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Manuscripts and other related correspondence may be made to :

Dr. Tarun Kumar Gahlot
Editor, Journal of Camel Practice and Research
67, Gandhi Nagar West
Near Lalgarh Palace
Bikaner-334001, INDIA

Mobile : 0091-9414137029
Email : tkcamelvet@yahoo.com
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Scope of Journal of Camel Practice and Research

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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INTERNATIONAL YEAR OF CAMELIDS 2024 CONCLUDES

The International Year of Camelids 2024 (IYC2024) was organised across the world with a great enthusiasm. The chronology of events can be viewed on the website of FAO. The concrete recommendations about the ongoing and future targets of research and production aspects of camelids need to be elaborated. Some countries like India needs serious efforts to increase an alarming decline in the population of camels. Lokhit Pashu-Palak Sansthan (LPPS) led by Dr Ilse Kohler Rollefson raised the issue with the government and suggested few measures also to stop a sharp decline in the camel population, hitherto no possible solution has come up. Many events took place in different countries in the IYC2024. Dr. U. Wernery has also organised a one day dromedary scientific symposium at CVRL, Dubai and the abstracts of papers presented are published in this issue of JCPR. There are many camel scientists and vets who are continuously working with camels or camelids even after their superannuation age. They have made a great contribution towards camel health, production, reproduction and science. The IYC2024 should have felicitated such outstanding camel scientists or vets in this year to make the camelids specific year more relevant and meaningful. The conferences and workshops on camelids were organised at India, Saudi Arabia, USA, Morocco and Kuwait in IYC2024. Hopefully, the proceedings of these conferences and workshops will be made available to the readers who could not participate in these events. I really appreciate the North American Camel Ranch Owners Association (NACROA) in the IYC2024 for making tours of several countries involved in camel research, tourism and entrepreneurship. Douglas Baum and Valeri Crenshaw of NACROA not only updated their members but also facilitated other interested camel lovers through a virtual tour of their visits via facebook. I foresee that NACROA would become a useful knowledge updation bridge between cameleers in USA and the institutes engaged in camel research elsewhere through the information garnered by their visits.

The present issue of JCPR is a plethora of scientific informations on camelids. It has review papers on acid-base status, antimicrobial peptides, camel milk processing opportunities and analysis of articles published in JCPR in past 28 years through SCOPUS database. An emerging topic of Camel Assisted Services (CAS) in treatment, education and support programmes finds a place in this issue. Research on aquaporin 9 in different genital organs, larvicidal potency of many drugs against nasal bots, histomorphological peculiarities of tongue, intestinal coccidians in dromedary calves, classification of hepatic tumours, dexmedetomidine anaesthesia, *Salvadora oleoides* leaves feeding, comparison of camel milk with small ruminants, traits of Jalori breed and genomic analysis of domestic and wild Bactrian camels are other important research published in this issue.

The Journal of Camel Practice and Research (JCPR) now completes a journey of 31 years with the release of December issue. The members of the editorial board of JCPR join me in thanking all the authors and readers who provided a continuous support to the JCPR. I am sure that such a support would continue in the future also.

Wishing you all a Merry Christmas and Happy New Year

(Dr. Tarun Kumar Gahlot)
Editor

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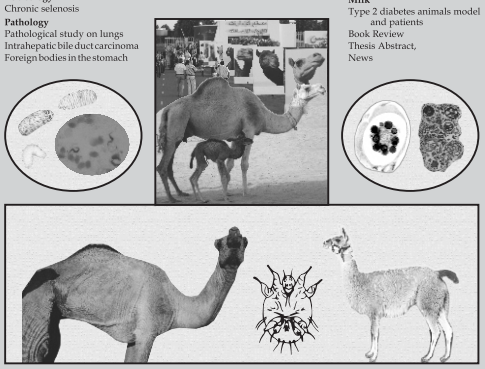

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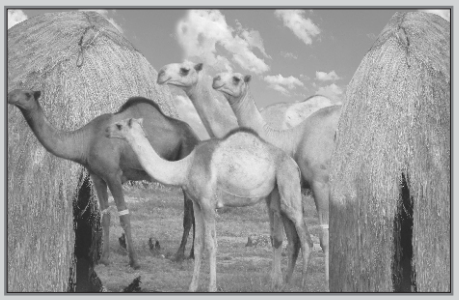

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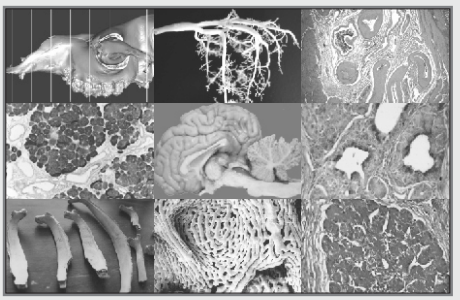

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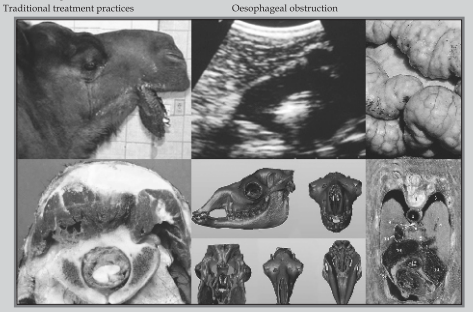

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ANALYSIS OF ARTICLES PUBLISHED IN THE JOURNAL OF CAMEL PRACTICE AND RESEARCH OVER A 28-YEAR PERIOD BASED ON SCOPUS DATABASE

Marwa H. Hassan[1] and Ashraf M. Abu-Seida[2,3]

[1]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, PO: 22110, Jordan

[2]Galala University, Animal Research Facility, Suez, Egypt

[3]Department of Surgery, Anaesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, Egypt

ABSTRACT

The Journal of Camel Practice and Research (JCPR) provides a unique platform for publishing submissions on New World and Old World camelids. This study analyses and categorises the characteristics of articles published in JCPR from 1996 to 2023, with a focus on the top 10 most cited articles. The top ten-cited papers were identified by their topic, year of publication, category, authors, institution, country and citations. Scopus database was used to determine details of the documents and their citations on April 3, 2024. A total of 1203 documents were published in JCPR during a 28-year span (1996-2023), with an average of 42.9 articles each year. All of these documents related to agricultural and biological sciences. These publications comprised 1031 original articles (85.7%), 82 conference papers (6.9%), 36 editorials (2.9%), 30 review articles (2.5%), 11 notes (0.9%), 8 brief surveys (0.7%), 4 erratum (0.3%) and one letter (0.1%). The top five years for camel research publication in JCPR were 1998 (62 documents), 2013 (61), 2021 (59), 2014 (58) and 2011 (57). Out of 56 countries, the top five concerned with JCPR publishing were India, Saudi Arabia, Egypt, United Arab Emirates and Iran. In terms of document counts, the most active researchers in JCPR, in decreasing order of ranking, are: Wernery, U., Gahlot, T.K., Kinne, J., Tharwat, M. and Faye, B.; affiliations: King Faisal University, ICAR-National Research Centre on Camel, Bikaner, College of Veterinary and Animal Science, Bikaner, Central Veterinary Research Laboratory- Dubai and Rajasthan University of Veterinary and Animal Sciences; sponsors: Deanship of Scientific Research-King Faisal University, King Abdulaziz City for Science and Technology, National Natural Science Foundation of China, Deanship of Scientific Research-King Saud University and Science Foundation of Inner Mongolia. The total number of citations for JCPR publications was 4721 in 2,678 documents, with an average of 3.9 citations per document. All of the top-cited documents were original research articles derived from United Arab Emirates (n= 3), India (n= 2), France, Argentina, Germany, United States of America and Iran (n= one article each). The most-cited papers studied camel milk, interdisciplinary production and fertility diagnostics, as well as pathological abnormalities. In conclusion, this bibliometric study is the first attempt at a multi-parameter analysis of the JCPR publications, including citation count. Authors could use the reported data to help them choose their future research projects and make a lasting contribution to the field of camelids health and production. It gives the editorial team information into the types of articles that JCPR readers find interesting, which will aid in the development of side ideas to eventually increase penetrance and the journal's quality.

Key words: Affiliation, bibliometrics, camelids, citation, funders, Journal of Camel Practice and Research

"Bibliometrics" refers to a collection of quantitative tools for analysing academic publications (Bellis, 2009). It employs statistical and mathematical approaches to track the overall trends of research in a certain topic (Zhu *et al,* 2021; Hernández-González *et al,* 2022).

"Citation analysis" is the most frequent bibliometric approach used in library and information science. Furthermore, all study domains employ bibliometrics tools to assess the influence of their research, a researcher, research group, institution, region, or publication (Pena-Cristobal *et al,* 2018; Toom, 2018).

The amount of citations received by an article is one way to assess its effect among its readers (Dhua *et al,* 2021). Citations, in which one publication refers to prior works, are the normal way for authors to recognise the source of their techniques, ideas

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and discoveries and they are frequently cited as an indicator of a study's value. Eugene Garfield released the Science Citation Index (SCI) 50 years ago, which was the first systematic attempt to track citations in scientific publications (Van Noorden *et al,* 2014). The number of citations a publication receives does not always reflect the quality of the research or the relevance of its authors (Cheek *et al,* 2006), but it has been suggested that articles with the most citations may be able to generate changes in practice, controversy, discussion and more research (Lefaivre *et al,* 2011).

Other things can distort citation numbers. The number of citations has grown, for example, while older works have had more time to accumulate citations. Biologists quote each other's work more frequently than physicists. Not all fields generate the same amount of articles. When determining the worth of a work, modern bibliometricians avoid using simple approaches such as counting citations and instead prefer to compare counts for articles of similar age and in relevant subjects (Van Noorden *et al,* 2014).

Although, camelids are understudied in scientific research, there has been a promising increase in camel research over the past five years (Kandeel *et al,* 2023; Abu-Seida *et al,* 2024). The JCPR is an exclusive journal that publishes submissions about New World and Old World camelids. Based on a recent study, JCPR is considered the top source of camel research all over the world (Abu-Seida *et al,* 2024). This journal provides an excellent platform for publishing camelid material in order to identify research gaps and keep camelid practitioners and academics up to speed on the most recent findings. JCPR is published by Camel Publishing House, India and its main subject is agricultural and biological sciences, category of animal science and zoology. The coverage on Scopus is extended from 1996 to 2023.

There have been many bibliometric evaluations of articles in the fields of veterinary medicine (Colombino *et al,* 2021; İnan, 2024), but the overall category of camel health and management has received little attention internationally (Kandeel *et al,* 2023; Masebo *et al,* 2023; Abu-Seida *et al,* 2024).

To our knowledge, no study has bibliometrically analysed the documents published in JCPR. With this backdrop, we aimed to identify, analyse and categorise the essential characteristics of the articles published in JCPR from 1996 to 2023, with particular focus on the top ten cited articles using Scopus database.

Materials and Methods

On April 3, 2024, a sources search was done to discover the documents finally published in JCPR and indexed in Scopus® using the search term "Journal of Camel Practice and Research". Scopus database was used to determine details of the documents and their citations. Regarding the top ten-cited articles, the individual article attributes were tabulated in a Microsoft Excel® spreadsheet. The year of publication, document type, topic, authors, affiliations, country of origin, sponsor and citations were collected. Descriptive statistics of all collected data were conducted. A full day was spent gathering all the data (3/4/2024). The top 10 highly-cited articles in JCPR were selected and analysed.

Results and Discussion

Journal of Camel Practice and Research published a total of 1203 documents during a 28-year span (1996-2023), with an average of 42.9 articles each year. All of these documents related to agricultural and biological sciences. These publications comprised 1031 original articles (85.7%), 82 conference papers (6.9%), 36 editorials (2.9%), 30 review articles (2.5%), 11 notes (0.9%), 8 brief surveys (0.7%), 4 erratum (0.3%) and one letter (0.1%), as shown in (Fig 1). The top 5 years for camel research publication in JCPR were 1998 (62 documents), 2013 (61), 2021 (59), 2014 (58) and 2011 (57).

Table 1 lists the top 5 authors, affiliations, countries and sponsors who contributed to JCPR publications during a 28-year period, according to the Scopus database. Out of 56 countries, the top 5 concerned with JCPR publishing were India, Saudi Arabia, Egypt, United Arab Emirates and Iran.

On April 3, 2024, the total number of citations for JCPR publications was 4721 in 2,678 documents, with an average of 3.9 citations per document. The document types cited the articles published in JCPR included; 2191 original articles, 235 reviews, 147 book chapters, 63 conference papers and 13 books. In terms of language, JCPR-published papers were mentioned in documents written in English (2,596), French (23), Spanish (20), Chinese (15), German (7), Persian (7), Turkish (5), Bosnian (3), Croatian (3) and Portuguese (3).

Table 2 displays the top 5 most-cited authors, affiliations, countries and sponsors who contributed to JCPR articles during a 28-year period, as determined by the Scopus database. The cited sources included; journals (2498), books (157), conference proceedings (13) and book Series (10). The top 5 most

cited journals for publications published in JCPR were: Journal of Camel Practice and Research (468 citations), Tropical Animal Health and Production (91), Indian Journal of Animal Sciences (58), Veterinary Practitioner (49) and Animal Reproduction Science (42).

Table 3 shows the top 10 most-cited documents of all categories published during the research period. The top 10 most-cited documents garnered a total of 449 citations till April 3, 2024. All of these documents were original research articles published between 1996 and 2007. These articles derived from United Arab Emirates (n= 3), India (n= 2), France, Argentina, Germany, United States of America and Iran (n= one article each).The top article was sourced from India and quoted 71 times, while the tenth article was derived from the United States of America and cited 36 times (Table 3).

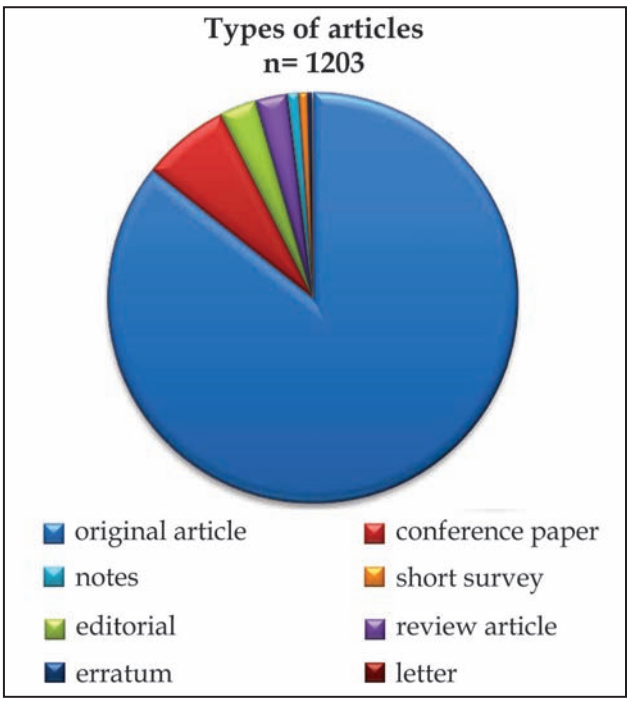

Fig 1. Type of articles published in JCPR during a 28-year period based on Scopus database and their distribution.

After 30 years of establishment, JCPR has become a well-known international publication in the field of camel practice and research. Our results indicate the unique nature and widespread of JCPR all over the world and its positive impact in the field of camelids health and production.

There are several databases which provide the bibliometric information for an article. Web of Science, CrossRef, Scopus and Google Scholar are some of the most widely used sites. Each has a different catchment region and it has been noticed that the number of citations they reveal for a given article at any one moment might alter (Kulkarni *et al*, 2009). Elsevier Corporation owns "Scopus" which is a citation database that includes bibliographic information, abstracts and citations of academic journal articles. Scopus covers a wide range of research topics, including science, technology, medicine, social sciences and the humanities. Therefore, we preferred Scopus database in the present bibliometric study.

According to our findings, the bulk (85.7%) of JCPR's published content throughout the study period is comprised of original works. As a result, it comes as no surprise that all of the top 10 most-cited papers are original articles. The present data also show that JCPR is steadily improving its share of

Table 1. The top 5 authors, affiliations, countries and funders shared in articles published in JCPR during a 28-year period based on Scopus database.

No	Authors		Affiliations		Countries		Funders	
	Name	Number of articles	Name	Number of articles	Name	Number of articles	Name	Number of articles
1	Wernery U.	83	King Faisal University	135	India	289	Deanship of Scientific Research, King Faisal University	9
2	Gahlot T.K.	64	ICAR - National Research Centre on Camel, Bikaner	114	Saudi Arabia	248	King Abdulaziz City for Science and Technology	6
3	Kinne J.	36	College of Veterinary and Animal Science, Bikaner	102	Egypt	141	National Natural Science Foundation of China	6
4	Tharwat M.	34	Central Veterinary Research Laboratory- Dubai	90	United Arab Emirates	132	Deanship of Scientific Research, King Saud University	5
5	Faye B.	33	Rajasthan University of Veterinary and Animal Sciences, Bikaner, India	64	Iran	91	Natural Science Foundation of Inner Mongolia	4

Table 2. The top 5 most highly cited authors, affiliations, countries and funders who contributed to JCPR articles during a 28-year period, as determined by Scopus database.

No	Authors		Affiliations		Countries		Funders	
	Name	Number of citationss	Name	Number of citations	Name	Number of citations	Name	Number of citations
1	Faye B.	94	King Faisal University	154	Saudi Arabia	433	Deanship of Scientific Research, King Saud University	57
2	Wernery U.	97	ICAR - National Research Centre on Camel, Bikaner	126	India	425	National Natural Science Foundation of China	43
3	Tharwat M.	54	Al Qassim University	106	Egypt	360	Deanship of Scientific Research, King Faisal University	27
4	Kataria A.K.	38	CIRAD	101	Iran	225	United Arab Emirates University	27
5	Khalafalla A.I.	36	King Saud University	99	United Arab Emirates	225	European Commission	23

Table 3. The top 10 most cited articles published in JCPR over a 27-year period based on Scopus database.

No	Title	Country of origin	Affiliation	Authors	Type of article	Year of publication	Citations (as on April 3, 2024)
1	Effect of camel milk on glycemic control, risk factors and diabetes quality of life in type-1 diabetes: A randomised prospective controlled study	India	National Research Centre on Camel, Bikaner	Agrawal *et al*	Original article	2003	71
2	Selected vitamins and fatty acid patterns in dromedary milk and colostrum	Germany	University of Veterinary Medicine Hannover Foundation	Stahl *et al*	Original article	2006	53
3	Ultrasonographic changes of the reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy.	United Arab Emirates	Veterinary Research Centre, Abu Dhabi	Tibary and Anouassi	Original article	1996	51
4	Camel milk, the white gold of the desert	United Arab Emirates	Central Veterinary Research Laboratory	Wernery	Original article	2006	47
5	Electroejaculation in llama (*Lama glama*)	Argentina	Universidad de Buenos Aires	Director *et al*	Original article	2007	39
6	Milk yield performance of dromedaries with an automatic bucket milking machine	United Arab Emirates	Central Veterinary Research Laboratory	Wernery *et al*	Original article	2004	39
7	Investigations on a new pathological condition of camels in Ethiopia	France	Campus International de Baillarguet	Roger *et al*	Original article	2000	39
8	Studies on normal haematological and biochemical parameters of turkmen camel in Iran	Iran	Shiraz University, Shir	Rezakhani *et al*	Original article	1997	38
9	Effect of raw camel milk in type 1 diabetic patients: 1 Year randomised study	India	S.P. Medical College, Bikaner	Agrawal *et al*	Original article	2005	36
10	Approach to diagnosis of infertility in camelids: Retrospective study in alpaca, lamas and camels	United States of America	Washington State University	Tibary *et al*	Original article	2001	36

original papers. Similarly, a recent analysis indicated that JCPR is the top source for publishing camel research from throughout the world (Abu-Seida *et al,* 2024).

It's natural that the bulk of the articles (289, 24.02%) published in JCPR would have come from Indian institutes. This is owing to the country's large populations of camelids and scholars who study them as well as JCPR is recognised as the official publication of camel research and practice in India.

Saudi Arabia is the second-largest publisher of JCPR articles (248, 20.62%). The observed result is consistent with prior research on national contributions to camel research (Kandeel *et al,* 2023; Abu-Seida *et al,* 2024). Also, it was no surprise that Saudi Arabia was among the top 5 nations, affiliations and supporters. Saudi Arabia has achieved significant progress in higher education, particularly in research, development and knowledge generation during the last decade. The Saudi government is working hard to offer adequate funds to the education industry and construct new academic institutions (Pavan, 2016). Funding agencies and research organisations have significant roles in furthering scientific research (Azeem *et al,* 2021). Furthermore, Saudi Arabia has been classified as having one of the greatest proportion of camels (Faye, 2020). Although, Egypt is classified as a camel nation, it is experiencing falling growth and has a smaller percentage of camel research than India and Saudi Arabia (Faye, 2020). The present rankings include a disclaimer. It is not permissible to generalise these ratings and evaluate research capabilities and outcomes only on the basis of these data. As a result, these findings are particular to JCPR and do not provide broad conclusions regarding the authors, institutions' or countries, research output.

The citation impact metric assesses the number of citations in scientific works. It is the sum of citations to publications divided by the number of articles (Moed, 2010). JCPR averages 3.9 citations per document. A recent research reported a similar finding (Abu-Seida *et al,* 2024). JCPR-published articles were cited in publications written primarily in English, but also in French, Spanish, Chinese, German, Persian, Turkish, Bosnian, Croatian and Portuguese. A notable fraction implies that JCPR's popularity goes beyond nations with an English-speaking population and has a worldwide reach. In addition, it is unsurprising that English is the dominant language in camel research citations. English is the global language of science and the vast majority of title sources use it in their published works (Abu-Seida *et al,* 2024).

The top ten-most cited articles are all original and come from various nations and affiliations. This is significant because original publications on key areas of study are more likely to be cited repeatedly. The most-cited papers studied camel milk (S. No. 1,2,4,6 and 9), interdisciplinary production and fertility diagnostics (S. No. 3, 5 and 10), as well as pathological abnormalities (S. No. 7 and 8). Therefore, researchers in the field of camelids must pay attention to these topics in their future research

The present observations on citation analysis have offered critical insights into the citation requirements. The publications with the most citations focus on hotspots for study and have a long-term influence on camel research and practice. This sort of study can be conducted at frequent intervals to depict a trend rather than simply a snapshot in order to be informative. In this regard, highly-cited articles differ significantly from 'regular' cited papers. Typically, they are written by a large number of scientists, with worldwide participation (Aksnes, 2003).

Readers are cautioned to be aware of the study's limitations. The authors want to provide a brief summary of the citation analysis of JCPR. The length of time that has gone since an article was published might affect how many citations it receives. A newly published work on an interesting topic may acquire more citations in the future than one that is reviewed soon after publication. This problem has been somewhat addressed by focusing primarily on manuscripts published until 2023.

This research is the first attempt at a multi-parameter analysis of the JCPR publications, including citation count. Authors could use the reported data to help them choose their future research projects and make a lasting contribution to the field of camelids health and production. It gives the editorial team information into the kinds of articles that JCPR readers find interesting, which will aid in the development of side ideas to eventually increase penetrance and the journal's quality.

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ACID-BASE STATUS IN CAMELS-A COMPREHENSIVE REVIEW AND ANALYSIS

Nawal Mohamed Elkhair[1]

[1]Department of Biomedical Sciences, College of Veterinary Medicine, King Faisal University, 31982, Al-Ahsa, Saudi Arabia
[1]Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, 13314, Shambat, Sudan

ABSTRACT

The study was aimed to analyse healthy and diseased camels' acid-base and electrolyte profiles to understand how these variations can lead to metabolic, systemic and respiratory disorders. The normal blood pH of camels ranges between 7.35 and 7.45 (7.44 ± 1.04). If this range is exceeded or decreased, it may cause metabolic and respiratory acidosis alkalosis, which may hinder the normal physiological functions of the body organs. Acid-base disorders have been observed in association with severe diseases such as myocardial infarction, trypanosomiasis, tick paralysis, tick-borne diseases, helminthic infections, bent neck syndrome, barter syndrome, Gitelman syndrome, Liddle syndrome, Glucocorticoid-remediable aldosteronism, pneumonia, bronchitis, left ventricular contractility, asthma, syncope, interstitial lungs disease and Peripheral and circumoral paresthesia. It was concluded that these imbalances can impact the immune system function in camels and increase susceptibility to infections, while fluctuation in essential ion levels may cause paralysis by affecting muscle contractions and relaxation processes.

Key words: Acid-base stauts, camel

The term "acid-base balance" describes the acidity and alkalinity of blood needed to sustain biological functioning (Zhang *et al,* 2022).

Camels show their physiological adaptations for arid environments and maintain their acid-base homeostasis by keeping the blood pH within narrow limits, ranging from 7.35 to 7.45 (Zouari *et al,* 2020). Compared to other animal species, camels have well-developed respiratory and renal systems to conserve water and maintain electrolyte balance (Wilson, 2012). The camel's kidneys excrete excess acids or bases in urine, contributing to acid-base equilibrium. The diet, primarily consisting of dry vegetation, can influence their acid-base status. However, they are adapted to efficiently utilise these components, ensuring their acid-base balance remains within normal limits under typical feeding conditions (Fesseha and Desta, 2020).

Body function depends on maintaining the proper acid-base balance and the arterial blood pH and pCO_2 greatly impact the pH levels of intracellular and interstitial fluid (Asopa *et al,* 2021).

An artery's blood sample assesses the pH level, or acid-base balance, as it carries oxygen-rich blood from the lungs into the body. The effectiveness of the lungs' ability to take in air and convert it into blood is measured by an arterial blood gas (ABG) test.

The initial value to be studied in assessing acid-base disorders is pH from ABG. It is followed by defining a main disturbance, figuring out the serum anion gap and assessing compensation. Metabolic acidosis, respiratory acidosis, metabolic alkalosis and respiratory alkalosis are the four basic acid-base diseases (Stegeman *et al,* 2020).

In the normal physiological state, the blood pH of healthy dromedary camels is about 7.44 ± 1.04. Clinically, metabolic acidosis is characterised by a pH of less than 7.35 and a low HCO^{3-} level. The anion gap aids in identifying the root cause of metabolic acidosis (Asopa *et al,* 2021). Strong ion difference (SID) was used to assess acid-base status in healthy camels and for the diagnosis of metabolic acidosis (Elkhair and Hartmann, 2010).

Uremia, diabetic ketoacidosis and salicylate poisoning can all result in an increased anion gap metabolic acidosis. It mainly happens in working camels as a result of concentrated overfeeding. Over the past few years, this ailment has become one of camels' most prevalent digestive issues. Acidotic camels display tympany, doughy rumen, anorexia, dehydration and recumbency (McCaffrey and Allinson, 2021).

Metabolic alkalosis in camels can be brought on by increased serum HCO^{3-} concentrations

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(overabundance of feed high in alkali), prolonged vomiting, or respiratory disorders, which impacts their acid-base equilibrium and lower arterial blood pH and shift it into the alkaline range (Elkhair *et al,* 2018).

When there is excess CO_2 in the blood, the blood pH drops, leading to respiratory acidosis in camels. Several environmental factors, including heat, dehydration, a high-fibre diet, exercise and heat stress, may contribute to respiratory acidosis in camels. Serum sodium, creatinine, urea and plasma hormone levels are significantly altered by dehydration, which affects the levels of ABGs (Mohamed *et al,* 2021).

Although camels' special digestive system allows them to absorb nutrients effectively, the fermentation of fibre can release volatile fatty acids and CO_2 that can enter the circulation. Respiratory acidosis can result from increased CO_2 generated by intense physical activity (Tharwat, 2021).

Respiratory alkalosis in camels occurs when the partial pressure of CO_2 decreases, leading to increased blood pH. It can be caused by panting, stress, hyperventilation at high altitudes, or pain or anxiety. Camels are adapted to hot and arid environments and their rapid breathing can result in excessive CO_2 loss (Elsayed, 2020).

This review study was aimed to examine healthy and diseased camels' acid-base and electrolyte profiles and to investigate how variations can contribute to metabolic, systemic and respiratory disorders.

Literature Review

Studies examining blood gases, acid-base and electrolyte profiles have demonstrated that the acid-base state in healthy dromedary camels varies with age. In comparison to older calves (11–21 days), younger calves (1-4 days) have lower potassium (K^+), higher sodium (Na^+) and higher chloride (Cl^-) concentrations. Older calves also have lower haemoglobin oxygen saturation (sO_2) levels and greater partial pressure of carbon dioxide (pCO_2). These results indicate an age-related influence on these parameters in healthy dromedary calves' first three weeks of life (Osman *et al,* 2023).

Subsequent investigations highlight the significance of trace elements for camel productivity and health, including copper, zinc, iron and selenium. Due to the potential effects of trace mineral toxicities or deficiencies on camel growth, metabolism and productivity, it is important to assess and maintain these (Abdelrahman, 2022).

Studies have indicated that blood pH in dehydrated or exercised camels stays within the normal range despite these stressors, demonstrating the critical function the acid-base balance plays in camel health. Camel survival in the face of stressors such as exercise and dehydration depends on maintaining the acid-base balance.

The blood pH remains within the usual range even in camels devoid of water, demonstrating the adaptability of camels to such circumstances. This equilibrium is essential to camels' overall physiological performance and homeostasis, enabling them to adjust to external stresses and preserve their health despite difficult conditions (Abdoun *et al,* 2012; Okab *et al,* 2012).

In camels, acid-base imbalances can result in Myocardial infarction (MI). Numerous factors that alter acid-base homeostasis in camels may give rise to MI. Studies indicated that modifications in strong electrolytes like sodium, potassium and chloride, as well as weak acids like proteins and phosphate, may be the cause of disruptions in the acid-base state of camels, including hyperchloraemic acidosis and hypoproteinaemic alkalosis (Elkhair and Hartmann, 2010).

The hallmark of metabolic acidosis is a drop in blood pH, which can bring several abnormalities. Furthermore, camel dehydration has been demonstrated to affect their acid-base balance, underscoring the complex connection between water deprivation and the physiological difficulties of these animals (Abdoun *et al,* 2012).

Acid-base imbalances can bring on several dangerous disorders. Conditions including uremia, diabetic ketoacidosis and significant loss of bicarbonate ions through the gastrointestinal system owing to diarrhoea can result in metabolic acidosis, which is defined by a lack of HCO_3^-, leading the blood to be too acidic (Seifter and Chang, 2017).

Conversely, disorders such as Cushing's disease and consuming large amounts of HCO_3^- or antacids can result in metabolic alkalosis, an excess of HCO_3^- that makes the blood excessively alkaline. These acid-base abnormalities can majorly impact health and may be a factor in the onset or aggravation of certain diseases (Seifter and Chang, 2017).

An important factor in camel trypanosomiasis is acid-base imbalances. Studies on *Trypanosoma evansi*-infected camels have demonstrated that these animals can have severe parasitemia, which can cause physiological alterations that impact acid-base

balance (Ahmadi-hamedani *et al,* 2014). Furthermore, research has demonstrated the effect of Trypanosoma infection on the oxidative status in camel blood, demonstrating notable alterations in antioxidant levels between infected and uninfected animals (Darwish *et al,* 2023).

Additionally, studies have shown that normocytic and normochromic anaemia, lymphocytosis and changes in acute-phase proteins like alpha-1 acid glycoprotein can occur in camels infected with trypanosomiasis. These findings highlight the intricate interactions between the disease and haematological parameters that affect the acid-base status of infected camels.

An acid-base imbalance and altered ABGs levels can exacerbate urinary tract infections (UTIs) and other urinary disorders, including cystitis, urine retention, hydronephrosis, red urine, renal masses, ruptured bladder and ruptured urethra in camels. Studies reveal that oxidative stress—associated with an acid-base imbalance—contributes to UTIs in dromedary camels.

A disruption in the normal redox state of cells caused by an acid-base imbalance can have harmful effects by producing reactive oxygen species (ROS), which can cause tissue damage and malfunction. The UTIs in camels may partly be caused by this oxidative stress-related disruption of the redox state (El-Deeb and Buczinski, 2015; Tharwat, 2023).

Acidosis and alkalosis in camels can result from electrolyte abnormalities through various pathways. Strong ion acidosis in the event of acidosis can be caused by certain electrolyte imbalances, such as hyponatremia (reduction in strong cation concentration) or increases in strong anions, such as L-lactate, D-lactate and ketoacids. High protein concentrations such as phosphate, albumin and globulin can also cause nonvolatile buffer ion acidosis. These abnormalities may cause metabolic acidosis in camels, detrimental to their acid-base equilibrium.

In camel calves, hyper D-lactatemia may result in metabolic alkalosis without dehydration. In addition, diseases such as pneumonia, severe pulmonary emphysema, depression of the respiratory center and left-sided heart failure can cause respiratory alkalosis. Alkalosis can result from these electrolyte imbalances and disruptions because they can upset the camels' acid-base balance (Ali *et al,* 2012; Constable *et al,* 2017).

Electrolyte abnormalities in camels are frequently caused by diseases and treatments that disrupt the body's normal fluid balance. The most prevalent electrolyte imbalance, hyponatremia, can be caused by several diseases that result in elevated antidiuretic hormone (ADH) levels and decreased circulating blood volume, such as hepatic cirrhosis and congestive heart failure. Conversely, hypernatremia is usually brought on by an excess of hypertonic saline, certain drugs such as lithium and excess fluid loss through the skin and gastrointestinal tract (Faye *et al,* 2018).

Methodology

Search Study

This review and literature search considered the most current publications and online abstracts on acid-base status in healthy and ill camels. Google Scholar, Springer, PubMed, Medscape, Medline and Science Direct were used in order of priority. The terms were carefully chosen from the research published between 2010 and 2024.

These keywords included were "Acid-base imbalance in camels," "Metabolic and respiratory acidosis in camels," "Metabolic and respiratory alkalosis in camels," "Electrolyte imbalance," and "ABGs and pH level in camels".

Selection criteria

Studies of camels who underwent acidosis and alkalosis due to acid-base, ABGs and electrolyte level imbalance included the identification of haemostatic parameters that are associated with acidic and basic conditions of the body and to reduce the rate of systemic and metabolic acidosis/ alkalosis, caused by environmental elements fluctuation. These studies were selected by following the inclusion and exclusion selection criteria.

Inclusion criteria

The study assessed relevance by including peer-reviewed articles from 2010-2024, reviews on metabolic acidosis and alkalosis and studies on hemostatic strategies for maintaining acid-base levels in camels to enhance robustness and replicability.

Exclusion criteria

The study excluded articles published in languages other than English due to unclear reporting of relevant information and ambiguity in its abstract.

Metabolic Acidosis

Hepatic Lipidosis

Metabolic acidosis in camels, particularly impacting pregnant and lactating camels, can result

in hepatic lipidosis, a disorder characterised by the buildup of hepatic fat in the liver and has been commonly seen in llamas and alpacas due to other metabolic abnormalities (Anderson *et al,* 1994; O'Conor Dowd, 2014).

Metabolic acidosis in camels leads to high fatty acid mobilisation, hepatic triglyceride synthesis and low-density lipoprotein secretion, causing hyperlipemia. Symptoms include lethargy, hypercholesterolemia, recumbency, ketonuria, weight loss and azotemia, causing liver dysfunction, muscle damage and anorexia (Foreman, 2019; Saun, 2023).

Helminth Infections

Metabolic acidosis can increase helminth infections by affecting the host's immune response and metabolic homeostasis, potentially impairing the host's ability to fight them. However, if it interferes with this immune response, it can create a favourable environment for helminth survival and growth (Wiria *et al,* 2014 and Kokova *et al,* 2021).

Tick paralysis

Metabolic acidosis has been observed in association with tick paralysis, in which ticks generate neurotoxins during feeding, gradually paralysing the host. Metabolic acidosis can exacerbate the neurological symptoms of tick paralysis by compromising the host's immune system and physiological homeostatic mechanisms (Cope, 2018).

A camel's physiological environment can drastically change by metabolic acidosis, impacting intracellular calcium levels and potassium channels. Muscle contraction and neuromuscular transmission are intimately related to variations in intracellular calcium concentrations. Tick neurotoxins alter potassium channels, influencing intracellular calcium levels and causing an imbalance that obstructs regular cellular functions. Increased intracellular calcium levels from abnormal potassium channel function can interfere with synaptic transmission, weaken muscles and ultimately cause the paralysis that tick-infested hosts experience (El-Aly *et al,* 2024; Chand *et al,* 2016).

Bent neck syndrome

Acidosis inhibits muscle function and disrupts the electrolyte balance, which can result in weakening and unusual spasms of the muscles. These muscular anomalies might show up as lateral deviation of the neck in bent-neck syndrome due to muscle imbalances and weakening brought on by metabolic disruptions (Al-Sobayil and Mousa, 2009).

Trypanosomiasis

Changes in pH resulting from metabolic acidosis could affect the production of cytokines and immune cell activity. These two factors are critical for building an effective response against infections. A weakened immune system may result from metabolic disorders and nutritional changes due to acidosis, leaving the host more vulnerable to parasite diseases like trypanosomosis; this causes the haematocrit count, haemoglobin and red blood cell count to decrease and leads to anaemia (Baldissera *et al,* 2015; de Aquino *et al,* 2021; Tharwat, 2021).

Metabolic alkalosis

Bartter syndrome

Bartter syndrome can lead to metabolic alkalosis in camels, a rare kidney disease characterised by potassium wasting, salt-wasting nephropathy and abnormal electrolyte levels that compromise renal tubular function and acid-base hemostasis, which is typified by elevated plasma HCO_3^- concentration and systemic pH (Heilberg *et al,* 2015; Mabillard and Sayer, 2018).

Hyperplasia of biliary epithelium

In camels, metabolic alkalosis can impair liver function, which may impact the control of bile secretion and production. Biliary epithelial cells may proliferate and multiply more readily in the liver due to cellular reactions brought on by metabolic alkalosis. Metabolic alkalosis-induced pH imbalances can affect gene expression and cellular communication pathways in the liver, possibly resulting in biliary epithelial hyperplasia. The biliary epithelium may thicken and expand due to this aberrant cellular response (Tharwat, 2020).

Gitelman syndrome

Electrolyte abnormalities like those seen in Gitelman syndrome in camels may be brought on by metabolic alkalosis. Metabolic alkalosis-induced pH imbalances can impact the kidneys' ability to reabsorb electrolytes like magnesium and potassium and decreased excretion of calcium. In camels with metabolic alkalosis, these electrolyte imbalances can lead to hypokalemia and metabolic alkalosis, which are important aspects of Gitelman syndrome (Mabillard and Sayer, 2018).

Liddle syndrome

Because of a mutation in epithelial sodium channels (ENaC) caused by metabolic alkalosis, the channel is always present on the apical membrane

of renal tubular cells, preventing its destruction by the ubiquitin-proteasome system. Raised ENaC levels on the membrane cause increased water retention, salt resorption and a condition similar to hyperaldosteronism. Increased salt reabsorption contributes to hypertension and abnormal electrolyte levels associated with Liddle syndrome (Rodby, 2023).

Glucocorticoid-remediable aldosteronism (GRA)

Metabolic alkalosis can upset electrolyte balance and acid-base homeostasis, which may impact aldosterone synthesis. Under the control of ACTH, ectopic aldosterone synthase activity in the adrenal cortex results from a chimeric gene duplication in GRA. Increased aldosterone production is a result of this dysregulation, which also contributes to the hypertension and electrolyte abnormalities typical with GRA (Halperin and Dluhy, 2014).

Respiratory acidosis

Pneumonia and Bronchitis

Decreased lung function caused by respiratory acidosis makes it more difficult for the camel to clear infections from its respiratory system. Increased body fluid acidity can also impede an ineffective defense against disease. Due to the combination of decreased immunity, compromised lung function and favorable conditions for bacterial proliferation caused by respiratory acidosis, the risk of pneumonia in camels has significantly increased (Nahed *et al,* 2016).

Reduced left ventricular contractility

Reduced actin-myosin interactions are necessary for the heart muscle to contract efficiently. Decrease calcium binding to troponin C, a crucial regulatory protein in muscle contraction, is linked to decreased left ventricular contractility during respiratory acidosis. Reduced left ventricular contractility results from the heart's diminished capacity to contract strongly due to these problems with calcium management and contractile mechanisms.

Additionally, despite the lower left ventricular contractility, respiratory acidosis causes hemodynamic alterations, such as increased venous return (which equals cardiac output). This compensatory mechanism maintains cardiac output despite the detrimental effect on contractility by raising heart rate and lowering systemic vascular resistance (Tharwat *et al,* 2014).

Asthma

Asthma exacerbations resulting in respiratory acidosis can cause hypercapnia, which can be made worse by therapeutic oxygen treatment. It can also alter the acid-base balance and elevate transcutaneous pCO_2 levels.

Respiratory acidosis, a common symptom of severe asthma, can lead to hypoxemia, circulatory compromise and lactic acidosis. Chronic hypocapnia can exacerbate acid-base imbalances, causing non-anion gap acidosis. Severe asthma exacerbations can also result in metabolic acid-base abnormalities, such as high anion gap or non-anion gap metabolic acidosis (Vasileiadis *et al,* 2019).

Respiratory alkalosis

Interstitial lungs disease

Respiratory alkalosis, a disorder causing excessive breathing and low blood carbon dioxide levels in camels can lead to interstitial lung disease due to its impact on the lungs. It can cause changes in blood pH, electrolyte levels and oxygen and carbon dioxide levels, potentially affecting lung tissue integrity and contributing to interstitial lung disease. It can also cause vasodilation and damage to fragile interstitial tissue (Brinkman and Sharma, 2018).

Syncope

In respiratory alkalosis, a drop in $paCO_2$ and an increase in pH levels that follow might impact cerebral blood flow and cause cerebral vasoconstriction; however, it is a rare condition reported in camels. Brain blood flow changes can cause syncope, dizziness and mental disorientation, among other neurological symptoms. Reduced cerebral blood flow from the lower $paCO_2$ levels may result in syncope or a brief loss of consciousness (Kohli *et al,* 2021).

Peripheral and circumoral paresthesia

Blood-ionised calcium levels can be impacted by respiratory alkalosis, characterised by a drop in $paCO_2$ and a rise in the following pH levels. Reduced levels of ionised calcium (Ca^{++}) in the extracellular fluid can result from alkalosis due to enhanced protein binding. This decrease in ionised calcium can have an impact on nerve excitability and function, resulting in symptoms such as circumoral paresthesia (tingling around the mouth) and peripheral paresthesia (tingling or numbness in the limbs), a very common symptom reported in respiratory alkalosis (James and Evans, 2023).

Discussion

Animals maintain homeostasis through physiological adjustments, including maintaining

acid-base balance. Blood function relies on maintaining this balance, with arterial blood pH and pCO_2 significantly influencing intracellular and interstitial fluid pH levels.Anion gap analysis can be used to detect metabolic acidosis, which is defined by a pH below 7.35 and low HCO_3^- levels. Healthy camels have a normal blood pH of 7.44 ± 1.04. Increased serum HCO_3^- concentrations, prolonged vomiting, or respiratory conditions cause metabolic alkalosis in camels, which affects their acid-base equilibrium and lowers arterial blood pH.

Heat, dehydration, a high-fibre diet, exercise, heat stress and respiratory disorders can all affect camels' blood levels of CO_2, which can change their plasma hormone levels, creatinine, urea and salt levels. It can result in respiratory acidosis in camels. Because of their high blood pH, quick breathing and reduced pCO_2, camels suffer respiratory alkalosis, which leads to significant CO_2 loss in hot and dry conditions.

Camel health depends on the proper acid-base balance, which maintains normal blood pH levels in the face of stresses like exercise and dehydration and ensures the animals' survival. Acid-base imbalances in camels, such as hypoproteinaemic alkalosis and hyperchloraemic acidosis, can result in alterations in strong electrolytes and weak acids, which can cause myocardial infarction.

Illnesses and treatments frequently disrupt fluid balance, leading to electrolyte imbalances in camels. Hypernatremia is brought on by excessive saline, lithium and fluid loss, whereas hyponatremia is typically caused by high ADH levels and decreased blood volume.

Strong ion acidosis brought on by hyponatremia or elevated anions and nonvolatile buffer ion acidosis brought on by high protein concentrations are two examples of electrolyte imbalances that can result in acidosis and alkalosis in camels. Reactive oxygen species (ROS) are produced when an acid-base imbalance damages and malfunctions tissue. The UTIs in camels may be caused by this disturbance linked to oxidative stress.

Metabolic acidosis in camels, especially in pregnant and lactating mothers, can lead to hepatic lipidosis, a liver fat buildup disorder triggered by high fatty acid mobilisation, elevated triglyceride synthesis and low-density lipoprotein secretion. It can impair the host's physiological homeostatic mechanisms and immune system; symptoms may arise; therefore, it increases the susceptibility to various pathogenic infections such as helminthic infections, tick-borne diseases, trypanosomosis and tick paralysis, a condition in which the host becomes paralysed as a result of neurotoxins that ticks release while eating.

In camels, metabolic alkalosis can affect bile secretion and production and the rare kidney disease Bartter syndrome. Biliary epithelial hyperplasia may cause the liver to thicken and enlarge. Moreover, metabolic alkalosis can contribute to electrolyte abnormalities like those seen in Gitelman syndrome by impairing the kidneys' capacity to reabsorb electrolytes like potassium and magnesium, resulting in hypokalemia and metabolic alkalosis.

Camels with respiratory acidosis suffer from impaired lung function, which may compromise their immunity to infections and increase their risk of pneumonia. Additionally, pneumonia is exacerbated by decreased left ventricular contractility and calcium binding to troponin C. Hypercapnia can result from asthma flare-ups and can get worse when oxygen therapy is administered. Acid-base abnormalities, hypoxemia and metabolic acid-base imbalances can also be caused by severe asthma.

Interstitial pulmonary diseases (IPDs) can result from respiratory alkalosis, a condition that causes camels to breathe excessively and have low blood carbon dioxide levels. The IPDs affect blood pH, electrolyte levels, O_2 and CO_2 levels, potentially affecting lung tissue integrity. Additionally, IPDs may affect cerebral blood flow, leading to syncope, vertigo and confusion. The IPDs also alters the levels of blood-ionised calcium, which excites nerves and produces symptoms including peripheral and circumoral paresthesia.

The acid-base status of camels is a complex issue due to the lack of published studies and the inability to accurately evaluate and compare findings.

Conclusion

This review study comprehensively analysed the acid-base status in healthy and diseased camels, revealing a complex interaction between ABGs, electrolytes, acidity and alkalinity. These interactions can lead to or exacerbate serious disorders in camels, such as metabolic and respiratory acidosis and alkalosis. Furthermore, these imbalances can impact the immune system, potentially increasing susceptibility to pathogenic infections. Additionally, fluctuating levels of essential ions may contribute to paralysis by affecting the smooth and skeletal muscle contractions and relaxation processes.

Recommendations

Comparative studies should analyse acid-base state variations in healthy camels and those with respiratory or renal dysfunction diseases. Long-term research should observe acid-base balance during physiological phases, using metabolic profiling to identify biomarkers and examining breed-specific variances and genetic variants.

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Conflict of Interest

Author declares no conflict of interest.

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ANTIMICROBIAL PEPTIDES OF CAMEL MILK - A REVIEW

Kun Dai[1], Ming Lu[2], Jie Bai[3], Shaobin Li[1] and Zhaohui Xie[2]

[1]Shool of Life Science, Yangtze Universty, Jingzhou, China

[2]School of Life Science and Bioengineering, Henan University of Urban Construction, Pingdingshan, China

[3]Institute of Animal Husbandry Research, Henan Academy of Agricultural Sciences, Zhengzhou, China

ABSTRACT

Camel milk is rich in bioactive peptides, lactoferrin, zinc, monounsaturated fatty acids and polyunsaturated fatty acids, among other health substances. This article reviews the mechanism of action of antimicrobial peptides, as well as the types and different effects of antimicrobial peptides obtained by different treatment methods in camel milk. The antibacterial peptides in camel milk can not only act alone, but also act in the form of complexes, such as camel recombinant chiral lactoferrin+lactoferrin+His-tag, which has inhibitory effects on plant bacterial pathogens; In addition, there are many common antimicrobial peptides, such as peptidoglycan recognition protein (PGRP), lactoferrin, immunoglobulin, etc., which have inhibitory effects on various bacteria. Although, many antimicrobial peptides have been found to play important roles in food and medicine, there are still more unknown aspects that need to be explored.

Key words: Antibacterial mechanism, antibacterial peptides, camel milk

Many studies have demonstrated the production and properties of peptides from milk proteins. Several bioactive peptides have good health effects on digestive, immune, cardiovascular and nervous systems. Whey proteins represent about 30% of the total proteins in camel's milk (Zhao *et al,* 2015). Whey proteins such as IgGs, Lf, lactoperoxidase, lysozyme and other enzymes are potent antimicrobial components in camel's milk (El-Agamy *et al,* 1992). Antimicrobial peptides (AMPs) are active small molecular peptides that can be produced by all organisms. They are an indispensable part of the innate immune system and can limit the growth of other microorganisms (Magana *et al,* 2020). Natural antimicrobial peptides have broad-spectrum killing activity against a variety of bacteria, yeasts, fungi, viruses and parasites (Huy *et al,* 2020). Antimicrobial peptides not only resist pathogens, but also possess properties, such as anti-inflammation, immune regulation, neutralising endotoxin and so on (Hee-Kyoung *et al,* 2017). Antimicrobial peptides act on different bacterial structural targets through a variety of mechanisms, hence the drug resistance is relatively rare (Browne *et al,* 2020). Various mechanisms of bacterial resistance to antimicrobial peptides have emerged, including modification of cell surface components, degradation of antimicrobial peptides and efflux of antimicrobial peptides (Milad *et al,* 2019). This review will mainly summarise the antibacterial peptides and their effects in camel milk.

Antibacterial peptides obtained from different treatments of camel milk

Different treatments of camel milk can make it play different roles (Fig 1). For example, the protein in camel milk is fermented to produce new peptides that exert antibacterial effects. Various studies have shown that camel milk is rich in antimicrobial peptides, which have an inhibitory effect on a variety of bacteria. Some studies have also found that the new peptides cultured from fermented camel milk can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* subspecies (Hussein *et al,* 2021). Algboory *et al* (2017) fermented camel milk using a mixed culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii.* The peptide concentration in fermented camel milk (0.483mg/mL) was three times higher than that of the same fresh milk before fermentation (0.156mg/mL). The concentration of peptides in the water extract was 0.435mg/mL. Bacterial fermentation of Iraqi camel milk has the potential to increase water-soluble peptides and enhance biological activity.

New peptides with antibacterial activity can also be produced through recombinant chimerism.

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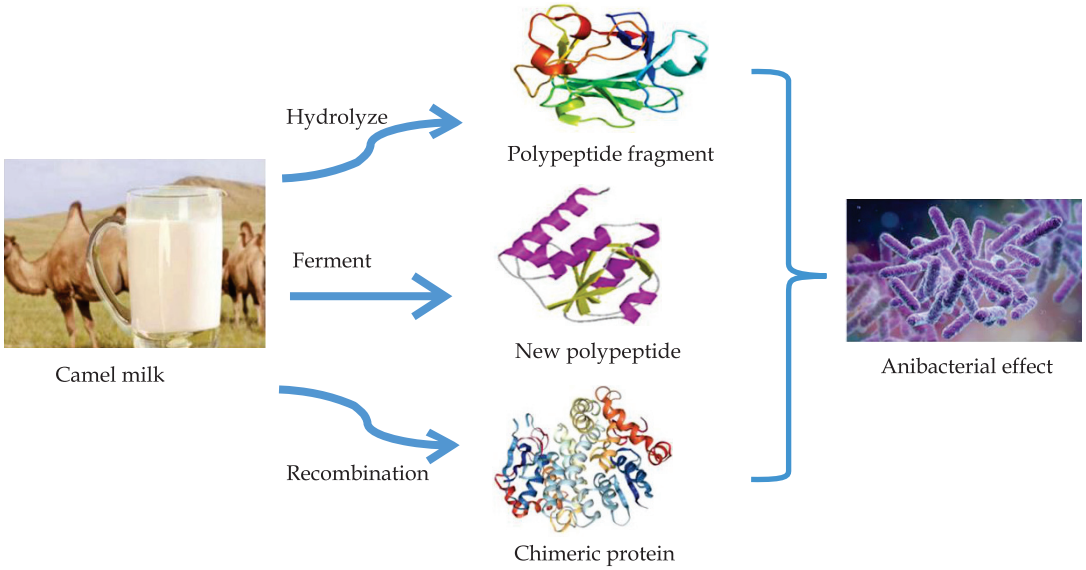

Fig 1. Camel milk obtains different antimicrobial peptides through different processing methods.

The LFA-LFC chimera has significant antibacterial properties against caries inducing bacteria and is not toxic to human gingival fibroblasts. Therefore, this peptide can serve as a safe alternative to other chemicals for the prevention and management of dental caries (Mohammadipour *et al,* 2021). Research has shown for the first time that the prepared camel recombinant chimeric lactoferrin+lactoferrin+His tag has good antibacterial activity against plant bacterial pathogens (Tanhaeian *et al,* 2018).

Different peptide fragments can also be produced through hydrolysis, which can exert antibacterial effects. *Trichosporon asahii ICVY021* were isolated from camel milk and found that it was able to inhibit *Kocuria rhizophila CIP* 53.45, through production of an extracellular heat-stable, proteinaceous antibacterial peptide,with partial amino acid sequences of PPFPK and CTHV(L/I)(K/Q) or TCHV(L/I)(K/Q), determined using LC/MS/MS. This peptide, named oranicin P16, was thought to impede the cell-division mechanism. Subsequent experiments also confirmed that the strain has the activity of anti-*K. rhizophila* (Soufian *et al,* 2020). It is to evaluate the potential antimicrobial effects of camel milk derived antimicrobial proteins and peptides against *Propionibacterium acnes* by micro broth dilution assay. The results showed that peptidoglycan recognition proteins PGRPs possess the strongest antimicrobial activity against *P. acnes* (Abu-qatouseh *et al,* 2019). Studies have shown that camel milk β-CN, the major protein of camel milk, can be hydrolysed with pepsin to increase its anti-bacterial activity. All fractions of hydrolysates exhibited some anti-bacterial activity and molecular mass fraction <1 kDa was the most active against both Gram- positive bacterial strains (Almi-Sebbane *et al,* 2018). The hydrolysates of camel cheese protein can be graded by ultrafiltration technology to obtain peptides with different molecular weight ranges, which have high antibacterial activity, such as anti *Listeria monocytogenes, Escherichia coli, Bacillus cereus, Staphylococcus argenteus* (Kumar *et al,* 2016). AMPs and proteins collected from camel milk have considerable antimicrobial and anti-inflammatory effects on *P. acnes* and would provide new trend toward applying these biological molecules in the management of acne vulgaris and other skin related infections (Shihab, 2019). Camel milk lactoferrin was used as the raw material and different AMPs were designed using bioinformatics methods. The most suitable AMP was screened according to the required standards. The designed peptide has antibacterial activity against *Pseudomonas aeruginosa, Baumannia* and *Staphylococcus aureus* (Khajeh *et al,* 2021). Lactoferrin (Lf) is an iron binding glycoprotein, which exists in different biological fluids and neutrophils of mammals. It has many functions, including protection from pathogen infections. The antibacterial activity of lactoferrin against *E. coli 0157: H7* was studied. The minimum inhibitory concentration (MIC) was determined by measuring the absorbance at 620nm. The minimum bactericidal concentrations (MBCs) were also measured. It was found that camel Lf was the most effective lactoferrin against *E. coli 0157: H7*, while camel and human lactoferrin had the lowest activity (Conesa *et al,* 2008).

Mechanism of action of antimicrobial peptides

Destruction of cell membrane function

The most basic mechanism of action of antimicrobial peptides is to destroy the bacterial plasma membrane structure, causing a large amount of water-soluble substances to seep out of the cell eventually leading to bacterial death (Dekker *et al*, 2001). The structural characteristics of antimicrobial peptide molecules are an important basis for ensuring the effectiveness of the aforementioned mechanisms. Bechinger (1997) proposed a model based on the interaction between antimicrobial peptide HNP2 and the hydrophobic and polar regions of biofilms, as well as the size of membrane pores formed. The model consists of 6 homologous dimers of defensin molecules forming a porous ring. Cheristensen *et al* (1988) studied the bactericidal mechanism of antimicrobial peptides using bilayer lipid membrane liposomes and believed that first, the positively charged nitroic acid of antimicrobial peptides and the negative charge formed by the bacterial cytoplasmic phospholipid molecules generate electrostatic attraction, causing the antimicrobial peptides to attach to the surface of the bacterial membrane. Then, the hydrophobic C-terminal is inserted into the hydrophobic region of the membrane and changes the conformation of the membrane. Multiple antimicrobial peptide molecules form ion channels on the membrane, leading to the loss of intracellular ions, especially the large escape of K+. Bacteria cannot maintain their normal osmotic pressure and die.

Inhibition of cellular respiration theory

Bobek *et al* (2003) used antibacterial peptide MUC7 extracted to act on common fungi, bacteria and cocci in clinical practice and found that MUC7 has a strong killing effect on both fungi and bacteria. In the ultrastructure, swelling, vacuolisation, ridge detachment and irregular arrangement of mitochondria were found, with unclear nuclear membrane boundaries and some nuclei ruptured and contents overflowed. This suggests that the mechanism of action of antimicrobial peptide MUC7 may be related to inhibiting tumour cell respiration. Fehlbaum *et al* (1996) also found that the mechanism of action of the antibacterial peptide tachy-plesin is related to the inhibition of mitochondrial related caspase7 and caspase6 proteins. Some researchers also believe that thanatin kills bacteria by inhibiting cellular respiration.

Inducing cell apoptosis

Mai *et al* (2001) injected the fusion antimicrobial peptide DP1 locally into solid tumours to study the effect of DP1 on the apoptosis of tumour cell line MCA20. They found that DP1 can quickly induce tumour cell apoptosis and reduce tumour volume. Chen *et al* (2001) treated prostate cancer cell line TSU with the antimicrobial peptide RGD tachyplesin and detected it using fluorescence immunoassay and Western blot hybridisation. The results showed that the expression of apoptosis related proteins caspase9, caspase8, caspase3 and Fas ligand increased, indicating that the antimicrobial peptide RGD tachyplesin can induce Fas related apoptosis. Therefore, it is inferred that inducing apoptosis

Table 1. Antibacterial peptides and their effects.

Antimicrobial peptides	Suppressed bacteria	Reference
FVVTPK, RGLVPL ELLPDMPLNQ APGPLVVPPVGPPPP PLPASGLL VMVSGVAGNPGA HPPGSGLL	*E. coli, S. aureus* subsp. aureus.	Hussein *et al* (2021)
lactoferrampin-lactoferricin [LFA-LFC]	*S. mutans, S. salivarius, S. sobrinus*	Mohammadipour *et al* (2021)
Camel recombinant chimeric lactoferricin + lactoferrampin +His-tag	plant bacterial, pathogens	Tanhaeian *et al* (2018)
PPFPK and CTHV(L/I)(K/Q) or TCHV(L/I)(K/Q)	*K. rhizophila*	Soufian *et al* (2020)
Lactoferrin, peptidoglycan recognition proteins (PGRPs) and immunoglobulins specific	*P. acnes* isolated	Abu-qatouseh *et al* (2019)
β-casein and β-CN hydrolysate	*E. coli, L. innocua, S. aureus*	Almi-Sebbane *et al* (2018)
Alcalase, α-Chymotrypsin	*L. monocytogenes, E. coli, B. cereus, S.aureus*	Kumar *et al* (2016)
peptidoglycan recognition proteins (PGRPs)	*P. acnes*	Shihab (2019)
Lactoferrin	*E. coli 0157:H7*	Conesa *et al* (2008)

may be one of the mechanisms of action of certain antimicrobial peptides.

Inhibition of cell wall formation

Harder *et al* (2001) found that antimicrobial peptides can inhibit the formation of bacterial cell walls, hinder bacterial growth and cause cell wall perforation, ultimately leading to bacterial death. Among the various mechanisms of action of antimicrobial peptides currently discovered, the membrane attack theory is considered to be the main mechanism of action of antimicrobial peptides. However, the same antimicrobial peptide may also exert its effects through multiple pathways. Antimicrobial cationic peptides can inhibit the formation of bacterial cell walls, preventing bacterial growth from maintaining normal cell morphology, but has no effect on already formed cell walls. Due to the different permeability of antimicrobial peptides to bacterial cell walls, the minimum lethal concentration of different antimicrobial peptides and the same antimicrobial peptide to different bacteria also varies greatly (Friedrich *et al,* 2000).

Effect on cancer cell cytoskeleton

At present, extensive research has been conducted on the killing effect of antimicrobial peptides on tumour cells and it has been found that the main effect of antimicrobial peptides on cancer cells cultured *in vitro* is to form pores on the membrane of cancer cells, cause leakage of contents and cause vacuolisation of mitochondria and detachment of cristae. The boundary of the nuclear membrane is unclear, with some cases of nuclear membrane damage, nuclear chromosome DNA breakage and inhibition of chromosome DNA synthesis, resulting in a certain degree of damage to the cytoskeleton. Antibacterial peptides can also stimulate the immune function and resist the invasion of cancer from the perspective of humoral immunity. Chen *et al* (2001) studied the anti-tumour mechanism of cecropin B, B1 and B3. Although, antimicrobial peptides have certain damage to the cytoskeleton of tumour cells and normal cells, the latter has a complete cytoskeleton system, fast repair and will not cause irreversible damage. Taghipour *et al* (2023) isolated camel milk protein components, casein and whey protein from antimicrobial peptides and hydrolysed them using pepsin, trypsin and these two enzymes. To screen peptides with antibacterial activity against breast cancer and pathogens. The peptides extracted from whey protein fractions using these two enzymes showed very good activity against MCF-7 breast cancer. The incomplete cytoskeleton of tumour cells, which cannot be repaired in a timely manner after the action of antimicrobial peptides, ultimately leads to death.

Application of antimicrobial peptides

Application of antimicrobial peptides in food

Antibacterial peptides have a strong killing effect on various Gram positive and negative bacteria in food (Tailor *et al,* 1997). Under acidic conditions, it has strong activity and can quickly inhibit the growth of microorganisms. It is suitable for most acidic foods. Proteases can quickly hydrolyse antimicrobial peptides consumed by humans and livestock and have no toxic side effects. Meanwhile, antimicrobial peptides have good thermal stability and solubility. In the process of food fermentation, antimicrobial peptides can effectively preserve certain bacterial communities (such as lactic acid bacteria) and can also cultivate or kill certain bacteria to prevent harmful bacteria. Antibacterial peptides can still maintain their unique physiological activity after hot processing.

Antibacterial peptides are also gradually being applied in the preservation of raw milk. In European countries, due to the distance between residential areas and pastures, milk needs to go through a long time during transportation in the hot summer. Insufficient refrigeration equipment can cause milk to spoil and cause great losses to producers. Adding a certain amount of antibacterial peptides to raw milk can effectively inhibit the spoilage bacteria produced in milk, extend the shelf life of milk without affecting its flavour.

Application of antimicrobial peptides in medicine

At present, more than 2500-3000 antimicrobial peptides have been isolated and identified, of which 261 have been confirmed to have anti-tumour activity (Wang, 2023). Antibacterial peptides or their precursor genes can be directly introduced into tumour cells, or these can be directly injected into the tumour to exert their effects. The high selectivity of the target audience of antibacterial peptides brings hope for the development of anticancer drugs, which may become a new type of peptide anti-tumour drugs that replace traditional surgery, radiotherapy and chemotherapy.

The currently used radiochemotherapy drugs can not only kill tumour cells, but also normal cells, with significant side effects. The development of the cytoskeleton system of tumour cells is incomplete and antimicrobial peptides can be inserted into the

cell plasma membrane, causing the microtubules of the cells to collapse, resulting in the dissolution of the bilayer and disruption of integrity. Antibacterial peptides can inhibit the growth of certain tumours with minimal toxic side effects and are harmless to normal cells, which brings great hope for the development of anti-tumour drugs. Antibacterial peptides can attack tumour cell membranes, forming pores on the cell membrane, causing a large amount of cell contents to seep out, ultimately causing tumour cells to fail to maintain normal osmotic pressure and die (Shai, 2002). Antibacterial peptides induce tumour cell death through the death receptor pathway (Chen *et al*, 2009) and mitochondrial pathway (Aarbiou *et al*, 2006). Antibacterial peptides can also damage the internal organelles of tumour cells, such as DNA breakage, mitochondrial damage and cytoskeleton breakage. The clinical application of antimicrobial peptides in anti-tumour has shown good prospects. At the same time, antimicrobial peptides can also enhance the body's immune system, resist tumour cell invasion and participate in cellular and humoral immunity. Due to its anti-tumour and antiviral properties, the development of skin antibiotics, antiviral drugs and anti-tumour drugs also has irresistible clinical application prospects, which will bring immeasurable significance to the development of disease treatment and medical health.

Conclusion

Antimicrobial peptides are an important component of biological innate immunity and have a wide range of killing effects on pathogens such as Gram negative bacteria, Gram positive bacteria, fungi and viruses, making them less susceptible to drug resistance. Camel milk antibacterial peptides are the most promising antimicrobial agents for addressing the challenge of multidrug-resistant bacteria and can be used alone or in combination with conventional antibiotics, antiviral drugs, or other antibacterial ingredients to achieve synergistic effects. Therefore, camel milk antimicrobial peptides are expected to become a new type of antibacterial growth promoter and immune modulator, applied in various industries, inducing or promoting the expression of antimicrobial peptides through nutritional regulation and increasing the concentration of local antimicrobial peptides in the mucosa, to achieve the goal of inhibiting pathogen invasion and improving the body's ability to resist infection, thereby reducing the use of antibiotics or dependence on antibiotics. Although, camel milk antimicrobial peptides have broad application prospects, research on camel milk antimicrobial peptides is still relatively limited, such as the toxicity, immunogenicity, pharmacodynamics and pharmacokinetics of camel milk antimicrobial peptides. The clinical trials of camel milk antimicrobial peptides are still limited to a certain aspect, with more basic research and less clinical and *in vivo* experiments. Therefore, in order to achieve the commercial application of camel milk antimicrobial peptides, a lot of basic work needs to be done.

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CAMEL MILK PROCESSING OPPORTUNITIES: A REVIEW

Ayaulym Rakhmatulina[1], Altynay Abuova[1,2], Arman Issimov[1,2,3] and Bernard Faye[4]

[1]Department of Mechanical Engineering and Robotics, Joldasbekov Institute of Mechanics and Engineering, Almaty, 050000, Kazakhstan

[2]Department of Food Technology, International Engineering Technological University, Almaty, 050000, Kazakhstan

[3]Department of Biology, K. Zhubanov Aktobe Regional University, Aktobe, 030000, Kazakhstan

[4]UMR SELMET, CIRAD-ES, TA/C 112A, Campus international de Baillarguet, 34398, Montpellier, France

ABSTRACT

Camel milk occupies a pivotal and essential position in the dietary customs of individuals residing in semi-arid and arid areas. Historically, the promotion and commercial distribution of camel milk have been negligible, primarily due to the absence of processing facilities in areas where camels are raised. Consequently, the consumption of untreated camel milk has been predominantly limited to nomadic households. However, owing to its health-enhancing effects, a substantial surge in the global demand for camel milk and its derivatives has been observed over the past two decades. This growing demand has prompted the dairy sector to introduce a diverse range of camel milk products, which are distinguished by their enhanced nutritional and functional properties. In contrast to products derived from bovine milk, the current market offers only a limited selection of food items sourced from camel milk. Recent advances in food processing technologies have enabled the production of an array of both dairy and non-dairy products derived from camel milk. This includes a variety of items such as powdered milk, cheese, yogurt, ice cream. Moreover, in certain regions, camel milk is incorporated into customary cuisine, serving as a key ingredient in local culinary practices like fermented milk, camel milk tea, or as a fundamental component in various meals. This review underscores the possibility of transforming camel milk into a range of dairy products by addressing its intrinsic functional constraints. This objective can be realised by adjusting the processing parameters and modifying its chemical composition through enrichment techniques. Furthermore, further research avenues may focus on enhancing product quality and exploring innovative processing techniques.

Key words: Camel milk, dairy products, food powder milk, food processing

Camel milk provides nutrition and food security, especially for populations residing in semi-arid and arid regions of Sub-Saharan Africa and Asian deserts. It possesses unique chemical characteristics and intrinsic functional properties that are distinct from the milk of other livestock. (Muthukumaran *et al,* 2023). Although, chemical composition of key nutrients in camel milk, such as water, protein, lactose and fat, aligns closely with that of cow's milk, there are significant differences in micronutrients. These include variations in immunoglobulin (IgG, IgA), vitamins (A, C), as well as mineral salts (Hammam, 2019; Mullaicharam, 2014). Furthermore, the molecular structure of major components in camel milk differs from that of bovine milk, posing a significant challenge for the dairy industry in transforming camel milk into valuable dairy products (Baig *et al,* 2022). Camel milk exhibits lower concentrations of carotene and short-chain fatty acids but higher quantities of long-chain fatty acids (Al-Nasseri *et al,* 2019). In recent years, there has been a significant increase in global interest and demand for camel milk and its dairy derivatives, attributed to their exceptional potential health benefits and health-enhancing properties (Konuspayeva *et al,* 2023). This trend has prompted the dairy sector to diversify its offerings, providing consumers with camel dairy products characterised by advanced nutritional and functional qualities. Since the 1960s, camel milk production has exhibited a consistent growth rate of 8.9% per year, leading to a significant 6.5-fold increase by 2024 (Faye and Corniaux, 2024) . This growth can be attributed to ensuring food security under challenging environmental conditions, increasing market demand due to perceived "medicinal properties," and the development of the camel dairy industry, which offers potential advantages for camel owners (Bilal *et al,* 2024; FAO, 2022; Faye and Konuspayeva, 2012, 2024).

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In general, milk sourced globally undergoes diverse processing methods employing contemporary technological approaches, aimed at extending its shelf life and producing functional dairy products that possess augmented dietetic value and health benefits. The conversion of milk into both non-fermented and fermented dairy products is a widespread practice, serving the dual purpose of preservation and enhancement of nutritional content to meet increasing demands. The technological and functional qualities of milk, encompassing aspects such as physical and chemical structure, foam generation potential, solubility, emulsifying properties, gelation ability and water holding capacity, are recognised as pivotal factors in milk processing techniques. These attributes play a significant role in the development of innovative food products originating from animal sources (Shokri *et al,* 2022).

The feasibility of utilising camel milk for the development of dairy products depends on its physicochemical and techno-functional properties (Konuspayeva and Faye, 2021). The functional properties of food are subject to the influence of processing technologies, food quality, utilisation practices, formulation and ultimately, their acceptability (Mahajan and Dua, 2002). The conversion of camel milk into processed products possess a significant challenge, requiring the application of appropriate technologies. Although, camel milk shares similar gross composition with bovine milk a range of factors including its distinctive chemical constitution, the presence of a complex colloidal system, inherent functionality, the dimensions of protein micelles and fat globules and the existence of antibacterial agents differs from bovine milk (Arain *et al,* 2023; Bornaz *et al,* 2009). As a result, processing camel milk and manufacturing its dairy products such as butter, yogurt, cheese and ice cream using technologies identical to those used for bovine milk products is reported to be challenging even when such products are produced, they exhibit poor quality (Seifu, 2023).

In recent time, the technological and functional properties of camel milk have attracted significant attention from researchers, owing to the inadequacy of standard technologies used for cow milk in processing camel milk into dairy products. Nevertheless, overcoming these challenges could be feasible through the application of innovative technologies, refinement of processing parameters and modification of its natural functionality by introducing synthetic compounds or fortifying camel milk. The aim of this review is to summarise the processing methods and functional capabilities of camel milk. It provides a thorough and current examination of the literature on camel milk products, highlighting recent advancements, processing limitations and prospects for enhancing camel dairy products.

Global production and technological advancements in camel milk industry

In 2022, global camel milk production was reported at approximately 4.11 million tons. However, estimates suggest actual outputs could be as high as 5.4 million tons annually, due to under reporting in remote areas where traditional herding dominates (FAO, 2022).

During the 1970s, the semi-automated milking process was first introduced in the former Soviet Union (Ermukhan, 1999) and later adopted in other parts of the world. This technology allowed for more efficient milking but required specific adjustments to cater to the unique anatomy and behaviour of camels (Ayadi *et al,* 2013; Ayadi *et al,* 2018). In 2002, a significant milestone was achieved in Dubai with the establishment of a modern camel dairy farm. This farm initially employed a single-camel milking stand, which was tested and optimised in Oman. The development of the herringbone milking system, which enables the simultaneous milking of 5 camels, represented a key advancement in milking efficiency and animal handling (Wernery *et al,* 2006; Wernery *et al,* 2004).

Innovations in milking system design have also included corridor and tandem systems, which provide camels with more space and make it easier for handlers to manage the animals during milking. These designs help prevent camels from sitting down during milking and allow calves easier access to the udder, which is important for the welfare of both dam and calf (Ayadi *et al,* 2015; Hammadi *et al,* 2010). However, these systems typically require larger milking parlours and more time to milk an equivalent number of animals than the more compact herringbone design. The introduction of the herringbone system necessitates specialised engineering solutions and a period of acclimation for the camels, but ultimately yields a more streamlined and efficient milking process (Nagy and Juhasz, 2016).

Camel milk production is not uniformly distributed globally; it is predominantly concentrated in Africa, the Middle East and parts of South Asia. The economic impact of camel milk is particularly

significant in arid and semi-arid regions where traditional cattle farming is less viable. The commercialisation of camel milk has provided substantial economic benefits to rural communities, supporting livelihoods and contributing to food security (Orazov *et al,* 2021).

The future of camel milk production faces several challenges, including the need for better breeding practices to enhance milk yield and quality and the adaptation of milking technologies to suit small-scale producers. Moreover, there is a growing need for research into the development of camel milk products, such as cheese and yogurt, which require specialised processing techniques due to the unique properties of camel milk (Ipsen, 2017; Jafarpour, 2017).

Factors influencing the gross composition of camel milk

Similar to other species, the principal determinants influencing milk content in camels include breed, seasonality, diet, parity and lactation. In recent study conducted by Nagy *et al* (2016), 2,332 milk samples from 7 camel breeds or ecotypes—Emirati, crossbreed-Emirati, Majaheem (black), Pakistani, Saudi, Saudi-crossbreed and Sudanese—were analysed. These camels, all from the same intensive dairy farm in the United Arab Emirates, exhibited significant variation in milk composition. The Pakistani breed demonstrated the highest fat content at 2.81%, while the Saudi-crossbreed had the lowest fat content at 2.3%. Protein concentrations were highest in the Emirati-crossbreed at 3.05% and lowest in the Saudi and Saudi-crossbreed at 2.85% and 2.87%, respectively. In contrast, the Saudi-crossbreed had the highest lactose content at 4.27%, whereas the Pakistani breed had the lowest lactose content at 3.7%. Additionally, (Konuspayeva, 2020) in their systematic review demonstrated that camel milk samples from Central Asian Bacterian breeds exhibited significantly higher concentrations of fat (4.87±0.77%) and total protein (3.86±0.57%) compared to those from other regions. Conversely, lower fat concentrations were observed in samples from West and North Africa, while protein concentrations were lower in samples from the West and Middle East Africa. Such variations can be caused, except camel management aspects mentioned earlier in this section, but by reason of physiological stage of all dairy species (Zhang *et al,* 2005). This variability factor was examined in both Bactrian camels and dromedaries (Ahmad *et al,* 2012). In most instances, the available data are reported on a monthly or even quarterly basis. However, the study by (Musaad *et al,* 2013), which collected data on a weekly basis, revealed a lower fat content between weeks 12 and 32, whereas other parameters exhibited a gradual and consistent decline throughout the lactation period.

The physiological stage has a greater influence on fat content compared to protein content, while lactose and ash content remain relatively stable. However, others (Bakheit *et al,* 2008), have noted a seasonal effect on lactose content. Overall, studies on the seasonal variability of camel milk composition often conflate this with the lactation stage, as the calving season for camels is typically concentrated within a 3-4 months of hot season (Shuiep *et al,* 2008) (Musaad *et al,* 2013; Zhao *et al,* 2015).

Camel milk processing and challenges

It is reported that, on average, camel milk shares similar chemical features with bovine milk (Table 1). Nevertheless, it is noted that molecular composition of proteins, distribution and content of fatty acids are documented to be distinct (Konuspayeva, 2020).

Cheese Production Process- Converting camel milk into cheese is regarded as challenging and has been deemed impractical (Merin *et al,* 2001). The amino acid composition and distribution of caseins in camel milk differ from those in cow milk. Specifically, camel milk casein exhibits a higher proportion of β-casein (65% compared to 39%), a lower percentage of α S1-casein (22% compared to 38%) and reduced κ casein (3.5% compared to 13%) in comparison to bovine milk caseins. This implies that camel milk cannot be coagulated traditionally due to its low concentration of the amino acid composition of κ-casein, which is responsible for the difficulty in coagulating camel milk. Camel milk has been documented to possess a higher whey protein to casein ratio in comparison to cow milk, contributing to the formation of a delicate curd mass easily digestible in the gastrointestinal tract (Shamsia, 2009). The casein in camel milk is characterised by a larger micelle size, with an average diameter of 380nm, in contrast to 150nm, 260nm and 180nm observed in cow, caprine and ovine milk, respectively (Bornaz *et al,* 2009). Small casein micelles in cow milk have been associated with improved gelation properties (Glantz *et al,* 2010). Consequently, the lower concentration of κ-casein (κ-CN), the elevated ratio of whey protein to casein and the greater micelle size found in camel milk have been identified as factors contributing to the difficulties in cheese production. These characteristics contribute to the formation of a softer

coagulum and decreased yields in cheese processing, as detailed in research conducted by Bornaz *et al* (2009), Konuspayeva *et al* (2014) and (Konuspayeva, 2020).

Butter Production Process- The fat percentage in camel milk, which varies between 1.2% and 6.4% (Konuspayeva, 2020) is similar to that of cow milk. However, the production of butter from camel milk is not a traditional practice and poses challenges when employing the same production technology as used for cow milk butter. High melting point (Berhe *et al,* 2013; Farah *et al,* 1989) of camel milk fat (41–43°C) complicates the churning process at temperatures optimal for cow milk, which is between 10 and 14°C. The conversion of camel milk into butter is hindered by the milk's limited propensity to form cream is attributed to a deficiency in agglutinin, smaller fat globule dimensions and a more robust membrane in fat globular (Berhe *et al,* 2013).

Camel milk is characterised by a higher concentration of long-chain fatty acids and a diminished quantity of short-chain fatty acids (Konuspayeva *et al,* 2008) This composition accounts for the higher melting gradient of camel milk butter which is attributable to the increased presence of long-chain fatty acids in its fatty acid composition. Nevertheless, the production of butter from camel milk is achievable with the right churning temperature and agitation technique. Berhe *et al* (2017) have shown that the robust agitation of fermented camel milk in a vertical motion, as opposed to the conventional back-and-forth method, at a higher churning temperature, led to the successful extraction of butter. This approach has been found to be efficient and exerts greater force to break the fat globule envelope, promoting the adhesion of the globules to each other. Farah *et al* (1989) also documented the successful production of butter from camel milk at churning temperatures ranging from 15 to 36°C. According to their findings, the optimal fat recovery in butter production, attaining 85%, was achieved when churning run at 25°C. However, the organoleptic property of camel butter has to be improved for encountering the consumers' expectation compared to the butter from other dairy species.

Yoghurt Production Process- The production of yogurt or other processed dairy products from camel milk is acknowledged to be challenging. The coagulum formed from dromedary milk lacks the desired curd formation and firmness; instead, the curd exhibits fragility and heterogeneity, composed of dispersed flakes (Attia *et al,* 2001). The challenge associated with camel milk yogurt stems from its runny consistency and fragile texture. The texture of yogurt is a crucial factor that affects its visual appeal, mouthfeel and general acceptance. Camel milk is recognised for its resistance to easy fermentation, primarily attributed to its antibacterial qualities. Despite this, it has been noted that commercial starter cultures can develop in camel milk. There are several approaches suggested to overcome challenges associated to the production of camel milk yogurt. Hashim *et al* (2009) demonstrated that the texture of camel milk yoghurt can be enhanced by augmenting the milk with alginate, calcium and gelatine. Moreover, Ibrahem and El Zubeir (2016) suggested that the firmnes of camel milk yoghurt texture can be enhanced by mixing camel milk with milk from other dairy livestock species. Additionally, Ifeanyi *et al* (2013) reported that *Lactobacillus bulgaricus* and *Streptococcus thermophilus* can be utilised as the most important starter culture bacteria in the fermentation process to stabilise the development of texture and flavour in yogurt.

Ice cream Production Process

Based on the literature review, commercial production of camel milk ice cream is currently established in the United Arab Emirates, Kazakhstan and Morocco (Konuspayeva and Faye, 2021). The production process includes heating camel milk to 80°C with continuous agitation to create foams, followed by cooling to 20°C and maintaining this temperature for 15 hours (El-Agamy, 2017). The approach of mixing camel milk with bovine milk can be used to make ice cream of high quality and sensory acceptability (Soni and Goyal, 2013). This is feasible mainly because camel milk and cow milk ice cream

Table 1. Comparative analysis of camel milk composition in relation to milk from other livestock species.

Species	Fat (%)	Total solids (%)	Lactose (%)	Ash (%)	Protein (%)	References
Camel	3.5	12.0	4.4	0.8	3.1	(Al Kanhal, 2010)
Cow	3.7	12.7	4.8	0.7	3.4	(Fox *et al,* 2015)
Ovine	7.4	19.3	4.8	1.0	4.5	(Fox *et al,* 2015)
Equine	1.21	13.2	6.4	0.42	2.1	(Jastrzebska *et al,* 2017)

possess similar sensory physicochemical properties (Jafarpour, 2017). Comparable processing parameters can be employed in the production of ice cream using camel milk as with bovine milk. However, this could yield a product with distinct storage stability and quality attributes (Ipsen, 2017). Additionally, it has been noted that incorporating natural ingredient and flavouring agents into the ice cream formulation enhances the nutritional value and sensory attributes of camel milk ice cream (Ahmed and El-Zubeir, 2015 and Ho *et al,* 2022).

Shelf life- The development of fermented milk products likely originated from the necessity to prolong milk's shelf life in the absence of refrigeration, coupled with their nutritional value and potential health advantages. Traditional fermented camel milk, distinct from camel milk cheese, butter and yoghurt, is a commonly available camel dairy product. This fermented milk is known by various names globally, such as *Dhanaan* in Ethiopia (Biratu and Seifu, 2016), *Suusac or susa* in Kenya and Somalia (Mwangi *et al,* 2016), *Gariss* in Sudan (Ahmed *et al,* 2010) and *Shubat* in Kazakhstan (Konuspayeva *et al,* 2023). The antimicrobial properties of the milk contribute to the reported relatively stable nature of fermented camel milk over an extended period at ambient temperatures.

Shubat is documented to exhibit heightened storage stability. Pastoralists in South and West Kazakhstan have noted that Shubat has a prolonged storage stability, remaining viable for several months, particularly when employing continuous back slopping. This method entails inoculating a fresh batch of milk with a sample from a previous batch, thereby extending its viability. Likewise, Sulieman *et al* (2006) reported that withdrawal of batch fermented accumulations and replacing with fresh camel milk can continue for several months. Notably, strains of potential probiotic lactic acid bacteria, isolated from fermented camel milk, exhibit bacteriocin activities capable of inhibiting pathogenic microbes. Additionally, yeasts are crucial in the fermentation process of camel dairy products, owing to their potent lipolytic and proteolytic enzymatic properties (Maurad and Meriem, 2008; Takeda *et al,* 2011).

Thermal processing applied to camel milk

The molecular characteristics of whey proteins can be altered by factors such as heating temperature, pH and salt content (NaCl) (Boye *et al,* 1995). Camel milk's lactoferrin (LF) and immunoglobulin G (IgG) exhibit higher heat resistance compared to their counterparts in bovine milk (El-Agamy, 2000). The thermal denaturation of camel milk whey proteins is contingent upon the physical state of the proteins. While liquid forms of camel and cow milk whey proteins share similar thermal resistance, the process of drying has been reported to diminish the thermal stability of camel milk whey protein due to the absence of β-lactoglobulin (β-LG) in camel milk (Laleye *et al,* 2008; Merin *et al,* 2001). Consequently, in the production of camel milk powder, it is advisable to apply modified thermal treatment and atomisation conditions. In the production of acidified milk products, the application of thermal treatment has been investigated through scanning electron microscopy. Specifically, in the production of *labneh* from camel milk, it was observed that a thermal treatment at 85°C for 30 minutes resulted in acidified milk protein gels with smaller particles. In contrast, a more extensive heat treatment at 90°C for 30 minutes caused casein particles to fuse, forming larger aggregates (Desouky *et al,* 2013). Unheated milk exhibited a protein matrix that was more open, loose and less dense. The higher abundance of α-lactalbumin (α-LA) in camel milk whey led to increased sensitivity in camel whey solubility to pH changes (Laleye *et al,* 2008), a phenomenon known to induce acid denaturation in bovine milk (Paulsson, Hegg and Castberg, 1985). Bovine α-LA forms aggregates in acidic conditions, unlike β-lactoglobulin (β-LG), which aggregates upon heating in both alkaline and acidic environments (Boye *et al,* 1995). Camel serum albumin (SA) has demonstrated lower heat sensitivity compared to SA from bovine or buffalo milk. Notably, denaturation of camel SA at 100 °C for 20 minutes was found to be comparable to the denaturation of bovine and buffalo SA heated at 85 °C for 20 minutes (El-Agamy, 2000). However, the fouling properties, specifically the adherence of milk proteins to heated surfaces, in camel milk have been primarily attributed to α-LA and SA, whereas β-LG is identified as the main foulant in cow milk (Felfoul *et al,* 2015).

Pasteurisation–The pasteurisation process for camel milk requires specific conditions and indicators. Previous research indicates that alkaline phosphatase, commonly utilised for cow milk (Rankin *et al,* 2010), is not a suitable marker for camel milk pasteurisation due to its heat resistance even at 90°C (El-Agamy, 2000). Consequently, it is. proposed using either glutamyltranspeptidase or leucine arylamidase as reliable indicators for camel milk pasteurisation (Loiseau *et al,* 2002).

In a growing body of literature, the indicators for pasteurisation vary considerably (Alhaj *et al,* 2013; Hassan *et al,* 2007; Ibtisam and Marowa, 2009; Rahman *et al,* 2012). It should also be noted that there are no international standards established for the pasteurisation processes of camel milk.

According to Wernery *et al* (2007) should camel milk exposed to pasteurisation at 72°C for 20 minutes, gamma-glutamyl transferase might be the most suitable indicator. On the other hand, Lorenzen *et al* (2011) reported that gamma-glutamyl transferase remained detectable in pasteurised camel milk, suggesting that lactoperoxidase might serve as a more fitting indicator for pasteurisation. Previous study (Tayefi-Nasrabadi *et al,* 2011) verified that camel lactoperoxidase exhibits less heat resistance compared to its bovine counterpart. To date, comprehensive studies in this area remain inadequate, despite the introduction of pasteurised camel milk to the global market. This uncertainty regarding a suitable pasteurisation indicator for camel milk poses a challenge in establishing an international standard (Konuspayeva *et al,* 2023). Therefore, the industrial-scale pasteurisation of camel milk may be conducted improperly, leading to potential inaccuracies in its heat treatment.

Sterilisation- Despite efforts by private companies, the sterilisation of camel milk through extremely high-temperature processing has not yet been successfully implemented. Research focusing on the heat resistance of casein proteins and whey, vitamins, fat globules and other components of camel milk is anticipated to aid in developing a technical resolution for this challenge (Farah, 1986; Farah and Atkins, 1992; Hattem *et al,* 2011; Kherouatou *et al,* 2003; Momen *et al,* 2019).

Camel milk undergoes separation into two distinct phases following high thermal processing. Thermal processing at 90°C for 5 minutes, 85°C for 5 minutes, 75°C for 5 minutes, 72°C for 30 seconds, 65°C for 30 minutes (Farah, 1986) and at 72°C for 15 seconds and 90, 80 and 63°C for 30 minutes (Hattem *et al,* 2011) have shown that whey proteins in camel milk are highly sensitive and begin to denature. Various methods were experimented with to stabilise camel milk proteins after high thermal processing. These included the addition of bovine k-casein, disodium hydrogen orthophosphate, calcium chloride, sodium hydroxide, sodium dihydrogen phosphate anhydrous, however these attempts yielded unsatisfactory outcomes (Alhaj *et al,* 2011). Further work is required to establish the production of camel milk processed under high thermal conditions.

Therapeutic applications of camel milk

The acknowledgment of camel milk as a suitable alternative to cow milk in human nutrition has been widespread and enduring across various regions worldwide. Various bioactive compounds can be extracted from milk and its derivatives, offering the potential to combat various diseases (Dziuba and Dziuba, 2014). Camel milk exhibits antiviral, antimicrobial properties and has demonstrated potential effects in mitigating tuberculosis and diabetes (Singh *et al,* 2017). The inhibitory effects of camel milk against *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* are attributed to the presence of lactoperoxidase, hydrogen peroxide and lysozyme, respectively. Additionally, the growth of *Salmonella typhimurium* is hindered by camel milk's lactoferrin, which binds iron, rendering it unavailable for bacterial growth (El Sayed *et al,* 1992; Ochoa and Cleary, 2009). Notably, camel lactoferrin has demonstrated greater efficiency in inhibiting Hepatitis C Virus (HCV) entry into human leukocytes compared to human or bovine lactoferrin (Redwan and Tabll, 2007). Fermented camel milk beverage known as *Shubat*, traditionally consumed in Kazakhstan, has been documented to elicit virus-inhibiting properties against both ortho- and paramyxoviruses (Chuvakova *et al,* 2000). These properties endure following storage and the suggested antiviral effectiveness of *Shubat* is believed to be associated with the presence of metabolic byproducts and sialic conjugates derived from yeasts and lactic acid bacteria. In other study, camel milk has been used to treat male patients who suffered from tuberculosis. The outcomes related to clinical, bacteriological and radiological characteristics demonstrated a more pronounced improvement in the group supplemented with camel milk in comparison to the control group (Mal *et al,* 2006). The elevated concentration of insulin-like substances (Su *et al,* 2024), notably half-cystine, in camel milk (Beg *et al,* 1986), the impact of camel milk's small-sized immunoglobulins on b-cells (Agrawal *et al,* 2007) and the absence of coagulation of camel milk in the human stomach collectively, contribute to the hypoglycemic effect observed in individuals with type 1 diabetes (Agrawal *et al,* 2004). This effect has been noted in humans, rats (Dikhanbayeva *et al,* 2021; Sahani *et al,* 2005) and alloxan-induced diabetic dogs (Sboui *et al,* 2010). Furthermore, several studies have demonstrated the potential therapeutic effects

of camel milk in treating rheumatoid arthritis and asthma (Arab *et al,* 2017; Bakhtiari *et al,* 2022). These findings suggest that camel milk consumption may elicit anti-inflammatory actions and could be used as an adjunctive therapeutic approach for managing these conditions.

Prospects for the production of camel milk powder

The conversion of liquid camel milk into powder represents the most effective method for preserving camel milk, particularly when it is produced in distant regions with limited transportation and preservation facilities (Konuspayeva *et al,* 2021). Typically, two contemporary processing technologies employed for the production of camel milk powder include spray-drying and freeze-drying (Konuspayeva and Faye, 2021).

Recently, a laboratory-scale study was conducted to determine the optimal freeze-drying conditions, with the objective of assessing the stability of camel milk powder and its components under various temperature settings using a freeze-drying method (Aralbayev, 2022; Zhang *et al,* 2020). Findings revealed that the resulting camel milk powder exhibited enhanced stability at a humidity level of 11.3%. Likewise, another study investigated the impact of drying methods, particularly freeze-drying, on the nutritional value of camel milk compared to fresh camel milk. The study findings suggest that drying technology effectively preserved the comparative nutritional content and enhanced the stability of milk components, such as minerals and vitamins (Ibrahim and Khalifa, 2015). In the context of spray-drying method, Sulieman *et al* (2014) investigated the comparative physical characteristics of camel and cow milk powder produced using this technology. The findings highlighted that spray-drying markedly prolonged the shelf life of camel milk powder, primarily by eliminating the milk's water content.

In a recent study, a nutritional content of camel milk, including fatty acid and vitamin C profile has been further investigated under spray-drying and freeze-drying technologies (Habtegebriel *et al,* 2018), (Aralbayev, 2022). The findings indicated that the production output of milk powder is affected by variables like the feed flows, temperature and the airflow of the processing apparatus. It was observed that elevated temperatures and increased airflow correlated with a notable reduction in the vitamin C concentration (Habtegebriel *et al,* 2018). In a recent study, the milk was subjected to fractionation through acid and enzymatic coagulation, followed by either freeze drying or spray drying coupled to gamma irradiation (Harizi *et al,* 2023). This process enabled the production of dried milk fractions that exhibited a higher total phenolic content and greater antioxidant activities compared to corresponding skim milk. Authors concluded that gamma radiation within the range of 5–11 kGy may be employed to improve the preservation of powdered milk. However, it is important to note that implementing this technology in the dairy industry requires a substantial investment to install expensive milk roller driers and spray driers. Furthermore, employing spray-drying method for producing milk powder requires substantial energy consumption. Consequently, the development of a camel dairy industry calls for immediate actions to improve reproductive characteristics of camels and to establish extensive camel farming operations. This should be complemented by an efficient and widespread collection network linking these farms.

The use of ultrafiltration can be also applicalable in the processing of camel milk in terms of fractionation and concentration of milk components (Kashaninejad *et al,* 2021). It is known that the physicochemical properties of camel milk differ from those of bovine milk, particularly regarding protein type and concentration. For instance, camel milk exhibits a higher whey protein to casein ratio compared to bovine milk. Additionally, the distribution of casein micelles in camel milk is broader, with a greater prevalence of larger micelles than is observed in bovine milk (Bornaz *et al,* 2009). Therefore, the production of concentrated milk *via* ultrafiltration in the manufacturing of various dairy products depends on the efficacy of the membrane filtration process and the alterations in milk constituents that occur during the procedure (Grandison *et al,* 2000). The most important limitation of the ultrafiltration process for complex fluids is the decreased membrane efficiency due to fouling phenomena and concentration polarisation. During the initial minutes of the process, concentration polarisation alters the solute rejection pattern, exacerbates fouling and markedly reduces permeate flux (Rao, 2002). Nonetheless, recent study demonstrated that, although, the physicochemical properties of camel milk differ significantly from cow milk, the dynamic behaviour of the fouling resistances and permeate flux during the ultrafiltration process is similar to bovine milk (Kashaninejad *et al,* 2021).

Additionally, in a more recent study the effect of high hydrostatic pressure technique was demonstrated to affect microbial load of camel milk (Aljasass *et al,* 2023). In their study, camel milk treated at 300 MPa for 5 minutes at 40°C reduced the total bacterial count to below 10^3 CFU/mL, maintaining this low count for 15 days when stored at 3°C. In contrast, the microbial load in untreated samples rapidly increased to spoilage levels within approximately one week. This approach could be used as an alternative to heat treatment and preserve nutrient and health values of camel dairy products. However, high pressure treatment up to 400 MPa was found to cause a clotting phenomenon in camel milk but not in cow and goat milk (Aljasass *et al,* 2023). This phenomenon could be explained by the content of proteins and minerals of camel milk. High-pressure treatment of bovine milk is known to destabilise casein micelles, leading to a reduction in their average diameter (Anema, 2008). Moreover, mean casein content and casein fraction of camel milk lower than those in bovine milk. Furthemore, it was identified that each of the 4 primary casein fractions in bovine milk has corresponding fractions in camel milk. However, significant differences were observed between the casein profiles of the two milk types (Laleye *et al,* 2008). Consequently, these variances in milk composition could influence their coagulation response under high-pressure treatment conditions (Aljasass *et al,* 2023).

Conclusion and perspectives

Camel milk and its derived products constitute an essential source of sustenance and economic livelihood for communities residing in exceptionally challenging environmental conditions, where traditional livestock farming is substantially impeded. Unlike conventional dairy sources, camel milk provides vital nutrition in arid and semi-arid regions, demonstrating its critical role in food security and community resilience.

The processing of camel dairy products diverges markedly from traditional dairy methods, presenting unique challenges that stem from the inherent biochemical properties of camel milk. These challenges include the adaptation of pasteurisation and fermentation techniques to accommodate the high mineral content and unique protein configuration of camel milk. Innovations in processing technology have enabled the production of camel milk derivatives such as pasteurised milk, milk powder, yogurt, butter and cheese. However, each product requires specific adjustments to standard dairy processing protocols to preserve the nutritional value and improve the sensory attributes of the final products. In addition to the technological challenges discussed mentioned above, maintaining the hygienic quality of camel milk used during the processing poses a significant hurdle. This is particularly pronounced when using pasteurised camel milk, which can lead to clotting issues, making raw milk a preferred choice. Several surveys have indeed indicated that the hygienic condition of raw camel milk is frequently inadequate. For instance, in Kenya, it has been reported that approximately 75% of camel milk collected across the country exceeds the acceptable limits for total bacterial count (10^6 CFU/ml) and enterobacteria (5×10^4 CFU/ml) (Kaindi *et al,* 2011 and Kaindi and Njage, 2020). In Ethiopia, Adugna (2013) reported a coliform count and total bacterial of log 2.9 ± 2.3 and 5.2 ± 1.9 CFU/ml, respectively, indicating inadequate hygiene (Adugna *et al,* 2013). The substandard hygienic conditions of camel milk are attributed not only to improper milking practices but also to transportation and storage conditions (Yam *et al,* 2014).

Enhancement of organoleptic properties- To address the distinct taste and textural characteristics of camel milk products, researchers have explored various biochemical modifications. These include altering the fat and protein content and adjusting the enzymatic activity during processing. Such modifications not only enhance the organoleptic properties of camel milk products but also aim to standardise these characteristics to appeal to a broader consumer base.

Persistent limitations and research directions- Despite significant advancements, considerable processing limitations remain, underscored by the persistent variability in camel milk composition. The study and development of camel milk products thus represent a dynamic and evolving research area with immense potential. Ongoing investigations are required to refine processing techniques and develop protocols that can reliably produce high-quality camel milk products. Furthermore, there is a need for comprehensive research focusing on the scalability of production methods and the integration of novel technologies that can support the industrial processing of camel milk. For example, optimising processing techniques for camel milk through the commercial implementation of high-pressure treatment for preservation, as an alternative to thermal methods and in the development and

production of diverse dairy products derived from camel milk.

Further research should be done to investigate the use of veterinary drugs in camels and their residual effect in milk. To date, most discoveries in veterinary drug development have not addressed the specific conditions for administering these drugs to lactating camels.

Future perspectives and global implications- Looking forward, the camel dairy industry must not only address the technical challenges of milk processing but also embrace innovative strategies that cater to specific dietary and medical needs. This includes the development of specialised products such as lactose-free camel milk, high-calcium formulas and hypoallergenic dairy products suitable for consumers with specific health conditions. Moreover, expanding the range of flavoured and specialty camel milk products can enhance market penetration and consumer acceptance both locally and globally. Furthemore, establishing an international standard for camel milk and its products, including guidelines for pasteurisation and microbiological quality, is crucial. This need arises from the absence of national standards for processed camel milk and its derivatives in many camel milk-producing countries. This poses a significant barrier to the trade of camel milk, particularly its export to international markets. Despite, the rising demand for camel milk and its products in Europe and North America, driven primarily by perceived health benefits, consumers in these regions face limited access to camel milk and its associated products due to the absence of quality standards. Consequently, the importation of camel milk to these countries is prohibited (Seifu, 2023).

To compete effectively with products derived from other milk-producing species, camel milk products must meet stringent quality and safety standards. Establishing international guidelines and regulatory frameworks can facilitate the broader acceptance and integration of camel milk into global dairy markets. Additionally, strategic marketing efforts and consumer education are crucial in highlighting the unique benefits of camel milk, thereby fostering a sustainable market demand. As the camel milk industry continues to grow, its contributions to food security, economic stability and dietary diversity will become increasingly significant, particularly in regions most affected by climate change and arid conditions. The future of camel milk and its derivatives lies in leveraging both scientific research and technological innovations to overcome existing challenges and unlock new opportunities in global dairy markets.

Author Contributions

A.R., A.A.: Conceptualisation, funding acquisition, data curation, methodology, formal analysis, investigation. A.K., A.I., B.F.: supervision, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

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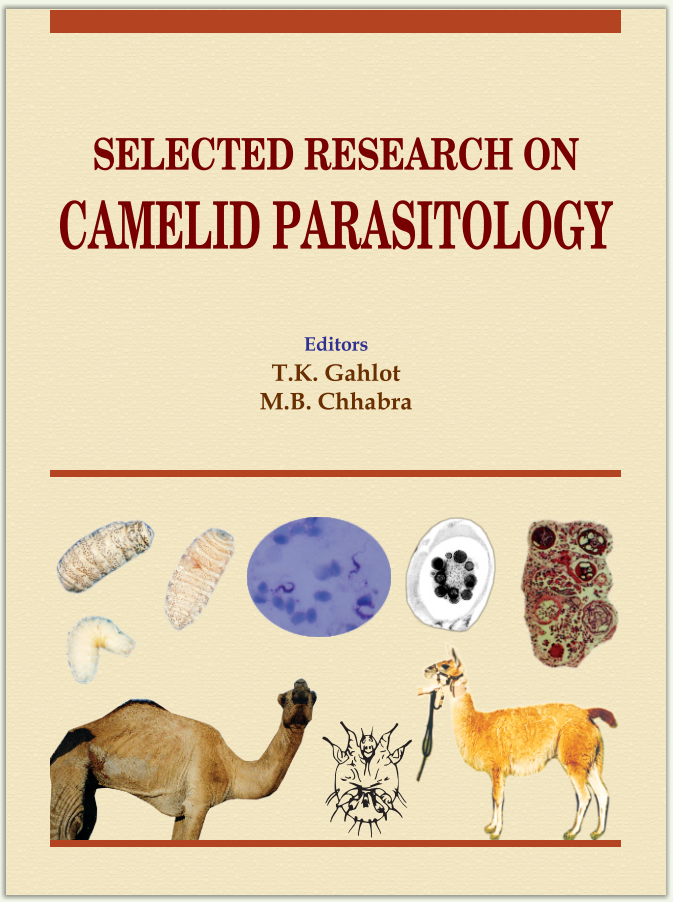

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AQUAPORIN 9'S CELLULAR DISTRIBUTION IN THE TESTIS AND EPIDIDYMIS OF CAMELS (*Camelus dromedarius*) DURING AND AFTER RUTTING SEASON

Abdelhay M. Ali
Department of Anatomy, College of Veterinary Medicine, King Faisal University, Postal code: 31982 Al-Ahsa, Saudi Arabia

ABSTRACT

Numerous cell types of the male genital system involved in fluid transport have tiny intrinsic membrane proteins called aquaporins (AQPs). These proteins are necessary to create the ideal luminal environment for sperm formation, maturation, and preservation. Aquaporin 9 (AQP9) allows water to move quickly across the epithelium. The current work used immunohistochemistry to clarify the expression of AQP9 in the testis (cranial and caudal parts and rete testis) and epididymis (caput, corpus, and cauda parts) of dromedary camels in the rutting and non-rutting seasons throughout the year. The results demonstrate that testicular gonocytes, Leydig cells, and rete testis and also the epididymal epithelial cells often exhibit a moderate immunoreaction to AQP9 antibodies during the mid-rutting season. However, these cells also show much higher expression levels at the beginning and end of this season. Throughout the non-rutting season, these organs exhibit intense immunoreactive staining of the AQP9 protein. In conclusion, transmembrane water and neutral solute transport via AQP9 is an essential physiological route in the testis and epididymis of the dromedary camels for spermiogenesis.

Key words: Aquaporin 9, distribution, Dromedary camel, epididymis, testis

Most living things, including people, animals, plants, and even lower species, contain the thirteen subtypes (AQP0–12) of the essential transmembrane protein family known as aquaporins (AQPs) (Azad *et al,* 2021; Shivaraj *et al,* 2017; Verkman, 2012). They regulated numerous physiological processes within cells, such as cell migration and proliferation, body water homeostasis, exocrine fluid secretion, and the transport of nutrients and other functional molecules into cells, along with the removal of metabolic residues (Ribeiro *et al,* 2021; Meli *et al,* 2018; Yu *et al,* 2014; Verkman, 2012). Many AQPs in the male reproductive system may be essential to the ordinary course of reproductive processes (Calamita *et al,* 2001). Furthermore, AQPs have been proposed as potential biomarkers for sperm freezability and fertility in the future (Yeste *et al,* 2017).

AQP9, one of the AQPs found in the male reproductive tract, is thought to play a significant role in the apical pathway for transmembrane fluxes of water and other solutes, including purines, pirimidines, carbimides, and polyols, which are collectively known as aquaglyceroporins (Matsuzaki *et al,* 2002; Tsukaguchi *et al,* 1998). AQP9's expression was inestigated in the testis and epididymis of several species, such as humans, bovines, buffalo bulls, wild ruminant species, dogs, agouti, rats, and adult mice (Oberska *et al,* 2024; Martinez-Madrid *et al,* 2023; Mohamed *et al,* 2022; Schimming *et al,* 2021; Domeniconi *et al,* 2007; Badran and Hermo, 2002; Pastor-Soler *et al,* 2001).

No data exist about this protein's expression in the camel's male reproductive organs. Thus, the current study's goal was to precisely locate AQP9 in various parts of the dromedary camel's testis and epididymis, to investigate the expression of these proteins which vary throughout the rutting season, and to compare this expression to that of non-rutting seasons using immunohistochemistry method (IHC).

Materials and Methods

Sampling

All procedures involving animal samples were conducted under the strict animal protocol approved by the ethical committee of King Faisal University. Thirty-six mature, healthy local bread dromedary camels (aged 4≥ years old or older) from the Al Omran abattoir in Al-Ahsa, Saudi Arabia, provided samples taken every two months for a year. Tissue samples were taken from the testes (cranial and caudal portions of the testis and rete testis) and caput,

SEND REPRINT REQUEST TO ABDELHAY M. ALI email: arhamallha@kfu.edu.sa

corpus, and cauda of the epididymis. Samples were kept in 10% buffered formalin for the IHC procedure.

IHC procedure

After being fixed in formalin, tissue samples were dehydrated in graded ethanol, cleaned via xylene, and embedded inside the paraffin wax. Sections of 5 µm were cut with a microtome and placed on Superfrost slides. Slides were stained using the avidin-biotin-peroxidase complex method after dewaxing and rehydrating (Adeghate *et al,* 2001). Antigen retrieval was carried out in a microwave oven for fifteen minutes using 0.01M PBS (pH 7.4). Subsequently, the sections were cooled to 25◦ C and given another PBS wash. 3% hydrogen peroxide was used for 30 minutes to suppress endogenous peroxidase. Goat serum (10%) was used for 20 minutes after three rounds of washing in PBS to prevent non-specific responses. After applying the primary antibody, polyclonal rabbit anti-AQP9 (Abcam, dilution 1:200, Cambridge, Cambridgeshire, UK), and the sample was incubated in a wet chamber for the whole night. The sections were treated with biotin-labeled secondary antibody and avidin-horseradish peroxidase (HRP). Dibutyl phthalate polystyrene xylene (DAB) was utilized to identify the positive staining. Section counter-staining was done using haematoxylin stain. The negative control sections follow the identical protocol except for skipping the primary antibody. Slides were examined under light microscopy for immunohistological investigations, and photomicrographs were taken.

Results

In all examined animals, immunohistochemical staining showed the existence of AQP 9 in the testis and epididymis of dromedary camels throughout both the rutting and non-rutting seasons. Tables 1 and 2 represented the localisation and intensity of AQP9 in these organs throughout the rutting and non-rutting seasons, respectively.

Rutting season

At the onset of the rutting season (October), the dromedary camel's testis and epididymis had varying reactions to the AQP 9 antibodies (Figs 1 & 2). In the testis, AQP 9 was localised moderately in the gonocyte and Sertoli cells lining the seminiferous tubules and the interstitial cells (Leydig cells) at the cranial portion and reti testis of the testis, while the protein expressed very strongly in the caudal portion (Fig 1A, 1B, 1C). The stereocilia pseudostratified columnar epithelium of the epididymis showed varying intensities of AQP9 immunoreactivity: weak in caput, very strong in the corpus, and strong in the cauda (Fig 2A, 2B, 2C).

Table 1. Showing AQP9 localisation in various parts of the dromedary camel's testis and epididymis during the rutting season.

Part / Month	T cr	T caud	T ret	EH	EB	ET
October	+ +	+ + + +	+ +	+	+ + + +	+ + +
December	+ +	+ +	+ +	+ +	+ +	+ +
February	+ + +	+ + +	+ +	+ + +	+ + +	+ + +

T cr, cranial part of the testis; T caud, caudal part of the testis; T ret, rete testis; EH, caput of epididymis, EB, corpus of epididymis, and ET, cauda of epididymis; +, weak reaction; + +, moderate reaction; + + +, strong reaction; + + + +, very strong reaction.

Table 2. Showing AQP9 distribution in various parts of the dromedary camel's testis and epididymis during the non-rutting season.

Part / Month	T cr	T caud	T ret	EH	EB	ET
April	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
June	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
August	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

T cr, cranial part of the testis; T caud, caudal part of the testis; T ret, rete testis; EH, caput of epididymis, EB, corpus of epididymis, and ET, cauda of epididymis; +, weak reaction; + +, moderate reaction; + + +, strong reaction; + + + +, very strong reaction.

In the middle of the rutting season (December), the lining epithelial cells of the seminiferous tubules and Leydig cells of the testis and the pseudostratified epithelium of the epididymis displayed moderate staining to the AQP 9 protein (Figs 1D, 1E, 1F, 2D, 2E, 2F). The epididymal sperm in the body and tail also had a positive reaction to this protein (Figs 2E, 2F).

At the end period of the rutting season (February), the lining epithelium of the seminiferous tubules and Leydig cells in the testis, the epithelial cells of all parts of the epididymis, and the epididymal sperm showed expressed AQP 9 strongly, while the rete testis epithelium remained moderately reactive to this protein (Fig 1G, 1H, 1I, 2G, 2H, 2I).

Non-rutting season

In the non-rutting season (April- September), the epithelial cells of the seminiferous tubules and interstitial cells in all parts of the testis and epididymis' epithelium in the dromedary camel displayed strong responses to the AQP 9 antibody with greater affinity in April (Figs 3 & 4). During this phase, epididymal sperm was highly reactive to AQP 9 antibody (Figs 3 & 4).

Fig 1. A photomicrograph of the dromedary camel's testis taken during the rutting season. In October, it showed a moderate immunoreactive of AQP9 in the lining epithelium (arrow) and Leydig cells (red arrow) of the cranial (A) and reti testis (C) parts, while the caudal part (B) showed extreme reaction to the AQP9 antibody. In December, all parts of the testis displayed moderate staining (D, E, F). At the end of the rutting season (February), the lining epithelium of the seminiferous tubules in the testis showed expressed AQP 9 strongly, while the rete testis epithelium remained moderately reactive to this protein (G, H, I). Negative control for the cranial and caudal parts of the testis and rete testis (J, K, L).

Fig 2. A photomicrograph of the dromedary camel's epididymis at the rutting season's beginning, middle, and end. In October, different levels of AQP9 immunoreactivity were seen in the lining epithelium (arrow): faint in the head, highly expressed in the body, and intense in the tail (A, B, C). In December, the AQP 9 protein showed notable staining in the lining epithelium of the epididymis's head, body, and tail (arrow) (D, E, F). The epididymal sperm in the body and tail reacted positively to this protein (arrowhead) (E, F). In February, the epididymal sperm (arrowhead) and the lining epithelium of all parts of the epididymis (arrow) demonstrated significantly expressed AQP 9 (G, H, I). Negative control for the epididymis's head, body, and tail (J, K, L).

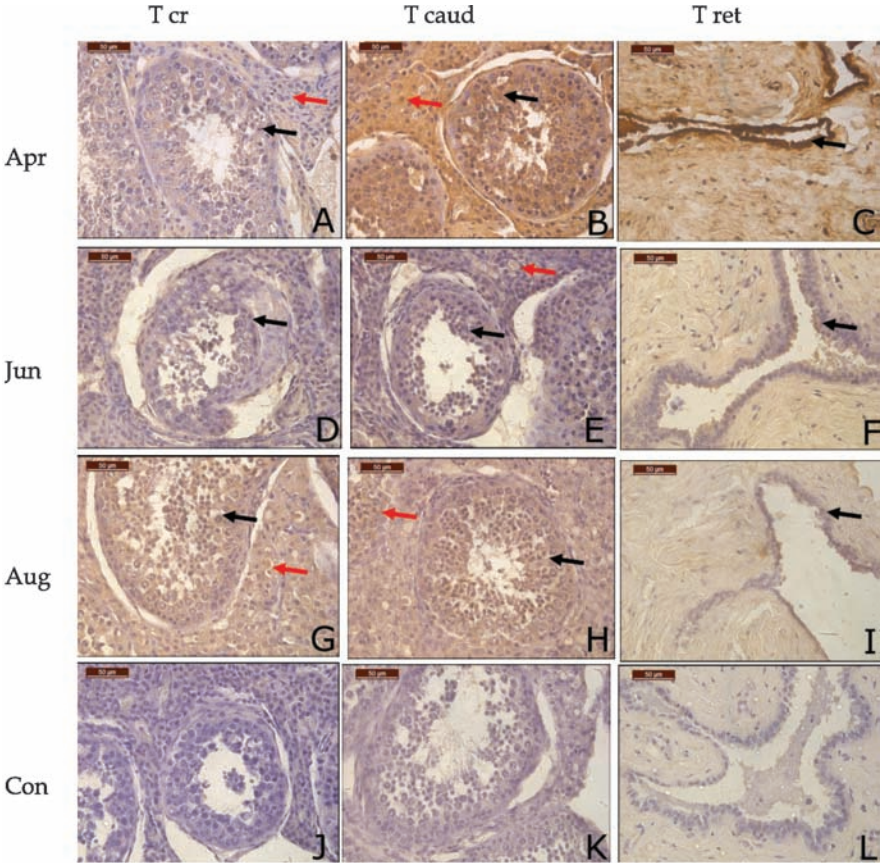

Fig 3. Fig 1: A photomicrograph of the dromedary camel's testis throughout the non-rutting season. In April, AQP9 protein expressed highly significant immunoreactive in the lining epithelium (arrow) and Leydig cells (red arrow) in the caudal (B) and reti testis (C) parts, while in the cranial part (A) is strong. The rest of the period shows a very strong in all parts of the testis (D, E, F, G, H, I). Negative control for the cranial and caudal parts of the testis and rete testis (J, K, L).

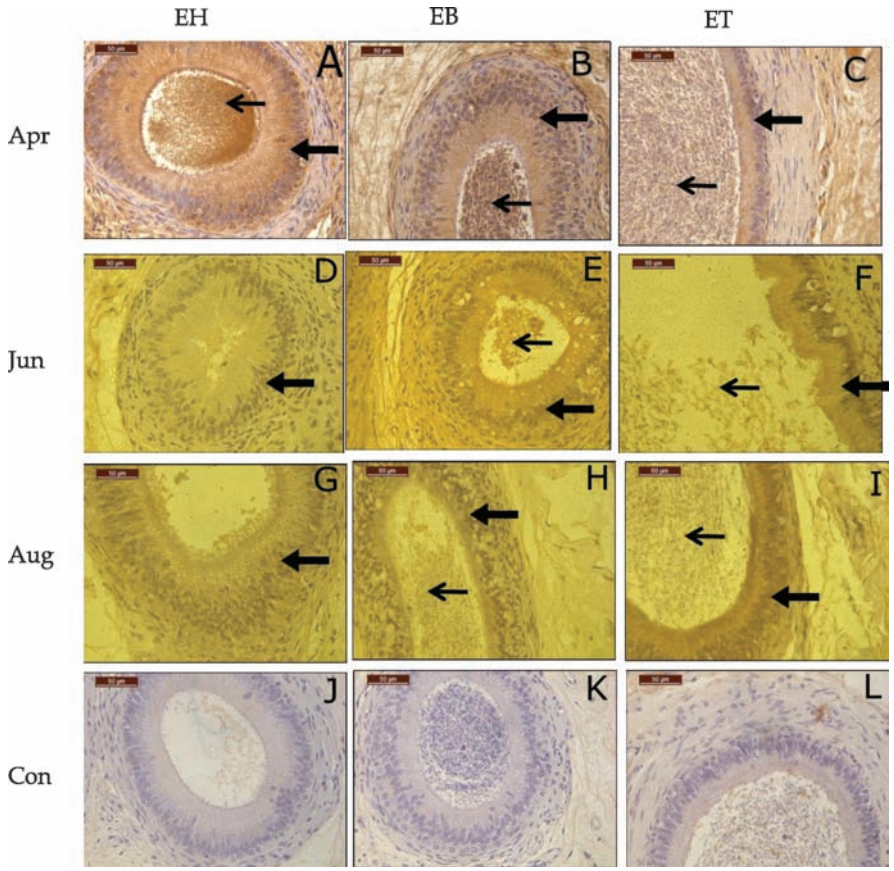

Fig 4. A photomicrograph of the dromedary camel's epididymis throughout the non-rutting season. In April, extreme levels of AQP9 immunoreactivity were seen in the lining epithelium (arrow) and epididymal sperm (arrowhead) in the head, body, and tail (A, B, C). The remaining time duration exhibits a strong in the lining epithelium and luminal sperm of each of the epididymis's parts (D, E, F, G, H, and I). Negative control for the epididymis's head, body, and tail (J, K, L).

Discussion

Our groundbreaking study, the first of its kind, delves into the AQP9 protein expressed in the male genital tract of camels. This research, which unveils the protein's localisation within the target cells, promises to illuminate the intricate roles of AQP9 in crucial fluid secretion and absorption processes, particularly in relation to spermatogenesis, sperm transition, and sperm maturation. The study is also the first to reveal the presence and distribution of aquaporins 9 in the testis and epididymis of dromedary camels, with a segment-specific distribution throughout the year. These findings have significant implications for our understanding of the male reproductive system of this animal and underscore the need for further research in this area.

The current study, conducted with meticulous attention to detail and precision, reveals that testicular and epididymal epithelial cells typically exhibit moderate immunoreaction to AQP9 antibodies in the middle of the rutting season. However, these cells also express significantly higher levels at the beginning and end of this season. Meanwhile, the AQP9 protein shows intense immunoreactive staining in these organs throughout the non-rutting season.

Among the results of this research is the expression of AQP9 in the gonocyte, Sertoli cells and Leydig cells of camel's testis, which, following several publications, that documented the expression of this protein in the testis of mammal species (Oberska *et al,* 2024; Martinez-Madrid *et al,* 2023; Mohamed *et al,* 2022; Schimming *et al,* 2021; Arena *et al,* 2011; Badran and Hermo, 2002; Nicchia *et al,* 2001; Elkjær *et al,* 2000). At the same time, other investigations reported that AQP9 was not found in the testicles of humans, dogs, or mice (Hashem, 2010; Domeniconi *et al,* 2007; Ko *et al,* 1999; Tsukaguchi *et al,* 1999). While the reasons behind these variations remain unclear, they underscore the urgent need for additional research on AQP9 in the dromedary camel and other mammal species to draw definitive conclusions regarding the function of aquaporin in the testis.

According to Setchell (1994), water is crucial in transporting sperm through the epididymis. In addition, transepithelial water flow in the male reproductive tract is critical for proper fertility (Domeniconi *et al,* 2008). As agreed upon by published investigations in mammals (Oliveira *et al,* 2013, 2005; Domeniconi *et al,* 2008, 2007; Badran and Hermo, 2002), the epididymal epithelium of the camel is considerably positive for AQP9 with variety in the distribution in the different parts in both seasons. Thus, possibly explained by the increased AQP9 levels during the slow increase in spermatozoa concentration from caput to cauda, they contribute to the hyperosmolar environment that maintains sperm quiescent. Following similar observations, (Belleannée *et al,* 2009) proposed that AQP9 is the primary water route in the mammalian epididymis.

According to these findings, AQP9 dispersion changed throughout the year in the testis and epididymis of the dromedary camel, which might be associated with variability in androgen and estrogen components across the whole year, as described in camel by Mohamed *et al* (2018). Furthermore, the seasonal variation studied by Althnaian in the camel and Oliveira and colleagues on a big fruit-eating bat confirmed the current research distribution (Althnaian, 2023; Oliveira *et al,* 2013). This seasonal variation could have significant implications for our understanding of the male reproductive system, particularly about the impact of environmental factors on the expression of AQP9.

To sum up, the findings strongly suggest that AQP9 is crucial for the differentiation and maturation of spermatozoa in the testis and epididymis. This implies that AQP9 could potentially serve as a unique biomarker for male fertility and infertility, and a valuable predictor of sperm quality.

Conflict of interest

None declared

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CAMEL-ASSISTED SERVICES (CAS): TREATMENT, EDUCATION AND SUPPORT PROGRAMMES

Carlos Iglesias Pastrana[1], Francisco Javier Navas González[1], Juan Vicente Delgado Bermejo[1], Douglas Baum[2], Valeri Crenshaw[3], Malin Larsson[4], Jenny Brachmann[5], Christina Adams[6], Samantha Arevalo[7], Michael Kaufmann[7] and David Argüelles Capilla[8]

[1]Department of Genetics, Faculty of Veterinary Sciences, University of Cordoba, Cordoba, Spain
[2]Camel owner 'Texas Camel Corps', Texas, USA/Independent researcher-documentarian
[3]Camel owner 'Shamrock Farms', Manhattan, USA/Secretary General, North American Camel Ranch Owners Association (NACROA)
[4]MSc inAnimal Science, Swedish University of Agricultural Sciences, Sweden/MSc in Environmental Psychology, Swedish University of Agricultural Sciences, Sweden
[5]Camel owner/Member of 'Altweltkamele e.V.', Löwenberger Land, Germany
[6]CEO and Co-founder of 'Nomad Ventures',Tennessee, USA/Independent Researcher and Author
[7]Green Chimneys Farm and Wildlife Center/Sam and Myra Ross Institute, Brewster, New York, USA
[8]Department of Animal Medicine and Surgery, Faculty of Veterinary Sciences, University of Cordoba, Cordoba, Spain

ABSTRACT

The increasing presence of camels in domestic settings has facilitated the implementation of camel-assisted services (treatment, education and support programmes) with satisfactory outcomes. However, their specific use for interventions that benefit human physical and psychosocial health requires further empirical investigation. This work reviews the characteristics of camels that make them suitable for these assisted services, such as their tranquil temperament, social character and unique locomotion. These assisted services can promote human and animal welfare and contribute to the sustainable conservation of zoogenetic resources. Nevertheless, more specific studies are needed to evaluate the detailed impact of these assisted services on human and animal health. Additionally, addressing the accessibility challenges is crucial, as costs are often not covered by insurance, making them less affordable.

Key words: Animal welfare, animal-assisted service, domestic camel, functional valorisation,human well-being, human-animal interaction

Historically, domestication arose from the human need for efficient food production (Hayden, 2009; Mota-Rojas *et al,* 2021) and as support in warfare (Hediger, 2017). Recently, there has been a rise in breeding animals for therapeutic, educational and recreational purposes through guided human-animal interactions (O'haire *et al,* 2015; Walsh, 2009). These interactions yield benefits across physical, social, emotional and cognitive domains for humans while promoting the psychological health of animals.

The latest 'Five Domains Model' incorporates human-animal interactions into animal welfare assessments, emphasising the need for animals to seek positive interactions with the environment, other non-human animals and humans (Mellor *et al,* 2020). The International Association of Human-Animal Interaction Organisations (IAHAIO) advocates that only domestic animals should participate in assisted services designed for human benefit (Jegatheesan, 2014), as they are more accustomed to human presence, minimising stress-related behaviours that could compromise both animal welfare and human safety.

The term 'animal-assisted interventions' (AAI) encompasses three categories: animal-assisted therapy (AAT), animal-assisted education (AAE) and animal-assisted activities (AAA). The new framework includes 'animal-assisted services' (AAS) as the overarching term, with subcategories such as 'animal-assisted treatment' (AATx), 'animal-assisted education' (AAE) and 'animal-assisted support programmes' (AASP) (Binder *et al,* 2024).

In AATx, animals participate in goal-directed activities to improve human physical, social, emotional, or cognitive functions, requiring licensed therapists and trained animals. AAE involves structured educational interactions, necessitating educators and child development specialists (Binder *et al,* 2024). AASP aims to enhance quality of life through recreational interactions, requiring activity coordinators and animal welfare professionals (Binder *et al,* 2024; Kruger and Serpell, 2010).

SEND REPRINT REQUEST TO CARLOS IGLESIAS PASTRANA email: ciglesiaspastrana@gmail.com

Common animals in AAS include dogs, cats, birds, horses and small ruminants (Martos-Montes *et al,* 2015). While donkeys and camelids are used in these activities, they are undervalued due to misconceptions about their temperament (González *et al,* 2019) and limited financial support for their inclusion. Health insurance companies and authorities may not be willing to subsidise animal-assisted services that involve camels due to the lack of scientifically proven impacts.

The present review work aims to create a conceptual framework for valorisation programmes for domestic camels (dromedaries or one-humped camels and Bactrian or two-humped camels) in animal-assisted services. This review aligns with the bottom-up approach to integrative validity, as proposed by Chen (2010), which emphasises viability as the key evaluation criterion for determining whether a program is practical, functional and evaluable. Systematic empirical research will enhance camel-assisted services (referred to as CAS from here after), addressing ethical concerns and promoting biological conservation by assigning these animals a new sustainable role (Glenk, 2017; McCune *et al,* 2020). In regions where domestic camels hold socio-cultural significance, their inclusion in therapy or educational programmes may also strengthen connections to local traditions and practices.

Theoretical foundations and previous experiences in CAS

Over the past three decades, there has been an increasing socio-economic interest in camel breeding and production due to their sustainability in the face of climate change and desertification (Faye, 2020). As the domestic camel population increases, it becomes imperative for caretakers to recognise these animals' behavioural needs to facilitate safe human-animal interactions that promote welfare. In domestic settings where camels are predominantly utilised for interactive experiences, this understanding is particularly essential. While traditional uses of camels in historically significant breeding regions focus on food production and transport, in countries where camel breeding is relatively nascent, such as in Europe and America, the emphasis often shifts towards leisure and fostering individual human-animal bonds.

Camels are group-living animals that display cooperative behaviours crucial for their survival in harsh environments (Brandlová *et al,* 2013). Their social structures and the dynamics within camel herds can strengthen social bonds and improve group cohesion, even though they engage in fewer close-contact interactions than other social animal species (Ward and Webster, 2016). This proclivity for cooperation underscores their social intelligence, which may enhance their engagement in structured activities and group settings, further highlighting their adaptability to therapeutic contexts where cooperation and focus are pivotal for successful outcomes. Moreover, given their evolutionary history in arid regions, camels have developed physiological adaptations, such as lower metabolic rates and energy requirements (Dittmann *et al,* 2014; Hoter *et al,* 2019; Nelson *et al,* 2015). These traits contribute to their generally calm temperament (Henry *et al,* 2010), which, along with their physical robustness and endurance (Soman and Tinson, 2016), makes them suitable for therapeutic settings where a reassuring presence is vital. Additionally, the minimal presence of natural predators contributes to their non-flight responses (Deel, 2014; Irwin, 2010). In any case, it remains crucial to ensure that these inherent characteristics do not result in overworking the animals.

Variability in camel behaviour and reactivity can arise from factors such as sex, age and phaneroptics. In particular, in leisure-oriented tourism activities, male dromedaries are often seen as more cautious and reactive than females, but castration, training and desensitisation protocols can help develop proactive coping behaviours from a young age (Pastrana *et al,* 2021; Pastrana *et al,* 2024). Furthermore, traits such as coat and eye colour have been reported to influence camel social dynamics and leadership behaviour. These findings are attributed to the pleiotropic effects of genes governing phaneroptical traits on the development and functioning of neural structures. Specifically, dromedaries exhibiting a variable proportion of white fur and significant iris depigmentation or heterochromia tend to be more submissive, potentially impairing their leadership abilities. Conversely, darker, younger and heavier individuals are more inclined to lead group movements (Iglesias Pastrana *et al,* 2021).

Given their social nature, the presence of conspecifics also fosters proactive coping behaviours, which not only facilitates group training but also enhances the comfort of the camels during collective activities (Pastrana *et al,* 2021; Pastrana *et al,* 2024). Understanding the social dynamics, including intraherd social hierarchies, enables handlers and caregivers to strategically utilise group structures in

training sessions and routine management activities. This approach allows for the selection of camels based on dominance or leadership traits, depending on the specific functional objectives (Schulte and Klingel, 1991).

Concerning the human-driven settings in which these animals are reared for various functional purposes, camels produce less noise and odour compared to conventional livestock, thereby enhancing the sensory experience of visitors at camel facilities (Gole and Hamido, 2020). The hypoallergenic nature of camel hair fibres may also reduce allergic reactions among those interacting with them (Fazal-ur-Rehman *et al,* 2024). Moreover, the rising demand for camel milk, particularly among health-seeking consumers, further underscores the appeal of camel-assisted treatments (Gahlot and Adams, 2023).

Based on this theoretical framework, domestic camels are gradually gaining popularity among professional groups dedicated to developing animal-assisted services (Kinoshita and Kaufmann, 2023; Lidfors *et al,* 2023). Larsson and Brothers (2019) conducted a survey that revealed camels have been actively included in animal-assisted services (AAS) in Australia, Austria, Germany and the United States for over ten years. The study involved individuals aged 15 to 24 and focused on educational objectives, therapeutic riding and treatments for emotional and cognitive conditions (autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), depression, separation anxiety, irritability and sadness). Results indicated improvements in overall well-being, attention, prosocial behaviour, physical coordination and self-esteem among human participants. However, there is limited information on the impact of these activities on the camels themselves, highlighting the need for future research to ensure their welfare is adequately assessed.

From a delimited perspective of animal welfare, Majchrzak *et al* (2015) found that tourist camel rides positively influenced camel welfare, evidenced by decreased cortisol levels, indicating reduced stress compared to non-participating dromedaries.

Utilitarian principles in CAS

This section discusses the potential utilitarian fundamentals and functional objectives of CAS to guide health and education professionals, as well as AAS practitioners, in recognising the diverse therapeutic, educational and recreational contributions of camels. It is important to note that no single form of AAS is universally superior for specific groups; individualised assessments are necessary to determine the appropriateness of assisted services for each person. The effectiveness of AAS is multifactorial and varies based on the specific context of its application. While an activity may focus on a particular objective, it also requires other important participant skills due to the surrounding environment. A thorough analysis and evaluation of each activity can uncover additional requirements, including motor, cognitive, social, communication, emotional regulation and sensory skills.

Camel-assisted treatment (CATx).

Functional potentialities associated to patients' familiarity with camels

A. Novelty: In regions where camel breeding is still emerging, these animals may be perceived as unique or exotic, capturing the attention of patients more effectively. The distinctive characteristics and size of camels can engage patients' interest, reducing disengagement during therapy and fostering trust. This, in turn, can help improve social interaction skills with unfamiliar elements (Allison and Ramaswamy, 2016; Heidicke, 2021).

B. Exposure: The exotic appearance of camels may initially intimidate patients, especially those with anxiety disorders (Firmin *et al,* 2016). However, camel-assisted treatment sessions can provide a controlled setting where patients are gradually exposed to anxiety-inducing stimuli under the guidance of trained therapists. This exposure, combined with emotional regulation techniques, may help patients manage their anxiety. Interestingly, some patients may even experience unexpected relaxation around camels, possibly due to the lack of preconceived negative preconceptions.

C. Recognition and flexibility: For individuals familiar with camels, these animals can facilitate better communication, encouraging self-expression and dialogue. Familiarity with camel body language, facial expressions and vocalisations also enhances patients' ability to recognise the emotional states of others, thereby promoting socialisation (Alwahaibi *et al,* 2023; Ramadan *et al,* 2018; VanFleet *et al,* 2015; Volodin *et al,* 2022). Patients also learn to interpret subtle cues such as avoidance behaviours and are encouraged to develop flexible thinking, adapting to changing situations and recognising when to withdraw from uncontrollable circumstances (Kaufmann *et al,* 2019).

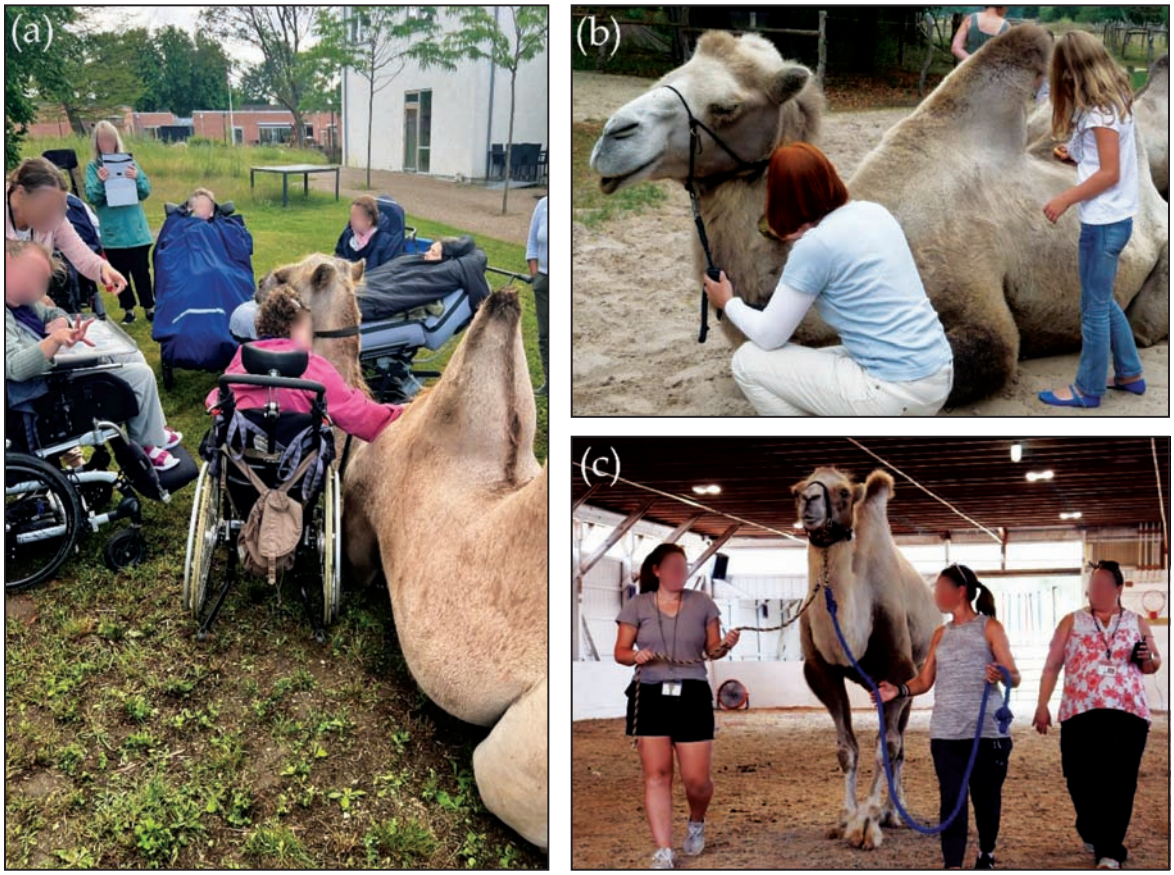

Fig 1. Illustrative scenes of camel-assisted treatment programmes, showcasing therapeutic interactions between camels and participants (a) A therapeutic session for individuals with functional diversity using wheelchairs, with the camel in sternal recumbency, facilitating accessibility (©Henrik Møller); (b) Two participants brushing a camel in sternal recumbency as part of a sensory and therapeutic interaction (©Malin Larsson); (c) An instructional activity teaching participants how to handle camels, focusing on developing confidence (©Green Chimneys Farm and Wildlife Centre/Sam and Myra Ross Institute).

Functional potentialities associated to camel social behaviour

A. **Non-demanding socialising:** Camels, while needing social connections, do not always engage in direct interaction unless for specific purposes like reproduction or dominance (Mohammed *et al,* 2020). Their ability to maintain well-being through passive cohabitation (Heidicke, 2021; Kikusui *et al,* 2006) can be advantageous for individuals with ASD, who may struggle with forming social connections and experience distress in isolation. The presence of camels offers a therapeutic opportunity for ASD patients to experience social engagement without pressure, potentially easing feelings of isolation and encouraging gradual interaction (Beetz, 2017).

B. **Calmness:** Camels naturally display a calm demeanor in non-threatening environments, an evolutionary trait linked to energy conservation. This innate calmness, paired with the intriguing nature of camels, makes them suitable candidates for animal-assisted treatment sessions targeting individuals with ASD or ADHD.

Functional potentialities associated to the camel morphology/size and locomotion

A. **Sternal recumbency:** When camels lie in sternal recumbency (with their chest and abdomen on the ground), their necks position their heads at a height similar to that of a medium-sized human. This posture reduces the perceived threat of the camel's size and minimises the risk of sudden movements (Niehaus, 2022), making interactions safer, particularly for patients developing social skills and confidence. It also facilitates secure engagement for individuals with physical disabilities, such as wheelchair users (Fig 1).

B. **Humps:** With the animal in sternal recumbency, the hump(s) can serve as an additional support mechanism for improving postural control.

C. **Size:** The substantial size of camels may facilitate nonverbal communication between the patient

Fig 2. Representative images of camel-assisted education programmes, depicting educational sessions where camels are integrated as part of the learning process (a) A session on safe camel handling techniques, promoting proper interaction with the animals (©Green Chimneys Farm and Wildlife Centre/Sam and Myra Ross Institute); (b) A group camel handling activity, emphasising the importance of cohesion and coordination (©Doug Baum); (c) An environmental education session using camels as model animals with primary school children (©Ursula Schulz); (d) A specific moment from an environmental education programme, where the instructor explains the unique callosities of camels, highlighting their adaptations (©Carlos Iglesias Pastrana).

and the animal, which could be particularly useful in crafting treatment programmes for narcissistic personality disorder (Abrams, 2013).

D. **Empowerment:** Interacting with and managing a large animal like a camel, under professional guidance, can help boost self-esteem. Patients are given specific tasks and recognised for their bravery, which fosters empowerment (Fig 1) (Granger and Kogan, 2000). These experiences can help socially excluded individuals overcome feelings of marginalisation through meaningful engagement with the animal.

E. **Locomotion:** The camel's unique four-beat gait mirrors natural human pelvic movement, making it beneficial for physiotherapy (Ni, 2020). However, difference between Bactrian and dromedary camels impact therapeutic riding experiences:

   a. Dromedary camels offer varying stability depending on seating position, though there is a lack of studies on optimal saddle configurations.

   b. Bactrian camels provide greater stability to the rider due to their two humps. In addition, riding between the humps of a Bactrian camel allows direct contact between the rider and the camel's soft and warm fur, which might have a therapeutic impact. Furthermore, when riding a Bactrian camel, the cranial hump can assist patients in developing visual orientation and offer tactile stimulation to the hands, arms and upper trunk, as well as the caudal hump may provide support and stimulation for the patient's back, promoting improved movement symmetry.

Other indirect avenues

Although, research in this area is still in its early stages, preliminary findings suggest a potential therapeutic benefit of camel milk for patients with

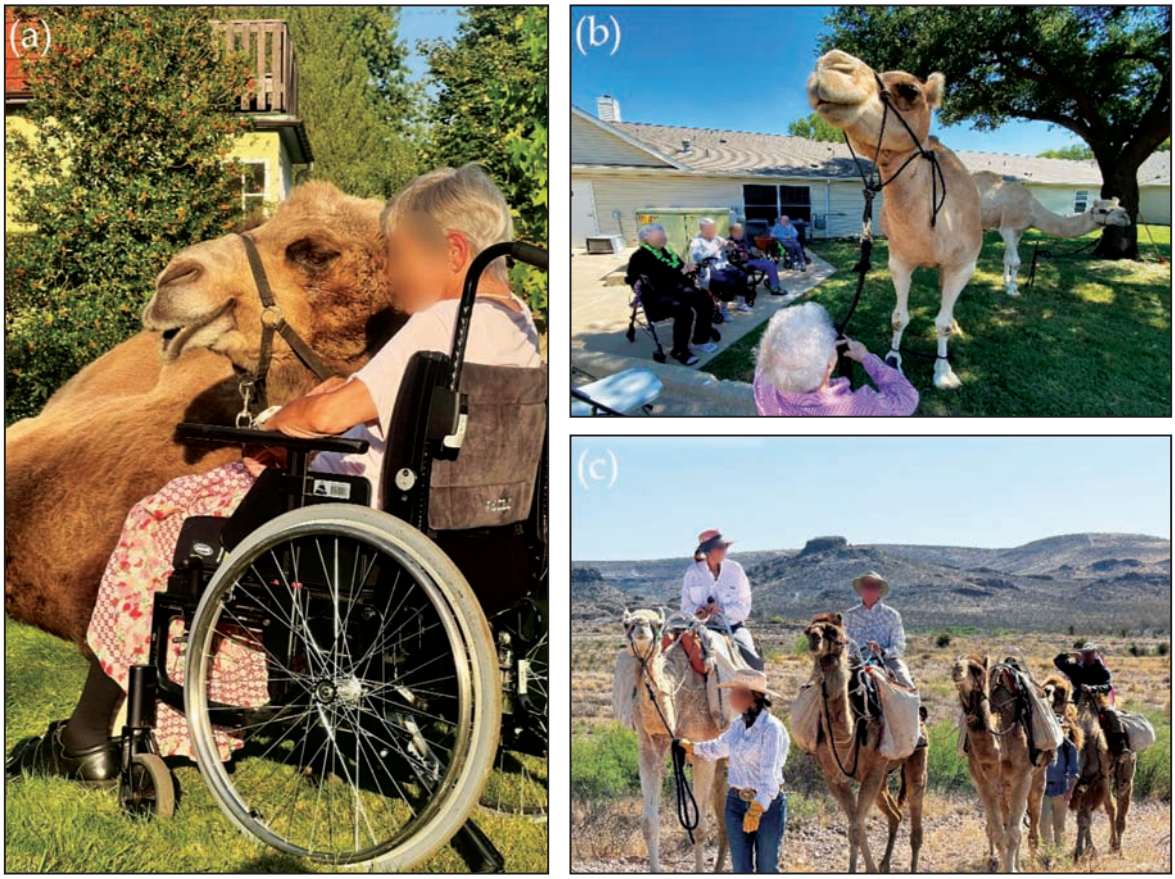

Fig 3. Scenes from camel-assisted support programmes (a) An interaction between an elderly individual and a camel in sternal recumbency, designed to foster emotional connection and provide comfort (©Henrik Møller); (b) Camels during a nursing home visit (©Doug Baum); (c) Camel trekking activity, which offers participants both physical engagement and emotional support, emphasising the therapeutic benefits of outdoor experiences with camels (©Doug Baum).

ASD. According to scores on the Childhood Autism Rating Scale (CARS) and the Social Response Scale (SRS), individuals with ASD who consume camel milk demonstrate significant improvements(Adams, 2013; Adams, 2019; Al-Ayadhi and Elamin, 2013; Al-Ayadhi *et al,* 2015). Further investigation is strongly recommended to enhance understanding of the effects of camel milk on neurological disorders.

Camel-assisted education (CAE)

A. Meeting strangers and self-reflection: Preparing for camel interactions involves familiarising oneself with the animal through activities like trekking and grooming. Participants learn to bond with the camel by securely fitting gear and interacting through gentle touch and verbal cues (Fig 2). Emphasising calmness and empathy fosters self-reflection and encourages kindness towards others (Altschiller, 2011; Heidicke, 2021).

B. Trust in strangers (humans and animals): For many, interacting with a camel may be a first-time experience, requiring trust in the camel handler's guidelines and the animals themselves to ensure a safe interaction (Fig 2) (Altschiller, 2011; Heidicke, 2021).

C. Listening and following instructions: Given their size and strength, camels can pose risks if not handled correctly. Camel handlers need to emphasise safety protocols, making it crucial for visitors to pay attention to grasp the safety instructions (Fig 2) (Altschiller, 2011; Heidicke, 2021).

D. Responsibility and self-efficacy: Engaging in tasks such as grooming, cleaning and feeding camels may help participants develop a sense of responsibility and empathy (Altschiller, 2011).

E. Group work: Camelback riding is typically done in groups to prevent undesirable reactions in camels due to anxiety from being separated from their conspecifics. Additionally, camels are accustomed to walking in a caravan (Riemer and Förster, 2021).

This reinforces the importance of group cohesion for safety and enhances the overall experience (Fig 2) (Altschiller, 2011).

F. Environmental and cultural education (Heidicke, 2021; Irwin, 2010; Khan et al, 2003): Camels can serve as effective models for environmental education in several ways (Fig 2):

- **a. Environmental adaptations:** Teaching about camels' adaptations to arid environments, including water conservation and heat tolerance.
- **b. Habitat conservation**: Raising awareness about conserving the ecosystems where camels thrive.
- **c. Sustainability:** Promoting sustainable resource use for communities that rely on camels for transportation and food.
- **d. Local culture and traditions:** Exploring the socio-cultural significance of camels in communities, as well as their historical roles, such as their importance on the Silk Road.

Camel-assisted support programmes (CASP)

Animal-assisted support programmes extend beyond entertainment, aiming to improve the physical and emotional well-being of participants in various settings:

A. Guided visits: Offering camel-guided visits to geriatric homes, hospitals, or prisons may introduce a multisensory experience that breaks the monotony of long-term stays. The presence of camels stimulates socialisation, encourages gentle physical activity and evokes positive emotions, improving overall well-being (Fig 3).

B. Camel trekking tours: Camel trekking tours, set in rural environments, allow participants to reconnect with nature and unwind from daily routines. The calming, rhythmic motion of riding a camel further promotes relaxation (Fig 3).

Ethics and safety of CAS

The implementation of CAS requires careful consideration of ethics and safety, ensuring proper management of both human participants and camels. CAS must adhere to established standards, set clear objectives and well-defined measurable outcomes in areas such as health, well-being, or education, be subject to regular oversight and be conducted by professionals with proper training.

Key points include:

1. Ethical considerations:
   a. Activities must be conducted in a respectful and non-exploitative manner, ensuring the welfare of camels and informed consent from participants. Camels should be viewed as co-therapists/co-workers and the human-camel relationship should be based on mutual respect (Clark *et al,* 2020).
   b. Handlers must be trained to recognise signs of stress and body language in camels, ensuring ethical and appropriate handling practices. Understanding signs of stress and body language in camels is further crucial to determine when an animal may need to be temporarily removed from a session or retired altogether (Brelsford *et al,* 2020; Murthy *et al,* 2015; Ng and Fine, 2019).
   c. Camel handlers must have AAS-specific training, including handling best practices, zoonosis prevention and professional conduct. Additionally, access to continuing education and maintaining recognised handling credentials are crucial to ensure that handlers stay updated on best practices and standards, fostering a safer and more effective environment for all participants (Kerulo *et al,* 2020; Linder *et al,* 2017; Stewart, 2014; Stewart *et al,* 2013).
   d. Animal-assisted service teams and facilities should be submitted to thorough and regular evaluations to assess their competences to remain in these activities (Binfet and Hartwig, 2019; Kerulo *et al,* 2020; Murthy *et al,* 2015; Serpell *et al,* 2020).
2. Safety considerations:
   a. The physical, mental and emotional safety of human participants, especially those in vulnerable situations, must be ensured through clear communication (e.g., information about the nature of the activities, potential risks and expectations) and personalised activities (American Veterinary Medical Association, 2024).
   b. The safety of camels is equally important. To provide camels with adequate training, routine care and rest periods, camels should be closely and routinely monitored for signs of stress or discomfort (Benaissa and Iglesias Pastrana, 2024; Hamad *et al,* 2022), ensuring that they are not placed in situations that could cause them undue stress or harm and guaranteeing that equipment like saddles or harnesses fits properly.

c. Facilities should be well-designed and maintained, adhering to animal welfare regulations and public safety guidelines and accommodating participants' needs (e.g., ramps, wide doorways and adaptable equipment for individuals with various disabilities) (American Veterinary Medical Association, 2024).

d. Involvement of veterinarians in the planning and maintenance of CAS programmes is crucial for ensuring adequate hygienic maintenance to minimise the risk of exposure to zoonotic pathogenic agents and prevent the dissemination of diseases (Boyle *et al,* 2019).

Actually, one of the primary challenges in maintaining a high level of animal care in CAS may be the availability of veterinarians with specialised knowledge about camels, particularly in new 'camel countries' (i.e., the nations where camels have been recently introduced and are contributing to the diversification of the livestock industry, especially in Western regions), where such expertise is often limited. Veterinarians involved in CAS programmes must not only possess general animal health knowledge but also be well-versed in camel-specific management strategies, including environmental adaptations. This is especially relevant when designing CAS programmes in areas with climate conditions that differ significantly from the camels' native habitats. Environmental factors such as temperature and humidity levels have a substantial impact on camel's thermal comfort. Inadequate housing systems can exacerbate health risks, further emphasising the importance of veterinary expertise and proper facility design to meet camels' specific climatic requirements (Faraz *et al,* 2024).

3. Recommendations for selection and training of camels participating in CAS:

   a. Camels selected to participate as co-therapists or co-workers in CAS with different groups and functional objectives should be adult, ideally socially mature and carefully selected based on their temperament, health status, physical constitution and ability to adapt to different environments (Fredrickson-MacNamara *et al,* 2006; Linder *et al,* 2017; Murthy *et al,* 2015; Winkle *et al,* 2020).

   b. For animals with partially unknown life histories, such as camels acquired from circuses, thorough ethological evaluation is crucial to determine their suitability for AAS. Previous and unknown events in the animal's life could condition the occurrence of unwanted/altered reactions to stimuli. This evaluation should assess the camel's behaviour towards humans, reactions to sounds and movements, interactions with other camels and responses to commands, as well as signs of stress and escape behaviours. Additionally, it is essential for animal handlers to receive proper training and supervision to ensure safe and responsible interactions, with a solid understanding of camel behaviour and welfare to anticipate and mitigate potential hazards during activities.

   c. An ethical training approach should combine both positive and negative reinforcement to enhance camels' adaptability and behaviour. For positive reinforcement, it is crucial to strike a balance by using food rewards judiciously and intermittently, while also incorporating other forms of positive reinforcement, such as verbal praise or tactile rewards, to maintain motivation without creating dependency solely on food. In the case of negative reinforcement (e.g., utilisation of a lead rope and apply gentle tension to guide the camel, promptly releasing pressure as soon as the camel begins to move), its ethical use is essential to ensure the safety of the camels and humans involved. To gain a further, comprehensive understanding of the fundamental principles for managing and training camelids, we can refer to a dedicated methodology known as CAMELIDynamics (Bennett, 2022).

Evaluation of CAS

Programme evaluation involves investigating the outcomes of AAS to assess their merit, value, or success in producing desired changes (Ballesteros, 2001). Key evaluation concepts include efficacy, effectiveness and efficiency (Echeburúa and Corral, 2001; González, 2019; Seligman, 1995). Additionally, important points to consider are utility, viability, addressing social needs and precision (Chen, 2010).

Before evaluating CAS, a thorough need assessment is essential to identify the target population, understand their specific challenges and determine how CAS can effectively address these needs. A systematic approach to CAS evaluation includes the following steps:

1. Programme design and implementation: Begin with a detailed assessment of the programme's design and implementation. This includes evaluating the selection and training of camels, the structure of the CAS, the qualifications of facilitators and adherence to established ethical standards and best practices.
2. Outcome measurement: Employ a variety of outcome measures across physical, psychological and social domains to gauge the impact of CAS. This includes changes in emotional regulation, social interactions, physical functioning and overall quality of life. Utilising standardised assessment tools for both quantitative and qualitative records ensures a comprehensive understanding of programme outcomes. Additionally, measuring the well-being and behavioural changes of camels provides a holistic view of the programme's impact.
3. Data analysis and interpretation: Rigorous analysis of data is crucial for drawing meaningful conclusions. Both quantitative and qualitative statistical analysis (Pandey *et al,* 2024) offers a comprehensive understanding of AAS effects, guiding future programme refinements and research directions.
4. Participant feedback: Gathering participant feedback is integral to the evaluation process. Surveys, interviews and focus groups can yield valuable insights into perceptions of programme efficacy, satisfaction and areas for improvement. Feedback from camel handlers, clients, parents, family members, teachers and school administrators can provide a broader perspective on the programme's effectiveness and operational challenges.
5. Long-term follow-up: Evaluating the sustainability of outcomes over time is critical for assessing the lasting impact of CAS. Long-term follow-up enables tracking of participants' progress beyond the CAS period, identifying potential challenges or relapses. Incorporating long-term monitoring of both participants and camels ensures sustained benefits from the service and allows for timely addressing of any emerging issues.

The calm demeanor and social intelligence of camels suggests that these animals can create a supportive and calming environment for human participants in AAS. Their ability to work as a team in challenging conditions further highlights their adaptability to therapeutic settings, where cooperation and attentiveness are vital for achieving successful outcomes.

Potential benefits from camel-assisted services (CAS) include various advantages for human patients and the camels themselves. For human patients, physical benefits may involve improved balance, muscle strength, coordination, postural control, increased range of motion and stimulated sensory integration. Psychosocial benefits can lead to better socialisation, enhanced self-esteem, self-confidence, self-discipline and self-efficacy, along with improved cognitive abilities (attention and concentration) and reduced anxiety and stress. Educationally, participants can develop camel-handling skills applicable in other contexts and gain knowledge of animal care, environmental stewardship and cultural sciences.

For camels, involvement in CAS may enhance their physical health through increased activity, provide mental and emotional enrichment to combat boredom and reduce stress and ensure they receive dedicated attention and care from their trainers and caregivers, promoting their overall well-being.

While CAS is an emerging field, there is a pressing need for further scientific research to validate their long-term efficacy, effectiveness, efficiency and sustainability.

Animal, camel and human health professionals, alongside education specialists, are uniquely positioned to contribute to CAS initiatives. Expertise of those delivering community services and supporting the scientific evaluation and documentation of both the benefits and potential risks associated with CAS is crucial.

Authors' contribution

Carlos Iglesias Pastrana conceived the study design and led the project. All authors contributed equally to the critical review and revision of the manuscript.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EFFECT OF FEEDING SALVADORA OLEOIDES LEAVES ON GROWTH AND NUTRIENT UTILISATION IN CAMEL CALVES

Mamta Laxmi[1], R. Nehra[1], R.K. Sawal[2], R.K. Dhuria[1] and A. Sahoo[2]

[1]Department of Animal Nutrition, Rajasthan University of Veterinary and Animal Sciences, Bikaner

[2]ICAR-National Research Centre on Camel, Bikaner, India

ABSTRACT

Study was conducted to evaluate extent of incorporation of *Salvadora oleoides* leaves on growth and nutrient utilisation in the diet of camel calves. Feeding trial of 60 days duration was followed by digestibility trial of 7 days duration. Twelve growing female camels having similar age (1-2 years) and body weight were selected and distributed randomly into three groups of four each (T_1, T_2 and T_3). T_1 group received basal roughage diet containing crop residues of groundnut (*Arachis hypogea*) and guar (*Cymopsis tetragonaloba*) in equal proportions. Treatment groups T_2 and T_3 group were provided basal roughage along with 5% and 12.5% *Salvadora oleoides* leaves (on dry basis). Live weight, ADG, nutrients intake, rumen fermentation and haemato-biochemical parameters were monitored. Observations revealed no significant change in live weight change and digestibility of dietary nutrients. However, significant effect was observed with respect to protozoal numbers; concentration of NH3N, total nitrogen and total VFA concentration. Results indicated that intake of dry matter, organic matter and crude protein significantly ($P \leq 0.01$) improved when supplemented at 5% level. However, digestibility of nutrients, haemato-biochemical parameters, water intake and faecal pellet attributes of animals were not affected. *Salvadora oleoides* leaves can be used as a cheaper feed resource at a level of 5% of diet due to its availability in the arid region wherein it can be pruned and fed as fresh or in dry form without any adverse effect.

Key words: Camel calves, *Salvadora oleoides* leaves

Camel, a unique animal species of desert ecosystem is adapted to sustain on a variety of feeds and fodders like grasses, tree leaves, crop residues and agro-industrial by products (Nagpal *et al*, 2002). The feeding system involving conventional forages seems inadequate and thus, it is imperative to use other non-conventional resources like leaves from trees and bushes as they form substantial biomass and could be of potential use in animal feed industry. A good and less expensive supply of protein and minerals is provided by trees leaves (Moyo *et al,* 2012; Sahoo and Sawal, 2021). *Salvadora oleoides* found in the dry arid regions of India (Khatak *et al,* 2010), locally known meetha jal, bada jal, pilu etc; it has immense ecological, economical as well as ethno-medicinal value. Salvadora with its remarkable drought resistance and high nutritional value, stands out as an excellent fodder option for animals in arid regions. Studies consistently show improvements in growth performance, milk production, digestibility, nutrient utilisation and overall health in animals fed with *Salvadora persica* leaves highlight the plant's utility in enhancing livestock productivity and sustainability in challenging environments (Priyanka *et al,* 2024). Leaves are bluish green and have leathery appearance (Garg *et al,* 2013), they have been found to contain a variety of chemical components including carbohydrates, alkaloids, steroids, glycosides, saponins, tannins, triterpenes, mucilage, lipids and oil (Arora *et al,* 2015). It also possesses anti-inflammatory, analgesic, antiulcer, anthelmintic, antibacterial and antifungal properties (Arora *et al,* 2015). Feeding experiments in cows have revealed 12% increase in milk yield and improved milk fat content by 1.5% indicating salvadora could enhance both the quantity and quality of milk produced (Khan *et al,* 2016). Ahmed *et al* (2017) reported 18% increase in milk production in buffaloes; additionally, there was improvement in the overall health and body condition of the which was attributed to the higher nutritional content of the *Salvadora persica* foliage. El-Shaer and Tawfik (2020) identified the anthelmintic properties of *Salvadora persica*, noting a significant reduction in parasite load and improved health and weight gain

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in treated camels. The present study was done with the objective to evaluate nutritional value of *Salvadora oleoides* and extent of utilisation in the diet of camel.

Materials and Methods

Twelve growing female camels of similar body weight and age (1-2 years), uniform conformation was selected from camel herd of ICAR-NRCC, Bikaner and randomly distributed into 3 groups of 4 each and fed experimental diets for 60 days followed by 7 days digestibility trial. The animals were housed in hygienic, well-ventilated shed with sandy floor, asbestos roofing, equipped with mangers for individual feeding. Incorporation of fresh leaves of *Salvadora oleoides* was assessed on the basis of palatability, intake of nutrients, body weight change and practical nutritional worth. Rumen fermentation attributes i.e. ruminal fluid pH, ammonia nitrogen, total nitrogen and TCA precipitable nitrogen, total protozoal count and total volatile fatty acid were estimated. Blood samples were also collected by jugular venipuncture and estimated for haemato-biochemical parameters to ascertain physiological health status of animal.

The camels were given prophylactic dose of anthelmintic and allowed to acclimatise for a period of one month prior to conduct of the experiment. Control group T_1 received combination of Guar and groundnut crop residue (1:1) was replaced with *Salvadora oleoides* leaves at 5% (T_2) and 12.5% (T_3) group (on dry basis).

Feeds offered, residues and faeces collected during the study along with leaves of *Salvadora oleoides* and *Salvadora persica* were analysed as per AOAC (2016). Phytochemical fractions were evaluated in different plant, viz. Total phenols (Hagerman *et al,* 2000), non-tannin phenolics by Folin-Ciocalteu method (Makkar, 2003) and saponin (Hiai *et al,* 1976).

The rumen liquor pH was estimated using digital pH meter, ammonia nitrogen content was estimated by Conway diffusion disc method (Conway, 1962). Rumen liquor was analysed for total nitrogen and TCA precipitable nitrogen by Kjeldahl procedure (AOAC, 2016). Total volatile fatty acid concentration was determined using Markham apparatus (Barnett and Reid, 1957). Haemato-biochemical parameters were estimated to ascertain physiological status of camel. Data generated during the studies was analysed by using randomised block design (Snedecor and Cochran, 1994).

Results and Discussion

Evaluation of both the available species of Salvadora revealed that *Salvadora persica* contained higher ether extract, total ash, acid insoluble ash, lignin content whereas it contained, lower organic matter, protein NDF and ADF and Calcium though phosphorus content was similar among species. Chemical composition of the feeds consumed by the animals (Table 1) revealed that the basal feeds were high in protein content. However, Salvadora leaves from both the species contained higher protein than the basal feed provided, it was low in NDF and ADF content but high in calcium and phosphorus content compared to groundnut and *Cymopsis tetragonaloba* crop residues. Relative to crude protein content observed in the present study, higher protein content has been reported earlier (Chaudhary, 2015). Variation could be due to season of sampling or leaves maturity. Higher content of total phenols, total tannin, condensed tannin and hydrolysable tannin have been reported in Khejri (*Prosopis cineraria*) leaves and Pala (*Zizyphus nummularia*) leaves which are also used for animal feeding in arid regions (Kumari

Table 1. Chemical and phyto chemical fractions of experimental feeds (% DM basis).

Feed stuff	Groundnut chara*	Guar chara**	*Salvadora oleoides*	*Salvadora persica*
DM	91.28	90.58	32.02	34.7
OM	91.25	90.2	82.71	78.78
CP	8.51	7.13	10.91	10.29
EE	0.976	1.15	1.2	1.85
TA	8.75	9.80	17.28	21.21
NDF	47.7	47.00	31.2	25.6
ADF	35.80	37.00	17.2	14.8
AIA	1.91	2.30	6.24	10.72
Lignin	4.50	6.00	1.6	2.6
Ca	1.68	0.89	8.80	7.5
P	0.52	0.17	0.65	0.63
Phytochemical fraction				
Total Polyphenols	1.38	0.65	3.87	3.89
Non-Tannin Phenolics	0.53	0.42	1.19	1.21
Total Tannin	0.38	0.09	2.85	2.86
Condensed Tannin	0.32	0.27	0.80	0.86
Hydrolysable Tannin	0.53	0.22	2.06	2.09
Saponin	3.12	2.96	5.16	5.75

**Arachis hypogea* crop residue, ***Cymopsis tetragonaloba* crop residue, Salvadora leaves.

et al, 2023). However, the cultivated legume crop residues contained lower quantities as tree forages accumulate more phenolic constituents as a defense mechanism against browsing by herbivores (Salminen and Karonen, 2011).

Dry matter intake in the treatment groups (Table 2) was observed to be higher in T_2 which decreased in T_3 which could be due to presence of fresh leaves in the form of soft twigs. However, DMI and OMI were observed to be similar when expressed on metabolic body size. Dry matter consumption (kg/d) was lower ($P<0.05$) in camels fed 12.5% tree leaves as compared to T_2 group which might be attributed to the effect of tannins on voluntary feed intake. Similar findings were reported by Olafadehan *et al* (2014). Crude protein intake improved significantly ($P\leq0.05$) with increase in *Salvadora oleoides* leaves in the diet due to supplementation of leaves with higher protein content. The findings of present investigation regarding CPI was in accordance with earlier observations on neem leaves in the diet of goats (Dida *et al,* 2019). Water consumption was observed to improve with supplementation of Salvadora leaves though the differences were not significant.

Table 2. Effect of feeding salvadora leaves on DMI and digestibility of nutrients.

Attributes	T_1	T_2	T_3	SEM	P value
Initial weight (kg)	366.52	371.0	370.32	17.5	0.987
Final weight (kg)	385.97	391.5	390.12	18.27	0.963
Daily gain Kg/d	0.324	0.343	0.330	0.031	0.978
DMI kg/d	6.17a	6.27b	6.19a	0.015	0.001
DMI kg/100kgBW	1.70	1.69	1.71	0.010	0.672
DMI g/kgW0.75	74.29	74.40	75.40	0.317	0.316
OMI (g/kgW0.75)	66.49	66.53	67.43	0.250	0.242
CP Intake (kg/d)	0.487^a	0.497^b	0.512^c	0.003	0.000
CP Intake (g/ kgW0.75)	5.90^a	5.89^a	6.18^b	0.048	0.002
Water intake L/d	24.90	27.05	29.10	0.822	0.104
Water intake L/100kg BW	6.77	7.18	8.27	0.372	0.253
Water intake L/ kgW0.75	0.30	0.32	0.33	0.007	0.128
Digestibility of nutrients (%)					
DM	61.00	64.39	62.17	0.946	0.364
OM	63.45	65.02	62.82	1.371	0.828
CP	67.15	69.26	68.61	0.665	0.455
EE	49.04	53.90	52.81	1.116	0.180
NDF	41.18^b	32.55^a	30.32a	1.706	0.006
ADF	31.59	29.10	28.76	0.726	0.236
TCHO	52.72	48.03	46.87	1.613	0.320
DCP	5.30	6.04	5.85	0.196	0.299
TDN	59.20	56.37	55.77	1.516	0.473
NR	10.19	8.46	8.68	0.454	0.255

Figures bearing different superscripts differ significantly, ($P\leq0.05$), ($P\leq0.01$).

Digestibility of Nutrients and Nutrient Intake

Live weight of animals was observed to be similar among the groups at the beginning and at end of 60 day feeding trial. Change observed was similar among groups reflecting diet had a insignificant effect on the growth of calves. Studies on impact of *Salvadora persica* as fodder in goats fed 25% and 50% salvadora showed an increase in average daily weight gain by 15% and 22%, respectively, relative to control reflecting that incorporating *Salvadora persica* leaves in the diet significantly enhanced growth performance in goats (Ali *et al,* 2012).

Digestibility of nutrients (Table 2) indicate that dry matter, organic matter crude protein, ether extract it improved at 5% level of inclusion however at higher levels the advantage faded off. Digestibility of NDF decreased significantly in T_2 and further in T_3, similar trend was observed with respect to ADF and total carbohydrates. DCP intake improved at 5% level of inclusion; however, it decreased when Salvadora leaves was incorporated at higher level, whereas TDN content decreased in T_2 and further in T_3 reflecting energy utilisation was affected due to incorporation of leaves in the diet. Evaluation of nutritional worth revealed that DCP content in the diet improved with supplementation of Salvadora leaves at lower level of 5%; however, TDN content reduced marginally at T_2 and incrementally in T_3 reflecting energy available in the diet was not efficiently utilised. Contrary to the present finding digestibility dry matter, crude protein and crude fibre improved significantly in goats fed Salvadora leaves and found that 20% and 40% of diets Rahman *et al* (2015). Weight of the faecal pellet (Table 3) was observed to be higher in the supplemented groups and density of faecal pellet decreased though the observations were non-significant. Length was observed to be similar but the diameter increased reflecting increase in size. Considering higher intake of DM; DM voided and its retention was less in T_2 which reflected better digestion then in other groups. Though the digestibility values in the present study were observed to be similar, it could be inferred that at a low level of 5% of the diet, supplementation of salvadora leaves supports better utilisation of dietary protein and energy as well.

Rumen Fermentation Pattern

Protozoal numbers (Table 4) decreased incrementally (P≤0.05) with increase in salvadora leaves in the diet of camel calves possibly due to defaunating action of the leaf metabolites. Mean values of pH were found to vary among treatments, however, no trends could be inferred as they were statistically similar among treatments. Singh *et al* (2011) studied effect of tannin rich Pakar (*Ficus infectoria*) leaves in goats and observed no difference in pH of rumen liquor of both the groups. Ammonia nitrogen in the present study was decreased in T_2 but partly recovered in T_3 suggesting that it was utilised in T_2 for microbial protein synthesis which is evident from the TCA nitrogen concentration, whereas higher levels might have suppressed rumen function. Vaithiyanathan *et al* (2007) assessed the effect of feeding different levels of tannin-containing *Prosopis cineraria* leaves in lambs and kids and observed lower ruminal ammonia nitrogen. Total nitrogen content in the rumen liquor was observed to increase at low level of supplementation, however, at higher level of supplementation it was further decreased which could be due to decrease in microbial activities contributing to the solubilisation. NPN content was marginally higher at low level of supplementation but were statistically similar among groups.

Table 3. Different attributes of faecal pellets in different treatment groups.

Attributes	T_1	T_2	T_3	SEM	P value
Faecal pellet weight (gm)	1.75	1.95	1.89	0.092	0.706
Faecal pellets volume (cm^3)	29.42	35.52	35.03	2.390	0.558
Density (gm/cm^3)	0.0581	0.0555	0.0567	0.001	0.626
Faecal pellet length (cm)	2.49	2.52	2.48	0.061	0.971
Faecal pellet diameter (cm)	3.95	4.20	4.06	0.065	0.315
DM intake	6.17a	6.27b	6.19a	0.015	0.001
DM voided	2.48	2.07	2.35	0.091	0.164
DM retained	3.69	4.18	3.82	0.098	0.092

Figures bearing different superscripts differ significantly, (P≤0.05), (P≤0.01).

Table 4. Rumen fermentation pattern in camel calves supplemented *Salvadora oleoides* leaves.

Attributes	T_1	T_2	T_3	SEM	P value
Total protozoal count ($*10^3$)	6.70^b	5.72^{ab}	5.18^a	0.284	0.059
pH	6.45	6.29	6.82	0.181	0.538
TVFA (meq/dl)	8.08^a	9.41^b	8.54^{ab}	0.247	0.053
NH3N(mg/dl)	5.83^b	2.33^a	4.08^{ab}	0.583	0.016
Total Nitrogen (mg/dl)	49.77a	56.38^b	51.72^a	1.158	0.022
NPN (mg/dl)	36.01	42.85	37.02	1.532	0.137
TCA-N (mg/dl)	13.53	14.7	13.76	0.969	0.902

Figures bearing different superscripts differ significantly, (P≤0.05), (P≤0.01)

Haemato-Biochemical Attributes

Haemato-biochemical attributes (Table 5) were recorded at the beginning and after 4 fortnights of feeding of experimental diets. Haemoglobin content in all the treatment groups was observed to be higher after feeding in 4 fortnights; however, differences among the groups due to diet were not evident. Similar observations were recorded on feeding of phytochemical rich diets containing *Prosopis cineraria / Zizyphus nummularia* leaf containing diets (Poonia *et al*, 2022). Similar results were observed for packed cell volume (%). Differences were not observed due to period and treatment in the values of Mean Corpuscular Haemoglobin. Glucose content marginally improved after 4 fortnights however, difference due to treatment were not evident. SGOT values decreased with supplementation before

Table 5. Effect of feeding *Salvadora oleoides* leaves on blood biochemical profile.

Attributes	Fortnight	T_1	T_2	T_3	SEM	P value
Haemoglobin (g/dl)	0	8.65	8.8	8.45	0.336	0.766
	4	12.2	12.3	14	0.639	0.136
Packed Cell Volume (%)	0	25.8	26.2	26.8	1.117	0.819
	4	37.42	39.15	40.57	1.42	0.336
MCH (pg/cell)	0	12.40	12.13	12.04	0.235	0.551
	4	12.31	12.73	12.85	0.224	0.305
Glucose (mg/dl)	0	90.75	89.5	107.75	4.514	0.189
	4	96.75	102.25	110.75	2.926	0.141
SGOT (IU/dl)	0	75.21	66.02	60.50	3.260	0.182
	4	69.46	84.13	80.40	7.827	0.766
SGPT (IU/dl)	0	8.615	9.22	8.71	0.498	0.889
	4	8.96	7.96	11.78	0.811	0.132
Blood Urea Nitrogen (mg/dl)	0	21.25	14.75	16.75	1.368	0.135
	4	27.75	25.75	22.00	3.382	0.812
Serum Protein (mg/dl)	0	6.98	7.45	7.42	0.762	0.968
	4	6.98	4.45	5.04	0.655	0.278
Ca (mg/dl)	0	8.64	8.38	9.11	0.368	0.755
	4	8.78	8.88	8.86	0.373	0.994
P (mg/dl)	0	3.80	4.24	4.00	0.170	0.615
	4	3.73	4.29	3.85	0.195	0.505

feeding, however, after 4 fortnights values improved but differences among the groups were not observed. Similarly, no definite pattern was observed in case of SGPT either due to period or treatment. Blood urea levels decreased with supplementation of 5% salvadora leaves before and after end of 4 fortnights; however, the values were higher after 4 fortnights reflecting better nitrogen recycling in animals. Serum protein was observed to be higher in the salvadora supplemented groups though the values were statistically similar, however, at the end of 4 fortnights, values decreased but non-significantly. Serum calcium and phosphorus levels were also observed to be similar among periods and treatments; they were in the normal range of experimentation. Non-significant difference in values of haematological and biochemical parameters at the start and end of trial in treatment supplemented with 5% and 12.5% *Salvadora oleoides* leaves as compared to control group. The results of haemato-biochemical attributes are in accordance with Dey *et al* (2015) and Sireesha *et al* (2021). Ali and Ahmed (2019) observed enhanced health and immunity in camels supplemented with 25% *Salvadora persica* leaves, including higher haemoglobin levels and reduced incidence of common ailments.

Conclusion

Findings of the present study corroborate that inclusion of *Salvadora oleoides* leaves in the diet of camel resulted in improvement in dry matter, organic matter and crude protein intake. The leaves of *Salvadora oleoides* can be used as a cheaper feed resource at a level of 5% and 12.5% of the diet as it could efficiently maintain feed intake, body weight and rumen fermentation pattern without any adverse effects on the digestibility and haemato-biochemical parameters of camels but 5% level of incorporation of *Salvadora oleoides* leaves proved more beneficial since safe limits for consumption of saponins depends on several factors such as source of saponin, type of saponins and many others due to which it is likely that less growth response was elicited when higher levels of *Salvadora oleoides* leaves was fed. As *Salvadora oleoides* tree is abundantly available in the arid region of western Rajasthan and Gujarat, it could be utilised as a browse species/ dietary supplement as fresh forage/ dry leaves in the dietary of camel without any adverse effect.

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CLINICO-PHYSIOHAEMODYNAMIC AND HAEMATO-BIOCHEMICAL EVALUATION OF DEXMEDETOMIDINE IN COMBINATION WITH BUTORPHANOL AND KETAMINE ANAESTHESIA IN CAMELS (*Camelus dromedarius*)

K. Nath[1], P. Bishnoi[2], S. Palecha[2] and S.D. Narnaware[1]
[1]ICAR- National Research Centre on Camel, Bikaner, India
[2]Department of Surgery and Radiology, College of Veterinary and Animal Science, Bikaner, India

ABSTRACT

The study was conducted to evaluate the preanaesthetic effects of two doses of dexmedetomidine (2.5 µgm kg^{-1} and 4 µgm kg^{-1}) in combination with butorphanol (0.05 mg Kg^{-1}) and ketamine HCl (2 mg Kg^{-1}) administered intravenously in camels. Prospective randomised crossover experimental trials were conducted in 6 camels divided in two groups (n=6): D1BK and D2BK based on the dose of dexmedetomidine 2.5 µgm kg^{-1} and 4 µgm kg^{-1}, respectively. Clinico-physiological parameters were recorded at T 0 (pre-administration), at induction T -I as well as at T- 15, 30, 45, 60, 90, 120 and T- 180 minutes post-administration. Haemato-biochemical parameters were recorded at 0 min (pre-administration) and at induction T- I, at T- 30, 60, 120 as well as T- 180 minutes post-administration. All data were statistically analysed. The results revealed that the induction of anaesthesia was quicker in D2BK group (0.99± 0.02 min) than that in D1BK group (1.03 ± 0.020 min). The duration of anaesthesia, recovery time and complete recovery time 62.71 ± 2.07, 63.71± 2.08 and 93.59 ± 1.63 min, respectively were significantly ($P<0.01$) longer in D2BK group 44.61±1.37, 45.64 ± 1.37 and 71.08 ± 2.38 min, respectively than those in D1BK group. The quality of anaesthesia and degree of analgesia were significantly better ($P<0.05$) in D2BK group than those in D1BK group. Rectal temperature and blood pressure showed non-significant changes at different time intervals in both groups ($P>0.05$). Respiration rate decreased significantly ($P<0.05$) in D2BK group. Heart rate and pulse rate decreased significantly ($P<0.05$) in both D1BK and D2BK groups. Haemato- biochemical parameters showed non-significant variations during the period of study within the group and between the groups at different time intervals ($P>0.05$). In conclusion, dexmedetomidine is a clinically useful and safe to be employed as pre-anaesthetic drug in combination with butorphanol- ketamine anaesthesia in camels.

Key words: Anaesthesia, butorphanol, camel, dexmedetomidine, ketamine, preanaesthetic

In India dromedary camel is reared primarily for carting, draught, agricultural operation and transportation therefore, these are more prone to various injuries and surgical disorders, hence requires good anaesthesia to carry out any surgical interventions. Use of preanaesthetic prior to induction of anaesthesia improves the quality of anaesthesia and also reduces the adverse effect of individual anaesthetic agent. Dexmedetomidine is a new generation potent alpha-2 adrenoceptor agonist with the highest affinity for alpha-2 adrenoceptor, allowing its application in relatively high doses for sedation and analgesia without the unwanted vascular effects resulting from stimulation of α1 receptors (Ebert *et al,* 2000; Kuusela *et al,* 2001). Studies on safe sedative combination of dexmedetomidine, ketamine and butorphanol for minor procedures in dogs have been done (Imboden *et al,* 2023). Opoids are the most commonly used analgesics to supplement anaesthetics as they have synergistic effect with the alpha-2 agonist (Chabot-Dore *et al,* 2015). Butorphanol is a synthetically derived opioid agonist-antagonist analgesic of the phenanthrene series, with a potency of about 4 to 7 times that of morphine (Bush *et al,* 2011). It provides analgesia and mild sedation but does not depress respiratory and cardiovascular function unless high dose rates are used (Muir, 1998). Medetomidine-ketamine and medetomidine-ketamine-butorphanol combinations were used for the field anaesthesia of free-ranging dromedary

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camels (*Camelus dromedarius*) in Australia (Boardman *et al,* 2014). Sedative, analgesic, behavioural and clinical effects of intravenous nalbuphine-xylazine combination in camels were also studied previously (Khalil *et al,* 2019). Ketamine butorphanol (Nath *et el,* 2024) and ketamine, diazepam and butorphanol were used as total intravenous anesthesia in camels (Almubarak, 2012). Premedication, sedation and adequate anaesthetics were suggested for camels in field conditions. In new previous studies, it is imperative to find a combination of preanaesthetics and anaesthetics which can provide satisfactory anaesthesia and muscle relaxation in camels. The present study was therefore, aimed to investigate pre-anaesthetic effects of two doses different of dexmedetomidine in combination with butorphanol and ketamine HCl anaesthesia in dromedary camels.

Materials and Methods

The experimental protocol was approved by the institutional ethics committee of National Research Centre on Camel (ICAR) Bikaner, India. Six adult healthy male camels with a mean body weight of 435.38 ± 11.22 kg and aged 4-5 years were selected and managed under uniform feeding and managemental conditions. All camels were used in a randomised crossover design with an interval of 14 days between two treatments on same animal. Food and water were withheld for 24 and 12 hours, respectively prior to the experiment. The experiment was performed outdoors in a quiet environmental condition. 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.

The preanaesthetic effect of two doses of dexmedetomidine[1] (2.5 µgm kg^{-1} b.wt and 4.0 µgm kg^{-1} b.wt) were evaluated in combination with butorphanol[2] (0.05 mg Kg^{-1}) and ketamine HCl[3] (2 mg Kg^{-1}) in two groups (n=6 in each group); D1BK and D2BK. The dose of dexmedetomidine was standardised and selected after conducting pilot trials. Dexmedetomidine was administered intravenously 10 minutes prior to the intravenous administration of butorphanol and ketamine HCl in each group. The dose of butorphanol and ketamine HCl were selected based on earlier studies (Boardman *et al,* 2014: Ahmed *et al,* 2015).

1. Dexmedetomidine - Dextomid (200mcg/ml), Neon Laboratories Ltd. andheri (East), Mumbai, India – 400093
2. Butorphanol- Butodol (2mg/ml) Neon Laboratories Ltd. andheri (East), Mumbai, India – 400093
3. Ketamine – Aneket ((50mg/ml), Neon Laboratories Ltd. andheri (East), Mumbai, India – 400093)

Anaesthesia was evaluated by subjective assessment of median scores of various reflexes. These reflexes including relaxation of jaw, palpebral reflex, response to intubation, response to pin prick and bone prick as well as pedal reflex were recorded at T-0 minute (pre-administration), at induction T –I, at T-15, 30, 45, 60, 90, 120 and 180 minutes post-administration of dexmedetomidine, butorphanol and ketamine. Quality of anaesthesia was evaluated by observing behavioural response of camel for various reflexes based on a 0 to 3 scoring scale *viz*. (0): intact (1): mild response; (2): moderate response; (3): good response (Singh *et al,* 2013) (Table 1).

The physio-haemodynamic parameters including 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), at T- I (induction), T-15, 30, 45, 60, 90 and 120 and 180 minutes post-administration of dexmedetomidine, butorphanol and ketamine.

Haemato-biochemical parameters were studied in blood sample collected at T-0 minute (pre administration) and at induction T-I, at T-30, 60, 120 and 120 minutes post-administration of dexmedetomidine butorphanol and ketamine. Haematological parameters *viz*. haemoglobin (Hb), packed cell volume (PCV), total leucocyte count (TLC), total erythrocyte count (TEC), differential leucocyte count (DLC) were estimated using automated haemato-analyser (IDEXX Vet Test, IDEXX Laboratories Inc. Westbrook United State). Biochemical parameters *viz*. glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), serum urea nitrogen (SUN) and creatinine were estimated with the help of semi-auto analyser (LT -100, Labtech Healthcare India Pvt Ltd) using commercial kits. The cortisol level (µg/dL) as a stress marker hormone was estimated using cortisol ELISA kits.

Statistical analysis

The data were presented as mean ± standard error of mean and median (Range) in parametric and non parametric statistical analysis, respectively by using SPSS software version 20 (IBM SPSS Statistics 20). 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 induction of anaesthesia in D2BK group (0.99 ± 0.02 min) was earlier than that of D1BK group (1.03 ± 0.020 min). The duration of anaesthesia, recovery time and complete recovery time were significantly (P<0.01) prolonged in D2BK 62.71 ± 2.07 min, 63.71 ± 2.08 and 93.59 ± 1.63 min, respectively than those of the D1BK group 44.61±1.37, 45.64 ± 1.37 and 25.85 ±0.97 min and 71.08 ± 2.38 min, respectively (Fig 1).

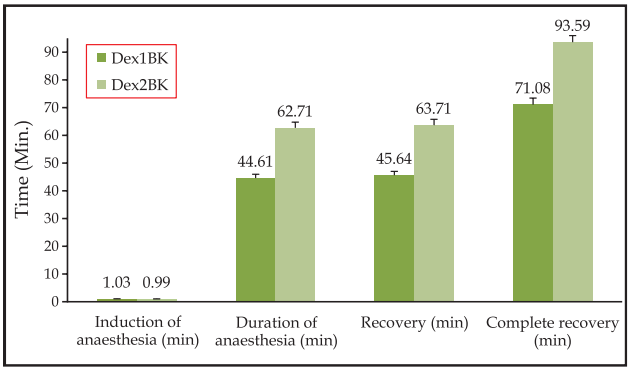

Fig 1. Induction, duration, recovery and complete recovery from dexmedetomidine, butorphanol and ketamine HCl anaesthesia in camels.

Good jaw relaxation was recorded in both D1BK and D2BK groups at induction but jaw tone appeared early in the D1BK group. Jaw relaxation score was significantly high (P<0.05) in D2BK group compared to that seen in the D1BK group particularly at 60, 90 and 120 min time interval (Table 2). Very weak to no palpebral reflex was recorded at induction of anaesthesia and up to 30 min interval in D1BK, whereas, no palpebral reflex was recorded at induction of anaesthesia and up to 45 min interval in D2BK group. The palpebral reflex score was significantly higher (P<0.05) at 60 min in

Table 1. Numeric scoring system used for used for recording of various reflexes and response.

Parameters	Scale			
	0	1	2	3
Jaw relaxation	Not allowing opening the jaws	Resistant to open the jaw and closed quickly	Less resistance to open the jaw and closed quickly	No resistance and jaw remain open
Palpebral reflex	Intact and strong reflex (quick blink)	Intact and strong reflex (slow response)	Very weak reflex (occasional response)	Abolished reflex (No response)
Intubation	Not permitting entry of tube in mouth	Allow entry but chewing	Difficult intubation	Easy intubation
Pedal reflex	Intact and strong refax (strong withdrawal)	Intact but weak reflex (slow response)	Intact but very light reflex (slow and occasional response)	Reflex abolished
Response to bone prick pin prick	Strong reaction (strong withdrawal)	Weak reaction (slow response)	Slow and occasional response	No reaction

Table 2. Median value of jaw relaxation, palpebral reflex, intubation, pedal reflex and pin prick in camels of different groups.

Parameters	Groups	Time intervals								
		0	I	15	30	45	60	90	120	180
Jaw relaxation	D1BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	3^{bA}	2^{abA}	1^{abA}	0^{aA}	0^{aA}
	D2BK	0^{a}	3^{bA}	3^{bA}	3^{bA}	3^{bA}	3^{bB}	1.5^{abB}	1^{abAB}	0^{aA}
Palpebral reflex	D1BK	0^{aA}	2.5^{bA}	2.5^{bA}	2.5^{bA}	2^{abA}	1.5^{abA}	1^{abA}	0^{aA}	0^{aA}
	D2BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	3^{bA}	2.5^{bB}	1.5^{abA}	1^{abB}	0^{aA}
Intubation	D1BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	3^{bA}	1^{abA}	1^{abA}	0^{aA}	0^{aA}
	D2BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	3^{bA}	3^{abB}	1^{abA}	0^{aA}	0^{aA}
Pedal reflex	D1BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	2^{abA}	1^{abA}	1^{abA}	0^{aA}	0^{aA}
	D2BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	3^{bB}	3^{abB}	1.5^{abA}	0^{aA}	0^{aA}
Pin prick	D1BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	$2.5a^{bA}$	1.5^{abA}	0^{aA}	0^{aA}	0^{aA}
	D2BK	0^{aA}	3^{bA}	3^{bA}	3^{bA}	3^{bA}	3^{abB}	2^{abB}	0^{aA}	0^{aA}

Variables with different superscript small letters differ significantly (P<0.05) within group
Variables with different superscript capital letters differ significantly (P<0.05) among different groups

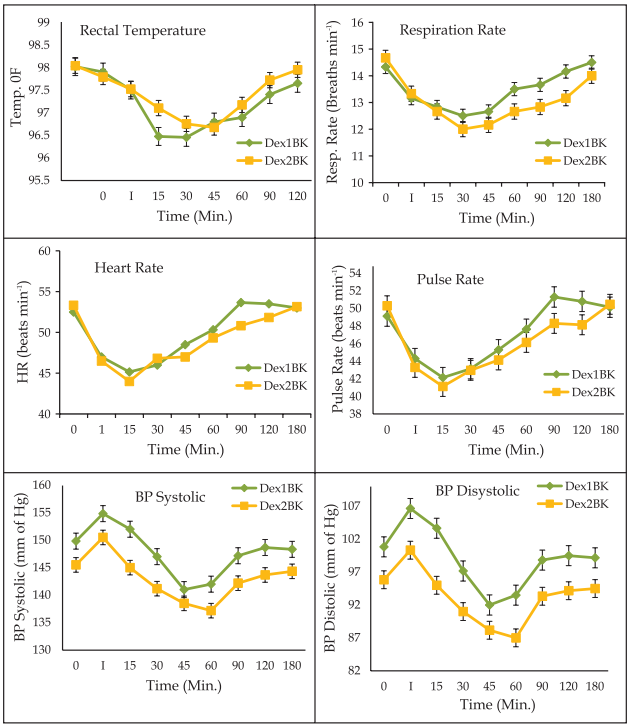

Fig 2. Physio-haemodynamic parameters in camels of D1BK and D2BK groups.

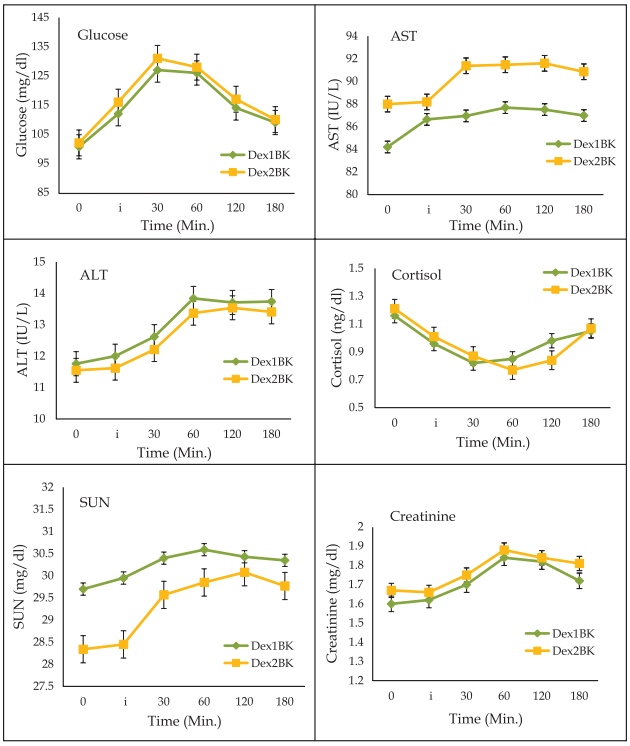

Fig 3. Biochemical parameters in camels of D1BK and D2BK groups.
AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, SUN: Serum urea nitrogen

D2BK group than that in the D1BK group (Table 2). Easy intubation without coughing could be done at induction of anaesthesia in all the animals of both D1BK and D2BK groups. Pedal reflex was abolished at induction of anaesthesia and remained so up to 30 min in D1BK group and up to 60 min in D2BK group. Significantly higher ($P<0.05$) pedal reflex score was recorded, respectively at 45 and 60 min in D2BK and D1BK groups (Table 2). Good degree of analgesia was recorded in both groups at induction but significantly higher ($P<0.05$) degree of analgesia was observed at 60 and 90 min in D2BK group than that recorded in the D1BK group (Table 2).

Early induction and prolonged duration of anaesthesia recorded in D2BK group might be attributed to the higher dose of dexmedetomidine in D2BK. Dose dependent duration of anaesthesia and recovery from anaesthesia in camels of D2BK group are well corroborated with the observations of Kuusela *et al* (2001) in dogs where significantly prolonged recovery time was recorded with a higher dose level of dexmedetomidine. Similarly, shorter weak time, down time and longer recovery time have also been reported at higher doses of dexmedetomidine in dogs (Santosh *et al*, 2012)

The combined effect of dexmedetomidine, butorphanol and ketamine caused good jaw muscles relaxation, depressed palpebral reflex and easy intubation in camels. This is well articulated with the fact that alpha 2 -agonists produce profound muscle relaxation when used alone or in combination with opioids (Ahmad *et al*, 2011) and Ketamine HCl (Ko *et al*, 2000). Alpha-2 agonists cause muscle relaxation by inhibition of alpha-2 adrenoceptors in the spinal cord (Branson *et al*, 1993). Pedal reflex in the present study is attributed to the increased nociceptive threshold due to combined effects of dexmedetomidine, butorphanol and ketamine HCl. Dose dependent analgesic effect of dexmedetomidine is mediated spinally (Hayashi *et al*, 1995) as it inhibits the release of substance P from the dorsal horn of the spinal cord (Savola *et al*, 1991). The analgesic property of ketamine HCl reduces sensitisation of pain pathways and leads to better pain control (Kee *et al*, 1997). Antinociceptive effect of butorphanol is due to partial agonist effects and mu receptor involvement (Garner *et al*, 1997).

Rectal temperature decreased non-significantly up to 45 and 60 min, respectively in D1BK and D2BK groups. Differences in RT were non-significant among groups (Table 3). Decrease in RT in both groups might be attributed to reduction in muscular activity, decrease in metabolic rate, muscle relaxation along

with depression of thermo regulatory system. Alpha-2 adrenergic agonist has been reported to induce prolonged depression of thermoregulation. (Ponder and Clarke, 1980). Decreased rectal temperauare has also been reported after midazolam, ketamine puke rate (PR), butorphanol with dexmedetomidine in dogs (Santosh *et al,* 2013) and dexmedetomidine in uraemic goats (Kumar *et al,* 2013).

Respiration rate (RR) decreased non-significantly in D1BK group whereas, in D2BK group RR decreased significantly (P<0.05) at 15, 30, 45, 60 and 90 min compared to base line thereafter increased non-significantly. Differences in RR were non-significant among groups (Table 3). Decrease in repialistion 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) and/or depressing action on respiratory center in central nervous system (Hall *et al,* 2001). Significant respiratory depressant effect in D2BK group receiving a higher dose of dexmedetomidine is well articulated with the findings of Ahmad *et al* (2011) in dogs. Non-significant variation in RR observed in D1BK group is in accordance with the dose dependent effect of dexmedetomidine seen in dogs (Sabbe *et al,* 1994).

In both D1BK and D2BK groups heart rate (HR) and PR decreased significantly (P<0.05) at 15 min and thereafter, heart rate and pulse rate increased non-significantly. Differences in HR and PR were non-significant among groups (Table 3). Bradycardia is a common effect of administration of alpha-2 agonists and ketamine as a result of reflex vagal activity and alpha-2 mediated decrease in norepinephrine release from CNS leading to inhibition of sympathetic tone (Selmi *et al,* 2004). Ketamine HCl usually stimulates the cardiovascular function causing increase in HR and blood pressure (Kumar *et al,* 2014). The dexmedetomidine countered the cardiovascular effect of ketamine. Alpha-2 agonist-induced vasoconstriction and direct increase in the release of acetylcholine from parasympathetic nerves in the heart might be responsible for bradycardia (MacDonald and Virtanen, 1992) Similarly significant bradycardia had also been reported following dexmedetomidine, ketamine and opioid administration in dogs (Balreta *et al,* 2011) and after

Table 3. Mean±SE values of physio-haemodynamic parameters in camels of both the groups.

Parameters	Groups	Time intervals								
		0	I	15	30	45	60	90	120	180
Rectal Temperature (°F)	D1BK	98.03 ± 0.50	97.91 ± 0.49	97.51 ± 0.53	96.48 ± 0.45	96.46 ± 0.53	96.80 ± 0.42	96.90 ± 0.45	97.41 ± 0.50	97.66 ± 0.50
	D2BK	98.05 ± 0.43	97.80 ± 0.40	97.53 ± 0.38	97.11 ± 0.33	96.76 ± 0.35	96.68 ± 0.25	97.18 ± 0.26	97.73 ± 0.40	97.96 ± 0.36
Respiration Rate (Breaths min^{-1})	D1BK	14.33^{abA} ± 0.55	13.16^{abA} ± 0.47	12.83^{abA} ± 0.70	12.50^{aA} ± 0.71	12.66^{abA} ± 0.71	13.50^{abA} ± 0.56	13.66^{abA} ± 0.49	14.16^{abA} ± 0.30	14.50^{bA} ± 0.42
	D2BK	14.67^{bA} ± 0.42	13.33^{abA} ± 0.66	12.66^{aA} ± 0.49	12.00^{aA} ± 0.71	12.16^{aA} ± 0.51	12.66^{aA} ± 0.65	12.83^{aA} ± 0.71	13.16^{abA} ± 0.60	14.00^{abA} ± 0.48
Heart rate (beats min^{-1})	D1BK	52.5^{bcA} ± 2.47	47.0^{abcA} ± 2.11	45.16^{aA} ± 2.02	46.0^{abA} ± 2.26	48.5^{abcA} ± 1.89	50.33^{abcA} ± 2.07	53.66^{cA} ± 2.29	53.5^{cA} ± 1.91	53.0^{cA} ± 2.30
	D2BK	53.33^{bA} ± 2.29	46.5^{abA} ± 1.91	44.0^{aA} ± 2.30	46.83^{abA} ± 2.56	47.0^{abA} ± 2.18	49.33^{abA} ± 1.98	50.83^{abA} ± 1.85	51.83^{bA} ± 2.08	53.16^{bA} ± 2.09
Pulse rate (beats min^{-1})	D1BK	$49.16b^{cA}$ ±2.56	44.33^{abcA} ± 2.26	42.17^{aA} ± 1.85	43.17^{abcA} ± 2.21	45.33^{abcdA} ± 1.74	47.67^{abcA} ± 2.06	51.16^{cA} ± 2.38	51^{cA} ± 2.06	50.17^{cA} ± 2.1
	D2BK	50.33^{bA} ± 2.33	43.67^{abA} ± 2.33	41.16^{aA} ± 1.88	43^{abA} ± 1.85	44.33^{abcA} ± 2.04	46.33^{abA} ± 2.07	48.33^{bA} ± 1.81	48.5^{bA} ± 2.27	50.33^{bA} ± 2.59
Blood pressure systolic (mm Hg)	D1BK	149.83 ± 6.34	160.16 ± 6.26	163.5 ± 6.55	153.67 ± 6.55	150.16 ± 5.79	147.16 ± 5.43	146.33 ± 5.32	147.66 ± 5.43	148.66 ± 5.95
	D2BK	145.5 ± 6.03	156.66 ± 6.70	159.83 ± 7.62	151.83 ± 5.71	146.66 ± 6.10	141.16 ± 5.91	143.16 ± 4.85	145.16 ± 5.56	145.0 ± 5.31
Blood pressure Diastolic (mm Hg)	D1BK	100.83 ± 4.35	110.16 ± 6.05	113.16 ± 6.69	103.83 ± 5.44	101.0 ± 5.79	97.5 ± 4.74	97.0 ± 4.94	97.33 ± 4.57	98 .5 ± 5.11
	D2BK	95.83 ± 5.61	107.66 ± 6.24	109.66 ± 7.77	101.0 ± 5.5	97.0 ± 5.85	94.16 ± 5.31	93.33 ± 4.77	95.0 ± 5.60	95.16 ± 5.1

[a,b,c] Values bearing different superscripts differ significantly (P<0.05) within groups.
[AB] Values bearing different superscripts differ significantly (P<0.05) among different groups.

administration of xylazine HCl and dexmedetomidine in camel calves (Samimi *et al,* 2020). Butorphanol has also been reported to cause mild lowering of HR with minimum cardiovascular effects (Greene *et al,* 1990)

Systolic and diastolic blood pressure increased initially at 15 min interval in both D1BK and D2BK groups and later followed decreasing trend (Table 3). The initial increase the SBP and DBP followed by a decrease in both groups is similar to previous reports following administration of dexmedetomidine along with propofol in dogs (Singh *et al,* 2020). This biphasic effect on arterial blood pressure, with an initial rise in blood pressure followed by subsequent reduction in blood pressure might be attributed to combined effect of dexmedetomidine and ketamine, alpha-2 agonists causes initial vasoconstriction and increased blood pressure mediated by the α2 b-subtype adrenoceptors and later causes decreased sympathetic tone and low blood pressure (Kamibayashi and Maze, 2000). The sympatho-mimetic action of ketamine also elevates arterial blood pressure (Kumar *et al,* 2014)

In the present study, complete blood count values remained within the normal clinical range in both groups of camels however, non-significant changes were observed at different time periods. Hb, PCV, TEC and TLC decreased non-significantly in both the groups. DLC values fluctuated within the normal clinical range throughout the period of observations in both D1BK and D2BK groups (Table 4). The decrease in Hb, PCV, TEC and TLC values during the period of sedation could be 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 also have attributed the decrease in Hb, erythrocyte, PCV and TLC (Wagner *et al,* 1991). Decrease in Hb and PCV have also been reported after administration of xylazine, butorphanol and ketamine HCl combination in dogs (Sika, 2013). The initial increase in neutrophils and a corresponding decrease in lymphocytes recorded in the present study may be associated with initial excitement due to handling of the animals and stress caused by preanaesthetic drug and subsequent stimulation release of epinephrine leading to the release of neutrophils from bone marrow (Rosin, 1981). Similar findings have also been reported following administration of dexmedetomidine in dogs (Ahmad *et al,* 2011).

In both D1BK and D2BK groups, the blood glucose increased non-significantly up to 60 and 120 min, respectively. Later on, the values decreased but remained above base line in both groups (Table 5). Increase in blood glucose level observed in both

Table 4. Mean ±SE values of haematological parameters in camels of D1BK and D2BK groups.

Parameters	Groups	Time intervals					
		0	I	30	60	120	180
HB (gm/dl)	D1BK	12.70 ± 0.42	12.51 ± 0.45	12.30 ± 0.46	12.15 ± 0.43	12.20 ± 0.43	12.25 ± 0.35
	D2BK	12.86 ± 0.31	12.85 ± 0.37	12.38 ± 0.37	12.21 ± 0.39	12.13 ± 0.40	12.31 ± 0.31
TEC ($x10^6$ cu. mm^{-1})	D1BK	8.00 ± 0.38	7.83 ± 0.42	7.50 ± 0.40	7.42 ± 0.40	7.61 ± 0.42	7.64± 0.37
	D2BK	7.83 ± 0.46	7.69 ± 0.47	7.55 ± 0.43	7.50 ± 0.42	7.36 ± 0.44	7.45 ± 0.42
TLC ($X10^3$ cu. mm^{-1})	D1BK	13.75 ± 0.36	13.72 ± 0.30	13.57 ± 0.32	13.32 ± 0.41	13.38 ± 0.41	13.50 ± 0.34
	D2BK	13.51 ± 0.34	13.44 ± 0.30	13.36 ± 0.26	13.14 ± 0.33	13.13 ± 0.28	13.32 ± 0.32
PCV (%)	D1BK	28.15 ± 1.70	28.08 ± 1.65	26.67 ± 1.39	25.7 ± 1.242	26.77 ± 1.671	27.62 ± 1.50
	D2BK	27.45 ± 1.64	27.23 ± 1.53	25.8 ± 1.32	24.38 ± 1.16	25.35 ± 1.219	26.42 ± 1.29
DLC							
Neutrophills (%)	D1BK	51.50 ± 1.05	51.83 ± 0.70	52.5 ± 0.67	52.33 ± 0.88	52.50± 1.33	52.00± 0.63
	D2BK	52.00 ± 1.41	52.33 ± 0.76	53.50 ± 0.76	54.00 ± 0.73	52.33 ± 1.20	52.17 ± 1.13
Lymphocyte (%)	D1BK	42.67 ± 1.17	42.33 ± 0.95	42.00 ± 0.85	42.33 ± 0.88	41.83 ± 1.24	42.33 ± 0.66
	D2BK	41.17 ± 1.75	41.5 ± 1.14	40.17 ± 1.24	40.00 ± 0.68	41.33 ± 1.28	42.00 ± 1.12
Monocyte (%)	D1BK	3.33 ± 0.33	3.16 ± 0.47	2.66 ± 0.21	2.50 ± 0.22	3.50 ± 0.56	3.00 ± 0.25
	D2BK	4.16 ± 0.30	3.83 ± 0.54	3.66 ± 0.33	4.00 ± 0.25	4.33 ± 0.33	3.66 ± 0.33
Eocinophills (%)	D1BK	2.50 ± 0.42	2.67 ± 0.42	2.83 ± 0.60	2.83 ± 0.54	2.17 ± 0.30	2.83 ± 0.47
	D2BK	2.67 ± 0.42	2.33 ± 0.33	2.67 ± 0.33	2.00 ± 0.36	2.00 ± 0.20	2.17 ± 0.40

HB; Haemoglobin, TEC: Total erythrocyte count, TLC: Total leucocyte count, PCV: Packed cell volume

Table 5. Mean±SE values of biochemical parameters in camels of D1Bk and D2BK groups.

Parameters	Groups	Time intervals					
		0	I	30	60	120	180
Blood glucose (gm/dl)	D1BK	100.66 ± 7.57	109.16 ± 8.0	123.83 ± 8.31	125.66 ± 9.15	115.5 ± 7.02	111.83 ± 6.87
	D2BK	103.0 ± 8.94	112.0 ± 9.61	119.16 ± 8.78	124.0 ± 8.96	127.67± 59.97	114.66 ± 18.41
AST (IU/L)	D1BK	84.2 ± 4.56	86.64 ±5.21	86.95 ±5.10	87.68 ±5.1	87.51 ± 5.03	86.98 ± 4.88
	D2BK	87.99 ± 5.88	88.18 ± 6.04	91.38 ± 5.63	91.47 ± 4.57	91.60 ± 5.32	90.85 ± 5.09
ALT (IU/L)	D1BK	11.76 ± 0.77	12.00 ± 0.80	12.62 ± 0.64	13.84 ± 0.75	13.71 ± 0.76	13.74 ± 0.77
	D2BK	11.55 ± 0.73	11.62 ± 0.73	12.21 ± 0.77	13.37 ± 0.85	13.54 ± 0.75	13.41 ± 0.76
ALP (IU/L)	D1BK	94.5 ± 5.69	94.67 ± 6.79	95.5 ± 5.82	97.5 ± 6.44	96.66 ± 5.99	95.5 ± 6.17
	D2BK	93.67 ± 5.41	94.5 ± 5.39	95.83 ± 5.39	97.66 ± 4.5	96.16 ± 4.16	95.0 ± 5.34
SUN (mg/dl)	D1BK	29.70 ± 1.57	29.95 ± 1.47	30.40 ± 1.75	30.59 ± 1.45	30.43 ± 1.01	30.35 ± 1.50
	D2BK	28.34 ± 1.23	28.45 ± 1.38	29.57 ± 1.37	29.85 ± 1.02	30.08 ± 1.45	29.77 ± 1.22
Creatinine (mg/dl)	D1BK	1.09 ± 0.14	1.12 ± 0.11	1.18 ± 0.11	1.23 ± 0.10	1.26 ± 0.09	1.21 ± 0.12
	D2BK	1.01 ± 0.12	1.03 ± 0.13	1.15 ± 0.13	1.24 ± 0.10	1.25 ± 0.10	1.22± 0.11
Cortisol (µg/dL)	D1BK	1.16 ± 0.11	0.96 ± 0.09	0.82 ± 0.09	0.85 ± 0.10	0.98 ± 0.08	1.05 ± 0.11
	D2BK	1.21 ± 0.11	1.01 ± 0.10	0.87± 0.07	0.77 ± 0.07	0.84 ± 0.09	1.07± 0.08

AST: Aspartate aminotransferase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, SUN: Serum urea nitrogen

groups might be attributed to increased hepatic glucose production, decreased glucose utilisation by body cells, decreased membrane transport and reduced insulin 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,* 1997). Increased blood glucose had been reported following administration of dexmedetomidine, butorphanol and ketamine in dogs (Verma *et al,* 2018). A non-significant increase was observed in AST, ALT and ALKP activity up to 60 min in both D1BK and D2BK groups. Thereafter, values decreased but remained above base line in both the groups (Table 5). Transient increase in ALT, AST and ALKP 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 SUN and creatinine levels up to 60 min in both groups then, followed decreasing trend but remained above base line value. The increase in SUN and creatinine values in the 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 SUN and creatinine levels have also been reported following xylazine and propofol anaesthesia in dogs (Surbhi *et al,* 2010) and dexmedetomidine, butorphanol and ketamine in dogs (Verma *et al,* 2018).

A non-significant decrease was observed in cortisol level up to 60 min in both D1BK and D2BK groups, later on followed increasing trend (Table 5). Decrease in the level of cortisol in both groups might be attributed to direct inhibitory neuroendocrine response or indirect sedative and analgesic properties of dexmedetomidine which obtund the stress response when administered systemically as evidenced in a previous study (Raekallio *et al,* 2005). Alpha-2 agonists have been reported 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).

Based on the present study, it is concluded that 4 µg/kg b.wt dexmedetomidine along with butorphanol and ketamine resulted in quicker induction, better anaesthesia of prolong duration with better analgesia than 2.5 µg/kg b.wt dexmedetomidine, butorphanol and ketamine without alarming changes in physio-haemodynamic and haemato-biochemical profiles. However, dexmedetomidine at a dose of 2.5 µg/kg b.wt with butorphanol and ketamine showed good analgesia and anaesthesia and can be used for shorter period procedures.

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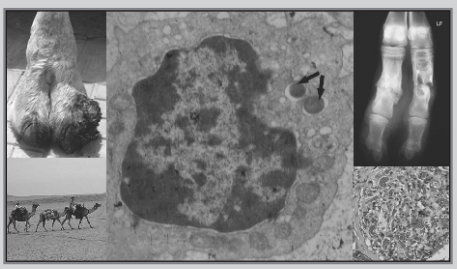

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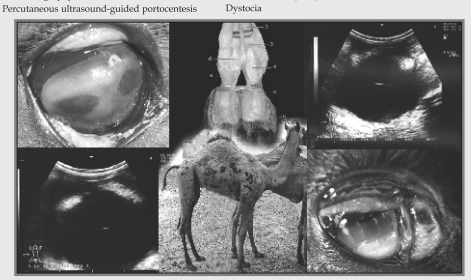

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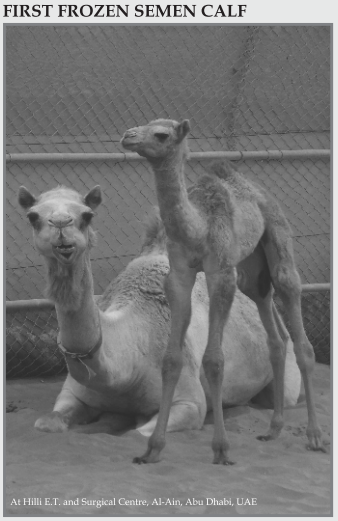

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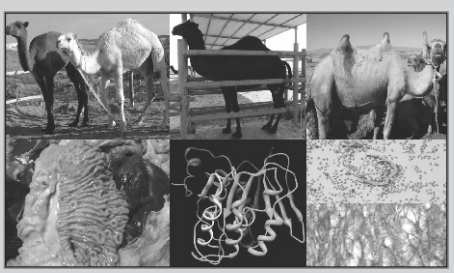

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COMPARATIVE LARVICIDAL POTENCY OF IVERMECTIN, DORAMECTIN, MOXIDECTIN, AND EPRINOMECTIN AGAINST DROMEDARY CAMEL NASAL BOTS (*Cephalopina titillator*)

Yehia Aljasim[1], Ahmed Aljazzar[2], Mohammad Al-Sabi[3], Sameer Alhojaily[1], Ibrahim Albokhadaim[1] and Mahmoud Kandeel[1,4]

[1]Department of Biomedical Sciences, [2]Department of Pathology, College of Veterinary Medicine, King Faisal University, 31982 Al-Ahsa, Saudi Arabia

[3]Department of Basic Veterinary Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid 22110, Jordan

[4]Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt

ABSTRACT

This study evaluates the efficacy of four commonly used antiparasitic drugs, i.e. ivermectin, doramectin, moxidectin, and eprinomectin against *C. titillator* third stage larvae (L3) in dromedary camels. In order to determine and compare the half-maximal inhibitory concentration (IC50) values of ivermectin, doramectin, moxidectin, and eprinomectin for controlling nasal myiasis in dromedary camels, present study was conducted on L3 collected from 200 naturally infested camels from the Eastern Province of Saudi Arabia. Upon slaughter, L3 were collected from the nasopharynx of the infested camels and were incubated in DMEM supplemented with 50% foetal calf serum and penicillin/streptomycin (100 IU). This media was mixed with serially diluted concentrations of ivermectin, doramectin, eprinomectin, before moxidectin from 1 mg/ml to 125 ng/ml. The larvicidal effect of the four drugs was inferred by the IC50 values that were calculated using nonlinear fitting of dose-response inhibition equations. The IC50 values indicated that ivermectin was the most potent drug with an IC50 of 0.0735 ± 0.016 µg/ml, followed by doramectin (0.249 ± 0.116 µg/ml), eprinomectin (0.46 ± 0.24 µg/ml), and moxidectin (11.96 ± 2.21 µg/ml). The efficacy of ivermectin, doramectin and eprinomectin was significantly higher than that of moxidectin. Ivermectin and doramectin could be considered more potent drugs for treating camel nasal myiasis. The results have important implications for the development of effective treatment protocols for managing parasitic infestations in camel populations.

Key words: Camel nasal myiasis, *Cephalopina titillator*, doramectin, eprinomectin, IC50, ivermectin, moxidectin

Camel's nasal myiasis is caused by the larvae of the *Cephalopina titillator* fly, which belongs to the *Oestridae* family (Angulo-Valadez *et al,* 2010). Camels that are infested with *C. titillator* exhibit a range of respiratory symptoms, including mucous discharge, inflammation of the mucous membrane, irritation, respiratory issues and tissue injury. The presence of these larvae in camels lead to significant economic setbacks (Taylor *et al,* 2007).

Prevalence of the second (L2) and third-stage larvae (L3) of the genus *C. titillator* are responsible for the infestation of dromedary camels (*Camelus dromedarius)* (Fatani and Hilali, 1994). The study indicated that the number of sick camels reached its highest point twice a year, in February and September, and that L3 are found far more frequently than L2. In addition, the research suggested that camels should be treated with larvicidals twice a year, in February and September, to eliminate the infestations (Fatani and Hilali, 1994). Another study was conducted in Sudan by removing fly larvae from the camels' nasal canals after they had been slaughtered and exposing the larvae to the following treatments: 2% pumpkin, 7.5% garlic and peppermint, 30% Lupinus, and 0.15% ivermectin (Khater, 2014). According to their findings, using the natural pumpkin oil rather than ivermectin to treat fly larvae was less hazardous to the camel. Nevertheless, ivermectin was found more effective, even though it induced undesirable side effects to the treated camels (Khater, 2014).

Avermectins are a group of 16-membered macrocyclic lactone compounds that can be naturally

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derived or semi-synthetically produced in labs. They originate from the fermentation of the soil bacterium *Streptomyces avermitilis*. Remarkably effective against a broad range of both internal and external parasites, Avermectins have extensive uses in various sectors, such as veterinary and human medicine, farming, horticulture, and in managing pests in an assortment of agricultural goods and ornamental plants (MacNeil *et al,* 1992; Siddique *et al,* 2014). The Avermectins target the gamma-aminobutyric acid (GABA) receptors and Glutamate-gated chloride (GluCl) related to neurotransmission in parasites. These Avermectins are believed to possess neurotoxic properties (Abongwa *et al,* 2017; Crump and Omura, 2011; Parisi *et al,* 2019). The neurotransmitter GABA is responsible for the opening of the organism's chloride ion channels, which leads to an increase in the number of chloride ions in the cell.

The primary objective of this research was to evaluate and compare the larvicidal efficacy of four commonly used antiparasitic drugs—ivermectin, doramectin, moxidectin, and eprinomectin—against *C. titillator* larvae. Specifically, the study was aimed to determine the half-maximal inhibitory concentration (IC50) of each drug to identify the most potent treatment for controlling camel nasal myiasis.

Materials and Methods

The source of the larvae

The study was conducted on L3 collected from 200 naturally infested camels, all of which were slaughtered at the Omran slaughterhouse in Al-Ahsa, Eastern Province, Saudi Arabia, between October 2021 and December 2021. These camels, belonging to a local breed, were at least 8 years old. No information about prior antiparasitic treatment was available for the sampled camels. Given the presence of parasite fauna in the infected animals and the absence of dead larvae in the examined camel heads, it is highly unlikely that these camels had received any form of treatment for the prevention of *C. titillator* infestation.

All procedures were approved by the King Faisal University ethics committee (approval number KFU-REC-2022-NOV-ETHICS308).

Collection of larvae

Upon slaughter, the nasopharynx of the slaughtered camels were carefully inspected to detect the presence of *C. titillator* larvae by making incisions along the pharynx, allowing full access to the areas where larvae are typically found. Once detected, the larvae were carefully collected manually. and were immediately placed into a sterile plastic container with a tight-fitting lid to maintain a controlled environment free from external contaminants.

The containers were transported to the laboratory within one hour after collection to ensure their viability until further processing. Excess mucuos or blood on the larvae was gently removed using sterile saline solution.

Preparation of incubation media and drug solutions

The incubation medium consisted of Dubbilco's Modified Essential Medium (DMEM, Molequle-ON®, New Zealand) supplemented with 50% foetal calf serum (FCS) and 100 IU Penicillin/Streptomycin antibiotics (Molequle-ON®, New Zealand) (Khater 2014). This was termed as basic medium. In this basic medium, 1 mg of each the following drugs was dissolved to produce stock solutions of the drugs: ivermectin, doramectin, moxidectin, and eprinomectin obtained from commercial sources. From this stock solutions, serial dilutions of each of the experimented drugs were prepared to produce final concentrations of 0.5, 0.25, 0.1, 0.01, 0.001, 0.0005, 0.00025 and 0.000125 mg/ml (Khater *et al,* 2013). These media with the serially diluted drugs were later termed as experimental media.

Larvicidal assays

Five ml of the experimental media were placed in sterile petri dishes, in which 5 L3 were placed. The control group consisted of 5 larvae placed in sterile petri dishes with basic medium, free of drugs. The dishes were incubated at 37°C and 60% relative humidity until the death of the larvae in the experimental media. To protect the larvae from the toxic effect of its products, the media was changed every 6 hours. During the media changing process, the mortality of the larvae was inferred by observation of flaccid body of the larvae with complete absence of movement upon stimulation. The experiment was repeated three times.

The IC50 of the experimented drugs was determined by plotting dose-response values using GraphPad Prism software (version 8), by applying dose-response-inhibition equations. The measured IC50 indicated the concentration of the drug needed to kill half of the larvae (Aykul and Martinez-Hackert, 2016). The values of IC50 were obtained by applying nonlinear regression models using GraphPad Prism software on the results of dead larvae (Lyles *et al,* 2008; Swift, 1997).

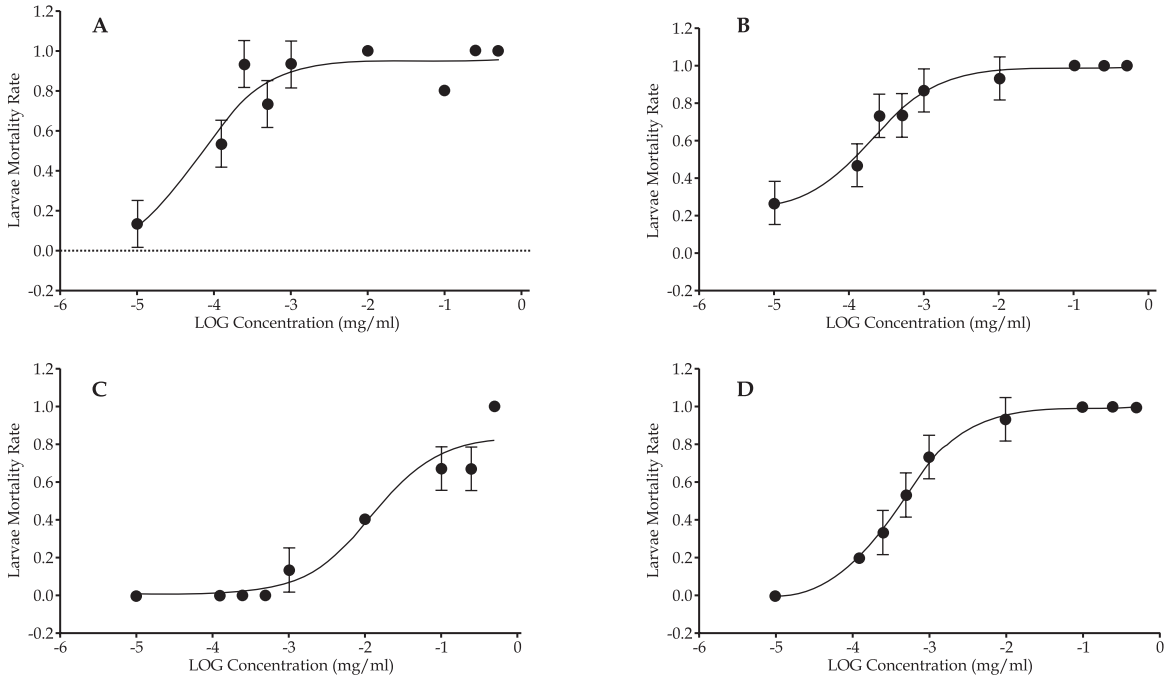

Fig 1. The dose-response curve for the treatment of *Cephalopina titillator* third stage larvae with different concentrations of ivermectin (A), doramectin (B), moxidectin (C), and eprinomectin (D). The X-axis is the log drug concentration (mg/ml). The Y-axis is the fraction of dead larvae after 12 hours after treatment.

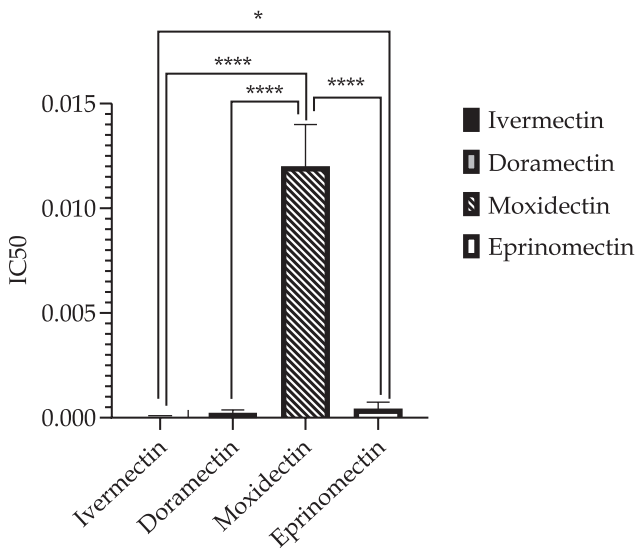

Fig 2. Bar chart showing the Mean and SD of the fraction of dead larvae after treatments. The number of asterisks (*) indicate the power of the statistically significant differences between the groups they are connected to, in which one asterisk is equivalent to ($p < 0.05$) and four asterisks is equivalent to ($p < 0.0001$).

Results and Discussion

IC50 of the experimented drugs

A gradient increase in the fraction of dead larvae was evident with increasing concentrations of all drugs tested (Fig 1). Larvae in the control group remained viable until the end of the experiment period. Additionally, the low standard deviations across different concentrations indicated that the results were consistent across the three replicates of the trials. Nonlinear regression models were applied with an IC50 of 0.0735±0.016 µg/ml for ivermectin, 0.249± 0.116 µg/ml for doramectin, 11.96±2.21 µg/ml for moxidectin, and 0.46±0.24 µg/ml for eprinomectin.

Comparison of the estimated IC50

Fig 2 depicts the fraction of dead larvae after treatment with four different drugs; ivermectin, doramectin, moxidectin, and eprinomectin. By comparing the data from all drugs, ivermectin and doramectin showed the highest efficacy, while moxidectin exhibited the lowest efficacy.

The IC50 values of ivermectin, doramectin and eprinomectin were significantly lower than that of moxidectin ($p<0.0001$), and the IC50 value of ivermectin was significantly lower than that of eprinomectin ($p<0.05$).

By enhancing GluCl ion channels in larvae, Avermectins are thought to be neurotoxic to the larvae (Salman *et al,* 2022). Increased permeability to chloride ions and hyperpolarisation of nerve cells results in the paralysis and death of the larvae. These medications also increase the activity of GABA-gated chloride channels. Due to the absence of GluCls channels in mammals and the reduced

affinity for other mammalian chloride channels, mammals are often unaffected. GABA-gated channels in the mammalian CNS are unaffected by these medications because they typically do not cross the blood-brain barrier in small concentrations (Bloomquist, 1996; Raymond-Delpech *et al,* 2005). Therefore, using minimum, yet effective Avermectin concentrations can be assessed by employing IC50 as a pharmacological metric of the drug potency (Aykul and Martinez-Hackert, 2016).

In this study, ivermectin had a IC50 value of 0.0735 µg/ml, with a standard deviation of 0.016, rendering a very small concentration of it sufficient to inhibit 50% of the *C. titillator*. Comparatively, the IC50 of doramectin was 0.249 µg/ml, with a standard deviation of 0.116. Despite this higher IC50 value than that of ivermectin, doramectin still demonstrated high effectiveness against *C. titillator* larvae. Moxidectin exhibited significantly less effectiveness than the other drugs in reducing the viability of *C. titillator* larvae, while eprinomectin provided a middle range of effectiveness between the tested drugs. Nonetheless, the results of the present study showed a dose-dependent effect of tested drugs on *C. titillator* larvae, with increasing concentrations leading to a higher number of dead larvae. The low standard deviations of the drugs' IC50 signified that the results were consistent across the replicates of the drugs' trials, adding credibility to the findings.

In a previous study which used dipping or fumigation techniques, doramectin and lavender oil showed high efficacy against the larval stages of *C. titillator*; both achieving 100% mortality within 30 hours (Khater *et al,* 2013). The later results showed that doramectin was particularly effective, outperforming essential oils like camphor and onion (Khater *et al,* 2013). In addition, using larvae immersion technique, ivermectin was effective in killing *C. titillator* larvae at low concentrations of 0.15% (Khater, 2014). In comparison, our study effectively simulated the natural habitat by permitting the larvae to reside in a nutrient medium infused with the medications. In addition, we provided detailed IC50 values, which were not reported previously. The stable viability of larvae in the control group ensured that the employed basic media and the incubation conditions supports the lives of the larvae *in vitro* for prolonged periods and that the observed lethal effects were due to the used avermectins. To the best of our knowledge, this study was unique in producing an effective *in vitro* model for evaluating the efficacy of the drugs against *C. titillator* larvae.

Camel nasal myiasis is a common disease among dromedary camels. *C. titillator* infestation was recorded in camels in Iraq (Shamsi *et al,* 2023), Libya (Abd El-Rahman, 2010), Saudi Arabia (Banaja and Ghandour, 1994), Sudan (Musa *et al,* 1989), Iran (Oryan *et al,* 2008) and Jordan (Sharrif *et al,* 1998), which shows its endemicity wherever camels are present. The results of the present study collectively provide valuable insights into the relative effectiveness of the tested drugs against *C. titillator* larvae. Given the widespread nature of *C. titillator* infestation and its significant impact on the health and well-being of affected camels, the findings of this study can be usefully integrated into broader parasitic control and management practices. This would not only aid in alleviating the immediate suffering of the infested animals but also contribute to the sustainable health and productivity of camel populations in the affected regions, with potentially positive implications for the communities that depend on them.

The study provides valuable insights into the comparative efficacy of four drugs; ivermectin, doramectin, moxidectin, and eprinomectin, against *C. titillator* larvae in dromedary camels. Among these drugs, ivermectin exhibited the highest larvicidal potency, followed closely by doramectin then eprinomectin, whereas moxidectin was significantly less effective. The dose-dependent effects of these drugs highlighted the varying degrees of effectiveness, with ivermectin requiring the lowest concentration to achieve IC50 to *C. titillator* larvae. The findings suggest that ivermectin and doramectin could be considered more potent options for treating camel nasal myiasis caused by *C. titillator* larvae. Given the widespread nature of this parasitic infestation and its impact on camel health and productivity, these results have significant implications for the development of effective treatment protocols. Further studies are recommended to validate these findings under field conditions and to explore the long-term effects of these treatments on camel health and productivity.

AUTHORS' CONTRIBUTIONS: Conceptualisation: Y.A., M.A. and M.K.; methodology: Y.A., A.A., M.A. and M.K.; software: M.K.; formal analysis: M.A. and M.K.; resources: M.A. and M.K.; data curation: Y.A., M.A. and M.K., project administration: S.A., and I.A., writing original draft preparation: Y.A. and M.K.; writing, review and editing: A.A., S.A., I.A. and M.K.

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Declarations

There is no conflict of interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability and sharing policy

Available in manuscript. Further details are available upon request.

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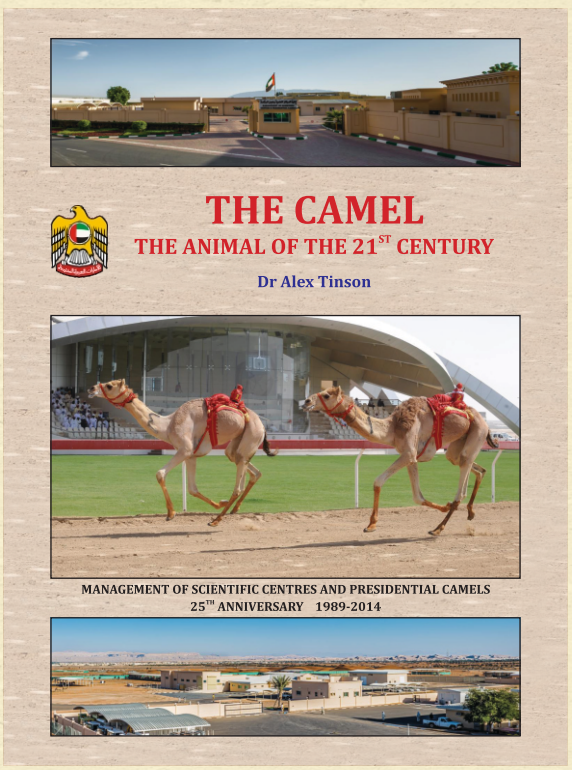
THE CAMEL

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.



Author
Dr Alex Tinson

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HISTOMORPHOLOGICAL PECULIARITIES OF THE TONGUE OF INDIAN DROMEDARY CAMEL (*Camelus dromedarius*)

Pankaj Kumar Thanvi, Sanjeev Joshi, Ashok Dangi, Aruna Panwar and Tanvi Mahajan
Department of Veterinary Anatomy, College of Veterinary and Animal Science, Bikaner
Rajasthan University of Veterinary and Animal Sciences, Bikaner, India

ABSTRACT

The present study was conducted on 10 tongues of recently dead adult camels (*Camelus dromedarius*), irrespective to age and sex, who were free from any pathological condition of tongue. Histologically, the tongue of camel consisted of mucosa, muscle layers, glands, blood vessels and nerves. The mucosa consisted of keratinised stratified squamous epithelium and lamina propria. Each papilla consisted of a central connective tissue core of lamina propria covered by keratinised stratified squamous epithelium. The keratinisation was more on the mechanical papillae as compared to gustatory papillae. The taste buds were present in the epithelium of the gustatory papillae. The filiform papillae were slender, elongated and sharp pointed structures. The lenticular papillae were round in shape and surrounded by a prominent papillary groove. The conical papillae were of 2 types. The small conical papilla was curved and blunt with a flat or rounded tip and large papilla was conical with a pointed tip. The fungiform papillae were small rounded button shaped and were of 2 types *viz.* dome shape and bud shape. Circumvallate papillae were large, rounded elongated structures. Each papilla was completely separated from thick annular fold by a deep gustatory groove. The lamina propria was composed of loosely interwoven collagen, reticular and elastic fibres, separating the epithelium from muscles. The bulk of tongue consisted of longitudinally, transversely and obliquely arranged intrinsic muscles which were essentially of striated type. Gustatory glands were associated with circumvallate papillae and were serous in nature. The glands of the root region and lateral margins of the torus were of mucous variety. The lyssa was composed of adipose tissue and striated muscle fibres.

Key words: Dromedary camel, histomorphology, tongue

The tongue assists in prehension, handles the movement of food during mastication and swallowing, dilates the airway during inspiration and is an organ of vocalisation in humans as well as in animals. The tongue is also used for grooming by licking the body coat and suckling in young ones. The tongue is an important sense organ, richly supplied with nerve endings for touch as well as with special endings for taste and chemical senses (Nonidez and Windle, 1953). The lingual structure is modified to play different abilities, for example feeding input, control and ingestion of nutrition molecules. The morphological appearance, prevalence, orientation and structure of lingual papillae are modified in accordance with the nutritional requirements, the types of nutritional particles accessible and the various environmental conditions (Farrag *et al,* 2022). It is a musculo – hydrostatic organ due to typical arrangement of longitudinal and transverse or oblique muscle fibres (Gilbert *et al,* 2007). The detailed description on the histology of tongue is meager. Scarce work on the microscopic anatomy of tongue of dromedary camel evoked interest to undertake the present study

Materials and Methods

The present study was conducted on 10 tongues of recently dead adult camels (*Camelus dromedarius*), irrespective to age and sex, who were free from any pathological condition of tongue. For the histological studies, small pieces (2-3 mm size) of tongue were taken from the representative areas and were preserved either in 10% formalin or Zenker's solution or Bouin's fluid for 48 hrs, 12hrs and 18 hrs, respectively. The tissues were washed overnight in running tap water, dehydrated in ascending order of alcohol (50%, 70%, 90% and then Absolute I, II and III), cleared in cedar wood oil and finally impregnated with paraffin. Paraffin blocks were prepared, numbered and stored at 4°C in refrigerator. Sections of 6–8-micron thickness were made by rotary microtome then taken on albuminised slides and

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kept overnight in hot air oven at 36°C and finally staining for the lining epithelium, connective tissue, musculature and various papillae of the tongue.

Results and Discussion

The tongue of the camel is a musculo-membranous organ consisted of mucosa, muscle layers, glands, blood vessels and nerves. Mucosa covered the entire tongue and consisted of epithelium and lamina propria. The epithelium covering the tongue was keratinised stratified squamous epithelium composed of basal, spinosum, granulosum, lucidum and corneum layers which was in accordance with the findings of Qayyum and Beg (1975) in goat, Stinson and Calhoun (1993) in domestic animals, El-Sharaby *et al* (2012) in camel, Choudhury *et al* (2013) in musk deer, El-Bakary and Abumandour (2017) in Egyptian water buffalo and Alhasson *et al* (2024) in buffalo. The cells in the deep layer of epithelium were elongated and the nucleus was situated at the base of cell whereas, cells towards the surface became flat. The loss of nuclei with weakly stained by eosin, in the most superficial layer of the epithelium was also observed (Fig 1). The epithelium and stratum corneum layers were thicker on dorsal surface of tongue than the ventral surface. The findings were also supported by Dhingra and Barnwal (1980) in buffalo, Stinson and Calhoun (1993) in domestic animals and partially supported by El-Bakary and Abumandour (2017) in Egyptian water buffalo. The stratum corneum was thickest on all type of papilla.

The mean thickness of the tongue epithelium at the dorsal surface of the tip was 617.177 ± 28.64 µm, at the ventral surface of the tip was 462.622 ± 17.801 µm, at the torus 647.311 ± 21.832 µm and at the root 356.332 ± 28.259 µm. The mean keratin thickness at the dorsal surface was 106.011 ± 9.006 µm, at the ventral surface of the tip was 43.235 ± 1.850 µm, at the torus was 188.373 ± 9.304 and at root was 116.825 ± 6.998 µm.

Papilla

The mucosa of dorsal surface and ventral margins of the tip was modified to form lingual papillae which were named according to their characteristic morphology *viz.* filiform, lenticular, conical, fungiform and circumvallate papillae. These papillae were classified into 2 groups according to their function *viz.* mechanical papillae and gustatory papillae which were also suggested by Eerdunchaolu *et al* (2001) in Bactrian camel, Farrag *et al* (2022) in water buffalo and Alhasson *et al* (2024) in buffalo.

Mechanical Papilla

Mechanical papillae were covered with a much thicker layer of keratin. The filiform, lenticular and conical papillae were mechanical papillae. Similar findings were noticed by Eerdunchaolu *et al* (2001) in Bactrian camel.

Filiform papillae

Filiform papillae were the dominant type of papillae on the tongue (Fig 2). These were slender, elongated, sharp pointed structures consisting of a central connective tissue core covered by keratinised stratified squamous epithelium, which was in conformity with the findings of Dellmann (2006) in domestic animals and contrary with the findings of Dhingra and Barnwal (1980) in buffalo according to which it did not possess any connective tissue core. The primary connective tissue core gave rise to several small secondary papillae but these did not extend up to the tip of the papillae, which was also reported by Labh and Mitra (1975) in goat, Lahkar *et al* (1992) in giraffe and Trautmann and Fiebiger (2002) in domestic animals. The epithelial coat was raised into a single keratinised apex. The filliform papillae were taller and thicker towards the *fossa linguae* and their height and thickness decreased towards the lingual apex. The lamina propria was also present in these small secondary papillae but did not extend up to the tip of the papillae. The epithelial coat was raised into a single large keratinised cone and covered by a highly keratinised epithelium as also mentioned by Qayyum *et al* (1988) in camel, Lahkar *et al* (1992) in giraffe and Dellmann (2006) in domestic animals.

The mean length of filiform papillae was 904.473 ± 63.162 µm, mean breadth was 351.375 ± 29.064 µm and mean keratin thickness was 84.111 ± 7.444 µm.

Lenticular papillae

The lenticular papillae were round in shape and were covered by stratified squamous epithelium with a thick layer of *stratum corneum* (Fig 3) which was in agreement with the observation of Lahkar *et al* (1992) for the tongue of a giraffe. The papillae were surrounded by a prominent papillary groove. The connective tissue core was dense with secondary projections and consisted of collagen fibres, reticular fibres, blood vessels and nerves which was in congruence with the findings of Dhingra and Barnwal (1980) in buffalo.

The mean length of the lenticular papillae was 4196.857 ± 249.291, breadth was 1797.847 ± 199.622 and keratin thickness was 84.111 ± 7.444 µm.

Table 1. Thickness of epithelium of different regions of tongue (in micron).

S. No.	At root		At *Torus lingaue*		At Ventral of Tip		At Dorsal of Tip	
	Epithelium	Keratin	Epithelium	Keratin	Epithelium	Keratin	Epithelium	Keratin
1.	391.920	132.445	616.267	195.606	542.909	49.653	683.694	120.537
2.	404.442	114.647	610.901	198.121	544.896	40.083	605.063	116.564
3.	524.748	171.235	651.66	199.324	471.941	34.312	697.785	154.391
4.	294.749	101.273	574.336	147.58	432.044	51.836	720.596	137.7
5.	410.661	121.310	708.325	211.484	446.043	40.853	613.164	96.511
6.	258.820	104.802	744.904	226.485	433.221	36.654	697.383	110.652
7.	266.882	102.192	711.7	213.604	397.377	41.609	546.362	76.723
8.	275.802	106.863	707.322	204.131	521.83	47.441	460.819	67.658
9.	391.257	125.021	548.506	140.291	413.694	41.609	527.997	86.041
10.	344.043	88.462	599.192	147.11	422.268	48.309	618.908	93.338
Mean	356.332	116.825	647.311	188.373	462.622	43.235	617.177	106.011
SD	89.363	22.098	69.040	29.422	56.293	5.8505	90.573	28.479
SE	28.259	6.988	21.832	9.304	17.801	1.850	28.641	9.006

Table 2. Length, width and thickness of keratin of filiform, lenticular and conical papillae (mechanical papillae) of tongue (in micron).

S. No.	Filliform papillae			Lenticular papillae			Conical papillae		
	Length	Width	Thickness of keratine	Length	Width	Thickness of keratine	Length	Width	Thickness of keratine
1.	805.078	253.029	54.251	4684.293	852.177	122.757	3220.193	1217.827	352.906
2.	1218.197	493.756	99.2	3179.056	1381.516	308.018	3150.465	1451.601	296.424
3.	955.51	217.213	93.338	3440.371	2077.514	146.236	4081.198	2320.307	240.037
4.	815.042	352.219	122.106	5138.751	2189.499	409.445	7228.575	4936.584	364.946
5.	650.346	224.071	72.261	3699.068	2312.078	186.23	4580.254	3816.802	179.257
6.	774.708	346.19	54.949	4735.745	942.231	131.875	3084.049	2014.903	213.142
7.	594.743	356.829	76.633	3585.616	1742.425	624.278	2864.551	2297.15	108.375
8.	825.263	265.385	65.367	4072.754	2456.013	179.265	4112.544	1915.732	183.834
9.	1092.185	404.827	104.371	5342.635	2478.236	456.156	5269.733	2966.423	274.493
10.	1313.661	600.237	98.64	4090.285	1546.783	257.254	4185.875	2657.493	231.818
Mean	904.473	351.375	84.111	4196.857	1797.847	282.151	4177.744	2559.482	244.523
SD	199.738	91.908	23.540	788.329	631.260	176.911	1395.011	1186.404	84.636
SE	63.162	29.064	7.444	249.291	199.622	55.944	441.141	375.174	26.764

Conical Papillae

The surface of conical papillae was covered by stratified squamous epithelium with a coat of keratin (Fig 4), which was in uniformity with the findings of Labh and Mitra (1975) in goat and Lahkar *et al* (1992) in giraffe. The connective tissue core was present below the epithelium. It consisted of collagen fibres and reticular fibres. The connective tissue core carried numerous low secondary papillae which was also reported by Labh and Mitra (1975) in goat. The conical papillae were of 2 types depending upon their shape and size i.e. small conical and large conical. In small conical papillae, connective tissue core was short cylindrical, curved and blunt with a flat or rounded tip which was in close agreement with the observation of Dhingra and Barnwal (1980) in buffalo tongue. In large papilla, the connective tissue core was conical with a pointed keratinised process. The giant conical papillae in the goat were greatly enlarged (Labh and Mitra, 1975). Most of them had a blunt conical dermal core but in some it was short and cylindrical (Fig 4).

The mean length was 4177.744 ± 441.141 µm, breadth was 2559.482 ± 375.174 µm and keratin thickness of the conical papillae was 244.523 ± 26.764 µm.

Table 3. Length, width and thickness of keratin of fungiform and circumvallet papillae (gustatory papillae) of tongue (in micron).

S. No.	Fugiform papillae			Circumvalate papillae			
				Median wall		Lateral wall	
	Length	Width	Thickness of keratine	Thickness of Epithelium	Thickness of keratine	Thickness of Epithelium	Thickness of keratine
1.	2247.763	1475.272	259.981	142.324	28.465	349.049	41.609
2.	1853.536	1188.88	209.735	163.92	22.33	357.585	24.262
3.	2831.975	1751.359	497.862	164.131	22.33	264.261	22.33
4.	2201.819	1475.272	593.431	153.717	24.826	285.79	22.638
5.	1429.798	964.322	370.146	194.986	28.465	292.473	20.553
6.	2096.786	1195.467	416.422	165.873	44.737	286.177	29.422
7.	2021.251	1233.377	33.701	208.045	34.211	344.375	30.689
8.	2367.801	2414.987	51.836	155.864	32.015	282.169	19.158
9.	3248.545	3397.71	93.708	159.053	35.793	243.943	22.484
10.	1578.114	1841.301	94.883	102.395	23.538	173.165	29.773
Mean	904.473	351.375	84.111	161.030	29.671	287.898	26.291
SD	199.738	91.908	23.540	20.786	7.250	40.116	7.016
SE	63.162	29.064	7.444	6.5733	2.292	12.685	2.218

Table 4. Length and width of taste buds (In Micron).

S. No.	Length	Width
1.	158.311	101.239
2.	153.311	75.906
3.	153.107	93.338
4.	154.189	81.621
5.	150.023	80.726
6.	175.292	85.678
7.	143.173	83.633
8.	136.766	81.749
9.	149.792	75.906
10.	119.15	90.627
Mean	149.311	85.042
SD	10.635	8.202
SE	3.3632	2.593
Correlation	0.013	

Gustatory Papilla

In gustatory papillae, keratin layer was comparatively thin and taste buds were present. The fungiform and circumvallate papillae were gustatory papillae. Eerdunchaolu *et al* (2001) had similar finding in Bactrian camel.

Fungiform Papilla

The fungiform papillae were small rounded button-shaped structures with rounded free top which was supported by a short cylindrical neck. Similar findings were seen by Dhingra and Barnwal (1980) in buffalo but they also observed narrow and shallow trench around the papilla which was not encountered in the dromedary tongue in present study. The fungiform papillae were of 2 types on the basis of morphological differences in shape, i.e. dome shaped and bud shaped. The epithelium covering the papilla on the top was very thin with a very thin keratin coat, which was in agreement with the report of Dhingra and Barnwal (1980) in buffalo and Dellmann (2006) in domestic animals but in disagreement with the findings of Labh and Mitra (1967) in goat. The taste buds were present in the epithelium of the upper surface of these papillae, which resembled with the finding of Eerdunchaolu *et al* (2001) in Bactrian camel and Dellmann (2006) in domestic animals but contradictory with the observations of Labh and Mitra (1967) in goat. The number of taste buds was very less, which was supported by Trautmann and Fiebiger (2002) and Dellmann (2006) in cattle and horses. The side walls of the papillae were devoid of taste buds. The connective tissue core was consisted of collagen fibres and reticular fibres which simulated the findings of Dellmann (2006) in domestic animals. The fungiform papillae present on the ventral surface of tip of tongue were comparatively small with thiner epithelium and keratin coat and devoid of taste buds which was not in harmony with the observations of Labh and Mitra (1967) in goat. No secondary papillae were present in the papillae of on the ventral surface (Fig 5).

The mean length of fungiform papilla was 2187.739 ± 63.162 µm, breadth was 1693.794 ± 29.064 µm and keratin thickness was 262.170 ± 7.444 µm.

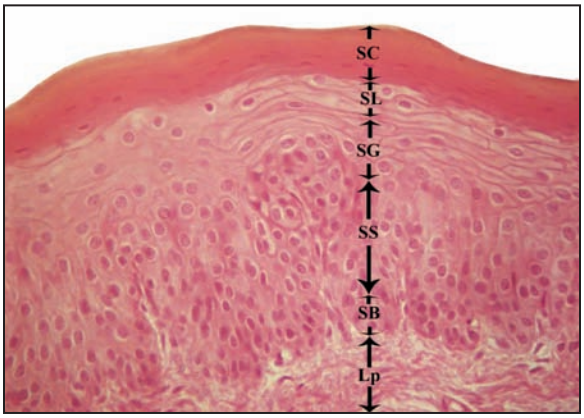

Fig 1. Photomicrograph of dorsal surface of tongue of camel showing different layers of epithelium. SC - Stratum corneum, SL - Stratum lucidum SG - Stratum granulosum, SS - Stratum spinosum, SB - Stratum basal, Lp - Lamina propria. (H & E stain, 400X).

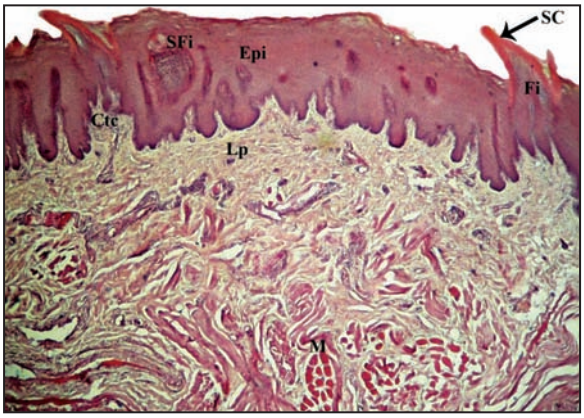

Fig 2. Photomicrograph of dorsal surface of tongue of camel showing filiform papillae. Fi - Filiform papilla, SFi - Secondary filiform papilla, Ctc - Connective tissue core, Epi - Epithelium, Lp - Lamina propria, M - Muscles, SC - Stratum cornium. (H & E stain, 40X).

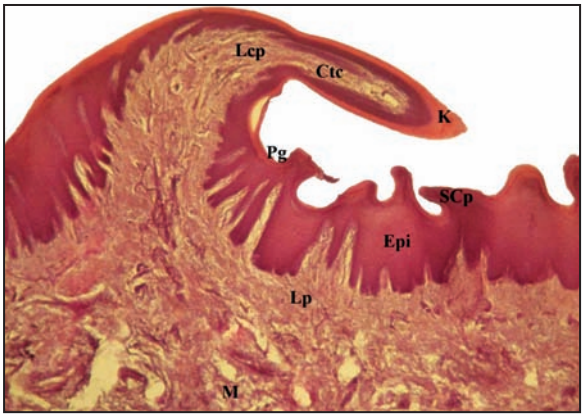

Fig 3. Photomicrograph of dorsal surface of tongue of camel showing lenticular papilla. Lcp - Lenticular papilla, Ctc - Connective tissue core, K - Keratin, Epi - Epithelium, Lp- Lamina propria, M - Muscles, Scp - Small conical papilla, Pg - Papillary groove. (H & E stain, 40X).

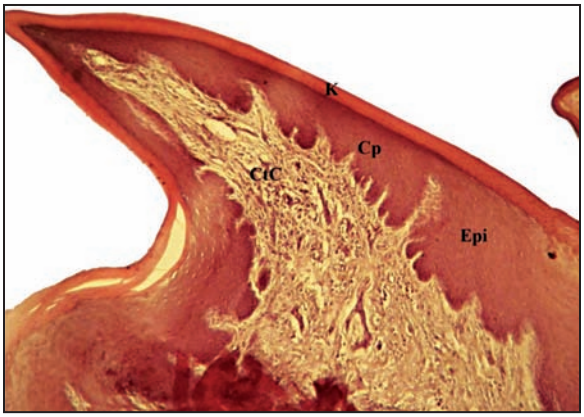

Fig 4. Photomicrograph of dorsal surface of tongue of camel showing large conical papilla. Cp - Conical papilla, Ctc - Connective tissue core, K - Keratin, Epi - Epithelium. (H & E stain, 100X).

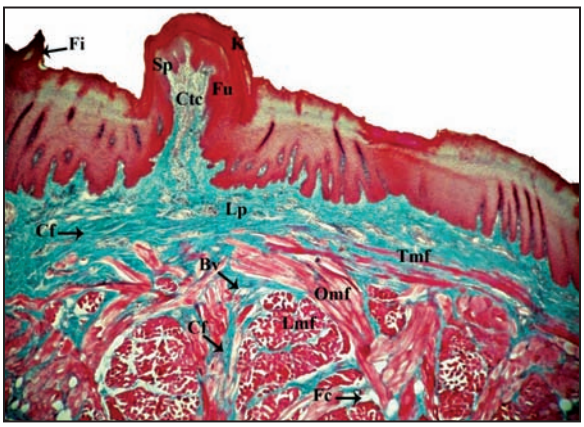

Fig 5. Photomicrograph of dorsal surface of tongue of camel showing bud shaped fungiform papilla. Fu - Fungiform papilla, Ctc - Connective tissue core, K - Keratin, Cf - Collagen fibres, Lp - Lamina propria, Lmf - Longitudinal muscle fibre, Tmf - Transverse muscle fibre, Omf - Oblique muscle fibre, Fc - Fat cell, Bv - Blood vessel. (Masson's Trichrome stain, 40X).

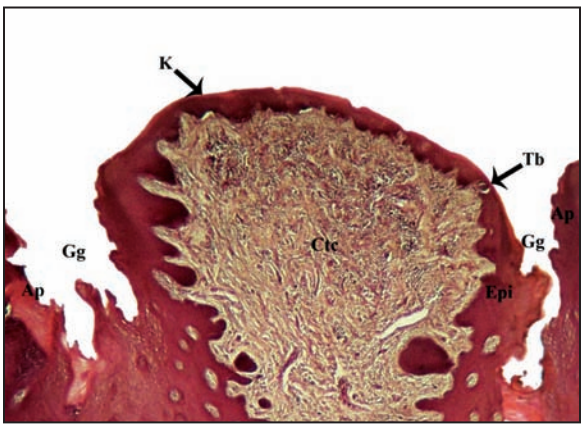

Fig 6. Photomicrograph of dorsal surface of tongue of camel showing a longitudinal section of circumvallate papilla. Ctc - Connective tissue core, K - Keratin, Tb - Taste bud, Epi - Epithelium, Ap - Annular pad, Gg - Gustatory groove. (H & E stain, 100X).

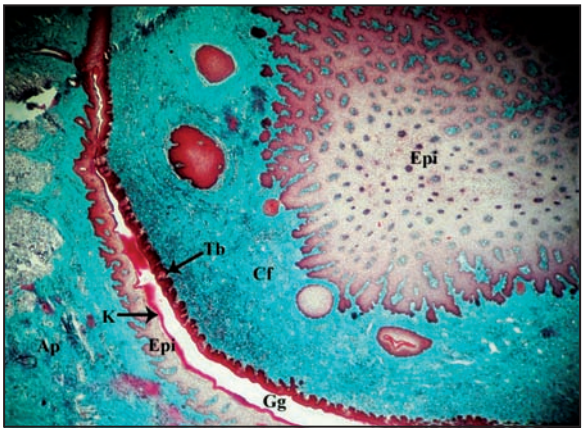

Fig 7. Photomicrograph of dorsal surface of tongue of camel showing transverse section of circumvallate papilla. Cf - Collagen fibre, K - Keratin, Tb - Taste bud, Epi - Epithelium, Ap - Annular pad, Gg - Gustatory groove. (Masson's Trichrom stain, 40X).

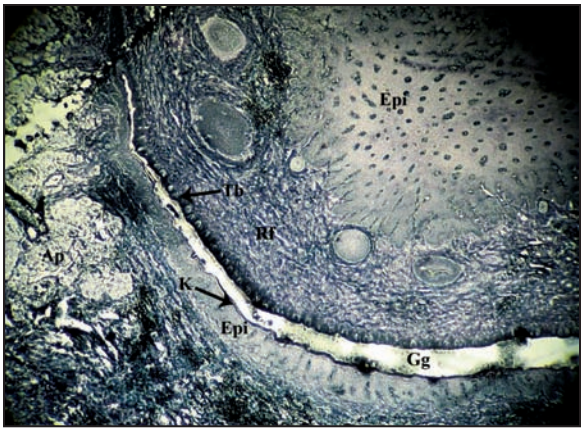

Fig 8. Photomicrograph of dorsal surface of tongue of camel showing transverse section of circumvallate papilla. Rf - Reticular fibres, K - Keratin, TB - Taste bud, Epi - Epithelium, Ap - Annular pad, Gg - Gustatory groove. (Gomori's stain for reticulum, 40X).

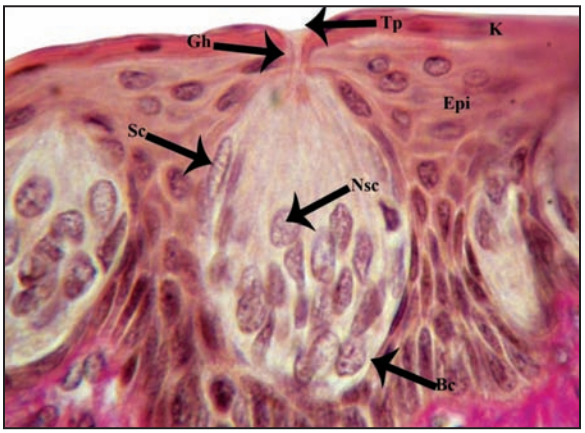

Fig 9. Photomicrograph of epithelium of circumvallate papilla of camel showing taste bud. Tp - Taste pore, Sc - Supportig cells, Bc - Basal cell, Nsc - Neuro-sensory cells, Gh - Gustatory hair, Epi - Epithelium, K – Keratin. (Van Geison's stain, 1000X).

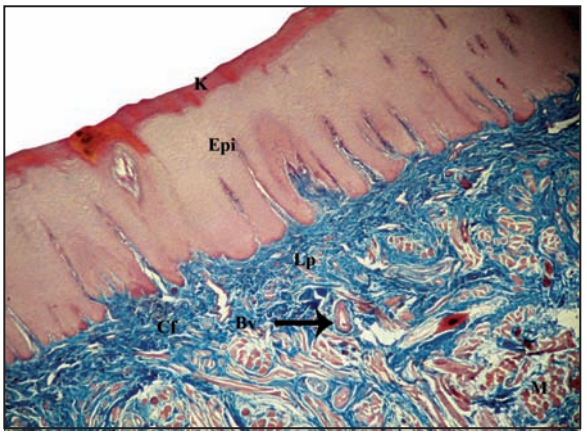

Fig 10. Photomicrograph of the tongue of camel showing different layers of the tongue. Epi - Epithelium, Lp - lamina propria, Cf - collagen fibres, M - Muscle layer, K - Keratin, BV - Blood vessel. (Selective demonstration of different connective tissue fibres stained by Silver & Orcin, 100X).

Circumvallate papillae

Circumvallate papillae were large, rounded elongated structures. Each papilla was completely separated from thick annular fold by a deep gustatory groove, an epithelial lined cleft or moat which was in consonance with the reports of Labh and Mitra (1967) in goat, Dhingra and Barnwal (1980) in buffalo, Trautmann and Fiebiger (2002) and Dellmann (2006) in domestic animals. The papillae were lined with moderately keratinised stratified squamous epithelium. The epithelium of papillary surface was less keratinised as compared to that of the surrounding annular pad which was in congruence with the observations of El-Sharaby *et al* (2012) in camel. Numerous small or large elongated taste buds were observed along the entire length of epithelium of papillary surface of the gustatory groove; which was in accordance with the findings of Trautmann and Fiebiger (2002) in horse, ruminants and swine and El-Sharaby *et al* (2012) in camel. No taste buds were found on dorsal surface of papillae and in the lateral walls of the groove, which was in conformity with the findings of Trautmann and Fiebiger (2002) in domestic animals. However, according to El-Sharaby *et al* (2014) taste buds were located in the medial and lateral epithelium of both primary and secondary grooves as well as in the dome epithelium in dog. The connective tissue core consisted of collagen and reticular fibres present under the epithelium. The core of connective tissue extended into the epithelium at few places and forming the secondary papillae, which was also reported by Labh and Mitra (1967) in goat and

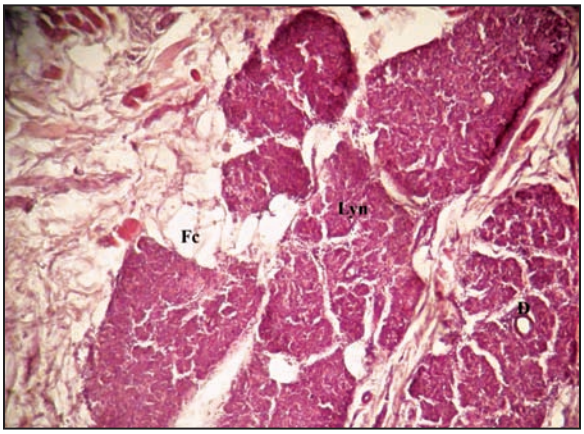

Fig 11. Photomicrograph of root of the tongue of camel showing lymph nodes. Lyn - Lymph node, D - Duct of lymph node, Fc- Fat cells. (H & E stain, 100X).

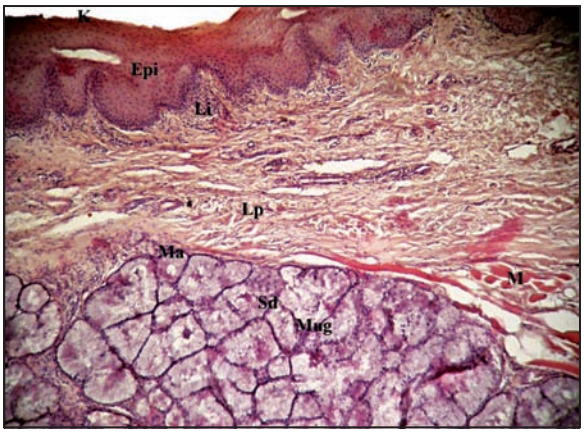

Fig 13. Photomicrograph of tongue of camel showing mucous glands. Mug - Mucous gland, Lp - Lamina propria, Epi - Epithelium, Li - Lymphatic infiltration, K - Keratin, M – Muscle fibres, Ma - Mucous acini, Sd - Serous demilune. (H & E stain, 40X).

Trautmann and Fiebiger (2002) in domestic animal. The openings of the lingual glands were also observed in the lateral wall of the gustatory groove, which was also favoured by Dellmann (2006) in domestic animals and El-Sharaby *et al* (2012) in camel (Figs 6, 7 and 8).

The mean keratin thickness of medial wall was 29.671 ± 2.292 µm, at lateral wall was 26.291 ± 2.218 µm, mean thickness of epithelium of medial wall was 161.030 ± 6.573 µm and at lateral wall was 287.898 ± 12.685 µm.

Taste buds

The taste buds were ovoid or spindle shape structures present along the entire length of the epithelium of the medial wall of the circumvallate papillae gustatory groove and the epithelium of the upper surface of the fungiform papillae which was in congruence with the observations of Dhingra and

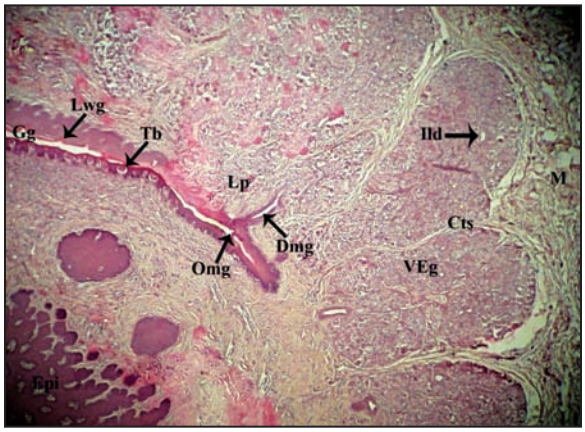

Fig 12. Photomicrograph of circumvallate papilla of the tongue of camel showing Von Ebner's glands. VEg - Von Ebner's gland, CtS - Connective tissue septum, Lp - Lamina propria, M – Muscle layer, Tb - Taste bud, Gg - Gustatory groove, Lwg – Lateral wall of gustatory groove, Epi - Epithelium, Ild - Intra lobular duct of the gland, Dmg - Duct of mucous gland, Omg - Opening of mucous gland in gustatory groove. (H & E stain, 40X).

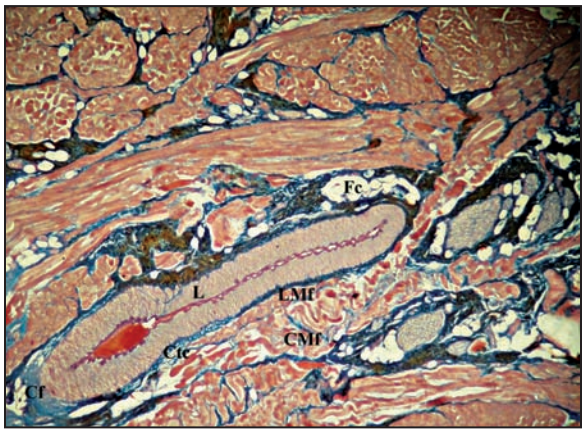

Fig 14. Photomicrograph of tongue of camel showing transverse section of lyssa. L - Lyssa, Ctc - Connective tissue capsule, LMf - Longitudinal muscle fibres, CMf - Circular muscle fibres, Fc - Fat cells, Cf - Collagen fibres. (Selective demonstration of different connective tissue fibres by Silver & Orcin, 40X).

Barnwal (1980) in buffalo and El-Sharaby *et al* (2012) in camel. These were arranged in single layer and occupied the entire thickness of the epithelium. These were extended from the lamina propria to a little opening, the taste pore, on the free epithelial surface. The long axis of taste bud was vertical to the surface (Figs 6, 7 and 8).

Each taste bud consisted of 3 types of cells, *viz.* basal cells, supporting cells and neuro-epithelial sensory cells or gustatory cell. The basal cells were arranged at the base of taste buds. The supporting cells were arranged like the sticks of a barrel and formed an outer envelope for the bud. The gustatory

cells occupied the central portion of the bud; these were spindle-shaped and possessed a large spherical nucleus near the middle of the cell. The gustatory cells extended the microvilli or fine gustatory hair into the taste pore. These observations were in conformity with the findings of Trautmann and Fiebiger (2002) in domestic animals (Fig 9).

The mean height of taste buds was 149.311 ± 3.3632 μm in and mean width was 85.042 ± 2.593 μm.

Lamina Propria

The lamina propria was a large layer of connective tissue which separated the innermost layer of epithelial cells from the muscle tissue. It was extended in the epithelium in a variable pattern to form connective tissue core of papillae and intermingled with the striated muscle fibres which was also confirmed by Deore *et al* (2002) in goat. It was composed of loosely interwoven collagen fibres, reticular fibres and elastic fibres along with the connective tissue cells (Fig 2, 5, 13). This observation was simulated with the findings of Dhingra and Barnwal (1980) and Alhasson *et al* (2024) in buffalo. The collagen and reticular fibres were abundant but elastic fibres were very few which was in agreement with Ramayya *et al* (2000) in goat. Lingual glands were present in the lamina propria which was also described by the Stinson and Calhoun (1993) in domestic animals, Lahkar *et al* (1992) in giraffe and El-Sharaby *et al* (2012) in camel. The lamina propria was highly vascular containing arteries, capillaries and veins which was in harmony with the observation of Jain (1975) in goat (Fig 5 and 10). Large lymph nodes were observed in lamina propria of the root of the tongue which was also investigated by Zidan and Pabst (2020) in camel (Fig 11). Small nodules were also seen in the lamina propria just below the epithelium and in some areas, it was in the form of diffused lymphatic tissue. Large numbers of fat cells were also observed in the lamina propria which was also reported by Trautmann and Fiebiger (2002) in domestic animals.

Muscles

The bulk of the tongue consisted of intrinsic muscles which were essentially of striated type found beneath the lamina propria, which was in conformity with the observations of Dhingra and Barnwal (1980) in buffalo, Stinson and Calhoun (1993) and Trautmann and Fiebiger (2002) in domestic animals, Choudhury *et al* (2013) in musk deer and El-Bakary and Abumandour (2017) in Egyptian water buffalo. The muscular mass consisted of longitudinally, transversely and obliquely arranged bundles of striated muscles fibres which interlaced with each other which were in consonance with the reports of Jain (1975) in goat, Dhingra and Barnwal (1980), Alhasson *et al* (2024) in buffalo and Stinson and Calhoun (1993) in domestic animals. Large amount of adipose tissue and numerous blood vessels were present between the muscle fibres, which simulated to the findings of Dhingra and Barnwal (1980) in buffalo and Trautmann and Fiebiger (2002) in domestic animals. In between the muscle fibres, collagen fibres were also present (Fig 2, 5, 10).

Lingual Glands

The lingual glands were of 2 types, *viz.* gustatory and mucous variety.

Gustatory Glands or Von Ebner's glands

Gustatory glands were associated with circumvallate papillae. These were serous in nature and arranged in lobes, which was also reported by Dhingra and Barnwal (1980) in buffalo, Lahkar *et al* (1992) in giraffe, Narasimhan *et al* (1999) in goat and Biradar and Ramkrishna (2000) in sheep. The glands were partially located in the lamina propria and the muscular layer, which was in consonance with the observation of Dhingra and Barnwal (1980) in buffalo. The latter extended between the lobes and partially covering the same. Each lobe consisted of several lobules. Thin connective tissue septa containing collagen and reticular fibres divided the lobes into lobules, this was in uniformity with the findings of Parida and Das (1991) in domestic ruminants. Each lobule consisted of several secretary units, the serous acini. Each acinus was spherical in shape and lined by tall columnar epithelium with basal nuclei which simulated the observations of Parida and Das (1991) in domestic ruminants but according to Narasimhan *et al* (1999) the acini were lined by pyramidal cells with indistinct cell boundaries in goat. The apical portion of each cell was filled with secretary granules. The cells rested on the basement membrane. The myo-epithelial cells with elongated nuclei were present in between the basement membranes of the acini partially in agreement with the findings of Stinson and Calhoun (1993) in domestic animals. The serous demilunes were also observed in between the serous acini (Fig 12).

Mucous Glands

The glands of the root region and lateral margins of the torus linguae were of mucous variety

with occasional serous demilunes which was in close agreement with the reports of Qayyum and Beg (1975) and Narasimhan *et al* (1999) in goat. The glands were of the acinar type and oval in shape and the acini showed large lumen lined by the pyramidal cells, which was not in agreement with the reports of Parida and Das (1991) in domestic ruminants according to which the mucous acini were more rectangular than spherical in shape and the acinar cells were cuboidal to columnar. The nucleus was rounded and situated at the base of the cell. The acini were densely packed. In between the acini myoepithelial cells with elongated nuclei were observed (Fig 13).

The intralobular and interlobular ducts were lined with simple cuboidal epithelium. The epithelium of the interlobular ducts was lined by two layered cuboidal epithelia. Terminal ducts of serous gland opened on the outer wall of the gustatory groove of the circumvallate papillae. The terminal ducts of the mucous glands opened directly on the dorsum of the tongue. The glands were absent in the tip region. These findings were in harmony with the findings of Narasimhan *et al* (1999) in goat.

Lyssa

The lyssa of the camel was highly vascular, resembled with the finding of Sultana *et al* (2017) in dog. Transverse section showed that the lyssa was composed of adipose tissue and striated muscle fibre which was also observed by Sultana *et al* (2017) in dog. It was enclosed by a dense connective tissue capsule formed mainly of collagen fibres which was also supported by Sultana *et al* (2017) in dog. The connective tissue capsule was surrounded by longitudinal and circular muscle fibres. The body of lyssa was elongated and consisted of adipose tissue and isolated striated muscle fibres and collagen fibres were found in between the adipose tissue. In the cranial third of the lyssa, a pyramidal rod formed by the adipose tissue and encircled by a fine capsule of connective tissue was observed. This rod was attached to the ventral edge of the lyssa, also favoured by Besoluk *et al* (2006) in dog and cat and Sultana *et al* (2017) in dog. In caudal part, body was divided in small - small parts and enclosed by the connective tissue separately (Fig 14).

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INTESTINAL COCCIDIANS IN DROMEDARY CALVES – AN ANALYSIS OF NECROPSY AND PARASITOLOGICAL RESULTS

Rolf K. Schuster, Saritha Sivakumar and J. Kinne
Central Veterinary Research Laboratory, Dubai, United Arab Emirates

ABSTRACT

A total of 1,593 camel calves were sent for necropsy and subsequent parasitological examination to the Central Veterinary Research Laboratory between January 2017 and June 2024. This article reports findings of intestinal coccidians in neonatal and young calves up to an age of 12 months. The content of rectum or small colon was examined for the presence of *Cystoisospora orlovi* and *Eimeria* spp. using the floatation method. Samples of diarrhoeic calves were examined for *Cryptosporidium* oocysts. Out of 1,437 samples examined with the floatation method, 94 were positive for *C. orlovi* and 55 samples contained *Eimeria* spp. oocysts. *Cryptosporidium* oocysts were detected in 72 out of 972 examined samples. The majority of *C. orlovi* oocysts were detected in camel calves in an age group up to 4 weeks of age, while *Cryptosporidium* oocysts were diagnosed mainly in 3 to 8 weeks old animals. The youngest calf with an *Eimeria* infection was 30 days old but the majority of *Eimeria* positive camels had a body weight between 100 and 220 kg. Out of 886 samples from necropsied adult dromedaries in the same time period, 74 were positive for *Eimeria* oocysts and *C. orlovi* was found in one sample only. Examination of 42 diarrhoeic samples for *Cryptosporidium* oocysts gave negative results.

Key words: *Camelus dromedarius, Cryptosporidium, Cystoisospora orlovi, Eimeria,* necropsy

Among the *Eimeria* species in Old World camelids, five were named in the literature (*E. cameli*, *E. rajasthani*, *E. dromedarii*, *E. bactriani* and *E. pellerdyi*) but the validity of the latter two species remained obscure (Schuster, 2018; Dubey and Schuster, 2018). Little is known about *Cystoisospora orlovi*. Oocysts of this species were described for the first time from camels in Kazakhstan by Cygankov (1950) and were later found also in dromedaries in India (Raisinghani *et al,* 1987), the UAE (Kinne *et al,* 2001, 2002), Kenya (Younan *et al,* 2002) and recently also in Iraq (Al-Yasari *et al,* 2024). *Cryptosporidium* spp. is another group of intestinal coccidians that are found in camels. Contrary to the above mentioned species, the genus *Cryptosporidium* with more than 40 described species has a broad host spectrum and some of the species are zoonotic (Ryan *et al,* 2022). *Cryptosporidium* spp. findings in dromedaries from Iran, Iraq, Kuwait, Saudi Arabia, Algeria, Egypt, Ethiopia and Australia and in Bactrian camels from Azerbaijan and China were recently reviewed by Elmahallawy *et al* (2023). Noaman *et al* (2022) recently published a review paper on enteric protozoa, however, none of these published articles specifically dealt with camel calf coccidians in their first months of life.

The aim of this study was to analyse parasitological findings of intestinal coccidians in dromedary calves of up to 12 months of age necropsied at the Central Veterinary Research Laboratory in Dubai, United Arab Emirates between 2017 and 2024.

Materials and Methods

During an eight and a half years (90 month) period between January 2017 and June 2024, total of 1,593 dromedary calves were necropsied at the Central Veterinary Research Laboratory in Dubai. The majority of samples were sent from a large camel dairy farm. Of these calves, 1,437 samples (faecal samples or colon content) were submitted to the department of parasitology. Samples from stillborn calves or calves less than 4 days of age were excluded.

All samples were examined with the simple floatation method using saturated $NaCl/ZnCl_2$ solution in tubes for the detection of *Eimeria* spp. and *Cystoisospora orlovi* oocysts. Specimens of liquid or pasty content (n=972) were stained in addition with the carbol-fuchsin method after Heine (1982) known as the negative staining (Potters and van Esbroeck, 2010). With samples positive for *Cryptosporidium*

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oocysts, acid fast staining or modified Ziehl-Neelsen staining (Rekha *et al,* 2016) was performed to confirm the correctness of the result. It has to be mentioned that the carbol-fuchsin staining will also detect *Cystoisospora* oocysts when their concentration in the feacal smear is high enough.

Unfortunately, the age was not indicated on the submission forms for all of the calves. Body weight and sex was taken from necropsy protocols.

In the same time span, 886 faecal samples from necropsied adult dromedaries were also examined with the floatation method.

Results and Discussion

During the 90 month observation period, *C. orlovi* oocysts (Figs 1, 2) were detected in 94 samples and 72 samples were positive for *Cryptosporidium* oocysts (Figs 3, 4). In three calves, a mixed infection between *C. orlovi* and *Cryptosporidium* oocysts was diagnosed. During the same time period, *Eimeria* oocysts were detected in 55 samples. A species determination revealed the presence of 3 species, *E. cameli* (Fig 5), *E. rajasthani* (Fig 6) and *E. dromedarii* (Fig 6). The distribution of the detected coccidians throughout the observation period is shown in Tables

Table 1. Detection of *Cystoisospora orlovi* oocysts in necropsied dromedary calves (n=1437) in Dubai between 2017 and 2024.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2017	2		1		3					1	1		8
2018	1	2											3
2019	3	1	7	1									12
2020	3	5	2	1			1			1			13
2021		4	5	1								2	12
2022	2	3	4	5	2					1		1	18
2023	1	7	6									1	14
2024	2	2	2	5	2								13
2017-2024	**14**	**24**	**27**	**13**	**7**	**0**	**1**	**0**	**0**	**3**	**1**	**4**	**94**

Table 2. Detection of *Cryptosporidium oocysts* in necropsied dromedary calves (n=972) in Dubai between 2017 and 2024.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2017			3		1	1							5
2018	1	1	1				1						4
2019	1	6	2	8	4	2							23
2020	1	3	3										7
2021			1	1								1	3
2022			1		1	1	1					1	5
2023		2	2	4	4				1				13
2024			1	3	6	2							12
2017-2024	**3**	**12**	**14**	**16**	**16**	**6**	**2**	**0**	**1**	**0**	**0**	**2**	**72**

Table 3. Detection of Eimeria oocysts in necropsied dromedary calves (n=1437) in Dubai between 2017 and 2024.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2017								1					1
2018	2		1		1								4
2019	1		3	1		1				1			7
2020						2							2
2021	1								1				2
2022	1		2	2		1	1					2	9
2023	5							1				1	7
2024	1			4	10	8							23
2017-2024	**11**	**0**	**6**	**7**	**11**	**12**	**1**	**2**	**1**	**1**	**0**	**3**	**55**

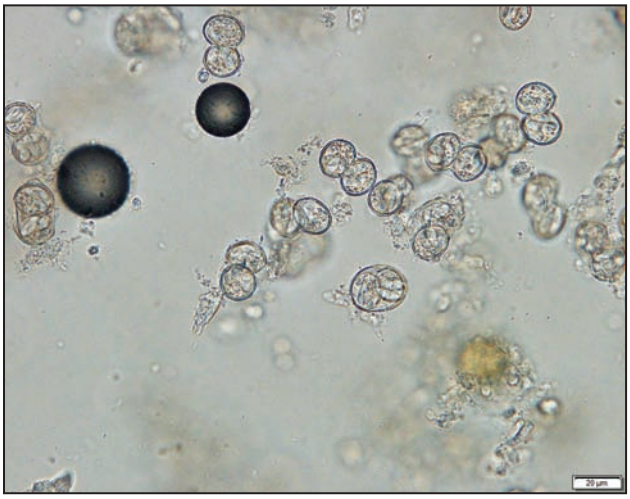

Fig 1. *Cystoisospora orlovi* concentrated by floatation. The majority of oocysts are already sporulated when excreted with the faeces.

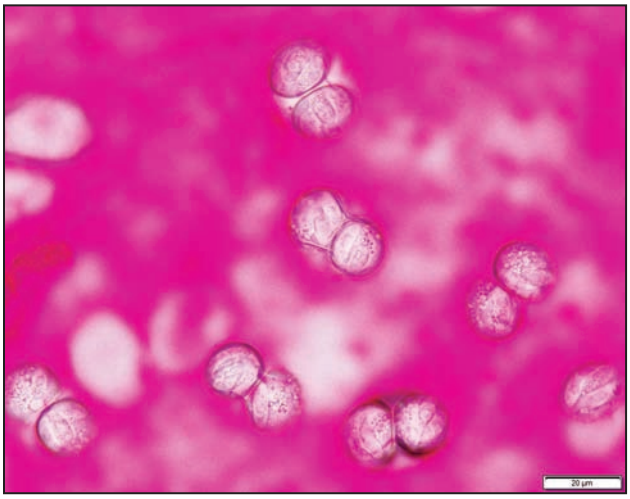

Fig 2. *Cystoisospora orlovi* in a faecal smear stained with carbol-fuchsin. In the dried smear, the very thin oocyst wall embeds the slightly ellipsoid sporocysts tight and produces an image of a digit eight.

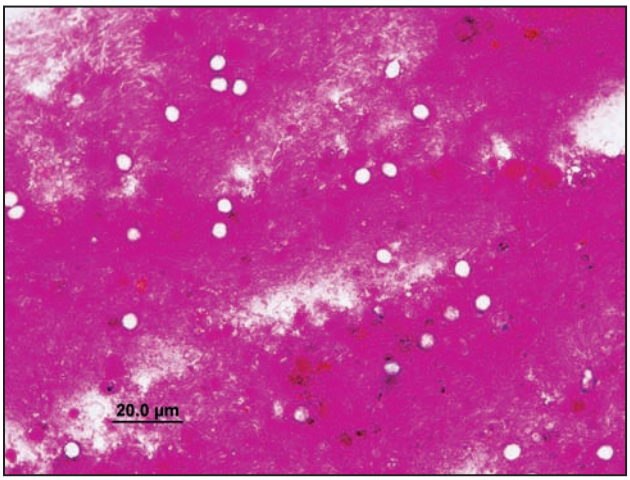

Fig 3. *Cryptosporidium* oocyst in a faecal smear stained with carbol-fuchsin (negative staining). In a freshly prepared and dried smear the round oocysts appear as colourless spots on a red background.

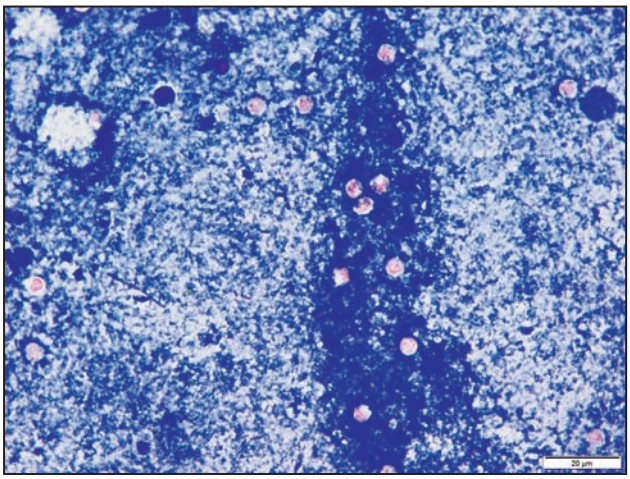

Fig 4. *Cryptosporidium* oocyst in a faecal smear (Ziehl-Neelsen staining). In this staining, oocysts are pink to red on a blue background.

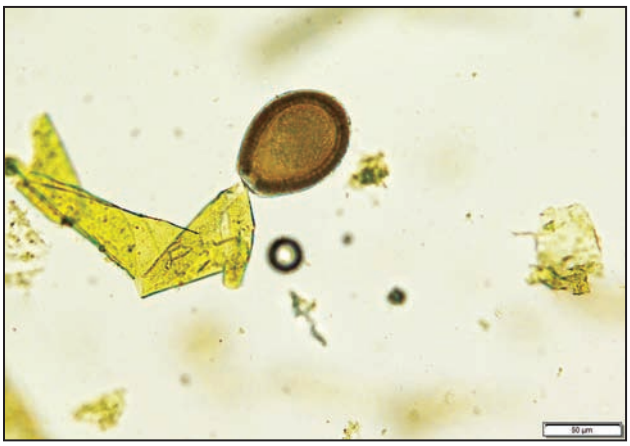

Fig 5. *Eimeria cameli*. The large brown oocyst from dromedary camelids resemble *Eimeria macusaniensis* of South American camelids.

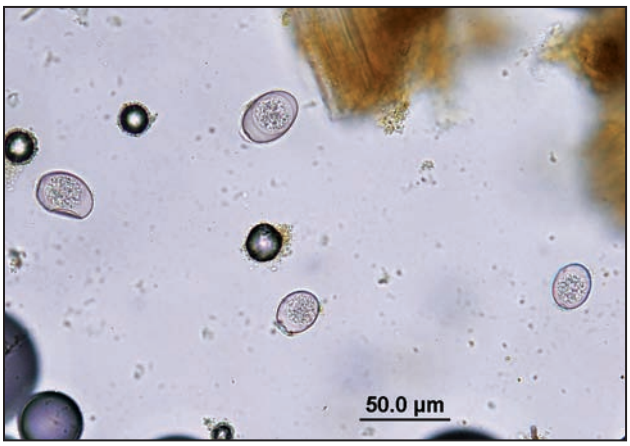

Fig 6. *Eimeria rajasthani* (larger sized oocyst with a pole cap) and smaller sized oocysts of *E. dromedarii*

1 to 3. Most of the positive cases were diagnosed in the first half of each calendar year.

According to the statement on age on the requisition form, *C. orlovi* was detected in calves that died in an age between 10 to 50 days (Table 4). The age of 24 calves was not stated on the requisition form but according to the body weight of the carcasses at necropsy (23-47 kg), these were less than two months old (Table 5). *Cystoisospora* oocysts were found in 40 female and 53 male dromedary calves. Cystoisosporosis as primary cause or in combination with white muscle disease, pneumonia or meningitis was responsible for the death of 80 calves.

Table 4. Detection of *Cryptosporidium* and *Cystoisospora* oocysts in in necropsied dromedary calves of different age in Dubai between 2017 and 2024 (not all the requisition forms indicated the age of the carcass).

Age (days)	Cryptosporidium	Cystoisospora
up to 20	4	12
21-30	18	51
31-40	3	3
41-50	6	2
50-60	8	0
older 60	7	0
not indicated	26	26
Total	72	94

Table 5. Detection of *Cystoisospora, Cryptosporidium* and Eimeria oocysts in necropsied dromedary calves of different body weight in Dubai between 2017 and 2024.

Body weight (kg)	*Cystoisospora*	*Cryptosporidium*	*Eimeria*
up to 30	15	10	0
31-40	48	22	0
41-60	31	31	5
61-80	0	6	5
81-100	0	0	7
100-120	0	3	9
120-140	0	0	29
Total	94	72	55

The age of calves with a patent *Cryptosporidium* infection ranges from 18 to 210 days (Table 4). For 26 carcasses age was not reported but the body weight range of these animals are given in table 5. Thirty-one calves were males and 24 were females. For 47 of the 72 infected calves, cryptosporidiosis was concluded as the cause of death.

The *Eimeria* oocysts were found mainly in older camel calves with a body weight ranging from 49 to 230 kg (Table 5). In 15 out of the 55 cases, the *Eimeria* infection was fatal. Eight of these fatal cases occurred in April, May and June 2024.

In the same time period, 886 samples from necropsied adult dromedaries were examined. Of these, 74 were positive for *Eimeria* oocysts and *C. orlovi* was found in one sample only. Examination of 42 diarrhoeic samples for *Cryptosporidium* oocysts gave negative results.

Our results showed the significance of the 3 coccidian pathogens in young dromedary calves in the United Arab Emirates. In general, little is known about the parasite fauna of dromedary calves in their first months of life. This is because this age group is not available when parasitological examinations are done with material taken from abattoirs (Mahmoud *et al,* 2009; Tafti *et al,* 2009; Razafi *et al,* 2009). In other studies, the youngest animals were grouped in the category less than one year (Yakhchali and Moradi, 2012; Bouragba, 2020) or less than five years (Sazmand *et al,* 2011; Maxamhud, 2023) or coccidian findings in juvenile camels were subjects of case reports (Wang *et al,* 2008; Gu *et al,* 2016; Zahedi *et al,* 2018).

All 3 reported protozoans have a faecal-oral way of transmission. Although in our material, the overall prevalence with *Eimeria*, *Cystoisospora* and *Cryptosporidium* oocysts was relatively low and amounted to 3.8, 6.6 and 7.4%, respectively. Most of the detected cases were seen in the first half of the year (Tables 1 to 3). This is because of the main calving period fallowing in spring and climatic peculiarities. Freshly excreted *Eimeria* oocysts need to sporulate outside the host. According to Gerlach (2008) the duration of *E. cameli* sporulation at room temperature lasts 6 to 8 days. It shortens under warmer conditions. Under natural conditions in the UAE desert with air temperatures in summer reaching more than 50°C, bring the temperature at the surface of the soil up to 65°C. Under such conditions, camel faecal piles lose 60% of its weight due to evaporation of water within 6 hours and oocysts will die. *Cryptosporidium* spp. and *C. orlovi* sporulate already in the gut of the infected camel and are infectious when they are excreted but due to their thin oocyst wall, they are extremely susceptible to high temperatures and desiccation. Frequent *Eimeria* infections were detected between April and June 2024. Most probably, this was an aftermath of heavy rainfalls in March, April and May 2024.

In the study, percentage of *Eimeria* positive calves was lower than in adult dromedaries examined in the same time period. A study of *Eimeria* prevalence

in racing camels at an age up to six years in Dubai revealed a prevalence of *E. cameli* between 7.7 and 17.5%. *E. rajasthani* and *E. dromedarii* were less frequent showing a prevalence of 1.5-7.4 and 1.9-4.8%, respectively (Dubey and Schuster, 2018). Comparable *Eimeria* prevalence was reported from Iran (Sazmand *et al,* 2012), Nigeria (Mahmuda *et al,* 2014), Uganda (Jesca *et al,* 2018) and Egypt (El Khabaz *et al,* 2019).

While *Eimeria* species of camels can be distinguished by the morphology of their oocysts the exact species inventory of *Cryptosporidium* spp. can be done only with molecular tools since most of the 14 different species that have been found in farm animals have similar sizes and only *C. muris* can be distinguished by larger oocyst measurements (7.5x5.6 µm). In our study, we found *Cryptosporidium* oocysts in 74 out 972 examined camel calves without species identification with the majority of positive samples being diagnosed between the months February and May. Other studies reported much higher prevalences of 55.0% (Jawad and Jasim 2016), 56.0% (Hussin *et al,* 2015), 58.0% (Saidi *et al,* 2022) or even 66.0% (Hasan *et al,* 2021).

Some *Cryptosporidium* species are zoonotic and in addition to camels, Hussin *et al* (2015) found *Cryptosporidium* oocysts in 56.0% of examined camel breeders or in their families. These results however, are more than dubious. A molecular study carried out in the UAE residents revealed 26 out of 134 stool samples positive for *Cryptosporidium* spp. (ElBakri *et al,* 2014).

In a large molecular study involving 476 Bactrian camels the coccidians identified were *C. andersoni, C. bovis, C. hominis, C. occultus, C. parvum* and *C. ubiquitum* (Chao *et al,* 2020). Other studies detected *C. muris* (Abdel-Wahab and Abdel-Maogod 2011, Wang *et al,* 2021; Zhang *et al,* 2021). Part of our *Cryptosporidium* samples were sequenced and the species found were *C. parvum, C. hominis* and *C. meleagridis* (Procter *et al,* 2024).

C. orlovi is the least known coccidian species in camels. The occurrence of *C. orlovi* in the UAE is known since 2001 (Kinne *et al,* 2001; 2002) and a review of annual reports of the Central Veterinary Research Laboratory in Dubai between 2005 and 2016 revealed findings of *Cystoisospora* oocysts in 72 out of 2,885 and in 12 out of 76,969 examined faecal samples from dromedary calves and adult camels, respectively (Schuster *et al,* 2017). As shown in this study, the number of cases of camel calf cystososporosis in the following years between 2017 and 2024 has increased (94/1.437). As in the previous study, the majority of cases were found in an age group between 21 and 30 days. Subsequently, the majority of cases were detected in the main calving period between January and May. The route of infection of camel calves with this parasite is still unknown.

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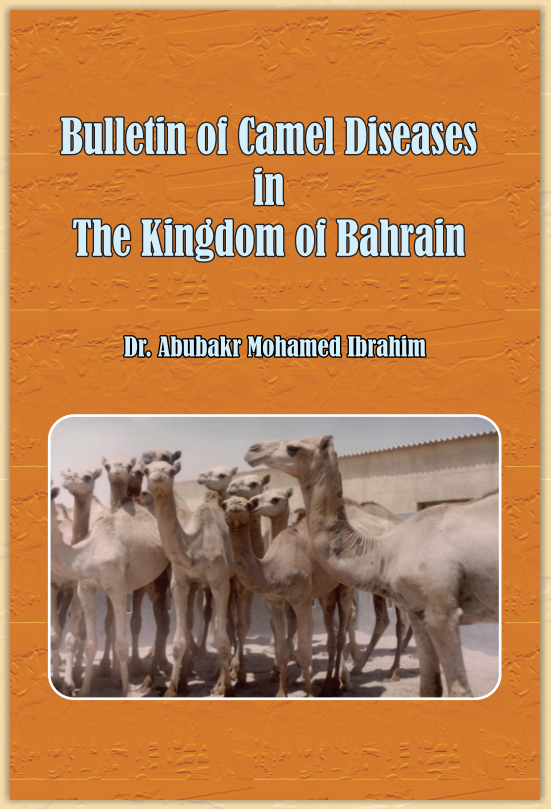
Bulletin of Camel Diseases in The Kingdom of Bahrain

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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MORPHOLOGICAL CLASSIFICATION OF HEPATIC TUMOURS IN ONE-HUMPED CAMEL (*Camelus dromedarius*)

Hala M.F. El Miniawy[1], Ashraf M. Abu-Seida[2,3], Gehan G. Ahmed[4] and Marwa A. Abd ElMagid[4]

[1]Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[2]Department of Surgery, Anaesthesiology & Radiology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[3]Animal Research Facility, Galala University, New Galala City, Suez, Egypt
[4]Department of Pathology, Animal Health Research Institute, Giza, Egypt

ABSTRACT

Camels rarely develop hepatic tumours. Forty six livers with tumour lesions from camels that had been slaughtered over a period of 3 years in the biggest abattoirs in Cairo, Egypt, were used in this investigation. The morphological and histological analyses of the hepatic lesions gave rise to a sufficient overview of the possible tumours that could develop in camel livers, whether they were neoplastic or not. Out of 46 cases, 40 cases (87%) had non-neoplastic tumours and six cases (13%) had neoplastic tumours. Out of 40 non-neoplastic tumours, 25 camels with hepatic hydatidosis constituted 54.3% of all hepatic tumours in the examined livers. Chronic hepatitis with hyperplasia or granulomatous lesions (n = 9, 19.5%), abscesses (n = 5, 10.8%) and hepatic lipomatosis (n = 1, 2.2%) were among the other non-neoplastic tumours. Cavernous haemangioma (n = 2, 4.4%), hepatocellular carcinoma (n = 2, 4.4%), cholangiocarcinoma (n = 1, 2.2%) and leiomyoma (n = 1, 2.2%), were among the neoplastic lesions that were noted. In conclusion, the majority of the one-humped camels' hepatic tumours were non-neoplastic. However, there were also reports of neoplastic liver tumours, such as cavernous haemangioma, cholangiocarcinoma, hepatocellular carcinoma and leiomyoma. Lipogranuloma and hepatic lipomatosis in camels have been identified histologically.

Key words: Camel, carcinoma, hepatic tumours, liver

Camels have a significant impact on a number of areas, including the preservation of ecosystems, biodiversity, food security, economic growth, adaptation to climate change and cultural and social aspects (Abu-Seida *et al,* 2024).

A significant amount of camel production is lost due to camel liver disorders, which also result in the condemnation of many livers in slaughterhouses. Although, camel liver can be affected by many different diseases, the most common causes are toxic substances, infectious diseases, parasitic hepatitis, fatty liver and tumours (Belina *et al,* 2015).

Several hepatic tumours have been recorded in camels including osteolipomatous metaplasia (Al-Sadi, 1994), liver abscess (Aljameel *et al,* 2014), benign mesenchymal hepatic tumours like lipoma, cavernous haemangioma and leiomyoma (El-Mahdy *et al,* 1997; Rezaie *et al,* 2015), as well as hydatidosis (Shoulah *et al,* 2023; Tharwat *et al,* 2023). However, the most common neoplastic tumour recorded is cholongiocarcinoma (Birincioğlu *et al,* 2008).

In Egypt, a recent study revealed unusual multiple primary hepatic tumours (prevalence 7/988, 0.7%) in dromedary camels. These tumours included one case each of cholangiocarcinoma-leiomyosarcoma, haemangiosarcoma-cholangiocarcinoma-leiomyoma, myelolipoma-osseous metaplasia, lymphosarcoma and 3 cases of leiomyomas (Elmaghraby *et al,* 2023).

Camelids are understudied in scientific studies (Abu-Seida *et al,* 2024). Moreover, neoplasia has been infrequently reported in Old World camelids (Ibrahim *et al,* 2023; Zabady *et al,* 2024). Therefore, this study described the morphological classification of hepatic tumours in 46 dromedary camels in Egypt.

Materials and Methods

This study was carried out on 46 livers obtained from camels that had been slaughtered for human consumption over 3 years in the main Cairo abattoirs (El- Basatin, El- warak and Kerdasa Abattoirs). These livers were thoroughly inspected and any tumour lesion was subjected to thorough

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gross and histological examination. Specimens from the hepatic tumours were preserved in 10% neutral buffered formalin for histopathology examination. The specimens were processed by paraffin embedding technique, sectioned at 4µ thickness and stained by the routine H&E stain and some histochemical stains according to Bancroft *et al* (2008). Special stains like Ziehl Nilsen and PAS were used to confirm granulomas as well as Van Giesson's stain was applied to confirm hepatic leiomyoma in the examined camels.

Results and Discussion

The recorded non-neoplastic and neoplastic tumours in the examined livers are shown in table 1. Out of 46 hepatic tumours, 40 cases, representing 87% of the total recorded hepatic tumours, had non-neoplastic tumours. However, neoplastic tumours were recorded in 6 cases representing 13% of the total recorded hepatic tumours.

Histopathology findings

Non-neoplastic tumours

In the present study, the most common lesion was hepatic hydatidosis (n=25 cases, 54.3%). Two cases of hydatidosis were associated with neoplastic changes like leiomyoma and cholangiocarcinoma.

Gross examination of hepatic hydatidosis revealed single or multiple cysts superficially or embedded within the hepatic parenchyma. They were unilocular cysts, reaching diameter 3-8 cm and irregular in shape with corrugated surface. It contained little amount of fluid with opaque or transparent capsule. In some cases, calcification resulted in gritting sound on cut section. Histological examination revealed that the hepatic tissue being replaced by cysts that appeared as thick eosinophilic lamellate layer surrounded by mononuclear cells, mostly of lymphocytic type and fibrous connective tissue capsule infiltrated by lymphocytes, macrophages, eosinophils and few plasma cells (Fig 1).

Liver abscesses were recorded in 5 cases. Gross examination revealed the presence of multiple subcapsular nodules elevated beyond the capsular surface of different size and surrounded by fibrous capsule. On cut section, large amount of creamy exudates discharged. Histological examination showed the presence of eosinophilic central necrotic area containing cellular debris with foci of mineralisation surrounded by pyogenic membrane (Fig 2).

Chronic hepatitis was recorded in 9 cases. It showed hepatic fibrosis with prominent biliary hyperplasia (n=6 cases) or granulomatous reaction (n=3 cases). In case of fibrosis, the fibrous connective tissue proliferation began mainly from the portal triads and became insinuated between hepatic lobules or pericellular with bile ductules hyperplasia and mononuclear cells infiltration (Fig 3).

Chronic hepatitis with granulomatous reaction was distinguished in two forms; the first one incited around old hydatid cyst with calcified centre forming calcosphere. Around this centre, there were mononuclear cells and giant cells aggregations (Fig 4). The second one was an eosinophilic granuloma formed of central area of necrosis containing living and died eosinophils surrounded by mononuclear cells, giant cells and eosinophils. Both types of granulomas were negative for Ziehl Nilsen and PAS stains.

One case showed characteristic granulomatous reaction called "lipogranuloma". Grossly, the liver appeared enlarged, pale yellowish in colour. There were several whitish nodules embedded in the parenchyma. Histologically, the liver showed diffuse fatty change. All the hepatocytes appeared vacuolated with fatty cyst formation. The hepatic fatty degeneration incited a granulomatous reaction began with small group of degenerated hepatocytes and appeared vacuolated with pyknotic nuclei and surrounded by macrophages and lymphocytes. A large granuloma formed of central necrosis with foamy centre (Fig 5A). It was surrounded by macrophages, a giant cell, eosinophils and lymphocytes (Fig 5B).

A case of hepatic lipomatosis was detected as a large separated mass over the liver (Fig 6). It composed of 3 compartments connected with each other by connective tissue strands. On cut section, the mass appeared whitish chalky and friable with fibrous tissue in between. Histologically, there was adipose tissue with large areas of necrosis and calcification. Mononuclear cells and foreign body giant cells were infiltrated (Fig 6).

Neoplastic tumours

The neoplastic tumours were reported in 6 cases representing 13% of the total examined livers as shown in table 1.

There were two cases of cavernous haemangiomas among the recorded benign tumours of the liver. Upon gross examination of the hepatic haemangiomas, a single, ovoid, dark brown mass

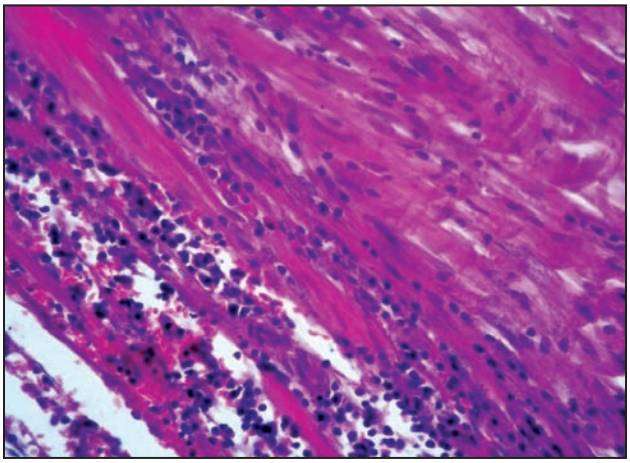

Fig 1. Photomicrograph from liver hydatidosis showing the fibrous capsule of the cyst infiltrated by lymphocytes, macrophages and eosinophils (H & E, X 400).

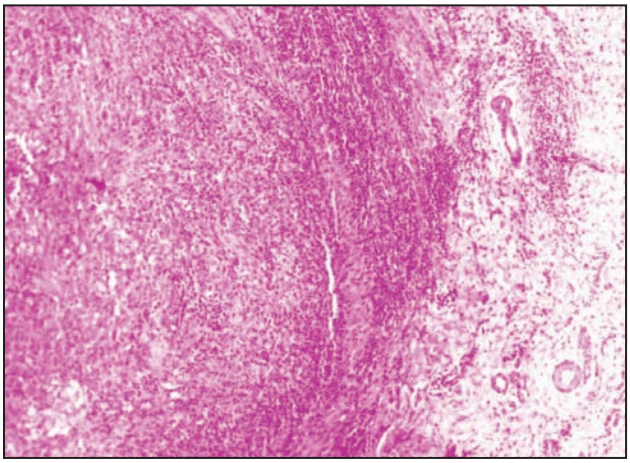

Fig 2. Photomicrograph of a liver abscess showing central liquefactive necrosis and surrounded by pyogenic membrane (H & E, X 100).

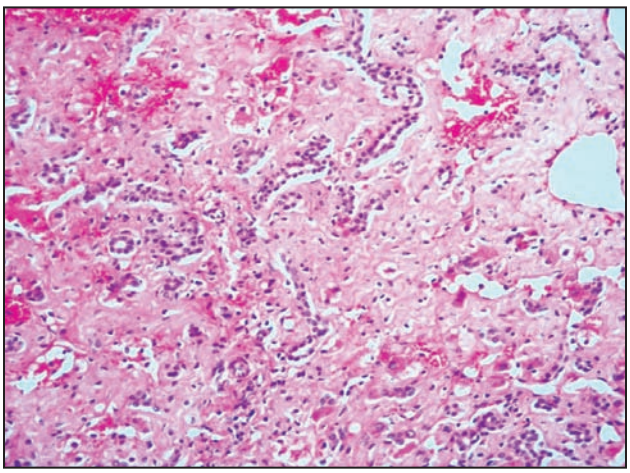

Fig 3. Photomicrograph of biliary hyperplasia showing biliary epithelial proliferation arranged in acini, tubules or branched tubules and in solid masses (H & E, X 400).

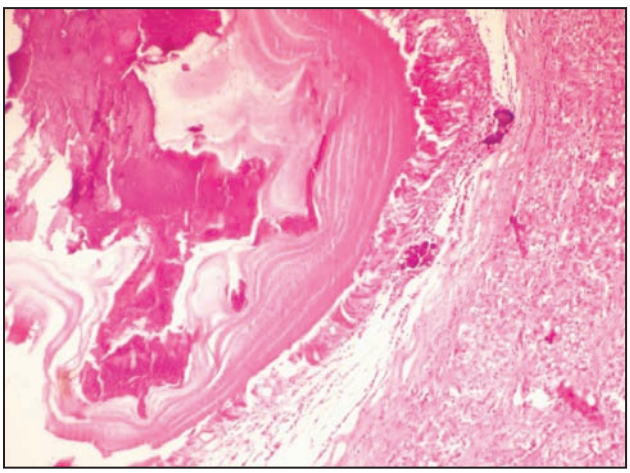

Fig 4. Photomicrograph of chronic hepatitis showing calcified hydatid cyst forming calcosphere. There were mononuclear cells and giant cells aggregation around the centre (H & E, X 200).

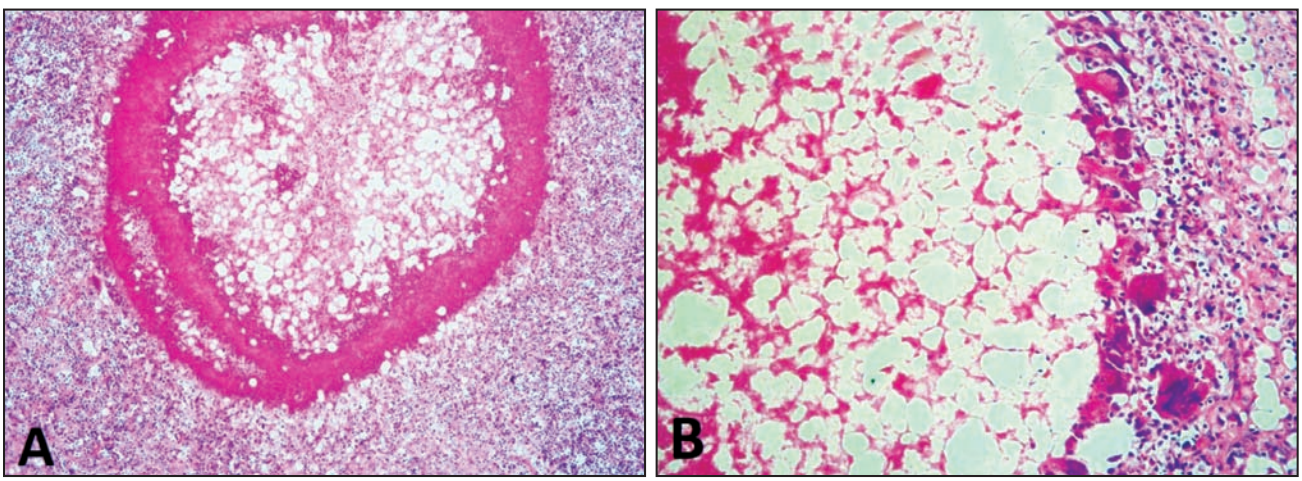

Fig 5. (A) Photomicrograph of lipogranuloma in the liver showing a large granulome formed of central necrosis with foamy centre (H & E, X 100). (B) Photomicrograph of lipogranuloma in the liver showing foamy centre surrounded by macrophages, giant cells, eosinophils and lymphocytes (H & E, X 400).

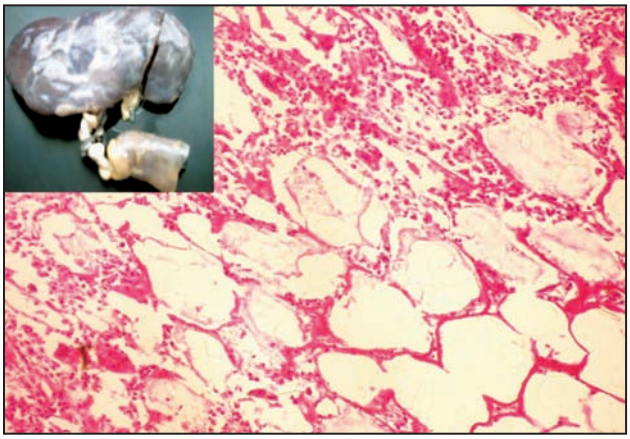

Fig 6. Small box: Photomacrograph of hepatic lipomatosis showing large, hard and separated mass over the liver. Photomicrograph of hepatic lipomatosis showing adipose tissue infiltrated with mononuclear cells and foreign body giant cells infiltration (H & E, X 200).

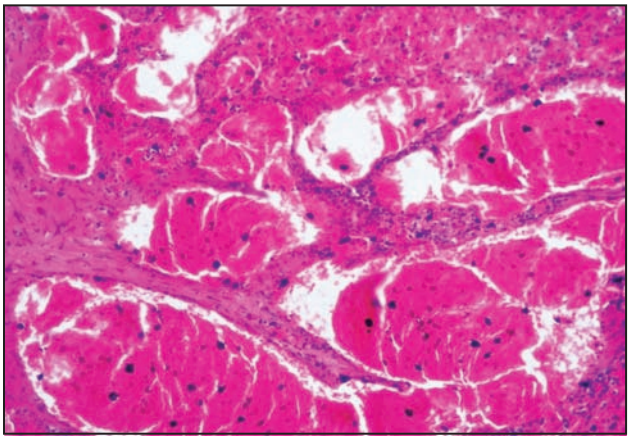

Fig 7. Photomicrograph from hepatic cavernous haemangioma. The tumour showing multiple thick-walled channels lined by flat endothelial cells and filled with blood (H & E, X 400).

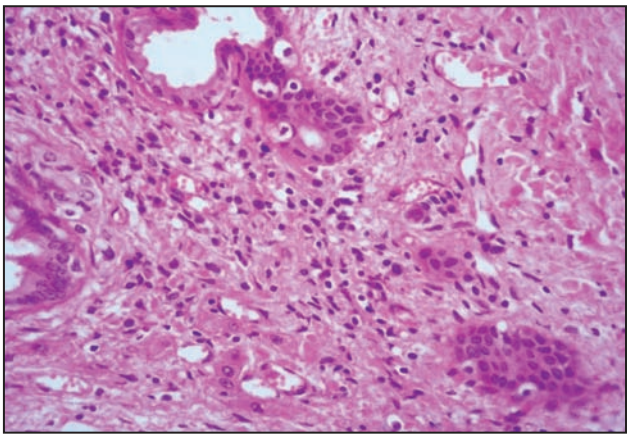

Fig 8. Photomicrograph from cholangiocarcinoma showing neoplastic cells invading the surrounding connective tissue stroma (H & E, X 400)

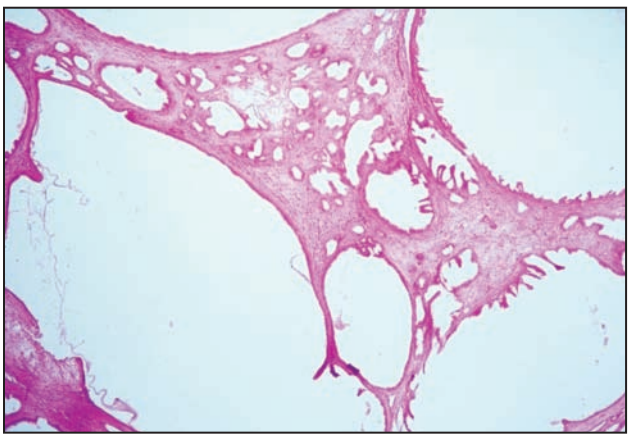

Fig 9. Photomicrograph of cystic cholangiocarcinoma showing cysts of variable sizes with papillary projections into the lumen (H & E, X 100).

was visible. Its diameter varied from 4 to 5 cm and its consistency was semi-hard. It was positioned above the liver's surface. Blood seeped from the slashed ends of the section. The histological features included numerous blood-filled channels that were bordered by flat endothelial cells of different sizes (Fig 7). Hemosiderin pigment was also observed in conjunction with a sizable area of bleeding.

Table 1. Types of non-neoplastic and neoplastic hepatic tumours in the examined camels (n=46).

	Hepatic tumours in camels	No	%
Non-neoplastic hepatic tumours	Hydatid cyst	25	54.3
	Chronic hepatitis with hyperplasia or granulomatous reaction	9	19.5
	Abscess	5	10.8
	Lipomatosis	1	2.2
Neoplastic hepatic tumours	Haemangioma	2	4.4
	Cholangiocarcinoma	2	4.4
	Hepatocellular carcinoma	1	2.2
	Leiomyoma	1	2.2
Total		46	100

The malignant hepatic tumours comprise 2 types, Cholangiocarcinoma and hepatocellular carcinoma. Cholangiocarcinoma was recorded in 2 cases. The liver was grossly enlarged, solid and showed several nodules deep in the parenchyma and on the serosal surface, all of which were grayish white and centrally depressed. According to histology, the tumour was made up of acini, which have varying-sized lumens and sometimes form papillae. With numerous mitotic figures and enlarged hyperchromatic nuclei, the neoplastic cells displayed

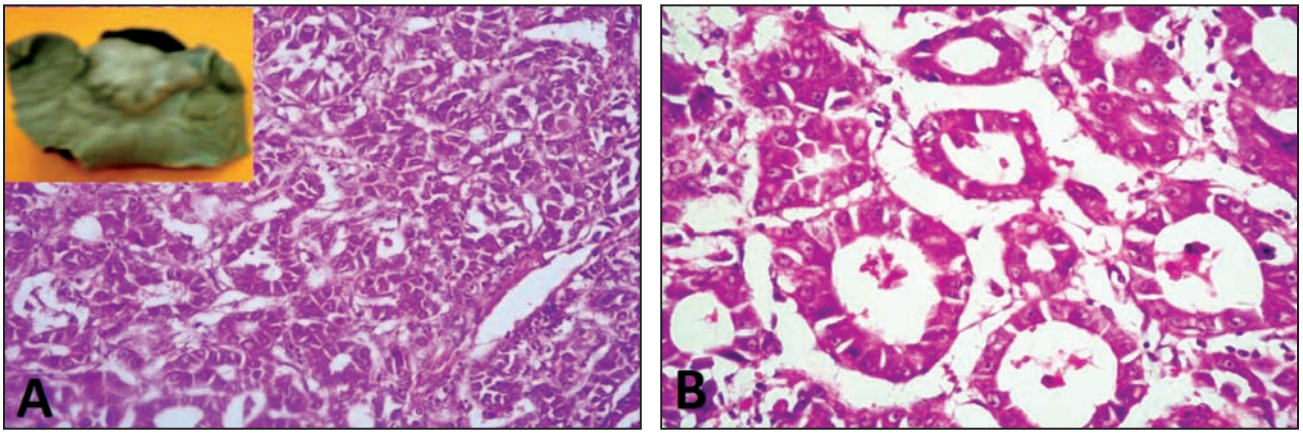

Fig 10. Small box: Photomacrograph of hepatocellular carcinoma showing multiple nodular overgrowths elevated on the hepatic surface. (A)The hepatocellular carcinoma photomicrograph. While some tumour cells formed irregular clusters, the majority of the tumour cells were grouped in acini and trabeculae (H & E, X 200). (B) Hepatocellular carcinoma: photomicrograph displaying significant pleomorphism. The nuclei showed vesicular appearance, hyperchromaticity and enlargement, with chromatin margination containing one or more conspicuous nucleoli (H & E, X 400).

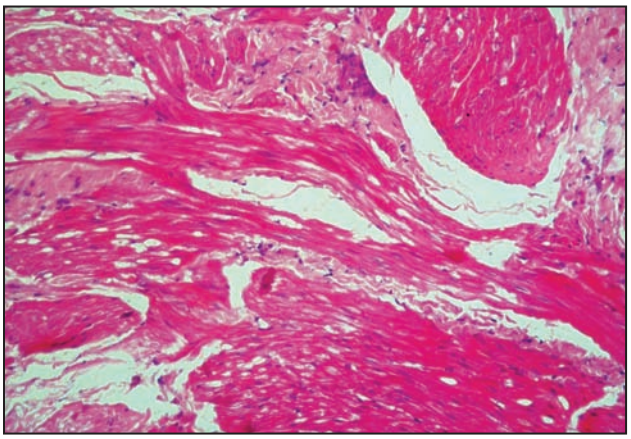

Fig 11. Photomicrograph from leiomyoma in liver. The tumour showing interlacing fibres and strands of spindle shaped smooth muscle cells (H & E, X 400).

remarkable pleomorphism. A portion of the cancerous cells were grouped together without lumens. Typically, fibrous connective tissues infiltrated with mononuclear cells separates the neoplasm's epithelial components (Fig 8). The second case demonstrated cystic cholangiocarcinoma, which was characterised by variable-volume cysts bordered with one to several layers of malignant biliary epithelium (Fig 9).

Hepatocellular carcinoma was recorded in one case. In terms of gross anatomy, the liver displayed a massive overgrowth with an uneven form that was partially immersed in the parenchyma and partially elevated on the hepatic surface (Fig 10). It was pale brown and the overgrowth on the area that had been sliced had the liver's texture. Different histological patterns were often present in the tumour. According to Fig 10A, the 3 main diagnostic groups were solid, adenoid and trabecular. The most prevalent histological type of the tumour, with the neoplastic cells arranged in plates of varying thickness, was called the trabecular pattern. The acini's lumens varied in size in the adenoid form of the tumour and some of them included proteinaceous material. Malignancy criteria were prevalent. A thin capsule of connective tissue enclosed the tumour (Fig 10B).

The leiomyoma case manifested as bounded regions that were firm and pale pink. Under histological examination, the fibres were arranged in bundles that ran in different directions; some showed a circular arrangement, while others showed longitudinal and oblique directions. The examination revealed interlacing fibres and strands of spindle-shaped smooth muscle fibres with an elliptically shaped nucleus (Fig 11). Van Giesson's stain verified the tumour.

Hepatic tumours in dromedary camels are rarely recorded in the veterinary literature (El-Mahdy *et al,* 1997; Birincioğlu *et al,* 2008; Klopfleisch *et al,* 2009; Elmaghraby *et al,* 2023). Therefore, this study recorded the common hepatic tumours in 46 one-humped camels. Nevertheless, there are numerous reports of neoplasia in South American camelids, including lymphoma (Cebra *et al,* 1995; Irwin, 2001; Sartin *et al,* 2004), oral neoplasia (McCauley *et al,* 2000; Step *et al,* 2003), malignant gastrointestinal neoplasia (Sartin *et al,* 1997), congenital hepatic neoplasia (Watt *et al,* 2001), cutaneous and mucocutaneous neoplasia (Rogers *et al,* 1997; Schulman *et al,* 2003), pulmonary neoplasia (Ramos-Vara and Miller, 2002; Ramos-Vara *et al,* 2004) and intraocular neoplasia (Hendrix *et al,* 2000).

Regarding hepatic non-neoplastic tumours, the incidence of such lesions was higher than the neoplastic one. Similar finding was previously reported by Al-Ani *et al* (1998).

Hepatic hydatidosis was the most common non-neoplastic tumour recorded in the present study. Similar result was reported in several studies (Al-Ani *et al,* 1998; Lotfi *et al,* 1994).

The second most common hepatic non-neoplastic tumour was chronic hepatitis which was reported in 9 cases. Such lesions were mainly associated with biliary hyperplasia. The hyperplastic biliary change was attributed to the blockage of large bile duct or to the effect of unidentified hepatotoxic agent (Hamir and Smith, 2002).

In the present study, hepatic lipomatosis was recorded in one case. This finding was in close resemblance to that described in cattle around the intestinal wall (Aydin and Gulbahar, 1995) and in llama around the liver (Klopfleisch *et al,* 2009).

Interestingly, hepatic lipogranuloma was recorded, for the first time, in one of the examined livers. Nevertheless, lipogranuloma was recorded in human with hepatitis C and fatty liver disease (Zhu *et al,* 2010) and in dogs with portosystemic shunt (Isobe *et al,* 2008).

Although, cavernous haemangioma is considered a hamartoma, it was diagnosed in two cases in our study. While cavernous haemangioma was reported previously in a higher incidence than that recorded in this study (El-Mahdy *et al,* 1997). In cows, the incidence of cavernous haemangioma was higher and reached to 3% (Betini and Marcato, 1991).

In the current study, cholangiocarcinoma was detected in two cases. The tumour was composed of cells that retained a resemblance to biliary epithelium. Similar findings were recorded in an earlier study (Cullen and Popp, 2002).

Hepatocellular carcinoma was recorded in one liver. To the best of our knowledge, no previous record of such tumour was reported in camels.

Among the neoplastic tumours detected in this study, hepatic leiomyoma that was recognised in only one case. In contrast, higher incidence of hepatic leiomyoma was recorded in a previous study (El-Mahdy *et al,* 1997). This could be attributed to the difference in number of examined livers.

In conclusion, the majority of the one-humped camels' hepatic tumours were non-neoplastic. However, there were also reports of neoplastic liver tumours, such as cavernous haemangioma, cholangiocarcinoma, hepatocellular carcinoma and leiomyoma. It's interesting to note that the majority of hepatic neoplasms diagnosed in cases of hepatic hydatidosis may provide insight into how long-term irritation affects the development of neoplasms in camels.

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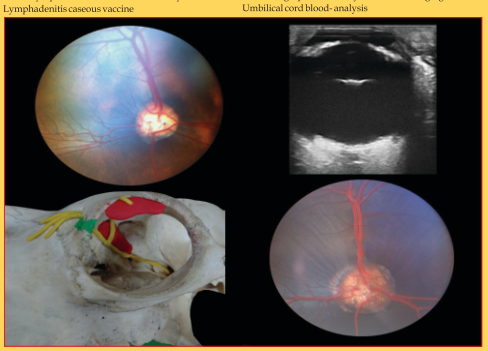

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email: tkcamelvet@yahoo.com

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THE PRESENCE OF AQUAPORIN 9 IN THE VAS DEFERENS AND PROSTATE GLAND OF CAMELS (*Camelus dromedarius*) DURING AND AFTER RUTTING SEASON

Abdelrahman MA Elseory[1,2]

[1]Department of Anatomy, College of Veterinary Medicine, King Faisal University, Saudi Arabia, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia

[2]Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum. PO Box: 32, Postal code: 13314, Shambat, Khartoum North, Sudan

ABSTRACT

Aquaporins (AQPs) are small intrinsic membrane proteins found in many cell types of the male genital system that are involved in fluid transport. These proteins are required to provide the optimal luminal environment for sperm production, maturation, preservation and immigration. Aquaporin 9 (AQP9) is expressed in the vas deferens and prostate gland, among other parts of the male reproductive system in mammals and it permits water to pass through the epithelium rapidly. The current study employed immunohistochemistry to elucidate the expression of AQP9 in the vas deferens (initial, middle and ampullary parts) and prostate gland (corpus and disseminated parts) in dromedary camels over the year's rutting and non-rutting seasons. The outcomes showed that the lining epithelium and luminal spermatozoon of the vas deferens expressed AQP9 protein moderately to weak in the beginning and middle of the rutting season. This expression peaked at the end of the season and continued through the first period of the non-rutting season. The distribution showed erratic patterns in the middle months and ended with a mild reaction to APQ9 antibodies in September. In the prostate gland, AQP9 protein fluctuated relatively little over the year. In conclusion, AQP9-mediated transmembrane water and neutral solute transport is a vital physiological pathway for sperm immigration in the dromedary camel's vas deferens. Also, a low protein expression level in the prostate gland can mean that the cells there are normal.

Key words: Aquaporin 9, distribution, Dromedary camel, prostate gland, vas deferens

Once sperm exit the seminiferous tubules, they travel through the excurrent ducts (efferent ducts, epididymis and vas deferens), where the luminal fluid composition changes gradually and lumen concentration rises noticeably (Jones and Murdoch, 1996; Mahmud *et al,* 2015). The epithelium of the prostate gland produces a large amount of the prostatic fluid. Sperm receives nourishment and protection from this fluid, which supports their motility, viability and an inherent determinant of male fertility (Verze *et al,* 2016). Comparably, the shape of the accessory sex glands with other factors like sexual behaviour, desire and environmental variables was connected with the seasonal variations in camel semen quality (Al-Bulushi *et al,* 2019).

The aquaporins (AQPs) family of vital transmembrane proteins controls cell movement and proliferation, the balance of water in the body, the production of exocrine fluid, the entry of nutrients and other valuable molecules into cells and the elimination of metabolic waste products (Shivaraj *et al,* 2017; Meli *et al,* 2018; Azad *et al,* 2021; Ribeiro *et al,* 2021). Several AQPs in the male reproductive system may be necessary for regular reproduction. They may be biomarkers for sperm freezability and fertility (Yeste *et al,* 2017; Calamita *et al,* 2001; Althnaian, 2023; Elseory, 2024).

AQP9 is a prominent apical route of transmembrane fluxes of water and other solutes called aquaglyceroporins channel expressed in the vas deferens, efferent ducts and epididymis, among other parts of the male reproductive system (Tsukaguchi *et al,* 1998; Matsuzaki *et al,* 2002; Domeniconi *et al,* 2007).

Numerous investigations conducted over the last ten years have documented the expression of AQP9y in the male genital system of many species, mainly in the testis and epididymis (Schimming

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et al, 2015; Schimming *et al,* 2021; Mohamed *et al,* 2022; Martinez-Madrid *et al,* 2023; Oberska *et al,* 2024). However, the location of this protein in the vas deferens and male accessory glands has not been well studied (Pastor-Soler *et al,* 2001; Domeniconi *et al,* 2007; Jian-bo *et al,* 2008).

There is a paucity of information on AQP9's expression in the male reproductive system of camels. Our aim is to precisely locate AQP9 in various regions of the vas deferens and prostate gland of dromedary camels during the rutting season and non-rutting seasons using immunohistochemistry (IHC).

Materials and Methods

Sample collection

King Faisal University's ethical committee accepted the stringent animal protocol that was followed for every step involving animal samples. Samples were taken every two months for a year from eighteen mature, healthy local bread dromedary camels (aged 4≥ years old or older) at the Al Omran slaughterhouse in Al-Ahsa, Saudi Arabia. Tissue samples were obtained from the initial, body and ampulla of the vas deferens and the prostate gland (corpus and disseminated parts). For the IHC process, samples were stored in 10% buffered formalin.

IHC method

Tissue samples were fixed in formalin, dried in graded ethanol, washed in xylene and embedded in paraffin wax. Sections of 5 mμ were cut using a rotary microtome and put on Superfrost slides. Slides were dewaxed and rehydrated before being stained using the avidin-biotin-peroxidase complex technique (Adeghate *et al,* 2001). Using 0.01M PBS (pH 7.4), antigen retrieval was done in a microwave oven for fifteen minutes. The pieces were washed with PBS and chilled to 25°C. Endogenous peroxidase was inhibited for half an hour with 3% hydrogen peroxide. Goat serum (10%) was utilised for 20 minutes following three rounds of washing in PBS to prevent non-specific reactions. The material was incubated in a wet chamber for the whole night after applying the primary antibody, polyclonal rabbit anti-AQP9 (Abcam, dilution 1:200, Cambridge, Cambridgeshire, UK). Biotin-labeled secondary antibodies and avidin-horseradish peroxidase (HRP) were applied to the sections. Dibutyl phthalate polystyrene xylene (DAB) was used to determine the positive staining. Haematoxylin stain was used for section counter-staining. Except for omitting the primary antibody, the negative control sections adhere to the same methodology. Immunohistological examinations were performed and slides were photographed using light microscopy.

Results

Table 1 and Fig 1 display the strength of AQP9 in the different parts of the vas deferens and prostate gland during the rutting and non-rutting seasons.

Table 1. Showing the immunoreactive distribution of AQP9 in dromedary camels' vas deferens and prostate gland regions during the rutting and non-rutting seasons.

Part / Month	October	December	February	April	June	August
DI	++	+	+++	+++	+	++
DM	+	+	+++	++	+++	+
DA	++	+	+++	+++	++	++
PC	+	+	+	+	+	+
PD	++	+	++	+	++	++

DI, initial ductus deferens; DM, middle ductus deferens; DA, ampullary ductus deferens; PC, corpus prostate; PD, disseminated prostate; +, weak reaction; ++, moderate reaction; +++, strong reaction.

The vas deferens

Throughout the year, the different parts of the camel's vas deferens expressed AQP9 in diverse ways (Fig 1A).

Early in the rutting season (October), there was a range of moderate to mild reactivity to AQP9 antibodies. The lining epithelium of the initial and ampullary parts of the vas deferens was shown to be moderately immunoreactive. Meanwhile, the epithelial cells and luminal spermatozoon in the middle part showed weak expression (Fig 2).

AQPP9 immunolocalisation in the epithelial cells along the vas deferens and the luminal sperm demonstrated a mild reactivity in December, the middle of this season (Fig 2). In contrast, the reaction was strong in February, near the end of the meeting period (Fig 2).

During the non-rutting season, which spanned from April to September, AQP9 was expressed in all parts of the vas deferens, with its reactivity showing significant variations. In April, the lining epithelium cells in the initial and ampullary parts exhibited strong immunoreactivity to AQP9 antibodies. The reaction in the epithelial cells of the middle part was moderate at that time (Fig 3). The reaction fluctuated in June, the midpoint of this season, with a faint reaction in the initial part, a strong reaction in the

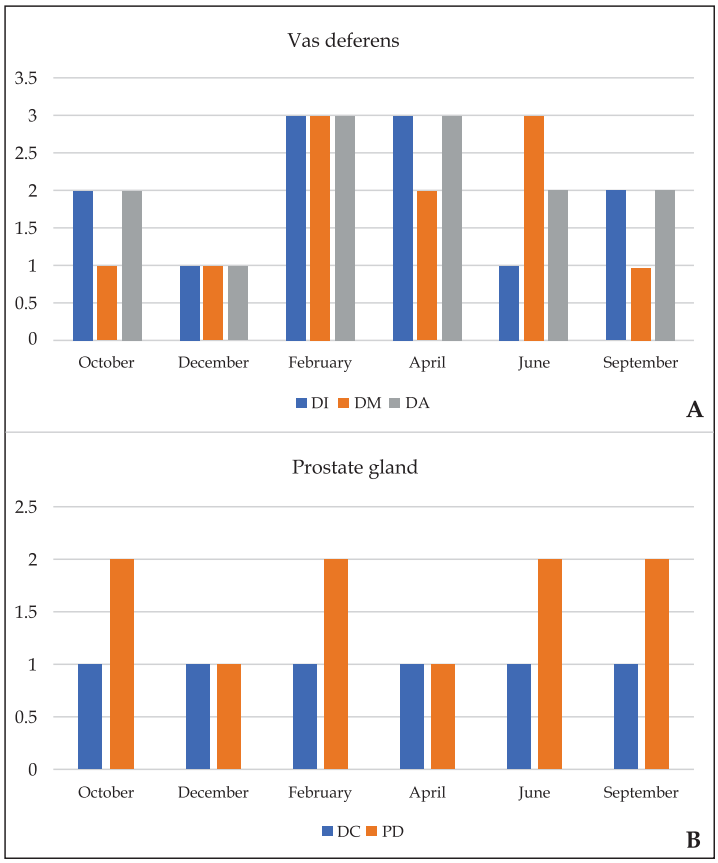


Fig 1. Showing the distribution of AQP9 in the different parts of the dromedary camel's vas deferens (A) and prostate gland (B) throughout the rutting and non-rutting seasons. VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; PC, corpus prostate; PD, disseminated prostate; 1, weak reaction; 2, moderate reaction; 3, strong reaction.

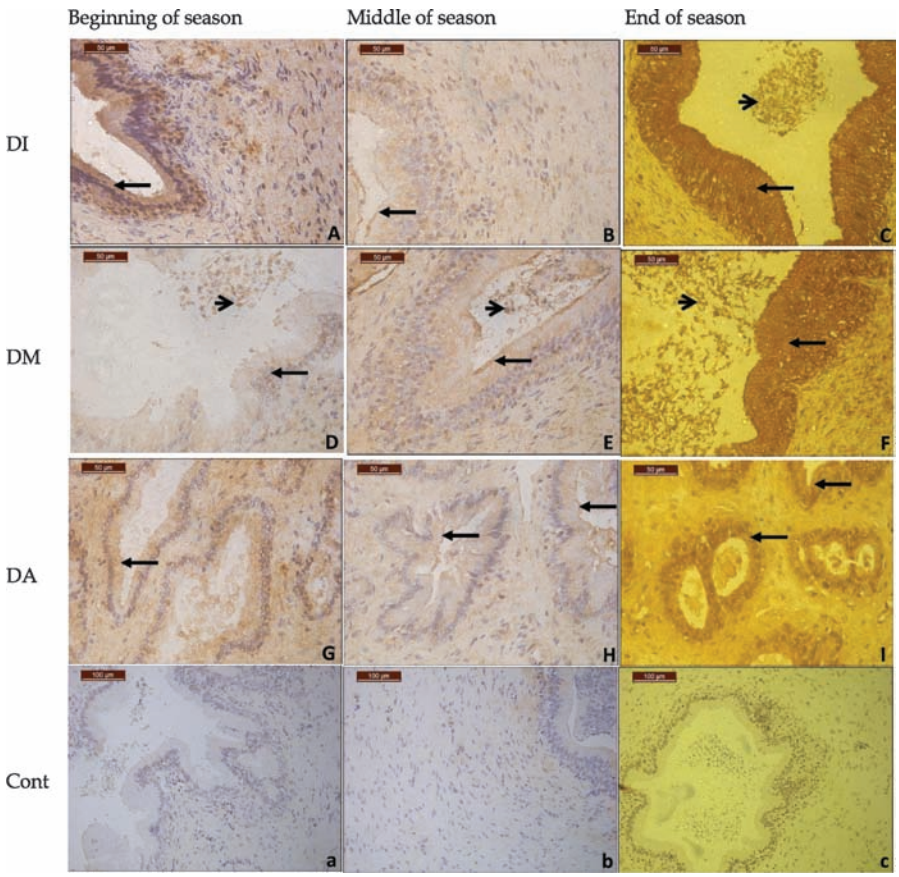


Fig 2. AQP9 immunolocalisation in the different parts of the camel's vas deferens during the rutting season. The reaction was observed variously in the epithelial cells (arrow). The response to AQP9 antibodies at the beginning of this season was between moderate and weak reactions (A, D, G). It reacted moderately in the middle of this period (B, E, H). Meanwhile, AQP9 immunoreaction at the end of the season clarified an extreme reaction (C, F, I). The luminal sperm shows a similar reaction (arrowhead). X40. (a), (b) and (c) negative control 20X. VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; Cont, negative control.

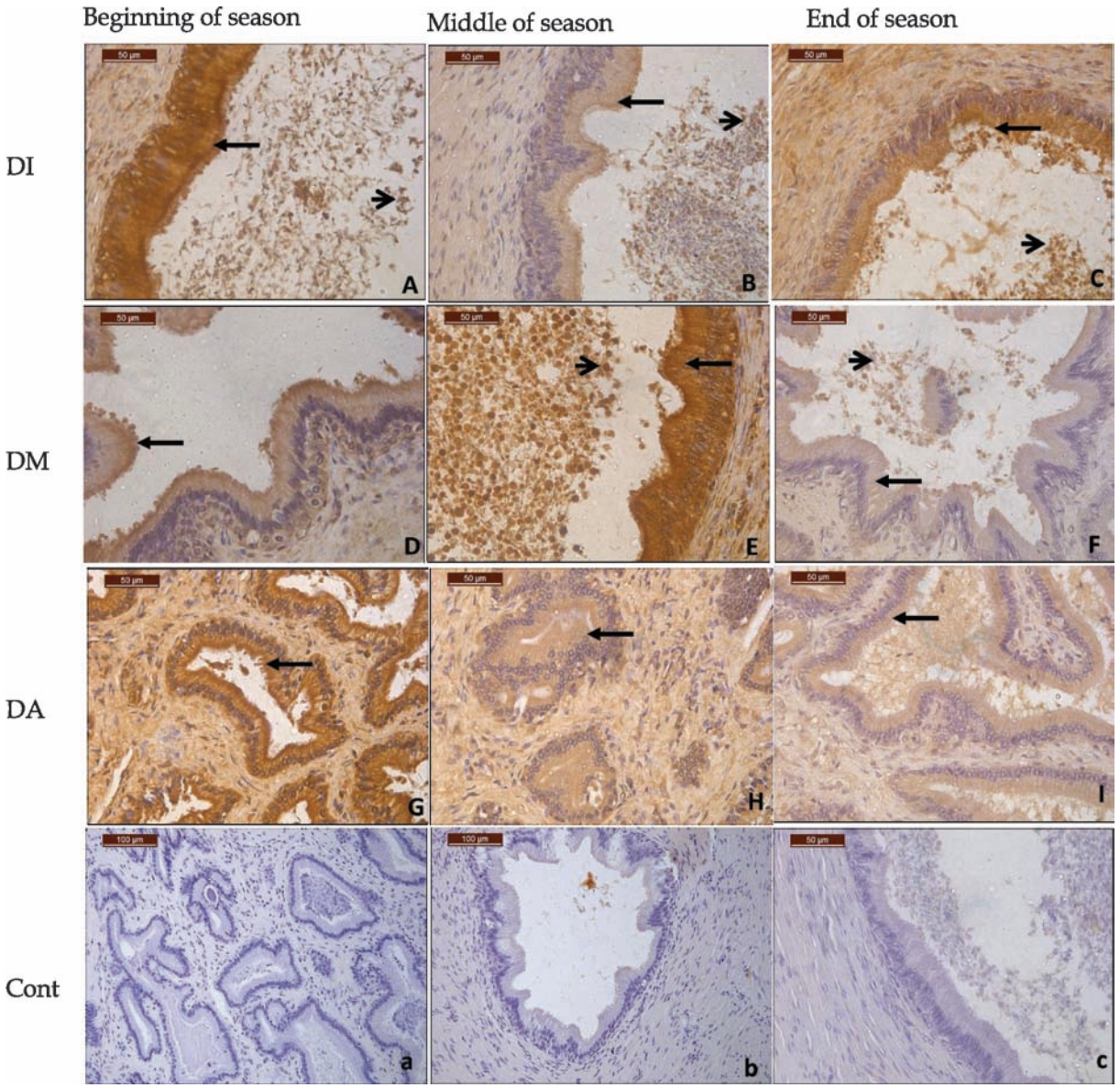


Fig 3. AQP9 immunolocalisation in the different parts of the vas deferens throughout the non-rutting season of the dromedary camel. Different reactions were seen in the epithelial cells (arrow). At the start of this season, the AQP9 immunoreactivity is generally strong (A, D, G). In the middle of this season, its reaction is inconsistent in the different parts (B, E, H). A moderate reaction is observed at the end of the season (C, F, I). A similar response is seen in the luminal sperm (arrowhead). X40. (a), (b) and (c) negative control 20X. VI, initial vas deferens; VM, middle vas deferens; VA, ampullary vas deferens; Cont, negative control.

second part and a moderate reaction at the ampulla (Fig 3). In August, when the season was almost over, the epithelium of the organ revealed a mild reactivity (Fig 3).

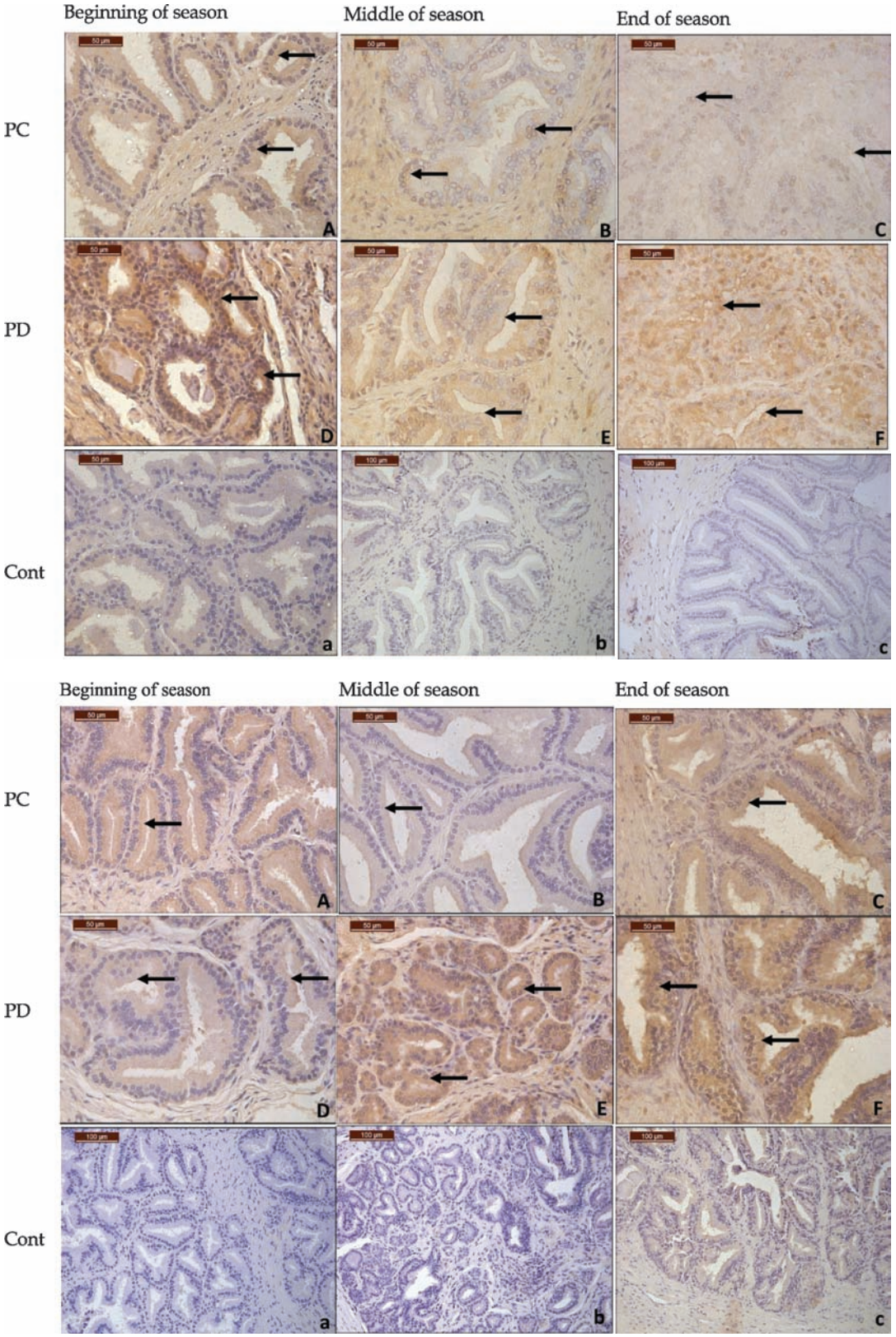
The prostate gland

In dromedary camels, AQP9 was slightly different throughout the rutting and non-rutting seasons in the corpus and disseminated parts of the prostate gland (Fig 1B). When exposed to AQP9 antibodies, the epithelial cells in both parts of the gland had mild to moderate immunoreaction (Figs 4, 5).

Discussion

The epithelial cells of the male reproductive tract carry fluid and electrolytes, drastically altering the luminal environment where spermatozoa form, store up and are passed. These activities occur in different parts of the male reproductive system (Clulow *et al*, 1998). According to Pastor-Soler *et al* (2001), the AQP9 is a water channel that permits the passage of neutral solutes in addition to water.

The present study is the demonstration of the seasonal variations of AQP9's dispersion in the



Figs 4 & 5. AQP9 immunolocalisation in the prostate gland of dromedary camels throughout both the rutting and non-rutting seasons in the corpus and disseminated parts of the gland. The epithelial cells (arrow) in both parts exhibit weak to moderate AQP9 positivity. The expression of the protein in the gland does not significantly change throughout the year. X40. (a), (b) and (c) negative control 20X. Pc, corpus prostate; PD, disseminated prostate; Cont, negative control.

dromedary camel's vas deferens and prostate gland over one year. In the early period of the rutting season, which is reported to start in October and end in April (El-Shoukary *et al,* 2020; Tibary and El Allali, 2020), the lining epithelium of the vas deferens' initial and ampullary parts are moderately immunoreactive to AQP9 antibodies, while the middle region's luminal spermatozoon and epithelial cells are expressed weakly. All these parts showed mild reactivity throughout the middle meeting period. On the other hand, during the last period of this season, AQP9 protein clarified strong reactivity across all parts, marking the season's peak. During the non-rutting period, the vas deferens protein displayed significant immunoreactivity for AQP9 in the first three periods for the remainder of the year. The second third displayed inconsistent patterns, while the last had a modest response to antibodies.

These findings in the vas deferens of dromedary camels corroborated the discovery of AQP9 in the vas deferens of animals by Pastor-Soler *et al* (2001) in rats, Domeniconi *et al* (2007) in dogs and Oberska *et al* (2024) in bovines. Additionally, it validated an earlier theory that said the vas deferens appears to do much more than transport spermatozoa out of the epididymis; in humans, it enhances sperm survival by protecting them from complement, proteases and reactive oxygen species (Ezer and Robaire, 2002). However, in monkeys, sperm are retained until fully charged and can travel at high speeds (Horst *et al,* 1999).

Furthermore, as noted by Stevens *et al* (2000), camels exhibit regional variations in the tissular distribution and cellular-specific location of the AQP9, as well as in the structure and functioning of the vas deferens.

However, it is essential to note that the mechanism underlying the transepithelial fluid in the vas deferens remains unknown. This intriguing area requires further research and understanding, presenting an exciting opportunity for future discoveries.

In the current study, dromedary camels' AQP9 protein in the corpus and disseminated parts of the prostate gland oscillated very little during both rutting and non-rutting seasons. The detection of AQP9 in the mammalian prostate gland (Pastor-Soler *et al,* 2001; Domeniconi *et al,* 2007; Jian-bo *et al,* 2008) was in consonance to the results of this study.

The limited distribution and seasonal variations observed in camel AQP9 may be related to early findings that testosterone significantly upregulates this protein in the prostate, where its overexpression indicates problems in the gland's cells. This reaffirms the potential of AQP9 as a unique therapeutic target for various malignancies, underscoring the importance and impact of our work in this field (Chen *et al,* 2016; Bründl *et al,* 2018; Kushwaha *et al,* 2023).

Conclusion

The present study concluded that the AQP9 expression varies during the non-rutting season and increases at the end of the rutting season. It also exhibits varying responsiveness in distinct regions of the vas deferens. In contrast, the prostate gland has no significant distribution or seasonal variation. The results indicated the significance of this protein, particularly for sperm via luminal immigration in the vas deferens. Furthermore, the consistent low protein expression in the prostate gland throughout the year was a positive, healthy sign, reassuring the health of the gland.

Conflict of interest

None declared

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PHYSICOCHEMICAL CHARACTERISTICS AND MICROBIOLOGICAL QUALITY OF CAMEL MILK IN TUNISIAN ARID LANDS : A COMPARISON WITH GOAT AND SHEEP MILK

Zammouri Amal[1,2], Imen Fguiri[1], Manel Ziadi[3], Samira Arroum[1], Mohamed Dbara[1], Mohamed Hammadi[1] and Touhami Khorchani[1]

[1]Laboratory of livestock and Wild Life Institute of Arid Lands (IRA Medenine). 4119. Medenine. Tunisia
[2]University of Gabès, Faculty of Sciences of Gabès, 6072, Gabès, Tunisia
[3]Laboratory of Microbial Technology and Ecology (LETMi), National Institute of Applied Sciences and Technology (INSAT), BP 876, 1080 Tunis. Tunisia

ABSTRACT

The aim of this study was to compare the physicochemical parameters and the microbiological quality of milk samples of 3 different species in Tunisian arid lands *viz.* camel, goat and sheep. The results for milk characteristics showed significant differences among the 3 species. Camel milk was the the richest in total proteins (38,6 ±0.07 g/l), the highest ash content (8,92 g/l±0,49), the most acid pH (pH 6.40±0.03) and the lowest density (1.02±0.2), whereas sheep milk was characterised by the greatest average value dry matter (151,17±2,60 g/l) and the highest fat content (52±0,55 g/l). Likewise, the microbial quality of camel milk was higher than that of sheep and goat milk based on total counts of coliform (TCC), yeast and molds (Y/M) and lactic bacteria (LAB). Although, the microbial analysis of total mesophilic aerobic bacteria (FMAT) revealed an exceeds of standard criteria, suggesting that all samples may contain higher levels of microbial contaminants. To reduce this contamination, several measures must be taken.

Key words: Arid region, camel, goat, milk quality, sheep, Tunisia

In Tunisia, sheep, goats and camels dominate livestock in the arid pastoral zones of the south (Khaldi *et al,* 2022). These species are vital for providing goods and services such as milk, meat, wool and transportation, making up a key part of the agricultural sector. Raw milk from these animal species has been a subject of interest due to its unique composition and potential health benefits.

The FAO reports that the global milk production is approximately 3.15 million tons of which cow milk represents over 85% with a growing interest in consuming raw milk from other species.

For exemple, camel milk production has notably increased since 1962, growing annually by 7% to reach a worldwide production of 6.6 million tons. This growth rate surpasses that of cow milk twofolds and that of sheep and goat milk threefold (Konuspayeva *et al,* 2022). The global demand for camel milk could be attributed to its diverse bioactive compounds, flavour and potential health benefits (Seifu, 2022; Mahamat Ahmat *et al,* 2023).

Indeed, the production of milk worldwide is influenced by various livestock that plays a crucial role in the livelihoods of many communities in arid regions characterised by extreme heat and limited water resources.

Numerous studies have demonstrated that a variety of variables appear to be significant in influencing the quality of these milk. The animal species (Yasmin *et al,* 2020), milking practices (Atigui *et al,* 2023), animal health and lactation stage (Chamekh *et al,* 2020; Mollica *et al,* 2021), season (Dhaoui *et al,* 2019; Mollica *et al,* 2021) and feeding practices (Mollica *et al,* 2021; Laameche *et al,* 2024) are some examples of these factors.

Physico-chemical properties such as fat, protein, lactose and mineral composition play a crucial role in determining the nutritional quality of milk. Additionally, the microbiological quality of milk is important for assessing its safety and shelf life (Atigui *et al,* 2023).

Understanding the differences and similarities among these different types of milk can provide

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valuable insights into their nutritional value and suitability for human consumption.

By comparing these properties among camel, goat and sheep milk, we can gain a better understanding of their unique characteristics and potential health benefits.

In this study, the focus is on comparing the physico-chemical properties and microbiological quality of raw camel milk with that of goat and sheep milk in Tunisian arid lands.

Materials and Methods

2.1. Biological Material

A total of 32 samples of raw milk were obtained by mixing the milk of several females. Milk was obtained from 3 species : camel milk (*Camelus dromedarius*, n=22), goat (*Capra hircus,* n =5), sheep (*Ovis aries,* n = 5). Camel and goat milk were obtained from herds belonging to the Wildlife and Livestock Laboratory, Arid lands Institute (IRA, Médenine), Tunisia while sheep milk was collected from a farm in the region of Medenine (Southeast of Tunisia).

The milk samples were collected in sterile bottles and then transferred to the laboratory under aseptic conditions.

2.2. Physical and chemical analyses

The physical characterstics of raw milk including density, pH and dornic acidity were measured immediately after arrival of samples at the laboratory. The pH was measured using a digital pH meter (model WTW 422), the dornic acidity was measured by titrimetric method as described by the AOAC International (Latimer, 2016) and the density was determined at 20°C using a lactodensimeter accompanied by a thermometer.

According to the international standard, the dry matter of raw milk was determined by loss on drying at 105°C for 3 hours (IDF, 2010) and the ash content was determined after incineration of the dry matter at 550°C until complete combustion of organic matter (AOAC, 2012). The fat content was determined through acid-butyrometric analysis (IDF, 2009) and the total protein content was determined by the Kjeldahl method (IDF, 2014).

2.3. Microbiological analysis

In order to determine the microbiological quality of raw milk, 1 ml from each sample was taken and then diluted with 9 ml of physiologique water. According to the international standard (ISO, 2020), further decimal dilutions were made from this dilution and plated on appropriate media.

Plate count agar (PCA, Merck, Germany) was used to isolate total aerobic mesophilic bacteria (TMAB). Incubation was performed at 30°C for 72 hrs (ISO, 2013). Sabouraud chloramphenicol (Pronadisa) was used to detect and enumerate yeast and molds (YM). Incubation was carried out at 25°C for 3–5 days (ISO, 2008).

Violet red bile agar (AppliChem) was used to quantitatively detect total coliformes (TCC). Seeding was done in a double layer and the samples were incubated at 30°C for 24 to 48 hours (ISO, 2006).

De Man-Rogosa-Sharpe (MRS) agar (Scharlau Chemie, S.A.) was used to detect and enumerate lactic acid bacteria (LAB).The agar plates were incubated at 30°C for 48 hours to allow the growth of LAB colonies (ISO, 2007).

2.4. Statistical analysis

Statistical analysis was conducted by comparing the averages of different parameters across species being studied. The significant differences between means were determined by one-way analysis of variance ANOVA and followed by the Tukey-Kramer test to correct the P values for multiple comparisons using GraphPad Prism 8.4.3 software package.

Results and Discussion

3.1. Physicochemical properties of raw milk

Results showed the physical characteristics of the milk samples (Table 1). Statistical analysis revealed no significant differences in pH, acidity and density among the three types of milk tested ($p>0.05$).

The pH values for the various samples ranged between 6.6 and 6.48. The pH of camel milk was lower than that of milk from other species. The average pH value of the collected goat milk was in the order of 6.6. This result was in agreement with the findings of Fguiri *et al* (2017). The pH results of camel milk (6.4) and of sheep milk (6.42) showed similair values observed by Singh *et al* (2017).

According to the litterature, milk from small ruminants has a pH range of 6.5 to 6.8 (Khaldi *et al,* 2022) while camel milk has a pH range of 6.2 to 6.5 (Seifu, 2022). The high vitamin C content and the presence of certain organic acids in camel milk contributes to its lower pH (Almoosawi and Almahdawi, 2023).

Additionally, measuring the pH and acidity of milk samples could be a critical indicator of animal

health and the hygienic quality of the milk (Gagara *et al*, 2022).

As shown in table 1, sheep milk had the highest dornic acidity values followed by goat milk and then by camel milk with values 19.58, 18.6 and 18.3, respectively. Previous studies (Elbagerma *et al,* 2014; Khaldi *et al,* 2022) have reported differences in the acidity value of goat, sheep and camel milk compared to the current research findings. However, other research have indicated comparable acidity findings. The values of titratable acidity in goat milk were in line with that reported by Otmane *et al (*2022). The titratable acidity values of sheep milk were similar to the findings of Asif (2010) and the values of titratable acidity in camel milk were similar to that reported by El-Hatmi (2015). Indeed, the acidity of milk could be influenced by various factors such as the presence of lactic acid bacteria, temperature, nature of forages and lactation stage (Alaoui *et al,* 2019; Laameche *et al,* 2024).

Table 1. Physical characteristics of camel, goats and sheeps milk in the Tunisian arid land.

Properties	Source of milk		
	Camel	Goat	Sheep
pH	6, 4± 0,3	6,6±0,6	6.48±0.3
Acidity (°D)	18 ,3±1,36	18,6±3,75	19.58±1.12
Density	1.02 ±0.2	1.03 ±0.06	1.04 ±0.05

The value of density obtained of camel milk is lower than those of milk from the other species. This value was ranged from 1.026 to 1.035, similar to previous density camel milk readings, averaging around 1.029 (Seifu, 2022). The density of milk is indeed influenced by various factors. The fat content, total solids and temperture are major factors influencing the density of milk (Parmar *et al,* 2021).

Table 2 shows the chemical composition of camel milk compared with sheep and goat milk. The results of compositional analyses revealed a significant difference among the 3 species ($P<0.001$).

Compared with goat and sheep milk, camel milk had the highest ($P < 0.001$) ash (8.92 g/l) and protein contents (38.6 g/l). The present observations are consistent with the finding of Bouhaddaoui *et al* (2019) for Moroccan camel milk and El-Hatmi (2015) for Tunisian camel milk. In contrast to these findings, Yasmin *et al* (2020) and Khaldi *et al* (2022) found that camel milk had the lowest ash and protein content compared to sheep milk and goat milk.

In this respect the diversity of ash content values in camel milk may be influenced by such facors like hydration status of the camel and the stage of lactation (Bouhaddaoui *et al,* 2019). Also, ash content in camel milk could be a good source of minerals in the human diet like sodium, chloride and calcium (Konuspayeva *et al,* 2022; Vincenzetti *et al,* 2022).

Table 2. Average chemical composition (g/l) of camel, goat and sheep milk in the Tunisian arid land.

Composition (g/l)	Source of milk		
	Camel	Goat	Sheep
Dry matter	107,17 ±1,58^{c}	143,46 ±2,17^{b}	151,17 ±2,60^{a}
Ash	8,92 ±0,49^{a}	8,76 ±0,48^{b}	7,57 ±0,97^{c}
Fat	33,6 ±0,11^{c}	41,3 ±0,75^{b}	52 ±0,55^{a}
Protein	38,6 ±0.07^{a}	24,9 ±0.17^{c}	35,85 ±4.21^{b}

The data is given as the mean±SD.
Values with different alphabetic signify a statistically significant variation between the means ($p<0.05$).

Additionally, the difference in protein content could be attributed to the genetic variation among these animal species. (Yasmin *et al,* 2020). Also, factors such as the differences in breed and geographical region, type of pasture and herd management could also influence the protein content of milk (Faye *et al,* 2010; Bouhaddaoui *et al,* 2019; Seifu, 2022).

Likewise, the protein content could affect the nutritional value and the technological suitability of milk (Seifu, 2022). The camel milk is therefore, more beneficial for human nutrition because of its higher protein content and essential amino acids compared to goat milk and sheep milk. Furthermore, the protein in camel milk is easier to digest, making it a suitable option for those with lactose intolerance or digestive issues (Swelum *et al,* 2021).

The concentration of dry matter in milk samples collected from the 3 speices reavealed that the dry matter in sheep milk (151.17 g/l) was higher than that in camel (107.17 g/l) and goat milk (143.46 g/l) at highly significant ($p<0.001$) level. The dry matter is low in camel milk as compared with the other milks goat and sheep. These results are similar to those findings reported by Yasmin *et al* (2020) and Khaldi *et al* (2022). Many other studies have shown that sheep milk has the greatest average dry matter value among all ruminant milks, especially camel milk (Bornaz *et al,* 2009; Asif, 2010; Elbagerma *et al,* 2014; El-Hatmi, 2015; Vincenzetti *et al,* 2022). Indeed, a number of factors, including breed, nutrition, lactation stage and individual genetics could affect the dry matter content of milk (Seifu, 2022). Similarly, it was shown

that the amount of water consumed by camels and the amount of solids in their milk were inversely correlated (Alaoui *et al,* 2019; Seifu, 2022).

The amount of fat content in sheep milk (52g/l) was higher ($p<0.001$) than that in the milk of other species. In previous studies, the fat content in sheep milk (Dhaoui *et al,* 2019) and in goat milk (Arroum *et al,* 2016; Ayeb *et al,* 2016; Fguiri *et al,* 2017) was found to be higher compared to finding (41.3 g/l) in the present study. However, our findings was higher to those reported by Sboui *et al* (2016) for goat milk and similair to that obtained by Khaldi *et al* (2021) for sheep milk. These variations in literature could be attributed to various factors such as breed variations, feeding practices, environmental factors, milk processing and lactation stage (Dhaoui *et al,* 2019; Chamekh *et al,* 2020; Mollica *et al,* 2021) .

Likewise, camel milk had the lower fat (33.6g/l) content than ovine and caprine milk samples. This value was in the range of the fat content values registered previously for Tunisian camel milk (Sboui *et al,* 2009; Khaldi *et al,* 2021; Hamouda *et al,* 2022). According to the literature (Bulca and Sarikoç, 2016; Seifu, 2023; Kumar *et al,* 2016), the fat in camel milk is characterised by a higher proportion of unsaturated fatty acids than milk from other species. The high content of long-chain unsaturated fatty acids (C14-C18) in camel milk could be advantageous for reducing risk factors associated with cardiovascular diseases (Karaman *et al,* 2022; Chamekh *et al,* 2023). Additionally, camel milk has a lower carotene content compared to other types of milk. Due to its low carotene concentration, camel milk is noticeably white (Swelum *et al,* 2021; Seifu, 2023).

3.2. Microbiological features

The overall microbiological quality of camel, sheep and goat raw milk were presented in table 3. Our data shows that significant differences were observed in the microbial load among the different types of milk. Except for total aerobic mesophilic flora, which surpasses the French regulatory limit on the hygiene of milk and dairy products (> 5x 10^5 CFU/ml), all of the examined samples met AFNOR (2001) criteria.

In this regard, based on the high contents of lactic acid bacteria (LAB) and the lowest count of total mesophilic aerobic bacteria (TMAB), yeasts (Y), molds (M) and total coliforms (TCC), camel milk had a better microbiological quality than goat milk and sheep milk.

The presence of high concentration of total germs in raw milk could be an indice of the poor hygienic quality and inadequate sanitation practices during milking (Atigui *et al,* 2023). Meanwhile, the presence of lactic acid bacteria (LAB) in milk contributes to the improvement of its quality (Arroum *et al,* 2023) by converting lactose into lactic acid, which act as preservatives and enhances flavour (Seifu, 2022).

The average TMAB, TCC, LAB, Y/M counts of camel, goat and sheep milk observed in the present study was lower than the values reported by Fguiri *et al* (2017). Concerning sheep milk, our findings were closer to those advanced in the literature by (Khaldi *et al,* 2022). For camel milk, it's also reported that our results are lower than cited by Alaoui *et al* (2019) and higher than the values found by Karaman *et al* (2022). Therefore, based on this study and previous research, the bacterial count in camel milk was found lower compared to the sheep and goat milk.

According to many authors (El-Hatmi, 2015; Fguiri *et al,* 2017; Alaoui *et al,* 2019; Swelum *et al,* 2021; Karaman *et al,* 2022), these results could be explained by the presence of certain components in raw camel milk, such as a high lysozyme concentration and vitamin C cotent that inhibit the growth of germs. Additionaly, its could be due to its soluble proteins (lactoferrin, lactoperoxidase and immunoglobulins) having antimicrobial properties .

Indeed, the high content of these compounds in camel milk, contributing to its lower bacterial load

Table 3. Average of various flora (cfu mL^{-1}) enumerated in raw camel, goat and sheep milk in the Tunisian arid land.

Flora cfu ml^{-1} / Source of milk	LAB	TCC	TMAB	YM
Camel milk	8,8± 0.20 10^3 c	0, 2 ± 0.12 10^2 b	6, 5 ± 0.6 10^8 b	2, 18± 0.20 10^2 c
Sheep milk	4,20 ±0.26 10^4 a	1,5 ±0.1510^2 a	6,9 ± 0.4 10^8 b	4,3 8± 0.6 10^2 b
Goat milk	3,5 8± 0.31 10^4 b	1,6 ± 0.34 10^2 a	3 ± 0.2 10^9 a	5,9 8± 0. 310^2 a

The data is given as the mean±SD.
Value with different superscript signify a statistically significant variation between the means ($p<0.05$).

compared to other milk, suggests that it could be a valuable resource for developing antimicrobial agents and fermented products. However, the relatively high total mesophilic aerobic bacteria (TMAB) in camel milk, reaching 6, 5 ± 0.6 108 UFC/ml, highlights the importance of strict hygiene measures throughout the camel milk value chain.

In this study, the investigation of physico-chemical composition and bacteriological properties of camel milk from Tunisian arid lands compared to that of local goat and sheep milk, revealed significant differences in the milk characteristics among the studied species.

The analysis of the camel milk revealed the highest concentrations of protein and ash content, while, the sheep milk showed the highest concentrations of fat and dry matter. These differences in composition between camel, sheep and goat milk have important implications for their nutritional value and technological properties. The high protein and ash content of camel milk make it a valuable raw material for the dairy industry and a nutritious food for human consumption.

On the other side, the microbial content revealed a best microbiological quality for camel milk compared to goat and sheep milk. However, the total counts of FMAT for camel milk and other types of milk were below the acceptable limits and did not conform to official standards. This indicates the necessity of applying good sanitary practices and implementing proper hygiene throughout the entire milk production process.

This comparative milk study could help in understanding the composition, nutritional profile, safety and quality of camel, goat and sheep milk. This provides a solid foundation to guide consumer choices and develop new dairy products.

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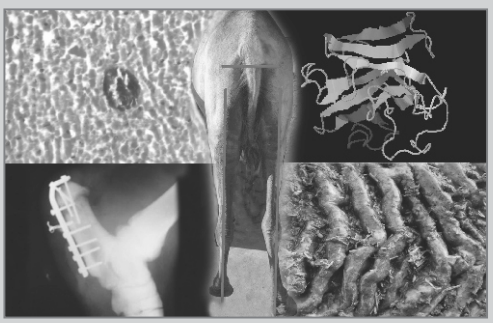

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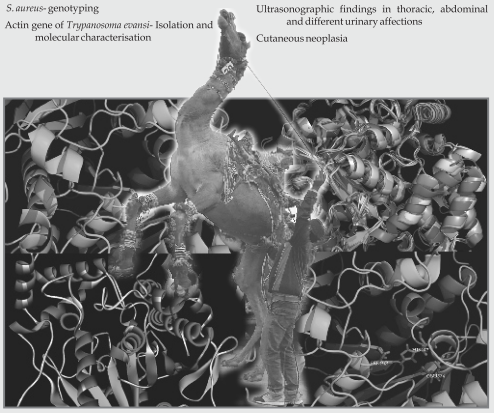

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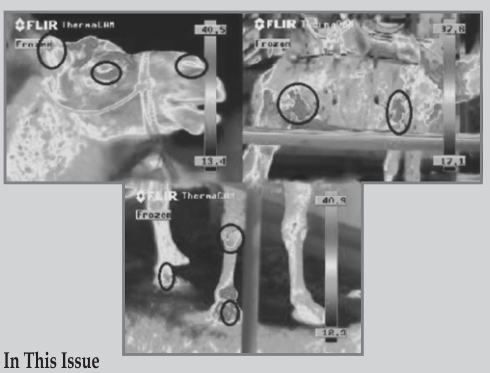

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STATUS, MORPHOMETRIC CHARACTERISTICS, MILK PRODUCTION AND QUALITY OF JALORI BREED CAMEL

S.C. Mehta, S.S. Dahiya and R.K. Sawal
ICAR-National Research Centre on Camel, Post Box-07, Bikaner 334001, Rajasthan

ABSTRACT

The Jalori breed of camel is one among the 9 registered camel breeds of the country. The breeding tract encompasses the Jalore and Sirohi districts of Rajasthan. In order to know the status of the breed, 3187 camels belonging to 188 camel breeders of 75 villages in the Jalore and Sirohi districts of Rajasthan were covered in the study. The population of Jalori camels in the breeding tract was estimated to be 7323 heads. The extent of crossbreeding was estimated to be 14.42%. The ratio of breedable male to female was estimated to be 1:18. In order to define the phenotype of the breed, the morphometric measurements were recorded for 628 adult camels, and on average the adult Jalori male and female camels measured 199.12 ± 1.73 and 199.12 ± 0.60 cm height at withers, 205.72 ± 2.12 and 207.58 ± 0.66 cm heart girth, and 155.01 ± 1.38 and 154.90 ± 0.40 cm body length, respectively. The average body weight of adult Jalori camels of 4 years of age was recorded as 452.50±24.98 kg, and that of ≥5 years was recorded as 510.67±17.71 kg. In order to record the milk production potential, 37 female camels were recorded continuously at the farmer's doorstep, and the average per day milk production from a female was recorded as 4858.52±26.671 ml and the percent fat in the milk was estimated to be 3.70±0.02. The Open Nucleus Breeding programme with its nucleus at the government research centre and associated herds with the camel owners may lead to significant improvement in the milk production potential of the animals. Continuous policy support and awareness programmes will not only help the camel owners in maintaining the Jalori camel with diverse livestock species under optimum production but will also boost their morale and bring happiness in them.

Key words: Characterisation, conservation, Jalori camel, milk production, milk quality

The nodal agency for the registration of domestic animal biodiversity in the country is the ICAR-National Bureau of Animal Genetic Resources, Karnal. The Bureau has registered 9 breeds of camel in the country *viz.* Bikaneri, Jaisalmeri, Kutchi, Mewari, Marwari, Jalori, Malvi, Mewati and Kharai. The Jalori breed of camel has been registered by the Bureau with the Accession No. INDIA_CAMEL_1700_JALORI_02004. The Domestic Animal Diversity Information System (DAD-IS) of the Food and Agriculture Organisation of the United Nations has also included the Jalori breed of camel among the 11 breeds of dromedary listed for the country. In spite of all these, the breed's phenotype and production potential remain undocumented. The population of livestock cannot sustain unless it has some utility to the stakeholders, which may be through the draught power or the production of meat, milk, hair, dung, urine etc. Recently, the production and properties of non-bovine milk has attracted the attention of researchers and the policy makers (Faye and Konuspayeva, 2012; Bekhit *et al,* 2022). Hence, in order to know the current status of the Jalori breed, it has become essential to characterise them at the phenotypic level as well as at molecular level. The molecular characterisation of this breed using microsatellite markers (Sharma *et al,* 2018) and hair production and quality of breed (Mehta and Dahiya, 2021) has already been reported. This paper highlights the morphometric characteristics, population status, milk production potential and milk quality of Jalori camels maintained under extensive system of management by the farmers in the breeding tract.

Materials and Methods

Survey of the breeding tract

In order to know the present status of the breed with its production potential and associated parameters, a total of 3187 camels belonging to 75 villages of Ahore (12), Jalore (12), Sayala (2), Sanchore (10), Raniwara (7), Bhinmal (11) tehsils of Jalore district and Sirohi (3) and Pindwara (18) tehsils of

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Sirohi districts of Rajasthan were covered in the study (Fig 1). Individual animals of each herd were meticulously judged for the typical features. The phenotype of important body parts was described. Body measurements of 14 body parameters were recorded for 628 adult camels with measuring tape. The body weight was taken for a total of 45 camels utilising commercial weigh bridge. Only healthy camels were measured. Pregnant females were not included in the study and body weight was taken in the morning before the animals were allowed to go for grazing. These animals were reared under extensive system of management.

Milk production

Thirty-seven (37) she-camels were continuously recorded in the field area for their milk production potential. Weekly recording of milk yield was carried out at the farmers door step. No special feed was given. Health was monitored throughout the lactation and milk production of only healthy animals was recorded. A few animals were dropped in the process. As per the practice adopted in the breeding tract, the farmers allowed the calves to suckle in the evening and thereafter the legs of the female were tied and thus disallowing further suckling. In the morning, the camel owners milked the animals. Later the calves were allowed to be with them for few hours and then calves were separated and taken for grazing. The morning milk production was measured and this is estimated to be about a half of the milk the animal produce. The analysis of milk quality was carried out using MilkoScan (FOSS) at the ICAR-National Research Centre on Camel, Bikaner (Rajasthan). However, the monthly fat estimation was carried out at the village level by utilising the Electronic Milk Tester (REIL) available at the milk cooperative societies following the standard protocol defined by the company.

Statistical Analysis

The statistical analysis was carried out by utilising SPSS version 26 (IBM Corp., 2019). The population data of the breed were extrapolated by utilising the percent availability of pure-bred animals of the breed in respective district and the total population of camels in the district as per latest livestock census (Livestock Census, 2019).

Results and Discussion

Origin and distribution of Jalori camel

The Jalori camel derives its name from the place of rearing, i.e. Jalore district of Rajasthan. The three districts surrounding Jalore, i.e. Barmer, Jodhpur and Pali constitutes a major portion of the Thar Desert in the country. Thus, spreading of camel from the Thar Desert to the Jalore and Sirohi appears natural extension to cater the human needs of baggage and transport in the region. The Jalore region is generally plain but has scattered hills, rocks and at some places it is dotted with sand dunes and ridges. Thus, geographically the hard land of Jalore and Sirohi districts has Thar desert in north, hills in the southeast and Rann of Kachchh in the southwest direction. Owing to adaptation and interbreeding, probably the Jalori camel has originated. The Jalori camels are reared mainly by the Dewasi community located in the region.

The geographical distribution of the breed encompasses chiefly the Jalore and Sirohi districts of Rajasthan. The breeding tract extends in east from 72°58′ to 71°3′ longitude and in north from 24°22′ to 25°22′ latitude with fair vegetation and average annual rainfall ranging from 40 to 58 cm. Average elevation of the breeding tract from main sea level ranges from about 268 metres to 321 metres. The breeding tract of Jalori camel in northwest is closely placed with the breeding tract of Jaisalmeri camel and in the northeast with the breeding tract of Marwari camel. The Rann of Kachchh, which is known for the Kachchhi breed of camel is attached with the breeding tract in the southwest. The town Sanchore is like a junction point between the Rajasthan and Gujarat in the extreme western part of the country (Fig 1).

Status of Jalori Camel

The status of a particular breed with respect to its age-wise and sex-wise distribution in different zones of the breeding tract is very important. As per the Livestock Census 2019 (GoI), the total population of the camel in the entire breeding tract is 8551, out of which about 44% population belonging to 188 households was covered in the present survey (Table 1). The morphometric traits were recorded for 628 adult camels. Individual camel was judged for the breed characteristics (Fig 2 and 3) and it was observed that in the Jalore district, Jalori camels were 86.08 % and in Sirohi district, the Jalori camels were 85.03 %. The majority of the crossbreds were showing the features of Bikaneri breed which is preferred because of better look and physique (Mehta and Sahani, 2006). The overall population of Jalori camels in the breeding tract was estimated to be 7323. Relatively smaller population figure of 5023 for the breed has been mentioned by the breed-wise report of livestock and Poultry (2019). The difference in the two figures could

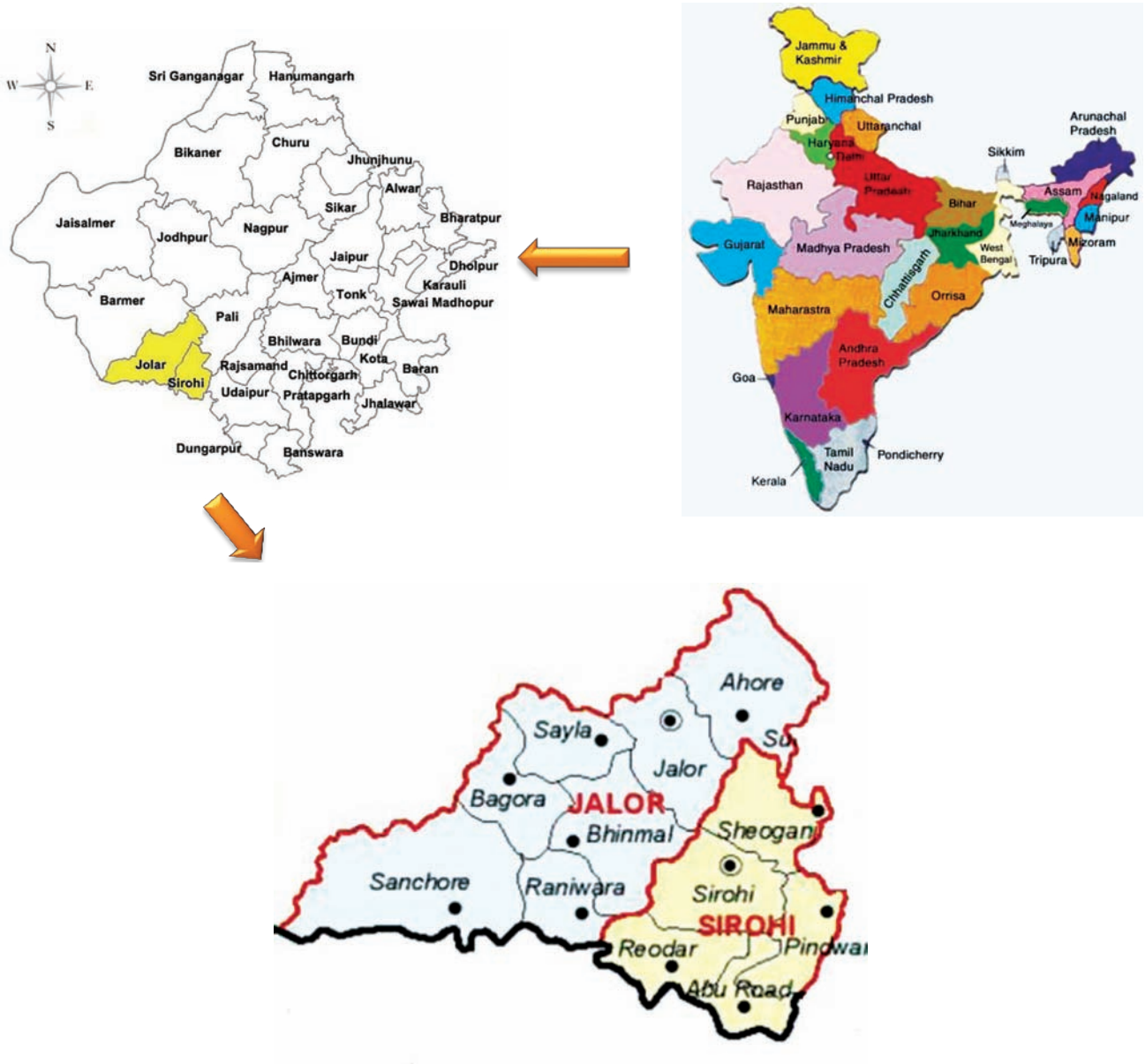

Fig 1. Geographical location of the breeding tract of Jalori breed of camels.

Fig 2. Adult Jalori Male.

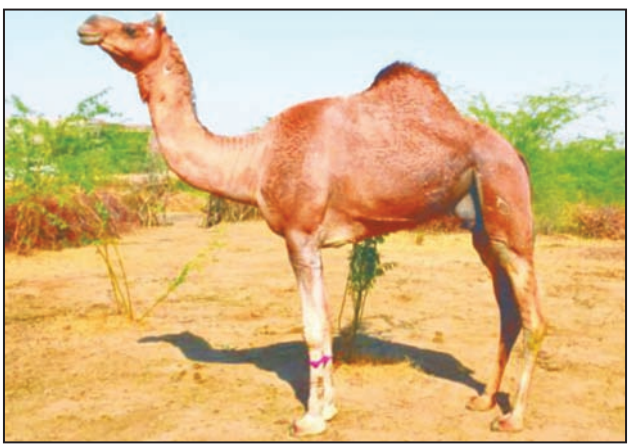

Fig 3. Adult Jalori Female.

be due to difference in the timings of conducting the census and the study.

The age-wise and sex-wise population of Jalori camels covered under the survey and status in the two districts and the breeding tract as a whole is presented in table 2. In the present survey, the ratio of breedable males to females was 1:18. The situation is alarming and indicates the danger of loss of genetic variation in the future generations. However, the ratio presented in the Breed-wise Report of Livestock and Poultry (2019) is 1: 2.25. Again, the difference in the two ratios could be due to the difference in the timings of conducting the census and the study. However, the present study has been carried out scientifically and appears more realistic as the surplus males of breedable age are no longer being retained by the camel owners due to the reduction in the draught utility of the camel, usually seen in all draught species of livestock.

General appearance and physical characteristics

Body Colour

The predominant colour of Jalori camel varies from light brown to dark brown. When the calves are born, the body colour is generally lighter in shade and the hairs are curly. The body colour gets darker and the curls, they open with increase in age. The body colour of the Jalori camels is quite close to that of Jaisalmeri camel (Mehta and Sahani, 2006).

Table 1. Status of Jalori Camel in the breeding tract.

District	Camel Population*	Population Covered	Jalori Camels				Jalori (%)	Estimated Population Jalori
			≤1 year	1-4 Years	Adults	Total		
Jalore	4962	1954	416	410	856	1682	86.08	4271
Sirohi	3589	1770	349	368	788	1505	85.03	3052
Total	8551	3724	765	778	1644	3187	85.58	7323

* Livestock Census 2019

Table 2. Age-wise and sex-wise population of Jalori camels covered under the study.

Sex	Age Group-wise Number of Camels Covered			
	1 Year	1-4 Years	>4 Years	Total
Male	336	293	88	717
Female	429	485	1556	2470
Total	765	778	1644	3187

Head

The head in Jalori camel is medium in size and is well carried on a thin neck. The eyes are prominent. Unlike the Bikaneri camel, in Jalori camels, the forehead is not dome shaped and has no "Stop", which is a name given to a depression on the frontal bone at the upper edge meeting the parietal bone. The supraorbital foramen, which is in the form

Table 3. Morphometric measurements (cm) of adult Jalori Males (N = 69).

S. No.	Character	Male (N=69)		Female (N=559)	
		Mean ± S.E.	Range	Mean ± S.E.	Range
1	Heart girth	205.72 ± 2.12	140-240	207.58 ± 0.66	142-240
2	Body length	155.01 ± 1.38	110-184	154.90 ± 0.40	108-184
3	Height at wither	199.12 ± 1.73	163-230	199.12 ± 0.60	133-230
4	Tail length	54.45 ± 0.75	40-66	54.99 ± 0.28	38-68
5	Neck length	103.54 ± 1.16	72-125	105.47 ± 0.43	70-124
6	Face length	46.75 ± 0.43	35-53	46.38 ± 0.15	35-54
7	Distance between eyes	22.58 ± 0.24	18-27	22.09 ± 0.08	16-27
8	Ear length	11.22 ± 0.13	8-14	10.85 ± 0.04	7-14
9	Fore leg length	149.33 ± 0.85	125-172	149.86 ± 0.22	125-175
10	Hind leg length	160.7 ± 0.92	136-182	161.18 ± 0.28	135-182
11	Foot pad (L/W)				
	i. Fore (Length)	18.4 2± 0.23	12-23	18.9 2± 0.08	12-23
	(Width)	19.43 ± 0.23	13-24	19.95 ± 0.08	13-24
	ii. Hind (Length)	16.77 ± 0.27	10-22	17.23 ± 0.09	10-23
	(Width)	17.80 ± 0.27	11-23	18.27 ± 0.09	11-24

of a deep fissure at the rostromedial margin of the orbit, is normal in depth as compared to the Bikaneri camels where it is deep (Rathore, 1986; Mehta and Sahani, 2006). The muzzle is narrow and mostly pointed in camels of Jalore district but rounded in the camels of Sirohi district. Ears are up-right and set well apart. The typical adaptive feature of desert camel, the "Jheepra" character, i.e. the luxuriant growth of hairs on eye lashes, ears and around the neck, which is often observed in Bikaneri camels is absent in Jalori camel. The lower lip is not droopy as seen in Kachchhi camels (Rathore, 1986; Mehta and Sahani, 2006).

Body and Stature

It is a medium sized breed of camel. The Jalori camels are of active temperament and have thin neck and legs. The body hairs are coarse in quality and medium in length (Mehta and Dahiya, 2021). The Jalori camels appear close to Bikaneri and Jaisalmeri camels and little bigger than the Mewari camels (Rathore, 1986; Mehta and Sahani, 2006).

Udder

The milk vein is small to medium in size. The udder is mostly round in shape. There are four quarters and each quarter has a small cone shaped teat with two canals in it. The shape of the udder is quite similar to other Indian dromedary breeds (Mehta and Sahani, 2006).

Morphometric characteristics

The morphometric measurements of adult male and female camels of Jalori have been presented in Table 3. The Jalori camels of 4 year and above age were considered adult as the camels of both sexes attain puberty at this age and the permanent incisors start erupting. However, the camel continues to gain weight significantly over previous year till 8 years of age (Mehta *et al,* 2010; Mehta, 2008), which is generally noticed by the presence of prominent canines in the month. However, the morphometric measurements of Jalori camel were observed to be close to Bikaneri and Jaisalmeri camels and little higher than that of Mewari camels (Rathore, 1986; Mehta and Sahani, 2006).

Growth

The body weight of healthy Jalori camels from birth to adulthood were recorded and have been presented in table 4. The body weight figures presented by Mehta *et al* (2010) for Bikaneri, Jaisalmeri, Kachchhi and Arab-cross camels are relatively lower than the one recorded for Jalori camels in the present study but the difference could well be accounted to significant difference in the samples size among the two studies. Still, the growth of the Jalori camels maintained by the farmers in the extensive system can be rated as excellent.

Table 4. Body Weight (kg) of Jalori Camel.

Age	Mean ± Standard Error	Range
≤1 Year	260.64±0.00 (1)	160-160
2 Years	282.50±22.93 (12)	170-470
3 Years	328.89±21.24 (9)	220-420
4 Years	452.50±24.98 (8)	360-590
≥5 Years	510.67±17.71(15)	380-650

Figures in parenthesis indicate number of animals.

Milk Production

Since, there had been lot of attention on the production of non-bovine milk in last few years (Faye and Konuspayeva, 2012; Bekhit *et al,* 2022), the weekly recording of the milk production was carried out in the breeding tract and the results have been presented in table 5. The average per day milk yield in Jalori camels was observed as 4.86 litres. The milk production potential of Indian dromedary breeds is now a well-studied parameter. The milk production potential of Mewari camels in the breeding tract has been quantified and it was reported that on an average 3.39 litres of milk per female is being sold in the market per day and it was extrapolated that if the Mewari camel model of milk production is adopted, it is expected that the milk collection is expected to increase by about 8-times its present level and the share of camel milk in the total milk produced in the state may reach to 2.5% (Mehta *et al,* 2009). Faye and Konuspayeva (2012) presented that about 16.9% of milk consumed by human comes from species other than cattle. However, a comprehensive picture of non-bovine milk and its sources and properties has been presented by Bekhit *et al* (2022). On the basis of FAOSTAT data, the authors presented that in the year 2022, goat, sheep, and camel milk represented 2.3%, 1.2%, and 0.4% of the world milk production. This clearly indicates the growing importance of camel milk. The average milk production of Bikaneri and Kachchhi camels from 2-teats was reported as 3606.31±64.59 ml at the organised farm (Mehta *et al,* 2011) and the average daily milk production from 2-teats was reported as 2.7±0.05 litre in Bikaneri, 3.2±0.07 litre in Kachchhi and 2.6±0.08 litre in Mewari breed of camel at the organised farm (Mehta *et al,* 2015). Though the 2-teat milk production at an

organised farm cannot directly be compared with the present observation where the 4-teat milking with some restriction on calf suckling is in practice as well there were differences in the parity of animals, feeding and grazing management; nevertheless, the milk production potential of Jalori camels can be rated as quite comparable or little better than other Indian breeds. The milk yield was almost comparable for about 9 months indicating very good persistency of lactation. This is in agreement with the findings of Mehta *et al* (2015) where the persistency of lactation was reported as 76.20, 67.07, 55.67 and 35.87% when calculated for lactation length of 10, 12, 14 and 16 months, respectively. The highest average per day milk for a month was 5.38 litres and it was 3.6 litres per day in the 14th month of lactation. It is clear from the data (Table 5) that the Jalori camels are producing on an average 10 litres of milk per day and during peak months the production is still higher. Mehta *et al* (2015) also reported that highest individual average daily milk yield from 2 teats was 8.06 litre in indigenous camel breeds and the peak yield was observed in fifth month. The selection of elite animals for breeding (Mehta *et al,* 2014) and proper feed supplementation can further increase the milk production and add to the income of camel farmers in the breeding tract. The camel milk in the breeding tract is being sold for human consumption and is generally used for the preparation of tea and coffee. However, ICAR-National Research Centre on Camel, Bikaner has prepared a variety of products from camel milk, the preparation and sale of such products may further add to the income of the farmers.

Table 5. Average per day milk yield of Jalori camels in the breeding tract.

Month of Lactation	Milk Production (ml)		
	N	Mean±Std. Error	Range
1	77	4977.27±95.187	3500-7000
2	89	5148.88±79.271	4000-7250
3	110	5381.09±83.614	4000-7250
4	89	5143.26±106.142	2000-7250
5	94	4938.83±80.365	3000-6500
6	112	5040.09±66.477	2500-6500
7	114	5129.39±60.261	2250-6500
8	105	5145.24±72.783	1000-6500
9	101	5165.84±78.371	3000-6250
10	108	4546.48±75.511	3000-6000
11	99	3985.05±73.121	2500-6000
12	79	3623.67±69.214	1500-5250
13	13	3596.15±151.220	2500-4500
14	3	3666.67±166.667	3500-4000
Pooled	1193	4858.52±26.671	1000-7250

N - Number of records.

The month-wise per cent fat content was recorded at the field level for a total of 376 records (Table 6). The analysis of milk quality was also carried out and the concentration of fat (Table 6), SNF (Solid Not Fat), protein, lactose and ash was recorded as 3.70 %, 7.6 %, 2.76%, 4.01% and 0.81%, respectively. The pH was recorded as 6.4. The milk composition of Jalori camels comfortably meets the standards defined by the FSSAI (Food Safety and Standards Authority of India) for camel milk (Fat 2% and SNF 6.0%) vide its notification dated June 1, 2017.

Table 6. Month-wise fat content (%) in Jalori camel milk.

Month of Lactation	N	Mean ± Std. Error	Range
1	10	3.47±0.18	2.60-4.60
2	32	3.58±0.12	2.60-5.40
3	7	3.84±0.14	3.10-4.30
4	44	3.73±0.08	2.40-4.70
5	64	3.75±0.06	2.40-4.70
6	68	3.83±0.06	2.80-4.70
7	60	3.83±0.06	2.80-4.70
8	46	3.54±0.08	2.00-4.60
9	31	3.49±0.09	2.00-4.10
10	12	3.56±0.16	2.40-4.20
11	1	3.60±0.00	3.60-3.60
12	1	3.60±0.00	3.60-3.60
Total	376	3.70±0.02	2.00-5.40

N - Number of records.

Improvement and Conservation

The Jalori camels are used for riding, camel dancing, camel carts, camel safari and other aspects of camel draught utility. These activities need to be supported and encouraged by converting this unorganised business into organised one and by modernising them. The other important source of income to the camel farmers in the tract is through sale of camel milk and surplus animals. The Open Nucleus Breeding programme for increasing the milk production with its nucleus at the government farm or research centre and associated herds with the camel owners may lead to significant improvement in the production potential of the animals and increase their income. An integrated rotational grazing system, silvi-pasture development programme along with proper nutritional and health care support will facilitate the camel owners in rearing the camels *in situ*. Continued policy support and awareness programme will not

only help the camel owners in maintaining the Jalori camel with diverse livestock species under optimum production but also will boost their morale and bring happiness in them.

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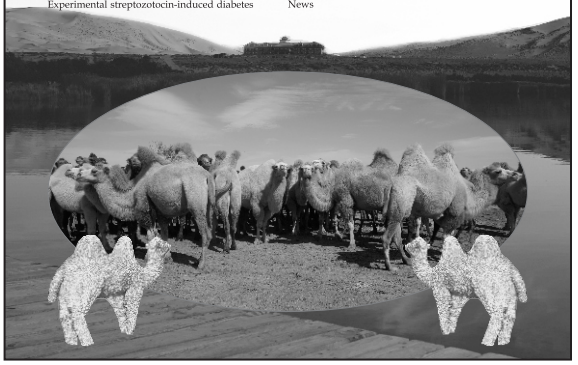

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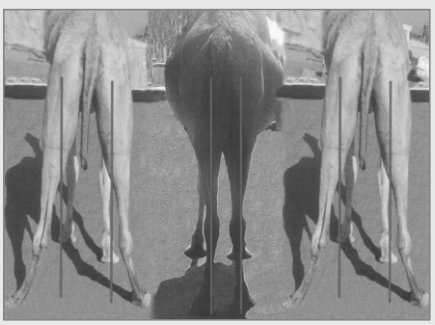

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GENOME-WIDE COMPARATIVE ANALYSES REVEAL SELECTION SIGNATURES UNDERLYING ADAPTATION IN DOMESTIC BACTRIAN AND WILD TWO-HUMPED CAMEL

Siriguleng[1#], Aodungaowa[2#], Wutaodao[1], Zhenji Li[3], Chaijin[4], Duhao[5], Shana Huang[6], Hangfei Gao[6], Naqin[6], Lixian Li[7], Wei Cao[8], Liang Ming[9], Jirimutu[5*], Alateng baolige[5*] and Yi Heng[5*]

[1]Ured Rear Banner Livestock and Aquatic Workstation, Inner Mongolia 015543, China
[2]Animal Disease Prevention and Control Center of Ured Rear Banner, Inner Mongolia 015543, China
[3]Bayannur City Animal Husbandry Service Center, Inner Mongolia 015000, China
[4]Agriculture, Animal Husbandry and Science and Technology Bureau of Ured Rear Banner, Inner Mongolia 015543, China
[5]Ured Rear Banner People's Government, Inner Mongolia 015543, China
[6]Inner Mongolia Agricultural and Animal Husbandry Technology Promotion Center, Inner Mongolia 010013, China
[7]Urad Rear Banner Green Industry Coordination and Development Center, Inner Mongolia 015543, China
[8]Bayannur Agricultural and Animal Husbandry Science Researc h Institute, Inner Mongolia 015199, China
[9]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, College of Food Science and Engineering, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, 010018, China
#First authors, *correspondence authors

ABSTRACT

Bactrian camels are vital large mammals that adapt well to the harsh environment of the desert. Genome-wide selection signatures can provide insights into natural and/or artificial selection and reveal functional genes related to biological characteristics and/or phenotypes. Here, we investigated genomic diversity and structure, and identified selection signatures of domestic Bactrians from China and Mongolia (IMG_D and MG_D) and wild two-humped camels (MG_W). The average sequencing depth reached 12.40× for each population, and more than 4.01, 3.58, and 2.70 million single-nucleotide polymorphisms (SNPs) were detected covering all autosomes and the X chromosome in the IMG_D, MG_D, and MG_W. The population structure suggested gene flows between IMG_D and MG_D, but no strong signal migration between the domestic and wild two-humped camels. Following the F_{ST} and θ_{π} approaches, candidate evolving genes in the camel lineage were significantly enriched in insulin secretion, insulin signaling pathway, lipid metabolism, immune system, and adaptation for desert, which may be the target of selection in domestic Bactrians and wild two-humped camels during the breeding and survival process. Furthermore, screened candidate genes, including ABCC8, KCNJ11, FFAR1, PRKACB, CREB1, PRKACB, ACACA, and SLC2A4, were associated with insulin pathways and putatively related to insulin resistance. We also identified candidate genes and KEGG pathways associated with olfactory transduction and environmental adaptation, implying a greater desert adaptation capacity in Bactrian camels. In conclusion, the present study provides a greater understanding of genome diversity and variations associated with adaptive and biological characteristics in Bactrian camels.

Key words: Domestic Bactrian, selection signature, genome-wide, wild two-humped camel

The Bactrian and dromedary camels are economically important livestock in desert and semi-desert areas, providing meat, milk, and wool and serving as a vital mode of transportation for local residents (Saipolda, 2004). According to current Food and Agriculture Organisation (FAO) statistics only 5% are Bactrian (two-humped) camels (Sikkema *et al,* 2019). Bactrian camels (*Camelus bactrianus*) are the last domesticated large mammals whose ancestors were primarily in Northeast and Central Asia approximately 4450 years ago (Ming *et al,* 2020). Today, more than 90% of Bactrian camels are distributed across China, Mongolia, and Kazakhstan, numbering about 952,000 (Ming *et al,* 2022), and formed different breeds or populations from different geographical locations, which have constituted an extensive genetic resource pool. In addition, wild two-humped camels (*Camelus ferus*), with morphological similarities to its domestic counterpart, is a critically endangered ungulate that inhabits Central Asia's

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desert ecosystems (Yadamsuren *et al,* 2019) and stores genetic resources differently from domestic Bactrian camels. In recent years, genetic diversity in domestic Bactrians and wild two-humped camels have been recognised as an important attribute (Ming *et al,* 2020; Ming *et al,* 2017; Yi *et al,* 2017; Ming *et al,* 2021), and the conservation of camels' genetic diversity is urgent for improving production and balancing the ecological environment (Burger *et al,* 2019).

Through long-term natural and artificial selection, such as climate change, environmental pressure, human migration and socioeconomic practices, Bactrian camels shaped their unique adaptability to harsh environmental conditions in desert and semi-desert areas—cold, hot, arid, poor grazing, and even food shortages. They can withstand extreme thirst and hunger for prolonged periods (Ali *et al,* 2019). Long-term selection leads to different phenotypic structures in the Bactrian camel population across world regions (Ming *et al,* 2022). These changes have left their footprint on specific regions of the organism genome as the selection signature associated with adaptive and productive phenotypes (Bigham *et al,* 2010; Lv *et al,* 2014). Detecting selection signatures can shed light on the processes involved in genome evolution and determine functional gene or genomic regions associated with unique biological characteristics and economic traits (Hayes *et al,* 2009; Nielsen, 2005).

Domestic Bactrians and wild two-humped camels belong to the genus Camelus. However, they originated from different ancestors and underwent different evolutionary processes (Ji *et al,* 2009; Jirimutu *et al,* 2012). To explore their genetic variance and identify candidate regions and genes related to important traits, we resequenced whole genomes belonging to 39 domestic Bactrians from China and combined whole genome sequencing from NCBI (National Centre for Biotechnology Information) database of domestic Bactrians and wild two-humped camels in Mongolia to detect a within and between domestic and wild comparative selection signature analysis. We aimed to provide a theoretical basis for improving economically important traits in Bactrian camels and further insight into the mechanisms underlying adaptation to extreme desert environments.

Materials and Methods

Ethics statement

The procedures and protocols were approved by the animal care committee of the Camel Protection Association of Inner Mongolia. The research was supported by the Review Committee for the Use of Human or Animal Subjects of the Food Science and Engineering College of Inner Mongolia Agricultural University (Hohhot, China). All experimental procedures used in this study were conducted in compliance with the ARRIVE guidelines (https://arriveguidelines.org).

Sample collection

Thirty nine Gobi Red Bactrian camel samples (IMG_D) were sampled from Bayan Nur city, Inner Mongolia, China. The Gobi Red Bactrian is an ancient population raised for milk, wool, and meat production. Notably, Gobi Red Bactrian has undergone a long-term natural and artificial selection and is characterised by high milk production (3.6-4.2 kg/day), red coats, and excellent wool quality (long fibre, fine velvet, and high yield). We collected 5 mL of blood in EDTA anticoagulant tubes from each camel's jugular vein after disinfection treatment, which was then stored at -80°C until further processing.

DNA extraction and sequencing

Blood sample DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions. The quality and integrity of DNA were detected by the OD260/280 ratio and a 2% agarose gel electrophoresis. The DNA concentration was controlled using the Quant-iT PicoGreen dsDNA Reagent Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Libraries were prepared using Illumina kits and then sequenced on the Illumina HiSeq platform (Illumina; CA, USA) with the standard paired-end mode. By combining the raw data by sequencing with the genome data from the database, filtration was performed as follows: (1) Reads containing adapter sequences were removed; (2) discarded bases with consecutive quality <20 for both ends of the sequencing read; (3) removed the final length of the sequencing read, which was <50 bp; (4) removed reads with >5% of unknown bases. We based all the bioinformatic analyses on the filtration data (clean reads) from the Illumina quality control filter.

Data Availability

The datasets generated and analysed during the persent study are available with the National Centre for Biotechnology Information (NCBI) Sequence Read Archive (SRA) GenBank repository, data-base under accession number PRJNA890442 (https://www.

ncbi.nlm.nih.gov/sra/PRJNA890442). Additionally, whole genome resequencing data from 16 Mongolian domestic Bactrians (MG_D) and 13 Mongolian wild two-humped camels (MG_W) were obtained from Sequence Read Archive at the NCBI (SAMN06759127-132, 134, 136, 141-145, 162-163, 169-178, 182-184, 187).

Read alignment and variant calling and annotation

We used the Burrows–Wheeler Aligner (v0.7.9a, MEM) (Li and Durbin, 2009) to map clean reads to the reference genome assembly of the wild two-humped camel (*Camelus ferus*, VSZR00000000; Assembly: GCA_009834535.1) (Ming *et al,* 2020). Duplicates reads were discarded using the Picard MarkDuplicates tool (v1.115). Statistics of alignment rate, coverage, and sequencing depth were performed on the deduplicated data. The sample alignment rate reflects the similarity between the sample sequencing data and the reference genome. The coverage depth directly reflects the homogeneity of the sequencing data and homology of the reference sequence. We followed the Genome Analysis Toolkit (GATK) (DePristo *et al,* 2011) pipeline for variant calling and ANNOVAR (Wang *et al,* 2010) was performed to assign SNPs.

Phylogenetic and population structure

In order to ensure the reliability of subsequent population analyses, raw variants were filtered according to the following criteria: minor allele frequency >5%, the proportion of the sample covered by SNP to the total sample >90%, and variants with <20% of individuals missing genotypes. We calculated the distance matrix with Treebest software (Vilella *et al,* 2009) and constructed a phylogenetic tree by the neighbor-joining method. We identified the top four principal components accounting for variation in the dataset. The population ancestry was inferred by Admixture (v1.3.0)v (Alexander *et al,* 2009) based on Bayesian mathematical models. Migration events among camel populations were inferred using TreeMix (v1.12) (Pickrell and Pritchard, 2012). We estimated the optimal number of ancestral clusters K with the cross-validation error. The filtered SNP set was also used to estimate genome-wide linkage disequilibrium (LD). The LD decay was calculated with PopLDdecay (Zhang *et al,* 2019) using default parameters.

Calculation of θπ and Fst

We used a sliding-window approach (100 kb windows sliding in 50 kb steps) quantify polymorphism levels (θπ, pairwise nucleotide variation as a measure of variability), and genetic differentiation (FST) between domestic Bactrian and wild two-humped camels.

Identification of selected regions

To detect regions associated with selective sweep, we calculated the distribution of the θπ ratios (θπ, wild/ θπ, domestic) and FST values according to Li *et al* (2013). We simultaneously used empirical procedures and selected windows with significantly low and high θπ ratios (the 5% left and right tails) and significantly high FST values (the 5% right tail) of the empirical distribution as regions with strong selective sweep signals along the genome, which could harbour genes that underwent a selective sweep.

Functional enrichment analysis

Functional classification of GO (Genen Ontology) categories was performed using Blast2GO (Conesa and Götz, 2008). We also tested each set of putative positively selected genes for overrepresentation of the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways. The P values were adjusted by FDR (False Discovery Rate) and the adjusted P value cut-off was 0.05.

Results

Genome data analysis and SNP identification

The present study reports on the whole genome sequencing of the Gobi Red Bactrian camel originating from Inner Mongolia, China. We characterised the genetic variations of combined Mongolian domestic and wild two-humped camel whole genome data from the National Centre for Biotechnology Information (NCBI). All methods in this study were performed in accordance with the relevant guidelines and regulations. We integrated and aligned 2×125 bp of paired-end reads, more than 1200 Gb, using Burrows–Wheeler Aligner (BWA) (Li and Durbin, 2009) software for the wild two-humped camel reference genome assembly. The average sequencing coverage rate reached 96.84%, and we thus inferred that the sequencing data covered most of the genome.

We achieved an average sequencing depth of 12.40× for each population and more than 97.77% of the sequence reads were mapped to the reference genome, indicating that the sequencing data covered most of the genome, and high-quality sequences were obtained (Table S1). The number of detected single-nucleotide polymorphisms (SNPs) for the IMG_D, MG_D, and MG_W camels were 4.01, 3.58, and 2.70

Table S1. Whole genome resequencing for 39 domestic Bactrians from China, 16 domestic Bactrians from Mongolia and 13 wild two-humped camels from Mongolia.

NO.	Sample	Clean reads	Clean Bases (bp)	Sequencing depth (×)	Coverage Rate (%)	Mapping Rate (%)	Singletons mapped(%)
1	IMG_D_1	233,377,308	34,163,102,124	13.25	98.5	98.28	0.36
2	IMG_D_2	235,673,208	34,590,568,017	12.54	98.59	98.13	0.33
3	IMG_D_3	230,639,824	33,926,116,838	12.00	98.28	98.05	0.33
4	IMG_D_4	230,481,092	33,914,522,791	12.17	98.48	98.27	0.31
5	IMG_D_5	259,457,164	37,977,083,433	14.48	98.56	98.07	0.35
6	IMG_D_6	233,882,908	34,060,358,933	13.47	98.53	98.05	0.40
7	IMG_D_7	272,247,348	40,076,051,109	13.85	98.42	98.37	0.30
8	IMG_D_8	240,660,096	35,427,587,595	12.72	98.38	98.34	0.35
9	IMG_D_9	229,351,106	33,535,939,131	13.01	98.44	97.97	0.37
10	IMG_D_10	226,821,290	33,183,779,029	13.03	98.48	98.11	0.36
11	IMG_D_11	258,208,284	37,749,957,795	14.35	98.45	98.23	0.37
12	IMG_D_12	235,661,296	34,400,249,280	13.50	98.60	98.15	0.39
13	IMG_D_13	264,040,292	38,610,372,980	14.92	98.51	97.96	0.39
14	IMG_D_14	248,890,494	36,440,198,986	14.15	98.58	98.17	0.35
15	IMG_D_15	250,601,884	36,896,180,928	12.92	98.33	98.12	0.32
16	IMG_D_16	252,689,076	37,150,798,850	13.00	98.48	98.25	0.33
17	IMG_D_17	275,330,276	406,12,701,892	14.85	98.52	98.15	0.32
18	IMG_D_18	262,921,260	38,802,491,675	14.22	98.6	98.04	0.32
19	IMG_D_19	247,285,228	36,320,848,848	14.02	98.49	98.22	0.33
20	IMG_D_20	250,795,352	36,841,958,919	13.35	98.46	98.08	0.37
21	IMG_D_21	235,153,794	34,517,978,197	13.25	98.43	98.17	0.36
22	IMG_D_22	256,840,936	37,753,221,093	13.90	98.60	98.00	0.37
23	IMG_D_23	264,183,004	38,901,619,901	13.52	98.67	98.17	0.34
24	IMG_D_24	256,203,234	37,783,618,394	13.57	98.45	98.13	0.31
25	IMG_D_25	253,721,376	37,403,517,863	13.63	98.49	98.17	0.33
26	IMG_D_26	255,661,860	37,518,440,629	14.26	98.53	98.08	0.34
27	IMG_D_27	234,668,174	34,419,215,228	13.25	98.44	97.97	0.33
28	IMG_D_28	227,115,944	33,337,568,585	12.98	98.33	98.28	0.30
29	IMG_D_29	250,766,240	36,816,697,912	14.00	98.47	97.97	0.33
30	IMG_D_30	230,007,272	33,729,098,117	13.09	98.29	98.26	0.32
31	IMG_D_31	245,052,612	35,970,636,344	13.76	98.42	97.92	0.35
32	IMG_D_32	270,646,976	39,673,054,313	10.05	98.16	98.16	0.33
33	IMG_D_33	238,430,504	34,959,568,806	13.36	98.39	97.83	0.37
34	IMG_D_34	261,378,634	38,477,622,495	13.28	98.25	98.16	0.32
35	IMG_D_35	227,745,594	33,402,148,164	12.97	98.45	98.24	0.33
36	IMG_D_36	225,840,872	33,059,604,073	13.07	98.33	98.30	0.33
37	IMG_D_37	241,568,264	35,400,380,850	13.65	98.31	98.18	0.36
38	IMG_D_38	228,566,354	33,490,388,190	12.97	98.54	98.06	0.34
39	IMG_D_39	245,314,832	35,830,593,946	13.82	98.42	98.08	0.34
40	IMG_D_40	234,122,566	34,479,066,182	12.59	98.41	98.31	0.30
Average		245,550,096	36,040,122,711	13.37	98.45	98.14	0.34
1	MG_D_126	206,602,240	24,317,444,074	11.09	92.82	97.28	0.73
2	MG_D_127	186,533,826	21,566,060,531	9.96	95.38	97.66	0.61

3	MG_D_135	218,518,034	26,477,212,053	12.27	98.27	97.75	0.57
4	MG_D_136	244,234,498	29,608,862,005	13.61	98.23	97.12	0.67
5	MG_D_137	229,412,608	27,216,482,487	12.60	94.11	97.8	0.63
6	MG_D_138	210,662,068	24,344,621,671	11.12	89.67	97.56	0.78
7	MG_D_139	189,265,650	21,954,426,844	10.12	89.00	97.84	0.72
8	MG_D_140	230,032,266	28,051,883,744	12.99	98.21	97.70	0.50
9	MG_D_141	214,667,082	26,189,095,619	12.11	89.71	97.62	0.57
10	MG_D_142	228,685,732	27,870,865,144	12.85	89.5	97.59	0.59
11	MG_D_143	210,397,592	25,808,492,572	11.89	98.34	97.55	0.51
12	MG_D_144	232,321,890	28,331,227,058	13.00	98.28	97.31	0.64
13	MG_D_148	213,008,706	25,962,184,933	12.00	98.66	97.89	0.60
14	MG_D_149	192,585,844	22,324,278,455	10.22	89.14	97.41	0.95
15	MG_D_150	229,141,296	27,907,655,650	12.98	88.40	97.97	0.55
16	MG_D_153	211,826,506	25,881,058,295	11.96	98.29	97.71	0.56
Average		215,493,490	25,863,240,696	11.92	94.13	97.61	0.64
1	MG_W_088	291,402,732	28,755,444,143	13.32	98.48	97.82	0.61
2	MG_W_089	353,854,564	35,214,965,866	16.29	98.94	97.73	0.58
3	MG_W_090	298,721,994	29,131,818,611	13.46	98.55	97.29	0.59
4	MG_W_091	281,696,446	27,426,967,391	12.69	97.49	97.61	0.63
5	MG_W_092	248,797,732	23,578,570,077	10.85	93.77	97.54	0.59
6	MG_W_093	215,445,682	21,161,872,442	9.52	97.89	97.84	0.54
7	MG_W_095	187,680,432	18,403,139,067	8.18	97.63	97.14	0.58
8	MG_W_097	325,823,712	32,357,429,084	14.70	98.80	97.64	0.51
9	MG_W_102	236,433,494	23,506,551,016	10.62	98.53	97.67	0.52
10	MG_W_103	231,188,056	22,934,262,953	10.25	98.4	97.43	0.58
11	MG_W_104	299,329,996	29,557,118,508	13.51	98.91	97.73	0.52
12	MG_W_105	282,630,858	28,023,934,404	12.82	98.77	97.78	0.51
13	MG_W_106	193,435,104	19,247,570,617	8.71	97.08	97.26	0.58
Average		265,110,831	26,099,972,629	11.92	97.94	97.58	0.59

IMG_D: Gobi Red Bactrian from Inner Mongolia, China. MG_D: Mongolian domestic Bactrian camel, from Mongolia. MG_W: Mongolian wild two-humped camel, from Mongolia.

million variants using the Genome Analysis Toolkit. The Ts/Tv ratio of the SNPs was 2.16, 2.21, and 2.17, respectively (Table 1), which agreed with previous research on the domestic Bactrian camel (2.18) (Ming *et al,* 2020), but were slightly lower than wild two-humped camels (2.26) and dromedaries (2.31 and 2.34) (Fitak *et al,* 2016; Khalkhali-Evrigh *et al,* 2018).

Table 1. Summary of identified variants for domestic Bactrians from China and Mongolia, and wild two-humped camels from Mongolia.

Populations	IMG_D	MG_D	MG_W
Number of SNPs	4,011,826	3,584,401	2,703,238
Ts/Tv	2.16	2.21	2.17
Heterozygous SNPs	2,326,859	2,043,109	1,811,169
Heterozygous ratio (%)	0.58	0.57	0.67
Homozygous SNPs	1,684,967	1,541,292	892,069
Homozygous ratio (%)	0.42	0.43	0.33

IMG_D: Gobi Red Bactrian from Inner Mongolia, China. MG_D: Mongolian domestic Bactrian camel from Mongolia. MG_W: Mongolian wild two-humped camel from Mongolia.

We also identified heterozygous 2,326,859, 2,043,109, and 1,811,169 SNPs in the IMG_D, MG_D, and MG_W populations. The heterozygosity ratio was similar in domestic Bactrian camels from China and Mongolia, and slightly higher in wild two-humped camels across whole genomes. Furthermore, the SNPs were classified at the chromosome level (Table 2).

SNP annotation and functional classification.

In the high-quality SNPs from three Bactrian camel populations, most of which were located in intergenic regions (57.79-56.87%), only 0.11% were located in exonic regions (Table S3). This intergenic

region was the most mutated in the Bactrian camel genome, whereas the exonic regions had fewer mutations. Compared to domestic Bactrian camel populations, fewer non-synonymous (12,555) and synonymous (14,774) SNPs in wild two-humped camel were localised within exons, resulting in a non-synonymous/synonymous ratio of 0.8498 (Table 2).

Table 2. The distribution of SNPs in the camel whole genome.

Populations		IMG_D	MG_D	MG_W
Intergenic[a]		2,318,481	2,038,605	1,548,855
ncRNA[b]		1,757	1,629	840
UTR[c]		59,785	56,829	40,340
Intronic		1,588,212	1,444,238	1,083,026
Splicing		188	182	137
exonicg	Synonymous	26,424	21,520	14,774
	Non-synonymous	17,963	17,687	12,555
	Stop altering	251	238	187

[a] Including "intergenic", "upstream", and "downstream" given by ANNOVAR.
[b] Including "ncRNA_exonic", "ncRNA_intronic", "ncRNA_splicing", and "ncRNA_UTR".
[c] Including "UTR5" and "UTR3".

In addition, the non-synonymous SNPs were classified and mapped to the KEGG pathway. Interestingly, 524 genes from IMG_D and 492 genes from MG_D were significantly enriched in the olfactory transduction (ko04740) pathway, whereas only 441 genes from MG_W were enriched in this pathway (Table S4). These results indicated that olfactory transduction was vital to artificial selection during the domestication of Bactrian camels.

Phylogenetic analysis

Principle component analysis (PCA)

To examine the genetic relationship among and within the 3 camel populations, we first completed a PCA analysis. The first and second eigenvectors clearly distinguished the domestic Bactrians and wild two-humped camels (Fig 1a). Unexpectedly, IMG_D and MG_D clustered together, indicating a close genetic relationship due to either close geographic proximity or deliberate intercrossing. Although, IMG_D and MG_D belong to the domestic population, they have distinct morphological characteristics and breeding histories. MG_D is an ancient breed bred by Mongolians and adapted to cold environments. IMG_D is known for its red coat colour and outstanding wool quality.

Phylogenetic tree

Furthermore, a phylogenetic tree was constructed with the filtered SNP using the neighbour-joining algorithm. The NJ (Neighbouring Tree) tree clustered the 3 studied populations into separate genetic groups confirming their genetic distinction (Fig 1b). Consistent with the PCA results, two individuals of MG_D clustered with the IMG_D, confirming a close genetic relationship or gene flow between IMG_D and MG_D Bactrian camels due to close geographic proximity.

Population genetic structure

To estimate the proportion of shared genetic ancestry and/or levels of admixture, we performed a population structure analysis with admixture (Alexander *et al,* 2009) (Fig 1c). The cross-validation procedure supported that K = 2 was optimal (Fig S1), showing a clear division between the wild two-humped camels (MG_W), and domestic Bactrian camels (MG_D and IMG_D). By contrast, domestic Bactrian camels from China and Mongolia were grouped from K = 2 to K = 3 (Fig 1c). Evident introgression of IMG_D camels into MG_D was observed. Consistent with the results of PCA and NJ, the admixture analysis further confirmed, with higher resolution, the intermixed genetic makeup of the two domestic Bactrian camel populations. Another method to infer the camels' population tree was TreeMix (Pickrell and Pritchard, 2012). It is worth mentioning that there was no strong migration signal between the domestic and wild two-humped camels (Fig 2a); however, gene flows from dromedaries to domestic Bactrian camels were identified.

Linkage disequilibrium (LD) analysis

The PopLDdecay software was used to explore genome-wide patterns of LD in each camel population by the default parameters. The IMG_D and MG_D populations had similar and lower LD values, suggesting a relatively early origin of the domestic Bactrian camels. The MG_W population had higher LD values, indicating that they were derived from a relatively small ancestral population (<1000) (Fig 2b).

Genome-wide selection signature analysis

The differentially selected genes and genomic regions by selection signatures have been identified, which are vital to revealing the genetics of economic and adaptive traits. To better understand the underlying genetics of their unique biological properties, the adaptation among domestic and wild two-humped camel populations, the fixation index

Table S2. Genome-wide summary of SNPs from IMG_D, MG_D and MG_W.

Chromosome	SNP Count			Chromosome	SNP Count		
	IMG_D	MG_D	MG_W		IMG_D	MG_D	MG_W
1	660985	555214	349038	20	261828	220856	128018
2	661441	534063	333531	21	166683	139387	91001
3	568323	431077	331244	22	168384	141202	81472
4	360613	298968	231269	23	209926	174802	105974
5	467046	380748	251238	24	199467	157019	108360
6	495467	390327	265460	25	241030	173302	113146
7	392311	327206	222000	26	202939	164138	99326
8	429930	320694	231109	27	159647	137692	92239
9	450362	389531	256474	28	57163	46799	28755
10	371975	315930	202625	29	176777	138295	96807
11	508255	434003	253039	30	181285	136771	94817
12	328280	259162	231019	31	150705	140119	91803
13	421560	303863	189248	32	184404	144969	80215
14	441653	342608	210909	33	130749	110245	77526
15	265101	227771	160315	34	140414	99646	73064
16	349410	268003	167139	35	214226	179007	106212
17	259301	225785	154052	36	320949	262025	194201
18	183787	160376	124721	X	351632	289319	263155
19	318083	250401	166088				

IMG_D: Gobi Red Bactrian from Inner Mongolia, China. MG_D: Mongolian domestic Bactrian camel, from Mongolia. MG_W: Mongolian wild two-humped camel, from Mongolia.

Table S3. Numbers and distribution of SNPs in different camel populations.

Population	IMG_D		MG_D		MG_W	
	Number	Per cent(%)	Number	Per cent(%)	Number	Per cent(%)
Total	4011826	100	3584401	100	2703238	100
UTR5	15765	0.39	15894	0.44	10640	0.39
UTR3	44020	1.10	40935	1.14	29700	1.10
UTR5;UTR3	65	0	59	0	47	0
exonic	42008	1.05	41562	1.16	29084	1.08
splicing	188	0	182	0.10	137	0.01
exonic;splicing	24	0	24	0	18	0
upstream	30087	0.75	29197	0.81	20233	0.75
downstream	31088	0.77	28945	0.81	20653	0.76
upstream;downstream	1306	0.03	1273	0.04	892	0.03
intronic	1588212	39.59	1444238	40.29	1083026	40.06
intergenic	2257306	56.27	1980463	55.25	1507969	55.78
ncRNA_exonic	346	0.01	330	0.01	238	0.01
ncRNA_splicing	1	0	1	0	1	0
ncRNA_intronic	1410	0.04	1298	0.04	601	0.02

IMG_D: Gobi Red Bactrian from Inner Mongolia, China. MG_D: Mongolian domestic Bactrian camel, from Mongolia. MG_W: Mongolian wild two-humped camel, from Mongolia.

(FST), and quantifying polymorphism level (θπ) tests involving IMG_D vs. MG_W, MG_D vs. MG_W, and IMG_D vs. MG_D were performed in 100 kb windows with a 50 kb sliding step. We selected the top 5% of candidate genes for further analysis. Up to 233 genes were positively selected for FST (IMG_D vs MG_W) comparison, 665 genes for FST (MG_D vs MG_W) comparison, and 576 genes for the FST (IMG_D vs MG_D) comparison (Fig 3); these candidates were distributed across different chromosomes.

Functional analysis of candidate genes

Furthermore, candidate genes from domestic Bactrians (IM_D and MG_D) and wild two-humped camels were mapped to the KEGG pathways (Table S8). A few genes were significantly enriched in the adipocytokine signaling pathway (ko04920), insulin signaling pathway (ko04910), insulin secretion (ko04911), B cell receptor signaling pathway (ko04662), IL-17 signaling pathway (ko04657), Th17 cell differentiation (ko04659), fatty acid biosynthesis and degradation (ko00071), glycerophospholipid metabolism (ko00564), glycerolipid metabolism (ko00561), circadian entrainment (ko04713), plant–pathogen interaction (ko04626), and carbohydrate metabolism (ko00030) (Fig S2). These enriched signaling pathways had significant enrichment ($P<0.05$). The findings suggested that insulin signaling, lipid metabolism and the immune system relate to desert adaptation could be the target of

Table 3. Significant KEGG pathway enrichment for candidate genes in domestic Bactrians and wild two-humped camels.

Pathway Hierarchy	KEGG Pathway	Accession Code	Tota Genes	P-value	Gene Name
Endocrine system	Adipocytokine signaling pathway	ko04920	7	4.80×10^{-3}	AKT2, NFKBIE, ACSBG2, NFKBIA, NFKB1, PPARGC1A, MAPK10
	Insulin signaling pathway	ko04910	9	4.69×10^{-2}	EIF4EBP1, PPARGC1A, CBLC, PRKACB, SLC2A4, PDE3B, AKT3, ACACA, PIK3CD
	Insulin secretion	ko04911	16	8.32×10^{-4}	ABCC8, PRKCB, KCNJ11, FFAR1, PCLO, CREB1, PRKCA, RAPGEF4, PRKACB, KCNMB2, ADCY8, GNAQ, CAMK2G, CREB3L2, ADCY1, CACNA1D
Immune system	B cell receptor signaling pathway	ko04662	8	1.10×10^{-3}	PPP3CA, PIK3CD, NFKBIE, AKT2, PLCG2, LOC102517806, NFKB1, NFKBIA
	Th1 and Th2 cell differentiation	ko04658	8	3.76×10^{-3}	NFKBIE, JAK1, NFKB1, NFKBIA, IL12A, PPP3CA, MAML3, MAPK10
	Fc epsilon RI signaling pathway	ko04664	5	2.80×10^{-2}	PLCG2,PIK3CD,PRKCA,MAPK10, AKT2
	IL-17 signaling pathway	ko04657	6	2.95×10^{-2}	MUC5AC, USP25, TNFAIP3, NFKBIA, NFKB1, MAPK10
	T cell receptor signaling pathway	ko04660	7	3.14×10^{-2}	PIK3CD, PPP3CA, PAK4, NFKBIA, NFKB1, AKT2, NFKBIE
	Th17 cell differentiation	ko04659	7	3.32×10^{-2}	RORA, MAPK10, PPP3CA, NFKBIA, NFKB1, JAK1, NFKBIE
	Toll-like receptor signaling pathway	ko04620	6	4.29×10^{-3}	MAPK10, PIK3CD, AKT2, NFKBIA, NFKB1, IL12A
Lipid metabolism	Fatty acid degradation	ko00071	3	3.07×10^{-2}	ECI1, ACADL, ACSBG2
	Glycerophospholipid metabolism	ko00564	6	2.57×10^{-2}	DGKB,DGKH, PHOSPHO1, DGKD, DGKI, CDS1
	Glycerolipid metabolism	ko00561	4	3.48×10^{-3}	DGKB, DGKI, DGKH, DGKD
Environmental adaptation	Circadian entrainment	ko04713	14	3.73×10^{-2}	PRKCA, PRKACB, ADCY8, GNAQ, CREB1, CACNA1H, ADCY1, CACNA1D, CAMK2G, ITPR1, GNB1, PRKG2, GNG7, PRKCB
	Plant–pathogen interaction	ko04626	1	1.49×10^{-2}	HSP90AA1
	Circadian rhythm - fly	Ko04711	1	4.24×10^{-2}	GSK3B
Carbohydrate metabolism	Pentose phosphate pathway	ko00030	2	4.21×10^{-2}	TALDO1,DERA

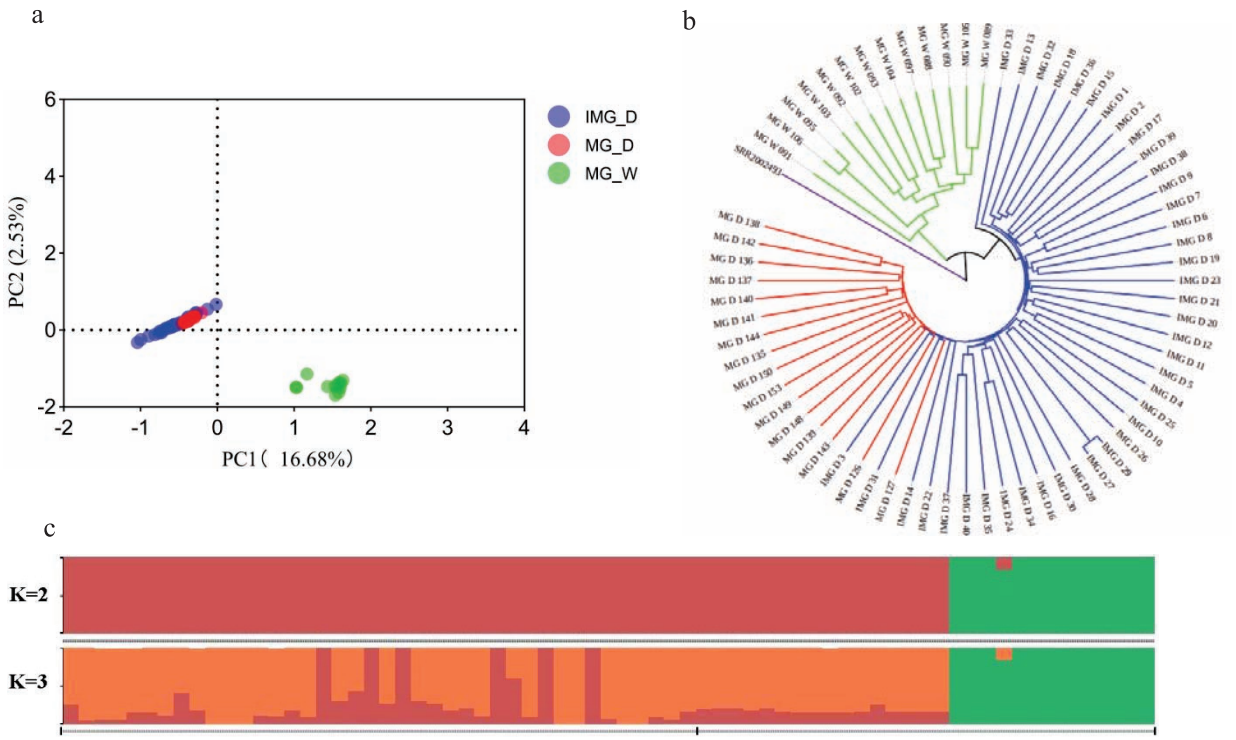

Fig 1. Population genetics analyses of Bactrian camels on genome-wide SNPs. (a) Principal component analysis (PCA) results of 3 Bactrian camel populations. (b) NJ tree constructed using p-distances between individuals. (c) Admixture analysis assuming different numbers of ancestry K. The proportion of an individual's genome assigned to each ancestry is represented by different colours.

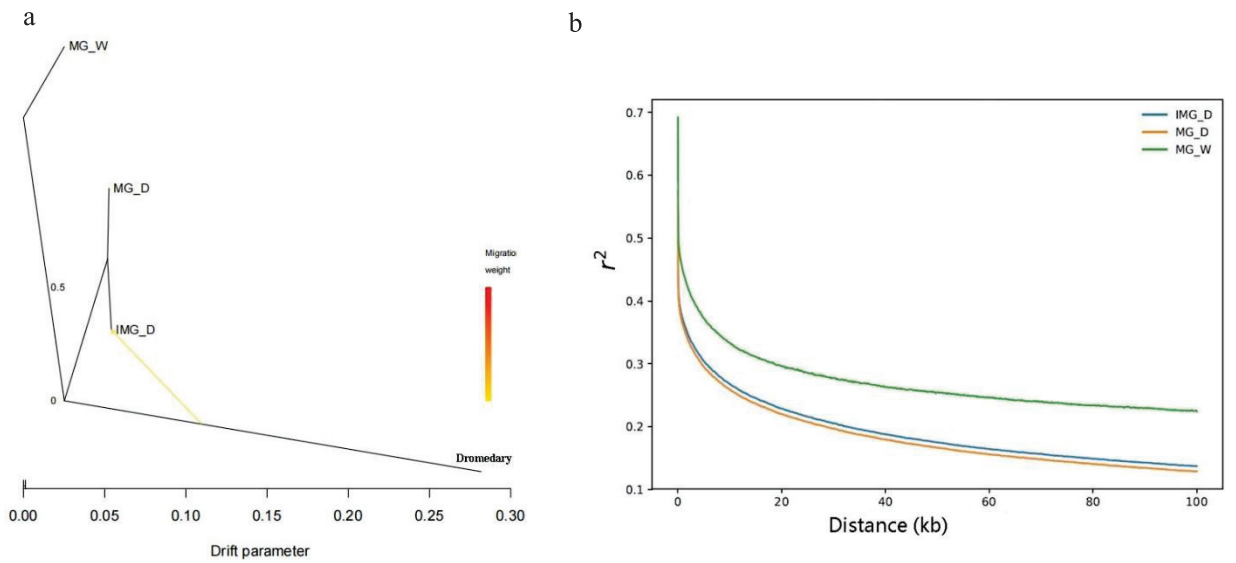

Fig 2. TreeMix analysis of migration events m (a) and decay of linkage disequilibrium (LD) in the Bactrian camel populations with one line per population (b).

selection in domestic Bactrians and wild two-humped camels during the breeding and survival process (Table 3).

Candidate genes related to insulin secretion and insulin signaling pathways

Bactrian camels exhibit insulin resistance, which maintains high blood sugar levels in their body. There are 16 functional candidate genes involved in the insulin secretion pathway and 9 in the insulin signaling pathway (Table 3, Fig 4). The changes in the ABCC8 and KCNJ11 genes disrupt the KATP channel's potentiality and regulate the secretion of insulin, thereby maintaining glucose homeostasis

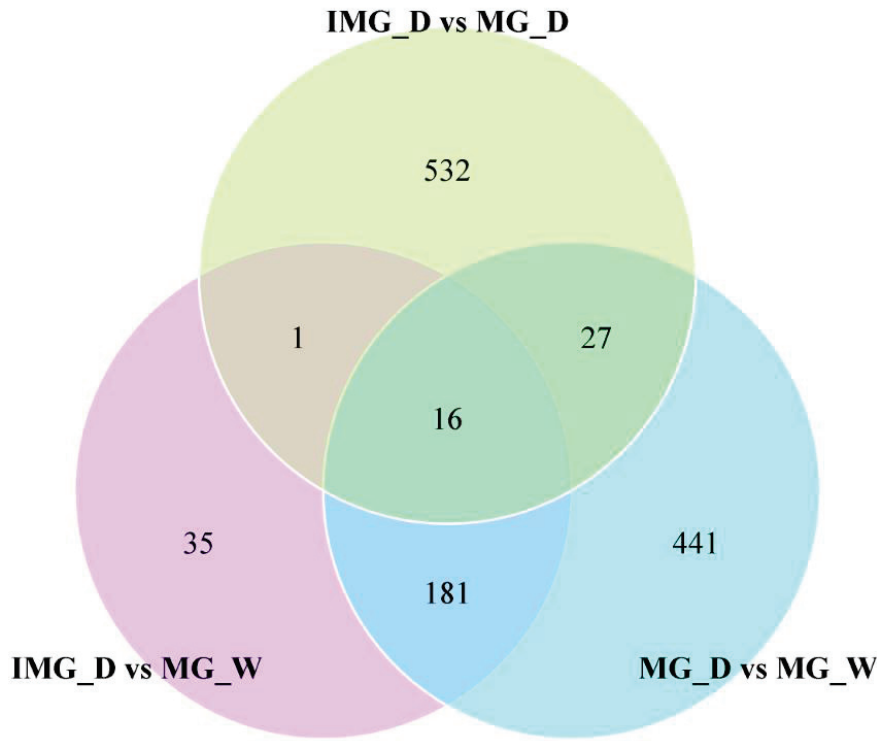

Fig 3. Venn diagram showing comparative analysis of candidate genes among different Bactrian camel populations.

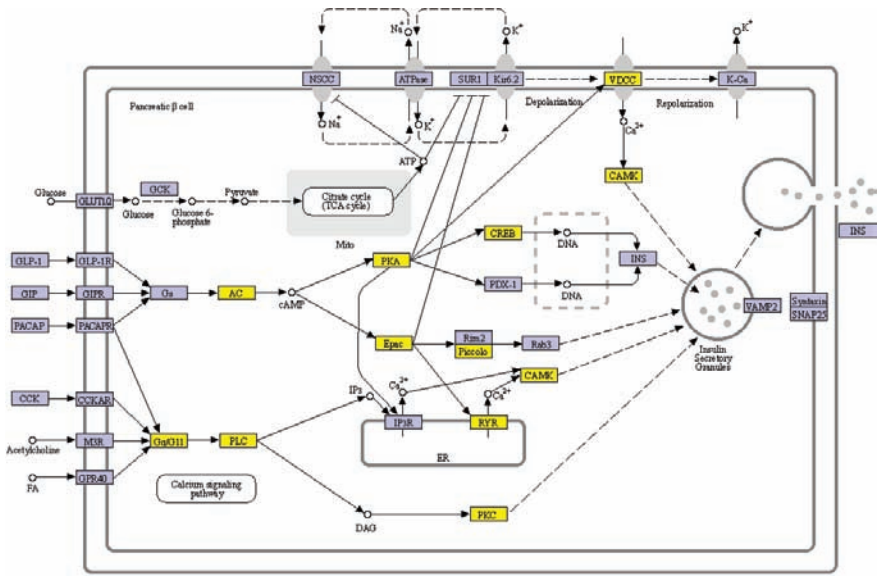

Fig 4. Insulin secretion pathway. Candidate genes are marked in yellow.

(Edghill *et al,* 2010; Reddy *et al,* 2021). The FFAR1 receptor is a long-chain fatty acid G-protein coupled receptor widely distributed in the pancreas and central nervous system. It can act on islet β cells to promote insulin secretion and activate islet α cells to secrete glucagon, regulating gastrointestinal endocrine cells and adjusting glycolipid levels (Walker *et al,* 2011). The CREB1 gene promotes insulin synthesis and secretion. The central gene PRKACB is involved in the insulin secretion pathway. The ACACA gene controls the secretion of insulin, and the GNAS gene is an important regulator of insulin secretion's capacity for pancreatic beta cells (Dalle *et al,* 2011; Taneera *et al,* 2019; Beale, 2013). Insulin resistance is also associated with SLC2A4 genes. During the Bactrian camels' evolution, these insulin-relevant pathways and the candidate genes under selection are similar to the previous study, explaining insulin resistance in Bactrian camels (Jirimutu *et al,* 2012).

Candidate genes related to environmental adaptation

The desert environment where Bactrian camels live is harsh, and a lack of food and water resources is common. In order to survive in this environment, Bactrian camels have developed a unique environmental adaptation mechanism. A few genes subject to selection were associated with circadian entrainment or rhythm—fly pathways (Fig 5), such as the PRKACB gene, which indirectly affects cell proliferation and differentiation; CACNA1H may regulate intracellular processes such as contraction, secretion, neurotransmission, and gene expression. ADCY1 is involved in the regulatory processes of the central nervous system (Chen *et al,* 2013; Santos-Cortez *et al,* 2014). In addition, limited protein-coding genes are involved in plant–pathogen interaction pathways, such as HSP90AA1 aids protein folding and quality control for many 'client' proteins (Zuehlke *et al,* 2015).

Discussion

Domestic animal characteristics result from a high-intensity artificial selection over a short period for wild ancestral species. In the fields of evolutionary

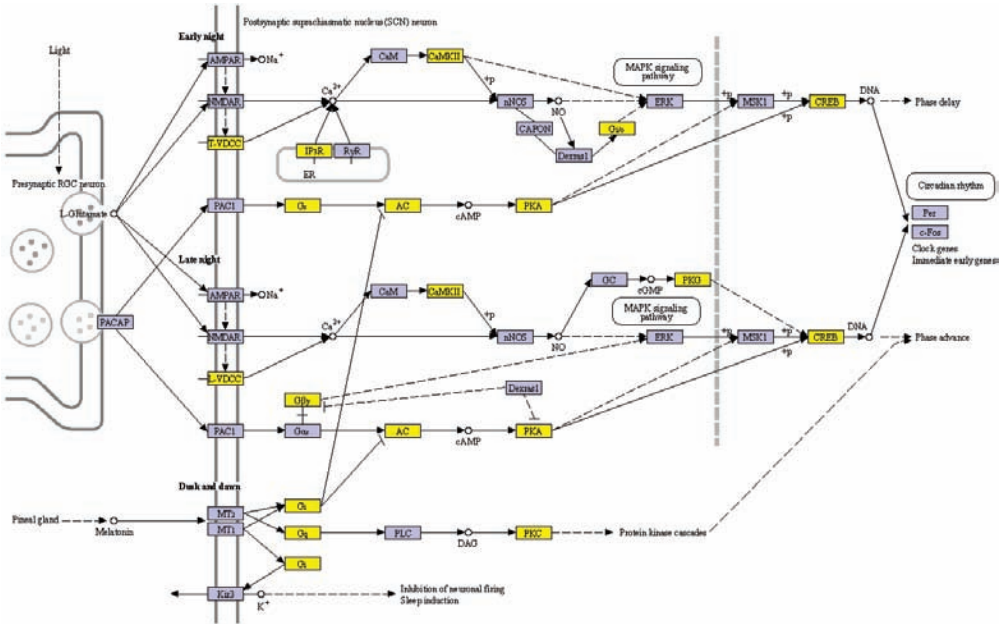

Fig 5. Circadian entrainment pathway. Candidate genes are marked in yellow.

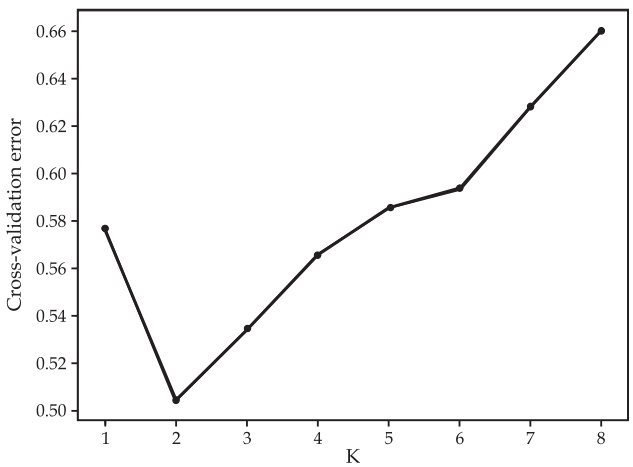

Fig S1. Cross-validation errors in the ADMIXTURE analysis. The number of ancestry K was assumed from 1 to 3 and K = 2 is the optimum number.

genetics and genomics, many theories and methods for testing natural selection at the gene and genome levels have been developed. Artificial selection is much stronger than natural selection; therefore, evolutionary genomic detection methods detect artificial selection signals more effectively (Jensen *et al,* 2007). In the present study, the whole genome variations of domestic Bactrians and wild two-humped camels were characterised. Genome-wide selection signatures were also performed between domestic and wild two-humped camels, providing vital genomic information under the influence of natural and artificial selection. From the genomic structure, due to their close geographic location and genetic relationship, gene flow exists between domestic Bactrian camel populations from China's Inner Mongolia and Mongolia (Fig 1a,b). However, gene flow signaling is not strong in the domestic and wild two-humped camel populations mainly due to their independent maternal origin (Ji *et al,* 2009) and the limited survival environment (Taklamakan Desert, Arjin Mountains in the Lop Nur Lake region, and the Great Gobi Strictly Protected Area 'A') (Yadamsuren *et al,* 2019) of wild two-humped camels, which may also lead to the relatively pure preservation of their genomes.

Bactrian camels are an important animal species in the Gobi Desert of China and Mongolia. Long-term evolution and natural selection have resulted in unique biological characteristics for adapting to harsh desert environments, including cold and hot resistance, anti-starvation, and a strong immune system. The analysis showed that a large number of positive candidate genes in Bactrian camels were involved in circadian entrainment pathways (ko 04713), plant–pathogen interactions (ko04626), and circadian rhythm—fly pathways (ko04711). These enrichment pathways involved in the environmental adaptation hierarchy may explain Bactrian camels' unique desert environment adaptability. It is worth mentioning that HSP90AA1, a member of the HSP protein (heat-shock proteins) family, completes positive selection in domestic Bactrian camels from IMG_D and MG_D, which relates to the temperature of living camels. The function of HSP is to protect cells from heat shock by resisting the denaturation of cellular proteins (Feder and Hofmann, 1999). In

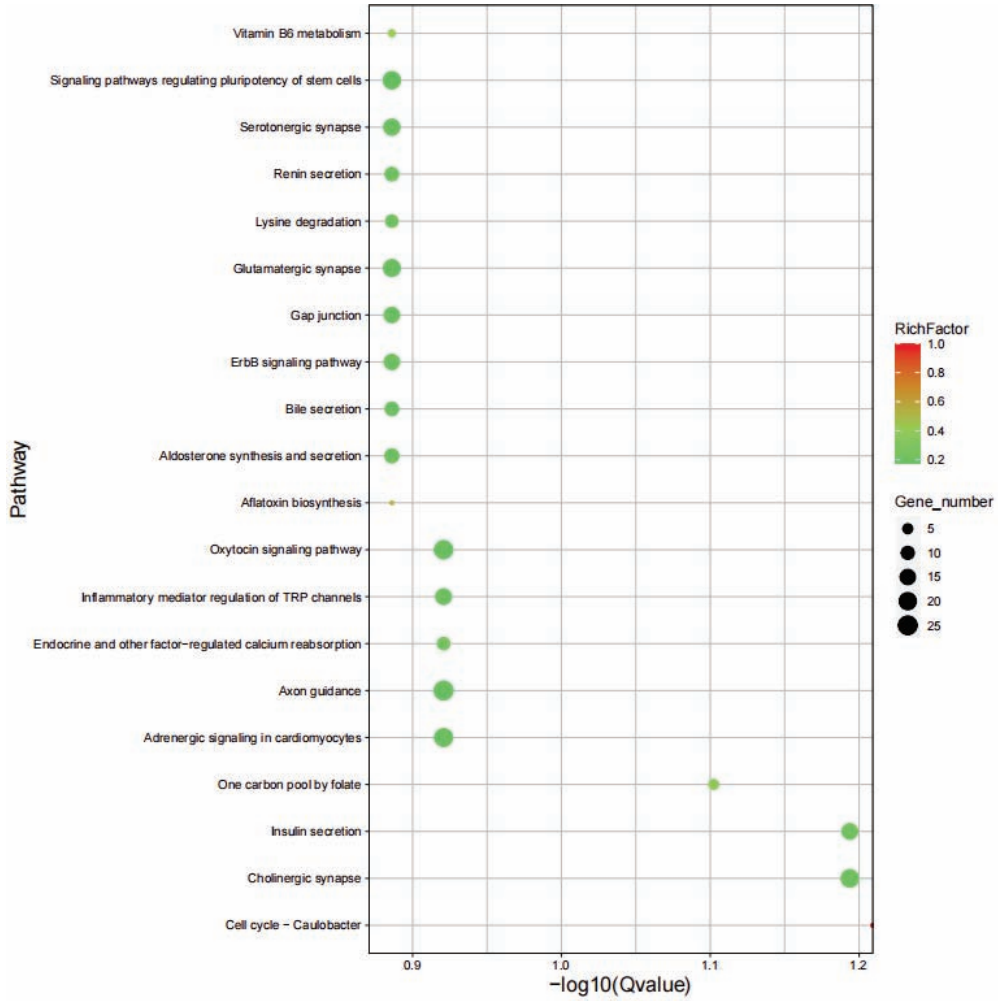

Fig S2. Significantly enrichment KEGG pathways canditate genes from domestic Bactrians and wild two-humped camels.

China's Inner Mongolia, the desert's temperature in summer is as high as 40 degrees, whereas in Mongolia, even in summer, the desert temperature is not as high. Therefore, this gene has been differentiated in Bactrian camel populations in Inner Mongolia and Mongolia.

Blood glucose levels in camels are higher than those of other animals (Al-Ali *et al,* 1988). In general, the blood glucose level of camels in a normal state (7.1 ± 0.3mmol/L) was higher than ruminants (2.5-3.5 mmol/L) and monogastric animals (3.5-5.0 mmol/L). The insulin content in camel blood (5 ± 1μU/mL) was lower than in sheep (12 ± 2μU/mL) and horses (7 ± 1μU/mL) (Elmahdi *et al,* 1997). Research has shown that the high level of blood glucose and low level of insulin in camels may be caused by their insulin resistance (Kaske *et al,* 2001; Guo *et al,* 2021). Insulin binding to receptors results in the tyrosine phosphorylation of insulin receptor substrates (IRS) by the insulin receptor tyrosine kinase (INSR). This process allows IRS association with the regulatory subunit of phosphoinositide 3-kinase (PI3K), which activates Akt. Insulin is mainly passed through the PI3K/Akt pathway to maintain the balance of glucose and lipid metabolism (Jensen *et al,* 2007; Taniguchi *et al,* 2006). In the present study, some candidate genes such as ABCC8, KCNJ11, FFAR1, PRKACB, CREB1, PRKACB, ACACA, SLC2A4, and AKT were enriched in the insulin signaling (ko04910) and secretion pathways (ko04911) between domestic Bactrian and wild two-humped camels, which may result in insulin responsiveness. According to reports, hibernating brown bears (Nelson *et al,* 2014) and reindeer (Elmahdi *et al,* 1997) that normally live during the snowy months or when food supplies are low also exhibit insulin resistance, which may benefit their survival in harsh conditions, similar to camels.

Bactrian camels have also well-developed, extraordinarily strong, and sensitive olfactory senses that can detect odours over distances up to 3 km

away. If they are downwind, the distance will be longer, at dozens of kilometers away. The KEGG pathway analysis of non-synonymous variations showed that more functional genes were enriched in the olfactory transduction (ko04740) pathway of domestic Bactrians (IMG_D and MG_D) than in wild two-humped camels (MG_W) (Fig 3). In wild two-humped camels, only 3 distribution areas have relatively simple vegetation; therefore, they do not need to distinguish many odours, and their olfactory receptors may be relatively small compared with domestic Bactrians. For these reasons, olfactory receptors are strengthened in wild two-humped camels and were an artificial selection during the domestication of domestic Bactrian camels, as confirmed by previous findings (Jirimutu *et al,* 2012).

Furthermore, several significant pathways associated with lipid metabolism and the immune system have been analysed. Candidate genes such as ECI1, ACADL, ACSBG2, DGK, and CDS1 were enriched in fatty acid degradation and glycerophospholipid metabolism. The protein encoded by the ECI1 gene is a key mitochondrial enzyme involved in the β-oxidation of unsaturated fatty acids. The ACADL gene is responsible for the beta-oxidation of fatty acids within the mitochondria. The ACSBG2 gene indirectly acts upstream or within the fatty acid metabolic process. Diacylglycerol kinases (DGKs) are regulators of the intracellular concentration of the second messenger diacylglycerol (DAG) and thus play a key role in cellular processes (Riese *et al,* 2016). The CDS1 gene encodes an enzyme that regulates the amount of phosphatidylinositol available for signaling by catalysing the conversion of phosphatidic acid to CDP-diacylglycerol (Huang and Freter, 2015). These genes may enhance a camel's energy storage and production capacity in the desert. In addition, seven significant immune systems related to KEGG pathways were identified, and 19 candidate genes were enriched in these pathways. These genes may be related to Bactrian camels' unique immune system, which allows them to adapt to the changing desert environment.

Conclusions

The present study provided comprehensive insights into the candidate regions for signatures of positive selection in the genome of domestic Bactrians from China and Mongolia and wild two-humped camels from Mongolia. Several candidate genes in have been identified 3 camel populations, which have essential roles in metabolism, insulin resistance, olfactory transduction, environmental adaptation, and other characteristics. These results provide evidence of selection in camels for adapting to the harsh arid conditions of desert environments and may provide some perspective on disease-resistance research.

Competing Interests

The authors declare that they have no competing interests.

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CVRL DROMEDARY SCIENTIFIC SYMPOSIUM

Tuesday, 10.12.2024			
WELCOME RECEPTION (opening ceremony) - 8:00 - 8:45			
Session	**Time**	**Authors**	**Presentation**
Surgery / Orthopaedic	9:00 - 9:30	Matthew De Bont	Modern aspects of surgeries in dromedary camel
Parasitology	9:30 - 10:00	Christiana Hebel	Severe infestation of dromedary camels with Sarcoptes scabiei dromedarii and its treatment
	10:00 - 10:30	Rolf Schuster	Cryptosporidium and Cystoisospora in camel calves
Herd and Farm Management	10:30 - 11:00	Sven Hammer	Management of Bactrian camel in Germany - (virtual)
COFFEE BREAK (11:00 - 11:15)			
Herd and Farm Management	11:15 - 11:45	Peter Nagy - Jutka Juhasz	Constant and new challenges of animal health on a large -scale camel dairy farm over two decades
	11:45 - 12:15	Annika Müller	Morphology and advanced examination techniques of the dromedary camel udder
	12:15 - 12:45	Marine Chemin	Morphological features of dromedary camel placenta
LUNCH BREAK (12:45 - 2:00)			
Herd and Farm Management	2:00 - 2:30	Fatma Al Mheiri	Catheterisation of female dromedary camel urinary bladder
Regenerative Camel mèdicine	2:30 - 3:00	Sunitha Joseph	Establishment of two permanent dromedary camel cell lines for virus isolation
	3:00 - 3:30	Ahsan Ul Haq	Application of stem cells in dromedary therapy
	3:30 - 4:00	Mohammed Al Qassim	The oestrous cycle in the Camelus dromedarius and the physiology behind induced ovulation
	4:00 - 4:30	Lulu Skidmore	Challenges of short and long term preservation of dromedary camel (Camelus dromedarius) embryos and semen

PREFACE

2024 has been designated as the International Year of Camelids, paying homage to camels, the pivotal role they played in the history of human civilisation in many parts of the world, their unique characteristics, cultural significance and ecological importance. As the centre of Camel research in the Middle East, the Central Veterinary Research Laboratory (CVRL) in Dubai, United Arab Emirates invited camel researchers to a dromedary symposium on 10-12-2024 at the Dubai Police Academy. The program of the symposium is diverse with 12 lectures on many dromedary camel subjects. Archaeological research has shown that the camel family evolved in North America millions of years ago and was domesticated around 3000 years ago on the Arabian Peninsula. The main species include three Old World Camel species and four New World Camelids.

The organiser from CVRL thank Dr. TK Gahlot, Chief Editor of Journal of Camel Practice and Research (JCPR) to publish the extended abstracts of the lectures in a separate section of JCPR.

U. Wernery

ABSTRACTS OF THE PAPERS PRESENTED

MORPHOLOGY AND ADVANCED EXAMINATION TECHNIQUES OF THE DROMEDARY UDDER*

A. Mueller[2], U. Wernery[1], J. Kinne[1], P. Nagy[3], J. Juhasz[3], M. De Bont[4], C. Booth[4], P. Azmanis5, A. Appelt[1], T. Wittek[2]

[1]Central Veterinary Research Laboratory, P.O. Box 597, 0000 Dubai, UAE,
[2]Vetmeduni Vienna, Clinic for Ruminants, Veterinärplatz 1, 1210 Wien, Austria, [3]Emirates Industry for Camel Milk Products, Al Ain Road- Um Nahad 3 area, 0000 Dubai, UAE,
[4]Dubai Camel Hospital, Al Marmum-Al Ain Road, 0000 Dubai, UAE,
[5]Dubai Falcon Hospital, Al Saada St, Zaabeel 2, 0000 Dubai, UAE

The aim of the present study was to examine the mammary gland of dromedary camels using measurements, injection casts, frozen sections, ultrasonography, endoscopy and radiography. Ultrasonography and endoscopy were used to diagnose pathological conditions of the mammary gland. For this purpose, 25 udders from camel carcasses, submitted to CVRL in Dubai for necropsy were examined.

Forty nine udders were obtained from a local slaughterhouse for further examinations. These udders were analysed using ultrasonography and radiography, with and without contrast medium, cast with injection of resin, gelatine or paraffin and frozen sections of native or casted udders were examined.

Additionally, 11 lactating dromedary camels were selected for ultrasonographic examination of udder. The injection casts and frozen section of the udder of the dromedaries shows a separation between the two teat canals and the two glandular complexes of each quarter. The structure of the teat and the parenchyma shows that the dromedary has specific anatomic structures which are important to know for application of machine milking and for improving.

Udder Health

Radiography, in contrast, did not prove a useful tool visualisation of internal udder structures. In addition, it did not allow interpretation due to the overlapping soft tissue. Radiography, therefore, seemed to be the least feasible as diagnostic tool for camel udder and teats.

The ultrasonographic and endoscopic examination proved a feasible tool for non-invasive diagnostics as well suitable for diagnostics for udder health in non-sedated animals. The examinations of camels retrained in a chute were easy to perform. However, additional examinations were necessary to evaluate the patholog through ultrasonographic and endoscopic images of the udder and teat of camels.

*extract from thesis submitted for the degree of DOCTOR MEDICINAE VETERINRIAE to the University of Veterinary Medicine in Vienna, Austria.

SEVERE INFESTATION OF DROMEDARY CAMELS WITH SARCOPTES SCABIEI

Christiana Hebel, Dr. med. Vet., MSc One Health

Central Veterinary Research Laboratory, Dubai, UAE

This case report details a notable outbreak in the United Arab Emirates, highlighting the importance of a One Health approach in managing such diseases. A camel farm in the UAE experienced a significant outbreak of sarcoptic mange. One camel, displaying severe skin lesions and intense pruritus, was brought to a treatment facility. Upon examination, the camel was diagnosed with a heavy infestation of *S. scabiei* mites. The outbreak spread rapidly, affecting both the resident camels and the human handlers at the facility. Despite wearing protective gear, several workers developed intense pruritic skin lesions consistent with pseudoscabies.

Both the infected camels and the affected humans received appropriate treatments for scabies. However, controlling the outbreak in the camel population proved to be challenging. The mites are highly contagious and can easily spread among animals, even with stringent hygiene measures. Additionally, the thick hides of camels can make it difficult to eradicate the mites completely, increasing the risk of reinfection.

Sarcoptic mange is a highly contagious disease that can affect a wide range of mammals, including human beings, which are considered dead end hosts. While camel-to-human transmission is not as common as in other species, this case demonstrates the potential for such infections to occur. The outbreak underscores the importance of early detection, strict hygiene practices, and quarantine measures to prevent the spread of the disease.

CHALLENGES IN SHORT- AND LONG-TERM PRESERVATION OF DROMEDARY CAMEL (*Camelus dromedarius*) EMBRYOS AND SEMEN

JA Skidmore, CM Malo and BP Mulligan

Camel Reproduction Centre, PO Box 79914, Dubai, UAE

Corresponding author- JA Skidmore: luluskidmore@cvrl.ae

The growing interest in camels for racing, milk production and beauty competitions has generated enthusiasm to breed more genetically superior animals by using assisted reproductive techniques. Protocols for embryo transfer and artificial insemination of fresh camel embryos and semen are now well established; yielding pregnancy rates of 65-75% for embryo transfer and 45 - 50% for artificial insemination. However, short- and long-term preservation of embryos and semen becomes important when there are not enough synchronised recipients at the time of transfer/insemination, or should embryos/semen need to be transported between farms or even different countries.

Short and long-term preservation of embryos.

Short-term liquid-phase storage of embryos at 37°C room temperature and 4°C is possible for up to 3 days with similar overall pregnancy rates ranging between 40 – 50%, but success depends on the quality of the embryos. For instance, high-grade embryos can survive cooling and culture, whereas low quality embryos improve in culture but do not survive prolonged cooling. Catalase, an antioxidant necessary for the decomposition of H_2O_2, added to the embryo holding media before cooling has been shown to have a beneficial effect on pregnancy rates for embryos held at 4°C for up to 48 h (-CAT 18% vs +CAT 64%).

Unlike in other species, however, cryopreservation of camelids embryos is still a challenge. Recent advances in vitrification of camel embryos have yielded pregnancy rates of 46%, although success rates depend on size and quality of the embryos. Smaller (300 - 500µm), good quality (Grade 1 or 2) embryos freeze better than larger embryos (44% vs 33%, respectively). Attempts to reduce the size of large embryos by aspirating the contents of the blastocoel did not have any beneficial effect: only 1/6 embryos aspirated resulted in a pregnancy.

Short and long-term preservation of semen

Short-term preservation of camel semen at 4°C for 24 h has also been achieved, yielding pregnancy rates of 40-60%. In addition, catalase added to diluted semen after chilling it to 5°C in 2 h, improved motility rates,

decreased percentage of dead and abnormal sperm over the storage period of 5 days and increased pregnancy rates from 22.2% to 37.5%.

Despite many attempts, cryopreservation of camel semen still remains an enigma. The quality and volume of a semen ejaculate is generally low making it difficult to obtain enough viable sperm for freezing. However, although quality and motility of semen samples can be improved using single-layer centrifugation over a colloid, this tends to lower sperm recoveries thereby further reducing the numbers of spermatozoa available for freezing. In our method of freezing semen, a final concentration of 3% glycerol in green buffer is the cryoprotectant of choice, straws are frozen at 1 cm above liquid nitrogen and thawed at 60°C for 10 sec. Post thaw motility rates can vary between 20 - 40% but pregnancy rates still remain <10%.

Whilst considerable advances have been made in all these techniques, they still present a challenge to ongoing research.

MODERN ASPECTS OF SURGERY IN THE DROMEDARY CAMEL

Matthew (Thijs) de Bont
Head of Surgery, Dubai Camel Hospital, Dubai, UAE

Traditionally surgery in the dromedary camel has by and large been performed under field conditions, meaning that the range and complexity of procedures has been limited to those that can be performed without full aseptic technique and in a relatively short period of time. Camels have been immobilised with manual restraint, aided by a combination of local anaesthesia, sedation and in some cases total intravenous anaesthesia.

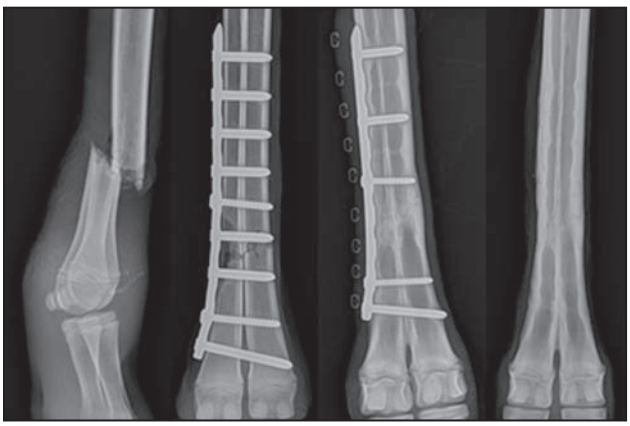

Fig 1. Repair of a complete open metatarsal fracture in a calf with a 4.5mm Narrow LCP in the various stages of healing, in a dromedary calf.

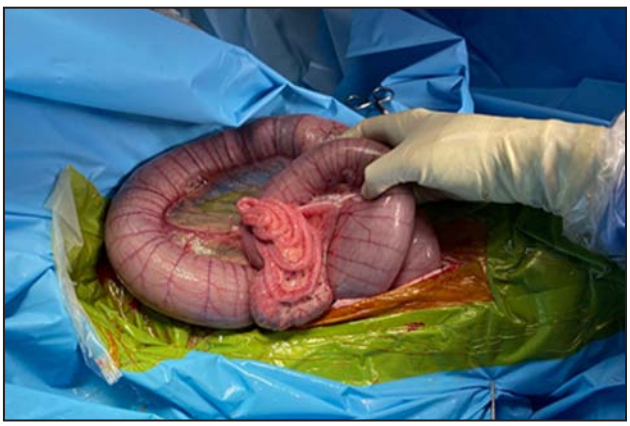

Fig 3. Exteriorisation of the spiral colon and a portion of the large colon via a ventral midline incision, in a dromedary calf.

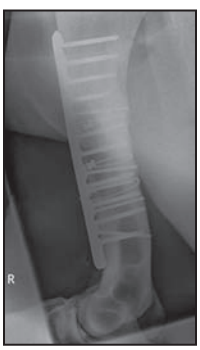

Fig 2. Repair of a right sided long oblique humerus fracture with a 5.5mm Broad LCP and stainless steel cables, in an adult dromedary camel.

The opening of our state of the art and fully equipped camel speciality hospital in Dubai has allowed for the development of a vast array of modern procedures, which have quickly become routine. Hospital facilities have allowed for the adaptation of well-established techniques from equine surgery to camels, in addition to the development of unique techniques specifically for camel breed related presentations.

Incorporation of multi-modal anaesthesia, where camels are intubated and maintained on isoflurane gas in combination with continuous rate infusions (ketamine/xylazine/lidocaine), as well as a full range of large animal monitoring equipment, allows for our animals to be safety anaesthetised in either sternal, lateral and dorsal recumbency for prolonged periods. Camels in our hospital are routinely anaesthetised for 4-5 hours at a time.

Abdominal surgical techniques are now routinely performed in dorsal recumbency through a ventral midline linea alba incision. This allows for a

more complete exploration of the abdomen, with almost the entire abdominal cavity open to palpation. Direct visualisation by exteriorisation of a significant portion of the small intestine, large colon, caecum and spiral colon is also easily achieved. Enterotomies and intestinal content evacuation are routinely performed, in addition to advanced resection and anastomosis techniques, including both hand sewn end to end jejunojejunal and stapled side to side jejunocaecal anastomoses. Caesarean section is also performed via a ventral midline approach.

Fracture repair techniques and in particular the utilisation of locking compression plates (LCP), has allowed for a wide range of repair of both long bone and complex mandibular fractures. Repair of humerus fractures in racing camels is now routine, which in many cases allows for a return to full limb function. Further commonly performed repairs involve the metacarpus/metatarsus and the proximal phalanx. Less common presentations which undergo repair include fractures of the olecranon, radius and tibia.

Techniques for amputation including upper limb, lower limb and digit amputations of both the fore and hind limbs, incorporating primary closure of the skin have been developed. Further arthroscopic and laparoscopic techniques, as well as surgical approaches to urogenital problems, oromaxillary surgery and advanced dental extraction techniques are routinely performed.

Although rapid advances have been made over the last years, the opening of several more speciality hospitals employing Board Certified Specialist large animal surgeons will allow for further modernisation of camel surgery, improving outcomes and ultimately animal welfare for years to come.

CATHETERISATION OF FEMALE DROMEDARY CAMEL URINARY BLADDER

Fatma Almheiri[1], Christiana Hebel[1], Jutta Friker[2]
[1]Central Veterinary Research Laboratory, Dubai, UAE
[2]Ludwig-Maximilians University, Munich, Germany

The collection of urine from female camels using a catheter is a rarely practiced technique. This is probably due to the anatomical peculiarities of the camel's urinary and sexual tracts. Without a certain amount of practice and knowledge of these particularities, it is not possible to perform catheterisation in a short period of time.

As with all domestic mammals, the urethra is significantly shorter in females than in males. This is an advantage that is utilised for the collection of urine. Hygienic urine collection is important for the diagnosis of any pathological changes of kidney and urinary bladder. The annular fold formed by the mucous membrane of the vagina around the external urethral ostium (Fig 1) and the sub urethral diverticulum (Fig 2) are disadvantageous for easy insertion of a catheter. The annular fold formed by the mucous membrane around the ostium urethrae externum, which is only found in camels, forms a second obstacle. This annular fold must be seen in connection with the small and frequently deposited volumes of urine.

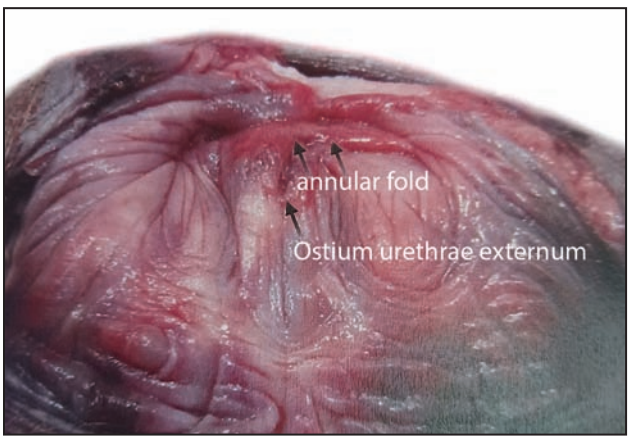

Fig 1. Ostium urethrae externum (Single arrow) and annular fold (double arrows) in the vagina of a female camel.

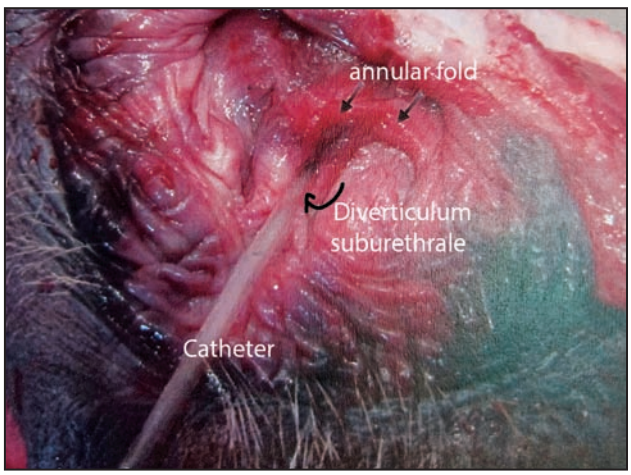

Fig 2. Placement of the urethral catheter close to the diverticulum suburethrde (Twisted arrow).

The special anatomical conditions are visualised with pictures, described and explained bwlow. The procedure for catheterising the urethra for urine collection is explained.

By knowing the basics and explaining the procedure, an important step can be taken in the animal health of female camels.

MORPHOLOGICAL FEATURES OF DROMEDARY CAMEL PLACENTA

M. Chemin[1], C. Hebel[2], J. Kinne[2], U. Wernery[2], J. Juhasz[3], P. Nagy[3]

[1]Warsaw University of Life Sciences, Poland; [2]Central Veterinary Research Laboratory, UAE;
[3]Emirates Industries for Camel Milk & Products, UAE

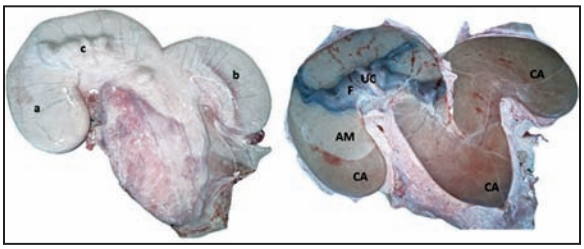

Fig 1. Side by side photograph of the pregnant uterus. Right side, uterus was opened to reveal placenta structures.

a: left horn, b: right horn, c: outline of foetus, CA: chorio-allantois, AM: amnion seen through transparent chorio-allantois, F: foetus seen through transparent CA and AM, UC: umbilical cord seen though transparent CA and AM

Understanding the dromedary camel's reproductive system is essential for sustaining the species' productivity, and the placenta plays a crucial role in supporting the development and birth of a healthy calf. This study provides a detailed examination of the placenta, highlighting its anatomical structures and their functions.

Necropsies performed at the Central Veterinary Research Laboratory in Dubai on a dead pregnant camel, revealed a non-deciduate, diffuse, and epitheliochorial placenta, similar to those found in equines.

The placenta contains several key components: the chorion, allantois, amnion, and a unique epidermal membrane. The chorion facilitates nutrient and gas exchange between mother and foetus through microvilli in contact with the maternal endometrium. The allantois, rich in blood vessels, vascularizes the chorion and amnion, and encapsulates the allantoic fluid. The amnion creates a protective, fluid-filled environment for the foetus, keeping it safe from outside traumas and temperature changes. The epidermal membrane, a thin layer unique to camelids, encases the foetus without obstructing airways after birth. This comprehensive anatomical understanding of the camel placenta provides essential insights for improving reproductive health and productivity in camel farming.

THE OESTROUS CYCLE IN THE CAMELUS DROMEDARIUS AND THE PHYSIOLOGY BEHIND INDUCED OVULATION

Mohammad AlQassim

Central Veterinary Research Laboratory, Dubai, UAE

Everything in the Arabian camel (*Camelus dromedarius*) is unique and spectacular, even the oestrous cycle's physiology. The oestrous cycle in the dromedary camel is seasonal polyoestrous, and ovulation is unspontaneous. It is defined by a recruitment and growth phase, maturation and stability phase, and regression phase. In the last three decades, the physiological mechanism behind induced ovulation in the dromedary camel has been deeply investigated, identifying a protein in the camel bull seminal plasma that is essential for ovulation. The protein, also known as ovulation inducing factor (OIF) is a neuronal growth factor classified in the beta group. Studies have illustrated its potent effect on the hypothalamic-pituitary-gonadal (HPA) axis in both Old World and New World camelids, increasing the ovulation rate, the size of the corpus luteum, and prolongation of the luteal phase with higher plasma progesterone levels. Different hypotheses have been developed discussing the delivery of OIF to the midbrain via copulation induced vaginal abrasion and its high lipid solubility, allowing it to pass into the vasculature to be delivered to the hypothalamus by systemic blood circulation. However, the mechanism by which it elects its effects on the HPA axis is still to be investigated. Understanding the seasonality of the oestrous cycle, the different stages, and the associated androgenic hormones is critical to a successful clinical approach to oestrous cycle manipulation for successful ovulation, fertilisation and embryo implantation.

ON INTESTINAL COCCIDIANS IN DROMEDARY CALVES – AN ANALYSIS OF NECROPSY AND PARASITOLOGICAL RESULTS

Rolf K. Schuster, Saritha Sivakumar and J. Kinne

Central Veterinary Research Laboratory, Dubai, UAE

A total of 1,593 camel calves were sent for necropsy and subsequent parasitological examination to the Central Veterinary Research Laboratory between January 2017 and June 2024. This article reports findings of intestinal coccidians in neonatal and young calves up to an age of 12 months. The content of rectum or small colon was examined for the presence of Cystoisospora orlovi and Eimeria spp. using the flotation method. Samples of diarrheic calves were examined for Cryptosporidium oocysts. Out of 1.437 samples (Tab. 1) examined with the flotation method, 94 were positive for *C. orlovi* and 55 samples contained Eimeria spp. oocysts. Cryptosporidium oocysts were detected in 72 out of 972 examined samples. The majority of *C. orlovi* oocysts were detected in camel calves in an age group up to four weeks of age, while Cryptosporidium oocysts were diagnosed mainly in three to eight weeks old animals. The youngest calf with an Eimeria infection was 30 days old but the majority of Eimeria positive camels had a body weight between 100 and 220 kg. Of 886 samples from necropsied adult dromedaries in the same time period, 74 were positive for Eimeria oocysts and *C. orlovi* was found one sample only. Examination of 42 diarrheic samples for Cryptosporidium oocysts gave negative results. Of 886 samples from necropsied adult dromedaries examined in the same time period, 74 were positive for Eimeria oocysts and *C. orlovi* was found in one sample only. Examination of 42 diarrheic samples for Cryptosporidium oocysts gave negative results.

Table 1. Frequency of intestinal coccidians in dromedary calves in Dubai between 2017 and 2024

Parasites	Examined samples (N)	Positives (n)	in %
Cystoisospora orlovi	1,437	94	6.54
Eimeria spp.	1,437	55	3.83
Cryptosporidium spp.	972	72	7.41

ESTABLISHMENT OF TWO PERMANENT DROMEDARY CAMEL CELL LINES FOR VIRUS ISOLATION

S. Joseph, N.A.G. Patteril, S.K. Elizabeth, R. Muhammed and U. Wernery

Central Veterinary Research Laboratory, PO Box 597, Dubai, UAE

Email: cvrl@cvrl.ae

Central Veterinary Research Laboratory (CVRL) established two permanent cell lines 25 years ago named Dubca (Dubai Camel) and CaKi (Camel Kidney) from a two-month-old camel fetus. Small pieces of skin and kidney were aseptically removed from the camel foetus and their cells were grown in culture flasks. Cells of the 7th passage were immortalised by infecting them with SV 40 (Simian virus) and cloned in soft agars. These high-quality permanent camel cell lines, free of Mycoplasma and viruses, particularly foot-and-mouth disease virus (FMDV) exhibited stable genotype and phenotypic characteristics. Therefore, the American Type Culture Collection (ATCC), USA accepted the authenticated and well-characterised Dubca cells, while the Collection of Cell Lines in Veterinary Medicine (CCLV) in Germany accepted the CaKi cells. These two permanent camel cell lines can be used by any researcher interested in viral diseases in camels or other animal species. The newest metagenomics analysis of viromes of dromedary camel fecal samples detected genomes of 8 different virus families, but not all of them produce cytopathic effects on cell cultures. Dubca and CaKi cells are susceptible to infection with viruses of several different families and are used for virus diagnosis and vaccine production. Virus isolation from camel tissues was successful for 12 different viral families which were camelpox virus, parapoxviruses, coronavirus, hepatitis E virus, West Nile virus, FMD from Bactrian camels, Newcastle Disease virus, MERS-CoV, astroviruses, bocaparvovirus, picobirna virus and circovirus.

MANAGEMENT OF BACTRIAN CAMEL IN GERMANY

Sven Hammer

Naturschutz-Tierpark Görlitz, Zittauerstr. 43, D-02826 Görlitz, Germany,
s.hammer@tierpark-goerlitz.de

Bactrian camels have been kept in German zoos and circuses for over 200 years. Over the last 25 years, private individuals have also become increasingly interested in keeping them. There are even tourist facilities offering hikes, camelback riding, animal-assisted therapy and products for Bactrian camels. Since the climatic requirements for Bactrian camels in Germany are different, the illnesses and associated therapies cannot always be directly derived from the experience in their home countries. Bactrian camels are subject to animal health regulations during transport, which are applied differently in Europe (TB, BTV, brucellosis, pseudotuberculosis). Due to government import controls, many of the world's known infectious diseases of Old World Camels (OWC) do not occur in Germany. The main focus of caring for Bactrian camels in Germany is on nutrition, husbandry methods, training, endoparasites and skin.

In order to compensate for the seasonal differences in the quality of roughage (grass, hay), supplementary feed is given. Vitamin and mineral supplements are particularly necessary for growing young animals, working animals, in the last third of pregnancy and for lactating females. For example, in most parts of Germany there is a selenium deficiency in the soil. Mineral and vitamin supplies should be available *ad libitum* as powder and administered as a feed supplement when there is an increased need. The combined supply of inorganic and organic mineral compounds (selenium, zinc, manganese, copper) is crucial. The salt requirements of OWC are significantly greater than those of other domestic animals, so free access to powdered livestock salt (NaCl) must be ensured separately from mineral and vitamin supplements. Due to very energy-rich pastures, Bactrian camels in Germany tend to be overweight. Therefore, access to fresh grass should be limited and straw should be available at all times to avoid compartment acidosis. Regular supply of leaves and twigs, also to promote tooth wear, is important.

The minimum husbandry guidelines for camels in Germany are binding. Since bulls can become very aggressive during the rut, the possibility of "hands-off" housing during the rutting season is necessary. A roof with a soft ground surface for comfortable bedding must be always available to each camel. In Germany it rains a lot and permanent wetness, despite fur, is not beneficial for the health of the Bactrian camel. Insufficient space, not separating the bull in time, and weaning calves under one year of age often lead to problems. The use of GnRH vaccines has been used in many zoological institutions in recent years to avoid such problems.

The specific handling of the animals (within the institution, in public, in traffic) requires adapted implementation and is subject to national safety guidelines and animal protection. Therefore, attention should be paid to targeted training (medical training, laying down, putting on a halter, etc.)

Infections with endoparasites that is overlooked or incorrectly treated leads to deaths in Bactrian camels in Germany. In addition to individual collected faecal samples over several days, the diagnosis must also include an evaluation by floatation, sedimentation and larval migration tests. In addition to the treatment of endoparasites (repeated after 14 days and rule of thumb dosage: 1.5 to 2 times the cattle dosage), pasture management and hygiene management are crucial. New research approaches, a look at the composition and influence (e.g.: combined use of anthelmintics) of the nemabion.

Due to the long fur of the Bactrian camels, skin infections are often overlooked in winter and cannot be diagnosed and treated in the early stages. In Germany, there are often multifactorial causes that can change during therapy. In addition to classics such as mange and zinc deficiency, mycotic infections are becoming more common. Therefore, the combined treatment against possible causes has proven to be effective. Infestation by black flies in the summer months is a growing problem, sometimes associated with allergic reactions. Unfortunately, repellents only have a very limited effect.

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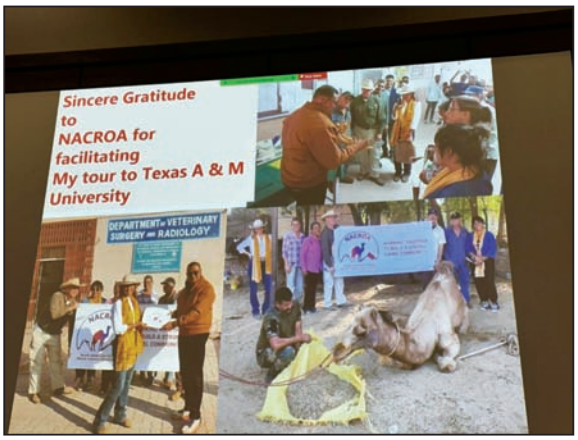
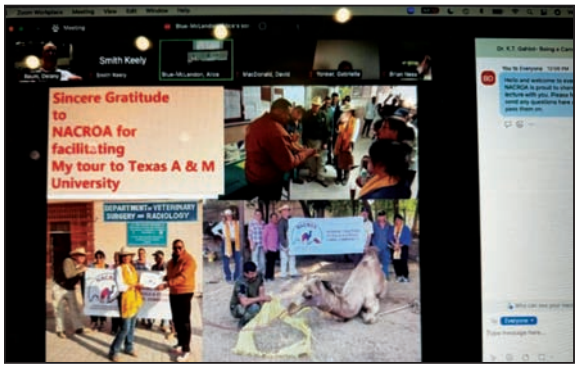
Wernery U, Kinne J, Schuster RK (2014) Camelid Infectious Disorders. OIE, Paris, p 500

DR. T.K. GAHLOT DELIVERED A LECTURE ON CAMEL SURGERY AND GIFTED CAMEL BOOKS AND JOURNAL OF CAMEL PRACTICE AND RESEARCH IN TEXAS A&M UNIVERSITY

Dr. T.K. Gahlot, Editor, JCPR delivered a lecture at Texas A&M University, Texas, USA on 18th September 2024 on "An overview of camel clinic facilities and camel surgery in India" in the International Year of Camelids 2024, both physical and virtual mode. Thanks to the North American Camel Ranch Owner Association (NACROA), specially Douglas Baum and Valeri Crenshaw for organising and facilitating my lecture and visit. Special thanks to Dr Alice Blue McLendon, Clinical Associate Professor in the department of Veterinary Physiology and Pharmacology, Texas A&M University, Texas, USA for arranging the lecture in the University and showing all the facilities at Department of Veterinary Surgery.





NEW STEM CELL LABORATORY OPENS IN UAE

Hortman Stem Cell Laboratory launches with prominent collaborations in healthcare innovation

Hortman Stem Cell Laboratory, the first GMP hybrid stem cell banking and molecular laboratory in the UAE, has officially launched its cutting-edge services in stem cell banking, regenerative medicine, and molecular and genomics testing. This milestone marks a pivotal moment for healthcare innovation in the region, with Hortman at the forefront of advanced molecular and genomics testing, stem cell banking, and regenerative medicine.

A key highlight was the signing of multiple Memorandums of Understanding (MOUs) with prominent partners, including the Central Veterinary Research Laboratory (CVRL), represented by Dr. Ali Ridha, Director General; Dubai Camel Hospital (DCH), represented by Mohammad Al Beloushi, Director; Germany's Marga-and-Walter-Boll-Laboratory for Cardiac Tissue Engineering (MWBL), represented by Prof. Dr. Kurt Pfannkuche, Principal Investigator; Eppendorf, represented by Akshay Kadalilae, Managing Director, Eppendorf Middle East and Africa; and the Manipal Academy of Higher Education (MAHE), Dubai Campus, represented by Dr. Sudhindra Shamanna, Academic President. Representing Hortman Stem Cell Laboratory was Dr. Fatma Al Hashimi. These partnerships will accelerate the development of advanced stem cell products, further establishing Hortman as a leader in medical innovation.

The launch of Hortman Stem Cell Laboratory marks a new chapter for healthcare in Dubai, with a focus on sustainable innovation and global collaborations. The lab hopes to play a key role in the UAE's Dubai 2030-2050 vision, contributing to the country's growth as a hub for world-class healthcare and scientific research.

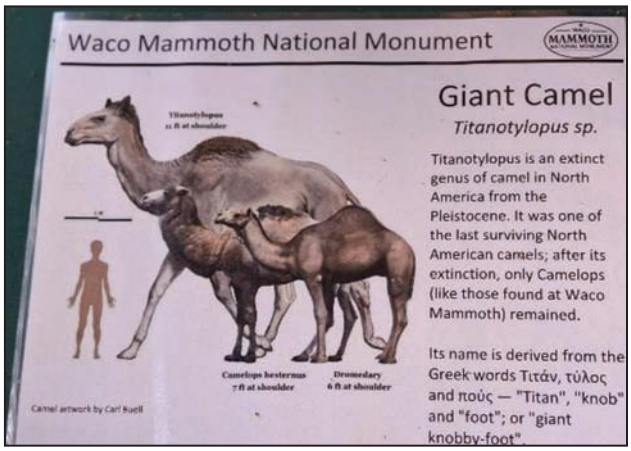
(Coutesy: The Khaleej Times; Tue, Oct 08, 2024)

CAMEL URINE AS A POTENTIAL SOURCE OF BIOACTIVE MOLECULES SHOWING THEIR EFFICACY AGAINST PATHOGENS: A SYSTEMATIC REVIEW

Authors searched three databases in order to point out relevant articles (Web of Science, Scopus and Google Scholar) until December 2022. Research articles of interest evaluating the antimicrobial effects of camel urine were selected. Overall, camel urine furnished promising antibacterial activities against gram-positive bacteria, namely Staphylococcus aureus (30 mm), Bacillus cereus (22 mm), Bacillus subtilis (25 mm) and Micrococcus luteus (21 mm), as well as gram-negative bacteria, especially Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter cloacae, andSalmonella spp., without forgetting its efficiency on Mycobacterium tuberculosis as well. The excretion also showed its potency against H1N1 virus, vesicular stomatitis virus and middle east respiratory syndrome coronavirus. Similarly, the camel urine featured strong antifungal activity against Candida albicans, Aspergillus niger, Aspergillus flavus and dermatophytes with a minimal inhibitory concentration of 0.625 µg/ml against Trichophyton violaceum, 2.5 µg/ml against Microsporum canis and 1.25 µg/ml against Trichophyton rubrum and Trichophyton mentagrophytes. This comprehensive review will be valuable for researchers interested in investigating the potential of camel urine in the development of novel broad-spectrum key molecules targeting a wide range of drugresistant pathogenic microorganisms.

Ressmi Amina, Raqraq Habiba, Barguigua Abouddihaj, Camel urine as a potential source of bioactive molecules showing their efficacy against pathogens: A systematic review,Saudi Journal of Biological Sciences, Volume 31, Issue 5,2024,103966, ISSN 1319-562X, https://doi.org/10.1016/j.sjbs.2024.103966.

EXPLORING CAMELS OF NORTH AMERICA WITH NACROA
GIANT CAMEL REMAINS AT WACO MAMMOTH NATIONAL MONUMENT



Mr. Douglas Baum and Ms.Valeri Crenshaw facilitated my tour to the Waco Mammoth National Monument (WMNM). I learnt here about archeological evidence of last surviving North American Camels. Giant camel (Titanotylopus Sp.) is an extinct genus of camel in North America from the Pleistocene. The skeleton found at WMNM was perhaps one of the last surviving North American camels.

Excavation at the site commenced in 1978 and was put on hold in 2001. Leaders within the City of Waco, Waco Mammoth Foundation, and Baylor University recognised the importance of preservation for the site. A successful fundraising campaign resulted in the Dig Shelter opening to the public on December 5, 2009. It is only one of two climate-controlled dig sites in the United States. The President Barack Obama proclaimed the site as Waco Mammoth National Monument through the Antiquities Act on July 10, 2015. This designation provides the protections needed to continue the site's education and preservation missions.

In 2009, the site containing the bones of a bull, a female, two juvenile mammoths, and the camel opened to the public. The attention the mammoths drew earned Waco standing in the international scientific community and increased tourism and commerce for the city. Most importantly, the mammoth discovery unlocked a fascinating part of Central Texas environmental history. Paleontologists have unearthed so far 24 Columbian mammoths, three camels, and a few other Ice Age creatures at what is now known as Waco Mammoth National Monument.

VISIT TO CAMP VERDE WITH NACROA

Camp Verde was a frontier post by the United States Army in July 8, 1855. It was head quarters in 1856 for 40 camels sent by secretary of war Jefferson Davis to be adapted and used in a system of overland communication with the west, which later proved impracticable. It was surrendered to the confederate government in 1861 and reoccupied in 1865 by the United States Army but later abandoned in April 1, 1869. I saw the murals at Ingram, Texas. Mural depicts the surprising arrival of camels at the U.S. Army's Camp Verde in the county. These were painted on the wall of a lumber company in Ingram, Texas. There were restaurants, post office and general stores at the site of Camp Verde showing good memoirs of presence of camels in USA.

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SUBJECT INDEX

INSTRUCTIONS TO CONTRIBUTORS

(Effective from the year 2025)

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

The Journal of Camel Practice and Research (JCPR) is a triannual journal (April, August and December issues) published in the English language by the Camel Publishing House, 67, Gandhi Nagar West, Near Lalgarh Palace, Bikaner, 334 001 (India). It is in offset print size of 20.5x27.5 cm in two columns with a print area of 17x22 cm. It will be known as **Journal of Camel Practice and Research** with **Volume number** on yearly basis and **Number** on issues per volume basis (in exceptional cases there can be more than three issues in a volume). The editorial policies of JCPR are established by the editor-in-chief and is detailed in this section. Views expressed in papers published in JCPR represent the opinions of the author(s) and do not necessarily reflect the official policy of the author's affiliated institution, or the editor-in-chief.

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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Editor in Chief of JCPR would consider retracting a publication, if: • They have clear evidence that the findings are unreliable, either as a result of major error (eg, miscalculation or experimental error), or as a result of fabrication (eg, of data) or falsification (eg, image manipulation) • It constitutes plagiarism • The findings have previously been published elsewhere without proper attribution to previous sources or disclosure to the editor, permission to republish, or justification (i.e., cases of redundant publication) • It contains material or data without authorisation for use • Copyright has been infringed or there is some other serious legal issue (eg, libel, privacy) • It reports unethical research • It has been published solely on the basis of a compromised or manipulated peer review process • The author(s) failed to disclose a major competing interest (aka, conflict of interest) that, in the view of the editor, would have unduly affected interpretations of the work or recommendations by editors and peer reviewers.

Publication Ethics and Malpractice Statement

JCPR is a peer-reviewed journal and ensures the highest standards of publication ethics. All parties involved in the act of publishing process (editors, authors, reviewers and the publisher) have to agree upon standards of ethical behaviour. The accepted principles of Publication Ethics and Publication Malpractice Statement based on the Code of Conduct and Best Practice Guidelines for Journal Editors of the Committee on Publication Ethics – COPE (available at http://publicationethics.org/) are followed.

Submission of manuscript: Manuscripts should be submitted in word files to **Dr. Tarun Kumar Gahlot,** Editor, Journal of Camel Practice and Research at tkcamelvet@yahoo.com or editorjcpr@camelsandcamelids.com by online submission portals available at www.indianjournals.com or www.camelsandcamelids.com. The figures can be submitted preferably as a high pixel JPEG or other format. The manuscript should be accompanied by a covering note and author consent letter and ethical statement declaration from the author responsible for correspondence. It should also contain a statement that manuscript has been seen and approved by all co-authors. Editor and members of the editorial board are not responsible for the opinions expressed by authors and reserves the right to reject any material or introduce editorial changes. Material will be accepted for publication on the understanding that it has not been published in any other form and is not being considered elsewhere. Any material once accepted for publication may not be republished in any form without prior permission of the author. **Single blind peer review policy** is used for the manuscripts submitted and the quality standards are maintained by the JCPR editorial board and by authors who submit manuscripts for publication. A preprint PDF is provided to the author in correspondence for verification of contents and corrections, if any.

Ethical declarations in research form is an important step while submitting a manuscript to the Journal of Camel Practice and Research (JCPR). Author has to look into several questions and statements before submission. These are listed below:

Pre-submission considerations for authors

- Manuscript should have been read and approved by all the authors. All the authors mentioned in the manuscript should have agreed for authorship and it's order. An author consent letter, duly signed should accompany the manuscript as per the given format (Author in correspondence can do it). (The authorship criteria should be based on the ICMJE guidelines.)
- Full names, institutional affiliations, highest degree obtained by the authors, e-mail address (in some cases, ORCiD ID and social media handles - Facebook, Twitter, or LinkedIn) need to be clearly mentioned on the title page.
- The corresponding author, who takes full ownership for all the communication related to the manuscript, should be designated and his/her detailed institutional affiliation (including the postal address, telephone number, fax number, and e-mail address) should be provided.
- In the research papers, JCPR expects a guarantor (who could be a senior research scientist or one of the author), he/she may be responsible for the integrity of the manuscript (including ethics, data handling, reporting of results, and study conduct), would communicate with the journal if any technical clarifications related to the manuscript are required, and would handle similar responsibilities.

All the authors need to agree on the name(s) included in the Acknowledgement section.

Manuscript submission related declarations: JCPR expects authors to declare the following:

- The manuscript in part or in full has **not been submitted or published anywhere**. In other words, the authors

should ensure that the manuscript is not a duplicate publication.

- The manuscript **will not be submitted elsewhere** until the editorial process is completed.
- If any part of the manuscript contains previously published content (figures/tables), authors should submit a **statement of permission to reproduce the material** signed by the author(s) and publishers concerned.
- When submitting material related to commercial products, it may, in some circumstances, be appropriate for the author to forward a copy of the contribution to the manufacturers before publication. This is to verify the correctness of the contents of the section in the manuscript that describes the new device/product.
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- If the manuscript is based on a dataset that has been the basis of another manuscript, authors should **maintain transparency** in such cases and should declare that by an appropriate reference.
- If there is a data set associated with the manuscript, provide information about where the data supporting the results or analyses presented in the paper can be accessed. Where applicable, this should include the hyperlink to publicly archived datasets, DOI, or other persistent identifier associated with the data set(s).

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If your study involves animals, and also if your manuscript includes case reports/case series, you need to provide the following:

- Authors must provide the **name of the ethical approval committee/Institutional Review Board** they have obtained consent from along with approval number/ID.
- Authors must state that **written informed consent was obtained** from the owners of the animals or head of the veterinary institutes where the research was carried out.

Declarations specific to article types

We have looked at the declarations related to manuscript submission and when your study involves human (for example-feeding camel milk to the diabetic human patients or autistic patients) or animal subjects. Let us now turn to specific article types and the declarations you need to prepare when submitting them.

1. **Clinical trials and research papers:** All clinical trials and planned research should be approved by the institutional ethics committee and should also be approved through an appropriate research committee of the institute (It should be depicted in the acknowledgement section).
2. **Reviews:** Reviews do not need any ethical approvals or informed consent. However, JCPR expects that the review should not be older than 4 decades.
3. **Short Communications or Case Reports:** These are rare clinical reports or new diagnoses or a new technique (pilot trials), usually published with or without abstract and keywords. These article types do not exceed 2-3 pages of the journal.

Other important declarations related to funding, conflicts of interest, and more

Apart from the declarations we have discussed, there are others that authors need to consider. Let us take a look at them:

1. **Describing new taxa:** Authors must provide relevant documents and unique digital identifier for manuscripts that describe new taxa or species. They should also declare that the relevant guidelines have been followed for algae, fungi and plants, zoological taxa, bacteria, and viruses. Registration numbers for the new species (for e.g. from **MycoBank** for fungi or **ZooBank** for zoological species) should be stated in the manuscript. New virus names should be sent to the relevant study groups for consideration before publication in a journal.
2. **Authors' contribution**: The individual contributions of authors to the research work and writing of the manuscript should be specified in this section; for example, who conceived the study design, who did the data acquisition, who performed the experiments, who did the data analysis, who wrote the manuscript, etc. Authors should check journal-specific guidelines to declare the authors' contribution.
3. **Acknowledgements:** Anyone who does not meet the authorship criteria, such as people who provided technical help, institutional/department head who provided general support, or scientific writers who assisted with the preparation of the manuscript content, should be acknowledged. Even if the authors have no one to acknowledge, usually JCPR expects authors to include this section in the manuscript and write "Not applicable."
4. **Funding:** All sources of funding for the research work and their role (if at all) in the design of the study and collection, analysis, interpretation of data, and in writing the manuscript should be declared. Provide the name(s) of the funding agency/agencies along with the grant number(s). If the study did not receive any funding, report the same.
5. **Competing interests/conflict of interest:** All financial and non-financial competing interests must be declared by the authors. Non-financial competing interests include a declaration of political, personal, religious, ideological, academic, and intellectual competing interests. Authors from pharmaceutical companies, or other commercial organisations that sponsor clinical trials, should declare these as competing interests on submission.

Authors should declare any personal conflict of interest including any association with consultancies; employment details; participation in advocacy groups; stock or share ownership, and any financial details with regard to grants; fees; honoraria, reimbursements royalties, and any registered patents. They should also declare any institutional conflict of interest, i.e. if their employer has any financial interest in or is in conflict with the subject matter or materials discussed in

the manuscript. If there is no disclosure, add the following statement: "No potential conflict of interest was reported by the authors."

It is mandatory and important that the authors declare all the above-mentioned statements to avoid un-submission of the manuscript. These declarations ensure ethical publication publication of the manuscript. JCPR expects from all the authors of manuscripts to read these practices involving transparency and integrity in the guidelines from the webpage of www.camelsandcamelids.com in the Instructions to Contributors section.

Initial Checks

The JCPR staff and in-house editorial team perform an initial quality check to identify potential issues such as:

- Competing interests
- Compliance with editorial policies and ethical standards
- Financial disclosures
- Data availability

Submissions may be returned to authors for changes or clarifications at this stage.

Editorial Review

After completing internal checks, each new submission is assigned to an Academic Editor (Usually Editor in Chief) with relevant subject matter expert. The editor reviews the manuscript against our publication criteria and determines whether reviews from additional experts are required to evaluate the manuscript. The Editor in Chief decides about further handling of the manuscript by usually a member of the Editorial Board of JCPR, but occasionally a Guest Editor is invited to serve instead.

Peer Review

The Editor in Chief selects reviewers, whether internal or external, based on expertise, publication history, and past reviews, and invites them to provide feedback on the manuscript. After agreeing to review, external peer reviewers typically have 10 days to submit their review. The journal office will follow up with late reviewers and keep you informed if there are any delays. **JCPR uses single-blind peer review policy**. Reviewers remain anonymous in our submission system.

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- Major revision
- Minor revision
- Accept

Decisions are communicated to the corresponding author in a formal letter, along with reviewer feedback and any other requirements from the journal office.

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In most cases, the revised manuscript is re-assigned to the original referee. The Editor in Chief may make a new decision based on their own assessment of the revised manuscript and your response to reviewers, or request additional input from external peer reviewers.

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PROTEOMIC CHARACTERISATION OF SERUM DURING THE BREEDING CYCLE IN MALE BACTRIAN CAMELS

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]

[1]Key Laboratory of Dairy Biotechnology and Bioengineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, China

[2]Inner Mongolia Institute of Camel Research, Badanjiran, Inner Mongolia, China

[3]Alxa League Institute of Animal Husbandry, Alxa, Inner Mongolia, China

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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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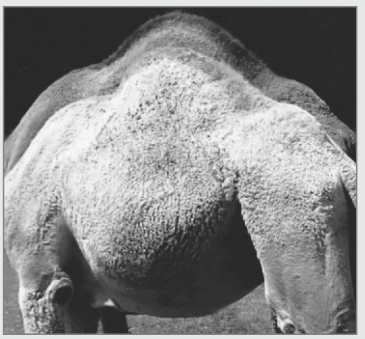

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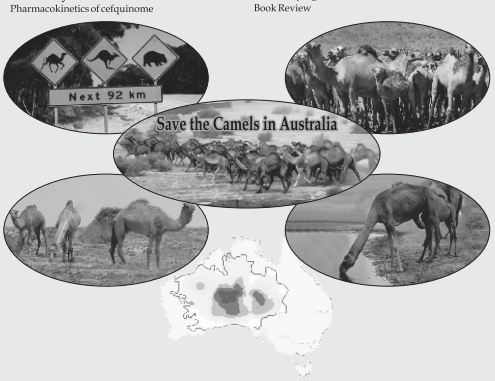

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