time intervals in both the groups. Biochemical parameters *viz*, blood glucose, AST, ALT, ALKP, serum urea nitrogen, creatinine and cortisol showed non-significant changes at various time intervals in both groups. The changes observed in physiological and haemato-biochemical parameters were transient and within the normal range in both the groups. It was concluded that dexmedetomidine 4 µg/kg b.wt (Dex_2) produced better sedation without significant alterations in vital parameters and therefore, may be employed safely for sedation and analgesia in camels.

Key words: Analgesia, camel, dexmedetomidine, sedation

An Indian dromedary camel is reared primarily for carting, draft, agricultural operation and transportation therefore are more prone to various types of injuries and surgical disorders. Drug-induced sedation and analgesia are used commonly in dromedaries for restraining, diagnostic procedures, to perform minor surgeries and as a preanaesthetic drug. There are various class of sedatives which have been used alone or as preanaesthetic agents to induce sedation and anaesthesia in camels. The α-2 adrenergic agonist are most commonly used sedative for this purpose eg xylazine, detomidine, etc. However, these have cardiopulmonary effects after intravenous administration in camels. The duration and intensity of these effects depend on the type and dose of α-2 agonist used (Khalil *et al*, 2019). Recently dexmedetomidine has become popular in canines and other animals as a sedative due to its high α2:α1 selectivity (Kamibayashi and Maze, 2000). It is considered as a full α-2 agonist, allowing the use of relatively high doses without the unwanted vascular effects resulting from stimulation of α-1 adrenoceptors (Ebert *et al*, 2000). It causes both sedation and analgesia with negligible respiratory and cardiovascular side effects (Salarian *et al*, 2016). In dogs, it produces dose-dependent sedation and analgesia (Kuusela *et al*, 2000). Use of dexmedetomidine as sedative in dromedary camels has not been reported previously. The present study is therefore undertaken to investigate sedative, physio-haemodynamic and haemato-biochemical effects of dexmedetomidine in male dromedary camels.

Materials and Methods

The experimental trials were conducted after approval of the experimental protocol by the Institutional Ethics Committee of National Research Centre on Camel.

The study was conducted on 6 adult male camels with a mean body weight of 435.38 ± 11.22

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kg and aged between 4-5 years in a randomised crossover design with an interval of 14 days between two treatments. Food and water were withheld for 24 and 12 hours, respectively prior to experiments. Camels were kept restrained in sitting position/ sternal recumbency with both fore limbs tied together and allowed for 15 minutes to be stabilised after restraining.

Two sedative doses of dexmedetomidine *viz*. 2.5 µgm kg^{-1} b.wt and 4.0 µgm kg^{-1} b.wt for achieving satisfactory sedation with fair to good analgesia were selected during pilot trials. The study was based on doses of dexmedetomidine administered hence selected adult male dromedary camels (n=6, each group) were divided into two groups, i.e. Dex_1 (dexmedetomidine 2.5 µg/kg b.wt) and Dex_2 (dexmedetomidine 4 µg/kg b.wt). Sedation and analgesia were evaluated by subjective analysis of median scores of sedation and analgesia within group and in between groups. Quality of sedation and analgesia was evaluated by observing sedative response and analgesia based on behavioural scoring method on a 0-3 scale (Table 1). The observations were recorded at T-0 minute (pre-administration) and at T -5, 15, 30, 45, 60, 90 and 120 minutes (min), post- administration.

The physio-haemodynamic parameters, i.e. rectal temperature (RT), respiration rate (RR), heart rate (HR), pulse rate (PR), systolic and diastolic blood pressure (SBP and DBP) were recorded at T-0 minute (pre-administration) and at T- 5, 15, 30, 45, 60, 90 and 120 min post-administration of drug.

Haemato-biochemical parameters were studied from blood sample collected at T-0 minute (pre administration) and at T-5, 15, 30, 45, 60, 90 and 120 min post-administration of drug. Haematological parameters *viz*. haemoglobin, packed cell volume, total leucocyte count, total erythrocyte count and differential leucocyte count were estimated using automated haemato-analyser (IDEXX Vet Test, IDEXX Lab). Biochemical parameters *viz*. glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, serum urea nitrogen and creatinine were estimated with the help of semi-auto analyser (LT-100) using commercial kits. The cortisol level as a stress marker hormone was estimated as per standard procedures.

The data were presented as mean ± standard error of mean and median (Range) in parametric and non parametric statistical analysis, respectively.

Analysis of Variance (ANOVA), and Duncan's multiple range test (DMRT) were used to compare the means at different time intervals among groups and paired t test was used to compare the means at different time interval with respective base values (Snedecor and Cochran, 1994). The subjective data generated from the sedation scores were analysed using non parametric Kruskal- Wallis test. A value of $P < 0.05$ was considered significant.

Results and Discussion

The onset of sedation in Dex_2 group (6.10 ± 0.44 min) was recorded earlier than Dex_1 group (6.85 ± 0.45 min). The duration of sedation and complete recovery time was significantly ($P<0.01$) longer in Dex_2 (40.05 ± 1.47 min and 64.32 ± 1.72 min, respectively) than Dex_1 group (25.85 ±0.97 min and 43.14 ± 1.13 min, respectively) (Fig 1). The recovery was smooth and uncomplicated in both the groups. Moderate to good sedation was recorded in camels of Dex_1 group whereas, good sedation was recorded in Dex_2 group. The Dex_2 group camels showed marked sedation with significantly higher ($P<0.05$) sedation score particularly at 15, 45 and 90 min time points than Dex_1 group. The Dex_1 group camels exhibited moderate to good analgesia. Whereas, Dex_2 group camels showed good analgesia. The extent of muscle relaxation was significantly higher ($P<0.05$) in Dex_2 group particularly at 5 and 45 min time points than Dex_1 group (Table 2). Sedative effects of dexmedetomidine are attributed to its action on supraspinal autoreceptors in pons (Ahmed *et al,* 2018). Dexmedetomidine inhibited the release of substance P from the dorsal horn of the spinal cord, leading to primary analgesic effects (Kending *et al,* 1991). Dose dependent sedation by dexmedetomidine in Dex_1 and Dex_2 groups was in accordance with the findings of previous studies in dogs (Sabbe *et al,* 1994; Santosh *et al,* 2012). Kuusela *et al* (2001) reported that time to

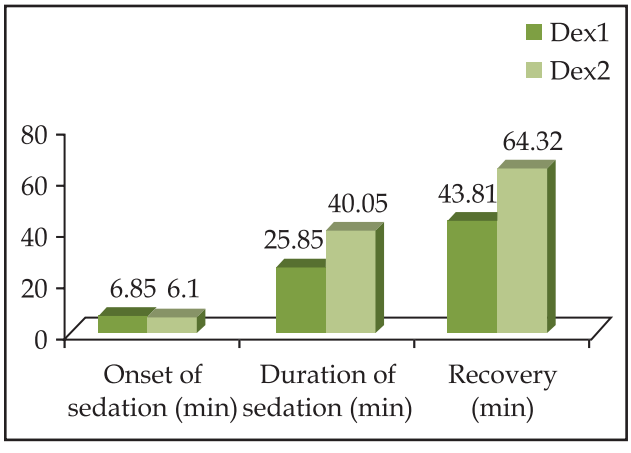

Fig 1. Onset of sedation, duration of sedation and recovery time after dexmedetomidine administration in camels.

Table 1. Description of sedation and analgesia score* in camel.

Score	Grade	Sedative response	Analgesia
0	No	Alert, normal carriage of head and neck, tongue and lower lip apposition and normal postural tone	strong reaction to bone prick
1	Poor	Slight reduction in alertness, mild head dropping, mild protrusion of tongue, Dropping of lower lip, slightly relaxed postural tone	weak reaction to bone prick
2	Fair	Moderately reduced alertness, moderate head dropping or deviation to one side, moderate protrusion of tongue and dropping of lower lip	occasional response to bone prick
3	Good	No alertness, deviation in posture, severe head dropping or deviated and rest on the back, severe protrusion of tongue, dropping of lower lip	no response to bone prick

*Adopted and modified (Khalil *et al*, 2019).

Table 2. Median values of sedation and analgesia score in camels of Dex_1 and Dex_2 group (n=6).

Parameters	Groups	Time interval							
		0	5	15	30	45	60	90	120
Sedation	Dex_1	0^{aA}	1.5^{abA}	2.5^{bA}	2.5^{bA}	1.5^{abA}	1^{abA}	0^{aA}	0^{aA}
	Dex_2	0^{aA}	2^{abB}	3^{bA}	3^{bA}	2.5^{bB}	2^{abA}	1^{abB}	0^{aA}
Analgesia	Dex_1	0^{aA}	1^{abA}	2.5^{bA}	2.5^{bA}	1^{abA}	1^{abA}	0^{aA}	0^{aA}
	Dex_2	0^{aA}	2^{acB}	3^{cA}	3^{cA}	2.5^{bcB}	1.5^{abcA}	0.5^{abA}	0^{aA}

Variables with different superscript in small letters differ significantly (P<0.05) within groups.

Variables with different superscript in capital letters differ significantly (P<0.05) among different groups.

Table 3. Mean±SE values of physio-haemodynamic parameters in camels of Dex_1 and Dex_2 group (n=6).

Parameters	Groups	Time interval							
		0	**5**	**15**	**30**	**45**	**60**	**90**	**120**
Rectal temperature (°F)	Dex_1	98.78 ± 0.56	98.76 ± 0.53	98.5 ± 0.44	97.93 ± 0.54	98.15 ± 0.49	98.28 ± 0.48	98.46 ± 0.48	98.26 ± 0.44
	Dex_2	98.45 ± 0.36	98.48 ± 0.30	98.08 ± 0.41	97.75 ± 0.45	97.33 ± 0.47	97.8 ± 0.50	97.66 ± 0.41	97.93 ± 0.42
Respiration rate (breaths min^{-1})	Dex_1	14.5 ± 0.67	14.0 ± 0.93	13.16 ± 0.79	13.5 ± 0.84	13.16 ± 0.90	13.33 ± 0.33	14 ± 0.77	14.16 ± 0.70
	Dex_2	14.16 ± 0.94	13.33 ± 0.95	12.17 ± 0.94	12.00 ± 0.77	11.67 ± 0.49	11.5 ± 0.67	12.83 ± 0.87	13.67 ± 0.84
Heart rate (beats min^{-1})	Dex_1	55.16 ± 3.14	52.16 ± 3.07	48.66 ± 2.98	48.0 ± 2.47	51.66 ± 2.47	52.00 ± 3.09	53.66 ± 2.81	55.66 ± 2.89
	Dex_2	54.66^{cA} ± 2.81	49.5^{bc} ± 2.04	43.33a ± 2.1	41.8a ± 2.34	44.33^{a} ± 2.81	46.66^{ab} ± 2.49	48.5^{abc} ± 2.18	53.16^{bc} ± 2.3
Pulse rate (beats min^{-1})	Dex_1	52.0 ± 2.59	49.16 ± 2.58	46.33 ± 2.92	46.16 ± 2.93	49.33 ± 3.25	49.66 ± 3.06	50.50 ± 3.77	52.33 ± 3.21
	Dex_2	51.83^{b} ± 3.14	46.0^{abc} ± 2.19	40.5^{a} ± 1.91	39.16a ± 1.66	42.5^{a} ± 2.43	43.67^{ab} ± 2.33	45.8^{abc} ± 2.0	50.33^{bc} ± 2.22
Blood pressure systolic (Mm Hg)	Dex_1	145.66 ± 4.97	152.33 ± 5.34	155.5 ± 5.35	145.16 ± 3.83	140.5 ± 4.16	143.0 ± 4.96	144.83 ± 4.76	145.16 ± 4.70
	Dex_2	144.33 ± 4.20	154.16 ± 5.36	156.33 ± 4.93	148.0 ± 5.68	140.83 ± 4.90	142.0. ± 5.13	143.5 ± 4.16	144.16 ± 3.51
Blood pressure diastolic (Mm Hg)	Dex_1	96.0 ± 4.80	102.83 ± 5.24	103.83 ± 5.81	94.67 ± 4.03	91.5 ± 4.0	93.5 ± 4.63	95.3 ± 4.8	95.83 ± 4.4
	Dex_2	95.33 ± 4.31	104.83 ± 5.34	106.83 ± 4.72	98.16 ± 5.64	91.83 ± 4.77	93.16 ± 4.72	94.16 ± 4.0	95.0 ± 3.6

sternal recumbency and walking were significantly prolonged in dogs treated with higher dose level, compared with the 2 lower dose levels in dogs.

Rectal temperature (RT) decreased non-significantly up to 30 and 45 min, respectively in Dex_1 and Dex_2 groups (Table 3). Decrease in rectal

Table 4. Mean ±SE values of haematological parameters in animals of Dex_1 and Dex_2 groups (n=6).

Parameters	Groups	Time interval					
		0	15	30	60	90	120
HB (g/dL)	Dex_1	12.73 ± 0.37	12.43 ± 0.32	12.31 ± 0.33	12.56 ± 0.30	12.53 ± 0.27	12.63 ± 0.41
	Dex_2	12.50 ± 0.34	12.3 ± 0.47	12.15 ± 0.30	12.10 ± 0.30	12.05 ± 0.27	12.21 ± 0.25
PCV (%)	Dex_1	27.71 ± 1.12	26.63 ± 0.92	25.93 ± 1.01	26.45 ± 1.19	26.61 ± 1.22	26.95 ± 1.00
	Dex_2	27.68 ± 0.98	27.00 ± 0.96	26.1 ± 0.89	25.66 ± 1.01	26.30 ± 0.93	26.86 ± 0.97
TEC (10^6/mm^3)	Dex_1	7.85 ± 0.46	7.79 ± 0.46	7.54 ± 0.52	7.30 ± 0.48	7.61 ± 0.45	7.59 ± 0.43
	Dex_2	7.93 ± 0.51	7.91 ± 0.52	7.50 ± 0.52	7.22 ± 0.49	7.12 ± 0.47	7.30 ± 0.48
TLC (10^3/mm^3)	Dex_1	13.26 ± 0.51	13.28 ± 0.51	13.10 ± 0.50	13.02 ± 0.54	13.21 ± 0.55	13.16 ± 0.57
	Dex_2	13.50 ± 0.45	13.49 ± 0.48	13.35 ± 0.50	13.22± 0.45	13.27 ± 0.44	13.30 ± 0.44
DLC							
N (%)	Dex_1	50.33 ± 0.80	50.67 ± 0.71	51.16 ± 1.01	50.66 ± 0.49	49.83 ± 0.70	50.00 ± 1.15
	Dex_2	51.66 ± 0.84	51.00 ± 0.44	51.83 ± 0.60	53.16 ± 1.24	52.16 ± 0.70	50.66 ± 0.49
L (%)	Dex_1	45.50 ± 1.17	45.50 ± 1.18	45.00 ± 1.41	46.50 ± 0.84	47.00 ±1.0	46.16 ± 1.47
	Dex_2	44.00 ± 0.89	44.33 ± 0.55	43.83 ± 0.60	42.50 ± 1.05	43.33 ± 0.76	44.16 ± 0.60
M (%)	Dex_1	2.83 ± 0.47	2.16 ± 0.47	2.00 ± 0.25	1.66 ± 0.21	2.00 ± 0.25	2.33 ± 0.33
	Dex_2	2.33 ± 0.33	2.00 ± 0.25	1.83 ± 0.40	2.33 ± 0.21	2.00 ± 0.25	2.33 ± 0.21
E (%)	Dex_1	2.00 ± 0.44	2.33 ± 0.33	2.83 ± 0.47	2.00 ± 0.25	2.33 ± 0.42	2.50 ± 0.42
	Dex_2	2.00 ± 0.25	2.66 ± 0.33	2.5 ± 0.34	2.00 ± 0.36	2.50 ± 0.42	2.83 ± 0.40

temperature in both groups might be attributed to reduction in muscular activity, decrease in metabolic rate, muscle relaxation with depression of thermo-regulatory system. Alpha-2 adrenergic agonist was reported to induce prolonged depression of thermoregulation (Ponder and Clarke, 1980). Similar observations after dexmedetomidine administration in dogs (Ahmad *et al,* 2011) and sheep (Monsang, 2011) were reported.

Respiration rate (RR) decreased non-significantly in animals of Dex_1 and Dex_2 groups (Table 3). Decrease in respiration rate might be due to depression of respiratory centres through stimulation of supra-spinal adrenoceptors following systemic administration of the alpha 2 agonist drug (Prado *et al,* 1999). A decreased RR results due to depressing action on respiratory centre in central nervous system (Hall *et al,* 2001). Decreased RR was also reported following administration of dexmedetomidine along with butorphanol in dogs (Surbhi *et al,* 2010) and dexmedetomidine along with propofol in dog (Singh *et al,* 2020).

In animals of Dex_1 group, heart rate and pulse rate decreased non-significantly whereas in animals of Dex_2 group, heart rate and pulse rate decreased significantly ($P<0.05$) at 15, 30 and 45 min from the base line value with a peak decrease at 30 min interval. Thereafter, heart rate and pulse rate increased significantly ($P<0.05$) at 120 min interval in animals of Dex_2 group (Table 3). Bradycardia was observed in animals of both Dex_1 and Dex_2 groups which is attributed to vasoconstriction property of alpha-2 agonists leading to reflex bradycardia (Congdon *et al,* 2011). Similarly, bradycardia was reported following dexmedetomidine administration in goats (Kastner *et al,* 2005) and camel calves (Samimi *et al,* 2020). Systolic and diastolic blood pressure increased initially at 5 and 15 min interval in both Dex_1 and Dex_2 groups and later followed decreasing trend with a peak low at 45 min. interval (Table 3).The increasing trend in the systolic and diastolic blood pressure followed by a decrease in both groups was similar to previous reports following administration of dexmedetomidine along with propofol in dog (Singh *et al,* 2020). This biphasic effect of dexmedetomidine on arterial blood pressure, with an initial rise in blood pressure followed by subsequent reduction in blood pressure might be attributed to change in plasma concentration of dexmedetomidine as reported earlier (Flaherty, 2013). Cardiovascular effects of alpha-2 agonists causes initial vasoconstriction and increased blood pressure mediated by the α2 b-subtype adrenoceptors and later causes decreased sympathetic tone and blood pressure (Kamibayashi and Maze, 2000).

In present study, complete blood count values remained within the normal clinical range in both groups of camels, however, non-significant changes

Table 5. Mean±SE values of biochemical parameters in animals of Dex_1 and Dex_2 group (n=6).

Parameters	Groups	Time interval					
		0	15	30	60	90	120
Blood glucose (gm/dl)	Dex_1	99.16 ± 6.22	117.5 ± 7.36	124.33 ± 7.55	121.16± 7.93	112.83 ± 9.7	107.16 ± 8.19
	Dex_2	102.5 ±6.08	119.83 ± 7.84	127.16 ± 8.46	127.83± 9.40	120.5 ± 9.91	111.50 ± 8.97
AST (IU/L)	Dex_1	90.82 ± 4.64	90.95 ± 6.09	92.71 ± 4.93	94.78 ± 5.96	94.63 ± 5.49	93.48 ± 5.11
	Dex_2	92.57 ± 5.92	94.08 ± 5.32	94.77 ± 5.66	95.25 ± 4.89	95.10 ± 5.27	94.55 ± 5.0
ALT (IU/L)	Dex_1	11.41 ±0.87	11.72 ± 0.92	11.81 ± 0.96	13.09 ± 0.80	12.94 ± 0.78	12.86 ± 0.77
	Dex_2	11.37 ± 0.89	11.85 ± 0.79	12.0 ± 0.70	13.36 ± 0.70	13.32 ± 0.68	13.34 ± 0.81
ALP (IU/L)	Dex_1	92.66 ± 5.89	92.83± 6.05	93.5 ± 6.13	95.5 ± 5.61	94.5± 5.64	93.83 ± 5.10
	Dex_2	94.33 ± 5.15	94.5± 5.05	96.0 ± 5.41	97.0 ± 5.24	96.5 ± 5.99	95.66 ± 6.07
SUN (mg/dl)	Dex_1	29.52 ± 1.76	29.55 ± 1.75	29.97 ± 1.89	30.78 ± 1.82	30.53 ± 1.74	29.83 ± 1.76
	Dex_2	29.46 ± 2.14	29.61 ± 2.21	29.68 ± 2.16	30.43 ± 1.98	30.61 ± 1.90	30.33 ± 1.91
Creatinine (mg/dl)	Dex_1	1.10 ± 0.15	1.13 ± 0.16	1.18± 0.14	1.20 ± 0.15	1.18 ± 0.14	1.15 ± 0.24
	Dex_2	1.06 ± 0.12	1.10 ± 0.13	1.15± 0.12	1.22 ± 0.12	1.23 ± 0.11	1.17 ± 0.12
Cortisol (µg/dL)	Dex_1	1.2 ± 0.14	1.11 ± 0.15	0.98 ± 0.12	0.90 ± 0.12	0.96± 0.09	1.06 ± 0.09
	Dex_2	1.16 ± 0.15	1.09± 0.14	0.97± 0.14	0.92 ± 0.12	0.97± 0.10	1.07± 0.08

were observed during the recording at different time periods. Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC) and toal leucocyte count (TLC) decreased non-significantly in both the groups (Table 4). It was attributed to shifting of body fluid from extravascular compartment to intravascular compartment in order to maintain normal cardiac output in the animals (Wagner *et al,* 1991). Pooling of circulatory blood cells in the spleen or other reservoirs secondary to decreased sympathetic activity might lead to a decrease in Hb, erythrocyte, PCV and TLC (Wagner *et al,* 1991). Decrease in Hb and PCV after administration of dexmedetomidine was also reported in dogs (Gupta, 2010) and sheep (Monsang, 2011). The initial increase in neutrophils and a corresponding decrease in lymphocytes recorded in present study might be associated with initial excitement due to handling of the animals and stress caused by the preanaesthetic drug and subsequent stimulation release of epinephrine leading to the release of neutrophils from bone marrow (Rosin, 1981). Similar findings were also reported following administration of dexmedetomidine in dogs (Ahmad *et al,* 2011).

In animals of Dex_1 and Dex_2 groups blood glucose increased non-significantly up to 30 and 60 min, respectively and later on, values decreased but remained above base line in both the groups (Table 5). Increase in blood glucose level observed in animals of both the groups might be attributed to increased hepatic glucose production, decreased glucose utilisation by body cells, decreased membrane transport and reduced plasma concentrations which are mediated by activation of α2-adrenoceptors present in the β-cells of pancreatic islets exerting a negative control of basal insulin release (Burton *et al,* 1977). Similar findings has also been reported following administration of medetomidine/ dexmedetomidine butorphanol followed by propofol anaesthesia in canine orthopaedic patients (Gupta, 2010), buffaloes (Malik *et al,* 2011) and sheep (Monsang, 2011). A non significant increase in aspartate amino transferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) activity was observed up to 60 min in both Dex_1 and Dex_2 groups. Thereafter, values decreased but remained above base line in animals of both the groups (Table 5). Transient increase in ALT, AST and ALP levels might be associated with increased cell membrane permeability in response to haemodynamic changes induced by anaesthetic agents as result of oxidative transformation of these drugs in the liver during the process of elimination (Verma *et al,* 2018). A non significant increase was observed in serum urea nitrogen and creatinine level up to 60 min in animals of Dex_1 and up to 90 min in animals of Dex_2 group, later followed decreasing trend but remained above base line value. The increase in BUN and creatnine values in present study might be attributed to the temporary inhibitory effects of anaesthetic drugs on the renal blood flow and consequent decrease in glomerular filtration rate and increased urea production in liver (Kinjavdekar *et al,* 2000). Increased BUN and creatinine was also

reported following midazolam-dexmedetomidine in dogs (Santosh, 2011), xylazine propofol anaesthesia in dog (Surbhi *et al,* 2010) and dexmedetomidine, butorphanol and ketamine in dogs (Verma *et al,* 2018).

A non significant decrease in cortisol level was observed up to 60 min in animals of both Dex_1 and Dex_2 groups but later on followed an increasing trend (Table 5). Decrease in the level of cortisol in animals of both the groups might be attributed to direct inhibitory neuroendocrine response or indirect sedative and analgesic properties of dexmedetomidine which decreases the stress response when administered systemically as evidenced in previous studies (Raekallio *et al,* 2005). Alpha-2 agonists were known to influence the pituitary response and may decrease adrenocorticotropic hormone output (Masala *et al,* 1985). Similarly decreased cortisol level has also been reported following dexmedetomidine with etomidate and sevoflurane administration in dogs (Bisht *et al,* 2018).

It is concluded that the quality of sedation and analgesia remained dose dependent in animals of present study. Dexmedetomidine 4 µg/kg b.wt (Dex_2) produced a better sedation and analgesia. Transient changes recorded in physio-haemodynamic and haemato-biochemical profiles were within normal range, suggesting no deleterious effects on function of vital organs. Use of dexmedetomidine was found safe for sedation and analgesia in camels.

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CAMEL MIDDLE EAST NETWORK (CAMENET)

Middle East Network (CAMENET) was launched as a camel sector development initiative, to assist members in improving the prevention and control of camel diseases in the Middle East and GCC countries. Currently, the network includes nine members, i.e. United Arab Emirates (UAE), the Kingdom of Saudi Arabia (KSA), Jordan, Oman Sultanate, Iraq, Kuwait, Qatar, and the Kingdom of Bahrain. As one of the recommendations of the FAO/WOAH global framework for the progressive control of transboundary animal diseases (GF-TADs) a meeting on camel diseases, held in Abu Dhabi in February 2016, a network focusing on camel health and breeding in the Middle East Region, the Camel Middle East Network (CAMENET), was established. Abu Dhabi Agriculture and Food Safety Authority (ADAFSA) was assigned to build and host CAMENET. During the official visit of World Organisation for Animal Health (WOAH) Director General to the United Arab Emirates in March 2022, the Director General of ADAFSA and Director General of WOAH recognised the importance to have a revitalised CAMENET in the Region. In this context, it was planned to review and update the objectives and membership of CAMENET and establish its Technical Committee as communicated by Rashid Mohamed Al Mansoori, Chairman, Camel Middle East Network. The next stage of the CAMENET network's work is considered the most important part of the post-establishment phase and is concerned with planning focused activities and attracting possibilities. This network would help bringing the expected qualitative change in the promising roles of camels in the region through well-prepared strategies, which would help eliminating diseases thus it will promote not only health but would augment the innovative developments in camel related animal production and food security.

(Personal Communication to Dr. T.K. Gahlot)

PASTEURISED OR UNPASTEURISED CAMEL MILK CONSUMPTION

Camel milk has been consumed for centuries due to its medicinal and healing properties. The present study aims to investigate the consumption patterns of camel milk and perceived benefits and risks among adults in the United Arab Emirates. A self-administered online questionnaire was developed in English and Arabic languages and was completed online by 852 adults. Socio-demographic characteristics, camel milk consumption patterns and perceived knowledge of the benefits and risks of camel milk were investigated. About 60 % of the participants have tried drinking camel milk, but only a quarter (25.1 %) were regular consumers. The most consumed camel milk products after fresh milk were yoghurt and flavoured milk. The most popular additions to camel milk were honey, turmeric and sugar. Most consumers had less than one cup of camel milk per day (57.0 %). Camel milk consumers preferred it over other types of milk due to its nutritional value (66.4 %) and medicinal properties (39.3 %). Among consumers, 58.4 % reported consuming unpasteurised camel milk. Reasons included the belief that it is fresher (87.2 %), better for the immune system (41.6 %), and higher in nutrients (39.2 %). Overall, participants had inadequate knowledge about the health benefits of camel milk (7.11 ± 5.3 out of 25). Males and camel milk consumers had a significantly higher knowledge about the health benefits of camel milk compared to females and non-consumers ($P < 0.05$). Although positive perceptions were common, misperceptions appear to be prevalent among non-consumers. Consuming unpasteurised camel milk is a major public health concern, thus national regulations are essential.

(Source: Cheikh Ismail L, Osaili TM, Mohamad MN, Zakaria H, Ali A, Tarek A, Ashfaq A, Al Abdouli MA, Saleh ST, Daour RA, AlRajaby R, Stojanovska L, Al Dhaheri AS. Camel milk consumption patterns and perceptions in the UAE: a cross-sectional study. J Nutr Sci. 2022 Jul 21;11:e59. doi: 10.1017/jns.2022.55. PMID: 35912304; PMCID: PMC9305078)

INSTRUCTIONS TO CONTRIBUTORS

(Effective from year 2023)

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

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

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Le Hai[1], Rendalai Si[2], Fu-Cheng Guo[1], Jing HeI, Li Yi[1], Liang Ming[1], Jun-Wen Zhou[3], La Ba[3], Rigetu Zhao[3] and Rimutu Ji[1,2]

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Abstract and Key words: A single paragraph of no more than 2,500 keystrokes (characters plus spaces) that summarises the results in an understandable form using statistical evidence (P-values). Abbreviations are defined at first use in the ABSTRACT and again in the body of the manuscript. List up to 6 key words in alphabetical order and separated by a comma. Capitalise only proper nouns. Do not use abbreviations. Place the key words at the end of the Abstract.

Text: The proper text of the paper should commence after key words. The text should be divided into sections with headings, introduction, materials and methods, results, discussion, tables/illustrations and references.

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Materials and Methods: Should contain details regarding materials and brief account of procedures used. The manuscript must include a statement of institutional animal care and use or ethical committee, or country-specific equivalent, approval of all animal procedures. This statement should appear as the first item in MATERIALS AND METHODS and should specify which publicly available animal care and use standards were followed. A clear description of all biological, analytical and statistical procedures is required with each section denoted by a short descriptive title (i.e., Animals and sampling, Tests used, Treatment, Experimental design and analysis, etc). Materials used must include the product name and vendor at first mention. When a commercial product is used as part of an experiment, the manufacturer name and location must be given parenthetically and the generic name should be used subsequently. No ™, ®, or © symbols should be used. Sex, breed, age, species are included in the animal descriptions. Provide evidence of assay validation, or suitable published reference, as well as inter/intra-assay CV, as needed. Appropriate statistical methods should be used with experimental unit defined. Numbers of biological and experimental replicates should be stated. State the threshold for significance ($P < 0.05$) and definition of tendency if used. In case reports, the case record sheet should also be included in materials and methods.

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

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

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

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

Thesis: Rathod Avni. Therapeutic studies on sarcopticosis in camels (Camelus dromedarius). Unpublished Masters Thesis (MVSc), Rajasthan Agricultural University, Bikaner, Rajasthan, India. 2006.

Commercial booklets: Anonymous/Name. Conray-Contrast Media. IIIrd Edn., 1967; pp 12-15, May and Baker Ltd., Dagenham, Essex, England.

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

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

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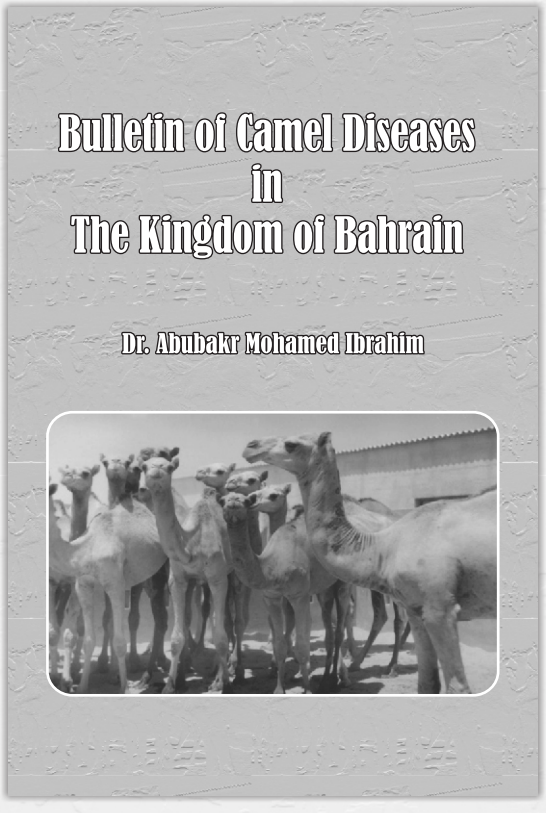
Bulletin of Camel Diseases in The Kingdom of Bahrain

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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